Characterization of bacterial communities in soybean cultivated soils of Manitoba

By

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A Thesis Submitted to the Faculty of Graduate Studies of
The University of Manitoba
In partial Fulfillment of the Requirements of the Degree of

Doctor of Philosophy

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Abstract

Soybean (*Glycine max* L.) is a legume plant that serves as a rich source of oil and protein. Soybean is well-known for its symbiotic relationship with the bacteria *Bradyrhizobium japonicum*, which supplies the plant with reduced nitrogen. Although the molecular aspects of this symbiotic relationship are well understood, the structure of bacterial communities in the root-associated soils and the factors influencing their composition have not been extensively studied. This study aims to characterize the bacterial communities of soybean grown in two different field experiments in Manitoba (Carman and Kelburn) with four different crop rotations: continuous soybean (CS), soybean-canola (SCa), soybean-corn (SCo), and soybean-wheat-canola-corn (SWCC) from 2017 to 2021. Soil samples were collected four times during the growing season; before planting (BP), emergence (VE), beginning of seed fill (R5), and full maturity (R8). DNA was extracted from the soil samples and subjected to 16S rRNA gene sequencing. In a separate experiment, subsamples were cultured to preserve a local culture collection and to isolate phosphate-solubilizing bacteria (PSB) through targeted culturing. 16S rRNA gene sequencing revealed that soil bacterial diversity was primarily influenced by soil type, with crop rotation inducing short-term changes. Despite environmental variability, crop rotations significantly affected bacterial diversity and composition. Culturing resulted in a total of 262 bacterial strains being added to a culture library. Eight strains, including *Paraburkholderia strydomiana*, *Paraburkholderia graminis*, and *Burkholderia ambifaria*, were identified for their ability to solubilize $Ca_3(PO_4)_2$. *Paraburkholderia strydomiana* strains promoted seedling growth, whereas *P. graminis* and *B. ambifaria* had negative effects on soybean seedlings. These findings enhance our understanding of bacterial diversity in crop rotation systems and suggest directions for future synthetic community research.

Acknowledgments

I would like to first express my sincere gratitude to Dr. Ivan Oresnik for his belief in my abilities and for offering me this opportunity. His mentorship, which began even before I commenced my studies, enabled me to overcome challenges and encouraged me to think independently. I would like to extend my thanks to my committee members, Dr. Georg Hausner, Dr. Matthew Bakker, and Dr. Yvonne Lawley for the guidance and input through the years. I also want to acknowledge my funding sources; Manitoba Pulse & Soybean Growers, and SEGS, University of Manitoba.

I am grateful to the past and present members of the Oresnik lab for fostering a pleasant working environment. Special thanks to Dr. Sabhjeet Kaur and Dr. Justin Hawkins for their immense support and mentorship, as well as Patricia Ordoñez and Zeel Patel for their assistance with sample collection and tedious culturing. I also want to thank the members of the Lawley lab, especially Stephanie Dheilly and Chathuri Weerasekara, for their help. My appreciation extends to the entire microbiology department, staff, and my colleagues for creating a friendly and enjoyable atmosphere.

I would like to thank the Vice Chancellor of the University of Jaffna, the Dean of the Faculty of Agriculture, and the Head of the Department of Agricultural Chemistry for granting me study leave to pursue my Ph.D. I also appreciate the staff of the Department of Agricultural Chemistry for their continued support.

My gratitude goes out to the families of Dr. Loganathan, Nickson and Dr. Jeyachandran families for their invaluable support since I moved to Winnipeg. A long list of friends in Winnipeg and back home also deserve appreciation for their support throughout my journey.

Lastly, I would like to thank my family; with their constant encouragement, I am here. I am deeply grateful to my dad, mother-in-law, siblings, and brother-in-law for their unconditional support and trust. A special thank you to my husband, Shayanthan Selvarajan, for always standing by me and being my pillar of strength. Last but not least, I want to thank my wonderful children, Karunika and Aathan, for their patience and understanding. I am truly fortunate to have such amazing kids in my life.

Dedication

This thesis is lovingly dedicated to my family, and in memory of my late mother, Yogarani Sivagnanam, and my late father-in-law, Rayappu Soosaipillai Selvarajan.

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List of Abbreviation

ACC deaminase	1-aminocyclopropane-1-carboxylic acid deaminase
ANI	Average Nucleotide Identity
ANOVA	Analysis of Variance
BP	Before Planting
CS	Continuous Soybean
dDDH	digital DNA-DNA Hybridization
ef_lda	effect size_linear discriminant analysis
LEfSe	Linear Discriminant Analysis Effect Size
MPSG	Manitoba Pulse and Soybean Growers' Association
NetCoMi	Network Construction and Comparison for Microbiome Data
OTU	Operational Taxonomic Unit
PBS	Phosphate Buffered Saline
PCoA	Principal Coordinate Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
PGP	Plant Growth-Promoting
PGPR	Plant Growth-Promoting Rhizobacteria
P_i	Inorganic Phosphate
P_o	Organic Phosphate
PSB	Phosphate Solubilizing Bacteria
PVK	Pikovskaya
R5	Beginning Seed Fill; a soybean growth stage
R8	After harvest
RAST	Rapid Annotations Using the Subsystems Technology
RDP	Ribosomal Database Project
SCa	Soybean-Canola
SCo	Soybean-Corn
SWCC	Soybean-Wheat-Canola-Corn
SynCom	Synthetic Microbial Community
TYGS	Type Strain Genome Server
VE	Emergence; a soybean vegetative growth stage

Chapter 1: Literature Review

This chapter was originally published in the journal Frontiers in Agronomy

Shayanthan A, Ordoñez PAC and Oresnik IJ (2022) The Role of Synthetic Microbial Communities (SynCom) in Sustainable Agriculture. Front. Agron. 4:896307. doi: 10.3389/fagro.2022.896307.

