Characterization of *C. debilis* GB1 a Thermophilic Facultative Anaerobe Capable of Lending Aerotolerance in Co-culture with *C. thermocellum*

by

Scott Wushke

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Scott Wushke

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Supervisory Committee

Dr. Richard Sparling (Department of Microbiology, University of Manitoba)

Supervisor

Dr. Nazim Cicek (Department of Biosystems Engineering, University of Manitoba)

Committee member

Dr. Karen Brassinga (Department of Microbiology, University of Manitoba)

Committee member

Abstract

Cellulosic ethanol production is mainly described as an anaerobic process. Through use of aerobic selective pressure, using inoculate from samples taken from a farm in South-Eastern Saskatchewan, Canada, we were able to create highly cellulolytic aerotolerant enrichments which produced both hydrogen and ethanol. We were able to recreate this highly cellulolytic aerotolerant phenotype using *C. thermocellum* DSM 1237 and one of the *Caldibacillus debilis* strains isolated from the aerotolerant enrichments, strain GB1.

Caldibacillus debilis GB1 is a thermophilic facultative anaerobe. *C. debilis* GB1 displayed a physiology distinct from that of the type strain Tf which is an obligate aerobe. Under oxygen limiting conditions, both GB1 and Tf produce end-products lactate, acetate, formate and CO_2; however, GB1 alone produces ethanol.

A key feature necessary for aerotolerant growth of the co-culture with *C. thermocellum* was the capacity of *C. debilis* GB1 to grow both aerobically and anaerobically. In order to characterize GB1, we sequenced and annotated the genome then used high throughput proteomics to characterise the protein expression changes between aerobic and anaerobic metabolism. We found *C. debilis* GB1 has typical mixed fermentation pathways and aerobic/anaerobic regulation similar to those found in *E. coli* K-12 and *B. cereus*.

In order to explain the lack of ethanol production in the type strain, *C. debilis* Tf, we did a genomic comparison of both strains Tf and GB1, focusing on the genes concerning pyruvate metabolism. In particular, there were amino acid changes in the

aldehyde-alcohol dehydrogenase ADHE that could lead to inactivity in strain Tf. This genomic evidence, plus Tf having physiology consistent with an *adhE* deletion mutant in the closely related *Geobacillus thermoglucosidasius,* leads us to propose that the *adhE* gene is either differently regulated or inactive in *C. debilis* Tf.

From our work, we showed that *C. debilis* GB1 allowed us to further define and characterize the physiology of the genus *Caldibacillus*, find a new aerotolerant mode of mode of Consolidated bioprocessing using *C. thermocellum*, and further understanding of aerobic/anaerobic regulation in thermophilic facultative anaerobes.

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Thank you to Ariadne Valadares who suffered through so much proof reading for me.

Dedication

To my parents, who never stopped believing in me.

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Chapter 2: Wushke S, Levin DB, Cicek N, Sparling R. 2013. "Characterization of Enriched Aerotolerant Cellulose-Degrading Communities for Biofuels Production Using Differing Selection Pressures and Inoculum Sources". *Canadian Journal of Microbiology*. 59:679-683. http://dx.doi.org/10.1139/cjm-2013-0430.

Chapter 3: Wushke S, Levin DB, Cicek N, Sparling R. 2015. "Characterization of the Facultative Anaerobe *Caldibacillus debilis* GB1 and its Use in a Designed Aerotolerant, Cellulose Degrading, Co-Culture with *Clostridium thermocellum*". *Applied Environmental Microbiology*. 7:35-15. http://dx.doi.org/10.1128/AEM.00735-15.

Chapter 1: Developing Consortia for Ethanol Production from Consolidated Bioprocessing

1.1. Introduction and Justification for Use of Consolidated Bioprocessing

The establishment of a sustainable and environmentally conscious economy is essential for combating the effects of man-made climate change. Biofuels are an obvious and logical replacement for fossil fuels given that they can create a closed-carbon-loop, resulting in reduced Green House Gas (GHG) emissions (DeCicco *et al.* 2013). One biofuel production process that looks particularly attractive is Consolidated Bioprocessing (CBP). A one-step hydrolysis and fermentation process, CBP uses lignocellulolytic substrates (e.g. wood, and wood waste, straw and other structural residues from food crops) to create ethanol or hydrogen. Currently, this process is not cost competitive, but if the process could be done effectively, it is projected to be one of the least expensive forms of ethanol production (Lynd *et al.* 2005, Olsen *et al.* 2012, Jouzani *et al.* 2015). In order to make CPB work as an economically viable process, we need to study multiple modes of operation, with different and unique bacteria in order to find the most economically viable production conditions (Jouzani *et al.* 2015, Olson *et al.* 2015). At this time, CBP is not operating at its theoretical maximum economic efficiency (Lynd *et al.* 2005; Jouzani *et al.* 2015). Making CBP financially beneficial is required before the adoption of this technology becomes sufficiently attractive to potential users. It is not clear whether this process can be optimized using a single organism, or require several organisms working in concert. It is also unclear whether there may be further improvements

from not yet discovered organisms or consortia (Jouzani *et al.* 2015). There is intense research into CBP using both pure cultures (Jouzani *et al.* 2015) and co-cultures consisting of cellulose degraders partnered with non-cellulolytic organisms (Hann *et al.* 2015). In order to further develop CBP, it is important to continue to bio-prospect, and to characterize novel organisms and organism combinations under conditions that may encourage CBP. As we characterize the physiological aspects of these organisms, it is also important to apply high throughput omics techniques. These techniques will help build the foundation of knowledge required to generate new hypotheses, as well as allow us to make inferences about other organisms and their abilities in order to fully explore all CBP options. (Olson *et al.* 2015).

1.2. The Need for Biofuels in 2016

Non-renewable fuels are a major producer of GHG emissions. Not only do they contribute to climate change, but the manipulation and burning of non-renewable fuels contributes a plethora of additional pollutants such as sulfur and BTEX (benzene, toluene, ethylbenzene, xylene) hydrocarbons (Kampa *et al.* 2008, Demirbas *et al.* 2009). Transitioning to renewable sources of fuel is an essential step in minimizing future increases of net GHG production as they can offset the CO_2 produced through combustion (DeCicco *et al.* 2013), theoretically decreasing net CO_2 emissions, as shown in Figure 1.1.

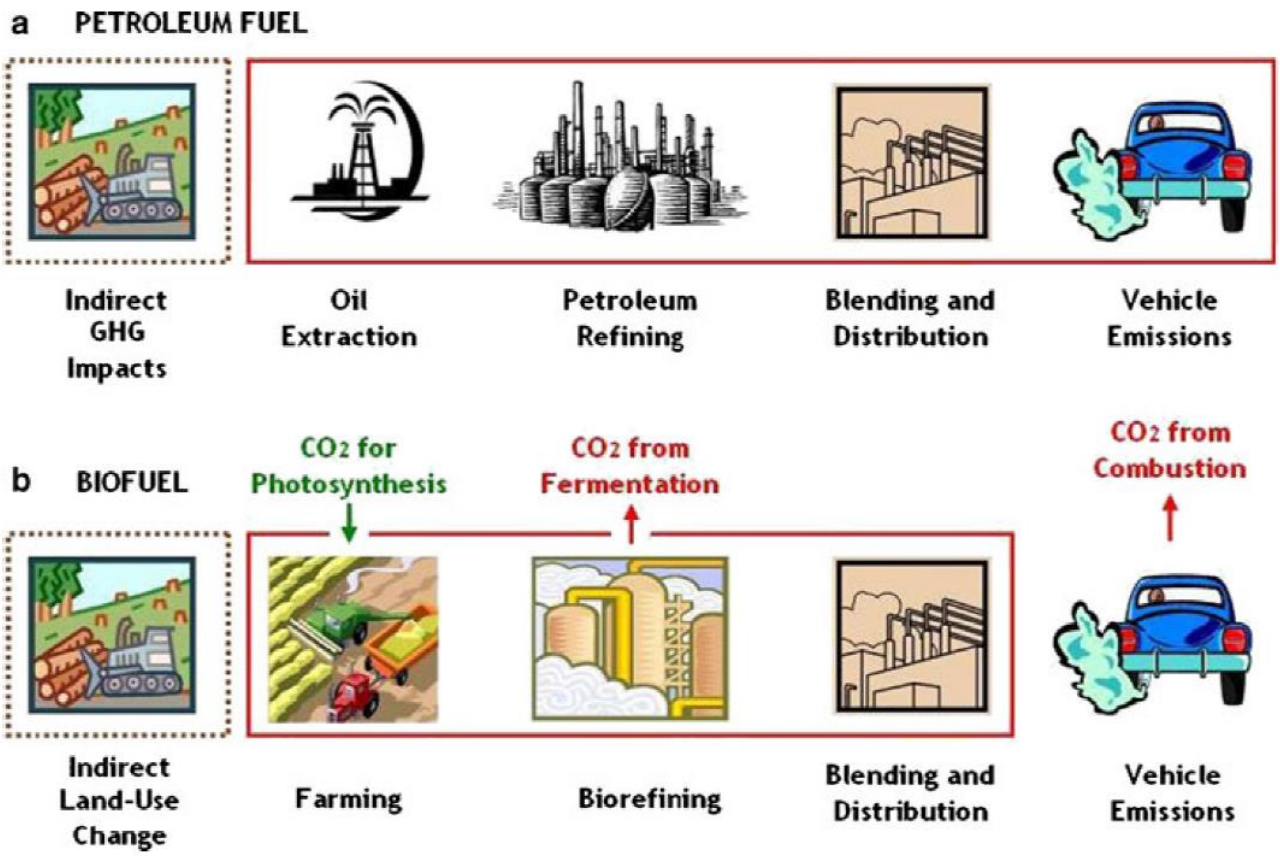


Figure 1.1. Carbon emission diagram where the CO_2 is both the input and output offsetting net CO_2 emission when using biofuels (b) when compared to petroleum fuel (a). (Source: DeCicco *et al.* 2013)

Due to their potential environmental and economic benefits, biofuel and biofuel co-product production is currently a highly active area of research with major interest involving, but not limited to: production of bio-oils (Koutinas *et al.* 2014), bioalcohols (Wheals *et al.* 1999), and bioplastics (Noda *et al.* 2010). In order to entice large private-sector entities to begin mass production of biofuels, an emphasis on the economic benefits is necessary. There are many examples of biofuel producing industries operating in an economically viable capacity (Wang *et al.* 2012, Koller *et al.* 2010, Olagunju *et al.* 2008). Bio-oils are well established at the economic scale, and have been shown to be economically viable with reliance on agricultural crops such as canola or palm plants (Kwon *et al.* 2015, Johari *et al.* 2015). However, due to the displacement of food or feed producing crops, such practices are being called into question. There is also intense research into bio-oils from microalgae, which can be grown anywhere in a controlled environment, and do not displace land to the same degree as crops (Gao *et al.* 2015). Bioplastics such as PHA (polyhydroxyalkanoates) and PHB (polyhydroxybutyrate) are both mass-produced and intensively researched (Koller *et al.* 2010). They utilize a variety of organic substrates such as sugar, glycerol, and acetate, and produce a wide selection of bioplastics depending on substrate, organism, and media condition (Koller *et al.* 2010). Ethanol and mixed solvents [acetone butanol plus ethanol (ABE)] are two prominent examples of bioalcohol production resulting in profit (Chen *et al.* 2015, Kong *et al.* 2015). Historically, food products such as wheat and barley have been the prominent substrates used in bioalcohol production, but there has recently been more research done in non-food bioalcohol production, such as lignocellulose to avoid ethical

concerns about increasing food prices due to crop displacement and to further increase total biofuel productivity by expanding substrates (Christian *et al.* 2010, Jouzani *et al.* 2015,).

There is room for further developments and increases in economic viability on several fronts in bioalcohol production using CBP (Lynd *et al.* 2005, Jouzani *et al.* 2015). The creation of biofuels and related co-products is a dynamic process that can utilize and create its own distinct waste streams. Efficiently utilizing every organic waste stream for cost effectiveness is important. Many of these processes create low value, high volume outputs, but there is potential to link these processes to the production of high value, low volume products such as resins from lignin (Demirbas *et al.* 2009) or high value bioplastics for specific purposes (Chen *et al.* 2009). Currently, the study of biofuels is a highly active area of research for both economic and environmental reasons, and there is a strong political encouragement to make biofuels displace fossil fuels. To what extent this is possible remains unclear (Solomon *et al.* 2015. Doshi *et al.* 2016, Chen *et al.* 2016).

1.3. Ethanol and Hydrogen

Ethanol and hydrogen are common microbial biofuels (Balat *et al.* 2011, Levin *et al.* 2004) produced by a wide range of microorganisms, and as such are a common focus of study within the realm of biofuels (Jouzani *et al.* 2015, Ghimire *et al.* 2015). Chemically, hydrogen and ethanol offer several advantages and disadvantages for use as biofuels. In vehicles for instance, hydrogen can be 60-75%

efficient through conversion directly into electricity by proton exchange membranes (Lamy *et al.* 2016), giving it a distinct advantage over internal combustion engines that typically have an upper limit of ~37% efficiency (Ferguson *et al.* 2015). However, hydrogen is considered difficult to work with due to its chemical properties such as causing hydrogen embrittlement (Daw *et al.* 1983). It is also a small molecule prone to leaking when being stored or transported using piping (Okazaki *et al.* 2003). The use of hydrogen through conventional combustion makes it less competitive than other flammable gases such as methane or propane, gases with better compressibility factors then hydrogen, which significantly reduce the cost of storage (Das *et al.* 1996). Up until now, little infrastructure has been developed around hydrogen as a fuel, making consumer adoption difficult without further investment in compatible infrastructure (Singh *et al.* 2015). There is still active research into hydrogen production, but it may be less favored over other biofuel types (Singh *et al.* 2015).

Ethanol production is appealing because it can use already existing infrastructure and established protocols for production allowing easy adoption by the consumer (Chen *et al.* 2015). The fact that ethanol is a liquid fuel that can be integrated into existing fuels, such as gasoline, directly by blending (Wheals *et al.* 1999) gives it a significant advantage as progress can then be incremental. For example, fuel for a car could vary anywhere from 1% to 100% ethanol (Coelho *et al.* 2006, Yüksel *et al.* 2004). While the physical properties of ethanol may be less than ideal compared to other biofuels, the relative simplicity of production from familiar processes, and integration into existing gasoline infrastructure, make it an ideal start point for biofuel production (Yüksel *et al.* 2004, Sarkar *et al.* 2012). A single ethanol

production factory can produce millions of liters of ethanol per year making ethanol production a consumptive process with regard to inputs. In order to meet demand the amount of ethanol produced will have to increase further. Choosing the right feedstock for the process is essential, as the substrate will be needed in large quantities (Sarkar *et al.* 2012). Using an inexpensive, plentiful substrate would greatly benefit the overall process cost and strengthen the economic case for ethanol production (Sarkar *et al.* 2012). Moving away from old technology, designated first generation biofuels, which can rely on relatively expensive ingredients, such as wheat, corn or other food crops, is absolutely essential in order to create inexpensive, environmentally friendly, ethical biofuels (Lynd *et al.* 2005, Jouzani *et al.* 2015).

1.4. First, Second, and Third Generation Biofuels

In an effort to develop ethanol, several different approaches are generally considered and designated as first, second, and third generation. First generation biofuels are typically defined as being derived from easily hydrolyzed starches, usually grain feed stocks, fermented using yeast (e.g. *Saccharomyces cerevisiae*) to produce ethanol (Lynd *et al.* 2005. Olson *et al.* 2012, Jouzani *et al.* 2015). A prominent example of this is wheat and corn ethanol production in the United States (U.S.) (Pimentel *et al.* 2005). First generation biofuels are produced from food crops, using processes derived from the brewing and distilling industry. Some would argue that first generation biofuels inflate food prices and creating a moral dilemma, an example of this is corn subsidies for biofuel in the U.S. (Piesse *et al.* 2009). Inflated

food prices could contribute to starvation and malnutrition in low-income families of poor countries (Christian *et al.* 2010).

Second generation biofuels are derived from lignocellulosic feed-stocks that undergo some form of chemical, physical, or biochemical treatment. This results in the hydrolysis of cellulose into sugar monomers that can be fermented by yeast using more traditional brewing and distilling methods to produce ethanol (Lynd *et al.* 2005, Jouzani *et al.* 2015). An example of second generation biofuels is the use of enzymatically hydrolysed corn stover (Humbird *et al.* 2011).

Third generation biofuels would be derived from the simultaneous hydrolysis and fermentation of a lignocellulosic substrate using an organism or several organisms together that are capable of simultaneous cellulose hydrolysis and fermentation. Due to the possibility for the third generation of biofuels to be a one step process, often referred to as consolidated bioprocessing (CBP), third generation biofuels are seen as providing the lowest costs of operation. However, that potential has not yet been realized, and as such CBP remains an active area of research (Lynd *et al.* 2005, Olson *et al.* 2012, Jouzani *et al.* 2015).

1.4.1. Feedstock for Consolidated Bioprocessing

A recurring theme in production of biofuel and biofuel co-product production is the importance of using low cost feed-stocks that require minimal pre-treatment. Low cost and easy to integrate feed-stocks can come from sources such as marginal lands or waste streams from other process (Lynd *et al.* 2005, Jouzani *et al.* 2015). For

ethanol or hydrogen, as stated previously, lignocellulose is particularly attractive as there are known organisms that are both highly cellulolytic and produce ethanol and hydrogen as end products such as *Clostridium thermocellum* or *Caldicellulosiruptor bescii* (Jouzani *et al.* 2015).

Another factor is the size and geographic availability of these feedstocks. For ethanol production the required output can be in the millions of liters of throughput per year for a single site meaning inputs will be very large, as such transportation costs, both in financial and environmental terms, become a factor (Lynd *et al.* 2005). To be attractive as a biofuel substrate, a lignocellulosic feedstock must meet the following criteria: high abundance, wide geographical availability, low cost of production or recovery from a waste stream, and potential for value added co-products derived from lignin (Lynd *et al.* 2005, Jouzani *et al.* 2015). Some notable examples of feedstocks that have been considered are recycled paper/cardboard, pulp and paper industry wastes, straw, switchgrass, hemp, and wood chips (Moreau *et al.* 2015, Schuster *et al.* 2013, Agbor *et al.* 2014, Horisawa *et al.* 2015).

1.4.2. Lignocellulosic Composition

The primary constituents of lignocellulose are lignin, cellulose, and hemicellulose, which for a substrate like plant biomass make up roughly 20%, 40 %, and 30% respectively (Agbor *et al.* 2014). Cellulose is composed of glucose molecules in β1-4 linked chains while hemicellulose is typically comprised of a xylose-linked backbone. The overall goal of CBP is conversion of cellulose, which

represents the bulk of hydrolysable biomass, and hemicellulose polymers into sugar monomers and simultaneous fermentation into biofuels. The hydrolysis of the cellulose (typically into cellobiose or glucose) is considered the crucial rate-limiting step, and is a core feature of research into biofuel production from lignocellulosic substrates (Lynd *et al.* 2005). Furthermore, it is essential to hydrolyze and ferment all the sugars. For instance, hemicellulose is comprised typically of a xylose back-bone that can be decorated with many different sugars such as arabinose and mannose (Olsen *et al.* 2012). Depending on the organism, it may not be able to fully use the hydrolysate or fully hydrolyse the lignocellulose sugar components. This is an issue observed in C. *thermocellum.* While it is highly cellulolytic, it is unable to use pentose sugars derived from hemicellulose hydrolysis (Lynd *et al.* 2005), causing a significant draw back. Although some of the sugars contribute to <1% of the possible total sugars, this can be economically significant at scale. The third component is lignin, which is a non-repeating polymer comprised of various different subunits including *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Agbor *et al.* 2014). Under conditions relevant to CBP, lignin is not fermented into bio-products to any great extent and can be toxic to cells especially when solubilised (Parisutham *et al*. 2014). It appears to be potentially profitable to make ethanol from the cellulose and hemilcellulose (Lynd *et al.* 2012); however, the addition of a high value product such as a lignin derived resin or biofuel related co-product would indeed greatly increase the economic viability of the entire process.

1.5. Typical Mixed Acid Fermentation of Bacteria and Ethanol Production

While the process of ethanol production from simple sugars using yeast is a well-known and efficient process, yeast do not fulfill the other demands of CBP using lignocellulosic substrates. Yeast do not naturally express cellulases and usually do not co-utilise xylose, glucose, and/or cellobiose (Wei *et al.* 2015). Bacteria that produce ethanol typically show lower ethanol efficiency, total yield, and tolerance when compared to yeast such as *Pichia stipites* or *Saccharomyces cerevisiae* (Olson *et al.* 2015), with the notable exception of *Zymomonas mobilis*, which is used due to its ability to ferment xylose efficiently to ethanol (Scopes *et al.* 1986). Bacteria commonly produce ethanol in the context of mixed acid fermentation producing some combination of lactate, acetate, formate, ethanol, CO_2, hydrogen, and succinate (Förster *et al.* 2014). This type of metabolism is seen in a wide variety of organisms, notably *Clostridiaceae, Enterobacteriaceae,* and *Bacillaceae* (Penning *et al.* 2006, Rachman *et al.* 1997, Nakano *et al.* 1997). Many of the bacteria aggressively studied in regards to biofuels produce some permutation of mixed acid fermentation, such as *C. thermocellum* (outlined in figure 1.2), *Caldicellulosiruptor spp.*, *Thermoanaerobacter spp.* and *Geobacillus thermoglucosidasius.* With ethanol production being the goal, it makes sense to characterize potential CBP candidates for end-product production looking for organisms that have high ethanol output, and do not produce competing end-products such as H_2 or lactate (Olson *et al.* 2015). Olson *et al.* (2015) compare pyruvate metabolism and possible modes of ethanol production, which are inextricably linked to their mixed acid production profile. While organisms

have been studied and modified to produce high yields of ethanol in organisms that are innate ethanol producers, Olsen *et al.* (2015) state that current work moving forward should focus on three things: i) increasing volumetric productivity; ii) increasing total titre; and iii) sufficiently understanding the high ethanol producing gene complement to be able to transfer it to low/non ethanol producers.

How an organism determines its end-product ratios can be quite complex and will be determined by the: i) gene complement; ii) regulation at the transcriptional, translational, and enzymatic level; and iii) intracellular concentrations of metabolites and co-factors. An example of this is ethanol production in members of the genus *Thermoanaerobacter*, as discussed by Verbeke *et al.* (2013), and their different *adh* genes. It appears based on the species analysed that they have between 5 and 9 annotated *adh* genes. There are also distinct types of alcohol dehydrogenases that have been characterized: *adhA*, *adhB*, and *adhE*. The propensity to synthesize ethanol through a given *adh* is in part determined by whether they are NADH-dependent or NADPH-dependent, as well as the intercellular concentration of each co-factor. The intercellular concentration of NADPH and NADH can in turn change depending on what pathways are used to break down a substrate and the composition of the substrate. It is absolutely essential to understand ethanol production at a systems level in order to manipulate physiological conditions and, thanks to genetic engineering tools, the gene complement of a strain in order to enhance ethanol production.

1.6. Organisms for Potential Use in Consolidated Bioprocessing

In order for a culture to be capable of CBP from lignocellulose substrates, it must simultaneously be cellulolytic and produce biofuels. Many organisms are studied in relation to CBP including *Caldicellulosiruptor spp.* (Chung *et al.* 2015), *C. thermocellum* (Biswas *et al.* 2015), *C. phytofermentans* (Zuroff *et al.2013), C. cellulolyticum* (Petitdemange *et al.* 1984), as well as many others (Olsen *et al.* 2012, Jouzani *et al.* 2015). Not only are these organisms studied individually, but also in combination with other non-cellulolytic partners (Jouzani *et al.* 2015).

1.6.1. *C. thermocellum* Role in Consolidated Bioprocessing

Among the most studied organisms for consolidated bioprocessing of lignocellulosic substrates is *C. thermocellum*, a thermophilic cellulolytic strict anaerobe capable of mixed acid fermentation and hydrogen production (Lynd *et al.* 2005, Olsen *et al.* 2012). *C. thermocellum* has shown high rates of cellulose hydrolysis from a variety of cellulosic substrates (Lynd *et al.* 2002, Jouzani *et al.* 2015), making it an attractive candidate for use in CPB. A property of *C. thermocellum* that allows for such a high cellulose degradation rate is an external organelle known as the cellulosome (Beguin *et al.* 1998), which acts as a scaffold for cellulolytic proteins, as well as proteins to bind to cellulosic substrates to enable a close interaction between the cell and substrate.

C. thermocellum has been studied extensively for both its ethanol and hydrogen production. While *C. thermocellum* is a major model organism, it is not a

perfect fit for CBP for ethanol as there are several drawbacks, namely: i) lack of aerotolerance; ii) inability to degrade many sugar substrates found in lignocellulose including pentoses; iii) poor ethanol production and tolerance, and iv) production of a range of fermentation products including amino acids (Ng *et al.* 1977, Lynd *et al.* 2005, Papanek *et al.* 2015, Biswas *et al.* 2015). Furthermore, *C. thermocellum* can be rather difficult to maintain in pure culture (Mori 1995), partially due to its poor inoculation efficiencies and intolerance to redox perturbation (Ng *et al.* 1977).

C. thermocellum displays a rather typical mixed acid fermentation profile (Figure 1.2) with major end-products: ethanol, lactate, formate, acetate, H_2, CO_2, and formate being produced. This makes *C. thermocellum* of potential interest in both hydrogen and ethanol production process (Levin *et al.* 2006, Rydzak *et al.* 2015). Efforts to enhance ethanol production have been successful, but can also lead to other unintended consequences as these pathways form a complex network of interactions. There are several examples of this: i) the removal or deactivation of the hydrogenases decreases H_2 and increases ethanol production, but also decreases the tolerance of *C. thermocellum* to ethanol due to redox balancing issues (Biswas *et al.* 2015), and ii) the elimination of formate to direct more electrons toward other end-products by deleting pyruvate formate lyase genes does increase ethanol production, but the elimination of formate production hinders biosynthesis (Rydzak *et al.* 2015). As the major non-beneficial end-products are eliminated, the cells begin to produce previously minor end-products in greater quantities leading to the synthesis of products such as amino acids, pyruvate, malate, fumarate, isobutanol, and butanediol (Rydzak *et al.* 2015).

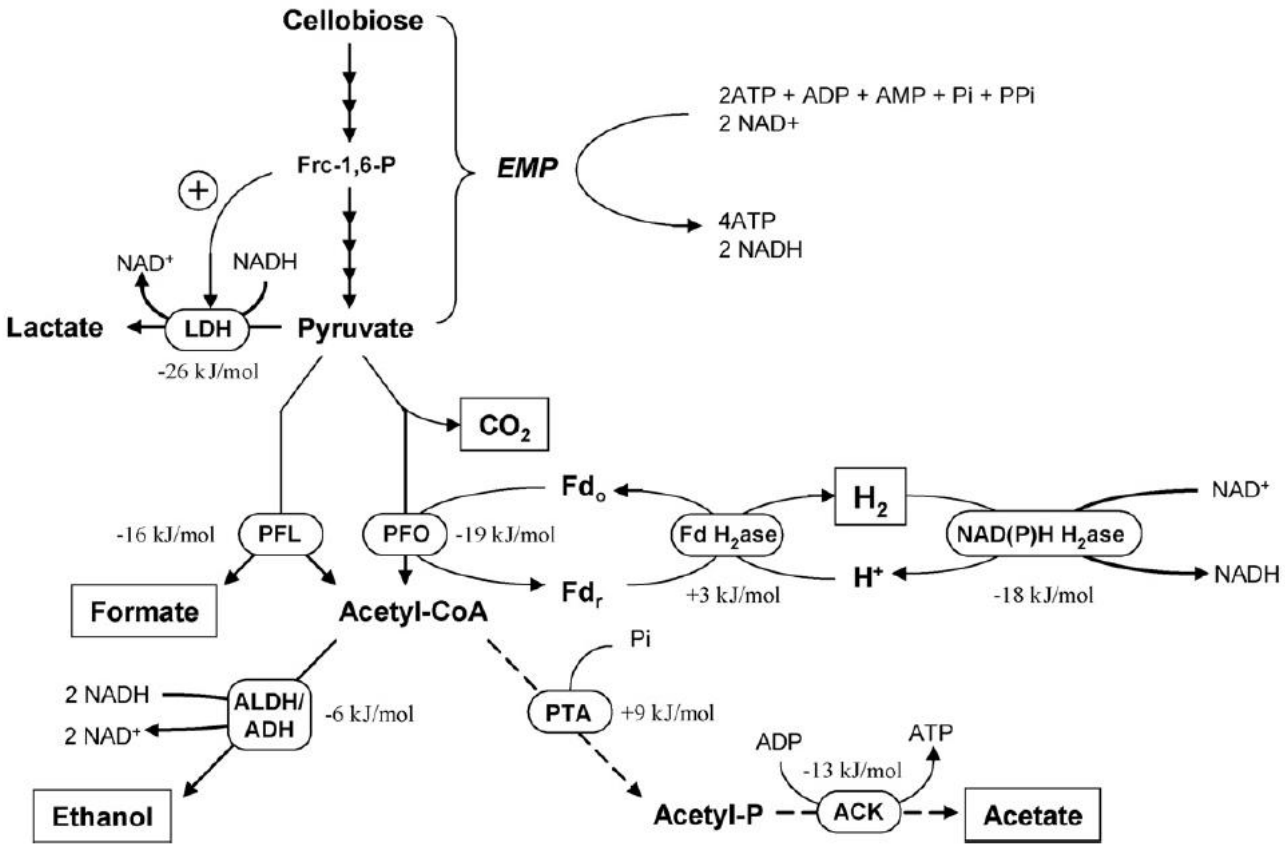


Figure 1.2. Pyruvate metabolism leading to mixed acid fermentation in *C. thermocellum* (source: Rydzak *et al.* 2009)

1.6.2. *Caldicellulosiruptor* Role in Consolidated Bioprocessing

Many *Caldicellulosiruptor spp.* have the ability to simultaneously degrade and use cellulose and hemi-cellulose (Blumer-Schuette *et al.* 2011). Several have been sequenced and analysis has shown that they vary in their hydrolytic abilities. Cellulolytic members of this genus have their own unique benefits such as being hydrogen producers, wide substrate utilization range, and complete use of sugars in lignocellulose (Abreu *et al.* 2013). There are drawbacks as well, such as requiring strictly anoxic conditions and typically only producing small amounts of ethanol (<=40% theoretical maximum) (Cha *et al.* 2013). One of the most prominent differences between *Caldicellulosirupter sp.* and *C. thermocellum* is the presence of a cellulosome in the latter organism. Its presence is both an advantage and disadvantage as the cellulosome can be highly effective in hydrolyses due to the physical proximity of hydrolysis and hydrolysis product removal. There may be cases where it is difficult for the cellulosome apparatus and cell to get physically close to the substrate, therefore individually secreted cellulases may be more effective (Beguin *et al.* 1998). *Caldicellulosiruptor sp.* has no such organelle and secretes their cellulolytic enzymes out into the medium, or they are bound to the cell's surface (Brunecky *et al.* 2013, Morrell-Falvey *et al.* 2015). The fact that *Caldicellulosiruptor sp.* are robust cellulolytic organisms with a distinct metabolism (Figure 1.3) and physiology from other cellulose degraders, such as *C. thermocellum,* increases the options when optimization of CBP is considered.

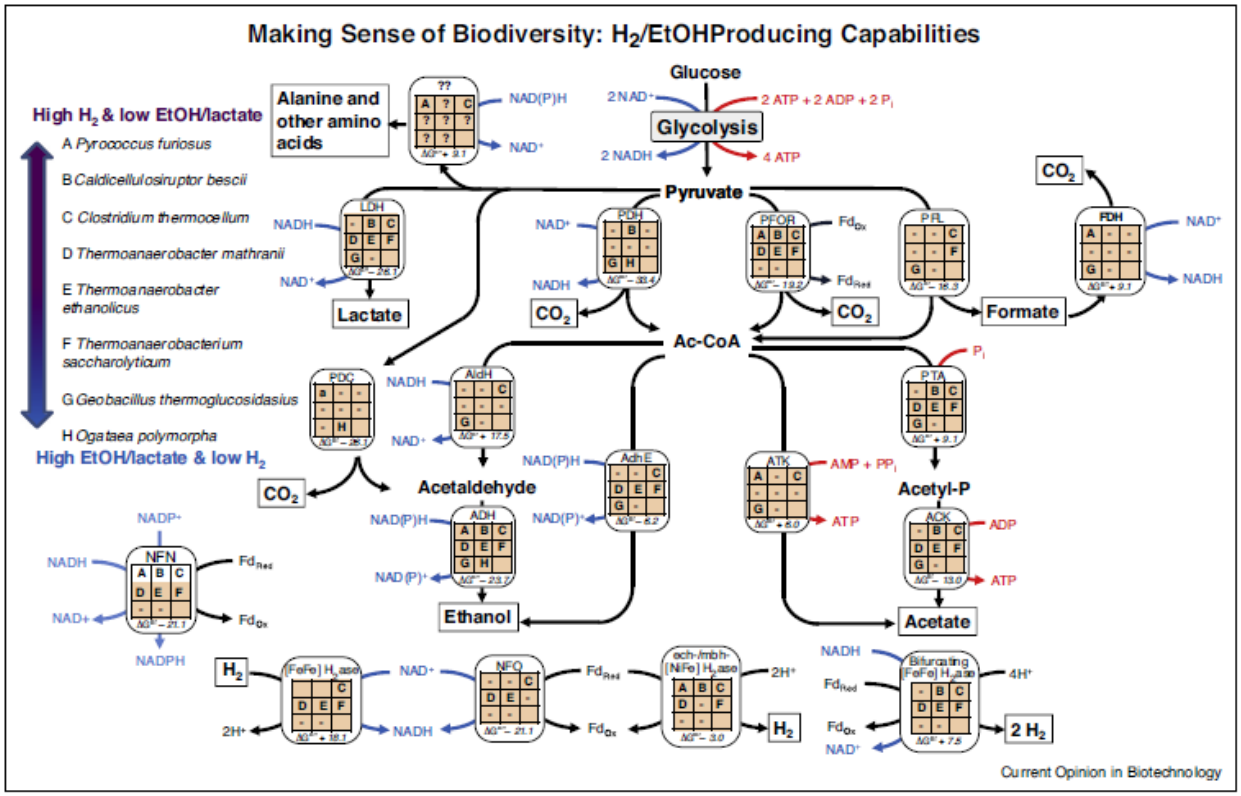
1.6.3. *C. phytofermentans* Role in Consolidated Bioprocessing

C. phytofermentans represents a mesophilic model for CBP compared to *C. thermocellum* and *C. caldicellulosiruptor* which are thermophilic (Warnick *et al.* 2002, Tolonen *et al.* 2009). Being thermophilic is generally considered a net positive over being mesophilic as high temperatures can be more favorable for H_2 production (Van Groenestijn *et al.* 2002), allow distillation of alcohols during production, and alleviate contamination issues (Lovitt *et al.* 1984). Sometimes being mesophilic can be beneficial as tolerance to high concentrations of alcohols may be more difficult at increased temperatures (Lovitt *et al.* 1984). *C. phytofermentans* allows pairing with well-studied mesophilic organisms such as yeast expanding the possibility and potential conditions for CBP to function when co-culturing is considered (Zuroff *et al.* 2013).

Warnick *et al.* (2002) show that lignocellulosic degradation is mainly due to one glycosyl hydrolase in *C. phytofermentans.* Petit *et al.* (2015) recently published a genome and transcriptome analysis on *C. phytofermentans* where their goal was to understand lignocellulosic biomass conversion into ethanol. They found *C. phytofermentans* was able to efficiently convert sugars into biomass, and that this was likely due to several factors: i) having an *adh* which creates ethanol from either NADPH or NADH, ii) effectively utilising pyrophosphate dependent enzymes to squeeze as much energy out of glycolysis as possible, iii) having the ability to easily get rid of reducing equivalents, and iv) creating more energy per sugar consumed due to the creation of a sodium gradient.

1.6.4. *C. cellulolyticum* Role in Consolidated Bioprocessing

C. cellulolyticum is a mesophilic strict anaerobe that is capable of producing hydrogen and ethanol from lignocellulosic substrates (Petitdemange *et al.* 1984). Typically, yields of ethanol are poor as a variety of end products compete for electrons and carbon. This has been remedied through genetic engineering and targeting of genes associated with the production of lactic acid (Li *et al.* 2012). Recently, Gaida *et al.* (2016) genetically engineered *C. cellulolyticum* to produce high yields of *n*-butanol by taking the *n*-butanol producing genes from *C. acetobutylicum* and putting them into *C.cellulolyticum*. While there is a focus on ethanol, thoroughly understanding organisms at a systems level should allow for genetic modification of organisms to produce a variety of desired end-products from CBP.



A, B, C, and G: from Carere CR, Rydzak T, Verbeke TJ, Cicek N, Levin DB, Sparling R: Linking genome content to biofuel production yields: a meta-analysis of major catabolic pathways among select H2 and ethanol-producing bacteria. *BMC Microbiol* 2012, **12**:295. **D** and **E** are from Verbeke TJ, Zhang X, Henrissat B, Spicer V, Rydzak T, Krokhin OV, Fristensky B, Levin DB, Sparling R: Genomic Evaluation of *Thermoanaerobacter* spp. for the Construction of Designer Co-cultures to Improve Lignocellulosic Biofuel Production. *PLOS ONE* 2013, **8**: e593625758. **F**: while based on Shaw *et al.* (2008), was complemented by manual search and BLAST to confirm that the NFOR is related to *nfnAB*, that the *hyd* is related to the bifurcating hydrogenases. A further Fe–Fe hydrogenase was observed in the genome. The absence of other genes was confirmed both from the annotation as well as BLAST anchored in *C. thermocellum* and *T. thermohydrosulfuricum* WC1, including lack of membrane bound RNF-type NFO. ***nfnAB***: Presence/absence was based on Wang S, Huang H, Moll J, Thauer RK: NADP+ reduction with reduced ferredoxin and NADP+ reduction with NADH are coupled via an electron bifurcating enzyme complex in Clostridium kluyveri. *J Bacteril* 2010, **192**: 5115–23. For *C. thermocellum* it was through the analysis by Rydzak T, Grigoryan M, Cunningham ZJ, Krokhin OV, Ezzati P, Cicek N, Levin DB, Wilkins JA, Sparling R: Insights into electron flux through manipulation of fermentation conditions and assessment of protein expression profiles in *Clostridium thermocellum*. Appl Microbiol Biotechnol 2014, **98**: 6497–6510. With respect to **B** and **F** it was through BLAST and side-by-side location of both genes needed for *nfnAB*. The annotated genes in F corresponding to *nfnAB* were TheetDRAFT_0838 and 0839. **Lower case (a) for PDC**: based on Ma K, Hutchins A, Sung SJS, Adams MWW: Pyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon, *Pyrococcus furiosus*, functions as a CoA-dependent pyruvate decarboxylase. *Proc Natl Acad Sci U S A* 1997, **94**: 9608–13. ***O. polymorpha***: based on Ravin NV, Eldarov Ma, Kadnikov VV, Beletsky AV, Schneider J, Mardanova ES, Smekalova EM, Zvereva MI, Dontsova Oa, Mardanov AV, *et al.*: Genome sequence and analysis of methylotrophic yeast Hansenula polymorpha DL1. *BMC Genomics* 2013, **14**:837. Amino acid synthesis indicates organisms where this phenotype has been observed. Question marks indicate that amino acid production has not been reported in these organisms.

Figure 1.3. Comparison of mixed acid fermentation of several hydrogen and ethanol producers relevant to CBP production of biofuels. (Source: Olson *et al.* 2015)

1.7. Designed Co-cultures for CBP

One attractive method for adding features and enhancing processes such as CBP from lignocellulosic substrates is adding co-culture partners alongside a strong cellulose degrader such as *C. thermocellum* (Lynd *et al.* 2005, Jouzani *et al.* 2015). There has been co-culture work using cellulolytic organisms with non-cellulolytic organisms (Hann *et al.* 2015, Jouzani *et al.* 2015). The combination of a cellulolytic organism with non-cellulose degrading species (sp.) in general has been shown to improve the rate of cellulose hydrolysis end-product yields (Odom & Wall 1983, Geng *et al.* 2010, Fang 2010). Pairing two cellulolytic organisms may provide the added benefit of their cellulases working synergistically (Irwin *et al.* 1993, Arun *et al.* 2014), and perhaps even create a high titre of cellulases depending on how expression is regulated (Zhang *et al.* 2005). Jouzani *et al.* (2015) outlines several promising co-culture arrangements and a few examples of the recent work on these co-cultures have been included in this thesis.

1.7.1. Co-cultures of *C. thermocellum* with *Thermoanaerobacter sp.* or *Thermoanaerobacterium sp.*

C. thermocellum co-cultures with various *Thermoanaerobacter sp.*, and the related genus *Thermoanerobacterium*, have been studied for over 20 years (Mori 1995), and continue to be studied as they offer many benefits over pure *C. thermocellum.* Benefits for CBP of a *C. thermocellum-Thermoanaerobacter sp.* co-culture include: i) vitamin cross-feeding which can have a positive effect on both

ethanol production and growth (He *et al.* 2011), ii) increased substrate usage range (Fang 2010), iii) assistance in hydrolysis of lignocellulolytic substrates (Verbeke *et al.* 2013), and iv) increased ethanol production efficiency and yield (Fang 2010). While co-cultures can be a benefit this is not always true; introduction of a high lactate producer could compete for sugars and may divert carbon and electrons away from biofuel related products, therefore it is essential to characterize the properties of prospective co-culture partners (Verbeke *et al.* 2011). The production of many of these unwanted mixed acid fermentation products can be offset with the use of genetic engineering. Shaw *et al.* (2008) removed the ability of *Thermoanaerobacterium saccharolyticum* to produce lactate and acetate through genetic engineering, and showed an improvement in ethanol total yield of ~40%. In the study by Argyros *et al.* (2011) they delete genes necessary for the production of lactate and acetate in *C. thermocellum* and use the genetically engineered *T. saccharolyticum* created by Shaw *et al.* 2008. This resulted in 92g/l of avicel cellulose consumed producing 32g/l of ethanol which is over double the amount produced when compared to non-genetically engineered co-culture under identical conditions and 75% of the theoretical maximum. This is a significant improvement over typical *Thermoanaerobacter* or *Thermoanaerobacterium sp.* containing co-cultures (Jiang *et al.* 2013).

1.7.2. *C. besci* and *T. maritma* Co-Cultures

Aberu *et al.* (2016) show co-cultures of *C. besci* and *T. maritima* are both excellent hydrogen producers. *C. besci* can be utilized in CBP process as it is highly cellulolytic (Chung *et al.* 2015). *C. besci* and *T. maritima* co-cultures act in synergy and produce superior amounts of total hydrogen on cellobiose and xylose compared to pure cultures of *C. besci*. Aberu *et al.* (2016) show that the co-culture was effective in fermenting a raw lignocellulose substrate as well as, but not necessarily better than, pure cultures. Finding a good co-culture partner to enhance CBP is partly trial and error, and partly understanding these organisms at a systems level. While this co-culture did not appear to greatly enhance the overall process, perhaps a change in conditions or different strains might.

1.7.3. Co-cultures under Aerobic Conditions

Brethauer *et al.* (2014) demonstrated a co-culture where *Trichoderma reesei* hydrolyse the lignocellulosic substrate aerobically and *Saccharomyces cerevisiae* and *Pichia stipites* then work to ferment the free sugars into ethanol. Zuroff *et al.* (2013) use *C. phytofermentans*, a strict anaerobe and a yeast in a co-culture, to show cellulose degradation and ethanol synthesis under microaerobic conditions demonstrating a new and interesting mode of CBP where conditions are simultaneously aerobic and ethanologenic. This is unlike Breathaur *et al.* (2014) where two macro-environments exist by developing a multilayered biofilm on the surface of an oxygen diffusible membrane reactor. In the layer adjacent to the

membrane, the aerobic cellulolytic *T. reesei* consumes the oxygen and provides cellulases for saccharification, while the yeast in a further layer ferments the simple sugars to ethanol. In theory aerobic ethanol production is possible as the enzymatic complement to produce ethanol is not air sensitive, making fermentative growth possible aerobically (Bulder *et al.* 1964). Elimination of the need to generate anaerobic conditions prior to CBP would remove a level of complexity making the process simpler and possibly cheaper.

1.8. Bioprospecting for Consolidated Bioprocessing

While there is a variety of organisms currently being studied in relation to CBP, it is important to keep expanding our list of organisms that could be used effectively either through finding new organisms, or revisiting known, but poorly characterized organisms with respect to CBP (Zuroff *et al.* 2012). Searching for new organisms has the added benefit of isolating and characterizing organisms that may be interesting for novel properties unrelated to biofuels as well. Additionally, while genetic engineering approach can and have been used to optimize existing strains relevant to CBP, such as for example *C. thermocellum or T. mathranii* (Rydzak *et al.* 2015, Yao *et al.* 2010), there may be properties in yet to be discovered organisms that would provide an even better starting point for genetic optimization. Many of the cellulolytic organisms used in CBP have strictly anaerobic requirements such as *C. thermocellum, Caldicellulosiruptor sp.,* and *C. phytofermentans*. It makes sense to create enrichment conditions for cultures that current CBP organisms are weak in, such as vitamin requirement (Mori 1995, Fang 2010) or oxygen tolerance. Some

weaknesses for an organism may be quite difficult to overcome with genetic engineering due to the complexity or innate characteristics that are directly at odds with a desired phenotype. New organism(s) may have distinct advantages over *C. thermocellum* in pure culture, such as higher ethanol production (Papanek *et al.* 2015), ease of manipulation in pure culture (Mori 1995), high ethanol tolerance (Brown *et al.* 2011), and wide substrate usage (Izquierdo *et al.* 2014). Additionally, understanding the enrichments greatly increase our knowledge of how to create a robust lignocellulolytic designer consortiums for CBP, and the possible interactions of these organisms in their natural environment (Abdel-Rahman *et al.* 2016).

Overall there has been some development in the ability to create mutants in biofuels organisms to both increase our understanding and to optimize them for biofuel production in CBP (Papanek *et al.* 2015). Understanding of biofuel production at systems level is incomplete, and there is great diversity in core metabolisms; see figure 1.3 (Olson *et al.* 2015). The possibility remains that there may be better suited organisms, hence interest in bioprospecting and understanding new organisms for CBP (Arora *et al.* 2015)

1.9. Oxygen Removal/Oxygen Tolerance

Many biofuel-producing cellulolytic bacteria, including *C. thermocellum*, lack aero-tolerance and have not been shown to tolerate greater than 2% O_2 in the atmosphere (Kato *et al.* 2004, Ng *et al.* 1977). While members of the genus *Clostridium* are typically described as strict anaerobes, some members, including *C.*

straminisolvens and *C. intestinalis* encode genes required for aerotolerance, and have been shown to grow in microaerobic environments (Kato *et al.* 2004, Lee *et al.* 1989, O'Brien & Morris 1971). Lack of oxygen tolerance observed in some *Clostridia* may be due to regulatory mechanisms rather than to not having the genetic potential to combat oxygen toxicity. Indeed, when *perR*, an oxygen responsive regulator, has been deleted in *C. acetobutylicum*, this genetic deletion was shown to lend aerotolerance (Hillmann *et al.* 2009).

Aerotolerance in CBP can be useful for two main reasons i) lack of complexity in design of system, if oxygen does not have to be kept out and ii) eliminates the need to make the media or substrate anaerobic in a continuous feeding CBP set up, nor maintain a constant anaerobic environment during cell growth. Previously, it has been reported that co-cultures containing a *Clostridium* species paired with facultative aerobes can lead to oxygen removal in a sealed vessel followed by fermentation in both designed and enriched cultures (Tran *et al.* 2010, Miyazaki *et al.* 2008). Tran *et al.* (2010) used a designed *Bacillus subtilis* and *Clostridium butylicum* culture to achieve oxygen removal, followed by acetone-butanol-ethanol production. Miyazaki *et al.* (2008) and Ronan *et al.* (2013) show enrichment cultures containing *Geobacillus/Caldibacillus* related organisms could cause oxygen removal followed by ethanogenesis on a cellulosic substrate. Co-cultures created by Zuroff *et al.* (2013) containing *Clostridium phytofermentans* and yeast achieve aerotolerance via respiratory protection under microaerobic conditions. Environments that are aerated, such as compost, can be highly cellulolytic (Mohee *et al.* 1998, Fathallh *et al.* 2012) but typically are not fermentative when aerobic (Dionisi *et al.* 2015). From the

examples listed above, we know that creating an aerotolerant highly cellulolytic fermentative culture through bio-prospecting is conceptually possible, either by finding a new suitable organism or finding the appropriate partner for a known strictly anaerobic cellulose degrader within the co-culture.

1.10. *Geobacillus* and *Caldibacillus* in CBP

Representatives of the genus *Geobacillus* have been found in highly cellulolytic enrichments (Ronan *et al.* 2013, Miyazaki *et al.* 2008). Miyazaki *et al.* (2008) characterize enrichments with a strain of *Geobacillus sp.* designated kpuB3 which is closely related to *Caldibacillus debilis* by 16S rRNA gene sequence similarity. *Geobacillus* and the former member of *Geobacillus, Caldibacillus,* which have more recently been split from that genus, are thermophilic facultative anaerobes or obligate aerobes that grow well at ~60ºC (Coorevits *et al.* 2012). Of the more recently created genera, is the single species genus *Caldibacillus.* It has been described as an obligate aerobe (Banat *et al.* 2004), and will become more important in the subsequent chapters of this thesis. *Geobacillus* can display typical mixed acid fermentation making lactate, acetate, formate, CO_2, and ethanol (Cripps *et al.* 2009). *Geobacillus* do not produce H_2, making them good potential candidates for ethanol production, as H_2 generation would directly compete for electrons. As *Geobacillus sp.* produce end-products that compete with ethanol production there has been interest in metabolically engineering them to produce ethanol at a higher efficiency (Cripps *et al.* 2009) on sugar substrates. Some *Geobacillus* also possess the innate characteristic of

high ethanol tolerance (Fong *et al.* 2006), which may be difficult to create through genetic modification. *Geobacillus* have been found growing along with *C. thermocellum* (Ronan *et al.* 2013, Miyazaki *et al.* 2008) in enrichments, therefore, a designer co-culture should be possible. A designer co-culture containing *C. thermocellum* and a *Geobacillus sp.* could be highly cellulolytic and may show benefits in ethanol production or culturing requirements over *C. thermocellum* in pure culture. Furthermore if the organism paired with *C. thermocellum* is facultatively anaerobic, then it could allow for respiratory protection analogous to *C. phytofermentase* and yeast by Zuroff *et al.* (2013). An aerotolerant designed co-culture has not yet been demonstrated for cultures involving *C. thermocellum*.

1.11. Omics for Microbial Characterization and Predictive Leverage

Current high throughput Omics techniques are widely used for the purposes of hypothesis building (Zoldoš *et al.* 2013). The employment of metagenomics can also be used to look for genes, organisms, and communities of interest (for example looking at microbial communities from ruminants, Dube *et al.* 2015). Martins *et al.* (2013) employed metagenomics to show that their lignocellulosic compost is being degraded by bacterial cellulases likely produced by organisms in the *Clostridiales* and *Actinomycetales*. Knowing the mode of degradation and the organisms involved in the degradation provides invaluable information for bioprospecting, as these organisms can now be targeted directly for isolation. Likewise, their cellulase genes can be cloned and expressed in other organisms. Instead of investigating natural

environments, another tactic is to create the desired environmental conditions, and then select for an adapted community. Yu *et al.* (2015) sought to analyse switchgrass degrading enrichment communities and the effect of preservation over time. The component members necessary for switchgrass degradation in the enrichment might be ideal for switchgrass degradation compared to other organisms that were not selected for on switchgrass

Omics including genomics, transcriptomics, and proteomics offer valuable information about an organism's inner workings (Mclean *et al.* 2013), and can be used for strain characterization related to biofuels (Verbeke *et al.* 2013). A well-annotated genome can provide insight into the potential end-product generated, substrate usage, auxotrophies, and presence of other biofuel related genes (Sczesnak *et al.* 2011, Verbeke *et al.* 2013). The transcriptome and proteome can give insight into the expression levels of biofuel related genes at the translational and protein level (Verbeke *et al.* 2014). As a systems level understanding of an organism is increased, such as ethanol production in *Thermoanaerobacter sp.* mentioned in section 1.5.1, this allows for further exploitation of *Thermoanaerobacter sp.* for alcohol production (Shaw *et al.* 2008, Olsen *et al.* 2015). This same approach can be applied to any other microorganism of interest.

Omics information is strengthened and verified by physiological measurements of the organism in question; in this way the annotation quality and predictive power of Omics increases over time (Munir *et al.* 2016, Rydzak *et al.* 2012). Carere *et al.* (2012) and Olsen *et al.* (2015) used genome analysis to predict organisms that may have good biofuel properties, essentially bioprospecting *in silico*.

This type of work will become more powerful and accurate as annotation quality improves and the number of genomes sequenced increase.

An example of this is Olson *et al.* 2015 (see figure 1.3); depending on the genome complement, one can hypothesize whether a 100% maximum theoretical ethanol yield is possible and/or likely based on the analysis of genome content. For instance, an organism that uses pyruvate formate lyase (PFL) for pyruvate fermentation will have difficulty creating the 100% maximum theoretical ethanol yield as electrons would be tied up in formate. One could choose an organism that expresses PFL, and enhance ethanol through the deletion of this gene through genetic modification (Rydzak *et al.* 2015). Through genome analysis as exemplified in figure 1.3, hypotheses can be generated about the abilities of organisms that have been sequenced. But looking at and understanding the gene complement of an organism is only the first step, we must also understand which genes are being translated and transcribed. Dai *et al.* (2015) analysed the metatranscriptome of the cow rumen to look for cellulases, allowing us to see which cellulase genes are actually being expressed at the transciptomic level. Knowing which genes are actually expressed is essential to build hypotheses and understand cellulose degradation in organisms at a systems level.

1.12. Conclusion

There can be both an environmental and economic benefit to biofuel production (Lynd *et al.* 2005). It makes sense to further develop CBP by first bioprospecting and characterising organisms that counter weakness in organisms typically used in CBP. Creating a robust set of organisms that can exploit a range of different operating modes for CBP will be a critical step in making CBP cost effective. At the same time, it is important to create tools for genetic modification and begin optimizing promising organisms. As these tools are developed they can then be applied to new organisms found through bioprospecting. Finding, optimising, and understanding organisms should be considered an iterative process, which should lead to incremental improvements in biofuel technology over time. Furthermore the pace at which it is iterated should improve with technological advances such as high throughput omics.

1.13. Thesis Objectives:

I set out to find new organism(s) suitable for CBP, or that would be able to act synergistically with those currently used. The goal was to identify and characterize organism(s) or co-culture partners that would excel at CBP and possess aerotolerant characteristics. The general workflow was as follows:

Chapter 2

Objective: Find and describe aerotolerant cellulolytic fermentative consortia.

This Objective was reached through the following tasks:

1) Bioprospect for organisms using conditions to enrich for: i) highly cellulolytic cultures; ii) robust cultures (transferable/ platable); and iii) functions that complement CBP (minimal media, aerotolerance).
2) Identify and purify organism(s) within these enrichments using: i) DGGE to identify genera and tentatively identify species and ii) tentative identifications to aid in colony isolation.
3) Characterize the physiology of enrichments and isolates, leading to the selection of a champion enrichment culture.

Chapter 3

Objective: Determine the minimum consortium with the aerotolerant phenotpye and characterize the enrichment, designer co-culture and *C. debilis* GB1.

This Objective was reached through the following tasks:

4) Recreate the most promising enrichment found in Chapter 2 from individual isolated strains and characterized organisms from pure culture.
5) Characterize the novel strain providing co-culture aerotolerance, *C. debilis* GB1, physiologically against its type strain Tf by comparing growth, end-products and substrate usage characteristics.

Chapter 4

Objective: Characterize enrichment isolate GB1 using genomics and proteomics.

This Objective was reached through the following tasks:

6) Characterize *C. debilis* GB1 under aerobic and anaerobic growth by: i) sequencing genome of constituent (*C. debilis* GB1) whose sp. has not been previously sequenced; ii) using comparative proteomics to understand regulation and expression of core metabolism, and iii) comparing and contrasting proteome information with physiological information from Chapters 2 and 3.

Chapter 5

Objective: To understand the observed physiological differences in GB1 and Tf at the genomic level.

This Objective was reached through the following tasks:

7) Characterize the type strain of *C. debilis* Tf against our isolate *C. debilis* GB1 at the genomic level by: i) using comparative genomics to understand differences between strains and ii) comparing and contrasting genome comparison information with physiological information from chapters 2 and 3.

1.14. Work not Included in the Thesis Chapters

I also spent time in NZ bioprospecting for cellulolytic enrichments, this work lead to isolation of *F. pennivorans* DYC, work on this bacterium was tangential to my thesis goals and is attached within the appendices (Appendix A).

Chapter 2: Characterization of Enriched Aero-tolerant Cellulose Degrading Communities for Biofuels Production Using Differing Selection Pressures and Inoculum Sources[1]

2.1. Abstract

Ethanol production from direct cellulose fermentation has mainly been described as a strictly anaerobic process. The use of air tolerant organisms or consortia for this process would reduce the need for pre-reduction of the medium and also permit continuous feed of aerobic feedstock. To this end, moderately thermophilic (60 ^{o}C) consortia of fermentative, cellulolytic bacteria were enriched from 3 distinct environments (manure, marsh, and rotten wood) from a farm in South-Eastern Saskatchewan, Canada. Community phenotypic and metabolic profiles were characterized. Selection methods, including direct plating under an aerobic atmosphere and repeated passaging, were designed to select for robust, stable aero-tolerant cellulose degrading communities. Several of the isolated communities exhibited an increase in total cellulose degradation (up to 2g/l cellulose) and total ethanol yield when compared to a monoculture of *C. thermocellum* DSMZ 1237 under batch conditions. Due to stringent selection conditions, low diversity enrichments were found, and many appeared to be binary cultures via denaturing gradient gel electrophoresis (DGGE) analysis. On the basis of 16S rRNA gene sequencing, aerobic conditions selected for a mix of organisms highly related to *C.*

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[1] Contributing authors: **[a]Scott Wushke, [b]David Levin, [b]Nazim Cicek, [b]Richard Sparling.** 2013 *Can J Microbiol* **59:**679-683 Contributions: [a]First author experimental design, cell growth and end-product [b]Lab space, equipment, funding, and research guidance.

thermocellum and *Geobacillus* species, while anaerobic conditions led to the development of consortia containing strains related to *C. thermocellum* with strains from either the genus *Geobacillus* or the genus *Thermoanaerobacter*. The presence of a *Geobacillus*-like species appeared to be a prerequisite for aero-tolerance of the cellulolytic enrichments, a highly desired phenotype in lignocellulosic CBP (consolidated bioprocessing).

2.2. Introduction

There is current interest expressed in cellulose containing biomass as a feed-stock for biofuel production (Demain *et al.* 2005, Islam *et al.* 2006). Environmental degradation of recalcitrant substrates such as lignocellulosic biomass is a complex process typically involving complex microbial communities (Wongwilaiwalin *et al.* 2010). These multi-species integrated communities may possess several benefits over pure cultures with respect to hydrolysis of lignocellulosic substrates. In fact, the combination of a cellulolytic organism with non-cellulose degrading species (sp.) has been shown to improve the rate of cellulose hydrolysis, end-product yields, and aerotolerance (Odom & Wall 1983, Geng *et al.* 2010, Fang 2010, Tran *et al.* 2010).

Clostridium thermocellum, a model organism for consolidated bioprocessing, has one of the highest rates of cellulose hydrolysis reported (Lynd *et al.* 2002) and is able to convert cellulosic biomass into several different products including H_2 and ethanol (Islam *et al.* 2009), but is a strict anaerobe. Growth analyses of cellulose degraders such as *C. thermocellum* have shown an excess of hydrolyzed sugars in

early exponential phase (Islam *et al.* 2009). When cellulolytic organisms are grown with other non-cellulolytic fermentative organisms, the excess sugars can be utilized by the non-cellulolytic organism, which can positively affect end-product yields (Fang 2010, Geng *et al.* 2010). It is expected that a co-culture of a cellulose degrader with specific partners could enhance end-product production in the co-culture and has the potential to speed up the rate of cellulose hydrolysis via product removal (Sharma 1991, Geng *et al.* 2010). For example, several studies have also shown enhanced lignocellulosic fermentation by co-culture of a *Thermoanaerobacter* sp. with *C. thermocellum* (Fang 2010).

Several attempts have been made to develop thermophilic lignocellulosic co-cultures with aero-tolerance. These communities can include a *Geobacillus* sp. that is either aerobic or facultatively anaerobic (Miyazaki *et al.* 2008). A facultatively anaerobic organism in culture with a strict anaerobe not only alleviates the need for pre-reduction of a medium, but could also serve as a pre-treatment for the substrate (Tran *et al.* 2010). Besides increasing end-product yields, providing aero-tolerance, and increasing cellulose degradation rates, there are several reports of cross-feeding essential vitamins and amino acids in microbial communities, which may further enhance the co-culture's growth capability (Mori 1995, Fang 2010). Previous work by Miyazaki *et al.* (2008) demonstrated the possibility of a strict anaerobe and facultative anaerobe creating a highly cellulolytic oxygen depleting thermophilic co-culture, an attempt to isolate a minimal functional community using aerotolerance, thermophilic conditions, and cellulose hydrolysis as selection pressures to achieve a discrete highly

cellulolytic aerotolerant thermophilic co-culture containing a strict anaerobe would represent novel work.

The current paper describes low diversity consortia capable of cellulolytic growth in the presence of an oxygen-containing atmosphere. The aim of this study was to select and characterize mixed cultures of cellulose degrading bacteria from environmental samples, and identify robust aero-tolerant cultures for future study.

2.3. Materials and Methods

2.3.1. Inocula and Enrichment Conditions

Samples including marsh soil, fresh cow feces, and rotted wood were collected from a farm site in South-Eastern Saskatchewan, Canada (Latitude, 50.359802; longitude, 101.919297; collected June 1, 2009). Samples, both liquid and solid, were collected aerobically, and stored in sealed containers without airspace at 4 ^{o}C. Sample liquid, or a slurry created using distilled-deionized water (diH_20) for solid samples (1 mL), was inoculated using conditions described in Table 2.1 (column 3).

2.3.2. Variation in Growth Medium and Selection Conditions

Sealed Balch tubes (26 mL) and 110 mL serum bottles supplied by Bellco Glass Inc. and Fisher Scientific, respectively, were used to carry out the experiments. Tubes containing10 mL of medium were prepared and adjusted to pH 7.2. Media preparation and protocols for anaerobic culturing were as described by Islam *et al.*

(2006). Unless otherwise specified, a modified 1191 medium, referred to as M-1191 medium (Islam *et al.* 2006), containing only 0.76 g/L yeast extract (YE), was used. Whatman paper #1 (2 g/L) was used as carbon and energy source, unless otherwise specified. Cellulose overlay plates were made by placing 55 mm Whatman paper disks on top of solidified M-1191 medium containing 15% agar. Czapek peptone medium (ATCC 522) was also prepared under aerobic conditions for conditions outlined in Table 2.1. Different selection pressures (Table 2.1, column 3) were used in an attempt to select for different co-cultures. After initial selection conditions were applied, samples were passaged 6 times anaerobically and 3 times aerobically (when possible) in sealed Balch tubes to obtain stable cultures. Tubes that tested positive for growth on cellulose were then plated anaerobically (and aerobically when possible) on Whatman paper overlays. Anaerobic conditions for plating were produced using a GasPak anaerobic container system. Cellulolytic colonies were then re-streaked 3 times and transferred back into anaerobic broth cultures. Pure cultures were obtained through plating and re-streaking on cellobiose plates.

2.3.3. Experimental Setup

All cultures were inoculated at 10% v/v from exponentially growing source cultures. Strong positive cellulolytic activity was revealed with the disappearance of 2g/L Whatman paper after 48 hours. All experiments were carried out with three independent replicates (i.e. in triplicate). Samples (1 mL) were taken for end-product

analyses at 24 hours (h) post-inoculation (pi) from cellobiose cultures and at 48 h from cellulose cultures and stored at -20 ^{o}C until analysed.

2.3.4. Sugar and End-Product Analyses

Cellobiose, glucose, lactate, formate, acetate, and ethanol were measured by high pressure liquid chromatography (HPLC) using a Dionex, ICS 3000 system equipped with a Bio-Rad Aminex-87H column, and run at 30 ^{o}C, 0.75mL/min, with 0.02 mM sulfuric acid. A Shodex 101 Refractive Index detector was used on all compounds being analysed. Measurements for CO_2, H_2, O_2, were taken on a Multiple Gas Analyzer #1 Gas Chromatograph (GC) System Model 8610-0070 (SRI Instruments, Torrance, CA) with a Thermal Conductivity Detector (TCD), and using argon as the carrier gas. Columns and methods were used as previously described by Rydzak *et al.* (2009).

2.3.5. DNA extraction and PCR Amplification

DNA extractions from cultures were performed using a Promega Wizard Genomic Extraction Kit. PCR of the genomic DNA with 16S rRNA gene primers 341F (5'-CCTACGGGA GGCAGCAGCGCCCGGGGCGCGCCCCGGGCGGGGCGGGGGCACGGGGGG-3') and 907R (5'-CCGTCAATTCMTTTGAGTT T-3'; (Sanchez *et al.* 2007). Pure cultures, when isolated from single colonies, were identified by amplification and sequencing of the 1533 bp fragment of the 16S rRNA gene using the 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCCGCA-3') primers (Verbeke *et al.* 2011).

2.3.6. Denaturing Gradient Gel Electrophoresis (DGGE)

A Denaturing Gradient Gel Electrophoresis (DGGE) protocol, as described by Muyzer *et al.* (1993), was used with some modifications. PCR products were separated on the Bio-Rad D-Code system with 40%-70% denaturing gradient polyacrylamide gel for 18 h at 60 ^{o}C, 80 Volts (V). Bands were then excised, soaked in 50 µL ddH_2O for 4 h, and centrifuged for 1 min at 19400g. The supernatant was then used as template for amplification and sequencing to identify bacteria.

2.3.7. DNA Sequencing and Phylogenetic Analyses

Sequencing of re-amplified DNA from excised DGGE bands was completed through Macrogen (Rockville, USA). Sequences were trimmed manually, and aligned for a consensus sequence using Bio-Edit. Sequences were run through NCBI BLAST (http://blast.ncbi.nlm.nih. gov/Blast.cgi) to determine possible matches and identify tentative species based on sequence identity to the known 16S rRNA sequence database. (KC291677-KC291704)

2.4. Results and Discussion

2.4.1. Culture Enrichment

Environmental samples were inoculated into Balch tubes or directly onto plates under conditions described in Table 2.1. Stable enrichments were stored at -80 ^{o}C for long-term storage and at 4 ^{o}C for working stocks. These served as sources of inocula for additional experiments including DGGE analysis and anaerobic end-product data.

2.4.2. Analysis of Microbial Community

Based on DGGE, followed by band excision and sequencing, a tentative identification of the strains present in each enrichment was made, DGGE gel is shown in figure 2.1. Table 2.2 lists the different enriched cultures with the tentative identifications of bacteria via 16S rRNA gene sequence analyses. Table 2.3 lists end-products produced by enrichments under anaerobic conditions in M-1191 media with cellulose as the substrate. All stable enrichments contained a strain related to *C. thermocellum* (97-100% nucleotide sequence identity), suggesting that a *C. thermocellum*-related organism may be the key member in cellulose degrading communities under these conditions (Kato *et al.* 2004, Shiratori *et al.* 2009). All of the cultures that could degrade cellulose on aerobic overlay plates contained members highly related to *Geobacillus* sp., suggesting that the metabolism of these organisms created a sufficient oxygen gradient on the cellulose to allow the growth of the anaerobic cellulolytic member (*C. thermocellum* in this case).

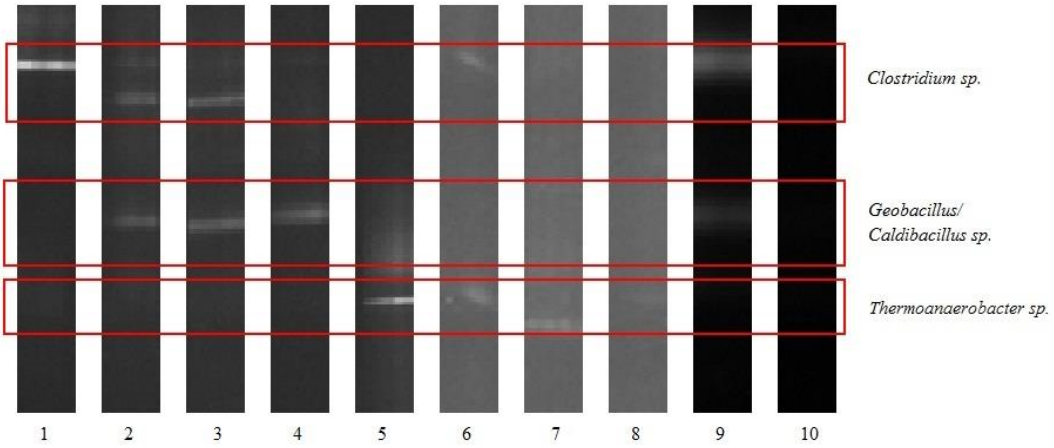

Figure 2.1. Denaturing gradient gel electrophoresis gel bands of isolated co-cultures. Red boxes represent areas of excision where amplification and viable sequences were obtained. Gels: 1, Clostridium thermocellum; 2, CNR1; 3, BN; 4, B4-1; 5, CN; 6, N2; 7, CP; 8, WS; 9, N4; and 10, N4P. For description of samples, please see Table 2.1.

Table 2.1. Samples and selection conditions

Sample	Culture Name	Strategies Employed [a]
Marsh	B4-1	Repeated anaerobic subculture
Marsh	BN	Low nutrient defined medium (1191 aerobic no YE)
Marsh	CP	Aerobic modified czapek peptone media
Marsh	N4	Aerobic plating/ anaerobic incubation
Marsh	N4P	Repeated aerobic sub culturing + aerobic plating
Marsh	N2	Direct anaerobic plating
Cow Feces	CNR1	Repeated anaerobic sub culturing
Cow feces	CN	Anaerobic plating
Wood Slurry	WS	Repeated anaerobic sub culturing

[a]Standard medium is M-1191 with cellulose unless indicated otherwise.

Table 2.2. List of cellulolytic consortium and organisms within identified by DGGE 16s rRNA fragment analysis

	Principal organisms present based on DGGE analysis			
Culture name[a]	No.	Commonly cellulolytic [b]	Not commonly cellulolytic[b]	Aerotolerance test[f]
Aerobic selection pressures				
BN	2	*C. thermocellum*[c]	*Caldibacillus debilis*	+
CP	3	*C. thermocellum*[d]	*Clostridium caenicola* , *Thermoanaerobacterium sp.*	-
N4	4	*C. thermocellum*	*Caldibacillus debilis* , *Geobacillus thermoglucosidasius*,*Geobacillus Y4.1MC1*	+
N4P	4	*C. thermocellum, Clostridium stercorarium*[e]	*Caldibacillus debilis* , *G. thermoglucosidasius*	+
Anaerobic selection pressures				
B4-1	2	*Clostridium thermocellum*	*Cadibacillus debilis*	+
CN	2	*C. thermocellum*	*Thermoanaerobacter wiegelii*	-
CNR1	2	*C. thermocellum*	*T. wiegelii*	-
N2	2	*C. thermocellum*	*Caldibacillus debilis*	+
WS	3	*C. thermocellum*	*Caldibacillus debilis, G. thermoglucosidasius*	+

[a]Please see Table 2.1 for details regarding cultures.

[b]Closest species identified in the NCBI database by BLAST analysis, based on a 97% or greater 16S rRNA nucleotide sequence identity. Due to the short 16S rRNA sequence length used, BLAST analyses of some fragments resulted in ambiguous species identification.

[c]BLAST analysis of this 16S rRNA sequence also resulted in 97% or greater sequence identity to *Clostridium clariflavum*.

[d]BLAST analysis of this 16S rRNA sequence also resulted in 97% or greater sequence identity with *Clostridium spp.* strain TG60-1, *Clostridium thermosuccinogenes*.

[e]BLAST analysis of this 16S rRNA sequence resulted in less than 97% sequence identity with this species.

[f]The test for aerotolerance consists of plating a stable coculture aerobically on cellulose overlay plates. A positive (+) result constitutes presence of at least one colony-forming unit.

Table 2.3. End products from anaerobic batch cultures for comparison against *Clostridium thermocellum* DSMZ 1237 in monoculture

Microorganisim/Consortia Name (Anaerobic)	End-products expressed in mol/mol glucose equivalent consumed (total yield in mmol)						Total glucose equivalents consumed (mmol)
	Formate	Acetate	Ethanol	Lactate	CO_2	H_2	
2g/l Cellobiose used as substrate							
C. debilis (isolated from B4-1)	1.8	1	0.8 (4.72)	0	0	0 (0)	5.9
G. thermoglucosidasius (isolated from N4P)	0.2	0.1	0.3 (3.51)	1.6	0.1	0 (0)	11.7
2g/l Cellulose used as substrate							
C. thermocellum DSMZ 1237	0.6	1	1.1 (5.9)	0.3	1.6	2.2 (11.9)	5.4
B4-1	0.6	1.2	0.5 (5.1)	0.3	0.7	0.9 (9.2)	10.2
BN	0.9	0.7	0.8 (10)	0.1	0.4	0.3 (3.8)	12.5
N2	0.9	0.7	0.8 (9.1)	0.5	0.6	0.6 (6.8)	11.4
N4	0.7	0.6	0.6 (6.2)	0.9	0.8	0.4 (4.2)	10.4
N4P	1.2	1	0.9 (8.5)	0.1	0.6	0.5 (4.7)	9.4
WS	0	0.3	0.5 (6)	1.2	0.8	0.7 (8.4)	12
CNR1	0	0.2	0.4 (4.2)	1.3	0.5	0.1 (1.0)	10.4
CN	0	0.3	0.3 (3.1)	1.4	0.7	0.6 (6.2)	10.3

Note: All measurements were conducted in triplicate with variations less than 15%. All cocultures were grown on cellulose excess except for the noncellulolytic *Geobacillus spp.* monocultures, which used cellobiose

Anaerobic plating did not result in the isolation of any cellulolytic strains in pure culture. This could be due to a dependence of the wild strains related to *C. thermocellum* on one or more companion species in the enrichments isolated, under the conditions used in this work. Cellulolytic colonies on cellulose overlay plates appear to contain a minimum of 2 species via DGGE analysis (N4, N4P, CP, N2), and none of the identified pure colonies on cellobiose plates matched either the *C. thermocellum* phenotype, or contained 16S rRNA gene sequences matching *C. thermocellum*. Instead, the 16S rRNA gene sequence for individual colonies matched *Geobacillus*, *Caldibacillus*, *Thermoanaerobacterium*, and *Thermoanaerobacter spp*.. The 16S rRNA sequence analyses did, however, identify species in the co-cultures that matched *Geobacillus* species, and aerobic plating on cellobiose resulted in the isolation of a pure strain of 2 *Geobacillus* species. 16S rRNA gene sequence analyses of the two pure isolates of *Geobacillus* revealed 99% nucleotide sequence identities with *G. debilis* strain tf (Banat *et al.* 2004), now called *Caldibacillus debilis* (Coorevits *et al.* 2012) and *G. thermoglucosidasius* NCIMB 11955 (Cripps *et al.* 2009*).*

The ability of the *C. thermocellum*-*Caldibacillus debilis* enrichment to carry out cellulose degradation under aerobic conditions suggests that it might be the minimal functional community for aero-tolerant cellulose. Many *Geobacillus* species have been shown to produce biofuels from starch or glucose, and have been genetically modified to produce high amounts of ethanol (Cripps *et al*. 2009). A subset of *Geobacillus* species possess properties the may be useful for biofuel production, are facultatively anaerobic (Banat *et al.* 2004), possess glycosyl

hydrolases (Shalom *et al.* 2002), possess sugar metabolisms that complement lignocellulosic fermentations (Banat *et al.* 2004), display low lactate production (Martin *et al.* 1992), and are highly tolerant of ethanol (Fong *et al.* 2006). Many examples of *Geobacillus* species with some of these features are described in the literature, although none have been tested for all of these features at the same time.

Members of the genus *Thermoanaerobacter*, strictly anaerobic thermophilic Firmicutes, have also been explored for their biofuel potential, and several studies have shown that co-cultures containing *C. thermocellum* and *Thermoanaerobacter ethanolicus* 39E display several advantages over a monocultures of *C. thermocellum* for the production of biofuels from lignocellulosic substrates (Fang 2010). Studies have also suggested vitamin cross-feeding between a *Thermoanaerobacter* sp. and *C. thermocellum,* showing a mutualistic relationship between them (Mori 1995). Other environmentally isolated co-cultures may also have mutualistic relationships (Mori 1990). Strictly anaerobic thermophilic members of the genus *Clostridium* within the consortia such as *C. stercorarium, C. clariflavum,* and *C. caenicola* have also been isolated in cellulosic enrichments (Izquierdo *et al*. 2010, Shiratori *et al.* 2009).

Different end-product synthesis patterns were displayed by enrichments under anaerobic conditions (Table 2.3). Some enrichments produced formate, lactate, ethanol, acetate, H_2, CO_2, all of which are synthesized by known strains of *C. thermocellum* (Rydzak *et al*. 2009). The highest ethanol concentrations produced by the enrichments were observed with the enrichments that appeared to be binary-cultures BN, N2, N4, and N4P in which a bacterium highly similar to *C. debilis* was the complementary member of the community. Enrichments observed to produce high

lactate concentrations contained bacteria similar to *G. thermoglucosidasius* and *Thermoanaerobacter sp.*, both of which are known lactate producers (Cook 2000, O-thong *et al.* 2008, Cripps *et al.* 2009). From the perspective of the total production of ethanol, the *C. thermocellum*-*Caldibacillus debilis* containing enrichments out-performed *C. thermocellum* mono-cultures (Table 2.2), possibly due to increased substrate conversion (see Table 2.3), *C. thermocellum* had a higher specific yield of ethanol, displaying higher yields of ethanol/mol glucose equivalent consumed. With respect to H_2, *C. thermocellum* mono-cultures outperformed enrichments in both total production as well as specific yields.

In our work, only two species appeared to be able to complement the *C. thermocellum*-like species in enrichments under conditions used, one related to a *Geobacillus* sp. under aerobic conditions and one related to *Thermoanaerobacter* sp. under anaerobic conditions. Most of the samples had direct interaction with the soil, from which *Geobacillus* species have been isolated. Samples derived from cow feces did not yield aero-tolerant enrichments (Marchant *et al.* 2002), and did not give rise to a *Geobacillus* sp. within their co-cultures. Enrichment CP, which appears to be composed of strict anaerobes, was passaged aerobically and presumably these anaerobes were able to overcome the oxygen due to the low solubility of atmosphere at 60ºC in media, however when tested for the ability to plate out aerobically on cellulose overlay plates no Colony forming units were observed.

An argument for a mutualistic relationship between the organisms related to *Caldibacillus debilis* and *C. thermocellum* under aerobic growth can also be made. The *C. debilis* is dependent upon *C. thermocellum* to hydrolyze cellulose to

fermentable sugars, while *C. thermocellum* is dependent upon *C. debilis* to consume oxygen and create an anaerobic microenvironment (Tran *et al.* 2010). A likely scenario for the aero-tolerant cellulolytic communities isolated is the formation of a biofilm whereby an oxygen gradient is formed to allow conditions ideal for a *C. thermocellum*-like organism's growth. Similar cooperation has been described for methanogens (Kato *et al.* 1993). Previous work by Islam *et al.* (2006) under similar conditions report cellulose above ~1.1g/l (~6.1mmol glucose equivalent) was not degraded, enrichment total glucose equivalents consumed (Table 2.3) show roughly a doubling in total cellulose degradation and consumption, demonstrating potential benefits of enrichments over monoculture work using C. *thermocellum.*

2.5. Conclusion

The results of this study show the potential for enrichments containing a *C. thermocellum*-like species and *Geobacillus*-like species to simplify consolidated bioprocessing by removing the requirement for pre-reducing the medium or substrate. Several of the enrichments exhibited a higher total cellulose conversion, and increased total ethanol yield. Future studies will concentrate on degradation rate and efficiency of production optimization within these systems. Isolation of what appears to be the minimal functional community containing *C. thermocellum* which achieves aerotolerant cellulolytic thermophilic growth to produce biofuels represents the first step in creating an aerotolerant lignocellulosic designer co-culture for CBP. Further studies need to be completed to determine whether this aero-tolerant phenotype is

specific to B4-1 and similar enrichments or a general theme that may be possible with other *Geobacillus* sp. paired with other strict anaerobes.

Chapter 3: Characterization of the Facultative Anaerobe *Caldibacillus debilis* GB1 and its use in a Designed Aerotolerant, Cellulose Degrading, Co-Culture with *Clostridium thermocellum*[2]

3.1. Abstract

Development of a designer co-culture that can achieve aerotolerant ethanogenic biofuel production from cellulose can reduce the costs of maintaining anaerobic conditions during industrial consolidated bioprocessing (CBP). To this end, a strain of *Caldibacillus debilis* isolated from an air-tolerant cellulolytic consortium, which included a *Clostridium thermocellum* strain, was characterized and compared with the *C. debilis* type strain. Characterization of the isolate *C. debilis* GB1 and comparisons with the type strain *C. debilis* revealed significant physiological differences including: i) the absence of anaerobic metabolism in the type strain and ii) different end-product synthesis profiles under the experimental conditions used. The designer co-cultures displayed unique responses to oxidative conditions, including an increase in lactate production. We show here that when the two species were cultured together, the non-cellulolytic facultative anaerobe *C. debilis* GB1 provided respiratory protection for *C. thermocellum*, allowing for synergistic utilization of cellulose even under an aerobic atmosphere.

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[2] Contributing authors: **[a]Scott Wushke, [b]David Levin, [b]Nazim Cicek, [b]Richard Sparling.** 2015. *Appl. Environ.* **81:**5567-73 Contributions: [a]First author experimental design, cell growth and end-product [b]Lab space, equipment, funding, and research guidance.

3.2. Introduction

Isolation and characterization of organisms with the ability to degrade and ferment cellulose is an essential step towards enhancing biofuel production via consolidated bioprocessing (Levin *et al* 2009). Designing a culture that could achieve both cellulose degradation and ethanol production under non-reduced conditions would reduce the costs and complexity of culturing strictly anaerobic bacteria, as this would eliminate the need for maintaining a reduced environment. *Clostridium thermocellum* is perhaps the best-studied model organism capable of consolidated bioprocessing (CBP) under anaerobic conditions (Lynd *et al.* 2005, Biswas *et al.* 2014). Co-culturing a cellulolytic organism with a non-cellulolytic partner capable of producing desired end-products at high rates and yields has been shown to improve overall biofuel production capability via CBP (Geng *et al.* 2010) and increased cellulose hydrolysis rates (Geng *et al.* 2010, Sharma 1991). Many biofuel-producing cellulolytic bacteria, including *C. thermocellum*, lack aero-tolerance and have not been shown to tolerate greater than 2% O_2 in the atmosphere (Kato *et al.* 2004, Ng *et al.* 1977). While members of the genus *Clostridium* are typically described as strict anaerobes, some members, including *C. straminisolvens*, *C. acetobutylicum*, and *C. intestinalis* encode genes required for aerotolerance, and have been shown to grow in microaerobic environments (Kato *et al.* 2004, Lee *et al.* 1989, O'Brien & Morris 1971). Moreover, genetic modification of *C. acetobutylicum* resulted in a significant increase in aerotolerance such that the recombinant strain was able to grow in the presence of atmospheric concentrations of oxygen (Hillmann *et al.* 2009).

Previously, it has been reported that co-cultures containing a *Clostridium* species paired with facultative aerobes can lead to oxygen removal in a sealed vessel followed by fermentation (Miyazaki *et al.* 2008, Ronan *et al.* 2013, Tran *et al.* 2010) or achieve aerotolerance via respiratory protection under microaerobic conditions (Zuroff *et al.* 2013). The importance of biofilms and the use of mixed cultures to help lignocellulosic degradation has also been highlighted recently (Zuroff & Curtis 2012). Ronan *et al.* (2013) highlighted the potential of stable, apparently aerotolerant, cellulolytic communities in fermenting cellulose to ethanol. Wushke *et al.* (Chapter 2) also described a stable enrichment culture, B4-1, capable of cellulolytic growth and fermentation in the presence of oxygen, that included *Caldibacillus debilis*-like and *C. thermocellum*-like bacteria. The *C. debilis* strain was subsequently isolated and named GB1.

The present paper aims to: i) characterize the *C. debilis* GB1 against the type strain, *C. debilis* Tf^{t} DSM 16016 and ii) demonstrate that the aerotolerant phenotype observed in the enrichment culture B4-1 can be achieved using a co-culture of *C. debilis* GB1 and *C. thermocellum* DSM 1237. To this end, we compared aerobic and anaerobic growth and end-product formation of *C. debilis* GB1, isolated from the cellulolytic aerotolerant ethanol-producing enrichment B4-1, against the type strain *C. debilis* Tf^{t} DSM 16016 isolated by Banat *et al.* (2004). Furthermore, the enrichment culture B4-1, primarily comprised of a *C. thermocellum*-like strain and *C. debilis* GB1, was compared against a co-culture containing *C. thermocellum* DSM 1237 and *C. debilis* GB1 under both aerobic and anaerobic conditions.

3.3. Materials and Methods

3.3.1. Culturing Method

Cultures of *C. thermocellum* DSM 1237 and *C. debilis* Tf^t DSM 16016 (hereafter referred to as *C. thermocellum* 1237 and *C. debilis* 16016, respectively) were obtained from the DSM culture collection. Enrichment culture B4-1 and *C. debilis* GB1, isolated from B4-1, were derived from previous work (Chapter 2). Modified 1191 medium (Islam *et al. 2006*), with a lower concentration of yeast extract (0.76 g/l) and pH adjusted to 7.2, was used for all experiments. Various sugars were used as carbon and energy source (at 2 g/L), including xylose, glucose, ribose, sucrose, arabinose, rhamnose, mannose, maltose, cellobiose, sorbitol, xylan, trehalose, as specified in the methods used for different experiments (see below).

Sealed Balch tubes (26 ml) and 110 ml serum bottles supplied from Bellco Glass Inc. (Vineland, NJ) and Fisher Scientific (Toronto, ON) respectively, were used to carry out experiments. Balch tubes were used for all substrate utilization experiments (Table 3.1) to compare *C. debilis* GB1 against *C. debilis* 16016. Anaerobic Balch tubes and serum bottles were prepared using the methodology described by Islam *et al.* (2006), while aerobic tubes and serum bottles were prepared using atmosphere in the headspace with no addition of the reductant sodium sulfide. Experiments designed to compare aerobic and anaerobic conditions (Table 3.1) utilized serum bottles with 10 ml media and 100 ml headspace.

3.3.2. Cell Growth

Prior to each experiment, cells were passaged once under the relevant condition to allow adaption to substrate and/or, in the case of co-cultures, to allow adaption to growth together. All experiments were carried out in triplicate using a 10% inoculum. All cultures were grown at 60ºC unless otherwise stated. Cultures grown for end-product sampling were grown with sampling points at 48 hours post-inoculation (h pi) (Table 3.1) in sealed aerobic Balch tubes contained 10 ml of media and 17 ml headspace. For Table 3.3 and Table 3.4, cultures were grown in serum bottles using 10 ml medium with 100 ml headspace to ensure oxygen excess throughout growth. Shaking (75 rpm) was used to equilibrate the medium with the gas phase. Sampling for end-products was taken at 72 h pi and 168 h pi. All cultures in Table 3.3 were grown under carbon excess conditions (5 g/L) with either cellulose or cellobiose. All cultures grown aerobically on cellulose were transferred with a cut-off pipet tip, as an intact biofilm appeared to enhance inoculation efficiency. Aerobiosis was also visually assessed during cell growth under relevant conditions via the pink colour of resazurin dye in the medium.

3.3.3. Physiological Characterization

Optical density OD_{600} was used to monitor cell growth in liquid media with soluble substrates. When the cells were grown on cellulose, substrate degradation, pH, end-product synthesis data, and protein concentrations were used to verify growth.

Protein concentrations were estimated by Bradford Assay (Bradford 1976), using a NanoDrop 1000 spectrometer.

3.3.4. Sugar and End-Product Analysis

Culture samples (1 ml) were collected after 48 hpi and stored at -20 ºC until analysed. Concentrations of cellobiose, glucose, ribose, trehalose, sorbitol, rhamnose, arabinose, maltose, mannose, sucrose lactate, formate, acetate, ethanol were measured by high pressure liquid chromatography (HPLC) using a Dionex ICS 3000 system equipped with a Bio-Rad Aminex-87H column, and run at 30 ºC, 0.75 ml/min, with 0.02 mM sulfuric acid. A Shodex 101 Refractive Index detector was used on all compounds being analysed.

Measurements of CO_2, H_2, and O_2 concentrations were determined using a Multiple Gas Analyzer #1 Gas Chromatograph (GC) System Model 8610-0070 (SRI Instruments, Torrance, CA), equipped with a Thermal Conductivity Detector (TCD), and using argon as the carrier gas. Columns and methods were used as previously described by Rydzak *et al*. (2009).

Welch's t-test was used to determine if ethanol production was significantly changed ($p<0.05$) in table 3.3 (Ruxton 2006). Glucose equivalents consumed were estimated from end-products produced using the formula: glucose equivalents = (C1/6) + (C2/3) + (C3/2). C1= formate and CO_2, C2= acetate and ethanol, and C3= lactate (Ellis *et al.* 2012).

3.3.5. Bioinformatic Analyses

The *C. thermocellum* DSM 1237 (NC_009012) and *C. debilis* 16016 (NZ_ARVR01000000) genomes were viewed and genes searched using IMG/er (Markowitz *et al.* 2008).

3.3.6. Culture Purity Confirmation

Monoculture purity was determined via 16S rRNA gene PCR amplification and sequencing using primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCCGCA-3'), and consistency of end-product production phenotype from literature and previous experiments (Chapter 2, Rydzak *et al.* 2009). The purity of the co-cultures (i.e. that they contained only *C. thermocellum* DSM 1237 and *C. debilis* 16016) was confirmed by PCR using the 16S rRNA interspacer primers, ITS-F 5'-GTCGTAACAAGGT AGCCGTA-3' and ITS-Reub 5'-GCCAAGGCATCCACC-3'. The resulting amplicons were compared against the interspacer profiles of the pure cultures using agarose gel electrophoresis (Cardinale *et al.* 2004).

3.4. Results

3.4.1. *Caldibacillus* Strain Comparison

C. debilis GB1 was deposited at the DSMZ and designated DSM 29516. Comparison of the *C. debilis* 16016 and *C. debilis* GB1 16S rRNA and *cpn60* gene sequences (16016 locus tags A3EQDRAFT_10000, A3EQDRAFT_00206; and GB1 GenBank accession numbers KF652080, KP259875) reveal high sequence identities (99.9%) for both. Growth of both strains on cellobiose at 60 °C revealed that strain GB1 grew similarly to 16016, both strains reaching similar cell densities as determined by OD_{600} (0.13), and total protein (Table 3.1). Growth of both strains on cellobiose was also measured and found to be similar at different temperatures (50, 55, 60, 65, and 70 ºC) as seen in Table 3.2. The growth rates for strain GB1 and 16016 were found to be optimal between 55 and 60 ºC (Table 3.2). Due to the low OD_{600} and high amount of cell lysis after reaching stationary typical of *C. debilis*, total protein/ml was used as an additional indicator of growth (Table 3.1).

Table 3.1. Substrate comparison of *C. debilis* strain 16016 and GB1 grown under static conditions in sealed Balch tubes on M-1191 at 48h post innoculation

Substrate	16016						GB1					
	Growth		End-Products (mM)				Growth		End-Products (mM)			
	Final pH[1]	µg protein/ml[2]	Lac[3]	Ac[3]	Form[3]	EtOH[3]	Final pH[1]	µg protein/ml[2]	Lac[3]	Ac[3]	Form[3]	EtOH[3]
Aerobic[4,5]												
cellobiose	5.5	118	0.6	14.4	6.3	-	5.5	106	-[4]	8.9	9.6	9.0
xylose	5.4	165	0.8	14.7	4.7	-	5.8	72	0.1	7.2	8.4	-
Anaerobic												
cellobiose	7.2	-	-	-	-	-	5.4	143	-	3.7	9.3	6.6
xylose	6.7	-	-	-	-	-	5.3	125	-	3.9	8.2	5.4

Tubes were incubated for 48h, for aerobic conditions. O_2 was measured by GC to ensure excess even at the end of the experiment. Also, the medium stayed pink indicating that the resazurin redox indicating dye was oxidized throughout the experiment.

[1]Standard deviation =<±0.1; [2]Standard deviation =<±6 µg; [3]Standard deviation =<±15%; [4]End-products of the media control were subtracted a dash (-) represents concentrations = or < Yeast extract controls. 16016 produced 0.1 mM lactate, 1.5 mM acetate and 0.9 mM formate and GB1 produced 0.1 mM lactate, 1.5 mM acetate, 0.5 mM formate; [5]Strain 16016 and GB1 also grew on xylose, ribose, glucose, mannose, cellobiose, sucrose, maltose tehalose under aerobic conditions

Table 3.2. Doubling Time of C. debilis GB1 and 16016 Between 50 and 70 ºC

Temperature (ºC)	GB1	16016
	Doubling Time (h)[1]	
50	6.25	6.00
55	1.78	2.18
60	1.71	3.00
65	3.29	2.96
70	5.33	3.00

[1]Standard deviation <=3%

A comparison of sugar utilization and end-product synthesis by strains GB1 and 16016 under aerobic and anaerobic conditions is summarized in Table 3.1. An increase in total protein concentrations, decrease in pH, and increase in end-product synthesis (when compared to the no substrate control) reveals that both strains 16016 and GB1 can grow aerobically on cellobiose, xylose, glucose, ribose, sucrose, mannose, maltose and trehalose, but not on sorbitol, arabinose, rhamnose or xylan.

The *Caldibacillus* genus, as presently described, indicates that they are obligate aerobes (Coorevits *et al.* 2012). While *C. debilis* 16016 was indeed confirmed to be a strict aerobe under the conditions tested, the novel isolate, strain GB1, was capable of both aerobic and anaerobic growth on the carbon sources tested: xylose and cellobiose (Table 3.1). Different end-product synthesis patterns were observed under aerobic and anaerobic growth for strain GB1. During anaerobic growth conditions (Table 3.1), *C. debilis* GB1 produced a higher acetate concentration while ethanol and formate formation remained at comparable levels (with the exception of xylose and ribose with which the GB1 cells did not produce any ethanol). Note that H_2 and CO_2 were not measured for experiments performed in Table 3.1.

A comparison of GB1 growth under anaerobic versus aerobic conditions, with shaking (Table 3.3), revealed that formate as an end-product was completely eliminated, and only acetate and CO_2 production was observed under aerobic conditions.

Table 3.3. End-Products (mmol/L of cell culture) After 72 and 168 Hours of Incubation at 60 ºC on M-1191 (shaken at 75 rpm)[1]

Microorganism/Consortia name	End products (mmol/L of cell culture)						O_2 consumed[4,7] (mmol/l of cell culture)	O_2 %[7]	Redox Balance
	Lactate[6]	Acetate[6]	Formate[6]	Ethanol[6, 8]	CO_2[6]	H_2[6]			
Anaerobic[5]									
C. debilis GB1 anaerobic [2]	0.1	5.5	10.1	6.5	BD	BD[3]	-		0.77
C. thermocellum anaerobic	1.5	5.1	2.0	4.2	8.7	15.9	-		0.80
C. thermocellum + *C. debilis* GB1 anaerobic	0.5	3.8	5.0	6.3	4.5	6.7	-		0.73
B4-1 anaerobic	2.6	7.2	10.7	11.0	5.6	4.1	-		0.84
Aerobic5									
C. debilis GB1 aerobic	BD	2.5/4.0	BD	BD	7.5/41.2	BD	6.1/35.4	19.7/13.6	1.22/1.16
C. thermocellum + *C. debilis* GB1 aerobic	6.6/1.3	8.7/BD	4.4/6.1	5.5/BD	13.2/86.2	12.4/9.3	8.0/97.0	19.3/0.8	0.46/90.5
B4-1 aerobic	2.4/4.2	12.4/17.3	4.5/5.2	3.8/3.8	48.2/41.2	9.2/11.5	32.0/34.0	16.4/16.0	1.31/1.01

[1] End-products produced from yeast extract control were subtracted from each condition (GB1 aerobically: CO_2=0.8 mmol/L of cell culture and O_2= 15 mmol/L of cell culture, GB1 anaerobically: 0.1 mM lactate, 1.5mM acetate and 0.4 mM formate), *C. thermocellum* did not produce any appreciable end-products from the media control; [2]All cultures were grown with cellulose excess (5g/L), with the exception of the pure culture of *C. debilis* GB1 where cellobiose (5 g/L) was used; [3] BD=below quantification limit (0.1 mM/L of cell culture); [4] t_0 has 100.7 mmol/L of cell culture of O_2 available; [5] only 72h shown for anaerobic samples as no change was observed at 168 hours, for aerobic contions measurements at 72h/168h is given; [6] standard deviation <= ± 10% for monocultures and =<±20% for co-cultures; [7] standard deviation <= ± 25%;

[8]Welch's t-test found a statically significant ($p<=0.05$) difference in ethanol production between *C. thermocellum* alone and *C. thermocellum* in co-culture under anaerobic conditions (20).

3.5. *Caldibacillus debilis* Plus *Clostridium thermocellum* Co-Culture

A picture of the designer co-culture grown aerobically on cellulose overlay plates can be found in Appendix B within the appendices.

Interspacer analysis (Cardinale *et al.* 2004) on sample points taken for co-culture experiments (Table 3.3) showed each co-culture was comprised of its component organisms and no contaminating organisms were detected.

Table 3.3 also compares the end-product synthesis patterns under aerobic and anaerobic conditions of the designed co-culture of *C. debilis* plus *C. thermocellum*, and monocultures of the two bacteria used in the designed co-culture against the initial B4-1 enrichment culture. The designed co-culture produced the same aerotolerant cellulolytic phenotype as the environmental enrichment culture B4-1 growing under aerobic conditions, with both co-cultures producing the end-products formate, acetate, lactate, ethanol, CO_2, and H_2 in varying concentrations at 72 h pi. Maintenance of aerobic conditions using shaking (Table 3.3) was confirmed visually (the pink colour of the resazurin dye) throughout growth, and at sample points by detection of excess oxygen using a GC.

The enrichment and designer co-cultures showed a similar response to oxygen for certain end-products. Both co-cultures display a decrease in formate and ethanol concentrations, and an increase in CO_2, acetate, and H_2 concentrations when compared to anaerobic cultures. Both the designer co-culture and B4-1 show a shift

towards lactate production, an aerotolerant fermentative pathway, under aerobic conditions when compared against the lactate production of the monoculture.

Further incubation under aerobic atmosphere (168 h pi) resulted in further metabolism of both substrate and fermentation end-products by the designer co-culture mostly to the respirative product CO_2. The reduction in concentrations of several end-products between 72 and 168 h pi in the aerobic co-culture is consistent with respiration of the fermentative end-products after the consumption of the sugars. Due to this apparent ability to consume end-products in co-culture, the ability to oxidize fermentation end-products was verified in pure culture using *C. debilis* (Table 3.3). Acetate and ethanol were oxidized, consistent with the reduction in O_2, while formate was only metabolised at higher levels in the presence of cellobiose.

3.6. Discussion

3.6.1. *Caldibacillus* Strain Comparison

First described as *Geobacillus debilis* in 2004 by Banat *et al.*, the type strain, DSM 16016 was characterized as Gram variable, rod shaped, spore forming, and obligatory aerobic organism with a temperature range of 50 – 70 º C. Upon re-evaluation of its taxonomic classification, it was renamed as *Caldibacillus debilis*, becoming the type strain for the new genus (Coorevits *et al.* 2012). Previous characterization of *C. debilis* strains showed the ability to grow aerobically on several substrates, including formate (Banat *et al.* 2004) in one strain, but none had been found to grow in the absence of oxygen. Our observed end-product profiles reveal

mixed fermentative and respiratory metabolism under aerobic atmosphere even though the type strain, 16016, was indeed an obligate aerobe (Table 3.1). While carbon sources were similar for both strains, aqueous end-product concentrations between strains were different (Table 3.1). When grown in static tubes in the presence of air, the redox indicator resazurin stayed oxidized throughout the experiment, both strains consumed oxygen but displayed incomplete respiration by the synthesis of acetate and formate. Strain 16016 (but not GB1) also synthesized lactate as a major end-product. GB1 produced ethanol under aerobic (resazurin oxidized to pink) or anaerobic conditions making it more attractive as an aerobic partner for CBP than 16016.

The decrease and subsequent elimination of formate production by GB1 in response to exposure of higher soluble O_2 (Table 3.3) concentrations likely stems from the fact that pyruvate formate lyase (PFL) is inactivated in the presence of O_2 (Takahashi *et al. 1987*). End-product data (Table 3.3) further suggests that PFL is used under anaerobic conditions, whereas pyruvate dehydrogenase (PDH) is most likely used under aerobic conditions, consistent with members of the closely related genus *Geobacillus* (Cripps *et al.* 2009). Analysis of the *C. debilis* 16016 genome revealed copies of genes encoding both of these enzymes. A copy of *adhe* an enzyme linked to high ethanol production (Peng *et al.* 2008) was also found in *C. debilis* 16016 but did not seem to be active under the growth conditions tested.

Typically, while under aerobic conditions, organisms produce CO_2 and H_2O, however, *C. debilis* produces acetate as well. Aerobic acetate production is commonly used to generate ATP and deal with excess acetyl-CoA generated by high carbon flux

through glycolysis (Farmer & Liao 1998). A unique characteristic that makes C. *debilis* GB1 desirable for biofuels production via CBP is low lactate production under all conditions tested, which is rare in wild-type thermophilic bacilli, such as *Geobacillus* species (Cripps *et al.* 2009, Tang *et al.* 2009, Danner *et al.* 1998). While both *C. debilis* GB1 and strain 16016 can grow aerobically using respirofermentative metabolism (Zigha *et al.* 2007), only GB1 maintains a low lactate phenotype.

Representatives of the thermophilic bacilli genera *Ureibacillus, Aneurinibacillus, Brevibacillus, Geobacillus,* and *Alicyclobacillus* (Fortina *et al.* 2001) have variable phenotypic characteristics regarding anaerobic metabolism, with both strictly aerobic and/or facultative anaerobic members being represented. Here we show a facultatively anaerobic strain within the genus *Caldibacillus*. Anaerobic fermentative metabolism make *C. debilis* GB1, with only low levels of lactate production, an interesting candidate for designer co-culture development to process lignocellulosic substrates in either aerobic or anaerobic conditions via CBP.

3.6.2. *Caldibacillus debilis* Plus *Clostridium thermocellum* Co-Culture

The enrichment culture B4-1 displayed an aerotolerant cellulolytic phenotype that is of interest for CBP (Chapter 2). Subsequent isolation and characterization of *C. debilis* GB1 from this enrichment demonstrated that *C. debilis* GB1 is most likely the organism lending functional aerotolerance to the enrichment. A co-culture of *C. debilis* GB1 and *C. thermocellum* 1237 was tested for cellulose degradation and ethanol production under non-reduced conditions. We compared the designed co-

culture with the original B4-1 enrichment containing *C. debilis* GB1 and an uncharacterized *C. thermocellum* strain as major components for their capacity to degrade cellulose. While both a pink colour and excess oxygen were observed throughout, *C. debilis* may reduce local concentrations of oxygen via respiration and/or via oxygen scavenging enzymes creating a reduced microenvironment for *C. thermocellum*. One possible requirement for this anaerobic micro-environment creation may be a solid substrate for biofilm creation (Zuroff & Curtis 2012). Indeed, there are several products generated from the co-cultures under aerobic conditions that require enzymes that are sensitive to oxygen, including H_2 and formate (Rydzak *et al.* 2009).

Figure 3.1 shows total ethanol, glucose equivalents consumed and ethanol yield per glucose. Previous studies of *C. thermocellum* growing on 1191 media on cellulose shows 6.1 mM of glucose equivalents are converted to end-products (Ronan *et al.* 2013, Biswas *et al.* 2015) in batch cultures, our experiments show 5.2mM glucose equivalents consumed. *C. thermocellum* under our conditions produces 4.2mM ethanol similar to other work in similar conditions (Islam *et al.* 2009, Biswas *et al.* 2015). The enrichment and defined co-cultures appear to act synergistically under aerobic conditions to allow for greater end-product production than either of the *C. debilis* or *C. thermocellum* monocultures. Synergies have been observed before in co-cultures containing *C. thermocellum* (He *et al.* 2011).

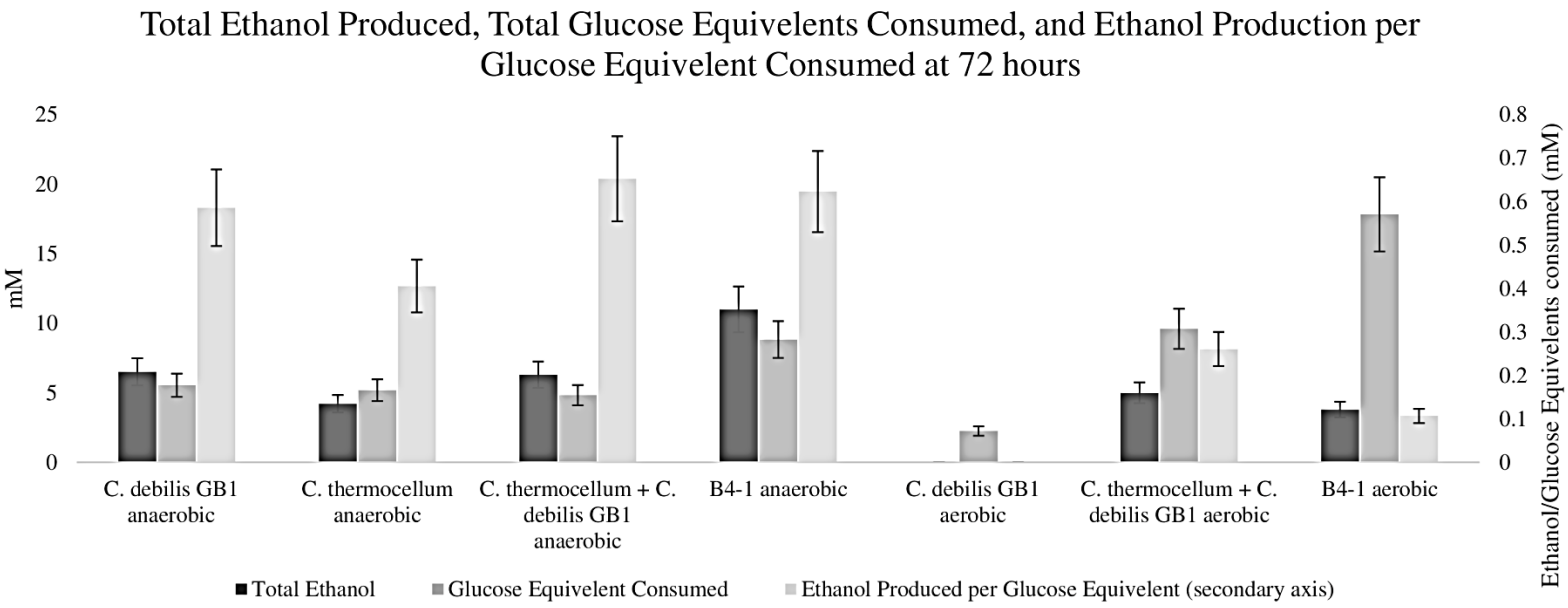


Figure 3.1. Comparison of total ethanol produced, total glucose equivalents consumed, and efficiency of ethanol produced per glucose consumed at 72 hours

Typically lignocellulosic ethanol production is rate-limited due to cellulose degradation rate (Levin *et al.* 2009, Lynd *et al.* 2005). The increase in total carbon consumed under co-culture conditions may be due to the ability of *C. debilis* to continue to metabolize cellobiose released through the action of the cellulosomes even after *C. thermocellum* has stopped growing due to low pH (under anaerobic conditions final pH~5.3, data not shown), alleviation of nutrient deficiencies, and/or consumption of end-products for utilization in respiration leading to a pH that does not limit growth of *C. thermocellum* (under aerobic conditions final pH of ~ 6.2, data not shown). Increasing total carbon consumed in CBP systems is a key element as an increase in total ethanol produced may enhance the economic viability of this process (Lynd *et al.* 2005).

