

**Transcriptomic profiling of the PhzIR quorum sensing, Gac- and PtrA-  
regulons of *Pseudomonas chlororaphis* PA23**

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## Abstract

The biocontrol agent *Pseudomonas chlororaphis* PA23 protects canola (*Brassica napus*) against infection by the necrotrophic fungus *Sclerotinia sclerotiorum*. This bacterium secretes an arsenal of antifungal (AF) compounds including pyrrolnitrin, phenazines, hydrogen cyanide together with degradative enzymes that are believed to work in concert to suppress the pathogen. Production of these compounds is governed by a complex regulatory pathway that includes the GacA/GacS two component system, the PhzI/PhzR QS system, and a novel LTTR regulator, called PtrA. The focus of this research was to elucidate the PhzRI, Gac and PtrA regulons of *P. chlororaphis* PA23. Transcriptomic profiling of *gacA*-, *gacS*- and *ptrA*-mutants, two QS-deficient strains, PA23-AHL and PA23-*phzR*, and the wild type was undertaken using RNA-sequencing. It was discovered that these regulators each control between 5-7% of the PA23 genome. Decreased expression of genes responsible for the biosynthesis of phenazines, pyrrolnitrin, HCN and degradative enzymes together with elevated siderophore gene expression was observed in all five strains, relative to WT expression. The largest number of differentially expressed genes fell into the COG category for unknown function (S). Comparative analysis of the QS-deficient strains revealed that the regulon of the AHL-deficient PA23-AHL was larger than that of PA23-*phzR*; these findings were attributed to the presence of a second uncharacterised QS system in PA23 named CsaI/CsaR. For genes under QS control, regulation may occur directly through PhzR binding to an upstream “*phz* box” or indirectly through control of other regulators. Only 30 genes contained putative *phz* boxes in the promoter region, suggesting that a large proportion of the Phz regulon is governed indirectly. Consistent with this notion, several transcriptional

regulators including CsaR, the H-NS type regulator MvaT and sigma factors RpoS, RpoN and RpoD showed differential expression in one or both of the QS-deficient strains.

The GacS and GacA transcriptomes shared a surprisingly low degree (42.8%) of overlap, which is unexpected since they comprise a two-component signal transduction system. Interestingly, there was a marked overlap (37.8%) between the PtrA and Gac regulons. The phenotype of a *ptrA* mutant closely resembles that of the *gac*-deficient strains and plasmid-borne *gacS* is able to partially complement a *ptrA* mutant. Thus, we were somewhat surprised to discover no change in expression of either *gacS* or *gacA* in the *ptrA* mutant background. At present, more questions than answers remain regarding the mechanisms underlying PtrA control.

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## List of Abbreviations

2-OH-PHZ – 2-hydroxy-phenazine

AF – antifungal

AHL – acyl-homoserine lactone

AI – autoinducer

Amp – ampicillin

ANR – anaerobic regulator of arginine deiminase and nitrate reductase

bp – base pair

cDNA – complementary DNA

COG – Cluster of Orthologous Groups

Csa (gene) – cell surface alterations

DNA – deoxyribonucleic acid

FeCl<sub>3</sub> – ferric chloride

FNR – fumarate and nitrate reductase regulator

FPKM – fragments per kilobase of transcript per million mapped reads

Gac (gene) – global activator of antibiotic and cyanide

Gm – gentamicin

HCl – hydrochloric acid

HCN – hydrogen cyanide

LTTR- lysR-type transcriptional regulator

mRNA – messenger RNA

NaOAc – sodium acetate

NGS – next generation sequencing

ORF – open reading frame

PCA – phenazine-1-carboxylic acid

PCR – polymerase chain reaction

PDA – potato dextrose agar

PG – phloroglucinol

PHZ – phenazine

Pip – piperacillin

PLT – pyoluteorin

(p)ppGpp – guanosine pentaphosphate

PRN – pyrrolnitrin

PsrA – Pseudomonas sigma regulator A

PtrA – Pseudomonas transcriptional regulator A

QS – quorum sensing

RBS – ribosome binding site

Rif – rifampicin

RNA – ribonucleic acid

RNAP – RNA polymerase

RNA-Seq – RNA-sequencing/ short-read massively parallel sequencing

rRNA – ribosomal RNA

Rsm (gene) – regulator of secondary metabolism

SR – stringent response

SSR – Sclerotinia stem rot

Tc – tetracycline

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# 1. Introduction

## 1.1. Canola (*Brassica napus*), plant disease and biocontrol

Canola is a cultivar of rapeseed, selected for its low erucic acid content that is used to produce edible oils, biodiesel and high protein animal feed. Canada is the 2<sup>nd</sup> largest producer of canola (*Brassica napus*), as such, it contributes over \$26 billion annually to the Canadian economy (Canola Council of Canada, 2016a), making management of disease to ensure optimal growth and yield a priority.

Commercial crops are highly susceptible to disease as they are grown in monoculture, encouraging the spread of pathogens (Canola Council of Canada, 2016b). Current strategies to reduce incidence of disease and the spread of pathogens include crop rotation, the application of petrol-derived pesticides/foiar fungicides and the use of pathogen-resistant cultivars (Canola Council of Canada, 2016b). Chemical-based pesticides usually have unwanted side effects leading to concerns over safety as they leach into the surrounding environment (Arias-Estévez *et al.*, 2008). Another disadvantage of chemical pesticides is that they are not always pathogen specific; therefore, they can have adverse effects on beneficial organisms in the environment.

Resistant cultivars are not available for every pathogen and crop rotation may not be a viable approach as some pathogens can persist in the soil for several years. An alternative strategy for disease management is the use of biocontrol organisms to suppress growth and subsequent spread of pathogens. Biocontrol is defined as the use of one or more organisms to control the growth of other, undesirable organisms in an ecosystem (Weller, 2007).

## **1.2. *Sclerotinia sclerotiorum* and fungal stem rot in Canola**

*Sclerotinia sclerotiorum* (Lib.) de Bary is a soil-borne, necrotrophic ascomycete fungus capable of causing disease in over 400 plant species (Purdy, 1979). Agriculturally relevant crops such as soybean, peas and beans, as well as oilseed crops including sunflower and canola are also susceptible to infection (Hegedus & Rimmer, 2005). *S. sclerotiorum* disease is characterized by either stem rot or white mould, based on which plant is infected (Purdy, 1979). In canola, *S. sclerotiorum* causes fungal stem rot, which is characterized by the formation of grey lesions on infected plant tissue and by the formation of resting bodies known as sclerotia. These resilient structures are composed of melanised hyphae and can survive under harsh environmental conditions for several years (Canola Council of Canada, 2016b). Sclerotia formation makes crop rotation an ineffective method for controlling *Sclerotinia* stem rot. When conditions are right, such as high humidity and a continuous period of soil moisture, apothecia are formed on the soil surface and ascospores are released into the air. These ascospores germinate on senescing petals which land on leaves, causing infection (Hegedus & Rimmer, 2005). *Sclerotinia* stem rot (SSR) can cause more than 50% yield loss per infected plant under conducive conditions (Canola Council of Canada, 2016b). Current strategies to combat *S. sclerotiorum* include the application of foliar fungicides and crop rotation, with the latter having limited value.

## **1.3. *Pseudomonas chlororaphis* PA23**

*Pseudomonas chlororaphis* PA23 is a gram negative fluorescent pseudomonad first isolated from soybean root-tips in 1998 (Savchuk & Fernando, 2004). PA23 was found to display antagonistic activity towards *S. sclerotiorum* under both laboratory and field

conditions (Fernando *et al.*, 2007). The biocontrol capability of PA23 is due to direct pathogen inhibition mediated by antibiotics and degradative enzymes (Fernando *et al.*, 2007; Selin *et al.*, 2010) as well as through indirect priming of the plant defense response (Duke *et al.*, 2017). The production of antibiotic compounds in *P. chlororaphis* PA23 is controlled by a complex regulatory pathway composed of several regulators including the GacA/GacS two component system, the PhzI/PhzR QS system, the stationary phase sigma factor RpoS and its TetR regulator, PsrA (Pseudomonas Sigma Regulator A), the anaerobic regulator ANR, and a LysR-type regulator called PtrA (Klaponski *et al.*, 2014; Manuel *et al.*, 2012; Nandi *et al.*, 2016; Poritsanos *et al.*, 2006; Selin *et al.*, 2010, 2012, 2014).

## **1.4. Antibiotic production by *P. chlororaphis* PA23**

### **1.4.1. Phenazines**

Naturally occurring PHZs are nitrogen-containing heterocyclic compounds produced by bacteria. The first PHZ to be identified, pyocyanin was originally observed and isolated from infected wound dressings (Fordos, 1859). Historically thought to be uniquely produced by *Pseudomonas* species, these compounds are naturally secreted by a wide range of bacteria including *Streptomyces spp.* (Turner & Messenger, 1986). PHZs are of interest as they have multiple roles and functions, contributing to biofilm and rhizosphere community development (Mavrodi *et al.*, 2006), in addition to possessing antibiotic (Turner & Messenger, 1986) and biocontrol properties (Mavrodi *et al.*, 2006).

*P. chlororaphis* PA23 produces two types of PHZs, namely 2-hydroxyphenazine (2-OH-PHZ) and phenazine-1-carboxylic acid (PCA) (Zhang *et al.*, 2006). In PA23, surprisingly, *phz*- biosynthetic mutants exhibit enhanced AF activity against *S. sclerotiorum*

(Selin *et al.*, 2010). PA23 control of *S. sclerotiorum* has been primarily attributed to PRN, another antibiotic compound produced by this bacterium (Selin *et al.*, 2010). In the *phz*-mutant, PRN is elevated 2-fold due to the common shikimic acid pathway used for synthesis of both PHZ and PRN (Selin *et al.*, 2010). The elevated PRN levels result in higher fungal suppression. While they play a lesser role in AF activity, PHZs contribute to biofilm production (Selin *et al.*, 2010). As such, these compounds are expected to confer an ecological advantage by aiding in the colonization and persistence of bacterial cells in the rhizosphere and soil (Mazzola *et al.*, 1992).

PHZs are believed to exert their toxic effect through a redox-cycling mechanism, which mediates the formation of radical oxidative species and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Mavrodi *et al.*, 2006). A number of pseudomonads carry PHZ-encoding genes with a core biosynthetic locus consisting of *phzABCDEFG* (Haas & Keel, 2003; Mavrodi *et al.*, 2006). The production of PHZs in PA23 is positively regulated by the GacA/S two-component system, the PhzI/R QS system, the stationary phase sigma factor RpoS and its regulator, PsrA (Selin *et al.*, 2012).

#### **1.4.2. Pyrrolnitrin**

3-chloro-4-(2-nitro-3-chlorophenyl)-pyrrole or pyrrolnitrin (PRN) is an antibiotic first isolated from *Pseudomonas pyrrocinia* by Arima *et al.*, 1964. PRN displays antibiotic properties, mostly against dermatophytic fungi (Tripathi & Gottlieb, 1969) and has been found to be an effective AF agent against *S. sclerotiorum* (Selin *et al.*, 2010). This biocontrol compound is derived from tryptophan and the reaction is catalyzed by the well-conserved *prnABCD* operon (Hammer *et al.*, 1997).

PRN inhibits ATP, DNA, RNA and protein biosynthesis (Nose & Arima, 1969) as well as endogenous and exogenous respiration (Tripathi & Gottlieb, 1969). This compound similarly displays antibiotic properties against gram-positive bacteria by disrupting the cell membrane and affecting cellular transport (Nose & Arima, 1969).

PRN has been shown to be the primary antifungal (AF) agent found in PA23, as PRN-deficient strains display no AF activity (Selin *et al.*, 2010). Production of PRN is positively regulated by the GacA/S two component system, the PhzI/R QS system and is negatively regulated by the stationary phase sigma factor, RpoS and its regulator, PsrA (Manuel *et al.*, 2012).

### **1.4.3. Hydrogen cyanide**

Hydrogen cyanide (HCN) is a toxic, volatile compound commonly produced by fungi. In the bacterial world, only certain pseudomonads and *Chromobacterium violaceum* have been shown to produce HCN (Knowles, 1976). The precursor for HCN production in bacteria is glycine, and HCN synthesis is enhanced by the addition of glycine to the growth media (Castric, 1977; Knowles, 1976; Nandi *et al.*, 2017). The cyanide ion is able to inhibit metalloenzymes such as copper-containing cytochrome *c* oxidases, but it is not selective towards fungi (Haas & D efago, 2005). In PA23, HCN displays nematocidal activities and is therefore hypothesized to aid in environmental persistence, which is important for biocontrol (Nandi *et al.*, 2015). Moreover, in a lettuce model of infection, HCN was found to contribute to PA23 control of *S. sclerotiorum* infection (Nandi *et al.*, 2017). HCN synthesis is governed by the *hcnABC* operon which is under control of the GacA/GacS, QS and the anaerobic



regulator transcriptional regulator ANR (Laville *et al.*, 1998; Nandi *et al.*, 2016; Pessi & Haas, 2000).

#### 1.4.4. Siderophores

One of the limiting nutrients in soil is iron, particularly in aerated, neutral or alkaline soils where  $\text{Fe}^{3+}$  is poorly soluble (Haas & Défago, 2005). Fluorescent pseudomonads inhibit the growth of other bacteria and fungi by the production of siderophores, which are iron chelating molecules with a high affinity for  $\text{Fe}^{3+}$  (Raaijmakers *et al.*, 2002). Pseudomonads are able to produce two types of siderophores, namely pyoverdine (or pseudobactin) and pyochelin; these molecules chelate the  $\text{Fe}^{3+}$  ion and are involved in uptake by the cell (Kloepper *et al.*, 1980). In iron limiting environments, siderophores are able to deprive pathogens with less avid iron uptake systems by sequestering  $\text{Fe}^{3+}$  (Haas & Défago, 2005).

The importance of siderophores was demonstrated against *Fusarium* wilt by the pyoverdine-producing *Pseudomonas putida* B10 strain. In greenhouse conditions, *P. putida* B10 suppressed diseases such as *Fusarium* wilt and take-all (a root disease). This suppression was lost with the addition of iron to the soil, which represses siderophore production in B10 (Haas & Défago, 2005; Kloepper *et al.*, 1980). While the exact contribution of siderophores to PA23 biocontrol remains unknown, hyper-siderophore producing *gac* and *ptrA* mutants that are otherwise deficient in exoproduct secretion exhibit no AF activity (Poritsanos *et al.*, 2006; Selin *et al.*, 2010) Thus, siderophore production alone is not sufficient for antagonism of *S. sclerotiorum*.

#### **1.4.5. Degradative enzymes**

Fluorescent pseudomonads produce several lytic enzymes including proteases, lipases, cellulases, chitinases and glucanases (Chin-A-Woeng et al., 2003; Pal & McSpadden Gardener, 2006). These enzymes are believed to contribute significantly to biocontrol activities as they are capable of hydrolyzing a wide variety of polymeric compounds such as chitin and cellulose (Neiendam Nielsen & Sørensen, 1999). Lim *et al.*, (1991) demonstrated that for *Pseudomonas stutzeri* YPL-1; lytic enzymes chitinase and laminerase were responsible for biocontrol activity against the fungal pathogen *Fusarium solani*.

Nematicidal activity observed against the root-knot nematode *Meloidogyne incognita* by *P. protegens* CHA0 diminished in protease-deficient (*aprA*- and *gacA*-deficient) strains, suggesting that proteases play an important role in biocontrol activity (Siddiqui *et al.*, 2005). PA23 secretes protease, lipase and chitinase; however, the role of these enzymes in PA23 biocontrol has yet to be determined.

#### **1.5. Regulation of antibiotic production by *P. chlororaphis* PA23**

Production of secondary metabolites in PA23 is carried out by a complex regulatory cascade. Regulatory elements include the GacA/GacS two component system, the Phz QS system, RpoS its response regulator PsrA, the stringent response and the transcriptional regulator ANR (Figure 1).

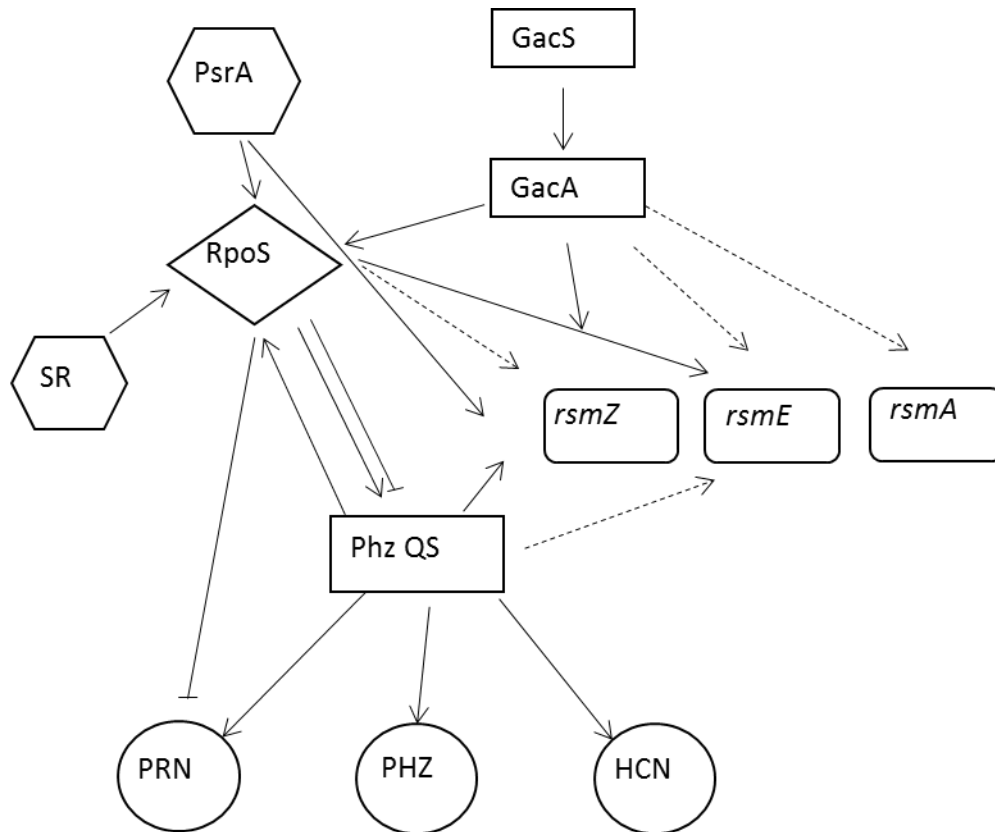


Figure 1: Current model of the genetic regulatory cascade governing production of exoproducts involved in biocontrol in *Pseudomonas chlororaphis* PA23. As described in the text, an unknown signal causes autophosphorylation of GacS and subsequent phosphotransfer to GacA. GacA induces expression of the small non-coding RNAs RsmZ (and RsmY and X, not shown). These RNAs antagonize the translational repressors, RsmA and RsmE. Similarly, GacA induces expression of the alternative sigma factor RpoS. RpoS is positively modulated by PsrA and the Stringent Response (SR). RpoS inhibits expression of PRN and PhzR, while positively regulating expression of PhzI. The PHZ QS system positively regulates expression of RpoS, RsmZ, RsmE as well as the production of PRN and PHZ. Symbols:  $\uparrow$ , positive effect;  $\perp$ , negative effect; solid lines, direct effect; dashed lines, indirect effect. Modified from Selin *et al.*, (2014).

### 1.5.1. Gac-Rsm system

The Gac-Rsm system (Global activator of Antibiotic and Cyanide; Regulator of Secondary Metabolism) is involved in the post translational regulation of secondary metabolites in plant beneficial and root colonizing bacteria (Brencic *et al.*, 2009; Laville *et al.*, 1992). In PA23, this system works in concert with other regulatory elements such as QS, RpoS, PsrA and the stringent response to control expression of biocontrol traits (Selin *et al.*, 2014). The GacA/GacS two component signal transduction system consists of GacS which is a membrane bound sensor kinase, and GacA, a cytoplasmic response regulator (Laville *et al.*, 1992). In response to an unknown signal, GacS undergoes autophosphorylation and phosphotransfer to GacA. Phosphorylated GacA activates transcription of target genes, which include the small non-coding RNA molecules, *rsmX*, *rsmY* and *rsmZ* (Chancey *et al.*, 1999; Heeb & Haas, 2001). These non-coding RNAs function in concert with RsmA and RsmE to control expression of secondary metabolites at the post-transcriptional level. RsmA and RsmE complex with the ribosomal binding site (RBS) of target mRNA preventing subsequent translation (Kay *et al.*, 2006). *rsmX*, *rsmY* and *rsmZ* contain multiple stem-loop structures that are able to bind to, and titrate out the repressor proteins freeing the RBS and allowing translation to take place (Brencic *et al.*, 2009; Haas & Défago, 2005; Kay *et al.*, 2006).

Studies of *P. protegens* CHA0 found that single mutations in *rsmE* or *rsmA* resulted in a slight increase in *hcnA*- (hydrogen cyanide), *aprA*- (exoprotease) and *phlA*- (2,4-diacetylphloroglucinol) translational *lacZ* fusion activity (Reimann *et al.*, 2005). However, an *rsmArsmE* double mutant showed a strong increase in expression as well as suppression of a *gacS* mutation. As such, both RsmA and RsmE are able to repress translation of target genes. The double mutant also showed a decrease in the expression of *rsmX*, *rsmY* and *rsmZ*;

therefore, RsmA and RsmE may influence the transcription of *rsmX*, *rsmY* and *rsmZ* (Kay *et al.*, 2006; Reimann *et al.*, 2005).

The GacA/S two component system is further modulated by RetS and LadS, two orphan sensor kinases that interact with GacS and affect regulation of downstream genes (Records & Gross, 2010). RetS binds to, and forms heterodimers with GacS, preventing autophosphorylation and subsequent transcription of the small RNA molecules. However, in the presence of an unknown signal, RetS and GacS form homodimers, enabling activation of the GacS-GacA cascade (Goodman *et al.*, 2009; Records & Gross, 2010). LadS is believed to activate the transcription of the small RNAs, leading to translation of secondary metabolites; however, the mechanism by which it interacts with GacS is currently unknown (Goodman *et al.*, 2004; Ventre *et al.*, 2006). In *P. aeruginosa*, studies have revealed that RetS and LadS play an important role in virulence. RetS positively regulates genes involved in an acute infection; while LadS controls genes involved in the chronic state of infection and establishment of bacteria within the host (Goodman *et al.*, 2009; Ventre *et al.*, 2006). While *retS* and *ladS* homologs have been identified in PA23, their role in biocontrol has not yet been explored.

In PA23, mutations in *gacA* and *gacS* show decreased production of secondary metabolites such as PHZ, PRN, HCN and proteases. This is validated by the loss of AF activity observed in both in vitro and in greenhouse analyses (Poritsanos *et al.*, 2006; Selin *et al.*, 2014).

### 1.5.2. Stringent response (SR), RpoS and PsrA

Gene transcription in bacteria is regulated by sigma factors. There are 24 sigma factors in *Pseudomonas*, of which 19 regulate extracytoplasmic function (Potvin *et al.*, 2008). These alternative sigma factors regulate genes in response to different conditions such as stress, nutrient-limiting conditions and changes in developmental stage (Feklístov *et al.*, 2014). RpoS ( $\sigma^{38}/\sigma^S$ ), also known as the stationary phase sigma factor, regulates the transcription of genes required for resistance to oxidative stress and starvation (Battesti *et al.*, 2011). RpoS was first discovered in *Escherichia coli*, and was found to directly and indirectly regulate 10% of the *E. coli* genome (Weber *et al.*, 2005). In *Pseudomonas* however, RpoS plays less of a general regulatory role and is involved in the production of virulence factors and antibiotics (Jorgensen *et al.*, 1999; Sarniguet *et al.*, 1995; Suh *et al.*, 1999; Venturi, 2003).

RpoS is induced and cellular concentrations increase drastically at the onset of stationary phase being regulated at the transcriptional, post-transcriptional and post-translational level (Battesti *et al.*, 2011; Venturi, 2006). Increasing levels of RpoS compete with RpoD and other sigma factors for binding to the core of RNA polymerase, changing the binding affinity of the RNAP, and altering expression of genes (Venturi, 2003).

In biocontrol pseudomonads, *rpoS*-null mutants display an aberrant stress response as well as altered production of secondary metabolites including PHZs, PRN, pyoluteorin, HCN and acyl-homoserine lactones (AHLs) (Haas & Keel, 2003; Oh *et al.*, 2013; Sarniguet *et al.*, 1995; Suh *et al.*, 1999; Venturi, 2003). Effects of *rpoS* deletion are species dependant; for example, in *P. fluorescens* Pf-5 and *P. protegens* CHA0, an *rpoS*-null mutant shows a lack of PRN synthesis (Haas & Défago, 2005; Sarniguet *et al.*, 1995; Venturi, 2003). While in *P.*

*chlororaphis* PA23, an *rpoS* mutant exhibits enhanced AF activity due to increased PRN, lipase and protease production (Manuel *et al.*, 2012). Moreover, an eight-fold decrease in production of PHZ and expression of *phzA*, a gene found in the PHZ-biosynthesis operon, was observed in this background (Manuel *et al.*, 2012).

RpoS is tightly controlled by a number of regulators including the GacA/GacS two component system, the PHZ QS system and the TetR regulator PsrA (Pseudomonas Sigma Regulator A) (Venturi, 2006). PsrA upregulates expression by binding to the *rpoS* promoter, -30 to -59 bp upstream of the transcriptional start site in *P. putida* and *P. aeruginosa* (Venturi, 2003). *rpoS* transcription was found to be 50% reduced in a *psrA* mutant compared to WT in both *P. putida* and *P. aeruginosa* (Venturi, 2003). PsrA represses its own expression through PsrA recognition sites found in its promoter region in *P. chlororaphis* and *Pseudomonas syringae* (Chin-A-Woeng *et al.*, 2005; Venturi, 2003). PsrA also regulates the production of AHLs in *P. aeruginosa*, *P. putida* and *P. chlororaphis* (Chin-A-Woeng *et al.*, 2005).

The stringent response (SR) is another RpoS-linked regulatory system overseeing PA23 biocontrol (Manuel *et al.*, 2012). Nutrient limitation is the cue that activates the SR and results in production of the alarmone (p)ppGpp (Potrykus & Cashel, 2008). When (p)ppGpp complexes with RNAP, it induces a conformation change enabling alternative sigma factors, including RpoS, to be more competitive for binding. SR mutants of PA23 closely resemble *rpoS* mutants, exhibiting enhanced AF activity due to elevated protease and PRN production (Manuel *et al.*, 2012).

### 1.5.3. Quorum sensing

Quorum sensing (QS) is a population-density dependent regulatory mechanism used to launch a coordinated response that would be difficult for a single bacterium to achieve (Juhas *et al.*, 2005). QS systems are widespread among both gram positive and gram negative bacteria, and rely on small diffusible signalling molecules to regulate gene expression (Miller & Bassler, 2001). These systems were first discovered in the luminous *Vibrio fischeri* and *Vibrio harveyi*, in which the luciferase operon (*luxCDABE*) used for light production was found to be under QS control (Miyamoto *et al.*, 2002).

Gram-negative bacterial QS systems are comprised of a LuxI-type autoinducer (AI) synthase and a LuxR-type transcriptional regulator. The AI synthase produces AI molecules, which in the case of gram-negative bacteria, are AHLs that are secreted by the cell. Once a threshold concentration has accumulated, the AI molecules bind to the transcriptional regulator, allowing dimerization. The dimerized regulator binds to a conserved sequence known as the *lux*-box which is situated upstream of target genes, allowing activation of transcription (Haas & Keel, 2003; Juhas *et al.*, 2005; Miller & Bassler, 2001).

In *P. chlororaphis*, the Phz QS system consists of the transcriptional regulator PhzR and the AHL synthase PhzI (Miller & Bassler, 2001). In PA23, the Phz QS system regulates production of secondary metabolites, biofilm formation and it also exhibits cross-regulation with RpoS (Selin *et al.*, 2012). The Phz-AHL complex activates the *phz* operon and other target genes by binding to a conserved upstream sequence known as the “*phz*-box” (Miller & Bassler, 2001). Both *phzR*- and AI-deficient PA23 strains exhibit reduced AF activity due to



decreased production of PHZ, PRN and proteases (Selin *et al.*, 2010). A model depicting PhzIR regulation of gene expression in PA23 is presented in Figure 2.

A second QS system called CsaR/CsaI (Cell Surface Alteration) was identified in the closely related *P. chlororaphis* 30-84 (Zhang & Pierson, 2001). This QS system is not involved in the regulation of secondary metabolites or biocontrol genes, instead, it controls cell surface properties and biofilm formation (Zhang & Pierson, 2001). CsaR/CsaI has been shown to play an important role in the rhizosphere competence of plants as well as biosynthesis of cell surface proteins (Juhas *et al.*, 2005; Zhang & Pierson, 2001).

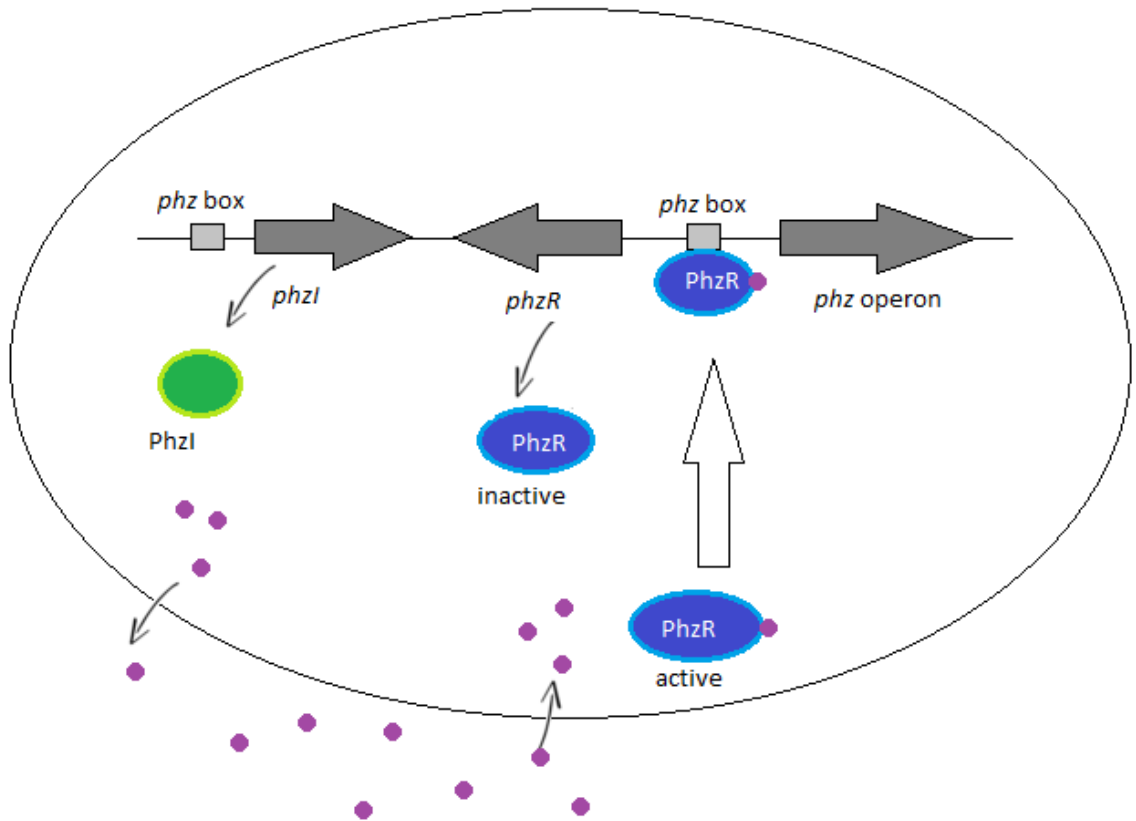


Figure 2: Mechanism of PhzR/PhzI QS regulation. The AI synthase (PhzI) produces AHLs that are secreted by the cell. Once a threshold concentration of the molecule has accumulated, the AIs bind to the transcriptional regulator PhzR, allowing dimerization. The dimerized regulator binds to a conserved sequence known as the *phz*-box which is situated upstream of the target genes, allowing activation of transcription.

#### 1.5.4. Anaerobic regulator of arginine deiminase and nitrate reductase (ANR)

Anaerobic metabolic pathways play a significant role in enabling bacteria to adapt to different environments found in the rhizosphere. The Anaerobic regulator of arginine and Nitrate Reductase (ANR) belongs to the Fumerate and Nitrate Reductase (FNR) family of transcriptional regulators, and was first discovered in *E. coli* where it complemented an *fnr*-deletion mutant (Sawers, 1991). The *anr* gene is 51% identical with *fnr* at the amino acid level and is required for the anaerobic growth of *P. aeruginosa* on nitrate and arginine (Zimmermann *et al.*, 1991). The FNR family of regulators can respond to various stimuli to regulate expression of target genes, including oxygen concentration, nitric oxide, carbon monoxide and cyclic nucleotides (Körner *et al.*, 2003).

In *P. aeruginosa* and *P. fluorescens*, ANR dimerizes in the presence of low oxygen concentrations ( $< 5\mu\text{mol}$ ) (Laville *et al.*, 1998; Sawers, 1991). The dimeric form binds to a cofactor, a  $4[4\text{Fe-4S}]^{2+}$  cluster (Spiro, 1994), activating transcription of target genes. ANR binds to a well-conserved sequence known as the *anr*-box ( $5'$ -TTGAT-N<sub>4</sub>-ATCAA- $3'$ ), located  $-41.5$  upstream of the transcriptional start site (Spiro, 1994). When oxygen levels are  $> 5\mu\text{mol}$ , the  $4[4\text{Fe-4S}]^{2+}$  is converted to  $[2\text{Fe-2S}]^{2+}$ , inactivating ANR (Zimmermann *et al.*, 1991). ANR works in concert with other regulatory elements such as QS and GacA/S to regulate production of secondary metabolites in *Pseudomonas* spp. (Pessi & Haas, 2000).

In *P. chlororaphis* PA23, loss of ANR has a dramatic effect on physiology due to decreased expression of both antibiotic and regulatory genes (Nandi *et al.*, 2017). ANR was found to be essential for production of HCN, PRN, PHZ, degradative enzymes and AHLs (Nandi *et al.*, 2017). Interestingly, the *anr*-mutant bears striking resemblance to the *phzR*-

deficient strain, and PhzR was found to negatively regulate *anr*; whereas ANR positively regulates PhzR and PhzI (Nandi *et al.*, 2017).

### **1.5.5. LysR-type transcriptional regulators**

The LysR-type transcriptional regulator (LTTR) family is the largest known group of prokaryotic transcriptional regulators. LTTRs are ubiquitous in bacteria, with orthologues also found in archaea and eukarya (Maddocks & Oyston, 2008). The family namesake derives from a *lysA* transcriptional activator called LysR, the best characterized protein at the time of study (Stragier *et al.*, 1983). A wide range of traits are governed by LTTRs including QS, biofilm formation, metabolism, attachment and virulence (Maddocks & Oyston, 2008). These regulators control gene expression at the transcriptional level for a variety of single or operonic genes. While target genes are usually divergently transcribed, they can be located elsewhere on the chromosome (Hernandez-Lucas *et al.*, 2008; Heroven & Dersch, 2006) and LTTRs are autoregulatory in nature (Maddocks & Oyston, 2008).

LTTRs are an average of 330 amino acids in length (Maddocks & Oyston, 2008). This family of regulators exhibits a great deal of structural similarity; they are comprised of a co-factor-binding C terminus and a helix-turn-helix (HTH) DNA-binding N terminus (Maddocks & Oyston, 2008; Pérez-Rueda & Collado-Vides, 2000). The HTH motif is important for DNA binding in bacteria, appearing in all LTTRs as well as approximately 95% of other DNA binding proteins (Huffman & Brennan, 2002; Maddocks & Oyston, 2008). LTTRs require a co-inducer to regulate transcription, which is often a product or an intermediate of the target metabolic pathway (Celis, 1999; van Keulen *et al.*, 1998; Maddocks & Oyston, 2008; Picossi *et al.*, 2007). These regulators can exert positive or negative effects on target gene expression

through binding to the activation-binding site (ABS) or regulatory binding site (RBS), respectively. The RBS is typically located between positions -65 and +20 bp and contains an LTTR box, an imperfect palindrome with a T-N<sub>11</sub>-A consensus sequence (Parsek *et al.*, 1994). The ABS lies near the -35 promoter region and lacks a conserved binding sequence (Maddocks & Oyston, 2008). A model for LTTR regulation is depicted in Figure 3. The co-inducer plays an important role in determining the affinity of the LTTR for the binding site. An inactive LTTR dimer can only bind to the RBS; however, in the presence of a co-inducer, a second LTTR dimer can bind to the ABS. The interaction between the two LTTR dimers causes DNA bending. In complex with co-inducer, the LTTR tetramer changes conformation, leading to relaxation of the DNA. This in turn allows for the stable binding of RNAP to the promoter and transcription of the target gene (Maddocks & Oyston, 2008).

#### **1.5.5.1 *Pseudomonas* transcriptional regulator A**

In PA23, an isolate displaying no AF activity was identified during a transposon mutagenesis study. Sequence analysis showed that the transposon had interrupted a gene encoding a novel LTTR which was named PtrA (P*seudomonas* transcriptional regulator A) (Klaponksi *et al.*, 2014; Poritsanos, 2005). Phenotypic analysis revealed reduction in production of antibiotics, degradative enzymes, AI molecules and biofilm formation (Klaponksi *et al.*, 2014).

As mentioned earlier, LTTRs often regulate divergently transcribed genes (Maddocks & Oyston, 2008). In PA23, the gene located directly upstream of *ptrA* encodes a short chain dehydrogenase (*scd*). A number of observations suggest that *scd* is not involved in PtrA-mediated regulation of PA23 biocontrol. First, expression of this gene is unchanged in the

*ptrA*-deficient strain (Shah *et al.*, 2016). Second, mutational inactivation of *scd* resulted in no change in phenotype or expression of biocontrol genes (Klaponksi, 2014; Shah *et al.*, 2016). Third, complementing the *ptrA* mutant in-trans with the *scd* gene does not alter the phenotype (Klaponksi *et al.*, 2014; Shah *et al.*, 2016).

To fully appreciate the impact of PtrA on PA23 physiology including secondary metabolite production, iTRAQ (Iso**baric** T**ag** for R**elative** and A**bsolute** Q**uantification**) analysis was used for proteomic profiling. This analysis revealed that the quantity of protein important for biocontrol, including PRN and PHZ, was much lower in the *ptrA*-deficient strain compared to the WT (Klaponksi *et al.*, 2014). This was consistent with the differences in the secondary metabolite production observed in this strain (Klaponksi *et al.*, 2014; Shah *et al.*, 2016). Interestingly, complementing the *ptrA* mutant *in trans* with the *gacS* gene partially restored the wild-type phenotype such as antifungal activity and PHZ production (Klaponksi *et al.*, 2014; Shah *et al.*, 2016). Consequently, there appears to be a link between these two regulators overseeing PA23 biocontrol (Figure 4).

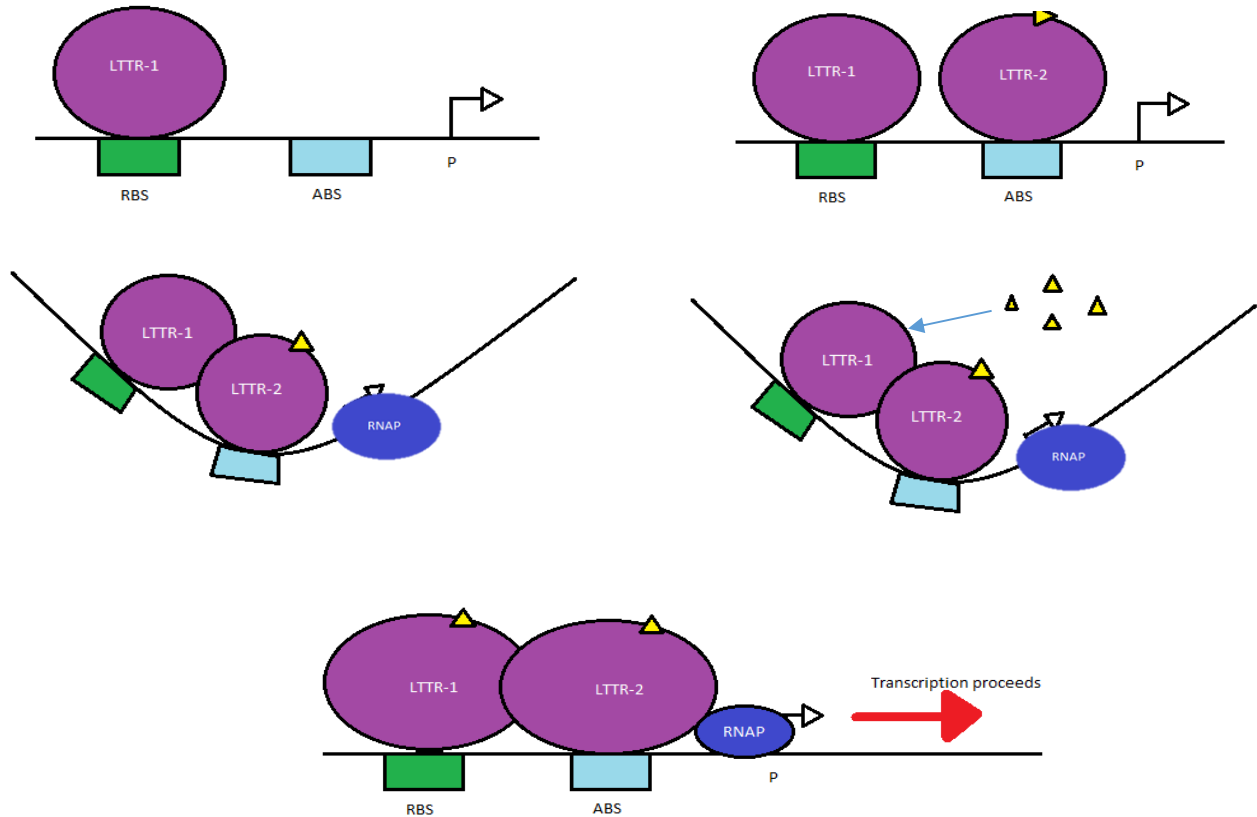


Figure 3: A schematic representation of the role of DNA bending and LTTR binding at the ABS and RBS sites in LTTR-dependent transcriptional activation. (1) Shows one LTTR dimer (LTTR-1) bound at the RBS. In the absence of co-inducer, an LTTR dimer is unable to bind to the ABS. Subsequently, RNAP can't bind to the promoter region (P). (2) In the presence of co-inducer, LTTR-2 changes conformation and binds to the ABS. (3) Protein-protein interaction between LTTR-1 and LTTR-2 causes the DNA to bend, RNAP is unable to transcribe target gene in the absence of interaction with the LTTR tetramer. (4) Shows the co-inducer binding to the LTTR tetramer, causing a second conformational change and the DNA bend relaxing; (5) The LTTR tetramer is consequently brought into contact with the RNA polymerase at the promoter, stabilising RNAP- binding and activating transcription of the target gene.

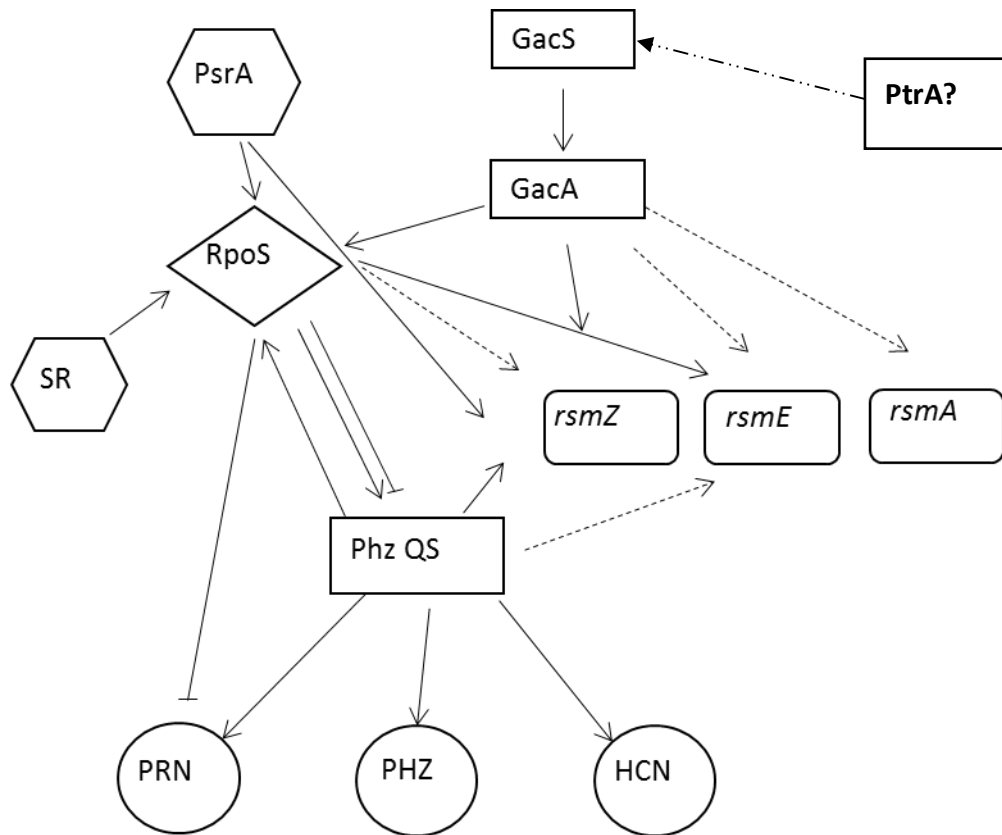


Figure 4. Revised model of the genetic regulatory cascade governing production of exoproducts involved in biocontrol in *Pseudomonas chlororaphis* PA23 (Figure 1). As described in text and Figure 1, the regulation of secondary metabolites involved in biocontrol is under the control of several regulators. Including the GacA/GacS two component system, the Phz QS system, the stationary sigma factor RpoS, and its regulator PsrA and the Stringent Response (SR).

PtrA, a LysR type regulator is also found to be involved in the production of secondary metabolites in PA23. While previous complementation studies indicate that GacS seems to interact with PtrA. The mechanism of this interaction is yet to be determined. Symbols: ↑, positive effect; ⊥, negative effect; solid lines, direct effect; dashed lines, indirect effect. Modified from Selin *et al.*, (2014) and Figure 1.



## 1.6. Thesis Objectives

In *P. chlororaphis* PA23, a complex network of regulatory factors oversees expression of compounds required for biological control. Governing elements include the GacS-GacA two-component system, the PhzRI QS system, and the novel LTTR PtrA. The extent to which these global regulators impact PA23 gene expression is largely unknown; therefore, the objectives of this research are as follows:

1. To define the transcriptomes of the AHL-deficient, *phzR*-, *gacS*-, *gacA*-, *ptrA* mutants through RNA-seq analysis.
2. To compare the transcriptional profiles of the AHL-deficient and *phzR*-mutant strains to reveal genes regulated by one or both of these QS components.
3. To investigate how a loss of *phzR*, and AHL production affects expression of other PA23 regulators.
4. To determine the degree of overlap between the Gac and PtrA regulons through comparative analysis of the *gacA*-, *gacS*- and *ptrA*-mutant transcriptomes.

## 2. Materials and Methods

### 2.1. Bacterial strains and growth conditions

Bacterial strains used in this study are outlined in Table 1. *E. coli* strains were cultured on Lennox Luria Bertani (LB) media (Difco Laboratories, Detroit, MI) at 37°C. *P. chlororaphis* PA23 and *Chromobacterium violaceum* CV026 were cultured and maintained on LB media (Difco) at 28°C. Pseudomonas Isolation Agar (PIA; Difco) was used as a selection media for *P. chlororaphis* PA23. *S. sclerotiorum* was cultured and maintained on Potato Dextrose Agar (PDA; Difco) at 22°C. Antibiotics were purchased from Research Products International Corp. (Prospect, IL) and supplemented at the following concentrations: piperacillin (Pip; 40 µg/mL), gentamicin (Gm; 20 µg/mL), tetracycline (Tc; 15 µg/mL) for *P. chlororaphis* PA23 and ampicillin (Amp; 100 µg/mL), Gm (15 µg/mL) and Tc (15 µg/mL) for *E. coli*. For phenotypic assays and cDNA library synthesis, strains were cultured in M9 Minimal Salts Media (M9; Difco) supplemented with 1 mM MgSO<sub>4</sub> and 0.2% glucose, from here on referred to as M9-Glc.

### 2.2. Nucleic acid manipulations

Isolation, purification, endonuclease digestion, ligation and all other manipulation of DNA was performed according to the protocols described in Sambrook *et al.* (1989). Polymerase Chain Reaction (PCR) was conducted following standard conditions outlined by Invitrogen Life Technologies and New England Biolabs (NEB) (Ipswich, USA) data sheets supplied with their buffer system and *Taq* polymerase.