This work was planned and drafted by Ambihai Shayanthan. AS, PO, and IO conceived and wrote the manuscript. All authors contributed to the article and approved the submitted version.

1.1 Trends in agricultural production

To meet the demand of the growing global population, agricultural production needs to increase by about 70% from its current level by 2050 (ELD Initiative, 2015; Singh et al., 2020). However, it is estimated that global food production will decrease by 12% over the next 25 years due to the degradation of agricultural lands (ELD Initiative, 2015). Following the second industrial revolution (early 20th century), traditional agricultural practices shifted towards using synthetic chemical fertilizers and pesticides to improve crop production (Dixon, 2018; Melillo, 2012). The intensive use of these agrochemicals has led to the deterioration of the quality of both the soil and the environment (Meena V. S. et al., 2017). Harnessing crop-associated microbiomes has been proposed to increase or sustain higher yields while maintaining overall soil health and fertility (Singh et al., 2020; Toju et al., 2018).

1.2 Current challenges of microbial inoculants

Over the past two decades, microbes with plant growth-promoting (PGP) traits have been isolated and used as inoculants to improve crop production (Banerjee et al., 2018; de Souza et al., 2020; Finkel et al., 2017). Microbes assist plant growth by enhancing nutrient acquisition through nitrogen fixation, phosphorus solubilization, and siderophore production or by producing plant growth-promoting substances (Chaudhary et al., 2021; Joshi et al., 2021; Olanrewaju et al., 2017; Saleem et al., 2018). In addition, microbial inoculants have the potential to suppress several pathogenic organisms (Abbasi et al., 2021; Olanrewaju et al., 2017; Yasmin et al., 2016). The main drawback of the microbial application is that these strategies often fail to yield consistent results because the plant-microbe association has not been considered with respect to various biotic and abiotic considerations that can affect the

outcome (de Souza et al., 2020; Finkel et al., 2017). For inoculums to be successful in the field, an in-depth knowledge of microbial abundance, diversity, and plant-microbe interactions is essential to be able to predict overall functionality (Chodkowski & Shade, 2017). The synthetic microbial community (SynCom) approach has become a promising technology as it integrates the concepts of microbial ecology and genetics. Advancements in high-throughput sequencing technologies and their associated bioinformatics tools have provided the opportunity to discover the complexities associated with plant-microbe interactions and the functionality they can provide to the plant.

1.3 Microbes at the plant-soil interface

Regardless of whether animals or plants are considered, microbial communities play vital roles in their respective ecosystems. The soil microbiome is defined as the microbial communities present in the soil and their encoded functions. Within the soil, microbes can be found as both free-living or in symbiotic relationships with higher organisms and are often considered key drivers of beneficial processes such as nutrient cycling and carbon sequestration (Fierer, 2017; Qiu et al., 2019; Wallenstein, 2017). Microorganisms that can form complex co-associations with plants obtain their carbon sources and other metabolites from the plant while performing these beneficial processes (Backer et al., 2018; Trivedi et al., 2020).

The interaction of microbes with plants occurs across their entire life cycle. These interactions can occur both above and below ground level. Whereas some are due to chance, many interactions are orchestrated by the plant. It can occur through vertical transfer, such as when endophytes living within a plant are transmitted via vascular connections or when

bacteria become incorporated within a developing seed and may play a role in seed germination and the development of a root system to aid in initial establishment and plant survival (Mitter et al., 2016). In addition, plant roots interact with the soil and actively exude carbon-containing compounds that influence all microbial growth around a root. Bacteria drawn to the plant in this manner are horizontally transferred from bulk soil to the rhizosphere.

1.3.1 Rhizosphere microbiome

The rhizosphere is defined as the soil under the direct influence of root exudates (Hartman & Tringe, 2019; Moe, 2013; Reinhold-Hurek et al., 2015). This zone has been further subdivided into the endorhizosphere, the ectorhizosphere, and the rhizoplane. The endorhizosphere consists of the zone of tissue in the plant root that can be occupied by microorganisms (Mcnear, 2013). The endorhizoshpere is delineated by the rhizoplane, which is the surface of the root, and beyond this is the ectorhizosphere, which is influenced by root exudation and rhizodeposition (Reinhold-Hurek et al., 2015).

The bulk soil microbiome is a potential source of inoculants for the rhizosphere microbiome. The composition of the rhizosphere microbiome is structured differently from that of the soil microbiome (Crecchio et al., 2018). This differentiation is initiated by plants through root exudates that attract specific microbes to the rhizosphere to support plant growth and development (Vives-Peris et al., 2020; Wallenstein, 2017). By regulating the secretion of signaling compounds and activation of plant immune responses, the plant can influence the recruitment of a subset of microbes from the rhizosphere to attach to the rhizoplane and subsequently to move from the rhizoplane to the endosphere (Hacquard et al., 2015; Hartman & Tringe, 2019). In general, it has been observed that plants modify their rhizosphere to