Islam *et al.* (2006) showed high lactate production by *C. thermocellum* 1237 with high substrate loading, and during stationary phase. The shift to lactate production in *C. thermocellum* may be an indicator of cellular stress or of metabolic activity with limited growth (Rydzak *et al.* 2009). Cellular and/or oxidative stress may be important factors leading to high lactate in the designer and enriched co-cultures at 72 h pi. The genome of *C. thermocellum* DSM 1237 contains several annotated genes that may be involved in oxygen tolerance, including copies of alkyl hydroperoxidase, superoxide dismutase, and Mn-catalase. These genes may be necessary to deal with reactive oxygen species and reducing low levels of oxidative stress. It is possible that these can only be expressed while growing under microaerobic conditions, as provided by conditions of co-culture with *C. debilis.*

C. thermocellum has previously been shown to consume lactate (Rydzak *et al.* 2009). The ability of the co-culture to consume the fermentative products (lactate, acetate, ethanol) at 168pi appears to be an additive effect of the co-culture, since *C. debilis* GB1 appears to be capable of both acetate and ethanol oxidation (Table 3.4). While back consumption of ethanol is not ideal for CBP, the novelty of (micro) aerobic cellulosic degradation to produce ethanol makes it an interesting subject of study.

Table 3.4. Aerobic end-product (mmol/l of cell culture) consumption/production by *C. debilis* GB1 at 0, 72, and 168 hours of incubation at 60ºC on M-1191 medium (shaken 75rpm)1

Substrate	End products (mmol/L of cell culture)					O_2 consumed[3,6] (mmol/l of cell culture)
	Lactate[4]	Acetate[4]	Formate[4]	Ethanol[4]	CO_2[5]	
Lactate[2]	21.7/21.8/22.2	BD[7]	BD	BD	BD/BD/BD	BD /0.8/ BD
Formate[2]	BD	BD	23.1/23.0/23.0	BD	BD/BD/ BD	BD /0.5/0.5
Acetate[2]	BD	21.7/21.0/15.7	BD	BD	BD/0.7/1.3	BD /6.3/11.0
Ethanol[2]	BD	BD/9.8/13.0	BD	21.3/11.7/6.4	BD/0.9/2.2	BD /3.4/8.9
Mixed end-products[8]	2.7/2.9/2.9	2.5/1.4/BD	2.9/3.2/3.0	2.9/0.4/0.3	BD/1.1/2.8	BD /11.3/19.1
Cellobiose with mixed end-products[8]	2.7/2.9/3.0	2.7/4.3/BD	2.9/3.2/1.6	2.7/0.5/0.2	BD/1.3/3.0	BD /13.7/18.5
Uninoculated mixed end-product control[8]	2.4	2.1	2.6	2.7	BD	BD

[1]End-products produced and oxygen consumed from yeast extract control were subtracted from each condition (GB1 aerobically: CO_2=0.8 mmol/L of cell culture and O_2= 15 mmol/L of cell culture, GB1 anaerobically: 0.1 mM lactate, 1.5mM acetate and 0.4 mM formate), *C. thermocellum* did not produced any appreciable end-products from the media control [2]Addition of ~20.0 mM;[3] t_0 has 100.7 mmol/L of cell culture of O_2 available; [4]Standard deviation =<±10%; [5] Standard deviation =<±15%; [6]Standard deviation =<±10%[7] BD=below detection limit; [8] ~2.5 mM of each end-product and 1.1mM of cellobiose added where applicable.

While the aerotolerant phenotype is conserved in both the B4-1 enrichment culture and the designed co-cultures, there are some differences between the end-product synthesis patterns of the enrichment culture versus the designed co-culture. Comparison of the enrichment and designer cultures should be done with caution as there could be other undetected organisms within the enrichment culture (Chapter 2). Respiratory protection of an obligate anaerobe under aerobic conditions has previously been observed between *Clostridium phytofermentans* and yeast (Zuroff *et al.* 2013).

3.7. Conclusion

Previously described models for culturing of an obligate anaerobe and facultative anaerobe on a solid substrate includes oxygen depletion as the first step, followed by anaerobic fermentation (Miyazaki *et al.* 2008, Tran *et al.* 2010). In the current research, we observed simultaneous anaerobic fermentation and respiration. It is probable that the presence of *C. debilis* created an anaerobic micro-environment to allow *C. thermocellum* to ferment, even though the macro-environment remained aerobic (Table 3.3).

This study demonstrates that co-cultures containing only *C. debilis* and *C. thermocellum* possess both aero-tolerance and cellulolytic capabilities, even though *C. thermocellum* and the cellulosome are known to be aero-sensitive (Resch *et al.* 2013). This ability demonstrates potential CBP functionality under aerobic, anaerobic, or aero-transient conditions.

Chapter 4: Genome and proteome characterization of *Caldibacillus debilis* GB1 core metabolism under aerobic and anaerobic conditions[3]

4.1. Abstract

Caldibacillus debilis GB1 was isolated from a thermophilic aero-tolerant cellulolytic enrichment culture. GB1 shows a unique physiology of anaerobic growth when compared to the type strain Tf. There is a lack of representative proteomes of facultative anaerobic thermophilic *Bacillaceae*, exploring aerobic/anaerobic expression using high throughput techniques. The novel characteristics of GB1 compared to the type strain Tf, and ability to apply proteomic tools make GB1 of interest to study. The *C. debilis* GB1 genome was sequenced and annotated, and the proteome was characterized under aerobic and anaerobic conditions while grown on cellobiose. For the determination of the proteome, 2 dimensional liquid chromatography-mass spectrometry/mass spectrometry was used for protein separation and identification. iTRAQ (isobaric tag for relative and absolute quantification) based quantification was used to compare protein expression profiles, with special attention given to core metabolism and energy production and conversion pathways. The draft sequence of *C. debilis* GB1 contains a 3,340,752 bp chromosome

[3] Contributing authors: **Scott Wushke[a], Victor Spicer[b], Xiang Li Zhang[c], Brian Fristensky[c], Oleg V. Krokhin[b], David B. Levin[d], Nazim Cicek[d], Richard Sparling[d].** 2016 (manuscript in preparation)

Contributions: [a]First author experimental design, cell growth and end-product analysis; worked in concert with others authors in analysis of genome and proteome [b]proteomic tools and expertise, data analyses, and running samples on MS/MS; [c]Bioinformatic tools and expertise, genome assembly and 1st draft annotation; [d]Lab space, equipment, funding, research guidance.

and a 5,386 bp plasmid distributed over 49 contigs. Under aerobic conditions, production of proteins in glycolysis and pyruvate fermentation associated pathway profiles were down-regulated. Simultaneously, peptides corresponding to enzymes of the tricarboxylic acid cycle, pyruvate dehydrogenase, the electron transport chain, and oxygen scavenging pathways showed increased amounts. Under anaerobic conditions, protein production in fermentation from pyruvate was consistent with the generated end-products: formate, acetate, ethanol, lactate, and CO2. Under aerobic conditions both the respiratory product CO_2 and significant levels of acetate were produced. Production of CO_2 and acetate is consistent with incomplete respiration. This result is corroborated in the proteome as many of the proteins associated with end-product production were detected in high abundance with the notable exception of lactate dehydrogenase (found in low amount under anaerobic conditions only), which is consistent with a low lactate production phenotype. Through a comparison with gene expression profiles from *Escherichia coli* and *Bacillus cereus*, we show that global regulation of core metabolism pathways is rather similar in thermophilic and mesophilic facultative anaerobes of the Phyla Proteobacteria and Firmicutes.

4.2. Introduction

Co-culturing of bacteria has been found to have potential applications in consolidated bioprocessing (CBP) for ethanol production from lignocellulosic substrates by providing stability for cellulose degrading anaerobes, such as *C. thermocellum*, through oxygen protection (Ronan *et al.* 2013, Chapter 3, Lü *et al.*

2013). The first step in understanding interplay and possible synergies between aero-tolerant cellulolytic co-cultures is characterization of both cultures individually. Wushke *et al.* (Chapter 2 and 3) isolated and described a novel strain of *Caldibacillus debilis*, strain GB1, which provided such protective capability.

The genus *Caldibacillus* is currently represented by one species, *Caldibacillus debilis*. *C. debilis*, is a thermophilic *Bacillaceae* highly related to, yet distinct from, the members of the genus *Geobacillus*. *Caldibacillus*, was recently separated from the genus *Geobacillus* based on 16S rRNA sequence similarity (93%) and physiological differences such as polar lipid composition (Coorevits *et al.* 2012). While the type strain is an obligate aerobe (Coorevits *et al.* 2012), one strain is capable of both aerobic and anaerobic growth, partial respiration under aerobic conditions, producing CO_2 plus acetate, and of synthesizing a mixture of fermentation end-products under anaerobic conditions, including lactate, ethanol, formate, acetate, and CO_2 (Chapter 3).

The genome of the type strain *C. debilis* Tf (DSMZ 16016) has been sequenced as part of the Department of Energy (DoE) tree of life project, but the strain has not yet been described beyond its initial isolation paper (Banat *et al.* 2004). Strain GB1 described within the present paper is highly related to Tf but, contrary to strain Tf, is capable of ethanol production and anaerobic growth (Chapter 3). Since the GB1 strain is a facultative anaerobe, we wanted to characterize aerobic and anaerobic metabolism in *C. debilis* GB1 using proteomics. Wushke *et al.* (Chapter 3) describe strain GB1 from a physiological perspective; as such this information will be used to contextualize the results observed using proteomics.

The regulation of core metabolic processes and differences in gene expression profiles created via a transition from aerobic to anaerobic conditions is an area that has been described in depth due to its importance to bacterial physiology (Nakano & Zuber 1998), but only on a limited number of organisms. Furthermore, incomplete respiration of sugars has not been an issue investigated in these previous studies. Since *C. debilis* GB1 produces acetate even under fully aerobic conditions (Chapter 3), it may act as an interesting organism to compare against. The present study employs high-throughput, genome sequencing and annotation, proteomic analysis, to characterize aerobic and anaerobic catabolic pathways of *C. debilis* GB1. *C. debilis* GB1 shows a unique end-product profile compared to the type strain Tf and incomplete respiration. There are very few examples of proteomes exploring aerobic/anaerobic regulation in facultative thermophilic member of the *Bacillaceae* using liquid chromatographic-mass spectrometry techniques. Feng *et al.* 2007 employed classical 2D-page proteomic techniques on *Geobacillus thermodenitrificans* and discuss the genome and proteome in relation to aerobic and anaerobic metabolism, the authors did not generate their proteomes to investigate aerobic/anaerobic cross state changes. Since aerobic/anaerobic cross state comparisons were not preformed they could only make statements on the presence or absence of proteins with regard to respiration and fermentation. Because changes seen in expression within a proteome are also seen at the transcriptomic level, proteomes and transcriptomes have been directly compared before within the same organism (Munir *et al.* 2016, Fu *et al.* 2015) and between organisms (Verbeke *et al.* 2013). In order to understand aerobic and anaerobic metabolism of *C. debilis* in a broader

context, a comparison against another organisms is pertinent. Therefore a comparison of the *C. debilis* GB1 proteome set to a published *E. coli* K-12 transcriptomic data set (Partridge *et al.* 2006) under both anaerobic and aerobic growth conditions was done. We also discuss the *C. debilis* GB1 data set in light of aerobic and anaerobic regulation reported by Weber *et al.* (2006) for *E. coli* and by Van der Voort *et al.* (2009), and Nakano & Zuber (1998) for *Bacillus cereus.* The resultant comparison of organisms with similar core metabolisms and gene complements revealed that global regulation is similar between mesophilic and thermophilic representatives of the Firmicutes and Proteobacteria, respectively.

4.3. Materials and Methods

4.3.1. Cell Culturing and Harvesting

C. debilis strain GB1, DSM 29516, described in previous work (Chapter 3, Chapter 2) was grown on modified 1191 (M-1191) medium (Chapter 2, Islam *et al.* 2006) for all experiments. The M-1191 medium contained lower concentrations of yeast extract (0.76 g/L) compared with standard 1191 medium, and the medium pH was adjusted to 7.2. Sealed Balch tubes (26 mL) from Bellco Glass Inc. (Vineland, NJ) and 1 L Corning bottles supplied from Fisher Scientific (Toronto, ON), were used to carry out experiments. Anaerobic and aerobic environments in Balch tubes and Corning bottles were prepared as previously described by Wushke *et al.* (Chapter 3). Prior to each experiment, cells were passaged once under the relevant growth condition to allow adaption. All cultures were grown at 60 ºC with shaking at 75 rpm.

During aerobic growth, excess oxygen in the headspace of the cultures was periodically determined by GC analysis (Chapter 3). Throughout growth, a higher redox of the medium consistent with aerobiosis was determined visually during cell growth under relevant conditions thanks to the pink colour of resazurin dye in the medium. Growth curve experiments were carried out in triplicate using a 0.2% inoculum.

Cells grown for proteomic experiments were carried out in duplicate using a 0.2% inoculum. Cells were harvested at ~40μg total protein/mL corresponding to early-exponential phase. Presents of oxygen under aerobic conditions was confirmed via GC and the production of end-products consistent with previous experiments was confirmed via HPLC. At this point in growth, the biomass had reached 50 μg total protein per mL as measured by the Bradford protein assay method (Bradford, 1976). Fifty mL of culture were harvested by centrifugation at 7000 x g for 20 minutes at 4ºC to create cell pellets. These were stored at -80ºC for further processing.

4.3.2. DNA Isolation and Processing

For genomic DNA, a Promega Wizard Genomic Extraction Kit (Promega) was used and protocol followed as described by the manufacture. DNA was sent for sequencing at McGill University and Genome Quebec Innovation Centre using the 454s-GS-FLX-Titanium shotgun sequencing method similar to methods used by Verbeke *et al.* (2013). To improve gap closure and obtain higher quality data, the genome was re-sequenced using Illumina HiSeq 2000 paired end technology.

Multiple assembly pipelines combining both 454 and Illumina data were evaluated (Verbeke *et al.* 2013). Based on statistical metrics, we chose Newbler v2.6 for our assembly (Trong *et al.* 2009), which resulted in a genome of 3,340,752 bp distributed on 49 contigs, with a minimum depth of coverage of 19X. The sequence was then submitted to the Joint Genome Institute's Integrated Microbial Genomes Expert Review (IMG/ER) platform for gene annotation (Markowitz *et al.* 2008). Anomalies were identified and corrected manually. Further corrections were made by use of a naive assembler to identify and correct possible sequence errors based on the proteomic data (Verbeke *et al.* 2013). The final genome annotation was submitted to the National Center for Biotechnology Information (NCBI) under the GenBank accession number AZRV00000000.

4.3.3. Preparation of Cells for Protein Extraction

Cells were washed 3 times in 10 mL PBS buffer and frozen at -80 ºC. Cells were re-suspended in 10 mL SDT buffer (4% w/v SDS, 100 mM pH 7.6 Tris-HCl, 0.1M DTT), boiled for 15 minutes (min), and sonicated for 2 min continuously (Fisher Sonic Dismembrator-model 300). Unlysed cells and cell debris were removed by centrifugation at 13,500 x g for 15 min at room temperature, and the supernatant was collected. Amicon ultra Falcon tube centrifugation units, with a 10 kDa filter, were used to concentrate the protein lysate, which was washed 3 times with 10 mL of filter sterilized UA solution (8M urea in 0.1 M tris/HCL pH 8.5) at 4000 x g for 30 min and the flow-through discarded. Proteins were alkylated using 5 mL of IAA

solution (UA solution with iodoacetamide 0.5 M added) for 15 minutes. IAA solution was centrifuged to remove it from the Amicon ultra falcon tube centrifugation unit. The alkylated protein lysate was rinsed 2 times with 5 mL UA solution using centrifugation at 4000 g separated the liquid from the alkylated protein lysate, the flow through was discarded. ABC solution (ammonium bicarbonate 0.05 M) was used to re-suspend protein, and 50 µL were collected for total protein estimation using a NanoDrop and Bicinchononic Acid (BCA) Protein Assay Kit (Pierce Biotechnology, Rockford, IL). Total protein was then concentrated using centrifugation 4000 x g for 30 min, and re-suspended in a final working volume of 2 mL using ABC with trypsin added at a 1:10 ratio (trypsin: total protein). Trypsinization was carried out for 10 hours (h) at room temperature. Then 10% trifluoroacetatic acid (TFA) (20 µL) was added to acidify the sample, after which the sample was added to a filtration unit placed on a fresh Falcon conical tube, centrifuged at 4000 x g for 30 min, and the flow though collected. The filter washed using 200 µL of 0.5 M NaCl, centrifuged at 4000 x g for 30 min and the flow through collected. Peptide concentration was estimated using the NanoDrop, samples were frozen at -80 ºC for 24 hours, dried and concentrated using a roto-evaporation (Savant Speedvac-sc110). Samples were re-suspended in 270 µL of 0.1% TFA and desalted using C18 X-Terra column (1 × 100 mm, 5 µm, 100 Å; Waters Corporation, Milford, MA, USA) with 50% acetonitrile used to elute peptides. Desalted samples were frozen at −80°C and concentrated using roto-evaporation. Two biological samples (100 µg) from aerobically and anaerobically grown cells were then labelled with isobaric Tags for Relative and

Absolute Quantitation (iTRAQ) reagent (Applied Biosystems, Foster City, CA, USA) following the protocol described by the manufacturer.

4.3.4. Mass Spectrometry

Instruments and methods used for proteomic analyses were previously described (Rydzak *et al.* 2012). Two-dimensional liquid chromatography-mass spectrometry/mass spectrometry was used for peptide separation and identification. A QStar Elite mass spectrometer (Applied Biosystems, Foster City, CA) was used in standard MS/MS data-dependent acquisition mode with a nano-electrospray ionization source. The 1 second(s) survey MS spectra (m/z 400–1500) were collected, followed by three MS/MS measurements on the most intense parent ions (80 counts/s threshold, +2 to +4 charge state, m/z 100–1500 mass range for MS/MS), using the manufacturer's "smart exit" settings and iTRAQ settings.

4.3.5. Database Search, Protein Identification, and Statistical Analysis

Raw spectra WIFF files, a QSTAR file format, of unlabeled peptides were treated using standard script (Analyst QS 2.0) to generate text files in Mascot Generic File format (MGF) (Cottrell *et al.*1999) and ProteoWizard to generate mzML files (Kessner *et al.* 2008) MGF files containing the MS/MS spectra information for all 4 fractions were concatenated and submitted for protein identification using Global Proteome Machine's (GPM) X!Tandem (Craig *et al.* 2004) and an in-house, GPU-based peptide identification engine described by McQueen *et al.* (2012).

Standard QTOF settings were used for the search: 100 ppm and 0.4 Da mass tolerance for parent and fragment ions, respectively. Permitted amino acid modifications included constant carbamidomethylation of Cys. The XML output file from an X!tandem search and its corresponding iTRAQ reporter intensity collection is archived at: http://hs2.proteome.ca/gpm/archive/cdeb/BASERESULTS.xml.gz and http://hs2.proteome.ca/gpm/archive/cdeb/BASERESULTS-tic.gz. The iTRAQ reporter intensities as well as total ion current (TIC) on a peptide level were summed into their source proteins. TIC represents the summed intensity across the entire range of masses being detected at every point in the analysis. These sums were log2 transformed for simple differential analysis (Rydzak *et al.* 2012). All 3264 of the protein coding genes in the genome were used in the data dependent identification of MS/MS spectra. X!Tandem used a survival function of the 6732 assigned spectra to give an estimated peptide spectrum match false discovery rate (FDR) of 1.8% (Fenyö *et al*. 2010). Each protein measurement required a minimum of two peptide spectrum matches. We used our in-house data analysis system UNITY (Fu *et al.* 2015) to analyse and manipulate our proteomic data. The reporter ion (iTRAQ tag) intensities for each tryptic peptide identified (with expectation values < −1.5) were transformed by the log2 of the ratios (Z0 = tag116/tag114, Z1 = tag117/tag115, R0 = tag115/tag114, and R1 = tag117/tag116) to build overall peptide population distribution histograms, where aerobically grown replicates were labeled with tags 114 and 115, respectively, and anaerobically grown replicates were labeled with tags 116 and 117, respectively. Peptide level Z-scores are mapped as the distance from the population mean in units of standard deviation; initial protein-level Z-scores are

averages of the member peptide Z-score values. The Z-scores (R0, R1) contain information about the stability across biological replicates at the same growth state, while Z0 and Z1 represent the differences between the 2 growth states.

A simple algorithm was used to combine these with the differential data in (Z0,Z1), expressed as the difference between the magnitudes of vectors from the origin to points (Z0,Z1) and (R1,R0), scaled by the widths of their peptide histogram distributions (Rydzak *et al.* 2012). Then Z0, Z1, R0, R1 were normalized to give $Z0_{net}$, $Z1_{net}$, $R0_{net}$, and $R1_{net}$, respectively. The sign of the transformed values were determined by the angle subtended by a vector from the origin to the point ($Z0_{net}$,$Z1_{net}$). We denote this combined value of the vector difference as Zmag. A Zmag >± 1.65, represents the outer 10% of population, while a Zmag > ± 1.28, represents outer 20% of population of measured protein differences. These can be converted to fold change difference by taking the 2 to the power of the Zmag, as described by Rydak *et al.* (2012), with a 1.65 and 1.28 Zmag representing a 3.2 and 2.4 fold change respectively.

To enable analysis of smaller Zmag differences, we used a function, W_{stat}, to evaluate the statistical significance of a two-state two-replicate dataset on a protein-by-protein basis. We derived an overall measurement-system quality as:

Quality = (SD (Z0) / SD (Z1)) / (SD (R0) / SD (R1))

Where SD = Standard deviation

Then, to exploit this at an individual protein level, we used the following formula:

W_{stat} = Quality* √ ($Z0net^2$+$Z1net^2$)/ √ ($R0net^2$+$R1net^2$),

Where Z0net and Z1net are normalized Z0 and Z1.

The W_{stat} value denotes the "signal to noise" (S : N) ratio of the vector magnitudes of each respective cross-state and intra-replicate normalized value, scaled by the overall system quality. Monte-Carlo models were used to derive a function relating the W_{stat} value to the FDR. In our analysis, proteins with a $W_{stat} > 2.8$ have a FDR of 10% or less.

4.3.6. Comparison of *C. debilis* GB1 Proteome with *E. coli* K-12 Transcriptome

We compared our proteomic set from *C. debilis* GB1 to a transcriptomic data set from *E. coli* K-12 (Partridge *et al.* 2006) using the in-house data analysis system called UNITY (Fu *et al.* 2015, Verbeke *et al.* 2014). We chose the *E. coli* K-12 data set as it was in a machine readable format compatible with our methods, and compared the data sets using Z-scores and Zmag expression changes under aerobic and anaerobic conditions as these values are scaled against their mean, allowing us to make direct comparisons. Using data from Partridge *et al.* (2006) in our analysis allowed us to look at expression patterns that were not overtly discussed in the cited paper, and allowed us to compare their interpretation with ours.

4.4. Results and Discussion

4.4.1. General Genome Features

The *C. debilis* GB1 genome consists of a 3,340,752 bp draft genome (and a 5,483 bp plasmid) distributed on 49 contigs with a GC content of 51% (Appendix D). The GB1 genome encodes a total of 3,374 annotated genes, 3,264 protein coding genes, 110 RNA genes, including 8 ribosomal rRNA genes, and 85 Transfer (t)RNA genes. Gene encoding sequences account for 79.8% of the genome. Putative functions were assigned to 2,450 Open Reading Frames (ORFs), with the majority of the remaining 814 ORFs designated as hypothetical or conserved hypothetical gene products. There are 25 putative transposase associated genes. A putative origin of replication, *oriC*, was identified based on structures homologous to origins of replication identified in other Firmicutes. A DnaA-box like sequence (Cdeb_00348) and several genes encoding DNA replication enzymes that are known to be associated with *oriC*, such as DNA gyrase subunits A and B (Cdeb_00344, Cdeb_00343), and a primary replicative helicase (Cdeb_00367), were identified in close proximity to one another. This annotated genome was used for the analysis of the proteomes described below.

4.4.2. Growth Characteristics at the Proteomic Sampling Point

Cells were grown aerobically and anaerobically to early exponential phase to get representative protein profiles for eventual proteomic analyses. For aerobic conditions, cultures were shaken at 75 rpm and continuous presence of O_2 in the headspace was confirmed by GC analysis. The pH of the aerobic culture during early exponential phase was 6.95. Conversely, anaerobic conditions entailed flushing O_2 with N_2 gas and shaking at 75 rpm. The pH of the anaerobic culture during early exponential phase was 6.85. End-product analyses indicated that under anaerobic conditions, *C. debilis* GB1 synthesized lactate, formate, acetate, and ethanol, as previously described by Wushke *et al.* (Chapter 3). Wushke *et al.* (Chapter 3) show that aerobic cultures synthesized CO_2 and acetate, suggesting that their metabolism was carrying out incomplete respiration. The end-product profiles of cells grown for proteomics measurements matched the end-product profiles of those grown in chapter 3 as measured by HPLC and GC under aerobic and anaerobic conditions, under aerobic conditions O_2 was present and in excess, further the resazurin media redox indicator was pink confirming oxidizing conditions. Growth curves showing the harvesting points for total protein extractions for proteomics analyses are in Figure 4.1. As previous work by Wushke *et al.* (Chapter 3) detailed the physiology with respect to end-product production under aerobic and anaerobic conditions, the proteomic data could be placed in physiological context.

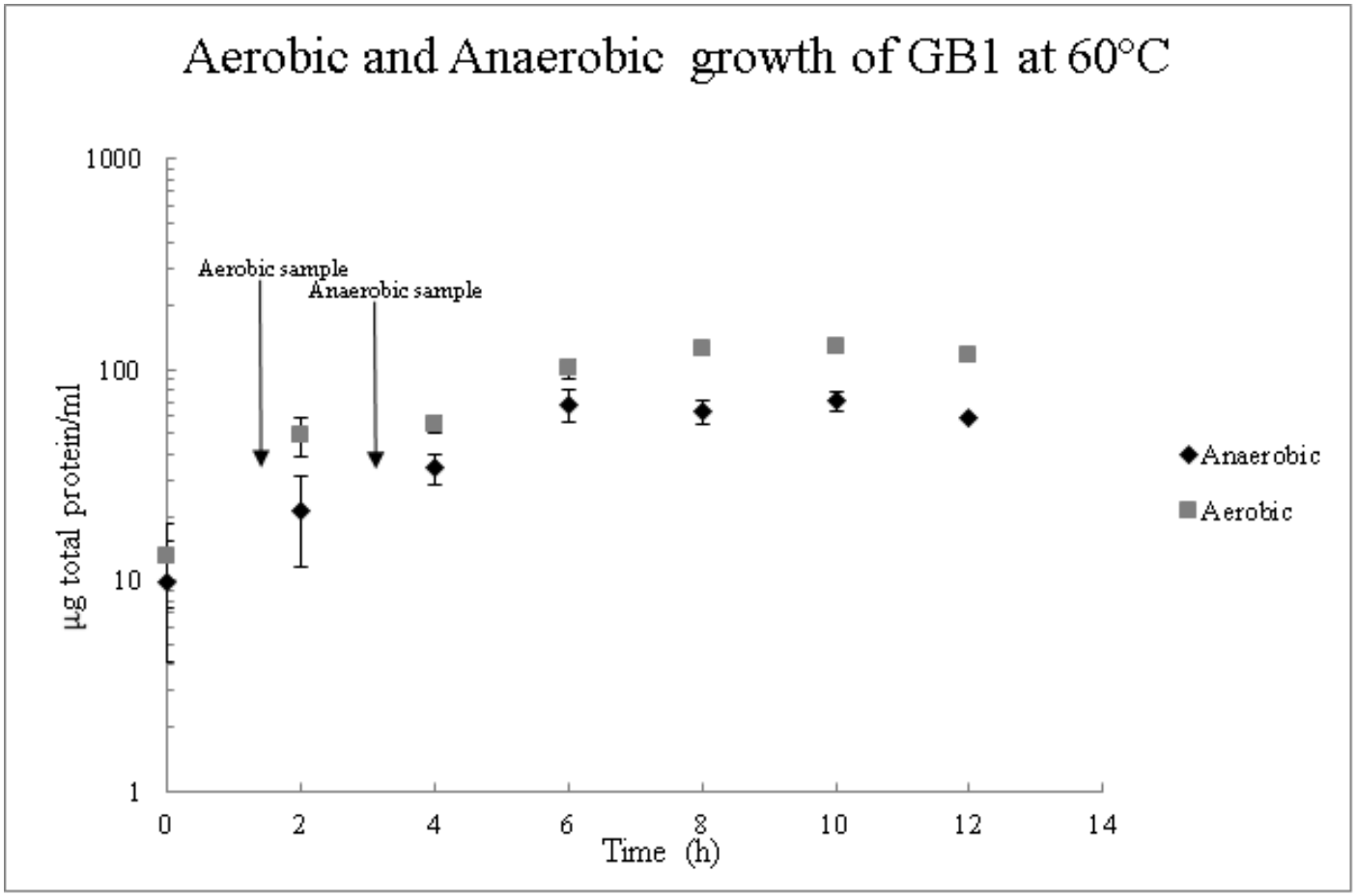


Figure 4.1. ***Caldibacillus debilis*** **GB1 growth curves at 60ºC shaken 75 rpm aerobically and anaerobically**

4.4.3. General Features of the Proteomes

Relative abundance of individual proteins between aerobic and anaerobic expression profiles were examined by z-score (Zmag is the combined vector of the 2 z-score replicates) and $\log_2$ (TIC). The Z0, Z1, R0, and R1 histograms showing their distributions, average, and standard deviations are in Figures 4.2, 4.3, 4.4, 4.5 and Table 4.1. Out of the 3,264 protein coding genes within the genome, 1,144 were detected in the pooled TICs (all 4 samples). 438 of the detected proteins could not be quantified as they were not detected in under both conditions and in both replicates. 706 could be quantified between conditions and given Zmag scores. These are listed in Table C.1, Appendix C and identified by their COG categories. Of the 706 proteins assigned Zmag values, 220 had W_{stat} values > 2.8, and therefore a FDR rate of < 10%. Correlation between the Z-scores of the cross state replicates (Z0net and Z1net in Figure 4.6) was good, with an R^2 of ~ 0.83.

The following sections describe the differences in protein amounts of specific pathways associated with catabolism in aerobically and anaerobically grown *C. debilis* GB1. For comparison and subsequent discussion (see section 4.4.12), transcriptomic data from *E. coli* K-12 (Partridge *et al.* 2006) for the equivalent functional genes are presented side-by-side on the same scale. An overview of the pathways with their average normalized expression values for each pathway in *E. coli* K-12 and *C. debilis* GB1 can be found in Table 4.2

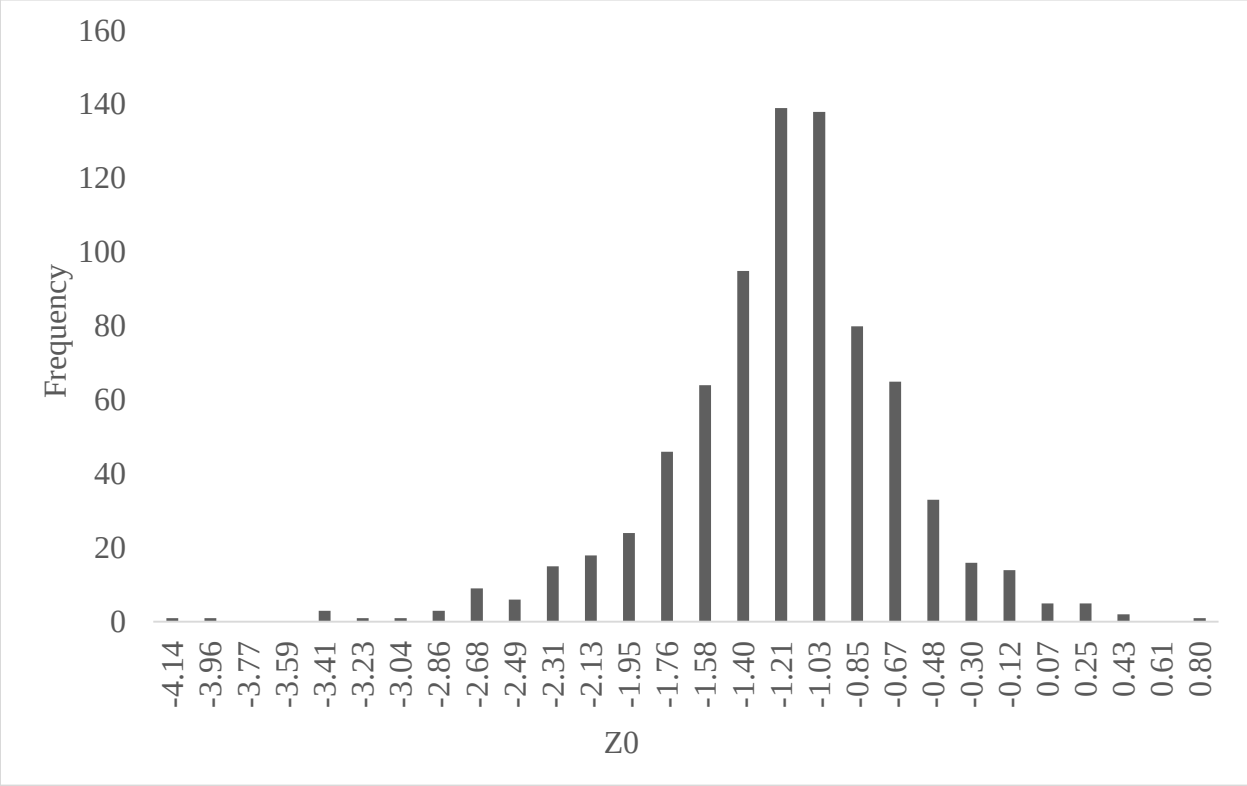


Figure 4.2. Histogram of Z0 values for cross state comparison (log_2(anaerobic tag-114/ aerobic tag-116) transformed into their corresponding Z-Scores)

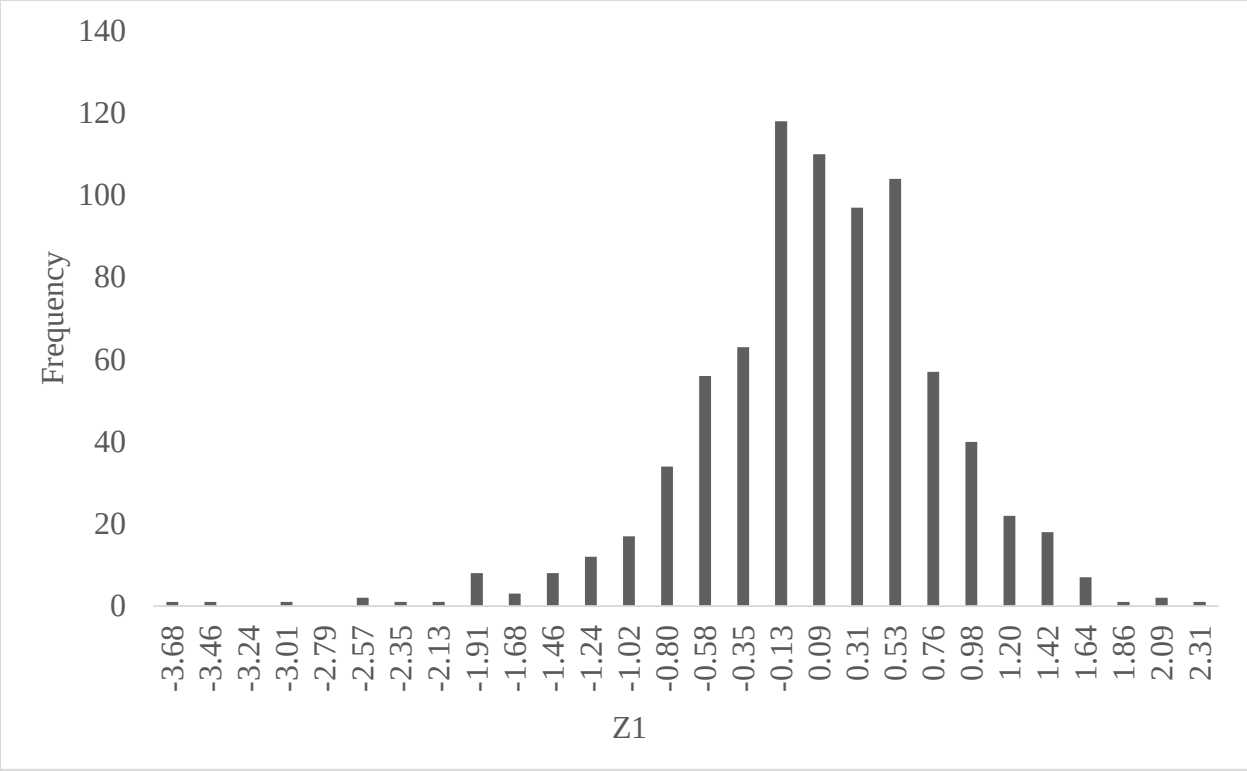


Figure 4.3. Histogram of Z1 values for cross-state comparison (log_2(anaerobic tag-115/ aerobic tag-117) transformed into their corresponding Z-Scores)

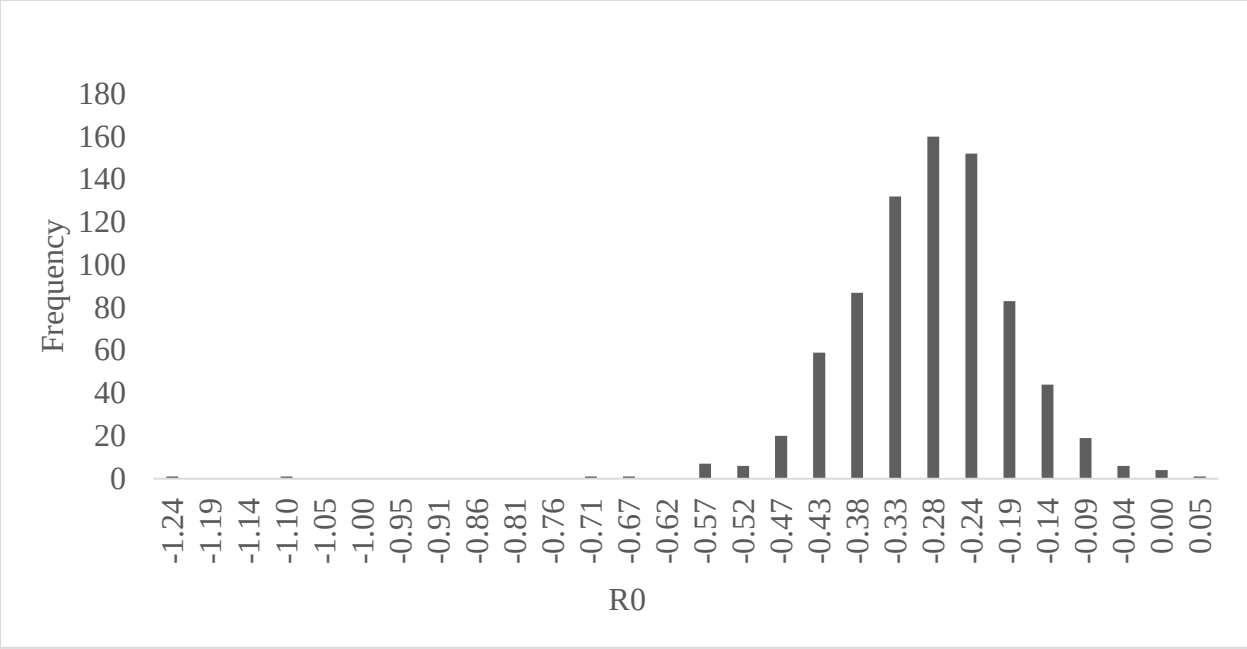


Figure 4.4. Histogram of R0 values (log2(anaerobic tag-114/ anaerobic tag-115) transformed into their corresponding Z-Scores) for aerobic replicates

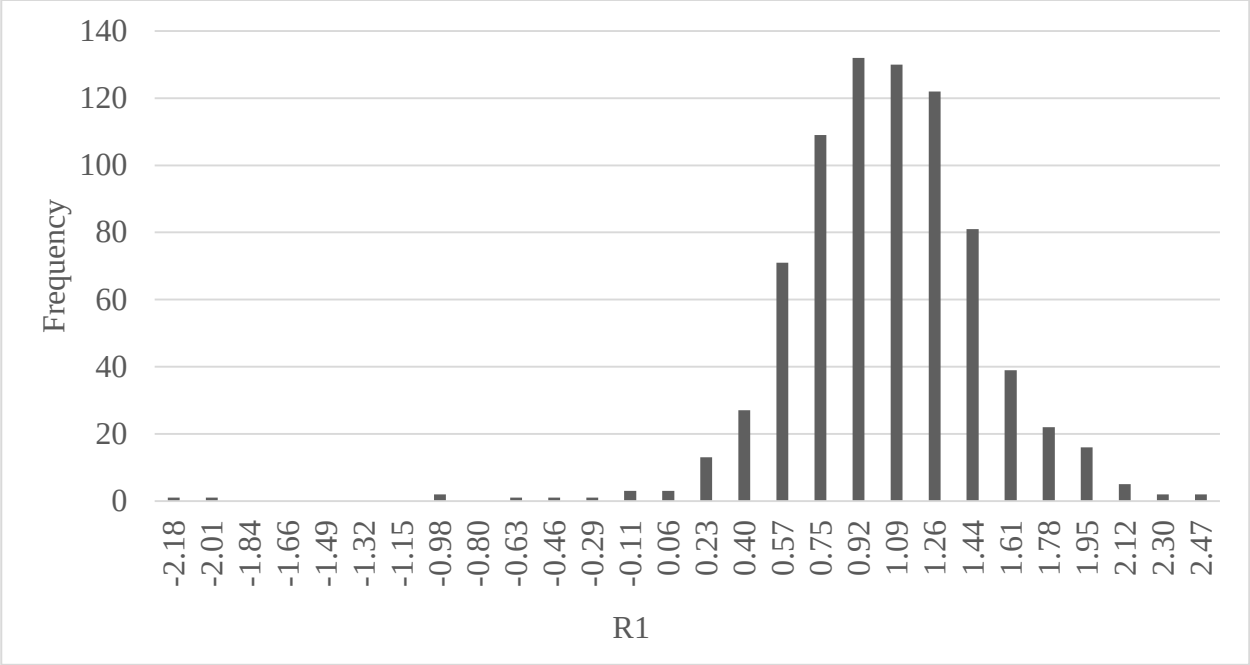


Figure 4.5. Histogram of R1 values (log_2(aerobic tag-116/aerobic tag-117) transformed into their corresponding Z-Scores) for anaerobic replicates

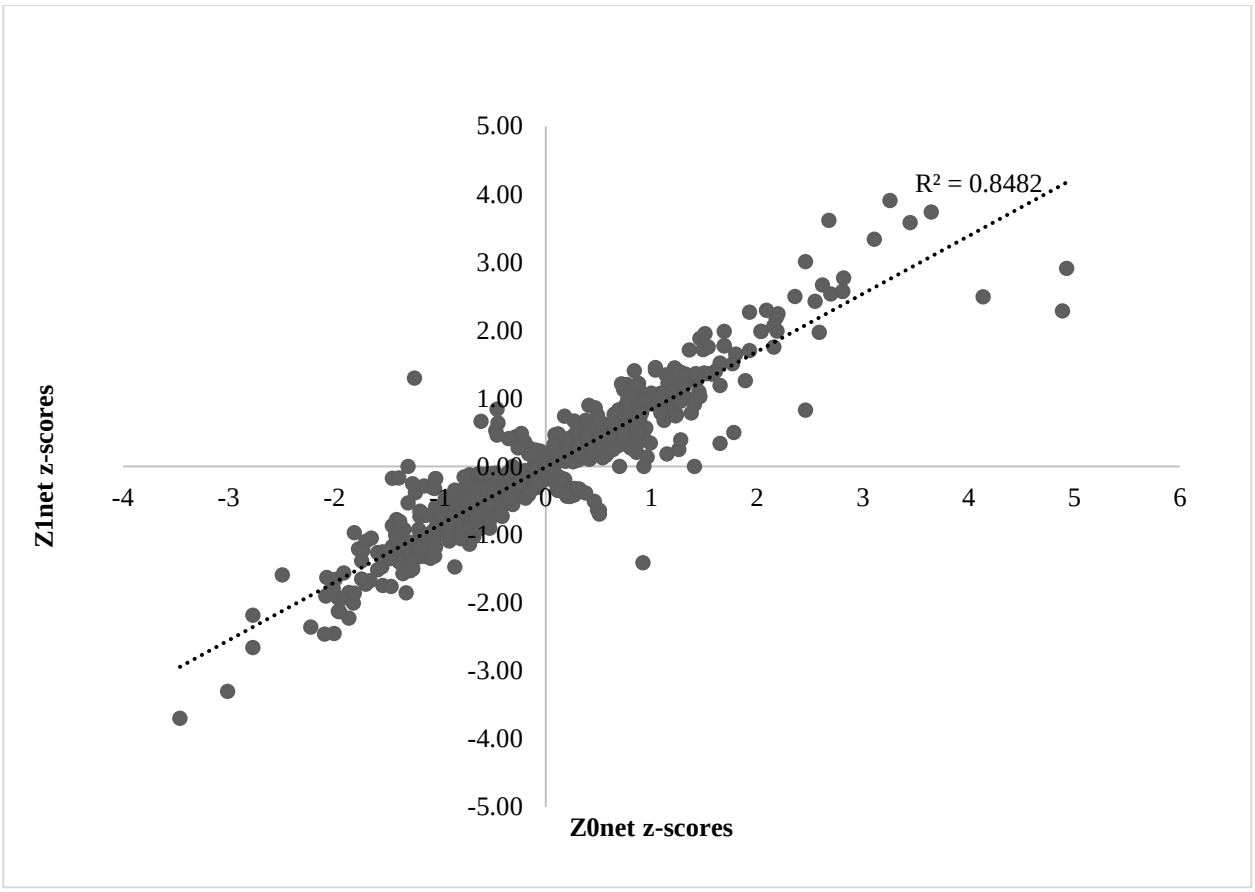


Figure 4.6. Correlation plot of cross state replicates (Z0net vs Z1net). Where $Z0_{net}$ and $Z1_{net}$ are the normalized Z0 and Z1.

Table 4.1. Average and standard deviations of Z0, Z1, R0, and R1

	Z0	Z1	R0	R1
Average	-1.30	-0.02	-0.31	0.96
Standard deviation	0.58	0.73	0.11	0.45

Z0, Z1 = Replicates comparing different states; R0, R1 = Inter-replicate differences

Table 4.2. Average Zmag and Z-Score for *C. debilis* GB1 and *E. coli* K-12 respectively

Pathway	*C. debilis* GB1 average Zmag[1]	*E. coli* K-12 average Z-score[2]	# of gene expression values measured for *C. debilis* (*E. coli* K-12)
Glycolysis	-0.49	-1.59	11 (11)
TCA	1.89	1.51	12(12)
ETC	1.1	0.78	13(12)
ROS	0.13	1.13	3(3)
Pyruvate fermentation (excluding PDH, ADH)	-0.56	-0.12	8(7)
PDH	0.85	1.95	8(8)
Cellobiose ABC transport	-1.2	-0.05	6(3)

[1]Zmag is the difference in expression between anaerobic/aerobic conditions by *C debilis*;

[2]Z-score is the expression difference between anaerobic/aerobic conditions of *E.coli* K-12 transcriptome;

4.4.4. Tricarboxylic Acid Cycle

The Tricarboxylic acid (TCA) cycle is an important source of reduced equivalents for ATP synthesis during aerobic growth, and should be highly expressed aerobically when compared to anaerobic conditions. On the basis of $\log_2$(TIC) the TCA cycle enzymes were highly expressed, with many being above the 50th percentile of expressed proteins (Table 4.1A). Upon deeper analysis, six enzymes (or subunits thereof) involved in the TCA cycle were highly (> 1.65) up-regulated and one was moderately up-regulated (> 1.28) in *C. debilis* GB1 cells under aerobic conditions (Table 4.3). Additionally, enzyme subunits *e.g.* Cdeb_02130, Cdeb_02131 of the TCA cycle were contiguous in the genome and showed similar changes with a Zmag of 3.34 and 2.29 respectively in the proteomes. When expression level differences were averaged for the entire TCA cycle (Table 4.2), it became clear that the TCA cycle was significantly up-regulated under aerobic conditions. Table 4.3 also illustrates the importance of grouping all of the elements of a pathway. This allows one to observe more subtle regulation at the pathway level. Indeed several of the changes seen, while they were rather large, were below our significance threshold cut-offs (± 1.65 and ± 1.28) at the individual gene level for the present experiments with *C. debilis*. However, the average Zmag values of the pathway clearly demonstrate an increase in the pathway expression of TCA cycle enzymes under aerobic conditions.

Table 4.3. Direct comparison of *C. debilis* GB1 Zmag and *E.coli* K-12 Z-scores for enzymes of central metabolism

Gene Product name	*C. debilis* GB1 Locus tags	*E. coli* K-12 tags	*C. debilis* GB1 Zmag[1]	*E. coli* K-12 Z-score[2]	*C. debilis* GB1 Average Log_2TIC[3]
Electron Transport Chain Locus Tags for Which a Zmag can be Calculated					
Complex II	Cdeb_02776, Cdeb_02777	b0723, b0724	0.9, 3.58	1.55, 2.03	10.15, 10.53
Complex III	Cdeb_00880	NA	1.18	NA	11.81
Complex IV	Cdeb_01033, Cdeb_01034, Cdeb_02460, Cdeb_00920, Cdeb_00921	b0978, b0734	2.27, 1.40, 1.72, 3.62, -0.5	-0.15, -0.72, 3.60, 4.04, -0.13	10.7, 10.82,10, 10.60, 11.45
ATP synthase	Cdeb_00529-Cdeb_00533	b3736, b2661, b3734, b3733, b3734, b3732	-0.39, -0.25, -0.18, 0.16, -0.32	-0.52, 1.73, -0.65, -0.98, -0.46	12.35, 11.57, 13.87, 10.04, 11.9
Reactive Oxygen Species Protection Locus Tags for Which a Zmag can be Calculated					
Catalase	Cdeb_00043	b3942	0.98	2.026	13.1
Peroxidase	Cdeb_01743	b2480	-0.27	-0.56	10.68
Peroxidase	Cdeb_03127	b1324	-0.31	1.93	11.09
Glycolysis and Gluconeogenesis Locus Tags for Which a Zmag can be Calculated					
Glucose 6-phosphate isomerase	Cdeb_03023	b4025	-0.96	-0.05	12.36
6-phosphofructoskinase	Cdeb_02916	b3916	-0.35	-1.18	9.86
Fructose-1, 6-bisphosphate aldolase	Cdeb_00505	b2096	-0.58	-5.41	13.54

Fructose-1-6 bisphosphatase	Cdeb_00508	b3925	0.31	-1.41	9.45
Glyceraldhyde-3-phosphate dehydrogenase	Cdeb_00261	b1779	-0.1	-2.53	15.93
1-3 phosphoglycerate kinase	Cdeb_00262	b2926	-0.23	-0.60	15.8
Phosphoglycerate mutase	Cdeb_00264	b3612	-1	-2.02	10.28
Enolase	Cdeb_00265	b2779	-0.74	-1.23	14.56
Pyruvate kinase	Cdeb_02915, Cdeb_02914	b1676	-0.45, -0.06	-0.47	11.04
Triose isomerase	Cdeb_00263	b3919	-1.23	-0.75	11.58
TCA Cycle Locus Tags for Which a Zmag can be Calculated					
Citrate synthase	Cdeb_02909	b0333	1.21	-0.58	11.86
Aconitate hydratase	Cdeb_02349	b1276	1.36	1.83	12.83
Isocitrate dehydrogenase	Cdeb_02908	b1136	2.53	3.05	11.17
Alpha-ketogultarate dehydrogenase	Cdeb_00961, Cdeb_00962, Cdeb_00776, Cdeb_02441	b0726, b0727, b0116	2.5, 0.69, 0.9, 0.99	1.40, 1.44, 2.31	10.92, 12.51, 9.81, 14.28
Succinyl-CoA synthase	Cdeb_02130, Cdeb_02131	b0729, b0728	3.34, 2.29	1.27, 1.44	10.68, 12.61
Succinate dehydrogenase	Cdeb_02777, Cdeb_02776	b0724, b0723	3.58, 0.9	2.03, 1.55	10.53, 10.15
Fumerate hydratase	Cdeb_01560	b1611	1.05	0.76	11.01
Malate dehydrogenase	Cdeb_02907	b3236	3.01	1.61	11.05
Pyruvate Metabolism Locus Tags for Which a Zmag can be Calculated					
PFL	Cdeb_01638, Cdeb_01637	b0903, b0902	-0.32, -0.31	-0.17, -0.68	14.16, 9.35
ADHE	Cdeb_01397	b1241	-1.65	-2.04	13.3

AK	Cdeb_03124	b2296	-0.43	-1.28	12.46
PTA	Cdeb_00469	NA[2]	-0.09	NA[2]	13.03
ADH	Cdeb_03022, Cdeb_00489, Cdeb_01569	b3011, b1478, b0356	0.53, -2.13, -0.05	1.01, 2.15, 0.19	10.45, 13.08, 12.72
Pyruvate Dehydrogenase Locus Tags for Which a Zmag can be Calculated					
E3 (EC:1.8.1.4)	Cdeb_02441	b0116	0.99	2.31	14.28
E2 (EC:2.3.1.12)	Cdeb_02440	b0115	1.36	2.26	14.3
E1 beta (EC:1.2.4.1)	Cdeb_02439	b0808	1.98	1.17	13.93
E1 alpha (EC:1.2.4.1)	Cdeb_02438	b0478	0.74	0.8	9.67
E2 (EC:2.3.1.12)	Cdeb_00779	b0115	0.59	2.26	11.02
E1 beta (EC:1.2.4.1)	Cdeb_00778	b0115	-0.2	2.26	10.58
E3 (EC:1.8.1.4)	Cdeb_00776	b0116	0.9	2.31	9.81
E2 (EC:2.3.1.12)	Cdeb_03165	b0115	0.5	2.26	11.03
Cellobiose ABC Transport Locus Tags for Which a Zmag can be Calculated					
Cellobiose-specific component IIA	Cdeb_01817	b1736	1.08	0.83	10.92
Cellobiose-specific component IIB	Cdeb_01067, Cdeb_02169	b1738, NA	-3.7, -1.38	0.05	10.23, 12.35
Cellobiose-specific component IIC	Cdeb_01068, Cdeb_02168, Cdeb_01814	NA, NA, b1737	-2.45, -1.86, 1.08	-1.03	12.03, 10.80, 11.14

[1]Zmag is the difference in expression between anaerobic/aerobic conditions of *C. debilis*; [2]Z-score is the expression difference between anaerobic/aerobic conditions of *E.coli* K-12 transcriptome; [3]TIC= Total ion current.

4.4.5. Glycolysis

Typically aerobic conditions are associated with down-regulation of glycolysis when compared to growth under anaerobic conditions (Partridge *et al.* 2006, Van Der Voort *et al.* 2009). However, no enzymes in *C. debilis* were highly down-regulated (<-1.65) or down-regulated moderately (< -1.28; Table 4.3). Nevertheless, when the Zmag values were averaged over the entire pathway (Table 4.2), a depression in the glycolysis pathway could be clearly observed. Furthermore, several glycolysis pathway proteins, encoded by genes located next to each other, and which may constitute operons, showed similar changes, indicating their expression levels may be linked. The $\log_2$TIC of the proteins involved in glycolysis indicate that these proteins are highly represented within the proteome, similar to other facultative anaerobic organisms (Van Der Voort *et al.* 2009). Under our conditions, aerobic metabolism of glucose yields higher amounts of energy and therefore, the cells need to flux less glucose when compared to anaerobic conditions to yield the same amount of conserved energy (Verbeke *et al.* 2014).

4.4.6. Pyruvate Metabolism and End-Product Synthesis

Pyruvate metabolism is essential for both aerobic growth and anaerobic growth. Typically the genes associated with fermentation are expressed anaerobically, while some genes linked to respiration (e.g. PDH) could be utilized both aerobically and anaerobically. Only two of the enzymes involved in pyruvate metabolism, alcohol/aldehyde dehydrogenase (ADHE) and aldehyde dehydrogenase (AlDH),

showed a high degree of down- regulation (<-1.65) under aerobic conditions (Table 4.3). An averaging of Zmag values in detected enzymes associated with pyruvate fermentation, excluding pyruvate dehydrogenase (PDH), shows an overall down-regulation of pyruvate fermentation pathways (Table 4.2). PDH is omitted due to its link with respiration (Nakano *et al.* 1997). Overall, we expect a depression in pathways linked to end-product synthesis and fermentation under aerobic conditions, as is typically observed in other facultative anaerobes (Van Der Voort *et al.* 2009). *C. debilis* produces acetate under aerobic growth conditions; therefore, we expect the components of pyruvate fermentation, such as phosphotransacetylase (PTA) and acetate kinase (AK), to show less of a depression aerobically. Indeed, *C. debilis* PTA and AK did not show Zmag changes above the moderate threshold of -1.28 with Zmag values of -0.09 and -0.43, respectively.

Pooled $\log_2$ (TIC) gives us a general estiment of protein proportiality within the cells regardless of conditions. We expect high pooled $\log_2$(TIC) values for the enzymes leading to the production of major end-products. When end-products synthesized under anaerobic versus aerobic conditions (Chapter 3) are considered, they appear to be consistent with protein expression and amounts. Enzymes associated with the synthesis of formate (Pyruvate formate lyase (PFL) and PFL activating enzyme: Cdeb_01638, Cded_01637), acetate (acetate kinase (AK): Cdeb_03124, phosphotransactylase (PTA):Cdeb_00469), and ethanol ADHE: Cdeb_01397) all had pooled $\log_2$(TIC) values above the 50^{th} percentile of expression. Trace amounts of lactate were formed. Lactate dehydrogenase (LDH: Cdeb_02710) LDH was observed just above the detection threshold under anaerobic conditions, and under the detection

threshold aerobically; therefore, its expression difference could not be determined through this proteomic analysis. Low expression of LDH, and the fact that when it is detected it has a very low log2(TIC), is consistent with the relatively low amounts of lactate detected compared to other end-products.

4.4.7. Oxygen Respiration

The *C. debilis* GB1 genome encodes the enzymes necessary for respiration including complex I (NADH:ubiquinone oxidoreductase), II (succinate dehydrogenase), III (cytochrome bc_1 complex) and IV (cytochrome c oxidase). However, we could not calculate Zmag for subunits of Complex I as peptides associated with proteins in Complex I did not meet our detection criteria threshold. We could calculate Zmag for several of the other components including genes encoding for Complex II:fumarate reductase (Cdeb_02776, Cdeb_02777), Complex III:cytochrome bc1 complex (Cdeb_00880), complex IV:cytochrome C oxidase (Cdeb_02460, Cdeb_00920, Cdeb_00921), as well as subunits for the high affinity Cytochrome bd oxidase complex (Cdeb_01033, Cdeb_01034), and the ATP synthase (Cdeb_00529-Cdeb_00533) proteins. Four individual genes encoding components of the electron transport chain were highly up-regulated (> 1.65), and one was moderately up-regulated (> 1.28), under aerobic conditions (Table 4.3). The overall combined $\log_2$TIC of enzymes associated with respiration were highly expressed, at levels similar to other core metabolic pathways, such as glycolysis or the TCA cycle. Several of the genes for which we were able to calculate Zmag values are found next

to each other in the genome, possibly forming operons, and shared similar trends in changes of expression levels under anaerobic versus aerobic conditions.

4.4.8. Reactive Oxygen Species Protection

Facultative anaerobes typically contain a gene complement for protection from reactive oxygen species (ROS) (Engelmann *et al.* 1995). The *C. debilis* GB1 genome was found to encode catalases, as well as superoxide dismutases and peroxidases. We were not able to quantify a Zmag for superoxide dismutase as it did not meet our detection threshold criteria. Although two distinct Mn-catalase genes are encoded in the GB1 genome, only one Mn-catalase (Cdeb_01630) was detected in the proteome (Table 4.3), but this gene product was not significantly up-regulated under aerobic conditions. Detection of only one catalase is consistent with other work where the copy of catalase being expressed at the transcriptome level is determined by whether the cells are in stationary or exponential growth phase (Engelmann *et al.* 1995). Two copies of peroxiredoxin were also detected in the proteome, but the expression levels of these gene products did not change significantly under aerobe versus anaerobic conditions.

The combined $\log_2$TIC of the Mn-catalase expression at high levels is consistent with an organism that is prepared for oxidative stress. The $\log_2$TIC of the peroxiredoxin gene products were much lower, indicating catalase may be the primary driver of hydrogen peroxide flux. In general, gene products related to ROS protection would be expected to increase average Zmag under aerobic conditions such as in

Bacillus cereus as studied by Van der Voort *et al.* (2009). However, likely due to the small number of proteins observed, this relationship was not detected

4.4.9. Pyruvate Dehydrogenase

Pyruvate dehydrogenase (PDH) can be associated with either respiratory or fermentative functions. Facultative anaerobes such as *E. coli*, and members of the genus *Bacillus*, also use alternative means of generating acetyl-CoA from pyruvate, such as PFL, and up-regulate PDH under aerobic conditions (Nakano *et al.* 1998, Partridge *et al* 2006). Typically PDH is encoded within operons; *C. debilis* possesses 3 operons. In *C. debilis* GB1, out of the 8 PDH operon associated genes for which we could calculate Zmag, only one PDH operon associated subunit (table 4.3). Cdeb_02439, showed high up-regulation (Zmag> 1.65), while a moderate increase (>=1.28) was observed for one other PDH operon associated gene product (Cdeb_02440) under aerobic conditions. Operon (Cdeb_02441-Cdeb_02438) also corresponds to the subunits that show the most change in Zmag when compared to other PDH operons. Although *C. debilis* GB1 genome appears to encode 3 full PDH operons, only one (Cdeb_02441-Cdeb_02438) is predominantly expressed, as indicated by the pooled $\log_2$(TIC) scores (table 4.3).

4.4.10. Other Highly Differentially-Expressed Pathways

While our primary focus was to elucidate core metabolism pathways in *C. debilis* GB1 under aerobic versus anaerobic conditions, we also observed other pathways that were highly differentially expressed aerobically (> ± 1.65). When arranged by KEGG category (Table 4.4) through the gene annotation program used, pathways with at least one gene product were assigned to a KEGG map and an overall Zmag change was calculated. As this is a broad analysis for the purpose of highlighting potential differences, the core aspect of this is to emphasize filled maps with possible large expression changes. We defined well filled maps as having 5 or more protein measurements. Many of the KEGG maps that were well filled, were found to undergo a high degree of change: an increase in aerobic expression Zmag> +1.65: fatty acid elongation, cysteine and methionine metabolism, carbon fixation pathways in prokaryotes, citrate cycle (TCA cycle), glyoxylate and dicarboxylate metabolism. Some of Zmag values from these KEGG maps can be misleading due to a large number of shared genes, for instance carbon fixation pathways in prokaryotes, and glyoxylate and dicarboxylate maps share a significant number of enzymes with the TCA cycle KEGG map. We did not see KEGG maps with a fill of 5 or more with a decrease in aerobic expression Zmag<-1.65. When COG categories are considered (Table 4.5) for the *C. debilis* dataset, the energy production and conversion category was the most changed with 16 proteins highly up-regulated aerobically and 2 highly down-regulated aerobically (± 1.65) out of 111.

Table 4.4. Overview of KEGG pathway regulation organized by largest to smallest # of proteins detected in map

Zmag[1,2]	# of proteins detected in map	# Genes in map	# Total possible genes in map	KEGG ID	PATHWAY
0.26	34	46	177	map00061	Fatty acid biosynthesis
-0.62	27	27	85	map00601	Glycosphingolipid biosynthesis - lacto and neolacto series
-0.41	20	21	42	map01057	Biosynthesis of type II polyketide products
1.26	18	21	55	map00280	Valine, leucine and isoleucine degradation
-0.53	17	17	45	map00942	Anthocyanin biosynthesis
-0.18	17	33	174	map00230	Purine metabolism
-0.62	16	18	39	map00513	Various types of N-glycan biosynthesis
-0.11	16	16	35	map00970	Aminoacyl-tRNA biosynthesis
-0.17	15	31	121	map00520	Amino sugar and nucleotide sugar metabolism
2.06	**15**	**17**	**51**	**map00720**	**Carbon fixation pathways in prokaryotes**
2.25	**15**	**18**	**27**	**map00020**	**Citrate cycle (TCA cycle)**
-0.51	14	14	22	map00522	Biosynthesis of 12-, 14- and 16-membered macrolides
0.47	14	15	98	map00680	Methane metabolism
-0.58	13	21	120	map00330	Arginine and proline metabolism
-0.2	13	15	35	map00550	Peptidoglycan biosynthesis
-0.09	13	21	107	map00240	Pyrimidine metabolism
-0.17	12	22	56	map00010	Glycolysis / Gluconeogenesis
0.36	11	18	82	map00071	Fatty acid metabolism
1.2	11	17	71	map00620	Pyruvate metabolism
-0.4	10	10	63	map00260	Glycine, serine and threonine metabolism
-0.68	9	12	45	map00030	Pentose phosphate pathway

-1.13	9	13	59	map00910	Nitrogen metabolism
-0.16	9	9	13	map00195	Photosynthesis
-0.63	8	10	45	map00250	Alanine, aspartate and glutamate metabolism
-0.1	8	9	63	map00906	Carotenoid biosynthesis
0.12	8	10	22	map00450	Selenocompound metabolism
1.46	8	13	89	map00380	Tryptophan metabolism
1.59	8	9	53	map00640	Propanoate metabolism
-0.62	7	8	20	map00603	Glycosphingolipid biosynthesis - globo series
-0.59	7	13	87	map00500	Starch and sucrose metabolism
-0.35	7	16	73	map00051	Fructose and mannose metabolism
-0.26	7	10	35	map00300	Lysine biosynthesis
0.11	7	7	15	map00965	Betalain biosynthesis
0.59	7	7	81	map00950	Isoquinoline alkaloid biosynthesis
1.24	7	13	63	map00480	Glutathione metabolism
1.69	**7**	**13**	**82**	**map00270**	**Cysteine and methionine metabolism**
-0.79	6	7	32	map00983	Drug metabolism - other enzymes
0.23	6	11	33	map00900	Terpenoid backbone biosynthesis
0.25	6	10	34	map00710	Carbon fixation in photosynthetic organisms
-0.92	5	13	50	map00052	Galactose metabolism
-0.53	5	5	33	map00622	Xylene degradation
0.14	5	5	39	map00540	Lipopolysaccharide biosynthesis
0.17	5	5	33	map00460	Cyanoamino acid metabolism
0.32	5	8	29	map00670	One carbon pool by folate
0.36	5	13	100	map00860	Porphyrin and chlorophyll metabolism

0.61	5	8	51	map00650	Butanoate metabolism
0.68	5	5	82	map00624	Polycyclic aromatic hydrocarbon degradation
1.65	**5**	**13**	**54**	**map00062**	**Fatty acid elongation**
2.39	**5**	**6**	**65**	**map00630**	**Glyoxylate and dicarboxylate metabolism**
-1.65	4	6	100	map00350	Tyrosine metabolism
-0.62	4	4	14	map00512	Mucin type O-Glycan biosynthesis
-0.62	4	6	38	map00604	Glycosphingolipid biosynthesis - ganglio series
-0.38	4	4	25	map00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis
-0.18	4	13	44	map00130	Ubiquinone and other terpenoid-quinone biosynthesis
0.1	4	6	70	map00360	Phenylalanine metabolism
0.29	4	4	22	map00430	Taurine and hypotaurine metabolism
0.43	4	11	26	map00790	Folate biosynthesis
0.72	4	8	75	map00361	Chlorocyclohexane and chlorobenzene degradation
1.26	4	5	44	map00623	Toluene degradation
1.78	4	5	12	map00190	Oxidative phosphorylation
1.89	4	7	61	map00310	Lysine degradation
-2.49	3	3	60	map00982	Drug metabolism - cytochrome P450
-0.62	3	3	11	map00563	Glycosylphosphatidylinositol(GPI)-anchor biosynthesis
-0.61	3	7	59	map00760	Nicotinate and nicotinamide metabolism
-0.43	3	10	24	map00524	Butirosin and neomycin biosynthesis
-0.13	3	3	76	map00940	Phenylpropanoid biosynthesis
0.38	3	7	20	map00730	Thiamine metabolism
0.5	3	4	14	map00253	Tetracycline biosynthesis
0.63	3	6	41	map00561	Glycerolipid metabolism

0.64	3	7	88	map00627	Aminobenzoate degradation
0.72	3	4	31	map00364	Fluorobenzoate degradation
1.04	3	4	28	map00290	Valine, leucine and isoleucine biosynthesis
-2.49	2	6	40	map00625	Chloroalkane and chloroalkene degradation
-2.49	2	6	47	map00626	Naphthalene degradation
-1.65	2	8	72	map00362	Benzoate degradation
-0.62	2	3	11	map00908	Zeatin biosynthesis
-0.38	2	2	73	map00941	Flavonoid biosynthesis
0.52	2	4	24	map00930	Caprolactam degradation
0.59	2	2	7	map00785	Lipoic acid metabolism
0.59	2	2	10	map00981	Insect hormone biosynthesis
0.71	2	2	23	map00643	Styrene degradation
1.93	2	2	51	map00120	Primary bile acid biosynthesis
-2.49	1	1	36	map00830	Retinol metabolism
-2.49	1	1	59	map00980	Metabolism of xenobiotics by cytochrome P450
-2.03	1	1	17	map00621	Dioxin degradation
-1.07	1	4	40	map00410	beta-Alanine metabolism
-0.62	1	7	44	map00600	Sphingolipid metabolism
-0.62	1	1	32	map00944	Flavone and flavonol biosynthesis
-0.44	1	4	47	map00400	Phenylalanine, tyrosine and tryptophan biosynthesis
-0.17	1	4	35	map00750	Vitamin B6 metabolism
0.31	1	1	54	map00590	Arachidonic acid metabolism
0.51	1	1	6	map00473	D-Alanine metabolism
0.59	1	2	39	map00340	Histidine metabolism

0.59	1	1	9	map00402	Benzoxazinoid biosynthesis
0.63	1	1	18	map00521	Streptomycin biosynthesis
0.72	1	1	52	map00943	Isoflavonoid biosynthesis
0.72	1	1	12	map00351	DDT degradation
0.88	1	1	2	map04660	T cell receptor signaling pathway
1.1	1	2	6	map00072	Synthesis and degradation of ketone bodies
1.22	1	4	56	map00053	Ascorbate and aldarate metabolism
1.22	1	4	21	map00531	Glycosaminoglycan degradation
1.93	1	4	18	map00281	Geraniol degradation
2.4	1	3	30	map00920	Sulfur metabolism
4.54	1	1	19	map00660	C5-Branched dibasic acid metabolism

[1]Zmag = combined vector of Z0net and Z1net;[2]**Bolded** maps signify a # of proteins detected of 5 or greater and the pathway Zmag is $-1.65 \geq Zmag \geq 1.65$

Table 4.5. Regulation of COG categories under aerobic vs anaerobic conditions in *C. debilis* GB1 sorted alphabetically

COG Map	Up	Down	Total
B "Chromatin structure and dynamics"	0	1	1
C "Energy Production and conversion"	**16**	**2**	**111**
D "Cell cycle control, cell division, chromosome partitioning"	0	1	34
E "Amino acid transport and metabolism"	4	7	185
F "Nucleotide transport and metabolism"	0	0	54
G "Carbohydrate transport and metabolism"	2	5	228
H "Coenzyme transport and metabolism"	0	1	58
I "Lipid transport and metabolism"	4	2	79
J "Translation, ribosomal structure and biogenesis"	1	2	160
K "Transcription"	2	3	208
L "Replication, recombination and repair"	0	0	159
M "Cell wall/membrane/envelope biogenesis"	1	1	132
N "Cell motility"	2	0	62
O "Post translation modification, protein turnover, chaperones"	1	1	90
P "Inorganic ion transport and metabolism"	2	2	133
Q "Secondary metabolites biosynthesis, transport and catabolism"	0	1	37
R "General function prediction only"	0	2	298

S "Function unknown"	1	1	254
T "Signal transduction"	2	1	112
U "Intracellular trafficking, secretion and vesicular transport"	1	0	66
V "Defense mechanisms"	0	0	54
X "Not in COGs"	3	1	1007
Z "Cytoskeleton"	0	0	0
Total changes	42	34	3522

Proteins with a <+1.65 or >-1.65 change in Zmag were considered up/ down regulated at a high level. This corresponds to the outermost 10% change corresponding to a fold difference of 3.2. Most changed COG category is **bolded**.

4.4.11. Core Metabolism Overview

Detailed analysis of the individual pathways (section 4.4.12-4.4.17) above is corroborated by KEGG map analysis in Tables 4.4 where filled pathways involved in aerobic and anaerobic metabolism are among the most changed. COG analysis also corroborate our detailed pathway analysis, Table 4.5 shows energy production and conversion has a high degree of changing expression as would be expected in an organism switching from respiration to substrate level phosphorylation.

A list of proteins with their corresponding W_{stat} value can be found in Table 4.1A, filtering W_{stat} by a 2.8 threshold give a higher quality (higher statistical confidence) data set. However, filtering our results leads to significant depopulation of pathways of interest; therefore, we found looking at significant changes of Zmag not filtered by W_{stat} gave us more usable results. Importantly, the overall pathway regulation patterns observed in Figure 4.7 are corroborated by the higher quality W_{stat} values. Figure 4.7 shows which core metabolism pathways were up- and down-regulated in a general sense, for *C. debilis* GB1 under aerobic versus anaerobic conditions. Each individual pathway is described in detail in the figure legend below.

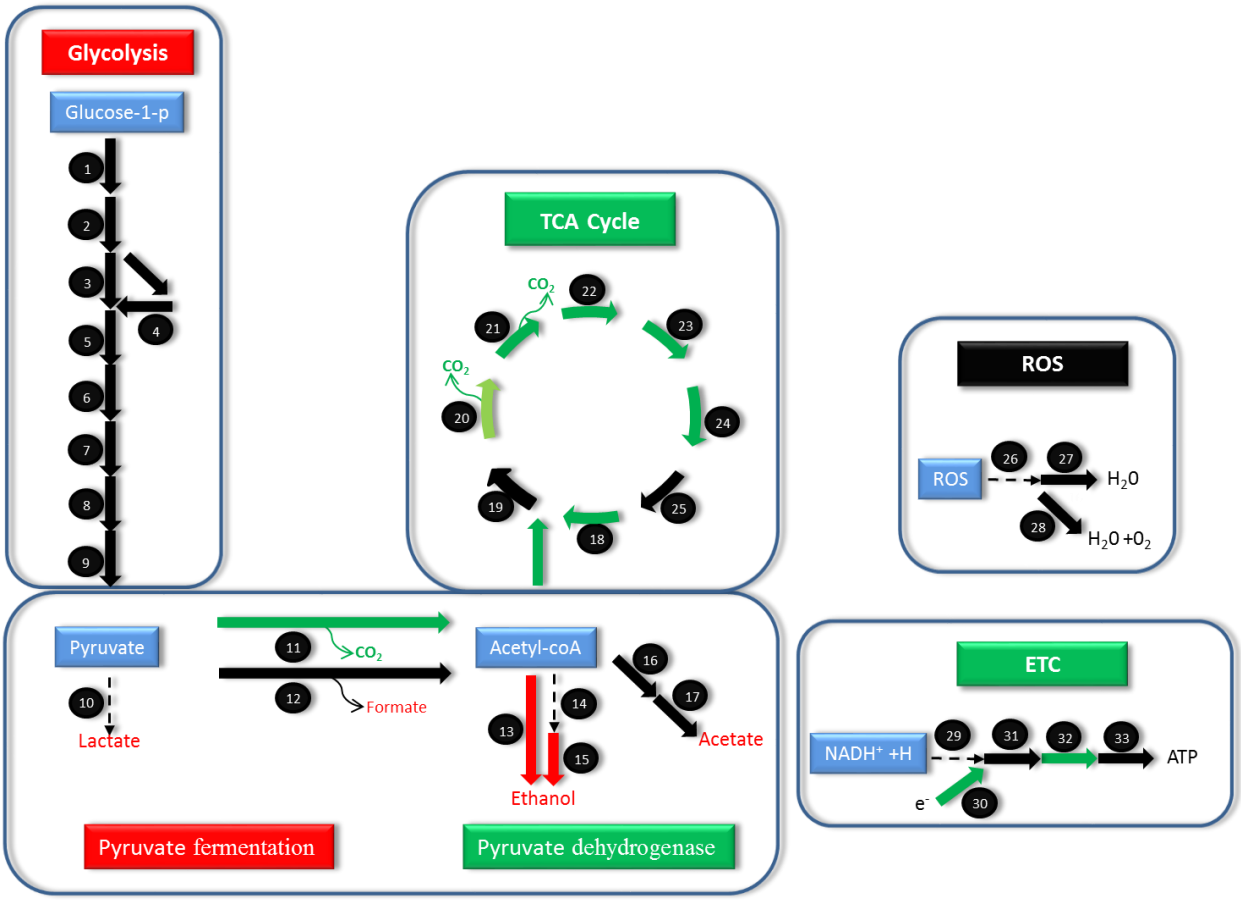


Figure 4.7. Comparative proteomic overview of core metabolism under aerobic vs anaerobic growth conditions in *C. debilis* GB1.

Individual protein colour scheme: Change of Zmag in one or more copies/subunits= ± 1.28: + light green(- orange); Change of Zmag in one or more copies/subunits=± 1.65: + dark green(- red); No statistically significant change -1.28<Zmag<1.28 black; Corresponding genes present in the genome, but no Zmag information= dash line.

Pathway colour scheme: Whelch's t-test was preformed to determine if there was a significant change in average Zmag at the pathway level, pathways that are found to increase significantly (p value< 0.05) are written in dark green, pathways that are found to decrease significantly (p value< 0.05) are written in red.

End-product colour scheme: Whelch's t-test was performed on end products produced under each condition (data taken from Chapter 3, end products that are found to increase significantly (p value< 0.05) are written in dark green, end products that are found to decrease significantly (p value< 0.05) are written in red.

Enzymes: 1) G-6-P isomerase, 2) 6-p phosphofructoskinase, 3) f-1, 6-P aldolase, 4) triose isomerase, 5) Glyceraldehyde 3-P dehydrogenase, 6) 1,3-P bisphosphoglycerate kinase, 7) Phosphoglycerate mutase, 8) enolase, 9) pyruvate kinase, 10) lactate dehydrogenase, 11) pyruvate dehydrogenase, 12) pyruvate formate lyase, 13) alcohol/aldehyde dehydrogenase, 14) aldehyde dehydrogenase, 15) alcohol

dehydrogenase,16) phosphotransacetylase, 17) acetate kinase, 18) citrate synthase, 19) aconitate hydratase, 20) isocitrate dehydrogenase, 21) alpha-ketogultarate dehydrogenase, 22) succinyl-CoA synthase, 23) succinate dehydrogenase, 24) fumerate hydratase 25), malate dehydrogenase 26), superoxide dismutase, 27) peroxidases, 28) catalase, 29) complex I, 30) complex II, 31) complex III, 32) complex IV, 33) ATP synthase/complex V

4.4.12. Comparison against the *E. coli* K-12 data set

Proteomic or transcriptomic have been utilized to make comparisons between species and between different published datasets (Verbeke *et al.* 2013, Fu *et al.* 2015). Comparing proteomes directly against transcriptomes has also been done and changes in expression were corroborative (Yang et al. 2012, Hahne *et al.* 2010, Munir *et al.* 2016) Since a suitable *Bacillus sp.* raw data set that could be integrated directly using our methods was not found, the *E.coli* K-12 data set provided a dataset that was compatible, and could be incorporated into our analysis engine. The difficulty in finding a closer related data set highlights the importance of reporting data in a similar fashion. Nevertheless, direct comparison against a well-studied organism from a different phylum, such as *E. coli* gives the ability to compare and contrast aerobic and anaerobic regulation across widely divergent organisms.

The physiological similarities between *C. debilis* GB1 and *E. coli* K-12 (both facultative anaerobes, with similar core carbohydrate metabolisms), the similarities between the experimental conditions used (anaerobic to aerobic conditions) and the compatibility of their data presentation with our in-house analysis system made the *E. coli* K-12 transcriptome dataset published by Partridge *et al.* (2006) an ideal model to compare with our *C. debilis* GB1 data set. Typically, there are major changes in gene and gene product expression when cells grown under aerobic and anaerobic conditions are compared, and these expression changes are usually associated with pathways involving energy production and conversion such as glycolysis, TCA, respiration, and pyruvate metabolism in *E. coli* K-12 (Partridge *et al.* 2006, Weber *et al.* 2006).

Glycolysis in *C. debilis* did not appear to change significantly at an individual enzyme level as protein expression changes were not as great as was expected on the basis of the *E. coli* K-12 data set by Partridge *et al.* (2006). At the pathway level, however, the direction of the changes in expression was consistent. Overall, down-regulation of glycolysis is consistent with an increase in energy per glucose and a reduction in glucose flux as seen in *E. coli* K-12 data set (Partridge *et al.* 2006).

At the pathway level, we noticed TCA expression under aerobic conditions in *C. debilis* was highly up-regulated and was similar to *E. coli* K-12 data set by Partridge *et al*. (Partridge *et al.* 2006). Overall an increase in flux through the TCA cycle is beneficial under aerobic conditions since the electrons generated can be used for ATP production via the ETC. Overall pyruvate fermentation was found to be consistent with aerobic growth of *E. coli* K-12, with the notable exception of ADH, which we directly compared (Partridge *et al.* 2006). ADH genes could be regulated differently or it could be due to experimental differences. The experimental design of Partridge *et al.* (2006) involved transitioning an anaerobic chemostat to aerobic conditions. It is possible trace alcohols were left over, and this caused an increase in ADH expression as a mechanism of uptake. Our *C. debilis* experiment was done in batch with aerobically grown inocula, and trace alcohols should not have been present under aerobic conditions. *C. debilis* produces acetate under aerobic conditions; the lack of significant change in the Zmag value of acetate kinase (-0.43) versus the moderate change in the z-score (-1.28) of the corresponding gene in *E. coli* K-12 corroborates this. Increased expression of PDH in *C. debilis* GB1 under aerobic conditions is consistent with the *E. coli* K-12 set for which we compared.

We expected an increase in genes associated with ROS under aerobic conditions to deal with oxidative stress. While facultative organisms typically express catalase in high amounts, as seen within our proteome, the magnitude of the change in expression was not statistically significant. Catalase was significantly up-regulated in in the Partridge *et al.* (2006) *E. coli* K-12 dataset. Overall the pooled $\log_2$(TIC) of gene products involved in ROS protection were consistent with the *E. coli* K-12 data set by Partridge *et al.* (2006) showing catalase is highly expressed.

Energy production and conversion is a core metabolic process in bacterial cells, and the ability to undergo respirative and fermentative metabolism is a key feature of energy production (Nakano *et al.* 1998, Partridge *et al* 2006). While each organism may have unique features with respect to gene content, expression, and physiology as a whole, many facultative anaerobes share expression patterns at the pathway level. To demonstrate this, we created a correlation plot of the average Zmag values of *C. debilis* GB1 proteins in the key catabolic pathways under aerobic versus anaerobic conditions to the average Z-scores of *E.coli* K-12 gene transcript profiles, from equivalent pathways, under aerobic and anaerobic conditions using data generated by Partridge et al. (2006).

A correlation plot (Figure 4.8) between *C. debilis* GB1 and the data set from *E. coli* K-12, from Partridge *et al.* (2006), show average changes (Zmag values in *C. debilis*, z-score in *E. coli* K-12) between anaerobic and aerobic conditions for the core catabolic pathways discussed in the sections above, with a high R^2 value of 0.54. When genes are plotted individually (Figure 4.9) a similar relationship is observed with correlation between expression changes in *C. debilis* GB1 and *E. coli* K-12

showing a R^2 of 0.41, confirming the validity of the use of pathway averages in Figure 4.8.

Each pathway appears to follow the same pattern of up regulation and down regulation in both *E. coli* K-12 and *C. debilis* GB1.We have included two notable exceptions: i) ADH's appear to be regulated oppositely, likely due to trace ethanol being present under the *E.coli* K-12 growth and sampling conditions as discussed above; and ii) the Cellobiose ABC transporters, as the IIB-cellobiose specific component is depressed (Zmag -3.7) in GB1 but not in *E.coli* K-12 (z-score 0.05) aerobically. A discordance between cellobiose ABC transporters is not surprising since they were grown using two related, but different, substrates (glucose for *E. coli* K-12 and cellobiose for *C. debilis* GB1).

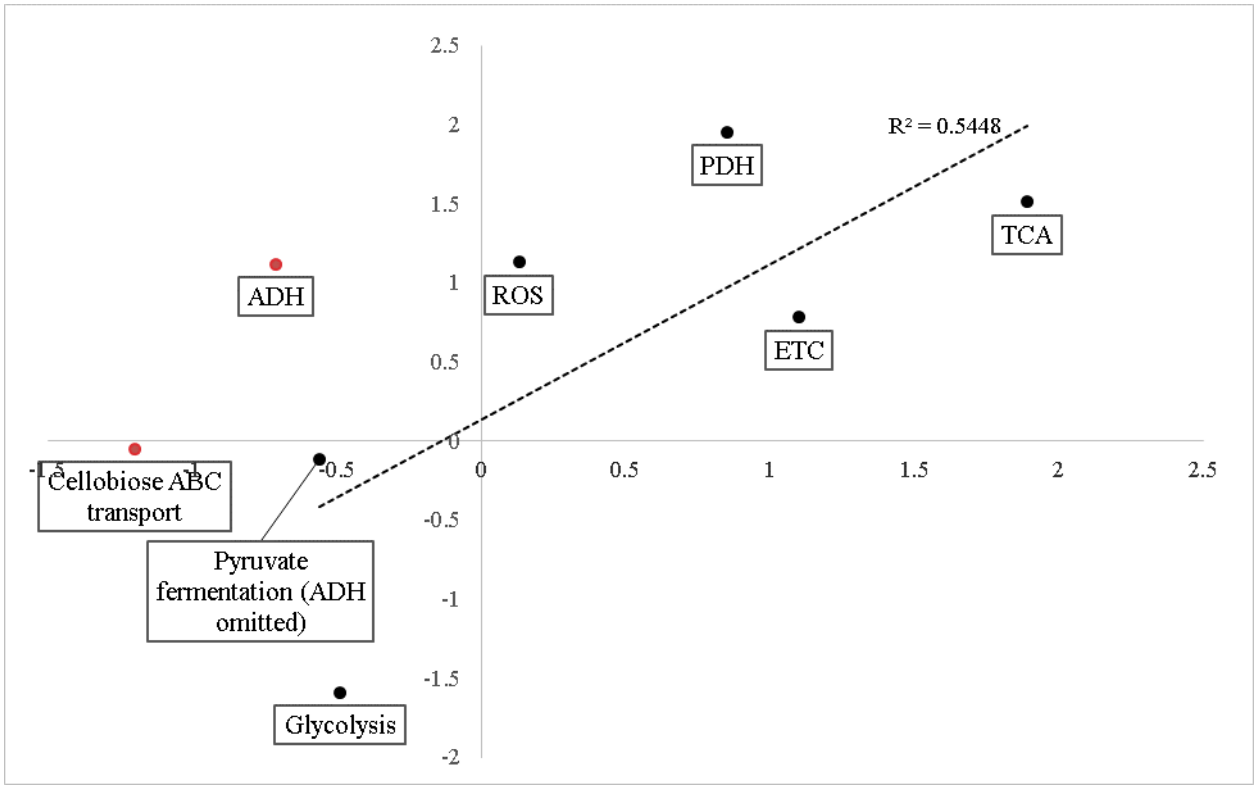


Figure 4.8. Average Zmag (x-axis) values and Z-scores (y-axis) comparing anaerobic to aerobic conditions of *C. debilis* (x-axis) and *E. coli* K-12 (y-axis) core metabolic pathways (listed in Table 4.2). Dots in red indicate gene expression changes that are discordant

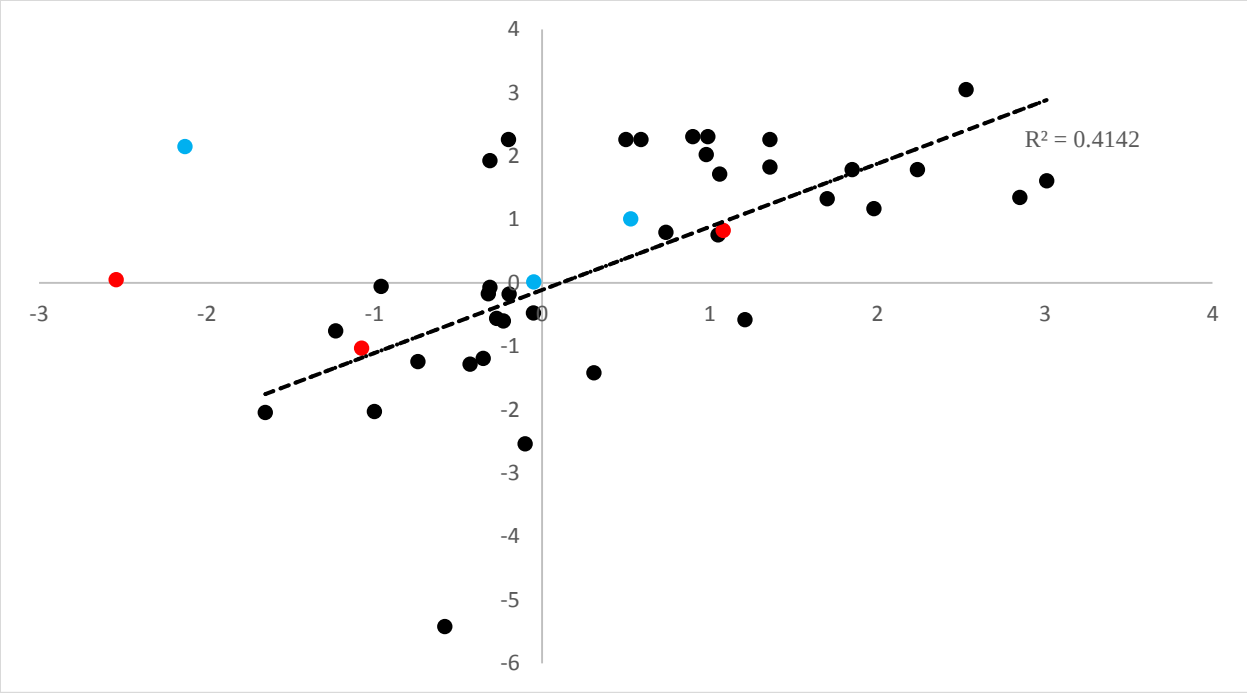


Figure 4.9. Zmag (x-axis) values and Z-scores (y-axis) comparing anaerobic to aerobic conditions of *C. debilis* (x-axis) and *E. coli* K-12 (y-axis) core metabolic pathways (listed in Table 4.3). Dots in black represent ETC, ROS, TCA, PDH, pyruvate fermentation (ADH omitted), and glycolysis. Dots in red (cellobiose ABC transport) and blue (ADH) indicate gene expressing changes that are discordant. R2 displayed in chart is for the black dots.

When we consider core metabolic regulation in response to aerobic and anaerobic conditions of the facultative anaerobe, *B. cereus* (Van Der *et al.* 2009), and studies on the regulation of *E. coli* K-12 (Partridge *et al.* 2006, Weber *et al.* 2006), the regulation pattern is rather similar in that increases in TCA, ETC, PDH expression under aerobic conditions and a decrease in glycolysis and fermentation associated genes are observed. Our data compared by our proteomic/transcriptomic methods closely matches observations made by others using various functional genomic methods (Partridge *et al.* 2006, Van Der Voort *et al.* 2009).

4.5. Conclusion

Our goal was to understand *C. debilis* central metabolism in regards to aerobic and anaerobic growth using high throughput techniques to give us insight into the inner workings of core metabolism in *C. debilis*. Comparing against *E. coli* K-12, a mesophilic Proteobacteria, allowed us to understand *C. debilis*, a thermophilic *Firmicute*, core metabolism in a broader context. We quantified the changes in protein expression levels using 2D –LC-MS-MS in *C. debilis* GB1 under aerobic versus anaerobic conditions. Although some variability between biological replicate samples was observed, the trends established by quantitative and statistical analyses revealed significant changes in many metabolic pathway gene products under aerobic versus anaerobic conditions. Even in pathways that did not show many proteins with a significant change in expression (Zmag= ±1.65, representing a 3.2 fold change in expression) at the level of the individual proteins, the average Zmag values across pathways showed general trends that would be expected in changes from aerobic to

anaerobic metabolism. We further compared changes in protein expression levels in *C. debilis* GB1 to changes in gene (transcription) expression levels observed in *E. coli* K-12 under similar conditions, and found very similar changes at the pathway level, as well as at the level of specific genes and gene products that one would not expect to be correlated. These data would indicate that there are common, global regulatory changes in diverse facultative bacteria: Gram positive (Firmicutes) versus Gram negative (Proteobacteria); mesophilic versus thermophilic. There are many studies comparing different organisms using omics to compare closely related strains or species (Lidbury *et al.* 2016, Goltsman *et al.* 2009). Comparing and understanding how novel and different organisms act in relation to well-studied organisms such as *E. coli* is important. Many of the gene annotation and gene functions have been tested in *E. coli*, therefore one can have greater confidence in understanding the data in *E.coli* compared to most other organisms. This study sought to understand anaerobic and aerobic regulation in *C. debilis* GB1 and this information is better understood in the context of the well-studied model organisms such as *E. coli*.

Chapter 5: Genomic Comparison of Facultatively Anaerobic and Obligately Aerobic *Caldibacillus debilis* Strains GB1 and Tf Helps Explain Physiological Differences[4]

5.1. Abstract

Caldibacillus debilis strains GB1 and Tf display distinct phenotypes. *C. debilis* GB1 is capable of anaerobic growth, and can synthesize ethanol while *C. debilis* Tf cannot. To help provide insights on these physiological differences, the genome of strain GB1 was sequenced. Comparison of the GB1 and Tf genome sequences revealed that the genomes were highly similar in gene content and showed a high level of synteny. At the genome scale, there were several large sections of DNA that appeared to be from lateral gene transfer to the GB1 genome, including: several cryptic prophage regions both internal and at contig ends, ~223,088bp of conjugation equipment, and a ~5kb plasmid. Tf did have unique genetic content but at a much smaller scale, 300 genes in Tf verses 857 genes in GB1 that matched at ≤90% sequence similarity. Gene complement and copy number of genes for the glycolysis, tricarboxylic acid (TCA) cycle, and electron transport chain (ETC) pathways were identical in both Tf and GB1. In addition, branched fermentation pathway genes were

[4] Contributing Authors: **Scott Wushke[a], Brian Fristensky[c], Xiang Li Zhang[c], Vic Spicer[b], Oleg V. Krokhin[b], David B. Levin[d], Nazim Cicek[d], Richard Sparling[d].** 2016 (Manuscript in preparation).

Contributions: [a]First author experimental design, cell growth and end-product analysis; worked in concert with others authors in analysis of genome and proteome [b]proteomic tools and expertise, data analyses, and running samples on MS/MS; [c]Bioinformatic tools and expertise, genome assembly and 1st draft annotation; [d]Lab space, equipment, funding, research guidance.

present in both strains, suggesting that both contain the gene complement necessary for both aerobic and anaerobic growth. Indeed, while Tf is an obligate aerobe, it possesses the gene complement for an anaerobic life style (*ldh, ak, pta, adhE, pfl*). There are only several minor genetic differences observed in regions of some key genes that may help explain some of the physiological differences (e.g. minor differences in *adhE* sequence between the strains). However, genomic comparisons did not reveal direct evidence explaining why strain Tf is not capable of growth in the complete absence of oxygen. This suggests that, as a species, *C. debilis* other strains should be expected to have the potential for anaerobic growth.

5.2. Introduction

Caldibacillus debilis GB1 was isolated from a co-culture found to convey aerotolerance to *C. thermocellum* when co-cultured aerobically on cellulose (Chapter 2). The recently defined, single species genus *Caldibacillus* has been the subject of only a few physiological studies in pure culture (Banat *et al.* 2004; Chapter 3). One major difference between these strains is the ability of strain GB1 (but not the type strain Tf) to grow anaerobically and synthesize ethanol under the conditions tested. *Caldibacillus debilis* Tf was also unable to create a micro-aerophilic environment suitable for co-culturing with the strict anaerobe *C. thermocellum*, contrary to what was observed with *C. debilis* GB1 (Chapter 3). We believe that the inability of Tf to grow anaerobically, or at low O_2 concentrations may affect its ability to create the reduced anaerobic microenvironment needed for *C. thermocellum* to thrive.

Previously, Wushke *et al.* (Chapter 3) compared the physiological profile of two strains of *C. debilis*, GB1 and Tf. They showed near identical characteristics in growth rate, temperature optimum, substrate utilization profile, and types of end-products produced under aerobic and oxygen-limiting conditions (with the notable exception of ethanol production). A *C. debilis* Tf draft genome has been generated and annotated by the Joint Genome Institute (JGI) (Grigoriev *et al.* 2012), but there has not been a detailed genome analysis published. The *Caldibacillus debilis* GB1 genome was previously sequenced and its proteome was analyzed with respect to aerobic and anaerobic growth (Chapter 4). Due to several major physiological differences, including anaerobic growth and ethanol synthesis, we hypothesized that a comparison of the genomes of the GB1 and Tf genome sequences, with a focus on core metabolism, could explain the observed physiological differences.

5.3. Materials and Methods

5.3.1. Cell Culturing

The methods for *Caldibacillus debilis* strain GB1 DSM 29516 and *C. debilis* Tf DSM 16016 cultures were described previously (Chapter 2 and 3). *C. debilis* strains were grown on modified 1191 (M-1191) medium (Islam *et al.* 2006) with a lower concentration of yeast extract (0.76 g/L) and the pH was adjusted to 7.2, for all experiments. Sealed Balch tubes (26 mL) from Bellco Glass Inc. (Vineland, NJ) and 1L Corning bottles supplied from Fisher Scientific (Toronto, ON), were used to carry

out experiments. Anaerobic and aerobic environments in Balch tubes and Corning bottles were prepared as previously described by Wushke *et al.* (Chapter 3).

5.3.2. Genomic Comparison

The GB1 genome was sequenced and annotated as previously described (Chapter 4). The GB1 and Tf genome sequences can both be accessed through the National Center for Biotechnology Information (NCBI) under the catalogue numbers AZRV00000000 and ARVR00000000, respectively. Joint Genome Institute (JGI) Integrated Microbial Genomes (IMG) Expert Review (IMG/ER) was used to compare the bacterial genomes (Markowitz *et al*. 2008). NUCmer plots were generated through IMG/ER. Gview was used to create genome-scale comparative images (Petkau *et al.* 2010). Protein and DNA alignments were done with Multiple Sequence Alignment Tool (MUSCLE) (Edgar *et al.* 2004). NCBI Blast was used create a 2D model of domains and key amino acids involved in function (Johnson *et al*. 2008). 3D models of the ADHE proteins were created using RAPTORX (Källberg et al. 2012).

5.3.3. Protein Extraction

Samples for protein extraction were collected during early exponential in aerobic growth shaken at 150rpm for both strains GB1 and Tf. Approximately ~200 mg cell pellets were washed twice in phosphate buffered saline (PBS) buffer then re-suspended in 1 mL of lysis buffer [1% sodium dodecyl sulfate (SDS), 100 mM dithiothreitol (DTT)] in 100 mM ammonium bicarbonate (ABC)). The mixture was

vortexed for 30 seconds (sec), then placed in boiling water for 5 minutes (min), and then cooled for 5 min. Samples were then sonicated three times with 15 sec pulses, and 1 min breaks in-between then were centrifuged at 16000 x g for 20 min. Supernatants containing the proteins were kept for further processing, and stored at -80 ºC. Aliquots (20 µL) of the protein containing supernatants were diluted with 180 µL of 100 mM ABC buffer. Twenty (20) µL of 500 mM iodoacetic acid (IAA) were added, vortexed for 5 sec and stored in the dark for 45 min to complete alkylation. To quench excess IAA, 33 µL of 100mM DTT was added. Then, 150 µL of ABC were added to bring total volume to ~380 µL to reduce the SDS concentration in order to allow digestion. Trypsin (4 µg) was added, and $CaCl_2$ was added to a final concentration of 10 mM. Samples were incubated for 12 hours at 37 ºC to allow trypsinization to occur. Samples were then dried using roto-evaporation (Savant Speedvac-sc110), and 200 µL of 3 M KCl was added and vortexed for 10 min, then were spun at 16000 x g for 25 min and supernatants were gently removed from the precipitate (containing the SDS, which is incompatible mass spectrometry methods). The samples were diluted 2 fold with 0.5% trifluoroacetic acid (TFA) then desalted by HPLC using a C18 column as described previously (Chapter 4).

5.3.4. Mass Spectrometry Methods

One-dimensional liquid chromatography followed by mass spectrometry (1D-LC-MS) proteomics were used to identify proteins by their peptide sequences, as previously described by Gungormusler-Yilmaz *et al.* (2014). Proteomic profiles of the

C. debilis strains GB1 and Tf grown to early exponential phase on cellobiose were compared using an in-house data analysis system called UNITY, as previously described (Fu *et al.* 2015) was used. The XML output file from an X!tandem search and its corresponding TIC intensity's can be found at http://hs2.proteome.ca/thegpmcgi/plist.pl?path=/gpm/archive/C.debilis_GB1/GB1A-BASERESULTS.xml&proex=-1&npep=0 and http://hs2.proteome.ca/thegpm-cgi/plist.pl?path=/gpm/archive/C.debilis_GB1/GB1B-BASERESULTS.xml&proex=-1&npep=0 and http://hs2.proteome.ca/thegpm-cgi/plist.pl?path=/gpm/archive/C.debilis_GB1/DSM1606AX-BASERESULTS.xml&proex=-1&npep=0.

5.3.5. Proteogenomics

To enhance the genome sequence and annotation, a proteogenomic approach was used. We have described, previously, the use of proteomic data to identify and correct improperly annotated genes using a naïve assembler (Chapter 4) using the same methodology as Verbeke *et al.* (2014). In this study two proteomes were generated, one for each, GB1 and Tf, during early exponential under aerobic conditions on cellobiose. We used these proteomic data to verify unique regions and genes missing in the *C. debilis* GB1 and Tf genome by analyzing each strains proteome with respect to both the GB1 and Tf genomes.

5.4. Results and Discussion

5.4.1. GB1 and Tf Genome Comparison

A summary of the GB1 and Tf genomes containing 49 and 41 contigs respectively can be found in Table 5.1. The genome sequences of *C. debilis* strains GB1 and Tf are highly similar in both gene identity and genome synteny (2354 genes in matching cassettes, where a cassette consists of blocks of associated genes in sets of 2 or more). GB1 has 2570 genes matching Tf with a sequence identity ≥90%. Figure 5.1 shows a NUCmer plot demonstrating their synteny produced using IMG/ER. When the genome sequences of the two strains were compared, there were 853 genes in GB1 that did not match with a sequence identity greater than or equal to 90% in Tf. Since these strains primarily have highly related genes, matches with ≤90% sequence identity were considered to be unique. The majority of unmatched genes found in GB1 were hypothetical (408). Many of the unmatched genes could be attributed to three structural differences: i) the addition of 207 genes on contig 4, which appear to form a conjugative element; ii) the addition of a full length genome of a cryptic prophage genome on contig 16, as well as several partial copies associated with the end of various contigs, and iii) a phage-related circular plasmid found as contig 31. There were also 16 transposase associated genes dispersed through the genome. Figure 5.2 shows a graphical representation of the GB1 and Tf unique genomes with genes matching at ≤90% sequence similarity produced by GView. In contrast to GB1, Tf has only 300 unique genes that do not match sequences in the GB1 genome at a ≤90% sequence similarity.