**Table 1. Bacterial strains, plasmids and oligonucleotides sequences**

Strains	Relevant genotypes	Source or reference
<b><i>Pseudomonas chlororaphis</i></b>		
PA23	Phz <sup>+</sup> Rif <sup>R</sup> WT (soy bean plant isolate)	Savchuk & Fernando, 2004
PA23- <i>ptrA</i>	Phz-Rif <sup>R</sup> <i>ptrA</i> ::Tn5-OT182 genomic fusion	Klaponski <i>et al.</i> , 2014
PA23- <i>gacA</i>	Gm <sup>R</sup> marker inserted into the <i>gacA</i> gene	Selin <i>et al.</i> , 2014
PA23- <i>gacS</i>	Phz <sup>-</sup> Rif <sup>r</sup> <i>gacS</i> ::Tn5-OT182 genomic fusion	Poritsanos <i>et al.</i> , 2006
PA23- <i>phzR</i>	Gm <sup>R</sup> marker inserted into <i>phzR</i> gene	Selin <i>et al.</i> , 2012
PA23--AHL	PA23 carrying pME6863 (AI-)	Selin <i>et al.</i> , 2012
<b><i>Escherichia coli</i></b>		
DH5 $\alpha$	<i>supE44</i> $\Delta$ <i>U169</i> ( $\phi$ 80 <i>lacZ</i> $\Delta$ <i>M15</i> ) <i>hadR17</i> <i>recA1</i> <i>endA1</i> <i>gyrA96</i> <i>thi-1</i> <i>relA1</i>	Gibco
DH5 $\alpha$ pir	DH5 $\lambda$ pir lysogen of DH5 $\alpha$	House <i>et al.</i> , 2004
<b><i>Chromobacterium violaceum</i></b>		
CVO26	Autoinducer synthase ( <i>cviI</i> ) mutant from <i>C. violaceum</i> ATCC 31532, autoinducer biosensor	Latifi <i>et al.</i> , 1995
<b>Plasmids</b>		
pUCP23	Broad-host range vector; Amp <sup>R</sup> , Gm <sup>R</sup>	West <i>et al.</i> , 1994
pUCP22- <i>gacA</i>	1.65-kb fragment containing <i>gacA</i> and <i>uvrC</i> from <i>P. protegens</i> CHA0 in pUCP22	Shah <i>et al.</i> , 2016
pUCP23- <i>gacS</i>	3.1-kb fragment containing <i>gacS</i> in pUCP23	Poritsanos <i>et al.</i> , 2006
pUCP22- <i>ptrA</i>	2.2-kb fragment containing <i>ptrA</i> in pUCP22	Klaponski <i>et al.</i> , 2014
pUCP22- <i>rpoS</i>	1.3-kb fragment containing <i>rpoS</i> in pUCP22	Poritsanos <i>et al.</i> , 2006
pUCP22- <i>psrA</i>	950-bp fragment containing <i>psrA</i> in pUCP22	Selin, 2012
pUCP22- <i>rsmA</i>	190-bp fragment containing <i>rsmA</i> in pUCP22	Selin, 2012
pUCP22- <i>rsmE</i>	600-bp fragment containing <i>rsmE</i> in pUCP22	Selin, 2012
pUCP23- <i>rsmZ</i>	400-bp fragment containing <i>rsmZ</i> in pUCP23	Selin, 2012
pUCP23- <i>phzR</i>	1.68-kb fragment containing <i>phzR</i> in pUCP23	Selin <i>et al.</i> , 2012

pUCP22- <i>retS</i>	2.9-kb fragment containing <i>retS</i> in pUCP22	Shah <i>et al.</i> , 2016
pUCP22- <i>ladS</i>	2.8-kb fragment containing <i>ladS</i> in pUCP22	Shah <i>et al.</i> , 2016
<b>Oligonucleotide sequences</b>		
tet FWD	5'- ACCCGTCCTGTGGATTCTCTA-3'	Shah <i>et al.</i> , 2016
new ptrA TL start FWD	5'- GCAAGCAAGCTTCGACGCGATACAACTGGC-3'	Shah <i>et al.</i> , 2016
scd-pKNOCK FWD	5'- TATTGGATCCTTCCACGCTCTTGCGTA-3'	Shah <i>et al.</i> , 2016
scd-pKNOCK REV	5'- TATTCTCGAGCCAACGGCACCATAGGTTCA-3'	Shah <i>et al.</i> , 2016
retS-F2	5'- GACGGATCCAGCGCCGCGCATAGTTAT-3'	Shah <i>et al.</i> , 2016
retS-R2	5'- ATGAAGCTTGGCGCAAACCTCACAGCG-3'	Shah <i>et al.</i> , 2016
ladS-F-BamHI	5'- GAGTGGATCCAAACCAATAACAGG-3'	Shah <i>et al.</i> , 2016
ladS-R-HindIII	5'- CCAGAAGCTTAGTTAAGCACCC-3'	Shah <i>et al.</i> , 2016
gacS RT-PCR FWD	5'- TGGTCAGCCTGGTGTATC-3'	Shah <i>et al.</i> , 2016
gacS RT-PCR REV	5'- TGTCTTCGTGTTCTTCTTCG-3'	Shah <i>et al.</i> , 2016
rpoS RT-PCR FWD	5'- TGGCTTTCCGAATTGACC-3'	Shah <i>et al.</i> , 2016
rpoS RT-PCR REV	5'- CAGACGCTTGAGACCTTC-3'	Shah <i>et al.</i> , 2016
prnA RT-PCR FWD	5'- CTGTCGTCGTGCTTTCTG-3'	Shah <i>et al.</i> , 2016
prnA RT-PCR REV	5'- GATCTCGGCGTTGAATGC-3'	Shah <i>et al.</i> , 2016
phzI RT-PCR FWD	5'- GCGATGCCGTTGTTCTGG-3'	Shah <i>et al.</i> , 2016
phzI RT-PCR REV	5'- AGCCGTTTCGTAGTGGACTC-3'	Shah <i>et al.</i> , 2016
phzR RT-PCR FWD	5'- GAATCCTTGGCTTCAGACC-3'	Shah <i>et al.</i> , 2016
phzR RT-PCR REV	5'- ATCAGGCGGCTAACTACG-3'	Shah <i>et al.</i> , 2016
psrA RT-PCR FWD	5'- CCATCTTCATGCGTCTTCTG-3'	Shah <i>et al.</i> , 2016

psrA RT-PCR REV	5'- ATGTAGCGGCGGAATACC-3'	Shah <i>et al.</i> , 2016
rsmZ RT-PCR FWD	5'- TGCGGTATGAAAGTTGTCTATTTG-3'	Shah <i>et al.</i> , 2016
rsmZ RT-PCR REV	5'- ATCCTTGATGGTTGTGTCTATCC-3'	Shah <i>et al.</i> , 2016
rsmE RT-PCR FWD	5'- GAAAGCATAAATATCGGTGAC-3'	Shah <i>et al.</i> , 2016
rsmE RT-PCR REV	5'- CGTTGGTAGATTTCTTCGC-3'	Shah <i>et al.</i> , 2016
phzA RT-PCR FWD	5'- GACTGGCAATGGCACAAC-3'	Shah <i>et al.</i> , 2016
phzA RT-PCR REV	5'- GCAATAACCTTCGGGATAACC-3'	Shah <i>et al.</i> , 2016
gacA RT-PCR FWD	5'- CTGGTGTTC AAGTCATTCC-3'	Shah <i>et al.</i> , 2016
gacA RT-PCR REV	5'- AAGATACGGTAACGGTAGG-3'	Shah <i>et al.</i> , 2016
rsmA RT-PCR FWD	5'- ATGCTGATTCTGACTCGTC-3'	Shah <i>et al.</i> , 2016
rsmA RT-PCR REV	5'- GCACCGCTACCTCTTTAG-3'	Shah <i>et al.</i> , 2016
rpoB RT-PCR FWD	5'- CGTGTTCCTGCCGCTATC-3'	Shah <i>et al.</i> , 2016
rpoB RT-PCR REV	5'- GCCGCAACCGAACTACC- 3'	Shah <i>et al.</i> , 2016
ptrA-RTpcr F3	5'- ACCTGGAGCAATATGGCGAG-3'	Shah <i>et al.</i> , 2016
ptrA-RTpcr R3	5'- TGCTGGTGATAGAGCCACTC-3'	Shah <i>et al.</i> , 2016
retS-RTpcr F2	5'- AGCACCACGTCGAAGTAGTCGC-3'	Shah <i>et al.</i> , 2016
retS-RTpcr R2	5'- ACAACGACACCTGCCGCAAG-3'	Shah <i>et al.</i> , 2016
ladS-RTpcr F1	5'- AGAGGTAATCGAGCAGGCAGCG-3'	Shah <i>et al.</i> , 2016
ladS-RTpcr R1	5'- GCTCAAACCTGTGCGACCAGGTG-3'	Shah <i>et al.</i> , 2016
Up1-RTpcr F	5'- GCCACCGAAATAGGCGCAAC-3'	Shah <i>et al.</i> , 2016
Up1-RTpcr R	5'- CCAACAACCGCCATGTCGAAC-3'	Shah <i>et al.</i> , 2016
Up2-RTpcr F	5'- TTGCTCGAAGCGCACTTCAC-3'	Shah <i>et al.</i> , 2016
Up2-RTpcr R	5'- AGATCCTCTACGTCAGCAAGCC-3'	Shah <i>et al.</i> , 2016

Up3-RTper F

5'- ATTGTGGGTTCTTGCGGCTG-3'

Shah *et al.*,  
2016

Up3-RTper R

5'- CTCTGCGGGATCGGCTTCACCATGAGCCTG-  
3'

Shah *et al.*,  
2016

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### 2.3. RNA extraction and cDNA library synthesis

Six PA23 strains were used for cDNA library synthesis, including the PA23 WT, *ptrA*-, *gacA*-, *gacS*-, *phzR*-mutants and an AI-deficient strain. Three biological replicates of each strain were cultured in 30 ml of M9-Glc. Cells were harvested at early stationary phase ( $OD_{600}$  1.20-1.50) by pelleting for 10 min at 6000 rpm at 4°C followed by flash freezing in liquid nitrogen. Pellets were stored at -80°C for up to a week. Total RNA was extracted using the Fermentas Plant RNA extraction kit per manufacturer's instructions. Residual genomic DNA was removed by treatment with TURBO RNAase-free DNase I (Ambion, Carlsbad, USA) according to manufacturer's instructions. RNA concentration was verified using a NanoVue spectrophotometer (GE Healthcare), and quality was measured using an Agilent 2100 Bioanalyzer with Agilent RNA 6000 Pico and Nano Chips (Agilent Technologies; Santa Clara, CA, USA). Only RNA samples with an  $A_{260}/A_{280}$  of between 1.8 and 2.0, and RIN values above 7 with a concentration greater than 250 ng/ $\mu$ l were used in subsequent steps.

cDNA libraries were constructed using the alternative HTR protocol described by Kumar et al. (2012) adapted for bacterial RNA. Ribosomal RNA was depleted using the MicroExpress kit (Ambion, Carlsbad, USA) as per manufacturer's instructions. Fragmentation time was reduced to 10 min and the number of cycles for final PCR amplification of the libraries was adjusted to 12 (Kumar *et al.*, 2012). Final cDNA libraries with ligated adaptors were size-selected to fall between 250 and 500 bp using the E-Gel® electrophoresis system (Invitrogen). cDNA quantity was measured using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Thermo Fisher, Rockford, USA) with a Nanodrop 3300 (Thermo Fisher). cDNA was validated using Agilent Bioanalyser High Sensitivity DNA Chips (Agilent Technologies) at three points: i) after first and second strand cDNA synthesis; ii)

after the final PCR amplification of the libraries; and iii) after size selection with the E-Gel® electrophoresis system. 100-bp single-end RNA-sequencing was carried out at Génome Québec (Montreal, Canada) on the Illumina HiSeq 2000 platform with a multiplex value of 9.

## 2.4. Data analysis

Sequenced reads were analysed by FastQC to determine quality (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>); the Trimmomatic tool (Bolger *et al.*, 2014) enabled removal of low quality reads and barcode adapters. Trimmomatic parameters for maximizing mapping efficiency to the *P. chlororaphis* PA23 genome were determined using reports generated by FastQC. Raw reads were then mapped to the genome via Tophat2 v.2.1.0 (Trapnell *et al.*, 2012) in the high-sensitivity mode, with the *P. chlororaphis* PA23 reference annotation from NCBI (gi: accession no. NZ\_CP008696) as a guide. Of total reads, 93.08% mapped to the *P. chlororaphis* PA23 genome across samples (Table 2). Utilising the mapped reads, a transcriptome was constructed to identify novel transcripts employing the cufflinks and cuffmerge tools within the Cufflinks v.2.2.1 suite (Trapnell *et al.*, 2012). Open reading frames (ORFs) within transcript sequences were identified with Transdecoder (<https://transdecoder.github.io/>). Default parameters were adjusted to identify ORFs that were a minimum of 50 amino acids long. Protein identification was achieved through BLAST (Altschul *et al.*, 1990); using the *Pseudomonas* specific databases in NCBI and Uniprot (taxid: 286). Functional analysis was carried out via Cluster of Orthologous Groups (COG) analysis. COG categories were assigned to the translated transcript sequences through the Conserved Domain Database (CDD) and the batch web-CD search tool (Marchler-Bauer *et al.*, 2011; Marchler-Bauer & Bryant, 2004). Cuffquant and



cuffnorm in the Cufflinks package were employed with default settings to normalise read counts to Fragments Per Kilobase of transcript per Million mapped reads (FPKM) values. Significantly differentially expressed genes were identified as those with a corrected p-value  $\leq 0.05$  and a q-value  $\leq 0.05$  with cuffdiff in the Cufflinks package at default settings. This output enabled generation of gene expression profiles for each strain. The  $\log_2$  fold change values  $\geq 1.5$  or  $\leq -1.5$  were selected based on previous analysis carried out by Shemesh *et al.*, (2007) on the *Streptococcus mutans* transcriptome.

Table 2. RNA-seq library reads mapped to the *Pseudomonas chlororaphis* PA23

<b>PA23 genome Sample (Replicate)</b>	<b>Total reads</b>	<b>Reads mapped to PA23 genome</b>	<b>PA23 mapping %</b>
<b>PA23-WT (1)</b>	4,662,579	4,057,409	87.00
<b>PA23-WT (2)</b>	4,722,434	4,333,007	91.80
<b>PA23-WT (3)</b>	4,485,424	4,109,800	91.60
<b>PA23-AHL (1)</b>	5,940,843	5,476,912	92.20
<b>PA23-AHL (2)</b>	2,940,079	2,759,435	93.90
<b>PA23-AHL (3)</b>	9,850,960	9,133,727	92.70
<b>PA23-<i>phzR</i> (1)</b>	6,569,876	6,092,833	92.70
<b>PA23-<i>phzR</i> (2)</b>	9,330,419	8,631,717	92.50
<b>PA23-<i>phzR</i> (3)</b>	7,937,214	7,468,960	94.10
<b>PA23-<i>ptrA</i> (1)</b>	6,851,661	6,496,563	94.80
<b>PA23-<i>ptrA</i> (2)</b>	1,760,770	1,655,995	94.00
<b>PA23-<i>ptrA</i> (3)</b>	7,366,478	6,923,730	94.00
<b>PA23-<i>gacA</i> (1)</b>	4,037,986	3,826,940	94.80
<b>PA23-<i>gacA</i> (2)</b>	13,876,934	12,832,421	92.50
<b>PA23-<i>gacA</i> (3)</b>	4,536,095	4,299,912	94.80
<b>PA23-<i>gacS</i> (1)</b>	8,778,628	8,225,980	93.70
<b>PA23-<i>gacS</i> (2)</b>	6,928,274	6,528,373	94.20
<b>PA23-<i>gacS</i> (3)</b>	5,875,151	5,538,573	94.30

## 2.5. Phylogenetic analysis of PtrA

Genetic sequences for *ptrA* (EF054873.1) homologs were obtained through BLAST. The accession number for *ptrA* (EF054873.1) was used as a query against Pseudomonadales (taxid:72274) sequences in the non-redundant nucleotide collection (nr/nt) employing the blastn algorithm. For amino acid sequences, this query was used against the non-redundant protein database using the blastp algorithm. Forty-one amino acid and forty nucleic acid sequences were selected with E-values of  $<2 \times 10^{-100}$ . All sequences were aligned using Mafft servers (Kato & Standley, 2013). Phylogenetic trees were constructed with the MEGA6 program package (Tamura *et al.*, 2013). For amino acid sequences, the Maximum likelihood method based on the Le-Gascuel 2008 model (Le & Gascuel, 2008) was employed with genetic distances estimated using the JTT model. Bootstrap analysis with a thousand substitutions was used to test the trees. The amino acid sequence for a LTTR from *S. marcescens* (gi: 759524346) was included as an out-group for phylogenetic tree construction. For nucleotide sequences, the Maximum likelihood method based on the Tamura-Nei model (Tamura & Nei, 1993) was applied, with genetic distances estimated through the Maximum composite likelihood approach. Bootstrap analysis with a thousand substitutions was utilized to test the trees. The nucleotide sequence for a LTTR from *Serratia marcescens* strain RSC-14 (gi: 926475601) was included as an out-group for phylogenetic tree construction.

## 2.6. Plasmid construction

For complementation analysis, pUCP22-*retS* and pUCP22-*ladS* were generated as follow: *retS* was PCR amplified from PA23 genomic DNA using primers *retS*-F2 and *retS*-R2. The 2.9-kb amplicon was digested with *Bam*HI & *Hind*III before cloning into the same sites

of pUCP22. The *ladS* gene was amplified using primers *ladS-F-BamHI* and *ladS-R-HindIII*. The 2.8-kb *ladS*-containing fragment was digested with *BamHI* & *HindIII* and cloned into pUCP22 digested with the same enzymes. pUCP22-*retS* and pUCP22-*ladS* were verified through sequence analysis.

## **2.7. Phenotypic assays**

### **2.7.1. Antifungal assay**

Radial diffusion assays were performed to measure AF activity against *S. sclerotiorum*. Strains were cultured overnight at 28°C and adjusted to an OD<sub>600</sub> of 1. A 5-μL aliquot of culture was spotted on to a 1/5 PDA plate which was incubated for 24 hours at 28°C. After incubation, an agar plug containing fungal mycelia was placed in the middle of the plate followed by incubation at 22°C for 72 hours. Inhibition was assessed by measuring the zone of clearing between the fungal growth and the edge of the bacterial colony as described by Poritsanos *et al.* (2006). Five replicates were analysed for each strain, and the experiment was repeated three times.

### **2.7.2. Autoinducer detection assay**

For qualitative analysis of AHL production, cultures were spotted onto LB agar seeded with *C. violaceum* CV026. CV026 has a mutation in *cviI*; consequently it generates neither AHL nor the QS controlled pigment violacein (Latifi *et al.*, 1995). However, in the presence of exogenous AHLs with carbon lengths ranging from C<sub>4</sub>-C<sub>8</sub>, a deep purple halo forms around the bacterial colony. For these assays, a 5-μL volume of overnight culture was spotted onto the CV026 plates. After incubation for 24-48 hours at 28°C, the zone of purple pigment was

measured (Poritsanos *et al.* 2006). Five replicates were analysed for each strain, and the experiment was repeated three times.

### **2.7.3. Protease assay**

Protease production was measured qualitatively by spotting bacteria onto a 2% skim milk agar plate. In the presence of protease, the skim milk is broken down, leaving a zone of lysis surrounding the colony. Strains were cultured overnight at 28°C and adjusted to an OD<sub>600</sub> of 1. A 5-µl aliquot of culture was spotted onto the plate and incubated for 24-36 hours at 28°C, after which the diameter of the colourless zone was measured according to Poritsanos *et al.* (2006). Five replicates were analysed for each strain, and the experiment was repeated three times.

### **2.7.4. Siderophore assay**

To analyze siderophore production, a 5-µl volume of overnight bacterial culture grown in M9-Glc was spotted onto Chrome Azurol S (CAS) media (Schwyn & Neilands, 1987). The diameter of the yellow zone surrounding the colonies, indicative of siderophore production, was measured following 24 hours of incubation at 28°C.

### **2.7.5. Chitinase assay**

Strains were tested for their ability to produce chitinase according to the protocol outlined by Wirth & Wolf (1990). Strains were cultured in M9-Glc broth until they reached early stationary phase (OD<sub>600</sub> 1.20-1.50). A 250-µl aliquot of cell free supernatant was incubated with equal parts of 0.1M NaOAc, pH 5.2 (250 µL) and carboxymethyl-chitin-

Remazol brilliant violet aqueous solution (250  $\mu$ L) (Blue Substrates, Göttingen, Germany). After incubation for 1 hour at either 28°C or 37°C, reactions were stopped by the addition of 250  $\mu$ l of 1M HCl. Mixtures were cooled on ice for 10 min, spun at 20,000xg for 10 min and absorbance readings were taken at 550nm. Three replicates were analysed for each strain and experiments were performed in triplicate.

## **2.8. Quantitative reverse transcriptase PCR (qRT-PCR)**

Quantitative reverse transcriptase PCR (qRT-PCR) was used to monitor expression of metabolite and regulatory genes involved in biocontrol. Triplicate cultures of PA23 and derivative strains were grown to early stationary phase in a 3ml volume of M9-Glc. Cells were harvested by incubating 500 $\mu$ l of culture with 2x volume of RNAprotect reagent (QIAGEN, Valencia, USA) for 5 min followed by centrifugation for 10 min at 6000 rpm. Pellets were stored at -20° for up to one week. Total RNA was extracted using the RNeasy Mini Kit (QIAGEN). Residual genomic DNA was removed by treatment with TURBO RNAase-free DNase I (Ambion). RNA concentrations were measured at absorbance 260 and 280 nm; only RNA samples with A260/A280 between 1.8 and 2.0 were used in subsequent steps. cDNA was generated by reverse transcription using the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific) and random hexamer primers in a 20- $\mu$ L total reaction volume. The following conditions were employed: initial heating at 25°C for 10 min, reverse transcription at 50°C for 15 min, and enzyme denaturation at 85°C for 5 min. Sequences for the PA33 genes of interest were obtained from GenBank (gi: NZ\_CP008696) and the primer sequences are listed in Table 1. PCR was performed using a CFX96 Connect™ Real-Time PCR Detection System (Bio-rad, Hercules, USA) and SsoFast™ EvaGreen® Supermix (Bio-rad).

The final 10- $\mu$ L volume mixture in each well contained 0.4  $\mu$ L of both forward and reverse primers (12 $\mu$ M), 1  $\mu$ L of 1:20 diluted cDNA, 5  $\mu$ L of SsoFast™ EvaGreen® Supermix and 3.4  $\mu$ L of nuclease-free water. PCR reaction conditions included an initial denaturation at 98°C for 2 min, followed by 39 cycles of 98°C for 5 s, 60°C for 30 s, and 60°C for 5 s. Melt-curve analysis was performed to evaluate the formation of primer dimers and other artefacts to validate results. Each reaction was performed in triplicate and experiments were repeated three times with three biological replicates. Relative gene expression was calculated using the  $\Delta\Delta$ Ct method as described by Livak & Schmittgen (2001), with *rpoB* as the reference gene and the CFXManager™ software (Bio-rad).

### 3. Results and discussion: transcriptomic analysis and characterisation of the PA23 QS regulon

#### 3.1. Quorum sensing controlled regulation in PA23

We have previously established that QS is essential for PA23 biocontrol. QS-deficient strains are devoid of antibiotic and degradative enzyme production and consequently, they are no longer capable of suppressing *Sclerotinia* (Selin *et al.*, 2012). To appreciate the global effect of QS on PA23 gene expression, transcriptomic analysis was conducted on two QS-deficient strains of PA23, namely PA23-AHL and PA23-*phzR*. All strains were cultured in M9-Glc to imitate the nutrient limiting conditions of the phyllosphere, and cells were harvested at early stationary phase, as production of secondary metabolites in PA23 occurs at this stage. In PA23 and derivatives, early stationary phase is  $OD_{600}$  was between 1.2-1.5. For both strains, only genes with a  $\log_2$  fold change value of  $\geq 1.5$  or  $\leq -1.5$ , a p-value  $\leq 0.05$  as well as a q-value  $\leq 0.05$  were considered significant. We discovered that between 5.5-7% of genes exhibit altered expression in strains lacking QS in comparison to the WT (Table 3).

In the PhzR-deficient strain, 350 genes were differentially expressed; 245 genes were downregulated and 105 genes were upregulated corresponding to 5.5% of the genome. In the AHL-deficient PA23-AHL, 290 genes were downregulated and 134 were upregulated for a total of 424 genes (approximately 7% of the genome) (Appendix Table 1 & Appendix Table 2).

The larger number of differentially expressed genes in PA23-AHL is likely the result of a second QS system in *P. chlororaphis*, called CsaI/CsaR (Zhang & Pierson, 2001). In *P. chlororaphis* 30-84, CsaI/CsaR governs expression of genes involved in cell surface properties and exoprotease production (Zhang & Pierson, 2001). While *csaI* and *csaR* are present in the PA23 genome (Loewen *et al.*, 2014), their role in PA23 physiology has not yet



been characterized. Because PA23-AHL was constructed with the plasmid pME6863, which carries the *aiiA* from *Bacillus* spp. all AHLs, including the products of both PhzI and CsaI are degraded by the AHL lactonase. Therefore, in PA23-AHL, two QS systems are disrupted instead of one, which we believe leads to a larger number of differentially regulated genes.

Our analysis revealed that 5.5-7% of the PA23 genome is under QS control. In *P. aeruginosa* PAO1, QS-modulated genes were reported to account for 6-10% of the genome (Schuster et al., 2003; Smith & Iglewski, 2003; Wagner et al., 2004). Discrepancies between studies have been attributed to environment, nutrient availability and growth phase (Wagner *et al.*, 2003). In all three studies, a larger number of *P. aeruginosa* genes were downregulated by the loss of QS, in keeping with findings presented herein. We discovered that in PA23-*phzR*, 70% of the differentially regulated genes showed decreased expression, while 30% were upregulated (Table 4). Similarly in PA23-AHL, 68.4% and 31.6% of genes displayed decreased and increased expression, respectively (Table 4). Thus for both PAO1 and PA23, QS is largely serving as a positive regulator of gene expression.

Table 3. Percentage of the genome regulated by LuxR-type QS systems in pseudomonads.

<b>Strain</b>	<b>% QS regulated genes</b>	<b>Reference</b>
<i>P. chlororaphis</i> PA23 <sup>a</sup>		
PA23- <i>phzR</i>	5.5%	This study
PA23-AHL	6.7%	This study
<i>P. aeruginosa</i> PAO1 <sup>b</sup>	6%	(Schuster et al., 2003)
<i>P. aeruginosa</i> PAO1 <sup>c</sup>	3%	(Hentzer et al., 2003)
<i>P. aeruginosa</i> PAO1 <sup>d</sup>	7%	(Wagner et al., 2003)

<sup>a</sup>log<sub>2</sub> fold change ≥1.5 or ≤ -1.5 (fold change ≥2.8 or ≤ -2.8)

<sup>b</sup> fold change ≥2.5 or ≤ -2.5

<sup>c</sup> fold change ≥5 or ≤ -5

<sup>d</sup> fold change ≥5 or ≤ -3, only selected PAO1 transcripts used

Table 4. Percentage of differentially expressed genes up- and down-regulated by PA23 QS-deficient strains.

<b>Strain</b>	<b>% upregulated genes</b>	<b>% downregulated genes</b>
PA23- <i>phzR</i>	30%	70%
PA23-AHL	31.6%	68.4%

### **3.2. Functional characterisation of differentially expressed genes in PA23-*phzR* and PA23-AHL**

Next, we sought to predict the functional role of these QS-controlled genes through Cluster of Orthologous Group (COG) analysis (Figure 5 and Figure 6). COG clusters are constructed using functional characterization based on prokaryotic and eukaryotic genomes (EuKaryotic Orthologous Groups- KOGs) (Tatusov *et al.*, 2003). According to their predicted function, the 350 differentially expressed genes in the *phzR*-mutant could be divided into 21 COG categories. Similarly, the 424 genes identified in PA23-AHL could be grouped into 23 COG categories. In PA23-AHL, a greater number of genes were down-regulated in every category except inorganic transport and metabolism (P), where a larger number of genes displayed increased expression. In PA23-*phzR*, every category exhibited a greater number of down-regulated genes except signal transduction mechanism (T). In this instance, an equal number of up- and down-regulated genes were observed.

The COG category for the unknown function group (S) had the largest number of genes, suggesting that many currently unidentified proteins play a role in governing global gene expression and biocontrol in PA23. To determine the role of these genes in PA23 physiology, better annotation and functional characterisation is required. Several genes characterised in this category were assigned to other COG categories based on BLASTP and further functional analysis. Unfortunately, this could not be accomplished for all of the genes in this category, as we were unable to predict functions or find well-characterised homologs in every case.

A major difference in the transcriptomic profiles of PA23-*phzR* and PA23-AHL is the number of genes downregulated in the COG categories for transcription (K) and cell wall/ membrane/ envelope biogenesis (M). More genes showed differential expression in the AI-deficient strain in both categories (K-35 genes, M-25 genes), compared to the *phzR*-mutant (K-13 genes, M-12 genes). These differences may in part be attributed to the presence of CsaI/ CsaR, as this QS system regulates genes associated with cell wall components (Zhang & Pierson, 2001). CsaI/ CsaR would also be expected to affect expression of transcriptional regulators involved in cell wall biosynthesis. Further analysis of annotated genes using KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways could provide insights on the function of unknown and unannotated genes in the genome.

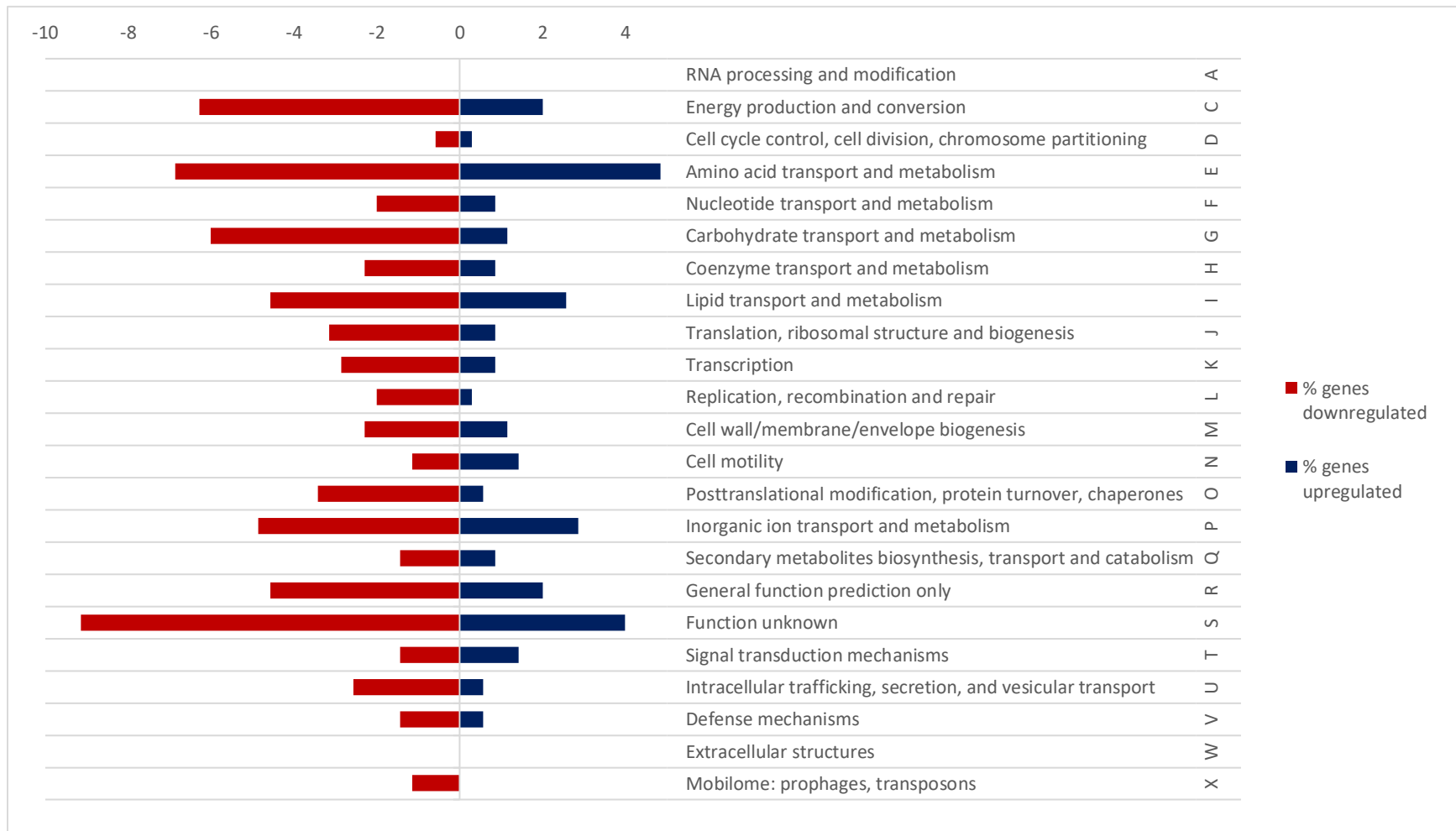


Figure 5: Functional analysis of differentially expressed genes in PA23-phzR in comparison to WT using Cluster of Orthologous Group (COG) analysis. The red bars indicate percent of differentially regulated genes that are down regulated, and the blue bars indicate percentage of genes that are up regulated in each category.

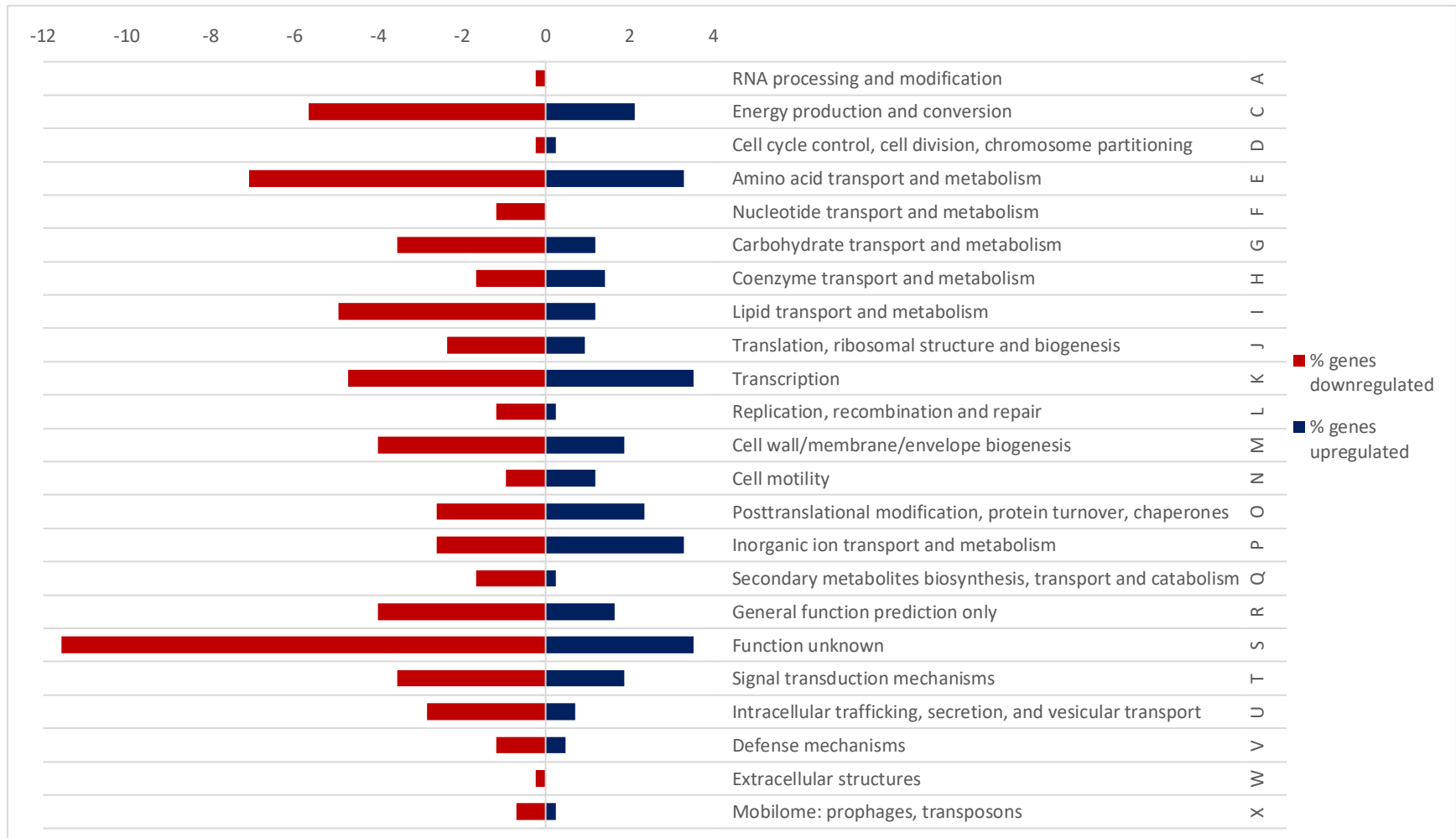


Figure 6. Functional analysis of differentially expressed genes in PA23-6863 in comparison to WT using Cluster of Orthologous Group (COG) analysis. The red bars indicate percent of differentially regulated genes that are down regulated, and the blue bars indicate percentage of genes that are up regulated in each category.

### 3.3. Role of QS in regulation of PA23 secondary metabolites

In both QS-deficient strains, a number of genes involved in secondary metabolite production were significantly down regulated; genes required for *phz* biosynthesis, *aprA* exoprotease and chitinase exhibited 4-fold or higher decreased expression compared to the WT (Figure 7 and Figure 8). Similarly, *prn* biosynthetic gene expression was reduced 1.9- and 4-fold in PA23-*phzR* and PA23-AHL, respectively (Figure 7 and Figure 8). A previous study conducted by Selin *et al.* (2012), found that AF activity in the PA23 QS-deficient strains was eliminated, which was attributed to decreased PRN, PHZ and protease production (Selin *et al.*, 2012). Thus, the transcriptomic data is in keeping with our previous phenotypic characterisation of strains lacking QS (Selin *et al.*, 2010, 2012)

Because chitinase production has not been previously linked to QS in PA23, chitinase assays were conducted to validate the RNA-seq findings (Table 5). Assays were performed at 28°C (optimum growth temperature for PA23) and 37°C (specified in the protocol). At both temperatures, chitinase production was almost completely abolished in the QS-deficient strains (Table 5).

Interestingly, expression of siderophore biosynthetic genes was elevated 3.6-fold in PA23-AHL and 2.7-fold in PA23-*phzR*. Moreover, a siderophore reductase gene (WP\_038634838) was downregulated by a log<sub>2</sub> fold change of 2 or more in the both strains. When spotted on CAS agar plates, the QS-deficient strains exhibited a 2.5-fold increase in siderophore production when compared to WT (Table 6). Collectively, these patterns of expression are consistent with the accumulation of iron-chelating molecules in the QS-deficient strains. The negative effect that QS plays on siderophore biosynthesis was observed



in a previous study of *P. aeruginosa* strains PAO1 and JB2, where the addition of (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone increased siderophore production (Ren *et al.*, 2005). This synthetic furanone acts as a QS antagonist by competing with AHL for binding to the QS receptor, disrupting AHL-mediated multicellular behaviour (Ren *et al.*, 2005).

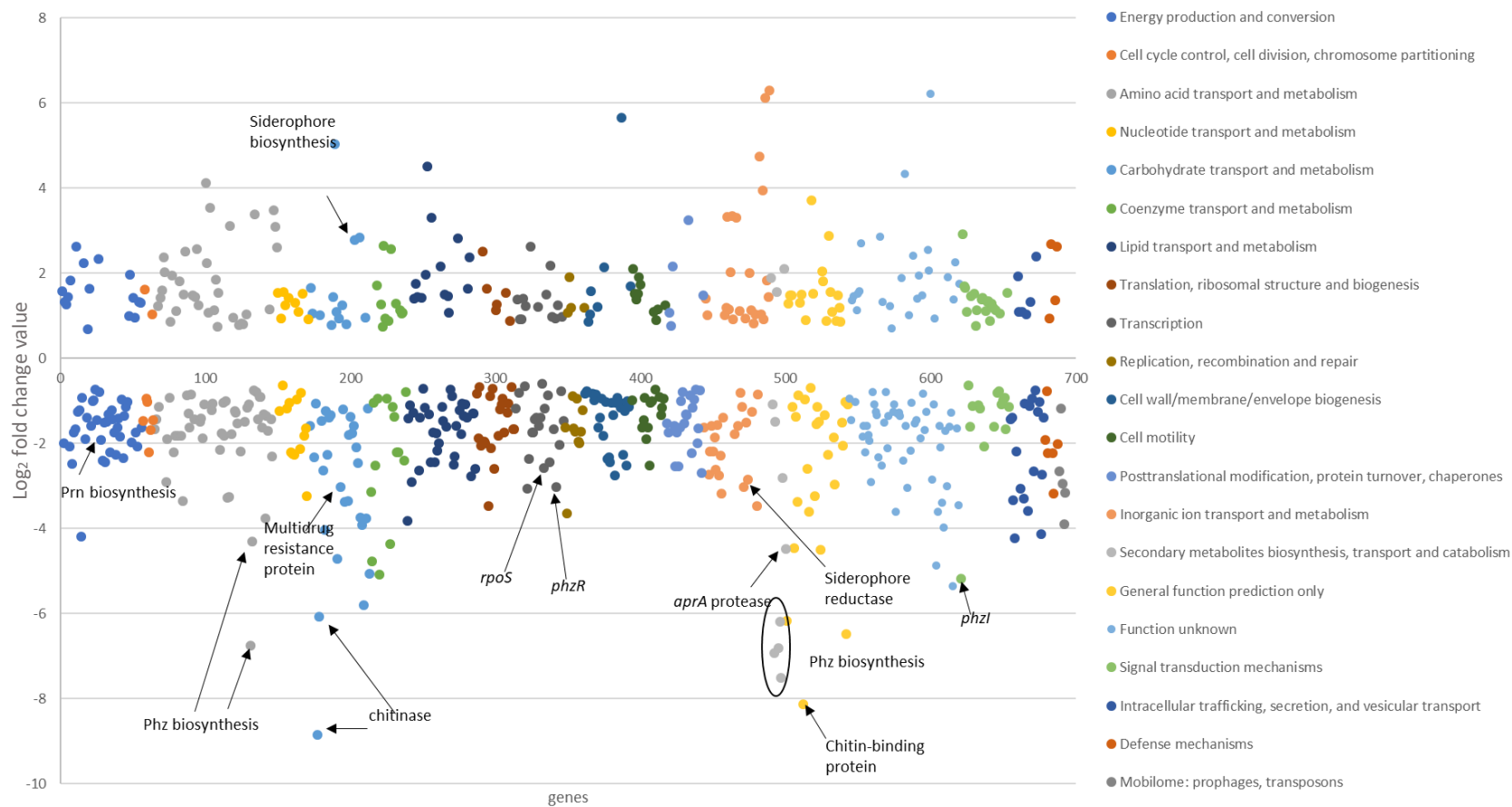


Figure 7. Differentially expressed genes in PA23-*phzR* when compared to PA23 WT. Differentially expressed genes with a p-value  $\leq 0.05$  and a q-value  $\leq 0.05$  are divided into functional categories based on Cluster of Orthologous Groups (COGs) (Tatusov et al., 2000). For the purpose of this study, only genes with a log<sub>2</sub> fold change  $\geq 1.5$  or  $\leq -1.5$  are considered significant. Genes important for the synthesis and regulation of biocontrol products are marked.

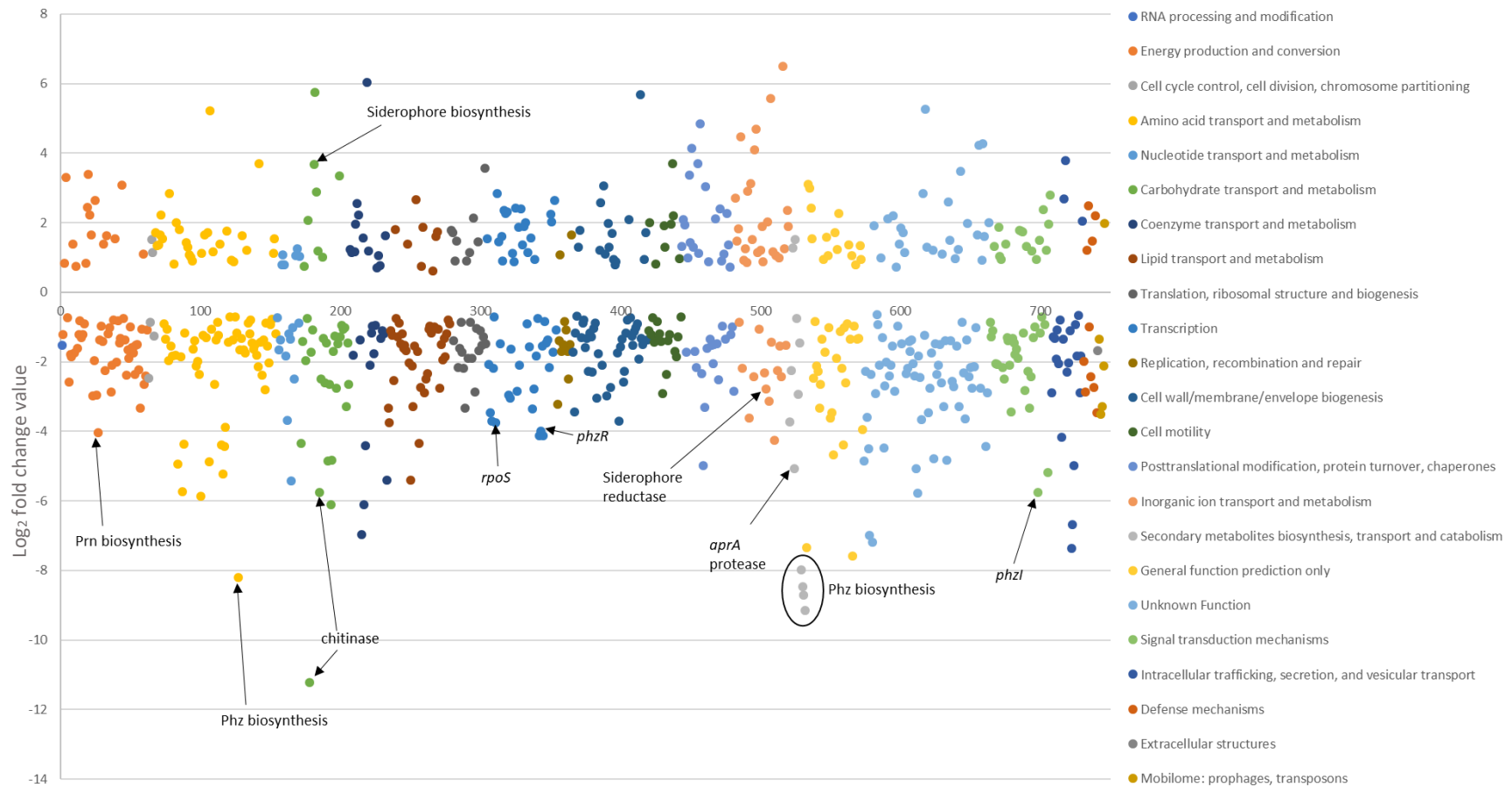


Figure 8. Differentially expressed genes in the AHL-deficient strain PA23-6863 compared to PA23 WT. Differentially expressed genes with a p-value  $\leq 0.05$  and a q-value  $\leq 0.05$  are divided into functional categories based on Cluster of Orthologous Groups (COGs) (Tatusov et al., 2000). For the purpose of this study, only genes with a log<sub>2</sub> fold change  $\geq 1.5$  or  $\leq -1.5$  are considered significant. Genes important for the synthesis and regulation of biocontrol products are marked.

Table 5: Chitinase activity in *Pseudomonas chlororaphis* PA23 and the QS-deficient strains at early stationary phase growing in M9-Glc at 28°C. Enzymatic activity was measured at 28°C and 37°C.

Strain	Absorbance at OD <sub>500</sub>	
	28°C <sup>a</sup>	37°C <sup>a</sup>
WT	0.20 (0.006)	0.22 (0.007)
PA23-AHL	0.00 (0.001) <sup>b</sup>	0.00 (0.001) <sup>b</sup>
PA23- <i>phzR</i>	0.00 (0.001) <sup>b</sup>	0.01 (0.002) <sup>b</sup>

Data set is a representative of three experimental replicates.

<sup>a</sup> Mean (standard deviation) of enzyme activity of three technical replicates

<sup>b</sup> Significantly different from WT (P<0.0001)

Table 6. Siderophore production by *Pseudomonas chlororaphis* PA23 and the QS-deficient strains.

<b>Strain</b>	<b>Zone of siderophore production (mm)<sup>a</sup></b>
<b>WT</b>	3.5 (0.2)
<b>PA23-AHL</b>	7.5 (0.1) <sup>b</sup>
<b>PA23-<i>phzR</i></b>	8.0 (0.3) <sup>b</sup>

<sup>a</sup> Mean (standard deviation) of enzyme activity of three technical replicates

<sup>b</sup> Significantly different from WT (P<0.0001).

### 3.4. QS directly and indirectly regulates gene expression in PA23

For genes under QS control, regulation may occur in one of two ways; directly through PhzR-AI binding to the promoter or indirectly owing to other regulators. For those in the former category, an activated PhzR dimer is believed to bind to a highly-conserved consensus sequence known as the *phz*-box, which has been identified upstream of the PA23 *phz*- and *prn*- biosynthetic operons (Selin *et al.*, 2012). In *P. aeruginosa*, only 7% of QS-regulated genes reportedly contain *las*- or *rhl*-boxes in the promoter region (Schuster *et al.*, 2003; Wagner *et al.*, 2003). To determine if a similar trend is observed in PA23, we analysed the genome for *phz*-boxes using RSA-tools (Medina-Rivera *et al.*, 2015). In PA23, 350 and 424 genes were differentially regulated in the PhzR- and AHL-deficient backgrounds, respectively. However, only 30 genes contained *phz*-boxes within the 500-bp region upstream of the translational start (Appendix Table 6). As discussed in detail below, a large number of transcriptional regulators display altered expression in the QS-deficient strains (Table 7). These findings support the hypothesis that a substantial proportion of the QS regulon is controlled indirectly (Table 7).

In total, 48 genes encoding TS regulators showed differential expression in one or both of the QS-deficient strains. The expression of 9 genes encoding regulators was altered in both strains (Table 7), while twenty-five genes exhibited altered expression in only PA23-AHL (Table 7) and four genes were differentially regulated by only PA23-*phzR*. Examples of prominent genes classified into each of these categories are provided in the following sections.

### **3.4.1. Regulatory genes showing differential expression in both QS-deficient strains**

Our transcriptomic analysis revealed that both QS-deficient strains differentially regulate expression of nine TS regulators (listed in Table 7), including a *csaR*-homologue, a *pspA*-homologue (suppressor of RpoN-dependant transcription) and RpoS (the stationary phase sigma factor). PhzR and PhzI are subject to positive autoregulation, which is typical of most QS systems (Juhas *et al.*, 2005).

### **3.4.2. Regulatory genes showing differential expression in PA23-*phzR***

Altered expression of four additional regulators is observed in PA23-*phzR*; the *lexA* repressor, and currently uncharacterised regulators from several different families (Table 7). Reduced expression (by a factor of -1.59 in comparison to WT) of the SOS repressor *lexA* (and to a lesser extent, *recA*) indicates that the Phz QS system may play a role in modulating the SOS response in PA23. Previous studies with *P. aeruginosa* have found that the SOS regulon consists of 15 genes, and is much smaller than that of *E. coli* SOS regulon (Cirz *et al.*, 2006).

### **3.4.3. Regulatory genes showing differential expression in PA23-AHL**

In addition, the AHL-deficient strain displays differential expression of twenty-four other regulators, including *mvaT* and *mvaV* homologues, *tfoX* competency protein, and sigma factors *rpoD* and *rpoE*, along with uncharacterised proteins from several different families.

As MvaT and MvaV have been reported to play a role in exoproduct secretion by both biocontrol and pathogenic pseudomonads, they will be discussed in more detail. In the absence of QS, the *mvaT* and *mvaV* homologues exhibit a 1.8- and 2.8-fold reduction in gene

expression, respectively (Table 7). MvaT and MvaV code for proteins which are functionally and structurally similar to H-NS family regulators (Baehler *et al.*, 2006). In *P. fluorescens* CHA0, biocontrol activity against *Pythium ultimum* is almost completely abolished in *mvaV* *mvaT* double mutants, and reduced in *mvaT* and *mvaV* single mutants (Baehler *et al.*, 2006). Surprisingly, MvaT and MvaV are repressors of most genes encoding exoproducts such as DAPG, HCN and exoproteases in CHA0, while positively modulating the production of PLT and siderophores. In *P. aeruginosa* PAO1, MvaT is a global regulator of virulence factors and biofilm formation, and is involved in transcriptional repression of QS (Venturi, 2006). Because expression of *mvaT* and *mvaV* was not significantly affected in PA23-*phzR*, it is possible that these genes are under control of the second QS system, CsaI/R. Further analysis of the MvaV- and MvaT- homologues is warranted as it may shed light on the interplay between the PHZ and Csa QS systems as well as the role of these two regulators in PA23 physiology.

Uncharacterized regulatory genes that may belong to the LuxR-, Fur-, H-NS histone-, FrmR-, MarR-, GntR-, AscN-, LacI-, TetR-, XRE-HTH, LysR-, and Rha-families were affected by QS, this indicates that Phz QS may be responsible for the regulation of the PA23 genome through the interaction with these putative regulators. Differential expression of these putative regulators may play a role in the modulation of the PA23 transcriptome by QS. Unfortunately, these genes encode proteins that are currently uncharacterised; consequently, their role in the PA23 regulatory cascade has yet to be determined.



#### 3.4.4. Sigma ( $\sigma$ ) Factors

Sigma ( $\sigma$ ) factors are another group of regulatory elements exhibiting differential expression in the PA23 QS-deficient strains. Sigma factors are responsible for numerous functions in the cell and frequently control expression of large regulons. In *P. aeruginosa* strain PAO1, 24 putative  $\sigma$  factors have been identified, of which 19 are alternative sigma factors of extracytoplasmic function (ECF) (Potvin *et al.*, 2008).

RpoD or  $\sigma^{70}$  is a major sigma factor in *Pseudomonas* that is homologous to  $\sigma^{70}$  in *E. coli*. This sigma is involved in the recognition and expression of a substantial number of housekeeping genes (Potvin *et al.*, 2008). RpoD-dependant expression of genes in *P. aeruginosa* is associated with growth-phase. Levels of RpoD are much higher during the logarithmic phase of growth, and decrease as the cells approach stationary phase (Fujita *et al.*, 1994). The opposite is observed for *rpoS* expression in *P. aeruginosa*; it increases at the onset of stationary phase, and is lower during the logarithmic phase (Fujita *et al.*, 1994). For our cDNA library synthesis, PA23 and its derivatives were harvested at early stationary phase (between OD<sub>600</sub>=1.20 -1.50). Based on a previous study by Fujita *et al.*, (1994), low *rpoD* expression would be expected at this stage of growth. Intriguingly, expression of two genes (WP\_016705108 and WP\_009048295) encoding  $\sigma^{70}$  exhibited a 1.5- and 2.3- log<sub>2</sub> fold increase when compared to WT (Table 7). No significant change was observed in PA23-*phzR* (Table 7).

RpoS is perhaps the best characterized alternative  $\sigma$  factor in *Pseudomonas spp.* (Bertani & Venturi, 2004; Potvin *et al.*, 2008; Selin *et al.*, 2012). In PA23, RpoS controls production of biocontrol products as an *rpoS* mutant exhibits increased and decreased levels of

PRN and PHZ, respectively, resulting in an overall increase in AF activity (Manuel *et al.*, 2012). Cross-regulation has been previously demonstrated between QS and RpoS, with RpoS positively regulating *phzI* and negatively regulating *phzR* (Selin *et al.*, 2012). *rpoS*, on the other hand, was found to be positively regulated by the Phz QS system (Selin *et al.*, 2012). These latter findings are in keeping with those presented herein; *rpoS* expression is reduced 3.7-fold in PA23-AHL and 2.7-fold in PA23-*phzR* (Table 7).

RpoN or  $\sigma^{54}$  has been found to play a role in motility, nutrient transport, formation of pili, mucoidy and regulates QS in *P. aeruginosa* PAO1 (Cases *et al.*, 2003; Heurlier *et al.*, 2003; Potvin *et al.*, 2008; Thompson *et al.*, 2003). In PA23, decreased expression of genes involved in flagellar biosynthesis (including *flgG*, *flgB* and *flgE*) was observed in both QS-deficient strains. Previous studies revealed that a loss of AI production results in decreased flagellar motility, whereas the *phzR* mutant was unchanged in comparison to the WT (Selin *et al.*, 2012). Thus, it is possible that the PhzI/R QS system does not directly modulate motility in PA23, rather the Csa system is involved. Interestingly, in *P. aeruginosa* PAO1, RpoN enhances expression of *rhII* and *rhIR* throughout all growth phases; whereas it has a negative effect on expression of *lasR* and *lasI* at low cell densities. (Heurlier *et al.*, 2003). In PA23, the role of RpoN in the production of secondary metabolites needs to be further examined to determine its place in the regulatory hierarchy.

Finally, two genes, (WP\_038633746 and WP\_009050411) encoding RpoE ( $\sigma^{24}$ ) subunits were up regulated by a factor of 1.6- and 1.9-fold in the PA23 AHL-deficient strain (Table 7). In *P. protegens* CHA0, RpoE influences tolerance towards osmotic stress, and controls production of exopolysaccharide and adaption to dry conditions (Schnider-Keel *et al.*,

2001). Since the PhzR-deficient strain showed no change in *rpoE* transcription, it is possible that QS regulation of RpoE is channelled through CsaI/R. As mentioned previously, in *P. chlororaphis* 30-84, the Csa system regulates production of cell surface components, including exopolysaccharides (Zhang & Pierson, 2001). The fact that RpoE governs expression of exopolysaccharide further supports the proposed link between CsaI/R and RpoE.