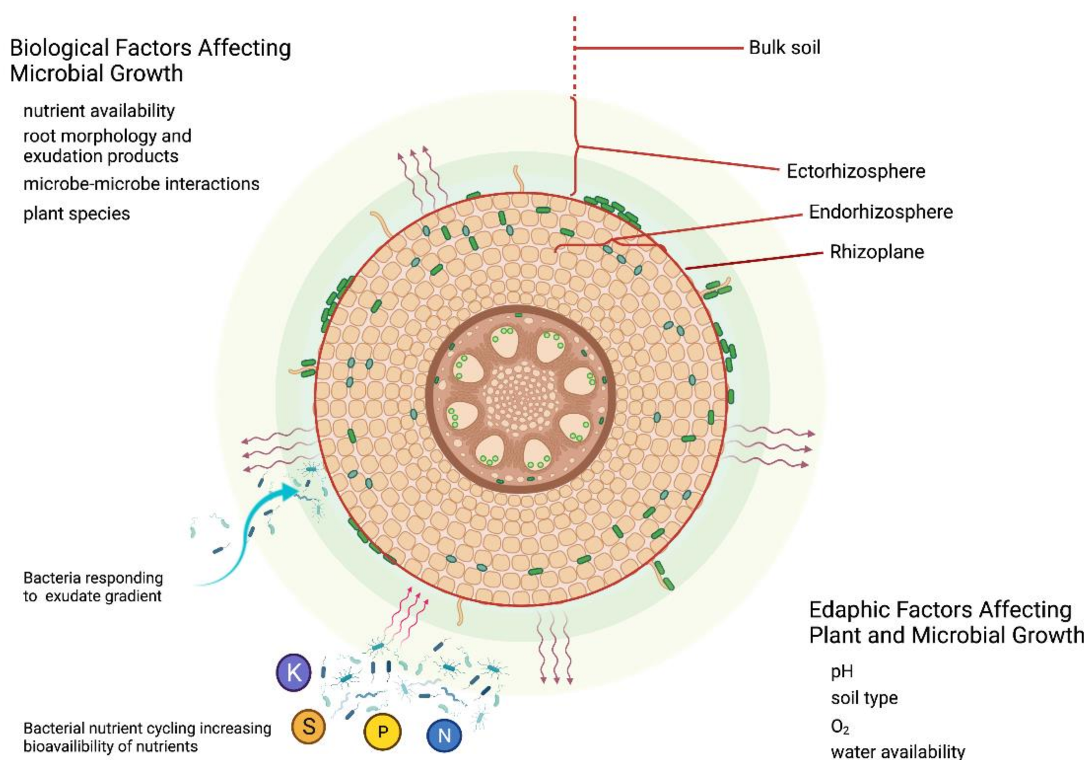

Figure 1. Root rhizosphere and factors affecting microbiome development.
A diagrammatic representation of a root cross-section. Factors affecting microbial and plant growth are presented as tables (see text for details). The rhizosphere is depicted as two tones (green and pale yellow) surrounding the root, representing an exudation gradient. The endorhizosphere, rhizoplane, ectorhizosphere, and bulk soil are highlighted using red brackets and lines. Endophytic bacteria and bacteria living on the rhizoplane are depicted as green rods. Plant exudation is represented as purple wavy arrows emanating from the root surface. Microbial communities in the rhizosphere are depicted as responding to exudates (single blue wavy arrow), and as bacteria involved in nutrient cycling. Major nutrients are depicted as spheres with letters (N, nitrogen; P, phosphorus; K, potassium; S, sulfur). Wavy tan arrows represent nutrients and/or bacterial factors that benefit plant growth. This figure was created with Biorender.com.

attract organisms that have beneficial traits such as plant growth promotion, solubilization of nutrients, and inhibition of pathogen growth (Andreote et al., 2014).

When compared to bulk soil, the rhizosphere microbiome has a richer and functionally better-characterized microbiome. It was reported that the rhizosphere and root microbiomes of barley differentiated from the soil microbiomes as a gradient (Bulgarelli et al., 2015). The soil microbiomes showed higher bacterial richness and diversity compared with root samples, while the rhizosphere microbiota composition was intermediate between soil and root samples. Similarly, higher microbial richness was reported in the bulk soil surrounding the rhizosphere soil of maize (Walters et al., 2018). Additionally, it was observed that the rhizosphere microbial communities had a greater network connectivity than the bulk soil in maize and wild oat (Peiffer et al., 2013; Shi et al., 2016; Walters et al., 2018). Collectively, this suggests that roots can promote the development of niches with dominant taxa that favor greater interactions and more complex co-occurrence patterns over time.

1.3.2 Core microbiome

With up to 20-40% of a plant's photosynthate becoming root exudate (Lynch & Whipps, 1990), it is not surprising that plants encourage microbial growth and that changes in the exudation components can modify the composition of the associated microbial community (Vives-Peris et al., 2020; Wallenstein, 2017). Although many microorganisms can respond to plant exudates, it is becoming more evident that plants harbor a specific subset of microorganisms, termed the core microbiome, that is consistently associated with a particular plant host across a wide range of environments (Toju et al., 2018; Walters et al., 2018). The core microbiome has been shown to provide several functional benefits to plants that include, but are not limited to, enhancing plant mineral nutrient uptake and suppressing soil-borne

diseases (Banerjee et al., 2018; Lemanceau et al., 2017a; Singh et al., 2020). Additionally, it has also been observed that plants can also recruit transient microbes that vary in composition and abundance to alleviate environmental stress (Berg et al., 2020). Therefore, a better understanding of the crop microbiomes, and their interaction with hosts with a range of climatic and soil types is necessary to explore the full potential of the crop microbiome to support crop production.