Table 5.1. General genome features of *C. debilis* GB1 and Tf (source: IMG/er)

	GB1		Tf	
	Number	% of Total	Number	% of Total
DNA, total number of bases	3346235	1	3061346	1
DNA coding number of bases	2670185	0.798	2430336	0.7939
DNA G+C number of bases	1712659	51.18% 1	1579272	51.59% [1]
DNA scaffolds	49	1	41	1
CRISPR Count	5		8	
Genes total number	3374	1	2896	1
Protein coding genes	3264	0.9674	2800	0.9669
RNA genes	110	0.0326	96	0.0331
rRNA genes	8	0.0024	13	0.0045
5S rRNA	5	0.0015	5	0.0017
16S rRNA	2	0.0006	5	0.0017
23S rRNA	1	0.0003	3	0.001
tRNA genes	85	0.0252	58	0.02
Other RNA genes	17	0.005	25	0.0086
Protein coding genes with function prediction	2450	0.7261	2212	0.7638
without function prediction	814	0.2413	588	0.203
Protein coding genes with enzymes	708	0.2098	731	0.2524
w/o enzymes but with candidate KO based enzymes	71	0.021	53	0.0183
Protein coding genes connected to Transporter Classification	427	0.1266	154	0.0532
Protein coding genes connected to KEGG pathways3	837	0.2481	854	0.2949
not connected to KEGG pathways	2427	0.7193	1946	0.672
Protein coding genes connected to KEGG Orthology (KO)	1559	0.4621	1567	0.5411
not connected to KEGG Orthology (KO)	1705	0.5053	1233	0.4258
Protein coding genes connected to MetaCyc pathways	614	0.182	628	0.2169
not connected to MetaCyc pathways	2650	0.7854	2172	0.75
Protein coding genes with COGs3	1960	0.5809	1933	0.6675
with KOGs3	488	0.1446	501	0.173
with Pfam3	2524	0.7481	2325	0.8028
with TIGRfam3	981	0.2908	955	0.3298
with InterPro	1625	0.4816	1497	0.5169
with IMG Terms	431	0.1277	317	0.1095
with IMG Pathways	150	0.0445	113	0.039

with IMG Parts List	168	0.0498	142	0.049
with MyIMG Annotation	1	0.0003	1	0.0003
Genes in Biosynthetic Clusters	99	0.0293	94	0.0325
Fused Protein coding genes	71	0.021	64	0.0221
Protein coding genes coding signal peptides	144	0.0427	99	0.0342
Protein coding genes coding transmembrane proteins	841	0.2493	742	0.2562
COG clusters	1310	0.6684	1307	0.6762
KOG clusters	351	0.1791	355	0.1837
Pfam clusters	1812	0.7179	1764	0.7587
TIGRfam clusters	887	0.9042	889	0.9309

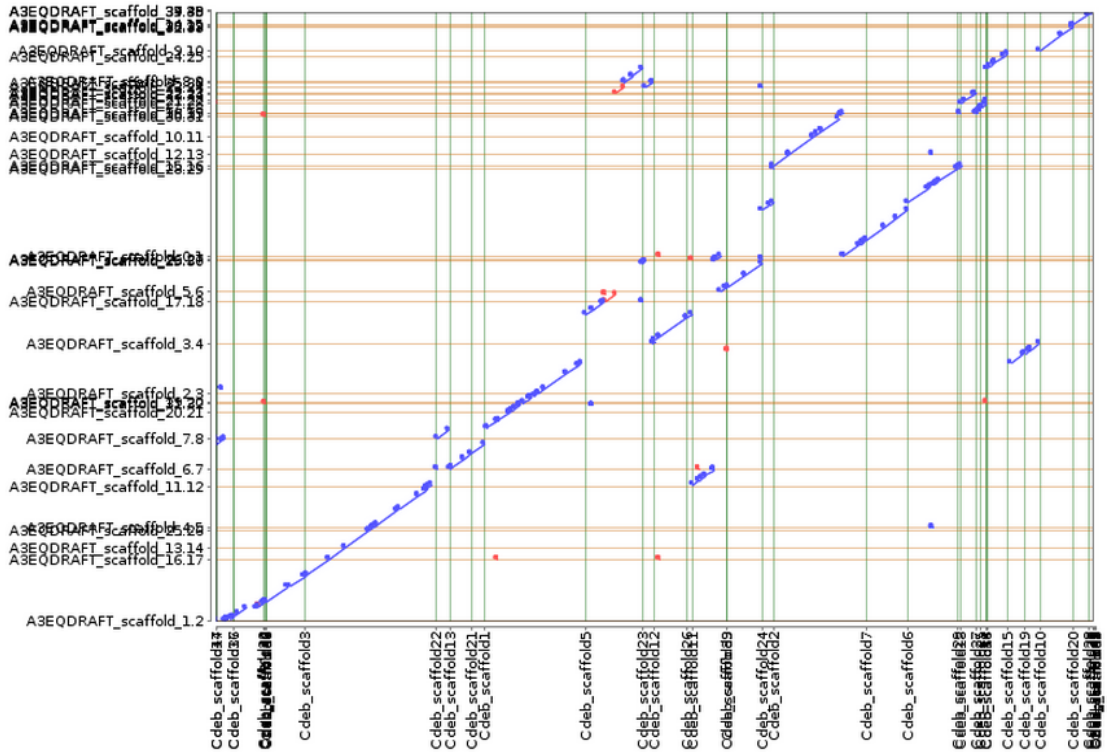
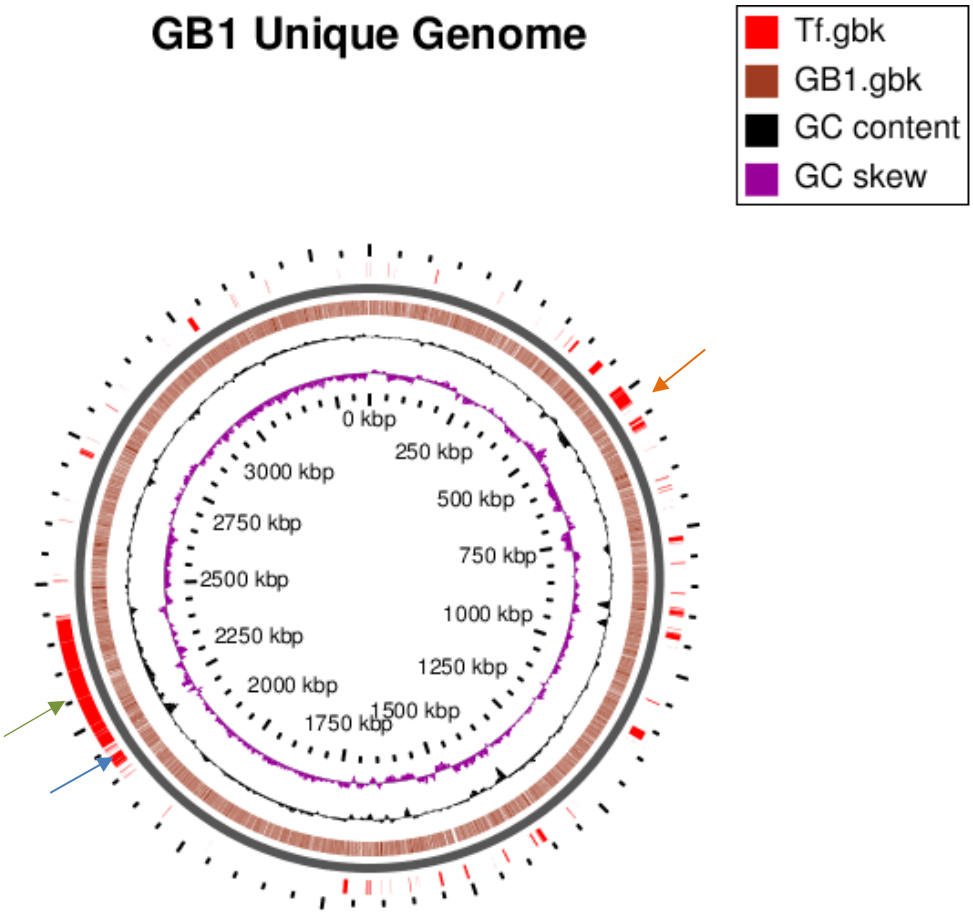


Figure 5.1. NUCmer comparison of *C. debilis* GB1 (x-axis) and Tf (y-axis) Genomes by Contig.
Blue lines are sequence that matches on the same DNA strand. Red lines represent DNA sequences that match on opposite DNA strand.

A)


GB1 Unique Genome
Tf.gbk
GB1.gbk
GC content
GC skew
0 kbp
250 kbp
500 kbp
750 kbp
1000 kbp
1250 kbp
1500 kbp
1750 kbp
2000 kbp
2250 kbp
2500 kbp
2750 kbp
3000 kbp

B)

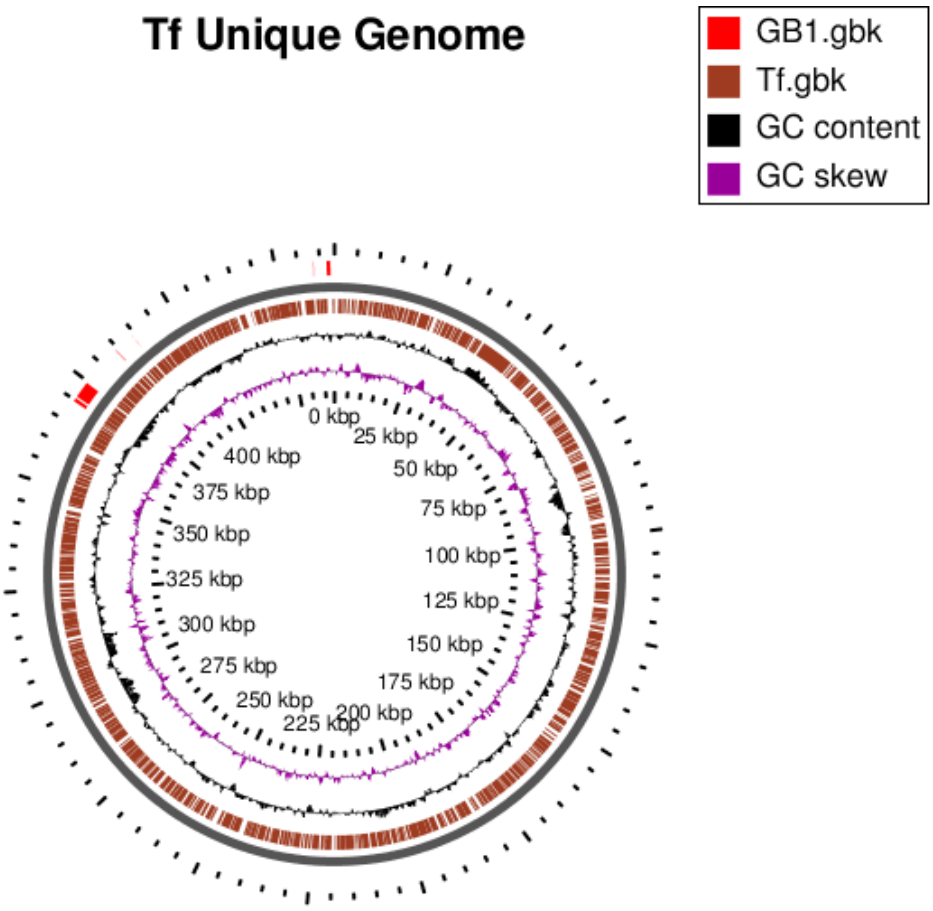


Figure 5.2. *C. debilis* GB1 (panel A) and Tf (Panel B). Gene bank file(.gbk) comparison of Unique Genome genes that match ≤90% sequence similarity to the corresponding strain are shown in red. In panel A, Contig 4 is shown with a green arrow, the full putative phage genome shown with orange arrow, plasmid shown with blue arrow.

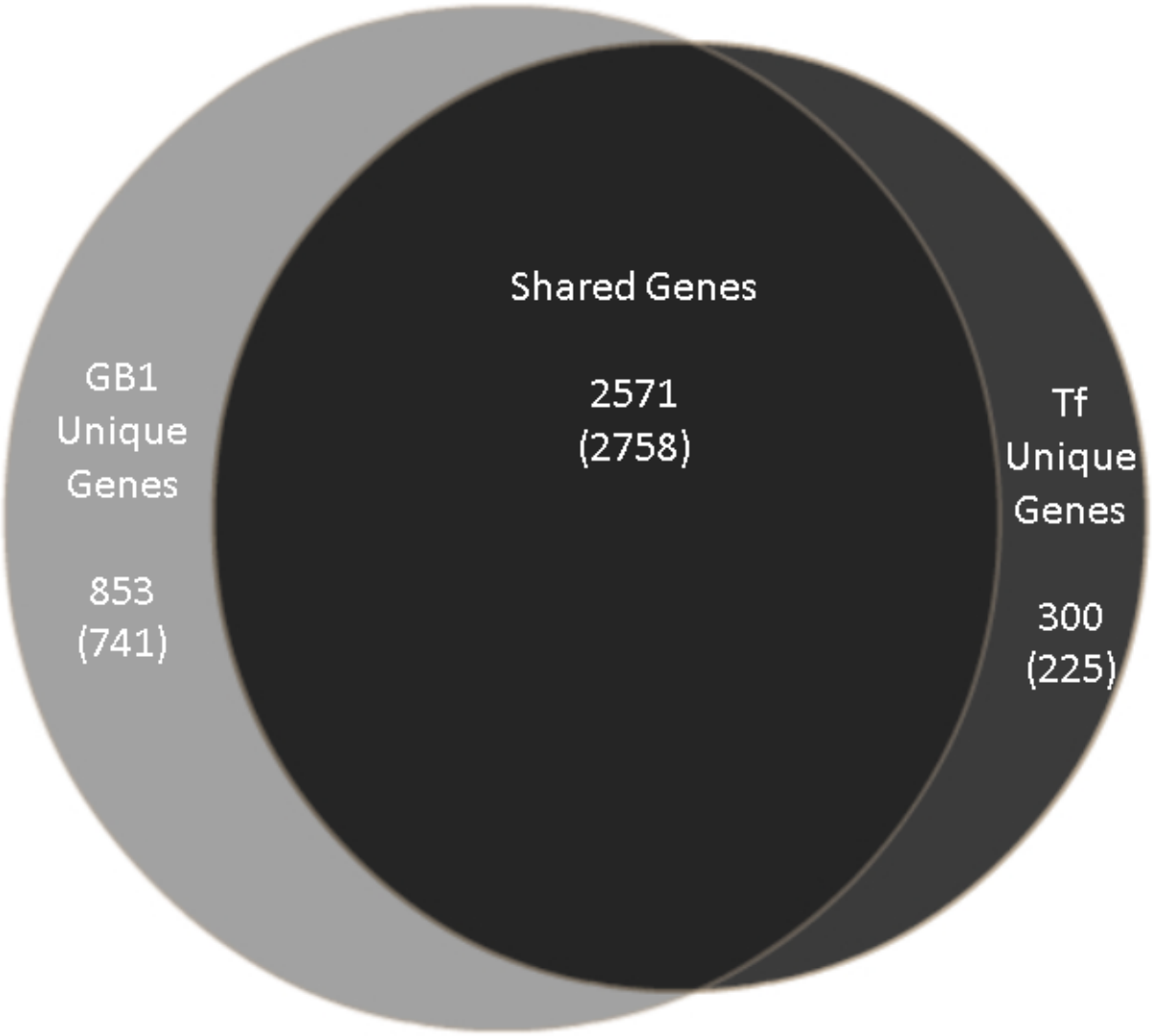


Figure 5.3. Venn diagram of genes unique to *C. debilis* strains at a ≤90% sequence similarity cut off. Brackets "()" refer to the number of genes shared when a ≤60% sequence similarity cut off is used.

Differences in tRNA complement have the potential to affect gene regulation at a global level. Sequence analyses of GB1 revealed that there are 25 tRNA genes on contig 4 that are associated with the conjugative element described above. Thanks in part to this addition, there are 85 tRNA genes in GB1 compared to only 58 in Tf, as shown in Table 5.2. All of the tRNA's unique to GB1 had anticodons that were shared by Tf and GB1, therefore the codon usage ability was not expanded. A change among tRNA availability could have an effect on translation regulation. The tRNA did not appear to be the cause of differences in anaerobic ability between the strains, as the added tRNA's did not appear to change the codons used.

Table 5.2. tRNA's unique to GB1

GB1 Locus Tag	TF Locus Tag	Gene Product Name	Anticodon
Cdeb_00070	A3EQDRAFT_00225	tRNA-Pro	GGG
Cdeb_00257	A3EQDRAFT_00784	tRNA-Arg	CCG
Cdeb_00720	A3EQDRAFT_00228	tRNA-Gln	TTG
Cdeb_00804	A3EQDRAFT_02250	tRNA-Ser	CGA
Cdeb_01241		tRNA-Phe	GAA
Cdeb_01242		tRNA-Gly	GCC
Cdeb_01243		tRNA-Pseudogene	CTG
Cdeb_01244		tRNA-Val	TAC
Cdeb_01245		tRNA-Trp	CCA
Cdeb_01246		tRNA-Asn	GTT
Cdeb_01247		tRNA-Ser	TGA
Cdeb_01248		tRNA-Ser	GGA
Cdeb_01249		tRNA-Ser	CGA
Cdeb_01250		tRNA-Ser	GCT
Cdeb_01251		tRNA-Gly	TCC
Cdeb_01252		tRNA-Lys	TTT
Cdeb_01256		tRNA-Thr	TGT
Cdeb_01257		tRNA-Pro	CGG
Cdeb_01258		tRNA-Leu	TAA
Cdeb_01259		tRNA-Glu	TTC
Cdeb_01260		tRNA-Pseudogene	???

Cdeb_01261		tRNA-Pseudogene	GAC
Cdeb_01262		tRNA-Pro	TGG
Cdeb_01263		tRNA-Asp	GTC
Cdeb_01264		tRNA-Gln	TTG
Cdeb_01265		tRNA-Leu	CAA
Cdeb_01299		tRNA-Pseudogene	???
Cdeb_01300		tRNA-Undetermined isotype	???
Cdeb_01446	A3EQDRAFT_02566	tRNA-Val	GAC
Cdeb_01786	A3EQDRAFT_00224	tRNA-Gly	TCC
Cdeb_01787	A3EQDRAFT_01732	tRNA-Pro	CGG
Cdeb_01788	A3EQDRAFT_00226	tRNA-Arg	ACG
Cdeb_01789	A3EQDRAFT_00227	tRNA-Leu	GAG
Cdeb_01790	A3EQDRAFT_00675	tRNA-Gln	TTG
Cdeb_01791	A3EQDRAFT_00229	tRNA-Glu	TTC
Cdeb_02265	A3EQDRAFT_01625	tRNA-Glu	TTC
Cdeb_02266	A3EQDRAFT_02905	tRNA-Ser	GCT
Cdeb_02267		tRNA-Asn	GTT
Cdeb_02268		tRNA-Ile	GAT
Cdeb_02269	A3EQDRAFT_02908	tRNA-Gly	TCC
Cdeb_02270	A3EQDRAFT_01621	tRNA-Phe	GAA
Cdeb_02271	A3EQDRAFT_01622	tRNA-Asp	GTC
Cdeb_02272	A3EQDRAFT_01623	tRNA-Met	CAT
Cdeb_02273	A3EQDRAFT_02912	tRNA-Ser	TGA

Cdeb_02274	A3EQDRAFT_02911	tRNA-Met	CAT
Cdeb_02275	A3EQDRAFT_02913	tRNA-Met	CAT
Cdeb_02276		tRNA-Ala	TGC
Cdeb_02277	A3EQDRAFT_02916	tRNA-Arg	ACG
Cdeb_02278	A3EQDRAFT_02333	tRNA-Leu	TAA
Cdeb_02279	A3EQDRAFT_02334	tRNA-Gly	GCC
Cdeb_02280	A3EQDRAFT_02919	tRNA-Leu	TAG
Cdeb_02281	A3EQDRAFT_02336	tRNA-Lys	TTT
Cdeb_02282	A3EQDRAFT_02921	tRNA-Val	TAC
Cdeb_02350	A3EQDRAFT_02141	tRNA-Lys	CTT
Cdeb_02360	A3EQDRAFT_02153	tRNA-Arg	CCT
Cdeb_02595	A3EQDRAFT_01613	tRNA-Leu	CAA
Cdeb_02596	A3EQDRAFT_01614	tRNA-Cys	GCA
Cdeb_02597	A3EQDRAFT_01615	tRNA-Gly	GCC
Cdeb_02598	A3EQDRAFT_01616	tRNA-Gln	TTG
Cdeb_02599	A3EQDRAFT_01617	tRNA-His	GTG
Cdeb_02600	A3EQDRAFT_01618	tRNA-Trp	CCA
Cdeb_02601	A3EQDRAFT_01619	tRNA-Tyr	GTA
Cdeb_02602	A3EQDRAFT_01620	tRNA-Thr	TGT
Cdeb_02603	A3EQDRAFT_02909	tRNA-Phe	GAA
Cdeb_02604	A3EQDRAFT_02910	tRNA-Asp	GTC
Cdeb_02605	A3EQDRAFT_02914	tRNA-Met	CAT
Cdeb_02606	A3EQDRAFT_01624	tRNA-Val	CAC

Cdeb_02607	A3EQDRAFT_02904	tRNA-Glu	TTC
Cdeb_02608	A3EQDRAFT_01626	tRNA-Ser	GGA
Cdeb_02609	A3EQDRAFT_01627	tRNA-Asn	GTT
Cdeb_02851	A3EQDRAFT_01342	tRNA-Arg	TCT
Cdeb_03031	A3EQDRAFT_00931	tRNA-Ala	GGC
Cdeb_03046	A3EQDRAFT_02331	tRNA-Ala	CGC
Cdeb_03047	A3EQDRAFT_02332	tRNA-Pro	TGG
Cdeb_03048	A3EQDRAFT_02917	tRNA-Leu	TAA
Cdeb_03049	A3EQDRAFT_02918	tRNA-Gly	GCC
Cdeb_03050	A3EQDRAFT_02335	tRNA-Leu	CAG
Cdeb_03051	A3EQDRAFT_02920	tRNA-Lys	TTT
Cdeb_03154	A3EQDRAFT_02907	tRNA-Ile	GAT
Cdeb_03155	A3EQDRAFT_02915	tRNA-Ala	TGC
Cdeb_03159	A3EQDRAFT_02906	tRNA-Asn	GTT
Cdeb_03160		tRNA-Thr	GGT
Cdeb_03172	A3EQDRAFT_01645	tRNA-Thr	CGT
Cdeb_03173	A3EQDRAFT_01646	tRNA-Tyr	GTA
Cdeb_03174	A3EQDRAFT_01647	tRNA-Gly	CCC
	A3EQDRAFT_00468	tRNA-Arg	TCT

5.4.2. Proteogenomics

The general features of the *C. debilis* GB1 genome have been described previously, and proteogenomics used to correct annotation start sites (Chapter 4). In this study we used a further single proteome for each strain, GB1 and Tf, during early aerobic growth (sampling point is shown in Figure 5.4) in order to: i) check for genes that were missed due to incomplete sequencing and/or annotation; and ii) check whether or not genes in regions which appeared unique to GB1 were expressed. Depth of coverage, however, was rather poor, with 704 and 473 proteins detected for GB1 and Tf respectively. Nevertheless, when the proteome of strain Tf was compared against the GB1 genome, 1 gene, LSU ribosomal protein L36P (Cdeb_03413), had peptide signals that had not been observed when the Tf genome analysed with the Tf proteome. Tf possessed the exact same DNA sequence but was partially overlapped by another gene. The lack of an ORF for this gene in Tf genome is likely due to a difference in the annotation methods used due to preference of an overlapping ORF. The GB1 proteome did not display any additionally detected proteins when analyzed against the Tf genome, suggesting its genome sequencing was more complete than the Tf genome sequence. The regions that appeared to be unique to GB1, a cryptic prophage region within contig 16 and conjugation equipment comprising contig 4, resulted in 5 and 3 proteins detected respectively. When the Tf proteome was used to check for GB1 genes in the regions that appeared to be from a lateral gene transfer, no proteins were found corresponding to these genes suggesting that the DNA which appears to be exogenously introduced into GB1 through lateral gene transfer is likely unique to GB1.

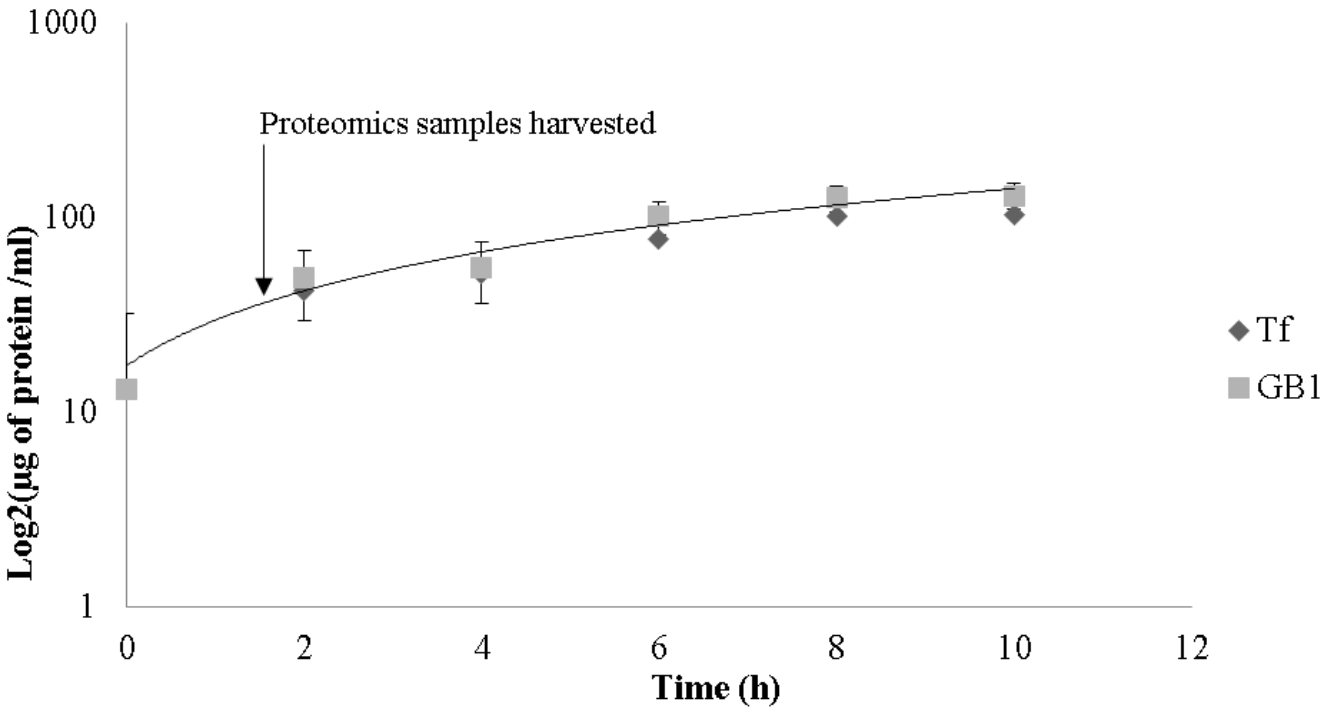


Figure 5.4. Growth curve and sampling point of *C. debilis* Tf and GB1 used for proteomics

5.4.3. Core Metabolic Genes Relative to Observed Phenotype

The primary reason for the present comparative study was to understand the significant differences in end-product synthesis profiles between the two strains. While both strains were able to produce lactate, acetate, formate, and CO_2 under oxygen-limiting conditions, the Tf strain lacked ethanol production under oxygen-limiting conditions, and showed an inability to grow anaerobically (Chapter 3). GB1, under oxygen-limiting conditions, showed the ability to produce ethanol and grow aerobically. Focusing on the genes responsible for core catabolic activity, the gene complement in strains GB1 and Tf were found to be identical and the genes appeared in similar genetic contexts in both genomes.

An overview of core metabolism in *C. debilis* GB1 can be found in our previous work Wushke *et al.* (Chapter 4). When the gene complement needed for pyruvate fermentation is considered, GB1 and Tf have the exact same complement and number of genes. A list of GB1 core metabolic genes and the corresponding locus tags for Tf can be found in Table 5.3. The differences between GB1 and Tf are in bold the pyruvate kinase (Cdeb_02915, Cdeb_02914) which was annotated as separate but adjacent genes with different annotated functions in GB1 and one gene, a pyruvate kinase, in Tf even though DNA in the coding region is 100% identical.

Table 5.3. Core metabolism locus tags of GB1 and Tf that matched with ≥90% identity, and predicted function as specified through KEGG maps built in IMG/ER

Gene Product name	*C. debilis* GB1 Locus tags	*C. debilis* Tf Locus tags
Electron Transport Chain Locus Tags		
Complex I	Cdeb_02701, Cdeb_02695	A3EQDRAFT_00873, A3EQDRAFT_00865
Complex II	Cdeb_02775, Cdeb_02776, Cdeb_02777	A3EQDRAFT_00449, A3EQDRAFT_00450, A3EQDRAFT_00451
Complex III	Cdeb_00880, Cdeb_00881, Cdeb_00882	A3EQDRAFT_02324, A3EQDRAFT_02325, A3EQDRAFT_02326
Complex IV	Cdeb_00920, Cdeb_00921, Cdeb_01033, Cdeb_01034, Cdeb_02460, Cdeb_02461, Cdeb_02462, Cdeb_02463	A3EQDRAFT_01157, A3EQDRAFT_01158A3EQDRAFT_01269, A3EQDRAFT_01270, A3EQDRAFT_01014, A3EQDRAFT_01015, A3EQDRAFT_01016, A3EQDRAFT_01017
ATP synthase	Cdeb_00527-Cdeb_00534	A3EQDRAFT_02012-A3EQDRAFT_02019
Reactive Oxygen Species Protection Locus Tags		
superoxide dismutase	Cdeb_00057, Cdeb_00690	A3EQDRAFT_00648, A3EQDRAFT_01719
Catalase	Cdeb_00043, Cdeb_01630	A3EQDRAFT_00110, A3EQDRAFT_01710
peroxidase	Cdeb_00209, Cdeb_00830, Cdeb_00953, Cdeb_01022, Cdeb_01743, Cdeb_02257, Cdeb_03127	A3EQDRAFT_00006, A3EQDRAFT_00737, A3EQDRAFT_01190, A3EQDRAFT_01259, A3EQDRAFT_01517, A3EQDRAFT_01874, A3EQDRAFT_02276
Glycolysis and Gluconeogenesis Locus Tags		

Phosphoglucomutase	Cdeb_01100	A3EQDRAFT_02658
Glucose 6-phsophate isomerase	Cdeb_03023	A3EQDRAFT_00922
6-phosphofructos kinase	Cdeb_02916	A3EQDRAFT_01497
Fructose-1, 6-bisphosphate aldolase	Cdeb_00505	A3EQDRAFT_02039
fructose-bisphosphatase	Cdeb_00508	A3EQDRAFT_02036
Glyceraldhyde-3-phosphate dehydrogenase	Cdeb_00261, Cdeb_02895	A3EQDRAFT_00788, A3EQDRAFT_01478
1-3 phosphoglycerate kinase	Cdeb_00262	A3EQDRAFT_00789
Phosphoglycerate mutase	Cdeb_00264	A3EQDRAFT_00791
enolase	Cdeb_00265	A3EQDRAFT_00792
Pyruvate kinase	**Cdeb_02915[a], Cdeb_02914[a]**	A3EQDRAFT_01496
Triose isomerase	Cdeb_00263	A3EQDRAFT_00790

Tricarboxylic Acid Cycle Locus Tags

Citrate synthase	Cdeb_02909	A3EQDRAFT_01492
aconitate hydratase	Cdeb_02349	A3EQDRAFT_02140
Isocitrate dehydrogenase	Cdeb_02908	A3EQDRAFT_01491
Alpha-ketogultarate dehydrogenase	Cdeb_00961, Cdeb_00962, Cdeb_00776, Cdeb_00637, Cdeb_02441	A3EQDRAFT_01199,A3EQDRAFT_01200, A3EQDRAFT_00335, A3EQDRAFT_01037, A3EQDRAFT_02497
Succinyl-CoA synthase	Cdeb_02130, Cdeb_02131	A3EQDRAFT_01441, A3EQDRAFT_01442
Succinate dehydrogenase	Cdeb_02775, Cdeb_02776, Cdeb_02777	A3EQDRAFT_00449, A3EQDRAFT_00450, A3EQDRAFT_00451
Fumerate hydratase	Cdeb_01560	A3EQDRAFT_01797
Malate dehydrogenase	Cdeb_02907	A3EQDRAFT_01490
Pyruvate Metabolism Locus Tags		
Pyruvate formate lyase	Cdeb_01638, Cdeb_01637	A3EQDRAFT_00102, A3EQDRAFT_00103
Bifunctional aldehyde/alcohol dehydrogenase	Cdeb_01397	A3EQDRAFT_01103
Acetate kinase	Cdeb_03124	A3EQDRAFT_01515
Phosphotransacetylase	Cdeb_00469	A3EQDRAFT_02234

Lactate dehydrogenase	Cdeb_02710	A3EQDRAFT_00857
Aldehyde dehydrogenase	Cdeb_00412, Cdeb_02563	A3EQDRAFT_02176, A3EQDRAFT_01581
Alcohol dehydrogenase	Cdeb_03022, Cdeb_00489, Cdeb_01569, Cdeb_01590,	A3EQDRAFT_00921, A3EQDRAFT_02052, A3EQDRAFT_02668, A3EQDRAFT_00146
Pyruvate Dehydrogenase Locus Tags		
E1 (EC:1.2.4.1)	Cdeb_02438, Cdeb_02439, Cdeb_00634, Cdeb_00635, Cdeb_03163, Cdeb_03164	A3EQDRAFT_00337, A3EQDRAFT_00338, A3EQDRAFT_01039, A3EQDRAFT_01040, A3EQDRAFT_01636, A3EQDRAFT_01637
E2 (EC:2.3.1.12)	Cdeb_02440, Cdeb_00636, Cdeb_03165	A3EQDRAFT_00336, A3EQDRAFT_01038, A3EQDRAFT_01638
E3 (EC:1.8.1.4)	Cdeb_02441, Cdeb_00637, Cdeb_00776	A3EQDRAFT_00335, A3EQDRAFT_01037, A3EQDRAFT_02497

Differences in annotated genes are show in **Bold**; [a]pyruvate kinase gene was split in GB1 and the adjacent ORF's were given different annotated functions.

5.4.4. Pyruvate Fermentation

When focusing on pyruvate fermentation pathways, small differences were observed in regions within 200bp up stream of a number of genes of interest shown in Figure 5.5: *adh* (Cdeb_03022, Cdeb_00489, Cdeb_01569, Cdeb_01590), *aldh*(Cdeb_00412, Cdeb_02563), *adhe* (Cdeb_01397), *ak* (Cdeb_03124), *pta* (Cdeb_00469), *pfl* (Cdeb_01637/01638) and *ldh* (Cdeb_02710). When examining the genes each appeared to have a putitive TATAAT box and transcription start site. Nevertheless, in some cases, the differences in the upstream regions between GB1 and Tf were extensive (*adh*), compared to others (*ak*, *pta*, *adhE*, *aldh*) as seen in Figure 5.5. Figure 5.6 shows the amino acid sequence alignments of genes in pyruvate fermentation that are not perfect matchs. Figure 5.7 is the *adhE* DNA alignment. The activity of key enzymes PFL, LDH, AK, ADHE could be verified by end-product analysis under oxygen-limited conditions, as their corresponding products were detected, with the exception of ethanol in Tf, even though the genome of strain Tf encodes ADHE. ADHE is the primary enzyme responsible for ethanol synthesis in many anaerobes (Carere *et al.* 2012).

Interpreting changes at the nucleotide level in and surrounding the core metabolism genes must be approached with caution as differences are also consistent with what would be expected in distinct strains (Hayashi *et al.* 2001). Indeed many of the differences noted between GB1 and Tf would not be expected to affect gene expression or activity (*i.e*. silent mutations, mutations in non-coding areas).

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *adh* in GB1 and Tf

```
Cdeb_03022           TGAAAAAATCCGACCAGTCCCCAGTTGATGGCCCCGATGATGGTTAAGACGAGACAAATT
A3EQDRAFT_00921      TGAAAAAATCCGACCAGTCCCCAGTTGATGGCCCCGATGATGGTTAAGACGAGACAAATT
                     ************************************************************

Cdeb_03022           CTTTGCAATACGCTCATGAAAAATCCTCCTTCGAATGATTATTACGGTACTTAGGATGGT
A3EQDRAFT_00921      CTTTGCAATACGCTCATGAAAAATCCTCCTTCGAATGATTATTACGGTACTTAGGATGGT
                     ************************************************************

Cdeb_03022           TAAAATTCACAAATTCTATGCATCGGAAATTTTCCATCCCGGTTTTCATGTATTAAAATA
A3EQDRAFT_00921      TAAAATTCACAAATTCTATGCATCGGAAATTTTCCATCCCGGTTTTCATGTATTAAAATA
                     ************************************************************

Cdeb_03022           TCGATGAGGAGGGATGAAAGA
A3EQDRAFT_00921      TCGATGAGGAGGGATGAAAGA
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *adh* in GB1 and Tf

```
Cdeb_00489           TTCTGTATTCCTCCTTTGCGGGATAATCAAGCCAAGTTCATTATAACTCGAAGGATATAA
A3EQDRAFT_02052      TTCTGTATTCCTCCTTTGCGGGATAATCAAGCCAAGCTCATTATAACTCGAAAAATGTGA
                     ********************************** **************  ** * *

Cdeb_00489           TTGTTCAACATTTCACAAACTATGTACATCATTCTTTCCAAGAATCGTAAATCTTGAA-T
A3EQDRAFT_02052      TTGTTCAACATTTCACAAACTATGTACATCATTCTTTCCAATAATCGTAAATCTTGAATT
                     ****************************************** *************** *

Cdeb_00489           TTTTTTGAATATGGGGCACTCCTCCATGGGAAAGGGCCGCAGGCGGGGCTCCCGCCGGAT
A3EQDRAFT_02052      TTTTTTGAATATGGGGCACTCCTCCATGGGAAAGGGCCGCAGGCGGGGTTCCCGCCGGAT
                     ********************************************** ***********

Cdeb_00489           CGATTTTTTCAAGGCCGCCGGG
A3EQDRAFT_02052      CGATTTTTTCAAGGCCGCCGG-
                     *********************
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *adh* in GB1 and Tf

```
Cdeb_01569          -------TCATTCTTTCCTCCCTTTCGAATTTACGGATGAATAGAAAATTCTTCATCTTT
A3EQDRAFT_02668     AAAGGAGACG----------------GGAAACACGGTCGGCTTGAAA----TTCGCTGAA
                           *                * *   ****  *  * ****    ***

Cdeb_01569          ATGGTAATCCCAGTCTCCGGCCGATGCAA----TTCCCCCTTCCGCTTTTTCTAATCGGC
A3EQDRAFT_02668     AAGGGATTTTTATCGTCCGG--AATGTAAACGCTTACTTATATCCTCTTTCCCGAACGAT
                    * ** * *   *   *****   *** **    ** *   *  *   *** *  * **

Cdeb_01569          GTT---------TCCAAACATTTTTTGAACA---AAACGATGGGCGGCCCAAGGCTTCCG
A3EQDRAFT_02668     GCTGGCTGCAGACGCGAACAAACCTTGAAAACTGCATCGCTGCATGGTGCAG------CG
                    * *           * ****    ***** *    * ** **   **  **       **

Cdeb_01569          G---CGTCCCCTTTCCCCGGATCAAGTCCTCCCATTTGTGTAAAATC----
A3EQDRAFT_02668     GAAAAACGACCTTCCTTTAAATAAGG--ATTGTATTCAAGGAGGAGAGAAA
                    *        **** *     ** * *   *   ***   * *  *
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *adh* in GB1 and Tf

```
Cdeb_01590          TGCTCCGATCCCTCCACTCCCGCAGGAAAATGAAAAAAAGAAGCGTTTTCGGTCATTCAT
A3EQDRAFT_00146     TGCTCCGATCCCTCCACTCCCGCAGGAAAATGAAAAAAAGAAGCGTTTTCGGTCATTCAT
                    ************************************************************

Cdeb_01590          AATACCCTTCGGTGGATTTGATGAAGGTTTTGAACTGTTCCGCGTCGTGGCCAAACCAGA
A3EQDRAFT_00146     AATACCCTTCGGTGGATTTGATGAAGGTTTTGAACTGTTCCGCGTCGTGGCCAAACCAGA
                    ************************************************************

Cdeb_01590          CTTCCGAATTCGTCTTTTTTGCATATCTTCTTATTTTTTCGACCGTCTTGGAAAATCCGA
A3EQDRAFT_00146     CTTCCGAATTCGTCTTTTTTGCATATCTTCTTATTTTTTCGACCGTCTTGGAAAATCCGA
                    ************************************************************

Cdeb_01590          TGGAATCGTAAAGGATCCCGG
A3EQDRAFT_00146     TGGAATCGTAAAGGATCCCGG
                    *********************
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *adhe* in GB1 and Tf.

```
Cdeb_01397          AGGACGATTCCCTCCTATTCTTGCTGGTC-GTTTTTTTCCCTTATGGTCATTTTTAGTCC
A3EQDRAFT_01103     AGGACGATTCCCTCCTATTCTTGTTGGTCAATTTTTGTCCCTTATGGTCATTTTTAGTCC
                    ********************** *****  ***** **********************

Cdeb_01397          CTCATGGGTAAAAATAAAAACTTCTTACACTCCTATTATATATGGGAAAGGGATCAAAGA
A3EQDRAFT_01103     CGCATGGGTAAAAATAAAAACTTCTTACACTCCTATTATATATGGGAAAGGGATCAAAGA
                    * ************************************************************

Cdeb_01397          TCGGAAAAGATATGACGAATTAGTGAAATAATTCTCAATCCGACGGGAATGCACCCGATT
A3EQDRAFT_01103     TCGGAAAAGATATGACGAATTAGTGAAATAATTCTCAATCCGACGGGAATGCACCCGGTT
                    ************************************************************ **

Cdeb_01397          CTTGCAGAAAGTTTGGCCAGAA
A3EQDRAFT_01103     CTTGCAGAAAGATTGGCCATA-
                    *********** ******* *
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *ak* in GB1 and Tf.

```
Cdeb_03124          TCCGTTTTCTCTCCTTTTTGCAATCGATCTATTGCTGAGACAGTTATGTGAATATATTCT
A3EQDRAFT_01515     TCCGTTTTCTCTCCTTTTTGCAATCGATCTATTGCTGAGACAGTTATGTGAATATATTCT
                    ************************************************************

Cdeb_03124          CAAAATTATGTCCTTAAACATTTAACCATTGTAGAAAGGTTTTTTCAACTGCTGAAACGA
A3EQDRAFT_01515     CAAAATTATGTCCTTAAACATTTAACCATTGTAGAAAGGTTTTTTCAACTGCTGAAACAA
                    ************************************************************ *

Cdeb_03124          ATTGTCACCGCTTTTACATAAAAAACAATTATTCCCATTAAAATTAATATTTTAAAAATT
A3EQDRAFT_01515     ATTGTCACCGCTTTTACATAAAAAACAATTATTCCCATTAAAATTAATATTTTAAAATTT
                    ************************************************************ **

Cdeb_03124          CAAATAAATAATTTCATAAAA
A3EQDRAFT_01515     CAAATAAATAATTTCATAAAA
                    **********************
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *aldh* in GB1 and Tf

```
Cdeb_00412           CCGGACGGAGCACCACGGGTTCGCC-----TCGCCTGTTCCGTCCGTATCCCTTCTGTTG
A3EQDRAFT_02176      -----CGGAGCACCACGGGTTCGCCTCCTTTCGCCTGTTCCGTCCGTATCCCTTCTGTTG
                          ********************     ******************************

Cdeb_00412           TAAGCGATTACAAGCGGGGCGTGGATCCCGCTTGGCGCATTTCAAAAAACTGTCCGGGTC
A3EQDRAFT_02176      TAAGCGATTACAAACGAGGCGTGGATCCCGCTTGGCGCATTTCAAAAAACTGCCCGGGTC
                     ************* ** ********************************** *******

Cdeb_00412           ATTTTTTTCCGTTTCCGTGAATCGAATAGCTTCAGGGGCATATAAAGATGAATGGAAACT
A3EQDRAFT_02176      ATTTTTTTCCGTTTCCGTGAATCGAATAGCTTCAGGGGCATATAAAGATGAATGGAAACT
                     ************************************************************

Cdeb_00412           TTTTCAAGAGAAAGGAGAATCGGCAA
A3EQDRAFT_02176      TTTTCAAGAGAAAGGAGAATCGGCAA
                     *************************
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *aldh* in GB1 and Tf

```
Cdeb_02563           TAAGGTCATTCCTCCTTCTTACGATATGGATTTTTTGCTGATTCAACCCGAATGCGCTTC
A3EQDRAFT_01581      TAAGGTCATTCCTCCTTCTTACGATATGGATTTTTTGCTGATTCAACCCGAATGTGCTTC
                     ************************************************** *****

Cdeb_02563           CCATCTCCCGTGCGGTAATGAAGAATCCTTTTCCGTCCCGGTCCCGGCGCCCGACGGAAC
A3EQDRAFT_01581      CCATCTCCCGTGCGGTAATGAAGAAGCCTTTTCCGTCCCGGTCCCGGCGCCCGACGGAAC
                     ************************ ********************************

Cdeb_02563           CAAGCGGAACCCGATGATATCGCTTTCAATATCCTCCGGCCTTTCTCCGCTTCCCTGCTA
A3EQDRAFT_01581      CAAGCGGAACCCGATGATATCGCTTTCAATATCCTCCGGCCTTTCTCCGCTTCCCTGCTA
                     ************************************************************

Cdeb_02563           CGTCATTTCCCCTCACCCCGC
A3EQDRAFT_01581      CGTCATTTCCCCTCACCCCGC
                     *********************
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *ldh* in GB1 and TF

```
Cdeb_02710           TGGCAATCTCCTTTCCCCTGCAAAGTCCGTTCCCTGATACGAAATCCGTCCGTCGGTGAT
A3EQDRAFT_00857      --GCAATCTCCTTTCCCCTGCAAAGTCCGTTCCCTGATACGAAATCCGTCCGTCGGTGAT
                       ************************************************************

Cdeb_02710           CCCGGATCCGTCCCGTGAAAGACGCAACGGAATCCCCTATTATTTTAACCAATCGACCCG
A3EQDRAFT_00857      CCCGGATCCGTCCCGTGAAAGACGCAACGGAATCCCCTATTATTTTAACCAATCGACCCG
                     ************************************************************

Cdeb_02710           CGGCAGCCAACGGTTTTGCATCCGGATTTGCAAATATTCGATCAATTCCGCTAAGAATTT
A3EQDRAFT_00857      CGGCAGCCAATGGTTTTGCATCCGGATTTGCAAATATTCGATCAATTCCGCCAAGAATTT
                     ********** ***************************************** ********

Cdeb_02710           TTCATATTCCCCGCTCCGTTC--
A3EQDRAFT_00857      TTCATATTCCCCGCTCCGTTCCA
                     **********************
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *pfl* activating enzyme in GB1 and Tf

```
Cdeb_01637           TCAAGATCACTCCAATAAATGAAATTGGGGGAAGAAGCACCGGCGTTTTGGGGGGAGAGA
A3EQDRAFT_00103      TCAAGATCACTCCAATAAATGAAATTGGGGGAAGAAGCACCGGCGTTTTGGGGGGAGAGA
                     ************************************************************

Cdeb_01637           TTCGAGGGCGCCGGTGCTCTTCCGGGAACGGGCCCGATTTTTCGGATCGGGAACCGGTTG
A3EQDRAFT_00103      TTCGAGGGCGCCGGTGCTCTTCCGGGAACGGGCCCGATTTTTCGGATCGGGAACCGGTTG
                     ************************************************************

Cdeb_01637           CCCGGCTTCCGGTTCATCGGTGTCCCTTGTTTTGCATAGCCATCGGTTTTTGGACGTCCG
A3EQDRAFT_00103      CCCGGCTTCCGGTTCATCGGTGTCCCTTGTTTTGCATAGCCATCGGTTTTTGGACGTCCG
                     ************************************************************

Cdeb_01637           GCGGCGTCAATCCGGATTCTT
A3EQDRAFT_00103      GCGGCGTCAATCCGGATTCTT
                     **********************
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *pfl* C-acetyltransferase in GB1 and Tf

```
Cdeb_01638           TTTTTTCATCCTCCTTAAATTATGTTCAATAGTGAGCATATTCGTTTAAAAAAATTTTGC
A3EQDRAFT_00102      TTTTTTCATCCTCCTTAAATTATGTTCAATAGTGAGCATATTCGTTTAAAAAAATTTTGC
```

```
                    ************************************************************

Cdeb_01638          TCAATATTGAACAATCTTTTTCTACCTTTATTATAGTACGATTTGATTGGTAAAGGAATA
A3EQDRAFT_00102     TCAATATTGAACAATCTTTTTCTACCTTTATTATAGTACGATTTGATTGGTAAAGGAATA
                    ************************************************************

Cdeb_01638          TCTGTTTCATAAAAATTCACTTATCTGTCAAATTTGAAGAAACCCTTGTTTATAAAGGTT
A3EQDRAFT_00102     TCTGTTTCATAAAAATTCACTTATCTGTCAAATTTGAAGAAACCCTTGTTTATAAAGGTT
                    ************************************************************

Cdeb_01638          TTATCCTGTTATTTTTTATCT
A3EQDRAFT_00102     TTATCCTGTTATTTTTTATCT
                    ********************
```

CLUSTAL multiple sequence alignment by MUSCLE of -200 bp upstream of *pta* in GB1 and TF

```
Cdeb_00469           ATAACTATAACATATTTTCAACATTTCCCATGTGGATATACTTTTGACAAATTGGTGGCA
A3EQDRAFT_02234      ------ATAACATATTTTCAACATTTCCCATGTGGATATACTTTTGACAAATTGGTGGCA
                           **************************************************

Cdeb_00469           GTTTTTTAACATTTTCCCCGTCCCTTTCTCCCCGTTC------GGGGAAGGGAAACGGGG
A3EQDRAFT_02234      GTTTTTTAACATTTTCCCCGTCCCTTTCTCCCCGTTCGGGGAAGGGGAAGGGAAACGGGG
                     ***********************************      *****************

Cdeb_00469           ATTCCCTGCCGTTTCCGGCCCCGGGGATGAGAAACTTGCAAATTTTTTCACAAATAGTAT
A3EQDRAFT_02234      ATTCCCTGCCGTTTCCGGCCCCGGGGATGAGAAACTTGCAAATTTTTTCACAAATAGTAT
                     ************************************************************

Cdeb_00469           GCTAAAGAAAGAAGGAGGACGAGACCA
A3EQDRAFT_02234      GCTAAAGAAAGAAGGAGGACGAGACCA
                     **************************
```

Figure 5.5. DNA alignments -200 regions upstream of adh, adhe, ak, aldh, ldh, pfl, and pta arranged alphabetically. Dashes "-" represent gaps in sequence that do not align; "*****" underneath indicates matching bp; Blank spaces " " underneath indicate positions that do not match

CLUSTAL multiple sequence alignment by MUSCLE of ADH in GB1 and Tf.

```
Cdeb_03022          MDSFVFQNPTKLIFGRGQIQALRDEIPKYGKKLLLVYGGGSIKRNGLYDEVMQILKEIGA
A3EQDRAFT_00921     MDSFVFQNPTKLIFGRGQIQALRDEIPKYGKKLLLVYGGGSIKRNGLYDEVMQILKEIGA
                    ************************************************************

Cdeb_03022          EVFELPGVEPNPRITTVRKGVDICKKEKIEFLLAVGGGSVIDCTKAIAAGAMYEGDPWDI
A3EQDRAFT_00921     EVFELPGVEPNPRITTVRKGVDICKKEKIEFLLAVGGGSVIDCTKAIAAGAMYEGDPWDI
                    ************************************************************

Cdeb_03022          VIKKAKPEAALPLGTVLTLAATGSEMNSGSVITNWETKEKYGWGSPLVYPKFSILDPVYT
A3EQDRAFT_00921     VVKKAKPEAALPLGTVLTLAATGSEMNSGSVITNWETKEKYGWGSPLVYPKFSILDPVYT
                    *:**********************************************************

Cdeb_03022          FTVPRDQTVYGIVDMMSHVFEHYFHHAANTPLQDRFCESLLTTIMETAPKLVDNLENYEY
A3EQDRAFT_00921     FTVPRDQTVYGIVDMMSHVFEHYFHHAANTPLQDRFCESLLTTIMETAPKLVDDLENYEY
                    ********************************************************:*****

Cdeb_03022          RETILYCGTMALNGMVQMGFRGDWATHNIEHAVSAAFDIPHGGGLAILFPNWMKHVLPVN
A3EQDRAFT_00921     RETILYCGTMALNGMVQMGFRGDWATHNIEHAVSAAFDIPHGGGLAILFPNWMKHVLPVN
                    ************************************************************

Cdeb_03022          PQRFKQLAVRVFGVDPANKTDEEAGLEGIERLREFWNSLGAPSRLRDYGIGEKDLPLLAD
A3EQDRAFT_00921     PQRFKQLAVRVFGVDPANKTDEEAGLEGIERLREFWNSLGAPSRLRDYGIGEKDLPLLAD
                    ************************************************************

Cdeb_03022          IAMKRGEFGNFKKLTRDDVLAIYRASL
A3EQDRAFT_00921     IAMKRGEFGNFKKLTRDDVLAIYRASL
                    ***************************
```

CLUSTAL multiple sequence alignment by MUSCLE of ADH in GB1 and Tf.

```
Cdeb_00489          MRAAVVSPDKDKLVEVKDVTLRPLQYGEALVDIEYCGVCHTDLHVAKGDFGHVPGRILGH
A3EQDRAFT_02052     MRAAVVSPDKDKLVEVKDVTLRPLQYGEALVDIEYCGVCHTDLHVAKGDFGHVPGRILGH
                    ************************************************************

Cdeb_00489          EGVGVVREIADGVTSLKVGDRVSIAWFYEGCGHCEYCVTGNETYCRSVKNAGYTVDGAMA
A3EQDRAFT_02052     EGVGVVREIADGVTSLKVGDRVSIAWFYEGCGHCEYCVTGNETYCRSVKNAGYTVDGAMA
                    ************************************************************

Cdeb_00489          EQCIVKADYAVKVPDGLDPKKATSITCAGVTTYKAIKVSDIKPGQWIVIYGVGGLGNLGV
A3EQDRAFT_02052     EQCIVKADYAVKVPDGLDPKKATSITCAGVTTYKAIKVSDIKPGQWIVIYGVGGLGNLGV
                    ************************************************************

Cdeb_00489          QYAKNVFNAKVIAVDINDEKLRLAKEVGADFTFNSLKGDPAQWIQEEFGGAHAAVVTSVS
A3EQDRAFT_02052     QYAKNVFNAKVIAVDINDEKLRLAKEVGADFTFNSLKGDPAQWIQEEFGGAHAAVVTSVS
                    ************************************************************

Cdeb_00489          KTAFNQAVHSVRPVSKVVGVGLPPETMDLEIVKTVLDGIQVVGSLVGTRKDLEEALMFAA
A3EQDRAFT_02052     KTAFNQAVHSVRPVSKVVGVGLPPETMDLEIVKTVLDGIQVIGSLVGTRKDLEEALMFAA
                    ****************************************:*******************

Cdeb_00489          EGKVDPVVQTRKLEEIRDIFREMEEGKIQGRMVIDMKK
A3EQDRAFT_02052     EGKVDPVVQTRKLEEIRDIFREMEEGKIQGRMVIDMKK
                    ***************************************
```

CLUSTAL multiple sequence alignment by MUSCLE of ADH in GB1 and Tf.

```
Cdeb_01569          MRIKAAVVHDKGENFRIEEVELSDLKRDEVLVKIVASGICHTDAVARDIGLTPFPAVLGH
A3EQDRAFT_01808     MRIKAAVVHDKGENFRIEEVELSDLKRDEVLVKIVASGICHTDAVARDIGLTPFPAVLGH
                    ************************************************************

Cdeb_01569          EGSGIVEKVGPDVKTIRPGDHVVISFASCGQCEHCLTGHPGACFKINELNFGGKDEDGDY
A3EQDRAFT_01808     EGSGIVEKVGPDVKTIRPGDHVVISFASCGQCEHCLTGHPGACFKINELNFGGKDEDGDY
                    ************************************************************

Cdeb_01569          RIFQNGRGVSTFFGQSSFATYAIAKERNVVKVDKDVDLALLSPLGCGIQTGAGTVLNKLK
A3EQDRAFT_01808     RIFQNGRGVSTFFGQSSFATYAIAKERNVVKVDKDVDLALLSPLGCGIQTGAGTVLNKLK
                    ************************************************************

Cdeb_01569          PAFGSSIAVYGCGAVGLSAVMAAKIAGCKHIVAVDIHQNRLDLAKELGATHVLNGKEADV
A3EQDRAFT_01808     PAFGSSIAVYGCGAVGLSAVMAAKIAGCKHIVAVDIHQNRLELAKELGATHVLNGKEADV
                    ****************************************:*******************

Cdeb_01569          VKEIKAVTEGGTHYAVETTGVPAVVRQSLQALRPLGQTAIVGVTPEMTIDVHNEIMAEGK
A3EQDRAFT_01808     VKEIKAVTEGGTHYAVETTGVPAVVRQSLKALRPLGQTAIVGVTPEMTIDVHNEIMAEGK
                    ****************************:*******************************

Cdeb_01569          TMMGVIEGDAVPKLFIPQLIAYYKKGLFPFDKLIRFYAFDEINKAFEDSAKGITVKPVIK
A3EQDRAFT_01808     TMMGVIEGDAVPKLFIPQLIAYYKKGLFPFDKLIRFYAFDEINKAFEDSAKGITVKPVIK
                    ************************************************************

Cdeb_01569          ISA
A3EQDRAFT_01808     ISA
                    ***
```

CLUSTAL multiple sequence alignment by MUSCLE of ADH in GB1 and Tf.

```
Cdeb_01590           MKIKSAVLRASGLERPYAKSQPIKIETLELDPPGYGEVLVQIKAANLCHSDLSVIDGSRP
A3EQDRAFT_00146      MKIKSAVLRASGLERPYAKSQPIKIETLELDPPGYGEVLVQIKAANLCHSDLSVIDGSRP
                     ************************************************************

Cdeb_01590           RPLPMALGHEAAGVVVEAGEGVEDLEPGDHVVCIFVPSCGQCLPCKEGRPALCEKGAEAN
A3EQDRAFT_00146      RPLPMALGHEAAGVVVEAGEGVEDLEPGDHVVCIFVPSCGQCLPCKEGRPALCEKGAEAN
                     ************************************************************

Cdeb_01590           GNGTLINGSRRLHKGDEPIHHHLGVSAFSEYAVLSRHSLVKVDPGIPFEKAALFGCAVIT
A3EQDRAFT_00146      GNGTLINGSRRLHKGDEPIHHHLGVSAFSEYAVLSRHSLVKVDPGIPFEKAALFGCAVIT
                     ************************************************************

Cdeb_01590           GVGAVVNTANIRLGSTVAIVGLGGVGLSALLGASAAGASRIIAVDINEAKLQKAKELGAT
A3EQDRAFT_00146      GVGAVVNTANIRLGSTVAIVGLGGVGLSALLGASAAGASRIIAVDINETKLQKAKELGAT
                     ************************************************:***********

Cdeb_01590           DTFNSRDKDVVEKIKAATNGGVDYAFETAGAVPAMEVAYAITRRGGTTVTTGLPHPEHHF
A3EQDRAFT_00146      DTFNSRDKEVVEKIKAATDGGVDYAFETAGAVPAMEVAYAITRRGGTTVTTGLPHPEHHF
                     ********:*********:****************************************

Cdeb_01590           SFPQVTLAAEERTIKGSYLGSCVPQRDIPRYIELFKQNRLPVDRLVSHIISLEEINEGFD
A3EQDRAFT_00146      SFPQVTLAAEERTIKGSYLGSCVPQRDIPRYIELFKQNRLPVDRLVSHIISLEEINEGFD
                     ************************************************************

Cdeb_01590           RLADGEAFRILVRF
A3EQDRAFT_00146      RLADGEAFRILVRF
                     **************
```

CLUSTAL multiple sequence alignment by MUSCLE of ADHE in GB1 and Tf.

```
Cdeb_01397           MAIVEKEVQKKTDHYQEIDALVKKGQEALNQFFLLDQEAVDRIVKEMALAGQKEHMRLAK
A3EQDRAFT_01103      MAIVEKEVQKKTDHYQEIDALVKKGQEALNQFFLLDQEAVDRIVKEMALAGQKEHMRLAK
                     ************************************************************

Cdeb_01397           LAYEETKRGVYEDKVIKNLFATEYIYHHIKYDKTVGIIKENVHEGIMEIAEPVGVIAGIT
A3EQDRAFT_01103      LAYEETKRGVYEDKVIKNLFATEYIYHHIKYDKTVGIIKENVHEGIMEIAEPVGVIAGIT
                     ************************************************************
```

```
Cdeb_01397          PVTNPTSTTMFKALIAMKTRNPIIFAFHPSAQQSSKEAARVMLEAAVKAGAPEHCILWIE
A3EQDRAFT_01103     PVTNPTSTTMFKALIAMKTRNPIIFAFHPSAQQSSKEAARVMLEAAVKAGAPEHCILWIE
                    ************************************************************

Cdeb_01397          HPAVEATRYLMKHPGISLILATGGAGMVKAAYSSGKPALGVGPGNVPCYIEKTADIKRAV
A3EQDRAFT_01103     HPAVEATRYLMKHPGISLILATGGAGMVKAAYSSGKPALGVGPGNVPCYIEKTADIKRAV
                    ************************************************************

Cdeb_01397          NDLILSKTFDNGMICASEQAVIIDKEIYAKTKNELKQNGCYFVTKEEKKKLEKIVIDENS
A3EQDRAFT_01103     NDLILSKTFDNGMICASEQAVIIDKEIYEKTKNELKQNGCYFVTKEEKKKLEKIVIDENS
                    *************************** ******************************

Cdeb_01397          CTINSAIVGMPAKVIADMAGIQVPEDTKILIAELEKVGPEEPLSREKLSPVLACYKVNST
A3EQDRAFT_01103     CTINSAIVGMPAKVIADMAGIQVPEDTKILIAELEKVGPEEPLSREKLSPVLACYKVNST
                    ************************************************************

Cdeb_01397          EEGFERAEQMLEFGGLGHSAVIHSNDEAVIREFGKRVKACRVIVNQPSSQGAIGDIYNAY
A3EQDRAFT_01103     EEGFERAEQMLEFGGLGHSAVIHSNDEAVIREFGKRVKACRVIVNQPSSQGAIGDIYNAY
                    ************************************************************

Cdeb_01397          IPSLTLGCGTFGGNSVSTNVGAVHLINKKTVARRRVNMQWFKVPPKIYFEKDATQYLSKM
A3EQDRAFT_01103     IPSLTLGCGTFGGNSVSTNVGAVHLINKKTVARRRVNMQWFKVPPKIYFEKDATQYLSKM
                    ************************************************************

Cdeb_01397          PDISRAFIVTDQGMVQHGYVDRVLYYLRKRPDYVHCEIFSEVEPDPSVDTVMKGVEMMFH
A3EQDRAFT_01103     PEISRAFIVTDQGMIQHGYVDRVLYYLRKRPDYVHCEIFSEVEPDPSVDTVMKGVEMMFH
                    *:************:*******************************************

Cdeb_01397          FQPDVIIALGGGSPLDAAKAMWLFYEHPETEFNGLKQKFLDIRKRVFKFPKLGRKAKLVA
A3EQDRAFT_01103     FQPDVIIALGGGSPLDAAKAMWLFYEHPETEFNGLKQKFLDIRKRVFKFPKLGRKAKLVA
                    ************************************************************

Cdeb_01397          IPTTSGSGSEVTSFAVITDKKLDIKYPLADYELTPDVAIIDPAYVMTVPKSVTADTGMDV
A3EQDRAFT_01103     IPTTSGSGSEVTSFAVITDKKLDIKYPLADYELTPDVAIIDPAYVMTVPKSVTADTGMDV
                    ************************************************************

Cdeb_01397          LTHAIEAYVSNMANDYTDGLAIKAIQLVFEYLPRAYKNGQDELAREKMHNASTIAGMAFS
A3EQDRAFT_01103     LTHAIEAYVSNMANDYTDGLAIKAIQLVFEYLPRAYKNGQDELAREKMHNASTIAGMAFS
                    ************************************************************
```

```
Cdeb_01397          NAFLGINHSLAHKLGGAFHIPHGRANAILMPHVIRYNATKPTKFVAFPKYEHFIADKRYA
A3EQDRAFT_01103     NAFLGINHSLAHKLGGAFHIPHGRANAILMPHVIRYNATKPTKFVAFPKYEHFIADKRYA
                    ************************************************************

Cdeb_01397          EIARILGLPAKTTEEGVESLIQAVIGLAKELEIPMSLEALGIDRDDFEKRVPELAELAFE
A3EQDRAFT_01103     EIARILGLPAKTTEEGVESLVQAVIGLAKELEIPMSLEALGIDRDAFEKKVHELAELAFE
                    ********************:************************ ***.* ********

Cdeb_01397          DQCTTANPKMPLVSELEEIYRQAYKGV
A3EQDRAFT_01103     DQCTTANPKMPLVSELEEIYRQAYKGV
                    ***************************
```

CLUSTAL multiple sequence alignment by MUSCLE of AlDH in GB1 and Tf.

```
Cdeb_00412          MKALNYINGAWCESKSGMTAPVINPADGKTIGHVTLSTEEDVRQAVRAAKAAQKEWALVP
A3EQDRAFT_02176     MKALNYINGAWCESKSGMTAPVINPADGKTIGHVTLSTEEDVRQAVRAAKAAQKEWALVP
                    ************************************************************

Cdeb_00412          APQRAEVLYKVGFLLKEKKERLARILTMEMGKVIEEARGEVQEGIDMAFYMAGEGRRLFG
A3EQDRAFT_02176     APQRAEVLYKVGFLLKEKKERLARILTMEMGKVIEEARGEVQEGIDMAFYMAGEGRRLFG
                    ************************************************************

Cdeb_00412          ETTPSELKDKFAMSVRVPVGVVGIITPWNFPIAIATWKSFPAIVAGNAVVWKPASETPLM
A3EQDRAFT_02176     ETTPSELKDKFAMSVRVPVGVVGIITPWNFPIAIATWKSFPAIVAGNAVVWKPASETPLM
                    ************************************************************

Cdeb_00412          AQELAKIFEEAGLPKGVFNVVNGTGPTVGSALVEHPDVRAISFTGSNEVGRGIAEKCGRL
A3EQDRAFT_02176     AQELAKIFEEAGLPKGVFNVVNGTGPTVGSALVEHPDVRAISFTGSNEVGRGIAEKCGRL
                    ************************************************************

Cdeb_00412          LKKVSLEMGGKNAVIVMDDADLSLAVEGILWSAFGTSGQRCTSCSRVIVHEKVKEELEER
A3EQDRAFT_02176     LKKVSLEMGGKNAVIVMDDADLSLAVEGILWSAFGTSGQRCTSCSRVIVHEKVKEELEER
                    ************************************************************

Cdeb_00412          LLEAMKTLKVGNGLDETVKVGPVINEAALKKIHEYVQIGKAEGARLLAGGEILAEGELAK
A3EQDRAFT_02176     LLEAMKTLKVGNGLDETVKVGPVINEAALKKIHEYVQIGKAEGARLLAGGEILAEGELAK
                    ************************************************************

Cdeb_00412          GFYYAPTLFTDVKPDMRIAKEEIFGPVLSVMRAGSLEEAIAINNAVDYGLSSAIFTRDVN
A3EQDRAFT_02176     GFYYAPTLFTDVKPDMRIAKEEIFGPVLSVMRAGSLEEAIAINNAVDYGLSSAIFTRDVN
                    ************************************************************

Cdeb_00412          RAFRAMRDLDTGIVYINAGTTGAEIHLPFGGTKGTGNGHRDSGTASLDVFTEWRSVYVDF
A3EQDRAFT_02176     RAFRAMRDLDTGIVYINAGTTGAEIHLPFGGTKGTGNGHRDSGTASLDVFTEWRSVYVDF
                    ************************************************************

Cdeb_00412          SGKLQRAQIDIDPESGREENPGSAQGDAGIGKEGGKA
A3EQDRAFT_02176     SGKLQRAQIDIDPESGREENPESAQGDAGIGKEGGKA
                    ********************* ***************
```

```
CLUSTAL multiple sequence alignment by MUSCLE of PFL activating enzyme in GB1 and
Tf.

Cdeb_01637           MVTGRIHSTESFGTVDGPGIRYVVFTQGCPLRCRYCHNPDTWKMNGGKEITVEEIIREVK
A3EQDRAFT_00103      MVTGRIHSTESFGTVDGPGIRYVVFTQGCPLRCRYCHNPDTWKMNGGKEITVEEIIREVK
                     ************************************************************

Cdeb_01637           DYLPFIESSGGGITVSGGEPLLQIEFLTELFKECKKLHIHTAIDTAGSCFSRRESFLKKL
A3EQDRAFT_00103      DYLPFIESSGGGITVSGGEPLLQIEFLTELFKECKKLHIHTSIDTAGSCFSRRESFLKKL
                     ****************************************:*******************

Cdeb_01637           EELLKYTDLILLDIKHIDREKHKNITGMDNDHILDFAKYLSDKKIPVWIRHVLVPGLTDF
A3EQDRAFT_00103      EELLKYTDLILLDIKHIDREKHKNITGMNNDHILDFAKYLSDKKIPVWIRHVLVPGLTDF
                     ****************************:*******************************

Cdeb_01637           DPDLKRLARFLRTLTNIEKIEVLPYHKLGVYKWKTLGIPYTLEHTEPPSEERVKNAEKIL
A3EQDRAFT_00103      DPDLKRLARFLRTLTNIEKIEVLPYHKLGVYKWKTLGIPYTLEHTEPPSEERVKNAEKIL
                     ************************************************************

Cdeb_01637           NSARLRVL
A3EQDRAFT_00103      NSARLRVL
                     ********
```

Figure 5.6. Pyruvate fermentation enzyme protein alignments that do not match perfectly through MUSCLE alignment (ADH ADHE, AlDH, PFL) arranged alphabetically "*" indicate a matching amino acid; "." Indicate semi-conservative; ":" indicate conservative; Blank spaces " " indicate non conservative; non-matching AA are highlighted in red.

5.4.5. Lactate Dehydrogenase

Many wild type thermophilic and mesophilic organisms synthesize lactate as a major fermentative end-product (Narayanan *et al.* 2004). Both strains of *Caldibacillus debilis* produce very little lactate, with lactate production typically comprising less than ~10% of carbon end-products (Chapter 3). Indeed, lactate dehydrogenase is not highly represented in the *C. debilis* GB1 proteome (Chapter 4), nor in the present study. In either strain, it was near the limit of detection when detected. Therefore low lactate production is correlated with low LDH expression levels. Lactate production can also be regulated allosterically, therefore the presence of LDH does not guarantee production of lactate (Rydzak et al. 2009) While indeed lactic acid production was low in both organisms, we do see differences in lactic acid production between the strains as well, with GB1 typically producing significantly less than Tf under comparable conditions (Chapter 3). Clear genetic reasons for difference in lactic acid production were not apparent.

5.4.6. Pyruvate Dehydrogenase

PDH in general can be used aerobically or anaerobically (Nakano *et al.* 1997). PDH can be important under anaerobic conditions in pyruvate metabolism. Other work on the related *G. thermoglucosidasius* focused on changing the PDH promoters to allow for higher PDH production under anaerobic conditions (Cripps *et al.* 2009). In *C. debilis* GB1 anaerobic growth does not appear to utilize PDH, as CO_2 appears only in trace concentrations anaerobically (Chapter 3). Previous work, Wushke *et al.* (Chapter 4), suggests that PDH is down regulated

anaerobically. While both Tf and GB1 have a PDH that could be utilized anaerobically, it appears that regulation limits its usefulness anaerobically. The differences between strains Tf and GB1 appear to be due to genes concerning anaerobic growth. PDH appears to be unimportant in determining the ability for these organisms to grow anaerobically.

5.4.7. Pyruvate Formate Lyase

When cultured under oxygen-limiting conditions both strains, GB1 and Tf, synthesize formate and acetate as end-products (Chapter 3). There is a difference in formate production under oxygen-limited conditions; significant differences in the upstream regions of PFL are observed. Production of formate and acetate alone from pyruvate cannot act as an electron sink for NADH generated by glycolysis. Under conditions where oxygen is present, Tf is able to get rid of the excess electrons generated through glycolysis via the electron transport chain (ETC). If typical glycolysis and PFL are the primary means by which acetyl-CoA is generated, then anaerobic growth is not possible unless an additional reduced compound is produced. Strain GB1 is capable of anaerobic growth because it is also able to synthesize ethanol. Typically expression of an aero-sensitive enzyme, such as PFL, is linked to anaerobic metabolism (Nakano *et al.* 1998), and the use of PFL can be important for biosynthesis (Rydzak *et al.* 2015). More likely, Tf represents a strain that has lost the ability to produce ethanol, preventing anaerobic growth. Tf still maintains regulation and expression of PFL as we see formate production under oxygen limiting conditions only. Since both strains have the proper gene complement, and demonstrate the ability to utilize PFL, as indicated by the presences of formate, the focus should be on ethanol production genes, since this is where there is a clear physiological difference.

5.4.8. Alcohol Aldehyde Dehydrogenase

The synthesis of ethanol appears to be the main mechanism used by *C. debilis* GB1 to dump excess electrons from glycolysis under anaerobic conditions, as lactic acid production seems to be limited in some way in both strains under the growth conditions used (Chapter 3 and 4). Ethanol synthesis may therefore be an anaerobic requirement in *C. debilis* GB1. Strains GB1 and Tf are highly similar organisms based on the fermentation pathway profile at the genome level. Both strains of *C. debilis* have a single copy of *adhe* as well as 4 and 2 discrete copies of *adh* and *aldh* respectivly. The *adh* genes have not been characterised for there specificity and could be playing a role in other functions. ADHE is associated with the bulk of ethanol production in many anaerobic organisms (Carere *et al.* 2012, Peng *et al.* 2008). Indeed, in the closely related *G. thermoglucosidasius,* an ADHE deletion mutant eliminates ethanol production despite the presence of both *adh* and *aldh* in their genome (Extance *et al.* 2013).

The lack of ethanol synthesis in *C. debilis* Tf could be understood through looking at the sequence of the bifunctional alcohol/aldehyde dehydrogenase (*adhE*) gene. While the genomes of both strains encoded a putative ADHE enzyme, detailed analyses revealed sequence differences in regions upstream, as well as within the coding sequence of the *adhE* gene. Figure 5.08 shows the protein alignment of ADHE. Nucleotide substitutions in the ADHE coding sequence, shown in Figure 5.10, result in different amino acids: GB1 → Tf = 269A→E, 482D→E, 495V→I, 825D → A, 829R → K, 831P→H. These amino acid changes were caused by a single nucleotide polymorphism in all but one case, V→I where two bp were changed. Changes 482D→E and 495V→I, appear to be benign mutations resulting in amino acids with similar properties. Both codon changes, 825 D→A and 829R→K, are seen in other functional

ADHE alignments and seem to be normal variations in coding sequence (Extance *et al.* 2013). 269A→E appears to be a missense mutation rather close to a putative catalytic cysteine at position 255. The 831P→H codon change is a missense mutation that appears to place a charged amino acid in an α-helix region on the outer shell of the Tf ADHE protein. The changes in the ADH domain of ADHE are not within any putative active site, cofactor binding-site, or known allosteric regulation site (Zheng *et al.* 2015). These amino acid substitutions affect a small loop connecting an α-helix region (Extance *et al.* 2013), and therefore could affect overall protein or multimeric protein structure. Figure 5.11 is a 2D representation of ADHE with domains and key sites specified generated through NCBI BLAST. Figure 5.12 and 5.13 are 3D models created in raptorX of ADHE in GB1 and Tf. Figure 5.13 show the domain alignments and the structural differences caused by the amino acid differences at positions 269 and 831.

Differences in *adhE* gene expression regulation or ADHE enzyme activity could result in the ethanol-minus Tf phenotype and explain the lack of anaerobic growth. While there are also differences in regions upstream of *adhE*, they did not appear to affect the promoter binding site.

Geobacillus thermoglucosidasius is similar to *C. debilis* GB1 in that it has the same major end products, similar core metabolism gene complement encoding ADH, AlDH and ADHE, the ability to grow anaerobically, and is a closely related genus that previously included *C. debilis.* A study by Extance *et al.* (2013) noted that gene knock-outs of *G. thermoglucosidasius* ADHE results in a lack of anaerobic growth and an ethanol-minus phenotype, very similar to the Tf phenotype. Taken together the results imply the phenotype differences observed when comparing GB1 and Tf are likely due to the differences in the amino acid sequence of the ADHE.

CLUSTAL multiple sequence alignment by MUSCLE of *adhE* coding DNA

```
Cdeb_01397          TTGGCCATCGTTGAAAAGGAAGTTCAGAAAAAAACCGATCATTACCAAGAAATCGATGCG
A3EQDRAFT_01103     TTGGCCATCGTTGAAAAGGAAGTTCAGAAAAAAACCGATCATTACCAAGAAATCGATGCG
                    ************************************************************

Cdeb_01397          TTGGTTAAAAAGGGACAAGAGGCATTGAACCAATTTTTCCTGCTGGATCAGGAAGCGGTT
A3EQDRAFT_01103     TTGGTTAAAAAGGGACAAGAGGCATTGAACCAATTTTTCCTGCTGGATCAGGAAGCGGTT
                    ************************************************************

Cdeb_01397          GACCGCATCGTGAAAGAAATGGCCCTTGCCGGGCAGAAGGAGCATATGCGGCTGGCCAAG
A3EQDRAFT_01103     GACCGCATCGTGAAAGAAATGGCCCTTGCCGGGCAGAAGGAGCATATGCGGCTGGCCAAG
                    ************************************************************

Cdeb_01397          CTGGCCTATGAGGAAACGAAGCGGGGAGTTTACGAAGATAAGGTGATCAAAAATTTGTTC
A3EQDRAFT_01103     CTGGCCTATGAGGAAACGAAGCGGGGAGTTTACGAAGATAAGGTGATCAAAAATTTGTTC
                    ************************************************************

Cdeb_01397          GCCACCGAATATATTTATCACCACATCAAATATGACAAAACCGTCGGCATCATCAAGGAA
A3EQDRAFT_01103     GCCACCGAATATATTTATCACCACATCAAATATGACAAAACCGTCGGCATCATCAAGGAA
                    ************************************************************

Cdeb_01397          AATGTTCATGAAGGCATCATGGAAATCGCCGAACCGGTGGGCGTCATCGCCGGCATAACC
A3EQDRAFT_01103     AATGTTCATGAAGGCATCATGGAAATCGCCGAACCGGTGGGCGTCATCGCCGGCATAACC
                    ************************************************************

Cdeb_01397          CCGGTCACCAACCCGACATCGACGACGATGTTCAAGGCGCTGATCGCCATGAAGACGAGA
A3EQDRAFT_01103     CCGGTCACCAACCCGACATCGACGACGATGTTCAAGGCGCTGATCGCCATGAAGACGAGA
                    ************************************************************

Cdeb_01397          AACCCGATCATTTTTGCTTTCCATCCGTCCGCCCAGCAGTCGAGCAAGGAAGCGGCCAGA
A3EQDRAFT_01103     AACCCGATCATTTTTGCTTTCCATCCGTCCGCCCAGCAGTCGAGCAAGGAAGCGGCCAGA
                    ************************************************************

Cdeb_01397          GTCATGCTGGAAGCGGCGGTGAAAGCCGGGGCCCCCGAACATTGCATCCTGTGGATCGAA
A3EQDRAFT_01103     GTCATGCTGGAAGCGGCGGTGAAAGCCGGGGCCCCCGAACATTGCATCCTGTGGATCGAA
                    ************************************************************

Cdeb_01397          CATCCCGCCGTGGAAGCCACCCGTTATTTAATGAAGCATCCGGGCATCTCCTTAATCCTT
A3EQDRAFT_01103     CATCCCGCCGTGGAAGCCACCCGTTATTTAATGAAGCATCCGGGCATCTCCTTGATCCTT
                    ********************************************************* ******

Cdeb_01397          GCCACCGGGGGAGCGGGCATGGTGAAAGCGGCTTACAGCTCCGGAAAACCGGCGTTAGGG
A3EQDRAFT_01103     GCCACCGGGGGAGCGGGCATGGTTAAAGCGGCTTACAGCTCCGGAAAACCGGCGTTAGGG
                    ********************** ************************************

Cdeb_01397          GTCGGCCCCGGAAATGTGCCGTGTTACATCGAAAAAACCGCCGACATCAAACGGGCGGTG
A3EQDRAFT_01103     GTCGGCCCCGGAAATGTGCCGTGTTACATTGAAAAAACCGCCGACATCAAACGGGCGGTG
                    ***************************** ******************************

Cdeb_01397          AACGATCTGATCCTTTCCAAAACCTTTGACAACGGCATGATTTGCGCATCGGAACAAGCG
A3EQDRAFT_01103     AACGATCTGATCCTTTCCAAAACCTTTGACAACGGCATGATTTGCGCATCGGAACAAGCG
                    ************************************************************

Cdeb_01397          GTTATCATCGACAAAGAAATTTACGCGAAAACGAAAAACGAATTGAAACAGAACGGCTGC
A3EQDRAFT_01103     GTTATCATCGACAAAGAAATTTACGAGAAAACGAAAAACGAATTGAAACAGAACGGCTGC
                    ************************ ***********************************

Cdeb_01397          TATTTCGTGACGAAGGAAGAAAAGAAAAAACTCGAAAAAATCGTCATCGATGAAAATTCT
A3EQDRAFT_01103     TATTTCGTGACGAAGGAAGAAAAGAAAAAACTCGAAAAAATCGTCATCGATGAAAATTCT
                    ************************************************************
```

```
Cdeb_01397          TGCACGATCAACAGCGCCATTGTCGGCATGCCAGCAAAAGTTATCGCCGATATGGCGGGA
A3EQDRAFT_01103     TGCACGATCAACAGCGCCATTGTTGGCATGCCAGCAAAAGTTATCGCCGATATGGCGGGA
                    ********************** ************************************

Cdeb_01397          ATCCAGGTGCCCGAAGATACAAAAATCCTGATTGCCGAGCTGGAAAAGGTGGGACCGGAA
A3EQDRAFT_01103     ATCCAGGTGCCCGAAGATACAAAAATCCTGATTGCCGAGCTGGAAAAGGTGGGACCGGAA
                    ************************************************************

Cdeb_01397          GAACCCCTTTCCCGGGAAAAATTGAGCCCCGTTTTGGCTTGTTATAAAGTGAACAGCACC
A3EQDRAFT_01103     GAACCCCTTTCCCGGGAAAAATTGAGCCCCGTTTTGGCTTGTTATAAAGTGAACAGCACC
                    ************************************************************

Cdeb_01397          GAAGAGGGATTTGAACGGGCGGAACAAATGTTGGAATTCGGCGGTTTGGGACACAGCGCA
A3EQDRAFT_01103     GAAGAGGGATTTGAACGGGCGGAACAAATGTTGGAATTCGGCGGTTTGGGACACAGCGCA
                    ************************************************************

Cdeb_01397          GTCATCCATTCCAACGATGAAGCGGTCATCCGGGAGTTCGGGAAACGGGTGAAGGCGTGC
A3EQDRAFT_01103     GTCATCCATTCCAACGATGAAGCGGTCATCCGGGAGTTCGGGAAACGGGTGAAGGCGTGC
                    ************************************************************

Cdeb_01397          CGGGTCATCGTCAATCAGCCCTCTTCCCAGGGAGCGATCGGGGATATTTACAACGCCTAT
A3EQDRAFT_01103     CGGGTCATCGTCAATCAGCCCTCTTCCCAGGGAGCGATCGGGGATATTTACAACGCCTAT
                    ************************************************************

Cdeb_01397          ATTCCGTCATTGACCCTCGGATGCGGCACCTTCGGCGGAAACTCGGTATCCACCAACGTC
A3EQDRAFT_01103     ATTCCGTCATTGACCCTCGGATGCGGCACCTTCGGCGGAAACTCGGTATCCACCAACGTC
                    ************************************************************

Cdeb_01397          GGGGCCGTGCATTTGATCAACAAGAAAACGGTGGCGAGAAGGCGGGTGAATATGCAGTGG
A3EQDRAFT_01103     GGGGCCGTGCATTTGATCAACAAGAAAACGGTGGCGAGAAGGCGGGTGAATATGCAGTGG
                    ************************************************************

Cdeb_01397          TTCAAAGTTCCGCCGAAAATTTATTTTGAAAAGGACGCCACCCAATATTTGTCGAAAATG
A3EQDRAFT_01103     TTCAAAGTTCCGCCGAAAATTTATTTCGAAAAGGACGCCACCCAATATTTGTCGAAAATG
                    ************************** ***********************************

Cdeb_01397          CCGGACATCTCCCGGGCGTTTATCGTCACCGATCAGGGGATGGTTCAACACGGCTATGTG
A3EQDRAFT_01103     CCGGAGATCTCCCGGGCGTTTATCGTCACCGATCAGGGGATGATACAACACGGCTATGTG
                    ***** ************************************ * ***************

Cdeb_01397          GACCGGGTATTGTATTATTTAAGAAAAAGGCCGGATTACGTCCACTGTGAGATTTTTTCC
A3EQDRAFT_01103     GACCGGGTATTGTATTATTTAAGAAAAAGGCCGGATTACGTCCACTGTGAGATTTTTTCC
                    ************************************************************

Cdeb_01397          GAAGTGGAGCCGGATCCCTCCGTGGATACGGTCATGAAAGGCGTGGAAATGATGTTCCAT
A3EQDRAFT_01103     GAAGTGGAGCCGGATCCCTCCGTGGATACGGTCATGAAAGGCGTGGAAATGATGTTCCAT
                    ************************************************************

Cdeb_01397          TTTCAGCCGGACGTCATTATCGCCCTCGGCGGGGGATCGCCGTTGGACGCGGCAAAAGCG
A3EQDRAFT_01103     TTTCAGCCGGACGTCATTATCGCCCTCGGCGGGGGATCGCCGTTGGACGCGGCAAAAGCG
                    ************************************************************

Cdeb_01397          ATGTGGCTGTTCTATGAACATCCTGAAACCGAGTTTAACGGATTGAAACAAAAATTTTTG
A3EQDRAFT_01103     ATGTGGCTGTTTTATGAACATCCAGAAACCGAGTTTAACGGATTGAAGCAAAAATTTTTG
                    *********** *********** ********************** ************

Cdeb_01397          GACATCCGCAAAAGGGTGTTCAAATTTCCGAAACTGGGGCGTAAGGCCAAACTGGTGGCC
A3EQDRAFT_01103     GACATCCGCAAAAGGGTGTTCAAATTTCCGAAACTGGGGCGAAAGGCCAAACTGGTGGCC
                    ****************************************** ******************

Cdeb_01397          ATCCCGACGACTTCCGGTTCCGGGTCCGAAGTGACGTCCTTTGCCGTGATCACGGACAAA
A3EQDRAFT_01103     ATCCCGACGACTTCCGGTTCCGGGTCCGAAGTGACGTCCTTTGCCGTGATCACGGACAAA
                    ************************************************************
```

```
Cdeb_01397          AAGCTGGACATCAAATATCCGTTGGCGGACTATGAATTGACGCCGGATGTGGCCATCATT
A3EQDRAFT_01103     AAGCTGGACATCAAATATCCGCTGGCGGACTATGAATTGACGCCGGATGTGGCCATCATT
                    ********************* ************************************

Cdeb_01397          GACCCGGCGTACGTGATGACCGTTCCGAAATCCGTCACCGCAGATACGGGAATGGATGTG
A3EQDRAFT_01103     GACCCGGCGTACGTGATGACCGTTCCGAAATCCGTCACTGCAGATACGGGAATGGATGTG
                    ************************************ ********************

Cdeb_01397          TTGACCCACGCCATCGAAGCTTATGTTTCCAACATGGCGAACGATTATACGGACGGATTG
A3EQDRAFT_01103     TTGACCCACGCCATCGAAGCTTATGTTTCCAACATGGCGAACGATTATACGGACGGATTG
                    ************************************************************

Cdeb_01397          GCCATCAAAGCCATTCAGCTTGTCTTTGAATATTTGCCCCGGGCGTATAAAAACGGCCAA
A3EQDRAFT_01103     GCCATCAAAGCCATTCAGCTTGTCTTTGAATATTTGCCCCGGGCGTATAAAAACGGCCAA
                    ************************************************************

Cdeb_01397          GATGAACTGGCGCGGGAAAAAATGCACAACGCGTCCACCATTGCCGGCATGGCTTTTTCC
A3EQDRAFT_01103     GATGAGCTGGCGCGGGAAAAAATGCACAACGCGTCCACCATTGCCGGCATGGCTTTTTCC
                    ***** ********************************************************

Cdeb_01397          AATGCCTTTTTGGGCATCAATCACAGTCTGGCCCATAAGCTCGGCGGCGCTTTCCATATC
A3EQDRAFT_01103     AATGCCTTTTTGGGCATCAATCACAGTCTGGCCCATAAGCTCGGGGGCGCTTTCCATATC
                    ******************************************** ***************

Cdeb_01397          CCCCACGGCAGGGCCAACGCCATTCTGATGCCCCACGTCATCCGGTATAACGCGACGAAG
A3EQDRAFT_01103     CCCCACGGCAGGGCCAACGCCATTCTGATGCCCCACGTCATCCGGTATAACGCGACGAAG
                    ************************************************************

Cdeb_01397          CCGACCAAGTTTGTCGCCTTCCCAAAATACGAACATTTTATCGCCGACAAGCGGTATGCG
A3EQDRAFT_01103     CCGACCAAGTTTGTCGCCTTCCCAAAATACGAACATTTTATCGCCGACAAGCGGTATGCG
                    ************************************************************

Cdeb_01397          GAAATCGCCAGGATTCTCGGACTGCCTGCGAAAACGACGGAGGAAGGGGTGGAAAGCTTG
A3EQDRAFT_01103     GAAATCGCCAGAATTCTCGGACTGCCTGCGAAAACGACGGAGGAAGGCGTGGAAAGCTTG
                    *********** ********************************** ************

Cdeb_01397          ATTCAGGCCGTGATCGGACTGGCGAAGGAATTGGAGATCCCGATGAGCCTGGAAGCCCTC
A3EQDRAFT_01103     GTTCAGGCCGTGATCGGACTGGCGAAGGAATTGGAGATTCCGATGAGCCTGGAAGCCCTC
                     ********************************** ********************

Cdeb_01397          GGCATCGATCGCGATGACTTCGAAAAGAGGGTGCCCGAACTGGCCGAGTTGGCTTTCGAA
A3EQDRAFT_01103     GGCATCGATCGCGATGCCTTCGAAAAGAAGGTGCACGAACTGGCCGAGTTGGCTTTCGAA
                    *************** *********** ***** *************************

Cdeb_01397          GACCAATGCACCACGGCGAACCCGAAAATGCCTTTAGTCTCGGAATTGGAAGAAATTTAT
A3EQDRAFT_01103     GACCAATGCACCACGGCGAACCCGAAAATGCCTTTAGTCTCGGAATTGGAAGAAATTTAT
                    ************************************************************

Cdeb_01397          CGGCAGGCGTATAAGGGCGTTTGA
A3EQDRAFT_01103     CGGCAGGCGTATAAGGGCGTTTGA
                    ************************
```

Figure 5.7. DNA alignments of AdhE coding region "*" underneath indicates matching bp; Blank spaces " " underneath indicate positions that do not match and the base pairs are highlighted in red.

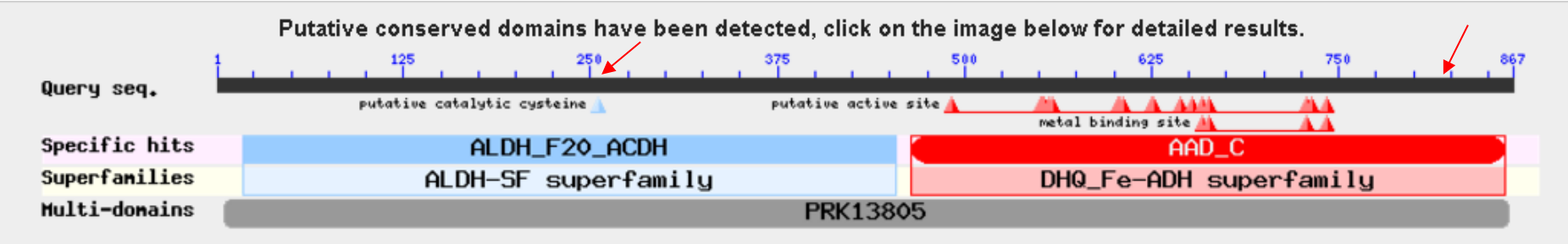
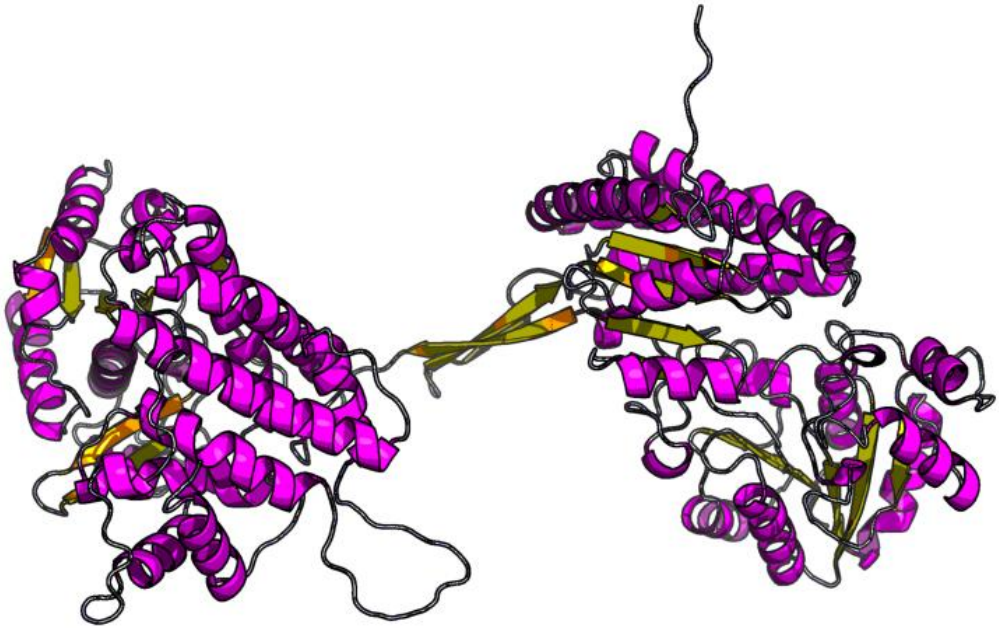


Figure 5.8. adhE (Cdeb_01397) domain and key amino acid sites as specified by NCBI BLAST AlDH domain shown in blue and ADH domain shown in red; Key amino acids in active sites and metal binding sites are shown by red arrows.

A)



B)

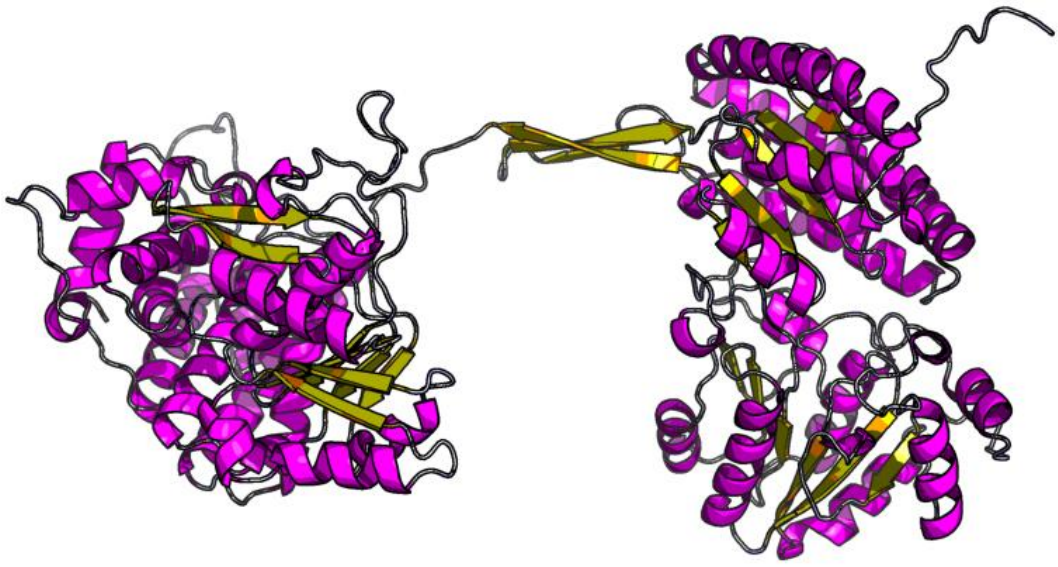
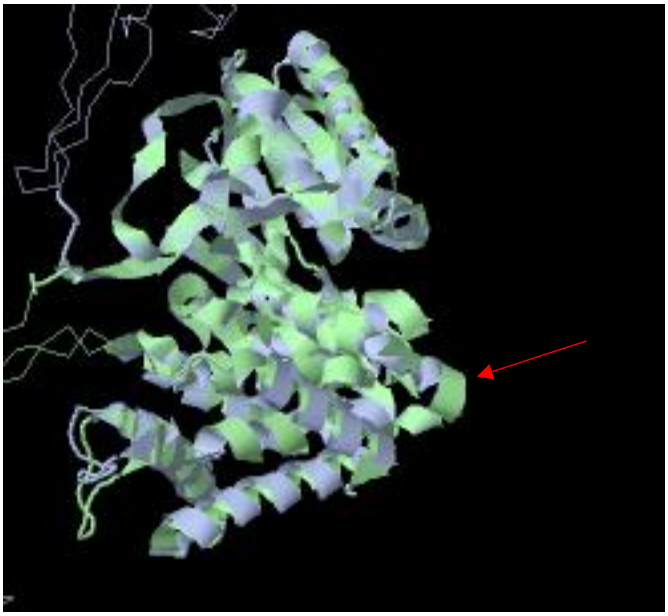


Figure 5.9. RaptorX modeled structure of A) ADHE (Cdeb_01397) and B)ADHE (A3EQDRAFT_01103) in GB1 and Tf The left side portion of the protein is the AlDH domain and the right side the ADH domain.

A)



B)

Figure 5.10. RaptorX modeled alignment structure of ADHE Cdeb_01397 A3EQDRAFT_01103 in GB1 (Green) and Tf (Blue) A) ADH domain; Red arrow points to AA position 831 where P→H in GB1 and Tf respectively B) AlDH domain; Red arrow points to AA position 269 where A→E in GB1 and Tf respectively.

5.4.9. Cellobiose Phosphotransferase Systems

Other differences of note between GB1 and Tf include the apparent differences in the gene complement of the Phosphotransferase (PT) systems, especially concerning PT systems with putative cellobiose specificity (Table 5.4). This is not surprising, due to the fact that strain GB1 was enriched and purified under conditions containing cellobiose (Chapter 2), while strain Tf was not (Banat *et al.* 2004). Furthermore *C. debilis* GB1 was isolated from a cellulolytic aerotolerant enrichment containing *C. thermocellum* to which it provided aerotolerance. Under enrichment conditions *C. debilis* GB1 and *C. thermocellum* would likely compete to get sugar resources.

Table 5.4.Comparison of C. debilis strains phosphotransferase systems with putative cellobiose specificity (source: IMG)

Protein Description	Tf	GB1
Phosphotransferase system cellobiose-specific component IIa	A3EQDRAFT_00255, A3EQDRAFT_00372, A3EQDRAFT_00890, A3EQDRAFT_00891, A3EQDRAFT_1303, A3EQDRAFT_01960	**Cdeb_02677**[1], Cdeb_02994, Cdeb_00155, Cdeb_00598, Cdeb_01069, Cdeb_01817, Cdeb_02167
Phosphotransferase system cellobiose-specific component IIb	A3EQDRAFT_00253, A3EQDRAFT_00892, A3EQDRAFT_01129, A3EQDRAFT_01301, A3EQDRAFT_01958	**Cdeb_02678, Cdeb_02995**, Cdeb_00156, Cdeb_01067, Cdeb_01426, Cdeb_01815, Cdeb_02169
Phosphotransferase system cellobiose-specific component IIc	A3EQDRAFT_00252, A3EQDRAFT_00893, A3EQDRAFT_01128, A3EQDRAFT_01302, A3EQDRAFT_01959	**Cdeb_02674, Cdeb_02993**, Cdeb_00157, Cdeb_01068, Cdeb_01425, Cdeb_01814, Cdeb_02168

[1]Locus tags of PTS subunits without a counterpart in Tf are given in **bold**

5.4.10. Respirofermentative Metabolism

Figure 5.11 displays the core pathways involved in growth of Tf under low concentrations of oxygen. Previous experiments done by Wushke *et al.* (Chapter 3) characterised end-product production on cellobiose aerobically and anaerobically for strains GB1 and Tf. Under conditions where oxygen concentrations are low Tf and GB1 both utilise respiration and fermentation, also called respirofermentative growth (Siso *et al.* 1996). During respirofermentative growth, fermentation products are produced, and the excess electrons from metabolism can then be used through the ECT to create ATP or used to create a reduced end-product. GB1 can either use its excess electrons to create ethanol and/or lactate and/or use them in ECT, based on end-product production under oxygen limiting conditions it appears to do all three. Tf when undergoing respirofermentative growth utilizes the ETC and produces lactate as the only reduced end-product. Tf fails to grow under anaerobic conditions most likely due to an inability to produce ethanol. While Tf could balance its electrons anaerobically by producing lactate alone, Tf does not do this. While Tf and GB1 both have additional discrete *adh* and *aldh* genes, they may not be involved in alcohol synthesis, Wushke *et al.* (Chapter 3) show GB1 is capable of consuming ethanol aerobically.

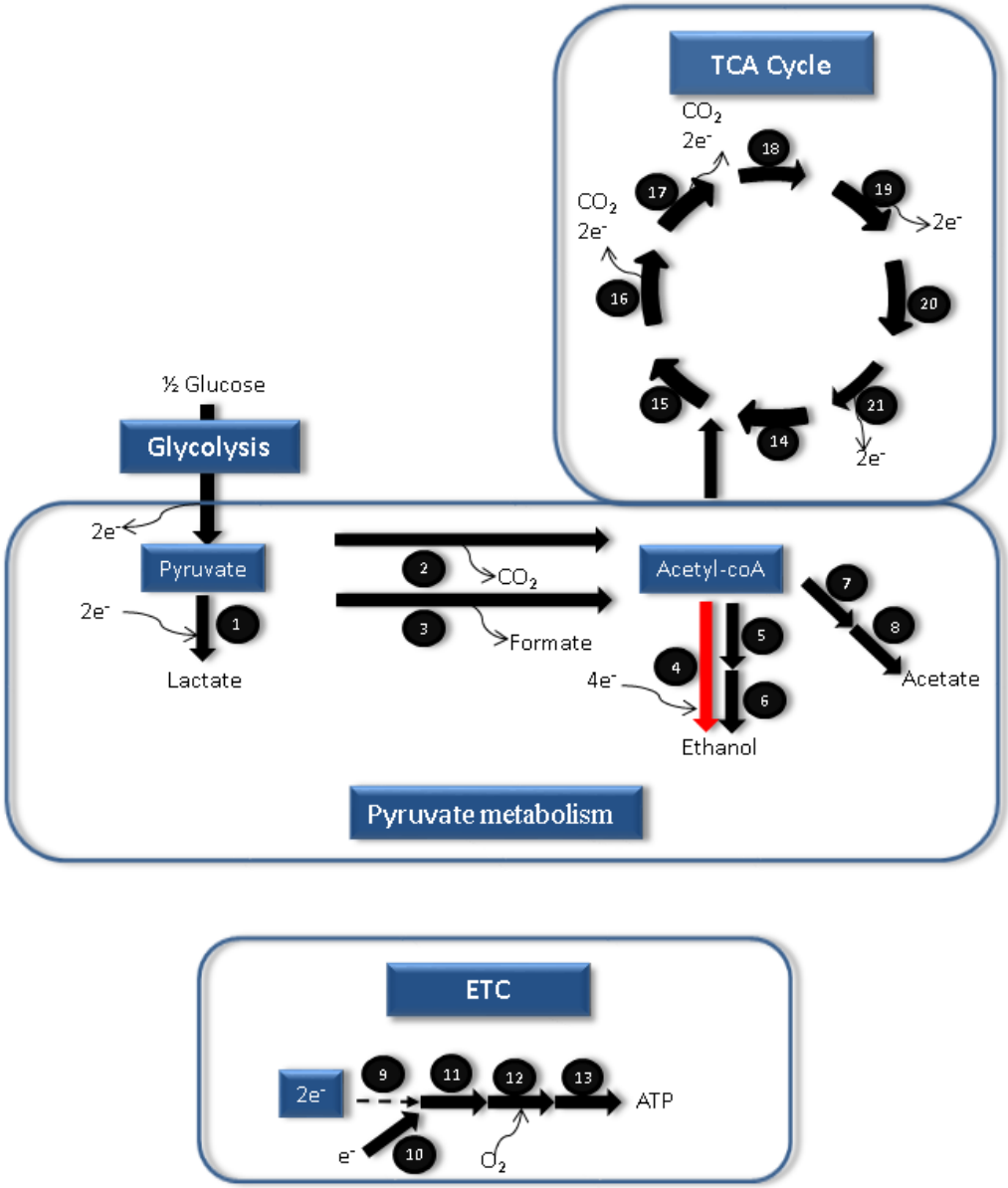


Figure 5.11. Respirofermentive metabolism *C. debilis* strains GB1 and Tf. enzymes 1) lactate dehydrogenase (LDH); 2) pyruvate dehydrogenase (PDH); 3) pyruvate formate lyase (PFL); 4) alcohol/aldehyde dehydrogenase (ADHE); 5) aldehyde dehydrogenase(ALDH); 6) alcohol dehydrogenase(ADH);7) phosphotransacetylase (PTA); 8) acetate kinase (AK); 9) complex I; 10) complex II; 11) complex III; 12) complex IV; 13) ATP synthase/complex; 14) citrate synthase; 15) aconitate hydratase; 16) isocitrate dehydrogenase; 17) alpha-ketogultarate dehydrogenase; 18) succinyl-CoA synthase; 19) succinate dehydrogenase; 21) fumerate hydratase; 22) malate dehydrogenase

5.5. Conclusion

No direct evidence was found to explain the lack of anaerobic metabolism and lack of ethanol production phenotypic differences by superficial examination of the overall annotated genome content and organization. However, closer examination of the gene sequences revealed evidence of differences within specific genes pertinent to anaerobic metabolism that could affect enzymatic activity. Tf is unable to produce ethanol under conditions where oxygen is limiting. The inability of Tf to produce ethanol is the likely cause of the lack of anaerobic growth phenotype. As ethanol production is typically produced through the ADHE enzyme in related genera, it is likely that the lack of ethanol production is caused by a defective *adhE* gene. (Extance *et al.* 2013). Indeed, the Tf strain phenotype closely matched an ADHE knockout phenotype in a related species, *G. thermoglucosidasius,* giving further credence to the hypothesis that ADHE expression or functionality is different in strain Tf. Differences in ADHE activity or expression is a plausible scenario leading to the observed phenotypic differences between Tf and GB1.The argument for differences in the ADHE activity is strengthened through observed amino acid changes in the ADHE seen in genome analysis.

Overall *C. debilis* GB1 and Tf are very similar organisms at the genetic level, in both synteny and gene complement, including core metabolism pathway genes. Through our genome analysis a gene complement consistent with fermentation (*pfl, ldh, ak, pta,* and *adhE*) was observed in Tf and GB1. Tf likely evolved from a strain that could ferment cellobiose. The ability of *C. debilis* GB1 to grow under anaerobic conditions and synthesize ethanol clearly distinguishes it from *C. debilis* Tf. The

genus description of strain GB1 should be amended to include anaerobic growth as a characteristic.

Chapter 6: General Discussion and Conclusions

6.1. Core Objectives

With the goals of reducing or slowing man-made climate change, and creating a sustainable economy/environment in mind, we must look at the obvious option of using non-fossil fuels to create a closed-carbon-loop in order to reduce GHG emissions (DeCicco *et al.* 2013). Converting lignocellulosic substrates into ethanol or hydrogen using Consolidated Bioprocessing (CBP) as a way of reducing carbon emissions is enticing. As this process is not currently cost competitive (Lynd *et al.* 2005, Olsen *et al.* 2012, Jouzani *et al.* 2015), and the optimal conditions and organisms for CBP have not yet been determined, discovering new organisms and expanding the operational parameters of CBP is essential to developing an optimal process.

With the goal of discovering a suitable organism for CBP, or one able to act synergistically with those currently used, in mind, we address our contributions through the objectives below:

Objective 1: Find and describe aerotolerant cellulolytic fermentative consortia.

A wide variety of environments on a Saskatchewan farm were sampled, and several highly cellulolytic enrichments that possessed aerotolerance traits that could expand the mode of operation of CBP were created. Several stable, transferable, and plateable cultures that exhibited high cellulose degradation and aerotolerance for further processing were generated. As a method, creating enrichments that exhibit a

specific set of community phenotypes, such as aerotolerant lignocellulolytic fermentation, is not novel in and of itself, and has been described in past literature (Miyazaki *et al.* 2008, and Ronan *et al.* 2013). However as the source inoculum and exact environmental conditions vary from one sampling site to another, it can yield novel organisms and enrichments that provide a starting point to isolating novel bacteria and/or novel strains. We generated the B4-1 enrichment, which showed cellulolytic abilities, aerotolerance, and better ethanol total-yield production then other comparable enrichments.

Objective 2: Determine the minimum consortium with the phenotype of interest and characterize its novel component(s)

We used the organism composition from B4-1 to create a designer culture showing the same ability as B4-1. Characterisation of the enrichment showed that this culture likely consisted of just two organisms related to *C. thermocellum* and *C. debilis.* An isolate of *C. debilis* GB1 from B4-1 was added to *C. thermocellum* DSMZ 1237 to create a co-culture. Chapter 3 definitively demonstrates that *C. debilis* GB1 alone is able to lend aerotolerance to *C. thermocellum* DSMZ 1237 and create an aerotolerant, highly cellulolytic co-culture. An aerobically fermentative co-culture containing *C. thermocellum* is a novel mode of operation for CBP.

Isolation and characterisation of *C. debilis* GB1 showed it was quite similar physiologically to type strain with the exception of exhibiting the ability to grow anaerobically and produce ethanol. The type strain, *C. debilis* Tf, however, could not replace the isolate in co-culture with *C. thermocellum.* It was not able to generate a microenvironment suitable for the growth of *C. thermocellum.*

Objective 3: Characterize *C. debilis* GB1 novel components using genomics and proteomics.