Table 7. Regulatory genes modulated by quorum sensing in *Pseudomonas chlororaphis* PA23.

Regulated by:	Regulator (type)	Protein ID	Log <sub>2</sub> fold change	
			AHL-	<i>phzR</i>
PA23-AHL	Transcriptional regulator	WP_038635479	-4.13	
	Transcriptional regulator	WP_009048394	-2.95	
	RNA polymerase subunit sigma-70	WP_009048295	2.32	1.41
	Transcriptional regulator (Cu(I) responsive transcriptional regulator)	WP_009046808	-2.19	
	XRE family transcriptional regulator	WP_007924514	-2.17	
	Transcriptional regulator (MvaV)	WP_009049335	-2.84	-0.78
	MarR family transcriptional regulator	WP_009046358	-2.08	
	H-NS histone (MvaT)	WP_007931174	-1.82	
	Cold-shock protein (CspD)	WP_009049775	-2.10	-0.82
	Transcriptional regulator	WP_009050371	-1.57	
	Competence protein TfoX	WP_009050884	-1.55	
	RNA polymerase sigma factor (RpoD)	WP_016705108	1.55	
	RNA polymerase subunit sigma 24 (RpoE)	WP_009050411	1.56	
	Cro/CI family transcriptional regulator	WP_023968513	1.60	
	GntR family transcriptional regulator	WP_038633317	1.88	
	RNA polymerase subunit sigma 24 (RpoE)	WP_038633746	1.90	
	RNA polymerase sigma factor	WP_016702389	2.00	
	TetR family transcriptional regulator	WP_009051542	2.03	
	AsnC family transcriptional regulator	WP_009051665	2.25	
	TetR family transcriptional regulator	WP_038631318	2.28	
	LacI family transcriptional regulator	WP_038631299	2.35	
	Cro/CI family transcriptional regulator (LexA, involved in SOS response)	WP_009049820	2.39	
	CdaR family transcriptional regulator	WP_016702462	2.41	
	ArsR family transcriptional regulator	WP_009047418	2.84	
	DNA-directed RNA polymerase subunit beta	WP_038635595	-1.72	-0.65
	PA23-AHL & PA23- <i>phzR</i>	Temperature acclimation protein B	WP_007931076	-3.36
GntR family transcriptional regulator		WP_009050459	-2.79	-3.06
DNA-binding protein		WP_009050695	-4.12	-2.04
AraC family transcriptional regulator		WP_028683816	-3.47	-1.18
TetR family transcriptional regulator		WP_009046585	-3.71	-1.66
LuxR family transcriptional regulator (CsaR)		WP_009048517	-3.05	-2.37
Uncharacterized protein (PspA)		WP_009048686	-1.63	-2.44
Rha family transcriptional regulator		WP_038633014	2.65	2.18

	PhzR	WP_038635113	-3.99	-3.03
	RNA polymerase sigma factor RpoS	WP_007924026	-3.74	-2.57
	TetR family transcriptional regulator	WP_007922198		1.51
PA23- <i>phzR</i>	LysR family transcriptional regulator	WP_016702918		2.63
	LexA repressor	WP_007921301		-1.59
	LysR family transcriptional regulator	WP_009051067		-1.74

### 3.5. Phz QS regulates *csaR*

Our transcriptomic analysis revealed that a connection exists between the Csa and Phz QS systems. In PA23-*phzR* and PA23-AHL, *csaR* was downregulated by a factor of 2.67 and 3.04 respectively; whereas, the expression of *csaI* was not altered in either strain. This hierarchical arrangement is similar to that observed in *P. aeruginosa* (de Kievit, 2002; Zhang & Pierson, 2001). *P. aeruginosa* employs two QS systems called Las and Rhl; however in this case, both the transcriptional activator (*rhlR*) and the AHL synthase (*rhlI*) genes are under control of the dominant Las system (Smith & Iglewski, 2003).

From our RNA-seq profiles, we observed a larger number of differentially expressed genes in the AI-deficient strain compared to the *phzR* mutant. As such, we predict that the Csa regulon is quite expansive and goes well beyond modulation of cell surface properties, which was originally reported (Zhang & Pierson, 2001). Future transcriptomic analysis of *csaI* and *csaR*-mutants should be conducted to reveal the scope of genes under Csa QS control. Such studies will undoubtedly uncover interactions with other regulators, adding another layer to the increasingly complex cascade governing expression of PA23 biocontrol factors.

## **4. Results and discussion: transcriptomic and phylogenetic analysis of the PtrA regulon**

### **4.1. Phylogenetic analysis of *ptrA* homologs reveals that it is well conserved among pseudomonads.**

PtrA is a novel LTTR found to be vital for PA23 biocontrol (Klaponski *et al.*, 2014). Disruption of *ptrA* results in strains that lack AF activity; consequently, they are unable to protect canola against Sclerotinia stem rot (Shah *et al.*, 2016). The LTTR family is the largest and most common group of regulators found in bacteria, with members functioning as either activators or repressors of single or operonic genes (Maddocks & Oyston, 2008).

Phylogenetic analysis was carried out to determine the degree of conservation of this LTTR among pseudomonads. The tree was constructed using 41 LTTR protein sequences homologous to PtrA. PtrA was found in the same clade as homologues from *P. chlororaphis* O6 and *P. fluorescens* Pf-5 (Figure 9). As closely related homologues of PtrA are found in biocontrol strains, they are expected to play a similar role in regulating genes responsible for production of AF compounds. Collectively, this analysis illustrates that a wide range of *Pseudomonas* species harbour a *ptrA* homologue (Figure 9). For both pathogenic and symbiotic pseudomonads, secreted products play a significant role in the virulence and biocontrol properties of these organisms. In addition, regulatory factors overseeing their expression are in many cases conserved. For example, the Gac two-component system and QS positively regulate production of secreted factors that play a key role in pathogenic and symbiotic interactions (Heeb and Haas 2001; Bassler, 2002). It is not surprising, therefore, to find that PtrA is highly conserved amongst *Pseudomonas* species.

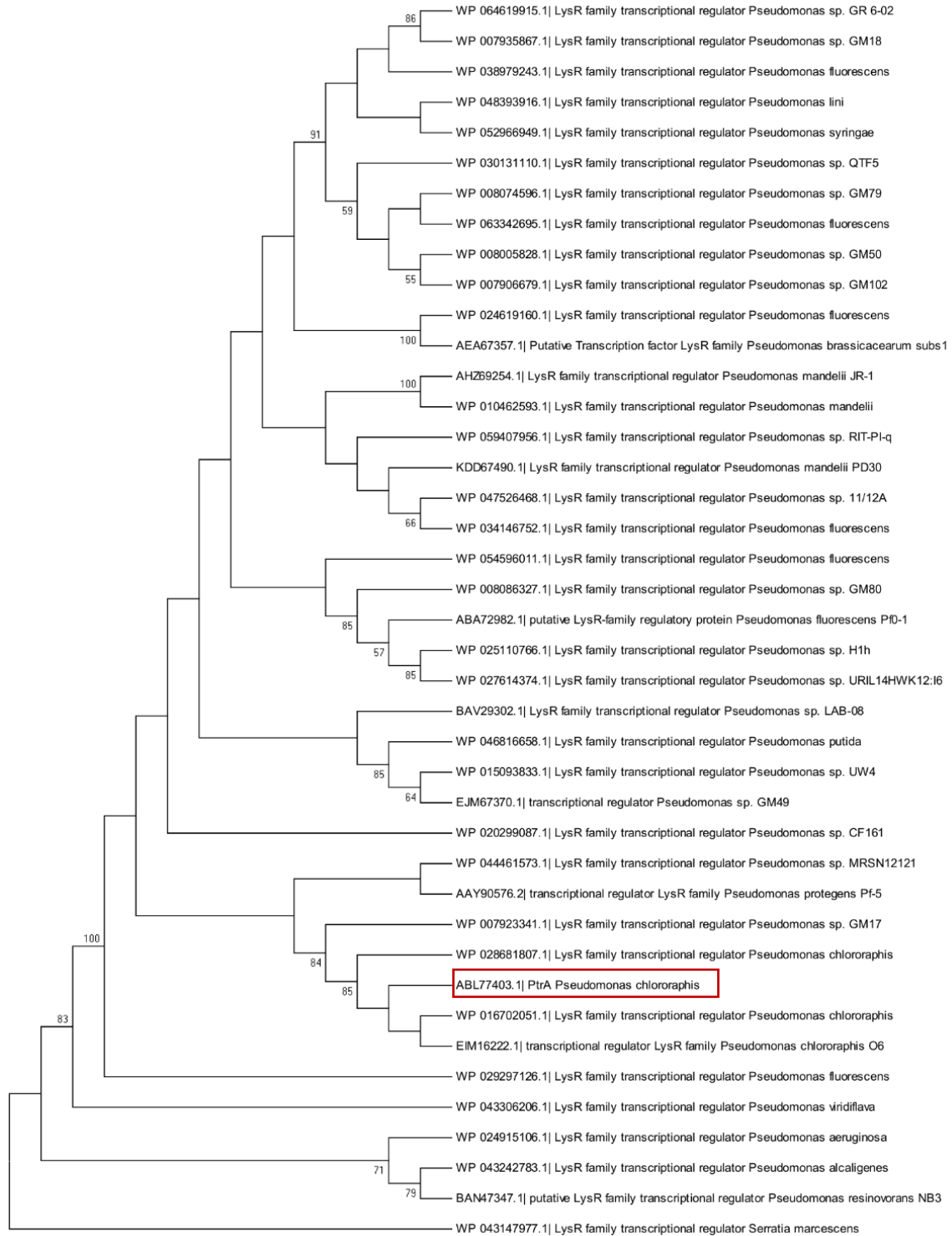


Figure 9. Molecular phylogenetic analysis of PtrA homologs by the Maximum Likelihood method. The evolutionary history was inferred using the Maximum Likelihood method based on the Le Gascuel Method (Le & Gascuel, 2008). This analysis involved 41 amino acid sequences including PtrA. The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is indicated next to the branches.



## 4.2. Elucidation and functional characterization of the PA23-*ptrA* regulon

Transcriptomic analysis was conducted on a *PtrA*-deficient strain (PA23-443) to examine the role of this regulator in PA23 global gene expression. All (*ptrA*-, *gacA*- and *gacS*-deficient) strains were cultured in M9-Glc to imitate the nutrient limiting conditions of the phyllosphere, and were harvested at early stationary phase (when cell OD<sub>600</sub> was 1.2-1.5) as production of secondary metabolites in PA23 occurs at this stage. Only genes with a log<sub>2</sub> fold change value of  $\geq 1.5$  or  $\leq -1.5$ , a p-value  $\leq 0.05$  as well as a q-value  $\leq 0.05$  were considered significant for this and subsequent analysis (section 4.6. Role of GacA and GacS in the PA23 regulatory cascade). In PA23-443, 460 genes exhibited differential expression; of these, 40.7% (187 genes) were up regulated and 59.3% (273 genes) were down regulated, collectively representing 7.2% of the genome (Appendix Table 3).

Next, we wanted to predict the functional role of these genes through COG analysis (Tatusov *et al.*, 2003). The 460 differentially expressed genes in PA23-443 were divided into 21 COG categories based on their predicted function as outlined in (Figure 10). Our transcriptomic findings indicate that most genes in the motility (N) COG category are up regulated in PA23-443 (Figure 10), in keeping with earlier iTRAQ and phenotypic analysis demonstrating that *PtrA* represses motility (Klaponksi *et al.*, 2014). A large number of genes in the secondary metabolite biosynthesis (Q) category were down regulated in the *ptrA*-deficient strain (discussed in more detail below). However, the largest number of differentially expressed genes appears in the unknown function (S) COG category. A similar finding was observed previously in our proteomic profiling of the *ptrA* mutant (Klaponksi *et al.*, 2014). To understand the role that these genes play in PA23, better annotation and functional characterisation is required.

Table 8. Percentage of differentially expressed genes up and down regulated in the PA23 *ptrA*- and *gac*-deficient strains.

<b>Strain</b>	<b>% upregulated genes</b>	<b>% downregulated genes</b>	<b>Total % of genome regulated</b>
<b>PA23-<i>ptrA</i></b>	40.65	59.35	7.2
<b>PA23-<i>gacS</i></b>	33.0	67.0	6.7
<b>PA23-<i>gacA</i></b>	32.39	67.61	6.2

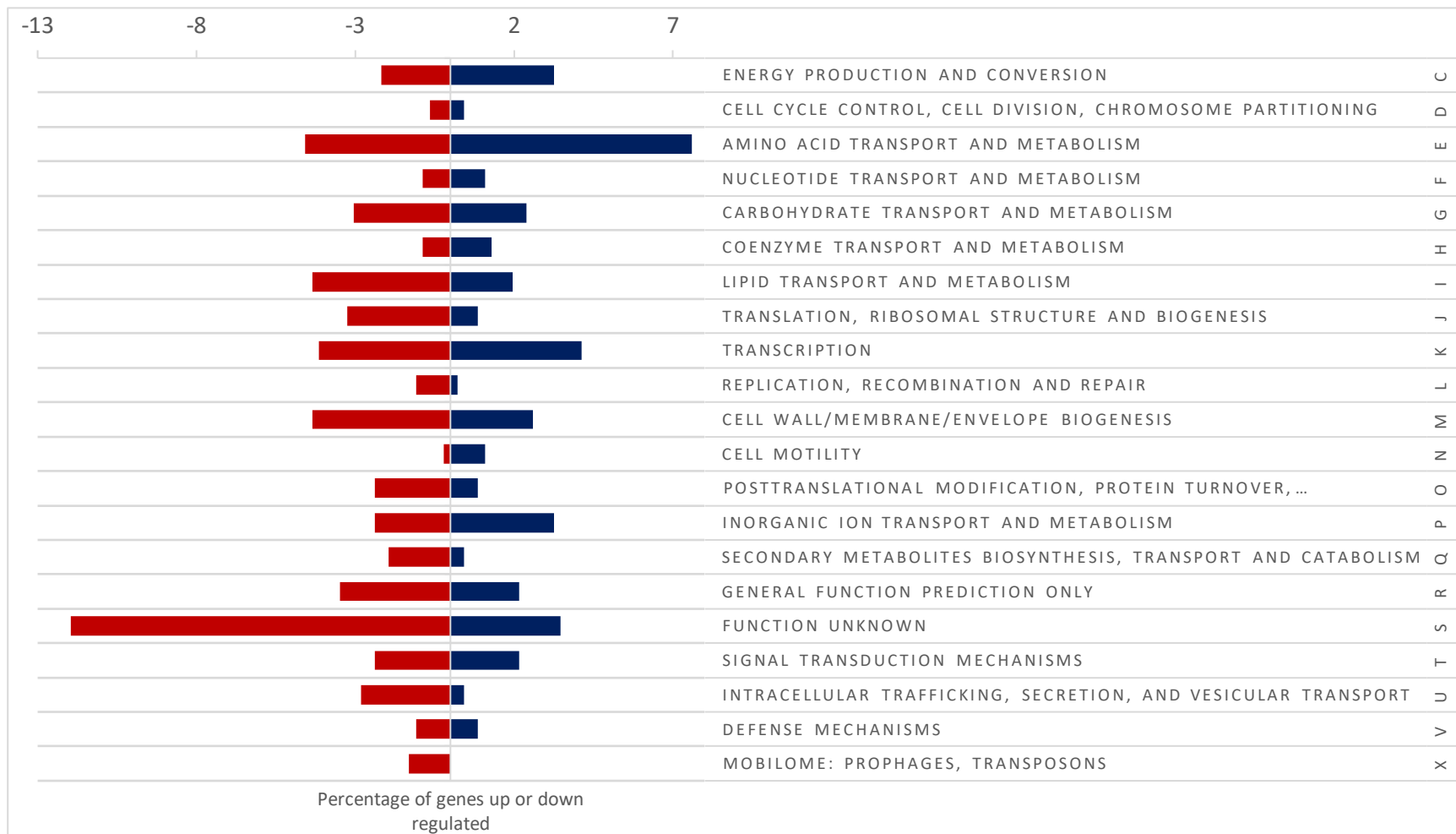


Figure 10. Percentage of up (blue) and down (red) regulated genes in PA23-*ptrA* compared to PA23 WT. Significantly differentially expressed genes with a log<sub>2</sub> fold change value of  $\leq -1.5$  and  $\geq 1.5$  were included. A total of 460 genes were identified and grouped using functional categories (COGs) (Tatusov et al., 2000) and the percentage of up and down regulated genes in each category is shown.

### 4.3. PtrA controls expression of biosynthetic and regulatory genes in PA23

PA23-443 lacks AF activity due to reduced antibiotic and enzyme production (Klaponksi *et al.*, 2014; Poritsanos, 2005; Shah *et al.*, 2016). Our transcriptomic analysis revealed that the genes involved in the production of secondary metabolites including those for PHZ biosynthesis, PRN biosynthesis, chitinase and protease were down regulated by a  $\log_2$  fold factor of at least 4 (Figure 11). Similar results were obtained in qPCR analysis, where *phzA* (PHZ) and *prnA* (PRN), exhibited a dramatic reduction in expression levels in the *ptrA* mutant background (Figure 11) (Shah *et al.*, 2016). In an earlier study, phenotypic and proteomic analysis revealed dramatically decreased PHZ, PRN and chitinase production in the *ptrA* mutant, consistent with our transcriptomic findings (Klaponksi, 2014). Moreover, genes for siderophore biosynthesis were increased  $\log_2$  fold factor of 5.5 in PA23-443, in keeping with the fact that the *ptrA*-mutant produces 3-fold more siderophore than the WT (Klaponksi *et al.*, 2014).

LTTRs tend to be autoregulatory in nature and usually control genes that are divergently transcribed (Schell, 1993). The gene immediately upstream of *ptrA*, encoding a short-chain dehydrogenase (*scd*), showed no change in expression in the absence of this LTTR (Figure 12). We complemented this analysis using qPCR to include three genes upstream of *scd* encoding a membrane protein (AIC18438.1; primers Up1), a hypothetical protein (AIC18437.1; primers Up2) as well as *nhaA* (AIC18435.1; primers Up3). In all 3 cases, no difference in transcript abundance was detected in the *ptrA*-mutant compared to the WT (Figure 12), similarly the RNA-seq data revealed no significant difference in the expression of these genes in the *ptrA*-mutant. Phenotypic analysis of an *scd* mutant revealed no change in

production of secondary metabolites (Klaponksi, 2014) or AHLs (Figure 13). Collectively these findings suggest that *scd* and neighbouring genes are not linked to PtrA control.

Our qPCR and whole genome transcriptomic analysis revealed a 9-fold and log<sub>2</sub> fold 2.2 increase in *ptrA* expression, respectively, in the mutant strain indicating that PtrA is subject to negative autoregulation (Schell, 1993; Shah *et al.*, 2016). With respect to other regulatory genes involved in PA23 biocontrol, expression of both *phzI*, encoding the AHL synthase and *phzR*, encoding the LuxR-type transcriptional regulator PhzR, were significantly downregulated in the *ptrA* mutant (Figure 11 and Figure 12). Reduced *phzI* expression coincides with lower AHL signal production by the *ptrA*-deficient strain (Figure 13). Previously, Selin *et al.*, (2012) demonstrated that both PhzR and AHL production is required for PA23 suppression of *S. sclerotiorum*. Interestingly, *psrA* transcription was modestly increased in our qPCR analysis but the RNA-seq findings showed only a log<sub>2</sub> fold elevation of 1.18, which was not considered as significant (Figure 12). Conversely, expression of the PsrA-regulated target gene *rpoS* was markedly reduced in the *ptrA* mutant background. Because RpoS is a negative regulator of PA23 biocontrol (Manuel *et al.*, 2012), the decrease in *rpoS* transcription was unexpected as the *ptrA* mutant is no longer capable of fungal suppression. Reduced expression may be the result of cross regulation occurring between the PhzRI QS system and RpoS (Selin *et al.*, 2012).

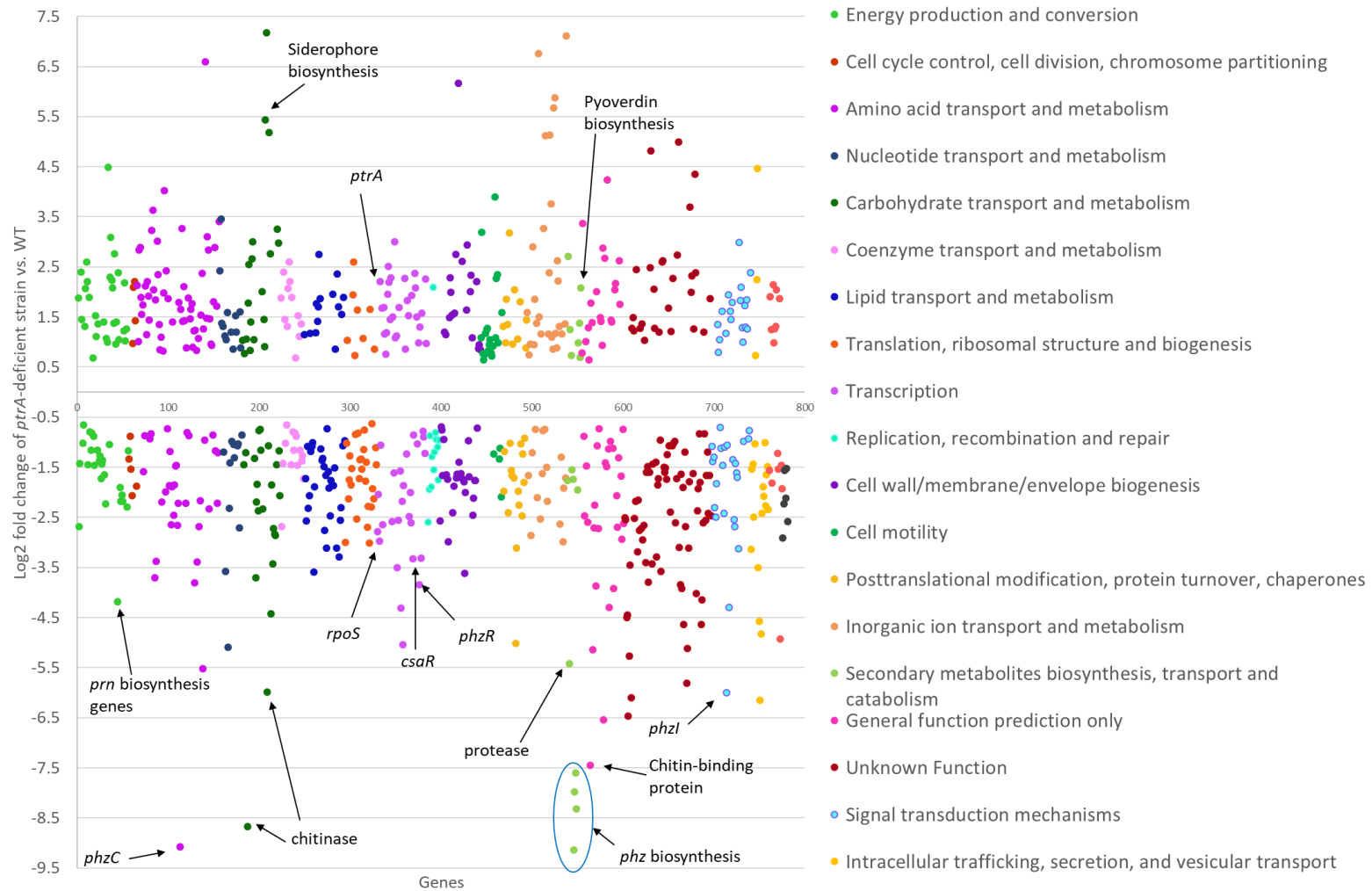


Figure 11. Differentially expressed genes in PA23-*ptrA* when compared to PA23 WT. Differentially expressed genes with a p-value  $\leq 0.05$  and a q-value  $\leq 0.05$  are divided into functional categories based on Cluster of Orthologous Groups (COGs) (Tatusov et al., 2000). For the purpose of this study, only genes with a  $\log_2$  fold change  $\geq 1.5$  or  $\leq -1.5$  are considered significant. Genes important for the synthesis and regulation of biocontrol products are marked.

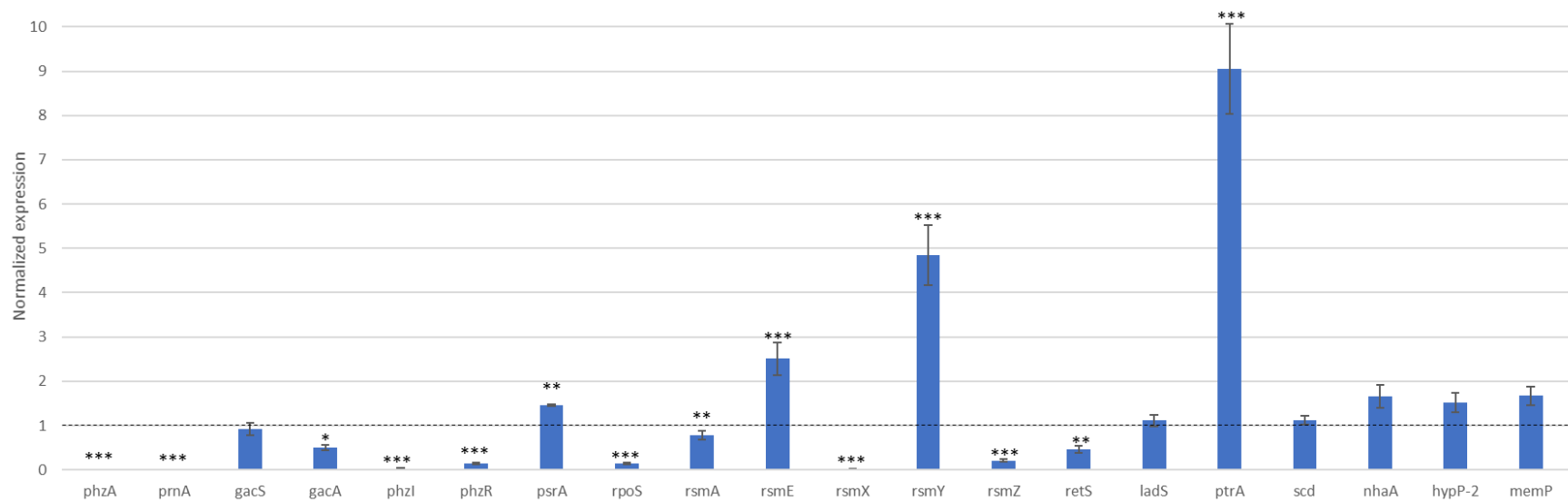


Figure 12. qRT-PCR fold change in gene expression in the *ptrA* mutant (443) compared to WT PA23. *rpoB* was used as a reference gene. Gene expression in the WT was normalized to 1.0 (indicated by the dotted line). For strains that differ significantly from WT, columns have been marked with an asterisk (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.0001$ )

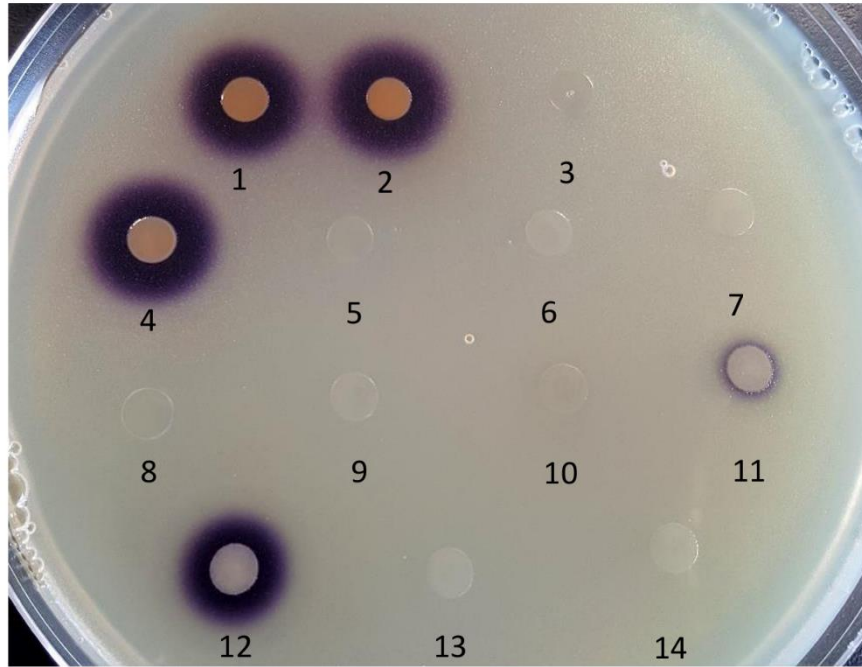


Figure 13. Autoinducer production by PA23, PA23-443 (*ptrA*-mutant) and derivative strains determined using *Chromobacterium violaceum* CVO26-seeded agar. Samples are as follows: 1, PA23 (pUCP22); 2, PA23-*scd* (pUCP22); 3, PA23-443 (pUCP22); 4, PA23-443 (pUCP22-*ptrA*); 5, PA23-443 (pUCP22-*rsmA*); 6, PA23-443 (pUCP22-*rsmE*); 7, PA23-443 (pUCP23-*rsmZ*); 8, PA23-443 (pUCP22-*rpoS*); 9, PA23-443 (pUCP22-*psrA*); 10, PA23-443 (pUCP23-*phzR*); 11, PA23-443 (pUCP22-*gacA*); 12, PA23-443 (pUCP23-*gacS*); 13, PA23-443 (pUCP22-*retS*), PA23-443 (pUCP22-*ladS*).



#### **4.4. Elucidation and functional characterization of the PA23-*gacA* and PA23-*gacS* regulon**

As mentioned earlier, the GacS-GacA two-component system plays an essential role in PA23 biocontrol (Poritsanos *et al.*, 2006; Selin *et al.*, 2014). Moreover Gac oversees expression of several other PA23 regulators including QS and it shares a regulatory link with PtrA (Poritsanos *et al.*, 2006; Selin *et al.*, 2014; Shah *et al.*, 2016). In order to fully appreciate the scope of the PA23 GacA- and GacS-regulons, transcriptomic profiling was undertaken. A total of 423 genes showed altered expression in the *gacS* mutant; of which 32.4% (137) genes were up regulated and 67.6% (286) genes were down regulated. For PA23-*gacA*, a total of 400 genes were differentially expressed, of which 34.7% (139) genes were up regulated and 65.3% (261) genes were down regulated (Appendix Table 4 & Appendix Table 5). We discovered that, compared to WT, approximately 6.5% and 6.1% of genes exhibit altered expression in the *gacS* and *gacA* mutants, respectively (Table 8). The Gac regulon was previously reported to constitute 10% and 15% of the *P. chlororaphis* 30-84 and *P. aeruginosa* M18 genome, respectively (Wang *et al.*, 2013; Wei *et al.*, 2013). Compared to our findings, the larger number of differentially regulated genes could be explained by differences in the experimental conditions employed including growth conditions, transcriptional profiling technique (RNA-seq versus microarrays) and data processing steps (both studies used different parameters for significance) (Wang *et al.*, 2013; Wei *et al.*, 2013).

Next, we sought to further characterise the function of the differentially expressed genes using COG analysis (Tatusov *et al.*, 2000) (Figure 14 and Figure 15). It is not surprising that the *gacA*- and *gacS*- regulons showed a high degree of overlap (Figure 16) since this system relies on both the GacS sensor kinase and the response regulator GacA (Haas &

Défago, 2005). The number of genes exhibiting altered expression and the COG profiles for both mutants were virtually identical, being grouped into 22 distinct COG categories. The largest number of genes was classified into the general prediction only (R) and the unknown function (S) categories (Figure 14 and Figure 15). More than 65% of the regulon in both strains was down regulated compared to the WT, underscoring the fact that this two-component system is largely serving as a positive regulator of gene expression in PA23.

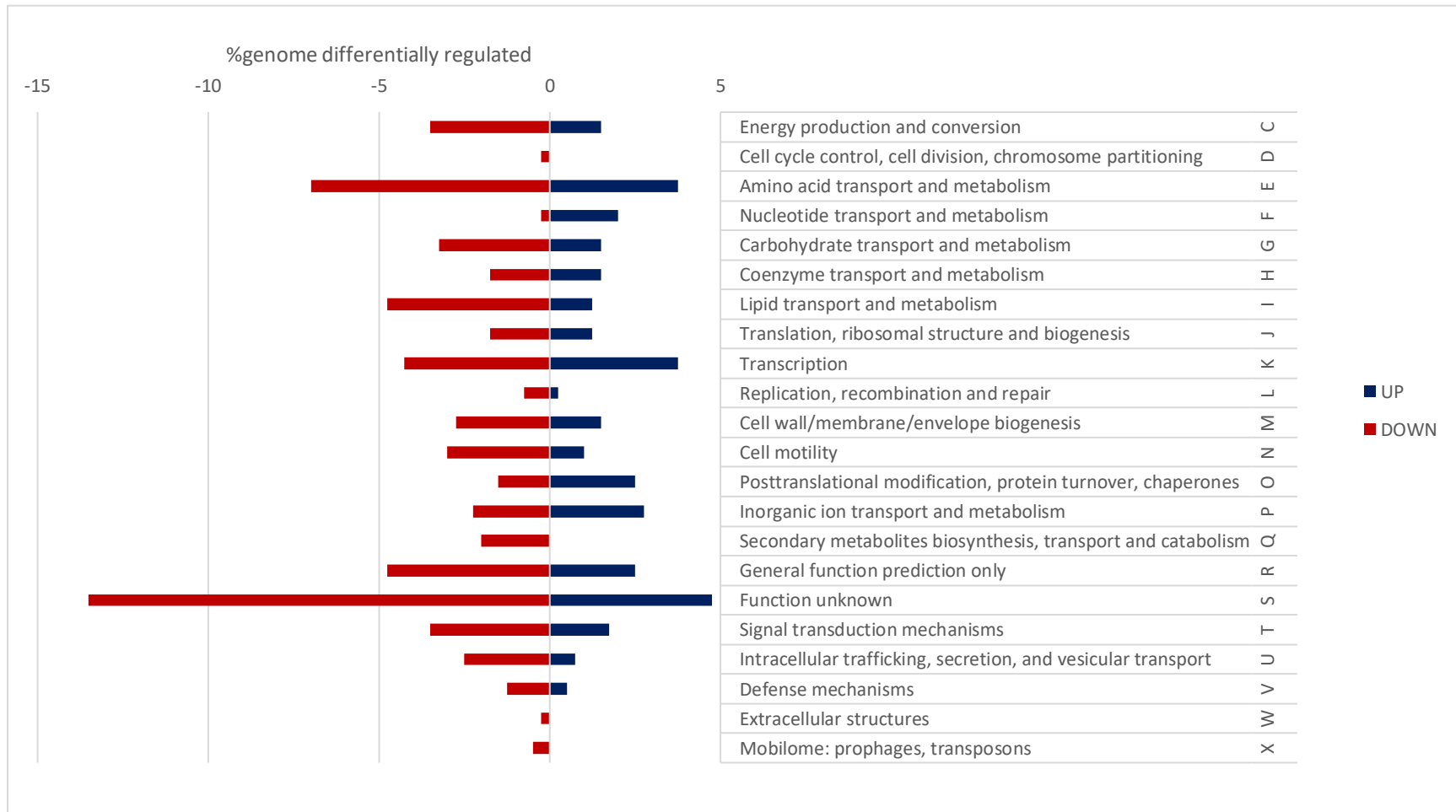


Figure 14. Percentage of up (blue) and down (red) regulated genes in PA23-*gacA* compared to PA23 WT. Significantly differentially expressed genes with a  $\log_2$  fold change value of  $\leq -1.5$  and  $\geq 1.5$  were selected. A total of 460 genes were identified and grouped using functional categories (COGs) (Tatusov et al., 2000). Percentage of total genes up or down regulated in each category are shown.

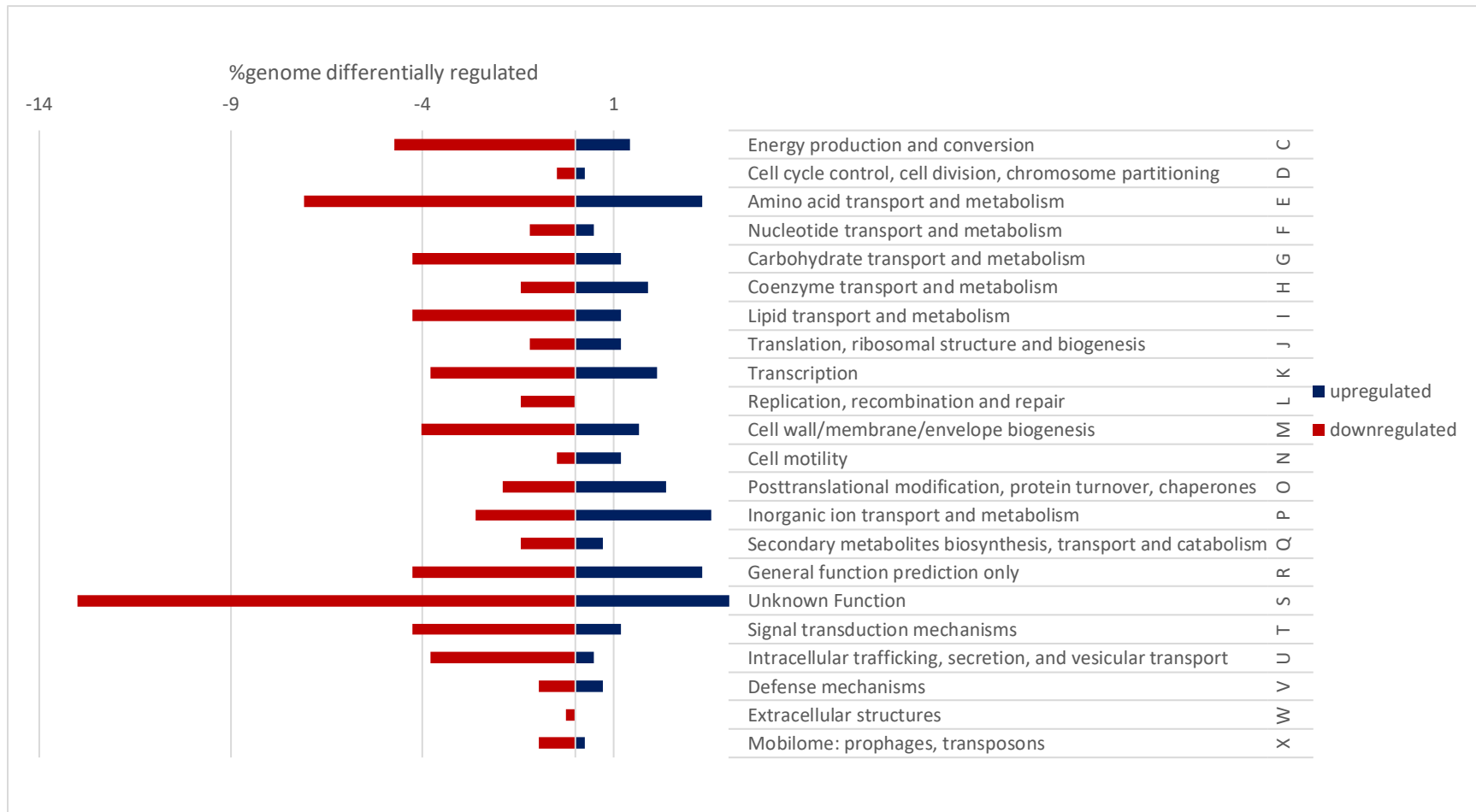


Figure 15. Percentage of up (blue) and down (red) regulated genes in PA23-*gacS* compared to PA23 WT. Significantly differentially expressed genes with a  $\log_2$  fold change value of  $\leq -1.5$  and  $\geq 1.5$  were selected. A total of 460 genes were identified and grouped using functional categories (COGs) (Tatusov et al., 2000). Percentage of total genes up or down regulated in each category are shown.

#### 4.5. Role of Gac regulon in controlling production of PA23 exoproducts

In both *gac*-mutants, a number of genes involved in secondary metabolite production were significantly down regulated. Expression of genes required for *phz*, *prn*, *hcn*, *aprA* exoprotease, and chitinase synthesis exhibited a  $\log_2$  fold  $\geq 5$  and  $\geq 4$  decrease in the *gacA*- and the *gacS* mutants, respectively, compared to the WT (Figure 16 and Figure 17).

Phenotypic analysis of the Gac-deficient strains revealed decreased AF activity due to diminished HCN, PRN, PHZ, protease and chitinase production (Poritsanos *et al.*, 2006; Poritsanos, 2005), consistent with the transcriptomic data presented herein (Figure 16 and Figure 17). Interestingly, genes for siderophore biosynthesis displayed increase in PA23-*gacA* by a  $\log_2$  fold factor of 2.4 and by a  $\log_2$  fold factor of 5.3- in PA23-*gacS*. Elevated gene expression is in keeping with enhanced siderophore production by the Gac-deficient strains (Poritsanos *et al.*, 2006).

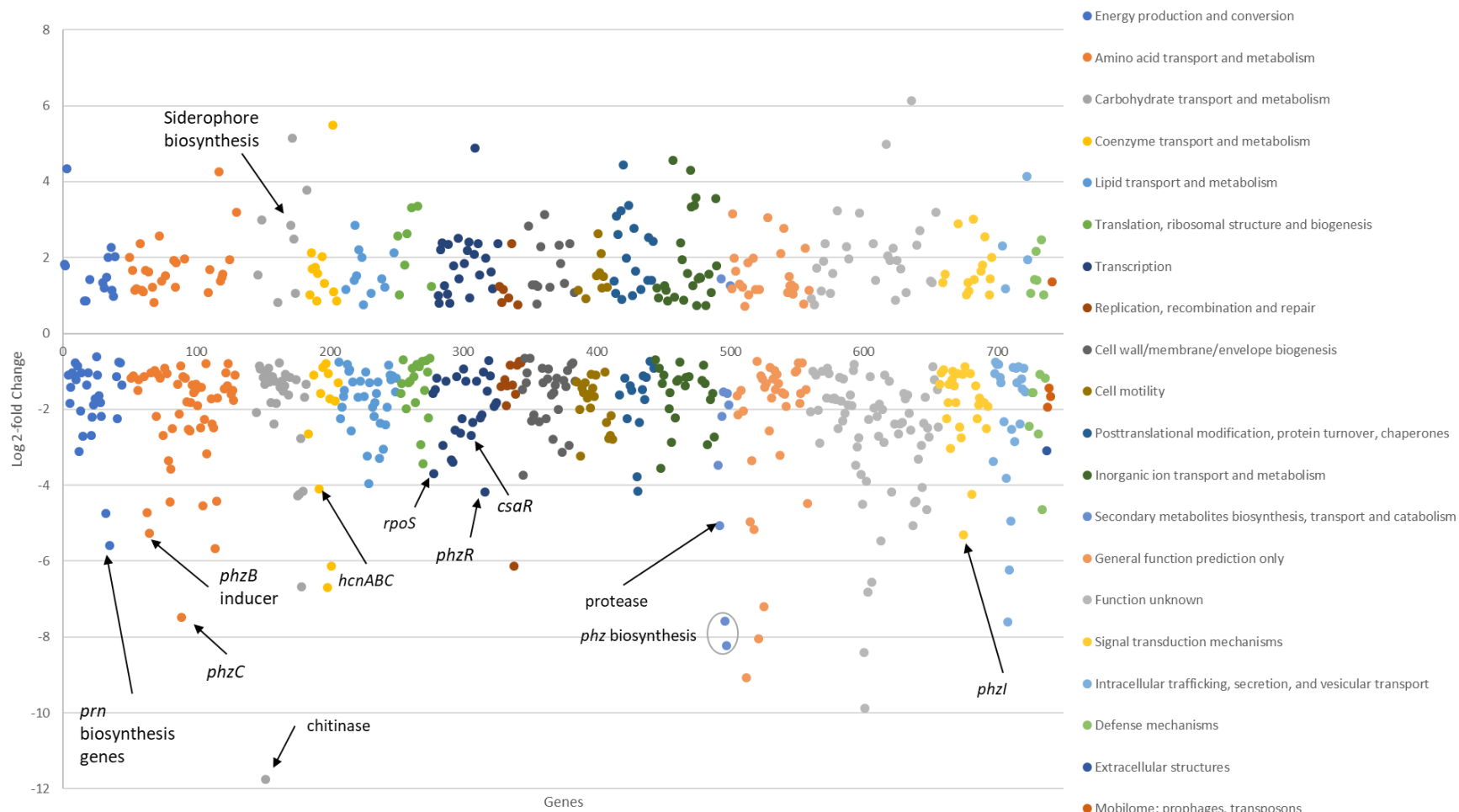


Figure 16. Differentially expressed genes in PA23-*gacA* when compared to PA23 WT. Differentially expressed genes with a p-value  $\leq 0.05$  and a q-value  $\leq 0.05$  are divided into functional categories based on Cluster of Orthologous Groups (COGs) (Tatusov et al., 2000). For the purpose of this study, only genes with a log<sub>2</sub> fold change  $\geq 1.5$  or  $\leq -1.5$  are considered significant. Genes important for the synthesis and regulation of biocontrol products are marked.

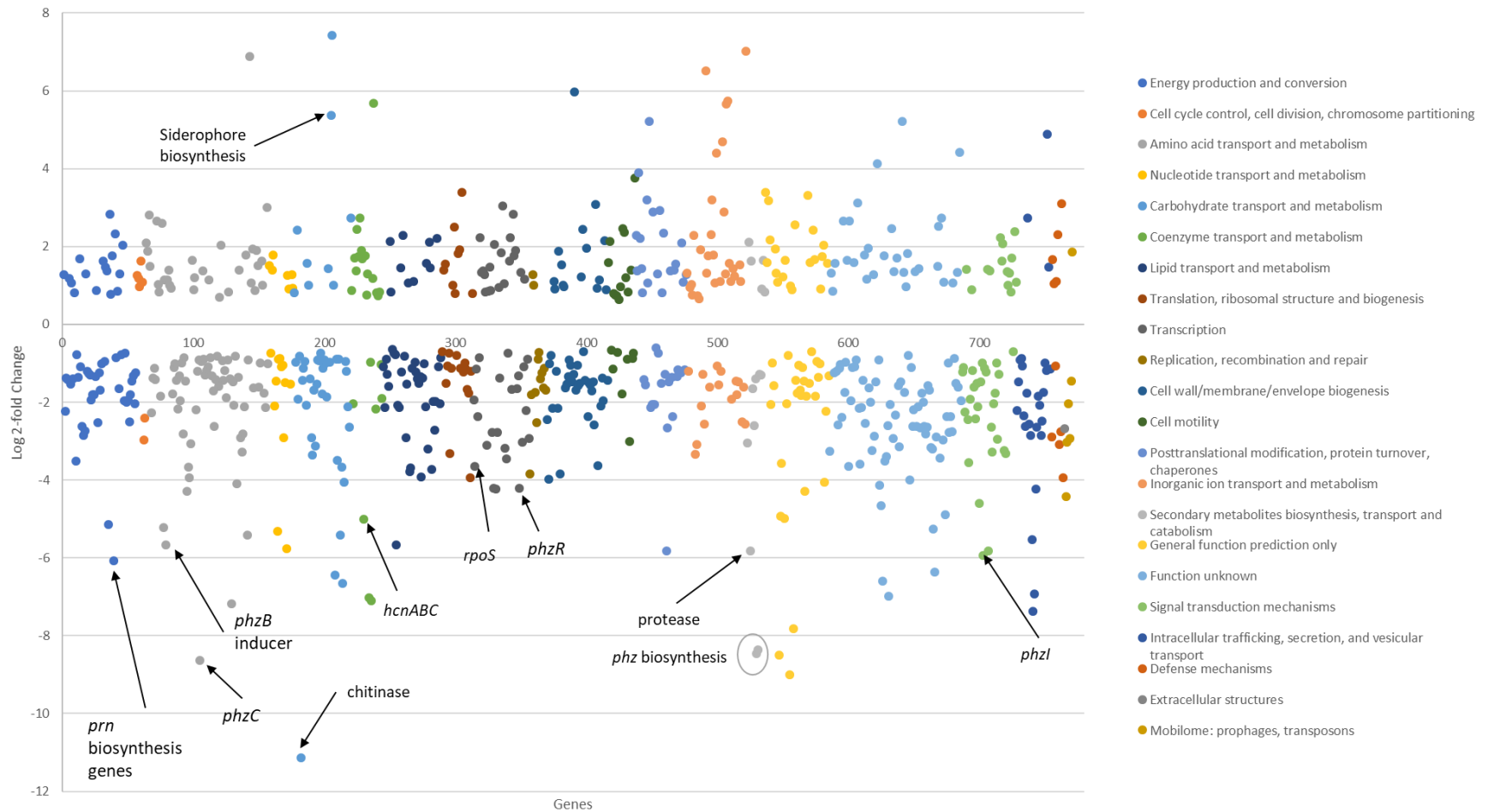


Figure 17. Differentially expressed genes in PA23-*gacS* when compared to PA23 WT. Differentially expressed genes with a p-value  $\leq 0.05$  and a q-value  $\leq 0.05$  are divided into functional categories based on Cluster of Orthologous Groups (COGs) (Tatusov et al., 2000). For the purpose of this study, only genes with a log<sub>2</sub> fold change  $\geq 1.5$  or  $\leq -1.5$  are considered significant. Genes important for the synthesis and regulation of biocontrol products are marked.

#### 4.6. Role of GacA and GacS in the PA23 regulatory cascade

Predictably, the categories for transcription (K) and signal transduction mechanisms (T) contained a large number of genes that were differentially expressed in both strains. As mutations in either *gacA* or *gacS* exert pleiotropic effects (Haas & Défago, 2005), altered expression of transcriptional regulators is not surprising. Genes involved in the PA23 regulatory cascade, including *phzR*, *rpoS* and *phzI* showed decreased expression by a log<sub>2</sub> fold factor of 3.0 and 4.0 in the *gacA*- and *gacS*-mutants, respectively (Figure 16 and Figure 17). Lack of AHL production by the Gac-deficient strains has been previously documented in PA23 (Poritsanos *et al.*, 2006; Selin *et al.*, 2014) and this can now be attributed to the decrease in expression of QS-related genes such as *phzI* and *phzR*. Interestingly, expression of the second QS regulatory gene *csaR* was decreased by a log<sub>2</sub> fold factor of 2.7- in PA23-*gacA*; but displayed no change in the *gacS*-mutant.

In *P. aeruginosa* PAO1, microarray analysis revealed an almost complete overlap between genes controlled by Gac and those regulated by RsmY and RsmZ (Brencic *et al.*, 2009). In genome-wide DNA-protein interaction studies, GacA interacted with only two genomic regions, both of which were located upstream of *rsmY* and *rsmZ*. These findings led the authors to conclude that the Gac system transduces regulatory signals to downstream genes by controlling expression of *rsmY* and *Z* exclusively (Brencic *et al.*, 2009). If the same holds true for PA23, the majority of genes under Gac control are being regulated indirectly.



#### 4.7. A regulatory link may exist between PtrA and GacS

The phenotype of the *gac* mutants closely resembles that of the *ptrA* mutant; therefore, we were interested to determine whether a connection exists between the Gac-Rsm system and PtrA. As mentioned earlier, the Gac-Rsm circuitry is complex and involves regulation at both the transcriptional and translational level. GacS is the sensor kinase which, upon binding to an unknown signal, undergoes autophosphorylation and phosphotransfer to the response regulator GacA (Lapouge *et al.*, 2008). Once phosphorylated, GacA activates expression of sRNAs, including RsmX, RsmY and RsmZ that titrate out the translational repressors RsmA and RsmE allowing expression of target genes (Lapouge *et al.*, 2008). GacS, GacA, RsmX, RsmY and RsmZ, therefore, positively regulate expression of AF compounds, while RsmA and RsmE exert a negative effect on biocontrol. qPCR analysis revealed a 50% reduction and no change in *gacA* and *gacS* expression, respectively, in PA23-443. When we examined the five Rsm genes, namely *rsmA*, *rsmE*, *rsmX*, *rsmY* and *rsmZ*, we discovered that *rsmX* and *rsmZ* expression was markedly down in the *ptrA* mutant; whereas *rsmY* was upregulated 4.8-fold compared to WT. In previous studies with *P. chlororaphis* 30-84, *rsmZ* was the only non-coding RNA to regulate secondary metabolite production (Wang *et al.*, 2013); therefore, increased expression of *rsmY* may not have an effect on PA23 biocontrol. Our analysis of genes encoding the repressor proteins, RsmA and RsmE, revealed 2.5-fold upregulation of *rsmE* compared to WT. A decrease in *rsmA* was observed; however, it was below what is expected to have biological relevance. Additionally, Wang *et al.*, (2013) demonstrated that overproduction of RsmA had no effect on production of antibiotics in *P. chlororaphis* 30-84; whereas RsmE was found to negatively regulate AHL production. Collectively, we discovered that in the *ptrA*-mutant background, two of the three positive regulators, namely *gacA* and

*rsmZ*, are down regulated while the negative regulator *rsmE* showed elevated expression (Figure 12). Thus, the overall pattern of Gac-Rsm gene expression is consistent with the *ptrA*-mutant phenotype. It was somewhat surprising to discover that *gacS* is not under PtrA transcriptional control, because full or partial complementation of the *ptrA* mutant occurs when *gacS* is provided *in trans* (Klaponksi *et al.*, 2014; Poritsanos, 2005; Shah *et al.*, 2016). Based on these findings, we believe that a PtrA deficiency is affecting GacS function rather than expression.