1.4 Major drivers of microbial diversity in plant ecosystem

1.4.1 Root exudates

Defining the major drivers for microbial diversity is a challenging task since plant-microbe interactions form a complex relationship. Several factors influence the composition of microbial communities, such as plants, microbe-microbe interaction, and edaphic factors. These factors influence the selection of microbes primarily through root exudates. Root exudates consist of a variety of chemicals, primary metabolites, and secondary metabolites (Rasmann & Turlings, 2016; Tsunoda & van Dam, 2017; Vives-Peris et al., 2020). Primary metabolites, such as the labile carbon of root exudates, increase the growth of fast-growing microorganisms with higher nutritional requirements, enabling them to outcompete slow-growing microorganisms with lower nutritional requirements (Terrazas et al., 2016). Several studies indicate that bacteria belonging to the phylum Proteobacteria, which are known to respond to labile carbon (Peiffer et al., 2013), are enriched in the rhizosphere compared to bulk soils (Aira et al., 2010; Chauhan et al., 2011; Lundberg et al., 2012; Peiffer et al., 2013; Zhang et al., 2020). Likewise, secondary metabolites trigger varying responses in organisms. Flavonoids, for example, attract symbionts in nodule formation, stimulate mycorrhizal spore

germination and hyphal branching, and influence quorum sensing in legumes (Philippot et al., 2013). By regulating the composition of root exudates, the microbial diversity in the plant ecosystem can be substantially altered.

1.4.2 Plant factors

Plant factors consist of the plant species, genotype, immune system, and physiological age, nutritional status, and pathogen infection (Hawkes et al., 2007; Overbeek & Elsas, 2008; Sharma & Verma, 2018; Vives-Peris et al., 2020; Zhalnina et al., 2018). Plant species strongly influence the structure of rhizosphere communities through differences in root morphology and exudation of different metabolites (Philippot et al., 2013). Colonization of different bacterial populations due to root exudates was observed in the rhizosphere of four plant species – wheat, maize, rape, and barrel clover (Haichar et al., 2008). Similarly, the activity and dynamics of the indigenous *Pseudomonas* spp. in the rhizosphere were significantly influenced by host plant species (Bergsma-Vlami et al., 2005).

Enrichment of antifungal microbial communities was reported in the barley rhizosphere after the infection of *Fusarium graminearum* (Dudenhoffer et al., 2016). A high nitrogen application rate increased the relative abundances of ammonia-oxidizing and denitrifying bacterial communities in maize rhizosphere (Zhu et al., 2016). It has also been reported that plant genotypes in *Arabidopsis thaliana* (Micallef et al., 2009), *Solanum tuberosum* (Inceoglu et al., 2010), grapevine (Berlanas et al., 2019), and *Zea mays* (Aira et al., 2010) influence the production of root exudates, thereby changing their microbial communities. Aira et al. reported that the rhizosphere microbial communities of two maize hybrids were strongly influenced by plant genotype (Aira et al., 2010). In contrast, a large-scale longitudinal study conducted in five fields with 27 maize inbred lines reported that plant

age was the strongest factor shaping the rhizosphere microbial community, followed by location and genotype (Walters et al., 2018). However, within a given field, plant genotype significantly influenced the richness of the microbiome (Peiffer et al., 2013). A study focused on the sugarcane microbiome under field conditions demonstrated that microbial communities were primarily influenced by the plant compartments, followed by the growing region, age, and crop variety (Hamonts et al., 2018). The influence of plant factors on the composition of microbes is obvious under the same environmental conditions.

1.4.3 Microbe-microbe interactions

In addition to host-microbe associations, microbe-microbe interactions also affect the structure of microbial communities in the rhizosphere (Bulgarelli et al., 2015). There are a wide range of microbe-microbe interactions ranging from synergistic to antagonistic which could shape the composition of the plant microbiota (Hacquard et al., 2015; Terrazas et al., 2016). Soil microbes can also affect the root exudation process by consuming primary root exudates or releasing secondary compounds to stimulate specific metabolite production (Canarini et al., 2019). Specific microbial taxa in the tomato rhizosphere were found to modify the chemical composition of root exudates; for example, acyl sucrose exudation was induced by *Bacillus subtilis* (Korenblum et al., 2020). Further, microbial interactions assist the host plant in mitigating several abiotic stresses through direct antagonism against pathogens or induction of systemic resistance by priming plants (Arif et al., 2020; Meena K. K. et al., 2017). Several microbes secret an enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, that regulates the level of stress hormone ethylene in the plant. Strains of *Arthrobacter* spp., *Bacillus* spp., and *Pseudomonas* spp. have been reported to enhance plant

growth through the production of ACC deaminase (Compant et al., 2019). This indicates the bi-directional relationship between plants and their microbial communities.

1.4.4 Edaphic factors

Edaphic factors such as pH, soil type, indigenous microflora, oxygen, nutrients, and light availability (Hacquard et al., 2015; Kaul et al., 2018) exert considerable impact on the developmental stage and physiological status of the host plant (Hacquard et al., 2015). A recent plant phytometer study with six plant species across diverse edaphic conditions and land use gradients indicates that changes in the changes in the bulk soil bacterial communities were the direct drivers of active bacterial communities (Vieira et al., 2020). The composition of the rhizosphere microbiome was strongly dictated by soil texture, water content, and soil type instead of plant properties and root exudates (Vieira et al., 2020). Another phytometer study was conducted on clonal oak saplings (*Quercus robur* L., clone DF159) under different field sites with similar climatic conditions. This study revealed that the effect of environmental factors was greater than the plant effect in shaping soil microbial communities. Similar microbial compositions were observed in sites with comparable pH, soil organic carbon, and C/N ratios (Habiyaremye et al., 2020). In contrast, similar rhizosphere communities were reported in three different fields having distinct physiochemical properties (Peiffer et al., 2013). Thus, plant-soil-microbe interaction is highly complex and their effect on the composition of the microbial community is determined by the interaction between them rather than each factor alone.