At the time of isolation a genome for *C. debilis* was not available. We sequenced *C. debilis* GB1 and annotated its genome. We were specifically interested in aerobic and anaerobic growth of GB1 and understanding GB1 aerobic/anaerobic expression in pure culture to shed light on the interactions of the *C. debilis* GB1 and *C. thermocellum* designer co-culture. The *C. debilis* GB1 proteome was characterized under aerobic and anaerobic conditions. Protein expression was found to change at the pathway level and was consistent with what one would expect in mesophilic facultative anaerobic organisms such as *E. coli* or *B. cereus* with similar core metabolism gene complements (chapter 4). Further examples of direct omic comparisons between such distantly related organisms within the literature as *C. debilis* and *E. coli* could not be found. Omic comparisons have been mainly performed among similar species or strains (Lidbury *et al.* 2016, Goltsman *et al.* 2009). The comparison of a novel organism to a distant, but well studied, organism from a different phylum shows that it is beneficial in understanding expression at the omics level.

The proteomic information for genes which were detected and measured corroborates physiology concerning central metabolism. As cross-state proteomes using high throughput techniques on thermophilic *Bacillaceae* is rare in current literature, the description of the aerobic and anaerobic metabolism of *C. debilis* GB1 contributed directly to our goal of understanding the role of GB1 in our cellulolytic aerotolerant designer co-culture.

Objective 4: Characterization of GB1 against the type strain at the genomic level

The *C. debilis* GB1 genome was sequenced, annotated, and compared directly to the type strain Tf. Comparing and contrasting these organisms allowed for a better understanding of the features in *C. debilis* GB1 which make it a good co-culture partner for CBP, while the type strain could not. Tf was not able to recreate the same aerotolerant cellulolytic co-culture when mixed with *C. thermocellum* as GB1 could. Understanding why Tf was limited to aerobic growth and did not synthesize ethanol is core in understanding why it could not protect *C. thermocellum* from oxygen. This contributed directly to the goals of understanding physiology through the lens of genomics and proteomics using the omics techniques to corroborate the results of the other.

6.2. Thesis Work in the Context of 2016

Pure culture work is a core aspect of CBP and being able to understand and optimise a single organism is objectively easier than understanding and optimizing organisms in a co-culture. For the study of co-cultures, it is possible to take a reductionist approach, and analyze each organism individually or to study them as a co-culture. Our experiments built on studying an enrichment, B4-1, which had the property of being aerotolerant and highly cellulolytic.

Large amounts of work has been put into studying *C. thermocellum,* it is beneficial that our designer co-culture worked with the well characterized strain of *C. thermocellum* (DSMZ 1237). *C. thermocellum* was the main driver of cellulose

degradation in these cultures as *C. debilis* was not cellulolytic. *C. debilis* had previously not been well characterized, therefore extensive work characterising this organism at the physiological level and omics level especially concerning core metabolism and aerobic/anaerobic growth was performed. *Geobacillus* and the closely related *Caldibacillus* appear to be naturally occurring co-culture partners with *C. thermocellum* as they are frequently found together in enrichments (Ronan *et al.* 2013, Miyazaki *et al.* 2008). Interestingly, deliberate co-cultures containing *C. thermocellum* and organisms related to *Geobacillus* for the purposes of CBP are relatively rare in the literature (Lu *et al.* 2013). Characterisation of *Geobacillus* and/or *Caldibacillus* in the context of CBP is relatively sparse even though they appear to have very attractive abilities and can be readily optimized (Cripps *et al.* 2009). Other organism such as *Thermoanaerobacter sp.* appear to be favored as co-culture partners for *C. thermocellum*, perhaps due to their capacity for robust growth. Indeed several strains of *C. thermocellum* were shown to be co-cultures (Freier *et al.* 1988). In deliberate co-cultures *Thermoanaerobacter sp.* can have the aptitude for increasing ethanol or hydrogen yields (Fang 2010). *Thermoanaerobacter sp.*may also be useful in co-culture with *C. thermocellum* because of their ability to hydrolyse some components of lignocellulosic substrates as they can be highly xylanolytic (Verbeke *et al.* 2013). They are also capable of fermenting xylose, a trait the *C. thermocellum* does not have. Some members of the *Geobacillus*, and more closely related genera, have similar abilities (Brumm *et al.* 2015, Cripps *et al.* 2009). *Geobacillus* related organisms also appear to have some of the highest innate ethanol tolerance of thermophiles (Fong *et al.* 2006) and the ability to lend aerotolerance to strict

anaerobes as alluded to by others (Zuroff *et al.* 2013) and demonstrated decisively through our work.

In the context of 2016, designer co-cultures appear to be a highly researched mechanism to improving CBP (Jouzani *et al.* 2015). In many cases, it tends to have a positive effect when compared to corresponding pure cultures. However, as the future is difficult to predict it is unclear what combination of organisms, and what mode an economically viable CBP process will take.

6.3. Future Work

A comprehensive omics analysis of the co-culture of *C. debilis* GB1 and *C. thermocellum* in a steady state is the next step in this project. There is a large amount of omics information on *C. thermocellum* in pure culture, and we have built up omics information on *C. debilis* GB1 in pure culture namely genomic and proteomic through our work. We could leverage the pure culture information in comparison to that from the co-culture to understand the interactions between its members. This would give us essential information on how these organisms work together. We attempted to harvest samples for omics from co-cultures grown using pH controlled fermenters but only achieved mitigated success (see Appendix E for likely causes). Collecting omics data on CBP co-cultures in a co-cultured state to determine co-culture interactions is the next step and logical final goal for this project.

6.4. Conclusion

This designed co-culture was built on previous bioprospecting work. While genetic modification tools are becoming incredibly powerful, it is important to build those genetic modifications on an organism that is suitable. In choosing an organism to genetically modify to be a high ethanol producer why not use an organism that is already ethanol tolerant? Theoretically it may be possible to build a highly cellulolytic, high ethanol producing organism from the ground up, but this may not be the easiest path. One could instead find an organism that already has a majority of desired features.

C. debilis GB1 appeared distinct from the type strain Tf with the former being reported, and confirmed through our work, as a strict aerobe. In side-by-side comparison with strain GB1, we have confirmed that Tf cannot grow in media that permit anaerobic growth of strain GB1. Our work here elucidates the anaerobic metabolism in *Caldibacillus* and that the strain Tf, while appearing to have the gene complement for ethanol and anaerobic metabolism, is limited due to difference in ADHE functionality. The characterisation of GB1 and Tf lead to the conclusion the *Caldibacillus* genus description should be amended to include anaerobic metabolism as a characteristic possible for members of the genus. With respect to co-culturing with the obligate anaerobe *C. thermocellum*, this ability to grow anaerobically as well as aerobically might be crucial in providing an appropriate environment to permit the growth of *C. thermocellum* under aerobic conditions.

The *C. debilis* GB1 is interesting as a candidate for co-culture with *C. thermocellum* for the purpose of ethanol production as *C. debilis* increases total substrate consumption, total ethanol production, ethanol production efficiency, and lends aerotolerance under required conditions. Facultative anaerobes make good co-cultures for strict anaerobes in CBP as they can counteract the toxic effects of oxygen making lab manipulation easier and increasing the inoculation efficiency, a topic seldom dealt with within the literature. Aerobicity is an interesting parameter to experiment with during CBP, and could be manipulated to push cell regulation or biofuel production in new ways. Zuroff *et al.* (2013) used microaerobic conditions to allow both a clostridium and yeast to grow under microaerobic conditions and produce ethanol. We were able to create conditions analogous to this with *C. thermocellum* and *C. debilis* GB1. While able to achieve aerotolerance in the co-cultures, the only benefits seen were to ethanol production under anaerobic conditions. The ability for *C. thermocellum* to grow, metabolise, and produce ethanol, and hydrogen under aerobic conditions with the help of GB1was definitively demonstrated through our work. However, while oxygen was present, the yields of ethanol during cellulose fermentation were lower when compared to the same co-culture under anaerobic conditions.

The Omics used to characterize and understand *C. debilis* gave general insight into aerobic/anaerobic regulation of *Caldibacillus*, and show how similar it is between Firmicutes versus Proteobacteria; mesophilic versus thermophilic bacteria. Furthermore the Omics information produced can also be used in the future for *in silico* bioprospecting.

In view of our work, *Geobacillus* and related thermophilic bacilli appear to be under exploited as CBP co-culture partners. Our characterisation of *C. debilis* enhances the ability for others to choose ideal co-culture partners from pools of interesting organisms for CBP

6.5. Future Perspectives

In order to make CBP work, we need to study different modes of operation with many different bacteria. A crucial element of this is the use of bioprospecting, with different organisms being tried under different operating modes. In concert with this, it is important to understand the core metabolism and regulation of these organisms in order to exploit them effectively under bioprocessing conditions. With respect to central metabolism, it has been demonstrated that *Caldibacillu*s works and acts similarly to other well studied facultative anaerobes: *E. coli* K-12 and *B. cereus*. Others have demonstrated genetic malleability in *Caldibacillus* related genera (Cripps et al. 2009), and in *Caldibacillus debilis* GB1 as well (Wan *et al.* 2013). The literature shows potential for facultative anaerobes to be obligate fermenters (Day *et al.* 2014) under aerobic conditions through either natural mutations and/or directed genetic modifications. Creation of a facultative anaerobe genetically modified to be an obligate fermenter with high ethanol production would be a highly beneficial co-culture partner for *C. thermocellum.* This could also be applied to other strictly anaerobic cellulolytic candidates for CBP under either anaerobic or oxygen limiting conditions. Indeed there has been some work on this with *G. thermoglucosidasius*

being used to create ethanol at high efficiency (Cripps *et al.* 2009). The approaches of genetic modification and co-culturing should dovetail, in the future there will be more studies on co-cultures where both members have been genetically modified for optimization (Argyros *et al.* 2011). The omics information created to understand organisms in pure culture should also be compared against omics data of co-cultures to further understand organism(s) interactions and how they can synergize.

CBP research may be very far, in terms of biofuel yields, from making the CBP processes work commerically; in many cases yields need to improve by large amounts (Jouzani *et al.* 2015). Incremental improvements of single digit percent would prevent rapid implementation of CBP. To achieve the goal of CBP quickly, we should instead focus on innovations that could cause large changes in production. However, such innovations are not entirely obvious at the present time. Experimenting with different CBP modes and organism combinations, and understanding the organisms involved, might permit further improvements in CBP.

Appendices

Appendix A: Physiological and Genomic Characterization of Fervidobacterium pennivorans strain DYC in Relation to the Type Strain[5]

A.1. Abstract

A strictly anaerobic thermophilic cellobiose degrading consortium from Ngatamariki Hot Spring in New Zealand was enriched. Repeated serial dilution of the enrichment culture led to the isolation of a strain of *Fervidobacterium pennivorans*, designated strain DYC. When fermentation profiles were analyzed, DYC was found to produce chromatographic profiles consistent with acetate, alanine, glutamate, H_2, and CO_2 as major end-products on 2g/l cellobiose in modified 1191 medium. The purified strain was sequenced by PacBio sequencing and assembled using Hierarchical Genome Assembly Process (HGAP) into a single 2.06Mbp circularized chromosome, annotated using genePRIMP, and submitted to Integrated Microbial Genomes Expert Review (IMG-er) platform. Comparison of *F. pennivorans* DYC against the type strain *F. pennivorans* DSM 9078 genome using dot plot revealed that these organisms are highly syntenic, and genome to genome distance calculator

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[5] Contributing authors: **Scott Wushke[a], Victor Spicer[b], Xiang Li Zhang[c], Brian Fristensky[c], Oleg V. Krokhin[b], David B. Levin[d], Nazim Cicek[d], Matthew Stott[d], Richard Sparling[d].** 2016 (manuscript in preparation)

Contributions: [a]First author experimental design, cell growth and end-product analysis; worked in concert with others authors in analysis of genome and proteome [b]proteomic tools and expertise, data analyses, and running samples on MS/MS; [c]Bioinformatic tools and expertise, genome assembly and 1st draft annotation; [d]Lab space, equipment, funding, research guidance.

analysis revealed that these organisms are likely the same species. Upon unique genome overlay using Gview, several large ~50kbp areas were distinct in each organism. The differences observed corresponded to differences noted in general physiology of substrate utilization, and end-product production for both the type strain 9078 and DYC. Of particular interest are differences in the Entner-Doudoroff pathway between the strains resulting in the inability of one strain to utilize gluconate. We produced genomic and proteomic data and interpreted it through the lens of our end-product data in order to understand the pathways likely involved in fermentation of sugar substrates.

A.2. Introduction

Fervidobacterium pennivorans is a strictly anaerobic thermophilic bacteria that falls within the order Thermotogales, and has the distinctive loose outer membrane known as a toga (Friedrich *et al.* 1996). *F. pennivorans* is typically isolated from hot springs and grows at an optimum temperature of ~70ºC (Friedrich *et al.* 1996). *F. pennivorans* strains have a wide substrate usage range allowing them to use many sugar monomers, polysaccharides, and complex protein substrates (Nam *et al.* 2002). There has been limited work on characterizing *Fervidobacterium* physiology. However, its ability to hydrolyze substrates, especially recalcitrant proteins, has been a prominent feature (Friedrich *et al.* 1996, Nam *et al.* 2002). *F. pennivorans* has been shown to produce end-products H_2, CO_2, lactate, acetate, and alanine (Ravot *et al.* 1996). The possibility of H_2 from sugars and recalcitrant protein substrates make *F.*

pennivorans a candidate worthy of characterization from a biofuels perspective. *F. pennivorans* has also been a source of thermostable enzymes such as pullulanases, keratinases (Bertoldo *et al.*1999, Nam *et al.* 2002). While major end-products from glucose fermentation have been described previously, at least in part (Ravot *et al.* 1996, Friedrich *et al.* 1996), there has been no attempt to complete a carbon and redox balance in the species *F. pennivorans. Fervidobacterium* core metabolic pathways has yet to be elucidated making it an interesting organism to characterize from a cell metabolism perspective, especially concerning alanine since alanine, as a major fermentation end-product, has been hypothesized to be a remnant of ancestral metabolism (Ravot *et al.* 1996). Alanine production as fermentation product does not appear to be rare as there are many examples of alanine production thermophiles and hyperthermophiles, which include *C. thermocellum, Pyrococcus furiosus,* and *Thermococcus kodakarensis* (Van Der Veen *et al.* 2013, Kengen *et al.* 1994, Nohara *et al.* 2014). While the production of alanine is well documented in *Thermatogales*, it is unclear whether the pathways used in *F. pennivorans* are similar to other thermophiles. We wished to characterize and compare our organism's fermentation profile and genetic complement to see if it fit conventional pathways of end-product production in hyperthermophiles. There has been limited work on the characterization of *F. pennivorans* as an organism (Ravot *et al.* 1996, Friedrich *et al.* 1996). Comparison of two closely related strains physiology through the lens of a genomic comparison can lead to furthering the understanding of core metabolism, especially if the gene complement between two strains is different concerning core metabolism. The chimeric nature of Thermotogales can lead to extensive differences at the whole

genome level between closely related members (Zhaxybayeva *et al.* 2009). The limited characterizing of metabolism, possibility for industrial usefulness, and chimeric genome properties make a genome and physiological comparison of two closely related strains of *F. pennivorans* interesting.

A.3. Materials and Methods

A.3.1. Isolation of DYC and Cell Culturing

Samples including soil and water collected from Ngatamariki Hot Springs in New Zealand in March, 2012. Samples were collected anaerobically and stored in sealed containers without airspace at 4ºC until inoculation. The base medium produced for all experiments was as previously described (Chapter 2), modified 1191 medium, with a lower concentration of yeast extract (0.76 g/l) and pH adjusted to 7.2. Sugars glucose, cellobiose, gluconate, xylose, and ribose were used as primary carbon and energy source (at 2 g/L) as specified for experiments. Sample liquid, or a slurry created using sample water and soil was inoculated at 10% in M-1191 medium in anaerobic Balch tubes with 2g/l cellobiose at 80ºC. Plating this enrichment did not result in colonies. The Ngatamariki Hot Springs enrichment was serial diluted to extinction 10 times in order to create a pure culture designated DYC.Cultures of *F. pennivorans* DSM 9087 were obtained from the DSM culture collection

A.3.2. Cell Growth and Measurement

Prior to each experiment cells were passaged once under the relevant condition to allow adaption to substrate. All experiments were carried out in triplicate using a 1% inoculum. All cultures were grown at 75ºC. Cultures grown for end-product sampling were grown with sampling points at 80 hours post-inoculation (h pi) in sealed aerobic Balch tubes containing 10 ml of media and 17 ml headspace. Optical density OD_{600} was used to monitor cell growth in liquid media with soluble substrates. Protein concentrations were estimated by Bradford Assay (Bradford 1976), using a NanoDrop 1000 spectrometer.

A.3.3. Sugar and End-Product Analysis

Culture samples (1 ml) were collected after 80 h pi and stored at -20ºC until analysed. Concentrations of cellobiose, glucose, gluconate, lactate, formate, acetate, ethanol, butyrate, and butanol were measured directly by high pressure liquid chromatography (HPLC) using a Dionex ICS 3000 system equipped with a Bio-rad Aminex-87H column, and run at 30ºC, 0.75 ml/min, with 0.02 mM sulfuric acid. A Shodex 101 Refractive Index Detector was used on all compounds being analysed. Several amino acids could be quantified indirectly on the same system by their conversion via the van Slyke reaction (Pleissner *et al.* 2010) to compounds that could be eluted, detected, and quantified with our system including: glycine, alanine, valine, leucine, isoleucine, methionine, serine, threonine, asparagine, glutamine, aspartic

acid, glutamic acid, and proline. Using this method, only peaks associated with glutamate and alanine were detected.

Measurements of CO_2, H_2, and O_2 concentrations were determined using a Multiple Gas Analyzer #1 Gas Chromatograph (GC) System Model 8610-0070 (SRI Instruments, Torrance, CA), equipped with a Thermal Conductivity Detector (TCD), and using argon as the carrier gas. Columns and methods were used as previously described by Wushke *et al*. (Chapter 3).

Carbon balance was estimated from end-products produced using the formula: glucose equivalents consumed = $(C_1/6) + (C_2/3) + (C_3/2) + (C_5*(5/6))$. C_1= CO_2, C_2= acetate, and C_3= alanine C_5=Glutamate (Wushke *et al.* 2015). This was then divided by the glucose equivalents consumed as measured by HPLC to give us a percent of carbon converted to end-products.

O/R index was calculated by dividing the oxidized major end products by the reduced products modulated by their oxidation numbers to give redox balance= $(2*CO_2)/H_2$ (Chapter 3)

A.3.4. Genome Sequencing

Cells were grown aerobically on M-1191 medium and cell pellets harvested during mid-exponential phase extraction of DNA. For genomic DNA a Promega wizard genomic extraction kit (Promega) was used and protocol followed as described by the manufacture. DNA was sequenced at Genome Quebec Innovation Centre by PacBio sequencing and assembled using Hierarchical Genome Assembly Process

(HGAP) into a single 2.1Mbp circularized chromosome, annotated using genePRIMP, then submitted NCBI under the accession # CP011393 and to Integrated Microbial Genomes Expert Review (IMG-er) platform.

A.3.5. Comparative Genomics

Comparative genomics were done using IMG (Markowitz *et al*. 2008), Gview (Petkau *et al.* 2010), Genome to Genome Distance Calculator (GGDC) (Meier-Kolthoff *et al.* 2013) using similar methods to those described previously by Wushke *et al.* (Chapter 5). We used MEGA4 for building our 16s rRNA gene tree (Tamura *et al.* 2011) using the neighbour joining method. Metacyc was used for pathway building of the publically available 9078 genome (Caspi *et al.* 2008).

A.3.6. Proteomics

Protein extraction, proteomic analysis, mass spectrometry, and data analysis using UNITY was done on *F. pennivorans* DSM 9078 during mid exponential using methods identical to those described by Wushke *et al*. (Chapter 5).

A.4. Results

A.4.1. Isolation and Initial Growth Characteristics

An enrichment was created using a 10% inoculum of Ngatamariki Hot Springs water in 1191 media with 2g/l cellobiose at 80ºC. This enrichment was serial diluted to extinction 10 times in order to create a pure culture. Both strains 9078 and DYC were pleomorphic and consistent with the literature (Friedrich *et al.* 1996) showing L-shaped rod pairs, large ~10um spheroid structures produced by swelling of the OM, and spheroid structures with elongated cells wrapped around in a yarn-like structure. Growth rate was not statistically different as measured by total protein, results not shown. *F. pennivorans DSM* 9078 and DYC grew well on cellobiose, glucose, and xylose, shown in Figure A.1 but did not grow on ribose under our conditions, data not shown. Addition of thiosulfate as a potential electron acceptor did not appear to stimulate growth to a statistically significant degree in either strain when added as an electron acceptor at 2mM along with glucose. Only 9078 appeared to grow on gluconate, although poorly, as seen in Figure A.1. Growth of 9078 on xylose was primarily homoacetogenic, growth of DYC on xylose was primarily homoalanineogenic.

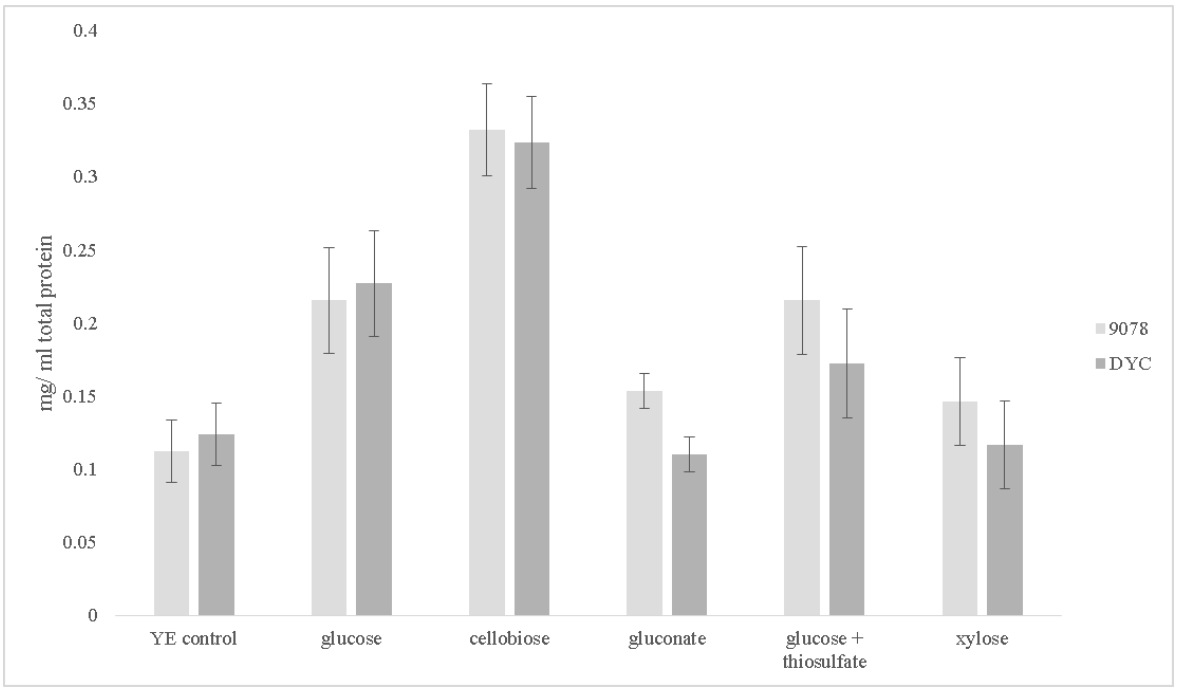


Figure A.1. 9078 and DYC max total protein at 24H pi

A.4.2. *F. pennivorans* DSM 9078 and DYC Relatedness

In order to determine the relatedness of strain DYC to other organisms, we employed four methods: i) compared the two copies of 16s rRNA in each organism and found a sequence similarity of 99% to the type strain of *Fervidobacterium pennivorans,* ii) used MEGA 4 to create a phylogenetic tree of related *Fervidobacterium* 16s rRNA species shown in Figure A.2, iii) used Dot plot with 9078 and *F. nosodum,* whose genome is also complete, to get an idea of synteny and relatedness which showed a much higher degree of synteny with 9078 then with *F. nosodum* at the whole genome level, results are shown in Figure A.3, iv) used GGDC analysis to determine that 9078 and DYC should indeed be considered the same species when considering the entirety of their genomes, results shown in Table A.1. The low formula 2 score (DDH >70%), in Table A.1, indicates that gene sequences have diverged and would not be considered the same species based on formula 2. This would be consistent with the isolation of these two strains from hot springs at distant geographic locations: the Azore Islands, Portugal for the type strain and New Zealand for strain DYC.

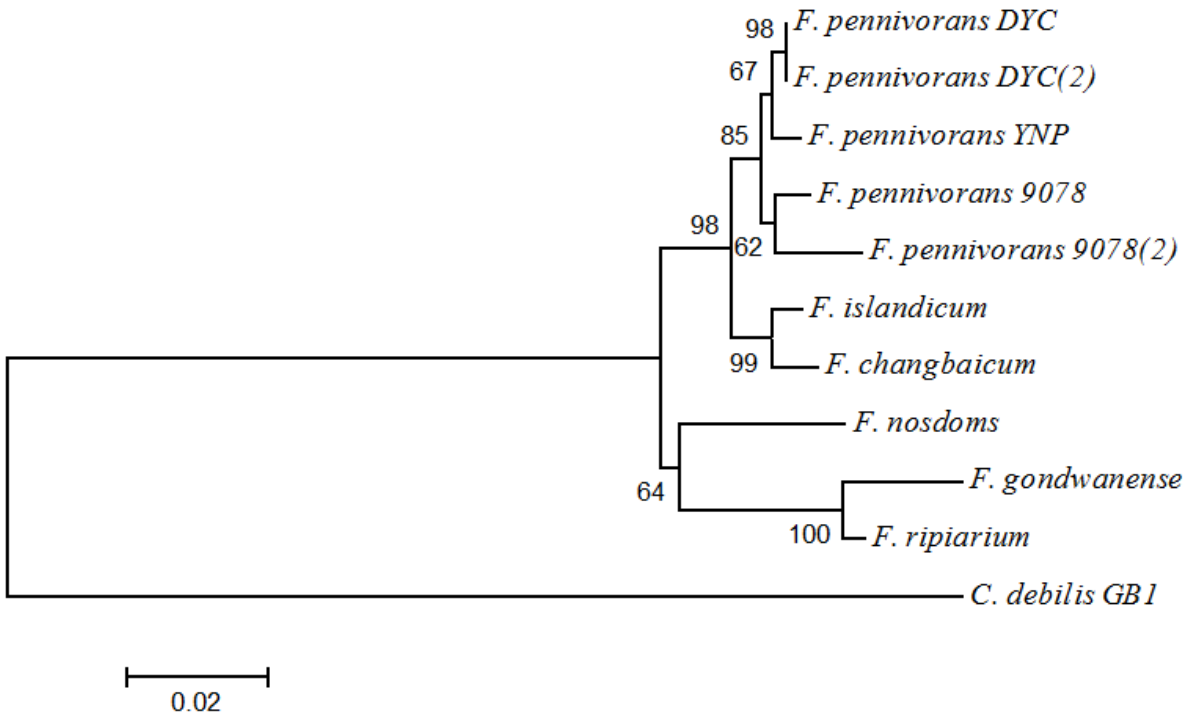
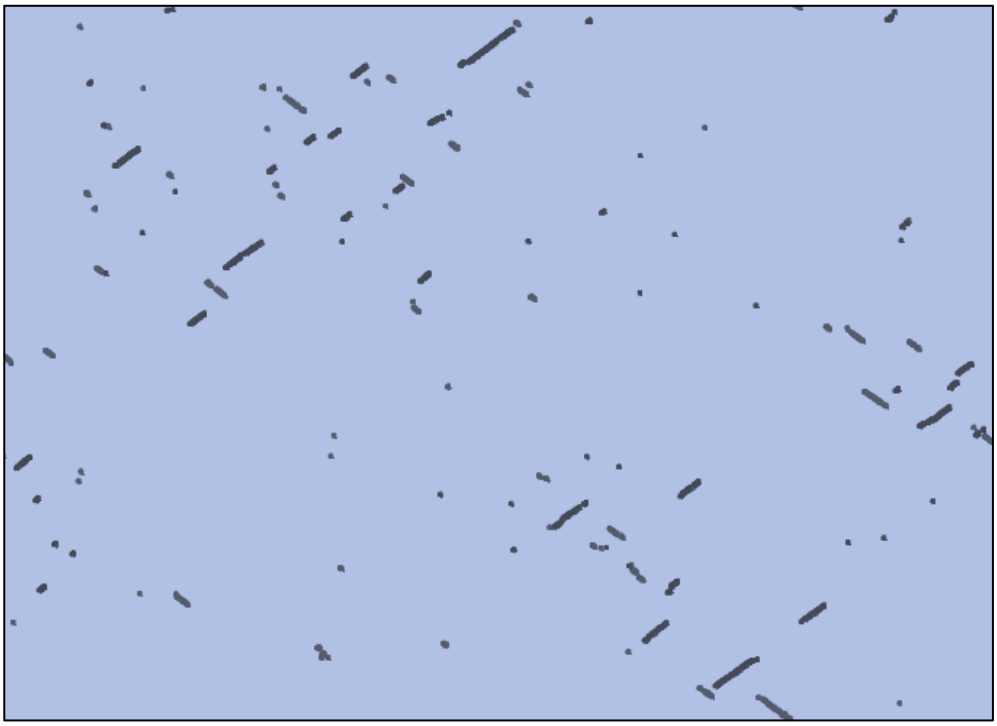


Figure A.2. 16s rRNA phylogenetic tree of Fervidobacterium using neighbor-joining in Mega4 Each strain of *F. pennivorans* has two 16S rRNA sequences

A)



B)

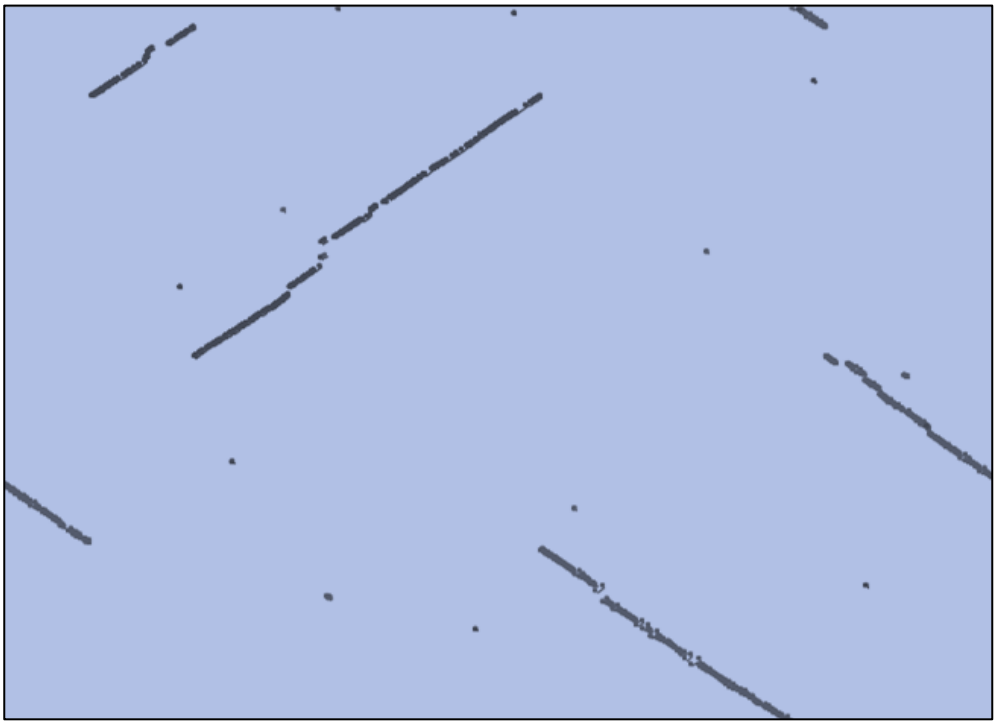


Figure A.3. NUCmer comparing A) DYC to *F. nosodum* and B) DYC to 9078

Table A.1. Genome to genome distance calculator analysis: DYC and 9078

Formula: 1 (HSP[1] length / total length)
DDH[2] > 70% (i.e., same species): 93.64%
DDH > 79% (i.e., same subspecies): 70.36%
Formula: 2 (identities / HSP length)
DDH > 70% (i.e., same species): 7.21%
DDH > 79% (i.e., same subspecies): 1.61%
Formula: 3 (identities / total length)
DDH > 70% (i.e., same species): 90.1%
DDH > 79% (i.e., same subspecies): 41.56%

[1]HSP= High scoring segmented pairs; [2]DDH= DNA/DNA hybridization

A.4.3. *F. pennivorans* DSM 9078 and DYC Genome Comparison

Table A.2 gives general statistics on 9078 and DYC including genome size, number of coding genes, and GC content. Strain 9078 and DYC were both assembled into one single circular chromosome and have a genome size of 2.16 and 2.06 Mbp respectively. Strain 9078 has 281 unique genes coding genes at a ≤60% sequence similarity with DYC, DYC has 123 unique genes at a ≤60% sequence similarity with 9078. The amount of unique genes increases significantly at a ≤90% sequence cut off to 595 and 398 for 9078 and DYC respectively. Figure A.4 is a Venn diagram of the shared and unique genes in DYC and 9078. Tables A.3 and A.4 list the genes unique to 9078 and DYC at a ≤60% sequence similarity cut off. Figure A.5 is a graphical representation of the unique genome of 9078 and DYC at ≤90% amino acid (AA) sequence similarity. Figure A.5 shows there are several large contiguous regions consistent with lateral or horizontal gene transfer as observed in other Thermotogales (Zhaxybayeva *et al.* 2009). A list of 9078 and the corresponding locus tags in DYC can be found in Table A.5. Pathways (Figures A.6, A.9, A.10, A.11, A.12) are based on the 9078 locus tags as it appeared to better fill out the pathways our study was interested in and bring attention to features in DYC that do not match.

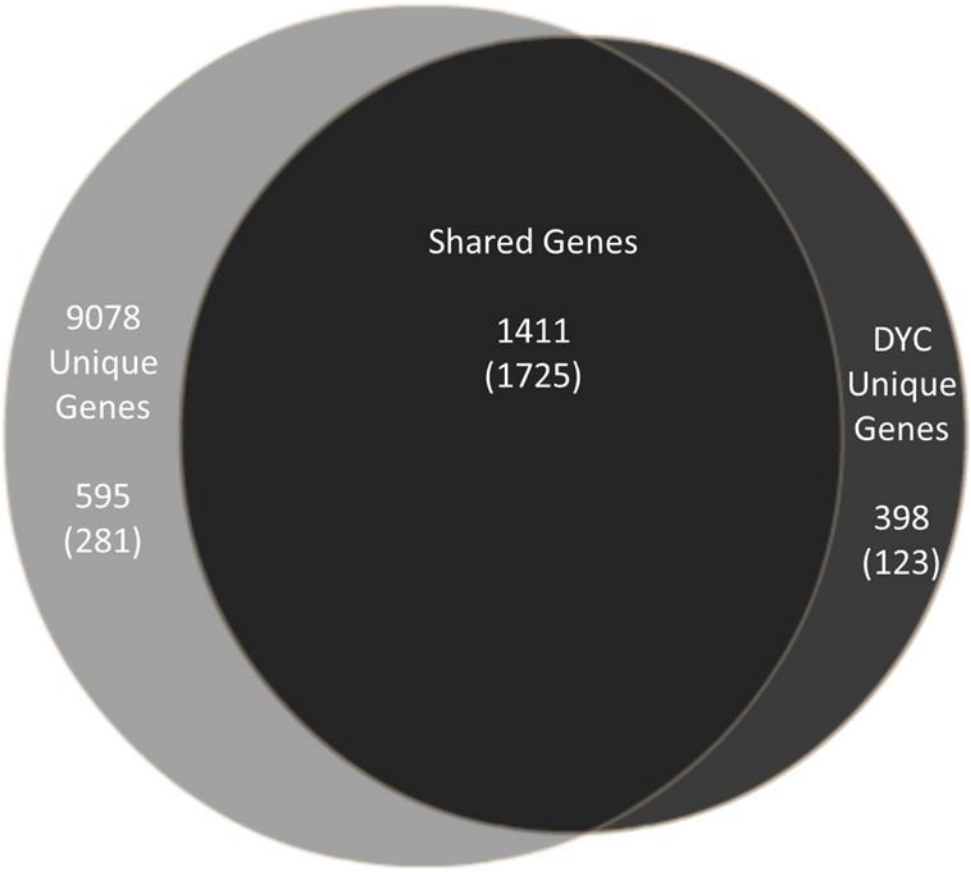


Figure A.4. Unique genes for 9078 and DYC at the ≤90% (≤60%) sequence similarity level

Table A.2. Genome summary of *F. pennivorans* strains 9078 and DYC

	9078		DYC	
	Number	% of Total	Number	% of Total
DNA, total number of bases	2166381	100.00%	2061852	100.00%
DNA coding number of bases	2002260	92.42%	1782593	86.46%
DNA G+C number of bases	842188	38.88% 1	802820	38.94% 1
DNA scaffolds	1	100.00%	1	100.00%
CRISPR Count	5		3	
Genes total number	2063	100.00%	1865	100.00%
Protein coding genes	2006	97.24%	1809	97.00%
Pseudo Genes	59	2.86%2	21	1.13%2
RNA genes	57	2.76%	56	3.00%
rRNA genes	7	0.34%	6	0.32%
5S rRNA	3	0.15%	2	0.11%
16S rRNA	2	0.10%	2	0.11%
23S rRNA	2	0.10%	2	0.11%
tRNA genes	48	2.33%	49	2.63%
Other RNA genes	2	0.10%	1	0.05%
Protein coding genes with function prediction	1621	78.57%	1464	78.50%
without function prediction	385	18.66%	345	18.50%
Protein coding genes with enzymes	614	29.76%	556	29.81%
w/o enzymes but with candidate KO based enzymes	5	0.24%	8	0.43%
Protein coding genes connected to Transporter Classification	110	5.33%	246	13.19%
Protein coding genes connected to KEGG pathways3	668	32.38%	621	33.30%
not connected to KEGG pathways	1338	64.86%	1188	63.70%
Protein coding genes connected to KEGG Orthology (KO)	1170	56.71%	1078	57.80%
not connected to KEGG Orthology (KO)	836	40.52%	731	39.20%
Protein coding genes connected to MetaCyc pathways	545	26.42%	493	26.43%
not connected to MetaCyc pathways	1461	70.82%	1316	70.56%

Protein coding genes with COGs3	1437	69.66%	1318	70.67%
with KOGs3	386	18.71%	355	19.03%
with Pfam3	1684	81.63%	1520	81.50%
with TIGRfam3	733	35.53%	694	37.21%
with InterPro	1718	83.28%	1050	56.30%
with IMG Terms	403	19.53%	325	17.43%
with IMG Pathways	132	6.40%	110	5.90%
with IMG Parts List	163	7.90%	125	6.70%
in internal clusters	267	12.94%	126	6.76%
in Chromosomal Cassette	2058	99.76%	1834	98.34%
Chromosomal Cassettes	90	-	154	-
Biosynthetic Clusters	7	-	3	-
Genes in Biosynthetic Clusters	60	2.91%	31	1.66%
Fused Protein coding genes	12	0.58%	32	1.72%
Protein coding genes coding signal peptides	43	2.08%	28	1.50%
Protein coding genes coding transmembrane proteins	602	29.18%	524	28.10%
COG clusters	1035	72.03%	993	75.34%
KOG clusters	297	20.67%	295	22.38%
Pfam clusters	1351	80.23%	1299	85.46%
TIGRfam clusters	670	91.41%	655	94.38%
Internal clusters	109		45	

Table A.3. Genes unique to 9078 when compared to DYC at a ≤60% sequence similarity cut off.

Locus Tag	Gene description
Ferpe_0018	PAS domain S-box-containing protein
Ferpe_0020	Transposase InsO and inactivated derivatives
Ferpe_0081	two-component system, OmpR family, phosphate regulon sensor histidine kinase PhoR
Ferpe_0084	ribonuclease HI
Ferpe_0103	Glycosyltransferase involved in cell wall bisynthesis
Ferpe_0107	Glycosyltransferase involved in cell wall bisynthesis
Ferpe_0108	oligosaccharide repeat unit polymerase
Ferpe_0109	Glycosyltransferase involved in cell wall bisynthesis
Ferpe_0111	hypothetical protein
Ferpe_0112	Glycosyltransferase involved in cell wall bisynthesis
Ferpe_0113	hypothetical protein
Ferpe_0114	Glycosyltransferase involved in cell wall bisynthesis
Ferpe_0115	glucose-1-phosphate thymidylyltransferase
Ferpe_0116	dTDP-4-amino-4,6-dideoxygalactose transaminase
Ferpe_0117	galactoside O-acetyltransferase
Ferpe_0118	dTDP-glucose 4,6-dehydratase
Ferpe_0120	hypothetical protein
Ferpe_0124	Transposase and inactivated derivatives
Ferpe_0128	hypothetical protein
Ferpe_0133	Lactate dehydrogenase
Ferpe_0134	gluconokinase
Ferpe_0135	GntR family transcriptional regulator, transcriptional repressor for pyruvate dehydrogenase complex
Ferpe_0136	2-dehydro-3-deoxyphosphogluconate aldolase / (4S)-4-hydroxy-2-oxoglutarate aldolase
Ferpe_0137	gluconokinase
Ferpe_0138	putative tricarboxylic transport membrane protein
Ferpe_0139	Tripartite-type tricarboxylate transporter, receptor component TctC

Ferpe_0140	Tripartite tricarboxylate transporter TctB family protein
Ferpe_0141	6-phosphogluconate dehydrogenase
Ferpe_0144	transcriptional attenuator, LytR family
Ferpe_0146	hypothetical protein
Ferpe_0149	mannose-1-phosphate guanylyltransferase
Ferpe_0152	ribokinase
Ferpe_0153	transcriptional regulator, LacI family
Ferpe_0154	monosaccharide ABC transporter substrate-binding protein, CUT2 family
Ferpe_0155	ribose transport system permease protein
Ferpe_0156	monosaccharide ABC transporter ATP-binding protein, CUT2 family
Ferpe_0157	D-ribose pyranase
Ferpe_0158	putative pyruvate formate lyase activating enzyme
Ferpe_0159	hypothetical protein
Ferpe_0161	hypothetical protein
Ferpe_0194	carbohydrate ABC transporter membrane protein 1, CUT1 family
Ferpe_0252	N-acetylglucosaminyl deacetylase, LmbE family
Ferpe_0254	hypothetical protein
Ferpe_0308	hypothetical protein
Ferpe_0356	hypothetical protein
Ferpe_0366	Transposase InsO and inactivated derivatives
Ferpe_0391	DNA-binding transcriptional regulator, MarR family
Ferpe_0403	Transposase
Ferpe_0410	Sugar kinase of the NBD/HSP70 family, may contain an N-terminal HTH domain
Ferpe_0451	hypothetical protein
Ferpe_0458	hypothetical protein
Ferpe_0480	flagellar protein FliO/FliZ
Ferpe_0512	UDP-GlcNAc:undecaprenyl-phosphate GlcNAc-1-phosphate transferase
Ferpe_0548	hypothetical protein
Ferpe_0549	hypothetical protein

Ferpe_0550	Protein of unknown function (DUF3298)
Ferpe_0554	type I restriction enzyme, S subunit
Ferpe_0555	type I restriction enzyme, R subunit
Ferpe_0556	hypothetical protein
Ferpe_0557	hypothetical protein
Ferpe_0560	hypothetical protein
Ferpe_0563	hypothetical protein
Ferpe_0564	DNA phosphorothioation system restriction enzyme
Ferpe_0565	hypothetical protein
Ferpe_0566	DNA sulfur modification protein DndD
Ferpe_0567	hypothetical protein
Ferpe_0568	Adenine specific DNA methylase Mod
Ferpe_0569	Type I site-specific restriction-modification system, R (restriction) subunit and related helicases
Ferpe_0572	hypothetical protein
Ferpe_0574	Outer membrane protein assembly factor BamB, contains PQQ-like beta-propeller repeat
Ferpe_0575	PAS domain S-box-containing protein
Ferpe_0578	Alpha/beta hydrolase family protein
Ferpe_0579	hypothetical protein
Ferpe_0581	glutamine amidotransferase
Ferpe_0584	CAAX protease self-immunity
Ferpe_0585	hypothetical protein
Ferpe_0586	hypothetical protein
Ferpe_0587	hypothetical protein
Ferpe_0588	putative transposase
Ferpe_0589	CAAX protease self-immunity
Ferpe_0590	hypothetical protein
Ferpe_0591	hypothetical protein
Ferpe_0592	CAAX protease self-immunity
Ferpe_0593	hypothetical protein
Ferpe_0619	prepilin-type N-terminal cleavage/methylation domain-containing protein

Ferpe_0657	Putative F0F1-ATPase subunit Ca2+/Mg2+ transporter
Ferpe_0665	Diguanylate cyclase, GGDEF domain
Ferpe_0667	signal peptidase II
Ferpe_0668	Cd2+/Zn2+-exporting ATPase
Ferpe_0669	transcriptional regulator, ArsR family
Ferpe_0670	Site-specific DNA recombinase
Ferpe_0671	hypothetical protein
Ferpe_0672	Recombinase zinc beta ribbon domain-containing protein
Ferpe_0677	hypothetical protein
Ferpe_0685	Transposase
Ferpe_0686	DNA replication protein DnaC
Ferpe_0689	Acetyltransferases, including N-acetylases of ribosomal proteins
Ferpe_0691	NAD+ synthase (glutamine-hydrolysing)
Ferpe_0695	thiamine diphosphokinase
Ferpe_0697	regulator of sigma E protease
Ferpe_0701	large conductance mechanosensitive channel
Ferpe_0702	Beta-propeller repeat-containing protein
Ferpe_0705	anaerobic ribonucleoside-triphosphate reductase activating protein
Ferpe_0706	serine O-acetyltransferase
Ferpe_0707	cysteine synthase A
Ferpe_0708	butyryl-CoA dehydrogenase
Ferpe_0709	electron transfer flavoprotein beta subunit
Ferpe_0710	electron transfer flavoprotein alpha subunit apoprotein
Ferpe_0712	Outer membrane protein assembly factor BamB, contains PQQ-like beta-propeller repeat
Ferpe_0713	hypothetical protein
Ferpe_0714	Outer membrane protein assembly factor BamB, contains PQQ-like beta-propeller repeat
Ferpe_0715	VanZ like family protein
Ferpe_0716	hypothetical protein
Ferpe_0717	Uncharacterized conserved protein, DUF58 family, contains vWF domain
Ferpe_0721	hypothetical protein

Ferpe_0729	Transposase
Ferpe_0730	DNA replication protein DnaC
Ferpe_0731	protein of unknown function
Ferpe_0732	hypothetical protein
Ferpe_0736	transcriptional attenuator, LytR family
Ferpe_0738	Predicted ATPase, AAA+ ATPase superfamily
Ferpe_0740	opine dehydrogenase
Ferpe_0741	Predicted cobalamin binding protein
Ferpe_0742	D-alanine-D-alanine ligase
Ferpe_0744	hypothetical protein
Ferpe_0745	hypothetical protein
Ferpe_0746	hypothetical protein
Ferpe_0747	Uncharacterized protein YydD, contains DUF2326 domain
Ferpe_0748	adenine-specific DNA-methyltransferase
Ferpe_0749	type III restriction enzyme
Ferpe_0785	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_0807	hypothetical protein
Ferpe_0808	multiple sugar transport system permease protein
Ferpe_0809	ABC-type sugar transport system, permease component
Ferpe_0810	hypothetical protein
Ferpe_0811	Gluconolactonase
Ferpe_0814	ABC-type glycerol-3-phosphate transport system, substrate-binding protein
Ferpe_0815	multiple sugar transport system substrate-binding protein
Ferpe_0816	Cellobiose phosphorylase
Ferpe_0817	beta-galactosidase
Ferpe_0818	transcriptional regulator, LacI family
Ferpe_0826	TusA-related sulfurtransferase
Ferpe_0827	tRNA 2-thiouridine synthesizing protein B
Ferpe_0828	tRNA 2-thiouridine synthesizing protein C
Ferpe_0829	tRNA 2-thiouridine synthesizing protein D
Ferpe_0830	hypothetical protein

Ferpe_0831	TusA-related sulfurtransferase
Ferpe_0847	transcriptional regulator, LacI family
Ferpe_0848	beta-galactosidase
Ferpe_0896	HDIG domain-containing protein
Ferpe_0905	hypothetical protein
Ferpe_0906	Predicted heme/steroid binding protein
Ferpe_1019	Uncharacterized lipoprotein YddW, UPF0748 family
Ferpe_1028	hypothetical protein
Ferpe_1056	Rod binding protein
Ferpe_1061	acyl-phosphate glycerol 3-phosphate acyltransferase
Ferpe_1062	hypothetical protein
Ferpe_1067	Predicted oxidoreductases of the aldo/keto reductase family
Ferpe_1068	hypothetical protein
Ferpe_1069	Transposase
Ferpe_1070	DNA replication protein DnaC
Ferpe_1071	hypothetical protein
Ferpe_1072	ATP-binding cassette, subfamily C
Ferpe_1073	ABC transporter transmembrane region
Ferpe_1074	ATP-binding cassette, subfamily C
Ferpe_1082	hypothetical protein
Ferpe_1101	hypothetical protein
Ferpe_1102	4-alpha-L-fucosyltransferase glycosyl transferase group 56
Ferpe_1103	Glucose-1-phosphate thymidylyltransferase
Ferpe_1104	dTDP-4-amino-4,6-dideoxygalactose transaminase
Ferpe_1105	galactoside O-acetyltransferase
Ferpe_1106	dTDP-glucose 4,6-dehydratase
Ferpe_1107	hypothetical protein
Ferpe_1109	Membrane protein involved in the export of O-antigen and teichoic acid
Ferpe_1110	Glycosyl transferases group 1
Ferpe_1111	Glycosyltransferases, probably involved in cell wall biogenesis
Ferpe_1112	osmoprotectant transport system substrate-binding protein

Ferpe_1113	osmoprotectant transport system permease protein
Ferpe_1114	osmoprotectant transport system ATP-binding protein
Ferpe_1115	osmoprotectant transport system permease protein
Ferpe_1127	Transposase
Ferpe_1133	S-layer homology domain-containing protein
Ferpe_1145	poly-gamma-glutamate synthesis protein (capsule biosynthesis protein)
Ferpe_1160	butyryl-CoA dehydrogenase
Ferpe_1161	electron transfer flavoprotein beta subunit
Ferpe_1162	electron transfer flavoprotein alpha subunit apoprotein
Ferpe_1163	Major Facilitator Superfamily protein
Ferpe_1170	Uncharacterized protein YvpB
Ferpe_1180	PQQ-like domain-containing protein
Ferpe_1199	H+/Cl- antiporter ClcA
Ferpe_1219	hypothetical protein
Ferpe_1223	hypothetical protein
Ferpe_1224	prepilin-type N-terminal cleavage/methylation domain-containing protein
Ferpe_1225	hypothetical protein
Ferpe_1226	Repeat domain-containing protein
Ferpe_1228	transcriptional regulator, LacI family
Ferpe_1229	ribose 5-phosphate isomerase B
Ferpe_1230	hypothetical protein
Ferpe_1231	alpha-glucosidase
Ferpe_1232	transketolase
Ferpe_1233	transketolase
Ferpe_1234	alcohol dehydrogenase
Ferpe_1235	EamA-like transporter family protein
Ferpe_1236	oligogalacturonide transporter
Ferpe_1237	multiple sugar transport system permease protein
Ferpe_1238	carbohydrate ABC transporter membrane protein 1, CUT1 family
Ferpe_1239	multiple sugar transport system substrate-binding protein
Ferpe_1342	Serine protease, subtilisin family

Ferpe_1343	Carboxypeptidase regulatory-like domain-containing protein
Ferpe_1360	RNAse III
Ferpe_1456	hypothetical protein
Ferpe_1461	peptide/nickel transport system permease protein
Ferpe_1476	hypothetical protein
Ferpe_1481	hypothetical protein
Ferpe_1483	AAA domain-containing protein
Ferpe_1488	predicted D-glycerate permease
Ferpe_1503	segregation and condensation protein B
Ferpe_1524	peptidoglycan L-alanyl-D-glutamate endopeptidase CwlK
Ferpe_1525	hypothetical protein
Ferpe_1526	Helix-turn-helix
Ferpe_1527	Dipeptidyl aminopeptidase/acylaminoacyl peptidase
Ferpe_1528	peptidoglycan L-alanyl-D-glutamate endopeptidase CwlK
Ferpe_1536	CRISPR-associated protein, Cmr2 family
Ferpe_1537	CRISPR-associated protein, Cmr1 family
Ferpe_1540	CRISPR-associated endoribonuclease Cas6
Ferpe_1568	hypothetical protein
Ferpe_1569	hypothetical protein
Ferpe_1570	hypothetical protein
Ferpe_1573	hypothetical protein
Ferpe_1575	hypothetical protein
Ferpe_1576	hypothetical protein
Ferpe_1577	hypothetical protein
Ferpe_1578	deoxyribodipyrimidine photo-lyase
Ferpe_1579	hypothetical protein
Ferpe_1580	AAA-like domain-containing protein
Ferpe_1585	hypothetical protein
Ferpe_1614	LAO/AO transport system kinase
Ferpe_1658	hypothetical protein
Ferpe_1666	hypothetical protein
Ferpe_1676	Type II secretory pathway, component PulD

Ferpe_1681	Iron-regulated ABC transporter permease protein SufD
Ferpe_1684	energy-coupling factor transport system substrate-specific component
Ferpe_1685	energy-coupling factor transport system permease protein
Ferpe_1686	energy-coupling factor transport system ATP-binding protein
Ferpe_1687	energy-coupling factor transport system ATP-binding protein
Ferpe_1708	energy-coupling factor transport system permease protein
Ferpe_1711	DNA methylase
Ferpe_1713	Transposase
Ferpe_1721	hypothetical protein
Ferpe_1727	phosphoribosyl-ATP pyrophosphatase
Ferpe_1730	regulatory protein, Fis family
Ferpe_1733	ATP-dependent exoDNAse (exonuclease V) beta subunit (contains helicase and exonuclease domains)
Ferpe_1745	hypothetical protein
Ferpe_1768	hypothetical protein
Ferpe_1803	transcriptional regulator, MarR family
Ferpe_1820	Na+-dependent transporter, SNF family
Ferpe_1821	hypothetical protein
Ferpe_1822	hypothetical protein
Ferpe_1850	Transposase
Ferpe_1859	Glycosyltransferase involved in cell wall bisynthesis
Ferpe_1860	hypothetical protein
Ferpe_1861	ADP-heptose:LPS heptosyltransferase
Ferpe_1862	hypothetical protein
Ferpe_1863	arabinose-5-phosphate isomerase
Ferpe_1864	Nucleoside-diphosphate-sugar pyrophosphorylase involved in lipopolysaccharide biosynthesis/translation initiation factor 2B, gamma/epsilon subunits (eIF-2Bgamma/eIF-2Bepsilon)
Ferpe_1895	cell division protein FtsW
Ferpe_1923	S-layer homology domain-containing protein
Ferpe_1929	Glyoxylase, beta-lactamase superfamily II
Ferpe_1932	Signal transduction histidine kinase

Ferpe_1937	putative pyruvate formate lyase activating enzyme
Ferpe_1951	protein of unknown function (DUF4897)
Ferpe_1957	PhoPQ-activated pathogenicity-related protein
Ferpe_1958	Cache 3/Cache 2 fusion domain-containing protein
Ferpe_1996	hypothetical protein
Ferpe_1997	Micrococcal nuclease (thermonuclease) homologs
Ferpe_1998	The GLUG motif-containing protein
Ferpe_2001	micrococcal nuclease
Ferpe_2021	multicomponent Na+:H+ antiporter subunit F
Ferpe_2039	hypothetical protein
Ferpe_2059	cell division protein FtsI (penicillin-binding protein 3)
Ferpe_2064	D-amino peptidase

Table A.4. Genes Unique to DYC when Compared to 9078 at a ≤60% Sequence Similarity cut Off

Locus Tag	Gene description
JM64_00410	hypothetical protein
JM64_00535	hypothetical protein
JM64_00540	hypothetical protein
JM64_00545	Cupin domain-containing protein
JM64_00785	CRISPR-associated protein, Cmr1 family
JM64_00905	hypothetical protein
JM64_01180	Polysaccharide deacetylase
JM64_01335	hypothetical protein
JM64_01585	hypothetical protein
JM64_01610	hypothetical protein
JM64_01615	Membrane protein involved in the export of O-antigen and teichoic acid
JM64_01620	Glycosyltransferase involved in cell wall bisynthesis
JM64_01625	Glycosyltransferase involved in cell wall bisynthesis
JM64_01635	hypothetical protein
JM64_01645	hypothetical protein
JM64_01650	hypothetical protein
JM64_01665	Fic/DOC family protein
JM64_01670	hypothetical protein
JM64_01680	hypothetical protein
JM64_01690	hypothetical protein
JM64_01695	Coenzyme F420 hydrogenase/dehydrogenase, beta subunit C terminus
JM64_01700	Polysaccharide pyruvyl transferase
JM64_01705	hypothetical protein
JM64_01720	hypothetical protein
JM64_01730	hypothetical protein
JM64_01735	Glycosyltransferase involved in cell wall bisynthesis
JM64_01740	hypothetical protein
JM64_01745	O-antigen ligase like membrane protein

JM64_01750	Glycosyltransferase involved in cell wall bisynthesis
JM64_01760	hypothetical protein
JM64_01780	Glycosyltransferase involved in cell wall bisynthesis
JM64_02145	LVIVD repeat-containing protein
JM64_02485	hypothetical protein
JM64_02490	Na+-transporting NADH:ubiquinone oxidoreductase subunit F
JM64_02495	Na+-transporting NADH:ubiquinone oxidoreductase subunit E
JM64_02750	Acyl-coenzyme A:6-aminopenicillanic acid acyl-transferase
JM64_02755	two component transcriptional regulator, LytTR family
JM64_02760	Histidine kinase
JM64_02765	hypothetical protein
JM64_02775	hypothetical protein
JM64_02825	hypothetical protein
JM64_02940	beta-galactosidase
JM64_03215	hypothetical protein
JM64_03220	Transposase and inactivated derivatives
JM64_03225	hypothetical protein
JM64_03280	hypothetical protein
JM64_03335	hypothetical protein
JM64_03375	transposase, IS4 family
JM64_03630	prepilin-type N-terminal cleavage/methylation domain-containing protein
JM64_03680	peptide/nickel transport system ATP-binding protein
JM64_03685	ABC-2 type transport system permease protein
JM64_03690	ABC-2 type transport system ATP-binding protein
JM64_03695	carbohydrate ABC transporter substrate-binding protein, CUT1 family
JM64_03825	hypothetical protein
JM64_03840	Mrr N-terminal domain-containing protein
JM64_03845	Mrr N-terminal domain-containing protein
JM64_03850	TaqI-like C-terminal specificity domain-containing protein
JM64_03855	SNF2 family N-terminal domain-containing protein
JM64_03860	UvrD-like helicase C-terminal domain-containing protein
JM64_03865	UvrD/REP helicase N-terminal domain-containing protein

JM64_03880	ATP-dependent DNA helicase RecG
JM64_03900	alkylhydroperoxidase AhpD family core domain-containing protein
JM64_03925	ATP-dependent DNA helicase RecG
JM64_03935	hypothetical protein
JM64_04055	anti-anti-sigma factor
JM64_04260	hypothetical protein
JM64_04425	carbohydrate ABC transporter substrate-binding protein, CUT1 family
JM64_04430	carbohydrate ABC transporter membrane protein 1, CUT1 family
JM64_04450	hypothetical protein
JM64_04720	hypothetical protein
JM64_05330	hypothetical protein
JM64_05340	Protein of unknown function (DUF4230)
JM64_05345	Protein of unknown function (DUF4230)
JM64_05975	Tocopherol cyclase
JM64_06240	hypothetical protein
JM64_06425	hypothetical protein
JM64_06635	peptide/nickel transport system ATP-binding protein
JM64_06640	peptide/nickel transport system ATP-binding protein
JM64_06645	peptide/nickel transport system permease protein
JM64_06650	peptide/nickel transport system permease protein
JM64_06655	peptide/nickel transport system substrate-binding protein
JM64_06725	hypothetical protein
JM64_06735	hypothetical protein
JM64_06740	UDP-galactopyranose mutase
JM64_07095	hypothetical protein
JM64_07290	Sugar kinase of the NBD/HSP70 family, may contain an N-terminal HTH domain
JM64_07295	putative multiple sugar transport system permease protein
JM64_07300	putative multiple sugar transport system ATP-binding protein
JM64_07305	monosaccharide ABC transporter substrate-binding protein, CUT2 family
JM64_07520	branched-chain amino acid transport system ATP-binding protein
JM64_07525	branched-chain amino acid transport system permease protein

JM64_07530	branched-chain amino acid transport system permease protein
JM64_07535	branched-chain amino acid transport system substrate-binding protein
JM64_07540	branched-chain amino acid transport system substrate-binding protein
JM64_07825	D-amino peptidase
JM64_08210	hypothetical protein
JM64_08235	peptide/nickel transport system substrate-binding protein
JM64_08240	peptide/nickel transport system permease protein
JM64_08245	peptide/nickel transport system permease protein
JM64_08250	peptide/nickel transport system ATP-binding protein
JM64_08255	peptide/nickel transport system ATP-binding protein
JM64_08260	BadF-type ATPase
JM64_08265	GntR family transcriptional regulator
JM64_08275	beta-N-acetylhexosaminidase
JM64_08280	Uncharacterized conserved protein YbbC, DUF1343 family
JM64_08285	peptide/nickel transport system substrate-binding protein
JM64_08290	peptide/nickel transport system permease protein
JM64_08300	peptide/nickel transport system permease protein
JM64_08305	oligopeptide transport system ATP-binding protein
JM64_08310	peptide/nickel transport system ATP-binding protein
JM64_08315	CubicO group peptidase, beta-lactamase class C family
JM64_08345	hypothetical protein
JM64_08865	2',3'-cyclic-nucleotide 2'-phosphodiesterase / 3'-nucleotidase
JM64_08880	hypothetical protein
JM64_08910	hypothetical protein
JM64_09725	Serine protease, subtilisin family
JM64_09730	hypothetical protein
JM64_09910	HD domain-containing protein
JM64_09915	hypothetical protein
JM64_09945	hypothetical protein
JM64_09950	hypothetical protein
JM64_09955	hypothetical protein
JM64_09960	hypothetical protein

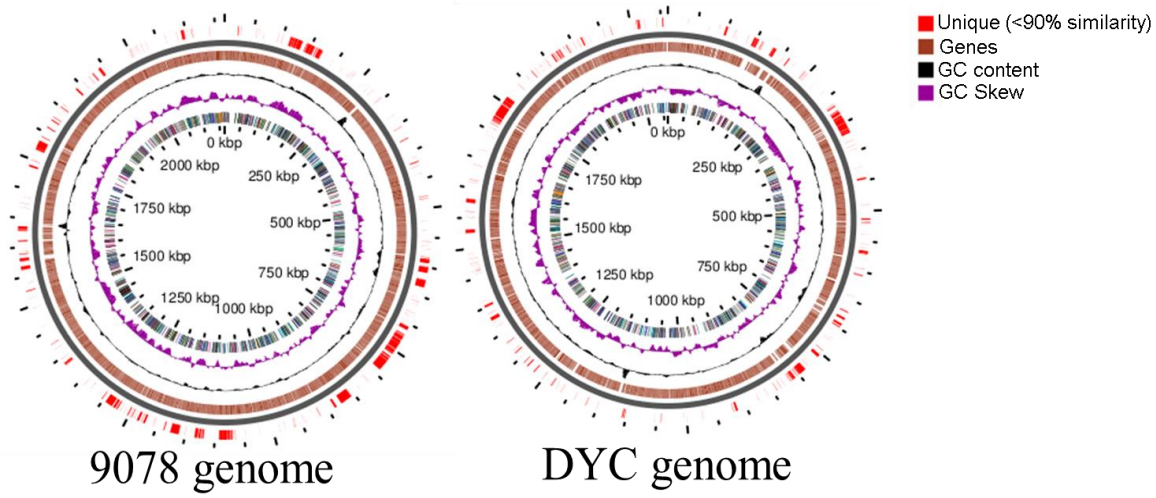


Figure A.5. 9078 and DYC unique genome analysis Areas in red are regions that do not match the corresponding strain at a ≤90% sequence similarity cut off

Table A.5. 9078 and DYC corresponding locus tags at a ≥60% sequence similarity

9078 Locus Tag	DYC Locus Tag	Gene description
Ferpe_0001	JM64_07830	chromosomal replication initiator protein DnaA
Ferpe_0002	JM64_07835	ferredoxin
Ferpe_0003	JM64_07840	hypothetical protein
Ferpe_0004	JM64_07845	nucleoside-binding protein
Ferpe_0005	JM64_07850	nucleoside ABC transporter ATP-binding protein
Ferpe_0006	JM64_07855	nucleoside ABC transporter membrane protein
Ferpe_0007	JM64_07860	nucleoside ABC transporter membrane protein
Ferpe_0008	JM64_07865	secondary thiamine-phosphate synthase enzyme
Ferpe_0009	JM64_07870	DNA-binding protein HU-beta
Ferpe_0010	JM64_07875	HDIG domain-containing protein
Ferpe_0011	JM64_07880	FecR family protein
Ferpe_0012	JM64_07885	Protein of unknown function (DUF3307)
Ferpe_0013	JM64_07890	HD domain-containing protein
Ferpe_0014	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0015	JM64_07895	adenylate cyclase
Ferpe_0016	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0017	JM64_07905	secondary thiamine-phosphate synthase enzyme
Ferpe_0019	JM64_07915	Fucose permease
Ferpe_0021	JM64_07920	hypothetical protein
Ferpe_0022	JM64_07925	hypothetical protein
Ferpe_0023	JM64_07930	hypothetical protein
Ferpe_0025	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0026	JM64_07940	chaperonin GroES
Ferpe_0027	JM64_07945	chaperonin GroEL
Ferpe_0028	JM64_07950	dihydrofolate reductase
Ferpe_0029	JM64_07955	translation elongation factor 2 (EF-2/EF-G)
Ferpe_0030	JM64_07960	hypothetical protein
Ferpe_0031	JM64_07965	Flagellar hook-length control protein
Ferpe_0032	JM64_07970	flagellar basal-body rod modification protein FlgD

Ferpe_0033	JM64_07975	flagellar hook protein FlgE
Ferpe_0034	JM64_07980	flagellar protein FlbD
Ferpe_0035	JM64_07985	chemotaxis protein MotA
Ferpe_0036	JM64_07990	chemotaxis protein MotB
Ferpe_0037	JM64_07995	flagellar FliL protein
Ferpe_0038	JM64_08000	flagellar motor switch protein FliM
Ferpe_0039	JM64_08005	flagellar motor switch protein FliN/FliY
Ferpe_0040	JM64_08010	broad-specificity cellobiase
Ferpe_0041	JM64_08025	heptaprenyl diphosphate synthase
Ferpe_0042	JM64_08030	hypothetical protein
Ferpe_0043	JM64_08035	imidazolonepropionase
Ferpe_0044	JM64_08040	dITPase
Ferpe_0045	JM64_08045	excinuclease ABC subunit A
Ferpe_0046	JM64_08050	hypothetical protein
Ferpe_0047	JM64_08055	magnesium transporter
Ferpe_0048	JM64_08060	leucyl-tRNA synthetase
Ferpe_0049	JM64_08065	SurA N-terminal domain-containing protein
Ferpe_0050	JM64_08070	voltage-gated potassium channel
Ferpe_0051	JM64_08075	methylthioribose-1-phosphate isomerase
Ferpe_0052	JM64_08080	seryl-tRNA synthetase
Ferpe_0053	JM64_08085	1-deoxy-D-xylulose-5-phosphate synthase
Ferpe_0054	JM64_08090	Uncharacterized conserved protein YloU, alkaline shock protein (Asp23) family
Ferpe_0055	JM64_08095	hypothetical protein
Ferpe_0056	JM64_08100	positive regulator of sigma(E), RseC/MucC
Ferpe_0057	JM64_08105	Lysophospholipase, alpha-beta hydrolase superfamily
Ferpe_0058	JM64_08110	flagellar assembly factor FliW
Ferpe_0059	JM64_08115	carbon storage regulator, CsrA
Ferpe_0060	JM64_08120	aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C
Ferpe_0061	JM64_08125	putative endonuclease
Ferpe_0062	JM64_08130	SsrA-binding protein

Ferpe_0063	JM64_08135	deoxyribose-phosphate aldolase
Ferpe_0064	JM64_01980	glucose-1-phosphate adenylyltransferase
Ferpe_0065	JM64_01975	glucose-1-phosphate adenylyltransferase
Ferpe_0066	JM64_01970	protein of unknown function (DUF4899)
Ferpe_0067	JM64_01965	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
Ferpe_0068	JM64_01960	ribosome recycling factor
Ferpe_0069	JM64_01955	undecaprenyl diphosphate synthase
Ferpe_0070	JM64_01950	phosphatidate cytidylyltransferase
Ferpe_0071	JM64_01945	alanyl-tRNA synthetase
Ferpe_0072	JM64_01940	rod shape-determining protein MreB
Ferpe_0073	JM64_01935	hypothetical protein
Ferpe_0074	JM64_01930	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_0075	JM64_01925	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
Ferpe_0076	JM64_01920	NDP-sugar epimerase, includes UDP-GlcNAc-inverting 4,6-dehydratase FlaA1 and capsular polysaccharide biosynthesis protein EpsC
Ferpe_0077	JM64_01915	molecular chaperone Hsp33
Ferpe_0078	JM64_01910	hypothetical protein
Ferpe_0079	JM64_01905	hypothetical protein
Ferpe_0080	JM64_01900	DNA-binding response regulator, OmpR family, contains REC and winged-helix (wHTH) domain
Ferpe_0082	JM64_01890	pyrimidine-nucleoside phosphorylase
Ferpe_0083	JM64_01885	Predicted protein (DUF2233)
Ferpe_0085	JM64_01875	Predicted PurR-regulated permease PerM
Ferpe_0086	JM64_01870	methylglyoxal synthase
Ferpe_0087	JM64_01865	23S rRNA (adenine2503-C2)-methyltransferase
Ferpe_0088	JM64_01860	Thioredoxin reductase
Ferpe_0089	JM64_01850	hypothetical protein
Ferpe_0090	JM64_01845	nucleotide sugar dehydrogenase
Ferpe_0091	JM64_01840	LSU ribosomal protein L21P
Ferpe_0092	JM64_01835	hypothetical protein

Ferpe_0093	JM64_01830	LSU ribosomal protein L27P
Ferpe_0094	JM64_01825	Riboflavin transporter FmnP
Ferpe_0095	JM64_01820	LSU ribosomal protein L13P
Ferpe_0096	JM64_01815	SSU ribosomal protein S9P
Ferpe_0097	JM64_01810	DNA primase
Ferpe_0098	JM64_01805	RNA polymerase primary sigma factor
Ferpe_0099	JM64_01800	putative ABC transport system permease protein
Ferpe_0100	JM64_01795	Periplasmic protein involved in polysaccharide export
Ferpe_0101	JM64_01790	Uncharacterized protein involved in exopolysaccharide biosynthesis
Ferpe_0102	JM64_01785	Sugar transferase involved in LPS biosynthesis (colanic, teichoic acid)
Ferpe_0104	JM64_01775	UDP-glucose 4-epimerase
Ferpe_0105	JM64_01770	UDP-2-acetamido-2,6-beta-L-arabino-hexul-4-ose reductase
Ferpe_0106	JM64_01765	UDP-N-acetylglucosamine 2-epimerase (non-hydrolysing)
Ferpe_0110	JM64_07020	Transposase and inactivated derivatives
Ferpe_0119	JM64_01590	Membrane protein involved in the export of O-antigen and teichoic acid
Ferpe_0121	JM64_01580	looped-hinge helix DNA binding domain-containing protein, AbrB family
Ferpe_0122	JM64_01575	hypothetical protein
Ferpe_0123	JM64_00355	DDE domain-containing protein
Ferpe_0125	JM64_01570	hypothetical protein
Ferpe_0126	JM64_01565	hypothetical protein
Ferpe_0127	JM64_01630	ATPase
Ferpe_0129	JM64_01550	UDP-N-acetylglucosamine 2-epimerase (non-hydrolysing)
Ferpe_0130	JM64_01545	hypothetical protein
Ferpe_0131	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0132	JM64_01540	UDP-N-acetylglucosamine 2-epimerase (non-hydrolysing)
Ferpe_0142	JM64_01530	hypothetical protein

Ferpe_0143	JM64_01540	UDP-N-acetylglucosamine 2-epimerase
Ferpe_0145	JM64_01530	Glycosyltransferase, catalytic subunit of cellulose synthase and poly-beta-1,6-N-acetylglucosamine synthase
Ferpe_0147	JM64_01525	hypothetical protein
Ferpe_0148	JM64_01520	putative hydrolase of the HAD superfamily
Ferpe_0150	JM64_01515	amidase
Ferpe_0151	JM64_01510	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase
Ferpe_0160	JM64_01505	Transglutaminase-like superfamily protein
Ferpe_0164	JM64_01485	GTP-binding protein
Ferpe_0165	JM64_01480	nicotinate-nucleotide adenylyltransferase
Ferpe_0166	JM64_01475	putative TIM-barrel protein, nifR3 family
Ferpe_0167	JM64_01470	probable phosphoglycerate mutase
Ferpe_0168	JM64_01465	hypothetical protein
Ferpe_0169	JM64_01460	spermidine/putrescine transport system ATP-binding protein
Ferpe_0170	JM64_01455	spermidine/putrescine transport system permease protein
Ferpe_0171	JM64_01450	spermidine/putrescine transport system permease protein
Ferpe_0172	JM64_01445	purine-nucleoside phosphorylase
Ferpe_0173	JM64_01440	methyl-accepting chemotaxis protein
Ferpe_0174	JM64_01435	tRNA modification GTPase trmE
Ferpe_0175	JM64_01430	flagellar biosynthesis protein FlhA
Ferpe_0176	JM64_01425	flagellar biosynthesis protein FlhF
Ferpe_0177	JM64_01420	flagellar biosynthesis protein FlhG
Ferpe_0178	JM64_01415	PilZ domain-containing protein
Ferpe_0179	JM64_01410	c-di-GMP-binding flagellar brake protein YcgR, contains PilZNR and PilZ domains
Ferpe_0180	JM64_01405	chemotaxis protein CheC
Ferpe_0181	JM64_01400	chemotaxis protein CheD
Ferpe_0182	JM64_01395	RNA polymerase, sigma 28 subunit, SigD/FliA/WhiG
Ferpe_0183	JM64_01390	hypothetical protein

Ferpe_0184	JM64_01385	hypothetical protein
Ferpe_0185	JM64_01380	hypothetical protein
Ferpe_0186	JM64_01375	electron transport complex protein RnfC
Ferpe_0187	JM64_01370	electron transport complex protein RnfD
Ferpe_0188	JM64_01365	electron transport complex protein RnfG
Ferpe_0189	JM64_01360	electron transport complex protein RnfE
Ferpe_0190	JM64_01355	electron transport complex protein RnfA
Ferpe_0191	JM64_01350	polyribonucleotide nucleotidyltransferase
Ferpe_0192	JM64_01345	Predicted Zn-dependent peptidase
Ferpe_0193	JM64_01340	ATP-dependent HslUV protease ATP-binding subunit HslU
Ferpe_0195	JM64_01330	carbohydrate ABC transporter membrane protein 2, CUT1 family
Ferpe_0196	JM64_01325	Na+/melibiose symporter
Ferpe_0197	JM64_01320	riboflavin kinase / FMN adenylyltransferase
Ferpe_0198	JM64_01315	DNA-(apurinic or apyrimidinic site) lyase /endonuclease III
Ferpe_0199	JM64_01310	flagellar hook-associated protein 2
Ferpe_0200	JM64_01305	flagellar protein FlaG
Ferpe_0201	JM64_01300	ribosome-binding factor A
Ferpe_0202	JM64_01295	HDIG domain-containing protein
Ferpe_0203	JM64_01290	two-component system, OmpR family, sensor kinase
Ferpe_0204	JM64_01285	arginyl-tRNA synthetase
Ferpe_0205	JM64_01280	GTP-binding protein
Ferpe_0206	JM64_01275	ATP-dependent Lon protease
Ferpe_0207	JM64_01270	tRNA threonylcarbamoyladenosine biosynthesis protein TsaE
Ferpe_0208	JM64_01265	glycerol kinase
Ferpe_0209	JM64_01260	Protein of unknown function (DUF2905)
Ferpe_0210	JM64_01255	Cell fate regulator YaaT, PSP1 superfamily (controls sporulation, competence, biofilm development)
Ferpe_0211	JM64_01250	DNA polymerase-3 subunit delta'

Ferpe_0212	JM64_01245	geranylgeranyl diphosphate synthase, type I
Ferpe_0213	JM64_01240	glycogen operon protein
Ferpe_0214	JM64_01235	hypothetical protein
Ferpe_0215	JM64_01230	endoribonuclease L-PSP
Ferpe_0216	JM64_01225	energy-coupling factor transport system ATP-binding protein
Ferpe_0217	JM64_01215	Uncharacterized membrane-anchored protein YitT, contains DUF161 and DUF2179 domains
Ferpe_0218	JM64_01210	methyl-accepting chemotaxis sensory transducer with Cache sensor
Ferpe_0219	JM64_01205	acetyl-CoA C-acetyltransferase
Ferpe_0220	JM64_08015	Transposase (or an inactivated derivative)
Ferpe_0221	JM64_01200	3-oxoacyl-[acyl-carrier-protein] reductase
Ferpe_0222	JM64_01195	polar amino acid transport system substrate-binding protein
Ferpe_0223	JM64_01190	thiamine transport system substrate-binding protein
Ferpe_0224	JM64_01175	Predicted arabinose efflux permease, MFS family
Ferpe_0225	JM64_01170	putative efflux protein, MATE family
Ferpe_0226	JM64_01165	Predicted arabinose efflux permease, MFS family
Ferpe_0227	JM64_01160	ATP-dependent Clp protease ATP-binding subunit ClpC
Ferpe_0230	JM64_01145	SSU ribosomal protein S6P
Ferpe_0231	JM64_01140	single-strand binding protein
Ferpe_0232	JM64_01135	SSU ribosomal protein S18P
Ferpe_0233	JM64_01130	cysteinyl-tRNA synthetase, unknown class
Ferpe_0234	JM64_01125	GTP-binding protein Era
Ferpe_0235	JM64_01120	DNA polymerase III, delta subunit
Ferpe_0236	JM64_01115	putative ABC transport system ATP-binding protein
Ferpe_0237	JM64_01110	Phosphopantetheine adenylyltransferase
Ferpe_0238	JM64_01105	replicative DNA helicase
Ferpe_0239	JM64_01100	apolipoprotein N-acyltransferase
Ferpe_0240	JM64_01095	dTDP-4-amino-4,6-dideoxygalactose transaminase
Ferpe_0241	JM64_01090	hypothetical protein

Ferpe_0242	JM64_01085	cation diffusion facilitator family transporter
Ferpe_0243	JM64_01080	LSU ribosomal protein L20P
Ferpe_0244	JM64_01075	large subunit ribosomal protein L35
Ferpe_0245	JM64_01070	translation initiation factor IF-3
Ferpe_0249	JM64_01050	UDP-N-acetylmuramyl pentapeptide phosphotransferase/UDP-N-acetylglucosamine-1-phosphate transferase
Ferpe_0250	JM64_01045	O-antigen ligase
Ferpe_0251	JM64_01040	Predicted peptidase
Ferpe_0253	JM64_01030	CrcB protein
Ferpe_0255	JM64_01020	hypothetical protein
Ferpe_0256	JM64_01015	hypothetical protein
Ferpe_0257	JM64_01010	cystathione beta-lyase
Ferpe_0258	JM64_01000	GMP synthase (glutamine-hydrolysing)
Ferpe_0259	JM64_00995	N-acetyl-beta-hexosaminidase
Ferpe_0260	JM64_00990	thiamine transport system permease protein
Ferpe_0261	JM64_00985	phosphomannomutase
Ferpe_0262	JM64_00980	phenazine biosynthesis protein PhzF family
Ferpe_0263	JM64_00975	phosphoesterase, MJ0936 family
Ferpe_0269	JM64_05390	methylenetetrahydrofolate--tRNA-(uracil-5-)-methyltransferase
Ferpe_0270	JM64_05385	putative efflux protein, MATE family
Ferpe_0271	JM64_05380	protein-L-isoaspartate(D-aspartate) O-methyltransferase
Ferpe_0272	JM64_05375	cation:H+ antiporter
Ferpe_0273	JM64_05370	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_0274	JM64_05365	glyceraldehyde-3-phosphate dehydrogenase (NAD+)
Ferpe_0275	JM64_07410	Transposase
Ferpe_0276	JM64_05360	phosphoglycerate kinase
Ferpe_0277	JM64_05355	triosephosphate isomerase
Ferpe_0278	JM64_05350	aspartate carbamoyltransferase
Ferpe_0279	JM64_05335	Glycosidase

Ferpe_0280	JM64_05320	Ubiquinone/menaquinone biosynthesis C-methylase UbiE
Ferpe_0281	JM64_05315	aspartate ammonia-lyase
Ferpe_0282	JM64_05310	iron-only hydrogenase maturation protein HydF
Ferpe_0283	JM64_05305	NitT/TauT family transport system substrate-binding protein
Ferpe_0284	JM64_05300	NitT/TauT family transport system ATP-binding protein
Ferpe_0285	JM64_05295	NitT/TauT family transport system permease protein
Ferpe_0286	JM64_05290	hypothetical protein
Ferpe_0287	JM64_05285	iron-only hydrogenase maturation protein HydE
Ferpe_0288	JM64_05280	hypothetical protein
Ferpe_0289	JM64_05275	iron-only hydrogenase maturation protein HydG
Ferpe_0290	JM64_05270	putative iron-only hydrogenase system regulator
Ferpe_0291	JM64_05265	NAD(P)-dependent iron-only hydrogenase catalytic subunit
Ferpe_0292	JM64_05255	NADH-quinone oxidoreductase subunit G
Ferpe_0293	JM64_05250	hypothetical protein
Ferpe_0294	JM64_05245	NagD protein
Ferpe_0295	JM64_05240	RNA polymerase, sigma 30 subunit, SigH
Ferpe_0296	JM64_05235	hypothetical protein
Ferpe_0297	JM64_05230	Predicted dehydrogenase
Ferpe_0298	JM64_05225	hypothetical protein
Ferpe_0299	JM64_05220	Peroxiredoxin
Ferpe_0300	JM64_05215	2-oxoglutarate ferredoxin oxidoreductase subunit beta
Ferpe_0301	JM64_05210	2-oxoglutarate ferredoxin oxidoreductase subunit alpha
Ferpe_0302	JM64_05205	indolepyruvate ferredoxin oxidoreductase beta subunit
Ferpe_0303	JM64_05200	indolepyruvate ferredoxin oxidoreductase alpha subunit
Ferpe_0304	JM64_05195	peptide/nickel transport system substrate-binding protein

Ferpe_0305	JM64_05190	succinyl-CoA synthetase beta subunit
Ferpe_0306	JM64_05185	succinyl-CoA synthetase alpha subunit
Ferpe_0307	JM64_05180	Uncharacterized membrane-anchored protein YitT, contains DUF161 and DUF2179 domains
Ferpe_0309	JM64_05170	aspartate semialdehyde dehydrogenase
Ferpe_0310	JM64_05165	diaminopimelate epimerase
Ferpe_0311	JM64_05160	dihydrodipicolinate synthase
Ferpe_0312	JM64_05150	dihydrodipicolinate reductase
Ferpe_0313	JM64_05145	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-acetyltransferase
Ferpe_0314	JM64_05140	aspartate kinase
Ferpe_0315	JM64_05130	acetyldiaminopimelate aminotransferase apoenzyme
Ferpe_0316	JM64_05125	energy-coupling factor transport system permease protein
Ferpe_0317	JM64_05120	hypothetical protein
Ferpe_0318	JM64_05115	hypothetical protein
Ferpe_0319	JM64_05110	N-acetyldiaminopimelate deacetylase
Ferpe_0320	JM64_05105	hypothetical protein
Ferpe_0321	JM64_05100	Predicted hydrolase of the alpha/beta superfamily
Ferpe_0322	JM64_05095	hypothetical protein
Ferpe_0323	JM64_05090	glycine hydroxymethyltransferase
Ferpe_0324	JM64_05085	Uncharacterized conserved protein, DUF1015 family
Ferpe_0325	JM64_05080	diadenylate cyclase
Ferpe_0326	JM64_05075	glucokinase
Ferpe_0327	JM64_05070	periplasmic chaperone for outer membrane proteins Skp
Ferpe_0328	JM64_05065	lipopolysaccharide export system ATP-binding protein
Ferpe_0329	JM64_05060	radical SAM-linked protein
Ferpe_0330	JM64_05055	two-component system, chemotaxis family, response regulator CheY
Ferpe_0331	JM64_05050	sigma-B regulation protein RsbU (phosphoserine phosphatase)

Ferpe_0332	JM64_05040	elongation factor P
Ferpe_0333	JM64_05035	Uncharacterized conserved protein YloU, alkaline shock protein (Asp23) family
Ferpe_0334	JM64_05030	NusB antitermination factor
Ferpe_0335	JM64_05025	hypothetical protein
Ferpe_0336	JM64_05020	dihydropteroate synthase
Ferpe_0337	JM64_05015	16S rRNA pseudouridine516 synthase
Ferpe_0338	JM64_05010	von Willebrand factor type A domain-containing protein
Ferpe_0339	JM64_05005	hypothetical protein
Ferpe_0340	JM64_05000	heat shock protein Hsp20
Ferpe_0341	JM64_04995	D-3-phosphoglycerate dehydrogenase
Ferpe_0342	JM64_04990	aspartate aminotransferase
Ferpe_0343	JM64_04985	DNA-binding transcriptional regulator, MocR family, contains an aminotransferase domain
Ferpe_0344	JM64_04980	butyrate kinase
Ferpe_0345	JM64_04975	phosphate butyryltransferase
Ferpe_0346	JM64_04970	butyrate kinase
Ferpe_0347	JM64_04965	hypothetical protein
Ferpe_0348	JM64_04960	2-oxoglutarate ferredoxin oxidoreductase subunit delta
Ferpe_0349	JM64_04955	2-oxoglutarate ferredoxin oxidoreductase subunit alpha
Ferpe_0350	JM64_04950	2-oxoglutarate ferredoxin oxidoreductase subunit beta
Ferpe_0351	JM64_04945	2-oxoglutarate ferredoxin oxidoreductase subunit gamma
Ferpe_0352	JM64_04940	hypothetical protein
Ferpe_0354	JM64_04930	IMP dehydrogenase
Ferpe_0355	JM64_04925	hypothetical protein
Ferpe_0357	JM64_04915	hypothetical protein
Ferpe_0358	JM64_04910	hypothetical protein
Ferpe_0359	JM64_04905	RNA-binding protein Hfq
Ferpe_0360	JM64_04900	GTP-binding protein HflX

Ferpe_0361	JM64_04895	Membrane proteins related to metalloendopeptidases
Ferpe_0362	JM64_04890	hypothetical protein
Ferpe_0363	JM64_04885	methionine adenosyltransferase
Ferpe_0364	JM64_04880	SSU ribosomal protein S20P
Ferpe_0365	JM64_04875	hypothetical protein
Ferpe_0367	JM64_04870	methyl-accepting chemotaxis sensory transducer with Cache sensor
Ferpe_0368	JM64_04865	histidine ammonia-lyase
Ferpe_0369	JM64_04860	cob(I)alamin adenosyltransferase
Ferpe_0370	JM64_04855	Protein N-acetyltransferase, RimJ/RimL family
Ferpe_0371	JM64_04850	protein of unknown function (DUF4911)
Ferpe_0372	JM64_04845	iron (metal) dependent repressor, DtxR family
Ferpe_0373	JM64_04840	uridine kinase
Ferpe_0374	JM64_04835	ribonuclease G
Ferpe_0375	JM64_04830	adenosylhomocysteinase
Ferpe_0376	JM64_04825	hypothetical protein
Ferpe_0377	JM64_04820	type IV pilus assembly protein PilB
Ferpe_0378	JM64_04815	cell division protein FtsZ
Ferpe_0379	JM64_04810	cell division protein FtsA
Ferpe_0380	JM64_04805	protein of unknown function (DUF4894)
Ferpe_0381	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0382	JM64_04775	DNA polymerase
Ferpe_0383	JM64_04780	iron (metal) dependent repressor, DtxR family
Ferpe_0384	JM64_04785	UDP-glucose 4-epimerase
Ferpe_0385	JM64_04790	putative ATPase
Ferpe_0386	JM64_04795	O-acetyl-ADP-ribose deacetylase (regulator of RNase III), contains Macro domain
Ferpe_0387	JM64_04800	Formylglycine-generating enzyme, required for sulfatase activity, contains SUMF1/FGE domain
Ferpe_0388	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0389	JM64_04765	ATP-binding cassette, subfamily B
Ferpe_0390	JM64_04760	ATP-binding cassette, subfamily B

Ferpe_0392	JM64_04750	hypothetical protein
Ferpe_0393	JM64_04745	Holliday junction DNA helicase subunit RuvB
Ferpe_0394	JM64_04740	hypothetical protein
Ferpe_0395	JM64_04735	YggT family protein
Ferpe_0396	JM64_04730	NAD+ kinase
Ferpe_0397	JM64_04725	phosphate uptake regulator, PhoU
Ferpe_0398	JM64_04715	Lipopolysaccharide export LptBFGC system, permease protein LptF
Ferpe_0399	JM64_04710	tRNA(Ile)-lysidine synthase
Ferpe_0400	JM64_04705	membrane protease FtsH catalytic subunit
Ferpe_0401	JM64_04700	Predicted thioesterase
Ferpe_0402	JM64_04695	Radical SAM superfamily enzyme YgiQ, UPF0313 family
Ferpe_0404	JM64_04690	hypothetical protein
Ferpe_0405	JM64_04685	peptide/nickel transport system ATP-binding protein
Ferpe_0406	JM64_04680	peptide/nickel transport system ATP-binding protein
Ferpe_0407	JM64_04675	peptide/nickel transport system permease protein
Ferpe_0408	JM64_04670	peptide/nickel transport system permease protein
Ferpe_0409	JM64_04665	peptide/nickel transport system substrate-binding protein
Ferpe_0411	JM64_04650	16S rRNA (uracil1498-N3)-methyltransferase
Ferpe_0412	JM64_04645	carbohydrate ABC transporter ATP-binding protein, CUT1 family
Ferpe_0413	JM64_04640	prevent-host-death family protein
Ferpe_0414	JM64_04635	Radical SAM superfamily enzyme, MoaA/NifB/PqqE/SkfB family
Ferpe_0415	JM64_04630	transcriptional regulator, TetR family
Ferpe_0416	JM64_04625	Arabinose efflux permease
Ferpe_0417	JM64_04620	S1 RNA binding domain protein
Ferpe_0418	JM64_04615	LSU ribosomal protein L31P
Ferpe_0419	JM64_04610	Methyl-accepting chemotaxis protein
Ferpe_0420	JM64_04605	diguanylate cyclase (GGDEF) domain-containing protein

Ferpe_0421	JM64_04600	glutamine amidotransferase
Ferpe_0422	JM64_04595	diaminopimelate decarboxylase
Ferpe_0423	JM64_04590	hypothetical protein
Ferpe_0424	JM64_04585	ATP-dependent Clp protease ATP-binding subunit ClpC
Ferpe_0425	JM64_04580	DNA repair protein RadA/Sms
Ferpe_0426	JM64_04575	EDD domain protein, DegV family
Ferpe_0427	JM64_04570	alkylhydroperoxidase AhpD family core domain-containing protein
Ferpe_0428	JM64_04565	DNA polymerase I
Ferpe_0429	JM64_04560	transporter family protein
Ferpe_0430	JM64_04555	chromate transporter
Ferpe_0431	JM64_04550	chromate transporter
Ferpe_0432	JM64_04545	Uncharacterized flavoproteins
Ferpe_0433	JM64_04540	Methyl-accepting chemotaxis protein
Ferpe_0434	JM64_04535	ferredoxin--NADP+ reductase
Ferpe_0435	JM64_04530	sulfide dehydrogenase (flavoprotein) subunit SudA
Ferpe_0436	JM64_04525	hypothetical protein
Ferpe_0437	JM64_04520	transcriptional regulator, LacI family
Ferpe_0438	JM64_04515	Beta-glucanase/Beta-glucan synthetase
Ferpe_0439	JM64_04510	hypothetical protein
Ferpe_0440	JM64_04505	beta-glucosidase
Ferpe_0441	JM64_04500	carbohydrate ABC transporter membrane protein 2, CUT1 family
Ferpe_0442	JM64_04495	carbohydrate ABC transporter membrane protein 1, CUT1 family
Ferpe_0443	JM64_04490	hypothetical protein
Ferpe_0444	JM64_04485	Uncharacterized protein conserved in bacteria
Ferpe_0445	JM64_04480	carbohydrate ABC transporter membrane protein 2, CUT1 family
Ferpe_0446	JM64_04475	carbohydrate ABC transporter membrane protein 1, CUT1 family
Ferpe_0447	JM64_04470	ABC-type glycerol-3-phosphate transport system, substrate-binding protein

Ferpe_0448	JM64_04465	ABC-type glycerol-3-phosphate transport system, substrate-binding protein
Ferpe_0449	JM64_04460	hypothetical protein
Ferpe_0450	JM64_04455	Beta-glucanase/Beta-glucan synthetase
Ferpe_0452	JM64_04445	carbohydrate ABC transporter substrate-binding protein, CUT1 family
Ferpe_0453	JM64_04440	glucosylceramidase
Ferpe_0454	JM64_04435	hypothetical protein
Ferpe_0455	JM64_04420	cell division protein FtsA
Ferpe_0456	JM64_04415	DNA mismatch repair protein MutS2
Ferpe_0457	JM64_04410	Predicted metal-dependent peptidase
Ferpe_0459	JM64_04400	undecaprenyl-diphosphatase
Ferpe_0460	JM64_04395	hypothetical protein
Ferpe_0461	JM64_04390	ATP-binding protein involved in chromosome partitioning
Ferpe_0462	JM64_04385	UDPglucose--hexose-1-phosphate uridylyltransferase
Ferpe_0463	JM64_04380	glycogen synthase (ADP-glucose)
Ferpe_0464	JM64_04375	Glyoxylase, beta-lactamase superfamily II
Ferpe_0465	JM64_04370	Rubredoxin
Ferpe_0466	JM64_04365	glutamyl-tRNA synthetase
Ferpe_0467	JM64_04360	cysteinyl-tRNA synthetase
Ferpe_0468	JM64_04355	prolyl-tRNA synthetase
Ferpe_0469	JM64_04350	tRNA-splicing ligase RtcB
Ferpe_0470	JM64_04345	Predicted arabinose efflux permease, MFS family
Ferpe_0471	JM64_04340	ornithine decarboxylase
Ferpe_0472	JM64_04335	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_0473	JM64_04330	tRNA pseudouridine38-40 synthase
Ferpe_0474	JM64_04325	hypothetical protein
Ferpe_0475	JM64_04320	23S rRNA (cytidine1920-2'-O)/16S rRNA (cytidine1409-2'-O)-methyltransferase
Ferpe_0476	JM64_04315	hypothetical protein

Ferpe_0477	JM64_04310	two-component system, chemotaxis family, sensor kinase CheA
Ferpe_0478	JM64_04305	purine-binding chemotaxis protein CheW
Ferpe_0479	JM64_04300	two-component system, chemotaxis family, response regulator CheY
Ferpe_0481	JM64_04290	flagellar biosynthetic protein FliP
Ferpe_0482	JM64_04285	cobalt-zinc-cadmium efflux system protein
Ferpe_0483	JM64_04280	NAD+ synthase (glutamine-hydrolysing)
Ferpe_0484	JM64_04275	hypothetical protein
Ferpe_0485	JM64_04270	hypothetical protein
Ferpe_0486	JM64_04265	phospholipid-binding protein, PBP family
Ferpe_0487	JM64_04255	thiamine biosynthesis protein ThiI
Ferpe_0488	JM64_04250	Rubrerythrin
Ferpe_0489	JM64_04245	fructose-bisphosphate aldolase, class II
Ferpe_0490	JM64_04240	acetate kinase
Ferpe_0491	JM64_04235	flagellar assembly protein FliH
Ferpe_0492	JM64_04230	type III secretion system ATPase, FliI/YscN
Ferpe_0493	JM64_04225	NAD-dependent deacetylase
Ferpe_0494	JM64_04220	peptide methionine sulfoxide reductase msrA/msrB
Ferpe_0495	JM64_04215	hypothetical protein
Ferpe_0496	JM64_04210	hypothetical protein
Ferpe_0497	JM64_04205	16S rRNA (adenine1518-N6/adenine1519-N6)-dimethyltransferase
Ferpe_0498	JM64_04200	hypothetical protein
Ferpe_0499	JM64_04195	tRNA-i(6)A37 thiotransferase enzyme MiaB
Ferpe_0500	JM64_04190	uridine kinase
Ferpe_0501	JM64_04185	Sugar kinase of the NBD/HSP70 family, may contain an N-terminal HTH domain
Ferpe_0502	JM64_04180	trehalose synthase (ADP-glucose)
Ferpe_0503	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0504	JM64_04175	carbohydrate ABC transporter substrate-binding protein, CUT1 family
Ferpe_0505	JM64_04170	carbohydrate ABC transporter membrane protein 1, CUT1 family

Ferpe_0506	JM64_04165	carbohydrate ABC transporter membrane protein 2, CUT1 family
Ferpe_0507	JM64_04160	fructokinase
Ferpe_0508	JM64_04155	sucrose-phosphate synthase
Ferpe_0509	JM64_04150	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase
Ferpe_0510	JM64_04145	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase
Ferpe_0511	JM64_04140	Phospho-N-acetylmuramoyl-pentapeptide-transferase
Ferpe_0513	JM64_04135	Uncharacterized conserved protein YqhQ
Ferpe_0514	JM64_04130	hypothetical protein
Ferpe_0515	JM64_04125	Stage V sporulation protein SpoVS
Ferpe_0516	JM64_04120	cysteine synthase A
Ferpe_0517	JM64_04115	serine O-acetyltransferase
Ferpe_0518	JM64_04110	Fur family transcriptional regulator, peroxide stress response regulator
Ferpe_0519	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0520	JM64_04105	Rubrerythrin
Ferpe_0521	JM64_04100	superoxide reductase
Ferpe_0522	JM64_04095	hypothetical protein
Ferpe_0523	JM64_04090	hypothetical protein
Ferpe_0524	JM64_04085	malate dehydrogenase (oxaloacetate-decarboxylating)
Ferpe_0525	JM64_04080	PAS domain S-box-containing protein/diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_0526	JM64_04075	2-phosphosulfolactate phosphatase
Ferpe_0527	JM64_04070	Iron-regulated ABC transporter ATPase subunit SufC
Ferpe_0528	JM64_04065	hypothetical protein
Ferpe_0529	JM64_04060	arsenite efflux membrane protein ArsB
Ferpe_0530	JM64_04050	hypothetical protein
Ferpe_0531	JM64_04040	Protein of unknown function (DUF1576)
Ferpe_0532	JM64_04035	hypothetical protein
Ferpe_0533	JM64_04030	Isochorismate hydrolase

Ferpe_0534	JM64_04025	hypothetical protein
Ferpe_0535	JM64_04020	conserved hypothetical protein
Ferpe_0536	JM64_04015	acetate CoA/acetoacetate CoA-transferase alpha subunit
Ferpe_0537	JM64_04010	acetate CoA/acetoacetate CoA-transferase beta subunit
Ferpe_0538	JM64_04005	3-aminobutyryl-CoA ammonia-lyase
Ferpe_0539	JM64_04000	3-keto-5-aminohexanoate cleavage enzyme
Ferpe_0540	JM64_03995	L-erythro-3,5-diaminohexanoate dehydrogenase
Ferpe_0541	JM64_03990	lysine 2,3-aminomutase
Ferpe_0542	JM64_03985	3-hydroxybutyryl-CoA dehydratase
Ferpe_0543	JM64_03980	hypothetical protein
Ferpe_0544	JM64_03975	Mismatch repair ATPase (MutS family)
Ferpe_0545	JM64_03965	beta-lysine 5,6-aminomutase alpha subunit
Ferpe_0546	JM64_03960	beta-lysine 5,6-aminomutase beta subunit
Ferpe_0551	JM64_03940	Protein of unknown function (DUF3298)
Ferpe_0552	JM64_03915	Uncharacterized conserved protein
Ferpe_0553	JM64_03930	type I restriction enzyme M protein
Ferpe_0558	JM64_03915	Uncharacterized membrane protein YkvA, DUF1232 family
Ferpe_0559	JM64_03910	NADPH-dependent 2,4-dienoyl-CoA reductase, sulfur reductase
Ferpe_0561	JM64_03895	hypothetical protein
Ferpe_0562	JM64_03890	7,8-dihydropterin-6-yl-methyl-4-(beta-D-ribofuranosyl)aminobenzene 5'-phosphate synthase
Ferpe_0570	JM64_03835	Restriction endonuclease
Ferpe_0571	JM64_03830	small redox-active disulfide protein 2
Ferpe_0573	JM64_03820	transcriptional regulator, ArsR family
Ferpe_0576	JM64_03800	hypothetical protein
Ferpe_0577	JM64_03795	PAP2 superfamily protein
Ferpe_0580	JM64_07020	Transposase and inactivated derivatives
Ferpe_0582	JM64_02900	Na+/melibiose symporter
Ferpe_0583	JM64_06325	MFS transporter, putative metabolite:H+ symporter

Ferpe_0594	JM64_03775	putative MFS transporter, AGZA family, xanthine/uracil permease
Ferpe_0595	JM64_03770	hypothetical protein
Ferpe_0596	JM64_03765	dTDP-4-amino-4,6-dideoxygalactose transaminase
Ferpe_0597	JM64_03760	hypothetical protein
Ferpe_0598	JM64_03755	DNA replication and repair protein RadC
Ferpe_0599	JM64_03750	septum formation protein
Ferpe_0600	JM64_03745	hypothetical protein
Ferpe_0601	JM64_03740	Glyoxylase, beta-lactamase superfamily II
Ferpe_0602	JM64_03735	hypothetical protein
Ferpe_0603	JM64_03730	RNAse R
Ferpe_0604	JM64_03725	DNA ligase (NAD+)
Ferpe_0605	JM64_03720	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase
Ferpe_0606	JM64_03715	hypothetical protein
Ferpe_0607	JM64_03710	hypothetical protein
Ferpe_0608	JM64_03705	competence protein ComFC
Ferpe_0609	JM64_03700	hypothetical protein
Ferpe_0610	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0611	JM64_03675	ABC transporter
Ferpe_0612	JM64_03670	peptide/nickel transport system permease protein
Ferpe_0613	JM64_03665	peptide/nickel transport system permease protein
Ferpe_0614	JM64_03660	Excinuclease ABC subunit C
Ferpe_0615	JM64_03655	CTP synthase
Ferpe_0616	JM64_03650	histidinol-phosphatase (PHP family)
Ferpe_0617	JM64_03645	elongation factor Ts
Ferpe_0618	JM64_03635	prepilin-type N-terminal cleavage/methylation domain-containing protein
Ferpe_0620	JM64_03625	hypothetical protein
Ferpe_0621	JM64_03615	Putative zinc- or iron-chelating domain-containing protein
Ferpe_0622	JM64_03610	Multimeric flavodoxin WrbA
Ferpe_0623	JM64_03605	fumarate hydratase subunit beta

Ferpe_0624	JM64_03600	fumarate hydratase subunit alpha
Ferpe_0625	JM64_03595	Na+-driven multidrug efflux pump
Ferpe_0626	JM64_03590	hypothetical protein
Ferpe_0627	JM64_03585	uncharacterized protein
Ferpe_0628	JM64_03580	hypothetical protein
Ferpe_0629	JM64_03575	branched-chain amino acid transport system permease protein
Ferpe_0630	JM64_03570	amino acid/amide ABC transporter membrane protein 1, HAAT family
Ferpe_0631	JM64_03565	branched-chain amino acid transport system ATP-binding protein
Ferpe_0632	JM64_03560	amino acid/amide ABC transporter ATP-binding protein 1, HAAT family
Ferpe_0633	JM64_03555	amino acid/amide ABC transporter substrate-binding protein, HAAT family
Ferpe_0634	JM64_03550	Acyl-CoA hydrolase
Ferpe_0635	JM64_03545	transcriptional regulator, TetR family
Ferpe_0636	JM64_03540	3-oxoacyl-[acyl-carrier-protein] synthase-3
Ferpe_0637	JM64_03535	protein of unknown function (DUF4438)
Ferpe_0638	JM64_03530	Nitroreductase
Ferpe_0639	JM64_03525	NADPH-dependent 2,4-dienoyl-CoA reductase, sulfur reductase
Ferpe_0640	JM64_03520	thiazole-adenylate synthase
Ferpe_0641	JM64_03515	hydroxymethylpyrimidine synthase
Ferpe_0642	JM64_03510	Sugar phosphate isomerase/epimerase
Ferpe_0643	JM64_03505	hydroxymethylpyrimidine/phosphomethylpyrimidine kinase
Ferpe_0644	JM64_03500	putative sigma-54 modulation protein
Ferpe_0645	JM64_03495	hypothetical protein
Ferpe_0646	JM64_03490	EDD domain protein, DegV family
Ferpe_0647	JM64_03485	EDD domain protein, DegV family
Ferpe_0648	JM64_03480	F-type H+-transporting ATPase subunit epsilon
Ferpe_0649	JM64_03475	ATP synthase F1 subcomplex beta subunit
Ferpe_0650	JM64_03470	F-type H+-transporting ATPase subunit gamma

Ferpe_0651	JM64_03465	ATP synthase F1 subcomplex alpha subunit
Ferpe_0652	JM64_03460	ATP synthase F1 subcomplex delta subunit
Ferpe_0653	JM64_03455	ATP synthase F0 subcomplex B subunit
Ferpe_0654	JM64_03450	F-type H+-transporting ATPase subunit c
Ferpe_0655	JM64_03445	ATP synthase F0 subcomplex A subunit
Ferpe_0656	JM64_03440	hypothetical protein
Ferpe_0658	JM64_03430	Xaa-Pro aminopeptidase
Ferpe_0660	JM64_03415	putative holliday junction resolvase
Ferpe_0661	JM64_03410	exonuclease RecJ
Ferpe_0662	JM64_03405	Imidazolonepropionase
Ferpe_0663	JM64_03400	glyoxylate reductase
Ferpe_0664	JM64_03395	Flavorubredoxin
Ferpe_0666	JM64_03385	BFD-like [2Fe-2S] binding domain.
Ferpe_0673	JM64_03115	transcription termination factor Rho
Ferpe_0674	JM64_03270	sulfide dehydrogenase (flavoprotein) subunit SudB
Ferpe_0675	JM64_03265	sulfide dehydrogenase (flavoprotein) subunit SudA
Ferpe_0676	JM64_03260	hypothetical protein
Ferpe_0678	JM64_03250	uncharacterized protein
Ferpe_0679	JM64_03245	ABC-2 type transport system ATP-binding protein
Ferpe_0680	JM64_03240	ABC-2 type transport system permease protein
Ferpe_0681	JM64_03235	ABC-2 type transport system permease protein
Ferpe_0682	JM64_03230	transcriptional regulator, LacI family
Ferpe_0683	JM64_04515	Beta-glucanase/Beta-glucan synthetase
Ferpe_0684	JM64_04505	Glycosyl hydrolase family 3 C-terminal domain-containing protein
Ferpe_0687	JM64_06605	peptide/nickel transport system permease protein
Ferpe_0688	JM64_09550	ATP-binding cassette, subfamily F, member 3
Ferpe_0690	JM64_09540	Endonuclease IV
Ferpe_0692	JM64_09535	hypothetical protein
Ferpe_0693	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0694	JM64_09535	Uncharacterized conserved protein
Ferpe_0696	JM64_09525	1-deoxy-D-xylulose 5-phosphate reductoisomerase