In *P. aeruginosa* PAO1, the orphan sensor kinases RetS and LadS modulate the Gac-Rsm circuitry (Ventre *et al.*, 2006; Goodman *et al.*, 2009). RetS blocks GacS autophosphorylation and subsequent activation of GacA, while LadS exerts a positive effect on Gac regulation (Goodman *et al.*, 2009; Ventre *et al.*, 2006). It is possible that PtrA is controlling expression of regulatory elements that impact signal transduction through the Gac system, such as RetS and LadS, and that overexpression of *gacS* is able to overcome this effect by a yet-to-be identified mechanism. To explore this possibility further, we analyzed whether *retS* and *ladS* transcription were under PtrA control. While qRT-PCR analysis revealed that expression of *retS* is reduced by 50% in PA23- 443, *ladS* was unchanged (Figure 12). Moreover, overexpression of RetS and LadS in the mutant background did not restore the WT phenotype (Table 9). Since RetS blocks GacS autophosphorylation and ultimately acts as an inhibitor of biocontrol, decreased RetS expression is expected to increase biocontrol, which is inconsistent with the *ptrA* mutant phenotype. Taken together, these findings suggest that while RetS and LadS are not involved, other currently uncharacterised orphan sensor kinases may play a role in PtrA-mediated regulation in PA23.

Table 9. Phenotypic analysis of PA23-*ptrA* complemented with *retS* and *ladS*

Strain	Zone (mm) <sup>a</sup>		
	Fungal inhibition	Protease activity	AHL production
PA23 (pUCP22)	5.50 (0.14) <sup>b</sup>	2.35 (0.21) <sup>b</sup>	4.6 (0.64) <sup>b</sup>
PA23-443 (pUCP22)	0 (0)	0 (0)	0 (0)
PA23-443 (pUCP22- <i>retS</i> )	0 (0)	0 (0)	0 (0)
PA23-443 (pUCP22- <i>ladS</i> )	0 (0)	0 (0)	0 (0)

<sup>a</sup> Mean (standard deviation) of enzyme activity of three technical replicates

<sup>b</sup> Significantly different from PA23-443 (P<0.0001).

## 5. Conclusions and future directions

The biocontrol capability of PA23 against *S. sclerotiorum* is due to direct pathogen inhibition mediated by antibiotics, such as PRN, HCN, PHZ, and degradative enzymes including proteases and chitinases (Poritsanos *et al.*, 2006). More recently, we have discovered that PA23 is capable of protecting against *S. sclerotiorum* through indirect priming of the plant defense response (Duke *et al.*, 2017). The production of antibiotic compounds in *P. chlororaphis* PA23 is controlled by a complex regulatory pathway composed of several regulators including the GacA/GacS two component system, the PhzI/PhzR QS system, RpoS, PsrA, the anaerobic regulator ANR, and a novel LysR-type regulator called PtrA (Klaponksi *et al.*, 2014; Manuel *et al.*, 2012; Nandi *et al.*, 2016; Poritsanos *et al.*, 2006; Selin *et al.*, 2010, 2012, 2014). Substantial cross-regulation occurs between the regulators themselves (Manuel *et al.*, 2012; Selin *et al.*, 2010; Selin *et al.*, 2012; Selin *et al.*, 2014).

Previous studies analyzing the regulatory network overseeing PA23 biocontrol have focused on expression of individual target genes (Manuel *et al.*, 2012; Poritsanos *et al.*, 2006; Selin *et al.*, 2010; Selin *et al.*, 2012; Selin *et al.*, 2014). While these studies are informative, they do not provide a complete picture of the regulatory networks involved. The focus of this research, therefore, was to elucidate the transcriptomes of three of the main regulatory systems involved in PA23 biocontrol, namely Phz QS, PtrA, and Gac. Through RNA sequencing, gene transcription at early stationary phase in the PA23 WT, an AHL-deficient strain, and *phzR*-, *gacA*-, *gacS*-, and *ptrA*-mutants was revealed. Between 5%-7% of the genome was found to be regulated by the PhzRI QS, PtrA, and Gac systems (Table 10). The largest number of differentially expressed genes fell into the COG category for unknown function (S). Better characterisation and functional analysis needs to be carried out to determine the role of these

genes in PA23 regulation. In addition, conducting transcriptomic analysis of the strains at different time points or physiological conditions could reveal further aspects of the genes governing regulation in PA23.

The first regulon to be explored in detail was that of the PhzRI QS system. A marked difference between the regulons of the AHL-deficient and *phzR*-mutant was observed, which was attributed to the presence of a second QS system named CsaI/CsaR. In *P. chlororaphis* 30-84, the Csa QS system regulates cell surface properties, proteases and rhizosphere competence (Zhang & Pierson, 2001). As expected, several of the genes down regulated in both QS-deficient strains were involved in secondary metabolite production, including those for PHZ, PRN, protease, and HCN biosynthesis. This transcriptional profile closely resembles the phenotype exhibited by the QS-deficient strains, validating the RNA-seq data. It was also discovered that in the absence of QS, genes involved in chitinase and siderophore production showed decreased and increased expression, respectively. Phenotypic analysis validated that chitinase is positively regulated by QS, while siderophores are repressed (Table 4 and Table 5). These biocontrol-related traits had not previously been linked to PA23 QS.

QS was found to both directly and indirectly affect regulation in PA23. Direct regulation is believed to occur when the activated PhzR dimer binds to a *phz*-box found upstream of the target gene. In PA23, the *phz*-box sequence was only found upstream of 30 genes, indicating that a large proportion of genes are indirectly regulated by the Phz QS system. Consistent with this notion, we identified 48 regulators that exhibited differential expression in either one or both of the QS-deficient strains (Table 7). Prominent examples include  $\sigma$  factors like RpoS, the QS regulator CsaR, the HN-S-type gene silencer MvaV together with several uncharacterised regulators.

The next regulon to be explored was the PtrA regulatory network. PtrA is a LTTR that exerts a pleiotropic effect in PA23, as strains lacking this protein produce markedly lower concentrations of biocontrol compounds (Klaponksi *et al.*, 2014; Poritsanos, 2005). Phylogenetic analysis showed that *ptrA* is well conserved among pathogenic and beneficial pseudomonads (Figure 9); as such, this LTTR is expected to play an important role in governing expression of both virulence and biocontrol factors. In PA23, both *phzA* and *prnA* exhibit decreased activity in the *ptrA* mutant, which is consistent with the lack of antibiotic production by these two strains (Selin *et al.*, 2010). A number of regulatory genes exhibited altered expression in the *ptrA* mutant. For example, *phzR*, *phzI*, *rpoS*, *rsmX* and *rsmZ* were all decreased in the PtrA-deficient strain; whereas the expression *rsmE* encoding the repressor RsmE was upregulated. For the most part, these gene expression profiles are in keeping with the PtrA phenotype. Through complementation analysis, we discovered that *gacS* is the only heterologous gene able to complement the *ptrA* mutant (Shah *et al.*, 2016). Thus, we were surprised to observe that *gacS* expression was unchanged in the *ptrA* mutant. Such findings indicate that PtrA is affecting GacS function rather than expression. Next we tested orphan sensor kinases RetS and LadS, that regulate GacS-dependant transcription in *P. aeruginosa* PAO1 (Ventre *et al.*, 2006). While expression of *retS* was reduced by 50% in PA23- 443; overexpression of RetS and LadS in the mutant background did not restore the WT phenotype. As such, these sensor kinases do not appear to be involved in PtrA regulation.

Finally, we explored the GacA and GacS regulatory networks through global RNA profiling. Interestingly, the two regulons only shared share a high degree of overlap and both exerted a global effect on PA23 transcription. We observed reduced expression of biocontrol genes, which is consistent with the previously reported decrease in production of secondary

metabolites (HCN, PRN, PHZ, protease) in both *gacS* and *gacA* mutants (Poritsanos *et al.*, 2006; Selin *et al.*, 2014). Overall, more than 65% of the regulon in both strains was down regulated compared to the WT; thus, for PA23, this two-component system is largely serving as a positive regulator of gene expression. Surprisingly, expression of *ptrA* was unaltered in both Gac-deficient strains. At present, the link to the Gac system and the mechanism whereby PtrA controls expression of biocontrol compounds remain an enigma.

Future studies should focus on the CsaRI QS system. As expression of *csaR* is reduced in both QS- and GacA-deficient strains, construction and characterisation of *csaR* and *csaI*-mutants could be used to determine regulatory links between the two QS systems and the Gac system in PA23. In *P. chlororaphis* 30-84, CsaIR aids in rhizosphere competence of the bacteria (Zhang & Pierson, 2001) and in *P. aeruginosa*, the Phz and Csa QS homologues, Las and Rhl exhibit cross regulation (Duan & Surette, 2007; Zhang & Pierson, 2001). Transcriptomic analysis through RNA-seq would reveal the global effect of the second QS system on PA23 physiology and regulation, as well as determining which exoproducts fall under Csa control. Studying the effect of Csa on rhizosphere competence of PA23 may provide further insight into the use of this bacterium as a PGPR (Plant Growth Promoting Rhizobacteria), along with its efficacy against fungal pathogens in the rhizosphere.

In addition, MvaV and MvaT are H-NS type proteins that alter exoproduct expression in *P. protegens* CHA0 (Baehler *et al.*, 2006). As decreased expression of *mvaV* and *mvaT* was found in the QS-deficient strains, it would be useful to determine the effect of these proteins on PA23 regulation and production of secondary metabolites, as previous studies with *P. aeruginosa* PAO1 reveal that *mvaV* and *mvaT* were involved in silencing expression of *rsmY* and *rsmZ* (Brencic *et al.*, 2009). As *rsmY* and *rsmZ* play an important role in the regulation of

biocontrol products in PA23, understanding the regulatory elements governing the expression of these non-coding RNAs through construction and characterisation of *mvaV* and *mvaT* mutants would open up new pathways in the complex regulatory system that governs PA23 metabolism.



Table 10. Percentage of differentially expressed genes up and down regulated in the PA23, QS, *ptrA*- and *gac*-deficient strains.

<b>Strain</b>	<b>% up-regulated genes</b>	<b>% down-regulated genes</b>	<b>Total % of genome regulated</b>
<b>PA23-<i>phzR</i></b>	30.0	70.0	5.5
<b>PA23-AHL</b>	31.53	68.47	6.7
<b>PA23-<i>ptrA</i></b>	40.65	59.35	7.2
<b>PA23-<i>gacS</i></b>	33.0	67.0	6.7
<b>PA23-<i>gacA</i></b>	32.39	67.61	6.2

## 6. Bibliography

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J.-C. & García-Río, L. (2008). The mobility and degradation of pesticides in soils and the pollution of groundwater resources. *Agric Ecosyst Environ* **123**, 247–260.
- Arima, K., Imanaka, H., Kousaka, M., Fukuta, A. & Tamura, G. (1964). Pyrrolnitrin, a new antibiotic substance, produced by *Pseudomonas*. *Agric Biol Chem* **28**, 575–576.
- Auffray, C., Behar, G., Bois, F., Bouchier, C., Da Silva, C., Devignes, M. D., Duprat, S., Houlgatte, R., Jumeau, M. N. & Lamy, B. (1995). [IMAGE: molecular integration of the analysis of the human genome and its expression]. *C R Acad Sci III* **318**, 263–72.
- Baehler, E., de Werra, P., Wick, L. Y., Péchy-Tarr, M., Mathys, S., Maurhofer, M. & Keel, C. (2006). Two novel MvaT-like global regulators control exoproduct formation and biocontrol activity in root-associated *Pseudomonas fluorescens* CHA0. *Mol Plant-Microbe Interact* **19**, 313–329.
- Battesti, A., Majdalani, N. & Gottesman, S. (2011). The RpoS-mediated general stress response in *Escherichia coli*. *Annu Rev Microbiol* **65**, 189–213.
- Bertani, I. & Venturi, V. (2004). Regulation of the N-acyl homoserine lactone-dependent quorum-sensing system in rhizosphere *Pseudomonas putida* WCS358 and cross-talk with the stationary-phase RpoS sigma factor and the global regulator GacA. *Appl Environ Microbiol* **70**, 5493–5502.
- Bolger, A. M., Lohse, M. & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120.
- Brencic, A., McFarland, K. A., McManus, H. R., Castang, S., Mogno, I., Dove, S. L. & Lory, S. (2009). The GacS/GacA signal transduction system of *Pseudomonas aeruginosa* acts exclusively through its control over the transcription of the RsmY and RsmZ regulatory small RNAs. *Mol Microbiol* **73**, 434–445
- Canola Council of Canada. (2016a). The economic impact of canola on the Canadian economy report for : Canola Council of Canada Winnipeg, Canada Research and analysis to inform your business decisions. Winnipeg.
- Canola Council of Canada. (2016b). Sclerotinia stem rot - Canola Council of Canada
- Cases, I., Ussery, D. W. & De Lorenzo, V. (2003). The  $\sigma_{54}$  regulon (sigmulon) of *Pseudomonas putida*. *Environ Microbiol* **5**, 1281–1293.
- Castric, P. A. (1977). Glycine metabolism by *Pseudomonas aeruginosa*: hydrogen cyanide biosynthesis. *J Bacteriol* **130**, 826–31.
- Celis, R. T. . (1999). Repression and activation of arginine transport genes in *Escherichia coli* K 12 by the ArgP protein. *J Mol Biol* **294**, 1087–1095.
- Chancey, S. T., Wood, D. W. & Pierson, L. S. I. (1999). Two-component transcriptional regulation of N-acyl-homoserine lactone production in *Pseudomonas aureofaciens*. *Appl Environ Microbiol* **65**, 2294–9.

- Chin-A-Woeng, T. F. C., Bloemberg, G. V. & Lugtenberg, B. J. J. (2003).** Phenazines and their role in biocontrol by *Pseudomonas* bacteria. *New Phytol* **157**, 503–523. Blackwell Science Ltd.
- Chin-A-Woeng, T. F. C., van den Broek, D., Lugtenberg, B. J. J. & Bloemberg, G. V. (2005).** The *Pseudomonas chlororaphis* PCL1391 sigma regulator PsrA represses the production of the antifungal metabolite phenazine-1-carboxamide. *Mol Plant-Microbe Interact* **18**, 244–253.
- Cirz, R. T., O’Neill, B. M., Hammond, J. A., Head, S. R. & Romesberg, F. E. (2006).** Defining the *Pseudomonas aeruginosa* SOS response and its role in the global response to the antibiotic ciprofloxacin. *J Bacteriol* **188**, 7101–10
- Croucher, N. J. & Thomson, N. R. (2010).** Studying bacterial transcriptomes using RNA-seq. *Curr Opin Microbiol* **13**, 619–24. Elsevier.
- Duan, K. & Surette, M. G. (2007).** Environmental regulation of *Pseudomonas aeruginosa* PAO1 Las and Rhl Quorum-Sensing Systems. *J Bacteriol* **189**, 4827–4836.
- Duke, K. A., Becker, M. G., Girard, I. J., Millar, J. L., Fernando, W. G. D., Belmonte, M. F. & de Kievit, T. R. (2017).** The biocontrol agent *Pseudomonas chlororaphis* PA23 primes *Brassica napus* defenses through distinct gene networks. *BMC Genomics* **73**, 1–16. BioMed Central.
- Feklistov, A., Sharon, B. D., Darst, S. A. & Gross, C. A. (2014).** Bacterial sigma factors: A historical, structural, and genomic perspective. *Annu Rev Microbiol* **68**, 357–376.
- Fernando, W. G. D., Nakkeeran, S., Zhang, Y. & Savchuk, S. C. (2007).** Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. *Crop Prot* **26**, 100–107.
- Fordos, J. (1859).** No Title. *Recl des Trav la Soc d’Emulation pour les Sci Pharm* **3**, 30.
- Fujita, M., Tanaka, K., Takahashi, H. & Amemura, A. (1994).** Transcription of the principal sigma-factor genes, *rpoD* and *rpoS*, in *Pseudomonas aeruginosa* is controlled according to the growth phase. *Mol Microbiol*.
- Goodman, A. L., Kulasekara, B., Rietsch, A., Boyd, D., Smith, R. S. & Lory, S. (2004).** A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. *Dev Cell* **7**, 745–754.
- Goodman, A. L., Merighi, M., Hyodo, M., Ventre, I., Filloux, A. & Lory, S. (2009).** Direct interaction between sensor kinase proteins mediates acute and chronic disease phenotypes in a bacterial pathogen. *Genes Dev* **23**, 249–259.
- Goodman, A. L., Kulasekara, B., Rietsch, A., Boyd, D., Smith, R. S. & Lory, S. (2004).** A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. *Dev Cell* **7**, 745–754.
- Goodman, A. L., Merighi, M., Hyodo, M., Ventre, I., Filloux, A. & Lory, S. (2009).** Direct interaction between sensor kinase proteins mediates acute and chronic disease phenotypes in a bacterial pathogen. *Genes Dev* **23**, 249–259.
- Haas, D. & Défago, G. (2005).** Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* **3**, 307–19.
- Haas, D. & Keel, C. (2003).** Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biological control of plant disease. *Annu Rev Phytopathol* **41**, 117–153.

- Hammer, P. E., Hill, D. S., Lam, S. T., Van Pée, K. H. & Ligon, J. M. (1997).** Four genes from *Pseudomonas fluorescens* that encode the biosynthesis of pyrrolnitrin. *Appl Environ Microbiol* **63**, 2147–54.
- Heeb, S. & Haas, D. (2001).** Regulatory roles of the GacS/GacA two-component system in plant-associated and other gram-negative bacteria. *Mol Plant-Microbe Interact* **14**, 1351–1363.
- Hegedus, D. D. & Rimmer, S. R. (2005).** *Sclerotinia sclerotiorum*: When ‘to be or not to be’ a pathogen? *FEMS Microbiol Lett* **251**, 177–184.
- Hernandez-Lucas, I., Gallego-Hernandez, A. L., Encarnacion, S., Fernandez-Mora, M., Martinez-Batallar, A. G., Salgado, H., Oropeza, R. & Calva, E. (2008).** The LysR-type transcriptional regulator LeuO controls expression of several genes in *Salmonella enterica* serovar Typhi. *J Bacteriol* **190**, 1658–1670.
- Heroven, A. K. & Dersch, P. (2006).** RovM, a novel LysR-type regulator of the virulence activator gene *rovA*, controls cell invasion, virulence and motility of *Yersinia pseudotuberculosis*. *Mol Microbiol* **62**, 1469–1483.
- Heurlier, K., Dénervaud, V., Pessi, G., Reimann, C. & Haas, D. (2003).** Negative control of quorum sensing by RpoN ( $\sigma$  54) in *Pseudomonas aeruginosa* PAO1. *J Bacteriol* **185**, 2227–2235.
- House, B. L., Mortimer, M. W. & Kahn, M. L. (2004).** New recombination methods for *Sinorhizobium meliloti* genetics. *Appl Environ Microbiol* **70**, 2806–15.
- Howell, C. R. & Stipanovic, R. D. (1979).** Control of *Rhizoctonia solani* on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. *Phytopathology* **69**, 480–482.
- Huffman, J. L. & Brennan, R. G. (2002).** Prokaryotic transcription regulators: more than just the helix-turn-helix motif. *Curr Opin Struct Biol* **12**, 98–106.
- Jorgensen, F., Bally, M., Chapon-Herve, V., Michel, G., Lazdunski, A., Williams, P. & Stewart, G. S. A. B. (1999).** RpoS-dependent stress tolerance in *Pseudomonas aeruginosa*. *Microbiology* **145**, 835–844.
- Juhas, M., Eberl, L. & Tümmler, B. (2005).** Quorum sensing: The power of cooperation in the world of *Pseudomonas*. *Environ Microbiol* **7**, 459–471.
- Katoh, K. & Standley, D. M. (2013).** MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* **30**, 772–80.
- Kay, E., Humair, B., Dénervaud, V., Riedel, K., Spahr, S., Eberl, L., Valverde, C. & Haas, D. (2006).** Two GacA-dependent small RNAs modulate the quorum-sensing response in *Pseudomonas aeruginosa*. *J Bacteriol* **188**, 6026–6033.
- van Keulen, G., Girbal, L., van den Bergh, E. R., Dijkhuizen, L. & Meijer, W. G. (1998).** The LysR-type transcriptional regulator CbbR controlling autotrophic CO<sub>2</sub> fixation by *Xanthobacter flavus* is an NADPH sensor. *J Bacteriol* **180**, 1411–7.
- de Kievit, T. R. (2002).** Role of the *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in *rhlI* regulation. *FEMS Microbiol Lett* **212**, 101–106.
- Klaponski, N. (2014).** Functional characterization of two divergently transcribed genes : *ptra* , encoding a LysR-type transcriptional regulator, and *scd*, encoding a short- chain dehydrogenase in *Pseudomonas chlororaphis PA23*. MSc thesis. University of Manitoba.

- Klaponiski, N., Selin, C., Duke, K., Spicer, V., Fernando, W. G. D., Belmonte, M. F. & de Kievit, T. R. (2014).** The requirement for the LysR-type regulator PtrA for *Pseudomonas chlororaphis* PA23 biocontrol revealed through proteomic and phenotypic analysis. *BMC Microbiol* **14**, 94.
- Kloepper, J. W., Leong, J., Teintze, M. & Schroth, M. N. (1980).** Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **286**, 885–886.
- Knowles, C. J. (1976).** Microorganisms and cyanide. *Bacteriol Rev* **40**, 652–80..
- Körner, H., Sofia, H. J. & Zumft, W. G. (2003).** Phylogeny of the bacterial superfamily of Crp-Fnr transcription regulators: exploiting the metabolic spectrum by controlling alternative gene programs. *FEMS Microbiol Rev* **27**, 559–92.
- Kumar, R., Ichihashi, Y., Kimura, S., Chitwood, D. H., Headland, L. R., Peng, J., Maloof, J. N. & Sinha, N. R. (2012).** A high-throughput method for Illumina RNA-Seq library preparation. *Front Plant Sci* **3**, 1–10.
- Lapouge, K., Schubert, M., Allain, F. H. T. & Haas, D. (2008, November 30).** Gac/Rsm signal transduction pathway of  $\gamma$ -proteobacteria: From RNA recognition to regulation of social behaviour. *Mol Microbiol*.
- Latifi, A., Winson, M. K., Foglino, M., Bycroft, B. W., Stewart, G. S. A. B., Lazdunski, A. & Williams, P. (1995).** Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in *Pseudomonas aeruginosa* PAO1. *Mol Microbiol* **17**, 333–343..
- Laville, J., Voisard, C., Keel, C., Maurhofer, M., Défago, G. & Haas, D. (1992).** Global control in *Pseudomonas fluorescens* mediating antibiotic synthesis and suppression of black root rot of tobacco. *Proc Natl Acad Sci U S A* **89**, 1562–6.
- Laville, J., Blumer, C., Von Schroetter, C., Gaia, V., Défago, G., Keel, C. & Haas, D. (1998).** Characterization of the *hcnABC* gene cluster encoding hydrogen cyanide synthase and anaerobic regulation by ANR in the strictly aerobic biocontrol agent *Pseudomonas fluorescens* CHA0. *J Bacteriol* **180**, 3187–96.
- Le, S. Q. & Gascuel, O. (2008).** An improved general amino acid replacement matrix. *Mol Biol Evol* **25**, 1307–1320.
- Lim, H. S., Kim, Y. S. & Kim, S. D. (1991).** *Pseudomonas stutzeri* Ypl-1 genetic transformation and antifungal mechanism against *Fusarium solani*, an agent of plant-root rot. *Appl Environ Microbiol* **57**, 510–516.
- Livak, K. J. & Schmittgen, T. D. (2001).** Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta CT}$  method. *Methods* **25**, 402–408.
- Loewen, P. C., Villeneuve, J., Fernando, W. G. D. & De Kievit, T. R. (2014).** Genome sequence of *Pseudomonas chlororaphis* strain PA23. *Genome Announc* **2**, e00689-14-e00689-14
- Maddocks, S. E. & Oyston, P. C. F. (2008).** Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. *Microbiology* **154**, 3609–3623.
- Manuel, J., Selin, C., Dilantha Fernando, W. G. & de Kievit, T. R. (2012).** Stringent response mutants of *Pseudomonas chlororaphis* PA23 exhibit enhanced antifungal activity against *Sclerotinia sclerotiorum* in vitro. *Microbiology* **158**, 207–216.
- Marchler-Bauer, A. & Bryant, S. H. (2004).** CD-Search: protein domain annotations on the fly.

*Nucleic Acids Res* **32**, W327–W331.

- Marchler-Bauer, A., Lu, S., Anderson, J. B., Chitsaz, F., Derbyshire, M. K., DeWeese-Scott, C., Fong, J. H., Geer, L. Y., Geer, R. C. & other authors. (2011).** CDD: a Conserved Domain Database for the functional annotation of proteins. *Nucleic Acids Res* **39**, D225–D229.
- Mavrodi, D. V., Blankenfeldt, W. & Thomashow, L. S. (2006).** Phenazine compounds in fluorescent *Pseudomonas* Spp. biosynthesis and regulation **44**, 417–445.
- Mazzola, M., Cook, R. J., Thomashow, L. S., Weller, D. M. & Pierson, L. S. (1992).** Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. *Appl Environ Microbiol* **58**, 2616–2624.
- Medina-Rivera, A., Defrance, M., Sand, O., Herrmann, C., Castro-Mondragon, J. A., Delerce, J., Jaeger, S., Blanchet, C., Vincens, P. & other authors. (2015).** RSAT 2015: Regulatory Sequence Analysis Tools. *Nucleic Acids Res* **43**, W50–W56. Oxford University Press.
- Miller, M. B. & Bassler, B. L. (2001).** Quorum sensing in bacteria. *Annu Rev Microbiol* **55**, 165–99.
- Miyamoto, C. M., Lin, Y. H. & Meighen, E. A. (2002).** Control of bioluminescence in *Vibrio fischeri* by the LuxO signal response regulator. *Mol Microbiol* **36**, 594–607.
- Nandi, M., Selin, C., Brassinga, A. K. C., Belmonte, M. F., Fernando, W. G. D., Loewen, P. C. & de Kievit, T. R. (2015).** Pyrrolnitrin and hydrogen cyanide production by *Pseudomonas chlororaphis* strain PA23 exhibits nematicidal and repellent activity against *Caenorhabditis elegans*. *PLoS One* **10**, e0123184.
- Nandi, M., Selin, C., Brawerman, G., Fernando, W. G. D. G. D. & De Kievit, T. R. (2016).** The global regulator ANR is essential for *Pseudomonas chlororaphis* strain PA23 biocontrol. *Microbiology* **162**, 2159–2169.
- Nandi, M., Selin, C., Brawerman, G., Fernando, W. G. D. & de Kievit, T. R. (2017).** Hydrogen cyanide, which contributes to *Pseudomonas chlororaphis* strain PA23 biocontrol, is upregulated in the presence of glycine. *Biol Control* **108**, 47–54.
- Neiendam Nielsen, M. & Sørensen, J. (1999).** Chitinolytic activity of *Pseudomonas fluorescens* isolates from barley and sugar beet rhizosphere. *FEMS Microbiol Ecol* **30**, 217–227.
- Nose, M. & Arima, K. (1969).** On the mode of action of a new antifungal antibiotic, pyrrolnitrin. *J Antibiot (Tokyo)* **22**, 135–143.
- Oh, S. A., Kim, J. S., Park, J. Y., Han, S. H., Dimkpa, C., Anderson, A. J. & Kim, Y. C. (2013).** The RpoS sigma factor negatively regulates production of IAA and siderophore in a biocontrol rhizobacterium, *Pseudomonas chlororaphis* O6. *plant Pathol J* **29**, 323–9.
- Pal, K. K. & McSpadden Gardener, B. (2006).** Biological control of plant pathogens. *Plant Heal Instr* **2**, 181–185.
- Parsek, M. R., Ye, R. W., Pun, P. & Chakrabarty, A. M. (1994).** Critical nucleotides in the interaction of a LysR-type regulator with its target promoter region. *J Biol Chem*, **269**, 11279–11284.
- Pérez-Rueda, E. & Collado-Vides, J. (2000).** The repertoire of DNA-binding transcriptional regulators in *Escherichia coli* K-12. *Nucleic Acids Res* **28**, 1838–47.
- Pessi, G. & Haas, D. (2000).** Transcriptional control of the hydrogen cyanide biosynthetic genes



- hcnABC* by the anaerobic regulator ANR and the quorum-sensing regulators LasR and RhlR in *Pseudomonas aeruginosa*. *J Bacteriol* **182**, 6940–6949.
- Picossi, S., Belitsky, B. R. & Sonenshein, A. L. (2007).** Molecular mechanism of the regulation of *Bacillus subtilis* *gltAB* expression by GltC. *J Mol Biol* **365**, 1298–1313.
- Poritsanos, N., Selin, C., Fernando, W. G. D., Nakkeeran, S. & De Kievit, T. R. (2006).** A GacS deficiency does not affect *Pseudomonas chlororaphis* PA23 fitness when growing on canola, in aged batch culture or as a biofilm. *Can J Microbiol* **52**, 1177–1188.
- Poritsanos, N. J. (2005).** Molecular mechanisms involved in secondary metabolite production and biocontrol of *Pseudomonas chlororaphis* PA23. MSc thesis. University of Manitoba
- Potrykus, K. & Cashel, M. (2008).** (p)ppGpp: still magical? *Annu Rev Microbiol* **62**, 35–51. Annual Reviews.
- Potvin, E., Sanschagrín, F. & Levesque, R. C. (2008).** Sigma factors in *Pseudomonas aeruginosa*. *FEMS Microbiol Rev* **32**, 38–55.
- Purdy, L. H. (1979).** *Sclerotinia sclerotiorum*: history, diseases and symptomatology, host range, geographic distribution, and impact. *Phytopathology* **69**, 875.
- Raaijmakers, J. M., Vlami, M. & de Souza, J. T. (2002).** Antibiotic production by bacterial biocontrol agents. *Antonie Van Leeuwenhoek* **81**, 537–47.
- Records, A. R. & Gross, D. C. (2010).** Sensor kinases RetS and LadS regulate *Pseudomonas syringae* type VI secretion and virulence factors. *J Bacteriol* **192**, 3584–3596.
- Reimann, C., Valverde, C., Kay, E. & Haas, D. (2005).** Posttranscriptional repression of GacS/GacA-controlled genes by the RNA-binding protein RsmE acting together with RsmA in the biocontrol strain *Pseudomonas fluorescens* CHA0. *J Bacteriol* **187**, 276–85.
- Ren, D., Zuo, R. & Wood, T. K. (2005).** Quorum-sensing antagonist (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone influences siderophore biosynthesis in *Pseudomonas putida* and *Pseudomonas aeruginosa*. *Appl Microbiol Biotechnol* **66**, 689–695.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989).** Molecular cloning: a laboratory manual. Mol cloning a Lab manual. Cold Spring Harbor Laboratory Press.
- Sarniguet, A., Kraus, J., Henkels, M. D., Muehlchen, A. M. & Loper, J. E. (1995).** The sigma factor sigma s affects antibiotic production and biological control activity of *Pseudomonas fluorescens* Pf-5. *Proc Natl Acad Sci U S A* **92**, 12255–9.
- Savchuk, S. C. & Fernando, W. G. D. (2004).** Effect of timing of application and population dynamics on the degree of biological control of *Sclerotinia sclerotiorum* by bacterial antagonists. *FEMS Microbiol Ecol* **49**, 379–388.
- Sawers, R. G. (1991).** Identification and molecular characterization of a transcriptional regulator from *Pseudomonas aeruginosa* PAO1 exhibiting structural and functional similarity to the FNR protein of *Escherichia coli*. *Mol Microbiol* **5**, 1469–1481.
- Schell, M. A. (1993).** Molecular biology of the LysR family of transcriptional regulators. *Annu Rev Microbiol* **47**, 597–626.
- Schnider-Keel, U., Lejbølle, K. B., Baehler, E., Haas, D. & Keel, C. (2001).** The sigma factor AlgU (AlgT) controls exopolysaccharide production and tolerance towards desiccation and osmotic

- stress in the biocontrol agent *Pseudomonas fluorescens* CHA0. *Appl Environ Microbiol* **67**, 5683–93.
- Schuster, M., Lostroh, C. P., Ogi, T. & Greenberg, E. P. (2003).** Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. *J Bacteriol* **185**, 2066–2079.
- Schwyn, B. & Neilands, J. B. (1987).** Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* **160**, 47–56.
- Selin, C., Habibian, R., Poritsanos, N., Athukorala, S. N. P., Fernando, W. G. D. & De Kievit, T. R. (2010).** Phenazines are not essential for *Pseudomonas chlororaphis* PA23 biocontrol of *Sclerotinia sclerotiorum*, but do play a role in biofilm formation. *FEMS Microbiol Ecol* **71**, 73–83.
- Selin, C., Fernando, W. G. D. & de Kievit, T. R. (2012).** The PhzI/PhzR quorum-sensing system is required for pyrolnitrin and phenazine production, and exhibits cross-regulation with RpoS in *Pseudomonas chlororaphis* PA23. *Microbiology* **158**, 896–907.
- Selin, C., Manuel, J., Fernando, W. G. D. & De Kievit, T. R. (2014).** Expression of the *Pseudomonas chlororaphis* strain PA23 Rsm system is under control of GacA, RpoS, PsrA, quorum sensing and the stringent response. *Biol Control* **69**, 24–33.
- Shah, N., Klaponski, N., Selin, C., Rudney, R., Fernando, W. G. D., Belmonte, M. F. & de Kievit, T. R. (2016).** PtrA Is Functionally Intertwined with GacS in Regulating the Biocontrol Activity of *Pseudomonas chlororaphis* PA23. *Front Microbiol* **7**, 1512.
- Shemesh, M., Tam, A. & Steinberg, D. (2007).** Differential gene expression profiling of *Streptococcus mutans* cultured under biofilm and planktonic conditions. *Microbiology* **153**, 1307–1317.
- Siddiqui, I. A., Haas, D., & Heeb, S. (2005).** Extracellular protease of *Pseudomonas fluorescens* CHA0, a biocontrol factor with activity against the root-knot nematode *Meloidogyne incognita*. *Appl Environ Microbiol* **71**, 5646–5649.
- Smith, R. S. & Iglewski, B. H. (2003).** *Pseudomonas aeruginosa* quorum sensing as a potential antimicrobial target. *J Clin Invest* **112**, 1460–1465.
- Spiro, S. (1994).** The FNR family of transcriptional regulators. *Antonie Van Leeuwenhoek* **66**, 23–36.
- Stragier, P., Richaud, F., Borne, F. & Patte, J. C. (1983).** Regulation of diaminopimelate decarboxylase synthesis in *Escherichia coli*. I. Identification of a *lysR* gene encoding an activator of the *lysA* gene. *J Mol Biol* **168**, 307–20.
- Stutz, E., Defago, G. & Kern, H. (1986).** Naturally occurring fluorescent pseudomonads involved in suppression of black root rot of tobacco. *Phytopathology* **76**, 181–185.
- Suh, S., Silo-suh, L., Woods, D. E., Daniel, J., West, S. E. H., Ohman, D. E. & Hassett, D. J. (1999).** Effect of *rpoS* mutation on the stress response and expression of virulence factors in *Pseudomonas aeruginosa* effect of *rpoS* mutation on the stress response and expression of virulence factors in *Pseudomonas aeruginosa* **181**, 3890–3897.
- Tamura, K. & Nei, M. (1993).** Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* **10**, 512–26.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013).** MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* **30**, 2725–9.



- Tatusov, R. L., Galperin, M. Y., Natale, D. A. & Koonin, E. V. (2000).** The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res* **28**, 33–36.
- Tatusov, R. L., Fedorova, N. D., Jackson, J. D., Jacobs, A. R., Kiryutin, B., Koonin, E. V, Krylov, D. M., Mazumder, R., Mekhedov, S. L. & other authors. (2003).** The COG database: an updated version includes eukaryotes. *BMC Bioinformatics* **4**, 41.
- Thompson, L. S., Webb, J. S., Rice, S. A. & Kjelleberg, S. (2003).** The alternative sigma factor RpoN regulates the quorum sensing gene *rhII* in *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* **220**, 187–195.
- Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., Pimentel, H., Salzberg, S. L., Rinn, J. L. & Pachter, L. (2012).** Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* **7**, 562–78.
- Tripathi, R. K. & Gottlieb, D. (1969).** Mechanism of action of the antifungal antibiotic pyrrolnitrin. *J Bacteriol* **100**, 310–8.
- Turner, J. M. & Messenger, A. J. (1986).** Occurrence, biochemistry and physiology of phenazine pigment production. *Adv Microb Physiol* **27**, 211–275.
- Ventre, I., Goodman, A. L., Vallet-Gely, I., Vasseur, P., Soscia, C., Molin, S., Bleves, S., Lazdunski, A., Lory, S. & Filloux, A. (2006).** Multiple sensors control reciprocal expression of *Pseudomonas aeruginosa* regulatory RNA and virulence genes. *Proc Natl Acad Sci U S A* **103**, 171–6.
- Venturi, V. (2003).** Control of *rpoS* transcription in *Escherichia coli* and *Pseudomonas*: why so different? *Mol Microbiol* **49**, 1–9.
- Venturi, V. (2006).** Regulation of quorum sensing in *Pseudomonas*. *FEMS Microbiol Rev* **30**, 274–291.
- Wagner, V. E., Bushnell, D., Passador, L., Brooks, A. I. & Iglewski, B. H. (2003).** Microarray analysis of *Pseudomonas aeruginosa* quorum-sensing regulons: effects of growth phase and environment. *J Bacteriol* **185**, 2080–95.
- Wagner, V. E., Gillis, R. J. & Iglewski, B. H. (2004).** Transcriptome analysis of quorum-sensing regulation and virulence factor expression in *Pseudomonas aeruginosa*. *Vaccine* **22**.
- Wang, D., Lee, S.-H., Seeve, C., Yu, J. M., Pierson, L. S. & Pierson, E. A. (2013).** Roles of the Gac-Rsm pathway in the regulation of phenazine biosynthesis in *Pseudomonas chlororaphis* 30-84. *Microbiologyopen* **2**, 505–524.
- Weber, H., Polen, T., Heuveling, J., Wendisch, V. F. & Hengge, R. (2005).** Genome-wide analysis of the general stress response network in *Escherichia coli*: sigmaS-dependent genes, promoters, and sigma factor selectivity. *J Bacteriol* **187**, 1591–603.
- Wei, X., Huang, X., Tang, L., Wu, D. & Xu, Y. (2013).** Global control of GacA in secondary metabolism, primary metabolism, secretion systems, and motility in the rhizobacterium *Pseudomonas aeruginosa* M18. *J Bacteriol* **195**, 3387–3400.
- Weller, D. M. (2007).** *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology* **97**, 250–256.
- West, S. E. H., Schweizer, H. P., Dall, C., Sample, A. K. & Runyen-Janecky, L. J. (1994).** Construction of improved *Escherichia-Pseudomonas* shuttle vectors derived from pUC18/19 and sequence of the region required for their replication in *Pseudomonas aeruginosa*. *Gene* **148-1**, 81–86.

- Wirth, S. J. & Wolf, G. A. (1990).** Dye-labelled substrates for the assay and detection of chitinase and lysozyme activity. *J Microbiol Methods* **12**, 197–205.
- Zhang, Y., Fernando, W. G. D., de Kievit, T. R., Berry, C., Daayf, F. & Paulitz, T. C. (2006).** Detection of antibiotic-related genes from bacterial biocontrol agents with polymerase chain reaction. *Can J Microbiol* **52**, 476–481.
- Zhang, Z. & Pierson, L. S. (2001).** A second quorum-sensing system regulates cell surface properties but not phenazine antibiotic production in *Pseudomonas aureofaciens*. *Appl Environ Microbiol* **67**, 4305–15.
- Zimmermann, A., Reimann, C., Galimand, M. & Haas, D. (1991).** Anaerobic growth and cyanide synthesis of *Pseudomonas aeruginosa* depend on *anr*, a regulatory gene homologous with *fnr* of *Escherichia coli*. *Mol Microbiol* **5**, 1483–1490.

## 7. Appendix

Appendix Table 1: Differentially expressed genes in PA23-AHL compared to WT.

COG category		Predicted function	Protein accession number	Gene locus	P-value	Q-value	log2 fold change
<b>RNA processing and modification</b>	A	Oligoribonuclease	WP_007921399	XLOC_000171	0.0020	0.0151	-1.52
<b>Energy production and conversion</b>	C	Cytochrome C oxidase subunit IV	WP_016702591	XLOC_000099	0.0001	0.0007	3.31
		Azurin	WP_009046707	XLOC_000192	0.0001	0.0007	-2.59
		Aldehyde dehydrogenase	WP_038630472	XLOC_000193	0.0004	0.0040	-1.78
		Methylmalonate-semialdehyde dehydrogenase	WP_009046853	XLOC_000243	0.0003	0.0032	-1.86
		Oxidoreductase	WP_038630684	XLOC_000407	0.0001	0.0007	-1.74
		NADH dehydrogenase	WP_009047568	XLOC_003085	0.0024	0.0174	-1.60
		Dihydrolipoyl dehydrogenase	WP_038631639	XLOC_003405	0.0001	0.0007	2.44
		Branched-chain alpha-keto acid dehydrogenase subunit E2	WP_038631641	XLOC_003406	0.0001	0.0007	3.39
		2-oxoisovalerate dehydrogenase	WP_028682773	XLOC_003407	0.0001	0.0007	2.23
		2-oxoisovalerate dehydrogenase	WP_038631643	XLOC_003408	0.0001	0.0007	1.66
		Cbb3-type cytochrome c oxidase subunit I	WP_009048795	XLOC_003544	0.0070	0.0400	-2.97
		Polyphosphate kinase	WP_009048999	XLOC_001234	0.0001	0.0007	-1.96
		Membrane protein	WP_038632572	XLOC_003693	0.0001	0.0007	2.65
		MFS transporter	WP_009049300	XLOC_001382	0.0001	0.0007	-2.96
		Flavin reductase/prnA/prnB/prnC	WP_016704027	XLOC_003884	0.0001	0.0007	-4.03
		NADH-quinone oxidoreductase subunit K	WP_009049788	XLOC_001599	0.0018	0.0136	-2.25
		FAD-dependent oxidoreductase	WP_038633983	XLOC_004126	0.0001	0.0012	1.62
		2-hydroxyacid dehydrogenase	WP_038634294	XLOC_001825	0.0018	0.0136	-2.87
		2Fe-2S ferredoxin	WP_007929527	XLOC_004452	0.0001	0.0007	-2.11
		Ferredoxin-NADP reductase	WP_038635265	XLOC_004622	0.0001	0.0007	1.55
		Cytochrome D ubiquinol oxidase subunit I	WP_009050974	XLOC_004623	0.0003	0.0032	-2.01
		Glutamate:protein symporter	WP_023967611	XLOC_004776	0.0060	0.0353	3.09
		Cytochrome oxidase subunit I	WP_009046264	XLOC_004792	0.0001	0.0007	-2.38
		Cytochrome c oxidase subunit 2	WP_009046263	XLOC_004793	0.0001	0.0007	-1.66
		Alcohol dehydrogenase	WP_009046258	XLOC_004797	0.0034	0.0229	-1.91
		ATP synthase subunit b	WP_007924504	XLOC_002333	0.0001	0.0007	-1.58
		ATP synthase gamma chain	WP_007924507	XLOC_002334	0.0001	0.0007	-1.75
		ATP synthase subunit beta	WP_009051697	XLOC_002335	0.0001	0.0007	-2.37
		ATP synthase epsilon chain	WP_009051696	XLOC_002336	0.0001	0.0007	-1.52
		Aldehyde dehydrogenase	WP_038636068	XLOC_004944	0.0001	0.0007	-2.24
		Cytochrome B561	WP_007932535	XLOC_002512	0.0001	0.0007	-3.34
		Glycerol-3-phosphate dehydrogenase	WP_016703438	XLOC_001995	0.0001	0.0007	-2.65
		Glycerol kinase	WP_009050562	XLOC_001993	0.0001	0.0007	-2.42
<b>Cell cycle control, cell division, chromosome partitioning</b>	D	Chromosome partitioning protein ParA	WP_009047182	XLOC_002968	0.0001	0.0007	-2.46
		Cell division protein FtsA	WP_007922131	XLOC_004491	0.0001	0.0007	1.52
<b>Amino acid transport and metabolism</b>	E	Arginine biosynthesis bifunctional protein ArgJ	WP_038634693	XLOC_004382	0.0003	0.0027	1.72
		Gamma-aminobutyrate transporter	WP_009046515	XLOC_000101	0.0001	0.0007	1.64
		MFS transporter	WP_028683803	XLOC_000103	0.0001	0.0007	2.22

		Amino acid ABC transporter substrate-binding protein	WP_009046527	XLOC_000108	0.0044	0.0280	1.53
		Branched-chain amino acid transporter permease subunit LivH	WP_009046714	XLOC_000196	0.0023	0.0166	-1.96
		4-hydroxy-tetrahydrodipicolinate reductase	WP_038630583	XLOC_000280	0.0001	0.0007	2.85
		Peptide ABC transporter ATP-binding protein	WP_009046965	XLOC_002862	0.0030	0.0205	-1.55
		Peptide ABC transporter substrate-binding protein	WP_009046968	XLOC_002865	0.0001	0.0007	-1.84
		Amino acid dehydrogenase	WP_007932382	XLOC_000357	0.0001	0.0007	-1.82
		Amino acid ABC transporter substrate-binding protein	WP_025806519	XLOC_000375	0.0001	0.0007	2.00
		Aromatic amino acid transporter	WP_009042698	XLOC_000634	0.0001	0.0007	-4.95
		ATPase AAA	WP_038581185	XLOC_000635	0.0001	0.0012	1.81
		GntR family transcriptional regulator	WP_009047750	XLOC_003154	0.0022	0.0163	-1.85
		Inducer of phenazine B	WP_038631049	XLOC_000690	0.0001	0.0007	-5.73
		Lysine transporter LysE	WP_016702183	XLOC_003173	0.0012	0.0100	-4.37
		Spermidine acetyltransferase	WP_051673124	XLOC_000889	0.0004	0.0036	-1.63
		Arginine N-succinyltransferase	WP_009048183	XLOC_003302	0.0017	0.0133	-2.11
		Arginine N-succinyltransferase	WP_038631375	XLOC_003303	0.0030	0.0208	-1.98
		Porin	WP_016702750	XLOC_003304	0.0001	0.0007	-2.35
		Transporter	WP_038631522	XLOC_003362	0.0090	0.0482	-5.86
		Lysine transporter LysE	WP_009048674	XLOC_001122	0.0046	0.0288	1.65
		DNA-binding protein	WP_009048753	XLOC_003537	0.0068	0.0391	1.71
		Transcriptional regulator	WP_038632162	XLOC_003545	0.0001	0.0007	-4.87
		Diaminobutyrate--2-oxoglutarate aminotransferase	WP_038636648	XLOC_003702	0.0001	0.0007	5.23
		Endonuclease	WP_038633790	XLOC_001653	0.0001	0.0007	-2.65
		Amino acid APC transporter	WP_038634442	XLOC_001891	0.0001	0.0007	-4.39
		Arginine deiminase	WP_009050421	XLOC_001892	0.0001	0.0007	-5.24
		Ornithine carbamoyltransferase	WP_009050422	XLOC_001893	0.0001	0.0007	-4.43
		Carbamate kinase	WP_016703410	XLOC_001894	0.0001	0.0007	-3.89
		Glycine cleavage system H protein	WP_009050426	XLOC_001897	0.0034	0.0226	1.75
		Phospho-2-dehydro-3-deoxyheptonate aldolase	WP_029526777	XLOC_002097	0.0001	0.0007	-8.20
		Gamma-glutamyltransferase	WP_016703609	XLOC_002101	0.0007	0.0064	-1.67
		HAD family hydrolase	WP_009050998	XLOC_004635	0.0055	0.0331	1.62
		Tryptophan synthase beta chain	WP_038635660	XLOC_002311	0.0001	0.0012	-1.65
		GntR family transcriptional regulator	WP_038635802	XLOC_002361	0.0033	0.0223	-1.75
		ABC transporter substrate-binding protein	WP_009051375	XLOC_002462	0.0001	0.0007	-1.64
		Methylenetetrahydrofolate reductase	WP_009051282	XLOC_004967	0.0007	0.0060	-1.80
		Glycine/betaine ABC transporter substrate-binding protein	WP_009051250	XLOC_004986	0.0008	0.0068	-2.15
		Aminoglycoside N(6')-acetyltransferase type 1	WP_038636173	XLOC_004987	0.0001	0.0007	3.71
		Sarcosine oxidase subunit delta	WP_009051222	XLOC_002539	0.0011	0.0093	-2.38
		Spermidine/putrescine ABC transporter substrate-binding protein	WP_016704058	XLOC_002567	0.0001	0.0007	-2.80
		Anthranilate phosphoribosyltransferase	WP_038636384	XLOC_002573	0.0001	0.0012	-1.55
		Glutamine synthetase	WP_007929953	XLOC_002679	0.0001	0.0007	-2.02
		Phenylalanine 4-monooxygenase	WP_007930098	XLOC_003143	0.0009	0.0075	1.55
<b>Nucleotide transport and metabolism</b>	F	Phosphoribosylamine--glycine ligase	WP_009046769	XLOC_002766	0.0012	0.0100	-1.67
		Uncharacterized protein	WP_009048128	XLOC_000878	0.0001	0.0007	-1.83
		Uncharacterized protein	WP_009048212	XLOC_003313	0.0019	0.0145	-3.68

		Uncharacterized protein	WP_038631987	XLOC_001109	0.0001	0.0007	-5.42		
		Nucleoside diphosphate kinase	WP_007929529	XLOC_004450	0.0001	0.0007	-2.50		
<b>Carbohydrate transport and metabolism</b>	G	Multidrug resistance protein B	WP_009049803	XLOC_003990	0.0018	0.0139	-4.34		
		Aquaporin Z	WP_009047606	XLOC_003103	0.0003	0.0027	-1.95		
		2-dehydro-3-deoxy-6-phosphogalactonate aldolase	WP_038631144	XLOC_000752	0.0001	0.0007	2.07		
		Chitinase	WP_009048115	XLOC_000870	0.0003	0.0027	-11.22		
		LacI family transcriptional regulator	WP_009043052	XLOC_000874	0.0001	0.0007	-1.71		
		Siderophore biosynthesis protein SbnG	WP_009049138	XLOC_003700	0.0001	0.0007	3.67		
		MFS transporter	WP_038632598	XLOC_003701	0.0001	0.0007	5.74		
		Polysaccharide deacetylase	WP_038632649	XLOC_003724	0.0001	0.0007	2.87		
		Chitinase	WP_009049243	XLOC_003753	0.0001	0.0007	-5.76		
		MFS transporter	WP_060550016	XLOC_001368	0.0042	0.0270	-2.49		
		Sorbose dehydrogenase	WP_038634274	XLOC_001820	0.0003	0.0032	-2.61		
		Sugar ABC transporter ATPase	WP_016703424	XLOC_004339	0.0001	0.0007	-4.84		
		Sugar ABC transporter permease	WP_009045231	XLOC_004340	0.0001	0.0007	-2.65		
		Sugar ABC transporter substrate-binding protein	WP_009050405	XLOC_004341	0.0001	0.0007	-6.11		
		Sugar isomerase	WP_016703422	XLOC_004342	0.0001	0.0007	-4.83		
		Glucokinase	WP_009050408	XLOC_004344	0.0001	0.0007	-1.70		
		Glycerol uptake facilitator GlpF	WP_009050561	XLOC_001992	0.0013	0.0107	-2.76		
		Sugar ABC transporter ATPase	WP_009050627	XLOC_004440	0.0001	0.0007	3.35		
		Glyceraldehyde-3-phosphate dehydrogenase	WP_009050409	XLOC_001882	0.0001	0.0007	-3.30		
		2-methylcitrate dehydratase	WP_038631169	XLOC_000781	0.0001	0.0007	-2.65		
<b>Coenzyme transport and metabolism</b>	H	Omega amino acid--pyruvate aminotransferase	WP_038630538	XLOC_000233	0.0001	0.0007	-1.80		
		Cyclic pyranopterin monophosphate synthase accessory protein	WP_009047097	XLOC_000372	0.0001	0.0007	1.95		
		Pyridoxine 5'-phosphate synthase	WP_038630664	XLOC_000389	0.0004	0.0036	2.55		
		Damage-inducible protein CinA	WP_023969838	XLOC_000467	0.0033	0.0223	2.23		
		MFS transporter	WP_009047472	XLOC_003055	0.0001	0.0007	-6.98		
		Alpha/beta hydrolase	WP_009047789	XLOC_000715	0.0011	0.0093	1.56		
		Tryptophan synthase subunit alpha	WP_009048364	XLOC_003383	0.0001	0.0007	-6.11		
		(2Fe-2S)-binding protein	WP_016701774	XLOC_001019	0.0001	0.0012	-4.41		
		Transporter	WP_009049200	XLOC_003732	0.0001	0.0007	6.04		
		Uncharacterized protein	WP_009049581	XLOC_003902	0.0001	0.0007	-2.10		
		Alpha/beta hydrolase	WP_028683000	XLOC_001688	0.0082	0.0450	-1.76		
		Dihydropterolate synthase	WP_009046931	XLOC_000285	0.0001	0.0012	1.62		
		(2Fe-2S)-binding protein	WP_016701774	XLOC_003431	0.0001	0.0012	-5.41		
		<b>Lipid transport and metabolism</b>	I	Poly(R)-hydroxyalkanoic acid synthase	WP_009046586	XLOC_002690	0.0001	0.0007	-3.34
				Poly(R)-hydroxyalkanoic acid synthase	WP_009046588	XLOC_002692	0.0001	0.0007	-3.76
				Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	WP_038630718	XLOC_000439	0.0001	0.0007	-2.54
				GDSL family lipase	WP_038630822	XLOC_003052	0.0001	0.0007	1.80
				Malonyl CoA-acyl carrier protein transacylase	WP_009047834	XLOC_000740	0.0007	0.0064	-1.62
				Acyl carrier protein	WP_003175607	XLOC_003180	0.0024	0.0171	-1.52
				Enoyl-CoA hydratase	WP_016704994	XLOC_003195	0.0003	0.0032	-1.68
3-oxoacyl-[acyl-carrier-protein] synthase 2	WP_041988549			XLOC_003283	0.0005	0.0049	-2.03		
Phosphatidylserine decarboxylase proenzyme	WP_038631548			XLOC_000962	0.0001	0.0007	-5.40		
Enoyl-[acyl-carrier-protein] reductase [NADH]	WP_038631995			XLOC_001112	0.0001	0.0007	-2.12		
Glucose-methanol-choline oxidoreductase	WP_038632521			XLOC_003682	0.0009	0.0075	-3.28		
AMP-binding protein	WP_038632834			XLOC_001401	0.0005	0.0049	-1.54		