1.5 Core microbiomes and their application potential

A core microbiome is comprised of microbes that are recruited by a plant regardless of the environment (Figure 2). These core microbiomes contain key microbial taxa carrying essential functional genes for the plant host (Astudillo-García et al., 2017; Berg et al., 2020; Bergsma-Vlami et al., 2005; Hacquard et al., 2015; Naylor et al., 2017; Toju et al., 2018; Trivedi et al., 2020; Vandenkoornhuyse et al., 2015). The functional redundancy of microbes, i.e., the coexistence of multiple taxa performing a particular biochemical function, allows for environmental variation without comprising plant host fitness (Lemanceau et al., 2017b; Louca et al., 2018). In addition, the network of interactions between organisms provides a buffer against disturbance by recruiting different microbial combinations to fulfill specific functions (Konopka et al., 2015). A dynamic functional community can be formed by focusing on the core microbiome instead of the highly complex native microbiota for further studies (Durán et al., 2021; Ramirez-Villacis et al., 2020).

Recent advances in high throughput sequencing and bioinformatic tools have enabled the discovery of the core microbiomes of different crops. Marker gene amplicon sequencing has been widely used to study microbial association with different plant parts over a range of environmental conditions (Durán et al., 2021; Hacquard et al., 2015). Through co-occurrence network analysis of the resulting genomic data, it is possible to identify a core microbiome. It also explores the positive or negative relationship between members based on their occurrence or abundance (Berg et al., 2020; Rodriguez et al., 2019; Xue et al., 2022). Further, the positions of microbes in the network can indicate their importance within the microbial community. Highly interactive members of the core microbiome, which are called "hub"

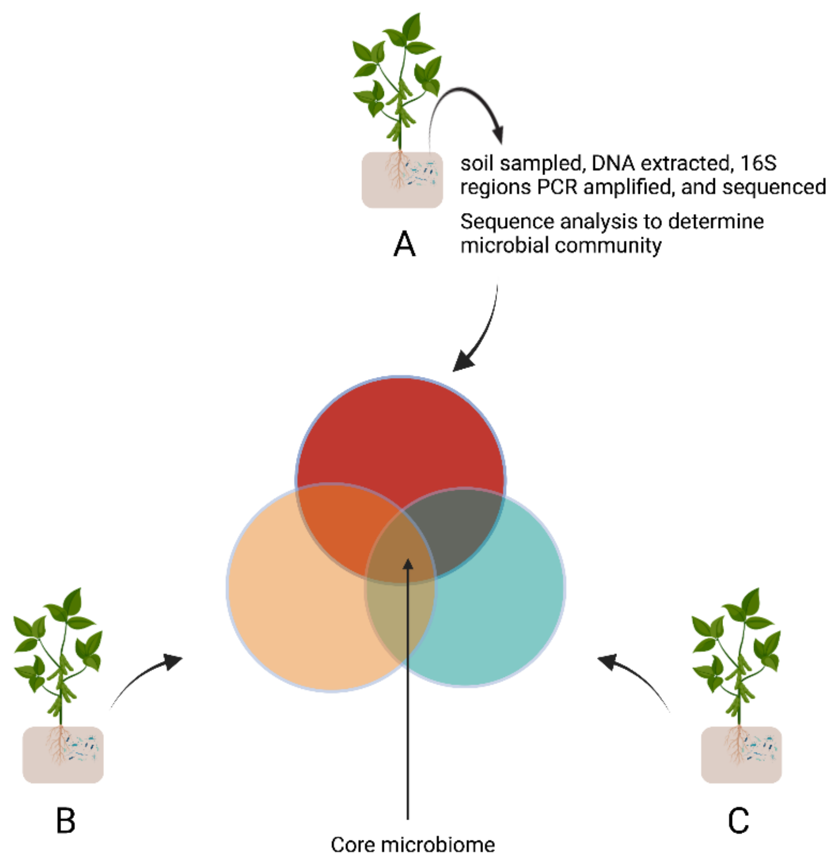

Figure 2. Determination of a core microbiome.
The same plants are grown in independent locations (A-C). The soil is then sampled, sequenced, and the bacterial population (colored circles) are determined and compared (Venn diagram). The intersection represents the plants' core microbiome. This figure was created with Biorender.com.

microbes, have been shown to have a strong influence in shaping the microbial communities of plant hosts (Agler et al., 2016; Muller et al., 2018; Trivedi et al., 2020).

Several studies have reported the taxonomy of core microbiomes in different crops (Table 1). Walters et al. (2018) found that seven bacterial operational taxonomic units (OTUs) were observed consistently in the maize rhizosphere at different ages and field conditions. All seven OTUs were taxonomically assigned to the phylum Proteobacteria with differences at the genus level. Likewise, the core microbiome of the citrus rhizosphere was identified through an extensive study of soil samples from twenty-three locations in eight citrus-producing countries across six continents (Xu et al., 2018). These studies show that the core microbiome can select for key members of the microbial community that can be screened in vitro for microbe-microbe interactions and putative functions (Lebeis, 2014).

Overall, there is general agreement in the literature that organisms do have strong associations with certain microbes. Many studies carry out their analysis at the genus level, which gives a descriptive analysis of what organisms can be present. In some cases, more functional metatranscriptomic studies can provide more insight into which species are present as well as what genes are being expressed under a given set of conditions. Together, these data are helping to develop hypotheses of how microbes might be affecting plant responses and are allowing work to be designed to ask key ecological questions regarding plant-microbe interactions to be asked more directly.