Ferpe_0698	JM64_09515	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
Ferpe_0699	JM64_09510	5'-nucleotidase /3'-nucleotidase /exopolyphosphatase
Ferpe_0700	JM64_09505	peptide deformylase
Ferpe_0703	JM64_03525	NADPH-dependent 2,4-dienoyl-CoA reductase, sulfur reductase
Ferpe_0704	JM64_00565	ribonucleoside-triphosphate reductase
Ferpe_0711	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0718	JM64_01000	GMP synthase (glutamine-hydrolysing)
Ferpe_0719	JM64_02475	L-ascorbate metabolism protein UlaG, beta-lactamase superfamily
Ferpe_0720	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0722	JM64_03260	hypothetical protein
Ferpe_0723	JM64_03265	sulfide dehydrogenase (flavoprotein) subunit SudA
Ferpe_0724	JM64_03270	sulfide dehydrogenase (flavoprotein) subunit SudB
Ferpe_0725	JM64_03115	transcription termination factor Rho
Ferpe_0726	JM64_03110	uracil phosphoribosyltransferase
Ferpe_0727	JM64_03105	peptide chain release factor 1
Ferpe_0728	JM64_03100	pilus retraction protein PilT
Ferpe_0733	JM64_01515	amidase
Ferpe_0734	JM64_01520	putative hydrolase of the HAD superfamily
Ferpe_0735	JM64_01530	Glycosyltransferase, catalytic subunit of cellulose synthase and poly-beta-1,6-N-acetylglucosamine synthase
Ferpe_0737	JM64_01550	UDP-N-acetylglucosamine 2-epimerase (non-hydrolysing)
Ferpe_0739	JM64_01565	hypothetical protein
Ferpe_0743	JM64_05715	Acetyl esterase/lipase
Ferpe_0750	JM64_03210	hypothetical protein
Ferpe_0751	JM64_03205	Site-specific DNA recombinase
Ferpe_0752	JM64_03200	hypothetical protein
Ferpe_0753	JM64_03195	23S rRNA (uracil1939-C5)-methyltransferase
Ferpe_0754	JM64_03190	MFS transporter, FSR family, fosmidomycin resistance protein

Ferpe_0755	JM64_03185	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_0756	JM64_03180	valyl-tRNA synthetase
Ferpe_0757	JM64_03175	dihydrofolate synthase / folylpolyglutamate synthase
Ferpe_0758	JM64_03170	ribosome-associated protein
Ferpe_0759	JM64_03165	5-methylthioadenosine/S-adenosylhomocysteine deaminase
Ferpe_0760	JM64_03160	hypothetical protein
Ferpe_0761	JM64_03155	peptide/nickel transport system substrate-binding protein
Ferpe_0762	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0763	JM64_03150	Peroxiredoxin
Ferpe_0764	JM64_03145	ferritin
Ferpe_0765	JM64_03140	hypothetical protein
Ferpe_0766	JM64_03135	Endonuclease V
Ferpe_0767	JM64_03130	possible tyrosine transporter P-protein
Ferpe_0768	JM64_03125	possible tyrosine transporter P-protein
Ferpe_0769	JM64_03120	hypothetical protein
Ferpe_0770	JM64_03115	transcription termination factor Rho
Ferpe_0771	JM64_03110	uracil phosphoribosyltransferase
Ferpe_0772	JM64_03105	bacterial peptide chain release factor 1 (bRF-1)
Ferpe_0773	JM64_03100	twitching motility protein PilT
Ferpe_0774	JM64_03095	DNA polymerase-3 subunit gamma/tau
Ferpe_0775	JM64_03090	tRNA dimethylallyltransferase
Ferpe_0776	JM64_03085	hypothetical protein
Ferpe_0777	JM64_03080	hypothetical protein
Ferpe_0778	JM64_03075	NTE family protein
Ferpe_0779	JM64_03070	S-layer homology domain-containing protein
Ferpe_0780	JM64_03065	PEGA domain-containing protein
Ferpe_0781	JM64_03060	transcriptional regulator, MarR family
Ferpe_0782	JM64_03055	Aspartyl aminopeptidase
Ferpe_0783	JM64_03050	UDP-N-acetylmuramate dehydrogenase

Ferpe_0784	JM64_03045	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_0786	JM64_03035	phosphoesterase RecJ domain-containing protein
Ferpe_0787	JM64_03030	purine-nucleoside phosphorylase
Ferpe_0788	JM64_01600	InsA N-terminal domain-containing protein
Ferpe_0789	JM64_03025	Predicted amino acid racemase
Ferpe_0790	JM64_03020	uncharacterized radical SAM protein YgiQ
Ferpe_0791	JM64_03015	L-glutamine synthetase
Ferpe_0792	JM64_03010	ferrous iron transport protein A
Ferpe_0793	JM64_03005	ferrous iron transport protein B
Ferpe_0794	JM64_03000	hypothetical protein
Ferpe_0795	JM64_02995	iron complex transport system substrate-binding protein
Ferpe_0796	JM64_02990	iron complex transport system permease protein
Ferpe_0797	JM64_02985	diaminohydroxyphosphoribosylaminopyrimidine deaminase
Ferpe_0798	JM64_02980	riboflavin synthase alpha chain
Ferpe_0799	JM64_02975	3,4-dihydroxy 2-butanone 4-phosphate synthase / GTP cyclohydrolase II
Ferpe_0800	JM64_02970	6,7-dimethyl-8-ribityllumazine synthase
Ferpe_0801	JM64_02965	ABC-2 type transport system ATP-binding protein
Ferpe_0802	JM64_02960	ABC-2 type transport system permease protein
Ferpe_0803	JM64_02955	protein of unknown function (DUF4382)
Ferpe_0804	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0805	JM64_02950	putative membrane protein
Ferpe_0806	JM64_02945	hypothetical protein
Ferpe_0812	JM64_04480	multiple sugar transport system permease protein
Ferpe_0813	JM64_04475	carbohydrate ABC transporter membrane protein 1, CUT1 family
Ferpe_0819	JM64_02935	Alpha/beta superfamily hydrolase
Ferpe_0820	JM64_02930	hypothetical protein
Ferpe_0821	JM64_02925	inorganic phosphate transporter, PiT family
Ferpe_0822	JM64_02920	Phosphoglycerate dehydrogenase

Ferpe_0823	JM64_02915	Predicted component of the ribosome quality control (RQC) complex, YloA/Tae2 family, contains fibronectin-binding (FbpA) and DUF814 domains
Ferpe_0824	JM64_02910	Excinuclease ABC subunit B
Ferpe_0825	JM64_02905	dihydrolipoamide dehydrogenase
Ferpe_0832	JM64_02900	Na+/melibiose symporter
Ferpe_0833	JM64_02895	Uncharacterized conserved protein YbjQ, UPF0145 family
Ferpe_0834	JM64_02890	ABC-2 family transporter protein
Ferpe_0835	JM64_02885	ABC-2 type transport system ATP-binding protein
Ferpe_0836	JM64_02880	transcriptional regulator, GntR family
Ferpe_0837	JM64_02875	hypothetical protein
Ferpe_0838	JM64_02870	dihydroorotase
Ferpe_0839	JM64_02865	dihydroorotate dehydrogenase electron transfer subunit
Ferpe_0840	JM64_02860	dihydroorotate dehydrogenase (NAD+) catalytic subunit
Ferpe_0841	JM64_02855	orotidine-5'-phosphate decarboxylase
Ferpe_0842	JM64_02850	orotate phosphoribosyltransferase
Ferpe_0843	JM64_02845	L-fuculose-phosphate aldolase
Ferpe_0844	JM64_02840	galactokinase
Ferpe_0845	JM64_02835	UDPglucose--hexose-1-phosphate uridylyltransferase
Ferpe_0846	JM64_02830	alpha-galactosidase
Ferpe_0849	JM64_02815	Predicted arabinose efflux permease, MFS family
Ferpe_0850	JM64_02810	ribonucleoside-diphosphate reductase class II
Ferpe_0851	JM64_02805	redox-sensing transcriptional repressor
Ferpe_0852	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0853	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0854	JM64_02800	Response regulator consisting of a CheY-like receiver domain and a Fis-type HTH domain
Ferpe_0856	JM64_02790	NADH:ubiquinone oxidoreductase 24 kD subunit
Ferpe_0857	JM64_02785	Iron only hydrogenase large subunit, C-terminal domain

Ferpe_0858	JM64_02780	Stage II sporulation protein E (SpoIIE)
Ferpe_0859	JM64_02770	hypothetical protein
Ferpe_0860	JM64_02745	iron-hydrogenase subunit gamma
Ferpe_0861	JM64_02740	iron-hydrogenase subunit beta
Ferpe_0862	JM64_02735	iron-hydrogenase subunit alpha
Ferpe_0863	JM64_02730	redox-sensing transcriptional repressor
Ferpe_0864	JM64_02725	carboxypeptidase Taq
Ferpe_0865	JM64_02720	magnesium chelatase family protein
Ferpe_0866	JM64_02715	ADP-ribose pyrophosphatase
Ferpe_0867	JM64_02710	PQQ-like domain-containing protein
Ferpe_0868	JM64_02705	23S rRNA (cytosine1962-C5)-methyltransferase
Ferpe_0869	JM64_02700	2Fe-2S iron-sulfur cluster binding domain-containing protein
Ferpe_0870	JM64_02695	Thioredoxin reductase
Ferpe_0871	JM64_02690	sarcosine oxidase subunit beta
Ferpe_0872	JM64_02685	response regulator receiver modulated diguanylate cyclase
Ferpe_0873	JM64_02680	zinc transporter, ZIP family
Ferpe_0874	JM64_02675	Arabinose efflux permease
Ferpe_0875	JM64_02665	Putative aminopeptidase FrvX
Ferpe_0876	JM64_02660	hypothetical protein
Ferpe_0877	JM64_02655	hypothetical protein
Ferpe_0878	JM64_02650	peptidyl-tRNA hydrolase, PTH1 family
Ferpe_0879	JM64_02645	large subunit ribosomal protein L25
Ferpe_0880	JM64_02640	ribose-phosphate pyrophosphokinase
Ferpe_0881	JM64_02635	bifunctional UDP-N-acetylglucosamine pyrophosphorylase / Glucosamine-1-phosphate N-acetyltransferase
Ferpe_0882	JM64_08015	InsA N-terminal domain-containing protein
Ferpe_0883	JM64_02630	hypothetical protein
Ferpe_0884	JM64_02625	pyruvate phosphate dikinase
Ferpe_0885	JM64_02620	Pimeloyl-ACP methyl ester carboxylesterase
Ferpe_0886	JM64_02615	3-oxoacyl-[acyl-carrier-protein] reductase

Ferpe_0887	JM64_02610	biotin transport system substrate-specific component
Ferpe_0888	JM64_02605	[acyl-carrier-protein] S-malonyltransferase
Ferpe_0889	JM64_02600	enoyl-[acyl-carrier protein] reductase II
Ferpe_0890	JM64_02595	3-hydroxyacyl-[acyl-carrier-protein] dehydratase
Ferpe_0891	JM64_02590	3-oxoacyl-[acyl-carrier-protein] synthase II
Ferpe_0892	JM64_02585	EDD domain protein, DegV family
Ferpe_0893	JM64_02580	acyl carrier protein
Ferpe_0894	JM64_02575	glycerophosphoryl diester phosphodiesterase
Ferpe_0895	JM64_02570	amino acid/amide ABC transporter substrate-binding protein, HAAT family
Ferpe_0897	JM64_02560	hypothetical protein
Ferpe_0898	JM64_02555	GTP cyclohydrolase I
Ferpe_0899	JM64_02550	Ribosomal protein L7Ae
Ferpe_0900	JM64_02545	Ribosomal protein S18 acetylase RimI
Ferpe_0901	JM64_02540	SpoIID/LytB domain protein
Ferpe_0902	JM64_02535	hypothetical protein
Ferpe_0903	JM64_02530	Uncharacterized conserved protein YloU, alkaline shock protein (Asp23) family
Ferpe_0904	JM64_02525	Uncharacterized conserved protein YloU, alkaline shock protein (Asp23) family
Ferpe_0907	JM64_02510	Na+-transporting NADH:ubiquinone oxidoreductase subunit B
Ferpe_0908	JM64_02505	Na+-transporting NADH:ubiquinone oxidoreductase subunit C
Ferpe_0909	JM64_02500	Na+-transporting NADH:ubiquinone oxidoreductase subunit D
Ferpe_0910	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_0911	JM64_02475	L-ascorbate metabolism protein UlaG, beta-lactamase superfamily
Ferpe_0912	JM64_02470	ATP-dependent RNA helicase DeaD
Ferpe_0913	JM64_02465	nicotinamidase/pyrazinamidase
Ferpe_0914	JM64_02460	hypothetical protein
Ferpe_0915	JM64_02455	5'-nucleotidase / UDP-sugar diphosphatase
Ferpe_0916	JM64_02450	Uncharacterized protein, Rmd1/YagE family

Ferpe_0917	JM64_02445	hypothetical protein
Ferpe_0918	JM64_02440	Glycosyltransferase involved in cell wall bisynthesis
Ferpe_0919	JM64_02435	FKBP-type peptidyl-prolyl cis-trans isomerase 2
Ferpe_0920	JM64_02430	Predicted arabinose efflux permease, MFS family
Ferpe_0921	JM64_02425	signal recognition particle subunit FFH/SRP54 (srp54)
Ferpe_0922	JM64_02420	SSU ribosomal protein S16P
Ferpe_0923	JM64_02415	hypothetical protein
Ferpe_0924	JM64_02410	16S rRNA processing protein RimM
Ferpe_0925	JM64_02405	tRNA (Guanine37-N(1)-) methyltransferase
Ferpe_0926	JM64_02400	hypothetical protein
Ferpe_0927	JM64_02395	LSU ribosomal protein L19P
Ferpe_0928	JM64_02390	signal peptidase I
Ferpe_0929	JM64_02385	hypothetical protein
Ferpe_0930	JM64_02380	LPP20 lipoprotein
Ferpe_0931	JM64_02375	hypothetical protein
Ferpe_0932	JM64_02365	Anti-sigma regulatory factor (Ser/Thr protein kinase)
Ferpe_0933	JM64_02360	hypothetical protein
Ferpe_0934	JM64_02355	HDIG domain-containing protein
Ferpe_0936	JM64_02345	ribosome maturation factor RimP
Ferpe_0937	JM64_02340	NusA antitermination factor
Ferpe_0938	JM64_02335	Fur family transcriptional regulator, ferric uptake regulator
Ferpe_0939	JM64_02330	competence protein ComEC
Ferpe_0940	JM64_02325	2,3-bisphosphoglycerate-independent phosphoglycerate mutase
Ferpe_0941	JM64_02320	aminopeptidase
Ferpe_0942	JM64_02315	hypothetical protein
Ferpe_0943	JM64_02310	Regulator of protease activity HflC, stomatin/prohibitin superfamily
Ferpe_0944	JM64_02305	Membrane protein implicated in regulation of membrane protease activity

Ferpe_0945	JM64_02300	HD-GYP domain
Ferpe_0946	JM64_02295	threonyl-tRNA synthetase
Ferpe_0947	JM64_02290	hypothetical protein
Ferpe_0948	JM64_02285	hypothetical protein
Ferpe_0949	JM64_02280	hypothetical protein
Ferpe_0950	JM64_02275	DNA helicase-2 / ATP-dependent DNA helicase PcrA
Ferpe_0951	JM64_02270	Uncharacterized conserved protein YecE, DUF72 family
Ferpe_0952	JM64_02265	RNA polymerase, sigma-24 subunit, RpoE
Ferpe_0953	JM64_02260	hypothetical protein
Ferpe_0954	JM64_02255	hypothetical protein
Ferpe_0955	JM64_02250	Radical SAM superfamily enzyme with C-terminal helix-hairpin-helix motif
Ferpe_0956	JM64_02240	S-layer homology domain-containing protein
Ferpe_0957	JM64_02235	ABC-2 type transport system permease protein
Ferpe_0958	JM64_02230	ABC-2 type transport system ATP-binding protein
Ferpe_0959	JM64_02225	ABC-2 type transport system permease protein
Ferpe_0960	JM64_02220	Phosphatidylserine/phosphatidylglycerophosphate/cardiolipin synthase
Ferpe_0961	JM64_02215	translation factor SUA5
Ferpe_0962	JM64_02210	ATP-dependent HslUV protease, peptidase subunit HslV
Ferpe_0963	JM64_02205	hypothetical protein
Ferpe_0964	JM64_02200	putative HD superfamily hydrolase of NAD metabolism
Ferpe_0965	JM64_02195	hypothetical protein
Ferpe_0966	JM64_02190	hypothetical protein
Ferpe_0967	JM64_02185	ornithine carbamoyltransferase
Ferpe_0968	JM64_02180	hypothetical protein
Ferpe_0969	JM64_02175	ribonuclease-3 family protein
Ferpe_0970	JM64_02170	CxxC motif-containing protein
Ferpe_0971	JM64_02165	Thioredoxin reductase
Ferpe_0972	JM64_02160	glycerol-3-phosphate dehydrogenase

Ferpe_0973	JM64_02155	thymidylate kinase
Ferpe_0974	JM64_02150	flagellar biosynthetic protein FliQ
Ferpe_0975	JM64_02140	5-methyltetrahydrofolate--homocysteine methyltransferase
Ferpe_0976	JM64_02135	hypothetical protein
Ferpe_0977	JM64_02130	hypothetical protein
Ferpe_0978	JM64_02125	hypothetical protein
Ferpe_0979	JM64_02120	alanyl-tRNA synthetase
Ferpe_0980	JM64_02115	glutamyl-tRNA synthetase
Ferpe_0981	JM64_02110	Predicted pyrophosphatase or phosphodiesterase, AlkP superfamily
Ferpe_0982	JM64_02105	maltose/maltodextrin transport system permease protein
Ferpe_0983	JM64_02100	carbohydrate ABC transporter membrane protein 1, CUT1 family
Ferpe_0984	JM64_02095	carbohydrate ABC transporter substrate-binding protein, CUT1 family
Ferpe_0985	JM64_02090	Predicted GTPase
Ferpe_0986	JM64_02085	hypothetical protein
Ferpe_0987	JM64_02080	Signal transduction histidine kinase
Ferpe_0988	JM64_02075	DNA-binding response regulator, OmpR family, contains REC and winged-helix (wHTH) domain
Ferpe_0990	JM64_02065	hypothetical protein
Ferpe_0991	JM64_02060	Histone acetyltransferase
Ferpe_0992	JM64_02055	hypothetical protein
Ferpe_0993	JM64_02050	Acetoin utilization deacetylase AcuC
Ferpe_0994	JM64_02045	ribose-5-phosphate isomerase
Ferpe_0995	JM64_02040	anti-sigma B factor antagonist
Ferpe_0996	JM64_02035	anti-sigma B factor antagonist
Ferpe_0997	JM64_02030	SOS-response transcriptional repressor, LexA
Ferpe_0998	JM64_02025	Archease protein family (MTH1598/TM1083)
Ferpe_0999	JM64_02020	DNA gyrase subunit A
Ferpe_1000	JM64_02015	methionyl-tRNA synthetase

Ferpe_1001	JM64_02010	2',3'-cyclic-nucleotide 2'-phosphodiesterase / 3'-nucleotidase
Ferpe_1002	JM64_02005	thymidine kinase
Ferpe_1003	JM64_02000	TatD DNase family protein
Ferpe_1004	JM64_01995	Holliday junction DNA helicase subunit RuvA
Ferpe_1005	JM64_01990	hypothetical protein
Ferpe_1006	JM64_08145	hypothetical protein
Ferpe_1007	JM64_08150	hypothetical protein
Ferpe_1008	JM64_08155	Histidine kinase-, DNA gyrase B-, and HSP90-like ATPase
Ferpe_1009	JM64_08160	outer membrane protein insertion porin family
Ferpe_1010	JM64_08165	N-glycosylase/DNA lyase
Ferpe_1011	JM64_08170	glutamate formiminotransferase
Ferpe_1012	JM64_08175	voltage-dependent potassium channel beta subunit, animal
Ferpe_1013	JM64_08180	alpha-mannosidase
Ferpe_1014	JM64_08185	N-acylglucosamine-6-phosphate 2-epimerase
Ferpe_1015	JM64_08190	FAD dependent oxidoreductase
Ferpe_1016	JM64_08195	carbohydrate ABC transporter substrate-binding protein, CUT1 family
Ferpe_1017	JM64_08200	carbohydrate ABC transporter membrane protein 1, CUT1 family
Ferpe_1018	JM64_08205	carbohydrate ABC transporter membrane protein 2, CUT1 family
Ferpe_1020	JM64_08215	Protein of unknown function (DUF4127)
Ferpe_1021	JM64_08220	glucokinase
Ferpe_1022	JM64_08225	transcriptional regulator, RpiR family
Ferpe_1023	JM64_08320	hypothetical protein
Ferpe_1024	JM64_08325	glucosamine--fructose-6-phosphate aminotransferase (isomerizing)
Ferpe_1025	JM64_08330	N-acetylglucosamine 6-phosphate deacetylase
Ferpe_1026	JM64_08335	hypothetical protein
Ferpe_1027	JM64_08340	MoxR-like ATPase
Ferpe_1029	JM64_08350	Uncharacterized conserved protein, DUF58 family, contains vWF domain

Ferpe_1030	JM64_08355	FOG: WD40 repeat
Ferpe_1031	JM64_08360	ADP-ribose pyrophosphatase
Ferpe_1032	JM64_08365	hypothetical protein
Ferpe_1033	JM64_08370	flagellar protein FliS
Ferpe_1034	JM64_08375	hypothetical protein
Ferpe_1035	JM64_08380	purine-binding chemotaxis protein CheW
Ferpe_1037	JM64_08390	hypothetical protein
Ferpe_1038	JM64_08395	23S rRNA pseudouridine955/2504/2580 synthase
Ferpe_1039	JM64_08400	adenosylhomocysteine nucleosidase
Ferpe_1040	JM64_08405	chemotaxis protein CheX
Ferpe_1041	JM64_08410	ribosome biogenesis GTPase A
Ferpe_1042	JM64_08415	uridylate kinase
Ferpe_1043	JM64_08420	DNA repair exonuclease SbcCD ATPase subunit
Ferpe_1044	JM64_08425	hypothetical protein
Ferpe_1045	JM64_08430	PhoH-like ATPase
Ferpe_1046	JM64_08435	DNA polymerase-3 subunit epsilon
Ferpe_1047	JM64_08440	flagellar motor switch protein FliG
Ferpe_1048	JM64_08445	flagellar M-ring protein FliF
Ferpe_1049	JM64_08450	uncharacterized protein
Ferpe_1050	JM64_08455	large subunit ribosomal protein L32
Ferpe_1051	JM64_08460	phosphate:acyl-[acyl carrier protein] acyltransferase
Ferpe_1052	JM64_08465	glucosamine--fructose-6-phosphate aminotransferase (isomerizing)
Ferpe_1053	JM64_08470	ATP-dependent Clp protease ATP-binding subunit ClpX
Ferpe_1054	JM64_08475	O-sialoglycoprotein endopeptidase
Ferpe_1055	JM64_08480	hypothetical protein
Ferpe_1057	JM64_08495	flagellar P-ring protein precursor FlgI
Ferpe_1058	JM64_08500	flagellar L-ring protein precursor FlgH
Ferpe_1059	JM64_08505	flagella basal body P-ring formation protein FlgA
Ferpe_1060	JM64_08900	Glycosyltransferase involved in cell wall bisynthesis

Ferpe_1063	JM64_08925	Phenylpropionate dioxygenase, large terminal subunit
Ferpe_1064	JM64_08930	SnoaL-like domain-containing protein
Ferpe_1065	JM64_08935	NAD(P)-dependent dehydrogenase, short-chain alcohol dehydrogenase family
Ferpe_1066	JM64_08940	Phytoene dehydrogenase-related protein
Ferpe_1075	JM64_08510	DNA gyrase subunit B
Ferpe_1076	JM64_08520	Protein of unknown function (DUF721)
Ferpe_1077	JM64_08525	branched-chain amino acid aminotransferase
Ferpe_1078	JM64_08530	threonylcarbamoyladenosine tRNA methylthiotransferase MtaB
Ferpe_1079	JM64_08535	Predicted transcriptional regulator containing CBS domains
Ferpe_1080	JM64_08540	1-phosphofructokinase
Ferpe_1081	JM64_08545	ABC-type cobalamin/Fe3+-siderophores transport system, ATPase component
Ferpe_1083	JM64_08555	L-aspartate aminotransferase apoenzyme
Ferpe_1084	JM64_08560	glycine dehydrogenase subunit 2
Ferpe_1085	JM64_08565	glycine dehydrogenase (decarboxylating) alpha subunit
Ferpe_1086	JM64_08575	aminomethyltransferase
Ferpe_1087	JM64_08580	nicotinate phosphoribosyltransferase
Ferpe_1088	JM64_08585	radical SAM family uncharacterized protein
Ferpe_1089	JM64_08590	hypothetical protein
Ferpe_1090	JM64_08595	rhomboid protease GluP
Ferpe_1091	JM64_08600	hypothetical protein
Ferpe_1092	JM64_08610	thiamine biosynthesis lipoprotein
Ferpe_1093	JM64_08615	pyruvate formate lyase activating enzyme
Ferpe_1094	JM64_08620	DNA polymerase-3 subunit alpha
Ferpe_1095	JM64_08625	ribulose-phosphate 3-epimerase
Ferpe_1096	JM64_08630	phosphate starvation-inducible protein PhoH
Ferpe_1097	JM64_08635	hypothetical protein
Ferpe_1098	JM64_08640	probable rRNA maturation factor
Ferpe_1099	JM64_08645	Glyoxylase, beta-lactamase superfamily II

Ferpe_1100	JM64_08650	polysaccharide pyruvyl transferase CsaB
Ferpe_1108	JM64_01595	Glycosyltransferase
Ferpe_1116	JM64_08655	NADH-quinone oxidoreductase subunit F
Ferpe_1117	JM64_08660	NADH-quinone oxidoreductase subunit E
Ferpe_1118	JM64_08665	NAD(P)-dependent iron-only hydrogenase diaphorase component flavoprotein
Ferpe_1119	JM64_08670	NADH:ubiquinone oxidoreductase 24 kD subunit
Ferpe_1120	JM64_08675	NADP-reducing hydrogenase subunit HndB
Ferpe_1121	JM64_08680	NADH-quinone oxidoreductase subunit E
Ferpe_1122	JM64_08685	Uncharacterized membrane protein
Ferpe_1123	JM64_08690	hypothetical protein
Ferpe_1124	JM64_08695	pyroglutamyl-peptidase I Cysteine peptidase. MEROPS family C15
Ferpe_1125	JM64_08700	Serine phosphatase RsbU, regulator of sigma subunit
Ferpe_1126	JM64_08705	alpha-galactosidase
Ferpe_1128	JM64_08710	DNA repair protein RecN (Recombination protein N)
Ferpe_1129	JM64_08715	cytidine deaminase
Ferpe_1130	JM64_08720	Hemolysin, contains CBS domains
Ferpe_1131	JM64_08725	PAS domain S-box-containing protein/diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_1132	JM64_08730	Alcohol dehydrogenase, class IV
Ferpe_1134	JM64_08740	two-component system, chemotaxis family, response regulator CheB
Ferpe_1135	JM64_08745	diacylglycerol kinase (ATP)
Ferpe_1136	JM64_08750	2-oxoglutarate ferredoxin oxidoreductase subunit gamma
Ferpe_1137	JM64_08755	2-oxoglutarate ferredoxin oxidoreductase, beta subunit
Ferpe_1138	JM64_08760	dCMP deaminase
Ferpe_1139	JM64_08765	methylated-DNA-[protein]-cysteine S-methyltransferase
Ferpe_1140	JM64_08770	Membrane carboxypeptidase (penicillin-binding protein)

Ferpe_1141	JM64_08775	hypothetical protein
Ferpe_1142	JM64_08780	nucleoside diphosphate kinase
Ferpe_1143	JM64_08785	Na+/H+ antiporter NhaC
Ferpe_1144	JM64_08790	Rubrerythrin
Ferpe_1147	JM64_08805	putative regulatory protein, FmdB family
Ferpe_1148	JM64_08810	cold-shock DNA-binding protein family
Ferpe_1149	JM64_08815	cold shock protein (beta-ribbon, CspA family)
Ferpe_1150	JM64_08820	hypothetical protein
Ferpe_1151	JM64_08825	Ribosome-associated heat shock protein implicated in the recycling of the 50S subunit (S4 paralog)
Ferpe_1152	JM64_08830	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_1153	JM64_08835	ATP-binding cassette, subfamily C
Ferpe_1154	JM64_08840	biotin synthase
Ferpe_1155	JM64_08845	flagellar biosynthetic protein FliR
Ferpe_1156	JM64_08850	flagellar biosynthetic protein FlhB
Ferpe_1157	JM64_08855	hypothetical protein
Ferpe_1158	JM64_08860	YhhN-like protein
Ferpe_1159	JM64_08870	hypothetical protein
Ferpe_1164	JM64_08885	Sugar (pentulose or hexulose) kinase
Ferpe_1165	JM64_08890	Serine protease, subtilisin family
Ferpe_1166	JM64_08895	protein of unknown function (DUF3783)
Ferpe_1167	JM64_08940	hypothetical protein
Ferpe_1168	JM64_08945	serine protease, ClpP class
Ferpe_1169	JM64_08950	hypothetical protein
Ferpe_1171	JM64_08950	hypothetical protein
Ferpe_1172	JM64_08955	LSU ribosomal protein L1P
Ferpe_1173	JM64_08960	LSU ribosomal protein L11P
Ferpe_1174	JM64_08965	transcription antitermination protein nusG
Ferpe_1175	JM64_08970	protein translocase subunit secE/sec61 gamma
Ferpe_1177	JM64_08980	large subunit ribosomal protein L33
Ferpe_1181	JM64_08995	putative redox protein
Ferpe_1182	JM64_09000	cytochrome c-type biogenesis protein

Ferpe_1183	JM64_09005	Thioredoxin-related protein
Ferpe_1184	JM64_09015	Glutaredoxin-like protein, YruB-family
Ferpe_1185	JM64_09020	thioredoxin reductase (NADPH)
Ferpe_1186	JM64_09025	Glutaredoxin-like domain protein
Ferpe_1187	JM64_09030	carboxylesterase
Ferpe_1188	JM64_09035	zinc transport system permease protein
Ferpe_1189	JM64_09040	zinc transport system ATP-binding protein
Ferpe_1190	JM64_09045	zinc transport system substrate-binding protein
Ferpe_1191	JM64_09050	Fur family transcriptional regulator, ferric uptake regulator
Ferpe_1197	JM64_09080	Predicted oxidoreductase
Ferpe_1198	JM64_09085	Radical SAM superfamily enzyme, MoaA/NifB/PqqE/SkfB family
Ferpe_1200	JM64_09090	pyruvate ferredoxin oxidoreductase beta subunit
Ferpe_1201	JM64_09095	pyruvate ferredoxin oxidoreductase, alpha subunit
Ferpe_1202	JM64_09100	pyruvate ferredoxin oxidoreductase, delta subunit
Ferpe_1203	JM64_09105	pyruvate ferredoxin oxidoreductase, gamma subunit
Ferpe_1204	JM64_09110	1,4-alpha-glucan branching enzyme
Ferpe_1205	JM64_09115	hypothetical protein
Ferpe_1206	JM64_09120	amino acid ABC transporter ATP-binding protein, PAAT family
Ferpe_1207	JM64_09125	amino acid ABC transporter membrane protein, PAAT family
Ferpe_1208	JM64_09130	amino acid ABC transporter substrate-binding protein, PAAT family
Ferpe_1209	JM64_09135	hypothetical protein
Ferpe_1210	JM64_09140	glycyl-tRNA synthetase beta chain
Ferpe_1211	JM64_09145	glycyl-tRNA synthetase alpha chain
Ferpe_1212	JM64_09150	hypothetical protein
Ferpe_1213	JM64_09155	hypothetical protein
Ferpe_1214	JM64_09160	hypothetical protein
Ferpe_1215	JM64_09165	Cell wall-associated hydrolases (invasion-associated proteins)

Ferpe_1216	JM64_09170	lactose/L-arabinose transport system substrate-binding protein
Ferpe_1217	JM64_09175	lactose/L-arabinose transport system permease protein
Ferpe_1218	JM64_09180	lactose/L-arabinose transport system permease protein
Ferpe_1220	JM64_09190	Acetyl esterase/lipase
Ferpe_1221	JM64_09195	hypothetical protein
Ferpe_1222	JM64_09200	hypothetical protein
Ferpe_1227	JM64_09205	succinyl-diaminopimelate desuccinylase
Ferpe_1240	JM64_09215	Predicted acetyltransferase
Ferpe_1241	JM64_09220	hypothetical protein
Ferpe_1242	JM64_09225	Predicted Fe2+/Mn2+ transporter, VIT1/CCC1 family
Ferpe_1246	JM64_09245	LSU ribosomal protein L34P
Ferpe_1247	JM64_09250	ribonuclease P protein component
Ferpe_1248	JM64_09255	hypothetical protein
Ferpe_1249	JM64_09260	YidC/Oxa1 family membrane protein insertase
Ferpe_1250	JM64_09265	spoIIIJ-associated protein
Ferpe_1251	JM64_09270	Cupin domain protein
Ferpe_1257	JM64_09300	stage V sporulation protein S
Ferpe_1258	JM64_00600	branched-chain amino acid transport system substrate-binding protein
Ferpe_1259	JM64_09305	tryptophan synthase beta chain
Ferpe_1260	JM64_09315	hypothetical protein
Ferpe_1261	JM64_09320	B12-binding domain/radical SAM domain protein, MJ_1487 family
Ferpe_1262	JM64_09325	Uncharacterized protein conserved in bacteria
Ferpe_1263	JM64_09330	hypothetical protein
Ferpe_1264	JM64_09335	tRNA pseudouridine synthase B
Ferpe_1265	JM64_09340	phenylalanyl-tRNA synthetase, alpha subunit
Ferpe_1266	JM64_09345	putative membrane protein
Ferpe_1267	JM64_09350	serine/threonine protein phosphatase 1
Ferpe_1268	JM64_09355	DNA topoisomerase-1

Ferpe_1269	JM64_09360	3'-5' exoribonuclease
Ferpe_1270	JM64_09365	alanine racemase
Ferpe_1271	JM64_09370	hypothetical protein
Ferpe_1272	JM64_09375	LSU ribosomal protein L28P
Ferpe_1273	JM64_09380	pyruvate kinase
Ferpe_1274	JM64_09385	hypothetical protein
Ferpe_1275	JM64_09390	glycine cleavage system H protein
Ferpe_1276	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_1277	JM64_09395	ATP-dependent DNA helicase RecG
Ferpe_1278	JM64_09400	putative N6-adenine-specific DNA methylase
Ferpe_1279	JM64_09405	hypothetical protein
Ferpe_1280	JM64_09410	23S rRNA (guanosine2251-2'-O)-methyltransferase
Ferpe_1281	JM64_09415	tetrapyrrole methylase family protein / MazG family protein
Ferpe_1282	JM64_09420	glycerol-3-phosphate dehydrogenase (NAD(P)+)
Ferpe_1283	JM64_09425	tRNA (guanine-N(7)-)-methyltransferase
Ferpe_1284	JM64_09430	hypothetical protein
Ferpe_1285	JM64_09435	NAD(P)H-hydrate epimerase
Ferpe_1286	JM64_09440	soluble lytic murein transglycosylase
Ferpe_1287	JM64_09445	leader peptidase (prepilin peptidase) / N-methyltransferase
Ferpe_1288	JM64_09450	Tetratricopeptide repeat-containing protein
Ferpe_1289	JM64_09455	Do/DeqQ family serine protease
Ferpe_1290	JM64_09460	TIGR00269 family protein
Ferpe_1291	JM64_09465	rod shape determining protein RodA
Ferpe_1292	JM64_09470	hypothetical protein
Ferpe_1295	JM64_09485	phosphoglucomutase
Ferpe_1296	JM64_09490	Uncharacterized protein conserved in bacteria
Ferpe_1297	JM64_09500	hypothetical protein
Ferpe_1298	JM64_09505	peptide deformylase
Ferpe_1299	JM64_09510	5'-nucleotidase /3'-nucleotidase /exopolyphosphatase

Ferpe_1300	JM64_09515	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
Ferpe_1301	JM64_09520	regulator of sigma E protease
Ferpe_1302	JM64_09525	1-deoxy-D-xylulose 5-phosphate reductoisomerase
Ferpe_1303	JM64_09530	thiamine diphosphokinase
Ferpe_1304	JM64_09535	Uncharacterized conserved protein YndB, AHSA1/START domain
Ferpe_1305	JM64_09540	Endonuclease IV
Ferpe_1306	JM64_09545	Acetyltransferases, including N-acetylases of ribosomal proteins
Ferpe_1307	JM64_09550	ATP-binding cassette, subfamily F, member 3
Ferpe_1308	JM64_09555	hypothetical protein
Ferpe_1309	JM64_09560	Permease of the drug/metabolite transporter (DMT) superfamily
Ferpe_1310	JM64_09565	SagB-type dehydrogenase domain-containing protein
Ferpe_1311	JM64_09570	phenylalanyl-tRNA synthetase beta subunit
Ferpe_1312	JM64_09575	hypothetical protein
Ferpe_1313	JM64_09580	hypothetical protein
Ferpe_1314	JM64_09585	hypothetical protein
Ferpe_1315	JM64_09590	1-acyl-sn-glycerol-3-phosphate acyltransferase
Ferpe_1316	JM64_09595	hypothetical protein
Ferpe_1317	JM64_09600	TPR repeat-containing protein
Ferpe_1318	JM64_09605	hypothetical protein
Ferpe_1319	JM64_09610	glycerol-3-phosphate acyltransferase PlsY
Ferpe_1320	JM64_09615	hypothetical protein
Ferpe_1321	JM64_09620	flagellar basal-body rod protein FlgG
Ferpe_1322	JM64_09625	flagellar basal-body rod protein FlgG
Ferpe_1323	JM64_09630	rod shape-determining protein MreB
Ferpe_1324	JM64_09635	Permease of the drug/metabolite transporter (DMT) superfamily
Ferpe_1325	JM64_09640	hypothetical protein
Ferpe_1326	JM64_09645	chemotaxis protein methyltransferase CheR
Ferpe_1327	JM64_09650	hypothetical protein

Ferpe_1328	JM64_09655	two-component system, chemotaxis family, sensor kinase CheA
Ferpe_1329	JM64_09660	two-component system, chemotaxis family, response regulator CheB
Ferpe_1330	JM64_09665	response regulator receiver modulated diguanylate cyclase
Ferpe_1331	JM64_09670	hypothetical protein
Ferpe_1332	JM64_09675	Predicted nucleoside-diphosphate sugar epimerases
Ferpe_1333	JM64_09680	hypothetical protein
Ferpe_1334	JM64_09685	phosphatidylglycerol:prolipoprotein diacylglycerol transferase
Ferpe_1335	JM64_09690	hypothetical protein
Ferpe_1336	JM64_09695	hypothetical protein
Ferpe_1337	JM64_07410	Transposase
Ferpe_1338	JM64_09700	hypothetical protein
Ferpe_1339	JM64_09705	methionyl-tRNA formyltransferase
Ferpe_1340	JM64_09710	TPR repeat-containing protein
Ferpe_1341	JM64_09720	ribokinase
Ferpe_1344	JM64_09735	endoglucanase
Ferpe_1345	JM64_09740	endoglucanase
Ferpe_1346	JM64_09745	septum site-determining protein MinC
Ferpe_1347	JM64_09750	putative hydrolases of HD superfamily
Ferpe_1348	JM64_09755	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_1349	JM64_09760	prepilin-type N-terminal cleavage/methylation domain-containing protein
Ferpe_1350	JM64_09765	hypothetical protein
Ferpe_1351	JM64_09770	hypothetical protein
Ferpe_1352	JM64_09775	methyl-accepting chemotaxis protein
Ferpe_1353	JM64_09780	hypothetical protein
Ferpe_1354	JM64_09785	Serine protease, subtilisin family
Ferpe_1355	JM64_09790	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_1356	JM64_09795	ATP-binding cassette, subfamily B

Ferpe_1357	JM64_09800	glutamate dehydrogenase (NAD(P)+)
Ferpe_1358	JM64_09805	Formate-tetrahydrofolate ligase
Ferpe_1359	JM64_09810	uncharacterized protein
Ferpe_1361	JM64_09820	integrase/recombinase XerD
Ferpe_1362	JM64_09825	GTP pyrophosphokinase
Ferpe_1363	JM64_09830	D-tyrosyl-tRNA(Tyr) deacylase
Ferpe_1364	JM64_09835	hypothetical protein
Ferpe_1365	JM64_09840	protein of unknown function (DUF4940)
Ferpe_1366	JM64_09845	serine/threonine-protein kinase RsbW
Ferpe_1367	JM64_09850	hypothetical protein
Ferpe_1368	JM64_09855	Soluble lytic murein transglycosylase and related regulatory proteins (some contain LysM/invasin domains)
Ferpe_1369	JM64_09860	iron complex transport system ATP-binding protein
Ferpe_1370	JM64_09865	uracil permease
Ferpe_1371	JM64_09870	5-(carboxyamino)imidazole ribonucleotide synthase
Ferpe_1372	JM64_09875	5-(carboxyamino)imidazole ribonucleotide mutase
Ferpe_1373	JM64_09880	phosphoribosylaminoimidazole-succinocarboxamide synthase
Ferpe_1374	JM64_09885	phosphoribosylformylglycinamidine synthase
Ferpe_1375	JM64_09890	phosphoribosylformylglycinamidine synthase
Ferpe_1376	JM64_09895	phosphoribosylformylglycinamidine synthase
Ferpe_1377	JM64_09900	hypothetical protein
Ferpe_1379	JM64_00005	HDIG domain-containing protein
Ferpe_1380	JM64_00010	23S rRNA pseudouridine1911/1915/1917 synthase
Ferpe_1381	JM64_00015	signal peptidase II
Ferpe_1382	JM64_00020	chemotaxis protein methyltransferase CheR
Ferpe_1383	JM64_00025	hypothetical protein
Ferpe_1384	JM64_00030	DNA-binding regulatory protein, YebC/PmpR family
Ferpe_1385	JM64_00035	ATP-dependent Clp protease, protease subunit
Ferpe_1386	JM64_00040	trigger factor
Ferpe_1387	JM64_00045	LSU ribosomal protein L17P

Ferpe_1388	JM64_00050	DNA-directed RNA polymerase subunit alpha
Ferpe_1389	JM64_00055	SSU ribosomal protein S4P
Ferpe_1390	JM64_00060	SSU ribosomal protein S11P
Ferpe_1391	JM64_00065	SSU ribosomal protein S13P
Ferpe_1392	JM64_00070	LSU ribosomal protein L36P
Ferpe_1393	JM64_00075	translation initiation factor IF-1
Ferpe_1394	JM64_00080	methionyl aminopeptidase
Ferpe_1395	JM64_00085	Adenylate kinase
Ferpe_1396	JM64_00090	protein translocase subunit secY/sec61 alpha
Ferpe_1397	JM64_00095	LSU ribosomal protein L15P
Ferpe_1398	JM64_00100	LSU ribosomal protein L30P
Ferpe_1399	JM64_00105	small subunit ribosomal protein S5
Ferpe_1400	JM64_00110	LSU ribosomal protein L18P
Ferpe_1401	JM64_00115	LSU ribosomal protein L6P
Ferpe_1402	JM64_00120	SSU ribosomal protein S8P
Ferpe_1403	JM64_00125	SSU ribosomal protein S14P
Ferpe_1404	JM64_00130	LSU ribosomal protein L5P
Ferpe_1405	JM64_00135	LSU ribosomal protein L24P
Ferpe_1406	JM64_00140	LSU ribosomal protein L14P
Ferpe_1407	JM64_00145	SSU ribosomal protein S17P
Ferpe_1408	JM64_00150	LSU ribosomal protein L29P
Ferpe_1409	JM64_00155	LSU ribosomal protein L16P
Ferpe_1410	JM64_00160	small subunit ribosomal protein S3
Ferpe_1411	JM64_00165	LSU ribosomal protein L22P
Ferpe_1412	JM64_00175	SSU ribosomal protein S19P
Ferpe_1413	JM64_00180	LSU ribosomal protein L2P
Ferpe_1414	JM64_00185	large subunit ribosomal protein L23
Ferpe_1415	JM64_00190	large subunit ribosomal protein L4
Ferpe_1416	JM64_00195	large subunit ribosomal protein L3
Ferpe_1417	JM64_00200	small subunit ribosomal protein S10
Ferpe_1418	JM64_00205	elongation factor Tu
Ferpe_1419	JM64_00210	elongation factor G

Ferpe_1420	JM64_00215	SSU ribosomal protein S7P
Ferpe_1421	JM64_00220	small subunit ribosomal protein S12
Ferpe_1424	JM64_00235	L-lactate dehydrogenase
Ferpe_1425	JM64_00240	Glycosidase
Ferpe_1427	JM64_00250	Predicted arabinose efflux permease, MFS family
Ferpe_1428	JM64_00255	Rubrerythrin
Ferpe_1429	JM64_00260	Thioredoxin domain-containing protein
Ferpe_1430	JM64_00265	penicillin-binding protein 2
Ferpe_1431	JM64_00270	tRNA nucleotidyltransferase (CCA-adding enzyme)
Ferpe_1432	JM64_00275	Calcineurin-like phosphoesterase
Ferpe_1433	JM64_00280	RNAse Z
Ferpe_1434	JM64_00285	LSU ribosomal protein L9P
Ferpe_1435	JM64_00290	release factor glutamine methyltransferase
Ferpe_1436	JM64_00295	Metal-sulfur cluster biosynthetic enzyme
Ferpe_1437	JM64_00300	phosphoglucosamine mutase
Ferpe_1438	JM64_07410	Transposase
Ferpe_1439	JM64_00305	TIGR00266 family protein
Ferpe_1440	JM64_00310	pyrimidine operon attenuation protein / uracil phosphoribosyltransferase
Ferpe_1441	JM64_00315	hypothetical protein
Ferpe_1442	JM64_00320	tRNA-guanine transglycosylase
Ferpe_1443	JM64_00325	Formiminotetrahydrofolate cyclodeaminase
Ferpe_1446	JM64_00330	putative efflux protein, MATE family
Ferpe_1447	JM64_00335	haloacid dehalogenase superfamily, subfamily IA, variant 1 with third motif having Dx(3-4)D or Dx(3-4)E
Ferpe_1448	JM64_00340	hypothetical protein
Ferpe_1449	JM64_00345	Protein of unknown function (DUF2922)
Ferpe_1450	JM64_00350	hypothetical protein
Ferpe_1451	JM64_00360	hypothetical protein
Ferpe_1452	JM64_00365	6-phosphofructokinase 1
Ferpe_1453	JM64_00370	1,4-alpha-glucan branching enzyme

Ferpe_1454	JM64_00375	hypothetical protein
Ferpe_1455	JM64_00380	hypothetical protein
Ferpe_1457	JM64_00390	Glycosidase
Ferpe_1458	JM64_00395	peptide/nickel transport system ATP-binding protein
Ferpe_1459	JM64_00400	peptide/nickel transport system ATP-binding protein
Ferpe_1460	JM64_00405	ABC-type dipeptide/oligopeptide/nickel transport system, permease component
Ferpe_1462	JM64_00415	ABC-type transport system, substrate-binding protein
Ferpe_1463	JM64_00420	hypothetical protein
Ferpe_1464	JM64_00425	type III pantothenate kinase
Ferpe_1465	JM64_00430	Radical SAM superfamily enzyme YgiQ, UPF0313 family
Ferpe_1466	JM64_00435	hypothetical protein
Ferpe_1467	JM64_00440	hypothetical protein
Ferpe_1468	JM64_00445	Cell division protein ZapA
Ferpe_1469	JM64_00450	glutamate racemase
Ferpe_1470	JM64_00455	UPF0042 nucleotide-binding protein
Ferpe_1471	JM64_00460	conserved hypothetical protein, cofD-related
Ferpe_1472	JM64_00465	hypothetical protein
Ferpe_1473	JM64_00470	transcriptional repressor NrdR
Ferpe_1474	JM64_00475	transcription elongation factor GreA
Ferpe_1475	JM64_00480	lysyl-tRNA synthetase, class II
Ferpe_1477	JM64_00490	hypothetical protein
Ferpe_1478	JM64_00495	NAD(P)-dependent dehydrogenase, short-chain alcohol dehydrogenase family
Ferpe_1479	JM64_00500	methyl-accepting chemotaxis protein
Ferpe_1480	JM64_00505	phosphoribosylaminoimidazolecarboxamide formyltransferase / IMP cyclohydrolase
Ferpe_1482	JM64_00515	DNA repair exonuclease SbcCD nuclease subunit
Ferpe_1484	JM64_07410	Transposase
Ferpe_1485	JM64_00525	UDP-glucose 4-epimerase

Ferpe_1486	JM64_00530	hypothetical protein
Ferpe_1487	JM64_00550	glycerate 2-kinase
Ferpe_1489	JM64_00555	tRNA threonylcarbamoyl adenosine modification protein YeaZ
Ferpe_1490	JM64_00560	DNA mismatch repair protein MutS
Ferpe_1491	JM64_00565	ribonucleoside-triphosphate reductase
Ferpe_1492	JM64_00570	anaerobic ribonucleoside-triphosphate reductase activating protein
Ferpe_1493	JM64_00575	amino acid/amide ABC transporter ATP-binding protein 2, HAAT family
Ferpe_1494	JM64_00580	amino acid/amide ABC transporter ATP-binding protein 1, HAAT family
Ferpe_1495	JM64_00585	amino acid/amide ABC transporter membrane protein 2, HAAT family
Ferpe_1496	JM64_00590	amino acid/amide ABC transporter membrane protein 1, HAAT family
Ferpe_1497	JM64_00595	amino acid/amide ABC transporter substrate-binding protein, HAAT family
Ferpe_1498	JM64_00600	amino acid/amide ABC transporter substrate-binding protein, HAAT family
Ferpe_1499	JM64_00605	phosphopantothenoylcysteine decarboxylase / phosphopantothenate--cysteine ligase
Ferpe_1500	JM64_00610	hypothetical protein
Ferpe_1501	JM64_00615	acetoin utilization protein AcuB
Ferpe_1502	JM64_00620	condensin subunit ScpA
Ferpe_1504	JM64_00630	MoxR-like ATPase
Ferpe_1505	JM64_00635	Protein of unknown function (DUF1292)
Ferpe_1507	JM64_00645	inorganic phosphate transporter, PiT family
Ferpe_1508	JM64_00650	adenine phosphoribosyltransferase
Ferpe_1509	JM64_00655	glucose-6-phosphate isomerase
Ferpe_1510	JM64_00660	hypothetical protein
Ferpe_1511	JM64_00665	dephospho-CoA kinase
Ferpe_1512	JM64_00670	hypothetical protein
Ferpe_1513	JM64_00675	NAD+ synthase
Ferpe_1514	JM64_00680	hypothetical protein

Ferpe_1515	JM64_00685	PAS/PAC sensor signal transduction histidine kinase
Ferpe_1516	JM64_00690	L-aspartate 1-decarboxylase
Ferpe_1517	JM64_00695	hypothetical protein
Ferpe_1518	JM64_00700	mannose-6-phosphate isomerase, type 1
Ferpe_1519	JM64_00705	acyl carrier protein
Ferpe_1520	JM64_00710	oligoendopeptidase F
Ferpe_1521	JM64_00715	GTP-binding protein LepA
Ferpe_1522	JM64_00720	small conductance mechanosensitive channel
Ferpe_1523	JM64_00725	tryptophanyl-tRNA synthetase
Ferpe_1529	JM64_00735	Reverse gyrase
Ferpe_1530	JM64_00740	DNA replication and repair protein RecF
Ferpe_1531	JM64_00745	hypothetical protein
Ferpe_1532	JM64_00760	CRISPR-associated protein, Cmr6 family
Ferpe_1533	JM64_00765	CRISPR-associated protein Cmr5
Ferpe_1534	JM64_00770	CRISPR-associated protein, Cmr4 family
Ferpe_1535	JM64_00775	CRISPR-associated protein, Cmr3 family
Ferpe_1538	JM64_00795	hypothetical protein
Ferpe_1539	JM64_00800	hypothetical protein
Ferpe_1541	JM64_00815	CRISPR-associated protein, Csx2 family
Ferpe_1542	JM64_00820	hypothetical protein
Ferpe_1543	JM64_00825	hypothetical protein
Ferpe_1544	JM64_00830	hypothetical protein
Ferpe_1545	JM64_00835	CRISPR-associated protein, Cas2 family
Ferpe_1546	JM64_00840	CRISPR-associated protein, Cas2 family
Ferpe_1547	JM64_00845	hypothetical protein
Ferpe_1548	JM64_00850	CRISPR-associated protein, Cas6 family
Ferpe_1549	JM64_00855	hypothetical protein
Ferpe_1550	JM64_00860	CRISPR-associated protein, Csm5 family
Ferpe_1551	JM64_00865	CRISPR-associated protein, Csm4 family
Ferpe_1552	JM64_00870	CRISPR-associated protein, Csm3 family
Ferpe_1553	JM64_00875	CRISPR-associated protein, Csm2 family

Ferpe_1554	JM64_00880	CRISPR-associated protein Cas10/Csm1, subtype III-A/MTUBE
Ferpe_1555	JM64_00885	CRISPR-associated protein, Cas1 family
Ferpe_1556	JM64_05795	Transposase
Ferpe_1557	JM64_00890	hypothetical protein
Ferpe_1558	JM64_00895	molecular chaperone DnaK
Ferpe_1559	JM64_00900	Glycosyl hydrolase 108
Ferpe_1560	JM64_00910	aspartate aminotransferase
Ferpe_1561	JM64_00915	Uncharacterized protein family UPF0029
Ferpe_1562	JM64_00920	hypothetical protein
Ferpe_1563	JM64_00925	RNA polymerase, sigma 54 subunit, RpoN/SigL
Ferpe_1564	JM64_00930	pseudouridine-5'-phosphate glycosidase
Ferpe_1565	JM64_00935	PrcB C-terminal
Ferpe_1566	JM64_00940	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_1571	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_1572	JM64_06415	translation initiation factor IF-2
Ferpe_1574	JM64_05155	Transposase (or an inactivated derivative)
Ferpe_1586	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_1588	JM64_05425	Predicted nuclease (RNAse H fold)
Ferpe_1589	JM64_05435	Uri superfamily endonuclease
Ferpe_1590	JM64_05440	Deoxyribodipyrimidine photo-lyase type II
Ferpe_1591	JM64_05445	Predicted membrane protein
Ferpe_1592	JM64_05450	Membrane protein of unknown function (DUF340)
Ferpe_1593	JM64_05455	asparaginyl-tRNA synthetase
Ferpe_1594	JM64_05460	Cell wall-associated hydrolases (invasion-associated proteins)
Ferpe_1595	JM64_05465	hypothetical protein
Ferpe_1596	JM64_05470	fructose-1,6-bisphosphatase II
Ferpe_1597	JM64_05475	kojibiose phosphorylase
Ferpe_1598	JM64_05480	beta-phosphoglucomutase
Ferpe_1599	JM64_05485	carbohydrate ABC transporter membrane protein 2, CUT1 family

Ferpe_1600	JM64_05490	Binding-protein-dependent transport system inner membrane component
Ferpe_1601	JM64_05495	carbohydrate ABC transporter substrate-binding protein, CUT1 family
Ferpe_1602	JM64_05500	transcriptional regulator, LacI family
Ferpe_1603	JM64_05505	Acetyltransferases
Ferpe_1604	JM64_05510	hypothetical protein
Ferpe_1605	JM64_05515	putative phosphotransacetylase
Ferpe_1606	JM64_05520	sulfur carrier protein
Ferpe_1607	JM64_05525	HDIG domain-containing protein
Ferpe_1608	JM64_05530	DNA polymerase-3 subunit beta
Ferpe_1609	JM64_05535	prepilin-type N-terminal cleavage/methylation domain-containing protein
Ferpe_1610	JM64_05540	ABC-2 type transport system ATP-binding protein
Ferpe_1611	JM64_05545	Protein N-acetyltransferase, RimJ/RimL family
Ferpe_1612	JM64_05555	hypothetical protein
Ferpe_1613	JM64_05560	Predicted oxidoreductase, contains short-chain dehydrogenase (SDR) and DUF2520 domains
Ferpe_1615	JM64_05570	methylmalonyl-CoA mutase, C-terminal domain
Ferpe_1616	JM64_05575	hypothetical protein
Ferpe_1617	JM64_05580	hypothetical protein
Ferpe_1618	JM64_05585	hypothetical protein
Ferpe_1619	JM64_05590	Type II secretory pathway, component PulD
Ferpe_1620	JM64_05595	hypothetical protein
Ferpe_1621	JM64_05600	hypothetical protein
Ferpe_1622	JM64_05605	hypothetical protein
Ferpe_1623	JM64_05610	hypothetical protein
Ferpe_1624	JM64_05615	Acetyl/propionyl-CoA carboxylase, alpha subunit
Ferpe_1625	JM64_05620	Oxaloacetate decarboxylase, gamma chain
Ferpe_1626	JM64_05625	Acetyl-CoA carboxylase, carboxyltransferase component
Ferpe_1627	JM64_05630	methylmalonyl-CoA epimerase
Ferpe_1628	JM64_05635	Cyclic nucleotide-binding domain-containing protein

Ferpe_1629	JM64_05640	hypothetical protein
Ferpe_1630	JM64_05645	protein translocase subunit secA
Ferpe_1631	JM64_05650	2-oxoglutarate ferredoxin oxidoreductase subunit alpha
Ferpe_1632	JM64_05655	2-oxoglutarate ferredoxin oxidoreductase subunit delta
Ferpe_1633	JM64_05660	oxaloacetate decarboxylase, beta subunit
Ferpe_1634	JM64_05665	beta-aspartyl-dipeptidase (metallo-type)
Ferpe_1635	JM64_05670	hypothetical protein
Ferpe_1636	JM64_05675	glycine C-acetyltransferase
Ferpe_1637	JM64_05680	threonine 3-dehydrogenase
Ferpe_1638	JM64_05685	cAMP-binding domain of CRP or a regulatory subunit of cAMP-dependent protein kinases
Ferpe_1639	JM64_05690	hydroxylamine reductase
Ferpe_1640	JM64_05700	Predicted phosphohydrolases
Ferpe_1641	JM64_05705	Predicted transcriptional regulator
Ferpe_1642	JM64_05710	Uncharacterized conserved protein YbgA, DUF1722 family
Ferpe_1643	JM64_05715	Acetyl esterase/lipase
Ferpe_1644	JM64_05720	D-alanyl-D-alanine carboxypeptidase / D-alanyl-D-alanine-endopeptidase (penicillin-binding protein 4)
Ferpe_1645	JM64_05725	BirA family transcriptional regulator, biotin operon repressor / biotin-[acetyl-CoA-carboxylase] ligase
Ferpe_1646	JM64_05730	putative peptidoglycan lipid II flippase
Ferpe_1647	JM64_05735	ribonucrease Y
Ferpe_1648	JM64_05740	regulatory protein
Ferpe_1649	JM64_05745	recombination protein RecA
Ferpe_1650	JM64_05750	2'-5' RNA ligase
Ferpe_1651	JM64_05755	CDP-diacylglycerol--glycerol-3-phosphate 3-phosphatidyltransferase
Ferpe_1652	JM64_05760	SSU ribosomal protein S12P methylthiotransferase
Ferpe_1653	JM64_05765	protein of unknown function (DUF4416)
Ferpe_1654	JM64_05770	hypothetical protein
Ferpe_1655	JM64_05780	hypothetical protein

Ferpe_1656	JM64_05785	Adenylosuccinate synthetase
Ferpe_1657	JM64_05790	acylphosphatase
Ferpe_1659	JM64_05800	aspartate kinase
Ferpe_1660	JM64_05805	Uncharacterized conserved protein, DUF39 family
Ferpe_1661	JM64_05810	hypothetical protein
Ferpe_1662	JM64_05815	methylenetetrahydrofolate reductase (NADPH)
Ferpe_1663	JM64_05820	L-cysteine desulfidase
Ferpe_1664	JM64_05825	PAS domain S-box-containing protein
Ferpe_1665	JM64_05830	oxaloacetate decarboxylase, beta subunit
Ferpe_1667	JM64_05840	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_1668	JM64_05845	16S rRNA m(2)G 1207 methyltransferase
Ferpe_1669	JM64_05850	energy-coupling factor transport system ATP-binding protein
Ferpe_1670	JM64_05855	type IV pilus assembly protein PilC
Ferpe_1671	JM64_05860	hypothetical protein
Ferpe_1672	JM64_05865	hypothetical protein
Ferpe_1673	JM64_05870	hypothetical protein
Ferpe_1674	JM64_05875	hypothetical protein
Ferpe_1675	JM64_05880	hypothetical protein
Ferpe_1677	JM64_05890	hypothetical protein
Ferpe_1678	JM64_05895	large conductance mechanosensitive channel
Ferpe_1679	JM64_05900	nitrogen fixation protein NifU
Ferpe_1680	JM64_05905	cysteine desulfurase / selenocysteine lyase
Ferpe_1682	JM64_05915	Iron-regulated ABC transporter membrane component SufB
Ferpe_1683	JM64_05920	Fe-S cluster assembly ATP-binding protein
Ferpe_1688	JM64_05925	STE24 endopeptidase
Ferpe_1689	JM64_05930	micrococcal nuclease
Ferpe_1690	JM64_05940	Protein of unknown function (DUF3798)
Ferpe_1691	JM64_05945	monosaccharide ABC transporter ATP-binding protein, CUT2 family
Ferpe_1692	JM64_05950	monosaccharide ABC transporter membrane protein, CUT2 family

Ferpe_1693	JM64_05955	monosaccharide ABC transporter membrane protein, CUT2 family
Ferpe_1694	JM64_05960	hypothetical protein
Ferpe_1695	JM64_05965	Serine aminopeptidase, S33
Ferpe_1696	JM64_05970	hypothetical protein
Ferpe_1697	JM64_05980	hypothetical protein
Ferpe_1698	JM64_05985	spermidine/putrescine transport system substrate-binding protein
Ferpe_1699	JM64_05155	Transposase (or an inactivated derivative)
Ferpe_1700	JM64_05990	nicotinate-nucleotide-dimethylbenzimidazole phosphoribosyltransferase
Ferpe_1701	JM64_05995	adenosylcobinamide kinase /adenosylcobinamide-phosphate guanylyltransferase
Ferpe_1702	JM64_06000	hypothetical protein
Ferpe_1703	JM64_06005	cobalamin-5'-phosphate synthase
Ferpe_1704	JM64_06010	adenosylcobyric acid synthase
Ferpe_1705	JM64_06015	adenosylcobinamide-phosphate synthase
Ferpe_1706	JM64_06020	L-threonine O-3-phosphate decarboxylase
Ferpe_1707	JM64_06025	cob(I)alamin adenosyltransferase
Ferpe_1709	JM64_06035	DNA adenine methylase
Ferpe_1710	JM64_06040	type II restriction enzyme
Ferpe_1712	JM64_06050	HEAT repeat
Ferpe_1714	JM64_08705	alpha-galactosidase
Ferpe_1715	JM64_06285	Predicted glycosyl hydrolase, GH43/DUF377 family
Ferpe_1716	JM64_06290	multiple sugar transport system permease protein
Ferpe_1717	JM64_06295	carbohydrate ABC transporter membrane protein 1, CUT1 family
Ferpe_1718	JM64_06300	multiple sugar transport system substrate-binding protein
Ferpe_1719	JM64_06305	transcriptional regulator, LacI family
Ferpe_1720	JM64_06310	hypothetical protein
Ferpe_1722	JM64_06320	Glycogen recognition site of AMP-activated protein kinase
Ferpe_1723	JM64_03300	PD-(D/E)XK nuclease superfamily protein

Ferpe_1724	JM64_03305	hypothetical protein
Ferpe_1725	JM64_03315	Transposase (or an inactivated derivative)
Ferpe_1726	JM64_03320	Glucan phosphorylase
Ferpe_1728	JM64_03325	hypothetical protein
Ferpe_1729	JM64_03330	CAAX protease self-immunity
Ferpe_1731	JM64_07020	Transposase (or an inactivated derivative)
Ferpe_1732	JM64_06060	Predicted oxidoreductase
Ferpe_1734	JM64_06070	UvrD-like helicase C-terminal domain-containing protein
Ferpe_1735	JM64_06075	hypothetical protein
Ferpe_1736	JM64_06080	8-oxo-dGTP diphosphatase
Ferpe_1737	JM64_06085	hypothetical protein
Ferpe_1738	JM64_06090	site-specific DNA-methyltransferase (adenine-specific)
Ferpe_1739	JM64_06095	MJ0570-related uncharacterized domain-containing protein
Ferpe_1740	JM64_06100	nicotinate-nucleotide pyrophosphorylase [carboxylating]
Ferpe_1741	JM64_06105	quinolinate synthetase
Ferpe_1742	JM64_06110	aspartate dehydrogenase
Ferpe_1743	JM64_06115	Outer membrane protein assembly factor BamB, contains PQQ-like beta-propeller repeat
Ferpe_1744	JM64_06120	hypothetical protein
Ferpe_1746	JM64_06125	maltose 6'-phosphate phosphatase
Ferpe_1747	JM64_06130	transcriptional regulator, XRE family with cupin sensor
Ferpe_1748	JM64_06135	spermidine synthase
Ferpe_1749	JM64_06140	adenosylmethionine decarboxylase proenzyme
Ferpe_1750	JM64_06145	hypothetical protein
Ferpe_1751	JM64_06150	lipoate-protein ligase A
Ferpe_1752	JM64_06155	16S rRNA (cytidine1402-2'-O)-methyltransferase
Ferpe_1753	JM64_06160	16S rRNA (guanine(966)-N(2))-methyltransferase RsmD
Ferpe_1754	JM64_06165	hypothetical protein

Ferpe_1755	JM64_06170	Triacylglycerol esterase/lipase EstA, alpha/beta hydrolase fold
Ferpe_1756	JM64_06175	Protein of unknown function (DUF3242)
Ferpe_1757	JM64_06180	RNase HII
Ferpe_1758	JM64_06185	thymidylate synthase (FAD)
Ferpe_1759	JM64_06190	hypothetical protein
Ferpe_1760	JM64_06195	methylenetetrahydrofolate dehydrogenase (NADP+) / methenyltetrahydrofolate cyclohydrolase
Ferpe_1761	JM64_06200	protein translocase subunit yajC
Ferpe_1762	JM64_06205	preprotein translocase subunit SecD
Ferpe_1763	JM64_06210	protein translocase subunit secF
Ferpe_1764	JM64_06215	K(+)-stimulated pyrophosphate-energized sodium pump
Ferpe_1765	JM64_06220	6-phosphofructokinase 1
Ferpe_1766	JM64_06225	Na+/melibiose symporter
Ferpe_1767	JM64_06235	Alcohol dehydrogenase, class IV
Ferpe_1769	JM64_06245	Nitroimidazol reductase NimA, pyridoxamine 5'-phosphate oxidase superfamily
Ferpe_1770	JM64_06255	Copper chaperone CopZ
Ferpe_1771	JM64_06260	Cu+-exporting ATPase
Ferpe_1772	JM64_06265	high-affinity iron transporter
Ferpe_1773	JM64_06270	diguanylate cyclase (GGDEF) domain-containing protein
Ferpe_1774	JM64_06280	6-phosphofructokinase 2
Ferpe_1775	JM64_06285	Predicted glycosyl hydrolase, GH43/DUF377 family
Ferpe_1776	JM64_06290	carbohydrate ABC transporter membrane protein 2, CUT1 family
Ferpe_1777	JM64_06295	carbohydrate ABC transporter membrane protein 1, CUT1 family
Ferpe_1778	JM64_06300	carbohydrate ABC transporter substrate-binding protein, CUT1 family
Ferpe_1779	JM64_06305	transcriptional regulator, LacI family
Ferpe_1780	JM64_06310	hypothetical protein

Ferpe_1781	JM64_06315	hypothetical protein
Ferpe_1782	JM64_06320	Glycogen recognition site of AMP-activated protein kinase
Ferpe_1783	JM64_06325	MFS transporter, putative metabolite:H+ symporter
Ferpe_1784	JM64_06330	hypothetical protein
Ferpe_1785	JM64_06335	hypothetical protein
Ferpe_1786	JM64_06340	hypothetical protein
Ferpe_1787	JM64_06345	hypothetical protein
Ferpe_1788	JM64_06350	Uncharacterized protein conserved in bacteria
Ferpe_1789	JM64_06355	histidine triad (HIT) family protein
Ferpe_1790	JM64_06360	hypothetical protein
Ferpe_1791	JM64_06365	Isoleucyl-tRNA synthetase
Ferpe_1792	JM64_06370	Response regulator containing CheY-like receiver, AAA-type ATPase, and DNA-binding domains
Ferpe_1793	JM64_06375	pullulanase
Ferpe_1794	JM64_06380	phosphate ABC transporter substrate-binding protein, PhoT family
Ferpe_1795	JM64_06385	phosphate ABC transporter membrane protein 1, PhoT family
Ferpe_1796	JM64_06390	phosphate ABC transporter membrane protein 2, PhoT family
Ferpe_1797	JM64_06395	phosphate ABC transporter ATP-binding protein, PhoT family
Ferpe_1798	JM64_06400	phosphate uptake regulator, PhoU
Ferpe_1799	JM64_06405	two-component system, OmpR family, response regulator
Ferpe_1800	JM64_06410	two-component system, OmpR family, sensor kinase
Ferpe_1801	JM64_06415	translation initiation factor IF-2
Ferpe_1802	JM64_06420	dihydroflavonol-4-reductase
Ferpe_1804	JM64_06440	phosphoenolpyruvate carboxykinase (ATP)
Ferpe_1805	JM64_06445	1-Cys peroxiredoxin
Ferpe_1806	JM64_06450	starch phosphorylase
Ferpe_1807	JM64_06455	hypothetical protein

Ferpe_1808	JM64_06460	LexA-binding, inner membrane-associated putative hydrolase
Ferpe_1809	JM64_06465	Heat shock protein. Metallo peptidase. MEROPS family M48B
Ferpe_1810	JM64_06470	D-alanine--D-alanine ligase
Ferpe_1811	JM64_06475	transcriptional regulator, LacI family
Ferpe_1812	JM64_06480	nicotinamide-nucleotide amidase
Ferpe_1815	JM64_07410	Transposase
Ferpe_1816	JM64_06495	hypothetical protein
Ferpe_1817	JM64_06500	ABC-2 type transport system permease protein
Ferpe_1818	JM64_06505	hypothetical protein
Ferpe_1819	JM64_06515	Fn3 associated
Ferpe_1823	JM64_06525	replication restart DNA helicase PriA
Ferpe_1824	JM64_06530	5,10-methylenetetrahydrofolate reductase
Ferpe_1825	JM64_06535	hypothetical protein
Ferpe_1826	JM64_06540	GAF domain-containing protein
Ferpe_1827	JM64_06545	Uncharacterized membrane protein
Ferpe_1828	JM64_06550	electron transport complex protein RnfB
Ferpe_1829	JM64_06555	DNA processing protein
Ferpe_1830	JM64_06560	pantothenate synthetase
Ferpe_1831	JM64_06565	alpha-glucosidase
Ferpe_1832	JM64_06570	exodeoxyribonuclease VII large subunit
Ferpe_1833	JM64_06575	hypothetical protein
Ferpe_1834	JM64_06580	hypothetical protein
Ferpe_1835	JM64_06585	peptide/nickel transport system ATP-binding protein
Ferpe_1836	JM64_06590	peptide/nickel transport system ATP-binding protein
Ferpe_1837	JM64_06595	peptide/nickel transport system substrate-binding protein
Ferpe_1838	JM64_06600	peptide/nickel transport system permease protein
Ferpe_1839	JM64_06605	peptide/nickel transport system permease protein
Ferpe_1840	JM64_06610	beta-1,4-mannooligosaccharide/beta-1,4-mannosyl-N-acetylglucosamine phosphorylase

Ferpe_1841	JM64_06620	endoglucanase
Ferpe_1842	JM64_06625	transcriptional regulator, LacI family
Ferpe_1843	JM64_06630	Beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase
Ferpe_1844	JM64_00170	Transposase
Ferpe_1845	JM64_06660	hypothetical protein
Ferpe_1847	JM64_06670	flagellin
Ferpe_1848	JM64_06675	Glycosyltransferase
Ferpe_1849	JM64_06680	flagellin
Ferpe_1851	JM64_06685	Glycosyltransferases involved in cell wall biogenesis
Ferpe_1852	JM64_06690	flagellin
Ferpe_1853	JM64_06695	L-serine dehydratase
Ferpe_1854	JM64_06700	L-serine dehydratase
Ferpe_1855	JM64_06705	urocanate hydratase
Ferpe_1856	JM64_06710	protein of unknown function (DUF4895)
Ferpe_1857	JM64_06715	uncharacterized protein
Ferpe_1858	JM64_06720	hypothetical protein
Ferpe_1866	JM64_06750	histidyl-tRNA synthetase
Ferpe_1867	JM64_06755	hypothetical protein
Ferpe_1868	JM64_06760	hypothetical protein
Ferpe_1869	JM64_06765	glucose-1-phosphate thymidylyltransferase
Ferpe_1870	JM64_06770	glutamine synthetase
Ferpe_1871	JM64_06775	transcriptional regulator, PadR family
Ferpe_1872	JM64_06780	Predicted Fe-Mo cluster-binding protein, NifX family
Ferpe_1873	JM64_06785	Putative regulator of cell autolysis
Ferpe_1874	JM64_06790	two component transcriptional regulator, LytTR family
Ferpe_1875	JM64_06795	hypothetical protein
Ferpe_1876	JM64_06800	molecular chaperone DnaJ
Ferpe_1877	JM64_06805	molecular chaperone GrpE
Ferpe_1878	JM64_06810	heat-inducible transcription repressor HrcA

Ferpe_1879	JM64_06815	Predicted permease, DMT superfamily
Ferpe_1880	JM64_06820	SSU ribosomal protein S2P
Ferpe_1881	JM64_06825	ABC-type amino acid transport substrate-binding protein
Ferpe_1882	JM64_06835	Response regulator containing a CheY-like receiver domain and an HD-GYP domain
Ferpe_1883	JM64_06840	hypothetical protein
Ferpe_1884	JM64_06845	GTP-binding protein
Ferpe_1885	JM64_06850	4-hydroxy-3-methylbut-2-enyl diphosphate reductase
Ferpe_1886	JM64_06855	cytidylate kinase
Ferpe_1887	JM64_06860	anti-sigma B factor antagonist
Ferpe_1888	JM64_06865	aspartyl-tRNA synthetase
Ferpe_1889	JM64_06870	transaldolase
Ferpe_1890	JM64_06875	NADH-FMN oxidoreductase RutF, flavin reductase (DIM6/NTAB) family
Ferpe_1891	JM64_06880	aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit A
Ferpe_1892	JM64_06885	aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B
Ferpe_1893	JM64_06890	hypothetical protein
Ferpe_1894	JM64_06895	oxygen-independent coproporphyrinogen-3 oxidase
Ferpe_1896	JM64_06905	UDP-N-acetylglucosamine-N-acetylmuramylpentapeptide N-acetylglucosamine transferase
Ferpe_1897	JM64_06910	UDP-N-acetylmuramate--L-alanine ligase
Ferpe_1898	JM64_06915	condensin subunit Smc
Ferpe_1899	JM64_06920	PAS domain S-box-containing protein
Ferpe_1900	JM64_06925	flagellar FliJ protein
Ferpe_1901	JM64_06930	Uncharacterized SAM-binding protein YcdF, DUF218 family
Ferpe_1902	JM64_06935	Membrane associated serine protease, rhomboid family
Ferpe_1903	JM64_06940	L-asparaginase
Ferpe_1904	JM64_06945	Predicted PurR-regulated permease PerM

Ferpe_1905	JM64_06950	ketopantoate hydroxymethyltransferase
Ferpe_1906	JM64_06955	oligoendopeptidase, pepF/M3 family
Ferpe_1907	JM64_06965	aldose 1-epimerase
Ferpe_1908	JM64_06970	PmbA protein
Ferpe_1909	JM64_06975	TldD protein
Ferpe_1910	JM64_06980	DNA mismatch repair protein MutL
Ferpe_1911	JM64_06985	hypothetical protein
Ferpe_1912	JM64_06990	hypothetical protein
Ferpe_1913	JM64_06995	flagellar basal-body rod protein FlgB
Ferpe_1914	JM64_07000	flagellar basal-body rod protein FlgC
Ferpe_1915	JM64_07005	flagellar hook-basal body complex protein FliE
Ferpe_1916	JM64_07010	hypothetical protein
Ferpe_1917	JM64_07015	SSU ribosomal protein S15P
Ferpe_1918	JM64_07025	S-adenosylmethionine--tRNA ribosyltransferase-isomerase
Ferpe_1919	JM64_07030	Holliday junction endonuclease RuvC
Ferpe_1920	JM64_07035	DNA polymerase-3 subunit alpha
Ferpe_1921	JM64_07040	protein translocase subunit secG
Ferpe_1922	JM64_07045	tyrosyl-tRNA synthetase
Ferpe_1924	JM64_07055	hypothetical protein
Ferpe_1925	JM64_07060	enolase
Ferpe_1926	JM64_07065	Glycosidase
Ferpe_1927	JM64_07070	UDP-N-acetylmuramoylalanine--D-glutamate ligase
Ferpe_1928	JM64_07075	DNA polymerase-4
Ferpe_1930	JM64_07085	VanZ like family protein
Ferpe_1931	JM64_07090	amino acid ABC transporter substrate-binding protein, PAAT family
Ferpe_1933	JM64_07100	Response regulator containing a CheY-like receiver domain and an HD-GYP domain
Ferpe_1934	JM64_07105	competence protein ComEA
Ferpe_1935	JM64_07110	hypothetical protein
Ferpe_1936	JM64_07115	endoglucanase

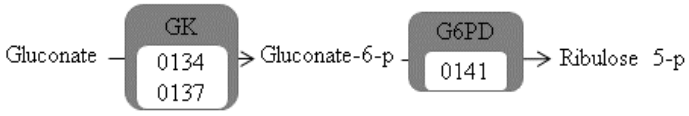
Ferpe_1938	JM64_07125	hypothetical protein
Ferpe_1939	JM64_07130	23S rRNA m(2)A-2503 methyltransferase
Ferpe_1940	JM64_07135	serine/threonine protein kinase
Ferpe_1941	JM64_07140	ribosome biogenesis GTPase
Ferpe_1942	JM64_07145	Glycosyltransferase involved in cell wall bisynthesis
Ferpe_1943	JM64_07150	hypothetical protein
Ferpe_1944	JM64_07155	hypothetical protein
Ferpe_1945	JM64_07160	hypothetical protein
Ferpe_1946	JM64_07165	C-terminal processing peptidase-3. Serine peptidase. MEROPS family S41A
Ferpe_1947	JM64_07170	tRNA (adenine57-N1/adenine58-N1)-methyltransferase
Ferpe_1948	JM64_07175	hypothetical protein
Ferpe_1949	JM64_07180	hypothetical protein
Ferpe_1950	JM64_07185	hypothetical protein
Ferpe_1952	JM64_07195	trk system potassium uptake protein TrkH
Ferpe_1953	JM64_07200	trk system potassium uptake protein TrkA
Ferpe_1954	JM64_07205	trk system potassium uptake protein TrkA
Ferpe_1955	JM64_07210	Response regulator containing CheY-like receiver, AAA-type ATPase, and DNA-binding domains
Ferpe_1956	JM64_07215	Glycosidase
Ferpe_1959	JM64_07230	hypothetical protein
Ferpe_1960	JM64_07235	transketolase
Ferpe_1961	JM64_07240	TIGR00255 family protein
Ferpe_1962	JM64_07245	hypothetical protein
Ferpe_1963	JM64_07250	guanylate kinase
Ferpe_1964	JM64_07255	DNA-directed RNA polymerase subunit omega
Ferpe_1965	JM64_07260	amidophosphoribosyltransferase
Ferpe_1966	JM64_07265	phosphoribosylglycinamide formyltransferase-1
Ferpe_1967	JM64_07270	phosphoribosylaminoimidazolecarboxamide formyltransferase / IMP cyclohydrolase
Ferpe_1968	JM64_07275	phosphoribosylamine--glycine ligase
Ferpe_1969	JM64_07280	phosphoribosylformylglycinamidine cyclo-ligase

Ferpe_1970	JM64_07285	LemA protein
Ferpe_1971	JM64_07310	conserved hypothetical protein
Ferpe_1972	JM64_07315	Predicted phosphoesterase, NUDIX family
Ferpe_1973	JM64_07320	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase
Ferpe_1974	JM64_07325	lon-related putative ATP-dependent protease
Ferpe_1975	JM64_07330	6-phosphofructokinase
Ferpe_1976	JM64_07335	hypothetical protein
Ferpe_1977	JM64_07340	tRNA uridine 5-carboxymethylaminomethyl modification enzyme
Ferpe_1978	JM64_07345	Murein DD-endopeptidase MepM and murein hydrolase activator NlpD, contain LysM domain
Ferpe_1979	JM64_07350	[LSU ribosomal protein L11P]-lysine N-methyltransferase
Ferpe_1980	JM64_07355	hypothetical protein
Ferpe_1981	JM64_07360	Uncharacterized conserved protein
Ferpe_1982	JM64_07365	23S rRNA (pseudouridine1915-N3)-methyltransferase
Ferpe_1983	JM64_07370	anti-sigma-28 factor, FlgM family
Ferpe_1984	JM64_07375	hypothetical protein
Ferpe_1985	JM64_07380	flagellar hook-associated protein 1 FlgK
Ferpe_1986	JM64_07385	flagellar hook-associated protein 3 FlgL
Ferpe_1987	JM64_07390	tRNA1(Val) A37 N6-methylase TrmN6
Ferpe_1988	JM64_07395	adenylosuccinate lyase
Ferpe_1989	JM64_07400	hypothetical protein
Ferpe_1990	JM64_07415	4-hydroxy-tetrahydrodipicolinate reductase
Ferpe_1991	JM64_07420	hypothetical protein
Ferpe_1992	JM64_07425	Cysteine synthase
Ferpe_1993	JM64_07430	D-ornithine 4,5-aminomutase S subunit
Ferpe_1994	JM64_07435	D-ornithine 4,5-aminomutase E subunit
Ferpe_1995	JM64_07440	delta-1-pyrroline-5-carboxylate dehydrogenase
Ferpe_1999	JM64_07450	hypothetical protein
Ferpe_2000	JM64_07455	hypothetical protein
Ferpe_2002	JM64_07465	hypothetical protein

Ferpe_2003	JM64_07470	alkaline phosphatase
Ferpe_2004	JM64_07475	Transglutaminase-like enzymes, putative cysteine proteases
Ferpe_2005	JM64_07480	L-alanine-DL-glutamate epimerase
Ferpe_2007	JM64_07500	peptide/nickel transport system substrate-binding protein
Ferpe_2008	JM64_07505	DNA-3-methyladenine glycosylase III
Ferpe_2009	JM64_07545	Rubrerythrin
Ferpe_2010	JM64_07550	glutamate synthase (NADPH/NADH) small chain
Ferpe_2011	JM64_07555	NADH-quinone oxidoreductase subunit D
Ferpe_2012	JM64_07560	NADH-quinone oxidoreductase subunit C
Ferpe_2013	JM64_07565	NADH-quinone oxidoreductase subunit B
Ferpe_2014	JM64_07570	NADH-quinone oxidoreductase subunit H
Ferpe_2015	JM64_07575	NADH-quinone oxidoreductase subunit M
Ferpe_2016	JM64_07580	multicomponent Na+:H+ antiporter subunit D
Ferpe_2017	JM64_07585	multicomponent Na+:H+ antiporter subunit C
Ferpe_2018	JM64_07590	multicomponent Na+:H+ antiporter subunit B
Ferpe_2019	JM64_07595	Uncharacterized MnhB-related membrane protein
Ferpe_2020	JM64_07600	multicomponent Na+:H+ antiporter subunit G
Ferpe_2022	JM64_07610	multicomponent Na+:H+ antiporter subunit E
Ferpe_2023	JM64_07615	pyridoxal phosphate synthase yaaE subunit
Ferpe_2024	JM64_07620	pyridoxal phosphate synthase yaaD subunit
Ferpe_2025	JM64_07625	methylmalonyl-CoA mutase, N-terminal domain
Ferpe_2026	JM64_07630	hypothetical protein
Ferpe_2027	JM64_07635	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine diphosphokinase
Ferpe_2028	JM64_07640	oxaloacetate decarboxylase, alpha subunit
Ferpe_2029	JM64_07645	hypothetical protein
Ferpe_2030	JM64_07650	Predicted nucleotidyltransferase
Ferpe_2031	JM64_07655	transcription-repair coupling factor
Ferpe_2032	JM64_07660	hypothetical protein
Ferpe_2033	JM64_07665	flagellar biosynthesis protein
Ferpe_2034	JM64_07670	hypothetical protein

Ferpe_2035	JM64_07675	peptide chain release factor 2
Ferpe_2036	JM64_07680	septum site-determining protein MinD
Ferpe_2037	JM64_07685	cell division topological specificity factor
Ferpe_2038	JM64_07690	hypothetical protein
Ferpe_2040	JM64_07705	Predicted O-methyltransferase
Ferpe_2041	JM64_07710	signal recognition particle-docking protein FtsY
Ferpe_2042	JM64_07715	hypothetical protein
Ferpe_2043	JM64_07720	3D (Asp-Asp-Asp) domain-containing protein
Ferpe_2044	JM64_07725	transcriptional regulator, BadM/Rrf2 family
Ferpe_2045	JM64_07730	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase
Ferpe_2046	JM64_07735	hypoxanthine phosphoribosyltransferase
Ferpe_2047	JM64_07740	transcriptional attenuator, LytR family
Ferpe_2048	JM64_07745	hypothetical protein
Ferpe_2049	JM64_07750	hypothetical protein
Ferpe_2051	JM64_07760	N-acetylglucosaminyldiphosphoundecaprenol N-acetyl-beta-D-mannosaminyltransferase
Ferpe_2052	JM64_07765	16S rRNA (guanine527-N7)-methyltransferase
Ferpe_2053	JM64_07770	Predicted dehydrogenase
Ferpe_2054	JM64_07775	6-pyruvoyltetrahydropterin/6-carboxytetrahydropterin synthase
Ferpe_2055	JM64_07780	DNA-directed RNA polymerase subunit beta'
Ferpe_2056	JM64_07785	DNA-directed RNA polymerase subunit beta
Ferpe_2057	JM64_07790	LSU ribosomal protein L12P
Ferpe_2058	JM64_07795	LSU ribosomal protein L10P
Ferpe_2060	JM64_07805	hypothetical protein
Ferpe_2061	JM64_07810	16S rRNA (cytosine1402-N4)-methyltransferase
Ferpe_2062	JM64_07815	carbamate kinase
Ferpe_2063	JM64_07820	phosphate butyryltransferase

The two strains of Fervidobacterium 9078 and DYC appear to be highly related, yet distinct. In Figure A.5 we noticed 2 large ~50Kbp tracks of DNA unique to each, as well as many small unique tracts of DNA, in some cases a single unique gene being added in an otherwise syntenic area. We assessed whether these unique genes enabled any different functionality from a core metabolism perspective using KEGG pathways overlay available through IMG/er. Several genes in DYC concerning core metabolism appeared to be missing when compared to 9078. Of note, the findings indicated that contained within a large tract of DNA unique to strain 9078 (Ferpe_0103- Ferpe_0194) appeared to have two key enzymes that may allow a full Entner-Doudoroff pathway to function, Gluconate kinase, and Gluconate 6-p dehydrogenase with locus tags Ferpe_0134/0137 and Ferpe_0141 respectively shown in Figure A.6.



GK= gluconate Kinase; G6PD= Gluconate 6-phosphate dehydrogenase

Figure A.6. Entner-Doudoroff pathway present only in 9078

A.4.4. *F. pennivorans* DSM 9078 and DYC End-Product Production

Both 9078 and DYC produce the same major end-product profiles from cellobiose metabolism chromatographically consistent with CO_2, H_2, acetate, alanine, and glutamate production. Major end-products on different substrates are shown in Figure A.7. Both alanine and glutamate were measured indirectly, via van Slyke reaction (Pleissner *et al.* 2010).There were many minor end-product peaks that were below quantification limits, some of them were responsive to the presence of sugar substrate being present such as ethanol, butanol, and butyrate; others were not responsive to sugar substrate being present such as peaks consistent with glycerol (or formate as they co-elute), and many unknown peaks at 10.1, 10.5, 13.1, 14.2, 18.51, 20.03, and 21.49 minutes. The peaks that were not responsive to sugar substrates were assumed to be the result of YE metabolism. Trace butyrate and butanol was observed in growth with 9078 under almost every growth condition when a sugar substrate was present and in DYC under growth on xylose, results shown in Table A.6. Trace ethanol (Table A.7) was produced in DYC under every sugar substrate (with the exception of xylose) and without a sugar substrate. In 9078 trace ethanol was only produced in the presence of cellobiose sugar substrate.

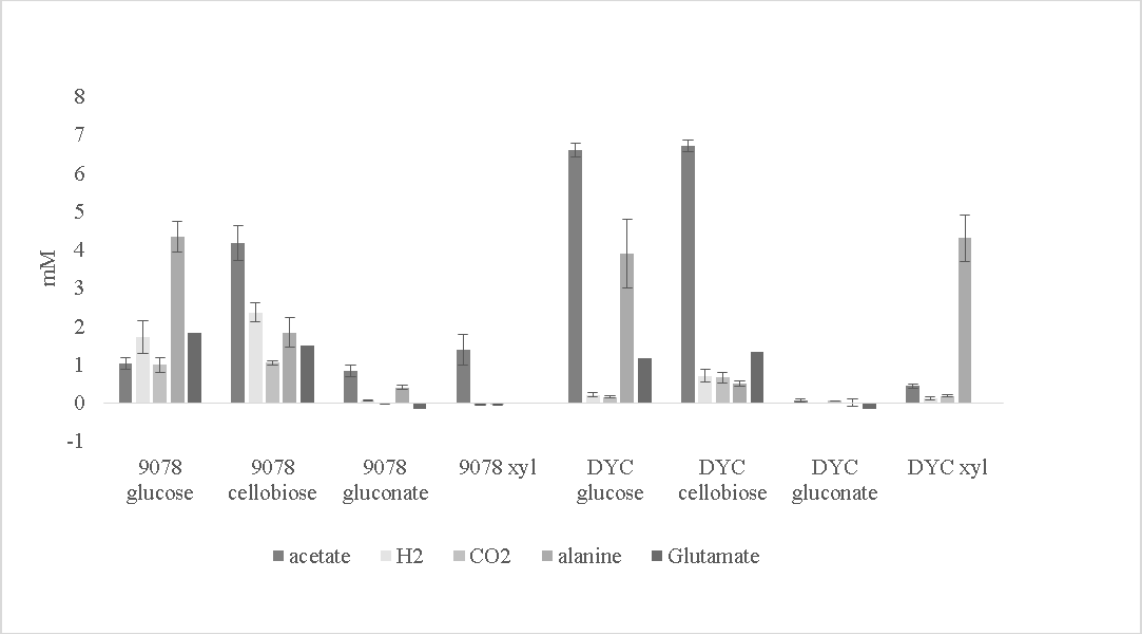


Figure A.7. 9078 vs DYC mM of major end-products yield after 80 hours PI

Table A.6. Presence of trace butyrate/butanol in fermentation profiles using different sugar substrates

Substrate	Trace butyrate	Trace butanol
9078 no sugar substrate	+	+
9078 glucose	+	+
9078 cellobiose	+	-
9078 gluconate	+	+
9078 xylose	+	+
DYC no sugar substrate	-	-
DYC glucose	-	-
DYC cellobiose	-	-
DYC gluconate	-	-
DYC xylose	+	+

Table A.7. Presences of trace ethanol in fermentation profiles using different sugar substrates

Substrate	Trace ethanol
9078 no sugar substrate	-
9078 glucose	-
9078 cellobiose	+
9078 gluconate	-
9078 xylose	-
DYC no sugar substrate	+
DYC glucose	+
DYC cellobiose	+
DYC gluconate	+
DYC xylose	-

A.4.5. Carbon and Redox Balance

Carbon balances, Figure A.8, slightly high and in the ~95-115% range. The only two major end-products which contributed to our redox calculation were CO_2 and H_2 as the other major end-products were redox neutral. O/R balances where an appreciable amount of H_2 and CO_2 were produced (<0.2mM) showed redox balances of 90%+ in all but two case, DYC on cellobiose which had the poorest redox balance of ~50%. As CO_2 and H_2 represented a small amount of total carbon and electrons in many cases the noise to signal ratio was quite high. When considering redox balance the most important observation we made is that the vast majority of end-products produced via sugar fermentation were O/R neutral.

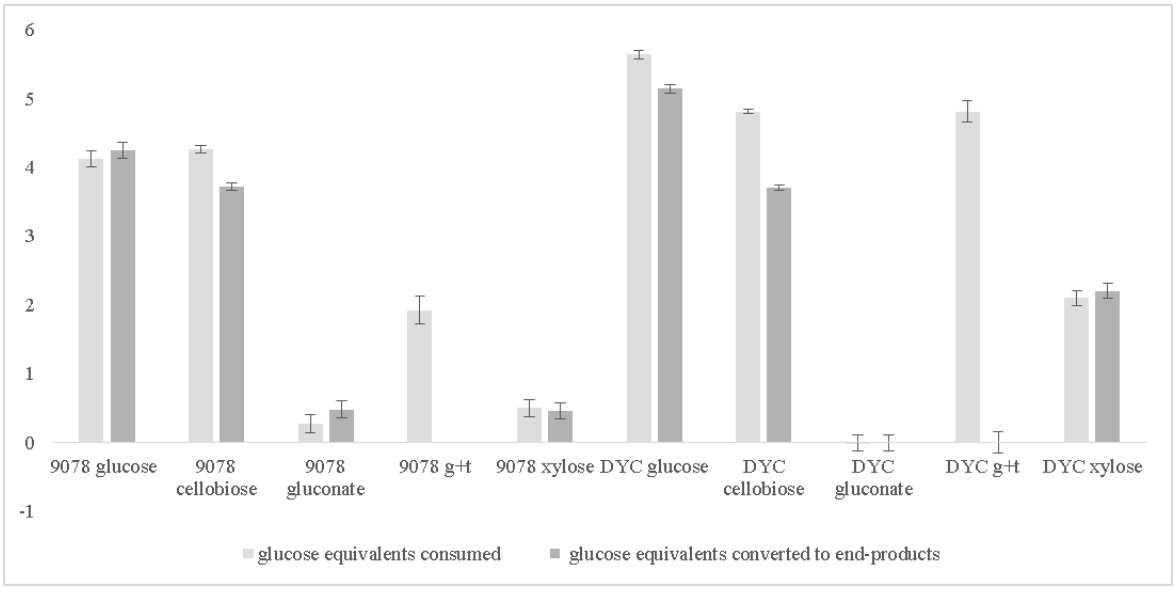
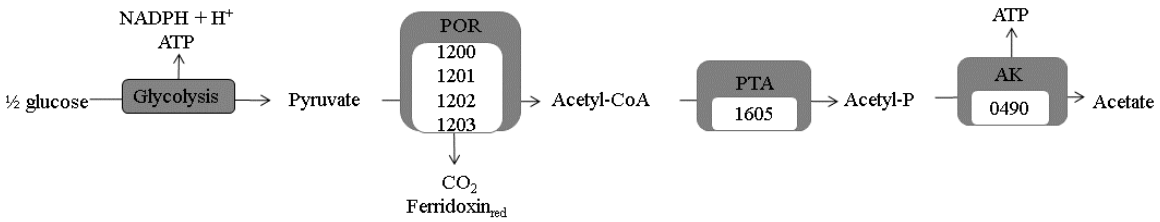


Figure A.8. 9078 vs DYC carbon balances at 80h pi

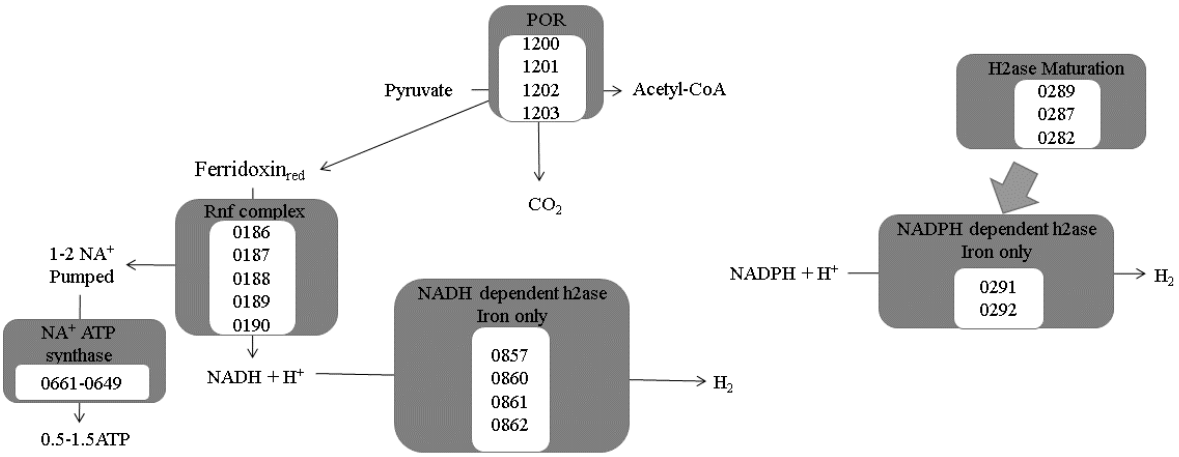
A.4.6. Gene Complement to Produce Major End-products of Interest

F. pennivorans appear to possess the necessary pathways to create the major products acetate, alanine, glutamate, H_2, and CO_2 (Shown in Figures A.9-A.12) and both DYC and 9078 appear to have the same gene complement regarding these products, only the 9078 locus tags are listed but the corresponding locus tag for DYC can be found in Table A.5.



POR= Pyruvate Oxidoreductase; PTA= phosphotransacetylase; AK= Acetate Kinase

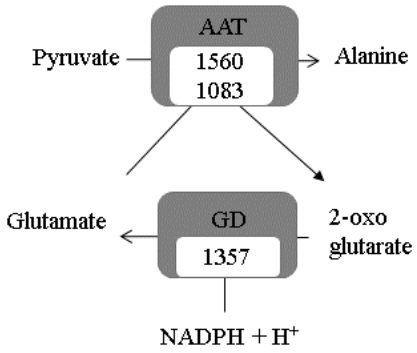
Figure A.9. Proposed pathway of acetate production in 9078 and DYC



POR= Pyruvate Oxidoreductase

Figure A.10. Proposed pathway of RNF and hydrogen production based on gene complement in 9078 and DYC.

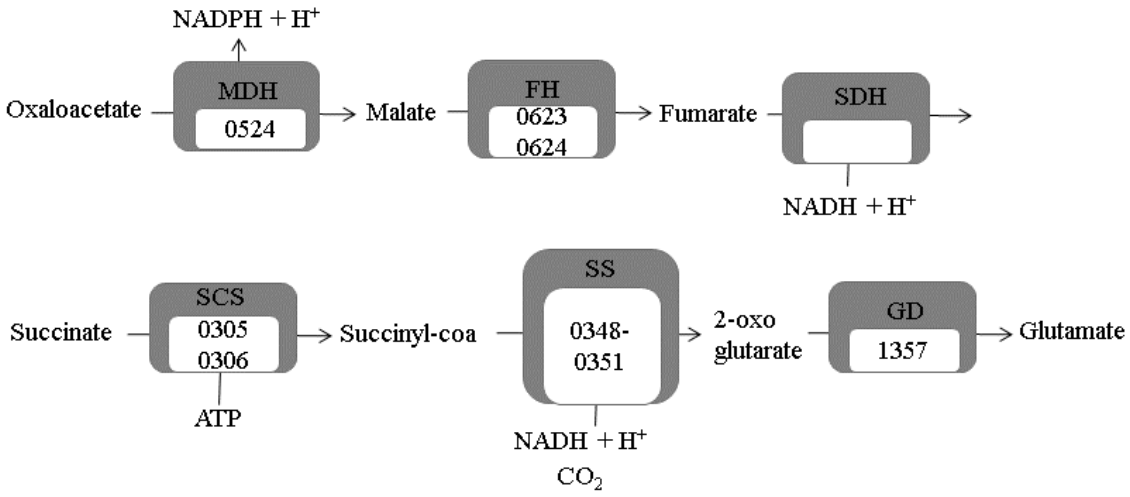
Kengen *et al.* (1994) proposes alanine formation in *Pyrococcus furiosus* via an alanine aminotransferase and glutamate/2 oxo-glutarate cycling via a glutamate reductase shown in Figure A.11. We were able to readily identify a glutamate reductase (Ferpe_1357) but had difficulty identifying an appropriate alanine aminotransferase (AAT). We identified 2 possible genes that could function as AAT and 9078, Ferpe_1560 and Ferpe_1083, which were annotated as aspartate aminotransferases with a 56% AA sequence similarity with *Pyrococcus furiosus's* alanine aminotransferase.



AAT=Alanine Ammonia Transferase; GD= Glutamate dehydrogenase

Figure A.11. Possible mechanism of alanine production in 9078 and DYC based off the mechanism for alanine production in *Pyrococcus furiosus* by Kengen *et al.* (1994)

The pathway used for glutamate production may not be as straight forward as in other organisms. *F. pennivorans* (both DYC and 9078) have an incomplete TCA cycle. *F pennivorans* is missing Citrate synthase, Aconitase, Isocitrate dehydrogenase, and Succinate dehydrogenase. *F. pennivorans* does not appear to produce glutamate via reverse TCA given that it is missing a succinate dehydrogenase gene or fumarate reductase to convert fumarate to succinate (Figure A.12). However Metacyc analysis shows that precursors to glutamate metabolism such as succinyl-CoA can be generated by other mechanisms like methylmalonyl-CoA mutase. *F. pennivorans* does have the genes to convert succinyl-CoA to 2-oxoglutarate and then to glutamate. Regardless of the source of production succinyl-CoA or succinate is necessary for glutamate synthesis, the mechanism for this is unclear.



MDH= Malate dehydrogenase; FH= Fumarate Hydratase; SDH= Succinate dehydrogenase; SCS=Succinate CoA Synthase; SS=Succinate synthase; GD= glutamate dehydrogenase.

Figure A.12. Missing SDH/FR in 9078 and DYC do not allow for synthesis of glutamate through reverse tricarboxylic acid cycle.

A.4.7. Proteomics

We analyzed the proteome of 9078 during exponential phase, Table A.8. We detected and gave TIC scores to 1690/2006 putative proteins giving us 84% proteomic coverage. While most of the highest expressed genes were involved in core metabolism, we were able to pick several highly expressed proteins in the top 20 that were of interest, including: i) ornithine carbamoyltransferase, which is involved in arginine fermentation, ii) serine-pyruvate aminotransferase, which may be involved in alanine production or serine degradation, and iii) a butanol specific alcohol dehydrogenase indicating potential for thermophilic butanol production. We noticed 9078 expressed several sulfide dehydrogenase associated genes amongst the lowest level of detected proteins. Strain 9078 appears to highly express genes needed for both AA fermentation and sugar fermentation. *F. pennivorans* has a small genome and may express a large portion (~ 84% of annotated genes were detected) of it in preparedness for changing conditions; indeed there are other examples of expression stability of thermophilic anaerobes under changing conditions (Verbeke *et al.* 2014).