		VdID	WP_009049637	XLOC_001532	0.0001	0.0007	2.65		
		AMP-binding protein	WP_038633666	XLOC_003998	0.0059	0.0351	-1.69		
		Acyl carrier protein	WP_038633949	XLOC_004114	0.0001	0.0012	-4.35		
		3-oxoacyl-ACP synthase	WP_009050008	XLOC_004115	0.0005	0.0045	-2.73		
		Probable lipid kinase YegS-like	WP_009050090	XLOC_004158	0.0052	0.0320	1.88		
		Glycerol acyltransferase	WP_009050240	XLOC_004248	0.0001	0.0012	-2.90		
		Acetyl-coenzyme A synthetase	WP_009050330	XLOC_004302	0.0001	0.0007	-2.49		
		Long-chain fatty acid--CoA ligase	WP_009050391	XLOC_004332	0.0001	0.0007	-2.35		
		Acetyl-CoA acetyltransferase	WP_038635179	XLOC_002140	0.0046	0.0288	1.62		
		Acyl-CoA dehydrogenase	WP_009051017	XLOC_004686	0.0001	0.0007	1.73		
		Glycerol acyltransferase	WP_009046219	XLOC_002323	0.0001	0.0007	-2.76		
		Serine/threonine protein phosphatase	WP_038636950	XLOC_004830	0.0001	0.0007	-1.50		
		Fatty acid methyltransferase	WP_016703983	XLOC_002348	0.0001	0.0007	-1.95		
		Short-chain dehydrogenase	WP_038636192	XLOC_004998	0.0001	0.0007	-1.86		
<b>Translation, ribosomal structure and biogenesis</b>	J	Prolyl-tRNA synthetase	WP_016705004	XLOC_000023	0.0012	0.0100	1.78		
		Transcriptional regulator	WP_009046631	XLOC_000150	0.0001	0.0007	1.71		
		Polyribonucleotide nucleotidyltransferase	WP_038630587	XLOC_000295	0.0001	0.0007	-2.17		
		Putative sigma(54) modulation protein	WP_007929872	XLOC_002883	0.0001	0.0007	-1.62		
		Elongation factor Ts	WP_009042383	XLOC_000428	0.0001	0.0007	-1.72		
		GNAT family acetyltransferase	WP_038630820	XLOC_000531	0.0023	0.0166	-2.19		
		Alanyl-tRNA synthetase	WP_009047809	XLOC_003174	0.0057	0.0340	-3.34		
		Ribosome modulation factor	WP_023968495	XLOC_003210	0.0001	0.0007	-1.89		
		Ribonuclease	WP_016704622	XLOC_001120	0.0086	0.0469	-1.90		
		Methionine aminopeptidase	WP_009048909	XLOC_003604	0.0081	0.0448	2.13		
		GTP-binding protein	WP_028683953	XLOC_003650	0.0001	0.0007	-2.87		
		Elongation factor P	WP_007931398	XLOC_004174	0.0001	0.0007	-1.67		
		7-cyano-7-deazaguanine synthase	WP_038636862	XLOC_004480	0.0001	0.0007	-1.53		
		ATP-dependent protease	WP_009046589	XLOC_002694	0.0001	0.0007	3.57		
		ATPase subunit HslU							
		<b>Transcription</b>	K	RNA polymerase sigma factor	WP_016705108	XLOC_002582	0.0001	0.0007	1.55
				MarR family transcriptional regulator	WP_009046358	XLOC_000026	0.0001	0.0007	-2.08
AraC family transcriptional regulator	WP_028683816			XLOC_002650	0.0001	0.0007	-3.47		
TetR family transcriptional regulator	WP_009046585			XLOC_002689	0.0009	0.0079	-3.71		
Transcriptional regulator	WP_009046808			XLOC_002789	0.0016	0.0123	-2.19		
RNA polymerase sigma factor RpoS	WP_007924026			XLOC_000448	0.0001	0.0007	-3.74		
ArsR family transcriptional regulator	WP_009047418			XLOC_003036	0.0026	0.0183	2.84		
Cro/C1 family transcriptional regulator	WP_023968513			XLOC_003219	0.0002	0.0017	1.60		
LacI family transcriptional regulator	WP_038631299			XLOC_000876	0.0018	0.0136	2.35		
TetR family transcriptional regulator	WP_038631318			XLOC_003289	0.0061	0.0360	2.28		
RNA polymerase subunit sigma-70	WP_009048295			XLOC_000950	0.0001	0.0007	2.32		
Transcriptional regulator	WP_009048394			XLOC_000993	0.0071	0.0402	-2.95		
LuxR family transcriptional regulator	WP_009048517			XLOC_003448	0.0004	0.0036	-3.05		
Uncharacterized protein	WP_009048686			XLOC_003507	0.0001	0.0007	-1.63		
CdaR family transcriptional regulator	WP_016702462			XLOC_003773	0.0081	0.0446	2.41		
Transcriptional regulator	WP_009049335			XLOC_001408	0.0001	0.0007	-2.84		
GntR family transcriptional regulator	WP_038633317			XLOC_001500	0.0004	0.0036	1.88		
Cold-shock protein	WP_009049775			XLOC_001584	0.0001	0.0007	-2.10		
Cro/C1 family transcriptional regulator	WP_009049820			XLOC_003999	0.0026	0.0186	2.39		
RNA polymerase subunit sigma-24	WP_038633746			XLOC_004046	0.0001	0.0012	1.90		



		RNA polymerase sigma factor	WP_016702389	XLOC_004106	0.0007	0.0060	2.00	
		Transcriptional regulator	WP_009050371	XLOC_004323	0.0003	0.0032	-1.57	
		RNA polymerase subunit sigma-24	WP_009050411	XLOC_001884	0.0040	0.0257	1.56	
		Temperature acclimation protein B	WP_007931076	XLOC_001901	0.0001	0.0007	-3.36	
		GntR family transcriptional regulator	WP_009050459	XLOC_001909	0.0001	0.0007	-2.79	
		H-NS histone/MvaT homologue	WP_007931174	XLOC_001963	0.0001	0.0007	-1.82	
		DNA-binding protein PhzR	WP_009050695	XLOC_002040	0.0001	0.0007	-4.12	
		Competence protein TfoX	WP_009050884	XLOC_002126	0.0004	0.0036	-1.55	
		Transcriptional regulator	WP_038635479	XLOC_002229	0.0001	0.0007	-4.13	
		XRE family transcriptional regulator	WP_007924514	XLOC_002338	0.0087	0.0470	-2.17	
		AsnC family transcriptional regulator	WP_009051665	XLOC_002346	0.0001	0.0007	2.25	
		TetR family transcriptional regulator	WP_009051542	XLOC_002409	0.0001	0.0007	2.03	
		DNA-directed RNA polymerase subunit beta	WP_038635595	XLOC_004754	0.0001	0.0007	-1.72	
		Rha family transcriptional regulator	WP_038633014	XLOC_003829	0.0001	0.0007	2.65	
<b>Replication, recombination and repair</b>	L	Methyltransferase domain protein	WP_038630393	XLOC_000100	0.0015	0.0120	-3.21	
		ATP-dependent DNA helicase	WP_028684106	XLOC_003734	0.0025	0.0177	-1.71	
		Uncharacterized protein	WP_007921116	XLOC_002393	0.0001	0.0007	-1.71	
		DNA polymerase III subunit epsilon	WP_038636295	XLOC_005008	0.0001	0.0007	-2.49	
		DNA gyrase subunit B	WP_007924471	XLOC_004819	0.0001	0.0007	-1.51	
		Ribonuclease H	WP_009048707	XLOC_001130	0.0076	0.0424	1.66	
<b>Cell wall/membrane/envelope biogenesis</b>	M	RND transporter	WP_009046338	XLOC_000017	0.0001	0.0007	-1.72	
		Transporter	WP_009046348	XLOC_000022	0.0001	0.0007	-3.43	
		Membrane protein	WP_038630568	XLOC_000270	0.0063	0.0367	1.77	
		Membrane-bound lytic murein transglycosylase F	WP_009042308	XLOC_002944	0.0001	0.0007	-1.60	
		Membrane protein	WP_028681745	XLOC_002967	0.0001	0.0007	-2.27	
		Membrane protein	WP_028681813	XLOC_000480	0.0001	0.0007	-2.31	
		Porin	WP_007927525	XLOC_000789	0.0001	0.0007	-3.05	
		Acyltransferase	WP_038636499	XLOC_003266	0.0001	0.0007	-1.77	
		Type I secretion protein TolC	WP_009048343	XLOC_000972	0.0017	0.0130	2.57	
		Membrane protein	WP_038632523	XLOC_001294	0.0001	0.0007	-2.10	
		Phospholipase	WP_038632608	XLOC_003705	0.0020	0.0148	3.07	
		Acriflavin resistance protein AcrA	WP_009049438	XLOC_003840	0.0001	0.0007	-2.98	
		Channel protein TolC	WP_038636749	XLOC_003928	0.0001	0.0007	1.99	
		Multidrug RND transporter	WP_038633661	XLOC_003992	0.0001	0.0007	-2.73	
		Hemolysin secretion protein D	WP_038633904	XLOC_001677	0.0001	0.0007	1.69	
		Membrane protein	WP_038634145	XLOC_004202	0.0001	0.0007	-1.73	
		UDP-N-acetylglucosamine 2-epimerase	WP_038634147	XLOC_001764	0.0014	0.0110	-3.71	
		Uncharacterized protein	WP_051673203	XLOC_004204	0.0024	0.0171	-1.56	
		Porin	WP_038634427	XLOC_004338	0.0001	0.0007	-2.58	
		Porin	WP_038634847	XLOC_002012	0.0016	0.0126	-2.28	
		RND transporter MFP subunit	WP_038634985	XLOC_002046	0.0039	0.0255	2.09	
		Polysaccharide biosynthesis protein	WP_009050917	XLOC_002146	0.0066	0.0382	-1.52	
		Glycosyltransferase	WP_038635733	XLOC_002345	0.0035	0.0231	-1.91	
		N-acetylmuramoyl-L-alanine amidase	WP_038635768	XLOC_002350	0.0018	0.0136	5.68	
			Glucans biosynthesis protein D	WP_016702744	XLOC_002958	0.0021	0.0157	1.72
	<b>Cell motility</b>	N	Chemotaxis protein	WP_009046693	XLOC_000180	0.0001	0.0007	2.00
			Chemotaxis protein	WP_038631529	XLOC_003363	0.0001	0.0007	-2.91
		General secretion pathway protein GspE	WP_009048770	XLOC_001151	0.0082	0.0450	1.92	
		Flagellar hook protein FlgE	WP_009050299	XLOC_004274	0.0001	0.0007	-2.37	
		Chemotaxis protein	WP_038634350	XLOC_001852	0.0002	0.0022	1.97	
		Chemotaxis protein	WP_038634354	XLOC_004312	0.0077	0.0429	3.70	

		Chemotaxis protein	WP_038634436	XLOC_001889	0.0001	0.0012	2.20
		Flagellar basal body rod protein FlgB	WP_016702521	XLOC_004277	0.0001	0.0007	-1.70
		Flagellar basal-body rod protein FlgG	WP_009047672	XLOC_000637	0.0001	0.0007	-1.86
<b>Posttranslational modification, protein turnover, chaperones</b>	O	Chaperone protein DnaK	WP_009046925	XLOC_000278	0.0001	0.0007	2.09
		Protein-disulfide isomerase	WP_038630382	XLOC_000093	0.0001	0.0007	1.95
		Probable Fe(2+)-trafficking protein	WP_007921798	XLOC_002666	0.0079	0.0437	-1.71
		ATP-dependent protease subunit HslV	WP_007925866	XLOC_002695	0.0001	0.0007	3.37
		Protein GrpE	WP_007920848	XLOC_000277	0.0001	0.0007	4.15
		Peptidyl-prolyl cis-trans isomerase	WP_007929845	XLOC_000341	0.0001	0.0007	-1.71
		Peptidyl-prolyl cis-trans isomerase	WP_007920626	XLOC_003105	0.0001	0.0007	-2.15
		Chaperone protein HtpG	WP_029526163	XLOC_000706	0.0001	0.0007	3.70
		Heat-shock protein	WP_007922004	XLOC_000845	0.0001	0.0007	4.85
		Peroxidase	WP_009048161	XLOC_000894	0.0001	0.0007	-2.33
		Glutathione S-transferase	WP_038631753	XLOC_003432	0.0001	0.0007	-4.99
		Alkyl hydroperoxide reductase	WP_007921754	XLOC_003780	0.0001	0.0007	-3.31
		Peptidylprolyl isomerase	WP_007932676	XLOC_001529	0.0064	0.0371	3.05
		Thiol:disulfide interchange protein	WP_038633534	XLOC_003950	0.0047	0.0293	-1.60
		Trigger factor	WP_009044653	XLOC_004022	0.0001	0.0007	-1.52
		Serine protease	WP_038633759	XLOC_004049	0.0001	0.0007	-2.06
		10 kDa chaperonin	WP_009050540	XLOC_004406	0.0001	0.0007	2.11
		Glutaredoxin	WP_007922425	XLOC_004415	0.0001	0.0007	-2.51
		Chaperone protein ClpB	WP_009050931	XLOC_004594	0.0001	0.0007	2.40
		Peroxidase	WP_009051386	XLOC_002452	0.0001	0.0007	2.26
		Probable thiol peroxidase	WP_038632154	XLOC_001174	0.0001	0.0007	-2.85
<b>Inorganic ion transport and metabolism</b>	P	Peptide ABC transporter substrate-binding protein	WP_038630318	XLOC_002581	0.0001	0.0007	2.72
		TonB-dependent receptor	WP_038630381	XLOC_002642	0.0001	0.0007	1.84
		TonB-dependent receptor	WP_038636418	XLOC_002754	0.0031	0.0210	4.47
		Alkaline phosphatase	WP_038630596	XLOC_000304	0.0022	0.0163	-2.18
		ABC transporter substrate-binding protein	WP_038631672	XLOC_001005	0.0001	0.0007	2.91
		Sulfite reductase	WP_009048796	XLOC_001169	0.0001	0.0007	-3.63
		Copper resistance protein CopC	WP_038632194	XLOC_003561	0.0001	0.0007	3.13
		Copper resistance protein CopB	WP_016704756	XLOC_003562	0.0001	0.0007	1.52
		ABC transporter substrate-binding protein	WP_038632351	XLOC_001230	0.0084	0.0458	-2.42
		Iron ABC transporter permease	WP_038632590	XLOC_003699	0.0001	0.0007	4.09
		TonB-dependent receptor	WP_038632604	XLOC_003703	0.0001	0.0007	4.69
		Peptide ABC transporter substrate-binding protein	WP_038633744	XLOC_004045	0.0001	0.0007	1.90
		Catalase-peroxidase	WP_038633847	XLOC_004084	0.0001	0.0007	-2.32
		ABC transporter substrate-binding protein	WP_009050227	XLOC_001802	0.0002	0.0022	-2.78
		TonB-dependent receptor	WP_038634433	XLOC_001886	0.0001	0.0007	2.02
		FAD-binding protein	WP_038634838	XLOC_002009	0.0001	0.0007	-3.14
		TonB-dependent receptor	WP_038634844	XLOC_002011	0.0003	0.0027	5.56
		Catalase	WP_009050970	XLOC_004621	0.0091	0.0488	-4.25
		Catalase	WP_016702645	XLOC_004732	0.0001	0.0007	-2.24
		SulP family inorganic anion transporter	WP_038635643	XLOC_004794	0.0079	0.0439	-1.55
		Carbonic anhydrase	WP_009046260	XLOC_004795	0.0001	0.0007	-2.42
		Zinc ABC transporter permease	WP_009051646	XLOC_004836	0.0001	0.0007	6.49
		Calcium transporter ChaC	WP_009051415	XLOC_002434	0.0001	0.0007	-1.53
		ABC transporter substrate-binding protein	WP_038635999	XLOC_002454	0.0001	0.0007	2.35
		Taurine ABC transporter substrate-binding protein	WP_009046457	XLOC_002630	0.0054	0.0329	1.89



<b>Secondary metabolites biosynthesis, transport and catabolism</b>	Q	Poly(3-hydroxyalkanoate) granule-associated protein PhaF	WP_009046584	XLOC_000134	0.0001	0.0007	-3.73	
		Poly(3-hydroxyalkanoate) depolymerase	WP_009046587	XLOC_002691	0.0001	0.0007	-2.24	
		Serine 3-dehydrogenase	WP_016702821	XLOC_000985	0.0001	0.0007	-5.06	
		Acyl-homoserine lactone acylase subunit beta	WP_038632200	XLOC_001187	0.0001	0.0007	1.52	
		NADP-dependent oxidoreductase	WP_009049328	XLOC_001403	0.0001	0.0007	-2.93	
		Phenazine biosynthesis protein	WP_009050819	XLOC_002096	0.0001	0.0007	-7.98	
		Isochorismatase	WP_009050821	XLOC_002098	0.0001	0.0007	-8.47	
		Phenazine biosynthesis protein	WP_038635119	XLOC_002100	0.0001	0.0007	-8.72	
		Phenazine biosynthesis protein	WP_009050818	XLOC_002095	0.0001	0.0007	-9.14	
	<b>General function prediction only</b>	R	Rhs family protein	WP_038630348	XLOC_002611	0.0001	0.0007	-7.34
		Alpha/beta hydrolase	WP_009046421	XLOC_000062	0.0060	0.0353	3.11	
		4-carboxymuconolactone decarboxylase	WP_009046984	XLOC_002874	0.0016	0.0126	2.99	
		Carbon-nitrogen hydrolase	WP_038630611	XLOC_000324	0.0001	0.0007	2.43	
		LrgA	WP_016701724	XLOC_000348	0.0086	0.0467	1.54	
		PsiE family protein	WP_009042237	XLOC_000360	0.0002	0.0017	-2.48	
		Carboxylesterase	WP_009047103	XLOC_002935	0.0002	0.0022	-2.11	
		Cytochrome D ubiquinol oxidase subunit II	WP_016702021	XLOC_002984	0.0001	0.0007	-2.27	
		Arylsulfate sulfotransferase	WP_009042529	XLOC_000517	0.0028	0.0194	-3.33	
		Putative hemagglutinin (DUF637)	WP_051429416	XLOC_003109	0.0080	0.0441	-2.64	
		Diaminopimelate decarboxylase	WP_009048261	XLOC_003333	0.0001	0.0012	-1.72	
		Na <sup>+</sup> /H <sup>+</sup> dicarboxylate symporter	WP_009048348	XLOC_000974	0.0007	0.0064	1.59	
		Sugar deacetylase	WP_016702950	XLOC_001239	0.0001	0.0007	-3.63	
		ABC transporter	WP_016702952	XLOC_001241	0.0001	0.0007	-3.46	
		Methyltransferase	WP_038632359	XLOC_003651	0.0013	0.0107	-4.68	
		ABC transporter ATP-binding protein	WP_016702438	XLOC_001421	0.0041	0.0265	1.71	
		Amidohydrolase	WP_009049582	XLOC_003903	0.0001	0.0007	-1.90	
		Peptidase C39	WP_038633471	XLOC_003930	0.0001	0.0007	2.26	
		Isomerase	WP_038633872	XLOC_004090	0.0002	0.0022	-2.18	
		Amidohydrolase	WP_038633980	XLOC_001697	0.0001	0.0007	-4.39	
		Uncharacterized protein	WP_038634153	XLOC_001765	0.0001	0.0012	-2.60	
		2,3-dihydro-3-hydroxyanthranilate isomerase	WP_016703606	XLOC_002099	0.0001	0.0012	-7.59	
		Nuclease	WP_028683814	XLOC_002657	0.0005	0.0049	-3.95	
<b>Unknown Function</b>		S	Uncharacterized protein	EIM12867	XLOC_000056	0.0001	0.0007	-4.84
			Uncharacterized protein	WP_038630325	XLOC_002590	0.0001	0.0007	-2.28
			Glycosyl hydrolase	WP_038636404	XLOC_000021	0.0001	0.0007	-3.60
			RebB like protein	WP_007930328	XLOC_000038	0.0007	0.0060	-4.50
			R body protein RebB-like protein	WP_009046382	XLOC_000043	0.0061	0.0360	-6.99
			Peptidoglycan-binding protein LysM	WP_009046477	XLOC_000086	0.0001	0.0007	-2.36
			Poly(3-hydroxyalkanoate) granule-associated protein PhaI	WP_009046583	XLOC_000133	0.0001	0.0007	-7.18
		Uncharacterized protein	WP_038630505	XLOC_002769	0.0005	0.0049	1.92	
		Autotransporter	WP_038630521	XLOC_000224	0.0001	0.0007	-2.91	
		Phosphatidylcholine-hydrolyzing phospholipase	WP_009046978	XLOC_002873	0.0001	0.0007	-2.09	
		Tail sheath protein	WP_038630744	XLOC_000457	0.0001	0.0007	-4.48	
		Phage tail protein	WP_007923973	XLOC_000458	0.0023	0.0169	-2.68	
		UPF0225 protein EY04_05815	WP_028681815	XLOC_003010	0.0022	0.0163	-2.00	
		Membrane protein	WP_016702126	XLOC_003058	0.0001	0.0007	2.10	
		Uncharacterized protein	WP_038636463	XLOC_003065	0.0001	0.0007	-2.40	
		Acetyltransferase	WP_038630891	XLOC_003073	0.0001	0.0007	-2.10	
		Metal-chelation protein CHAD	WP_038630895	XLOC_003075	0.0001	0.0007	-1.54	
		Uncharacterized protein	WP_038630933	XLOC_000599	0.0001	0.0007	2.20	
		Putative hemagglutinin (DUF637)	EJL96817	XLOC_000612	0.0007	0.0060	-2.85	
		Uncharacterized protein	WP_011060148	XLOC_003191	0.0093	0.0496	1.85	

Uncharacterized protein	WP_009042934	XLOC_000791	0.0001	0.0007	1.71
Uncharacterized protein	WP_009048113	XLOC_000868	0.0020	0.0151	-2.35
Membrane protein	WP_009048116	XLOC_000871	0.0006	0.0057	-2.18
Uncharacterized protein	WP_007925069	XLOC_000998	0.0019	0.0145	-1.70
Oxidase	WP_038631869	XLOC_003462	0.0001	0.0007	-2.06
Uncharacterized protein	WP_038632150	XLOC_001170	0.0001	0.0007	-2.41
Uncharacterized protein	WP_038632169	XLOC_003551	0.0001	0.0007	-5.08
Lipoprotein	WP_038632271	XLOC_003611	0.0001	0.0007	-5.78
Membrane protein	WP_038632611	XLOC_001325	0.0004	0.0040	-1.83
Porin	WP_025808788	XLOC_003710	0.0001	0.0007	-2.44
Bifunctional pyrazinamidase/nicotinamidase	ADO14993	XLOC_001373	0.0004	0.0040	-3.65
Uncharacterized protein	WP_009049346	XLOC_003785	0.0001	0.0007	2.84
Uncharacterized protein	WP_038633414	XLOC_001519	0.0001	0.0007	5.27
Uncharacterized protein	WP_038633491	XLOC_003935	0.0001	0.0007	-3.47
Spore coat protein	WP_009049832	XLOC_004007	0.0050	0.0310	-2.75
Spore coat protein	WP_038633677	XLOC_004009	0.0001	0.0007	-1.73
Membrane protein	WP_038633841	XLOC_004083	0.0001	0.0007	-4.80
Dialkylrecorsinol condensing enzyme	WP_009050009	XLOC_004116	0.0001	0.0007	-2.37
Uncharacterized protein (Fragment)	KPZ18068	XLOC_004139	0.0043	0.0272	-2.09
Membrane protein	WP_038634529	XLOC_004361	0.0001	0.0012	-2.60
Type IV secretion protein Rhs	WP_038634583	XLOC_004369	0.0001	0.0007	-3.28
Porin	WP_038634688	XLOC_001955	0.0001	0.0007	-4.83
Lon protease	WP_038634854	XLOC_002017	0.0001	0.0007	2.61
Uncharacterized protein	WP_009050754	XLOC_004521	0.0001	0.0007	-2.57
Uncharacterized protein	WP_009050755	XLOC_004522	0.0001	0.0007	-2.71
Uncharacterized protein	WP_009050883	XLOC_002125	0.0001	0.0007	-1.54
Phospholipid-binding protein	WP_009050906	XLOC_004586	0.0001	0.0007	-3.29
ABC transporter substrate-binding protein	WP_009051009	XLOC_004641	0.0017	0.0130	3.48
Uncharacterized protein	WP_051673205	XLOC_004653	0.0001	0.0007	-2.31
Peptidase C13	WP_038635515	XLOC_002247	0.0001	0.0007	-3.65
Membrane protein	WP_038635748	XLOC_002347	0.0001	0.0007	1.99
Uncharacterized protein	WP_023967792	XLOC_002375	0.0001	0.0007	-2.43
Hypothetical protein	WP_054617053	XLOC_002384	0.0002	0.0022	-2.16
Uncharacterized protein	WP_025807035	XLOC_002408	0.0011	0.0093	-2.77
Secondary thiamine-phosphate synthase enzyme	EIM17029	XLOC_004893	0.0007	0.0060	-2.13
ATPase	WP_025807119	XLOC_002466	0.0042	0.0267	-2.15
Uncharacterized protein	WP_009051217	XLOC_002543	0.0001	0.0007	1.66
Short-chain dehydrogenase	WP_016704711	XLOC_003541	0.0001	0.0007	4.22
Uncharacterized protein	WP_009049553	XLOC_003894	0.0002	0.0017	-2.77
Glutamine synthetase	EIM18812	XLOC_004837	0.0047	0.0293	4.26
Pyroloquinoline quinone biosynthesis protein PqqE	WP_009051193	XLOC_002552	0.0001	0.0007	1.60
Tail protein	WP_038630740	XLOC_000455	0.0001	0.0007	-4.43
Potassium channel protein	WP_038632026	XLOC_003505	0.0004	0.0040	-2.90
SAM-dependent methyltransferase	EXF47260	XLOC_001129	0.0051	0.0315	1.99
<b>Signal transduction mechanisms</b>	<b>T</b>				
Histidine kinase	WP_038630504	XLOC_002765	0.0001	0.0007	-2.06
LuxR family transcriptional regulator	WP_009046790	XLOC_000221	0.0001	0.0007	-2.07
Uncharacterized protein	WP_009046954	XLOC_000306	0.0001	0.0007	1.86
Crp/Fnr family transcriptional regulator	WP_038630893	XLOC_003074	0.0001	0.0007	-2.17
DeoR family transcriptional regulator	WP_016702287	XLOC_003124	0.0054	0.0329	-2.49
Anti-anti-sigma factor	WP_009047705	XLOC_000662	0.0001	0.0007	-2.10
Chemotaxis protein CheY	WP_038631017	XLOC_000663	0.0001	0.0007	-3.44
Protein kinase	WP_038631821	XLOC_003441	0.0001	0.0007	-1.78
Transporter	WP_038632357	XLOC_001235	0.0004	0.0036	-1.63
Diguanylate cyclase	WP_009049109	XLOC_001313	0.0001	0.0007	-1.90
Diguanylate phosphodiesterase	WP_028684076	XLOC_003708	0.0001	0.0007	1.79
Universal stress protein	WP_009049186	XLOC_003726	0.0002	0.0017	1.76
Histidine kinase	WP_016705133	XLOC_001339	0.0018	0.0139	1.74
Signal peptide protein	WP_038633336	XLOC_003900	0.0082	0.0452	-2.83
Chemotaxis protein CheY	WP_009049747	XLOC_003975	0.0002	0.0017	-2.27

		Diguanylate cyclase	WP_016701868	XLOC_001577	0.0001	0.0007	-3.34
		Histidine kinase	WP_009049953	XLOC_004086	0.0005	0.0049	-2.43
		Acyl-homoserine-lactone synthase	WP_038635110	XLOC_002094	0.0001	0.0007	-5.76
		Chemotaxis protein	WP_016703630	XLOC_004556	0.0021	0.0154	1.50
		Histidine kinase	WP_016703743	XLOC_004687	0.0001	0.0007	2.38
		Diguanylate cyclase	WP_038636150	XLOC_002522	0.0001	0.0007	-5.19
		Response regulator	WP_009045922	XLOC_002548	0.0016	0.0126	1.95
		Diguanylate cyclase	WP_038575258	XLOC_002554	0.0065	0.0378	2.80
<b>Intracellular trafficking, secretion, and vesicular transport</b>	U	Protein-export protein SecB	WP_009046533	XLOC_000111	0.0001	0.0007	-2.89
		ATPase	WP_038630518	XLOC_002782	0.0031	0.0210	-1.92
		Potassium ABC transporter	WP_038630522	XLOC_002785	0.0001	0.0007	-2.06
		ATPase					
		EvpB family type VI secretion protein	WP_038633339	XLOC_001503	0.0025	0.0177	-4.17
		ShlB family hemolysin secretion/activation protein	WP_041987697	XLOC_001737	0.0018	0.0139	2.69
		Filamentous hemagglutinin	WP_038634114	XLOC_001738	0.0001	0.0007	3.79
		Secretin	WP_029526712	XLOC_001903	0.0001	0.0007	-2.30
		Preprotein translocase subunit YajC	WP_007929510	XLOC_004463	0.0001	0.0007	-2.04
		Type VI secretion protein	WP_007930286	XLOC_002379	0.0031	0.0210	-7.35
		TssB2					
		Type VI secretion protein	WP_009051592	XLOC_002380	0.0001	0.0007	-6.68
		Type VI secretion protein	WP_007930278	XLOC_002383	0.0001	0.0007	-4.98
		Elongation factor G	WP_009051119	XLOC_004750	0.0001	0.0007	-1.83
		Elongation factor Tu	WP_016702644	XLOC_004749	0.0001	0.0007	-2.89
		Elongation factor Tu	WP_016702644	XLOC_004761	0.0001	0.0007	-1.83
	Uncharacterized protein	WP_038635388	XLOC_002204	0.0001	0.0007	2.04	
<b>Defense mechanisms</b>	V	Beta-lactamase	WP_038633878	XLOC_001672	0.0026	0.0186	-1.99
		DSBA oxidoreductase	WP_009046334	XLOC_000016	0.0001	0.0007	-2.86
		3-deoxy-D-manno-octulosonic-acid transferase	WP_007921448	XLOC_002717	0.0010	0.0086	2.49
		ABC transporter	WP_038630975	XLOC_003123	0.0001	0.0007	-2.44
		Transporter	WP_009049437	XLOC_003839	0.0001	0.0007	-2.74
		Hemolysin D	WP_028682887	XLOC_003931	0.0001	0.0007	2.21
	Hemolysin D	WP_009049804	XLOC_003991	0.0001	0.0012	-3.47	
<b>Extracellular structures</b>	W	Molecular chaperone	WP_028682799	XLOC_004005	0.0003	0.0027	-1.68
<b>Mobilome: prophages, transposons</b>	X	Tail protein	WP_038630732	XLOC_000451	0.0001	0.0007	-3.51
		Tail protein	WP_016701993	XLOC_000453	0.0001	0.0007	-3.30
		2-hydroxyacid dehydrogenase	WP_038630746	XLOC_000459	0.0001	0.0007	-2.12
		Uncharacterized protein	WP_038632916	XLOC_003806	0.0001	0.0007	1.97

Appendix Table 2: Differentially expressed genes in PA23-*phzR* compared to WT.

COG category		Predicted function	Protein accession number (NCBI)	Gene locus	P-value	Q-value	log2 fold change
<b>Energy production and conversion</b>	<b>C</b>	2-hydroxyacid dehydrogenase	WP_038631825	XLOC_003445	0.0001	0.0007	1.57
		2-oxoglutarate dehydrogenase	WP_038581009	XLOC_000700	0.0001	0.0007	-1.99
		MFS transporter	WP_009049300	XLOC_001382	0.0001	0.0007	-2.08
		Alcohol dehydrogenase	WP_038632862	XLOC_003788	0.0006	0.0057	1.83
		Alcohol dehydrogenase	WP_009046258	XLOC_004797	0.0084	0.0461	-2.47
		Aldehyde dehydrogenase	WP_009049718	XLOC_001565	0.0001	0.0007	-1.74
		ATP synthase subunit beta	WP_009051697	XLOC_002335	0.0001	0.0007	-1.66
		Branched-chain alpha-keto acid dehydrogenase subunit E2	WP_038631641	XLOC_003406	0.0001	0.0007	2.63
		(Fe-S)-binding protein	WP_016704739	XLOC_003546	0.0001	0.0012	-4.19
		Cytochrome C oxidase subunit IV	WP_016702591	XLOC_000099	0.0008	0.0072	2.25
		Cytochrome o ubiquinol oxidase subunit I	WP_009045490	XLOC_004483	0.0001	0.0007	-1.89
		Dihydrolipoyl dehydrogenase	WP_038631639	XLOC_003405	0.0001	0.0007	1.64
		Dihydrolipoyl dehydrogenase	WP_016703974	XLOC_004827	0.0049	0.0303	-1.59
		FAD-dependent oxidoreductase	WP_038633983	XLOC_004126	0.0001	0.0007	2.33
		Flavin reductase/prnA/prnB/prnC	WP_016704027	XLOC_003884	0.0026	0.0186	-1.92
		Glycerol kinase	WP_009050562	XLOC_001993	0.0001	0.0007	-2.39
		Glycerol-3-phosphate dehydrogenase	WP_016703438	XLOC_001995	0.0001	0.0007	-1.51
		Ferredoxin-NADP reductase	KWV82315	XLOC_000473	0.0001	0.0007	-2.43
		Malate dehydrogenase	WP_009046595	XLOC_000137	0.0001	0.0007	-2.22
		ATP synthase gamma chain	WP_007924507	XLOC_002334	0.0001	0.0007	-1.75
		Cytochrome B561	WP_007932535	XLOC_002512	0.0001	0.0007	-2.26
		Cytochrome C oxidase	WP_007929704	XLOC_004482	0.0001	0.0007	-1.63
		ATP synthase subunit b	WP_007924504	XLOC_002333	0.0001	0.0007	-1.84
		2Fe-2S ferredoxin	WP_007929527	XLOC_004452	0.0001	0.0007	-2.35
		NADH-quinone oxidoreductase subunit B	WP_007929186	XLOC_001593	0.0001	0.0007	1.97
		Succinyl-CoA ligase [ADP-forming] subunit beta	WP_009047768	XLOC_000702	0.0001	0.0007	-1.97
		Oxidoreductase	WP_038630684	XLOC_000407	0.0001	0.0007	-1.74
		CbbBc protein	EGH48104	XLOC_002636	0.0001	0.0007	-2.08
		Citrate synthase	WP_009047765	XLOC_003159	0.0001	0.0007	-1.62
		<b>Cell cycle control, cell division, chromosome partitioning</b>	<b>D</b>	Cell division protein FtsK	WP_036985053	XLOC_003980	0.0004
Cell division protein FtsZ	WP_007922129			XLOC_004490	0.0001	0.0007	-2.21
Cell division topological specificity factor	WP_007928666			XLOC_001797	0.0001	0.0007	-1.68
<b>Amino acid transport and metabolism</b>	<b>E</b>	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	WP_009047206	XLOC_002976	0.0001	0.0007	-1.67
		3-isopropylmalate dehydratase large subunit	WP_009043027	XLOC_000847	0.0001	0.0007	1.59
		3-isopropylmalate dehydratase small subunit	WP_016704914	XLOC_000848	0.0001	0.0007	2.37
		4-aminobutyrate aminotransferase	WP_009046384	XLOC_000045	0.0001	0.0007	2.03
		ABC transporter	WP_025806014	XLOC_001996	0.0001	0.0007	-2.90
		ABC transporter substrate-binding protein	WP_009048194	XLOC_000907	0.0001	0.0007	-1.90
		Acetylornithine deacetylase	WP_009049170	XLOC_003717	0.0012	0.0100	1.94
		Glutamate-pyruvate aminotransferase	WP_038634397	XLOC_001866	0.0001	0.0007	-2.21
		Amino acid ABC transporter permease	WP_009050566	XLOC_001997	0.0001	0.0007	-1.82
		Amino acid ABC transporter substrate-binding protein	WP_038636132	XLOC_004965	0.0021	0.0157	-1.81
		Amino acid ABC transporter substrate-binding protein	WP_009046527	XLOC_000108	0.0013	0.0107	1.81
		Amino acid ABC transporter substrate-binding protein	WP_016704434	XLOC_000130	0.0006	0.0057	-1.81

		Aromatic amino acid transporter	WP_009042698	XLOC_000634	0.0001	0.0007	-3.35
		Aminoglycoside N(6)-acetyltransferase type 1	WP_038636173	XLOC_004987	0.0001	0.0012	2.50
		Arginine deiminase	WP_009050421	XLOC_001892	0.0001	0.0007	-1.63
		Branched-chain alpha-keto acid dehydrogenase subunit E2	WP_038630629	XLOC_000359	0.0074	0.0418	2.57
		Cysteine desulfurase IscS	WP_009050642	XLOC_004457	0.0001	0.0007	-1.88
		Diaminobutyrate--2-oxoglutarate aminotransferase	WP_038636648	XLOC_003702	0.0001	0.0007	4.13
		DNA-binding protein	WP_009048753	XLOC_003537	0.0013	0.0107	2.24
		Gamma-glutamyl-gamma-aminobutyrate hydrolase	WP_038631481	XLOC_003346	0.0078	0.0433	3.55
		Gamma-glutamylputrescine synthetase	WP_009051380	XLOC_004926	0.0001	0.0007	1.86
		Gamma-glutamylputrescine synthetase	WP_009048287	XLOC_000943	0.0031	0.0210	1.55
		Aminomethyltransferase	WP_009051399	XLOC_002443	0.0001	0.0007	-2.14
		Glycine dehydrogenase (decarboxylating)	WP_038634450	XLOC_001898	0.0001	0.0007	-1.76
		GMP synthase [glutamine-hydrolyzing]	WP_016703480	XLOC_004438	0.0001	0.0007	-1.82
		Transcriptional regulator	WP_038632162	XLOC_003545	0.0001	0.0007	-3.28
		Leucyl aminopeptidase (Aminopeptidase T)	WP_038630987	XLOC_003139	0.0001	0.0007	-3.25
		LysR family transcriptional regulator	WP_009050031	XLOC_004132	0.0087	0.0470	3.11
		Methylenetetrahydrofolate reductase	WP_009051282	XLOC_004967	0.0016	0.0123	-1.78
		Ketol-acid reductoisomerase	WP_007959661	XLOC_004578	0.0001	0.0007	-1.53
		Glutamine synthetase	WP_007929953	XLOC_002679	0.0001	0.0007	-2.19
		Phospho-2-dehydro-3-deoxyheptonate aldolase	WP_029526777	XLOC_002097	0.0001	0.0007	-6.75
		Phospho-2-dehydro-3-deoxyheptonate aldolase	WP_038633423	XLOC_001520	0.0008	0.0072	-4.31
		Pilus assembly protein PilV	WP_025167063	XLOC_004362	0.0055	0.0333	3.38
		Sarcosine oxidase subunit beta	WP_009051223	XLOC_002538	0.0050	0.0307	-1.62
		Serine/threonine protein kinase	WP_009048515	XLOC_001051	0.0001	0.0007	-3.76
		L-threonine aldolase	WP_009051226	XLOC_005000	0.0002	0.0017	-1.71
		Urocanate hydratase	WP_016704424	XLOC_000119	0.0001	0.0007	-2.30
		(Fe-S)-binding protein	WP_016704911	XLOC_003263	0.0009	0.0075	3.47
		Allantoate amidohydrolase	WP_038631669	XLOC_001004	0.0017	0.0130	3.09
		Allantoate amidohydrolase	WP_038634005	XLOC_004131	0.0001	0.0007	2.62
<b>Nucleotide transport and metabolism</b>	<b>F</b>	3-carboxy-cis,cis-muconate cycloisomerase	WP_038630787	XLOC_000500	0.0016	0.0126	1.54
		Amidotransferase	WP_016704995	XLOC_000767	0.0001	0.0007	1.56
		Uncharacterized protein	WP_009048212	XLOC_003313	0.0007	0.0060	-2.20
		Inosine-5'-monophosphate dehydrogenase	WP_009050626	XLOC_004439	0.0001	0.0007	-2.25
		Adenylosuccinate lyase	WP_007929192	XLOC_001588	0.0001	0.0007	-2.26
		Ribose-phosphate pyrophosphokinase	WP_003171603	XLOC_002078	0.0001	0.0007	-2.13
		Nitrate reductase	WP_038631666	XLOC_001003	0.0060	0.0353	1.53
		Phosphoribosylformylglycinamide cyclo-ligase	WP_038634283	XLOC_001821	0.0001	0.0007	-1.82
		Phosphoribosylglycinamide formyltransferase	WP_009050271	XLOC_001822	0.0002	0.0017	-1.65
		Glutamine amidotransferase/protease	WP_009047437	XLOC_000516	0.0001	0.0007	-3.24
<b>Carbohydrate transport and metabolism</b>	<b>G</b>	2-methylcitrate dehydratase	WP_038631169	XLOC_000781	0.0001	0.0007	-1.58
		2-methylcitrate dehydratase	WP_009048962	XLOC_003636	0.0033	0.0223	1.65
		Glycerol uptake facilitator GlpF	WP_009050561	XLOC_001992	0.0028	0.0194	-2.32
		Chitinase	WP_009048115	XLOC_000870	0.0001	0.0007	-8.85
		Chitinase	WP_009049243	XLOC_003753	0.0001	0.0007	-6.08
		Glucokinase	WP_009050408	XLOC_004344	0.0001	0.0007	-2.63
		Glucose-6-phosphate 1-dehydrogenase	WP_023966786	XLOC_004336	0.0001	0.0007	-4.04
		Glutamine--fructose-6-phosphate aminotransferase [isomerizing]	WP_038635708	XLOC_002339	0.0005	0.0045	-2.27
		MFS transporter	WP_038632598	XLOC_003701	0.0001	0.0007	5.04

		MFS transporter	WP_009050506	XLOC_001969	0.0081	0.0448	-4.71
		Multidrug resistance protein B	WP_009049803	XLOC_003990	0.0002	0.0017	-3.03
		sn-glycerol-3-phosphate transporter	WP_007926732	XLOC_004911	0.0001	0.0007	-3.36
		Permease DsdX	WP_009050373	XLOC_004325	0.0008	0.0072	-3.35
		Phosphoglucomutase	WP_016703154	XLOC_004874	0.0001	0.0007	-1.79
		Pyruvate kinase	WP_009050499	XLOC_001966	0.0001	0.0007	-1.75
		Ribulose-phosphate 3-epimerase	WP_009051147	XLOC_002571	0.0001	0.0007	-1.59
		Siderophore biosynthesis protein SbnG	WP_009049138	XLOC_003700	0.0001	0.0007	2.78
		Sorbose dehydrogenase	WP_038634274	XLOC_001820	0.0001	0.0007	-2.08
		Sucrase	WP_016703507	XLOC_002037	0.0004	0.0040	-2.45
		Sugar ABC transporter ATPase	WP_009050627	XLOC_004440	0.0001	0.0007	2.84
		Sugar ABC transporter ATPase	WP_016703424	XLOC_004339	0.0001	0.0007	-3.74
		Sugar ABC transporter permease	WP_009045231	XLOC_004340	0.0001	0.0012	-3.91
		Sugar ABC transporter substrate-binding protein	WP_009050405	XLOC_004341	0.0001	0.0007	-5.80
		Sugar isomerase	WP_016703422	XLOC_004342	0.0001	0.0007	-3.76
		Glyceraldehyde-3-phosphate dehydrogenase	WP_009050409	XLOC_001882	0.0001	0.0007	-5.06
<b>Coenzyme transport and metabolism</b>	<b>H</b>	(2Fe-2S)-binding protein	WP_016701774	XLOC_001019	0.0001	0.0007	-3.14
		(2Fe-2S)-binding protein	WP_016701774	XLOC_003431	0.0001	0.0007	-4.78
		Adenosylhomocysteinase	WP_009045993	XLOC_004968	0.0001	0.0007	-2.53
		Alpha/beta hydrolase	WP_009047789	XLOC_000715	0.0006	0.0053	1.71
		Tryptophan synthase subunit alpha	WP_009048364	XLOC_003383	0.0001	0.0007	-5.09
		Cyclic pyranopterin monophosphate synthase accessory protein	WP_009047097	XLOC_000372	0.0001	0.0007	2.64
		MFS transporter	WP_009047472	XLOC_003055	0.0001	0.0007	-4.36
		Molybdenum import ATP-binding protein ModC	WP_038632869	XLOC_001420	0.0001	0.0007	2.57
		Octaprenyl-diphosphate synthase	WP_009045711	XLOC_002163	0.0012	0.0096	-2.20
		Omega amino acid--pyruvate aminotransferase	WP_038630538	XLOC_000233	0.0001	0.0007	-2.20
		Putative FtsK/SpoIIIE family	WP_009047019	XLOC_000344	0.0006	0.0053	-2.39
<b>Lipid transport and metabolism</b>	<b>I</b>	Glycerol acyltransferase	WP_009050240	XLOC_004248	0.0001	0.0007	-3.82
		3-oxoacyl-ACP synthase	WP_009050008	XLOC_004115	0.0006	0.0057	-2.91
		Acetyl-CoA acetyltransferase	WP_039968019	XLOC_001218	0.0001	0.0007	1.75
		Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	WP_038630718	XLOC_000439	0.0001	0.0007	-2.63
		VldD	WP_009049637	XLOC_001532	0.0001	0.0012	1.97
		AMP-binding protein	WP_038632314	XLOC_001216	0.0018	0.0136	4.52
		3-oxoacyl-[acyl-carrier-protein] synthase 2	WP_041988549	XLOC_003283	0.0024	0.0171	-2.44
		Choline dehydrogenase	WP_038631604	XLOC_003392	0.0042	0.0267	3.32
		Oxygen-dependent choline dehydrogenase	WP_016704223	XLOC_004982	0.0002	0.0022	-1.75
		Poly(R)-hydroxyalkanoic acid synthase	WP_009046588	XLOC_002692	0.0001	0.0007	-2.44
		Poly(R)-hydroxyalkanoic acid synthase	WP_009046586	XLOC_002690	0.0001	0.0007	-1.76
		Fatty acid methyltransferase	WP_016703983	XLOC_002348	0.0001	0.0007	-2.16
		Enoyl-CoA hydratase	WP_016704994	XLOC_003195	0.0002	0.0022	-1.99
		Gamma-carboxygeranyl-CoA hydratase	WP_016701921	XLOC_001611	0.0002	0.0017	2.17
		Glutathione peroxidase	WP_009047015	XLOC_000342	0.0010	0.0086	1.50
		Glycerol acyltransferase	WP_009046219	XLOC_002323	0.0001	0.0007	-1.60
		Long-chain fatty acid--CoA ligase	WP_009050391	XLOC_004332	0.0001	0.0007	-2.50
		Malonyl CoA-acyl carrier protein transacylase	WP_009047834	XLOC_000740	0.0003	0.0032	-2.31
		Membrane protein	WP_009051633	XLOC_004842	0.0001	0.0007	-1.58
		Methylcrotonoyl-CoA carboxylase	WP_038633668	XLOC_001610	0.0006	0.0053	2.81
		Acetyl-CoA carboxylase	WP_007921646	XLOC_002771	0.0001	0.0007	-1.79
		Peptide synthetase	WP_038633921	XLOC_004110	0.0001	0.0007	1.64
		Peptide synthase	WP_038634075	XLOC_004152	0.0001	0.0007	2.37



		Phosphatidylserine decarboxylase proenzyme	WP_038631548	XLOC_000962	0.0001	0.0007	-2.78
		Enoyl-[acyl-carrier-protein] reductase [NADH]	WP_038631995	XLOC_001112	0.0001	0.0007	-2.59
<b>Translation, ribosomal structure and biogenesis</b>	<b>J</b>	Acetyltransferase	WP_009046341	XLOC_000018	0.0002	0.0022	-1.88
		Elongation factor Ts	WP_009042383	XLOC_000428	0.0001	0.0007	-2.06
		GNAT family acetyltransferase	WP_038634977	XLOC_002043	0.0001	0.0007	2.52
		GTP-binding protein	WP_028683953	XLOC_003650	0.0001	0.0007	-1.95
		Ribosome-binding ATPase YchF	WP_009050794	XLOC_002081	0.0001	0.0007	-1.99
		Alanyl-tRNA synthetase	WP_009047809	XLOC_003174	0.0009	0.0075	1.64
		Glutaminase	WP_009048121	XLOC_003273	0.0001	0.0007	-3.48
		50S ribosomal protein L32	KNH46388	XLOC_003179	0.0001	0.0007	-2.12
		Conjugal transfer protein TraR	WP_007923643	XLOC_001033	0.0032	0.0216	-2.59
		tRNA (guanine-N(1)-)-methyltransferase	WP_007927679	XLOC_000396	0.0002	0.0017	-1.82
		Polyribonucleotide nucleotidyltransferase	WP_038630587	XLOC_000295	0.0001	0.0007	-1.75
		Prolyl-tRNA synthetase	WP_016705004	XLOC_000023	0.0029	0.0202	1.55
		tRNA sulfurtransferase	WP_016702616	XLOC_000114	0.0001	0.0007	-1.67
		Tyrosine--tRNA ligase	WP_038636398	XLOC_002578	0.0001	0.0007	-1.67
<b>Transcription</b>	<b>K</b>	GntR family transcriptional regulator	WP_009050459	XLOC_001909	0.0001	0.0007	-3.06
		LuxR family transcriptional regulator	WP_009048517	XLOC_003448	0.0002	0.0022	-2.37
		LysR family transcriptional regulator	WP_016702918	XLOC_001222	0.0001	0.0007	2.63
		LysR family transcriptional regulator	WP_009051067	XLOC_004709	0.0028	0.0197	-1.74
		Temperature acclimation protein B	WP_007931076	XLOC_001901	0.0001	0.0007	-1.74
		LexA repressor	WP_007921301	XLOC_003239	0.0032	0.0216	-1.59
		RNA polymerase sigma factor RpoS	WP_007924026	XLOC_000448	0.0001	0.0007	-2.57
		TetR family transcriptional regulator	WP_007922198	XLOC_001002	0.0004	0.0040	1.51
		Uncharacterized protein	WP_009048686	XLOC_003507	0.0001	0.0007	-2.44
		Rha family transcriptional regulator	WP_038633014	XLOC_003829	0.0004	0.0036	2.18
		TetR family transcriptional regulator	WP_009046585	XLOC_002689	0.0004	0.0036	-1.66
		PhzR	WP_038635113	XLOC_004545	0.0001	0.0007	-3.03
		DNA-binding protein	WP_009050695	XLOC_002040	0.0001	0.0007	-2.04
<b>Replication, recombination and repair</b>	<b>L</b>	ATP-dependent DNA helicase	WP_028684106	XLOC_003734	0.0004	0.0040	-1.63
		Competence protein ComEA	WP_009050160	XLOC_004201	0.0032	0.0216	-3.65
		DNA polymerase III subunit chi	WP_038630662	XLOC_002941	0.0003	0.0032	1.91
		DNA-binding protein HU, nu subunit	WP_007932138	XLOC_000383	0.0001	0.0007	-1.64
		DNA gyrase subunit B	WP_007924471	XLOC_004819	0.0001	0.0007	-1.72
		Uncharacterized protein	WP_007921116	XLOC_002393	0.0001	0.0007	-1.95
		ATP-dependent RNA helicase	WP_007931434	XLOC_004169	0.0001	0.0007	-1.99
		DeaD	WP_038630414	XLOC_000136	0.0061	0.0360	-1.71
<b>Cell wall/membrane/envelope biogenesis</b>	<b>M</b>	Channel protein TolC	WP_038636749	XLOC_003928	0.0007	0.0060	1.58
		Membrane protein	WP_028681745	XLOC_002967	0.0001	0.0007	-1.62
		Membrane protein	WP_038576794	XLOC_004272	0.0001	0.0007	-1.82
		Membrane protein	WP_009050551	XLOC_004412	0.0007	0.0064	2.15
		Membrane protein insertase YidC	WP_009051704	XLOC_002327	0.0001	0.0007	-2.36
		Acriflavin resistance protein	WP_009049438	XLOC_003840	0.0001	0.0007	-2.32
		AcrA	WP_038633661	XLOC_003992	0.0001	0.0007	-2.45
		Multidrug RND transporter	WP_007927525	XLOC_000789	0.0001	0.0007	-2.75
		Porin	WP_038635768	XLOC_002350	0.0018	0.0136	5.65
		N-acetylmuramoyl-L-alanine amidase	WP_009048213	XLOC_003314	0.0009	0.0079	-2.26
		Porin	WP_038634847	XLOC_002012	0.0043	0.0272	-2.51
		UDP-glucuronate 5-epimerase	WP_009048937	XLOC_003621	0.0027	0.0191	1.70
<b>Cell motility</b>	<b>N</b>	Chemotaxis protein	WP_038631780	XLOC_001034	0.0001	0.0007	2.11
		Chemotaxis protein	WP_038634436	XLOC_001889	0.0026	0.0186	1.53
		Chemotaxis protein	WP_009047950	XLOC_003231	0.0001	0.0007	1.53