Table 1. Studies related with core microbiome identification in agricultural crops

Crop	Composition of core microbiomes	Location	References
Common bean (*Phaseolus vulgaris*)	Nearly 70 % Proteobacteria (*Rhizobium*, *Bradyrhizobium*, *Burkholderia, Novosphingobium*, and *Sphingomonas*), Acidobacteria, Actinobacteria, Verrucomicrobia and Planctomycetes.	Colombia (North-west region)	(Pérez-Jaramillo et al., 2019)
Citrus	*Pseudomonas, Agrobacterium, Cupriavidus, Bradyrhizobium, Rhizobium, Mesorhizobium, Burkholderia, Cellvibrio, Sphingomonas, Variovorax,* and *Paraburkholderia*.	Eight citrus producing countries (Six continents)	(Xu et al., 2018)
Grape vine	*Bradyrhizobium, Steroidobacter, and Acidobacteria* spp.	New York (Suffolk County)	(Zarraonaindia et al., 2015)
Maize	*Agrobacterium*, Bradyrhizobiaceae, *Devosia*, Comamonadaceae, *Pseudomonas* and Sinobacteraceae.	New York (Urbana, Columbia, Aurora, Lancing and Ithaca)	(Walters et al., 2018)
Potato	*Bradyrhizobium, Sphingobium, Microvirga, Blastococcus* and SMB53.	Peru (Pazos, Sincos, and Sicaya)	(Pfeiffer et al., 2017)
Wheat	*Bradyrhizobium, Sphingomonadaceae, Massilia, Variovorax, Oxalobacteraceae, and Caulobacteraceae.*	United States (Inland Pacific Northwest)	(Schlatter et al., 2020)

1.6 Synthetic community (SynCom) approach in sustainable agriculture

The SynCom approach incorporates a synthetic biology approach that is coupled with the knowledge generated from microbial community analysis, metagenomic, and bioinformatic approaches that have become more accessible with the advent of next generation sequencing technologies. Understanding the dynamic interactions within microbial ecosystems is useful to engineer microbial consortia with robust, stable, and predictable behaviors (Mccarty & Ledesma-Amaro, 2019).

Briefly, SynComs are constructed by co-culturing multiple taxa under well-defined conditions to mimic the structure and function of a microbiome. The underlying principle is to reduce the complexity of the original microbial community while still preserving some of the essential interactions between the microbes and their hosts (de Souza et al., 2020; Kaminsky et al., 2019; Vorholt et al., 2017). The goal is to facilitate an increase in community stability through synergistic interactions between its members (de Souza et al., 2020). Several studies have reported that SynCom application enhanced plant growth under greenhouse conditions (Armanhi et al., 2021; Chai et al., 2021; Lee et al., 2021) as well as field conditions (Santhanam et al., 2015; Wang et al., 2021).

A SynCom can be constructed using either top-down or bottom-up approaches (Großkopf & Soyer, 2014). The top-down approach focuses on a functional definition for a community to characterize its structure and dynamics in detail. Toju et al., (2020) applied the functional core microbiome concept to discover the best combinations of species/strains that potentially maximize functionality at the community/ecosystem level. This method produces communities with natural representation and high reproducibility while lowering the chances of missing important species. However, the effectiveness is dependent on the ability to

measure species diversity in a complex community accurately. The bottom-up approach identifies common interaction patterns and processes among species. Paredes et al. (2018) used binary-association assays to design a SynCom for *Arabidopsis thaliana* that led to predictable plant phenotypes. Even though binary-association assays facilitates establishing causality, it requires technological advances to manage highly complex communities and increases the chances of missing important community members. Recently Kehe et al., (2019) introduced a microfluidic droplet-based platform, the kChip, to automatically construct SynComs with all possible microbe combinations using a set of species. This has made the SynCom approach more efficient and viable for large-scale studies, but it has limitations that may make it difficult to replicate for field trials.

1.6.1 Effective SynCom formulation

1.6.1.1 Identifying members of the SynCom

An effective SynCom can be produced by identifying functional communities through a top-down approach and then applying the bottom-up approach to study the interactions between the members of those communities. Genomic information and gene expression profiles could be used to select the microbes with beneficial functional traits or metabolic capability to design the best microbial combination for the microbial consortia (de Souza et al., 2020; Toju et al., 2018). Since multiple genes are responsible for important traits, such as colonization efficiency and prevalence, genomic analysis for multiple markers may be key to identifying relevant microbes (Cole et al., 2017; de Souza et al., 2016, 2020; de Souza et al., 2019; Levy et al., 2018). Computational tools can be used to screen for beneficial microbial candidates from existing genomic datasets, which would be less laborious than traditional methods (Finkel et al., 2017). Then, the SynCom could be constructed using a bottom-up

approach by addition, elimination, or substitution at the strain level (Liu et al., 2019a; Vorholt et al., 2017).

1.6.1.2 Maintaining Microbial Culture Collection

An extensive microbial culture collection is essential to building a SynCom since it is comprised of culturable microbes (Choi et al., 2021; de Souza et al., 2020; Finkel et al., 2017; Vorholt et al., 2017). The SynCom approach is initiated from the isolation of microbial cultures from the natural ecosystem and then formulated through manipulations of the selected microbiota to perform the desired functions for the host plants (de Souza et al., 2016). Since nearly % of bacteria are unculturable under normal conditions (Oberhardt et al., 2015), new approaches are necessary to generate extensive microbial collection. One approach is to use metagenomic analysis to identify appropriate media and culture conditions (Oberhardt et al., 2015). Also, high-throughput bacterial cultivation methods, such as the limiting dilution method (Zhang et al. 2019; 2021), cell sorting (Bai et al., 2015), and colony picking, (Armanhi et al., 2018) provide potential solutions for capturing diverse bacterial species on a large scale (Liu et al., 2019a).