Table A.8. *F. pennivorans* 9078 log2TIC expression value as measured by proteomics

COG	Locus Tag	Locus Description	Log2TIC
J	Ferpe_01979	LSU ribosomal protein L11P]-lysine	21.49
K	Ferpe_01362	(p)ppGpp synthetase, RelA/SpoT family	18.96
T	Ferpe_01362	(p)ppGpp synthetase, RelA/SpoT family	18.96
X	Ferpe_01453	1,4-alpha-glucan branching enzyme	17.47
E	Ferpe_00313	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate	20.52
G	Ferpe_00940	2,3-bisphosphoglycerate-independent	26.57
I	Ferpe_00067	2C-methyl-D-erythritol 2,4-cyclodiphosphate	22.17
C	Ferpe_00301	2-oxoacid:acceptor oxidoreductase, alpha subunit	22.19
C	Ferpe_00300	2-oxoacid:acceptor oxidoreductase, beta subunit,	21.36
C	Ferpe_01137	2-oxoglutarate ferredoxin oxidoreductase, beta	24.06
I	Ferpe_00536	3-oxoacid CoA-transferase, A subunit	23.84
I	Ferpe_00537	3-oxoacid CoA-transferase, B subunit	22.12
H	Ferpe_01760	5,10-methylene-tetrahydrofolate	23.42
E	Ferpe_01662	5,10-methylenetetrahydrofolate reductase	18.54
E	Ferpe_01824	5,10-methylenetetrahydrofolate reductase	18.47
J	Ferpe_01416	50S ribosomal protein L3, bacterial	24.55
J	Ferpe_01415	50S ribosomal protein L4, bacterial/organelle	25.01
F	Ferpe_00915	5'-nucleotidase/2',3'-cyclic phosphodiesterase	24.14
V	Ferpe_00915	5'-nucleotidase/2',3'-cyclic phosphodiesterase	24.14
F	Ferpe_01001	5'-nucleotidase/2',3'-cyclic phosphodiesterase	26.26
V	Ferpe_01001	5'-nucleotidase/2',3'-cyclic phosphodiesterase	26.26
H	Ferpe_00800	6,7-dimethyl-8-ribityllumazine synthase (EC	17.36
G	Ferpe_00141	6-phosphogluconate dehydrogenase,	21.24
H	Ferpe_00223	ABC transporter periplasmic binding protein,	22.97
M	Ferpe_00328	ABC-type (unclassified) transport system, ATPase	21.4
M	Ferpe_00236	ABC-type antimicrobial peptide transport system,	20.26
P	Ferpe_00216	ABC-type cobalt transport system, ATPase	18.24
R	Ferpe_00216	ABC-type cobalt transport system, ATPase	18.24
P	Ferpe_01669	ABC-type cobalt transport system, ATPase	20.09
R	Ferpe_01669	ABC-type cobalt transport system, ATPase	20.09
P	Ferpe_01686	ABC-type cobalt transport system, ATPase	18.94
R	Ferpe_01686	ABC-type cobalt transport system, ATPase	18.94

P	Ferpe_01687	ABC-type cobalt transport system, ATPase	19.51
R	Ferpe_01687	ABC-type cobalt transport system, ATPase	19.51
H	Ferpe_00316	ABC-type cobalt transport system, permease	17.7
H	Ferpe_01685	ABC-type cobalt transport system, permease	
E	Ferpe_00304	ABC-type dipeptide transport system, periplasmic	25.24
E	Ferpe_00409	ABC-type dipeptide transport system, periplasmic	27.5
E	Ferpe_00761	ABC-type dipeptide transport system, periplasmic	24.21
E	Ferpe_01462	ABC-type dipeptide transport system, periplasmic	24.43
E	Ferpe_01837	ABC-type dipeptide transport system, periplasmic	26.37
E	Ferpe_02007	ABC-type dipeptide transport system, periplasmic	14.78
P	Ferpe_00260	ABC-type Fe3+ transport system, permease	17.24
P	Ferpe_00795	ABC-type Fe3+-hydroxamate transport system,	19.93
P	Ferpe_00796	ABC-type Fe3+-siderophore transport system,	17.31
G	Ferpe_00982	ABC-type maltose transport systems, permease	23
P	Ferpe_01190	ABC-type metal ion transport system, periplasmic	19.56
P	Ferpe_01189	ABC-type Mn/Zn transport systems, ATPase	19.67
P	Ferpe_01188	ABC-type Mn2+/Zn2+ transport systems, permease	
V	Ferpe_00679	ABC-type multidrug transport system, ATPase	16.29
V	Ferpe_00801	ABC-type multidrug transport system, ATPase	24.27
V	Ferpe_00835	ABC-type multidrug transport system, ATPase	20.84
V	Ferpe_00958	ABC-type multidrug transport system, ATPase	19.03
V	Ferpe_01610	ABC-type multidrug transport system, ATPase	19.34
V	Ferpe_00389	ABC-type multidrug transport system, ATPase and	20.05
V	Ferpe_00390	ABC-type multidrug transport system, ATPase and	20.07
V	Ferpe_01072	ABC-type multidrug transport system, ATPase and	23.13
V	Ferpe_01073	ABC-type multidrug transport system, ATPase and	21.72
V	Ferpe_01153	ABC-type multidrug transport system, ATPase and	20.98
V	Ferpe_01356	ABC-type multidrug transport system, ATPase and	21.28
V	Ferpe_00959	ABC-type multidrug transport system, permease	
G	Ferpe_00157	ABC-type ribose transport system, auxiliary	
E	Ferpe_00170	ABC-type spermidine/putrescine transport system,	20.41
E	Ferpe_00171	ABC-type spermidine/putrescine transport system,	
G	Ferpe_00447	ABC-type sugar transport system, periplasmic	20.53
G	Ferpe_00448	ABC-type sugar transport system, periplasmic	21.78

G	Ferpe_00814	ABC-type sugar transport system, periplasmic	18.03
G	Ferpe_00815	ABC-type sugar transport system, periplasmic	22.42
G	Ferpe_01216	ABC-type sugar transport system, periplasmic	22.54
G	Ferpe_01239	ABC-type sugar transport system, periplasmic	14.33
G	Ferpe_01718	ABC-type sugar transport system, periplasmic	17.19
G	Ferpe_00808	ABC-type sugar transport system, permease	
G	Ferpe_00812	ABC-type sugar transport system, permease	
G	Ferpe_01218	ABC-type sugar transport system, permease	
G	Ferpe_01237	ABC-type sugar transport system, permease	
G	Ferpe_01716	ABC-type sugar transport system, permease	
G	Ferpe_00446	ABC-type sugar transport systems, permease	
G	Ferpe_00809	ABC-type sugar transport systems, permease	13.53
G	Ferpe_01217	ABC-type sugar transport systems, permease	
G	Ferpe_01600	ABC-type sugar transport systems, permease	
M	Ferpe_00099	ABC-type transport system, involved in	20.04
X	Ferpe_01624	Acetyl/propionyl-CoA carboxylase, alpha subunit	19.25
I	Ferpe_01626	Acetyl-CoA carboxylase, carboxyltransferase	25.91
J	Ferpe_00370	Acetyltransferases, including N-acetylases of	16.31
O	Ferpe_00370	Acetyltransferases, including N-acetylases of	16.31
X	Ferpe_01306	Acetyltransferases, including N-acetylases of	19.75
J	Ferpe_01611	Acetyltransferases, including N-acetylases of	21.9
O	Ferpe_01611	Acetyltransferases, including N-acetylases of	21.9
T	Ferpe_00015	Adenylate cyclase, family 3 (some proteins	
C	Ferpe_01132	Alcohol dehydrogenase, class IV	22.88
C	Ferpe_01234	Alcohol dehydrogenase, class IV	18.44
C	Ferpe_01767	Alcohol dehydrogenase, class IV	28.87
G	Ferpe_01126	Alpha-galactosidases/6-phospho-beta-glucosidases,	21.88
G	Ferpe_01714	Alpha-galactosidases/6-phospho-beta-glucosidases,	21.6
G	Ferpe_01231	Alpha-glucosidases, family 31 of glycosyl	18.85
G	Ferpe_01831	Alpha-glucosidases, family 31 of glycosyl	23.71
E	Ferpe_01206	amino acid ABC transporter ATP-binding protein,	22.16
E	Ferpe_01207	amino acid ABC transporter membrane protein,	21.05
K	Ferpe_01983	anti-sigma-28 factor, FlgM family	17.65
N	Ferpe_01983	anti-sigma-28 factor, FlgM family	17.65

J	Ferpe_01888	aspartyl-tRNA synthetase, bacterial type	25.4
C	Ferpe_00654	ATP synthase, F0 subunit c	24.26
C	Ferpe_00650	ATP synthase, F1 gamma subunit	23
P	Ferpe_01771	ATPase, P-type (transporting), HAD superfamily,	20.21
J	Ferpe_00207	ATPase, YjeE family	19
O	Ferpe_00227	ATPases with chaperone activity, ATP-binding	25.14
O	Ferpe_00424	ATPases with chaperone activity, ATP-binding	24.77
O	Ferpe_01385	ATP-dependent Clp protease, proteolytic subunit	24.46
O	Ferpe_00193	ATP-dependent protease HslVU, ATPase subunit	25.25
O	Ferpe_00962	ATP-dependent protease HslVU, peptidase subunit	19.96
R	Ferpe_01261	B12-binding domain/radical SAM domain protein,	18.81
H	Ferpe_01645	birA, biotin-[acetyl-CoA-carboxylase] ligase	22.13
G	Ferpe_00194	carbohydrate ABC transporter membrane protein 1,	19.71
G	Ferpe_00442	carbohydrate ABC transporter membrane protein 1,	
G	Ferpe_00505	carbohydrate ABC transporter membrane protein 1,	22.06
G	Ferpe_00813	carbohydrate ABC transporter membrane protein 1,	
G	Ferpe_00983	carbohydrate ABC transporter membrane protein 1,	22.11
G	Ferpe_01017	carbohydrate ABC transporter membrane protein 1,	
G	Ferpe_01238	carbohydrate ABC transporter membrane protein 1,	
G	Ferpe_01717	carbohydrate ABC transporter membrane protein 1,	
G	Ferpe_01777	carbohydrate ABC transporter membrane protein 1,	
G	Ferpe_00195	carbohydrate ABC transporter membrane protein 2,	24.26
G	Ferpe_00441	carbohydrate ABC transporter membrane protein 2,	18.01
G	Ferpe_00445	carbohydrate ABC transporter membrane protein 2,	
G	Ferpe_00506	carbohydrate ABC transporter membrane protein 2,	20.54
G	Ferpe_01018	carbohydrate ABC transporter membrane protein 2,	
G	Ferpe_01599	carbohydrate ABC transporter membrane protein 2,	
G	Ferpe_01776	carbohydrate ABC transporter membrane protein 2,	
T	Ferpe_00059	carbon storage regulator, CsrA	17.62
D	Ferpe_00072	cell shape determining protein, MreB/Mrl family	23.72
D	Ferpe_01323	cell shape determining protein, MreB/Mrl family	24.18
T	Ferpe_00180	Chemotaxis protein CheC, inhibitor of MCP	22.78
G	Ferpe_01471	conserved hypothetical protein, cofD-related	21.03
H	Ferpe_01471	conserved hypothetical protein, cofD-related	21.03

V	Ferpe_01555	CRISPR-associated protein, Cas1 family	
V	Ferpe_01545	CRISPR-associated protein, Cas2 family	
V	Ferpe_01546	CRISPR-associated protein, Cas2 family	
V	Ferpe_01548	CRISPR-associated protein, Cas6 family	17.92
V	Ferpe_01537	CRISPR-associated protein, Cmr1 family	20.31
V	Ferpe_01536	CRISPR-associated protein, Cmr2 family	21.07
V	Ferpe_01535	CRISPR-associated protein, Cmr3 family	19.27
V	Ferpe_01534	CRISPR-associated protein, Cmr4 family	22.19
V	Ferpe_01532	CRISPR-associated protein, Cmr6 family	19.9
V	Ferpe_01554	CRISPR-associated protein, Csm1 family	22.03
V	Ferpe_01553	CRISPR-associated protein, Csm2 family	22.61
V	Ferpe_01552	CRISPR-associated protein, Csm3 family	22.32
V	Ferpe_01551	CRISPR-associated protein, Csm4 family	20.95
V	Ferpe_01550	CRISPR-associated protein, Csm5 family	21.76
X	Ferpe_01541	CRISPR-associated protein, Csx2 family	22.62
E	Ferpe_01680	cysteine desulfurases, SufS subfamily	22.72
B	Ferpe_00993	Deacetylases, including yeast histone	20.44
Q	Ferpe_00993	Deacetylases, including yeast histone	20.44
E	Ferpe_01227	dipeptidase, putative	24.36
L	Ferpe_00999	DNA gyrase, A subunit	25.79
L	Ferpe_01075	DNA gyrase, B subunit	25.36
L	Ferpe_00604	DNA ligase, NAD-dependent	23.49
L	Ferpe_01920	DNA polymerase III, alpha chain, Gram-positive	22.49
L	Ferpe_01608	DNA polymerase III, beta subunit	25.93
L	Ferpe_00235	DNA polymerase III, delta subunit (EC 2.7.7.7)	22.18
L	Ferpe_00774	DNA polymerase III, subunit gamma and tau	21.71
L	Ferpe_01268	DNA topoisomerase I, bacterial	21.9
J	Ferpe_01384	DNA-binding regulatory protein, YebC/PmpR family	21.88
K	Ferpe_01384	DNA-binding regulatory protein, YebC/PmpR family	21.88
K	Ferpe_02055	DNA-directed RNA polymerase, beta' subunit/160	26.82
K	Ferpe_01964	DNA-directed RNA polymerase, subunit K/omega	18.82
M	Ferpe_00118	dTDP-glucose 4,6-dehydratase (EC 4.2.1.46)	23.32
M	Ferpe_01106	dTDP-glucose 4,6-dehydratase (EC 4.2.1.46)	22.57
I	Ferpe_00426	EDD domain protein, DegV family	23.6

I	Ferpe_00646	EDD domain protein, DegV family	21.63
I	Ferpe_00647	EDD domain protein, DegV family	20.92
I	Ferpe_00892	EDD domain protein, DegV family	26.11
C	Ferpe_00190	electron transport complex, RnfABCDGE type, A	
X	Ferpe_01828	electron transport complex, RnfABCDGE type, B	18.78
C	Ferpe_00186	electron transport complex, RnfABCDGE type, C	22.87
C	Ferpe_00187	electron transport complex, RnfABCDGE type, D	
C	Ferpe_00189	electron transport complex, RnfABCDGE type, E	19.81
C	Ferpe_00188	electron transport complex, RnfABCDGE type, G	20.98
L	Ferpe_00045	excinuclease ABC, A subunit	22.74
L	Ferpe_01832	Exonuclease VII, large subunit	20.44
N	Ferpe_01321	flagellar basal-body rod protein FlgG,	19.89
N	Ferpe_01155	Flagellar biosynthesis pathway, component FliR	
G	Ferpe_01596	fructose-1,6-bisphosphatase, class II	22.25
G	Ferpe_00489	fructose-1,6-bisphosphate aldolase, class II,	26.13
G	Ferpe_00167	Fructose-2,6-bisphosphatase	20.61
G	Ferpe_01889	fructose-6-phosphate aldolase, TalC/MipB family	24.47
G	Ferpe_00462	galactose-1-phosphate uridylyltransferase,	25.5
G	Ferpe_00845	galactose-1-phosphate uridylyltransferase,	20.01
G	Ferpe_00064	glucose-1-phosphate adenylyltransferase, GlgD	22.72
M	Ferpe_01869	glucose-1-phosphate thymidylylransferase, long	25.8
M	Ferpe_00115	glucose-1-phosphate thymidylyltransferase, short	23.6
E	Ferpe_01870	glutamine synthetase, type I	25.58
J	Ferpe_00980	glutamyl-tRNA synthetase, bacterial family	21.96
O	Ferpe_01184	Glutaredoxin-like protein, YruB-family	20.01
N	Ferpe_00145	Glycosyltransferases, probably involved in cell	20.99
N	Ferpe_00735	Glycosyltransferases, probably involved in cell	17.71
F	Ferpe_00258	GMP synthase (glutamine-hydrolyzing), C-terminal	20.86
F	Ferpe_00718	GMP synthase (glutamine-hydrolyzing), C-terminal	14.85
H	Ferpe_00496	HAD-superfamily hydrolase, subfamily IIB	20.65
R	Ferpe_00496	HAD-superfamily hydrolase, subfamily IIB	20.65
H	Ferpe_01868	HAD-superfamily hydrolase, subfamily IIB	23.33
R	Ferpe_01868	HAD-superfamily hydrolase, subfamily IIB	23.33
H	Ferpe_00148	haloacid dehalogenase superfamily, subfamily IA,	21.75

H	Ferpe_00734	haloacid dehalogenase superfamily, subfamily IA,	21.96
H	Ferpe_01447	haloacid dehalogenase superfamily, subfamily IA,	18.62
X	Ferpe_01008	Histidine kinase-, DNA gyrase B-, and HSP90-like	19.69
C	Ferpe_00292	hydrogenase, Fe-only	26.28
C	Ferpe_00862	hydrogenase, Fe-only	28.43
N	Ferpe_01003	hydrolase, TatD family	21.54
C	Ferpe_00623	hydro-lyases, Fe-S type, tartrate/fumarate	19.59
C	Ferpe_00624	hydro-lyases, Fe-S type, tartrate/fumarate	19.94
C	Ferpe_00303	Indolepyruvate ferredoxin oxidoreductase, alpha	21.45
K	Ferpe_00372	iron (metal) dependent repressor, DtxR family	23.43
K	Ferpe_00383	iron (metal) dependent repressor, DtxR family	20.15
C	Ferpe_00857	Iron only hydrogenase large subunit, C-terminal	24.73
P	Ferpe_00050	K+ transport systems, NAD-binding component	19.9
P	Ferpe_01953	K+ transport systems, NAD-binding component	24.41
X	Ferpe_01954	K+ transport systems, NAD-binding component	22.27
X	Ferpe_00121	looped-hinge helix DNA binding domain, AbrB	18.1
E	Ferpe_01853	L-serine dehydratase, iron-sulfur-dependent,	21.15
E	Ferpe_01854	L-serine dehydratase, iron-sulfur-dependent,	20.28
E	Ferpe_00541	lysine-2,3-aminomutase	25.26
J	Ferpe_01475	lysyl-tRNA synthetase, class II (EC 6.1.1.6)	25.54
O	Ferpe_00943	Membrane protease subunits, stomatin/prohibitin	25.2
J	Ferpe_01098	metalloprotein, YbeY/UPF0054 family	18.63
J	Ferpe_01394	methionine aminopeptidase, type I	19.96
I	Ferpe_02025	Methylmalonyl-CoA mutase, N-terminal	25.1
G	Ferpe_01692	monosaccharide ABC transporter membrane protein,	19.97
G	Ferpe_01693	monosaccharide ABC transporter membrane protein,	20.99
P	Ferpe_02018	Multisubunit Na+/H+ antiporter, MnhB subunit	18.66
P	Ferpe_02017	Multisubunit Na+/H+ antiporter, MnhC subunit	
P	Ferpe_02022	Multisubunit Na+/H+ antiporter, MnhE subunit	
P	Ferpe_02021	Multisubunit Na+/H+ antiporter, MnhF subunit	16.61
P	Ferpe_02020	Multisubunit Na+/H+ antiporter, MnhG subunit	18.96
C	Ferpe_00908	Na+-transporting NADH:ubiquinone oxidoreductase,	17.32
C	Ferpe_00909	Na+-transporting NADH:ubiquinone oxidoreductase,	
O	Ferpe_00493	NAD-dependent protein deacetylases, SIR2 family	19.31

C	Ferpe_00861	NADH:ubiquinone oxidoreductase, NADH-binding (51	28.41
C	Ferpe_01116	NADH:ubiquinone oxidoreductase, NADH-binding (51	25.35
C	Ferpe_00860	NADH-quinone oxidoreductase, E subunit	25.87
C	Ferpe_01117	NADH-quinone oxidoreductase, E subunit	21.73
E	Ferpe_01906	oligoendopeptidase, pepF/M3 family	23.19
E	Ferpe_00406	oligopeptide/dipeptide ABC transporter,	25.53
P	Ferpe_00406	oligopeptide/dipeptide ABC transporter,	25.53
E	Ferpe_01458	oligopeptide/dipeptide ABC transporter,	24.25
E	Ferpe_01459	oligopeptide/dipeptide ABC transporter,	23.99
P	Ferpe_01459	oligopeptide/dipeptide ABC transporter,	23.99
E	Ferpe_01835	oligopeptide/dipeptide ABC transporter,	26.08
P	Ferpe_01835	oligopeptide/dipeptide ABC transporter,	26.08
E	Ferpe_01836	oligopeptide/dipeptide ABC transporter,	25.81
X	Ferpe_01625	Oxaloacetate decarboxylase, gamma chain.	19.35
H	Ferpe_01464	pantothenate kinase, type III	22.11
O	Ferpe_01289	periplasmic serine protease, Do/DeqQ family	22.31
J	Ferpe_01265	phenylalanyl-tRNA synthetase, alpha subunit (EC	22.26
P	Ferpe_01797	phosphate ABC transporter ATP-binding protein,	19.65
P	Ferpe_01795	phosphate ABC transporter membrane protein 1,	
P	Ferpe_01796	phosphate ABC transporter membrane protein 2,	
T	Ferpe_01096	Phosphate starvation-inducible protein PhoH,	23.28
P	Ferpe_00397	phosphate uptake regulator, PhoU	20.28
P	Ferpe_01798	phosphate uptake regulator, PhoU	21.16
R	Ferpe_00263	phosphoesterase, MJ0936 family	21.12
R	Ferpe_00607	phosphoesterase, MJ0936 family	22.38
R	Ferpe_01562	phosphoesterase, MJ0936 family	22
R	Ferpe_00486	phospholipid-binding protein, PBP family	20.23
F	Ferpe_01374	phosphoribosylformylglycinamidine synthase, purS	16.61
F	Ferpe_01966	phosphoribosylglycinamide formyltransferase,	15.85
T	Ferpe_00056	positive regulator of sigma(E), RseC/MucC	17.65
G	Ferpe_00233	Predicted extracellular endo alpha-1,4	17.78
J	Ferpe_01263	Predicted GTPase, probable translation factor	24.03
N	Ferpe_01040	Predicted inhibitor of MCP methylation, homolog	19.97
S	Ferpe_01049	Predicted metal-binding, possibly nucleic	20.79

C	Ferpe_00907	Predicted NADH:ubiquinone oxidoreductase,	
X	Ferpe_01338	Predicted polymerase, most proteins contain PALM	15.4
S	Ferpe_01517	Predicted polymerase, most proteins contain PALM	21.27
O	Ferpe_00826	Predicted redox protein, regulator of disulfide	20.23
O	Ferpe_00831	Predicted redox protein, regulator of disulfide	20.76
R	Ferpe_01181	Predicted redox protein, regulator of disulfide	19.41
T	Ferpe_00932	Predicted transcriptional regulator, contains	21.3
J	Ferpe_01435	protein-(glutamine-N5) methyltransferase,	15.54
U	Ferpe_01762	protein-export membrane protein, SecD/SecF	24.88
J	Ferpe_01038	pseudouridine synthase, RluA family	20.38
J	Ferpe_01380	pseudouridine synthase, RluA family	18.97
G	Ferpe_01793	pullulanase, type I	21.21
F	Ferpe_00172	purine nucleoside phosphorylase I, inosine and	24.79
F	Ferpe_00787	purine nucleoside phosphorylase I, inosine and	23.31
V	Ferpe_00225	putative efflux protein, MATE family	15.9
V	Ferpe_00270	putative efflux protein, MATE family	18.17
V	Ferpe_01446	putative efflux protein, MATE family	16.77
R	Ferpe_01147	putative regulatory protein, FmdB family	
R	Ferpe_00394	pyridoxal phosphate enzyme, YggS family	21.86
C	Ferpe_01201	pyruvate ferredoxin oxidoreductase, alpha	26.87
C	Ferpe_01202	pyruvate ferredoxin oxidoreductase, delta	23.97
C	Ferpe_01203	pyruvate ferredoxin oxidoreductase, gamma	25.84
R	Ferpe_00627	RecB family nuclease, putative, TM0106 family	19.25
J	Ferpe_00374	ribonuclease, Rne/Rng family	22.01
J	Ferpe_00879	ribosomal protein L25, Ctc-form	24.32
J	Ferpe_01177	ribosomal protein L33, bacterial type	
J	Ferpe_01417	ribosomal protein S10, bacterial/organelle	22.79
J	Ferpe_01421	ribosomal protein S12, bacterial/organelle	19.04
J	Ferpe_01410	ribosomal protein S3, bacterial type	25.53
J	Ferpe_01399	ribosomal protein S5, bacterial/organelle type	24.3
J	Ferpe_01753	RNA methyltransferase, RsmD family	16.34
J	Ferpe_00411	RNA methyltransferase, RsmE family	18.99
K	Ferpe_00098	RNA polymerase sigma factor, sigma-70 family	22.02
K	Ferpe_00182	RNA polymerase, sigma 28 subunit, SigD/FliA/WhiG	20.22

K	Ferpe_00295	RNA polymerase, sigma 30 subunit, SigH	
X	Ferpe_01563	RNA polymerase, sigma 54 subunit, RpoN/SigL	17.92
K	Ferpe_00952	RNA polymerase, sigma-24 subunit, RpoE	18.86
J	Ferpe_01280	rRNA methylase, putative, group 3	20.67
K	Ferpe_00331	Serine phosphatase RsbU, regulator of sigma	23.53
T	Ferpe_00331	Serine phosphatase RsbU, regulator of sigma	23.53
K	Ferpe_01125	Serine phosphatase RsbU, regulator of sigma	19.23
T	Ferpe_01125	Serine phosphatase RsbU, regulator of sigma	19.23
U	Ferpe_00928	signal peptidase I, bacterial type	22.14
L	Ferpe_00670	Site-specific recombinases, DNA invertase Pin	
L	Ferpe_00751	Site-specific recombinases, DNA invertase Pin	14.97
C	Ferpe_01633	sodium ion-translocating decarboxylase, beta	20.9
C	Ferpe_01665	sodium ion-translocating decarboxylase, beta	15.94
K	Ferpe_00997	SOS-response transcriptional repressor, LexA	20.26
T	Ferpe_00997	SOS-response transcriptional repressor, LexA	20.26
C	Ferpe_00306	Succinyl-CoA synthetase, alpha subunit	
C	Ferpe_00305	Succinyl-CoA synthetase, beta subunit	
O	Ferpe_01679	SUF system FeS assembly protein, NifU family	18.14
G	Ferpe_00507	Sugar kinases, ribokinase family	21.6
G	Ferpe_01341	Sugar kinases, ribokinase family	16.94
G	Ferpe_01229	sugar-phosphate isomerases, RpiB/LacA/LacB	16.41
M	Ferpe_00144	transcriptional attenuator, LytR family	18.81
M	Ferpe_00736	transcriptional attenuator, LytR family	16.62
M	Ferpe_02047	transcriptional attenuator, LytR family	21.35
K	Ferpe_00573	transcriptional regulator, ArsR family	17.51
K	Ferpe_00669	transcriptional regulator, ArsR family	17.76
K	Ferpe_02044	transcriptional regulator, BadM/Rrf2 family	21.22
K	Ferpe_00836	transcriptional regulator, GntR family	17.01
K	Ferpe_00153	transcriptional regulator, LacI family	21.32
K	Ferpe_00437	transcriptional regulator, LacI family	20.72
K	Ferpe_00682	transcriptional regulator, LacI family	16.58
K	Ferpe_00818	transcriptional regulator, LacI family	16.95
K	Ferpe_00847	transcriptional regulator, LacI family	20.28
K	Ferpe_01228	transcriptional regulator, LacI family	17.86

K	Ferpe_01602	transcriptional regulator, LacI family	17.65
K	Ferpe_01719	transcriptional regulator, LacI family	19.18
K	Ferpe_01779	transcriptional regulator, LacI family	19.22
K	Ferpe_01811	transcriptional regulator, LacI family	21.82
K	Ferpe_01842	transcriptional regulator, LacI family	22.1
K	Ferpe_00781	transcriptional regulator, MarR family	22.79
K	Ferpe_01803	transcriptional regulator, MarR family	
X	Ferpe_01871	transcriptional regulator, PadR family	16.38
K	Ferpe_01022	transcriptional regulator, RpiR family	18.96
K	Ferpe_00415	transcriptional regulator, TetR family	15.76
X	Ferpe_00635	transcriptional regulator, TetR family	21.25
K	Ferpe_01747	transcriptional regulator, XRE family with cupin	20.74
X	Ferpe_02004	Transglutaminase-like enzymes, putative cysteine	23.14
G	Ferpe_01232	Transketolase, C-terminal subunit	18.47
G	Ferpe_01233	Transketolase, N-terminal subunit	18.62
X	Ferpe_00588	transposase, IS605 OrfB family, central region	
P	Ferpe_01952	Trk-type K+ transport systems, membrane	17.96
J	Ferpe_00399	tRNA(Ile)-lysidine synthetase, N-terminal	19.68
K	Ferpe_01874	two component transcriptional regulator, LytTR	19.01
T	Ferpe_01874	two component transcriptional regulator, LytTR	19.01
V	Ferpe_00555	type I site-specific deoxyribonuclease, HsdR	22.65
N	Ferpe_00377	Type II secretory pathway, ATPase PulE/Tfp pilus	23.22
U	Ferpe_00377	Type II secretory pathway, ATPase PulE/Tfp pilus	23.22
W	Ferpe_00377	Type II secretory pathway, ATPase PulE/Tfp pilus	23.22
X	Ferpe_01676	Type II secretory pathway, component PulD	23.05
N	Ferpe_01670	Type II secretory pathway, component PulF	19.34
U	Ferpe_01670	Type II secretory pathway, component PulF	19.34
W	Ferpe_01670	Type II secretory pathway, component PulF	19.34
N	Ferpe_01287	Type II secretory pathway, prepilin signal	
U	Ferpe_01287	Type II secretory pathway, prepilin signal	
N	Ferpe_00492	type III secretion system ATPase, FliI/YscN (EC	20.98
U	Ferpe_00492	type III secretion system ATPase, FliI/YscN (EC	20.98
M	Ferpe_00509	UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diamin	25.09
R	Ferpe_00158	Uncharacterized Fe-S protein PflX, homolog of	18.11

R	Ferpe_01937	Uncharacterized Fe-S protein PflX, homolog of	20.26
S	Ferpe_00522	Uncharacterized membrane protein, possible Na+	
C	Ferpe_01959	Uncharacterized oxidoreductases, Fe-dependent	23.1
R	Ferpe_00034	Uncharacterized protein, possibly involved in	17.58
P	Ferpe_00535	uncharacterized protein, YfiH family	21.75
G	Ferpe_00252	Uncharacterized proteins, LmbE homologs	17.98
L	Ferpe_00382	uracil-DNA glycosylase, family 4	18.01
X	Ferpe_00620	Vitamin B12 dependent methionine synthase,	19.25
M	Ferpe_00105	WxcM-like, C-terminal/NAD dependent	22.7
F	Ferpe_01285	yjeF C-terminal region, hydroxyethylthiazole	22.33
R	Ferpe_00464	Zn-dependent hydrolases, including glyoxylases	21.02
R	Ferpe_00601	Zn-dependent hydrolases, including glyoxylases	20.9
R	Ferpe_01099	Zn-dependent hydrolases, including glyoxylases	19.34
R	Ferpe_01929	Zn-dependent hydrolases, including glyoxylases	
J	Ferpe_02052	16S rRNA (guanine(527)-N(7))-methyltransferase	19.54
X	Ferpe_01668	16S rRNA m(2)G 1207 methyltransferase (EC	20.69
J	Ferpe_00924	16S rRNA processing protein RimM	21.69
I	Ferpe_01315	1-acyl-sn-glycerol-3-phosphate acyltransferases	22.28
V	Ferpe_01805	1-Cys peroxiredoxin (EC 1.11.1.15)	25.41
I	Ferpe_00696	1-deoxy-D-xylulose 5-phosphate reductoisomerase	19.05
I	Ferpe_01302	1-deoxy-D-xylulose 5-phosphate reductoisomerase	21.66
H	Ferpe_00053	1-deoxy-D-xylulose-5-phosphate synthase (EC	22.41
I	Ferpe_00053	1-deoxy-D-xylulose-5-phosphate synthase (EC	22.41
G	Ferpe_01774	1-phosphofructokinase	22.85
J	Ferpe_00753	23S rRNA (uracil-5-)-methyltransferase RumA	23.23
J	Ferpe_01939	23S rRNA m(2)A-2503 methyltransferase (EC	22.53
J	Ferpe_01650	2'-5' RNA ligase	23.02
H	Ferpe_02027	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine	20.74
C	Ferpe_00434	2-polyprenylphenol hydroxylase and related	18.62
H	Ferpe_00434	2-polyprenylphenol hydroxylase and related	18.62
C	Ferpe_00674	2-polyprenylphenol hydroxylase and related	17.84
H	Ferpe_00674	2-polyprenylphenol hydroxylase and related	17.84
C	Ferpe_00839	2-polyprenylphenol hydroxylase and related	
H	Ferpe_00839	2-polyprenylphenol hydroxylase and related	

M	Ferpe_01802	3-beta hydroxysteroid dehydrogenase/isomerase	
I	Ferpe_00890	3-hydroxymyristoyl/3-hydroxydecanoyl-(acyl	24.98
L	Ferpe_00699	3'-nucleotidase (EC 3.1.3.6)/5'-nucleotidase (EC	21.37
L	Ferpe_01299	3'-nucleotidase (EC 3.1.3.6)/5'-nucleotidase (EC	21.77
I	Ferpe_00221	3-oxoacyl-[acyl-carrier-protein] reductase (EC	23.05
Q	Ferpe_00221	3-oxoacyl-[acyl-carrier-protein] reductase (EC	23.05
R	Ferpe_00221	3-oxoacyl-[acyl-carrier-protein] reductase (EC	23.05
I	Ferpe_00886	3-oxoacyl-[acyl-carrier-protein] reductase (EC	26.66
Q	Ferpe_00886	3-oxoacyl-[acyl-carrier-protein] reductase (EC	26.66
R	Ferpe_00886	3-oxoacyl-[acyl-carrier-protein] reductase (EC	26.66
I	Ferpe_00636	3-oxoacyl-[acyl-carrier-protein] synthase III	22.86
I	Ferpe_01973	4-diphosphocytidyl-2-C-methyl-D-erythritol	20.45
I	Ferpe_00605	4-diphosphocytidyl-2-methyl-D-erithritol	19.78
I	Ferpe_00698	4-hydroxy-3-methylbut-2-en-1-yl diphosphate	17.71
I	Ferpe_01300	4-hydroxy-3-methylbut-2-en-1-yl diphosphate	20.91
J	Ferpe_01885	4-hydroxy-3-methylbut-2-enyl diphosphate	26.07
F	Ferpe_01371	5-(carboxyamino)imidazole ribonucleotide	15.91
F	Ferpe_01372	5-(carboxyamino)imidazole ribonucleotide mutase	
F	Ferpe_01039	5'-methylthioadenosine/S-adenosylhomocysteine	21.93
G	Ferpe_01452	6-phosphofructokinase	22.86
G	Ferpe_01765	6-phosphofructokinase	24.69
G	Ferpe_01975	6-phosphofructokinase (EC 2.7.1.11)	24.49
H	Ferpe_01636	8-amino-7-oxononanoate synthase	26.87
R	Ferpe_01504	AAA domain (dynein-related subfamily).	22.82
C	Ferpe_01817	ABC-2 type transporter.	17.38
P	Ferpe_01817	ABC-2 type transporter.	17.38
E	Ferpe_00222	ABC-type amino acid transport/signal	21.17
T	Ferpe_00222	ABC-type amino acid transport/signal	21.17
E	Ferpe_01881	ABC-type amino acid transport/signal	21.33
T	Ferpe_01881	ABC-type amino acid transport/signal	21.33
E	Ferpe_00629	ABC-type branched-chain amino acid transport	21.52
E	Ferpe_00631	ABC-type branched-chain amino acid transport	21.69
E	Ferpe_01258	ABC-type branched-chain amino acid transport	17.53
H	Ferpe_01081	ABC-type cobalamin/Fe3+-siderophores transport	21.67

P	Ferpe_01081	ABC-type cobalamin/Fe3+-siderophores transport	21.67
H	Ferpe_01369	ABC-type cobalamin/Fe3+-siderophores transport	20.1
P	Ferpe_01369	ABC-type cobalamin/Fe3+-siderophores transport	20.1
E	Ferpe_00407	ABC-type dipeptide/oligopeptide/nickel transport	23.26
P	Ferpe_00407	ABC-type dipeptide/oligopeptide/nickel transport	23.26
E	Ferpe_00408	ABC-type dipeptide/oligopeptide/nickel transport	21.83
P	Ferpe_00408	ABC-type dipeptide/oligopeptide/nickel transport	21.83
E	Ferpe_00612	ABC-type dipeptide/oligopeptide/nickel transport	21.46
P	Ferpe_00612	ABC-type dipeptide/oligopeptide/nickel transport	21.46
E	Ferpe_00613	ABC-type dipeptide/oligopeptide/nickel transport	21.94
P	Ferpe_00613	ABC-type dipeptide/oligopeptide/nickel transport	21.94
E	Ferpe_00687	ABC-type dipeptide/oligopeptide/nickel transport	17.85
P	Ferpe_00687	ABC-type dipeptide/oligopeptide/nickel transport	17.85
E	Ferpe_01460	ABC-type dipeptide/oligopeptide/nickel transport	24.75
P	Ferpe_01460	ABC-type dipeptide/oligopeptide/nickel transport	24.75
E	Ferpe_01461	ABC-type dipeptide/oligopeptide/nickel transport	22.4
P	Ferpe_01461	ABC-type dipeptide/oligopeptide/nickel transport	22.4
E	Ferpe_01838	ABC-type dipeptide/oligopeptide/nickel transport	24.35
P	Ferpe_01838	ABC-type dipeptide/oligopeptide/nickel transport	24.35
E	Ferpe_01839	ABC-type dipeptide/oligopeptide/nickel transport	25.37
P	Ferpe_01839	ABC-type dipeptide/oligopeptide/nickel transport	25.37
P	Ferpe_00283	ABC-type nitrate/sulfonate/bicarbonate transport	21.88
P	Ferpe_00284	ABC-type nitrate/sulfonate/bicarbonate transport	19.14
P	Ferpe_00285	ABC-type nitrate/sulfonate/bicarbonate transport	
E	Ferpe_01113	ABC-type proline/glycine betaine transport	
E	Ferpe_01115	ABC-type proline/glycine betaine transport	
O	Ferpe_00528	ABC-type transport system involved in Fe-S	22.01
C	Ferpe_00490	acetate kinase	25.32
I	Ferpe_00219	acetyl-CoA acetyltransferases	23.73
C	Ferpe_00634	Acetyl-CoA hydrolase	22.14
E	Ferpe_00315	acetyldiaminopimelate aminotransferase apoenzyme	22.24
R	Ferpe_00117	Acetyltransferase (isoleucine patch superfamily)	23.22
R	Ferpe_01105	Acetyltransferase (isoleucine patch superfamily)	24.25
J	Ferpe_00900	Acetyltransferases	22.21

X	Ferpe_01603	Acetyltransferases	22.12
D	Ferpe_00455	Actin-like ATPase involved in cell division	24.26
I	Ferpe_00893	acyl carrier protein	24.24
Q	Ferpe_00893	acyl carrier protein	24.24
I	Ferpe_01519	acyl carrier protein	19.76
Q	Ferpe_01519	acyl carrier protein	19.76
I	Ferpe_00542	Acyl dehydratase	22.81
I	Ferpe_01160	Acyl-CoA dehydrogenases	25.51
C	Ferpe_01657	Acylphosphatases	
I	Ferpe_01319	acyl-phosphate glycerol 3-phosphate	19.53
F	Ferpe_01508	adenine phosphoribosyltransferase (EC 2.4.2.7)	25.12
X	Ferpe_00568	Adenine specific DNA methylase Mod	22.55
H	Ferpe_01701	adenosylcobinamide kinase (EC	17.44
H	Ferpe_01705	adenosylcobinamide-phosphate synthase (EC	
H	Ferpe_00375	adenosylhomocysteinase (EC 3.3.1.1)	23.32
E	Ferpe_01749	adenosylmethionine decarboxylase proenzyme (EC	15.52
F	Ferpe_01395	Adenylate kinase (EC 2.7.4.3)	23.5
F	Ferpe_01988	adenylosuccinate lyase	22.82
F	Ferpe_01656	Adenylosuccinate synthetase (EC 6.3.4.4)	22.13
X	Ferpe_00866	ADP-ribose pyrophosphatase	16.91
F	Ferpe_01736	ADP-ribose pyrophosphatase	
M	Ferpe_01270	alanine racemase	22.39
J	Ferpe_00071	alanyl-tRNA synthetase (EC 6.1.1.7)	25.44
G	Ferpe_01907	aldose 1-epimerase (EC 5.1.3.3)	19.64
P	Ferpe_02003	Alkaline phosphatase	19.38
R	Ferpe_02003	Alkaline phosphatase	19.38
G	Ferpe_00846	Alpha-galactosidase	20.67
G	Ferpe_01013	Alpha-mannosidase	23.56
H	Ferpe_00913	Amidases related to nicotinamidase	19.84
R	Ferpe_00913	Amidases related to nicotinamidase	19.84
F	Ferpe_01965	amidophosphoribosyltransferase (EC 2.4.2.14)	21.04
E	Ferpe_01208	amino acid ABC transporter substrate-binding	20.04
T	Ferpe_01208	amino acid ABC transporter substrate-binding	20.04
E	Ferpe_01931	amino acid ABC transporter substrate-binding	18.51

T	Ferpe_01931	amino acid ABC transporter substrate-binding	18.51
E	Ferpe_00633	amino acid/amide ABC transporter	23.59
X	Ferpe_00895	amino acid/amide ABC transporter	22.69
E	Ferpe_01497	amino acid/amide ABC transporter	19.63
E	Ferpe_01498	amino acid/amide ABC transporter	17.93
E	Ferpe_00632	amino acid/amide ABC transporter ATP-binding	21.51
E	Ferpe_01493	amino acid/amide ABC transporter ATP-binding	20.08
E	Ferpe_01494	amino acid/amide ABC transporter ATP-binding	20.82
E	Ferpe_00630	amino acid/amide ABC transporter membrane	17.64
E	Ferpe_01495	amino acid/amide ABC transporter membrane	16.66
E	Ferpe_01496	amino acid/amide ABC transporter membrane	
E	Ferpe_01086	aminomethyltransferase (EC 2.1.2.10)	24.44
F	Ferpe_00704	anaerobic ribonucleoside-triphosphate reductase	18.35
F	Ferpe_01491	anaerobic ribonucleoside-triphosphate reductase	14.43
T	Ferpe_00995	anti-anti-sigma factor	21.25
T	Ferpe_00996	anti-anti-sigma factor	21.32
T	Ferpe_01887	anti-anti-sigma factor	
T	Ferpe_01366	Anti-sigma regulatory factor (Ser/Thr protein	21.96
M	Ferpe_00239	apolipoprotein N-acyltransferase	20.71
G	Ferpe_00224	Arabinose efflux permease	
G	Ferpe_00226	Arabinose efflux permease	15.66
X	Ferpe_00416	Arabinose efflux permease	17.05
G	Ferpe_00470	Arabinose efflux permease	16.55
G	Ferpe_00754	Arabinose efflux permease	
G	Ferpe_00849	Arabinose efflux permease	16.33
G	Ferpe_00920	Arabinose efflux permease	
G	Ferpe_01427	Arabinose efflux permease	19.58
X	Ferpe_00159	Archaeal ATPase.	21.28
X	Ferpe_00998	Archease protein family (DUF101/UPF0211).	17.75
J	Ferpe_00204	arginyl-tRNA synthetase (EC 6.1.1.19)	24.57
P	Ferpe_00529	arsenite efflux membrane protein ArsB (TC	
J	Ferpe_01593	asparaginyl-tRNA synthetase (EC 6.1.1.22)	24.75
E	Ferpe_00281	Aspartate ammonia-lyase	20.94
F	Ferpe_00278	aspartate carbamoyltransferase (EC 2.1.3.2)	22.53

R	Ferpe_01742	aspartate dehydrogenase	16.76
E	Ferpe_00314	aspartate kinase (EC 2.7.2.4)	22.51
E	Ferpe_01659	aspartate kinase (EC 2.7.2.4)	20.81
E	Ferpe_00309	aspartate semialdehyde dehydrogenase (EC	23.6
E	Ferpe_01560	Aspartate/tyrosine/aromatic aminotransferase	25.33
E	Ferpe_00782	Aspartyl aminopeptidase	25.38
J	Ferpe_00060	aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase	19.87
J	Ferpe_01891	aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase	24.67
J	Ferpe_01892	aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase	24.51
C	Ferpe_00655	ATP synthase F0 subcomplex A subunit	20.34
C	Ferpe_00653	ATP synthase F0 subcomplex B subunit	22.7
C	Ferpe_00651	ATP synthase F1 subcomplex alpha subunit	25.44
C	Ferpe_00649	ATP synthase F1 subcomplex beta subunit	26
C	Ferpe_00652	ATP synthase F1 subcomplex delta subunit	20.13
H	Ferpe_00369	ATP:cob(I)alamin adenosyltransferase	22.53
R	Ferpe_00688	ATPase components of ABC transporters with	17.65
R	Ferpe_01307	ATPase components of ABC transporters with	20.92
L	Ferpe_01128	ATPase involved in DNA repair	22
L	Ferpe_00385	ATPase related to the helicase subunit of the	16.69
D	Ferpe_00177	ATPases involved in chromosome partitioning	21.31
N	Ferpe_00177	ATPases involved in chromosome partitioning	21.31
D	Ferpe_00461	ATPases involved in chromosome partitioning	24.2
O	Ferpe_01053	ATP-dependent Clp protease ATP-binding subunit	22.22
L	Ferpe_01277	ATP-dependent DNA helicase RecG (EC 3.6.1.-)	19.77
X	Ferpe_01733	ATP-dependent exoDNAse (exonuclease V) beta	21.26
O	Ferpe_00206	ATP-dependent protease La	24.46
K	Ferpe_00851	AT-rich DNA-binding protein	23.36
K	Ferpe_00863	AT-rich DNA-binding protein	24.7
X	Ferpe_01145	Bacterial capsule synthesis protein PGA_cap.	16.45
D	Ferpe_01291	Bacterial cell division membrane protein	16.11
D	Ferpe_01895	Bacterial cell division membrane protein	18.22
L	Ferpe_00009	Bacterial nucleoid DNA-binding protein	27.05
J	Ferpe_00772	bacterial peptide chain release factor 1 (bRF-1)	21.31
X	Ferpe_02037	Bacterial trigger factor protein (TF)	20.63

G	Ferpe_00817	Beta-galactosidase	17.05
G	Ferpe_00848	Beta-galactosidase	21.33
X	Ferpe_00438	Beta-glucanase/Beta-glucan synthetase	19.06
X	Ferpe_00450	Beta-glucanase/Beta-glucan synthetase	19.37
G	Ferpe_01843	Beta-glucosidase/6-phospho-beta-glucosidase/beta-galactosidase	27.54
G	Ferpe_00440	Beta-glucosidase-related glycosidases	22.87
I	Ferpe_00891	beta-ketoacyl-acyl-carrier-protein synthase II	27.65
Q	Ferpe_00891	beta-ketoacyl-acyl-carrier-protein synthase II	27.65
G	Ferpe_01598	beta-phosphoglucomutase	18.12
R	Ferpe_01598	beta-phosphoglucomutase	18.12
S	Ferpe_01857	Beta-propeller domains of methanol dehydrogenase	18.8
X	Ferpe_00702	Beta-propeller repeat.	13.89
E	Ferpe_00257	Bifunctional PLP-dependent enzyme with	21.3
R	Ferpe_00257	Bifunctional PLP-dependent enzyme with	21.3
R	Ferpe_01154	Biotin synthase-related enzyme	17.72
E	Ferpe_01077	Branched-chain amino acid	22.32
H	Ferpe_01077	Branched-chain amino acid	22.32
G	Ferpe_00040	broad-specificity cellobiase (EC 3.2.1.21)	24.02
C	Ferpe_00344	butyrate kinase (EC 2.7.2.7)	25.45
C	Ferpe_00346	butyrate kinase (EC 2.7.2.7)	25.87
I	Ferpe_00708	butyryl-CoA dehydrogenase (EC 1.3.99.2)	23.15
X	Ferpe_01729	CAAX amino terminal protease family.	16.06
E	Ferpe_02062	Carbamate kinase	24.5
G	Ferpe_00412	carbohydrate ABC transporter ATP-binding	26.07
G	Ferpe_00452	carbohydrate ABC transporter substrate-binding	21.54
G	Ferpe_00504	carbohydrate ABC transporter substrate-binding	25.53
G	Ferpe_01016	carbohydrate ABC transporter substrate-binding	21.49
G	Ferpe_01601	carbohydrate ABC transporter substrate-binding	20.72
G	Ferpe_01778	carbohydrate ABC transporter substrate-binding	16.81
P	Ferpe_00242	cation diffusion facilitator family transporter	18.35
P	Ferpe_00482	cation diffusion facilitator family transporter	
I	Ferpe_01651	CDP-diacylglycerol--glycerol-3-phosphate	19.15
I	Ferpe_00070	CDP-diglyceride synthetase	18.45
D	Ferpe_00379	cell division protein FtsA	23.46

D	Ferpe_01430	Cell division protein FtsI/penicillin-binding	18.87
M	Ferpe_01430	Cell division protein FtsI/penicillin-binding	18.87
D	Ferpe_02059	Cell division protein FtsI/penicillin-binding	20.62
M	Ferpe_02059	Cell division protein FtsI/penicillin-binding	20.62
D	Ferpe_00378	cell division protein FtsZ	24.43
X	Ferpe_01468	Cell division protein ZapA.	16.86
X	Ferpe_01215	Cell wall-associated hydrolases	17.82
G	Ferpe_00816	Cellobiose phosphorylase	20.76
E	Ferpe_00875	Cellulase M and related proteins	
G	Ferpe_00875	Cellulase M and related proteins	
E	Ferpe_01344	Cellulase M and related proteins	24.47
G	Ferpe_01344	Cellulase M and related proteins	24.47
E	Ferpe_01345	Cellulase M and related proteins	24.52
G	Ferpe_01345	Cellulase M and related proteins	24.52
E	Ferpe_01936	Cellulase M and related proteins	25.2
G	Ferpe_01936	Cellulase M and related proteins	25.2
O	Ferpe_01876	chaperone protein DnaJ	21.03
O	Ferpe_01558	chaperone protein DnaK	25.1
O	Ferpe_00027	chaperonin GroL	29.16
N	Ferpe_00477	Chemotaxis protein histidine kinase and related	25.38
T	Ferpe_00477	Chemotaxis protein histidine kinase and related	25.38
N	Ferpe_00181	Chemotaxis protein; stimulates methylation of	21.95
T	Ferpe_00181	Chemotaxis protein; stimulates methylation of	21.95
N	Ferpe_01134	Chemotaxis response regulator containing a	23.62
T	Ferpe_01134	Chemotaxis response regulator containing a	23.62
N	Ferpe_00478	Chemotaxis signal transduction protein	23.02
T	Ferpe_00478	Chemotaxis signal transduction protein	23.02
N	Ferpe_01035	Chemotaxis signal transduction protein	20.37
T	Ferpe_01035	Chemotaxis signal transduction protein	20.37
P	Ferpe_01199	Chloride channel protein EriC	19.34
P	Ferpe_00430	Chromate transport protein ChrA	
P	Ferpe_00431	Chromate transport protein ChrA	
L	Ferpe_00001	chromosomal replication initiator protein DnaA	17.45
H	Ferpe_01707	cob(I)alamin adenosyltransferase	

H	Ferpe_01703	cobalamin-5'-phosphate synthase (EC 2.7.8.26)	
O	Ferpe_00026	Co-chaperonin GroES (HSP10)	24.57
K	Ferpe_01149	Cold shock proteins	16.27
K	Ferpe_01148	cold-shock DNA-binding protein family	21.71
L	Ferpe_01934	competence protein ComEA helix-hairpin-helix	
R	Ferpe_01812	competence/damage-inducible protein CinA	21.31
L	Ferpe_01502	condensin subunit ScpA	19.66
D	Ferpe_01898	condensin subunit Smc	22.74
S	Ferpe_00530	conserved hypothetical integral membrane protein	
S	Ferpe_01750	conserved hypothetical integral membrane protein	
S	Ferpe_00606	conserved hypothetical protein	19.07
K	Ferpe_01472	conserved hypothetical protein	21.54
X	Ferpe_01971	conserved hypothetical protein	22.97
M	Ferpe_01248	conserved hypothetical protein YidD	
J	Ferpe_01647	conserved hypothetical protein YmdA/YtgF	24.92
R	Ferpe_01647	conserved hypothetical protein YmdA/YtgF	24.92
C	Ferpe_01890	Conserved protein/domain typically associated	20.66
P	Ferpe_01770	Copper chaperone	18.04
D	Ferpe_00253	crcB protein	
P	Ferpe_00253	crcB protein	
V	Ferpe_01533	CRISPR type III-B/RAMP module-associated protein	15.07
O	Ferpe_01946	C-terminal processing peptidase-3. Serine	22.55
F	Ferpe_00615	CTP synthase (EC 6.3.4.2)	24.86
R	Ferpe_01251	Cupin domain.	22.26
X	Ferpe_01629	Cyclic nucleotide-binding domain.	21.05
T	Ferpe_01638	Cyclic nucleotide-binding domain.	19.54
E	Ferpe_01992	Cysteine synthase	23.81
E	Ferpe_00707	cysteine synthase A	20.72
E	Ferpe_00516	cysteine synthases	22.72
J	Ferpe_00467	cysteinyl-tRNA synthetase (EC 6.1.1.16)	23.99
F	Ferpe_01129	cytidine deaminase (EC 3.5.4.5)	20.92
F	Ferpe_01886	cytidylate kinase	22.23
C	Ferpe_01182	Cytochrome c biogenesis protein	
O	Ferpe_01182	Cytochrome c biogenesis protein	

F	Ferpe_00759	Cytosine deaminase and related metal-dependent	21
R	Ferpe_00759	Cytosine deaminase and related metal-dependent	21
R	Ferpe_00055	DAK2 domain fusion protein YloV	24.44
M	Ferpe_01810	D-alanine--D-alanine ligase (EC 6.3.2.4)	19.92
R	Ferpe_01810	D-alanine--D-alanine ligase (EC 6.3.2.4)	19.92
M	Ferpe_00742	D-alanine-D-alanine ligase and related ATP-grasp	18.07
R	Ferpe_00742	D-alanine-D-alanine ligase and related ATP-grasp	18.07
E	Ferpe_02064	D-aminopeptidase	20.71
I	Ferpe_00485	Dehydrogenases with different specificities	22.65
Q	Ferpe_00485	Dehydrogenases with different specificities	22.65
R	Ferpe_00485	Dehydrogenases with different specificities	22.65
I	Ferpe_01065	Dehydrogenases with different specificities	
Q	Ferpe_01065	Dehydrogenases with different specificities	
R	Ferpe_01065	Dehydrogenases with different specificities	
I	Ferpe_01478	Dehydrogenases with different specificities	21.92
Q	Ferpe_01478	Dehydrogenases with different specificities	21.92
R	Ferpe_01478	Dehydrogenases with different specificities	21.92
C	Ferpe_01995	delta-1-pyrroline-5-carboxylate dehydrogenase	23.33
F	Ferpe_01138	Deoxycytidylate deaminase	19.46
L	Ferpe_01578	Deoxyribodipyrimidine photolyase	
L	Ferpe_01590	Deoxyribodipyrimidine photo-lyase type II (EC	17.67
F	Ferpe_00063	deoxyribose-phosphate aldolase	22.95
H	Ferpe_01511	dephospho-CoA kinase	18.23
C	Ferpe_00521	desulfoferrodoxin ferrous iron-binding domain	23.52
I	Ferpe_01135	Diacylglycerol kinase	
F	Ferpe_01789	Diadenosine tetraphosphate (Ap4A) hydrolase and	21.64
G	Ferpe_01789	Diadenosine tetraphosphate (Ap4A) hydrolase and	21.64
R	Ferpe_01789	Diadenosine tetraphosphate (Ap4A) hydrolase and	21.64
H	Ferpe_00797	diaminohydroxyphosphoribosylaminopyrimidine	
E	Ferpe_00471	Diaminopimelate decarboxylase	22.46
E	Ferpe_00422	diaminopimelate decarboxylase (EC 4.1.1.20)	22.19
E	Ferpe_00310	diaminopimelate epimerase (EC 5.1.1.7)	19.34
T	Ferpe_00074	diguanylate cyclase (GGDEF) domain	21.8
X	Ferpe_00273	diguanylate cyclase (GGDEF) domain	19.94

T	Ferpe_00420	diguanylate cyclase (GGDEF) domain	19.79
T	Ferpe_00472	diguanylate cyclase (GGDEF) domain	22.04
T	Ferpe_00784	diguanylate cyclase (GGDEF) domain	24.32
T	Ferpe_00785	diguanylate cyclase (GGDEF) domain	22.01
T	Ferpe_01152	diguanylate cyclase (GGDEF) domain	24.91
V	Ferpe_01152	diguanylate cyclase (GGDEF) domain	24.91
T	Ferpe_01348	diguanylate cyclase (GGDEF) domain	16.97
T	Ferpe_01355	diguanylate cyclase (GGDEF) domain	21.67
T	Ferpe_01566	diguanylate cyclase (GGDEF) domain	20.08
T	Ferpe_01667	diguanylate cyclase (GGDEF) domain	20.48
T	Ferpe_01773	diguanylate cyclase (GGDEF) domain	20.44
E	Ferpe_00312	dihydrodipicolinate reductase (EC 1.3.1.26)	22.01
E	Ferpe_00311	dihydrodipicolinate synthase (EC 4.2.1.52)	23.08
M	Ferpe_00311	dihydrodipicolinate synthase (EC 4.2.1.52)	23.08
H	Ferpe_00028	Dihydrofolate reductase	17.8
C	Ferpe_00825	dihydrolipoamide dehydrogenase	25.03
F	Ferpe_00838	dihydroorotase (EC 3.5.2.3)	15.86
F	Ferpe_00840	dihydroorotate dehydrogenase (subfamily 1)	15.63
H	Ferpe_00336	dihydropteroate synthase	22.57
J	Ferpe_00497	dimethyladenosine transferase	20.06
E	Ferpe_01527	Dipeptidyl	23.25
O	Ferpe_00077	Disulfide bond chaperones of the HSP33 family	24.48
F	Ferpe_00044	dITPase (EC 3.6.1.-)	20.05
L	Ferpe_01709	DNA adenine methylase (dam)	19.42
X	Ferpe_01711	DNA methylase.	14.75
L	Ferpe_01910	DNA mismatch repair protein MutL	22.58
L	Ferpe_01490	DNA mismatch repair protein MutS	23.38
L	Ferpe_01738	DNA modification methylase	20.65
K	Ferpe_00749	DNA or RNA helicases of superfamily II	
L	Ferpe_00749	DNA or RNA helicases of superfamily II	
K	Ferpe_00564	DNA phosphorothioation system restriction enzyme	15.51
L	Ferpe_00564	DNA phosphorothioation system restriction enzyme	15.51
L	Ferpe_00428	DNA polymerase I (EC 2.7.7.7)	23.61
L	Ferpe_00097	DNA primase (EC 2.7.7.-)	21.71

L	Ferpe_01829	DNA protecting protein DprA	15.34
L	Ferpe_01482	DNA repair exonuclease	16.9
O	Ferpe_00425	DNA repair protein RadA	22.72
L	Ferpe_00598	DNA replication and repair protein RadC	
L	Ferpe_01530	DNA replication and repair protein RecF	22.18
L	Ferpe_00686	DNA replication protein	
L	Ferpe_00730	DNA replication protein	
L	Ferpe_01070	DNA replication protein	
L	Ferpe_00198	DNA-(apurinic or apyrimidinic site) lyase (EC	19.35
R	Ferpe_02008	DNA-3-methyladenine glycosylase III (EC 3.2.2.-)	17.71
K	Ferpe_01388	DNA-directed RNA polymerase subunit alpha (EC	25.57
K	Ferpe_02056	DNA-directed RNA polymerase subunit beta (EC	26.56
X	Ferpe_00977	DnaJ domain.	22.72
X	Ferpe_01759	DRTGG domain.	21.09
J	Ferpe_01363	D-tyrosyl-tRNA(Tyr) deacylase	19.75
C	Ferpe_00710	electron transfer flavoprotein alpha subunit	16.84
C	Ferpe_01162	electron transfer flavoprotein alpha subunit	23.55
C	Ferpe_00709	electron transfer flavoprotein beta subunit	21.97
C	Ferpe_01161	electron transfer flavoprotein beta subunit	23.95
G	Ferpe_01841	Endoglucanase	24.8
L	Ferpe_00690	Endonuclease IV (EC 3.1.21.-)	19.34
L	Ferpe_01305	Endonuclease IV (EC 3.1.21.-)	22.52
L	Ferpe_00766	Endonuclease V (EC 3.1.21.-)	18.46
V	Ferpe_00215	endoribonuclease L-PSP	20.76
G	Ferpe_01925	enolase (EC 4.2.1.11)	26.99
G	Ferpe_00136	Entner-Doudoroff aldolase	21.51
I	Ferpe_00743	Esterase/lipase	
Q	Ferpe_01187	Esterase/lipase	23.07
I	Ferpe_01220	Esterase/lipase	16.9
I	Ferpe_01643	Esterase/lipase	19.67
L	Ferpe_00824	Excinuclease ABC subunit B	19.36
L	Ferpe_00614	Excinuclease ABC subunit C	20.63
L	Ferpe_00661	exonuclease RecJ (EC 3.1.-.-)	23.3
F	Ferpe_00786	Exopolyphosphatase-related proteins	23.38

X	Ferpe_01015	FAD binding domain.	18.84
X	Ferpe_00033	fagellar hook-basal body proteins	22.18
N	Ferpe_01322	fagellar hook-basal body proteins	15.22
P	Ferpe_00792	Fe2+ transport system protein A	19.75
P	Ferpe_00518	Fe2+/Zn2+ uptake regulation proteins	19.63
P	Ferpe_00938	Fe2+/Zn2+ uptake regulation proteins	21
P	Ferpe_01191	Fe2+/Zn2+ uptake regulation proteins	16.95
C	Ferpe_00002	Ferredoxin	16.05
C	Ferpe_01120	Ferredoxin	22.01
P	Ferpe_00764	Ferritin-like protein	25.47
P	Ferpe_00793	ferrous iron transporter FeoB	22
O	Ferpe_01683	FeS assembly ATPase SufC	20.44
R	Ferpe_00402	Fe-S oxidoreductase	19.3
R	Ferpe_01465	Fe-S oxidoreductase	20.57
X	Ferpe_01226	FG-GAP repeat.	17.29
X	Ferpe_00089	Fibronectin type III domain.	19.44
O	Ferpe_01386	FKBP-type peptidyl-prolyl cis-trans isomerase	26.59
O	Ferpe_00919	FKBP-type peptidyl-prolyl cis-trans isomerases 2	
X	Ferpe_01059	flagella basal body P-ring formation protein	16.37
N	Ferpe_01058	Flagellar basal body L-ring protein	16.85
N	Ferpe_00037	Flagellar basal body-associated protein	22.23
N	Ferpe_01048	flagellar basal-body M-ring protein/flagellar	22.91
U	Ferpe_01048	flagellar basal-body M-ring protein/flagellar	22.91
N	Ferpe_01057	Flagellar basal-body P-ring protein	20.35
N	Ferpe_01913	flagellar basal-body rod protein FlgB	
N	Ferpe_01914	flagellar basal-body rod protein FlgC	
N	Ferpe_00175	flagellar biosynthesis protein FlhA	19.88
N	Ferpe_00491	Flagellar biosynthesis/type III secretory	20.43
U	Ferpe_00491	Flagellar biosynthesis/type III secretory	20.43
N	Ferpe_01156	flagellar biosynthetic protein FlhB	
N	Ferpe_00176	flagellar biosynthetic protein FlhF	21.84
N	Ferpe_00481	flagellar biosynthetic protein FliP	17.09
N	Ferpe_00974	flagellar biosynthetic protein FliQ	
N	Ferpe_01033	flagellar biosynthetic protein FliS	19.21

U	Ferpe_01033	flagellar biosynthetic protein FliS	19.21
N	Ferpe_00199	Flagellar capping protein	19.56
N	Ferpe_01900	flagellar export protein FliJ	
N	Ferpe_00032	Flagellar hook capping protein	
X	Ferpe_01986	flagellar hook-associated protein 3	18.54
N	Ferpe_01985	flagellar hook-associated protein FlgK	19.98
N	Ferpe_01915	flagellar hook-basal body complex protein FliE	
X	Ferpe_00031	Flagellar hook-length control protein	14.73
N	Ferpe_00035	Flagellar motor component	22.03
N	Ferpe_00036	Flagellar motor protein	19.5
N	Ferpe_01047	flagellar motor switch protein FliG	21.77
N	Ferpe_00038	flagellar motor switch protein FliM	19.01
T	Ferpe_00039	flagellar motor switch protein FliN	21.38
X	Ferpe_01847	Flagellin and related hook-associated proteins	16.76
N	Ferpe_01849	Flagellin and related hook-associated proteins	23.46
N	Ferpe_01852	Flagellin and related hook-associated proteins	19.78
X	Ferpe_01984	FlgN protein.	17.9
X	Ferpe_00755	FOG: GGDEF domain	22.7
R	Ferpe_01712	FOG: HEAT repeat	18.42
X	Ferpe_01030	FOG: WD40 repeat	17.96
M	Ferpe_00574	FOG: WD40-like repeat	15.16
M	Ferpe_00712	FOG: WD40-like repeat	23.47
M	Ferpe_00714	FOG: WD40-like repeat	
H	Ferpe_00757	folylpolyglutamate synthase/dihydrofolate	21.63
C	Ferpe_02015	Formate hydrogenlyase subunit 3/Multisubunit	19.72
P	Ferpe_02015	Formate hydrogenlyase subunit 3/Multisubunit	19.72
C	Ferpe_02016	Formate hydrogenlyase subunit 3/Multisubunit	18.49
P	Ferpe_02016	Formate hydrogenlyase subunit 3/Multisubunit	18.49
C	Ferpe_02014	Formate hydrogenlyase subunit 4	17.15
F	Ferpe_01358	Formate-tetrahydrofolate ligase (EC 6.3.4.3)	24.84
G	Ferpe_01080	Fructose-1-phosphate kinase and related	24.34
G	Ferpe_00019	Fucose permease	
G	Ferpe_00844	galactokinase	22.98
H	Ferpe_00212	Geranylgeranyl pyrophosphate synthase	20.76

G	Ferpe_00326	glucokinase (EC 2.7.1.2)	21.01
K	Ferpe_00326	glucokinase (EC 2.7.1.2)	21.01
X	Ferpe_00811	Gluconolactonase	16.99
M	Ferpe_01052	glucosamine--fructose-6-phosphate	24.87
G	Ferpe_00065	glucose-1-phosphate adenylyltransferase	22.25
M	Ferpe_01103	Glucose-1-phosphate thymidylyltransferase (EC	22.79
G	Ferpe_01509	Glucose-6-phosphate isomerase	25.23
J	Ferpe_01977	glucose-inhibited division protein A	22.82
E	Ferpe_01357	Glutamate dehydrogenase/leucine dehydrogenase	28.51
E	Ferpe_01011	glutamate formiminotransferase	25.95
M	Ferpe_01469	glutamate racemase (EC 5.1.1.3)	18.04
J	Ferpe_00466	glutamyl-tRNA synthetase (EC 6.1.1.17)	23.97
X	Ferpe_01186	Glutaredoxin-like domain protein	20.58
G	Ferpe_00274	glyceraldehyde-3-phosphate dehydrogenase (NAD+)	28.74
G	Ferpe_01487	glycerate 2-kinase (EC 2.7.1.165)	13.55
C	Ferpe_00208	glycerol kinase (EC 2.7.1.30)	22.4
X	Ferpe_00740	Glycerol-3-phosphate dehydrogenase	20.87
C	Ferpe_01282	Glycerol-3-phosphate dehydrogenase	22.26
I	Ferpe_00894	Glycerophosphoryl diester phosphodiesterase	22.23
E	Ferpe_01114	glycine betaine/L-proline transport ATP binding	
E	Ferpe_01275	glycine cleavage system H protein	19.07
E	Ferpe_01084	Glycine cleavage system protein P	26.07
E	Ferpe_01085	glycine dehydrogenase (decarboxylating) alpha	24.79
E	Ferpe_00871	Glycine/D-amino acid oxidases (deaminating)	20.06
E	Ferpe_00323	Glycine/serine hydroxymethyltransferase	25.75
G	Ferpe_00213	glycogen debranching enzyme GlgX	21.44
G	Ferpe_00463	glycogen synthase (ADP-glucose)	25.88
G	Ferpe_01806	glycogen/starch/alpha-glucan phosphorylases	24.18
G	Ferpe_00279	Glycosidases	24.14
G	Ferpe_01425	Glycosidases	25.11
G	Ferpe_01457	Glycosidases	16.34
G	Ferpe_01926	Glycosidases	23.99
G	Ferpe_01956	Glycosidases	
X	Ferpe_00130	Glycosyl transferases group 1.	19.81

X	Ferpe_01110	Glycosyl transferases group 1.	22.01
M	Ferpe_00103	Glycosyltransferase	19.22
M	Ferpe_00107	Glycosyltransferase	19.58
M	Ferpe_00109	Glycosyltransferase	22.74
M	Ferpe_00114	Glycosyltransferase	21.33
M	Ferpe_00508	Glycosyltransferase	23.93
M	Ferpe_00918	Glycosyltransferase	18.14
X	Ferpe_01848	Glycosyltransferase	21.8
M	Ferpe_01942	Glycosyltransferase	21.86
M	Ferpe_00112	Glycosyltransferases involved in cell wall	23.39
M	Ferpe_01060	Glycosyltransferases involved in cell wall	
X	Ferpe_01851	Glycosyltransferases involved in cell wall	22.82
M	Ferpe_01859	Glycosyltransferases involved in cell wall	19.54
J	Ferpe_01211	glycyl-tRNA synthetase alpha chain (EC 6.1.1.14)	23.11
J	Ferpe_01210	glycyl-tRNA synthetase beta chain (EC 6.1.1.14)	24.47
H	Ferpe_00799	GTP cyclohydrolase II	14.04
J	Ferpe_00234	GTP-binding protein Era	24.32
J	Ferpe_00360	GTP-binding protein HflX	21.99
J	Ferpe_01521	GTP-binding protein LepA	24.57
F	Ferpe_01963	guanylate kinase (EC 2.7.4.8)	20.68
R	Ferpe_01463	HD superfamily phosphohydrolases	25.39
X	Ferpe_00945	HD-GYP domain	21.87
O	Ferpe_00340	heat shock protein Hsp20	25.88
O	Ferpe_01809	Heat shock protein. Metallo peptidase. MEROPS	18.71
K	Ferpe_01878	heat-inducible transcription repressor HrcA	23.14
P	Ferpe_00668	heavy metal-(Cd/Co/Hg/Pb/Zn)-translocating	19.58
J	Ferpe_00475	hemolysin TlyA family protein	19.91
R	Ferpe_01130	Hemolysins and related proteins containing CBS	20.97
E	Ferpe_00368	histidine ammonia-lyase (EC 4.3.1.3)	21.87
E	Ferpe_00616	histidinol phosphate phosphatase HisJ family	22.06
R	Ferpe_00616	histidinol phosphate phosphatase HisJ family	22.06
J	Ferpe_01866	histidyl-tRNA synthetase (EC 6.1.1.21)	24.3
X	Ferpe_00991	Histone acetyltransferase	18.4
L	Ferpe_01004	Holliday junction DNA helicase subunit RuvA	20.67

L	Ferpe_00393	Holliday junction DNA helicase subunit RuvB	21.94
L	Ferpe_01919	Holliday junction endonuclease RuvC (EC	16.17
H	Ferpe_00641	hydroxymethylpyrimidine synthase	25.33
X	Ferpe_00003	hypothetical protein	25.49
X	Ferpe_00021	hypothetical protein	16.73
X	Ferpe_00022	hypothetical protein	23.59
X	Ferpe_00023	hypothetical protein	19.32
X	Ferpe_00030	hypothetical protein	22.6
X	Ferpe_00046	hypothetical protein	18.45
X	Ferpe_00049	hypothetical protein	19.5
X	Ferpe_00066	hypothetical protein	20.33
X	Ferpe_00073	hypothetical protein	20.06
X	Ferpe_00078	hypothetical protein	16.27
X	Ferpe_00079	hypothetical protein	20.54
X	Ferpe_00108	hypothetical protein	18.04
X	Ferpe_00111	hypothetical protein	23.68
X	Ferpe_00140	hypothetical protein	17.72
X	Ferpe_00183	hypothetical protein	14.04
X	Ferpe_00184	hypothetical protein	20.65
X	Ferpe_00185	hypothetical protein	18.63
X	Ferpe_00214	hypothetical protein	19.73
X	Ferpe_00241	hypothetical protein	18.41
X	Ferpe_00255	hypothetical protein	17.11
X	Ferpe_00256	hypothetical protein	17.66
X	Ferpe_00286	hypothetical protein	23.35
X	Ferpe_00288	hypothetical protein	18.34
X	Ferpe_00317	hypothetical protein	18.87
X	Ferpe_00320	hypothetical protein	15.99
X	Ferpe_00322	hypothetical protein	20.38
X	Ferpe_00347	hypothetical protein	22.42
X	Ferpe_00352	hypothetical protein	19.84
X	Ferpe_00356	hypothetical protein	20.86
X	Ferpe_00357	hypothetical protein	19.44
X	Ferpe_00362	hypothetical protein	18.3

X	Ferpe_00365	hypothetical protein	19.09
X	Ferpe_00371	hypothetical protein	18.79
X	Ferpe_00376	hypothetical protein	20.79
X	Ferpe_00392	hypothetical protein	19.8
X	Ferpe_00423	hypothetical protein	16.52
X	Ferpe_00439	hypothetical protein	23.09
X	Ferpe_00443	hypothetical protein	19.99
X	Ferpe_00449	hypothetical protein	19.18
X	Ferpe_00451	hypothetical protein	17.7
X	Ferpe_00458	hypothetical protein	21.17
X	Ferpe_00476	hypothetical protein	21.07
X	Ferpe_00495	hypothetical protein	16.49
X	Ferpe_00498	hypothetical protein	19.89
X	Ferpe_00514	hypothetical protein	16.63
X	Ferpe_00523	hypothetical protein	18.44
X	Ferpe_00532	hypothetical protein	19.84
X	Ferpe_00543	hypothetical protein	20.54
X	Ferpe_00548	hypothetical protein	20.5
X	Ferpe_00561	hypothetical protein	18.83
X	Ferpe_00566	hypothetical protein	20.49
X	Ferpe_00567	hypothetical protein	16.3
X	Ferpe_00576	hypothetical protein	21.18
X	Ferpe_00585	hypothetical protein	15.2
X	Ferpe_00593	hypothetical protein	19.07
X	Ferpe_00595	hypothetical protein	17.91
X	Ferpe_00597	hypothetical protein	22.1
X	Ferpe_00602	hypothetical protein	20.38
X	Ferpe_00637	hypothetical protein	23.04
X	Ferpe_00645	hypothetical protein	20.83
X	Ferpe_00765	hypothetical protein	18.26
X	Ferpe_00769	hypothetical protein	20.18
X	Ferpe_00777	hypothetical protein	19.91
X	Ferpe_00803	hypothetical protein	22.1
X	Ferpe_00810	hypothetical protein	17.27

X	Ferpe_00834	hypothetical protein	17.37
X	Ferpe_00867	hypothetical protein	21.47
X	Ferpe_00897	hypothetical protein	20.05
X	Ferpe_00929	hypothetical protein	17.89
X	Ferpe_00930	hypothetical protein	19.61
X	Ferpe_00931	hypothetical protein	23.13
X	Ferpe_00953	hypothetical protein	16.77
X	Ferpe_00954	hypothetical protein	18.66
I	Ferpe_00960	hypothetical protein	13.86
X	Ferpe_00965	hypothetical protein	23.56
X	Ferpe_00966	hypothetical protein	20
X	Ferpe_00968	hypothetical protein	25.21
X	Ferpe_00976	hypothetical protein	19.25
X	Ferpe_00978	hypothetical protein	21.41
X	Ferpe_00990	hypothetical protein	22.22
X	Ferpe_01020	hypothetical protein	16.83
X	Ferpe_01026	hypothetical protein	17.29
X	Ferpe_01028	hypothetical protein	17.43
X	Ferpe_01032	hypothetical protein	24.28
X	Ferpe_01037	hypothetical protein	21.84
L	Ferpe_01043	hypothetical protein	25.02
X	Ferpe_01044	hypothetical protein	21.28
X	Ferpe_01055	hypothetical protein	20.03
X	Ferpe_01089	hypothetical protein	20.94
X	Ferpe_01141	hypothetical protein	24.22
X	Ferpe_01157	hypothetical protein	15.39
X	Ferpe_01159	hypothetical protein	22.66
X	Ferpe_01166	hypothetical protein	18.54
X	Ferpe_01169	hypothetical protein	21.38
X	Ferpe_01171	hypothetical protein	19.73
X	Ferpe_01209	hypothetical protein	17.26
X	Ferpe_01213	hypothetical protein	18.92
X	Ferpe_01221	hypothetical protein	19.63
X	Ferpe_01222	hypothetical protein	15.33

X	Ferpe_01223	hypothetical protein	15.54
X	Ferpe_01225	hypothetical protein	16.06
X	Ferpe_01230	hypothetical protein	18.96
X	Ferpe_01260	hypothetical protein	18.67
X	Ferpe_01284	hypothetical protein	23.97
X	Ferpe_01292	hypothetical protein	21.16
X	Ferpe_01297	hypothetical protein	18.7
X	Ferpe_01312	hypothetical protein	18.28
X	Ferpe_01313	hypothetical protein	22.65
X	Ferpe_01318	hypothetical protein	19.23
X	Ferpe_01320	hypothetical protein	21.35
X	Ferpe_01327	hypothetical protein	20.13
X	Ferpe_01331	hypothetical protein	25.03
X	Ferpe_01333	hypothetical protein	21.9
X	Ferpe_01335	hypothetical protein	19.59
X	Ferpe_01340	hypothetical protein	17.49
X	Ferpe_01343	hypothetical protein	18.72
X	Ferpe_01350	hypothetical protein	16.48
X	Ferpe_01351	hypothetical protein	20.06
X	Ferpe_01353	hypothetical protein	16.31
X	Ferpe_01365	hypothetical protein	20.17
X	Ferpe_01377	hypothetical protein	20.92
X	Ferpe_01383	hypothetical protein	19.43
X	Ferpe_01432	hypothetical protein	19.1
X	Ferpe_01441	hypothetical protein	19.79
X	Ferpe_01454	hypothetical protein	19.56
X	Ferpe_01456	hypothetical protein	15.29
X	Ferpe_01467	hypothetical protein	18.39
X	Ferpe_01476	hypothetical protein	24.82
X	Ferpe_01477	hypothetical protein	15.64
X	Ferpe_01481	hypothetical protein	14.62
X	Ferpe_01483	hypothetical protein	19.85
X	Ferpe_01486	hypothetical protein	19.32
X	Ferpe_01500	hypothetical protein	18.96

X	Ferpe_01510	hypothetical protein	16.17
X	Ferpe_01531	hypothetical protein	15.54
X	Ferpe_01542	hypothetical protein	21.47
X	Ferpe_01543	hypothetical protein	22.02
X	Ferpe_01544	hypothetical protein	19.05
X	Ferpe_01557	hypothetical protein	21.98
X	Ferpe_01565	hypothetical protein	19.11
X	Ferpe_01573	hypothetical protein	20.67
X	Ferpe_01580	hypothetical protein	20.88
X	Ferpe_01595	hypothetical protein	18.64
X	Ferpe_01618	hypothetical protein	16.86
X	Ferpe_01653	hypothetical protein	19.61
X	Ferpe_01654	hypothetical protein	19.07
X	Ferpe_01672	hypothetical protein	21.82
X	Ferpe_01673	hypothetical protein	20.16
X	Ferpe_01674	hypothetical protein	19.77
X	Ferpe_01675	hypothetical protein	17.28
X	Ferpe_01690	hypothetical protein	22.74
X	Ferpe_01694	hypothetical protein	17.42
X	Ferpe_01697	hypothetical protein	18.93
X	Ferpe_01702	hypothetical protein	15.16
X	Ferpe_01735	hypothetical protein	18.99
X	Ferpe_01737	hypothetical protein	20.61
X	Ferpe_01768	hypothetical protein	17.69
X	Ferpe_01780	hypothetical protein	15.3
X	Ferpe_01790	hypothetical protein	20.83
X	Ferpe_01807	hypothetical protein	21.78
X	Ferpe_01816	hypothetical protein	19.45
X	Ferpe_01818	hypothetical protein	16
X	Ferpe_01825	hypothetical protein	17.56
X	Ferpe_01826	hypothetical protein	19.59
X	Ferpe_01833	hypothetical protein	21.99
X	Ferpe_01834	hypothetical protein	21.41
X	Ferpe_01845	hypothetical protein	21.99

X	Ferpe_01856	hypothetical protein	20.04
X	Ferpe_01858	hypothetical protein	18.35
X	Ferpe_01860	hypothetical protein	19.7
M	Ferpe_01861	hypothetical protein	19.82
X	Ferpe_01862	hypothetical protein	17.61
X	Ferpe_01875	hypothetical protein	16.53
X	Ferpe_01893	hypothetical protein	19.11
X	Ferpe_01911	hypothetical protein	19.98
X	Ferpe_01912	hypothetical protein	20.98
X	Ferpe_01916	hypothetical protein	27.09
X	Ferpe_01924	hypothetical protein	27.82
X	Ferpe_01935	hypothetical protein	20.55
X	Ferpe_01948	hypothetical protein	20.13
X	Ferpe_01950	hypothetical protein	20.48
X	Ferpe_01951	hypothetical protein	20.37
X	Ferpe_01991	hypothetical protein	18.39
X	Ferpe_01996	hypothetical protein	17.5
X	Ferpe_01999	hypothetical protein	19.8
X	Ferpe_02000	hypothetical protein	20.55
X	Ferpe_02032	hypothetical protein	18.34
X	Ferpe_02034	hypothetical protein	18.7
X	Ferpe_02038	hypothetical protein	21.7
X	Ferpe_02048	hypothetical protein	19.99
X	Ferpe_02049	hypothetical protein	20.25
F	Ferpe_02046	hypoxanthine phosphoribosyltransferase	22.42
Q	Ferpe_00043	imidazolonepropionase (EC 3.5.2.7)	20.43
Q	Ferpe_00662	Imidazolonepropionase and related	26.3
F	Ferpe_00354	inosine-5'-monophosphate dehydrogenase	23.15
M	Ferpe_01646	integral membrane protein MviN	16.35
J	Ferpe_00758	iojap-like ribosome-associated protein	23.23
H	Ferpe_00287	iron-only hydrogenase maturation protein HydE	20.09
R	Ferpe_00282	iron-only hydrogenase maturation protein HydF	21.79
H	Ferpe_00289	iron-only hydrogenase maturation protein HydG	24.14
O	Ferpe_00527	Iron-regulated ABC transporter ATPase subunit	20.37

O	Ferpe_01682	Iron-regulated ABC transporter membrane	21.04
O	Ferpe_01681	Iron-regulated ABC transporter permease protein	20.76
F	Ferpe_01634	isoaspartyl dipeptidase IadA	22.58
Q	Ferpe_00533	Isochorismate hydrolase	18.57
J	Ferpe_01791	Isoleucyl-tRNA synthetase (EC 6.1.1.5)	26.99
P	Ferpe_00272	K+-dependent Na+/Ca+ exchanger related-protein	19.38
H	Ferpe_01905	ketopantoate hydroxymethyltransferase (EC	21.09
C	Ferpe_00133	Lactate dehydrogenase and related dehydrogenases	24.48
H	Ferpe_00133	Lactate dehydrogenase and related dehydrogenases	24.48
R	Ferpe_00133	Lactate dehydrogenase and related dehydrogenases	24.48
C	Ferpe_00663	Lactate dehydrogenase and related dehydrogenases	24.9
H	Ferpe_00663	Lactate dehydrogenase and related dehydrogenases	24.9
R	Ferpe_00663	Lactate dehydrogenase and related dehydrogenases	24.9
M	Ferpe_02005	L-alanine-DL-glutamate epimerase and related	22.56
R	Ferpe_02005	L-alanine-DL-glutamate epimerase and related	22.56
O	Ferpe_01614	LAO/AO transport system ATPase	22.41
M	Ferpe_00701	large conductance mechanosensitive channel	18.25
M	Ferpe_01678	Large-conductance mechanosensitive channel	18.56
J	Ferpe_01903	L-asparaginase/archaeal Glu-tRNAGln	21.87
U	Ferpe_01903	L-asparaginase/archaeal Glu-tRNAGln	21.87
H	Ferpe_01516	L-aspartate 1-decarboxylase (EC 4.1.1.11)	17.65
E	Ferpe_01083	L-aspartate aminotransferase apoenzyme (EC	24.09
E	Ferpe_00941	Leucyl aminopeptidase (aminopeptidase T)	23.85
J	Ferpe_00048	leucyl-tRNA synthetase (EC 6.1.1.4)	25.21
E	Ferpe_00791	L-glutamine synthetase (EC 6.3.1.2)	22.76
M	Ferpe_00250	Lipid A core - O-antigen ligase and related	23.69
H	Ferpe_01751	Lipoate-protein ligase A	21.16
M	Ferpe_00667	lipoprotein signal peptidase	
U	Ferpe_00667	lipoprotein signal peptidase	
C	Ferpe_01424	L-lactate dehydrogenase	21.38
J	Ferpe_02058	LSU ribosomal protein L10P	25.36
J	Ferpe_01173	LSU ribosomal protein L11P	24.04
J	Ferpe_02057	LSU ribosomal protein L12P	26.55
J	Ferpe_00095	LSU ribosomal protein L13P	23.87

J	Ferpe_01406	LSU ribosomal protein L14P	24.22
J	Ferpe_01397	LSU ribosomal protein L15P	23.7
J	Ferpe_01409	LSU ribosomal protein L16P	24.63
J	Ferpe_01387	LSU ribosomal protein L17P	23.54
J	Ferpe_01400	LSU ribosomal protein L18P	23.11
J	Ferpe_00927	LSU ribosomal protein L19P	24.63
J	Ferpe_01172	LSU ribosomal protein L1P	25.39
J	Ferpe_00243	LSU ribosomal protein L20P	23.39
J	Ferpe_00091	LSU ribosomal protein L21P	24.59
J	Ferpe_01411	LSU ribosomal protein L22P	24.15
J	Ferpe_01405	LSU ribosomal protein L24P	20.85
J	Ferpe_00093	LSU ribosomal protein L27P	21.54
J	Ferpe_01272	LSU ribosomal protein L28P	21.52
J	Ferpe_01408	LSU ribosomal protein L29P	20.63
J	Ferpe_01413	LSU ribosomal protein L2P	25.23
J	Ferpe_01398	LSU ribosomal protein L30P	20.57
J	Ferpe_00418	LSU ribosomal protein L31P	19.39
J	Ferpe_01392	LSU ribosomal protein L36P	
J	Ferpe_01404	LSU ribosomal protein L5P	25.78
J	Ferpe_01401	LSU ribosomal protein L6P	25.76
J	Ferpe_01434	LSU ribosomal protein L9P	22.45
E	Ferpe_01706	L-threonine O-3-phosphate decarboxylase (EC	
I	Ferpe_00057	Lysophospholipase	22.8
Q	Ferpe_00599	MAF protein	20.77
G	Ferpe_00196	Major Facilitator Superfamily.	16.15
C	Ferpe_00524	Malic enzyme	21.07
I	Ferpe_00888	malonyl CoA-acyl carrier protein transacylase	25.65
G	Ferpe_00984	Maltose-binding periplasmic proteins/domains	24.95
M	Ferpe_00149	Mannose-1-phosphate guanylyltransferase	22.25
X	Ferpe_01281	MazG family protein	20.11
M	Ferpe_01140	Membrane carboxypeptidase (penicillin-binding	23.91
O	Ferpe_00400	membrane protease FtsH catalytic subunit (EC	25.79
O	Ferpe_00944	Membrane protein implicated in regulation of	19.97
M	Ferpe_00119	Membrane protein involved in the export of	16.27

M	Ferpe_01109	Membrane protein involved in the export of	16.03
X	Ferpe_00361	Membrane proteins related to	22.89
M	Ferpe_01978	Membrane proteins related to	17.98
H	Ferpe_01092	Membrane-associated lipoprotein involved in	22.91
R	Ferpe_01746	Metal-dependent hydrolase	
R	Ferpe_00562	Metal-dependent hydrolases of the beta-lactamase	15.74
E	Ferpe_01443	Methenyl tetrahydrofolate cyclohydrolase	23.83
H	Ferpe_00363	methionine adenosyltransferase (EC 2.5.1.6)	27.92
O	Ferpe_00494	methionine-R-sulfoxide	
J	Ferpe_01339	methionyl-tRNA formyltransferase	19.88
J	Ferpe_01000	methionyl-tRNA synthetase (EC 6.1.1.10)	25.63
N	Ferpe_00173	Methyl-accepting chemotaxis protein	23.65
T	Ferpe_00173	Methyl-accepting chemotaxis protein	23.65
N	Ferpe_00419	Methyl-accepting chemotaxis protein	25.27
T	Ferpe_00419	Methyl-accepting chemotaxis protein	25.27
X	Ferpe_00433	Methyl-accepting chemotaxis protein	22.53
N	Ferpe_01352	Methyl-accepting chemotaxis protein	24.45
T	Ferpe_01352	Methyl-accepting chemotaxis protein	24.45
N	Ferpe_01479	Methyl-accepting chemotaxis protein	22.83
T	Ferpe_01479	Methyl-accepting chemotaxis protein	22.83
N	Ferpe_00218	methyl-accepting chemotaxis sensory transducer	26.4
T	Ferpe_00218	methyl-accepting chemotaxis sensory transducer	26.4
N	Ferpe_00367	methyl-accepting chemotaxis sensory transducer	26.52
T	Ferpe_00367	methyl-accepting chemotaxis sensory transducer	26.52
H	Ferpe_00280	Methylase involved in ubiquinone/menaquinone	
N	Ferpe_01326	Methylase of chemotaxis methyl-accepting	18.46
T	Ferpe_01326	Methylase of chemotaxis methyl-accepting	18.46
N	Ferpe_01382	Methylase of chemotaxis methyl-accepting	22.67
T	Ferpe_01382	Methylase of chemotaxis methyl-accepting	22.67
G	Ferpe_00086	methylglyoxal synthase	21.42
I	Ferpe_01615	methylmalonyl-CoA mutase C-terminal domain	21.91
E	Ferpe_00051	methylthioribose-1-phosphate isomerase (EC	25.03
O	Ferpe_00865	Mg chelatase-related protein	19.36
X	Ferpe_01501	Mg/Co/Ni transporter MgtE (contains CBS domain)	24.48

P	Ferpe_00047	Mg2+ transporter (mgtE)	19.74
J	Ferpe_01078	MiaB-like tRNA modifying enzyme	20.54
X	Ferpe_01997	Micrococcal nuclease (thermonuclease) homologs	17.08
L	Ferpe_02001	Micrococcal nuclease (thermonuclease) homologs	17.41
X	Ferpe_00544	Mismatch repair ATPase (MutS family)	21.76
O	Ferpe_01877	Molecular chaperone GrpE (heat shock protein)	21.75
G	Ferpe_00156	monosaccharide ABC transporter ATP-binding	
G	Ferpe_01691	monosaccharide ABC transporter ATP-binding	23.25
G	Ferpe_00154	monosaccharide ABC transporter substrate-binding	
R	Ferpe_01027	MoxR-like ATPases	21.66
X	Ferpe_00534	Mpv17 / PMP22 family.	19.58
L	Ferpe_00456	MutS2 family protein	22.67
C	Ferpe_01143	Na+/H+ antiporter	20.16
G	Ferpe_00582	Na+/melibiose symporter and related transporters	
G	Ferpe_00832	Na+/melibiose symporter and related transporters	15.04
G	Ferpe_01766	Na+/melibiose symporter and related transporters	18.79
R	Ferpe_01820	Na+-dependent transporters of the SNF family	19.94
V	Ferpe_00625	Na+-driven multidrug efflux pump	16.35
X	Ferpe_00259	N-acetyl-beta-hexosaminidase	21.39
R	Ferpe_00319	N-acetyldiaminopimelate deacetylase (EC	22.68
G	Ferpe_01025	N-acetylglucosamine 6-phosphate deacetylase (EC	16.57
C	Ferpe_01118	NAD(P)-dependent iron-only hydrogenase	26.26
C	Ferpe_00291	NAD(P)-dependent iron-only hydrogenase catalytic	24.76
H	Ferpe_00483	NAD+ synthetase	23.67
R	Ferpe_00483	NAD+ synthetase	23.67
H	Ferpe_00691	NAD+ synthetase	22.6
R	Ferpe_00691	NAD+ synthetase	22.6
H	Ferpe_01513	NAD+ synthetase	19.71
C	Ferpe_02013	NADH:ubiquinone oxidoreductase 20 kD subunit and	20.36
X	Ferpe_00856	NADH:ubiquinone oxidoreductase 24 kD subunit	19.61
X	Ferpe_01119	NADH:ubiquinone oxidoreductase 24 kD subunit	20.35
C	Ferpe_01121	NADH:ubiquinone oxidoreductase 24 kD subunit	23.56
C	Ferpe_02012	NADH:ubiquinone oxidoreductase 27 kD subunit	18.74
C	Ferpe_02011	NADH:ubiquinone oxidoreductase 49 kD subunit 7	21.99

C	Ferpe_00622	NADPH-dependent FMN reductase.	18.13
E	Ferpe_02010	NADPH-dependent glutamate synthase beta chain	23.2
R	Ferpe_02010	NADPH-dependent glutamate synthase beta chain	23.2
H	Ferpe_00165	nicotinate-nucleotide adenylyltransferase (EC	20.58
H	Ferpe_01740	nicotinate-nucleotide pyrophosphorylase	18.45
H	Ferpe_01700	nicotinate-nucleotide-dimethylbenzimidazole	20.28
H	Ferpe_01087	Nicotinic acid phosphoribosyltransferase	25.04
C	Ferpe_00638	Nitroreductase	22.89
V	Ferpe_01031	NTP pyrophosphohydrolases including oxidative	21.38
R	Ferpe_00005	nucleoside ABC transporter ATP-binding protein	25.47
R	Ferpe_00006	nucleoside ABC transporter membrane protein	23.08
R	Ferpe_00007	nucleoside ABC transporter membrane protein	20.01
F	Ferpe_01142	nucleoside diphosphate kinase (EC 2.7.4.6)	24.45
M	Ferpe_00004	nucleoside-binding protein	24.66
M	Ferpe_00384	Nucleoside-diphosphate-sugar epimerases	22.3
X	Ferpe_01864	Nucleoside-diphosphate-sugar pyrophosphorylase	23.25
M	Ferpe_00090	nucleotide sugar dehydrogenase	26.54
L	Ferpe_01928	Nucleotidyltransferase/DNA polymerase involved	
K	Ferpe_00937	NusA antitermination factor	25.15
K	Ferpe_00334	NusB antitermination factor	15.88
D	Ferpe_00164	Obg family GTPase CgtA	22.65
L	Ferpe_00164	Obg family GTPase CgtA	22.65
M	Ferpe_00453	O-Glycosyl hydrolase	22.95
E	Ferpe_01520	Oligoendopeptidase F	24.73
X	Ferpe_00776	Organic solvent tolerance protein OstA	20.11
E	Ferpe_00967	ornithine carbamoyltransferase	27.51
F	Ferpe_00842	orotate phosphoribosyltransferase (EC 2.4.2.10)	
F	Ferpe_00841	orotidine-5'-phosphate decarboxylase (EC	18.01
J	Ferpe_01054	O-sialoglycoprotein endopeptidase (EC 3.4.24.57)	19.92
M	Ferpe_01009	Outer membrane protein/protective antigen OMA87	24.21
H	Ferpe_01830	pantothenate synthetase (EC 6.3.2.1)	21.79
K	Ferpe_00018	PAS domain S-box	
T	Ferpe_00018	PAS domain S-box	
T	Ferpe_01664	PAS domain S-box	20.67

X	Ferpe_01899	PAS domain S-box	25.77
X	Ferpe_00525	PAS domain S-box/diguanylate cyclase (GGDEF)	16.21
T	Ferpe_01131	PAS domain S-box/diguanylate cyclase (GGDEF)	21.79
X	Ferpe_01515	PAS/PAC sensor signal transduction histidine	23.17
X	Ferpe_00780	PEGA domain.	19.3
X	Ferpe_01466	Peptidase family M50.	17.56
J	Ferpe_00727	peptide chain release factor 1	20.06
J	Ferpe_02035	peptide chain release factor 2	23.28
J	Ferpe_00700	peptide deformylase	19.89
J	Ferpe_01298	peptide deformylase	20.44
J	Ferpe_00878	peptidyl-tRNA hydrolase	18.77
M	Ferpe_00327	periplasmic chaperone for outer membrane	21.17
O	Ferpe_00327	periplasmic chaperone for outer membrane	21.17
M	Ferpe_01112	Periplasmic glycine betaine/choline-binding	17.02
X	Ferpe_00100	Periplasmic protein involved in polysaccharide	24.06
O	Ferpe_01168	Periplasmic serine proteases (ClpP class)	24.45
F	Ferpe_00594	Permeases	21.27
E	Ferpe_01309	Permeases of the drug/metabolite transporter	16.8
G	Ferpe_01309	Permeases of the drug/metabolite transporter	16.8
R	Ferpe_01309	Permeases of the drug/metabolite transporter	16.8
E	Ferpe_01324	Permeases of the drug/metabolite transporter	
G	Ferpe_01324	Permeases of the drug/metabolite transporter	
R	Ferpe_01324	Permeases of the drug/metabolite transporter	
O	Ferpe_00299	Peroxiredoxin	20.91
O	Ferpe_00763	Peroxiredoxin	23.85
R	Ferpe_00262	phenazine biosynthesis protein PhzF family	21.5
J	Ferpe_01311	phenylalanyl-tRNA synthetase beta subunit (EC	25.22
P	Ferpe_01063	Phenylpropionate dioxygenase and related	14.39
R	Ferpe_01063	Phenylpropionate dioxygenase and related	14.39
P	Ferpe_01794	phosphate ABC transporter substrate-binding	16.27
S	Ferpe_00820	Phosphate transport regulator (distant homolog	23.47
P	Ferpe_00821	Phosphate/sulphate permeases	20.69
P	Ferpe_01507	Phosphate/sulphate permeases	
I	Ferpe_01051	phosphate:acyl-[acyl carrier protein]	24.35

G	Ferpe_01437	phosphoglucosamine mutase (EC 5.4.2.10)	22.06
H	Ferpe_00341	Phosphoglycerate dehydrogenase and related	28.21
R	Ferpe_00341	Phosphoglycerate dehydrogenase and related	28.21
H	Ferpe_00822	Phosphoglycerate dehydrogenase and related	23.04
R	Ferpe_00822	Phosphoglycerate dehydrogenase and related	23.04
G	Ferpe_00276	phosphoglycerate kinase (EC 2.7.2.3)	26.99
G	Ferpe_00261	Phosphomannomutase	24.91
G	Ferpe_01295	Phosphomannomutase	24.92
M	Ferpe_00511	Phospho-N-acetylmuramoyl-pentapeptide-transferase (EC	20.86
H	Ferpe_00237	Phosphopantetheine adenylyltransferase (EC	21.02
H	Ferpe_01499	phosphopantothenoylcysteine	24.11
F	Ferpe_01968	phosphoribosylamine--glycine ligase (EC	19.01
F	Ferpe_01373	Phosphoribosylaminoimidazolesuccinocarboxamide	18.33
X	Ferpe_01728	phosphoribosyl-ATP pyrophosphatase (EC	17.37
F	Ferpe_01969	phosphoribosylformylglycinamidine cyclo-ligase	18.92
F	Ferpe_01375	phosphoribosylformylglycinamidine synthase I	17.43
F	Ferpe_01376	phosphoribosylformylglycinamidine synthase II	19.71
H	Ferpe_00526	Phosphosulfolactate phosphohydrolase and related	19.43
R	Ferpe_00526	Phosphosulfolactate phosphohydrolase and related	19.43
C	Ferpe_00345	Phosphotransacetylase	26.67
C	Ferpe_02063	Phosphotransacetylase	25.78
Q	Ferpe_01066	Phytoene dehydrogenase and related proteins	18.56
N	Ferpe_00773	pilus retraction protein PilT	23.7
W	Ferpe_00773	pilus retraction protein PilT	23.7
X	Ferpe_00178	PilZ domain.	20.29
J	Ferpe_00191	polyribonucleotide nucleotidyltransferase	26.05
P	Ferpe_00767	possible tyrosine transporter P-protein (TC	18.86
P	Ferpe_00768	possible tyrosine transporter P-protein (TC	
M	Ferpe_01743	PQQ enzyme repeat.	
C	Ferpe_00150	Predicted acetamidase/formamidase	17.83
C	Ferpe_00733	Predicted acetamidase/formamidase	15.16
X	Ferpe_01240	Predicted acetyltransferase	19.31
I	Ferpe_01755	Predicted acetyltransferases and hydrolases with	15.05
R	Ferpe_00608	Predicted amidophosphoribosyltransferases	

E	Ferpe_00789	Predicted amino acid racemase	22.68
R	Ferpe_00127	Predicted ATPase (AAA+ superfamily)	
R	Ferpe_00732	Predicted ATPase (AAA+ superfamily)	19.61
R	Ferpe_00738	Predicted ATPase (AAA+ superfamily)	18.56
R	Ferpe_01525	Predicted ATPase (AAA+ superfamily)	19.12
R	Ferpe_01045	Predicted ATPase related to phosphate	22.8
J	Ferpe_01739	Predicted ATPases of PP-loop superfamily	
O	Ferpe_01974	Predicted ATP-dependent protease	23.47
R	Ferpe_00933	Predicted CoA-binding protein	18.98
C	Ferpe_00546	Predicted cobalamin binding protein	22.99
X	Ferpe_00741	Predicted cobalamin binding protein	20.09
G	Ferpe_00972	Predicted dehydrogenase	24.96
R	Ferpe_00297	Predicted dehydrogenases and related proteins	21.83
R	Ferpe_02053	Predicted dehydrogenases and related proteins	24.59
G	Ferpe_01488	predicted D-glycerate permease (TC 2.A.8.1.6)	
R	Ferpe_01488	predicted D-glycerate permease (TC 2.A.8.1.6)	
R	Ferpe_01677	Predicted dioxygenase	21.32
P	Ferpe_00873	Predicted divalent heavy-metal cations	
L	Ferpe_00061	Predicted endonuclease distantly related to	
K	Ferpe_00660	Predicted endonuclease involved in recombination	19.11
X	Ferpe_01123	Predicted enzyme of the cupin superfamily	17.29
R	Ferpe_00778	Predicted esterase of the alpha-beta hydrolase	20.23
R	Ferpe_00484	Predicted exporters of the RND superfamily	21.74
R	Ferpe_01943	Predicted exporters of the RND superfamily	22.77
R	Ferpe_00414	Predicted Fe-S oxidoreductases	23.41
R	Ferpe_01198	Predicted Fe-S oxidoreductases	22.18
J	Ferpe_00087	Predicted Fe-S-cluster redox enzyme	22.1
V	Ferpe_01724	Predicted flavin-nucleotide-binding protein	14.53
V	Ferpe_01769	Predicted flavin-nucleotide-binding protein	18.16
R	Ferpe_00421	Predicted glutamine amidotransferase	20.6
R	Ferpe_00581	Predicted glutamine amidotransferase	18.13
G	Ferpe_01715	Predicted glycosylase	21.09
G	Ferpe_01775	Predicted glycosylase	21.88
G	Ferpe_01840	Predicted glycosylase	22.71

N	Ferpe_00179	Predicted glycosyltransferase	20.4
R	Ferpe_00985	Predicted GTPase	27.25
J	Ferpe_01041	Predicted GTPases	21.22
R	Ferpe_00906	Predicted heme/steroid binding protein	
R	Ferpe_00321	Predicted hydrolase of the alpha/beta	
R	Ferpe_00819	Predicted hydrolase of the alpha/beta	19.98
R	Ferpe_00296	Predicted hydrolase of the HAD superfamily	16.83
F	Ferpe_01347	Predicted hydrolases of HD superfamily	20.52
R	Ferpe_01347	Predicted hydrolases of HD superfamily	20.52
H	Ferpe_01695	Predicted hydrolases or acyltransferases	18.91
R	Ferpe_01695	Predicted hydrolases or acyltransferases	18.91
S	Ferpe_00395	Predicted integral membrane protein	21.22
S	Ferpe_00715	Predicted integral membrane protein	
S	Ferpe_01930	Predicted integral membrane protein	
S	Ferpe_00041	Predicted membrane protein	
H	Ferpe_00094	Predicted membrane protein	20.29
X	Ferpe_00429	Predicted membrane protein	14.49
S	Ferpe_00805	Predicted membrane protein	17.15
I	Ferpe_01061	Predicted membrane protein	
S	Ferpe_01122	Predicted membrane protein	15.9
S	Ferpe_01266	Predicted membrane protein	15.77
S	Ferpe_01827	Predicted membrane protein	16.13
S	Ferpe_00513	Predicted metal-dependent enzyme	20.92
R	Ferpe_00161	Predicted metal-dependent hydrolase	
R	Ferpe_00556	Predicted metal-dependent hydrolase	14.58
R	Ferpe_01976	Predicted metal-dependent hydrolase with the	16.69
J	Ferpe_00979	Predicted metal-dependent hydrolases related to	22.59
R	Ferpe_00949	Predicted metal-dependent phosphoesterases (PHP	21.11
R	Ferpe_01007	Predicted metal-dependent phosphoesterases (PHP	17.41
O	Ferpe_01436	Predicted metal-sulfur cluster biosynthetic	
J	Ferpe_01278	Predicted N6-adenine-specific DNA methylase	18.17
T	Ferpe_00325	Predicted nucleic-acid-binding protein (contains	21.7
M	Ferpe_00076	Predicted nucleoside-diphosphate sugar	22.67
O	Ferpe_00076	Predicted nucleoside-diphosphate sugar	22.67

X	Ferpe_01332	Predicted nucleoside-diphosphate sugar	22.71
R	Ferpe_02030	Predicted nucleotidyltransferase	22.25
R	Ferpe_00147	Predicted nucleotidyltransferases	
J	Ferpe_01987	Predicted O-methyltransferase	17.68
X	Ferpe_02040	Predicted O-methyltransferase	20.85
R	Ferpe_01732	Predicted oxidoreductase	23.02
R	Ferpe_01012	Predicted oxidoreductases (related to	25.18
R	Ferpe_01197	Predicted oxidoreductases (related to	20.77
X	Ferpe_00251	Predicted peptidase	15.74
X	Ferpe_00083	Predicted periplasmic protein (DUF2233).	23.24
R	Ferpe_00085	Predicted permease	16.5
R	Ferpe_01904	Predicted permease	20.13
R	Ferpe_00802	Predicted permease (DUF2074).	21.74
M	Ferpe_00398	Predicted permeases	21.41
N	Ferpe_00398	Predicted permeases	21.41
S	Ferpe_00572	Predicted permeases	18.64
S	Ferpe_00876	Predicted permeases	
R	Ferpe_01212	Predicted permeases	
S	Ferpe_01241	Predicted permeases	13.63
J	Ferpe_00386	Predicted phosphatase homologous to the	
R	Ferpe_01972	Predicted phosphoesterase (MutT family)	21.3
R	Ferpe_00750	Predicted phosphohydrolases	
X	Ferpe_01640	Predicted phosphohydrolases	19.29
M	Ferpe_01024	Predicted phosphosugar isomerases	17.76
T	Ferpe_01470	Predicted P-loop-containing kinase	21.22
M	Ferpe_00116	Predicted pyridoxal phosphate-dependent enzyme	22.39
M	Ferpe_00240	Predicted pyridoxal phosphate-dependent enzyme	21.82
M	Ferpe_00596	Predicted pyridoxal phosphate-dependent enzyme	23.97
M	Ferpe_01104	Predicted pyridoxal phosphate-dependent enzyme	23.35
S	Ferpe_00092	Predicted ribosomal protein	17.89
R	Ferpe_00417	Predicted RNA binding protein (contains	22.54
R	Ferpe_01250	Predicted RNA-binding protein	23.54
R	Ferpe_00923	Predicted RNA-binding protein (contains KH	22.67
J	Ferpe_00823	Predicted RNA-binding protein homologous to	21.57

J	Ferpe_00868	Predicted SAM-dependent methyltransferases	22.93
H	Ferpe_00914	Predicted small molecule binding protein	18.24
K	Ferpe_00914	Predicted small molecule binding protein	18.24
R	Ferpe_02019	Predicted subunit of the Multisubunit Na+/H+	
F	Ferpe_00396	Predicted sugar kinase	20.35
F	Ferpe_00294	Predicted sugar phosphatases of the HAD	21.16
G	Ferpe_01863	Predicted sugar phosphate isomerase involved in	21.72
M	Ferpe_01863	Predicted sugar phosphate isomerase involved in	21.72
R	Ferpe_00401	Predicted thioesterase	22.21
X	Ferpe_01641	Predicted transcriptional regulator	17.58
X	Ferpe_01079	Predicted transcriptional regulator containing	20.01
K	Ferpe_01503	Predicted transcriptional regulator containing	21.88
R	Ferpe_00830	Predicted transporter component	18.94
G	Ferpe_00719	Predicted Zn-dependent hydrolases of the	15.07
G	Ferpe_00911	Predicted Zn-dependent hydrolases of the	22.71
R	Ferpe_00192	Predicted Zn-dependent peptidases	23.37
O	Ferpe_01612	Predicted Zn-dependent protease	22.15
R	Ferpe_01908	Predicted Zn-dependent proteases and their	23.66
R	Ferpe_01909	Predicted Zn-dependent proteases and their	24.83
N	Ferpe_00619	prepilin-type N-terminal cleavage/methylation	24.01
U	Ferpe_00619	prepilin-type N-terminal cleavage/methylation	24.01
W	Ferpe_00619	prepilin-type N-terminal cleavage/methylation	24.01
X	Ferpe_01224	prepilin-type N-terminal cleavage/methylation	18.38
X	Ferpe_01349	prepilin-type N-terminal cleavage/methylation	17.46
X	Ferpe_01609	prepilin-type N-terminal cleavage/methylation	15.32
J	Ferpe_01752	probable S-adenosylmethionine-dependent	21.04
M	Ferpe_01334	prolipoprotein diacylglyceryl transferase	19.8
J	Ferpe_00468	prolyl-tRNA synthetase (EC 6.1.1.15)	24.25
Q	Ferpe_01605	Propanediol utilization protein	22.05
X	Ferpe_01505	Protein of unknown function (DUF1292).	19.71
X	Ferpe_01723	Protein of unknown function (DUF1703)/Predicted	22.41
X	Ferpe_01756	Protein of unknown function (DUF3242).	20.18
L	Ferpe_01649	protein RecA	25.64
U	Ferpe_01630	protein translocase subunit secA	24.26