		Chemotaxis protein	WP_009046314	XLOC_000007	0.0001	0.0007	1.92		
		Chemotaxis protein CheW	WP_009050086	XLOC_004157	0.0001	0.0007	1.74		
		Basal-body rod modification protein FlgD	WP_009050300	XLOC_004275	0.0001	0.0007	-1.62		
		Flagellar basal-body rod protein FlgG	WP_009047672	XLOC_000637	0.0001	0.0007	-1.91		
		Flagellar basal body rod protein FlgB	WP_016702521	XLOC_004277	0.0001	0.0007	-1.63		
		Flagellar hook protein FlgE	WP_009050299	XLOC_004274	0.0001	0.0007	-2.53		
<b>Posttranslational modification, protein turnover, chaperones</b>	<b>O</b>	Probable thiol peroxidase	WP_038632154	XLOC_001174	0.0001	0.0007	-1.53		
		Alkyl hydroperoxide reductase	WP_038634787	XLOC_004413	0.0001	0.0007	-1.74		
		CAAX protease	WP_009047951	XLOC_003232	0.0018	0.0136	2.17		
		Co-chaperone protein HscB homolog	WP_038576126	XLOC_004454	0.0001	0.0007	-1.59		
		Glutathione S-transferase	WP_038631753	XLOC_003432	0.0001	0.0007	-2.53		
		Chaperone protein DnaK	WP_009046925	XLOC_000278	0.0001	0.0007	-1.75		
		Chaperonin 60 (Fragment)	WP_009050539	XLOC_004405	0.0001	0.0007	-2.54		
		Chaperone protein HscA homolog	WP_038634899	XLOC_004453	0.0001	0.0007	-1.63		
		10 kDa chaperonin	WP_009050540	XLOC_004406	0.0001	0.0007	-1.53		
		Peptidylprolyl isomerase	WP_007932676	XLOC_001529	0.0052	0.0317	3.25		
		Alkyl hydroperoxide reductase	WP_007921754	XLOC_003780	0.0001	0.0007	-2.23		
		Serine protease	WP_038633759	XLOC_004049	0.0001	0.0007	-1.99		
		Thiol:disulfide interchange protein	WP_038633534	XLOC_003950	0.0071	0.0402	-1.64		
		Trigger factor	WP_009044653	XLOC_004022	0.0001	0.0007	-2.70		
		<b>Inorganic ion transport and metabolism</b>	<b>P</b>	(2Fe-2S)-binding protein	AIC22009	XLOC_004414	0.0004	0.0036	-1.62
				ABC transporter substrate-binding protein	WP_038632351	XLOC_001230	0.0081	0.0446	-2.74
ABC transporter substrate-binding protein	WP_009050227			XLOC_001802	0.0001	0.0007	-2.18		
Alkaline phosphatase	WP_038630596			XLOC_000304	0.0081	0.0446	-1.58		
Nuclease PIN	WP_009047396			XLOC_003029	0.0001	0.0007	-2.19		
Arsenate reductase	WP_009050284			XLOC_001827	0.0018	0.0136	-1.87		
Calcium transporter ChaC	WP_009051415			XLOC_002434	0.0001	0.0007	-2.61		
Carbonic anhydrase	WP_009046260			XLOC_004795	0.0001	0.0007	-1.57		
Carbonic anhydrase	WP_009050866			XLOC_004568	0.0001	0.0007	-2.76		
Catalase	WP_016702645			XLOC_004732	0.0001	0.0007	-2.29		
Catalase	WP_009050970			XLOC_004621	0.0011	0.0089	-3.18		
Copper resistance protein CopC	WP_038632194			XLOC_003561	0.0001	0.0007	3.32		
Cystathionine gamma-synthase	WP_038635962			XLOC_002426	0.0001	0.0007	2.04		
Cytochrome biogenesis protein	WP_009042956			XLOC_003224	0.0067	0.0384	3.35		
Membrane protein	WP_038633477			XLOC_003932	0.0065	0.0378	-1.79		
Iron ABC transporter permease	WP_038632590			XLOC_003699	0.0001	0.0007	3.31		
Iron ABC transporter substrate-binding protein	WP_016703211			XLOC_002441	0.0001	0.0012	-1.59		
Ligand-gated channel protein	WP_028681659			XLOC_002855	0.0004	0.0036	-3.02		
Methionine ABC transporter substrate-binding protein	WP_009046270			XLOC_002302	0.0001	0.0007	-1.51		
FAD-binding protein	WP_038634838			XLOC_002009	0.0001	0.0007	-2.84		
Peptide ABC transporter substrate-binding protein	WP_038630318			XLOC_002581	0.0001	0.0007	2.01		
Sulfite reductase	WP_009048796			XLOC_001169	0.0001	0.0007	-3.47		
TonB-dependent receptor	WP_038636418			XLOC_002754	0.0031	0.0210	4.75		
TonB-dependent receptor	WP_038632604			XLOC_003703	0.0001	0.0007	3.95		
TonB-dependent receptor	WP_038634844			XLOC_002011	0.0003	0.0027	6.12		
Fe <sup>2+</sup> zn <sup>2+</sup> uptake regulation protein	WP_007928590			XLOC_003565	0.0001	0.0007	1.83		
Zinc ABC transporter permease	WP_009051646			XLOC_004836	0.0001	0.0007	6.29		
<b>Secondary metabolites biosynthesis, transport and catabolism</b>	<b>Q</b>			Acyl-homoserine lactone acylase subunit beta	WP_038632200	XLOC_001187	0.0001	0.0007	1.89
				Isochorismatase	WP_009050821	XLOC_002098	0.0001	0.0007	-6.92
				Peptide synthase	WP_038633919	XLOC_004109	0.0001	0.0007	1.56
				Phenazine biosynthesis protein	WP_009050818	XLOC_002095	0.0001	0.0007	-6.81
				Phenazine biosynthesis protein	WP_009050819	XLOC_002096	0.0003	0.0027	-6.20
		Phenazine biosynthesis protein	WP_038635119	XLOC_002100	0.0001	0.0007	-7.51		
		Poly(3-hydroxyalkanoate) granule-associated protein PhaF	WP_009046584	XLOC_000134	0.0001	0.0007	-2.81		
		Pyoverdine biosynthesis protein	WP_009050075	XLOC_004153	0.0001	0.0007	2.09		



<b>General function prediction only</b>	<b>R</b>	Serine 3-dehydrogenase	WP_016702821	XLOC_000985	0.0001	0.0007	-4.48
		2,3-dihydro-3-hydroxyanthranilate isomerase	WP_016703606	XLOC_002099	0.0001	0.0007	-6.18
		Amidohydrolase	WP_038633980	XLOC_001697	0.0001	0.0007	-4.45
		Arylsulfate sulfotransferase	WP_009042529	XLOC_000517	0.0031	0.0210	-3.38
		Carbon-nitrogen hydrolase	WP_038630611	XLOC_000324	0.0031	0.0210	1.51
		Chitin-binding protein	WP_009048114	XLOC_000869	0.0001	0.0007	-8.13
		Cytochrome D ubiquinol oxidase subunit II	WP_016702021	XLOC_002984	0.0001	0.0007	-2.60
		Diaminopimelate decarboxylase	WP_009048261	XLOC_003333	0.0007	0.0060	-3.61
		Glyoxalase	WP_009046877	XLOC_000259	0.0001	0.0007	3.71
		Hydrolase	WP_038632167	XLOC_003548	0.0043	0.0272	1.51
		Ring-cleavage extradiol dioxygenase	WP_016702948	XLOC_001237	0.0001	0.0007	-3.25
		Sugar deacetylase	WP_016702950	XLOC_001239	0.0001	0.0007	-1.55
		Methyltransferase	WP_038632359	XLOC_003651	0.0013	0.0107	-4.50
		Methyltransferase	WP_038633459	XLOC_001531	0.0001	0.0007	2.05
		Molybdenum cofactor sulfuryase	WP_038634095	XLOC_004156	0.0019	0.0142	1.81
		Nuclease	WP_028683814	XLOC_002657	0.0001	0.0007	-2.27
		Peptidase C39	WP_038633471	XLOC_003930	0.0001	0.0007	2.87
		Phosphoesterase	WP_009047660	XLOC_000629	0.0001	0.0012	1.56
		PKHD-type hydroxylase	WP_016701680	XLOC_000305	0.0001	0.0007	-1.87
		EY04_03685					
		PsiE family protein	WP_009042237	XLOC_000360	0.0003	0.0032	-2.96
Transcriptional regulator	WP_038635883	XLOC_004878	0.0042	0.0270	-1.50		
Thiamine biosynthesis protein ThiF	WP_016705302	XLOC_000425	0.0035	0.0231	-2.05		
Rhs family protein	WP_038630348	XLOC_002611	0.0001	0.0007	-6.48		
<b>Function unknown</b>	<b>S</b>	Membrane protein	WP_038630514	XLOC_000219	0.0039	0.0255	1.53
		Membrane protein	WP_038630794	XLOC_000514	0.0016	0.0126	1.58
		Membrane protein	WP_038635748	XLOC_002347	0.0001	0.0007	2.70
		Membrane protein	WP_009048116	XLOC_000871	0.0002	0.0017	-1.84
		Cytochrome D ubiquinol oxidase subunit III	WP_007928428	XLOC_002055	0.0001	0.0007	-1.58
		Uncharacterized protein	WP_009047832	XLOC_000738	0.0001	0.0007	-2.20
		Uncharacterized protein	WP_007925069	XLOC_000998	0.0023	0.0166	-1.58
		UPF0114 protein EY04_27105	WP_007924753	XLOC_004610	0.0001	0.0012	-1.80
		UPF0260 protein EY04_06945	KPW12423	XLOC_000589	0.0002	0.0017	-1.93
		Uncharacterized protein	WP_038632150	XLOC_001170	0.0001	0.0007	-2.91
		Sensor histidine kinase	KPX62819	XLOC_002871	0.0001	0.0007	-2.33
		Sulfuryase	WP_038636332	XLOC_002557	0.0001	0.0012	2.86
		Tail protein	WP_009046343	XLOC_002592	0.0011	0.0089	-2.52
		Type IV secretion protein Rhs	WP_038634583	XLOC_004369	0.0001	0.0007	-1.51
		2-oxoglutarate dehydrogenase subunit E1 [Pseudomonas fluorescens]	WP_047284630	XLOC_003160	0.0006	0.0057	-2.11
		Hydrolase/acyltransferase	EJZ57002	XLOC_000535	0.0001	0.0007	-3.60
		Pyrrroloquinoline quinone biosynthesis protein PqqE	WP_009051193	XLOC_002552	0.0001	0.0007	1.89
		Uncharacterized protein	WP_009048294	XLOC_003351	0.0032	0.0218	-2.41
		Glutamine synthetase	EIM18812	XLOC_004837	0.0047	0.0293	4.34
		Uncharacterized protein	WP_038636482	XLOC_003161	0.0001	0.0007	-1.59
		Lipoprotein	WP_038632271	XLOC_003611	0.0001	0.0007	-3.05
		Uncharacterized protein	WP_025807035	XLOC_002408	0.0001	0.0007	-2.17
		Uncharacterized protein	WP_038632989	XLOC_003825	0.0041	0.0263	2.41
		Membrane protein	WP_038633841	XLOC_004083	0.0001	0.0007	-1.52
		Uncharacterized protein	WP_011060148	XLOC_003191	0.0060	0.0355	1.96
		CrfX protein	WP_007927518	XLOC_000786	0.0001	0.0007	-1.87
		Uncharacterized protein	WP_047276289	XLOC_000722	0.0001	0.0007	-1.67
		Uncharacterized protein	WP_038630933	XLOC_000599	0.0001	0.0007	2.55
		PasA protein	WP_009047965	XLOC_000818	0.0001	0.0007	2.07
		Uncharacterized protein	WP_038633414	XLOC_001519	0.0001	0.0007	6.23
		Phosphatidylcholine-hydrolyzing phospholipase	WP_009046978	XLOC_002873	0.0001	0.0007	-2.85
		Poly(3-hydroxyalkanoate) granule-associated protein PhaI	WP_009046583	XLOC_000133	0.0001	0.0007	-4.87
		Porin	WP_038634688	XLOC_001955	0.0001	0.0007	-3.60
		Porin	WP_025807108	XLOC_002451	0.0001	0.0007	-1.58
		Potassium channel protein	WP_038632026	XLOC_003505	0.0003	0.0032	-2.08

		R body protein RebB-like protein	WP_009046381	XLOC_000042	0.0001	0.0007	-3.39
		R body protein RebB-like protein	WP_009046382	XLOC_000043	0.0001	0.0007	-3.98
		Bifunctional pyrazinamidase/nicotinamidase	ADO14993	XLOC_001373	0.0003	0.0032	-1.70
		Uncharacterized protein	EIM12867	XLOC_000056	0.0001	0.0007	-3.00
		SAM-dependent methyltransferase	EXF47260	XLOC_001129	0.0057	0.0342	1.91
		secondary thiamine-phosphate synthase enzyme [Pseudomonas chlororaphis O6]	EIM17029	XLOC_004893	0.0028	0.0197	-1.61
		Tail sheath protein	WP_038630744	XLOC_000457	0.0001	0.0007	-5.36
		Tripartite tricarboxylate transporter TctB	WP_038631417	XLOC_000921	0.0001	0.0007	2.26
		Uncharacterized protein	WP_051673205	XLOC_004653	0.0001	0.0007	-1.64
		Uncharacterized protein	WP_038632169	XLOC_003551	0.0001	0.0007	-3.44
		Uncharacterized protein	WP_038630505	XLOC_002769	0.0011	0.0089	1.76
<b>Signal transduction mechanisms</b>	<b>T</b>	Acyl-homoserine-lactone synthase	WP_038635110	XLOC_002094	0.0001	0.0007	-5.18
		Diguanylate phosphodiesterase	WP_028684076	XLOC_003708	0.0001	0.0007	2.93
		Chemotaxis protein	WP_038630410	XLOC_002683	0.0010	0.0086	1.70
		Chemotaxis protein	WP_016703630	XLOC_004556	0.0011	0.0093	1.65
		Crp/Fnr family transcriptional regulator	WP_038630893	XLOC_003074	0.0001	0.0007	-1.61
		Histidine kinase	WP_009048180	XLOC_000901	0.0020	0.0151	1.52
		Diguanylate cyclase	WP_038582632	XLOC_002713	0.0001	0.0007	-2.08
		Divalent cation transporter	WP_041984621	XLOC_001842	0.0057	0.0342	-1.60
		Histidine kinase	WP_016703421	XLOC_004343	0.0001	0.0007	-1.67
		Chemotaxis protein CheY	WP_016703129	XLOC_002372	0.0001	0.0007	1.55
<b>Intracellular trafficking, secretion, and vesicular transport</b>	<b>U</b>	Elongation factor G	WP_009051119	XLOC_004750	0.0001	0.0007	-3.34
		Elongation factor Tu	WP_016702644	XLOC_004749	0.0001	0.0007	-4.22
		Elongation factor Tu	WP_016702644	XLOC_004761	0.0001	0.0007	-2.19
		Filamentous hemagglutinin	WP_038634114	XLOC_001738	0.0001	0.0007	1.93
		GTP-binding protein TypA	WP_009046549	XLOC_000115	0.0001	0.0007	-3.05
		Type VI secretion protein TssB2	WP_007930286	XLOC_002379	0.0001	0.0007	-3.30
		Type VI secretion protein	WP_007930278	XLOC_002383	0.0001	0.0007	-3.59
		Preprotein translocase, SecG subunit	EIM15962	XLOC_002848	0.0001	0.0007	-2.65
		ShlB family hemolysin secretion/activation protein	WP_041987697	XLOC_001737	0.0037	0.0244	2.39
		Type VI secretion protein	WP_009051592	XLOC_002380	0.0001	0.0007	-4.12
		EvpB family type VI secretion protein	WP_038633339	XLOC_001503	0.0001	0.0007	-2.73
<b>Defense mechanisms</b>	<b>V</b>	Beta-lactamase	WP_038633878	XLOC_001672	0.0049	0.0303	-1.92
		DSBA oxidoreductase	WP_009046334	XLOC_000016	0.0001	0.0012	-2.23
		Hemolysin D	WP_028682887	XLOC_003931	0.0001	0.0007	2.68
		Transporter	WP_009049437	XLOC_003839	0.0001	0.0007	-2.22
		Hemolysin D	WP_009049804	XLOC_003991	0.0001	0.0007	-3.17
		Peptide ABC transporter permease	WP_038635565	XLOC_002266	0.0007	0.0064	2.63
		Uncharacterized protein	WP_047739265	XLOC_000272	0.0001	0.0007	-2.02
<b>Mobilome: prophages, transposons</b>	<b>X</b>	2-hydroxyacid dehydrogenase	WP_038630746	XLOC_000459	0.0001	0.0007	-2.66
		Phage assembly protein	WP_009047314	XLOC_000461	0.0009	0.0079	-2.95
		Tail protein	WP_038630732	XLOC_000451	0.0001	0.0007	-3.89
		Tail protein	WP_016701993	XLOC_000453	0.0001	0.0007	-3.17

Appendix Table 3: Differentially expressed genes in PA23-*ptrA* compared to WT.

COG category		Predicted function	Protein accession number (NCBI)	Gene locus	P-value	Q-value	log2 fold change		
<b>Energy production and conversion</b>	<b>C</b>	Succinate-semialdehyde dehydrogenase	WP_009046383	XLOC_000044	0.0001	0.0007	1.88		
		Azurin	WP_009046707	XLOC_000192	0.0001	0.0007	-2.68		
		ABC transporter permease	WP_038630628	XLOC_000358	0.0001	0.0007	2.40		
		3-isopropylmalate dehydrogenase	WP_009048099	XLOC_000849	0.0001	0.0007	2.07		
		C4-dicarboxylate ABC transporter substrate-binding protein	WP_038631415	XLOC_000920	0.0068	0.0389	2.60		
		Bifunctional glyoxylate/hydroxypyruvate reductase B	WP_028682556	XLOC_001380	0.0001	0.0007	2.20		
		Cytochrome C	WP_016703084	XLOC_001515	0.0084	0.0458	1.89		
		Glycerate dehydrogenase	WP_038635038	XLOC_002059	0.0071	0.0404	1.52		
		ATP synthase gamma chain	WP_007924507	XLOC_002334	0.0001	0.0007	-1.72		
		Cytochrome c5	EJL96380	XLOC_002403	0.0018	0.0136	-1.65		
		Cytochrome B561	WP_007932535	XLOC_002512	0.0001	0.0007	-2.23		
		Tricarboxylic transport membrane protein	WP_028681770	XLOC_003059	0.0001	0.0007	4.49		
		Dihydrolipoyl dehydrogenase	WP_038631639	XLOC_003405	0.0001	0.0007	2.29		
		Branched-chain alpha-keto acid dehydrogenase subunit E2	WP_038631641	XLOC_003406	0.0001	0.0007	3.09		
		2-oxoisovalerate dehydrogenase	WP_028682773	XLOC_003407	0.0001	0.0007	2.23		
		2-oxoisovalerate dehydrogenase	WP_038631643	XLOC_003408	0.0001	0.0007	2.09		
		Membrane protein	WP_038632572	XLOC_003693	0.0001	0.0007	2.76		
		Flavin reductase	WP_016704027	XLOC_003884	0.0001	0.0007	-4.18		
		FAD-dependent oxidoreductase	WP_038633983	XLOC_004126	0.0001	0.0007	2.38		
		2Fe-2S ferredoxin	WP_007929527	XLOC_004452	0.0001	0.0007	-1.90		
		Cytochrome C oxidase	WP_007929704	XLOC_004482	0.0001	0.0007	-2.19		
		Cytochrome o ubiquinol oxidase subunit I	WP_009045490	XLOC_004483	0.0001	0.0007	-2.06		
		Ubiquinol oxidase subunit II	WP_009050698	XLOC_004484	0.0001	0.0007	-2.17		
		Ferredoxin-NADP reductase	WP_038635265	XLOC_004622	0.0001	0.0007	2.18		
		Alcohol dehydrogenase	WP_009046258	XLOC_004797	0.0093	0.0496	-2.29		
		<b>Cell cycle control, cell division, chromosome partitioning</b>	<b>D</b>	Cell division inhibitor SuiA	WP_023968541	XLOC_003238	0.0003	0.0027	-1.54
				Nucleotidyltransferase	WP_038632725	XLOC_003750	0.0058	0.0346	-2.07
				Cell division protein FtsZ	WP_007922129	XLOC_004490	0.0001	0.0007	2.09
				Cell division protein FtsA	WP_007922131	XLOC_004491	0.0001	0.0007	2.21
				Rod shape-determining protein RodA	EIM17625	XLOC_004696	0.0080	0.0441	-1.88
<b>Amino acid transport and metabolism</b>	<b>E</b>	4-aminobutyrate aminotransferase	WP_009046384	XLOC_000045	0.0001	0.0007	2.13		
		Gamma-aminobutyrate transporter	WP_009046515	XLOC_000101	0.0001	0.0007	2.84		
		MFS transporter	WP_028683803	XLOC_000103	0.0001	0.0007	2.88		
		Amino acid ABC transporter substrate-binding protein	WP_009046527	XLOC_000108	0.0011	0.0089	1.90		
		ABC transporter permease	WP_009046564	XLOC_000122	0.0004	0.0040	1.56		
		Histidine ammonia-lyase	WP_009046567	XLOC_000124	0.0008	0.0068	1.74		
		3-dehydroquinate synthase	WP_038630416	XLOC_000141	0.0001	0.0007	-1.58		
		Amino acid ABC transporter substrate-binding protein	WP_009046713	XLOC_000195	0.0001	0.0007	2.12		
		Branched-chain amino acid ABC transporter permease	WP_038630474	XLOC_000197	0.0001	0.0007	2.20		
		Gamma-glutamyltranspeptidase	WP_038630553	XLOC_000255	0.0001	0.0007	1.91		

Branched-chain alpha-keto acid dehydrogenase subunit E2	WP_038630629	XLOC_000359	0.0022	0.0163	3.23
Amino acid ABC transporter substrate-binding protein	WP_025806519	XLOC_000375	0.0001	0.0007	3.63
Amino acid ABC transporter permease	WP_038630656	XLOC_000376	0.0031	0.0213	2.05
Aromatic amino acid transporter	WP_009042698	XLOC_000634	0.0001	0.0007	-3.70
ATPase AAA	WP_038581185	XLOC_000635	0.0001	0.0012	1.85
Inducer of phenazine B	WP_038631049	XLOC_000690	0.0001	0.0007	-3.38
3-isopropylmalate dehydratase large subunit	WP_009043027	XLOC_000847	0.0001	0.0007	3.01
3-isopropylmalate dehydratase small subunit	WP_016704914	XLOC_000848	0.0001	0.0007	2.34
Spermidine acetyltransferase	WP_051673124	XLOC_000889	0.0003	0.0027	-2.20
Gamma-glutamylputrescine synthetase	WP_009048287	XLOC_000943	0.0008	0.0068	1.98
Allantoate amidohydrolase	WP_038631669	XLOC_001004	0.0003	0.0027	4.02
Serine/threonine protein kinase	WP_009048515	XLOC_001051	0.0001	0.0007	-1.99
Lysine transporter LysE	WP_009048674	XLOC_001122	0.0001	0.0007	2.42
Acetyltransferase	WP_038632134	XLOC_001156	0.0001	0.0007	1.87
Phospho-2-dehydro-3-deoxyheptonate aldolase	WP_038633423	XLOC_001520	0.0001	0.0007	-1.87
Peptidase M19	WP_009049989	XLOC_001680	0.0001	0.0007	-2.65
Class V aminotransferase	WP_038633911	XLOC_001681	0.0001	0.0007	-1.89
Amino acid APC transporter	WP_038634442	XLOC_001891	0.0001	0.0007	-2.45
Arginine deiminase	WP_009050421	XLOC_001892	0.0001	0.0007	-1.85
ABC transporter	WP_025806014	XLOC_001996	0.0001	0.0007	-2.65
Amino acid ABC transporter permease	WP_009050566	XLOC_001997	0.0001	0.0007	-2.22
2-isopropylmalate synthase	WP_009050632	XLOC_002026	0.0001	0.0007	1.79
Phospho-2-dehydro-3-deoxyheptonate aldolase	WP_029526777	XLOC_002097	0.0024	0.0171	-9.07
Glutamate-1-semialdehyde 2,1-aminomutase	WP_009045779	XLOC_002238	0.0002	0.0017	1.65
Ethanolamine transporter	WP_009051053	XLOC_002249	0.0053	0.0324	3.27
Amino-acid acetyltransferase	WP_009051388	XLOC_002450	0.0001	0.0007	2.10
Transporter	WP_038575139	XLOC_002514	0.0009	0.0079	-2.22
Urease subunit beta	WP_009046740	XLOC_002746	0.0007	0.0060	1.88
Spermidine/putrescine ABC transporter permease	WP_009047162	XLOC_002961	0.0056	0.0336	1.65
Spermidine/putrescine ABC transporter substrate-binding protein	WP_009047165	XLOC_002963	0.0012	0.0100	1.77
Leucyl aminopeptidase (Aminopeptidase T)	WP_038630987	XLOC_003139	0.0001	0.0007	-3.80
Lysine transporter LysE	WP_016702183	XLOC_003173	0.0004	0.0036	-3.38
Arginine N-succinyltransferase	WP_038631375	XLOC_003303	0.0068	0.0391	-2.68
Porin	WP_016702750	XLOC_003304	0.0002	0.0017	-2.16
Branched-chain-amino-acid aminotransferase	WP_009048405	XLOC_003404	0.0001	0.0007	1.54
DNA-binding protein	WP_009048753	XLOC_003537	0.0008	0.0068	2.37
Transcriptional regulator	WP_038632162	XLOC_003545	0.0001	0.0007	-5.52
Diaminobutyrate--2-oxoglutarate aminotransferase	WP_038636648	XLOC_003702	0.0001	0.0007	6.59
4-hydroxyphenylpyruvate dioxygenase	WP_028682540	XLOC_003776	0.0001	0.0007	3.10
2,4-diaminobutyrate 4-aminotransferase	WP_038634057	XLOC_004146	0.0001	0.0007	2.84
Amino acid ABC transporter substrate-binding protein	WP_038634078	XLOC_004154	0.0008	0.0072	-2.33
HAD family hydrolase	WP_009050998	XLOC_004635	0.0024	0.0171	1.81
Aspartate ammonia-lyase	WP_007928510	XLOC_004853	0.0001	0.0007	2.89
Gamma-glutamylputrescine synthetase	WP_009051380	XLOC_004926	0.0001	0.0007	1.71
Amino acid ABC transporter substrate-binding protein	WP_038636132	XLOC_004965	0.0012	0.0096	-2.22
Aminoglycoside N(6')-acetyltransferase type 1	WP_038636173	XLOC_004987	0.0001	0.0007	3.40

<b>Nucleotide transport and metabolism</b>	<b>F</b>	Nitrate reductase	WP_038636416	XLOC_000161	0.0011	0.0089	2.42			
		Glutamine ABC transporter	WP_009047107	XLOC_000378	0.0021	0.0154	3.46			
		ATP-binding protein								
		Methionyl-tRNA formyltransferase	WP_025805252	XLOC_000693	0.0001	0.0007	-3.57			
		Nitrate reductase	WP_038631666	XLOC_001003	0.0042	0.0267	1.59			
		Uncharacterized protein	WP_038631987	XLOC_001109	0.0001	0.0007	-5.10			
		Purine phosphorylase	WP_038632729	XLOC_001355	0.0027	0.0188	-2.32			
		N(5)-hydroxyornithine	WP_038633913	XLOC_004108	0.0001	0.0007	1.52			
		transformylase PvdF								
		Nucleoside diphosphate kinase	WP_007929529	XLOC_004450	0.0001	0.0007	-2.72			
Glutamate 5-kinase	WP_007924743	XLOC_004605	0.0001	0.0007	1.61					
<b>Carbohydrate transport and metabolism</b>	<b>G</b>	Chitinase	WP_009048115	XLOC_000870	0.0001	0.0007	-8.67			
		Membrane protein	WP_038631513	XLOC_000954	0.0014	0.0110	1.78			
		MFS transporter	WP_038631586	XLOC_000977	0.0021	0.0154	2.55			
		AP endonuclease	WP_029526986	XLOC_001378	0.0001	0.0007	2.66			
		MFS transporter	WP_009049295	XLOC_001379	0.0001	0.0007	3.01			
		Glyceraldehyde-3-phosphate dehydrogenase	WP_009050409	XLOC_001882	0.0001	0.0007	-3.70			
		DeoR family transcriptional regulator	WP_009050563	XLOC_001994	0.0001	0.0007	-2.20			
		Inositol monophosphatase	WP_007929514	XLOC_002027	0.0004	0.0036	-1.85			
		Sucrase	WP_016703507	XLOC_002037	0.0023	0.0169	-2.37			
		PTS N-acetyl-D-glucosamine transporter	WP_038630669	XLOC_002946	0.0014	0.0110	2.01			
		Beta-(1-3)-glucosyl transferase	WP_038630710	XLOC_002974	0.0001	0.0007	-2.33			
		Siderophore biosynthesis protein SbnG	WP_009049138	XLOC_003700	0.0001	0.0007	5.43			
		MFS transporter	WP_038632598	XLOC_003701	0.0001	0.0007	7.17			
		Chitinase	WP_009049243	XLOC_003753	0.0001	0.0007	-5.99			
		ABC transporter substrate-binding protein	WP_023964376	XLOC_003984	0.0001	0.0007	5.18			
		Sugar ABC transporter permease	WP_007929618	XLOC_003986	0.0001	0.0007	2.77			
		Multidrug resistance protein B	WP_009049803	XLOC_003990	0.0073	0.0411	-4.43			
		Glucose-6-phosphate 1-dehydrogenase	WP_023966786	XLOC_004336	0.0001	0.0007	-1.85			
		Sugar ABC transporter ATPase	WP_016703424	XLOC_004339	0.0001	0.0007	-3.42			
		Sugar ABC transporter permease	WP_009045231	XLOC_004340	0.0001	0.0007	-2.72			
		Sugar ABC transporter substrate-binding protein	WP_009050405	XLOC_004341	0.0001	0.0007	-2.83			
		Sugar isomerase	WP_016703422	XLOC_004342	0.0001	0.0007	-2.87			
		PTS glucose transporter subunit IIB	WP_038634867	XLOC_004432	0.0010	0.0086	3.25			
		Sugar ABC transporter ATPase	WP_009050627	XLOC_004440	0.0001	0.0007	2.98			
		Ribose-5-phosphate isomerase A	WP_009051359	XLOC_004933	0.0040	0.0260	-2.07			
		<b>Coenzyme transport and metabolism</b>	<b>H</b>	Phosphomethylpyrimidine synthase	WP_009046670	XLOC_000160	0.0001	0.0007	1.96	
				Omega amino acid--pyruvate aminotransferase	WP_038630538	XLOC_000233	0.0001	0.0007	-2.78	
				Cyclic pyranopterin monophosphate synthase	WP_009047097	XLOC_000372	0.0001	0.0007	2.59	
				accessory protein						
				Molybdenum cofactor biosynthesis protein MoaE	WP_038630652	XLOC_000373	0.0004	0.0040	2.45	
				Pyridoxine 5'-phosphate synthase	WP_038630664	XLOC_000389	0.0001	0.0012	2.99	
				(2Fe-2S)-binding protein	WP_016701774	XLOC_001019	0.0001	0.0007	-4.04	
				Aminotransferase	WP_038636003	XLOC_002460	0.0001	0.0007	2.39	
MFS transporter	WP_009047472			XLOC_003055	0.0001	0.0007	-6.76			
Tryptophan synthase subunit alpha	WP_009048364			XLOC_003383	0.0001	0.0007	-5.17			
Molybdenum cofactor biosynthesis protein MoaD	WP_038633582			XLOC_003966	0.0025	0.0177	2.87			

<b>Lipid transport and metabolism</b>	<b>I</b>	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ	WP_007924058	XLOC_000435	0.0001	0.0007	-1.72		
		Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	WP_038630718	XLOC_000439	0.0001	0.0007	-2.58		
		Malonyl CoA-acyl carrier protein transacylase	WP_009047834	XLOC_000740	0.0001	0.0007	-2.32		
		3-oxoacyl-(Acyl carrier protein) synthase II (Modular protein)	WP_003175607	XLOC_000741	0.0087	0.0472	-2.06		
		Phosphatidylserine decarboxylase proenzyme	WP_038631548	XLOC_000962	0.0001	0.0007	-3.60		
		Enoyl-[acyl-carrier-protein] reductase [NADH]	WP_038631995	XLOC_001112	0.0001	0.0007	-1.90		
		Enoyl-CoA hydratase	WP_025808975	XLOC_001220	0.0048	0.0300	1.77		
		Acetoacetyl-CoA synthetase	WP_038633443	XLOC_001527	0.0001	0.0007	1.71		
		VldD	WP_009049637	XLOC_001532	0.0001	0.0007	2.75		
		CAIB/BAIF family CoA transferase	WP_038634439	XLOC_001890	0.0001	0.0007	1.86		
		Glycerol acyltransferase	WP_009046219	XLOC_002323	0.0001	0.0007	-2.76		
		Poly(R)-hydroxyalkanoic acid synthase	WP_009046586	XLOC_002690	0.0001	0.0007	-1.66		
		Poly(R)-hydroxyalkanoic acid synthase	WP_009046588	XLOC_002692	0.0001	0.0007	-3.12		
		3-oxoacyl-ACP synthase	WP_025805277	XLOC_003165	0.0001	0.0007	-1.59		
		3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase	WP_007931558	XLOC_003166	0.0001	0.0007	-1.91		
		Acyl carrier protein	WP_003175607	XLOC_003180	0.0002	0.0022	-2.47		
		Enoyl-CoA hydratase	WP_016704994	XLOC_003195	0.0005	0.0049	-1.76		
		3-oxoacyl-[acyl-carrier-protein] synthase 2	WP_041988549	XLOC_003283	0.0031	0.0210	-1.86		
		Acyl-CoA dehydrogenase	WP_009048955	XLOC_003632	0.0068	0.0389	1.95		
		Glucose-methanol-choline oxidoreductase	WP_038632521	XLOC_003682	0.0051	0.0315	-1.86		
		CDP-diacylglycerol--serine O-phosphatidyltransferase	WP_009049274	XLOC_003763	0.0011	0.0093	-1.51		
		3-oxoacyl-ACP synthase	WP_009050008	XLOC_004115	0.0010	0.0086	-3.11		
		Peptide synthase	WP_038634075	XLOC_004152	0.0001	0.0007	2.36		
		Acyl-CoA thioesterase	WP_038634144	XLOC_004190	0.0031	0.0210	1.70		
		Glycerol acyltransferase	WP_009050240	XLOC_004248	0.0001	0.0007	-3.30		
		Acetyl-coenzyme A synthetase	WP_009050330	XLOC_004302	0.0001	0.0007	-2.56		
		Long-chain fatty acid--CoA ligase	WP_009050391	XLOC_004332	0.0001	0.0007	-2.30		
		PvdL	WP_038634960	XLOC_004477	0.0049	0.0305	1.56		
		Acyl-CoA dehydrogenase	WP_009051017	XLOC_004686	0.0001	0.0007	1.89		
		<b>Translation, ribosomal structure and biogenesis</b>	<b>J</b>	Acetyltransferase	WP_009046341	XLOC_000018	0.0007	0.0060	-3.00
				Polyribonucleotide nucleotidyltransferase	WP_038630587	XLOC_000295	0.0001	0.0007	-2.13
				tRNA (guanine-N(1)-)-methyltransferase	WP_007927679	XLOC_000396	0.0005	0.0049	-1.76
Elongation factor Ts	WP_009042383			XLOC_000428	0.0001	0.0007	-1.86		
GNAT family acetyltransferase	WP_038630820			XLOC_000531	0.0034	0.0229	-1.53		
4-hydroxy-4-methyl-2-oxoglutarate aldolase	WP_009042919			XLOC_000784	0.0001	0.0007	1.94		
NADPH-dependent 7-cyano-7-deazaguanine reductase	WP_009047982			XLOC_000827	0.0001	0.0007	2.59		
Conjugal transfer protein TraR	WP_007923643			XLOC_001033	0.0050	0.0310	-2.53		
Amidase	WP_038633863			XLOC_001669	0.0023	0.0166	1.64		
RNA polymerase-binding transcription factor DksA	WP_007924645			XLOC_002129	0.0001	0.0007	-1.73		
Peptide deformylase	WP_009046227			XLOC_002318	0.0014	0.0110	-1.54		
RNA-binding protein	WP_007920867			XLOC_002847	0.0001	0.0007	-1.77		
50S ribosomal protein L32	KNH46388			XLOC_003179	0.0001	0.0007	-2.70		
Ribosome modulation factor	WP_023968495			XLOC_003210	0.0001	0.0007	-2.28		
Glutaminase	WP_009048121			XLOC_003273	0.0001	0.0007	-3.02		
Methionine aminopeptidase	WP_009048909			XLOC_003604	0.0040	0.0257	1.65		
GTP-binding protein	WP_028683953			XLOC_003650	0.0001	0.0007	-2.29		
Elongation factor P	WP_007931398			XLOC_004174	0.0001	0.0007	-1.92		



		7-cyano-7-deazaguanine synthase	WP_038636862	XLOC_004480	0.0001	0.0007	-2.13
<b>Transcription</b>	<b>K</b>	MarR family transcriptional regulator	WP_009046358	XLOC_000026	0.0001	0.0007	-2.78
		RNA polymerase sigma70	WP_009046377	XLOC_000037	0.0001	0.0007	-2.04
		RNA polymerase sigma factor RpoS	WP_007924026	XLOC_000448	0.0001	0.0007	-2.98
		LysR family transcriptional regulator	WP_028681807	XLOC_000487	0.0040	0.0257	2.21
		LysR family transcriptional regulator	WP_009047555	XLOC_000581	0.0003	0.0032	1.68
		MarR family transcriptional regulator	WP_007929752	XLOC_000623	0.0001	0.0007	-2.64
		AraC family transcriptional regulator	WP_048360720	XLOC_000627	0.0040	0.0260	1.69
		LacI family transcriptional regulator	WP_038631299	XLOC_000876	0.0058	0.0346	1.95
		RNA polymerase subunit sigma-70	WP_009048295	XLOC_000950	0.0001	0.0007	2.51
		ArsR family transcriptional regulator	WP_009048410	XLOC_001000	0.0067	0.0384	2.20
		Transcriptional regulator	WP_028682559	XLOC_001377	0.0001	0.0007	2.29
		Transcriptional regulator	WP_009049335	XLOC_001408	0.0001	0.0007	-2.58
		GntR family transcriptional regulator	WP_038633317	XLOC_001500	0.0025	0.0180	1.50
		Cold-shock protein	WP_009049775	XLOC_001584	0.0001	0.0007	-1.61
		Fis family transcriptional regulator	WP_038633652	XLOC_001603	0.0012	0.0100	3.00
		Temperature acclimation protein B	WP_007931076	XLOC_001901	0.0001	0.0007	-3.51
		GntR family transcriptional regulator	WP_009050459	XLOC_001909	0.0001	0.0007	-2.55
		DEAD/DEAH box helicase	WP_038634571	XLOC_001931	0.0002	0.0017	1.69
		H-NS histone	WP_007931174	XLOC_001963	0.0001	0.0007	-1.58
		DNA-binding protein	WP_009050695	XLOC_002040	0.0001	0.0007	-4.31
		Competence protein TfoX	WP_009050884	XLOC_002126	0.0001	0.0007	-2.18
		Transcriptional regulator	WP_038635479	XLOC_002229	0.0006	0.0053	-5.05
		LysR family transcriptional regulator	WP_009046306	XLOC_002277	0.0021	0.0154	-1.86
		AsnC family transcriptional regulator	WP_009051665	XLOC_002346	0.0001	0.0007	2.27
		AraC family transcriptional regulator	WP_016704212	XLOC_002529	0.0020	0.0151	1.82
		RNA polymerase sigma factor	WP_016705108	XLOC_002582	0.0001	0.0007	1.53
		AraC family transcriptional regulator	WP_028683816	XLOC_002650	0.0001	0.0007	-2.49
		TetR family transcriptional regulator	WP_009046585	XLOC_002689	0.0002	0.0022	-2.00
		Uncharacterized protein	WP_002554837	XLOC_003002	0.0001	0.0007	-2.61
		LysR family transcriptional regulator	WP_016703226	XLOC_003093	0.0013	0.0107	1.93
		LuxR family transcriptional regulator	WP_009048517	XLOC_003448	0.0018	0.0136	-3.32
		CdaR family transcriptional regulator	WP_016702462	XLOC_003773	0.0082	0.0450	2.38
		RNA polymerase subunit sigma-24	WP_038633746	XLOC_004046	0.0001	0.0007	2.08
		RNA polymerase sigma factor	WP_016702389	XLOC_004106	0.0064	0.0373	1.50
		PhzR	WP_038635113	XLOC_004545	0.0001	0.0007	-3.84
		LysR family transcriptional regulator	WP_009051067	XLOC_004709	0.0067	0.0384	-3.31
		AraC family transcriptional regulator	WP_038635586	XLOC_004729	0.0026	0.0183	1.58
		TetR family transcriptional regulator	WP_009051381	XLOC_004925	0.0058	0.0346	2.26
<b>Replication, recombination and repair</b>	<b>L</b>	Methyltransferase domain protein	WP_038630393	XLOC_000100	0.0090	0.0482	-2.60
		DNA-binding protein HU, nu subunit	WP_007932138	XLOC_000383	0.0001	0.0007	-1.94

		Uncharacterized protein	WP_007921116	XLOC_002393	0.0001	0.0007	-1.89
		DNA polymerase III subunit chi	WP_038630662	XLOC_002941	0.0002	0.0022	2.10
		Error-prone DNA polymerase	WP_038633547	XLOC_003957	0.0059	0.0351	-1.55
		DNA polymerase III subunit epsilon	WP_038636295	XLOC_005008	0.0001	0.0012	-1.75
<b>Cell wall/membrane/envelope biogenesis</b>	<b>M</b>	Transporter	WP_009046348	XLOC_000022	0.0001	0.0012	-2.57
		Outer membrane protein, Skp family	WP_007924063	XLOC_000433	0.0001	0.0007	-1.67
		Transporter	WP_007927520	XLOC_000787	0.0001	0.0007	-1.65
		Porin	WP_007927525	XLOC_000789	0.0001	0.0007	-1.74
		Membrane protein	WP_038632523	XLOC_001294	0.0005	0.0045	-2.99
		Hemolysin secretion protein D	WP_038633904	XLOC_001677	0.0001	0.0007	2.29
		Macrolide export ATP-binding/permease protein MacB	WP_009049986	XLOC_001678	0.0001	0.0007	1.99
		RND transporter	WP_038633906	XLOC_001679	0.0001	0.0007	1.54
		dTDP-glucose 4,6-dehydratase	WP_038634163	XLOC_001766	0.0077	0.0431	-2.41
		Porin	WP_038634847	XLOC_002012	0.0039	0.0252	-1.88
		Glucose dehydrogenase	WP_016703467	XLOC_002013	0.0001	0.0007	1.58
		RND transporter MFP subunit	WP_038634985	XLOC_002046	0.0005	0.0049	2.75
		Large-conductance mechanosensitive channel	WP_009045733	XLOC_002179	0.0001	0.0007	-1.72
		Membrane protein insertase YidC	WP_009051704	XLOC_002327	0.0001	0.0007	-1.64
		N-acetylmuramoyl-L-alanine amidase	WP_038635768	XLOC_002350	0.0018	0.0136	6.16
		Potassium transporter KefA	WP_009046633	XLOC_002708	0.0001	0.0007	-1.66
		Membrane-bound lytic murein transglycosylase F	WP_009042308	XLOC_002944	0.0001	0.0007	-1.62
		Glucans biosynthesis protein D	WP_016702744	XLOC_002958	0.0001	0.0007	2.62
		Lipoprotein	AAT46075	XLOC_002981	0.0001	0.0007	-1.74
		TrbB protein	WP_038630956	XLOC_003108	0.0016	0.0126	-1.79
		Acyltransferase	WP_038636499	XLOC_003266	0.0001	0.0007	-3.62
		Ion channel protein Tsx	WP_009048213	XLOC_003314	0.0032	0.0218	-2.00
		UDP-glucuronate 5-epimerase	WP_009048937	XLOC_003621	0.0035	0.0234	1.64
		Phospholipase	WP_038632608	XLOC_003705	0.0025	0.0180	2.93
		Acriflavin resistance protein AcrA	WP_009049438	XLOC_003840	0.0001	0.0007	-1.69
		Channel protein TolC	WP_016703028	XLOC_003888	0.0009	0.0079	2.00
		Channel protein TolC	WP_038636749	XLOC_003928	0.0001	0.0007	2.34
		Multidrug RND transporter	WP_038633661	XLOC_003992	0.0001	0.0007	-2.46
		Membrane protein	WP_038634145	XLOC_004202	0.0001	0.0007	-2.12
		Uncharacterized protein	WP_051673203	XLOC_004204	0.0008	0.0068	-1.77
		Membrane protein	WP_038576794	XLOC_004272	0.0001	0.0007	-1.76
		Membrane protein	WP_009050551	XLOC_004412	0.0006	0.0053	2.21
		<b>Cell motility</b>	<b>N</b>	Chemotaxis protein	WP_009046693	XLOC_000180	0.0001
General secretion pathway protein GspE	WP_009048770			XLOC_001151	0.0001	0.0012	3.90
Chemotaxis protein	WP_038634350			XLOC_001852	0.0001	0.0007	2.27
Chemotaxis protein	WP_038634436			XLOC_001889	0.0001	0.0007	2.34
Chemotaxis protein	WP_038631529			XLOC_003363	0.0001	0.0007	-2.09
Chemotaxis protein CheW	WP_009050086			XLOC_004157	0.0001	0.0007	1.59
<b>Posttranslational modification, protein turnover, chaperones</b>	<b>O</b>	Peptidyl-prolyl cis-trans isomerase	WP_007929845	XLOC_000341	0.0001	0.0007	-1.98
		Heat-shock protein	WP_007922004	XLOC_000845	0.0028	0.0194	1.86
		Probable thiol peroxidase	WP_038632154	XLOC_001174	0.0001	0.0007	-2.41
		Peptidylprolyl isomerase	WP_007932676	XLOC_001529	0.0067	0.0384	3.18
		Peptidyl-prolyl cis-trans isomerase	WP_007929634	XLOC_001604	0.0001	0.0007	-1.57
		Thioredoxin	EPA97025	XLOC_002431	0.0001	0.0007	-1.66
		Peptidyl-prolyl cis-trans isomerase	WP_007920626	XLOC_003105	0.0005	0.0045	-1.71
		CAAX protease	WP_009047951	XLOC_003232	0.0026	0.0183	2.04
		Glutathione S-transferase	WP_038631753	XLOC_003432	0.0001	0.0007	-5.02
		Alkyl hydroperoxide reductase	WP_007921754	XLOC_003780	0.0001	0.0007	-3.12
		Trigger factor	WP_009044653	XLOC_004022	0.0001	0.0007	-1.98
		Serine protease	WP_038633759	XLOC_004049	0.0001	0.0007	-2.26
		Calcium channel protein	WP_025805966	XLOC_004396	0.0053	0.0322	1.80



		10 kDa chaperonin Glutaredoxin	WP_009050540 WP_007922425	XLOC_004406 XLOC_004415	0.0001 0.0001	0.0007 0.0007	-2.47 -1.80
<b>Inorganic ion transport and metabolism</b>	<b>P</b>	Adenosine-3'(2'),5'- bisphosphate nucleotidase	WP_038630373	XLOC_000088	0.0011	0.0089	1.59
		Cation diffusion facilitator family transporter	WP_009046718	XLOC_000199	0.0007	0.0064	-1.66
		Sulfite reductase	WP_009048796	XLOC_001169	0.0001	0.0007	-2.87
		TonB-dependent receptor	WP_038633608	XLOC_001578	0.0032	0.0218	2.91
		Iron utilization protein	WP_038633927	XLOC_001685	0.0001	0.0007	1.86
		FAD-binding protein	WP_038634838	XLOC_002009	0.0001	0.0007	-2.11
		TonB-dependent receptor	WP_038634844	XLOC_002011	0.0003	0.0027	6.76
		Fur family transcriptional regulator	WP_009046276	XLOC_002299	0.0001	0.0007	1.51
		Phosphate-specific transport system accessory protein PhoU	WP_007928528	XLOC_002371	0.0093	0.0494	-2.54
		Calcium transporter ChaC	WP_009051415	XLOC_002434	0.0003	0.0027	-1.62
		Peptide ABC transporter substrate-binding protein	WP_038630318	XLOC_002581	0.0001	0.0007	3.26
		TonB-dependent receptor	WP_038636418	XLOC_002754	0.0031	0.0210	5.12
		Ligand-gated channel protein	WP_028681659	XLOC_002855	0.0001	0.0012	-1.50
		Nuclease PIN	WP_009047396	XLOC_003029	0.0003	0.0032	-2.18
		Copper resistance protein CopD	WP_038632192	XLOC_003560	0.0043	0.0272	2.39
		Copper resistance protein CopC	WP_038632194	XLOC_003561	0.0001	0.0007	5.13
		Copper resistance protein CopB	WP_016704756	XLOC_003562	0.0001	0.0007	3.76
		Iron ABC transporter permease	WP_038632590	XLOC_003699	0.0001	0.0007	5.67
		TonB-dependent receptor	WP_038632604	XLOC_003703	0.0001	0.0007	5.87
		Amino acid ABC transporter substrate-binding protein	WP_038636768	XLOC_004044	0.0001	0.0007	2.62
		Peptide ABC transporter substrate-binding protein	WP_038633744	XLOC_004045	0.0001	0.0007	2.22
		(2Fe-2S)-binding protein	AIC22009	XLOC_004414	0.0003	0.0027	-2.64
		Catalase	WP_009050970	XLOC_004621	0.0012	0.0100	-2.99
		Catalase	WP_016702645	XLOC_004732	0.0001	0.0007	-1.91
		Carbonic anhydrase	WP_009046260	XLOC_004795	0.0001	0.0007	-1.71
		Zinc ABC transporter permease	WP_009051646	XLOC_004836	0.0001	0.0007	7.11
		<b>Secondary metabolites biosynthesis, transport and catabolism</b>	<b>Q</b>	Poly(3-hydroxyalkanoate) granule-associated protein PhaF	WP_009046584	XLOC_000134	0.0001
Homogentisate 1,2- dioxygenase	WP_038630625			XLOC_000355	0.0001	0.0007	2.71
Serine 3-dehydrogenase	WP_016702821			XLOC_000985	0.0001	0.0007	-5.42
NADP-dependent oxidoreductase	WP_009049328			XLOC_001403	0.0001	0.0007	-1.55
Aldo/keto reductase	WP_038635010			XLOC_002053	0.0001	0.0007	-1.74
Phenazine biosynthesis protein	WP_009050819			XLOC_002096	0.0001	0.0007	-7.98
Isochorismatase	WP_009050821			XLOC_002098	0.0001	0.0007	-7.60
Phenazine biosynthesis protein	WP_038635119			XLOC_002100	0.0001	0.0007	-8.33
Poly(3-hydroxyalkanoate) depolymerase	WP_009046587			XLOC_002691	0.0001	0.0007	-1.95
Pyoverdine biosynthesis protein	WP_009050075			XLOC_004153	0.0001	0.0007	2.08
<b>General function prediction only</b>	<b>R</b>			Glyoxalase	WP_009046877	XLOC_000259	0.0001
		PsiE family protein	WP_009042237	XLOC_000360	0.0002	0.0022	-2.47
		Arylsulfate sulfotransferase	WP_009042529	XLOC_000517	0.0006	0.0057	-2.60
		Chitin-binding protein	WP_009048114	XLOC_000869	0.0001	0.0007	-7.44
		Na <sup>+</sup> /H <sup>+</sup> dicarboxylate symporter	WP_009048348	XLOC_000974	0.0001	0.0012	1.78
		Ring-cleavage extradiol dioxygenase	WP_016702948	XLOC_001237	0.0001	0.0007	-5.15
		Sugar deacetylase	WP_016702950	XLOC_001239	0.0001	0.0007	-2.71
		ABC transporter	WP_016702952	XLOC_001241	0.0001	0.0007	-2.26
		Amidohydrolase	WP_038633980	XLOC_001697	0.0001	0.0007	-3.86
		Methyltransferase	WP_038634054	XLOC_001717	0.0018	0.0136	2.01
		Uncharacterized protein	WP_038634153	XLOC_001765	0.0002	0.0017	-2.72
		Phenazine biosynthesis protein	WP_009050818	XLOC_002095	0.0001	0.0007	-9.14