1.6.1.3 Assessing SynCom performance

The effectiveness of SynComs can be quantitatively and qualitatively assessed with plant hosts under controlled environments using different axenic systems such as agar-based (highly artificial and uniformly controlled), clay-based (mimic soil), and FlowPot (autoclaved and washed peat) systems (Bai et al., 2015; Castrillo et al., 2017; Finkel et al., 2019; Liu et al., 2018; Paredes et al., 2018; Zhang et al., 2019). Axenic systems allow for detailed investigations of its components under controlled and reproducible conditions, which facilitate the establishment of causal links between genotypes and phenotypes. Changes can

also be made at the functional level by removing or adding specific functions via gene expression (Liu et al., 2019a). Further, the consequences of biotic or abiotic perturbations can be monitored at all levels (Liu et al., 2018; Melnyk et al., 2019).

Finally, an efficient SynCom could be tested under real field conditions to offset the limitations of the traditional approach. Assessment of a SynCom on plant phenotypic traits could be done through high-throughput phenotyping technologies as they offer multiple advantages such as automated, non-destructive and dynamic monitoring of morphological and physiological traits related to growth, yield, and performance throughout their entire lifecycle (Rouphael et al., 2018). This would facilitate an effective SynCom with more compatible, efficient, and adaptable microbes (Choi et al., 2021; Hart et al., 2018).

1.7 Current approaches in SynCom application

SynCom approaches have been used in experimental ecology and evolution studies to understand ecological interactions as well as ecological processes (Cairns et al., 2018; Castrillo et al., 2017; Finkel et al., 2017; Levy et al., 2018; Teixeira et al., 2021). The SynCom approach started being used to test evolutionary interactions in plant-microbe studies. Then, the focus has shifted towards the improvement of plant growth and production. Several studies have been conducted in the model plant *A. thaliana* as well as agricultural crops – maize, soybean, sorghum, and tomato – to understand plant-microbe interactions using SynComs under controlled environments (Table 2). The above studies reiterate that the SynCom approach is an effective tool for exploring plant-microbe and microbe-microbe interactions. Even though most of the SynCom experiments were conducted under controlled conditions, it gives valuable information about the interaction between each member in the community assemblage and identifying keystone members. For example, Niu et al. (2017)

reported that the removal of one species from the SynCom led to drastic changes in community composition. The simplicity of this approach allows repeated experiments to ensure reproducibility, which could prevent problems in future large-scale applications. Recently, the interest in the SynCom approach has been focused on improving crop yield by extending the research in the greenhouse to field conditions, which is an important milestone of the SynCom application. Despite there being a long way to go, the current application of SynCom indicates the possibilities to be incorporated into the large-scale application in the near future.

1.7.1 Understanding plant-microbe interaction

Bodenhausen et al. (2014) showed that host genotype influences the phyllosphere community composition and abundance using fifty-five *A. thaliana* plant mutants inoculated with a SynCom. Castrillo et al. (2017) studied the effect of plant P_i stress response on the *A. thaliana* immune system function and microbiome assembly a SynCom composed of thirty-five members. Niu et al. (2017) constructed a simplified seven-species SynCom from microbes associated with maize root to investigate the dynamics of root colonization, interspecies interactions, and the role of each member in the community. In another SynCom study, it was reported that root colonization was regulated by microbe-associated molecular patterns (MAMPs) -triggered immunity (Teixeira et al., 2021). The SynCom approach has also been used to examine the role of specialized metabolites on the colonization of bacteria in the *A. thaliana* rhizosphere (Voges et al., 2019). Thus, SynComs can effectively be used to explore plant-microbe interactions, which must be considered when using microbes in large-scale agricultural applications.

Table 2. SynCom approach used in different studies

Plant	Growth condition	SynCom size & origin	Objective	Reference
A. thaliana	Gnotobiotic system	7 strains (representatives of the most abundant phyla in the phyllosphere)	To identify plant genetic factors that influence community composition and/ or the bacterial abundance of the leaf-associated community.	(Bodenhausen et al., 2014)
A. thaliana	Growth chamber	38 (37 *A. thaliana* root associated strains and *E.coli*)	To study the colonization ability of isolated bacterial strains and the effect of exogenous application of salicylic acid on root microbiome assembly	(Lebeis et al., 2015)
A. thaliana	*In vitro*	35 (34 root associated strains that represent the taxonomic diversity and *E.coli*)	To study Pi stress on microbiome assembly (Castrillo *et al.*, 2017) and effect on immune system of *Arabidopsis* (Teixeira *et al.*, 2021)	(Castrillo et al., 2017; Teixeira et al., 2021)
A. thaliana	Hydroponic s-based gnotobiotic setup	22 (*A. thaliana* root-derived bacterial commensals)	To explore the role of root-specialized metabolites in rhizosphere bacterial assembly	(Voges et al., 2019)
Astragalus mongholicus	*In vivo* and greenhouse condition	Two SynComs 13 (disease-resistant bacterial community with 10 high- and three low-abundance bacteria enriched in diseased roots) 4 (composed of three high-abundance bacteria and one low-abundance bacterium)	To investigate the roles of low-abundance bacteria in the control of root rot disease	(Li et al., 2021)
Maize	Greenhouse	12 (maize seed-borne bacterial strains)	To assess the effect of SynCom on germination and seedling growth of maize	(Figueiredo dos Santos et al., 2021)
Maize	*In vitro* and Pot experiment	4 (desiccation-tolerant bacterial strains)	to test their effect on maize growth under normal and desiccated conditions.	(Molina-Romero et al., 2017)