U	Ferpe_01175	protein translocase subunit secE/sec61 gamma	
U	Ferpe_01763	protein translocase subunit secF	24.27
U	Ferpe_01921	protein translocase subunit secG	20.48
U	Ferpe_01396	protein translocase subunit secY/sec61 alpha	22.58
U	Ferpe_01761	protein translocase subunit yajC	23.18
O	Ferpe_00271	protein-L-isoaspartate(D-aspartate)	
J	Ferpe_00337	pseudouridine synthase	20.07
J	Ferpe_00473	pseudouridylate synthase I	21.83
R	Ferpe_00889	putative enoyl-(acyl-carrier-protein) reductase	27.03
X	Ferpe_00657	Putative F0F1-ATPase subunit (ATPase_gene1).	13.89
R	Ferpe_00964	putative HD superfamily hydrolase of NAD	22.07
X	Ferpe_00290	putative iron-only hydrogenase system regulator	19.41
G	Ferpe_01014	Putative N-acetylmannosamine-6-phosphate	
H	Ferpe_01894	putative oxygen-independent coproporphyrinogen	21.22
X	Ferpe_01873	Putative regulator of cell autolysis	19.29
H	Ferpe_02024	pyridoxal phosphate synthase yaaD subunit	26.33
H	Ferpe_02023	pyridoxal phosphate synthase yaaE subunit	24.85
E	Ferpe_01259	pyridoxal-phosphate dependent TrpB-like enzyme	20.1
F	Ferpe_01440	Pyrimidine operon attenuation protein/uracil	21.97
F	Ferpe_00082	pyrimidine-nucleoside phosphorylase	25.08
O	Ferpe_01124	pyroglutamyl-peptidase I (EC:3.4.19.3). Cysteine	19.9
G	Ferpe_01273	pyruvate kinase	24.94
G	Ferpe_00884	pyruvate phosphate dikinase (EC 2.7.9.1)	29.07
C	Ferpe_02028	Pyruvate/oxaloacetate carboxyltransferase	23.35
C	Ferpe_00302	Pyruvate:ferredoxin oxidoreductase and related	19.32
C	Ferpe_00349	Pyruvate:ferredoxin oxidoreductase and related	26.84
C	Ferpe_00350	Pyruvate:ferredoxin oxidoreductase and related	26.94
C	Ferpe_00351	Pyruvate:ferredoxin oxidoreductase and related	25.77
C	Ferpe_01136	Pyruvate:ferredoxin oxidoreductase and related	24
C	Ferpe_01200	Pyruvate:ferredoxin oxidoreductase and related	27.14
C	Ferpe_01631	Pyruvate:ferredoxin oxidoreductase and related	24.75
O	Ferpe_01093	Pyruvate-formate lyase-activating enzyme	19.28
H	Ferpe_02054	queuosine biosynthesis protein QueD	
H	Ferpe_01741	quinolinate synthetase (EC 2.5.1.72)	17.69

O	Ferpe_00678	radical SAM additional 4Fe4S-binding domain	
R	Ferpe_01088	radical SAM family uncharacterized protein	21.35
S	Ferpe_00329	radical SAM-linked protein	19.11
L	Ferpe_01823	replication restart DNA helicase PriA	18.54
L	Ferpe_00238	replicative DNA helicase	22.13
X	Ferpe_00854	Response regulator consisting of a CheY-like	21.71
X	Ferpe_01882	Response regulator containing a CheY-like	22.28
X	Ferpe_01933	Response regulator containing a CheY-like	22.48
X	Ferpe_01792	Response regulator containing CheY-like	24.12
X	Ferpe_01955	Response regulator containing CheY-like	23.34
K	Ferpe_00872	response regulator receiver modulated	22.41
T	Ferpe_00872	response regulator receiver modulated	22.41
T	Ferpe_01330	response regulator receiver modulated	23.7
K	Ferpe_00080	Response regulators consisting of a CheY-like	26.06
T	Ferpe_00080	Response regulators consisting of a CheY-like	26.06
K	Ferpe_00988	Response regulators consisting of a CheY-like	19.97
T	Ferpe_00988	Response regulators consisting of a CheY-like	19.97
K	Ferpe_01799	Response regulators consisting of a CheY-like	18.71
T	Ferpe_01799	Response regulators consisting of a CheY-like	18.71
V	Ferpe_00554	Restriction endonuclease S subunits	
L	Ferpe_01529	Reverse gyrase	23.48
H	Ferpe_00197	riboflavin kinase/FMN adenylyltransferase	21.98
H	Ferpe_00798	riboflavin synthase alpha chain (EC 2.5.1.9)	
G	Ferpe_00152	ribokinase	18.99
L	Ferpe_00084	Ribonuclease HI	17.41
J	Ferpe_01247	ribonuclease P protein component (EC 3.1.26.5)	18.35
F	Ferpe_00850	ribonucleoside-diphosphate reductase class II	25.53
G	Ferpe_00155	Ribose/xylose/arabinose/galactoside ABC-type	
G	Ferpe_00994	ribose-5-phosphate isomerase (EC 5.3.1.6)	21.85
E	Ferpe_00880	ribose-phosphate pyrophosphokinase	25.38
F	Ferpe_00880	ribose-phosphate pyrophosphokinase	25.38
J	Ferpe_00899	Ribosomal protein HS6-type (S12/L30/L7a)	19.69
J	Ferpe_01414	Ribosomal protein L23	24.08
J	Ferpe_01050	ribosomal protein L32	20.21

J	Ferpe_00244	ribosomal protein L35	21.68
J	Ferpe_01604	ribosome biogenesis GTPase YqeH	22.77
D	Ferpe_00205	ribosome biogenesis GTP-binding protein	22.54
J	Ferpe_00068	ribosome recycling factor	23.68
J	Ferpe_01941	ribosome small subunit-dependent GTPase A	22.62
R	Ferpe_01884	ribosome-associated GTPase EngA	23.65
X	Ferpe_01151	Ribosome-associated heat shock protein	18.57
J	Ferpe_00644	Ribosome-associated protein Y (PSrp-1)	24.99
J	Ferpe_00201	ribosome-binding factor A	22.45
G	Ferpe_00843	Ribulose-5-phosphate 4-epimerase and related	22.2
G	Ferpe_01095	ribulose-phosphate 3-epimerase	19.9
T	Ferpe_00359	RNA-binding protein Hfq	22.08
L	Ferpe_01757	RNase HII (EC 3.1.26.4)	18.43
K	Ferpe_01360	RNAse III (EC 3.1.26.3)	18.67
K	Ferpe_00603	RNAse R (EC 3.1.-.-)	24.89
J	Ferpe_01433	RNAse Z (EC 3.1.26.11)	20.86
C	Ferpe_00465	Rubredoxin	
C	Ferpe_00520	Rubrerythrin	25.54
X	Ferpe_00488	Rubrerythrin.	21.73
J	Ferpe_01918	S-adenosylmethionine--tRNA	21.53
J	Ferpe_02061	S-adenosyl-methyltransferase MraW	22.46
C	Ferpe_01310	SagB-type dehydrogenase domain	20.03
H	Ferpe_00008	secondary thiamine-phosphate synthase enzyme	18.6
H	Ferpe_00017	secondary thiamine-phosphate synthase enzyme	
X	Ferpe_01620	Secretin and TonB N terminus short domain.	17.63
D	Ferpe_01346	septum site-determining protein MinC	20.95
D	Ferpe_02036	septum site-determining protein MinD	23.31
E	Ferpe_00517	Serine acetyltransferase	16.17
X	Ferpe_00578	Serine hydrolase (FSH1).	21.41
E	Ferpe_00706	serine O-acetyltransferase	15.16
E	Ferpe_00342	Serine-pyruvate aminotransferase/archaeal	28.22
F	Ferpe_00342	Serine-pyruvate aminotransferase/archaeal	28.22
J	Ferpe_00052	seryl-tRNA synthetase (EC 6.1.1.11)	24.35
R	Ferpe_00626	Short-chain dehydrogenases of various substrate	20.85

U	Ferpe_00921	signal recognition particle subunit FFH/SRP54	22.78
U	Ferpe_02041	signal recognition particle-docking protein FtsY	24.01
T	Ferpe_00081	Signal transduction histidine kinase	23.55
T	Ferpe_00987	Signal transduction histidine kinase	20.07
T	Ferpe_01932	Signal transduction histidine kinase	20.7
T	Ferpe_01958	Signal transduction histidine kinase	22.03
L	Ferpe_00231	single-strand binding protein	19.69
L	Ferpe_01361	Site-specific recombinase XerD	18.06
X	Ferpe_00779	S-layer homology domain.	23.91
X	Ferpe_00956	S-layer homology domain.	23.1
X	Ferpe_01133	S-layer homology domain.	26.72
X	Ferpe_01923	S-layer homology domain.	26.21
X	Ferpe_00571	small redox-active disulfide protein 2	18.63
M	Ferpe_01522	Small-conductance mechanosensitive channel	23.45
X	Ferpe_01368	Soluble lytic murein transglycosylase and	16.31
E	Ferpe_01748	spermidine synthase	25.53
E	Ferpe_00169	spermidine/putrescine ABC transporter	23.64
E	Ferpe_01698	Spermidine/putrescine-binding periplasmic	20.97
X	Ferpe_00901	SpoIID/LytB domain	15.81
O	Ferpe_00062	SsrA-binding protein	18.84
J	Ferpe_01390	SSU ribosomal protein S11P	23.36
J	Ferpe_01652	SSU ribosomal protein S12P methylthiotransferase	22.76
J	Ferpe_01391	SSU ribosomal protein S13P	23.44
J	Ferpe_01403	SSU ribosomal protein S14P	
J	Ferpe_01917	SSU ribosomal protein S15P	22.2
J	Ferpe_00922	SSU ribosomal protein S16P	22.73
J	Ferpe_01407	SSU ribosomal protein S17P	24.32
J	Ferpe_00232	SSU ribosomal protein S18P	22.37
J	Ferpe_01412	SSU ribosomal protein S19P	23.39
J	Ferpe_00364	SSU ribosomal protein S20P	22.82
J	Ferpe_01880	SSU ribosomal protein S2P	25.11
J	Ferpe_01389	SSU ribosomal protein S4P	24.88
J	Ferpe_00230	SSU ribosomal protein S6P	23.84
J	Ferpe_01420	SSU ribosomal protein S7P	25.87

J	Ferpe_01402	SSU ribosomal protein S8P	24.29
J	Ferpe_00096	SSU ribosomal protein S9P	23.13
X	Ferpe_00858	Stage II sporulation protein E (SpoIIE).	22.09
O	Ferpe_01165	Subtilisin-like serine proteases	15.13
O	Ferpe_01342	Subtilisin-like serine proteases	22.46
O	Ferpe_01354	Subtilisin-like serine proteases	17.15
G	Ferpe_01236	sugar (Glycoside-Pentoside-Hexuronide)	
G	Ferpe_00134	Sugar (pentulose and hexulose) kinases	22.9
G	Ferpe_00137	Sugar (pentulose and hexulose) kinases	22.71
G	Ferpe_01164	Sugar (pentulose and hexulose) kinases	
G	Ferpe_00642	Sugar phosphate isomerases/epimerases	21.31
G	Ferpe_00583	Sugar phosphate permease	
G	Ferpe_01783	Sugar phosphate permease	17.03
M	Ferpe_00102	Sugar transferases involved in	20.39
E	Ferpe_00435	sulfide dehydrogenase (flavoprotein) subunit	20.02
R	Ferpe_00435	sulfide dehydrogenase (flavoprotein) subunit	20.02
E	Ferpe_00675	sulfide dehydrogenase (flavoprotein) subunit	13.52
R	Ferpe_00675	sulfide dehydrogenase (flavoprotein) subunit	13.52
E	Ferpe_00723	sulfide dehydrogenase (flavoprotein) subunit	
R	Ferpe_00723	sulfide dehydrogenase (flavoprotein) subunit	
C	Ferpe_00724	sulfide dehydrogenase (flavoprotein) subunit	
H	Ferpe_00724	sulfide dehydrogenase (flavoprotein) subunit	
O	Ferpe_00827	sulfur relay protein TusB/DsrH	23.66
H	Ferpe_01606	Sulfur transfer protein involved in thiamine	
L	Ferpe_00950	Superfamily I DNA and RNA helicases	21.1
L	Ferpe_00912	Superfamily II DNA and RNA helicases	18.65
M	Ferpe_02051	Teichoic acid biosynthesis proteins	20.1
X	Ferpe_01288	Tetratricopeptide repeat.	24.57
X	Ferpe_01317	Tetratricopeptide repeat.	18.74
X	Ferpe_01998	The GLUG motif.	20.52
L	Ferpe_01010	Thermostable 8-oxoguanine DNA glycosylase	20.94
V	Ferpe_01010	Thermostable 8-oxoguanine DNA glycosylase	20.94
H	Ferpe_00695	thiamine diphosphokinase	19.83
H	Ferpe_01303	thiamine diphosphokinase	19.3

H	Ferpe_00487	thiazole biosynthesis/tRNA modification protein	23.92
J	Ferpe_00487	thiazole biosynthesis/tRNA modification protein	23.92
H	Ferpe_00640	thiazole-adenylate synthase	25.94
X	Ferpe_01429	Thioredoxin domain-containing protein	17.12
O	Ferpe_00088	Thioredoxin reductase	17.63
O	Ferpe_00870	Thioredoxin reductase	20.01
O	Ferpe_00971	Thioredoxin reductase	24.41
O	Ferpe_01185	thioredoxin-disulfide reductase	23.87
O	Ferpe_01183	Thioredoxin-related protein	18.17
E	Ferpe_00540	Threonine dehydrogenase and related Zn-dependent	24.91
R	Ferpe_00540	Threonine dehydrogenase and related Zn-dependent	24.91
E	Ferpe_01637	Threonine dehydrogenase and related Zn-dependent	26.38
R	Ferpe_01637	Threonine dehydrogenase and related Zn-dependent	26.38
J	Ferpe_00946	threonyl-tRNA synthetase (EC 6.1.1.3)	25.73
F	Ferpe_01002	thymidine kinase (EC 2.7.1.21)	22.48
F	Ferpe_00973	thymidylate kinase (EC 2.7.4.9)	21.62
F	Ferpe_01758	thymidylate synthase (FAD) (EC 2.1.1.148)	23.56
S	Ferpe_01754	TIGR00153 family protein	23.79
S	Ferpe_01439	TIGR00266 family protein	22.92
J	Ferpe_01290	TIGR00269 family protein	21.77
R	Ferpe_01325	TIGR00725 family protein	21.64
K	Ferpe_01174	transcription antitermination protein nusG	24.78
K	Ferpe_01474	transcription elongation factor GreA	20.88
K	Ferpe_00725	transcription termination factor Rho	23.83
K	Ferpe_00770	transcription termination factor Rho	23.9
K	Ferpe_01473	transcriptional regulator NrdR	21.83
G	Ferpe_00410	Transcriptional regulator/sugar kinase	23.08
K	Ferpe_00410	Transcriptional regulator/sugar kinase	23.08
G	Ferpe_00501	Transcriptional regulator/sugar kinase	22.18
K	Ferpe_00501	Transcriptional regulator/sugar kinase	22.18
G	Ferpe_01021	Transcriptional regulator/sugar kinase	15.47
K	Ferpe_01021	Transcriptional regulator/sugar kinase	15.47
K	Ferpe_00135	Transcriptional regulators	20.12
K	Ferpe_00391	Transcriptional regulators	18.46

E	Ferpe_00343	Transcriptional regulators containing a	27.19
K	Ferpe_00343	Transcriptional regulators containing a	27.19
X	Ferpe_00160	Transglutaminase-like superfamily.	16.46
G	Ferpe_01960	Transketolase	26.4
J	Ferpe_00029	translation elongation factor 2 (EF-2/EF-G)	26.4
J	Ferpe_01419	translation elongation factor EF-G	26.35
J	Ferpe_00332	translation elongation factor P	23.4
X	Ferpe_00617	translation elongation factor Ts	26.24
J	Ferpe_01418	translation elongation factor TU	29.37
J	Ferpe_00961	translation factor SUA5	20.71
J	Ferpe_01393	translation initiation factor IF-1	18.77
J	Ferpe_01572	translation initiation factor IF-2	23.71
J	Ferpe_01801	translation initiation factor IF-2	23.96
J	Ferpe_00245	translation initiation factor IF-3	24.44
X	Ferpe_00014	Transposase and inactivated derivatives	
X	Ferpe_00016	Transposase and inactivated derivatives	
X	Ferpe_00020	Transposase and inactivated derivatives	
X	Ferpe_00025	Transposase and inactivated derivatives	
X	Ferpe_00131	Transposase and inactivated derivatives	
X	Ferpe_00220	Transposase and inactivated derivatives	
X	Ferpe_00275	Transposase and inactivated derivatives	18.59
X	Ferpe_00366	Transposase and inactivated derivatives	
X	Ferpe_00381	Transposase and inactivated derivatives	
X	Ferpe_00388	Transposase and inactivated derivatives	
X	Ferpe_00403	Transposase and inactivated derivatives	
X	Ferpe_00503	Transposase and inactivated derivatives	
X	Ferpe_00519	Transposase and inactivated derivatives	
X	Ferpe_00610	Transposase and inactivated derivatives	
X	Ferpe_00685	Transposase and inactivated derivatives	14.19
X	Ferpe_00693	Transposase and inactivated derivatives	
X	Ferpe_00711	Transposase and inactivated derivatives	
X	Ferpe_00720	Transposase and inactivated derivatives	
X	Ferpe_00729	Transposase and inactivated derivatives	
X	Ferpe_00762	Transposase and inactivated derivatives	

X	Ferpe_00804	Transposase and inactivated derivatives	
X	Ferpe_00852	Transposase and inactivated derivatives	
X	Ferpe_00910	Transposase and inactivated derivatives	
X	Ferpe_01069	Transposase and inactivated derivatives	
X	Ferpe_01127	Transposase and inactivated derivatives	
X	Ferpe_01276	Transposase and inactivated derivatives	
X	Ferpe_01337	Transposase and inactivated derivatives	
X	Ferpe_01438	Transposase and inactivated derivatives	
X	Ferpe_01484	Transposase and inactivated derivatives	
X	Ferpe_01556	Transposase and inactivated derivatives	
X	Ferpe_01571	Transposase and inactivated derivatives	
X	Ferpe_01574	Transposase and inactivated derivatives	
X	Ferpe_01586	Transposase and inactivated derivatives	
X	Ferpe_01699	Transposase and inactivated derivatives	
X	Ferpe_01713	Transposase and inactivated derivatives	
X	Ferpe_01725	Transposase and inactivated derivatives	
X	Ferpe_01731	Transposase and inactivated derivatives	
X	Ferpe_01815	Transposase and inactivated derivatives	
X	Ferpe_01844	Transposase and inactivated derivatives	
X	Ferpe_01850	Transposase and inactivated derivatives	
G	Ferpe_01597	Trehalose and maltose hydrolases (possible	23.3
M	Ferpe_00502	trehalose synthase (ADP-glucose) (EC 2.4.1.245)	26.84
G	Ferpe_00277	triosephosphate isomerase (EC 5.3.1.1)	24.92
J	Ferpe_02045	tRNA	22.33
J	Ferpe_00925	tRNA (Guanine37-N(1)-) methyltransferase (EC	20.84
J	Ferpe_01283	tRNA (guanine-N(7)-)-methyltransferase (EC	21.02
J	Ferpe_00775	tRNA dimethylallyltransferase	21.78
J	Ferpe_00174	tRNA modification GTPase trmE	21.99
J	Ferpe_01431	tRNA nucleotidyltransferase/poly(A) polymerase	21.35
J	Ferpe_01264	tRNA pseudouridine synthase B (EC 4.2.1.70)	22.32
J	Ferpe_01947	tRNA(1-methyladenosine) methyltransferase and	22.61
J	Ferpe_00269	tRNA:m(5)U-54 methyltransferase	19.94
J	Ferpe_00166	tRNA-dihydrouridine synthase	20.15
J	Ferpe_01442	tRNA-guanine transglycosylase (EC 2.4.2.29)	21.73

J	Ferpe_00499	tRNA-i(6)A37 thiotransferase enzyme MiaB	22.17
J	Ferpe_01523	tryptophanyl-tRNA synthetase (EC 6.1.1.2)	22.82
V	Ferpe_00553	type I restriction system adenine methylase	
X	Ferpe_00569	Type I site-specific restriction-modification	21.57
J	Ferpe_01922	tyrosyl-tRNA synthetase (EC 6.1.1.1)	24.22
X	Ferpe_00151	UDP-3-O-[3-hydroxymyristoyl] glucosamine	19.66
M	Ferpe_01485	UDP-glucose-4-epimerase	21.17
M	Ferpe_00075	UDP-N-acetylglucosamine	23.09
M	Ferpe_00881	UDP-N-acetylglucosamine	24.71
M	Ferpe_00106	UDP-N-acetylglucosamine 2-epimerase	23.84
M	Ferpe_00129	UDP-N-acetylglucosamine 2-epimerase	22.02
M	Ferpe_00132	UDP-N-acetylglucosamine 2-epimerase	
M	Ferpe_00737	UDP-N-acetylglucosamine 2-epimerase	21.98
M	Ferpe_01896	UDP-N-acetylglucosamine-N-acetylmuramylpentapeptide	22.06
M	Ferpe_00783	UDP-N-acetylmuramate dehydrogenase (EC	22.51
M	Ferpe_01897	UDP-N-acetylmuramate--L-alanine ligase (EC	21.97
M	Ferpe_01927	UDP-N-acetylmuramoylalanine--D-glutamate ligase	23.2
M	Ferpe_00510	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	23.45
M	Ferpe_00249	UDP-N-acetylmuramyl pentapeptide	
M	Ferpe_00512	UDP-N-acetylmuramyl pentapeptide	17.07
X	Ferpe_00621	Uncharacterised protein family (UPF0153).	18.36
I	Ferpe_00459	Uncharacterized bacitracin resistance protein	
S	Ferpe_00217	Uncharacterized conserved protein	22.41
T	Ferpe_00254	Uncharacterized conserved protein	17.32
S	Ferpe_00307	Uncharacterized conserved protein	
H	Ferpe_00318	Uncharacterized conserved protein	23.53
S	Ferpe_00324	Uncharacterized conserved protein	25.25
O	Ferpe_00387	Uncharacterized conserved protein	24.59
J	Ferpe_00469	Uncharacterized conserved protein	22.91
S	Ferpe_00539	Uncharacterized conserved protein	23.04
S	Ferpe_00558	Uncharacterized conserved protein	
R	Ferpe_00643	Uncharacterized conserved protein	26.21
S	Ferpe_00833	Uncharacterized conserved protein	20.19
H	Ferpe_00887	Uncharacterized conserved protein	

H	Ferpe_00898	Uncharacterized conserved protein	21.13
S	Ferpe_00916	Uncharacterized conserved protein	20.89
S	Ferpe_00951	Uncharacterized conserved protein	19.06
S	Ferpe_00992	Uncharacterized conserved protein	19
M	Ferpe_01100	Uncharacterized conserved protein	19.76
P	Ferpe_01144	Uncharacterized conserved protein	22.24
S	Ferpe_01150	Uncharacterized conserved protein	19.55
G	Ferpe_01204	Uncharacterized conserved protein	22.34
S	Ferpe_01304	Uncharacterized conserved protein	17.8
R	Ferpe_01314	Uncharacterized conserved protein	21.33
P	Ferpe_01428	Uncharacterized conserved protein	17.8
S	Ferpe_01514	Uncharacterized conserved protein	15.66
R	Ferpe_01561	Uncharacterized conserved protein	18.17
R	Ferpe_01589	Uncharacterized conserved protein	15.27
R	Ferpe_01613	Uncharacterized conserved protein	19.96
S	Ferpe_01642	Uncharacterized conserved protein	20.17
S	Ferpe_01655	Uncharacterized conserved protein	15.38
S	Ferpe_01660	Uncharacterized conserved protein	20.99
S	Ferpe_01661	Uncharacterized conserved protein	
E	Ferpe_01663	Uncharacterized conserved protein	19.85
O	Ferpe_01872	Uncharacterized conserved protein	20.66
R	Ferpe_01901	Uncharacterized conserved protein	19.91
S	Ferpe_01949	Uncharacterized conserved protein	20.65
S	Ferpe_01970	Uncharacterized conserved protein	19.4
S	Ferpe_01980	Uncharacterized conserved protein	16.06
X	Ferpe_01981	Uncharacterized conserved protein	22.07
J	Ferpe_01982	Uncharacterized conserved protein	19.56
P	Ferpe_02009	Uncharacterized conserved protein	20.11
X	Ferpe_01064	Uncharacterized conserved protein (DUF2358).	18.13
S	Ferpe_00717	Uncharacterized conserved protein (some members	
S	Ferpe_01029	Uncharacterized conserved protein (some members	18.42
P	Ferpe_00829	Uncharacterized conserved protein involved in	22.5
S	Ferpe_01990	Uncharacterized conserved protein related to	24.69
X	Ferpe_00010	uncharacterized domain HDIG	17.1

T	Ferpe_00202	uncharacterized domain HDIG	19.79
X	Ferpe_00896	uncharacterized domain HDIG	22.11
X	Ferpe_00934	uncharacterized domain HDIG	20.94
R	Ferpe_00948	uncharacterized domain HDIG	22.34
X	Ferpe_01097	uncharacterized domain HDIG	20.57
J	Ferpe_01269	uncharacterized domain HDIG	21.96
X	Ferpe_01379	uncharacterized domain HDIG	21.78
X	Ferpe_01607	uncharacterized domain HDIG	22.31
F	Ferpe_01564	Uncharacterized enzyme involved in pigment	22.77
R	Ferpe_00955	Uncharacterized Fe-S oxidoreductase	21.7
X	Ferpe_00200	Uncharacterized flagellar protein FlaG	19.8
X	Ferpe_00432	Uncharacterized flavoproteins	22.67
C	Ferpe_00664	Uncharacterized flavoproteins	25.83
X	Ferpe_00427	Uncharacterized homolog of	19.8
R	Ferpe_01938	Uncharacterized homolog of biotin synthetase	17.87
T	Ferpe_00210	Uncharacterized homolog of PSP1	20.55
U	Ferpe_02033	Uncharacterized homolog of the cytoplasmic	15.94
P	Ferpe_01242	Uncharacterized membrane protein	19.3
O	Ferpe_01090	Uncharacterized membrane protein (homolog of	
O	Ferpe_01902	Uncharacterized membrane protein (homolog of	17.19
I	Ferpe_00559	Uncharacterized NAD(FAD)-dependent	22.04
I	Ferpe_00639	Uncharacterized NAD(FAD)-dependent	23.78
I	Ferpe_00703	Uncharacterized NAD(FAD)-dependent	22.63
S	Ferpe_00042	Uncharacterized protein conserved in bacteria	16.24
S	Ferpe_00054	Uncharacterized protein conserved in bacteria	22.53
N	Ferpe_00058	Uncharacterized protein conserved in bacteria	
R	Ferpe_00138	Uncharacterized protein conserved in bacteria	19.1
C	Ferpe_00139	Uncharacterized protein conserved in bacteria	23.46
S	Ferpe_00333	Uncharacterized protein conserved in bacteria	20.99
S	Ferpe_00358	Uncharacterized protein conserved in bacteria	19.72
X	Ferpe_00444	Uncharacterized protein conserved in bacteria	18.69
R	Ferpe_00457	Uncharacterized protein conserved in bacteria	18.5
S	Ferpe_00460	Uncharacterized protein conserved in bacteria	16.8
S	Ferpe_00515	Uncharacterized protein conserved in bacteria	23.02

S	Ferpe_00747	Uncharacterized protein conserved in bacteria	17.23
S	Ferpe_00806	Uncharacterized protein conserved in bacteria	18.01
S	Ferpe_00807	Uncharacterized protein conserved in bacteria	
S	Ferpe_00903	Uncharacterized protein conserved in bacteria	22.27
S	Ferpe_00904	Uncharacterized protein conserved in bacteria	23.28
S	Ferpe_00917	Uncharacterized protein conserved in bacteria	18.25
S	Ferpe_00926	Uncharacterized protein conserved in bacteria	23.22
J	Ferpe_00936	Uncharacterized protein conserved in bacteria	20.49
S	Ferpe_00963	Uncharacterized protein conserved in bacteria	18.86
J	Ferpe_00969	Uncharacterized protein conserved in bacteria	18.51
S	Ferpe_01019	Uncharacterized protein conserved in bacteria	20.46
R	Ferpe_01091	Uncharacterized protein conserved in bacteria	21.77
S	Ferpe_01170	Uncharacterized protein conserved in bacteria	16.04
S	Ferpe_01205	Uncharacterized protein conserved in bacteria	21.51
S	Ferpe_01257	Uncharacterized protein conserved in bacteria	24.76
X	Ferpe_01262	Uncharacterized protein conserved in bacteria	21.95
R	Ferpe_01364	Uncharacterized protein conserved in bacteria	
R	Ferpe_01367	Uncharacterized protein conserved in bacteria	
R	Ferpe_01588	Uncharacterized protein conserved in bacteria	
O	Ferpe_01648	Uncharacterized protein conserved in bacteria	20.5
X	Ferpe_01788	Uncharacterized protein conserved in bacteria	24.49
M	Ferpe_01962	Uncharacterized protein conserved in bacteria	23.5
S	Ferpe_02042	Uncharacterized protein conserved in bacteria	19.79
S	Ferpe_02043	Uncharacterized protein conserved in bacteria	22.1
M	Ferpe_00101	Uncharacterized protein involved in	24.92
P	Ferpe_00828	Uncharacterized protein involved in the	21.76
S	Ferpe_00970	Uncharacterized protein with conserved CXXC	20.47
R	Ferpe_00981	Uncharacterized proteins of the AP superfamily	18.33
R	Ferpe_00790	uncharacterized radical SAM protein YgiQ	20.81
S	Ferpe_01961	Uncharacterized stress-induced protein	23.32
I	Ferpe_00069	undecaprenyl diphosphate synthase	21.07
J	Ferpe_01489	universal bacterial protein YeaZ	21.86
F	Ferpe_00726	uracil phosphoribosyltransferase (EC 2.4.2.9)	20.89
F	Ferpe_00771	uracil phosphoribosyltransferase (EC 2.4.2.9)	22.88

F	Ferpe_01370	uracil-xanthine permease	
F	Ferpe_00373	Uridine kinase	24.58
F	Ferpe_00500	uridine kinase (EC 2.7.1.48)	22.59
F	Ferpe_01042	uridylate kinase (EC 2.7.4.22)	23.71
E	Ferpe_01855	urocanate hydratase (EC 4.2.1.49)	26.31
X	Ferpe_01734	UvrD/REP helicase.	21.07
C	Ferpe_01764	vacuolar-type H(+)-translocating pyrophosphatase	21.65
J	Ferpe_00756	valyl-tRNA synthetase (EC 6.1.1.9)	26.08
E	Ferpe_00658	Xaa-Pro aminopeptidase	23.77
X	Ferpe_00600	Zc3h12a-like Ribonuclease domain.	18.41
E	Ferpe_00864	Zn-dependent carboxypeptidase	23.82
O	Ferpe_01688	Zn-dependent protease with chaperone function	20.5

A.5. Discussion

A.5.1. 9078 and DYC Genome Comparison

The 9078 and DYC genome comparison shows that 9078 and DYC are highly related strains of the same species. They both contain areas that are highly syntenic and regions which are unique, likely due to the chimeric nature of Thermotgales. This allowed for quite different gene complements between the organisms even for genes in core metabolism such as the number of gene copies in the pentose phosphate KEGG map unique to 9078, transketolases (Ferpe_1232, Ferpe_1233), and ribokinase (Ferpe_0152). Difference in pentose phosphate gene numbers could cause the differences we see on end-product production on xylose between the two strains. While lactate production was not observed in our strains under our conditions we also noted that only 9078 appears to have a lactate dehydrogenase (Ferpe_0133); again, this is a gene on a long contiguous region missing from DYC. Overall, 9078 and DYC appear to be quite similar yet distinct strains of the same species.

A.5.2. Gluconate metabolism

Typically gluconate has not been used as a growth substrate in Fervidobacterium, but Entner-Doudoroff activity has been demonstrated in other Thermotogales (Kengen *et al.* 1994, Cappelletti *et al.* 2014). While only 9078 was able to grow on gluconate, its growth was poor relative to its growth on other sugars. This suggests that the Entner-Doudoroff pathway is utilized to only a very limited

degree in 9078 under our conditions. As the end-products of gluconate growth were not more reduced, as would be expected with a more reduced sugar, it is likely that YE metabolism was used to accept the extra electrons from gluconate. This would also explain why 9078 exhibited poor growth as the YE was present in only very small amounts. The ability to grow on gluconate may be a good substrate to compare *Fervidobacterium sp.* on and has not been done previously in the literature.

A.5.3. Major end-products

9078 and DYC appear to have a rather typical mechanism for acetate fermentation, and pathways necessary for hydrogen production. They also may conserve energy through the use of a sodium gradient and the RNF system as many sodium coupled genes were observed in their genomes.

Pyroglutamate production has been reported in other thermophiles such as *C. thermocellum* (Holwerda *et al.* 2014). Glutamate has not been previously described as a major end-product of sugar metabolism in the genus *Fervidobacterium pennivorans*. Typically, glutamate links carbon and nitrogen metabolism in hyperthermophiles (Robb *et al.* 1992). We are unsure about the pathways involved in glutamate production for our strains.

Alanine production is well documented in hyperthermophiles as well as in the present genus (Cappelletti *et al.* 2014). There are very few sequences of confirmed alanine aminotransferases within hyperthermophiles. Given this, it was challenging to make judgement on the quality of the annotation. There are other alanine forming

proteins such as an archaea type serine-pyruvate aminotransferase (SPAT) in both DYC and 9078 that could also be involved in the creation of alanine. Conversely SPAT could be involved in serine fermentation, as a key aspect of this organism's physiology is protein degradation (Friedrich *et al.* 1996).

A.5.4. Lack of C1's and H_2

While our carbon and O/R balances were found to be in acceptable ranges in most of our conditions, the pathways built from the genome could not account for the stoichiometry we see in end-product production. The extreme example of this is 9078's growth on xylose which appears to be homoacetogenic and as such no C1's appear to be produced. Strain 9078 and DYC both appear to have a fully functioning and typical pentose phosphate pathway (although the number of gene copies differs considerably) and are able to produce glyceraldehyde 3-p for fermentation through glycolysis to pyruvate. However, production of acetate via POR should result in an excess of 1 CO_2 and 2 $ferridoxin_{red}$ equivalents therefore, CO_2 must be consumed or not produced at all as we do not detect any significant amounts under xylose growth when the only major product is acetate.

F. pennivorans does not appear to have the gene complement for a complete Wood-Ljungdahl pathway lacking formate hydrogen lyase (FHL), pyruvate formate lyase (PFL), and carbon monoxide dehydrogenase (CMD). Based on the annotation it should not be able to produce formate for methylate tetrahydrofolate (THF), or uptake CO_2 directly using the Wood-Ljungdahl pathway. This is an apparent contradiction as

the related *Thermotoga lettingae*, which also appears to lacks FHL, PFL, and CMD, upon genome analysis can grow on H_2, CO_2, and elemental sulfur. It is possible that CO_2 uptake might be through another pathway such as the glycine cleavage system, a shared feature among many Thermotogales. Zhang *et al.* (2002) propose the reverse TCA as the mechanism of carbon uptake in *Thermotoga maritima*, our organism does not appear to possess a complete TCA cycle based on annotation. Regardless of the annotation *F. pennivorans* requires some mechanism of converting CO_2 and excess electrons into acetate to explain our C1 to C2 ratio that is not 1:1. Other Thermotogales have been shown to be homo alanine producers using methanol when thiosulfate or elemental sulfate is present (Balk *et al.* 2002). C1 uptake pathways and alanine production have a pertinent connection. Serine is an intermediate of C1 uptake when glycine is methylated. Serine pyruvate ammonia transferase (SPAT), a shared feature of Thermotogales, could utilize serine and pyruvate to create alanine and 2-hydroxypyruvate which could then be cycled back to pyruvate. This would be a relatively straight forward mechanism for homoalanine through C1 metabolism seen in other Thermotogales and causes us to consider the possible role of SPAT in alanine creation under our conditions.

A.6. Conclusion

The *Fervidobacterium* genus, and the order *Thermotogales* in general, remains understudied especially where core metabolism is concerned. The isolation of two unique strains of *F. pennivorans* represents an opportunity to elucidate the inner workings of *Fervidobacterium* via side by side experiments interpreted through the lens of a genomic comparison. Our genetic comparison predicted DYC's inability to grow on gluconate, a feature confirmed via a physiological experiment. Using KEGG pathways and Metecyc we were able to model pathways for major end-products acetate, CO_2, and H_2. The conventional models for alanine and glutamate formation are less than satisfactory due to poor sequence identity of AAT and a missing SDH/FR respectively. The atypical fermentation profile (C1 and C2 ratio that is not 1:1) of *F. pennivorans* under homoacteogenic conditions causes us to look for a C1 uptake pathway in our genome, the complete Wood-Ljungdahl pathway appears to be absent based on annotation. There does appear to be other mechanisms of CO_2 uptake, though we are unable to create a satisfactory pathway.

While *F. pennivorans* does not appear to be an ideal candidate for biofuel production under our conditions, being able to understand and manipulate fermentation in hyperthermophilic organisms is a worthy goal and potential path to create new process of biofuel production. We were unsuccessful in elucidating satisfactory core metabolic pathways in our species, showing that there is still work to be done in regards to genome annotation and physiological experiments.

Appendix B: The Designer Co-culture Grown Aerobically on Cellulose Overlay Plates

Figure B.1. The designer co-culture grown aerobically on cellulose overlay plates

Appendix C: *C. debilis* Locus-Tags and Their Corresponding Log_2tic Expression Values Under Aerobic and Anaerobic Conditions Converted to Zmag

Table C.1. *C. debilis* Locus-tags and their corresponding log_2TIC expression values under aerobic and anaerobic conditions converted to Zmag

R0, R1 = Inter-replicate differences; Z0, Z1 = Replicates comparing different states; Z0net, Z1net = normalized Z0, Z1; Zmag = combined vector of Z0net and Z1net.

			Log_2(TIC)														
COG	locus	locus-description	anaerobic	anaerobic	aerobic	aerobic	Average	Z0	Z1	R0	R1	Z0net	Z1net	Zmag	R0net	R1net	WSTAT
			-114	-115	-116	-117	log_2TIC										
M	Cdeb_02028	LysM repeat	6.66	7.35	8.97	11.03	8.50	-2.31	-3.68	-0.69	-2.06	1.72	4.93	2.91	-3.36	-6.57	1.54
X	Cdeb_01222	Putative xylanase/chitin deacetylase	6.99	7.45	7.71	5.64	6.95	-0.72	1.81	-0.46	2.07	-1.02	-2.49	-1.59	-1.27	2.41	2.15
G	Cdeb_01721	Beta-glucosidase-related glycosidase	7.12	7.54	8.34	7.17	7.54	-1.22	0.37	-0.42	1.17	-0.16	-0.54	-0.29	-0.91	0.46	1.2
X	Cdeb_00864	stage IV sporulation protein A	7.14	7.60	9.07	11.25	8.77	-1.93	-3.65	-0.46	-2.18	1.07	4.89	2.29	-1.27	-6.83	1.57
F	Cdeb_02933	Adenylate kinase	7.61	7.95	9.59	8.60	8.44	-1.98	-0.65	-0.34	0.99	1.16	0.84	0.99	-0.18	0.07	16.12
H	Cdeb_03058	dihydropteroate synthase	7.80	8.25	9.11	7.32	8.12	-1.31	0.93	-0.45	1.79	0.00	-1.3	0.00	-1.18	1.8	1.31
G	Cdeb_00133	Maltose-binding periplasmic proteins/domain	7.81	8.16	10.46	10.08	9.13	-2.65	-1.92	-0.35	0.38	2.31	2.55	2.43	-0.27	-1.26	5.8
X	Cdeb_01288	LysM domain	7.95	8.38	9.04	8.18	8.39	-1.09	0.20	-0.43	0.86	-0.38	-0.31	-0.34	-1	-0.22	1.04
I	Cdeb_00490	acetyl-CoA acetyltransferase	7.96	8.46	9.79	8.72	8.73	-1.83	-0.26	-0.50	1.07	0.90	0.31	0.53	-1.64	0.24	1.25
E	Cdeb_01867	ABC-type dipeptide/oligopeptide/nickel transport	8.11	8.40	9.50	8.94	8.74	-1.39	-0.54	-0.29	0.56	0.14	0.69	0.31	0.27	-0.87	1.68
P	Cdeb_01867	ABC-type dipeptide/oligopeptide/nickel transport	8.11	8.40	9.50	8.94	8.74	-1.39	-0.54	-0.29	0.56	0.14	0.69	0.31	0.27	-0.87	1.68
L	Cdeb_01326	Topoisomerase IA	8.16	8.57	10.04	9.30	9.02	-1.88	-0.73	-0.41	0.74	0.98	0.95	0.96	-0.82	-0.48	3.12
E	Cdeb_00315	cysteine desulfurase	8.23	8.54	10.41	9.22	9.10	-2.18	-0.68	-0.31	1.19	1.50	0.88	1.15	0.09	0.5	7.44
H	Cdeb_03373	Delta-aminolevulinic acid dehydratase	8.29	8.61	10.97	9.26	9.28	-2.68	-0.65	-0.32	1.71	2.36	0.84	1.41	0	1.63	3.34
S	Cdeb_01750	Putative membrane protein	8.31	8.44	9.96	9.29	9.00	-1.65	-0.85	-0.13	0.67	0.59	1.11	0.81	1.73	-0.63	1.48
R	Cdeb_01757	MoxR-like ATPase	8.34	8.70	9.99	9.01	9.01	-1.65	-0.31	-0.36	0.98	0.59	0.38	0.47	-0.36	0.04	4.21
R	Cdeb_02051	nucleoside ABC transporter membrane protein	8.34	8.74	10.62	8.97	9.17	-2.28	-0.23	-0.40	1.65	1.67	0.27	0.67	-0.73	1.5	2.2
E	Cdeb_00291	L-proline dehydrogenase	8.34	8.70	10.39	9.31	9.19	-2.05	-0.61	-0.36	1.08	1.28	0.78	1.00	-0.36	0.26	7.34
N	Cdeb_02107	flagellar motor switch protein FliN	8.34	8.86	9.90	9.00	9.03	-1.56	-0.14	-0.52	0.90	0.43	0.15	0.25	-1.82	-0.13	0.54
T	Cdeb_02107	flagellar motor switch protein FliN	8.34	8.86	9.90	9.00	9.03	-1.56	-0.14	-0.52	0.90	0.43	0.15	0.25	-1.82	-0.13	0.54
C	Cdeb_02462	Heme/copper-type cytochrome/quinol oxidase,	8.40	8.99	11.93	11.72	10.26	-3.53	-2.73	-0.59	0.21	3.83	3.65	3.74	-2.45	-1.63	3.91

R	Cdeb_02618	beta-phosphoglucomutase	8.41	8.74	10.21	9.57	9.23	-1.80	-0.83	-0.33	0.64	0.84	1.08	0.95	-0.09	-0.7	4.21
X	Cdeb_00622	Protein of unknown function (DUF4230)	8.42	8.66	10.33	8.68	9.02	-1.91	-0.02	-0.24	1.65	1.03	-0.01	0.10	0.73	1.5	1.34
C	Cdeb_00920	Heme/copper-type cytochrome/quinol oxidase,	8.44	9.68	12.58	11.69	10.60	-4.14	-2.01	-1.24	0.89	4.88	2.68	3.62	-8.36	-0.15	1.45
X	Cdeb_00494	Acyl-CoA dehydrogenase	8.47	8.95	10.47	9.22	9.28	-2.00	-0.27	-0.48	1.25	1.19	0.32	0.62	-1.45	0.63	1.69
X	Cdeb_00471	transcription factor, RsfA family	8.47	8.65	10.65	11.74	9.88	-2.18	-3.09	-0.18	-1.09	1.50	4.14	2.49	1.27	-4.46	2.06
E	Cdeb_03014	ornithine aminotransferase	8.51	8.85	10.72	8.54	9.16	-2.21	0.31	-0.34	2.18	1.55	-0.46	0.84	-0.18	2.65	1.32
X	Cdeb_00867	Tryptophan RNA-binding attenuator protein	8.51	8.86	9.85	8.79	9.00	-1.34	0.07	-0.35	1.06	0.05	-0.14	-0.08	-0.27	0.22	0.93
U	Cdeb_02016	pilus retraction protein PilT	8.52	8.87	10.91	10.15	9.61	-2.39	-1.28	-0.35	0.76	1.86	1.69	1.77	-0.27	-0.43	10.76
K	Cdeb_02869	Response regulators consisting of a CheY-like	8.52	8.81	10.95	10.35	9.66	-2.43	-1.54	-0.29	0.60	1.93	2.04	1.98	0.27	-0.78	7.4
N	Cdeb_02016	pilus retraction protein PilT	8.52	8.87	10.91	10.15	9.61	-2.39	-1.28	-0.35	0.76	1.86	1.69	1.77	-0.27	-0.43	10.76
T	Cdeb_02869	Response regulators consisting of a CheY-like	8.52	8.81	10.95	10.35	9.66	-2.43	-1.54	-0.29	0.60	1.93	2.04	1.98	0.27	-0.78	7.4
P	Cdeb_00740	Rhodanese-related sulfurtransferase	8.53	9.07	9.43	8.21	8.81	-0.90	0.86	-0.54	1.22	-0.71	-1.2	-0.92	-2	0.57	1.46
S	Cdeb_00154	Uncharacterized conserved protein	8.56	8.87	10.09	9.19	9.18	-1.53	-0.32	-0.31	0.90	0.38	0.39	0.38	0.09	-0.13	7.49
O	Cdeb_02458	Uncharacterized protein required for cytochrome	8.56	8.95	11.45	11.07	10.01	-2.89	-2.12	-0.39	0.38	2.72	2.82	2.77	-0.64	-1.26	6.03
R	Cdeb_02337	Putative CoA-binding protein	8.57	8.89	10.22	9.96	9.41	-1.65	-1.07	-0.32	0.26	0.59	1.41	0.91	0	-1.52	2.19
E	Cdeb_02061	aspartate kinase	8.57	8.91	10.71	10.54	9.68	-2.14	-1.63	-0.34	0.17	1.43	2.16	1.76	-0.18	-1.72	3.26
E	Cdeb_02184	3-deoxy-D-arabinoheptulosonate-7-phosphate	8.58	8.99	9.75	8.62	8.99	-1.17	0.37	-0.41	1.13	-0.24	-0.54	-0.36	-0.82	0.37	1.43
H	Cdeb_01536	ferrochelatase	8.59	8.84	10.42	9.11	9.24	-1.83	-0.27	-0.25	1.31	0.90	0.32	0.54	0.64	0.76	2.09
X	Cdeb_02090	hypothetical protein	8.59	8.76	9.86	10.01	9.31	-1.27	-1.25	-0.17	-0.15	-0.07	1.65	0.34	1.36	-2.41	1.3
R	Cdeb_00443	Zn-dependent hydrolase, including glyoxylase	8.61	9.16	9.59	8.50	8.97	-0.98	0.66	-0.55	1.09	-0.57	-0.93	-0.73	-2.09	0.28	1.12
M	Cdeb_03198	glucosamine--fructose-6-phosphate	8.62	9.14	10.48	9.21	9.36	-1.86	-0.07	-0.52	1.27	0.95	0.05	0.22	-1.82	0.67	1.07
S	Cdeb_02516	Uncharacterized protein conserved in bacteria	8.63	8.97	10.31	9.31	9.31	-1.68	-0.34	-0.34	1.00	0.64	0.42	0.52	-0.18	0.09	8.27
C	Cdeb_02438	pyruvate dehydrogenase E1 component, alpha	8.63	9.09	11.71	9.25	9.67	-3.08	-0.16	-0.46	2.46	3.05	0.18	0.74	-1.27	3.26	1.9
I	Cdeb_02151	malonyl CoA-acyl carrier protein transacylase	8.63	8.97	10.19	9.29	9.27	-1.56	-0.32	-0.34	0.90	0.43	0.39	0.41	-0.18	-0.13	5.68
X	Cdeb_02466	YugN-like family	8.64	8.90	10.45	9.07	9.27	-1.81	-0.17	-0.26	1.38	0.86	0.19	0.40	0.55	0.91	1.8
U	Cdeb_02934	protein translocase subunit secY/sec61 alpha	8.67	8.97	10.46	8.97	9.27	-1.79	0.00	-0.30	1.49	0.83	-0.04	0.18	0.18	1.15	1.55
E	Cdeb_01923	shikimate dehydrogenase	8.70	9.11	9.78	8.82	9.10	-1.08	0.29	-0.41	0.96	-0.40	-0.43	-0.41	-0.82	0	1.56
X	Cdeb_02896	Cell wall-associated hydrolases	8.70	8.88	9.40	8.07	8.76	-0.70	0.81	-0.18	1.33	-1.05	-1.14	-1.09	1.27	0.8	2.24
E	Cdeb_02647	spermidine/putrescine ABC transporter	8.71	9.29	9.84	9.05	9.22	-1.13	0.24	-0.58	0.79	-0.31	-0.36	-0.33	-2.36	-0.37	0.43
C	Cdeb_01590	Zn-dependent alcohol dehydrogenase, class III	8.72	9.12	10.08	9.19	9.28	-1.36	-0.07	-0.40	0.89	0.09	0.05	0.07	-0.73	-0.15	0.3
P	Cdeb_00388	bacillibactin-binding protein	8.72	9.14	10.90	11.09	9.96	-2.18	-1.95	-0.42	-0.19	1.50	2.59	1.97	-0.91	-2.5	2.45
I	Cdeb_02712	Phosphatidylglycerophosphatase A	8.72	9.07	9.76	8.33	8.97	-1.04	0.74	-0.35	1.43	-0.47	-1.04	-0.70	-0.27	1.02	2.35
L	Cdeb_01967	ATPase related to the helicase subunit of the	8.74	9.20	9.80	8.19	8.98	-1.06	1.01	-0.46	1.61	-0.43	-1.41	-0.78	-1.27	1.41	1.69

I	Cdeb_03088	4-diphosphocytidyl-2-C-methyl-D-erythritol	8.75	9.09	10.29	8.97	9.28	-1.54	0.12	-0.34	1.32	0.40	-0.2	0.28	-0.18	0.78	1.21
F	Cdeb_01975	adenine phosphoribosyltransferase	8.77	9.21	10.03	8.97	9.25	-1.26	0.24	-0.44	1.06	-0.09	-0.36	-0.18	-1.09	0.22	0.73
S	Cdeb_00180	EDD domain protein, DegV family	8.77	9.30	10.06	9.40	9.38	-1.29	-0.10	-0.53	0.66	-0.03	0.09	0.05	-1.91	-0.65	0.1
I	Cdeb_01405	Acyl-coenzyme A synthetase/AMP-(fatty) acid	8.77	8.95	10.27	9.45	9.36	-1.50	-0.50	-0.18	0.82	0.33	0.64	0.46	1.27	-0.3	1.2
G	Cdeb_00508	fructose-1,6-bisphosphatase, class II	8.78	9.21	10.21	9.59	9.45	-1.43	-0.38	-0.43	0.62	0.21	0.47	0.31	-1	-0.74	0.9
X	Cdeb_00693	putative periplasmic solute-binding protein	8.81	9.29	9.43	8.87	9.10	-0.62	0.42	-0.48	0.56	-1.19	-0.61	-0.85	-1.45	-0.87	1.72
C	Cdeb_02460	cytochrome c oxidase, subunit II	8.82	9.39	11.28	10.52	10.00	-2.46	-1.13	-0.57	0.76	1.98	1.49	1.72	-2.27	-0.43	2.33
L	Cdeb_01976	exonuclease RecJ	8.83	9.07	10.69	9.82	9.60	-1.86	-0.75	-0.24	0.87	0.95	0.97	0.96	0.73	-0.2	3.9
Q	Cdeb_02761	Acyl-CoA synthetases (AMP-forming)/AMP-acid	8.83	9.20	11.12	9.88	9.76	-2.29	-0.68	-0.37	1.24	1.69	0.88	1.22	-0.45	0.61	5.46
I	Cdeb_02761	Acyl-CoA synthetases (AMP-forming)/AMP-acid	8.83	9.20	11.12	9.88	9.76	-2.29	-0.68	-0.37	1.24	1.69	0.88	1.22	-0.45	0.61	5.46
J	Cdeb_00229	bacterial peptide chain release factor 2 (bRF-2)	8.84	9.19	9.69	10.20	9.48	-0.85	-1.01	-0.35	-0.51	-0.79	1.32	1.02	-0.27	-3.2	1.04
C	Cdeb_02777	succinate dehydrogenase subunit B	8.84	9.20	12.31	11.78	10.53	-3.47	-2.58	-0.36	0.53	3.72	3.45	3.58	-0.36	-0.93	11.06
S	Cdeb_02491	mraZ protein	8.85	9.32	10.08	8.90	9.29	-1.23	0.42	-0.47	1.18	-0.14	-0.61	-0.29	-1.36	0.48	0.94
R	Cdeb_03259	Protein involved in sex pheromone biosynthesis	8.87	9.19	10.49	9.44	9.50	-1.62	-0.25	-0.32	1.05	0.53	0.3	0.40	0	0.2	6.62
L	Cdeb_02891	replicative DNA helicase loader DnaB	8.87	9.09	10.06	9.33	9.34	-1.19	-0.24	-0.22	0.73	-0.21	0.28	0.24	0.91	-0.5	0.73
C	Cdeb_01033	cytochrome bd-I ubiquinol oxidase subunit 1	8.88	9.29	11.74	10.75	10.17	-2.86	-1.46	-0.41	0.99	2.67	1.93	2.27	-0.82	0.07	8.7
S	Cdeb_02506	Uncharacterized protein conserved in bacteria	8.89	9.34	10.47	10.29	9.75	-1.58	-0.95	-0.45	0.18	0.47	1.24	0.76	-1.18	-1.7	1.39
V	Cdeb_02378	ABC-type multidrug transport system, ATPase and	8.89	9.21	10.07	9.29	9.37	-1.18	-0.08	-0.32	0.78	-0.22	0.07	-0.12	0	-0.39	1.29
O	Cdeb_01637	pyruvate formate-lyase 1-activating enzyme	8.89	9.25	10.32	8.95	9.35	-1.43	0.30	-0.36	1.37	0.21	-0.45	-0.31	-0.36	0.89	1.12
E	Cdeb_00874	3-dehydroquinate synthase	8.91	9.41	10.21	8.91	9.36	-1.30	0.50	-0.50	1.30	-0.02	-0.72	-0.12	-1.64	0.74	0.87
S	Cdeb_01929	Uncharacterized conserved protein	8.91	9.17	10.25	9.34	9.42	-1.34	-0.17	-0.26	0.91	0.05	0.19	0.10	0.55	-0.11	0.76
X	Cdeb_00981	Fructosamine-3-kinase	8.92	9.34	10.25	9.67	9.55	-1.33	-0.33	-0.42	0.58	0.03	0.41	0.11	-0.91	-0.83	0.73
K	Cdeb_00355	ParB-like partition protein	8.93	9.38	11.29	11.03	10.16	-2.36	-1.65	-0.45	0.26	1.81	2.19	1.99	-1.18	-1.52	3.21
J	Cdeb_00360	GTP-binding protein YchF	8.93	9.26	9.35	8.45	9.00	-0.42	0.81	-0.33	0.90	-1.53	-1.14	-1.32	-0.09	-0.13	26.23
X	Cdeb_02884	Putative cell wall binding repeat 2	8.93	9.45	11.65	10.56	10.15	-2.72	-1.11	-0.52	1.09	2.43	1.46	1.88	-1.82	0.28	3.35
D	Cdeb_02147	condensin subunit Smc	8.94	9.05	10.40	9.38	9.44	-1.46	-0.33	-0.11	1.02	0.26	0.41	0.33	1.91	0.13	0.55
C	Cdeb_00776	dihydrolipoamide dehydrogenase	8.98	9.36	10.74	10.17	9.81	-1.76	-0.81	-0.38	0.57	0.78	1.05	0.90	-0.55	-0.85	2.81
C	Cdeb_02130	succinyl-CoA synthetase (ADP-forming) alpha	8.99	9.51	12.38	11.84	10.68	-3.39	-2.33	-0.52	0.54	3.59	3.11	3.34	-1.82	-0.91	5.07
R	Cdeb_02430	N-acetyldiaminopimelate deacetylase	9.00	9.52	9.94	8.56	9.26	-0.94	0.96	-0.52	1.38	-0.64	-1.34	-0.93	-1.82	0.91	1.59
C	Cdeb_00675	Cytochrome c, mono- and diheme variant	9.01	9.60	11.74	9.70	10.01	-2.73	-0.10	-0.59	2.04	2.45	0.09	0.47	-2.45	2.35	1.57
X	Cdeb_01687	putative NAD(FAD)-dependent dehydrogenase	9.01	9.52	10.31	8.48	9.33	-1.30	1.04	-0.51	1.83	-0.02	-1.45	-0.17	-1.73	1.89	1.23
E	Cdeb_00463	spermidine synthase	9.02	9.17	9.90	8.48	9.14	-0.88	0.69	-0.15	1.42	-0.74	-0.97	-0.85	1.55	1	1.44
M	Cdeb_00947	D-alanyl-D-alanine carboxypeptidase	9.02	9.35	10.73	9.67	9.69	-1.71	-0.32	-0.33	1.06	0.69	0.39	0.52	-0.09	0.22	7.25

R	Cdeb_01774	AT-rich DNA-binding protein	9.03	9.52	10.40	9.57	9.63	-1.37	-0.05	-0.49	0.83	0.10	0.03	0.05	-1.55	-0.28	0.14
L	Cdeb_01986	Holliday junction DNA helicase subunit RuvB	9.04	9.32	10.65	9.75	9.69	-1.61	-0.43	-0.28	0.90	0.52	0.54	0.53	0.36	-0.13	4.26
M	Cdeb_02082	RIP metalloprotease RseP	9.04	9.37	10.05	9.18	9.41	-1.01	0.19	-0.33	0.87	-0.52	-0.3	-0.39	-0.09	-0.2	5.95
G	Cdeb_03133	putative sugar kinase	9.06	9.53	10.40	9.07	9.52	-1.34	0.46	-0.47	1.33	0.05	-0.66	-0.18	-1.36	0.8	0.91
L	Cdeb_00761	Exodeoxyribonuclease VII large subunit	9.06	9.28	10.92	10.23	9.87	-1.86	-0.95	-0.22	0.69	0.95	1.24	1.09	0.91	-0.59	3.13
N	Cdeb_00518	Methyl-accepting chemotaxis protein	9.06	9.32	11.74	11.43	10.39	-2.68	-2.11	-0.26	0.31	2.36	2.81	2.58	0.55	-1.41	5.27
T	Cdeb_00518	Methyl-accepting chemotaxis protein	9.06	9.32	11.74	11.43	10.39	-2.68	-2.11	-0.26	0.31	2.36	2.81	2.58	0.55	-1.41	5.27
S	Cdeb_00957	bacillopeptidase F	9.06	9.26	8.86	8.31	8.87	0.20	0.95	-0.20	0.55	-2.60	-1.32	-1.85	1.09	-0.89	4.5
G	Cdeb_02243	transcriptional regulator, DeoR family	9.07	9.41	11.49	9.53	9.88	-2.42	-0.12	-0.34	1.96	1.91	0.12	0.48	-0.18	2.17	1.91
K	Cdeb_00150	transcriptional regulator, RpiR family	9.07	9.33	10.88	10.09	9.84	-1.81	-0.76	-0.26	0.79	0.86	0.99	0.92	0.55	-0.37	4.3
K	Cdeb_02243	transcriptional regulator, DeoR family	9.07	9.41	11.49	9.53	9.88	-2.42	-0.12	-0.34	1.96	1.91	0.12	0.48	-0.18	2.17	1.91
L	Cdeb_00367	primary replicative DNA helicase	9.07	9.32	10.51	9.75	9.66	-1.44	-0.43	-0.25	0.76	0.22	0.54	0.34	0.64	-0.43	1.64
S	Cdeb_02399	Putative membrane protein	9.12	9.40	9.92	8.39	9.21	-0.80	1.01	-0.28	1.53	-0.88	-1.41	-1.11	0.36	1.24	2.8
X	Cdeb_01217	hypothetical protein	9.17	9.12	10.52	9.47	9.57	-1.35	-0.35	0.05	1.05	0.07	0.43	0.17	3.36	0.2	0.28
V	Cdeb_01026	ABC-type multidrug transport system, ATPase and	9.18	9.49	11.31	10.43	10.10	-2.13	-0.94	-0.31	0.88	1.41	1.23	1.32	0.09	-0.17	21.15
E	Cdeb_03205	L-serine dehydratase, iron-sulfur-dependent,	9.19	9.59	10.09	8.36	9.31	-0.90	1.23	-0.40	1.73	-0.71	-1.7	-1.10	-0.73	1.67	2.2
G	Cdeb_01849	PTS system unknown substrate IIC component, Gat	9.21	9.35	11.17	10.44	10.04	-1.96	-1.09	-0.14	0.73	1.12	1.43	1.27	1.64	-0.5	2.3
O	Cdeb_01902	chaperone protein DnaJ	9.21	9.60	10.63	9.55	9.75	-1.42	0.05	-0.39	1.08	0.19	-0.11	0.14	-0.64	0.26	0.69
C	Cdeb_02776	succinate dehydrogenase subunit A	9.22	9.68	11.68	10.01	10.15	-2.46	-0.33	-0.46	1.67	1.98	0.41	0.90	-1.27	1.54	2.2
O	Cdeb_01781	putative glycoprotease GCP	9.23	9.41	10.92	9.37	9.73	-1.69	0.04	-0.18	1.55	0.66	-0.09	0.24	1.27	1.28	0.8
X	Cdeb_01307	hypothetical protein	9.23	9.26	11.00	10.25	9.94	-1.77	-0.99	-0.03	0.75	0.79	1.3	1.01	2.64	-0.46	1.23
G	Cdeb_02446	Archaeal fructose-1,6-bisphosphatase and related	9.27	9.61	10.50	9.00	9.60	-1.23	0.61	-0.34	1.50	-0.14	-0.86	-0.35	-0.18	1.17	1.6
C	Cdeb_02565	putative oxidoreductase, LLM family	9.28	9.57	10.72	9.32	9.72	-1.44	0.25	-0.29	1.40	0.22	-0.38	-0.29	0.27	0.96	0.96
O	Cdeb_02545	Membrane protease subunit,	9.28	9.61	10.41	9.68	9.75	-1.13	-0.07	-0.33	0.73	-0.31	0.05	-0.12	-0.09	-0.5	1.34
T	Cdeb_00342	HD-GYP domain	9.29	9.47	10.86	10.09	9.93	-1.57	-0.62	-0.18	0.77	0.45	0.8	0.60	1.27	-0.41	1.5
J	Cdeb_01899	MiaB-like tRNA modifying enzyme	9.29	9.55	9.92	8.67	9.36	-0.63	0.88	-0.26	1.25	-1.17	-1.23	-1.20	0.55	0.63	4.41
P	Cdeb_02250	DNA-binding ferritin-like protein (oxidative	9.29	9.73	10.66	9.34	9.76	-1.37	0.39	-0.44	1.32	0.10	-0.57	-0.24	-1.09	0.78	0.94
I	Cdeb_02487	Acetyl-CoA carboxylase, carboxyltransferase	9.30	9.76	11.49	11.12	10.42	-2.19	-1.36	-0.46	0.37	1.52	1.8	1.65	-1.27	-1.28	2.84
P	Cdeb_01617	phosphonate ABC transporter, ATP-binding protein	9.31	9.58	10.55	10.34	9.95	-1.24	-0.76	-0.27	0.21	-0.12	0.99	0.34	0.45	-1.63	1.28
X	Cdeb_03228	Putative RNA-binding protein homologous to	9.32	9.73	10.32	9.77	9.79	-1.00	-0.04	-0.41	0.55	-0.53	0.01	-0.07	-0.82	-0.89	0.95
J	Cdeb_02923	pseudouridylate synthase I	9.33	9.59	10.62	10.47	10.00	-1.29	-0.88	-0.26	0.15	-0.03	1.15	0.19	0.55	-1.76	1.36
G	Cdeb_00599	Transcriptional regulator/sugar kinase	9.34	9.56	11.40	10.45	10.19	-2.06	-0.89	-0.22	0.95	1.29	1.16	1.22	0.91	-0.02	4.14
K	Cdeb_00599	Transcriptional regulator/sugar kinase	9.34	9.56	11.40	10.45	10.19	-2.06	-0.89	-0.22	0.95	1.29	1.16	1.22	0.91	-0.02	4.14

C	Cdeb_01583	succinate semialdehyde dehydrogenase	9.34	9.62	10.91	9.90	9.94	-1.57	-0.28	-0.28	1.01	0.45	0.34	0.39	0.36	0.11	3.26
V	Cdeb_02742	ABC-type multidrug transport system, ATPase	9.34	9.61	10.59	9.46	9.75	-1.25	0.15	-0.27	1.13	-0.10	-0.24	-0.15	0.45	0.37	0.97
P	Cdeb_00309	ABC-type metal ion transport system, ATPase	9.35	9.72	10.93	9.77	9.94	-1.58	-0.05	-0.37	1.16	0.47	0.03	0.12	-0.45	0.43	1.64
D	Cdeb_02002	septum site-determining protein MinD	9.36	9.83	10.47	9.09	9.69	-1.11	0.74	-0.47	1.38	-0.34	-1.04	-0.59	-1.36	0.91	1.45
V	Cdeb_00435	ABC-type multidrug transport system, ATPase and	9.36	9.77	10.80	10.06	10.00	-1.44	-0.29	-0.41	0.74	0.22	0.35	0.28	-0.82	-0.48	0.95
D	Cdeb_03138	Negative regulator of septation ring formation	9.37	9.67	10.83	9.88	9.94	-1.46	-0.21	-0.30	0.95	0.26	0.24	0.25	0.18	-0.02	4.25
I	Cdeb_00756	biotin carboxyl carrier protein	9.37	9.59	10.68	9.39	9.76	-1.31	0.20	-0.22	1.29	0.00	-0.31	0.00	0.91	0.72	0.58
X	Cdeb_01571	PucR C-terminal helix-turn-helix domain	9.37	9.92	10.45	10.28	10.01	-1.08	-0.36	-0.55	0.17	-0.40	0.45	0.42	-2.09	-1.72	0.48
R	Cdeb_02052	nucleoside ABC transporter membrane protein	9.38	9.42	11.17	9.98	9.99	-1.79	-0.56	-0.04	1.19	0.83	0.72	0.77	2.55	0.5	0.92
E	Cdeb_02225	asparagine synthase (glutamine-hydrolyzing)	9.38	9.71	11.40	10.87	10.34	-2.02	-1.16	-0.33	0.53	1.22	1.53	1.37	-0.09	-0.93	4.55
F	Cdeb_02531	Formate-tetrahydrofolate ligase	9.38	9.63	11.02	10.32	10.09	-1.64	-0.69	-0.25	0.70	0.57	0.89	0.71	0.64	-0.57	2.68
O	Cdeb_02576	Molecular chaperone (small heat shock protein)	9.38	9.69	10.93	9.15	9.79	-1.55	0.54	-0.31	1.78	0.41	-0.77	-0.56	0.09	1.78	1.06
X	Cdeb_00922	hypothetical protein	9.38	10.52	13.41	12.96	11.57	-4.03	-2.44	-1.14	0.45	4.69	3.26	3.91	-7.45	-1.11	1.65
S	Cdeb_03225	TIGR00255 family protein	9.39	9.61	10.81	10.29	10.03	-1.42	-0.68	-0.22	0.52	0.19	0.88	0.41	0.91	-0.96	1.48
X	Cdeb_02919	hypothetical protein	9.39	9.62	10.70	10.69	10.10	-1.31	-1.07	-0.23	0.01	0.00	1.41	0.00	0.82	-2.07	1.38
X	Cdeb_00286	hypothetical protein	9.39	9.50	10.56	9.96	9.85	-1.17	-0.46	-0.11	0.60	-0.24	0.58	0.37	1.91	-0.78	0.66
J	Cdeb_02952	Ribosomal protein L23	9.40	9.66	10.36	9.05	9.62	-0.96	0.61	-0.26	1.31	-0.60	-0.86	-0.72	0.55	0.76	2.43
R	Cdeb_02048	Dehydrogenases with different specificities	9.42	9.78	10.42	9.25	9.72	-1.00	0.53	-0.36	1.17	-0.53	-0.76	-0.63	-0.36	0.46	3.45
Q	Cdeb_02048	Dehydrogenases with different specificities	9.42	9.78	10.42	9.25	9.72	-1.00	0.53	-0.36	1.17	-0.53	-0.76	-0.63	-0.36	0.46	3.45
I	Cdeb_02048	Dehydrogenases with different specificities	9.42	9.78	10.42	9.25	9.72	-1.00	0.53	-0.36	1.17	-0.53	-0.76	-0.63	-0.36	0.46	3.45
X	Cdeb_02539	Prenyltransferase	9.42	9.86	11.27	10.48	10.26	-1.85	-0.62	-0.44	0.79	0.93	0.8	0.86	-1.09	-0.37	2.32
E	Cdeb_02296	Acetylornithine	9.43	9.84	11.02	10.34	10.16	-1.59	-0.50	-0.41	0.68	0.48	0.64	0.55	-0.82	-0.61	1.7
U	Cdeb_01982	protein translocase subunit yajC	9.44	9.64	10.87	10.16	10.03	-1.43	-0.52	-0.20	0.71	0.21	0.66	0.37	1.09	-0.54	1.24
R	Cdeb_01993	Obg family GTPase CgtA	9.45	9.61	10.43	9.98	9.87	-0.98	-0.37	-0.16	0.45	-0.57	0.46	-0.51	1.45	-1.11	0.87
J	Cdeb_02087	translation elongation factor Ts (EF-Ts)	9.45	9.62	11.23	9.71	10.00	-1.78	-0.09	-0.17	1.52	0.81	0.08	0.25	1.36	1.22	0.97
P	Cdeb_02873	copper ion binding protein	9.45	9.85	11.39	10.59	10.32	-1.94	-0.74	-0.40	0.80	1.09	0.96	1.02	-0.73	-0.35	3.9
X	Cdeb_00755	SpoIIIAH-like protein	9.45	9.91	9.50	10.62	9.87	-0.05	-0.71	-0.46	-1.12	-2.17	0.92	-1.41	-1.27	-4.52	1.09
C	Cdeb_00532	ATP synthase F1 subcomplex gamma subunit	9.46	9.82	10.94	9.92	10.04	-1.48	-0.10	-0.36	1.02	0.29	0.09	0.16	-0.36	0.13	1.72
R	Cdeb_02043	competence/damage-inducible protein cinA	9.47	9.83	10.86	9.85	10.00	-1.39	-0.02	-0.36	1.01	0.14	-0.01	0.04	-0.36	0.11	0.81
C	Cdeb_02907	malate dehydrogenase (NAD)	9.47	9.98	12.92	11.83	11.05	-3.45	-1.85	-0.51	1.09	3.69	2.46	3.01	-1.73	0.28	5.5
O	Cdeb_03039	htrA-like peptidase	9.47	9.82	11.08	10.40	10.19	-1.61	-0.58	-0.35	0.68	0.52	0.74	0.62	-0.27	-0.61	2.95
X	Cdeb_00677	dinuclear metal center protein, YbgI/SA1388	9.47	9.73	11.90	10.53	10.41	-2.43	-0.80	-0.26	1.37	1.93	1.04	1.42	0.55	0.89	4.56
K	Cdeb_00617	transcriptional regulator, LacI family	9.48	9.85	10.53	9.33	9.80	-1.05	0.52	-0.37	1.20	-0.45	-0.74	-0.58	-0.45	0.52	2.74

L	Cdeb_00344	DNA gyrase subunit B	9.48	9.75	11.14	9.94	10.08	-1.66	-0.19	-0.27	1.20	0.60	0.22	0.36	0.45	0.52	2.02
D	Cdeb_02185	Actin-like ATPase involved in cell division	9.48	9.67	11.11	10.30	10.14	-1.63	-0.63	-0.19	0.81	0.55	0.81	0.67	1.18	-0.33	1.74
X	Cdeb_02631	Protein of unknown function (DUF4085)	9.48	9.79	10.21	8.64	9.53	-0.73	1.15	-0.31	1.57	-1.00	-1.59	-1.26	0.09	1.33	3.06
X	Cdeb_03283	ABC-2 family transporter protein	9.48	9.76	10.20	8.65	9.52	-0.72	1.11	-0.28	1.55	-1.02	-1.54	-1.25	0.36	1.28	3.02
R	Cdeb_03216	ribosome small subunit-dependent GTPase A	9.49	9.76	11.25	9.98	10.12	-1.76	-0.22	-0.27	1.27	0.78	0.26	0.45	0.45	0.67	2.21
R	Cdeb_01436	Putative Zn-dependent protease	9.49	9.92	9.99	8.80	9.55	-0.50	1.12	-0.43	1.19	-1.40	-1.55	-1.47	-1	0.5	4.06
E	Cdeb_00844	glutamate dehydrogenase (NAD)	9.50	9.93	11.62	11.18	10.56	-2.12	-1.25	-0.43	0.44	1.40	1.65	1.52	-1	-1.13	3.12
L	Cdeb_02335	DNA topoisomerase IV, A subunit,	9.51	9.63	11.23	10.00	10.09	-1.72	-0.37	-0.12	1.23	0.71	0.46	0.57	1.82	0.59	0.96
S	Cdeb_02467	Uncharacterized protein with SCP/PR1 domain	9.51	9.71	11.13	10.05	10.10	-1.62	-0.34	-0.20	1.08	0.53	0.42	0.47	1.09	0.26	1.31
X	Cdeb_02011	hypothetical protein	9.51	10.05	10.91	10.29	10.19	-1.40	-0.24	-0.54	0.62	0.16	0.28	0.21	-2	-0.74	0.33
R	Cdeb_02214	flavoprotein, HI0933 family	9.52	9.67	10.91	9.33	9.86	-1.39	0.34	-0.15	1.58	0.14	-0.5	-0.26	1.55	1.35	0.55
S	Cdeb_00897	Uncharacterized protein conserved in bacteria	9.52	9.73	10.88	10.16	10.07	-1.36	-0.43	-0.21	0.72	0.09	0.54	0.22	1	-0.52	1.06
G	Cdeb_02916	6-phosphofructokinase	9.53	9.81	10.73	9.36	9.86	-1.20	0.45	-0.28	1.37	-0.19	-0.65	-0.35	0.36	0.89	1.53
E	Cdeb_01792	oligoendopeptidase, M3 family	9.53	9.84	11.02	10.38	10.19	-1.49	-0.54	-0.31	0.64	0.31	0.69	0.46	0.09	-0.7	2.33
M	Cdeb_02498	UDP-N-acetylmuramoylalanyl-D-glutamate--2,	9.53	9.93	10.93	10.29	10.17	-1.40	-0.36	-0.40	0.64	0.16	0.45	0.27	-0.73	-0.7	1.03
R	Cdeb_01663	Threonine dehydrogenase and related Zn-dependent	9.54	9.93	10.44	9.70	9.90	-0.90	0.23	-0.39	0.74	-0.71	-0.35	-0.50	-0.64	-0.48	2.15
E	Cdeb_01663	Threonine dehydrogenase and related Zn-dependent	9.54	9.93	10.44	9.70	9.90	-0.90	0.23	-0.39	0.74	-0.71	-0.35	-0.50	-0.64	-0.48	2.15
C	Cdeb_00961	2-oxoglutarate dehydrogenase, E1 component	9.54	9.98	12.38	11.76	10.92	-2.84	-1.78	-0.44	0.62	2.64	2.36	2.50	-1.09	-0.74	5.84
I	Cdeb_00780	heterodimeric methylmalonyl-CoA mutase small	9.54	9.87	11.20	10.52	10.28	-1.66	-0.65	-0.33	0.68	0.60	0.84	0.71	-0.09	-0.61	3.64
E	Cdeb_01778	oligopeptidase F	9.55	9.74	10.58	9.62	9.87	-1.03	0.12	-0.19	0.96	-0.48	-0.2	-0.31	1.18	0	0.96
S	Cdeb_01900	RNA methyltransferase, RsmE family	9.55	9.82	10.36	9.35	9.77	-0.81	0.47	-0.27	1.01	-0.86	-0.68	-0.76	0.45	0.11	5.14
X	Cdeb_03060	Disulfide bond chaperones of the HSP33 family	9.55	9.76	11.39	9.46	10.04	-1.84	0.30	-0.21	1.93	0.91	-0.45	0.64	1	2.11	0.95
S	Cdeb_00051	Uncharacterized protein conserved in bacteria	9.56	9.64	10.90	10.60	10.18	-1.34	-0.96	-0.08	0.30	0.05	1.26	0.25	2.18	-1.43	1.05
J	Cdeb_02400	tRNA-i(6)A37 thiotransferase enzyme MiaB	9.56	10.07	10.61	9.22	9.87	-1.05	0.85	-0.51	1.39	-0.45	-1.19	-0.73	-1.73	0.93	1.41
J	Cdeb_00512	bacterial peptide chain release factor 1 (bRF-1)	9.57	9.85	10.85	10.52	10.20	-1.28	-0.67	-0.28	0.33	-0.05	0.86	0.21	0.36	-1.37	1.32
X	Cdeb_01736	Dehydrogenases with different specificities	9.57	9.82	11.51	10.83	10.43	-1.94	-1.01	-0.25	0.68	1.09	1.32	1.20	0.64	-0.61	4.21
G	Cdeb_00615	UTP-hexose-1-phosphate uridylyltransferase	9.58	9.78	10.13	9.51	9.75	-0.55	0.27	-0.20	0.62	-1.31	-0.41	-0.73	1.09	-0.74	2.27
X	Cdeb_02397	hypothetical protein	9.59	9.89	10.84	10.18	10.13	-1.25	-0.29	-0.30	0.66	-0.10	0.35	0.19	0.18	-0.65	1.17
V	Cdeb_02380	ABC-type multidrug transport system, ATPase and	9.60	9.99	11.58	10.63	10.45	-1.98	-0.64	-0.39	0.95	1.16	0.82	0.98	-0.64	-0.02	4.82
S	Cdeb_02297	EDD domain protein, DegV family	9.61	10.19	9.95	9.41	9.79	-0.34	0.78	-0.58	0.54	-1.67	-1.09	-1.35	-2.36	-0.91	1.71
G	Cdeb_01095	PTS system D-mannose-specific IIC component, Fru	9.62	9.87	11.15	9.76	10.10	-1.53	0.11	-0.25	1.39	0.38	-0.19	0.27	0.64	0.93	0.82
K	Cdeb_03079	transcription-repair coupling factor	9.62	9.82	10.96	10.27	10.17	-1.34	-0.45	-0.20	0.69	0.05	0.57	0.17	1.09	-0.59	1
L	Cdeb_03079	transcription-repair coupling factor	9.62	9.82	10.96	10.27	10.17	-1.34	-0.45	-0.20	0.69	0.05	0.57	0.17	1.09	-0.59	1

M	Cdeb_01797	alanine racemase	9.62	9.89	10.97	9.47	9.99	-1.35	0.42	-0.27	1.50	0.07	-0.61	-0.21	0.45	1.17	1.06
S	Cdeb_00372	Uncharacterized protein conserved in bacteria	9.62	10.06	10.62	9.44	9.94	-1.00	0.62	-0.44	1.18	-0.53	-0.88	-0.68	-1.09	0.48	1.88
I	Cdeb_00781	heterodimeric methylmalonyl-CoA mutase large	9.62	9.92	11.85	10.79	10.55	-2.23	-0.87	-0.30	1.06	1.59	1.14	1.35	0.18	0.22	14.96
X	Cdeb_00080	Universal stress protein family	9.62	9.87	10.65	9.32	9.87	-1.03	0.55	-0.25	1.33	-0.48	-0.78	-0.61	0.64	0.8	1.94
X	Cdeb_02882	Protein of unknown function with PCYCGC motif	9.62	9.76	11.93	10.34	10.41	-2.31	-0.58	-0.14	1.59	1.72	0.74	1.13	1.64	1.37	1.9
K	Cdeb_00673	RNA polymerase, sigma 70 subunit, RpoD	9.63	10.07	10.89	10.34	10.23	-1.26	-0.27	-0.44	0.55	-0.09	0.32	0.17	-1.09	-0.89	0.51
E	Cdeb_01110	acetylornithine deacetylase or	9.63	9.99	10.64	9.40	9.92	-1.01	0.59	-0.36	1.24	-0.52	-0.84	-0.66	-0.36	0.61	3.03
M	Cdeb_00125	Putative glycosyl/glycerophosphate transferases	9.63	10.02	11.67	11.16	10.62	-2.04	-1.14	-0.39	0.51	1.26	1.5	1.37	-0.64	-0.98	3.64
J	Cdeb_02752	phenylalanyl-tRNA synthetase, alpha subunit	9.63	9.81	10.46	9.26	9.79	-0.83	0.55	-0.18	1.20	-0.83	-0.78	-0.80	1.27	0.52	1.8
X	Cdeb_02861	ATP-dependent Lon protease, bacterial type	9.63	9.86	11.45	9.94	10.22	-1.82	-0.08	-0.23	1.51	0.88	0.07	0.25	0.82	1.2	1.32
V	Cdeb_01025	ABC-type multidrug transport system, ATPase and	9.64	10.06	10.68	9.51	9.97	-1.04	0.55	-0.42	1.17	-0.47	-0.78	-0.61	-0.91	0.46	1.94
R	Cdeb_01865	haloacid dehalogenase superfamily, subfamily IA,	9.65	10.06	11.04	11.41	10.54	-1.39	-1.35	-0.41	-0.37	0.14	1.78	0.50	-0.82	-2.89	1.29
T	Cdeb_00365	Putative signaling protein consisting of a	9.65	9.89	11.19	10.45	10.30	-1.54	-0.56	-0.24	0.74	0.40	0.72	0.54	0.73	-0.48	2.05
I	Cdeb_02083	1-deoxy-D-xylulose 5-phosphate reductoisomerase	9.65	9.86	10.92	9.57	10.00	-1.27	0.29	-0.21	1.35	-0.07	-0.43	-0.17	1	0.85	0.72
K	Cdeb_02077	NusA antitermination factor	9.66	10.08	11.13	9.60	10.12	-1.47	0.48	-0.42	1.53	0.28	-0.69	-0.44	-0.91	1.24	1.05
L	Cdeb_03338	DNA polymerase III, subunit gamma and tau	9.66	9.92	11.01	10.39	10.25	-1.35	-0.47	-0.26	0.62	0.07	0.59	0.20	0.55	-0.74	1.4
V	Cdeb_01600	ABC-type multidrug transport system, permease	9.66	9.98	10.56	9.51	9.93	-0.90	0.47	-0.32	1.05	-0.71	-0.68	-0.69	0	0.2	10.69
X	Cdeb_02009	Sporulation related domain	9.66	10.07	11.72	11.41	10.72	-2.06	-1.34	-0.41	0.31	1.29	1.77	1.51	-0.82	-1.41	2.92
S	Cdeb_00346	S4 domain protein YaaA	9.68	10.13	10.17	9.26	9.81	-0.49	0.87	-0.45	0.91	-1.41	-1.22	-1.31	-1.18	-0.11	3.42
C	Cdeb_01556	ABC-type Na+ efflux pump, permease component	9.68	9.76	10.84	10.38	10.17	-1.16	-0.62	-0.08	0.46	-0.26	0.8	0.46	2.18	-1.09	0.75
P	Cdeb_00682	ABC-type Mn/Zn transport system, ATPase	9.68	10.09	9.92	8.74	9.61	-0.24	1.35	-0.41	1.18	-1.84	-1.86	-1.85	-0.82	0.48	5.99
P	Cdeb_01556	ABC-type Na+ efflux pump, permease component	9.68	9.76	10.84	10.38	10.17	-1.16	-0.62	-0.08	0.46	-0.26	0.8	0.46	2.18	-1.09	0.75
R	Cdeb_00800	Short-chain dehydrogenase of various substrate	9.69	10.02	10.17	9.03	9.73	-0.48	0.99	-0.33	1.14	-1.43	-1.38	-1.40	-0.09	0.39	10.79
K	Cdeb_01834	transcriptional regulator, GntR family	9.69	9.90	11.00	10.45	10.26	-1.31	-0.55	-0.21	0.55	0.00	0.7	0.00	1	-0.89	1.14
E	Cdeb_00439	oligopeptide/dipeptide ABC transporter,	9.69	9.92	10.75	9.68	10.01	-1.06	0.24	-0.23	1.07	-0.43	-0.36	-0.39	0.82	0.24	1.43
M	Cdeb_00614	UDP-galactose 4-epimerase	9.70	10.19	10.50	9.35	9.94	-0.80	0.84	-0.49	1.15	-0.88	-1.18	-1.02	-1.55	0.41	2
X	Cdeb_00656	hypothetical protein	9.70	10.04	11.50	11.47	10.68	-1.80	-1.43	-0.34	0.03	0.84	1.89	1.26	-0.18	-2.02	2.22
R	Cdeb_01493	Putative metal-sulfur cluster biosynthetic	9.72	9.98	11.47	10.21	10.35	-1.75	-0.23	-0.26	1.26	0.76	0.27	0.45	0.55	0.65	2.06
K	Cdeb_01423	Transcriptional regulator	9.72	10.18	10.59	9.95	10.11	-0.87	0.23	-0.46	0.64	-0.76	-0.35	-0.52	-1.27	-0.7	1.25
E	Cdeb_03137	Cysteine sulfinate desulfinase/cysteine	9.72	10.03	10.54	9.42	9.93	-0.82	0.61	-0.31	1.12	-0.84	-0.86	-0.85	0.09	0.35	7.23
K	Cdeb_02171	sigma 54 modulation protein	9.73	10.00	11.00	10.43	10.29	-1.27	-0.43	-0.27	0.57	-0.07	0.54	0.19	0.45	-0.85	1.23
T	Cdeb_02171	sigma 54 modulation protein	9.73	10.00	11.00	10.43	10.29	-1.27	-0.43	-0.27	0.57	-0.07	0.54	0.19	0.45	-0.85	1.23
R	Cdeb_03255	Putative enzyme related to lactoylglutathione	9.75	9.99	10.95	9.65	10.09	-1.20	0.34	-0.24	1.30	-0.19	-0.5	-0.31	0.73	0.74	1.12

G	Cdeb_02167	Phosphotransferase system cellobiose-specific	9.75	9.99	10.31	9.00	9.76	-0.56	0.99	-0.24	1.31	-1.29	-1.38	-1.33	0.73	0.76	3.9
L	Cdeb_02128	DNA topoisomerase I, bacterial	9.75	10.07	11.48	10.97	10.57	-1.73	-0.90	-0.32	0.51	0.72	1.18	0.92	0	-0.98	3.07
X	Cdeb_00877	TPR repeat/Tetratricopeptide repeat	9.75	10.11	11.29	9.95	10.28	-1.54	0.16	-0.36	1.34	0.40	-0.26	0.32	-0.36	0.83	1.15
M	Cdeb_00568	Putative glycosyl/glycerophosphate transferases	9.76	9.93	11.25	9.93	10.22	-1.49	0.00	-0.17	1.32	0.31	-0.04	0.11	1.36	0.78	0.43
J	Cdeb_03219	ribosomal RNA small subunit methyltransferase	9.76	10.04	11.16	10.20	10.29	-1.40	-0.16	-0.28	0.96	0.16	0.18	0.17	0.36	0	1.45
X	Cdeb_02452	hypothetical protein	9.76	9.99	11.33	11.04	10.53	-1.57	-1.05	-0.23	0.29	0.45	1.38	0.79	0.82	-1.46	1.88
N	Cdeb_02109	Flagellar basal body-associated protein	9.77	10.03	11.10	10.37	10.32	-1.33	-0.34	-0.26	0.73	0.03	0.42	0.11	0.55	-0.5	1.23
M	Cdeb_01800	Outer membrane lipoprotein-sorting protein	9.77	10.05	11.24	11.90	10.74	-1.47	-1.85	-0.28	-0.66	0.28	2.46	0.83	0.36	-3.52	1.52
K	Cdeb_02148	RNAse III	9.78	10.10	11.35	10.09	10.33	-1.57	0.01	-0.32	1.26	0.45	-0.05	0.15	0	0.65	1.51
E	Cdeb_02429	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate	9.78	10.13	11.88	9.24	10.26	-2.10	0.89	-0.35	2.64	1.36	-1.24	1.30	-0.27	3.65	1.09
S	Cdeb_02144	Uncharacterized protein conserved in bacteria	9.78	9.95	11.06	9.97	10.19	-1.28	-0.02	-0.17	1.09	-0.05	-0.01	-0.02	1.36	0.28	0.08
C	Cdeb_02908	isocitrate dehydrogenase (NADP)	9.78	10.19	12.47	12.22	11.17	-2.69	-2.03	-0.41	0.25	2.38	2.7	2.53	-0.82	-1.54	4.48
X	Cdeb_00573	hypothetical protein	9.78	10.03	10.82	10.22	10.21	-1.04	-0.19	-0.25	0.60	-0.47	0.22	-0.32	0.64	-0.78	1.12
H	Cdeb_00071	putative nicotinate phosphoribosyltransferase	9.80	10.24	10.94	9.75	10.18	-1.14	0.49	-0.44	1.19	-0.29	-0.7	-0.45	-1.09	0.5	1.37
S	Cdeb_02078	Uncharacterized protein conserved in bacteria	9.80	10.00	10.88	10.31	10.25	-1.08	-0.31	-0.20	0.57	-0.40	0.38	-0.39	1.09	-0.85	0.87
E	Cdeb_01108	oligopeptide/dipeptide ABC transporter,	9.81	10.17	10.64	9.19	9.95	-0.83	0.98	-0.36	1.45	-0.83	-1.36	-1.06	-0.36	1.07	3.07
H	Cdeb_03030	thiamine-phosphate diphosphorylase	9.81	10.28	11.41	10.95	10.61	-1.60	-0.67	-0.47	0.46	0.50	0.86	0.66	-1.36	-1.09	1.24
M	Cdeb_00678	4-hydroxy-3-methylbut-2-enyl diphosphate	9.81	10.28	10.86	9.47	10.11	-1.05	0.81	-0.47	1.39	-0.45	-1.14	-0.72	-1.36	0.93	1.62
P	Cdeb_02390	Cystathionine beta-lyase family protein involved	9.81	10.14	11.22	9.85	10.26	-1.41	0.29	-0.33	1.37	0.17	-0.43	-0.27	-0.09	0.89	1.12
I	Cdeb_00678	4-hydroxy-3-methylbut-2-enyl diphosphate	9.81	10.28	10.86	9.47	10.11	-1.05	0.81	-0.47	1.39	-0.45	-1.14	-0.72	-1.36	0.93	1.62
G	Cdeb_00793	glucose-6-phosphate 1-dehydrogenase	9.82	10.09	11.22	10.34	10.37	-1.40	-0.25	-0.27	0.88	0.16	0.3	0.22	0.45	-0.17	1.54
X	Cdeb_00705	hypothetical protein	9.82	10.00	11.26	9.37	10.11	-1.44	0.63	-0.18	1.89	0.22	-0.89	-0.44	1.27	2.02	0.84
K	Cdeb_01974	(p)ppGpp synthetase, RelA/SpoT family	9.83	10.00	11.16	9.26	10.06	-1.33	0.74	-0.17	1.90	0.03	-1.04	-0.18	1.36	2.04	0.92
T	Cdeb_01974	(p)ppGpp synthetase, RelA/SpoT family	9.83	10.00	11.16	9.26	10.06	-1.33	0.74	-0.17	1.90	0.03	-1.04	-0.18	1.36	2.04	0.92
I	Cdeb_02177	Acyl-coenzyme A synthetases/AMP-(fatty) acid	9.83	10.29	12.47	11.95	11.14	-2.64	-1.66	-0.46	0.52	2.29	2.2	2.24	-1.27	-0.96	4.34
D	Cdeb_00353	glucose-inhibited division protein A	9.84	10.06	10.85	10.05	10.20	-1.01	0.01	-0.22	0.80	-0.52	-0.05	-0.16	0.91	-0.35	1.16
R	Cdeb_01080	Short-chain alcohol dehydrogenase of unknown	9.86	10.17	11.65	11.27	10.74	-1.79	-1.10	-0.31	0.38	0.83	1.45	1.10	0.09	-1.26	2.88
J	Cdeb_00414	Acetyltransferase, including N-acetylase of	9.86	10.28	10.61	9.37	10.03	-0.75	0.91	-0.42	1.24	-0.97	-1.27	-1.11	-0.91	0.61	3.17
C	Cdeb_01034	cytochrome bd-I ubiquinol oxidase subunit 2	9.86	10.16	11.88	11.38	10.82	-2.02	-1.22	-0.30	0.50	1.22	1.61	1.40	0.18	-1	4.32
P	Cdeb_02635	ABC-type metal ion transport system, periplasmic	9.86	10.20	11.66	11.12	10.71	-1.80	-0.92	-0.34	0.54	0.84	1.2	1.00	-0.18	-0.91	3.43
R	Cdeb_01628	ABC-type uncharacterized transport system,	9.87	10.15	10.85	10.32	10.30	-0.98	-0.17	-0.28	0.53	-0.57	0.19	-0.33	0.36	-0.93	1.31
R	Cdeb_01924	ribosome biogenesis GTPase YqeH	9.87	10.09	11.48	10.51	10.49	-1.61	-0.42	-0.22	0.97	0.52	0.53	0.52	0.91	0.02	1.77
Q	Cdeb_01102	Imidazolonepropionase and related amidohydrolase	9.87	10.19	10.72	10.17	10.24	-0.85	0.02	-0.32	0.55	-0.79	-0.07	-0.24	0	-0.89	1.94

V	Cdeb_00172	ABC-type multidrug transport system, ATPase	9.88	10.36	11.26	10.23	10.43	-1.38	0.13	-0.48	1.03	0.12	-0.22	-0.16	-1.45	0.15	0.37
E	Cdeb_01043	Threonine synthase	9.90	10.16	11.00	9.99	10.26	-1.10	0.17	-0.26	1.01	-0.36	-0.27	-0.31	0.55	0.11	1.74
P	Cdeb_01449	Enterochelin esterase	9.90	10.26	11.54	10.73	10.61	-1.64	-0.47	-0.36	0.81	0.57	0.59	0.58	-0.36	-0.33	3.65
K	Cdeb_02787	Putative transcriptional regulator	9.91	10.23	11.43	9.86	10.36	-1.52	0.37	-0.32	1.57	0.36	-0.54	-0.44	0	1.33	1.06
J	Cdeb_01973	D-tyrosyl-tRNA(Tyr) deacylase	9.91	10.26	11.11	9.93	10.30	-1.20	0.33	-0.35	1.18	-0.19	-0.49	-0.31	-0.27	0.48	2.07
E	Cdeb_01048	agmatine deiminase	9.92	10.26	11.24	9.82	10.31	-1.32	0.44	-0.34	1.42	0.02	-0.64	-0.11	-0.18	1	1.37
L	Cdeb_00004	CRISPR-associated autoregulator, Cst2 family	9.92	10.10	11.47	10.52	10.50	-1.55	-0.42	-0.18	0.95	0.41	0.53	0.47	1.27	-0.02	1.15
L	Cdeb_01378	DNA ligase, NAD-dependent	9.92	10.13	10.94	10.33	10.33	-1.02	-0.20	-0.21	0.61	-0.50	0.23	-0.34	1	-0.76	0.95
G	Cdeb_01723	PTS system, N-acetylglucosamine-specific IIBC	9.93	10.13	11.41	10.72	10.55	-1.48	-0.59	-0.20	0.69	0.29	0.76	0.47	1.09	-0.59	1.43
K	Cdeb_02573	transcriptional regulator, LacI family	9.93	10.27	11.03	9.85	10.27	-1.10	0.42	-0.34	1.18	-0.36	-0.61	-0.47	-0.18	0.48	3
I	Cdeb_00496	methylmalonyl-CoA mutase C-terminal	9.93	10.25	12.51	11.89	11.15	-2.58	-1.64	-0.32	0.62	2.19	2.18	2.18	0	-0.74	9.08
C	Cdeb_03022	putative oxidoreductase, Fe-dependent alcohol	9.94	10.30	11.59	9.98	10.45	-1.65	0.32	-0.36	1.61	0.59	-0.47	0.53	-0.36	1.41	1.13
X	Cdeb_01216	hypothetical protein	9.94	10.25	11.92	11.45	10.89	-1.98	-1.20	-0.31	0.47	1.16	1.58	1.35	0.09	-1.07	3.97
G	Cdeb_00264	phosphoglycerate mutase	9.95	10.26	11.67	9.24	10.28	-1.72	1.02	-0.31	2.43	0.71	-1.42	-1.00	0.09	3.2	1.08
Q	Cdeb_00972	hypothetical protein	9.95	10.34	10.90	10.13	10.33	-0.95	0.21	-0.39	0.77	-0.62	-0.32	-0.45	-0.64	-0.41	2
X	Cdeb_00410	Aspartate/tyrosine/aromatic aminotransferase	9.95	10.14	11.15	9.79	10.26	-1.20	0.35	-0.19	1.36	-0.19	-0.51	-0.31	1.18	0.87	0.81
R	Cdeb_00424	Uncharacterized protein containing SIS (Sugar	9.96	10.17	11.53	11.11	10.69	-1.57	-0.94	-0.21	0.42	0.45	1.23	0.74	1	-1.17	1.85
S	Cdeb_00808	Uncharacterized conserved protein	9.96	10.28	10.79	9.93	10.24	-0.83	0.35	-0.32	0.86	-0.83	-0.51	-0.65	0	-0.22	9.63
S	Cdeb_01712	Putative membrane protein	9.96	10.38	11.14	10.68	10.54	-1.18	-0.30	-0.42	0.46	-0.22	0.36	0.28	-0.91	-1.09	0.65
G	Cdeb_02613	ABC-type sugar transport system, periplasmic	9.97	10.14	11.70	11.25	10.77	-1.73	-1.11	-0.17	0.45	0.72	1.46	1.03	1.36	-1.11	2.02
M	Cdeb_02193	UDP-N-acetylmuramate--L-alanine ligase	9.97	10.23	11.19	10.18	10.39	-1.22	0.05	-0.26	1.01	-0.16	-0.11	-0.13	0.55	0.11	0.75
J	Cdeb_02738	LSU ribosomal protein L35P	9.97	10.29	11.32	9.95	10.38	-1.35	0.34	-0.32	1.37	0.07	-0.5	-0.19	0	0.89	1.23
C	Cdeb_02456	pyruvate carboxylase	9.97	10.11	11.71	10.77	10.64	-1.74	-0.66	-0.14	0.94	0.74	0.85	0.79	1.64	-0.04	1.49
X	Cdeb_00174	hypothetical protein	9.97	10.22	11.06	10.45	10.43	-1.09	-0.23	-0.25	0.61	-0.38	0.27	-0.32	0.64	-0.76	1.02
K	Cdeb_01056	transcriptional regulator, TetR family	9.98	10.32	11.31	10.24	10.46	-1.33	0.08	-0.34	1.07	0.03	-0.15	-0.07	-0.18	0.24	1.11
X	Cdeb_03264	Protein of unknown function (DUF3048)	9.98	10.38	11.12	10.06	10.39	-1.14	0.32	-0.40	1.06	-0.29	-0.47	-0.37	-0.73	0.22	1.57
K	Cdeb_00160	transcriptional attenuator, LytR family	9.99	10.28	10.75	9.56	10.15	-0.76	0.72	-0.29	1.19	-0.95	-1.01	-0.98	0.27	0.5	5.3
R	Cdeb_02050	Putative Zn-dependent peptidase	10.00	10.25	11.08	10.50	10.46	-1.08	-0.25	-0.25	0.58	-0.40	0.3	-0.35	0.64	-0.83	1.04
J	Cdeb_00008	Asp-tRNAAsn/Glu-tRNAGln amidotransferase A	10.00	10.26	11.42	10.68	10.59	-1.42	-0.42	-0.26	0.74	0.19	0.53	0.32	0.55	-0.48	1.68
J	Cdeb_01481	tryptophanyl-tRNA synthetase	10.00	10.47	10.51	9.03	10.00	-0.51	1.44	-0.47	1.48	-1.38	-1.99	-1.66	-1.36	1.13	2.98
X	Cdeb_00783	Disulfide isomerase	10.00	10.45	11.73	11.00	10.80	-1.73	-0.55	-0.45	0.73	0.72	0.7	0.71	-1.18	-0.5	1.7
H	Cdeb_01537	uroporphyrinogen decarboxylase	10.02	10.29	11.17	9.90	10.35	-1.15	0.39	-0.27	1.27	-0.28	-0.57	-0.40	0.45	0.67	1.71
J	Cdeb_02975	cysteinyl-tRNA synthetase	10.02	10.44	11.53	10.05	10.51	-1.51	0.39	-0.42	1.48	0.34	-0.57	-0.44	-0.91	1.13	0.99

P	Cdeb_02638	ABC-type metal ion transport system, periplasmic	10.02	10.18	11.49	10.79	10.62	-1.47	-0.61	-0.16	0.70	0.28	0.78	0.47	1.45	-0.57	1.16
G	Cdeb_00600	monosaccharide ABC transporter substrate-binding	10.03	10.36	11.21	9.43	10.26	-1.18	0.93	-0.33	1.78	-0.22	-1.3	-0.53	-0.09	1.78	1.61
E	Cdeb_01105	ABC-type dipeptide/oligopeptide/nickel transport	10.03	10.36	10.24	8.94	9.89	-0.21	1.42	-0.33	1.30	-1.90	-1.96	-1.93	-0.09	0.74	7.96
P	Cdeb_01105	ABC-type dipeptide/oligopeptide/nickel transport	10.03	10.36	10.24	8.94	9.89	-0.21	1.42	-0.33	1.30	-1.90	-1.96	-1.93	-0.09	0.74	7.96
H	Cdeb_00457	phosphomethylpyrimidine kinase	10.04	10.37	11.74	9.18	10.33	-1.70	1.19	-0.33	2.56	0.67	-1.65	-1.05	-0.09	3.48	1.11
N	Cdeb_01065	methyl-accepting chemotaxis sensory transducer	10.04	10.29	11.28	10.50	10.53	-1.24	-0.21	-0.25	0.78	-0.12	0.24	0.17	0.64	-0.39	0.78
T	Cdeb_01065	methyl-accepting chemotaxis sensory transducer	10.04	10.29	11.28	10.50	10.53	-1.24	-0.21	-0.25	0.78	-0.12	0.24	0.17	0.64	-0.39	0.78
J	Cdeb_03086	endoribonuclease L-PSP	10.04	10.34	11.42	11.01	10.70	-1.38	-0.67	-0.30	0.41	0.12	0.86	0.32	0.18	-1.2	1.56
S	Cdeb_00938	EDD domain protein, DegV family	10.05	10.29	11.02	10.21	10.39	-0.97	0.08	-0.24	0.81	-0.59	-0.15	-0.30	0.73	-0.33	1.65
J	Cdeb_00515	translation factor SUA5	10.05	10.28	11.21	10.03	10.39	-1.16	0.25	-0.23	1.18	-0.26	-0.38	-0.31	0.82	0.48	1.05
G	Cdeb_01817	PTS system lichenan oligosaccharide-specific IIA	10.06	10.39	11.99	11.23	10.92	-1.93	-0.84	-0.33	0.76	1.07	1.09	1.08	-0.09	-0.43	7.56
P	Cdeb_02583	Putative periplasmic lipoprotein involved in	10.06	10.49	12.37	11.42	11.09	-2.31	-0.93	-0.43	0.95	1.72	1.22	1.45	-1	-0.02	4.58
K	Cdeb_00926	cold-shock DNA-binding protein family	10.07	10.25	11.36	10.42	10.53	-1.29	-0.17	-0.18	0.94	-0.03	0.19	0.08	1.27	-0.04	0.33
C	Cdeb_00778	branched-chain alpha-keto acid dehydrogenase E1	10.07	10.54	11.44	10.26	10.58	-1.37	0.28	-0.47	1.18	0.10	-0.42	-0.20	-1.36	0.48	0.65
R	Cdeb_02551	ABC-type uncharacterized transport system,	10.08	10.50	11.09	9.19	10.22	-1.01	1.31	-0.42	1.90	-0.52	-1.81	-0.97	-0.91	2.04	1.83
X	Cdeb_00589	amidohydrolase	10.08	10.38	10.80	9.61	10.22	-0.72	0.77	-0.30	1.19	-1.02	-1.08	-1.05	0.18	0.5	6.08
E	Cdeb_01871	oligopeptide/dipeptide ABC transporter,	10.09	10.55	12.55	12.18	11.34	-2.46	-1.63	-0.46	0.37	1.98	2.16	2.07	-1.27	-1.28	3.53
M	Cdeb_01908	GTP-binding protein LepA	10.09	10.44	11.37	9.94	10.46	-1.28	0.50	-0.35	1.43	-0.05	-0.72	-0.19	-0.27	1.02	1.49
H	Cdeb_03136	thiazole biosynthesis/tRNA modification protein	10.12	10.23	11.36	10.45	10.54	-1.24	-0.22	-0.11	0.91	-0.12	0.26	0.18	1.91	-0.11	0.33
M	Cdeb_00691	cell elongation-specific peptidoglycan	10.12	10.28	11.35	10.47	10.56	-1.23	-0.19	-0.16	0.88	-0.14	0.22	0.18	1.45	-0.17	0.39
G	Cdeb_00460	carbohydrate ABC transporter membrane protein 1,	10.13	10.48	11.99	11.39	11.00	-1.86	-0.91	-0.35	0.60	0.95	1.19	1.06	-0.27	-0.78	4.01
J	Cdeb_02947	LSU ribosomal protein L16P	10.13	10.37	11.64	11.10	10.81	-1.51	-0.73	-0.24	0.54	0.34	0.95	0.57	0.73	-0.91	1.88
G	Cdeb_00607	carbohydrate ABC transporter substrate-binding	10.15	10.44	11.89	10.97	10.86	-1.74	-0.53	-0.29	0.92	0.74	0.68	0.71	0.27	-0.09	7.68
J	Cdeb_02846	RNAse PH	10.16	10.29	11.66	10.69	10.70	-1.50	-0.40	-0.13	0.97	0.33	0.5	0.41	1.73	0.02	0.75
V	Cdeb_00112	ABC-type multidrug transport system, ATPase	10.16	10.57	11.45	10.17	10.59	-1.29	0.40	-0.41	1.28	-0.03	-0.58	-0.13	-0.82	0.7	1.17
X	Cdeb_03227	Putative RNA-binding protein homologous to	10.16	10.54	11.67	10.24	10.65	-1.51	0.30	-0.38	1.43	0.34	-0.45	-0.39	-0.55	1.02	1.06
S	Cdeb_00748	stage III sporulation protein AA	10.17	10.77	11.00	11.16	10.78	-0.83	-0.39	-0.60	-0.16	-0.83	0.49	-0.64	-2.55	-2.43	0.59
C	Cdeb_01560	fumarase, class II	10.17	10.48	12.22	11.15	11.01	-2.05	-0.67	-0.31	1.07	1.28	0.86	1.05	0.09	0.24	13.08
K	Cdeb_00638	Transcriptional regulator, contains sigma	10.18	10.49	11.44	9.73	10.46	-1.26	0.76	-0.31	1.71	-0.09	-1.07	-0.31	0.09	1.63	1.43
Q	Cdeb_01506	2-keto-4-pentenoate	10.18	10.55	11.73	11.41	10.97	-1.55	-0.86	-0.37	0.32	0.41	1.12	0.68	-0.45	-1.39	1.77
J	Cdeb_00898	asparaginyl-tRNA synthetase	10.18	10.58	11.19	9.54	10.37	-1.01	1.04	-0.40	1.65	-0.52	-1.45	-0.87	-0.73	1.5	2.01
C	Cdeb_00060	homodimeric glycerol 3-phosphate dehydrogenase	10.18	10.51	11.44	10.51	10.66	-1.26	0.00	-0.33	0.93	-0.09	-0.04	-0.06	-0.09	-0.07	1.88
K	Cdeb_01937	transcription elongation factor GreA	10.19	10.63	12.00	11.88	11.18	-1.81	-1.25	-0.44	0.12	0.86	1.65	1.19	-1.09	-1.83	1.9