		D-amino acid dehydrogenase	WP_038636119	XLOC_002511	0.0083	0.0456	2.87
		Rhs family protein	WP_038630348	XLOC_002611	0.0001	0.0007	-6.54
		4-carboxymuconolactone decarboxylase	WP_009046984	XLOC_002874	0.0033	0.0221	2.68
		Membrane protein	WP_009047479	XLOC_003057	0.0001	0.0007	4.24
		Diaminopimelate decarboxylase	WP_009048261	XLOC_003333	0.0054	0.0327	-4.30
		Methyltransferase	WP_038632359	XLOC_003651	0.0001	0.0012	-3.92
		Phosphoribosyl transferase	WP_038632651	XLOC_003725	0.0024	0.0171	2.04
		Peptidase C39	WP_038633471	XLOC_003930	0.0001	0.0007	2.17
		Isomerase	WP_038633872	XLOC_004090	0.0008	0.0068	-2.07
		Sugar ABC transporter ATP-binding protein	WP_038634153	XLOC_004205	0.0006	0.0053	-2.70
		UPF0060 membrane protein EY04_22005	WP_007932246	XLOC_004221	0.0009	0.0079	2.63
		23S rRNA (uracil(1939)-C(5))-methyltransferase RlmD	WP_038634290	XLOC_004264	0.0001	0.0007	1.76
		Exclusion suppressor FxsA	WP_007932702	XLOC_004407	0.0028	0.0194	-1.68
		Beta-lactamase	WP_038635452	XLOC_004671	0.0001	0.0007	-2.93
		Transcriptional regulator	WP_038635883	XLOC_004878	0.0001	0.0007	-2.62
<b>Unknown Function</b>	<b>S</b>	Sulfotransferase	WP_009046345	XLOC_000020	0.0001	0.0007	-2.50
		Glycosyl hydrolase	WP_038636404	XLOC_000021	0.0001	0.0007	-2.55
		RebB like protein	WP_007930328	XLOC_000038	0.0007	0.0060	-4.50
		R body protein RebB-like protein	WP_009046381	XLOC_000042	0.0001	0.0007	-4.45
		R body protein RebB-like protein	WP_009046382	XLOC_000043	0.0007	0.0060	-6.47
		Uncharacterized protein	EIM12867	XLOC_000056	0.0001	0.0007	-5.27
		Peptidoglycan-binding protein LysM	WP_009046477	XLOC_000086	0.0001	0.0007	-3.45
		Poly(3-hydroxyalkanoate) granule-associated protein PhaI	WP_009046583	XLOC_000133	0.0001	0.0007	-6.10
		Tail sheath protein	WP_038630744	XLOC_000457	0.0075	0.0420	-2.52
		Phage tail protein	WP_007923973	XLOC_000458	0.0002	0.0022	-2.18
		Uncharacterized protein	WP_038630933	XLOC_000599	0.0001	0.0007	2.44
		Putative hemagglutinin (DUF637)	EJL96817	XLOC_000612	0.0001	0.0007	-3.19
		Membrane protein	WP_007931481	XLOC_000698	0.0028	0.0194	-2.15
		Uncharacterized protein	WP_009047832	XLOC_000738	0.0001	0.0007	-2.76
		CrfX protein	WP_007927518	XLOC_000786	0.0001	0.0007	-2.72
		Uncharacterized protein	WP_009048113	XLOC_000868	0.0074	0.0418	-2.95
		Membrane protein	WP_009048116	XLOC_000871	0.0009	0.0075	-2.65
		Uncharacterized protein	WP_038632150	XLOC_001170	0.0001	0.0007	-3.40
		Porin	WP_038636600	XLOC_001214	0.0017	0.0133	-1.61
		Bifunctional pyrazinamidase/nicotinamidase	ADO14993	XLOC_001373	0.0015	0.0116	-3.80
		Single-stranded DNA-binding protein	AIC20617	XLOC_001444	0.0008	0.0072	-1.61
		Uncharacterized protein	WP_038636735	XLOC_001488	0.0011	0.0089	2.48
		Uncharacterized protein	WP_038633414	XLOC_001519	0.0001	0.0007	4.82
		Uncharacterized protein	WP_007923269	XLOC_001732	0.0001	0.0007	-3.43
		Membrane protein	WP_009050289	XLOC_001828	0.0001	0.0007	2.05
		Porin	WP_038634688	XLOC_001955	0.0001	0.0007	-3.29
		Cytochrome D ubiquinol oxidase subunit III	WP_007928428	XLOC_002055	0.0001	0.0007	-1.85
		Uncharacterized protein	WP_009050883	XLOC_002125	0.0001	0.0007	-1.73
		Peptidase C13	WP_038635515	XLOC_002247	0.0001	0.0007	-3.58
		UPF0391 membrane protein yjA	WP_003177151	XLOC_002293	0.0011	0.0089	2.60
		Membrane protein	WP_038635748	XLOC_002347	0.0001	0.0007	2.63
		Uncharacterized protein	WP_025807035	XLOC_002408	0.0001	0.0007	-1.60
		Porin	WP_025807108	XLOC_002451	0.0001	0.0007	-1.52
		ATPase	WP_025807119	XLOC_002466	0.0057	0.0340	-1.74
		Sulfurase	WP_038636332	XLOC_002557	0.0055	0.0333	1.68
		Tail protein	WP_009046343	XLOC_002592	0.0019	0.0142	-2.40
		Uncharacterized protein	WP_038630505	XLOC_002769	0.0001	0.0007	2.27
		Phosphatidylcholine-hydrolyzing phospholipase	WP_009046978	XLOC_002873	0.0001	0.0007	-2.61
		Pilin assembly protein	WP_009047183	XLOC_002969	0.0033	0.0223	-1.72

		Membrane protein	WP_009047384	XLOC_003022	0.0067	0.0384	2.73
		Membrane protein	WP_016702126	XLOC_003058	0.0001	0.0007	4.99
		Uncharacterized protein	WP_038636463	XLOC_003065	0.0001	0.0007	-3.09
		Acetyltransferase	WP_038630891	XLOC_003073	0.0002	0.0022	-3.84
		Metal-chelation protein CHAD	WP_038630895	XLOC_003075	0.0001	0.0007	-1.64
		Fusaric acid resistance protein	WP_038631055	XLOC_003156	0.0073	0.0411	-3.93
		2-oxoglutarate dehydrogenase subunit E1	WP_047284630	XLOC_003160	0.0031	0.0213	-1.91
		Uncharacterized protein	WP_038636482	XLOC_003161	0.0001	0.0007	-4.63
		Uncharacterized protein	WP_038631945	XLOC_003480	0.0031	0.0213	-2.68
		Uncharacterized protein	WP_038632169	XLOC_003551	0.0001	0.0007	-5.81
		Lipoprotein	WP_038632271	XLOC_003611	0.0001	0.0007	-5.11
		Uncharacterized protein	WP_038632500	XLOC_003680	0.0032	0.0216	-3.12
		Porin	WP_025808788	XLOC_003710	0.0001	0.0007	-1.79
		Uncharacterized protein	WP_038632989	XLOC_003825	0.0001	0.0007	3.70
		Uncharacterized protein	WP_038632995	XLOC_003827	0.0053	0.0324	1.99
		Cytotoxin	WP_038633468	XLOC_003929	0.0001	0.0007	2.32
		Uncharacterized protein	WP_038633491	XLOC_003935	0.0014	0.0110	-1.62
		Membrane protein	ETD38759	XLOC_003985	0.0001	0.0007	4.36
		RNA signal recognition particle 4.5S RNA	WP_028682047	XLOC_004065	0.0009	0.0079	2.38
		Membrane protein	WP_038633841	XLOC_004083	0.0001	0.0007	-4.02
		Dialkylrecorsinol condensing enzyme	WP_009050009	XLOC_004116	0.0001	0.0007	-2.37
		Uncharacterized protein (Fragment)	KPZ18068	XLOC_004139	0.0052	0.0317	-1.93
		Uncharacterized protein	WP_009050148	XLOC_004192	0.0035	0.0231	-1.80
		Uncharacterized protein	CDI89872	XLOC_004224	0.0039	0.0252	-4.64
		Membrane protein	WP_038634529	XLOC_004361	0.0014	0.0113	-4.14
		Type IV secretion protein Rhs	WP_038634583	XLOC_004369	0.0001	0.0007	-2.61
		Phospholipid-binding protein	WP_009050906	XLOC_004586	0.0001	0.0007	-2.55
		UPF0114 protein EY04_27105	WP_007924753	XLOC_004610	0.0004	0.0040	-1.66
		Uncharacterized protein	WP_051673205	XLOC_004653	0.0001	0.0007	-2.42
		Uncharacterized protein	WP_038635341	XLOC_004657	0.0006	0.0057	-1.66
		Uncharacterized protein	WP_038635640	XLOC_004789	0.0001	0.0007	1.86
		secondary thiamine-phosphate synthase enzyme	EIM17029	XLOC_004893	0.0011	0.0089	-2.47
<b>Signal transduction mechanisms</b>	<b>T</b>	Anti-anti-sigma factor	WP_009047705	XLOC_000662	0.0001	0.0007	-2.31
		Chemotaxis protein CheY	WP_038631017	XLOC_000663	0.0001	0.0007	-2.50
		Histidine kinase	WP_009047707	XLOC_000664	0.0001	0.0007	-1.83
		Histidine kinase	WP_038633447	XLOC_001528	0.0001	0.0012	1.61
		Divalent cation transporter	WP_041984621	XLOC_001842	0.0014	0.0113	-2.42
		Acyl-homoserine-lactone synthase	WP_038635110	XLOC_002094	0.0001	0.0007	-6.00
		Chemotaxis protein CheY	WP_016703129	XLOC_002372	0.0001	0.0007	1.94
		Diguanylate cyclase	WP_038636150	XLOC_002522	0.0001	0.0007	-4.29
		Transcriptional regulator	WP_007926150	XLOC_002565	0.0069	0.0393	1.62
		Chemotaxis protein	WP_038630410	XLOC_002683	0.0006	0.0053	1.79
		Crp/Fnr family transcriptional regulator	WP_038630893	XLOC_003074	0.0001	0.0007	-2.54
		LuxR family transcriptional regulator	WP_009047547	XLOC_003077	0.0067	0.0384	-2.68
		Universal stress protein	WP_038583860	XLOC_003237	0.0032	0.0218	-1.61
		Carbon storage regulator homolog	2JPP	XLOC_003271	0.0006	0.0053	-1.70
		Protein kinase	WP_038631821	XLOC_003441	0.0001	0.0007	-3.13
		Diguanylate phosphodiesterase	WP_028684076	XLOC_003708	0.0001	0.0007	2.99
		Universal stress protein	WP_009049186	XLOC_003726	0.0001	0.0012	1.83
		Chemotaxis protein CheY	WP_038633404	XLOC_003913	0.0003	0.0032	2.10
		Histidine kinase	WP_009050536	XLOC_004404	0.0007	0.0060	1.73
		Chemotaxis protein	WP_016703630	XLOC_004556	0.0003	0.0032	1.85
		Histidine kinase	WP_016703743	XLOC_004687	0.0001	0.0007	2.38
<b>Intracellular trafficking, secretion, and vesicular transport</b>	<b>U</b>	Protein-export protein SecB	WP_009046533	XLOC_000111	0.0001	0.0012	-3.14
		GTP-binding protein TypA	WP_009046549	XLOC_000115	0.0001	0.0007	-1.54
		Pilus assembly protein	WP_038630520	XLOC_000222	0.0004	0.0040	-2.50
		ShlB family hemolysin secretion/activation protein	WP_041987697	XLOC_001737	0.0050	0.0310	2.24
		Filamentous hemagglutinin	WP_038634114	XLOC_001738	0.0001	0.0007	4.46
		Uncharacterized protein	WP_038636832	XLOC_001932	0.0003	0.0027	-3.51

		Type VI secretion protein ImpA	WP_009051593	XLOC_002378	0.0002	0.0017	-4.57
		Type VI secretion protein	WP_009051592	XLOC_002380	0.0001	0.0007	-6.15
		Type VI secretion protein	WP_007930278	XLOC_002383	0.0001	0.0007	-4.82
		Pilus assembly protein	WP_028681576	XLOC_002784	0.0001	0.0007	-2.42
		Preprotein translocase, SecG subunit	EIM15962	XLOC_002848	0.0001	0.0007	-1.89
		Preprotein translocase subunit YajC	WP_007929510	XLOC_004463	0.0001	0.0007	-2.08
		Elongation factor Tu	WP_016702644	XLOC_004749	0.0001	0.0007	-2.27
		Elongation factor G	WP_009051119	XLOC_004750	0.0001	0.0007	-1.65
		Elongation factor Tu	WP_016702644	XLOC_004761	0.0001	0.0007	-2.34
<b>Defense mechanisms</b>	<b>V</b>	DSBA oxidoreductase	WP_009046334	XLOC_000016	0.0015	0.0116	-1.56
		ADP-ribose diphosphatase	WP_007931715	XLOC_000087	0.0002	0.0017	1.91
		Uncharacterized protein	WP_047739265	XLOC_000272	0.0001	0.0007	-1.82
		Beta-lactamase	WP_016702077	XLOC_000507	0.0049	0.0305	2.14
		Peptide ABC transporter permease	WP_038635565	XLOC_002266	0.0047	0.0293	2.04
		Transporter	WP_009049437	XLOC_003839	0.0001	0.0007	-1.53
		Hemolysin D	WP_028682887	XLOC_003931	0.0001	0.0007	1.86
		Hemolysin D	WP_009049804	XLOC_003991	0.0076	0.0424	-4.93
		Peroxisredoxin OsmC	WP_009046246	XLOC_004802	0.0001	0.0007	-1.92
<b>Mobilome: prophages, transposons</b>	<b>X</b>	Tail protein	WP_038630732	XLOC_000451	0.0001	0.0007	-2.92
		Tail protein	WP_016701993	XLOC_000453	0.0001	0.0007	-2.23
		2-hydroxyacid dehydrogenase	WP_038630746	XLOC_000459	0.0001	0.0007	-1.56
		Uncharacterized protein	WP_009047313	XLOC_000460	0.0049	0.0303	-2.12
		Phage assembly protein	WP_009047314	XLOC_000461	0.0038	0.0250	-1.52
		Integrase	WP_051673118	XLOC_002838	0.0028	0.0194	-2.58

Appendix Table 4: Differentially expressed genes in PA23-*gacA* compared to WT.

COG category		Predicted function	Protein accession number (NCBI)	Gene locus	P-value	Q-value	log2 fold change
<b>Energy production and conversion</b>	<b>C</b>	Cytochrome B558 subunit A	WP_038630383	XLOC_000096	0.0001	0.0007	1.82
		Cytochrome C oxidase	WP_038630384	XLOC_000097	0.0003	0.0027	1.77
		Cytochrome C oxidase subunit IV	WP_016702591	XLOC_000099	0.0001	0.0007	4.33
		Azurin	WP_009046707	XLOC_000192	0.0001	0.0007	-1.84
		(2Fe-2S)-binding protein	WP_009048695	XLOC_001125	0.0001	0.0007	-3.11
		Polyphosphate kinase	WP_009048999	XLOC_001234	0.0001	0.0007	-2.04
		MFS transporter	WP_009049300	XLOC_001382	0.0001	0.0007	-2.71
		Glycerol kinase	WP_009050562	XLOC_001993	0.0001	0.0007	-2.68
		Glycerol-3-phosphate dehydrogenase	WP_016703438	XLOC_001995	0.0001	0.0007	-2.20
		Aldehyde dehydrogenase	WP_007924088	XLOC_002248	0.0001	0.0007	-1.88
		Inorganic pyrophosphatase	WP_007933577	XLOC_002255	0.0001	0.0007	-1.72
		Acetyl-CoA hydrolase	WP_038635895	XLOC_002404	0.0031	0.0210	-1.65
		Cytochrome B561	WP_007932535	XLOC_002512	0.0001	0.0007	-1.83
		CbbBc protein	EGH48104	XLOC_002636	0.0001	0.0007	-2.19
		(Fe-S)-binding protein	WP_016704739	XLOC_003546	0.0025	0.0177	-4.75
		Alcohol dehydrogenase	WP_038632862	XLOC_003788	0.0003	0.0027	2.01
		Flavin reductase	WP_016704027	XLOC_003884	0.0001	0.0007	-5.60
		FAD-dependent oxidoreductase	WP_038633983	XLOC_004126	0.0001	0.0007	2.27
		Ferredoxin-NADP reductase	WP_038635265	XLOC_004622	0.0001	0.0007	2.02
		Cytochrome oxidase subunit I	WP_009046264	XLOC_004792	0.0001	0.0007	-2.25
<b>Cell cycle control, cell division, chromosome partitioning</b>	<b>D</b>	Chromosome partitioning protein ParA	WP_009047182	XLOC_002968	0.0001	0.0007	-1.70
<b>Amino acid transport and metabolism</b>	<b>E</b>	MFS transporter	WP_028683803	XLOC_000103	0.0001	0.0007	2.01
		Amino acid ABC transporter substrate-binding protein	WP_009046527	XLOC_000108	0.0024	0.0174	1.67
		4-hydroxy-tetrahydrodipicolinate reductase	WP_038630583	XLOC_000280	0.0001	0.0012	2.37
		Amino acid ABC transporter substrate-binding protein	WP_025806519	XLOC_000375	0.0001	0.0007	1.67
		Aromatic amino acid transporter	WP_009042698	XLOC_000634	0.0001	0.0007	-4.72
		ATPase AAA	WP_038581185	XLOC_000635	0.0006	0.0057	1.62
		Inducer of phenazine B	WP_038631049	XLOC_000690	0.0001	0.0007	-5.27
		Serine/threonine protein kinase	WP_009048515	XLOC_001051	0.0001	0.0007	-2.19
		5-carboxymethyl-2-hydroxymuconate isomerase	WP_009049318	XLOC_001396	0.0003	0.0027	2.56
		Endonuclease	WP_038633790	XLOC_001653	0.0011	0.0093	-2.69
		Phospho-2-dehydro-3-deoxyheptonate aldolase	WP_009050120	XLOC_001745	0.0034	0.0226	1.52
		Amino acid APC transporter	WP_038634442	XLOC_001891	0.0001	0.0007	-3.35
		Arginine deiminase	WP_009050421	XLOC_001892	0.0001	0.0007	-4.44
		Ornithine carbamoyltransferase	WP_009050422	XLOC_001893	0.0001	0.0007	-3.57
		Carbamate kinase	WP_016703410	XLOC_001894	0.0001	0.0007	-2.51
		Glycine cleavage system H protein	WP_009050426	XLOC_001897	0.0020	0.0151	1.92
		Serine dehydratase	WP_028683221	XLOC_001899	0.0004	0.0040	1.87
		2,3-diketo-5-methylthio-1-phosphopentane phosphatase	WP_009050576	XLOC_002002	0.0001	0.0007	-2.12
		Phospho-2-dehydro-3-deoxyheptonate aldolase	WP_029526777	XLOC_002097	0.0001	0.0007	-7.48
		Homocysteine methyltransferase	WP_038635794	XLOC_002360	0.0077	0.0431	1.96
		Spermidine/putrescine ABC transporter substrate-binding protein	EIM16703	XLOC_002461	0.0001	0.0007	-1.81

		ABC transporter substrate-binding protein	WP_009051375	XLOC_002462	0.0001	0.0007	-2.53
		Sarcosine oxidase subunit delta	WP_009051222	XLOC_002539	0.0001	0.0007	-2.57
		Spermidine/putrescine ABC transporter substrate-binding protein	WP_016704058	XLOC_002567	0.0001	0.0012	-1.82
		Anthranilate phosphoribosyltransferase	WP_038636384	XLOC_002573	0.0033	0.0221	-1.55
		Peptide ABC transporter substrate-binding protein	WP_009046968	XLOC_002865	0.0001	0.0007	-1.90
		GCN5 family acetyltransferase	WP_016701734	XLOC_002897	0.0001	0.0007	-2.49
		Leucyl aminopeptidase (Aminopeptidase T)	WP_038630987	XLOC_003139	0.0001	0.0007	-4.53
		GntR family transcriptional regulator	WP_009047750	XLOC_003154	0.0003	0.0032	-2.26
		Lysine transporter LysE	WP_016702183	XLOC_003173	0.0002	0.0022	-3.18
		Aromatic amino acid aminotransferase	WP_038631265	XLOC_003258	0.0001	0.0012	1.69
		Arginine N-succinyltransferase	WP_009048183	XLOC_003302	0.0049	0.0305	-1.71
		Arginine N-succinyltransferase	WP_038631375	XLOC_003303	0.0024	0.0174	-2.39
		Porin	WP_016702750	XLOC_003304	0.0001	0.0007	-2.50
		Transporter	WP_038631522	XLOC_003362	0.0001	0.0007	-5.67
		Transcriptional regulator	WP_038632162	XLOC_003545	0.0001	0.0007	-4.43
		GNAT family acetyltransferase	WP_038632322	XLOC_003641	0.0001	0.0007	-1.69
		Diaminobutyrate--2-oxoglutarate aminotransferase	WP_038636648	XLOC_003702	0.0001	0.0007	4.26
		D-alanine/D-serine/glycine permease	WP_038634833	XLOC_004426	0.0001	0.0007	1.56
		HAD family hydrolase	WP_009050998	XLOC_004635	0.0013	0.0107	1.94
		GntR family transcriptional regulator	WP_016703162	XLOC_004880	0.0071	0.0402	-1.59
		Amino acid ABC transporter substrate-binding protein	WP_038636132	XLOC_004965	0.0009	0.0075	-1.76
		Aminoglycoside N(6')-acetyltransferase type 1	WP_038636173	XLOC_004987	0.0001	0.0007	3.19
<b>Nucleotide transport and metabolism</b>	<b>F</b>	Methionyl-tRNA formyltransferase	WP_025805252	XLOC_000693	0.0008	0.0072	-4.82
		Membrane protein	WP_016704578	XLOC_001100	0.0001	0.0007	-1.76
		Uncharacterized protein	WP_038631987	XLOC_001109	0.0001	0.0007	-3.19
		Probable allantoinase	WP_016704390	XLOC_001788	0.0014	0.0113	-2.92
		Uncharacterized protein	WP_009048212	XLOC_003313	0.0009	0.0075	-3.23
		Oxidoreductase	WP_016703255	XLOC_003842	0.0039	0.0252	-5.76
		Nucleoside diphosphate kinase	WP_007929529	XLOC_004450	0.0001	0.0007	-1.79
		Zinc-binding protein	WP_007930180	XLOC_005033	0.0080	0.0441	-1.78
		Acetyltransferase	WP_038633713	XLOC_001637	0.0001	0.0007	5.68
<b>Carbohydrate transport and metabolism</b>	<b>G</b>	DSBA oxidoreductase	WP_038630323	XLOC_000015	0.0001	0.0007	-2.09
		Shikimate kinase	WP_009041824	XLOC_000140	0.0001	0.0007	1.54
		2-dehydro-3-deoxy-6-phosphogalactonate aldolase	WP_038631144	XLOC_000752	0.0001	0.0007	3.00
		Chitinase	WP_009048115	XLOC_000870	0.0071	0.0402	-11.76
		MFS transporter	WP_060550016	XLOC_001368	0.0002	0.0017	-1.82
		Sorbosone dehydrogenase	WP_038634274	XLOC_001820	0.0005	0.0045	-2.40
		DeoR family transcriptional regulator	WP_009050563	XLOC_001994	0.0001	0.0007	-1.84
		Fructose-bisphosphate aldolase, class II	WP_003229012	XLOC_002521	0.0001	0.0007	-1.52
		Maltooligosyl trehalose synthase	WP_038632179	XLOC_003555	0.0005	0.0049	-1.63
		Siderophore biosynthesis protein SbnG	WP_009049138	XLOC_003700	0.0001	0.0007	2.84
		MFS transporter	WP_038632598	XLOC_003701	0.0001	0.0007	5.14
		Polysaccharide deacetylase	WP_038632649	XLOC_003724	0.0001	0.0007	2.48
		Multidrug resistance protein B	WP_009049803	XLOC_003990	0.0013	0.0107	-4.29
		Sugar ABC transporter ATPase	WP_016703424	XLOC_004339	0.0001	0.0007	-4.24
		Sugar ABC transporter permease	WP_009045231	XLOC_004340	0.0001	0.0007	-2.77
		Sugar ABC transporter substrate-binding protein	WP_009050405	XLOC_004341	0.0001	0.0007	-6.67



		Sugar isomerase	WP_016703422	XLOC_004342	0.0001	0.0007	-4.17
		Phosphoglucosmutase	WP_016703154	XLOC_004874	0.0001	0.0007	-1.69
		Sugar ABC transporter ATPase	WP_009050627	XLOC_004440	0.0001	0.0007	3.78
<b>Coenzyme transport and metabolism</b>	<b>H</b>	Omega amino acid--pyruvate aminotransferase	WP_038630538	XLOC_000233	0.0001	0.0007	-2.65
		Cyclic pyranopterin monophosphate synthase accessory protein	WP_009047097	XLOC_000372	0.0001	0.0007	2.13
		Molybdenum cofactor biosynthesis protein MoaE	WP_038630652	XLOC_000373	0.0052	0.0317	1.70
		Alpha/beta hydrolase	WP_009047789	XLOC_000715	0.0005	0.0049	1.75
		Delta-aminolevulinic acid dehydratase	WP_009048237	XLOC_000926	0.0014	0.0113	1.58
		(2Fe-2S)-binding protein	WP_016701774	XLOC_001019	0.0001	0.0007	-4.10
		Bifunctional protein FolD	WP_009049852	XLOC_001625	0.0004	0.0036	-1.58
		D-amino acid dehydrogenase	WP_009051552	XLOC_002400	0.0001	0.0007	2.03
		MFS transporter	WP_009047472	XLOC_003055	0.0001	0.0007	-6.69
		Methyltryptophan oxidase	WP_038631474	XLOC_003341	0.0079	0.0439	-1.72
		Tryptophan synthase subunit alpha	WP_009048364	XLOC_003383	0.0001	0.0007	-6.13
		Transporter	WP_009049200	XLOC_003732	0.0001	0.0007	5.49
		Uncharacterized protein	WP_009049581	XLOC_003902	0.0001	0.0007	-1.79
<b>Lipid transport and metabolism</b>	<b>I</b>	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	WP_038630718	XLOC_000439	0.0001	0.0007	-2.25
		Glucose-1-phosphate cytidyltransferase	WP_009047679	XLOC_000644	0.0001	0.0007	-1.94
		Beta-ketoacyl-ACP synthase	WP_009047691	XLOC_000648	0.0001	0.0007	-1.65
		Malonyl CoA-acyl carrier protein transacylase	WP_009047834	XLOC_000740	0.0010	0.0082	-1.51
		Enoyl-[acyl-carrier-protein] reductase [NADH]	WP_038631995	XLOC_001112	0.0001	0.0007	-2.56
		VdID	WP_009049637	XLOC_001532	0.0001	0.0007	2.84
		CAIB/BAIF family CoA transferase	WP_038634439	XLOC_001890	0.0003	0.0027	1.51
		Glycerol acyltransferase	WP_036985434	XLOC_002063	0.0002	0.0022	-1.67
		4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	WP_009050791	XLOC_002076	0.0001	0.0007	2.21
		Acetyl-CoA acetyltransferase	WP_038635179	XLOC_002140	0.0013	0.0103	2.00
		Glycerol acyltransferase	WP_009046219	XLOC_002323	0.0001	0.0007	-1.66
		Poly(R)-hydroxyalkanoic acid synthase	WP_009046586	XLOC_002690	0.0001	0.0007	-3.23
		Poly(R)-hydroxyalkanoic acid synthase	WP_009046588	XLOC_002692	0.0001	0.0007	-3.95
		3-oxoacyl-ACP synthase	WP_025805277	XLOC_003165	0.0001	0.0007	-1.94
		3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase	WP_007931558	XLOC_003166	0.0001	0.0007	-2.18
		Enoyl-CoA hydratase	WP_016704994	XLOC_003195	0.0002	0.0017	-1.82
		3-oxoacyl-[acyl-carrier-protein] synthase 2	WP_041988549	XLOC_003283	0.0059	0.0348	-1.59
		Glucose-methanol-choline oxidoreductase	WP_038632521	XLOC_003682	0.0005	0.0049	-3.29
		AMP-binding protein	WP_038633666	XLOC_003998	0.0001	0.0007	-2.36
		3-oxoacyl-ACP synthase	WP_009050008	XLOC_004115	0.0005	0.0045	-3.04
		Glycerol acyltransferase	WP_009050240	XLOC_004248	0.0001	0.0007	-2.40
		Long-chain fatty acid--CoA ligase	WP_009050391	XLOC_004332	0.0004	0.0040	-1.94
		Acyl-CoA dehydrogenase	WP_009051017	XLOC_004686	0.0001	0.0007	2.13
		Acyl-CoA dehydrogenase	WP_009046307	XLOC_004773	0.0007	0.0064	-1.55
<b>Translation, ribosomal structure and biogenesis</b>	<b>J</b>	Prolyl-tRNA synthetase	WP_016705004	XLOC_000023	0.0001	0.0007	2.57
		Polyribonucleotide nucleotidyltransferase	WP_038630587	XLOC_000295	0.0001	0.0007	-1.58
		Methyltransferase	WP_038630795	XLOC_000515	0.0044	0.0280	1.80
		GNAT family acetyltransferase	WP_038630820	XLOC_000531	0.0024	0.0171	-1.98
		NADPH-dependent 7-cyano-7-deazaguanine reductase	WP_009047982	XLOC_000827	0.0001	0.0007	2.62
		GNAT family acetyltransferase	WP_038634977	XLOC_002043	0.0001	0.0007	3.32
		Poly(A) polymerase I	WP_009050896	XLOC_002133	0.0001	0.0007	-1.83

		ATP-dependent protease	WP_009046589	XLOC_002694	0.0001	0.0007	3.34
		ATPase subunit HslU					
		Ribosome modulation factor	WP_023968495	XLOC_003210	0.0001	0.0007	-2.93
		GTP-binding protein	WP_028683953	XLOC_003650	0.0001	0.0007	-3.44
		GNAT family acetyltransferase	WP_009049809	XLOC_003995	0.0025	0.0177	-1.51
		tRNA 2-thiocytidine biosynthesis protein TtcA	WP_009050288	XLOC_004268	0.0008	0.0068	-2.22
<b>Transcription</b>	<b>K</b>	MarR family transcriptional regulator	WP_009046358	XLOC_000026	0.0010	0.0086	-1.57
		RNA polymerase sigma factor RpoS	WP_007924026	XLOC_000448	0.0001	0.0007	-3.69
		LacI family transcriptional regulator	WP_038631299	XLOC_000876	0.0030	0.0205	2.21
		RNA polymerase subunit sigma-70	WP_009048295	XLOC_000950	0.0001	0.0007	2.39
		Transcriptional regulator	WP_009048394	XLOC_000993	0.0071	0.0402	-2.95
		MarR family transcriptional regulator	WP_028682073	XLOC_001670	0.0003	0.0027	2.35
		Temperature acclimation protein B	WP_007931076	XLOC_001901	0.0001	0.0007	-3.34
		GntR family transcriptional regulator	WP_009050459	XLOC_001909	0.0001	0.0007	-3.39
		DEAD/DEAH box helicase	WP_038634571	XLOC_001931	0.0001	0.0007	1.79
		DNA-binding protein	WP_009050695	XLOC_002040	0.0001	0.0007	-2.54
		AsnC family transcriptional regulator	WP_009051665	XLOC_002346	0.0001	0.0007	2.51
		AraC family transcriptional regulator	WP_028683816	XLOC_002650	0.0001	0.0007	-2.63
		TetR family transcriptional regulator	WP_009046585	XLOC_002689	0.0001	0.0007	-2.24
		AraC family transcriptional regulator	WP_038630635	XLOC_002906	0.0092	0.0490	1.85
		LysR family transcriptional regulator	WP_016703226	XLOC_003093	0.0007	0.0060	2.19
		Cro/C1 family transcriptional regulator	WP_023968513	XLOC_003219	0.0001	0.0007	2.42
		LuxR family transcriptional regulator	WP_009048517	XLOC_003448	0.0001	0.0012	-2.70
		Uncharacterized protein	WP_009048686	XLOC_003507	0.0001	0.0012	-2.34
		Transcriptional regulator	WP_038632860	XLOC_003787	0.0020	0.0151	2.08
		Regulatory protein	WP_025804620	XLOC_003830	0.0002	0.0017	4.89
		Cro/C1 family transcriptional regulator	WP_009049820	XLOC_003999	0.0027	0.0188	2.37
		RNA polymerase subunit sigma-24	WP_038633746	XLOC_004046	0.0029	0.0202	1.54
		MarR family transcriptional regulator	WP_016702496	XLOC_004254	0.0001	0.0012	-2.22
		Transcriptional regulator	WP_009050371	XLOC_004323	0.0001	0.0012	-2.15
		PhzR	WP_038635113	XLOC_004545	0.0001	0.0007	-4.19
		Diguanylate cyclase	WP_038636938	XLOC_004679	0.0001	0.0007	1.99
		LysR family transcriptional regulator	WP_009051067	XLOC_004709	0.0025	0.0180	-1.53
		Uncharacterized protein	AFK70866	XLOC_000548	0.0053	0.0324	1.63
		Transcriptional regulator	WP_009049335	XLOC_001408	0.0001	0.0007	-1.92
		H-NS histone	WP_007931174	XLOC_001963	0.0001	0.0007	-1.87
		Transcriptional regulator	WP_038635479	XLOC_002229	0.0001	0.0007	-1.83
		Rha family transcriptional regulator	WP_038633014	XLOC_003829	0.0002	0.0017	2.37
<b>Replication, recombination and repair</b>	<b>L</b>	Integration host factor	WP_003213368	XLOC_002377	0.0001	0.0007	-1.90
		Deoxyribonuclease	WP_009048119	XLOC_003272	0.0038	0.0247	2.37
		Competence protein ComEA	WP_009050160	XLOC_004201	0.0001	0.0007	-6.14
		DNA-3-methyladenine glycosylase	WP_016703412	XLOC_004345	0.0036	0.0239	-1.60
<b>Cell wall/membrane/envelope biogenesis</b>	<b>M</b>	Transporter	WP_009046348	XLOC_000022	0.0039	0.0252	-3.74
		Galactonate dehydratase	WP_038631146	XLOC_000753	0.0002	0.0017	2.83
		Porin	WP_007927525	XLOC_000789	0.0001	0.0007	-2.31
		Membrane protein	WP_038632523	XLOC_001294	0.0002	0.0017	-2.14
		GNAT family acetyltransferase	WP_038634191	XLOC_001773	0.0005	0.0045	-2.33
		RND transporter MFP subunit	WP_038634985	XLOC_002046	0.0021	0.0157	2.29



		N-acetylmuramoyl-L-alanine amidase	WP_038635768	XLOC_002350	0.0058	0.0344	3.13
		Peptidase M23	WP_009051601	XLOC_002374	0.0054	0.0329	-2.25
		Membrane-bound lytic murein transglycosylase F	WP_009042308	XLOC_002944	0.0001	0.0007	-1.63
		Membrane protein	WP_028681745	XLOC_002967	0.0001	0.0007	-2.79
		Lipoprotein	AAT46075	XLOC_002981	0.0002	0.0022	-1.56
		Acyltransferase	WP_038636499	XLOC_003266	0.0016	0.0126	-1.99
		Channel protein TolC	WP_038636749	XLOC_003928	0.0001	0.0007	2.33
		Curli production assembly protein CsgG	WP_009049702	XLOC_003956	0.0001	0.0007	1.84
		Multidrug RND transporter	WP_038633661	XLOC_003992	0.0001	0.0007	-3.13
		Porin	WP_038634427	XLOC_004338	0.0001	0.0007	-2.95
		Membrane protein	WP_009050551	XLOC_004412	0.0003	0.0027	2.37
<b>Cell motility</b>	<b>N</b>	Flagellar basal body protein	WP_009047671	XLOC_000636	0.0001	0.0007	-2.01
		Flagellar basal-body rod protein FlgG	WP_009047672	XLOC_000637	0.0001	0.0007	-3.24
		Flagellar P-ring protein	WP_016702270	XLOC_000639	0.0001	0.0007	-1.56
		Flagellar hook-basal body complex protein FlhE	WP_007922633	XLOC_000658	0.0001	0.0007	-1.69
		Flagellar hook-length control protein	WP_038631019	XLOC_000665	0.0001	0.0007	-1.60
		Flagellar basal body protein FlhL	WP_023968317	XLOC_000666	0.0001	0.0007	-1.96
		Cobalamin biosynthesis protein CobQ	WP_007922498	XLOC_000681	0.0001	0.0007	-1.67
		Chemotaxis protein	WP_038631780	XLOC_001034	0.0001	0.0007	1.52
		General secretion pathway protein GspE	WP_009048770	XLOC_001151	0.0014	0.0110	2.62
		Chemotaxis protein	WP_038633971	XLOC_001695	0.0001	0.0007	1.60
		Chemotaxis protein	WP_038634350	XLOC_001852	0.0002	0.0017	2.10
		Chemotaxis protein	WP_038631529	XLOC_003363	0.0001	0.0007	-2.34
		Flagellar hook protein FlgE	WP_009050299	XLOC_004274	0.0001	0.0007	-2.77
		Basal-body rod modification protein FlgD	WP_009050300	XLOC_004275	0.0001	0.0007	-2.68
		Flagellar basal-body rod protein FlgC	WP_009050301	XLOC_004276	0.0001	0.0007	-2.17
		Flagellar basal body rod protein FlgB	WP_016702521	XLOC_004277	0.0001	0.0007	-2.80
<b>Posttranslational modification, protein turnover, chaperones</b>	<b>O</b>	Protein GrpE	WP_007920848	XLOC_000277	0.0001	0.0007	3.10
		Chaperone protein DnaK	WP_009046925	XLOC_000278	0.0001	0.0007	2.60
		Peptidyl-prolyl cis-trans isomerase	WP_007929845	XLOC_000341	0.0001	0.0007	-1.62
		Chaperone protein HtpG	WP_029526163	XLOC_000706	0.0001	0.0007	3.24
		Heat-shock protein	WP_007922004	XLOC_000845	0.0001	0.0007	4.44
		Peptide methionine sulfoxide reductase MsrA	WP_038631679	XLOC_001007	0.0004	0.0040	1.99
		Probable thiol peroxidase	WP_038632154	XLOC_001174	0.0001	0.0012	-2.24
		Peptidylprolyl isomerase	WP_007932676	XLOC_001529	0.0029	0.0202	3.38
		ATP-dependent protease subunit HslV	WP_007925866	XLOC_002695	0.0005	0.0045	2.78
		2-alkenal reductase	WP_025806436	XLOC_002887	0.0093	0.0496	1.65
		Peptidyl-prolyl cis-trans isomerase	WP_007920626	XLOC_003105	0.0016	0.0123	-3.77
		Glutathione S-transferase	WP_038631753	XLOC_003432	0.0001	0.0007	-4.16
		Alkyl hydroperoxide reductase	WP_007921754	XLOC_003780	0.0001	0.0007	-2.35
		Serine protease	WP_038633759	XLOC_004049	0.0001	0.0007	-1.74
		10 kDa chaperonin	WP_009050540	XLOC_004406	0.0001	0.0007	2.52
		Chaperone protein ClpB	WP_009050931	XLOC_004594	0.0001	0.0007	2.42
<b>Inorganic ion transport and metabolism</b>	<b>P</b>	Sulfite reductase	WP_009048796	XLOC_001169	0.0001	0.0007	-3.56
		ABC transporter substrate-binding protein	WP_009050227	XLOC_001802	0.0001	0.0007	-1.98
		Arsenate reductase	WP_009050284	XLOC_001827	0.0083	0.0456	-1.56
		FAD-binding protein	WP_038634838	XLOC_002009	0.0001	0.0007	-2.87
		TonB-dependent receptor	WP_038634844	XLOC_002011	0.0003	0.0027	4.56
		Phosphate-specific transport system accessory protein PhoU	WP_007928528	XLOC_002371	0.0018	0.0139	-2.22
		Peptide ABC transporter substrate-binding protein	WP_038630318	XLOC_002581	0.0001	0.0007	2.38

		TonB-dependent receptor	WP_038630381	XLOC_002642	0.0001	0.0007	1.94
		Iron dicitrate transport regulator FecR	WP_038630799	XLOC_003042	0.0001	0.0007	1.59
		Copper resistance protein CopC	WP_038632194	XLOC_003561	0.0001	0.0007	4.30
		Copper resistance protein CopB	WP_016704756	XLOC_003562	0.0001	0.0007	3.32
		Iron ABC transporter permease	WP_038632590	XLOC_003699	0.0001	0.0007	3.38
		TonB-dependent receptor	WP_038632604	XLOC_003703	0.0001	0.0007	3.57
		Catalase	WP_009050970	XLOC_004621	0.0007	0.0060	-2.92
		Iron-hydroxamate ABC transporter substrate-binding protein	WP_038635543	XLOC_004719	0.0003	0.0032	1.56
		Catalase	WP_016702645	XLOC_004732	0.0001	0.0007	-1.74
		SulP family inorganic anion transporter	WP_038635643	XLOC_004794	0.0051	0.0315	-1.59
		Carbonic anhydrase	WP_009046260	XLOC_004795	0.0001	0.0007	-2.72
		Zinc ABC transporter permease	WP_009051646	XLOC_004836	0.0003	0.0032	3.56
		Sodium:proton antiporter	WP_009051281	XLOC_004969	0.0011	0.0089	1.79
<b>Secondary metabolites biosynthesis, transport and catabolism</b>	<b>Q</b>	Poly(3-hydroxyalkanoate) granule-associated protein PhaF	WP_009046584	XLOC_000134	0.0001	0.0007	-3.47
		Serine 3-dehydrogenase	WP_016702821	XLOC_000985	0.0001	0.0007	-5.07
		NADP-dependent oxidoreductase	WP_009049328	XLOC_001403	0.0001	0.0007	-2.18
		Aldo/keto reductase	WP_038635010	XLOC_002053	0.0001	0.0007	-1.54
		Isochorismatase	WP_009050821	XLOC_002098	0.0001	0.0007	-7.58
		Phenazine biosynthesis protein	WP_038635119	XLOC_002100	0.0001	0.0007	-8.23
		Poly(3-hydroxyalkanoate) depolymerase	WP_009046587	XLOC_002691	0.0001	0.0007	-1.58
		NADP-dependent oxidoreductase	WP_038631084	XLOC_003170	0.0055	0.0333	-1.89
<b>General function prediction only</b>	<b>R</b>	Glyoxalase	WP_009046877	XLOC_000259	0.0001	0.0007	3.16
		Carbon-nitrogen hydrolase LrgA	WP_038630611	XLOC_000324	0.0002	0.0022	1.99
		PsiE family protein	WP_016701724	XLOC_000348	0.0056	0.0338	1.65
		Ketosteroid isomerase	WP_009042237	XLOC_000360	0.0015	0.0116	-1.64
		Arylsulfate sulfotransferase	WP_009047112	XLOC_000380	0.0001	0.0007	-2.15
		Flagellar biosynthesis regulator FlhF	WP_009042529	XLOC_000517	0.0014	0.0113	-1.51
		Chitin-binding protein	WP_009047715	XLOC_000674	0.0001	0.0007	-2.04
		Na <sup>+</sup> /H <sup>+</sup> dicarboxylate symporter	WP_009048114	XLOC_000869	0.0001	0.0007	-9.06
		Sugar deacetylase	WP_009048348	XLOC_000974	0.0001	0.0007	1.85
		ABC transporter	WP_016702950	XLOC_001239	0.0059	0.0348	-4.96
		Methyltransferase	WP_016702952	XLOC_001241	0.0001	0.0007	-3.34
		Amidohydrolase	WP_038633459	XLOC_001531	0.0001	0.0007	1.98
		2,3-dihydro-3-hydroxyanthranilate isomerase	WP_038633980	XLOC_001697	0.0004	0.0040	-5.16
		Rhs family protein	WP_016703606	XLOC_002099	0.0002	0.0022	-8.04
		4-carboxymuconolactone decarboxylase	WP_038630348	XLOC_002611	0.0001	0.0007	-7.20
		Carboxylesterase	WP_009046984	XLOC_002874	0.0014	0.0110	3.05
		ATPase AAA	WP_009047103	XLOC_002935	0.0004	0.0036	-2.58
		Methyltransferase	WP_016705272	XLOC_003095	0.0042	0.0267	-1.70
		Phosphoribosyl transferase	WP_038632359	XLOC_003651	0.0001	0.0007	-3.21
		Amidohydrolase	WP_038632651	XLOC_003725	0.0018	0.0139	2.11
		Peptidase C39	WP_009049582	XLOC_003903	0.0001	0.0012	-1.56
		Membrane protein	WP_038633471	XLOC_003930	0.0001	0.0007	2.77
		Isomerase	WP_007929309	XLOC_003971	0.0001	0.0007	-1.61
		Molybdenum cofactor sulfurase	WP_038633872	XLOC_004090	0.0019	0.0142	-1.92
		Uncharacterized protein	WP_038634095	XLOC_004156	0.0069	0.0396	1.50
		Transcriptional regulator	WP_009051600	XLOC_004857	0.0068	0.0389	-1.84
		Ribosomal subunit interface protein	WP_038635883	XLOC_004878	0.0001	0.0007	-1.58
		Nuclease	WP_009047054	XLOC_000362	0.0001	0.0007	2.25
			WP_028683814	XLOC_002657	0.0003	0.0032	-4.48

Function unknown	S						
		Peptidoglycan-binding protein LysM	WP_009046477	XLOC_000086	0.0001	0.0007	-2.07
		Uncharacterized protein	WP_009042934	XLOC_000791	0.0001	0.0007	1.72
		Membrane protein	WP_009048116	XLOC_000871	0.0003	0.0032	-2.03
		Uncharacterized protein	WP_038632150	XLOC_001170	0.0001	0.0007	-2.88
		Acetyltransferase	WP_038634752	XLOC_001976	0.0010	0.0086	1.91
		Lon protease	WP_038634854	XLOC_002017	0.0001	0.0007	2.37
		Cytochrome D ubiquinol oxidase subunit III	WP_007928428	XLOC_002055	0.0001	0.0007	-1.70
		Peptidase C13	WP_038635515	XLOC_002247	0.0002	0.0022	-1.72
		Membrane protein	WP_038635748	XLOC_002347	0.0001	0.0007	1.57
		Uncharacterized protein	WP_023967792	XLOC_002375	0.0001	0.0007	-1.69
		DNA-binding protein	WP_038636014	XLOC_002469	0.0005	0.0049	-1.75
		Sulfurase	WP_038636332	XLOC_002557	0.0001	0.0007	3.23
		Tail protein	WP_009046343	XLOC_002592	0.0016	0.0126	-2.51
		Sensor histidine kinase	KPX62819	XLOC_002871	0.0002	0.0017	-1.78
		UPF0225 protein EY04_05815	WP_028681815	XLOC_003010	0.0031	0.0210	-2.30
		Porin	WP_025808788	XLOC_003710	0.0001	0.0007	-2.10
		Lipoprotein	WP_016705380	XLOC_003955	0.0001	0.0007	2.29
		Spore coat protein	WP_038633677	XLOC_004009	0.0001	0.0007	-1.89
		Phospholipid-binding protein	WP_038633836	XLOC_004078	0.0059	0.0351	1.97
		Methyltransferase	WP_007927856	XLOC_004103	0.0041	0.0263	-1.61
		DoxX family protein	WP_009050141	XLOC_004188	0.0060	0.0355	-1.89
		Fusaric acid resistance protein	WP_016703861	XLOC_004334	0.0008	0.0072	-3.47
		Type IV secretion protein Rhs	WP_038634583	XLOC_004369	0.0001	0.0007	-2.98
		Phospholipid-binding protein	WP_009050906	XLOC_004586	0.0001	0.0007	-2.75
		ABC transporter substrate-binding protein	WP_009051009	XLOC_004641	0.0029	0.0200	3.17
		Glycosyl hydrolase	WP_038636404	XLOC_000021	0.0001	0.0007	-3.72
		RebB like protein	WP_007930328	XLOC_000038	0.0007	0.0060	-4.50
		R body protein RebB-like protein	WP_009046381	XLOC_000042	0.0001	0.0007	-8.40
		R body protein RebB-like protein	WP_009046382	XLOC_000043	0.0001	0.0007	-9.88
		Uncharacterized protein	EIM12867	XLOC_000056	0.0001	0.0007	-3.90
		Poly(3-hydroxyalkanoate) granule-associated protein PhaI	WP_009046583	XLOC_000133	0.0001	0.0007	-6.83
		Ribonuclease 3	WP_009042306	XLOC_000387	0.0028	0.0194	-2.26
		Hydrolase/acyltransferase	EJZ57002	XLOC_000535	0.0007	0.0060	-6.55
		Uncharacterized protein	WP_038630933	XLOC_000599	0.0001	0.0007	2.36
		Putative hemagglutinin (DUF637)	EJL96817	XLOC_000612	0.0005	0.0045	-1.95
		Uncharacterized protein	WP_009042857	XLOC_000745	0.0067	0.0387	-2.19
		CrfX protein	WP_007927518	XLOC_000786	0.0001	0.0007	-2.71
		Uncharacterized protein	WP_009048113	XLOC_000868	0.0046	0.0288	-1.87
		Polyketide cyclase / dehydrase and lipid transport	WP_050899140	XLOC_001173	0.0001	0.0007	-5.47
		Membrane protein	WP_038632611	XLOC_001325	0.0011	0.0089	-2.01
		Bifunctional pyrazinamidase/nicotinamidase	ADO14993	XLOC_001373	0.0004	0.0036	-2.87
		Uncharacterized protein	WP_038633414	XLOC_001519	0.0001	0.0007	4.98
		Uncharacterized protein	WP_009045046	XLOC_001778	0.0042	0.0270	2.04
		Methyltransferase	WP_038634569	XLOC_001930	0.0001	0.0007	1.93
		Porin	WP_038634688	XLOC_001955	0.0001	0.0007	-4.18
		Type III chaperone ShcE	WP_038635350	XLOC_002198	0.0007	0.0064	2.24
		Uncharacterized protein	WP_025807035	XLOC_002408	0.0003	0.0032	-2.38
		Pyroloquinoline quinone biosynthesis protein PqqE	WP_009051193	XLOC_002552	0.0001	0.0007	1.92
		Polyketide cyclase	WP_038630319	XLOC_002583	0.0001	0.0007	-2.66
		Uncharacterized protein	WP_038630325	XLOC_002590	0.0001	0.0007	-2.39
		Uncharacterized protein	WP_038630505	XLOC_002769	0.0016	0.0126	1.70
		Phosphatidylcholine-hydrolyzing phospholipase	WP_009046978	XLOC_002873	0.0001	0.0007	-2.35
		Uncharacterized protein	WP_038636463	XLOC_003065	0.0001	0.0007	-1.58
		Acetyltransferase	WP_038630891	XLOC_003073	0.0001	0.0007	-1.72
		Membrane protein	WP_038631867	XLOC_003461	0.0012	0.0096	-1.62
		Oxidase	WP_038631869	XLOC_003462	0.0001	0.0007	-2.11
		Potassium channel protein	WP_038632026	XLOC_003505	0.0001	0.0007	-2.55
		Short-chain dehydrogenase	WP_016704711	XLOC_003541	0.0001	0.0007	6.12

		Cbb3-type cytochrome c oxidase subunit I	WP_009048795	XLOC_003544	0.0001	0.0007	-5.06
		Uncharacterized protein	WP_038632169	XLOC_003551	0.0001	0.0007	-4.47
		Lipoprotein	WP_038632271	XLOC_003611	0.0001	0.0007	-4.41
		Uncharacterized protein	WP_009049346	XLOC_003785	0.0003	0.0027	2.32
		Uncharacterized protein	WP_009049553	XLOC_003894	0.0004	0.0040	-3.31
		Cytotoxin	WP_038633468	XLOC_003929	0.0001	0.0007	2.72
		Uncharacterized protein	WP_038633491	XLOC_003935	0.0001	0.0007	-2.96
		Membrane protein	WP_038633841	XLOC_004083	0.0001	0.0007	-4.05
		Dialkylrecorsinol condensing enzyme	WP_009050009	XLOC_004116	0.0001	0.0007	-2.37
		Quinohemoprotein amine dehydrogenase	WP_038633961	XLOC_004119	0.0039	0.0255	-1.68
		Uncharacterized protein	CDI89872	XLOC_004224	0.0039	0.0252	-4.64
		Uncharacterized protein	WP_009050754	XLOC_004521	0.0001	0.0007	-2.49
		Uncharacterized protein	WP_009050755	XLOC_004522	0.0001	0.0007	-2.73
		Glutamine synthetase	EIM18812	XLOC_004837	0.0071	0.0402	3.19
		Secondary thiamine-phosphate synthase enzyme	EIM17029	XLOC_004893	0.0069	0.0396	-2.47
<b>Signal transduction mechanisms</b>	<b>T</b>	Uncharacterized protein	WP_009046954	XLOC_000306	0.0001	0.0007	1.56
		Histidine kinase	WP_016702256	XLOC_000656	0.0001	0.0007	-2.25
		Fis family transcriptional regulator	WP_038631011	XLOC_000657	0.0001	0.0007	-1.82
		Chemotaxis protein CheY	WP_038631017	XLOC_000663	0.0001	0.0007	-3.02
		Diguanylate cyclase	WP_009049109	XLOC_001313	0.0001	0.0007	-1.81
		Histidine kinase	WP_016705324	XLOC_001538	0.0047	0.0295	2.89
		Diguanylate cyclase	WP_016701868	XLOC_001577	0.0002	0.0017	-2.47
		AraC family transcriptional regulator	WP_028683224	XLOC_001906	0.0059	0.0348	-2.74
		Acyl-homoserine-lactone synthase	WP_038635110	XLOC_002094	0.0001	0.0007	-5.31
		Diguanylate cyclase	WP_038636150	XLOC_002522	0.0001	0.0007	-4.23
		Diguanylate cyclase	WP_038575258	XLOC_002554	0.0039	0.0252	3.01
		DeoR family transcriptional regulator	WP_016702287	XLOC_003124	0.0057	0.0342	-2.25
		Histidine kinase	WP_038631277	XLOC_003260	0.0001	0.0007	-1.87
		Protein kinase	WP_038631821	XLOC_003441	0.0001	0.0007	-1.71
		Diguanylate phosphodiesterase	WP_028684076	XLOC_003708	0.0001	0.0007	1.65
		Universal stress protein	WP_009049186	XLOC_003726	0.0002	0.0017	1.80
		Signal peptide protein	WP_038633336	XLOC_003900	0.0094	0.0499	-1.81
		Histidine kinase	WP_038636745	XLOC_003927	0.0001	0.0007	2.55
		Chemotaxis protein CheY	WP_009049747	XLOC_003975	0.0001	0.0007	-2.52
		Histidine kinase	WP_009049953	XLOC_004086	0.0006	0.0057	-1.92
		Histidine kinase	WP_016703743	XLOC_004687	0.0001	0.0007	2.00
<b>Intracellular trafficking, secretion, and vesicular transport</b>	<b>U</b>	Protein-export protein SecB	WP_009046533	XLOC_000111	0.0001	0.0007	-3.38
		ShlB family hemolysin secretion/activation protein	WP_041987697	XLOC_001737	0.0049	0.0303	2.30
		Secretin	WP_029526712	XLOC_001903	0.0001	0.0007	-2.33
		Type VI secretion protein ImpA	WP_009051593	XLOC_002378	0.0001	0.0007	-3.81
		Type VI secretion protein TssB2	WP_007930286	XLOC_002379	0.0056	0.0336	-7.60
		Type VI secretion protein	WP_009051592	XLOC_002380	0.0001	0.0007	-6.24
		Type VI secretion protein	WP_007930278	XLOC_002383	0.0001	0.0007	-4.93
		ATPase	WP_038630518	XLOC_002782	0.0001	0.0007	-2.52
		Type VI secretion protein	WP_038633332	XLOC_003899	0.0001	0.0007	-2.85
		Elongation factor Tu	WP_016702644	XLOC_004749	0.0001	0.0007	-2.40
		Biopolymer transporter ExbB	WP_009051571	XLOC_004868	0.0001	0.0007	-1.53
		Filamentous hemagglutinin	WP_038634114	XLOC_001738	0.0001	0.0007	4.15
		Uncharacterized protein	WP_038635388	XLOC_002204	0.0001	0.0007	1.94
<b>Defense mechanisms</b>	<b>V</b>	DSBA oxidoreductase	WP_009046334	XLOC_000016	0.0001	0.0007	-2.45
		Uncharacterized protein	WP_047739265	XLOC_000272	0.0001	0.0007	-1.57
		Cephalosporin hydroxylase	WP_038631001	XLOC_000645	0.0006	0.0053	-1.56
		Transporter	WP_038632752	XLOC_001359	0.0001	0.0007	2.17
		ABC transporter	WP_038630975	XLOC_003123	0.0001	0.0007	-2.64
		Hemolysin D	WP_028682887	XLOC_003931	0.0001	0.0007	2.46
		Hemolysin D	WP_009049804	XLOC_003991	0.0027	0.0188	-4.64
<b>Extracellular structures</b>	<b>W</b>	Molecular chaperone	WP_028682799	XLOC_004005	0.0002	0.0022	-3.08

<b>Mobilome: prophages, transposons</b>	<b>X</b>	Tail protein	WP_038630732	XLOC_000451	0.0008	0.0072	-1.95
		Phage assembly protein	WP_009047314	XLOC_000461	0.0047	0.0293	-1.67

Appendix Table 5: Differentially expressed genes in PA23-*gacS* compared to WT.