R	Cdeb_00580	Superfamily I DNA and RNA helicase	10.20	10.39	10.96	9.35	10.23	-0.76	1.04	-0.19	1.61	-0.95	-1.45	-1.17	1.18	1.41	2.05
K	Cdeb_00357	ParB-like partition protein	10.20	10.53	11.48	10.64	10.71	-1.28	-0.11	-0.33	0.84	-0.05	0.11	0.07	-0.09	-0.26	0.95
O	Cdeb_01743	Peroxiredoxin	10.20	10.53	11.29	10.70	10.68	-1.09	-0.17	-0.33	0.59	-0.38	0.19	-0.27	-0.09	-0.8	1.15
H	Cdeb_01535	protoporphyrinogen oxidase	10.22	10.51	11.76	10.15	10.66	-1.54	0.36	-0.29	1.61	0.40	-0.53	-0.46	0.27	1.41	1.01
E	Cdeb_00482	spermidine synthase	10.23	10.58	11.44	10.33	10.65	-1.21	0.25	-0.35	1.11	-0.17	-0.38	-0.25	-0.27	0.33	2.12
E	Cdeb_00736	glycine dehydrogenase (decarboxylating) alpha	10.23	10.68	11.82	10.60	10.83	-1.59	0.08	-0.45	1.22	0.48	-0.15	0.27	-1.18	0.57	0.83
E	Cdeb_01927	oligoendopeptidase, pepF/M3 family	10.23	10.67	10.41	9.28	10.15	-0.18	1.39	-0.44	1.13	-1.95	-1.92	-1.93	-1.09	0.37	5.17
L	Cdeb_02079	DNA polymerase III, alpha chain, Gram-positive	10.23	10.65	11.45	10.63	10.74	-1.22	0.02	-0.42	0.82	-0.16	-0.07	-0.11	-0.91	-0.3	0.4
N	Cdeb_02096	Chemotaxi protein histidine kinase and related	10.24	10.51	11.80	10.40	10.74	-1.56	0.11	-0.27	1.40	0.43	-0.19	0.29	0.45	0.96	0.96
T	Cdeb_02096	Chemotaxi protein histidine kinase and related	10.24	10.51	11.80	10.40	10.74	-1.56	0.11	-0.27	1.40	0.43	-0.19	0.29	0.45	0.96	0.96
D	Cdeb_00560	capsular exopolysaccharide family	10.24	10.61	10.81	9.82	10.37	-0.57	0.79	-0.37	0.99	-1.28	-1.11	-1.19	-0.45	0.07	8.09
G	Cdeb_00616	aldose 1-epimerase	10.25	10.63	10.28	9.31	10.12	-0.03	1.32	-0.38	0.97	-2.21	-1.82	-2.01	-0.55	0.02	11.31
L	Cdeb_02336	DNA topoisomerase IV subunit B	10.25	10.66	11.11	10.41	10.61	-0.86	0.25	-0.41	0.70	-0.78	-0.38	-0.54	-0.82	-0.57	1.89
D	Cdeb_00544	rod shape-determining protein MreB	10.25	10.60	11.88	9.99	10.68	-1.63	0.61	-0.35	1.89	0.55	-0.86	-0.69	-0.27	2.02	1.09
C	Cdeb_00148	glycolate oxidase, subunit GlcD	10.26	10.58	11.35	10.55	10.69	-1.09	0.03	-0.32	0.80	-0.38	-0.08	-0.17	0	-0.35	2.41
X	Cdeb_01748	Nucleotidyltransferase-like	10.26	10.53	11.56	9.53	10.47	-1.30	1.00	-0.27	2.03	-0.02	-1.39	-0.17	0.45	2.33	1.27
R	Cdeb_00351	Putative RNA-binding protein	10.28	10.64	12.14	10.90	10.99	-1.86	-0.26	-0.36	1.24	0.95	0.31	0.54	-0.36	0.61	3.07
R	Cdeb_02198	EMAP domain	10.28	10.59	10.98	9.65	10.38	-0.70	0.94	-0.31	1.33	-1.05	-1.31	-1.17	0.09	0.8	4.53
I	Cdeb_02917	acetyl-CoA carboxylase carboxyltransferase	10.28	10.59	11.17	10.35	10.60	-0.89	0.24	-0.31	0.82	-0.72	-0.36	-0.51	0.09	-0.3	5.59
O	Cdeb_01688	Glutaredoxin	10.28	10.87	10.25	9.45	10.21	0.03	1.42	-0.59	0.80	-2.31	-1.96	-2.13	-2.45	-0.35	2.66
X	Cdeb_01713	DinB superfamily	10.28	10.54	11.03	10.14	10.50	-0.75	0.40	-0.26	0.89	-0.97	-0.58	-0.75	0.55	-0.15	4.31
R	Cdeb_02981	Integral membrane protein (PIN domain	10.29	10.56	11.44	10.62	10.73	-1.15	-0.06	-0.27	0.82	-0.28	0.04	-0.11	0.45	-0.3	1.14
T	Cdeb_00178	Signal transduction histidine kinase	10.29	10.55	11.36	10.11	10.58	-1.07	0.44	-0.26	1.25	-0.41	-0.64	-0.51	0.55	0.63	1.98
T	Cdeb_00834	Signal transduction histidine kinase	10.29	10.58	11.67	10.91	10.86	-1.38	-0.33	-0.29	0.76	0.12	0.41	0.22	0.27	-0.43	1.83
G	Cdeb_02201	Cellulase M	10.32	10.35	11.67	10.08	10.61	-1.35	0.27	-0.03	1.59	0.07	-0.41	-0.17	2.64	1.37	0.3
F	Cdeb_03224	guanylate kinase	10.33	10.61	12.06	11.05	11.01	-1.73	-0.44	-0.28	1.01	0.72	0.55	0.63	0.36	0.11	5.23
R	Cdeb_00807	nudix-type nucleoside diphosphatase, YffH/AdpP	10.34	10.42	11.28	10.65	10.67	-0.94	-0.23	-0.08	0.63	-0.64	0.27	-0.42	2.18	-0.72	0.66
L	Cdeb_00807	nudix-type nucleoside diphosphatase, YffH/AdpP	10.34	10.42	11.28	10.65	10.67	-0.94	-0.23	-0.08	0.63	-0.64	0.27	-0.42	2.18	-0.72	0.66
X	Cdeb_01903	Molecular chaperone	10.34	10.73	11.97	11.06	11.03	-1.63	-0.33	-0.39	0.91	0.55	0.41	0.47	-0.64	-0.11	2.3
E	Cdeb_00737	glycine dehydrogenase (decarboxylating) beta	10.35	10.80	11.59	9.92	10.67	-1.24	0.88	-0.45	1.67	-0.12	-1.23	-0.38	-1.18	1.54	1.38
X	Cdeb_00963	hypothetical protein	10.35	10.69	11.45	9.84	10.58	-1.10	0.85	-0.34	1.61	-0.36	-1.19	-0.65	-0.18	1.41	1.9
F	Cdeb_02086	uridylate kinase (EC 2.7.4.22)	10.36	10.64	12.31	11.60	11.23	-1.95	-0.96	-0.28	0.71	1.10	1.26	1.18	0.36	-0.54	5.6
C	Cdeb_00779	branched-chain alpha-keto acid dehydrogenase E2	10.36	10.60	11.95	11.17	11.02	-1.59	-0.57	-0.24	0.78	0.48	0.73	0.59	0.73	-0.39	2.29

G	Cdeb_01814	PTS system, lactose/cellobiose family IIC	10.38	10.52	12.36	11.29	11.14	-1.98	-0.77	-0.14	1.07	1.16	1	1.08	1.64	0.24	2.01
S	Cdeb_03195	Uncharacterized protein conserved in bacteria	10.38	10.75	11.45	10.60	10.80	-1.07	0.15	-0.37	0.85	-0.41	-0.24	-0.31	-0.45	-0.24	2.03
C	Cdeb_03165	Pyruvate/2-oxoglutarate dehydrogenase complex,	10.38	10.65	11.90	11.19	11.03	-1.52	-0.54	-0.27	0.71	0.36	0.69	0.50	0.45	-0.54	2.41
R	Cdeb_01585	lipid kinase, YegS/Rv2252/BmrU family	10.39	10.62	11.52	11.30	10.96	-1.13	-0.68	-0.23	0.22	-0.31	0.88	0.52	0.82	-1.61	1.12
F	Cdeb_02525	Pyrimidine operon attenuation protein/uracil	10.39	10.71	11.80	10.97	10.97	-1.41	-0.26	-0.32	0.83	0.17	0.31	0.23	0	-0.28	2.74
I	Cdeb_01585	lipid kinase, YegS/Rv2252/BmrU family	10.39	10.62	11.52	11.30	10.96	-1.13	-0.68	-0.23	0.22	-0.31	0.88	0.52	0.82	-1.61	1.12
G	Cdeb_00453	Cellulase M	10.40	10.67	12.11	10.49	10.92	-1.71	0.18	-0.27	1.62	0.69	-0.28	0.44	0.45	1.43	1.08
U	Cdeb_02146	signal recognition particle-docking protein FtsY	10.41	10.69	11.99	10.38	10.87	-1.58	0.31	-0.28	1.61	0.47	-0.46	0.46	0.36	1.41	0.98
G	Cdeb_00813	phosphopentomutase	10.41	10.75	11.68	10.53	10.84	-1.27	0.22	-0.34	1.15	-0.07	-0.34	-0.15	-0.18	0.41	1.69
S	Cdeb_00095	Putative secreted protein	10.41	10.74	11.75	10.98	10.97	-1.34	-0.24	-0.33	0.77	0.05	0.28	0.12	-0.09	-0.41	1.47
J	Cdeb_00274	23S rRNA m(5)U-1939 methyltransferase	10.41	10.69	11.90	10.81	10.95	-1.49	-0.12	-0.28	1.09	0.31	0.12	0.19	0.36	0.28	1.58
P	Cdeb_02648	ABC-type Fe3+ transport system, periplasmic	10.41	10.70	12.19	11.03	11.08	-1.78	-0.33	-0.29	1.16	0.81	0.41	0.58	0.27	0.43	3.89
V	Cdeb_02432	ABC-type multidrug transport system, ATPase and	10.41	10.85	11.76	10.50	10.88	-1.35	0.35	-0.44	1.26	0.07	-0.51	-0.19	-1.09	0.65	0.88
U	Cdeb_02014	prepilin-type N-terminal cleavage/methylation	10.42	10.82	12.37	11.81	11.36	-1.95	-0.99	-0.40	0.56	1.10	1.3	1.20	-0.73	-0.87	3.26
N	Cdeb_02014	prepilin-type N-terminal cleavage/methylation	10.42	10.82	12.37	11.81	11.36	-1.95	-0.99	-0.40	0.56	1.10	1.3	1.20	-0.73	-0.87	3.26
R	Cdeb_02656	amidohydrolase	10.43	10.77	11.08	10.66	10.74	-0.65	0.11	-0.34	0.42	-1.14	-0.19	-0.47	-0.18	-1.17	2.12
N	Cdeb_02095	Chemotaxis signal transduction protein	10.43	10.77	11.57	10.16	10.73	-1.14	0.61	-0.34	1.41	-0.29	-0.86	-0.50	-0.18	0.98	1.98
T	Cdeb_02095	Chemotaxis signal transduction protein	10.43	10.77	11.57	10.16	10.73	-1.14	0.61	-0.34	1.41	-0.29	-0.86	-0.50	-0.18	0.98	1.98
E	Cdeb_02704	amino acid/amide ABC transporter membrane	10.44	10.67	11.86	11.39	11.09	-1.42	-0.72	-0.23	0.47	0.19	0.93	0.42	0.82	-1.07	1.53
E	Cdeb_03083	ribose-phosphate pyrophosphokinase	10.44	10.74	11.97	10.71	10.97	-1.53	0.03	-0.30	1.26	0.38	-0.08	0.17	0.18	0.65	1.25
F	Cdeb_03083	ribose-phosphate pyrophosphokinase	10.44	10.74	11.97	10.71	10.97	-1.53	0.03	-0.30	1.26	0.38	-0.08	0.17	0.18	0.65	1.25
J	Cdeb_00668	glycyl-tRNA synthetase alpha chain	10.44	10.76	11.59	10.59	10.85	-1.15	0.17	-0.32	1.00	-0.28	-0.27	-0.27	0	0.09	9.4
G	Cdeb_00597	Beta-glucosidase/6-phospho-beta-	10.45	10.97	12.19	11.02	11.16	-1.74	-0.05	-0.52	1.17	0.74	0.03	0.15	-1.82	0.46	0.86
J	Cdeb_03220	methionyl-tRNA formyltransferase	10.45	10.81	12.03	11.47	11.19	-1.58	-0.66	-0.36	0.56	0.47	0.85	0.63	-0.36	-0.87	2.24
J	Cdeb_00941	NOL1/NOP2/sun family putative RNA methylase	10.45	10.78	11.52	10.36	10.78	-1.07	0.42	-0.33	1.16	-0.41	-0.61	-0.50	-0.09	0.43	3.64
G	Cdeb_02172	PTS system IIA component, Glc family	10.47	10.84	10.77	9.33	10.35	-0.30	1.51	-0.37	1.44	-1.74	-2.08	-1.90	-0.45	1.04	5.2
V	Cdeb_02727	Type I restriction-modification system	10.47	10.82	11.56	10.30	10.79	-1.09	0.52	-0.35	1.26	-0.38	-0.74	-0.53	-0.27	0.65	2.57
G	Cdeb_00584	Alpha-mannosidase	10.48	10.80	11.91	10.71	10.98	-1.43	0.09	-0.32	1.20	0.21	-0.16	0.18	0	0.52	1.1
O	Cdeb_01783	universal bacterial protein YeaZ	10.48	10.73	11.60	10.72	10.88	-1.12	0.01	-0.25	0.88	-0.33	-0.05	-0.13	0.64	-0.17	1.1
K	Cdeb_01803	Superfamily II DNA and RNA helicase	10.49	10.74	12.23	10.94	11.10	-1.74	-0.20	-0.25	1.29	0.74	0.23	0.41	0.64	0.72	1.75
E	Cdeb_00918	Zn-dependent carboxypeptidase	10.49	10.85	12.02	10.70	11.02	-1.53	0.15	-0.36	1.32	0.38	-0.24	0.30	-0.36	0.78	1.14
L	Cdeb_01803	Superfamily II DNA and RNA helicase	10.49	10.74	12.23	10.94	11.10	-1.74	-0.20	-0.25	1.29	0.74	0.23	0.41	0.64	0.72	1.75
H	Cdeb_00447	2-amino-3-ketobutyrate coenzyme A ligase	10.49	10.86	12.39	10.72	11.12	-1.90	0.14	-0.37	1.67	1.02	-0.23	0.48	-0.45	1.54	1.42

J	Cdeb_01803	Superfamily II DNA and RNA helicase	10.49	10.74	12.23	10.94	11.10	-1.74	-0.20	-0.25	1.29	0.74	0.23	0.41	0.64	0.72	1.75
Q	Cdeb_03007	uncharacterized domain 1	10.50	10.84	11.75	11.46	11.14	-1.25	-0.62	-0.34	0.29	-0.10	0.8	0.28	-0.18	-1.46	1.19
D	Cdeb_00232	cell division ATP-binding protein FtsE	10.51	10.41	9.72	8.21	9.71	0.79	2.20	0.10	1.51	-3.62	-3.01	-3.30	3.82	1.2	2.56
X	Cdeb_00339	hypothetical protein	10.51	10.62	12.21	10.50	10.96	-1.70	0.12	-0.11	1.71	0.67	-0.2	0.37	1.91	1.63	0.61
R	Cdeb_01941	putative periplasmic solute-binding protein	10.52	10.87	11.21	10.12	10.68	-0.69	0.75	-0.35	1.09	-1.07	-1.05	-1.06	-0.27	0.28	8.38
K	Cdeb_01018	transcriptional regulator, LacI family	10.52	10.60	11.86	10.42	10.85	-1.34	0.18	-0.08	1.44	0.05	-0.28	-0.12	2.18	1.04	0.26
R	Cdeb_00392	Aldo/keto reductase, related to diketogulonate	10.53	10.91	12.24	11.61	11.32	-1.71	-0.70	-0.38	0.63	0.69	0.91	0.79	-0.55	-0.72	2.74
R	Cdeb_00885	Putative Zn-dependent protease	10.53	10.97	11.37	9.96	10.71	-0.84	1.01	-0.44	1.41	-0.81	-1.41	-1.07	-1.09	0.98	2.41
E	Cdeb_00093	Spermidine/putrescine-binding periplasmic	10.53	10.79	11.24	10.01	10.64	-0.71	0.78	-0.26	1.23	-1.03	-1.09	-1.06	0.55	0.59	4.04
M	Cdeb_02494	Cell division protein FtsI/penicillin-binding	10.54	10.84	11.82	11.11	11.08	-1.28	-0.27	-0.30	0.71	-0.05	0.32	0.13	0.18	-0.54	1.24
X	Cdeb_00161	Subtilisin-like serine protease	10.54	10.78	11.78	11.76	11.22	-1.24	-0.98	-0.24	0.02	-0.12	1.28	0.39	0.73	-2.04	1.29
X	Cdeb_02786	Protein of unknown function (DUF4352)	10.54	10.97	12.36	11.63	11.38	-1.82	-0.66	-0.43	0.73	0.88	0.85	0.86	-1	-0.5	2.38
R	Cdeb_00352	tRNA modification GTPase trmE	10.55	10.87	11.47	10.39	10.82	-0.92	0.48	-0.32	1.08	-0.67	-0.69	-0.68	0	0.26	8.04
R	Cdeb_00950	Dehydrogenases with different specificities	10.55	10.75	11.80	11.15	11.06	-1.25	-0.40	-0.20	0.65	-0.10	0.5	0.22	1.09	-0.67	0.87
E	Cdeb_03119	alanine dehydrogenase	10.55	10.91	12.09	11.04	11.15	-1.54	-0.13	-0.36	1.05	0.40	0.14	0.24	-0.36	0.2	2.24
Q	Cdeb_00950	Dehydrogenases with different specificities	10.55	10.75	11.80	11.15	11.06	-1.25	-0.40	-0.20	0.65	-0.10	0.5	0.22	1.09	-0.67	0.87
I	Cdeb_00950	Dehydrogenases with different specificities	10.55	10.75	11.80	11.15	11.06	-1.25	-0.40	-0.20	0.65	-0.10	0.5	0.22	1.09	-0.67	0.87
X	Cdeb_03061	Disulfide bond chaperones of the HSP33 family	10.55	10.83	11.86	11.55	11.20	-1.31	-0.72	-0.28	0.31	0.00	0.93	0.00	0.36	-1.41	1.39
K	Cdeb_03223	DNA-directed RNA polymerase subunit omega	10.56	10.67	11.86	11.41	11.13	-1.30	-0.74	-0.11	0.45	-0.02	0.96	0.14	1.91	-1.11	0.94
S	Cdeb_02045	Uncharacterized protein conserved in bacteria	10.56	10.91	11.88	10.57	10.98	-1.32	0.34	-0.35	1.31	0.02	-0.5	-0.10	-0.27	0.76	1.35
H	Cdeb_01531	lipoate-protein ligase	10.57	11.02	12.05	11.63	11.32	-1.48	-0.61	-0.45	0.42	0.29	0.78	0.48	-1.18	-1.17	1.09
X	Cdeb_02915	Pyruvate kinase	10.58	10.97	11.76	10.32	10.91	-1.18	0.65	-0.39	1.44	-0.22	-0.92	-0.45	-0.64	1.04	1.68
H	Cdeb_03028	hydroxyethylthiazole kinase	10.60	10.84	12.03	10.89	11.09	-1.43	-0.05	-0.24	1.14	0.21	0.03	0.08	0.73	0.39	0.56
H	Cdeb_01762	NAD+ synthetase	10.60	11.03	11.43	10.65	10.93	-0.83	0.38	-0.43	0.78	-0.83	-0.55	-0.68	-1	-0.39	2.02
J	Cdeb_02072	ribosome-binding factor A	10.60	10.89	11.17	9.39	10.51	-0.57	1.50	-0.29	1.78	-1.28	-2.07	-1.63	0.27	1.78	2.94
E	Cdeb_02684	ABC-type dipeptide/oligopeptide/nickel transport	10.61	11.03	11.56	10.46	10.92	-0.95	0.57	-0.42	1.10	-0.62	-0.81	-0.71	-0.91	0.3	2.31
Q	Cdeb_02149	acyl carrier protein	10.61	11.01	11.79	10.54	10.99	-1.18	0.47	-0.40	1.25	-0.22	-0.68	-0.39	-0.73	0.63	1.61
P	Cdeb_02684	ABC-type dipeptide/oligopeptide/nickel transport	10.61	11.03	11.56	10.46	10.92	-0.95	0.57	-0.42	1.10	-0.62	-0.81	-0.71	-0.91	0.3	2.31
I	Cdeb_02149	acyl carrier protein	10.61	11.01	11.79	10.54	10.99	-1.18	0.47	-0.40	1.25	-0.22	-0.68	-0.39	-0.73	0.63	1.61
R	Cdeb_03033	Putative ATPase of the ABC class	10.62	10.95	12.00	10.98	11.14	-1.38	-0.03	-0.33	1.02	0.12	0	0.00	-0.09	0.13	1.65
G	Cdeb_00603	carbohydrate ABC transporter substrate-binding	10.62	11.06	12.60	11.45	11.43	-1.98	-0.39	-0.44	1.15	1.16	0.49	0.75	-1.09	0.41	2.35
E	Cdeb_02706	amino acid/amide ABC transporter ATP-binding	10.62	10.95	12.06	11.17	11.20	-1.44	-0.22	-0.33	0.89	0.22	0.26	0.24	-0.09	-0.15	4.23
E	Cdeb_00775	leucine dehydrogenase	10.62	10.99	12.52	11.91	11.51	-1.90	-0.92	-0.37	0.61	1.02	1.2	1.11	-0.45	-0.76	3.88

L	Cdeb_02396	DNA mismatch repair protein MutS	10.62	10.92	11.91	11.02	11.12	-1.29	-0.10	-0.30	0.89	-0.03	0.09	0.05	0.18	-0.15	0.88
L	Cdeb_01021	DNA topoisomerase III, bacteria and conjugative	10.62	10.83	11.97	11.12	11.14	-1.35	-0.29	-0.21	0.85	0.07	0.35	0.16	1	-0.24	0.75
I	Cdeb_02363	Acyl-CoA hydrolase	10.62	10.94	11.45	9.66	10.67	-0.83	1.28	-0.32	1.79	-0.83	-1.77	-1.21	0	1.8	2.36
X	Cdeb_02914	Pyruvate kinase	10.65	11.03	11.99	11.00	11.17	-1.34	0.03	-0.38	0.99	0.05	-0.08	-0.06	-0.55	0.07	0.37
K	Cdeb_00509	transcription termination factor Rho	10.66	11.08	11.87	10.84	11.11	-1.21	0.24	-0.42	1.03	-0.17	-0.36	-0.25	-0.91	0.15	0.94
E	Cdeb_01063	carbamate kinase	10.66	11.00	11.30	9.97	10.73	-0.64	1.03	-0.34	1.33	-1.16	-1.43	-1.29	-0.18	0.8	4.88
J	Cdeb_02945	30S ribosomal protein S17	10.66	10.76	10.91	9.84	10.54	-0.25	0.92	-0.10	1.07	-1.83	-1.28	-1.53	2	0.24	2.41
V	Cdeb_02431	ABC-type multidrug transport system, ATPase and	10.66	11.01	12.47	11.36	11.38	-1.81	-0.35	-0.35	1.11	0.86	0.43	0.61	-0.27	0.33	4.9
X	Cdeb_01747	Protein of unknown function (DUF2614)	10.66	10.90	11.36	10.81	10.93	-0.70	0.09	-0.24	0.55	-1.05	-0.16	-0.41	0.73	-0.89	2.01
X	Cdeb_03127	Peroxiredoxin	10.66	11.00	12.07	10.61	11.09	-1.41	0.39	-0.34	1.46	0.17	-0.57	-0.31	-0.18	1.09	1.17
E	Cdeb_00788	peptidase T-like protein	10.67	10.92	11.73	10.44	10.94	-1.06	0.48	-0.25	1.29	-0.43	-0.69	-0.54	0.64	0.72	1.83
O	Cdeb_00831	ResB protein required for cytochrome c	10.68	10.98	11.92	10.45	11.01	-1.24	0.53	-0.30	1.47	-0.12	-0.76	-0.30	0.18	1.11	1.49
G	Cdeb_01501	carbohydrate ABC transporter substrate-binding	10.69	11.03	12.49	11.66	11.47	-1.80	-0.63	-0.34	0.83	0.84	0.81	0.82	-0.18	-0.28	7.62
F	Cdeb_00946	purine-nucleoside phosphorylase, family 1 (deoD)	10.69	10.82	12.33	11.66	11.38	-1.64	-0.84	-0.13	0.67	0.57	1.09	0.79	1.73	-0.63	1.45
X	Cdeb_02860	ATP-dependent protease La	10.69	10.94	12.10	11.24	11.24	-1.41	-0.30	-0.25	0.86	0.17	0.36	0.25	0.64	-0.22	1.28
Q	Cdeb_00104	putative enzyme involved in pigment biosynthesis	10.70	10.94	12.21	11.53	11.35	-1.51	-0.59	-0.24	0.68	0.34	0.76	0.51	0.73	-0.61	1.9
M	Cdeb_01862	Cell division protein FtsI/penicillin-binding	10.70	10.95	11.90	11.08	11.16	-1.20	-0.13	-0.25	0.82	-0.19	0.14	-0.16	0.64	-0.3	0.73
S	Cdeb_00027	Putative membrane protein	10.70	11.10	12.02	10.78	11.15	-1.32	0.32	-0.40	1.24	0.02	-0.47	-0.10	-0.73	0.61	1.08
X	Cdeb_00555	Superfamily II DNA/RNA helicase, SNF2 family	10.70	10.94	11.90	11.17	11.18	-1.20	-0.23	-0.24	0.73	-0.19	0.27	0.23	0.73	-0.5	0.81
E	Cdeb_03059	cysteine synthase	10.71	11.17	13.18	12.31	11.84	-2.47	-1.14	-0.46	0.87	2.00	1.5	1.73	-1.27	-0.2	4.23
J	Cdeb_01970	histidyl-tRNA synthetase	10.71	11.17	12.89	12.63	11.85	-2.18	-1.46	-0.46	0.26	1.50	1.93	1.70	-1.27	-1.52	2.68
E	Cdeb_02683	oligopeptide/dipeptide ABC transporter,	10.72	11.10	11.46	10.55	10.96	-0.74	0.55	-0.38	0.91	-0.98	-0.78	-0.87	-0.55	-0.11	4.85
P	Cdeb_02683	oligopeptide/dipeptide ABC transporter,	10.72	11.10	11.46	10.55	10.96	-0.74	0.55	-0.38	0.91	-0.98	-0.78	-0.87	-0.55	-0.11	4.85
X	Cdeb_02529	hypothetical protein	10.72	11.04	12.15	11.71	11.41	-1.43	-0.67	-0.32	0.44	0.21	0.86	0.42	0	-1.13	1.7
R	Cdeb_02433	RNase J1	10.74	11.14	12.01	10.71	11.15	-1.27	0.43	-0.40	1.30	-0.07	-0.62	-0.21	-0.73	0.74	1.3
R	Cdeb_01406	Putative metal-dependent hydrolase with the	10.74	11.02	11.24	10.09	10.77	-0.50	0.93	-0.28	1.15	-1.40	-1.3	-1.35	0.36	0.41	7.61
H	Cdeb_03222	phosphopantothenoylcysteine	10.74	11.21	12.18	10.98	11.28	-1.44	0.23	-0.47	1.20	0.22	-0.35	-0.28	-1.36	0.52	0.62
M	Cdeb_00123	Putative glycosyl/glycerophosphate transferases	10.74	11.03	11.27	10.50	10.89	-0.53	0.53	-0.29	0.77	-1.34	-0.76	-1.01	0.27	-0.41	6.82
X	Cdeb_00411	Aspartate/tyrosine/aromatic aminotransferase	10.74	11.04	12.54	11.70	11.51	-1.80	-0.66	-0.30	0.84	0.84	0.85	0.84	0.18	-0.26	8.22
L	Cdeb_03290	DNA repair exonuclease	10.75	10.94	12.26	11.08	11.26	-1.51	-0.14	-0.19	1.18	0.34	0.15	0.23	1.18	0.48	0.63
L	Cdeb_00680	Endonuclease IV	10.76	11.08	11.47	10.27	10.90	-0.71	0.81	-0.32	1.20	-1.03	-1.14	-1.08	0	0.52	6.42
G	Cdeb_00613	galactokinase	10.77	11.11	10.60	9.09	10.39	0.17	2.02	-0.34	1.51	-2.55	-2.77	-2.66	-0.18	1.2	6.75
G	Cdeb_00964	yjeF C-terminal region, hydroxyethylthiazole	10.77	11.06	11.77	10.38	11.00	-1.00	0.68	-0.29	1.39	-0.53	-0.96	-0.71	0.27	0.93	2.46

L	Cdeb_00238	Excinuclease ABC subunit B	10.77	11.10	11.94	11.08	11.22	-1.17	0.02	-0.33	0.86	-0.24	-0.07	-0.13	-0.09	-0.22	2.29
F	Cdeb_00499	CTP synthase	10.77	11.14	12.69	11.13	11.43	-1.92	0.01	-0.37	1.56	1.05	-0.05	0.23	-0.45	1.3	1.66
O	Cdeb_00654	Membrane-bound serine protease (ClpP class)	10.77	11.02	12.17	11.44	11.35	-1.40	-0.42	-0.25	0.73	0.16	0.53	0.29	0.64	-0.5	1.48
K	Cdeb_00165	transcriptional attenuator, LytR family	10.78	11.07	11.72	10.62	11.05	-0.94	0.45	-0.29	1.10	-0.64	-0.65	-0.64	0.27	0.3	4.91
T	Cdeb_03218	Serine/threonine protein phosphatase	10.78	11.05	12.16	11.68	11.42	-1.38	-0.63	-0.27	0.48	0.12	0.81	0.31	0.45	-1.04	1.57
J	Cdeb_02958	SSU ribosomal protein S7P	10.78	11.13	12.16	11.06	11.28	-1.38	0.07	-0.35	1.10	0.12	-0.14	-0.13	-0.27	0.3	0.99
K	Cdeb_03098	transcriptional regulator, AbrB family	10.79	11.08	10.65	9.47	10.50	0.14	1.61	-0.29	1.18	-2.50	-2.22	-2.36	0.27	0.48	13.2
E	Cdeb_00746	Xaa-Pro aminopeptidase	10.79	11.04	12.52	10.62	11.24	-1.73	0.42	-0.25	1.90	0.72	-0.61	0.66	0.64	2.04	0.96
H	Cdeb_02253	1,4-Dihydroxy-2-naphthoyl-CoA synthase	10.79	11.20	11.58	10.54	11.03	-0.79	0.66	-0.41	1.04	-0.90	-0.93	-0.91	-0.82	0.17	3.36
R	Cdeb_02141	RNA-binding protein (KH domain)	10.80	11.02	11.36	10.11	10.82	-0.56	0.91	-0.22	1.25	-1.29	-1.27	-1.28	0.91	0.63	3.56
E	Cdeb_00888	dihydrodipicolinate reductase	10.80	11.15	11.85	10.62	11.11	-1.05	0.53	-0.35	1.23	-0.45	-0.76	-0.58	-0.27	0.59	2.96
F	Cdeb_03266	adenylosuccinate lyase	10.80	11.08	12.24	11.37	11.37	-1.44	-0.29	-0.28	0.87	0.22	0.35	0.28	0.36	-0.2	2.18
I	Cdeb_00292	3-hydroxyacyl-CoA dehydrogenase	10.80	11.20	13.20	11.80	11.75	-2.40	-0.60	-0.40	1.40	1.88	0.77	1.20	-0.73	0.96	3.66
E	Cdeb_02237	oligopeptide/dipeptide ABC transporter,	10.81	11.17	11.89	10.66	11.13	-1.08	0.51	-0.36	1.23	-0.40	-0.73	-0.54	-0.36	0.59	2.62
F	Cdeb_00523	uracil phosphoribosyltransferase	10.81	11.13	11.65	10.99	11.15	-0.84	0.14	-0.32	0.66	-0.81	-0.23	-0.43	0	-0.65	2.82
P	Cdeb_02237	oligopeptide/dipeptide ABC transporter,	10.81	11.17	11.89	10.66	11.13	-1.08	0.51	-0.36	1.23	-0.40	-0.73	-0.54	-0.36	0.59	2.62
L	Cdeb_00343	DNA gyrase subunit A	10.82	11.12	12.24	11.34	11.38	-1.42	-0.22	-0.30	0.90	0.19	0.26	0.22	0.18	-0.13	3.15
C	Cdeb_02909	2-methylcitrate synthase/citrate synthase II	10.82	11.37	13.32	11.93	11.86	-2.50	-0.56	-0.55	1.39	2.05	0.72	1.21	-2.09	0.93	2.06
X	Cdeb_01870	Bacterial extracellular solute-binding protein,	10.82	11.54	13.71	13.51	12.40	-2.89	-1.97	-0.72	0.20	2.72	2.62	2.67	-3.64	-1.65	2.05
L	Cdeb_00348	chromosomal replication initiator protein DnaA	10.83	11.20	11.87	10.21	11.03	-1.04	0.99	-0.37	1.66	-0.47	-1.38	-0.81	-0.45	1.52	2
F	Cdeb_03272	GMP synthase (glutamine-hydrolyzing)	10.83	11.17	11.96	11.45	11.35	-1.13	-0.28	-0.34	0.51	-0.31	0.34	0.32	-0.18	-0.98	1
K	Cdeb_02076	Putative nucleic-acid-binding protein implicated	10.84	11.20	12.17	11.46	11.42	-1.33	-0.26	-0.36	0.71	0.03	0.31	0.10	-0.36	-0.54	1.04
S	Cdeb_03291	Uncharacterized conserved protein	10.84	11.13	12.33	11.48	11.45	-1.49	-0.35	-0.29	0.85	0.31	0.43	0.37	0.27	-0.24	3.19
R	Cdeb_02164	Aldo/keto reductase, related to diketogulonate	10.85	11.18	11.96	10.89	11.22	-1.11	0.29	-0.33	1.07	-0.34	-0.43	-0.38	-0.09	0.24	4.65
U	Cdeb_03132	signal peptide peptidase SppA, 36K type	10.86	11.16	11.97	11.43	11.36	-1.11	-0.27	-0.30	0.54	-0.34	0.32	-0.33	0.18	-0.91	1.09
N	Cdeb_00087	Methyl-accepting chemotaxis protein	10.86	11.10	12.75	11.64	11.59	-1.89	-0.54	-0.24	1.11	1.00	0.69	0.83	0.73	0.33	3.3
T	Cdeb_00087	Methyl-accepting chemotaxis protein	10.86	11.10	12.75	11.64	11.59	-1.89	-0.54	-0.24	1.11	1.00	0.69	0.83	0.73	0.33	3.3
S	Cdeb_00234	Uncharacterized protein conserved in bacteria	10.86	11.05	11.17	9.03	10.53	-0.31	2.02	-0.19	2.14	-1.72	-2.77	-2.18	1.18	2.57	2.51
O	Cdeb_03132	signal peptide peptidase SppA, 36K type	10.86	11.16	11.97	11.43	11.36	-1.11	-0.27	-0.30	0.54	-0.34	0.32	-0.33	0.18	-0.91	1.09
F	Cdeb_02671	5'-nucleotidase/2',3'-cyclic phosphodiesterase	10.88	11.14	11.66	10.73	11.10	-0.78	0.41	-0.26	0.93	-0.91	-0.59	-0.73	0.55	-0.07	4.25
T	Cdeb_01795	Growth inhibitor	10.88	11.17	12.05	10.50	11.15	-1.17	0.67	-0.29	1.55	-0.24	-0.95	-0.48	0.27	1.28	1.63
S	Cdeb_02660	Putative membrane protein (DUF2207)	10.88	11.15	11.68	10.34	11.01	-0.80	0.81	-0.27	1.34	-0.88	-1.14	-1.00	0.45	0.83	3.32
J	Cdeb_02088	ribosomal protein S2, bacterial type	10.88	11.09	12.51	11.46	11.49	-1.63	-0.37	-0.21	1.05	0.55	0.46	0.50	1	0.2	1.53

K	Cdeb_02969	transcription antitermination protein nusG	10.89	11.14	11.65	10.69	11.09	-0.76	0.45	-0.25	0.96	-0.95	-0.65	-0.79	0.64	0	3.91
L	Cdeb_00767	DNA replication and repair protein RecN	10.90	11.15	12.00	10.89	11.24	-1.10	0.26	-0.25	1.11	-0.36	-0.39	-0.37	0.64	0.33	1.6
T	Cdeb_02868	Signal transduction histidine kinase	10.90	11.08	12.74	11.59	11.58	-1.84	-0.51	-0.18	1.15	0.91	0.65	0.77	1.27	0.41	1.82
C	Cdeb_00880	Rieske Fe-S protein	10.90	11.25	13.15	11.92	11.81	-2.25	-0.67	-0.35	1.23	1.62	0.86	1.18	-0.27	0.59	6.15
V	Cdeb_01601	ABC-type multidrug transport system, ATPase	10.90	11.37	11.37	10.22	10.97	-0.47	1.15	-0.47	1.15	-1.45	-1.59	-1.52	-1.36	0.41	3.29
S	Cdeb_02434	Uncharacterized conserved small protein	10.91	11.24	11.27	10.49	10.98	-0.36	0.75	-0.33	0.78	-1.64	-1.05	-1.31	-0.09	-0.39	10.58
R	Cdeb_00670	CBS domain	10.92	11.24	11.85	10.92	11.23	-0.93	0.32	-0.32	0.93	-0.66	-0.47	-0.56	0	-0.07	25.16
E	Cdeb_01887	Zn-dependent dipeptidase, microsomal	10.92	11.17	12.02	11.10	11.30	-1.10	0.07	-0.25	0.92	-0.36	-0.14	-0.22	0.64	-0.09	1.3
T	Cdeb_02913	Phosphohistidine swiveling domain	10.92	11.38	11.92	11.18	11.35	-1.00	0.20	-0.46	0.74	-0.53	-0.31	-0.41	-1.27	-0.48	0.98
P	Cdeb_01567	K+ transport system, NAD-binding component	10.92	11.19	12.15	10.96	11.31	-1.23	0.23	-0.27	1.19	-0.14	-0.35	-0.22	0.45	0.5	1.22
J	Cdeb_02942	LSU ribosomal protein L5P	10.93	11.12	12.14	10.67	11.22	-1.21	0.45	-0.19	1.47	-0.17	-0.65	-0.33	1.18	1.11	0.9
R	Cdeb_00799	Zn-dependent hydrolase, including glyoxylase	10.95	11.33	12.51	11.57	11.59	-1.56	-0.24	-0.38	0.94	0.43	0.28	0.35	-0.55	-0.04	2.02
E	Cdeb_01030	cystathionine gamma-lyase	10.95	11.30	12.89	11.60	11.69	-1.94	-0.30	-0.35	1.29	1.09	0.36	0.63	-0.27	0.72	3.25
D	Cdeb_00233	cell division protein FtsX	10.95	11.05	11.57	11.02	11.15	-0.62	0.03	-0.10	0.55	-1.19	-0.08	-0.31	2	-0.89	1.18
G	Cdeb_01070	aryl-phospho-beta-glucosidase	10.96	11.11	11.65	10.47	11.05	-0.69	0.64	-0.15	1.18	-1.07	-0.91	-0.99	1.55	0.48	1.88
E	Cdeb_00434	peptidase T	10.96	11.23	11.42	10.37	11.00	-0.46	0.86	-0.27	1.05	-1.47	-1.2	-1.33	0.45	0.2	8.38
C	Cdeb_00062	glycerol kinase	10.96	11.36	13.45	12.16	11.98	-2.49	-0.80	-0.40	1.29	2.03	1.04	1.45	-0.73	0.72	4.84
C	Cdeb_00797	NADH:flavin oxidoreductase, Old Yellow Enzyme	10.96	11.34	11.29	10.14	10.93	-0.33	1.20	-0.38	1.15	-1.69	-1.66	-1.67	-0.55	0.41	7.51
G	Cdeb_00068	dihydroxyacetone kinase DhaK subunit	10.97	11.34	13.44	12.51	12.07	-2.47	-1.17	-0.37	0.93	2.00	1.54	1.75	-0.45	-0.07	12.05
G	Cdeb_00561	Capsular polysaccharide biosynthesis protein	10.99	11.21	12.03	11.27	11.38	-1.04	-0.06	-0.22	0.76	-0.47	0.04	-0.14	0.91	-0.43	1.02
G	Cdeb_01067	Phosphotransferase system cellobiose-specific	10.99	11.23	10.01	8.70	10.23	0.98	2.53	-0.24	1.31	-3.95	-3.46	-3.70	0.73	0.76	10.83
M	Cdeb_00561	Capsular polysaccharide biosynthesis protein	10.99	11.21	12.03	11.27	11.38	-1.04	-0.06	-0.22	0.76	-0.47	0.04	-0.14	0.91	-0.43	1.02
I	Cdeb_00294	Acyl-CoA dehydrogenase	10.99	11.42	13.55	12.46	12.11	-2.56	-1.04	-0.43	1.09	2.16	1.36	1.71	-1	0.28	5.34
M	Cdeb_00948	C-terminal peptidase (prc)	11.00	11.34	12.25	11.10	11.42	-1.25	0.24	-0.34	1.15	-0.10	-0.36	-0.19	-0.18	0.41	1.81
D	Cdeb_02055	DNA translocase FtsK	11.00	11.34	12.04	11.27	11.41	-1.04	0.07	-0.34	0.77	-0.47	-0.14	-0.26	-0.18	-0.41	2.38
K	Cdeb_00260	Transcriptional regulator, contains sigma	11.02	11.33	12.36	11.16	11.47	-1.34	0.17	-0.31	1.20	0.05	-0.27	-0.12	0.09	0.52	1.13
G	Cdeb_00116	fructokinase	11.03	11.26	12.87	11.51	11.67	-1.84	-0.25	-0.23	1.36	0.91	0.3	0.52	0.82	0.87	1.74
K	Cdeb_00116	fructokinase	11.03	11.26	12.87	11.51	11.67	-1.84	-0.25	-0.23	1.36	0.91	0.3	0.52	0.82	0.87	1.74
K	Cdeb_02183	transcriptional regulator, LacI family	11.03	11.46	12.37	11.36	11.56	-1.34	0.10	-0.43	1.01	0.05	-0.18	-0.09	-1	0.11	0.4
E	Cdeb_00223	Gamma-glutamyltransferase	11.03	11.39	11.79	10.89	11.28	-0.76	0.50	-0.36	0.90	-0.95	-0.72	-0.83	-0.36	-0.13	6.77
D	Cdeb_03348	ATPases involved in chromosome partitioning	11.03	11.31	12.29	10.92	11.39	-1.26	0.39	-0.28	1.37	-0.09	-0.57	-0.23	0.36	0.89	1.31
V	Cdeb_00395	ABC-type antimicrobial peptide transport system,	11.03	11.36	11.82	10.80	11.25	-0.79	0.56	-0.33	1.02	-0.90	-0.8	-0.85	-0.09	0.13	16.56
V	Cdeb_01398	Cation/multidrug efflux pump	11.03	11.34	12.27	11.24	11.47	-1.24	0.10	-0.31	1.03	-0.12	-0.18	-0.15	0.09	0.15	2.69

R	Cdeb_01421	glutamate synthase, NADH/NADPH, small subunit	11.04	11.42	11.91	10.84	11.30	-0.87	0.58	-0.38	1.07	-0.76	-0.82	-0.79	-0.55	0.24	4.05
E	Cdeb_01421	glutamate synthase, NADH/NADPH, small subunit	11.04	11.42	11.91	10.84	11.30	-0.87	0.58	-0.38	1.07	-0.76	-0.82	-0.79	-0.55	0.24	4.05
J	Cdeb_02935	LSU ribosomal protein L15P	11.04	11.36	12.63	11.56	11.65	-1.59	-0.20	-0.32	1.07	0.48	0.23	0.33	0	0.24	4.82
R	Cdeb_02393	RNA-binding protein Hfq	11.05	11.44	12.06	11.53	11.52	-1.01	-0.09	-0.39	0.53	-0.52	0.08	-0.20	-0.64	-0.93	1.01
G	Cdeb_02676	Beta-glucosidase/6-phospho-beta-	11.05	11.46	11.62	10.98	11.28	-0.57	0.48	-0.41	0.64	-1.28	-0.69	-0.94	-0.82	-0.7	2.93
J	Cdeb_02946	LSU ribosomal protein L29P	11.05	11.17	12.01	11.22	11.36	-0.96	-0.05	-0.12	0.79	-0.60	0.03	-0.13	1.82	-0.37	0.7
J	Cdeb_02964	ribosomal protein L7/L12	11.05	11.36	12.64	11.13	11.55	-1.59	0.23	-0.31	1.51	0.48	-0.35	0.41	0.09	1.2	1.07
J	Cdeb_00669	glycyl-tRNA synthetase beta chain	11.05	11.36	12.38	10.82	11.40	-1.33	0.54	-0.31	1.56	0.03	-0.77	-0.15	0.09	1.3	1.29
O	Cdeb_00314	FeS assembly protein SufD	11.06	11.28	12.53	10.93	11.45	-1.47	0.35	-0.22	1.60	0.28	-0.51	-0.38	0.91	1.39	0.76
X	Cdeb_02200	hypothetical protein	11.06	11.44	12.35	11.65	11.63	-1.29	-0.21	-0.38	0.70	-0.03	0.24	0.08	-0.55	-0.57	0.66
R	Cdeb_00445	Hemolysin and related protein containing CBS	11.07	11.39	12.67	11.71	11.71	-1.60	-0.32	-0.32	0.96	0.50	0.39	0.44	0	0	30
M	Cdeb_02503	UDP-N-acetylglucosamine-N-	11.07	11.31	12.14	11.03	11.39	-1.07	0.28	-0.24	1.11	-0.41	-0.42	-0.41	0.73	0.33	1.59
R	Cdeb_02419	Dehydrogenases with different specificities	11.08	11.45	12.27	11.74	11.64	-1.19	-0.29	-0.37	0.53	-0.21	0.35	0.27	-0.45	-0.93	0.86
Q	Cdeb_02419	Dehydrogenases with different specificities	11.08	11.45	12.27	11.74	11.64	-1.19	-0.29	-0.37	0.53	-0.21	0.35	0.27	-0.45	-0.93	0.86
I	Cdeb_02419	Dehydrogenases with different specificities	11.08	11.45	12.27	11.74	11.64	-1.19	-0.29	-0.37	0.53	-0.21	0.35	0.27	-0.45	-0.93	0.86
G	Cdeb_02168	PTS system, lactose/cellobiose family IIC	11.12	11.03	11.32	9.72	10.80	-0.20	1.31	0.09	1.60	-1.91	-1.81	-1.86	3.73	1.39	1.44
X	Cdeb_00645	drug resistance transporter, EmrB/QacA subfamily	11.12	11.48	12.53	11.37	11.63	-1.41	0.11	-0.36	1.16	0.17	-0.19	-0.18	-0.36	0.43	0.99
G	Cdeb_02748	Cellulase M	11.13	11.36	13.36	11.74	11.90	-2.23	-0.38	-0.23	1.62	1.59	0.47	0.86	0.82	1.43	2.19
E	Cdeb_00735	glycine cleavage system T protein	11.13	11.58	12.76	12.05	11.88	-1.63	-0.47	-0.45	0.71	0.55	0.59	0.57	-1.18	-0.54	1.35
L	Cdeb_02042	RecA protein	11.13	11.42	12.01	10.93	11.37	-0.88	0.49	-0.29	1.08	-0.74	-0.7	-0.72	0.27	0.26	5.91
M	Cdeb_02176	Membrane carboxypeptidase (penicillin-binding	11.13	11.57	12.53	11.59	11.71	-1.40	-0.02	-0.44	0.94	0.16	-0.01	0.04	-1.09	-0.04	0.32
M	Cdeb_00507	UDP-N-acetylglucosamine	11.14	11.46	12.38	11.59	11.64	-1.24	-0.13	-0.32	0.79	-0.12	0.14	0.13	0	-0.37	1.08
C	Cdeb_02921	Malic enzyme	11.15	11.41	12.14	11.10	11.45	-0.99	0.31	-0.26	1.04	-0.55	-0.46	-0.50	0.55	0.17	2.71
U	Cdeb_00256	ATP-dependent Clp protease proteolytic subunit	11.17	11.42	12.37	11.17	11.53	-1.20	0.25	-0.25	1.20	-0.19	-0.38	-0.27	0.64	0.52	1.12
G	Cdeb_01572	carbohydrate ABC transporter ATP-binding	11.17	11.41	12.44	11.67	11.67	-1.27	-0.26	-0.24	0.77	-0.07	0.31	0.15	0.73	-0.41	0.83
O	Cdeb_00256	ATP-dependent Clp protease proteolytic subunit	11.17	11.42	12.37	11.17	11.53	-1.20	0.25	-0.25	1.20	-0.19	-0.38	-0.27	0.64	0.52	1.12
E	Cdeb_01964	Cysteine sulfinate desulfinase/cysteine	11.18	11.44	12.53	10.62	11.44	-1.35	0.82	-0.26	1.91	0.07	-1.15	-0.28	0.55	2.07	1.17
M	Cdeb_00525	UDP-N-Acetylglucosamine 2-epimerase	11.19	11.49	12.55	11.48	11.68	-1.36	0.01	-0.30	1.07	0.09	-0.05	0.07	0.18	0.24	0.75
F	Cdeb_00741	ribonucleoside-diphosphate reductase class II	11.19	11.44	12.91	11.84	11.85	-1.72	-0.40	-0.25	1.07	0.71	0.5	0.60	0.64	0.24	2.76
O	Cdeb_02858	endopeptidase Clp ATP-binding regulatory subunit	11.19	11.52	12.85	11.97	11.88	-1.66	-0.45	-0.33	0.88	0.60	0.57	0.58	-0.09	-0.17	9.35
J	Cdeb_02739	LSU ribosomal protein L20P	11.20	11.59	12.47	11.39	11.66	-1.27	0.20	-0.39	1.08	-0.07	-0.31	-0.15	-0.64	0.26	1
X	Cdeb_01338	StbA protein	11.20	11.47	13.14	12.06	11.97	-1.94	-0.59	-0.27	1.08	1.09	0.76	0.91	0.45	0.26	5.56
R	Cdeb_00017	HAD-superfamily hydrolase, subfamily IIB	11.21	11.53	11.98	10.99	11.43	-0.77	0.54	-0.32	0.99	-0.93	-0.77	-0.85	0	0.07	37.5

E	Cdeb_01888	ABC-type dipeptide transport system, periplasmic	11.22	11.51	12.55	11.11	11.60	-1.33	0.40	-0.29	1.44	0.03	-0.58	-0.13	0.27	1.04	1.18
E	Cdeb_02235	ABC-type dipeptide/oligopeptide/nickel transport	11.22	11.51	12.54	11.73	11.75	-1.32	-0.22	-0.29	0.81	0.02	0.26	0.07	0.27	-0.33	1.33
C	Cdeb_00425	Inorganic pyrophosphatase/exopolyphosphatase	11.22	11.34	12.42	11.59	11.64	-1.20	-0.25	-0.12	0.83	-0.19	0.3	0.24	1.82	-0.28	0.42
P	Cdeb_02235	ABC-type dipeptide/oligopeptide/nickel transport	11.22	11.51	12.54	11.73	11.75	-1.32	-0.22	-0.29	0.81	0.02	0.26	0.07	0.27	-0.33	1.33
X	Cdeb_00591	Metal-dependent	11.22	11.58	12.33	11.58	11.68	-1.11	0.00	-0.36	0.75	-0.34	-0.04	-0.12	-0.36	-0.46	1.27
R	Cdeb_03292	Dehydrogenases with different specificities	11.23	11.54	12.54	11.49	11.70	-1.31	0.05	-0.31	1.05	0.00	-0.11	0.00	0.09	0.2	1.09
Q	Cdeb_03292	Dehydrogenases with different specificities	11.23	11.54	12.54	11.49	11.70	-1.31	0.05	-0.31	1.05	0.00	-0.11	0.00	0.09	0.2	1.09
J	Cdeb_02753	phenylalanyl-tRNA synthetase beta subunit	11.23	11.51	12.78	11.83	11.84	-1.55	-0.32	-0.28	0.95	0.41	0.39	0.40	0.36	-0.02	3.41
I	Cdeb_03292	Dehydrogenases with different specificities	11.23	11.54	12.54	11.49	11.70	-1.31	0.05	-0.31	1.05	0.00	-0.11	0.00	0.09	0.2	1.09
J	Cdeb_02949	ribosomal protein L22, bacterial type	11.24	11.58	12.41	11.36	11.65	-1.17	0.22	-0.34	1.05	-0.24	-0.34	-0.29	-0.18	0.2	3.36
J	Cdeb_03092	dimethyladenosine transferase	11.24	11.38	11.78	11.00	11.35	-0.54	0.38	-0.14	0.78	-1.33	-0.55	-0.86	1.64	-0.39	1.86
J	Cdeb_01911	ribosomal protein S20	11.25	11.60	12.01	11.78	11.66	-0.76	-0.18	-0.35	0.23	-0.95	0.2	-0.44	-0.27	-1.59	1.31
J	Cdeb_01059	arginyl-tRNA synthetase	11.26	11.66	12.35	11.50	11.69	-1.09	0.16	-0.40	0.85	-0.38	-0.26	-0.31	-0.73	-0.24	1.3
G	Cdeb_01498	Alpha-glucosidase, family 31 of glycosyl	11.27	11.40	11.99	10.90	11.39	-0.72	0.50	-0.13	1.09	-1.02	-0.72	-0.86	1.73	0.28	1.55
C	Cdeb_00530	ATP synthase F1 subcomplex delta subunit	11.27	11.65	12.61	10.75	11.57	-1.34	0.90	-0.38	1.86	0.05	-1.26	-0.25	-0.55	1.96	1.35
O	Cdeb_02983	ATPases with chaperone activity, ATP-binding	11.27	11.64	12.76	11.56	11.81	-1.49	0.08	-0.37	1.20	0.31	-0.15	0.22	-0.45	0.52	1.09
M	Cdeb_03330	D-alanyl-D-alanine carboxypeptidase	11.28	11.53	12.51	11.60	11.73	-1.23	-0.07	-0.25	0.91	-0.14	0.05	-0.08	0.64	-0.11	0.5
M	Cdeb_01804	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-	11.30	11.66	12.27	11.33	11.64	-0.97	0.33	-0.36	0.94	-0.59	-0.49	-0.54	-0.36	-0.04	4.6
M	Cdeb_01805	D-alanine--D-alanine ligase	11.30	11.62	12.51	11.91	11.84	-1.21	-0.29	-0.32	0.60	-0.17	0.35	0.24	0	-0.78	1.08
S	Cdeb_00671	Uncharacterized protein conserved in bacteria	11.30	11.63	12.77	11.69	11.85	-1.47	-0.06	-0.33	1.08	0.28	0.04	0.11	-0.09	0.26	2.23
R	Cdeb_00660	putative domain HDIG	11.31	11.59	11.84	10.85	11.40	-0.53	0.74	-0.28	0.99	-1.34	-1.04	-1.18	0.36	0.07	10.05
M	Cdeb_00124	Glycosyltransferase	11.31	11.72	12.92	11.60	11.89	-1.61	0.12	-0.41	1.32	0.52	-0.2	0.32	-0.82	0.78	1.07
J	Cdeb_03052	lysyl-tRNA synthetase, class II	11.31	11.51	12.44	11.60	11.72	-1.13	-0.09	-0.20	0.84	-0.31	0.08	-0.16	1.09	-0.26	0.62
R	Cdeb_00109	Putative dehydrogenase	11.32	11.58	12.55	11.67	11.78	-1.23	-0.09	-0.26	0.88	-0.14	0.08	-0.11	0.55	-0.17	0.61
E	Cdeb_02779	glycine betaine/L-proline transport ATP binding	11.32	11.53	12.40	11.02	11.57	-1.08	0.51	-0.21	1.38	-0.40	-0.73	-0.54	1	0.91	1.34
R	Cdeb_00792	Kynurenine formamidase	11.33	11.75	12.19	11.22	11.62	-0.86	0.53	-0.42	0.97	-0.78	-0.76	-0.77	-0.91	0.02	2.6
V	Cdeb_01413	ABC-type multidrug transport system, ATPase	11.33	11.59	12.88	12.24	12.01	-1.55	-0.65	-0.26	0.64	0.41	0.84	0.59	0.55	-0.7	2.28
R	Cdeb_02058	putative hydrolase of the metallo-beta-lactamase	11.34	11.70	12.78	11.52	11.84	-1.44	0.18	-0.36	1.26	0.22	-0.28	-0.25	-0.36	0.65	1.04
E	Cdeb_00522	serine hydroxymethyltransferase	11.34	11.66	12.55	11.75	11.83	-1.21	-0.09	-0.32	0.80	-0.17	0.08	-0.12	0	-0.35	1.17
C	Cdeb_02131	succinyl-CoA synthetase, beta subunit	11.34	11.86	14.11	13.44	12.69	-2.77	-1.58	-0.52	0.67	2.52	2.09	2.29	-1.82	-0.63	3.7
E	Cdeb_00821	diaminopimelate decarboxylase	11.35	11.56	12.37	11.98	11.82	-1.02	-0.42	-0.21	0.39	-0.50	0.53	0.51	1	-1.24	0.99
R	Cdeb_01667	Putative oxidoreductase	11.37	11.66	11.98	10.81	11.46	-0.61	0.85	-0.29	1.17	-1.21	-1.19	-1.20	0.27	0.46	6.92
L	Cdeb_02395	DNA mismatch repair protein MutL	11.38	11.68	12.23	11.44	11.68	-0.85	0.24	-0.30	0.79	-0.79	-0.36	-0.53	0.18	-0.37	4.59

D	Cdeb_02007	cell shape determining protein, MreB/Mrl family	11.38	11.61	12.56	11.15	11.68	-1.18	0.46	-0.23	1.41	-0.22	-0.66	-0.38	0.82	0.98	1.18
L	Cdeb_00347	DNA polymerase III, beta subunit	11.39	11.78	12.51	11.51	11.80	-1.12	0.27	-0.39	1.00	-0.33	-0.41	-0.37	-0.64	0.09	1.77
G	Cdeb_00582	PTS system, fructose-specific, IIB component/PTS	11.41	11.67	13.49	11.76	12.08	-2.08	-0.09	-0.26	1.73	1.33	0.08	0.33	0.55	1.67	1.65
J	Cdeb_02737	bacterial translation initiation factor 3	11.41	11.73	12.21	11.19	11.64	-0.80	0.54	-0.32	1.02	-0.88	-0.77	-0.82	0	0.13	19.55
X	Cdeb_00049	hypothetical protein	11.41	11.83	12.83	11.82	11.97	-1.42	0.01	-0.42	1.01	0.19	-0.05	0.10	-0.91	0.11	0.47
J	Cdeb_03142	SSU ribosomal protein S4P	11.42	11.77	12.90	11.61	11.93	-1.48	0.16	-0.35	1.29	0.29	-0.26	0.27	-0.27	0.72	1.1
T	Cdeb_00120	SH3 domain protein	11.43	11.83	12.69	12.46	12.10	-1.26	-0.63	-0.40	0.23	-0.09	0.81	0.27	-0.73	-1.59	1.01
G	Cdeb_00263	triosephosphate isomerase	11.46	11.92	12.26	10.67	11.58	-0.80	1.25	-0.46	1.59	-0.88	-1.73	-1.23	-1.27	1.37	2.26
J	Cdeb_02944	LSU ribosomal protein L14P	11.46	11.72	12.73	12.08	12.00	-1.27	-0.36	-0.26	0.65	-0.07	0.45	0.18	0.55	-0.67	1.14
R	Cdeb_01542	Diadenosine tetraphosphate (Ap4A) hydrolase and	11.47	11.88	12.34	11.76	11.86	-0.87	0.12	-0.41	0.58	-0.76	-0.2	-0.39	-0.82	-0.83	1.46
G	Cdeb_01542	Diadenosine tetraphosphate (Ap4A) hydrolase and	11.47	11.88	12.34	11.76	11.86	-0.87	0.12	-0.41	0.58	-0.76	-0.2	-0.39	-0.82	-0.83	1.46
F	Cdeb_01542	Diadenosine tetraphosphate (Ap4A) hydrolase and	11.47	11.88	12.34	11.76	11.86	-0.87	0.12	-0.41	0.58	-0.76	-0.2	-0.39	-0.82	-0.83	1.46
R	Cdeb_00059	Dehydrogenases with different specificities	11.50	11.84	11.77	10.94	11.51	-0.27	0.90	-0.34	0.83	-1.79	-1.26	-1.50	-0.18	-0.28	14.3
R	Cdeb_00860	ribosome-associated GTPase EngA	11.50	11.86	12.34	11.11	11.70	-0.84	0.75	-0.36	1.23	-0.81	-1.05	-0.92	-0.36	0.59	4.17
E	Cdeb_02653	L-arginine ABC transporter ATP-binding protein	11.50	11.80	12.00	10.80	11.53	-0.50	1.00	-0.30	1.20	-1.40	-1.39	-1.39	0.18	0.52	7.79
E	Cdeb_01422	glutamate synthase (NADPH) large subunit	11.50	11.85	11.75	10.88	11.50	-0.25	0.97	-0.35	0.87	-1.83	-1.35	-1.57	-0.27	-0.2	14.71
Q	Cdeb_00059	Dehydrogenases with different specificities	11.50	11.84	11.77	10.94	11.51	-0.27	0.90	-0.34	0.83	-1.79	-1.26	-1.50	-0.18	-0.28	14.3
I	Cdeb_00059	Dehydrogenases with different specificities	11.50	11.84	11.77	10.94	11.51	-0.27	0.90	-0.34	0.83	-1.79	-1.26	-1.50	-0.18	-0.28	14.3
E	Cdeb_02655	ABC-type amino acid transport/signal	11.51	11.84	12.47	11.11	11.73	-0.96	0.73	-0.33	1.36	-0.60	-1.03	-0.79	-0.09	0.87	2.96
T	Cdeb_02655	ABC-type amino acid transport/signal	11.51	11.84	12.47	11.11	11.73	-0.96	0.73	-0.33	1.36	-0.60	-1.03	-0.79	-0.09	0.87	2.96
K	Cdeb_02124	GTP-sensing transcriptional pleiotropic	11.52	11.86	12.80	11.55	11.93	-1.28	0.31	-0.34	1.25	-0.05	-0.46	-0.15	-0.18	0.63	1.54
E	Cdeb_01886	oligopeptide/dipeptide ABC transporter,	11.53	11.88	12.41	11.47	11.82	-0.88	0.41	-0.35	0.94	-0.74	-0.59	-0.66	-0.27	-0.04	7.54
H	Cdeb_02226	methionine adenosyltransferase	11.53	11.98	11.92	10.52	11.49	-0.39	1.46	-0.45	1.40	-1.59	-2.01	-1.79	-1.18	0.96	3.66
P	Cdeb_01886	oligopeptide/dipeptide ABC transporter,	11.53	11.88	12.41	11.47	11.82	-0.88	0.41	-0.35	0.94	-0.74	-0.59	-0.66	-0.27	-0.04	7.54
X	Cdeb_01904	Molecular chaperone	11.53	11.86	12.65	11.81	11.96	-1.12	0.05	-0.33	0.84	-0.33	-0.11	-0.19	-0.09	-0.26	2.75
G	Cdeb_02568	Putative nucleoside-diphosphate-sugar epimerase	11.54	11.91	13.03	12.22	12.18	-1.49	-0.31	-0.37	0.81	0.31	0.38	0.34	-0.45	-0.33	1.91
M	Cdeb_02568	Putative nucleoside-diphosphate-sugar epimerase	11.54	11.91	13.03	12.22	12.18	-1.49	-0.31	-0.37	0.81	0.31	0.38	0.34	-0.45	-0.33	1.91
F	Cdeb_00871	nucleoside diphosphate kinase	11.55	11.80	13.14	12.45	12.24	-1.59	-0.65	-0.25	0.69	0.48	0.84	0.63	0.64	-0.59	2.42
C	Cdeb_00861	glycerol 3-phosphate dehydrogenase (NAD(P)+)	11.55	11.83	13.33	12.67	12.35	-1.78	-0.84	-0.28	0.66	0.81	1.09	0.94	0.36	-0.65	3.97
J	Cdeb_02943	ribosomal protein L24, bacterial/organelle	11.56	11.85	12.77	11.52	11.93	-1.21	0.33	-0.29	1.25	-0.17	-0.49	-0.29	0.27	0.63	1.64
K	Cdeb_01794	Transcriptional accessory protein	11.57	11.93	12.74	11.68	11.98	-1.17	0.25	-0.36	1.06	-0.24	-0.38	-0.30	-0.36	0.22	2.32
R	Cdeb_02053	nucleoside ABC transporter ATP-binding protein	11.58	11.94	12.84	11.65	12.00	-1.26	0.29	-0.36	1.19	-0.09	-0.43	-0.20	-0.36	0.5	1.55
G	Cdeb_00462	Trehalose and maltose hydrolases (possible	11.60	11.92	12.84	11.76	12.03	-1.24	0.16	-0.32	1.08	-0.12	-0.26	-0.18	0	0.26	2.39

H	Cdeb_00866	GTP cyclohydrolase I	11.60	11.80	12.72	11.16	11.82	-1.12	0.64	-0.20	1.56	-0.33	-0.91	-0.55	1.09	1.3	1.24
R	Cdeb_02186	Gas vesicle protein	11.61	11.91	12.78	11.51	11.95	-1.17	0.40	-0.30	1.27	-0.24	-0.58	-0.37	0.18	0.67	1.97
G	Cdeb_03196	phosphoglucosamine mutase	11.61	11.94	12.98	11.89	12.11	-1.37	0.05	-0.33	1.09	0.10	-0.11	-0.10	-0.09	0.28	1.1
J	Cdeb_03332	seryl-tRNA synthetase	11.61	11.94	13.19	12.01	12.19	-1.58	-0.07	-0.33	1.18	0.47	0.05	0.15	-0.09	0.48	2.1
C	Cdeb_00533	ATP synthase F1 subcomplex beta subunit	11.62	11.92	12.87	11.17	11.90	-1.25	0.75	-0.30	1.70	-0.10	-1.05	-0.32	0.18	1.61	1.42
R	Cdeb_03211	DAK2 domain fusion protein YloV	11.63	11.89	13.07	12.19	12.20	-1.44	-0.30	-0.26	0.88	0.22	0.36	0.28	0.55	-0.17	1.59
X	Cdeb_00931	Scaffold protein Nfu/NifU N terminal/Virulence	11.65	11.99	12.97	11.87	12.12	-1.32	0.12	-0.34	1.10	0.02	-0.2	-0.06	-0.18	0.3	1.25
E	Cdeb_00090	ABC-type spermidine/putrescine transport system,	11.66	12.09	13.07	12.28	12.28	-1.41	-0.19	-0.43	0.79	0.17	0.22	0.19	-1	-0.37	0.57
L	Cdeb_02901	DNA polymerase I	11.66	11.97	12.82	12.05	12.13	-1.16	-0.08	-0.31	0.77	-0.26	0.07	-0.13	0.09	-0.41	1.39
X	Cdeb_01869	Bacterial extracellular solute-binding protein,	11.66	12.12	13.86	13.09	12.68	-2.20	-0.97	-0.46	0.77	1.53	1.27	1.39	-1.27	-0.41	3.24
X	Cdeb_02437	Putative cell-wall binding lipoprotein	11.66	12.00	13.01	12.30	12.24	-1.35	-0.30	-0.34	0.71	0.07	0.36	0.16	-0.18	-0.54	1.4
P	Cdeb_02872	copper-(or silver)-translocating P-type ATPase	11.68	12.05	14.45	13.20	12.85	-2.77	-1.15	-0.37	1.25	2.52	1.51	1.95	-0.45	0.63	8.25
J	Cdeb_02081	prolyl-tRNA synthetase	11.70	12.05	12.90	11.87	12.13	-1.20	0.18	-0.35	1.03	-0.19	-0.28	-0.23	-0.27	0.15	2.38
J	Cdeb_01996	ribosomal protein L27	11.71	11.90	12.81	12.36	12.20	-1.10	-0.46	-0.19	0.45	-0.36	0.58	0.46	1.18	-1.11	0.92
J	Cdeb_01969	aspartyl-tRNA synthetase	11.72	11.91	12.42	11.62	11.92	-0.70	0.29	-0.19	0.80	-1.05	-0.43	-0.67	1.18	-0.35	2
K	Cdeb_00833	Response regulators consisting of a CheY-like	11.73	11.97	13.36	12.47	12.38	-1.63	-0.50	-0.24	0.89	0.55	0.64	0.59	0.73	-0.15	2.46
T	Cdeb_00833	Response regulators consisting of a CheY-like	11.73	11.97	13.36	12.47	12.38	-1.63	-0.50	-0.24	0.89	0.55	0.64	0.59	0.73	-0.15	2.46
J	Cdeb_02978	glutamyl-tRNA synthetase, bacterial family	11.73	12.09	13.21	12.67	12.43	-1.48	-0.58	-0.36	0.54	0.29	0.74	0.46	-0.36	-0.91	1.77
P	Cdeb_01611	plasma-membrane calcium-translocating P-type	11.73	12.01	12.50	11.93	12.04	-0.77	0.08	-0.28	0.57	-0.93	-0.15	-0.37	0.36	-0.85	2.22
R	Cdeb_01044	intracellular protease, PfpI family	11.74	12.01	12.80	11.63	12.05	-1.06	0.38	-0.27	1.17	-0.43	-0.55	-0.49	0.45	0.46	2.36
E	Cdeb_02062	aspartate semialdehyde dehydrogenase	11.75	12.04	12.70	11.41	11.98	-0.95	0.63	-0.29	1.29	-0.62	-0.89	-0.74	0.27	0.72	3.07
C	Cdeb_00962	2-oxoglutarate dehydrogenase E2 component	11.76	12.13	13.63	12.52	12.51	-1.87	-0.39	-0.37	1.11	0.97	0.49	0.69	-0.45	0.33	4.23
I	Cdeb_00686	4-hydroxy-3-methylbut-2-en-1-yl diphosphate	11.76	11.95	13.08	11.83	12.16	-1.32	0.12	-0.19	1.25	0.02	-0.2	-0.06	1.18	0.63	0.33
F	Cdeb_00814	pyrimidine-nucleoside phosphorylase	11.77	12.12	12.98	11.67	12.14	-1.21	0.45	-0.35	1.31	-0.17	-0.65	-0.33	-0.27	0.76	1.81
X	Cdeb_01759	Glutamine synthetase	11.80	12.13	12.37	10.75	11.76	-0.57	1.38	-0.33	1.62	-1.28	-1.91	-1.56	-0.09	1.43	3.49
M	Cdeb_00579	UTP-glucose-1-phosphate uridylyltransferase	11.81	12.05	12.22	11.69	11.94	-0.41	0.36	-0.24	0.53	-1.55	-0.53	-0.91	0.73	-0.93	3.01
O	Cdeb_03064	membrane protease FtsH catalytic subunit	11.81	11.96	12.76	12.10	12.16	-0.95	-0.14	-0.15	0.66	-0.62	0.15	-0.30	1.55	-0.65	0.83
J	Cdeb_02174	tyrosyl-tRNA synthetase	11.83	12.04	12.67	12.45	12.25	-0.84	-0.41	-0.21	0.22	-0.81	0.51	-0.64	1	-1.61	1.1
L	Cdeb_03260	DNA ligase, NAD-dependent	11.84	12.19	13.29	12.12	12.36	-1.45	0.07	-0.35	1.17	0.24	-0.14	0.18	-0.27	0.46	1.13
J	Cdeb_03250	aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase	11.84	12.12	13.08	11.85	12.22	-1.24	0.27	-0.28	1.23	-0.12	-0.41	-0.22	0.36	0.59	1.34
O	Cdeb_01550	Parvulin-like peptidyl-prolyl isomerase	11.85	12.18	12.94	12.59	12.39	-1.09	-0.41	-0.33	0.35	-0.38	0.51	0.44	-0.09	-1.33	1.04
G	Cdeb_00791	6-phosphogluconate dehydrogenase	11.86	12.16	13.32	12.11	12.36	-1.46	0.05	-0.30	1.21	0.26	-0.11	0.17	0.18	0.54	1.08
C	Cdeb_02349	aconitate hydratase 1	11.86	12.23	13.98	13.24	12.83	-2.12	-1.01	-0.37	0.74	1.40	1.32	1.36	-0.45	-0.48	6.36

I	Cdeb_01486	3-oxoacyl-(acyl-carrier-protein) synthase III	11.86	12.15	12.41	11.51	11.98	-0.55	0.64	-0.29	0.90	-1.31	-0.91	-1.09	0.27	-0.13	11.57
J	Cdeb_02887	threonyl-tRNA synthetase	11.87	12.35	12.67	11.73	12.16	-0.80	0.62	-0.48	0.94	-0.88	-0.88	-0.88	-1.45	-0.04	1.87
J	Cdeb_02024	valyl-tRNA synthetase	11.88	12.21	13.21	12.30	12.40	-1.33	-0.09	-0.33	0.91	0.03	0.08	0.05	-0.09	-0.11	1.31
O	Cdeb_00313	Iron-regulated ABC transporter ATPase subunit	11.90	12.25	13.42	12.70	12.57	-1.52	-0.45	-0.35	0.72	0.36	0.57	0.45	-0.27	-0.52	2.5
E	Cdeb_01643	Leucyl aminopeptidase (aminopeptidase T)	11.93	12.23	13.22	12.66	12.51	-1.29	-0.43	-0.30	0.56	-0.03	0.54	0.13	0.18	-0.87	1.32
U	Cdeb_01977	protein translocase subunit secF	11.94	12.32	13.09	12.00	12.34	-1.15	0.32	-0.38	1.09	-0.28	-0.47	-0.36	-0.55	0.28	1.93
U	Cdeb_02143	signal recognition particle subunit FFH/SRP54	11.94	12.21	13.36	12.52	12.51	-1.42	-0.31	-0.27	0.84	0.19	0.38	0.27	0.45	-0.26	1.78
R	Cdeb_01527	ABC-type uncharacterized transport system,	11.96	12.32	13.16	12.57	12.50	-1.20	-0.25	-0.36	0.59	-0.19	0.3	0.24	-0.36	-0.8	0.88
J	Cdeb_00366	LSU ribosomal protein L9P	11.99	12.29	13.29	11.87	12.36	-1.30	0.42	-0.30	1.42	-0.02	-0.61	-0.11	0.18	1	1.31
F	Cdeb_00646	deoxyribose-phosphate aldolase	12.01	12.23	12.95	11.76	12.24	-0.94	0.47	-0.22	1.19	-0.64	-0.68	-0.66	0.91	0.5	1.96
J	Cdeb_02521	Isoleucyl-tRNA synthetase	12.03	12.36	13.24	12.11	12.44	-1.21	0.25	-0.33	1.13	-0.17	-0.38	-0.25	-0.09	0.37	2.38
J	Cdeb_02955	SSU ribosomal protein S10P	12.03	12.27	13.16	12.70	12.54	-1.13	-0.43	-0.24	0.46	-0.31	0.54	0.41	0.73	-1.09	1.03
X	Cdeb_00162	Subtilase family	12.03	12.48	13.64	12.78	12.73	-1.61	-0.30	-0.45	0.86	0.52	0.36	0.43	-1.18	-0.22	1.15
M	Cdeb_00903	penicillin-binding protein, 1A family	12.04	12.26	12.94	11.75	12.25	-0.90	0.51	-0.22	1.19	-0.71	-0.73	-0.72	0.91	0.5	2.13
C	Cdeb_00529	ATP synthase F0 subcomplex B subunit	12.04	12.28	13.20	11.87	12.35	-1.16	0.41	-0.24	1.33	-0.26	-0.59	-0.39	0.73	0.8	1.29
M	Cdeb_00559	Capsular polysaccharide biosynthesis protein	12.06	12.22	13.23	11.69	12.30	-1.17	0.53	-0.16	1.54	-0.24	-0.76	-0.43	1.45	1.26	0.9
D	Cdeb_02508	cell division protein FtsA	12.06	12.34	13.08	12.10	12.40	-1.02	0.24	-0.28	0.98	-0.50	-0.36	-0.42	0.36	0.04	3.7
O	Cdeb_02696	Thioredoxin reductase	12.07	12.33	13.11	12.39	12.48	-1.04	-0.06	-0.26	0.72	-0.47	0.04	-0.14	0.55	-0.52	1.35
J	Cdeb_02968	LSU ribosomal protein L11P	12.08	12.33	13.28	12.53	12.56	-1.20	-0.20	-0.25	0.75	-0.19	0.23	0.21	0.64	-0.46	0.82
T	Cdeb_00243	Hpr(Ser) kinase/phosphatase	12.09	12.41	12.75	11.79	12.26	-0.66	0.62	-0.32	0.96	-1.12	-0.88	-0.99	0	0	30
S	Cdeb_00468	Uncharacterized conserved protein	12.10	12.45	14.12	12.76	12.86	-2.02	-0.31	-0.35	1.36	1.22	0.38	0.68	-0.27	0.87	3.05
P	Cdeb_01616	phosphate/phosphite/phosphonate ABC transporter,	12.11	12.44	13.49	12.81	12.71	-1.38	-0.37	-0.33	0.68	0.12	0.46	0.23	-0.09	-0.61	1.68
P	Cdeb_01841	ABC-type Fe3+-hydroxamate transport system,	12.11	12.38	13.60	13.06	12.79	-1.49	-0.68	-0.27	0.54	0.31	0.88	0.52	0.45	-0.91	2
G	Cdeb_00110	alpha-phosphoglucomutase	12.12	12.41	13.48	12.72	12.68	-1.36	-0.31	-0.29	0.76	0.09	0.38	0.18	0.27	-0.43	1.67
P	Cdeb_00198	ABC-type enterochelin transport system,	12.12	12.53	13.63	12.79	12.77	-1.51	-0.26	-0.41	0.84	0.34	0.31	0.32	-0.82	-0.26	1.16
G	Cdeb_03023	Glucose-6-phosphate isomerase	12.14	12.55	12.95	11.79	12.36	-0.81	0.76	-0.41	1.16	-0.86	-1.07	-0.96	-0.82	0.43	3.22
C	Cdeb_03124	acetate kinase	12.17	12.43	13.26	12.10	12.49	-1.09	0.33	-0.26	1.16	-0.38	-0.49	-0.43	0.55	0.43	1.93
K	Cdeb_02928	DNA-directed RNA polymerase subunit alpha	12.18	12.53	13.06	12.06	12.46	-0.88	0.47	-0.35	1.00	-0.74	-0.68	-0.71	-0.27	0.09	7.68
J	Cdeb_02216	leucyl-tRNA synthetase	12.18	12.46	13.46	12.45	12.64	-1.28	0.01	-0.28	1.01	-0.05	-0.05	-0.05	0.36	0.11	0.41
X	Cdeb_00934	YtkA	12.20	12.46	13.32	12.53	12.63	-1.12	-0.07	-0.26	0.79	-0.33	0.05	-0.13	0.55	-0.37	1.09
L	Cdeb_00239	Excinuclease ABC subunit A	12.24	12.49	13.55	12.54	12.71	-1.31	-0.05	-0.25	1.01	0.00	0.03	0.00	0.64	0.11	0.1
P	Cdeb_00043	Mn-containing catalase	12.24	12.65	14.15	13.37	13.10	-1.91	-0.72	-0.41	0.78	1.03	0.93	0.98	-0.82	-0.39	3.32
G	Cdeb_00623	xylose repressor, XylR	12.25	12.41	13.09	12.54	12.57	-0.84	-0.13	-0.16	0.55	-0.81	0.14	-0.34	1.45	-0.89	1.05

K	Cdeb_00623	xylose repressor, XylR	12.25	12.41	13.09	12.54	12.57	-0.84	-0.13	-0.16	0.55	-0.81	0.14	-0.34	1.45	-0.89	1.05
L	Cdeb_02758	MutS2 family protein	12.25	12.54	13.41	12.60	12.70	-1.16	-0.06	-0.29	0.81	-0.26	0.04	-0.10	0.27	-0.33	1.34
C	Cdeb_01569	Zn-dependent alcohol dehydrogenase, class III	12.25	12.57	13.55	12.52	12.72	-1.30	0.05	-0.32	1.03	-0.02	-0.11	-0.05	0	0.15	1.62
J	Cdeb_02142	SSU ribosomal protein S16P	12.27	12.46	13.09	12.66	12.62	-0.82	-0.20	-0.19	0.43	-0.84	0.23	-0.44	1.18	-1.15	1.15
O	Cdeb_02125	ATP-dependent protease HslVU, ATPase subunit	12.31	12.64	13.46	12.44	12.71	-1.15	0.20	-0.33	1.02	-0.28	-0.31	-0.29	-0.09	0.13	5.74
E	Cdeb_00440	ABC-type dipeptide transport system, periplasmic	12.33	12.69	13.61	12.46	12.77	-1.28	0.23	-0.36	1.15	-0.05	-0.35	-0.13	-0.36	0.41	1.41
G	Cdeb_00242	N-acetylglucosamine 6-phosphate deacetylase	12.34	12.65	12.93	11.59	12.38	-0.59	1.06	-0.31	1.34	-1.24	-1.47	-1.35	0.09	0.83	5.01
E	Cdeb_02060	dihydrodipicolinate synthase	12.35	12.59	13.06	12.71	12.68	-0.71	-0.12	-0.24	0.35	-1.03	0.12	-0.35	0.73	-1.33	1.49
M	Cdeb_02060	dihydrodipicolinate synthase	12.35	12.59	13.06	12.71	12.68	-0.71	-0.12	-0.24	0.35	-1.03	0.12	-0.35	0.73	-1.33	1.49
N	Cdeb_00191	Flagellin and related hook-associated protein	12.36	12.52	13.56	12.74	12.80	-1.20	-0.22	-0.16	0.82	-0.19	0.26	0.22	1.45	-0.3	0.47
S	Cdeb_00758	Uncharacterized protein conserved in bacteria	12.38	12.49	13.11	12.29	12.57	-0.73	0.20	-0.11	0.82	-1.00	-0.31	-0.56	1.91	-0.3	1.18
D	Cdeb_02519	DivIVA domain	12.40	12.67	12.80	12.20	12.52	-0.40	0.47	-0.27	0.60	-1.57	-0.68	-1.03	0.45	-0.78	4.13
X	Cdeb_00227	Preprotein translocase subunit SecA (ATPase, RNA	12.40	12.71	13.77	12.53	12.85	-1.37	0.18	-0.31	1.24	0.10	-0.28	-0.17	0.09	0.61	1.05
G	Cdeb_00506	transaldolase	12.41	12.60	13.42	12.27	12.68	-1.01	0.33	-0.19	1.15	-0.52	-0.49	-0.50	1.18	0.41	1.24
J	Cdeb_02953	50S ribosomal protein L4, bacterial/organelle	12.42	12.60	13.15	12.71	12.72	-0.73	-0.11	-0.18	0.44	-1.00	0.11	-0.33	1.27	-1.13	1.29
F	Cdeb_03065	hypoxanthine phosphoribosyltransferase	12.43	12.77	13.25	12.83	12.82	-0.82	-0.06	-0.34	0.42	-0.84	0.04	-0.18	-0.18	-1.17	1.54
J	Cdeb_02929	30S ribosomal protein S11	12.43	12.75	13.35	12.52	12.76	-0.92	0.23	-0.32	0.83	-0.67	-0.35	-0.48	0	-0.28	5.87
G	Cdeb_01068	phosphotransferase system, cellobiose specific,	12.45	12.55	12.02	11.10	12.03	0.43	1.45	-0.10	0.92	-3.00	-2	-2.45	2	-0.09	3.92
J	Cdeb_02951	ribosomal protein L2, bacterial/organellar	12.45	12.73	13.72	13.01	12.98	-1.27	-0.28	-0.28	0.71	-0.07	0.34	0.15	0.36	-0.54	1.16
G	Cdeb_02169	Phosphotransferase system cellobiose-specific	12.48	12.51	13.15	11.25	12.35	-0.67	1.26	-0.03	1.90	-1.10	-1.74	-1.38	2.64	2.04	1.34
E	Cdeb_01822	Dipeptidyl	12.48	12.87	13.36	12.32	12.76	-0.88	0.55	-0.39	1.04	-0.74	-0.78	-0.76	-0.64	0.17	3.53
J	Cdeb_02959	SSU ribosomal protein S12P	12.52	12.78	13.86	13.13	13.07	-1.34	-0.35	-0.26	0.73	0.05	0.43	0.15	0.55	-0.5	1.27
J	Cdeb_03097	methionyl-tRNA synthetase	12.52	12.76	13.51	12.06	12.71	-0.99	0.70	-0.24	1.45	-0.55	-0.99	-0.74	0.73	1.07	1.9
I	Cdeb_01466	Enoyl-(acyl-carrier-protein) reductase (NADH)	12.54	12.91	13.59	12.88	12.98	-1.05	0.03	-0.37	0.71	-0.45	-0.08	-0.19	-0.45	-0.54	1.41
J	Cdeb_02068	polyribonucleotide nucleotidyltransferase	12.58	12.86	14.08	12.88	13.10	-1.50	-0.02	-0.28	1.20	0.33	-0.01	0.06	0.36	0.52	1.13
O	Cdeb_00317	Iron-regulated ABC transporter membrane	12.58	12.95	14.31	13.93	13.44	-1.73	-0.98	-0.37	0.38	0.72	1.28	0.96	-0.45	-1.26	2.39
R	Cdeb_01686	putative NAD(FAD)-dependent dehydrogenase	12.59	12.94	13.67	12.48	12.92	-1.08	0.46	-0.35	1.19	-0.40	-0.66	-0.51	-0.27	0.5	2.95
D	Cdeb_02509	cell division protein FtsZ	12.59	12.90	13.67	12.84	13.00	-1.08	0.06	-0.31	0.83	-0.40	-0.12	-0.22	0.09	-0.28	3.09
M	Cdeb_03084	UDP-N-acetylglucosamine	12.60	12.93	13.80	13.09	13.11	-1.20	-0.16	-0.33	0.71	-0.19	0.18	-0.18	-0.09	-0.54	1.04
J	Cdeb_02930	30S ribosomal protein S13	12.63	12.86	13.24	12.23	12.74	-0.61	0.63	-0.23	1.01	-1.21	-0.89	-1.04	0.82	0.11	3.95
P	Cdeb_00312	ABC-type metal ion transport system, periplasmic	12.66	13.05	14.40	13.38	13.37	-1.74	-0.33	-0.39	1.02	0.74	0.41	0.55	-0.64	0.13	2.82
C	Cdeb_02439	Pyruvate/2-oxoglutarate dehydrogenase complex,	12.70	13.19	15.36	14.47	13.93	-2.66	-1.28	-0.49	0.89	2.33	1.69	1.98	-1.55	-0.15	4.02
C	Cdeb_00469	phosphate acetyltransferase	12.70	12.92	13.55	12.94	13.03	-0.85	-0.02	-0.22	0.61	-0.79	-0.01	-0.09	0.91	-0.76	1.45

R	Cdeb_02040	putative HD superfamily hydrolase	12.71	13.09	13.51	12.62	12.98	-0.80	0.47	-0.38	0.89	-0.88	-0.68	-0.77	-0.55	-0.15	4.24
G	Cdeb_01816	6-phospho-beta-glucosidase	12.74	12.96	13.93	13.12	13.19	-1.19	-0.16	-0.22	0.81	-0.21	0.18	-0.19	0.91	-0.33	0.62
J	Cdeb_02954	50S ribosomal protein L3, bacterial	12.74	13.02	13.93	12.92	13.15	-1.19	0.10	-0.28	1.01	-0.21	-0.18	-0.19	0.36	0.11	1.6
E	Cdeb_02702	amino acid/amide ABC transporter	12.78	13.09	14.41	13.40	13.42	-1.63	-0.31	-0.31	1.01	0.55	0.38	0.46	0.09	0.11	10.23
E	Cdeb_01476	oligopeptidase F	12.78	13.12	13.87	12.28	13.01	-1.09	0.84	-0.34	1.59	-0.38	-1.18	-0.67	-0.18	1.37	1.95
J	Cdeb_03249	aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase	12.80	12.98	13.95	12.90	13.16	-1.15	0.08	-0.18	1.05	-0.28	-0.15	-0.20	1.27	0.2	0.54
X	Cdeb_01109	Protein of unknown function (DUF1177)	12.81	13.04	13.59	12.63	13.02	-0.78	0.41	-0.23	0.96	-0.91	-0.59	-0.73	0.82	0	2.88
J	Cdeb_02927	LSU ribosomal protein L17P	12.82	13.20	12.67	12.59	12.82	0.15	0.61	-0.38	0.08	-2.52	-0.86	-1.47	-0.55	-1.91	2.91
J	Cdeb_02965	LSU ribosomal protein L10P	12.85	13.06	13.98	12.90	13.20	-1.13	0.16	-0.21	1.08	-0.31	-0.26	-0.28	1	0.26	0.85
J	Cdeb_03345	SSU ribosomal protein S9P	12.86	13.06	13.74	13.03	13.17	-0.88	0.03	-0.20	0.71	-0.74	-0.08	-0.24	1.09	-0.54	1.33
E	Cdeb_01107	oligopeptide/dipeptide ABC transporter,	12.87	13.26	13.43	12.81	13.09	-0.56	0.45	-0.39	0.62	-1.29	-0.65	-0.92	-0.64	-0.74	3.21
P	Cdeb_01107	oligopeptide/dipeptide ABC transporter,	12.87	13.26	13.43	12.81	13.09	-0.56	0.45	-0.39	0.62	-1.29	-0.65	-0.92	-0.64	-0.74	3.21
J	Cdeb_02938	LSU ribosomal protein L18P	12.89	13.13	14.21	12.90	13.28	-1.32	0.23	-0.24	1.31	0.02	-0.35	-0.08	0.73	0.76	0.72
G	Cdeb_00708	carbohydrate ABC transporter substrate-binding	12.90	13.12	13.96	13.08	13.27	-1.06	0.04	-0.22	0.88	-0.43	-0.09	-0.20	0.91	-0.17	1.03
J	Cdeb_01945	alanyl-tRNA synthetase	12.90	13.24	13.94	13.05	13.28	-1.04	0.19	-0.34	0.89	-0.47	-0.3	-0.38	-0.18	-0.15	5.17
J	Cdeb_02950	SSU ribosomal protein S19P	12.92	13.15	13.18	12.65	12.98	-0.26	0.50	-0.23	0.53	-1.81	-0.72	-1.14	0.82	-0.93	3.42
E	Cdeb_01060	arginine deiminase	12.96	13.23	13.25	12.00	12.86	-0.29	1.23	-0.27	1.25	-1.76	-1.7	-1.73	0.45	0.63	6.87
J	Cdeb_01998	LSU ribosomal protein L21P	12.99	13.16	13.75	13.57	13.37	-0.76	-0.41	-0.17	0.18	-0.95	0.51	-0.70	1.36	-1.7	1.08
J	Cdeb_02939	ribosomal protein L6, bacterial type	13.08	13.25	14.06	13.38	13.44	-0.98	-0.13	-0.17	0.68	-0.57	0.14	-0.28	1.36	-0.61	0.86
C	Cdeb_03258	delta-1-pyrroline-5-carboxylate dehydrogenase	13.12	13.37	14.31	12.99	13.45	-1.19	0.38	-0.25	1.32	-0.21	-0.55	-0.34	0.64	0.78	1.27
J	Cdeb_02074	translation initiation factor IF-2	13.13	13.36	14.34	13.84	13.67	-1.21	-0.48	-0.23	0.50	-0.17	0.61	0.32	0.82	-1	1.06
G	Cdeb_02418	phosphoenolpyruvate--protein phosphotransferase	13.14	13.45	13.97	12.86	13.36	-0.83	0.59	-0.31	1.11	-0.83	-0.84	-0.83	0.09	0.33	7.51
E	Cdeb_01061	ornithine carbamoyltransferase	13.15	13.31	12.91	11.96	12.83	0.24	1.35	-0.16	0.95	-2.67	-1.86	-2.23	1.45	-0.02	4.88
J	Cdeb_00361	ribosomal protein S6	13.17	13.38	13.94	13.41	13.48	-0.77	-0.03	-0.21	0.53	-0.93	0	0.00	1	-0.93	1.48
R	Cdeb_00489	Zn-dependent alcohol dehydrogenase	13.29	13.59	13.25	12.18	13.08	0.04	1.41	-0.30	1.07	-2.33	-1.95	-2.13	0.18	0.24	22.02
M	Cdeb_03085	Uncharacterized protein, involved in the	13.30	13.57	13.94	13.06	13.47	-0.64	0.51	-0.27	0.88	-1.16	-0.73	-0.92	0.45	-0.17	6.19
G	Cdeb_00505	fructose-bisphosphate aldolase	13.31	13.48	14.26	13.10	13.54	-0.95	0.38	-0.17	1.16	-0.62	-0.55	-0.58	1.36	0.43	1.26
C	Cdeb_02440	Pyruvate/2-oxoglutarate dehydrogenase complex,	13.31	13.71	15.38	14.79	14.30	-2.07	-1.08	-0.40	0.59	1.31	1.42	1.36	-0.73	-0.8	3.88
S	Cdeb_00655	Uncharacterized protein conserved in bacteria	13.32	13.62	14.23	13.61	13.70	-0.91	0.01	-0.30	0.62	-0.69	-0.05	-0.19	0.18	-0.74	1.97
J	Cdeb_02548	arginyl-tRNA synthetase	13.34	13.68	13.50	12.57	13.27	-0.16	1.11	-0.34	0.93	-1.98	-1.54	-1.75	-0.18	-0.07	28.23
J	Cdeb_02967	ribosomal protein L1, bacterial/chloroplast	13.34	13.66	14.33	13.83	13.79	-0.99	-0.17	-0.32	0.50	-0.55	0.19	-0.32	0	-1	1.26
O	Cdeb_02857	trigger factor	13.34	13.60	14.33	13.22	13.62	-0.99	0.38	-0.26	1.11	-0.55	-0.55	-0.55	0.55	0.33	2.64
R	Cdeb_02150	3-oxoacyl-(acyl-carrier-protein) reductase	13.38	13.61	14.47	13.86	13.83	-1.09	-0.25	-0.23	0.61	-0.38	0.3	-0.34	0.82	-0.76	0.94

Q	Cdeb_02150	3-oxoacyl-(acyl-carrier-protein) reductase	13.38	13.61	14.47	13.86	13.83	-1.09	-0.25	-0.23	0.61	-0.38	0.3	-0.34	0.82	-0.76	0.94
J	Cdeb_02957	translation elongation factor 2 (EF-2/EF-G)	13.38	13.73	14.64	13.83	13.90	-1.26	-0.10	-0.35	0.81	-0.09	0.09	-0.09	-0.27	-0.33	0.65
I	Cdeb_02150	3-oxoacyl-(acyl-carrier-protein) reductase	13.38	13.61	14.47	13.86	13.83	-1.09	-0.25	-0.23	0.61	-0.38	0.3	-0.34	0.82	-0.76	0.94
R	Cdeb_01534	Threonine dehydrogenase and related Zn-dependent	13.40	13.54	13.83	12.63	13.35	-0.43	0.91	-0.14	1.20	-1.52	-1.27	-1.39	1.64	0.52	2.5
E	Cdeb_01534	Threonine dehydrogenase and related Zn-dependent	13.40	13.54	13.83	12.63	13.35	-0.43	0.91	-0.14	1.20	-1.52	-1.27	-1.39	1.64	0.52	2.5
M	Cdeb_00369	Membrane protein related to metalloendopeptidase	13.41	13.52	13.04	12.00	12.99	0.37	1.52	-0.11	1.04	-2.90	-2.09	-2.46	1.91	0.17	4.05
K	Cdeb_01071	Transcriptional antiterminator	13.43	13.57	13.51	12.52	13.26	-0.08	1.05	-0.14	0.99	-2.12	-1.46	-1.76	1.64	0.07	3.41
C	Cdeb_00531	ATP synthase F1 subcomplex alpha subunit	13.43	13.76	14.63	13.66	13.87	-1.20	0.10	-0.33	0.97	-0.19	-0.18	-0.18	-0.09	0.02	6.17
C	Cdeb_01397	acetaldehyde dehydrogenase	13.46	13.56	13.86	12.30	13.30	-0.40	1.26	-0.10	1.56	-1.57	-1.74	-1.65	2	1.3	2.14
C	Cdeb_02441	dihydrolipoamide dehydrogenase	13.47	13.76	15.31	14.59	14.28	-1.84	-0.83	-0.29	0.72	0.91	1.08	0.99	0.27	-0.52	5.24
J	Cdeb_02937	SSU ribosomal protein S5P	13.56	13.82	14.51	13.60	13.87	-0.95	0.22	-0.26	0.91	-0.62	-0.34	-0.46	0.55	-0.11	2.74
E	Cdeb_01519	Dipeptidyl	13.68	13.87	14.89	14.20	14.16	-1.21	-0.33	-0.19	0.69	-0.17	0.41	0.26	1.18	-0.59	0.73
J	Cdeb_02137	ribosomal protein L19, bacterial type	13.68	13.92	14.30	13.56	13.87	-0.62	0.36	-0.24	0.74	-1.19	-0.53	-0.79	0.73	-0.48	3.24
O	Cdeb_01768	Co-chaperonin GroES (HSP10)	13.69	14.03	14.53	14.19	14.11	-0.84	-0.16	-0.34	0.34	-0.81	0.18	-0.38	-0.18	-1.35	1.32
E	Cdeb_02352	Acetylornithine	13.74	13.98	14.37	13.70	13.95	-0.63	0.28	-0.24	0.67	-1.17	-0.42	-0.70	0.73	-0.63	2.8
C	Cdeb_01638	formate acetyltransferase 1	13.75	14.09	15.15	13.63	14.16	-1.40	0.46	-0.34	1.52	0.16	-0.66	-0.32	-0.18	1.22	1.2
G	Cdeb_00459	carbohydrate ABC transporter substrate-binding	13.79	13.98	15.16	14.48	14.35	-1.37	-0.50	-0.19	0.68	0.10	0.64	0.25	1.18	-0.61	1.06
J	Cdeb_02956	translation elongation factor 1A (EF-1A/EF-Tu)	13.81	14.15	14.75	13.93	14.16	-0.94	0.22	-0.34	0.82	-0.64	-0.34	-0.47	-0.18	-0.3	4.5
R	Cdeb_03025	Threonine dehydrogenase and related Zn-dependent	13.85	14.13	14.67	13.49	14.04	-0.82	0.64	-0.28	1.18	-0.84	-0.91	-0.87	0.36	0.48	4.49
E	Cdeb_03025	Threonine dehydrogenase and related Zn-dependent	13.85	14.13	14.67	13.49	14.04	-0.82	0.64	-0.28	1.18	-0.84	-0.91	-0.87	0.36	0.48	4.49
J	Cdeb_02948	SSU ribosomal protein S3P	13.90	14.03	14.82	14.01	14.19	-0.92	0.02	-0.13	0.81	-0.67	-0.07	-0.22	1.73	-0.33	0.83
L	Cdeb_00865	bacterial nucleoid protein Hbs	13.92	14.10	14.75	14.23	14.25	-0.83	-0.13	-0.18	0.52	-0.83	0.14	-0.34	1.27	-0.96	1.15
G	Cdeb_02385	transketolase	13.94	14.15	14.80	14.13	14.26	-0.86	0.02	-0.21	0.67	-0.78	-0.07	-0.23	1	-0.63	1.44
K	Cdeb_02961	DNA-directed RNA polymerase subunit beta'	13.96	14.28	15.11	13.95	14.33	-1.15	0.33	-0.32	1.16	-0.28	-0.49	-0.37	0	0.43	2.85
R	Cdeb_02054	nucleoside-binding protein	14.02	14.23	14.91	14.34	14.38	-0.89	-0.11	-0.21	0.57	-0.72	0.11	-0.28	1	-0.85	1.21
C	Cdeb_00578	glycerol 2-dehydrogenase (NAD+)	14.04	14.32	15.33	14.00	14.42	-1.29	0.32	-0.28	1.33	-0.03	-0.47	-0.12	0.36	0.8	1.17
Q	Cdeb_01485	3-oxoacyl-(acyl-carrier-protein) synthase II	14.19	14.41	14.66	13.87	14.28	-0.47	0.54	-0.22	0.79	-1.45	-0.77	-1.06	0.91	-0.37	3.63
I	Cdeb_01485	3-oxoacyl-(acyl-carrier-protein) synthase II	14.19	14.41	14.66	13.87	14.28	-0.47	0.54	-0.22	0.79	-1.45	-0.77	-1.06	0.91	-0.37	3.63
K	Cdeb_02962	DNA-directed RNA polymerase subunit beta	14.20	14.51	15.35	14.37	14.61	-1.15	0.14	-0.31	0.98	-0.28	-0.23	-0.25	0.09	0.04	8
G	Cdeb_00265	enolase	14.37	14.57	15.19	14.11	14.56	-0.82	0.46	-0.20	1.08	-0.84	-0.66	-0.74	1.09	0.26	2.07
E	Cdeb_01104	ABC-type dipeptide transport system, periplasmic	14.74	14.97	15.25	14.97	14.98	-0.51	0.00	-0.23	0.28	-1.38	-0.04	-0.23	0.82	-1.48	1.77
O	Cdeb_01767	chaperonin GroL	15.26	15.50	16.40	15.63	15.70	-1.14	-0.13	-0.24	0.77	-0.29	0.14	-0.20	0.73	-0.41	0.84
R	Cdeb_00796	Dehydrogenases with different specificities	15.27	15.58	16.45	15.83	15.78	-1.18	-0.25	-0.31	0.62	-0.22	0.3	0.26	0.09	-0.74	1.08

Q	Cdeb_00796	Dehydrogenases with different specificities	15.27	15.58	16.45	15.83	15.78	-1.18	-0.25	-0.31	0.62	-0.22	0.3	0.26	0.09	-0.74	1.08
I	Cdeb_00796	Dehydrogenases with different specificities	15.27	15.58	16.45	15.83	15.78	-1.18	-0.25	-0.31	0.62	-0.22	0.3	0.26	0.09	-0.74	1.08
G	Cdeb_00262	phosphoglycerate kinase	15.43	15.71	16.36	15.68	15.80	-0.93	0.03	-0.28	0.68	-0.66	-0.08	-0.23	0.36	-0.61	2.04
G	Cdeb_00261	glyceraldehyde-3-phosphate dehydrogenase (NAD+)	15.52	15.87	16.82	15.49	15.93	-1.30	0.38	-0.35	1.33	-0.02	-0.55	-0.10	-0.27	0.8	1.42
E	Cdeb_02686	ABC-type oligopeptide transport system,	15.97	16.15	16.51	15.72	16.09	-0.54	0.43	-0.18	0.79	-1.33	-0.62	-0.91	1.27	-0.37	2.41
E	Cdeb_02233	ABC-type dipeptide transport system, periplasmic	16.54	16.82	17.04	16.26	16.67	-0.50	0.56	-0.28	0.78	-1.40	-0.8	-1.06	0.36	-0.39	6.6

Appendix D: General Genome Features of *C. debilis* GB1 (Source: IMG/er)

Table D.1. General genome features of *C. debilis* GB1 (Source: IMG/er)

	Number	% of Total
DNA, total number of bases	3346235	1
DNA coding number of bases	2670185	0.798
DNA G+C number of bases	1712659	51.18
DNA scaffolds	49	1
CRISPR Count	5	
Genes total number	3374	1
Protein coding genes	3264	0.9674
RNA genes	110	0.0326
rRNA genes	8	0.0024
5S rRNA	5	0.0015
16S rRNA	2	0.0006
23S rRNA	1	0.0003
tRNA genes	85	0.0252
Other RNA genes	17	0.005
Protein coding genes with function prediction	2450	0.7261
without function prediction	814	0.2413
Protein coding genes with enzymes	708	0.2098
w/o enzymes but with candidate KO based enzymes	71	0.021
Protein coding genes connected to Transporter Classification	427	0.1266
Protein coding genes connected to KEGG pathways3	837	0.2481
not connected to KEGG pathways	2427	0.7193
Protein coding genes connected to KEGG Orthology (KO)	1559	0.4621
not connected to KEGG Orthology (KO)	1705	0.5053
Protein coding genes connected to MetaCyc pathways	614	0.182
not connected to MetaCyc pathways	2650	0.7854
Protein coding genes with COGs3	1960	0.5809
with KOGs3	488	0.1446
with Pfam3	2524	0.7481

with TIGRfam3	981	0.2908
with InterPro	1625	0.4816
with IMG Terms	431	0.1277
with IMG Pathways	150	0.0445
with IMG Parts List	168	0.0498
with MyIMG Annotation	1	0.0003
in paralog clusters	2255	0.6683
in Chromosomal Cassette	3374	1
Chromosomal Cassettes	623	-
Biosynthetic Clusters	10	-
Genes in Biosynthetic Clusters	99	0.0293
Fused Protein coding genes	71	0.021
Protein coding genes coding signal peptides	144	0.0427
Protein coding genes coding transmembrane proteins	841	0.2493
COG clusters	1310	0.6684
KOG clusters	351	0.1791
Pfam clusters	1812	0.7179
TIGRfam clusters	887	0.9042

Appendix E: *C. debilis* and *C. thermocellum* Co-culture Work in Fermenters

The ultimate goal of this work would be a comprehensive omics analysis of the co-culture of *C. debilis* GB1 and *C. thermocellum* in a steady state. Analysis of a co-culture in batch fermenters was initiated. These batch cultures showed a significant decrease in pH within the first 6 hours before any appreciable amount of metabolism from *C. thermocellum* occurred (hydrogen was a clear proxy for *C. thermocellum* metabolism as *C. debilis* does not produce hydrogen). We wanted to ensure we measured the co-culture in steady state so we employed the use of pH-controlled fermenters. We found that the aerobic co-cultures varied significantly in rate of end-product production and the length of time it took for metabolism to begin slowing

down, between 8-14 days. The large differences in aerobic fermenter replicates were likely due to two factors: i) differences in *C. thermocellum* lag time, although we made great efforts to employ consistency and ii) inability to control exact concentration of oxygen in headspace and liquid as aerobic systems were open air.

After several runs we approximated the time of the highest cellulose degradation rate to be ~20 hours anaerobically and ~27 hours aerobically. We decided to sample for omics at this point as we could be confident that *C. thermocellum* was metabolising and synthesising cellulases. We extracted samples for both transcriptomics and proteomics, however we were unsuccessful in getting good quality RNA or total protein. The difficulty in getting good quality RNA and total protein we attributed to i) interference of the solid cellulose substrate on the extraction process, ii) the possibility that cells were not in exponential growth, as the increase in cellulolytic rate between 10-27 hours appeared to be linear and not exponential, and iii) the possibility that a subpopulation of *C. debilis* was utilising dead cell biomass as a substrate secreting proteases, RNases, and DNases.

To alleviate these issues with consistency and omics sample quality several things could be done: i) finer control of the of oxygen concentration in the head space and liquid, ii) sampling for proteomics at earlier time point, perhaps 12-15h, and iii) utilize only as much cellulose substrate as would be degraded on the order of 20 hours to minimize its impact in omics extraction process.

In retrospect we should have taken a more incremental approach, first attempting to measure omics on co-cultures on plates or in batch experiments. Attempting to do omics on the co-culture from the fermenter as a starting point made

it clear we did not have the appropriate understanding of the co-culture dynamics at the time these experiments were done.

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