COG category		Predicted function	Protein accession number (NCBI)	Gene locus	P-value	Q-value	log2 fold change		
<b>Energy production and conversion</b>	<b>C</b>	Flavin reductase	WP_016704027	XLOC_003884	0.0001	0.0007	-6.07		
		(Fe-S)-binding protein	WP_016704739	XLOC_003546	0.0049	0.0303	-5.13		
		MFS transporter	WP_009049300	XLOC_001382	0.0001	0.0007	-3.52		
		Glycerol kinase	WP_009050562	XLOC_001993	0.0001	0.0007	-2.86		
		Glycerol-3-phosphate dehydrogenase	WP_016703438	XLOC_001995	0.0001	0.0007	-2.73		
		2-hydroxyacid dehydrogenase	WP_038634294	XLOC_001825	0.0013	0.0107	-2.62		
		Cytochrome B561	WP_007932535	XLOC_002512	0.0001	0.0007	-2.52		
		Cytochrome oxidase subunit I	WP_009046264	XLOC_004792	0.0001	0.0007	-2.50		
		Azurin	WP_009046707	XLOC_000192	0.0001	0.0007	-2.23		
		Aldehyde dehydrogenase	WP_038636068	XLOC_004944	0.0001	0.0007	-2.04		
		MFS transporter	WP_009046266	XLOC_004790	0.0002	0.0022	-1.99		
		Cytochrome D ubiquinol oxidase subunit I	WP_009050974	XLOC_004623	0.0005	0.0049	-1.95		
		Cysteine synthase	WP_009046265	XLOC_004791	0.0072	0.0409	-1.94		
		ATP synthase subunit b	WP_007924504	XLOC_002333	0.0001	0.0007	-1.93		
		Cytochrome c oxidase subunit 2	WP_009046263	XLOC_004793	0.0001	0.0007	-1.80		
		ATP synthase subunit beta	WP_009051697	XLOC_002335	0.0001	0.0007	-1.80		
		ATP synthase gamma chain	WP_007924507	XLOC_002334	0.0001	0.0007	-1.80		
		CbbBc protein	EGH48104	XLOC_002636	0.0001	0.0007	-1.68		
		2Fe-2S ferredoxin	WP_007929527	XLOC_004452	0.0002	0.0017	-1.65		
		Ferredoxin-NADP reductase	KWV82315	XLOC_000473	0.0001	0.0007	-1.54		
		Tricarboxylic transport membrane protein	WP_028681770	XLOC_003059	0.0071	0.0404	1.63		
		NADH-quinone oxidoreductase subunit B	WP_007929186	XLOC_001593	0.0001	0.0007	1.68		
		Alcohol dehydrogenase	WP_038632862	XLOC_003788	0.0010	0.0082	1.75		
		Ferredoxin-NADP reductase	WP_038635265	XLOC_004622	0.0001	0.0007	2.03		
		FAD-dependent oxidoreductase	WP_038633983	XLOC_004126	0.0001	0.0007	2.33		
		Membrane protein	WP_038632572	XLOC_003693	0.0001	0.0007	2.83		
		<b>Cell cycle control, cell division, chromosome partitioning</b>	<b>D</b>	Chromosome partitioning protein ParA	WP_009047182	XLOC_002968	0.0001	0.0007	-2.97
				Polyketide cyclase	WP_009048832	XLOC_003563	0.0017	0.0133	-2.40
				Cell division protein FtsA	WP_007922131	XLOC_004491	0.0001	0.0007	1.63
		<b>Amino acid transport and metabolism</b>	<b>E</b>	Phospho-2-dehydro-3-deoxyheptonate aldolase	WP_029526777	XLOC_002097	0.0002	0.0022	-8.64
Leucyl aminopeptidase (Aminopeptidase T)	WP_038630987			XLOC_003139	0.0003	0.0032	-7.18		
Inducer of phenazine B	WP_038631049			XLOC_000690	0.0001	0.0007	-5.67		
Transcriptional regulator	WP_038632162			XLOC_003545	0.0001	0.0007	-5.42		
Aromatic amino acid transporter	WP_009042698			XLOC_000634	0.0001	0.0007	-5.22		
Amino acid APC transporter	WP_038634442			XLOC_001891	0.0001	0.0007	-4.29		
Lysine transporter LysE	WP_016702183			XLOC_003173	0.0006	0.0053	-4.09		
Ornithine carbamoyltransferase	WP_009050422			XLOC_001893	0.0001	0.0007	-3.94		
Arginine deiminase	WP_009050421			XLOC_001892	0.0001	0.0007	-3.67		
Arginine N-succinyltransferase	WP_038631375			XLOC_003303	0.0081	0.0448	-3.29		
Carbamate kinase	WP_016703410			XLOC_001894	0.0001	0.0007	-3.07		
Arginine N-succinyltransferase	WP_009048183			XLOC_003302	0.0047	0.0295	-2.92		
Porin	WP_016702750			XLOC_003304	0.0001	0.0007	-2.82		
Endonuclease	WP_038633790			XLOC_001653	0.0004	0.0040	-2.81		
Sarcosine oxidase subunit delta	WP_009051222			XLOC_002539	0.0023	0.0166	-2.62		
Urocanate hydratase	WP_016704424			XLOC_000119	0.0001	0.0007	-2.28		
ABC transporter	WP_025806014			XLOC_001996	0.0001	0.0007	-2.20		
Serine/threonine protein kinase	WP_009048515			XLOC_001051	0.0001	0.0007	-2.17		
Glycine/betaine ABC transporter substrate-binding protein	WP_009051250			XLOC_004986	0.0004	0.0040	-2.14		
UPF0056 membrane protein	WP_016704550			XLOC_003477	0.0011	0.0093	-2.12		
GntR family transcriptional regulator	WP_009047750			XLOC_003154	0.0007	0.0064	-2.08		
Acetyltransferase, GNAT family	EIM14774			XLOC_001427	0.0012	0.0096	-1.97		
Peptide ABC transporter substrate-binding protein	WP_009046968			XLOC_002865	0.0001	0.0012	-1.89		
Amino acid dehydrogenase	WP_007932382			XLOC_000357	0.0001	0.0007	-1.85		



		Spermidine acetyltransferase	WP_051673124	XLOC_000889	0.0001	0.0012	-1.78
		Amino acid ABC transporter substrate-binding protein	WP_038636132	XLOC_004965	0.0013	0.0107	-1.76
		Sarcosine oxidase subunit beta	WP_009051223	XLOC_002538	0.0032	0.0216	-1.70
		Amino acid ABC transporter substrate-binding protein	WP_038634078	XLOC_004154	0.0009	0.0079	-1.64
		Cysteine desulfurase IscS	WP_009050642	XLOC_004457	0.0001	0.0007	-1.60
		Anthranilate phosphoribosyltransferase	WP_038636384	XLOC_002573	0.0001	0.0012	-1.59
		Arginine biosynthesis bifunctional protein ArgJ	WP_038634693	XLOC_004382	0.0010	0.0086	1.51
		HAD family hydrolase	WP_009050998	XLOC_004635	0.0052	0.0320	1.63
		Glycine cleavage system H protein	WP_009050426	XLOC_001897	0.0045	0.0283	1.65
		DNA-binding protein	WP_009048753	XLOC_003537	0.0056	0.0338	1.77
		Gamma-aminobutyrate transporter	WP_009046515	XLOC_000101	0.0001	0.0007	1.88
		Threonine aldolase	WP_009050320	XLOC_004291	0.0001	0.0007	1.90
		2,4-diaminobutyrate 4-aminotransferase	WP_038634057	XLOC_004146	0.0001	0.0007	1.94
		Taurine ABC transporter permease	WP_038630365	XLOC_002629	0.0001	0.0007	2.04
		4-aminobutyrate aminotransferase	WP_009046384	XLOC_000045	0.0001	0.0007	2.10
		Amino acid ABC transporter substrate-binding protein	WP_025806519	XLOC_000375	0.0001	0.0007	2.60
		4-hydroxy-tetrahydrodipicolinate reductase	WP_038630583	XLOC_000280	0.0001	0.0007	2.66
		MFS transporter	WP_028683803	XLOC_000103	0.0001	0.0007	2.82
		Aminoglycoside N(6)-acetyltransferase type 1	WP_038636173	XLOC_004987	0.0001	0.0007	3.00
		Diaminobutyrate--2-oxoglutarate aminotransferase	WP_038636648	XLOC_003702	0.0001	0.0007	6.89
<b>Nucleotide transport and metabolism</b>	<b>F</b>	Oxidoreductase	WP_016703255	XLOC_003842	0.0039	0.0252	-5.76
		Uncharacterized protein	WP_038631987	XLOC_001109	0.0001	0.0007	-5.33
		Uncharacterized protein	WP_009048212	XLOC_003313	0.0005	0.0045	-2.91
		Uncharacterized protein	WP_009048128	XLOC_000878	0.0001	0.0007	-2.10
		Nucleoside diphosphate kinase	WP_007929529	XLOC_004450	0.0001	0.0007	-1.54
		Phosphoribosylglycinamide formyltransferase 2	WP_038630671	XLOC_000394	0.0001	0.0007	1.51
		Amidotransferase	WP_016704995	XLOC_000767	0.0001	0.0007	1.78
<b>Carbohydrate transport and metabolism</b>	<b>G</b>	Chitinase	WP_009048115	XLOC_000870	0.0001	0.0012	-11.14
		Sugar ABC transporter substrate-binding protein	WP_009050405	XLOC_004341	0.0001	0.0007	-6.65
		Chitinase	WP_009049243	XLOC_003753	0.0001	0.0007	-6.44
		Sugar ABC transporter ATPase	WP_016703424	XLOC_004339	0.0009	0.0079	-5.42
		Sugar isomerase	WP_016703422	XLOC_004342	0.0001	0.0007	-4.06
		Sugar ABC transporter permease	WP_009045231	XLOC_004340	0.0001	0.0012	-3.68
		Multidrug resistance protein B	WP_009049803	XLOC_003990	0.0003	0.0027	-3.50
		Glyceraldehyde-3-phosphate dehydrogenase	WP_009050409	XLOC_001882	0.0001	0.0007	-3.35
		Glycerol uptake facilitator GlpF	WP_009050561	XLOC_001992	0.0050	0.0310	-3.13
		Sorbosone dehydrogenase	WP_038634274	XLOC_001820	0.0002	0.0017	-2.91
		Sucrase	WP_016703507	XLOC_002037	0.0036	0.0237	-2.64
		sn-glycerol-3-phosphate transporter	WP_007926732	XLOC_004911	0.0002	0.0017	-2.11
		MFS transporter	WP_060550016	XLOC_001368	0.0001	0.0007	-2.07
		2-methylcitrate dehydratase	WP_038631169	XLOC_000781	0.0001	0.0007	-1.93
		Aquaporin Z	WP_009047606	XLOC_003103	0.0001	0.0007	-1.86
		Membrane-bound PQQ-dependent dehydrogenase, glucose/quinone/shikimate family	WP_038575289	XLOC_002559	0.0006	0.0057	-1.78
		Methylglyoxal synthase	WP_023966804	XLOC_001883	0.0040	0.0260	-1.70
		DeoR family transcriptional regulator	WP_009050563	XLOC_001994	0.0001	0.0007	-1.53
		AP endonuclease	WP_029526986	XLOC_001378	0.0001	0.0007	1.56
		2-dehydro-3-deoxy-6-phosphogalactonate aldolase	WP_038631144	XLOC_000752	0.0001	0.0007	2.42
		Sugar ABC transporter ATPase	WP_009050627	XLOC_004440	0.0001	0.0007	2.74

		Siderophore biosynthesis protein SbnG	WP_009049138	XLOC_003700	0.0001	0.0007	5.36		
		MFS transporter	WP_038632598	XLOC_003701	0.0001	0.0007	7.43		
<b>Coenzyme transport and metabolism</b>	<b>H</b>	Tryptophan synthase subunit alpha	WP_009048364	XLOC_003383	0.0001	0.0007	-7.11		
		MFS transporter	WP_009047472	XLOC_003055	0.0001	0.0007	-7.02		
		(2Fe-2S)-binding protein	WP_016701774	XLOC_001019	0.0001	0.0007	-5.00		
		Uncharacterized protein	WP_009049581	XLOC_003902	0.0001	0.0007	-2.17		
		Omega amino acid--pyruvate aminotransferase	WP_038630538	XLOC_000233	0.0001	0.0007	-2.03		
		Amine oxidase	WP_038575284	XLOC_005020	0.0001	0.0007	-1.90		
		Alpha/beta hydrolase	WP_009047789	XLOC_000715	0.0006	0.0057	1.69		
		Dihydropteroate synthase	WP_009046931	XLOC_000285	0.0001	0.0007	1.71		
		Molybdenum cofactor biosynthesis protein MoaE	WP_038630652	XLOC_000373	0.0041	0.0263	1.74		
		D-amino acid dehydrogenase	WP_009051552	XLOC_002400	0.0002	0.0017	1.76		
		Damage-inducible protein CinA	WP_023969838	XLOC_000467	0.0073	0.0411	1.90		
		Cyclic pyranopterin monophosphate synthase accessory protein	WP_009047097	XLOC_000372	0.0001	0.0007	2.44		
		Pyridoxine 5'-phosphate synthase	WP_038630664	XLOC_000389	0.0001	0.0012	2.74		
		Transporter	WP_009049200	XLOC_003732	0.0001	0.0007	5.67		
<b>Lipid transport and metabolism</b>	<b>I</b>	Phosphatidylserine decarboxylase proenzyme	WP_038631548	XLOC_000962	0.0001	0.0007	-5.67		
		Glucose-methanol-choline oxidoreductase	WP_038632521	XLOC_003682	0.0027	0.0191	-3.92		
		Poly(R)-hydroxyalkanoic acid synthase	WP_009046586	XLOC_002690	0.0001	0.0007	-3.78		
		Glycerol acyltransferase	WP_009050240	XLOC_004248	0.0001	0.0007	-3.73		
		Poly(R)-hydroxyalkanoic acid synthase	WP_009046588	XLOC_002692	0.0001	0.0007	-3.69		
		3-oxoacyl-ACP synthase	WP_009050008	XLOC_004115	0.0008	0.0068	-3.20		
		Glycerol acyltransferase	WP_009046219	XLOC_002323	0.0001	0.0007	-2.90		
		Long-chain fatty acid--CoA ligase	WP_009050391	XLOC_004332	0.0001	0.0007	-2.72		
		Isovaleryl-CoA dehydrogenase	WP_016701919	XLOC_001609	0.0065	0.0378	-2.13		
		Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	WP_038630718	XLOC_000439	0.0001	0.0007	-2.13		
		AMP-binding protein	WP_038633666	XLOC_003998	0.0051	0.0315	-2.12		
		Enoyl-[acyl-carrier-protein] reductase [NADH]	WP_038631995	XLOC_001112	0.0001	0.0007	-2.07		
		Short-chain dehydrogenase	WP_038636192	XLOC_004998	0.0001	0.0007	-2.03		
		Acetyl-coenzyme A synthetase	WP_009050330	XLOC_004302	0.0001	0.0007	-1.99		
		Acyl-CoA dehydrogenase	WP_016703514	XLOC_004475	0.0079	0.0439	-1.98		
		Malonyl CoA-acyl carrier protein transacylase	WP_009047834	XLOC_000740	0.0004	0.0040	-1.59		
		Fatty acid methyltransferase	WP_016703983	XLOC_002348	0.0001	0.0007	-1.53		
		Enoyl-CoA hydratase	WP_016704994	XLOC_003195	0.0004	0.0040	-1.51		
		Peptide synthetase	WP_038633921	XLOC_004110	0.0001	0.0007	1.57		
		Peptide synthase	WP_038634075	XLOC_004152	0.0001	0.0007	2.11		
		3-hydroxyacyl-CoA dehydrogenase	WP_038631158	XLOC_000766	0.0001	0.0007	2.13		
		Acyl-CoA dehydrogenase	WP_009051017	XLOC_004686	0.0001	0.0007	2.21		
		Acetyl-CoA acetyltransferase	WP_038635179	XLOC_002140	0.0004	0.0040	2.28		
		<b>Translation, ribosomal structure and biogenesis</b>	<b>J</b>	GTP-binding protein	WP_028683953	XLOC_003650	0.0001	0.0007	-3.94
				GNAT family acetyltransferase	WP_038630820	XLOC_000531	0.0080	0.0443	-3.31
				Glutaminase	WP_009048121	XLOC_003273	0.0001	0.0007	-1.76
				Ribosome modulation factor	WP_023968495	XLOC_003210	0.0001	0.0007	-1.66
Polyribonucleotide nucleotidyltransferase	WP_038630587			XLOC_000295	0.0001	0.0007	-1.51		
Ribosomal RNA large subunit methyltransferase E	WP_007920869			XLOC_000283	0.0002	0.0017	1.54		
Amidase	WP_038633863			XLOC_001669	0.0009	0.0079	1.81		
GNAT family acetyltransferase	WP_038634977			XLOC_002043	0.0002	0.0022	1.91		
NADPH-dependent 7-cyano-7-deazaguanine reductase	WP_009047982			XLOC_000827	0.0001	0.0007	2.51		
ATP-dependent protease ATPase subunit HslU	WP_009046589			XLOC_002694	0.0001	0.0007	3.39		
<b>Transcription</b>	<b>K</b>	DNA-binding protein	WP_009050695	XLOC_002040	0.0001	0.0007	-4.23		



		PhzR	WP_038635113	XLOC_004545	0.0001	0.0007	-4.22
		GntR family transcriptional regulator	WP_009050459	XLOC_001909	0.0001	0.0007	-4.20
		RNA polymerase sigma factor RpoS	WP_007924026	XLOC_000448	0.0001	0.0007	-3.64
		TetR family transcriptional regulator	WP_009046585	XLOC_002689	0.0003	0.0032	-3.46
		AraC family transcriptional regulator	WP_028683816	XLOC_002650	0.0001	0.0007	-3.18
		Cold-shock protein	WP_009049775	XLOC_001584	0.0001	0.0007	-3.10
		LysR family transcriptional regulator	WP_009051067	XLOC_004709	0.0038	0.0250	-3.03
		Transcriptional regulator	WP_038635479	XLOC_002229	0.0001	0.0007	-2.94
		Temperature acclimation protein B	WP_007931076	XLOC_001901	0.0001	0.0007	-2.78
		Competence protein TfoX	WP_009050884	XLOC_002126	0.0002	0.0017	-2.77
		MarR family transcriptional regulator	WP_007929752	XLOC_000623	0.0001	0.0007	-2.36
		Transcriptional regulator	WP_009049335	XLOC_001408	0.0001	0.0007	-2.20
		MarR family transcriptional regulator	WP_009046358	XLOC_000026	0.0008	0.0068	-1.94
		Uncharacterized protein	WP_009048686	XLOC_003507	0.0001	0.0007	-1.67
		Transcriptional regulator	WP_009050371	XLOC_004323	0.0004	0.0036	-1.66
		Cro/CI family transcriptional regulator	WP_023968513	XLOC_003219	0.0002	0.0017	1.62
		RNA polymerase subunit sigma-24	WP_038633746	XLOC_004046	0.0005	0.0045	1.76
		LysR family transcriptional regulator	AIC22910	XLOC_002340	0.0081	0.0448	1.84
		RNA polymerase sigma factor	WP_016702389	XLOC_004106	0.0012	0.0100	1.90
		DEAD/DEAH box helicase	WP_038634571	XLOC_001931	0.0001	0.0007	2.06
		TetR family transcriptional regulator	WP_038631318	XLOC_003289	0.0067	0.0387	2.22
		RNA polymerase subunit sigma-70	WP_009048295	XLOC_000950	0.0002	0.0017	2.23
		Cro/CI family transcriptional regulator	WP_009049820	XLOC_003999	0.0006	0.0057	2.82
		AsnC family transcriptional regulator	WP_009051665	XLOC_002346	0.0001	0.0007	3.04
<b>Replication, recombination and repair</b>	<b>L</b>	Methyltransferase domain protein	WP_038630393	XLOC_000100	0.0001	0.0007	-3.84
		Uncharacterized protein	WP_007921116	XLOC_002393	0.0001	0.0007	-2.52
		DNA-binding protein HU, nu subunit	WP_007932138	XLOC_000383	0.0001	0.0007	-1.80
		Integration host factor	WP_003213368	XLOC_002377	0.0001	0.0007	-1.74
		Integrase	WP_038635495	XLOC_004681	0.0001	0.0007	-1.69
		DNA polymerase III subunit epsilon	WP_038636295	XLOC_005008	0.0001	0.0012	-1.63
<b>Cell wall/membrane/envelope biogenesis</b>	<b>M</b>	Transporter	WP_009046348	XLOC_000022	0.0001	0.0007	-3.99
		Membrane protein	WP_038632523	XLOC_001294	0.0017	0.0130	-3.84
		Multidrug RND transporter	WP_038633661	XLOC_003992	0.0001	0.0012	-3.63
		Acriflavin resistance protein AcrA	WP_009049438	XLOC_003840	0.0001	0.0007	-2.57
		RND transporter	WP_009046338	XLOC_000017	0.0001	0.0007	-2.44
		Membrane protein	WP_028681745	XLOC_002967	0.0001	0.0007	-2.37
		Porin	WP_007927525	XLOC_000789	0.0001	0.0007	-2.15
		Membrane protein	WP_028681813	XLOC_000480	0.0001	0.0007	-2.15
		Membrane protein	WP_038634145	XLOC_004202	0.0001	0.0007	-2.10
		Porin	WP_038634427	XLOC_004338	0.0001	0.0007	-1.96
		Acyltransferase	WP_038636499	XLOC_003266	0.0001	0.0007	-1.72
		Peptidase M23	WP_009051601	XLOC_002374	0.0002	0.0022	-1.70
		Porin	WP_038634847	XLOC_002012	0.0034	0.0226	-1.64
		dTDP-glucose 4,6-dehydratase	WP_038630376	XLOC_002641	0.0023	0.0169	-1.58
		TrbB protein	WP_038630956	XLOC_003108	0.0072	0.0407	-1.56
		Capsular biosynthesis protein	WP_009050914	XLOC_002145	0.0001	0.0007	-1.53
		Potassium transporter KefA	WP_009046633	XLOC_002708	0.0001	0.0007	-1.52
		Hemolysin secretion protein D	WP_038633904	XLOC_001677	0.0001	0.0007	1.54
		N-acetylmuramoyl-L-alanine amidase	WP_009048404	XLOC_000999	0.0001	0.0007	1.87
		Glucans biosynthesis protein D	WP_016702744	XLOC_002958	0.0008	0.0072	1.97

		Membrane protein	WP_009050551	XLOC_004412	0.0007	0.0064	2.15
		Lipoprotein	WP_016701700	XLOC_002875	0.0002	0.0022	2.45
		Channel protein TolC	WP_038636749	XLOC_003928	0.0001	0.0007	3.08
		N-acetylmuramoyl-L-alanine amidase	WP_038635768	XLOC_002350	0.0018	0.0136	5.98
<b>Cell motility</b>	<b>N</b>	Chemotaxis protein	WP_038631529	XLOC_003363	0.0001	0.0007	-3.01
		Chemotaxis protein	WP_016702200	XLOC_000710	0.0007	0.0060	-1.78
		Chemotaxis protein	WP_009046314	XLOC_000007	0.0003	0.0027	1.59
		Chemotaxis protein	WP_009046693	XLOC_000180	0.0001	0.0007	2.14
		Chemotaxis protein	WP_038634436	XLOC_001889	0.0001	0.0007	2.36
		Chemotaxis protein	WP_038634350	XLOC_001852	0.0001	0.0007	2.46
		Chemotaxis protein	WP_038634354	XLOC_004312	0.0074	0.0418	3.76
<b>Posttranslational modification, protein turnover, chaperones</b>	<b>O</b>	Glutathione S-transferase	WP_038631753	XLOC_003432	0.0001	0.0007	-5.83
		Alkyl hydroperoxide reductase	WP_007921754	XLOC_003780	0.0001	0.0007	-2.66
		Serine protease	WP_038633759	XLOC_004049	0.0001	0.0007	-2.37
		Peroxidase	WP_009048161	XLOC_000894	0.0001	0.0007	-2.14
		Probable thiol peroxidase	WP_038632154	XLOC_001174	0.0001	0.0007	-2.06
		Putative arginyl-tRNA--protein transferase	WP_007929216	XLOC_001582	0.0001	0.0012	-2.05
		Aspartyl beta-hydroxylase	WP_009047585	XLOC_000597	0.0004	0.0040	-1.55
		Serine protease	WP_009046353	XLOC_002594	0.0064	0.0371	-1.51
		10 kDa chaperonin	WP_009050540	XLOC_004406	0.0001	0.0007	1.56
		Chaperone protein ClpB	WP_009050931	XLOC_004594	0.0001	0.0007	2.10
		Chaperone protein DnaK	WP_009046925	XLOC_000278	0.0001	0.0007	2.21
		Protein-disulfide isomerase	WP_038630382	XLOC_000093	0.0001	0.0007	2.30
		ATP-dependent protease subunit HslV	WP_007925866	XLOC_002695	0.0014	0.0110	2.34
		Peptidylprolyl isomerase	WP_007932676	XLOC_001529	0.0075	0.0422	2.90
		Peroxidase	WP_009051386	XLOC_002452	0.0001	0.0007	2.94
		Chaperone protein HtpG	WP_029526163	XLOC_000706	0.0001	0.0007	3.20
		Protein GrpE	WP_007920848	XLOC_000277	0.0001	0.0007	3.90
		Heat-shock protein	WP_007922004	XLOC_000845	0.0001	0.0007	5.22
<b>Inorganic ion transport and metabolism</b>	<b>P</b>	Sulfite reductase	WP_009048796	XLOC_001169	0.0001	0.0007	-3.34
		ABC transporter substrate-binding protein	WP_038632351	XLOC_001230	0.0067	0.0387	-3.09
		Carbonic anhydrase	WP_009046260	XLOC_004795	0.0001	0.0007	-2.57
		FAD-binding protein	WP_038634838	XLOC_002009	0.0001	0.0007	-2.56
		Catalase	WP_016702645	XLOC_004732	0.0001	0.0007	-2.51
		ABC transporter substrate-binding protein	WP_009050227	XLOC_001802	0.0003	0.0027	-2.10
		Membrane protein	WP_038633477	XLOC_003932	0.0038	0.0247	-1.91
		(2Fe-2S)-binding protein	AIC22009	XLOC_004414	0.0003	0.0032	-1.81
		Calcium transporter ChaC	WP_009051415	XLOC_002434	0.0001	0.0007	-1.61
		SulP family inorganic anion transporter	WP_038635643	XLOC_004794	0.0067	0.0384	-1.61
		Nuclease PIN	WP_009047396	XLOC_003029	0.0002	0.0022	-1.55
		Hemin import ATP-binding protein HmuV	WP_038635171	XLOC_004584	0.0001	0.0007	1.53
		Sugar ABC transporter substrate-binding protein	WP_038632606	XLOC_003704	0.0001	0.0007	1.56
		Cystathionine gamma-synthase	WP_038635962	XLOC_002426	0.0001	0.0007	1.77
		Taurine ABC transporter substrate-binding protein	WP_009046457	XLOC_002630	0.0079	0.0437	1.78
		Iron utilization protein	WP_038633927	XLOC_001685	0.0001	0.0007	1.92
		ABC transporter substrate-binding protein	WP_038631672	XLOC_001005	0.0001	0.0007	2.28
		ABC transporter substrate-binding protein	WP_038635999	XLOC_002454	0.0001	0.0007	2.30
		Copper resistance protein CopB	WP_016704756	XLOC_003562	0.0001	0.0007	2.88
		Peptide ABC transporter substrate-binding protein	WP_038630318	XLOC_002581	0.0001	0.0007	3.19
		TonB-dependent receptor	WP_038636418	XLOC_002754	0.0031	0.0210	4.39
		Copper resistance protein CopC	WP_038632194	XLOC_003561	0.0001	0.0007	4.70
		Iron ABC transporter permease	WP_038632590	XLOC_003699	0.0001	0.0007	5.66
		TonB-dependent receptor	WP_038632604	XLOC_003703	0.0001	0.0007	5.73
		TonB-dependent receptor	WP_038634844	XLOC_002011	0.0003	0.0027	6.52
		Zinc ABC transporter permease	WP_009051646	XLOC_004836	0.0001	0.0007	7.02
<b>Secondary metabolites</b>	<b>Q</b>	Isochorismatase	WP_009050821	XLOC_002098	0.0001	0.0007	-8.46
		Phenazine biosynthesis protein	WP_038635119	XLOC_002100	0.0001	0.0007	-8.36

<b>biosynthesis, transport and catabolism</b>	Serine 3-dehydrogenase	WP_016702821	XLOC_000985	0.0001	0.0007	-5.82
	Poly(3-hydroxyalkanoate) granule-associated protein PhaF	WP_009046584	XLOC_000134	0.0001	0.0007	-3.04
	NADP-dependent oxidoreductase	WP_009049328	XLOC_001403	0.0001	0.0007	-2.59
	Alcohol dehydrogenase	WP_028683952	XLOC_001236	0.0001	0.0012	-1.65
	Acyl-homoserine lactone acylase subunit beta	WP_038632200	XLOC_001187	0.0001	0.0007	1.63
	Peptide synthase	WP_038633919	XLOC_004109	0.0001	0.0007	1.64
	DeoR family transcriptional regulator	WP_038631063	XLOC_000707	0.0001	0.0007	2.11
	<b>General function prediction only</b>	<b>R</b>				
2,3-dihydro-3- hydroxyanthranilate isomerase	WP_016703606	XLOC_002099	0.0062	0.0364	-9.01	
Ring-cleavage extradiol dioxygenase	WP_016702948	XLOC_001237	0.0003	0.0032	-8.49	
Rhs family protein	WP_038630348	XLOC_002611	0.0001	0.0007	-7.82	
Amidohydrolase	WP_038633980	XLOC_001697	0.0001	0.0007	-4.99	
Sugar deacetylase	WP_016702950	XLOC_001239	0.0048	0.0298	-4.93	
Methyltransferase	WP_038632359	XLOC_003651	0.0002	0.0017	-4.29	
Nuclease	WP_028683814	XLOC_002657	0.0007	0.0060	-4.06	
ABC transporter	WP_016702952	XLOC_001241	0.0001	0.0007	-3.57	
Lipoprotein	WP_041986442	XLOC_003464	0.0031	0.0213	-2.22	
PsiE family protein	WP_009042237	XLOC_000360	0.0006	0.0053	-2.06	
Uncharacterized protein	WP_038634153	XLOC_001765	0.0010	0.0082	-2.03	
Amidohydrolase	WP_009049582	XLOC_003903	0.0001	0.0007	-1.85	
Beta-lactamase	WP_038635452	XLOC_004671	0.0088	0.0474	-1.84	
1-carboxy-3-chloro-3,4- dihydroxycyclo hexa-1,5-diene dehydrogenase	WP_038631818	XLOC_003440	0.0031	0.0210	-1.82	
Cytochrome D ubiquinol oxidase subunit II	WP_016702021	XLOC_002984	0.0001	0.0007	-1.77	
Diaminopimelate decarboxylase	WP_009048261	XLOC_003333	0.0001	0.0007	-1.70	
Arylsulfate sulfotransferase	WP_009042529	XLOC_000517	0.0010	0.0086	-1.57	
Isomerase	WP_038633872	XLOC_004090	0.0029	0.0202	-1.51	
PvdJ/PvdD/PvdP-like protein	WP_038633908	XLOC_004107	0.0001	0.0007	1.57	
Ketosteroid isomerase	WP_007922537	XLOC_000094	0.0001	0.0007	1.58	
Molybdenum cofactor sulfurase	WP_038634095	XLOC_004156	0.0047	0.0293	1.60	
Methyltransferase	WP_038634054	XLOC_001717	0.0060	0.0355	1.66	
23S rRNA (uracil(1939)-C(5))- methyltransferase RlmD	WP_038634290	XLOC_004264	0.0001	0.0007	1.67	
Ribosomal subunit interface protein	WP_009047054	XLOC_000362	0.0001	0.0007	1.74	
Lysozyme	WP_038630887	XLOC_000572	0.0001	0.0007	1.93	
5-formyltetrahydrofolate cyclo- ligase	WP_007926741	XLOC_002437	0.0013	0.0103	2.04	
Carbon-nitrogen hydrolase	WP_038630611	XLOC_000324	0.0002	0.0017	2.16	
UPF0060 membrane protein EY04_22005	WP_007932246	XLOC_004221	0.0018	0.0139	2.42	
4-carboxymuconolactone decarboxylase	WP_009046984	XLOC_002874	0.0041	0.0265	2.56	
Glyoxalase	WP_009046877	XLOC_000259	0.0001	0.0007	3.18	
Peptidase C39	WP_038633471	XLOC_003930	0.0001	0.0007	3.32	
Alpha/beta hydrolase	WP_009046421	XLOC_000062	0.0050	0.0307	3.40	
<b>Unknown Function</b>	<b>S</b>					
Hydrolase/acyltransferase	EJZ57002	XLOC_000535	0.0034	0.0226	-6.98	
Poly(3-hydroxyalkanoate) granule-associated protein PhaI	WP_009046583	XLOC_000133	0.0001	0.0007	-6.60	
Lipoprotein	WP_038632271	XLOC_003611	0.0001	0.0007	-6.36	
Uncharacterized protein	WP_038632169	XLOC_003551	0.0001	0.0007	-5.25	
Membrane protein	WP_038633841	XLOC_004083	0.0001	0.0007	-4.89	
Uncharacterized protein	EIM12867	XLOC_000056	0.0001	0.0007	-4.66	
Glycosyl hydrolase	WP_038636404	XLOC_000021	0.0001	0.0007	-4.13	
Porin	WP_038634688	XLOC_001955	0.0001	0.0007	-4.01	
Peptidase C13	WP_038635515	XLOC_002247	0.0001	0.0007	-3.64	
Fusaric acid resistance protein	WP_038631055	XLOC_003156	0.0024	0.0171	-3.58	
Tail protein	WP_038630740	XLOC_000455	0.0002	0.0017	-3.52	
Uncharacterized protein	WP_009049553	XLOC_003894	0.0011	0.0089	-3.44	
Tail sheath protein	WP_038630744	XLOC_000457	0.0002	0.0022	-3.40	
Peptidoglycan-binding protein LysM	WP_009046477	XLOC_000086	0.0001	0.0007	-3.27	
Type IV secretion protein Rhs	WP_038634583	XLOC_004369	0.0001	0.0007	-3.23	

Uncharacterized protein	WP_038631945	XLOC_003480	0.0008	0.0068	-3.21
Oxidase	WP_038631869	XLOC_003462	0.0001	0.0007	-3.15
Membrane protein	WP_038632611	XLOC_001325	0.0001	0.0007	-3.14
Uncharacterized protein	WP_038633491	XLOC_003935	0.0001	0.0007	-2.97
Putative hemagglutinin (DUF637)	EJL96817	XLOC_000612	0.0001	0.0007	-2.92
Uncharacterized protein	WP_038632500	XLOC_003680	0.0034	0.0229	-2.90
Phospholipid-binding protein	WP_009050906	XLOC_004586	0.0001	0.0007	-2.87
Putative hemagglutinin (DUF637)	WP_051429416	XLOC_003109	0.0005	0.0049	-2.82
Membrane protein	WP_038634529	XLOC_004361	0.0001	0.0012	-2.77
Uncharacterized protein	WP_009050755	XLOC_004522	0.0001	0.0007	-2.75
Phosphatidylcholine-hydrolyzing phospholipase	WP_009046978	XLOC_002873	0.0001	0.0007	-2.67
Uncharacterized protein	WP_038636482	XLOC_003161	0.0001	0.0007	-2.65
Uncharacterized protein	WP_038630325	XLOC_002590	0.0001	0.0007	-2.63
Acetyltransferase	WP_038630891	XLOC_003073	0.0001	0.0007	-2.62
Uncharacterized protein	WP_025807035	XLOC_002408	0.0001	0.0007	-2.61
Phage tail protein	WP_007923973	XLOC_000458	0.0003	0.0032	-2.60
Membrane protein	WP_009048116	XLOC_000871	0.0004	0.0036	-2.57
Spore coat protein	WP_038633677	XLOC_004009	0.0001	0.0007	-2.53
Uncharacterized protein	WP_038632150	XLOC_001170	0.0001	0.0007	-2.39
Dialkylrecorsinol condensing enzyme	WP_009050009	XLOC_004116	0.0001	0.0007	-2.37
Uncharacterized protein	WP_009050754	XLOC_004521	0.0001	0.0007	-2.36
CrfX protein	WP_007927518	XLOC_000786	0.0001	0.0007	-2.34
ATPase	WP_025807119	XLOC_002466	0.0090	0.0484	-2.19
Metal-chelation protein CHAD	WP_038630895	XLOC_003075	0.0001	0.0007	-2.15
Porin	WP_025808788	XLOC_003710	0.0001	0.0007	-2.14
Excinuclease	WP_009046609	XLOC_002701	0.0086	0.0469	-2.11
Transcriptional regulator	WP_038630852	XLOC_000550	0.0020	0.0148	-2.05
DoxX family protein	WP_009050141	XLOC_004188	0.0050	0.0310	-1.99
Uncharacterized protein	WP_038636463	XLOC_003065	0.0001	0.0007	-1.99
Secondary thiamine-phosphate synthase enzyme	EIM17029	XLOC_004893	0.0012	0.0100	-1.96
Uncharacterized protein	WP_023967792	XLOC_002375	0.0001	0.0007	-1.96
Pilin assembly protein	WP_009047183	XLOC_002969	0.0024	0.0174	-1.78
Uncharacterized protein	WP_007923269	XLOC_001732	0.0001	0.0007	-1.75
Cytochrome D ubiquinol oxidase subunit III	WP_007928428	XLOC_002055	0.0001	0.0007	-1.74
Membrane protein	WP_038631867	XLOC_003461	0.0025	0.0177	-1.68
Methyltransferase	WP_007927856	XLOC_004103	0.0030	0.0208	-1.64
Serine/threonine protein phosphatase	WP_038636950	XLOC_004830	0.0001	0.0007	-1.62
Uncharacterized protein	WP_009048250	XLOC_003331	0.0067	0.0384	-1.57
Porin	WP_038636600	XLOC_001214	0.0012	0.0096	-1.57
Uncharacterized protein	WP_051673205	XLOC_004653	0.0001	0.0007	-1.52
Uncharacterized protein	WP_009042934	XLOC_000791	0.0001	0.0012	1.58
Sulfurase	WP_038636332	XLOC_002557	0.0072	0.0409	1.59
Histidine kinase	WP_038635178	XLOC_002139	0.0002	0.0017	1.64
PasA protein	WP_009047965	XLOC_000818	0.0001	0.0012	1.71
Uncharacterized protein	WP_038630513	XLOC_000218	0.0086	0.0469	1.77
Lipoprotein	WP_016705380	XLOC_003955	0.0002	0.0017	1.78
Methyltransferase	WP_038634569	XLOC_001930	0.0001	0.0007	1.82
RNA signal recognition particle 4.5S RNA	WP_028682047	XLOC_004065	0.0032	0.0218	1.95
Uncharacterized protein	WP_038630933	XLOC_000599	0.0001	0.0007	2.46
Uncharacterized protein	WP_038632989	XLOC_003825	0.0027	0.0188	2.52
Membrane protein	WP_038635748	XLOC_002347	0.0001	0.0007	2.65
Lon protease	WP_038634854	XLOC_002017	0.0001	0.0007	2.66
Cytotoxin	WP_038633468	XLOC_003929	0.0001	0.0007	2.73
Membrane protein	WP_009047384	XLOC_003022	0.0007	0.0060	3.13
ABC transporter substrate- binding protein	WP_009051009	XLOC_004641	0.0009	0.0079	4.13
Glutamine synthetase	EIM18812	XLOC_004837	0.0047	0.0293	4.42
Uncharacterized protein	WP_038633414	XLOC_001519	0.0001	0.0007	5.22
<b>Signal transduction mechanisms</b>	<b>T</b>				
Acyl-homoserine-lactone synthase	WP_038635110	XLOC_002094	0.0001	0.0007	-5.94
Diguanylate cyclase	WP_038636150	XLOC_002522	0.0001	0.0007	-5.83

		Diguanylate cyclase	WP_016701868	XLOC_001577	0.0001	0.0007	-4.61		
		Chemotaxis protein CheY	WP_038631017	XLOC_000663	0.0001	0.0007	-3.55		
		Histidine kinase	WP_009049953	XLOC_004086	0.0031	0.0210	-3.31		
		Crp/Fnr family transcriptional regulator	WP_038630893	XLOC_003074	0.0001	0.0007	-3.28		
		Chemotaxis protein CheY	WP_009049747	XLOC_003975	0.0002	0.0017	-3.24		
		Protein kinase	WP_038631821	XLOC_003441	0.0001	0.0007	-2.96		
		Histidine kinase	WP_038630504	XLOC_002765	0.0001	0.0007	-2.64		
		Anti-anti-sigma factor	WP_009047705	XLOC_000662	0.0001	0.0007	-2.45		
		Diguanylate cyclase	WP_009049109	XLOC_001313	0.0001	0.0007	-2.44		
		LuxR family transcriptional regulator	WP_009046790	XLOC_000221	0.0001	0.0007	-2.26		
		Diguanylate phosphodiesterase	WP_038632841	XLOC_001407	0.0064	0.0373	-2.11		
		DeoR family transcriptional regulator	WP_016702287	XLOC_003124	0.0045	0.0283	-2.04		
		Histidine kinase	WP_009047707	XLOC_000664	0.0001	0.0007	-1.93		
		Histidine kinase	WP_038631277	XLOC_003260	0.0001	0.0007	-1.77		
		Transporter	WP_038632357	XLOC_001235	0.0005	0.0045	-1.61		
		LuxR family transcriptional regulator	WP_009049205	XLOC_001341	0.0001	0.0007	-1.52		
		Universal stress protein	WP_009049186	XLOC_003726	0.0005	0.0049	1.62		
		Chemotaxis protein	WP_016703630	XLOC_004556	0.0009	0.0075	1.71		
		Histidine kinase	WP_038636745	XLOC_003927	0.0001	0.0007	2.08		
		Diguanylate phosphodiesterase	WP_028684076	XLOC_003708	0.0001	0.0007	2.24		
		Histidine kinase	WP_016703743	XLOC_004687	0.0001	0.0007	2.39		
<b>Intracellular trafficking, secretion, and vesicular transport</b>	<b>U</b>	Type VI secretion protein TssB2	WP_007930286	XLOC_002379	0.0019	0.0145	-7.37		
		Type VI secretion protein	WP_009051592	XLOC_002380	0.0001	0.0007	-6.93		
		Type VI secretion protein ImpA	WP_009051593	XLOC_002378	0.0092	0.0492	-5.52		
		Type VI secretion protein	WP_007930278	XLOC_002383	0.0001	0.0007	-4.24		
		Uncharacterized protein	WP_038636832	XLOC_001932	0.0054	0.0329	-2.86		
		Type VI secretion protein	WP_038633332	XLOC_003899	0.0001	0.0007	-2.85		
		ATPase	WP_038630518	XLOC_002782	0.0082	0.0452	-2.64		
		Uncharacterized protein	WP_009049566	XLOC_001502	0.0001	0.0007	-2.62		
		Secretin	WP_029526712	XLOC_001903	0.0001	0.0007	-2.55		
		Elongation factor Tu	WP_016702644	XLOC_004749	0.0001	0.0007	-2.48		
		Uncharacterized protein	WP_050899139	XLOC_001168	0.0094	0.0498	-2.34		
		Protein-export protein SecB	WP_009046533	XLOC_000111	0.0001	0.0007	-2.21		
		Potassium ABC transporter ATPase	WP_038630522	XLOC_002785	0.0002	0.0017	-2.07		
		Pilus assembly protein CpaB	WP_038630519	XLOC_002783	0.0002	0.0017	-1.85		
		Secretin	WP_038633645	XLOC_001590	0.0029	0.0202	-1.76		
		Elongation factor G	WP_009051119	XLOC_004750	0.0001	0.0007	-1.74		
		ShlB family hemolysin secretion/activation protein	WP_041987697	XLOC_001737	0.0016	0.0123	2.73		
		Filamentous hemagglutinin	WP_038634114	XLOC_001738	0.0001	0.0007	4.89		
		<b>Defense mechanisms</b>	<b>V</b>	Hemolysin D	WP_009049804	XLOC_003991	0.0001	0.0007	-3.95
				ABC transporter	WP_038630975	XLOC_003123	0.0001	0.0007	-3.08
DSBA oxidoreductase	WP_009046334			XLOC_000016	0.0002	0.0017	-2.90		
Transporter	WP_009049437			XLOC_003839	0.0001	0.0007	-2.76		
ADP-ribose diphosphatase	WP_007931715			XLOC_000087	0.0006	0.0057	1.66		
Peptide ABC transporter permease	WP_038635565			XLOC_002266	0.0026	0.0186	2.30		
Hemolysin D	WP_028682887			XLOC_003931	0.0001	0.0007	3.10		
<b>Extracellular structures</b>	<b>W</b>	Molecular chaperone	WP_028682799	XLOC_004005	0.0002	0.0017	-2.68		
<b>Mobilome: prophages, transposons</b>	<b>X</b>	Tail protein	WP_038630732	XLOC_000451	0.0001	0.0007	-4.42		
		Tail protein	WP_016701993	XLOC_000453	0.0001	0.0007	-3.03		
		Phage assembly protein	WP_009047314	XLOC_000461	0.0014	0.0113	-2.93		
		2-hydroxyacid dehydrogenase	WP_038630746	XLOC_000459	0.0001	0.0007	-2.03		
		Uncharacterized protein	WP_038632916	XLOC_003806	0.0001	0.0007	1.86		

Appendix Table 6: Genes found within 500bp of *phz*-box consensus sequence

Consensus <i>phz</i> -box sequence	Gene regulated	NCBI protein accession number	Locus of gene in PA23 genome		Distance between <i>phz</i> -box & gene (bp)
ctccGCCTGCCAGGCTGCCAGTGCAgcca	poly(3-hydroxyalkanoate) depolymerase	WP_009046587	363477	364334	197
gaccATCTACAAGCACTTCAAGTCCAaggc	class V aminotransferase	WP_007928066	1913015	1914148	226
caggCTCTGCCAGTGCTGAAAGATTTgaaa	hypothetical protein	WP_009042976	2080434	2080670	289
ctatGGCTGCAAGACCTACAAGGTGAggac	inositol 2-dehydrogenase	WP_009048469	2615209	2616219	244
gtagGTCTTCAAGCTCTTCCAGCTCAgacct	hypothetical protein	WP_009047258	3811919	3812293	162
caatGGCTGCCAGATCTGCCAGCGATtgag	hypothetical protein	WP_016705343	4155158	4155541	455
ggaaAGCTTCCAGTGCTCCAAGGGTTcggc	hypothetical protein	WP_016705366	4203921	4204325	350
gtacGGCTACCAGCGCTTCCAGGAAAcggg	histidine kinase	WP_009049751	4277114	4278925	347
ctccGGCTACGAGAACTTCGAGTTCGaagt	NADH dehydrogenase	WP_009049784	4322233	4322730	446
caagTACTTCAAGATCTGGGAGATGGcctg	TetR family transcriptional regulator	WP_009050042	4685290	4685859	439
ggctTTCTGCCAGGCCTTGAAGGCTGgcca	hypothetical protein	WP_009045296	5372164	5373102	46
cttgCGCTTCCAGGCCTTGAAGTTGCcatc	InaA protein	WP_016703354	5440879	5441592	207
cagcCGCTACCAGCGCTGGCAGGCCGagca	hypothetical protein	WP_009050677	5598091	5598330	405
aagcCCCTACCAGATCTTGCAGGTGCcaag	acyl-homoserine-lactone synthase ( <i>phzI</i> )	WP_016703600	5772051	5772641	56
taatCACTACAAGATCTGGTAGTTCCaccc	transcriptional regulator ( <i>phzR</i> )	WP_009045585	5772673	5773398	282
aaccTGCTCCAAGTCTTGGCAGCAGGgta	phospholipid-binding protein	WP_009050906	5884645	5885001	407
caatGGCTGCAAGCGCTGGTAGAGCTgata	hypothetical protein	WP_011911818	6161124	6163058	220
gcacGCCTTCGAGCACTTGTAGCACGgcac	DNA-binding protein	WP_007926162	6407533	6407820	336
cggcGGCTACCAGCTCTTGCAGAGTGatgg	zinc ABC transporter permease	WP_007927613	6523419	6524285	420
agtgTCCTTCTAGGCCTCGCAGGGCCgaga	biopolymer transporter ExbB	WP_009051571	6639569	6640555	55
ggccTTCTACAAGCTCTGCGAGCATAtgct	hypothetical protein	WP_009051265	6940569	6940866	112
gcctGGCTTCAAGGCCTACCAGCAGCaagt	sarcosine oxidase subunit beta	WP_007248069	6994708	6995958	410
gccaCGCTGCCAGGGCTTCCAGGTAAgccg	hypothetical protein	WP_016702880	2391284	2391547	411
cgggtTCTTCCAGTTCTTCCAGGGCGtagc	transporter	WP_009043524	2788733	2790193	288
tcgcAGCTTCCAGGTCTCAAAGTCAgagtc	exonuclease	WP_012071861	3622216	3622764	482
ccagACCTACGAGTACTTCAAGGGCGagga	lipoprotein	WP_009049700	4223087	4223746	251
gggcATCTGCCAGACCTACGAGTTCAcgt	ligand-gated channel	WP_009049908	4491292	4493418	330
ggccATCTCCCAGATCTTGAAGTACTtgcg	DSBA oxidoreductase	WP_007930404	4686731	4687321	435
aaggGCCTTCGAGGGCTTCAAGAGCAcgtc	acyl-CoA dehydrogenase	WP_009044914	4740205	4741362	145
gtggAACTACCAGATCTTGTAGTGATtagc	phenazine biosynthesis protein	WP_007923193	5773827	5774318	147