Maize	Gnotobiotic system	7 (Isolated from maize root representing three of the four most dominant phyla)	To study the dynamics of root colonization (Niu *et al.*, 2017) and the effect of microbial communities on heterosis of root biomass and other traits in maize (Wagner *et al.*, 2021)	(Niu et al., 2017; Wagner et al., 2020)
Maize	Greenhouse	17 (community-based isolates comprising 26 bacterial strains collected from sugarcane rhizosphere, endophytic root, and stalk)	To assess the SynCom performance on colonization and growth of maize (Armanhi *et al.*, 2018), explore the bacterial traits associated with successful colonization of plants (de Souza *et al.*, 2019) and study the impact of the SynCom on three commercial maize hybrids under drought stress (Armanhi *et al.*, 2021)	(Armanhi et al., 2018, 2021; de Souza et al., 2019)
Maize	Greenhouse	6 (*Bacillus* strains isolated from maize roots and leaves)	To examine their suppressive effect on fungal pathogen of maize	(Ali et al., 2021)
Potato	*In vitro* assays	Nine *Pseudomonas* strains isolated from the rhizosphere and shoots of field grown potato plants	To compare the disease inhibition capacity	(De Vrieze et al., 2018)
Soybean	Greenhouse and field	3 different SynComs were constructed from 12 isolates	To assess the influence of root associated microbes on host plant growth and nutrient acquisition.	(Wang et al., 2021)
Sorghum	Greenhouse	5 SynComs (36 bacterial strains isolated from soil and roots of sorghum growing fields with different combination)	To determine the effect of SynCom inoculation on the growth dynamics and microbial communities of four genotypes with different N status	(Chai et al., 2021)
Tobacco	*In vitro* and Field conditions	6 (native root-associated isolates from field-grown tobacco plants)	To study the effect of bacterial consortium on protection against a sudden wilt disease	(Santhanam et al., 2015)
Tomato	Greenhouse	4 (isolated from healthy tomato rhizospheric soil)	To explore the effect of SynCom on wilt disease suppression in tomato and underlying mechanism	(Lee et al., 2021)

1.7.2 Pathogen suppression

Pathogens are a major threat in agriculture as they can lead to complete yield loss. Several studies have reported that SynComs can be effectively used to suppress pathogenic organisms while improving crop performance (Ali et al., 2021; De Vrieze et al., 2018; Li et al., 2021; Santhanam et al., 2015, 2019). Li et al., (2021) constructed two SynComs by adding both high and low abundance bacteria isolated from diseased plants. Results indicated that high-abundance bacteria protected the host through plant growth promotion and inhibition of the pathogenic fungus, while low-abundance diseases enhanced plant-induced systemic resistance. It is important to note that SynComs showed a superior effect on disease suppression and growth promotion compared to the mono-inoculated plants (Ali et al., 2021; Li et al., 2021). Synergistic interactions between the members of SynComs facilitate improved plant protection and growth.

1.7.3 Crop improvement

Regardless of other benefits, crop productivity is always a prime concern. Inoculation with a SynCom constructed from sugarcane-associated microbes increased the biomass of maize plants compared to the uninoculated controls (Armanhi et al., 2018). The same SynCom also improved drought tolerance and reduced yield loss in maize (Armanhi et al., 2021). Another SynCom, composed of desiccation-tolerant bacteria, showed increased plant growth parameters such as dry weight of shoot and root, plant height, and plant diameter when compared with either non-inoculated control or mono-inoculated treatments (Molina-Romero et al., 2017). Further, Wang et al., (2021) reported that functionally assembled SynComs improved soybean yield up to 36% under field conditions. Thus, recent studies suggest that SynCom could be effectively incorporated in agriculture to enhance crop yield.

1.8 Are synthetic microbial communities a way forward?

Interest in rhizosphere research has continually grown exponentially since 1994 to the present day, with the term "plant microbiome" first being used as a key word in publications in 2011 (Oresnik et al., 2016). The application of microorganisms in agriculture has emerged as a promising, sustainable approach to improving crop production. The microbiome plays an essential role in several plant processes and soil fertility. Poor performance of microbial inoculants is a challenge in developing stable inoculants for agriculture. However, recent advances in high-throughput sequencing technologies create an opportunity to identify the core microbes associated with plants and facilitate the formation of effective SynComs.

Although the SynCom approach is a promising technology, several challenges must be addressed before it can be used in large-scale applications. Designing SynComs with hundreds of microbes is impractical due to a lack of industrial technologies and difficulties handling them. This issue can be addressed by constructing SynComs with microbes that have multiple beneficial traits and synergistic interactions. Nevertheless, keeping multiple species is challenging as medium composition plays a critical role in population dynamics. Stochastic events can also cause fluctuations of a population in mixed communities. Therefore, it will be necessary to monitor the population dynamics of a SynCom to ensure all members are functioning and having enough viable cell counts.

Prediction of SynCom interaction with host plant and soil microbes in the natural environment is challenging due to the influence of the native microbes. Thus, maintaining the long-term stability of SynCom is another task to be attained as introduced inoculants are exposed to an environment with competitive species. The SynCom may change over time due to genomic evolution and horizontal gene transfer. In addition, some microbes show

differential expression of genes with varying environmental conditions. Sustaining the community robustness and function over a timescale is a crucial aspect. Biosensors and marker gene technologies could be incorporated to trace the interaction and behaviors of introduced SynCom.

The ability to genetically modify or engineer host plants and microbes has increased dramatically over the last five years. Whereas in the past, there were relatively few microbial genetic model systems (Miller, 1991), the ability to sequence genomes as well as tools such as CRISPR/Cas9 have allowed the genetic modification of many diverse bacteria (Shelake et al., 2019; Rubin et al., 2021). With respect to developing a SynCom, this can lead to modifying certain community member(s) to allow desired interactions with target crops. Recently, it has been shown that endophytic bacteria could be engineered to contain inducible nitrogenase activity (Ryu et al., 2020), which in principle can be combined with plants that have been modified to produce signals for targeted regulation of bacterial genes (Geddes et al., 2019).

So far, most studies have been conducted in controlled systems which are opposite to diverse natural environments. Assessing their stability and plant performance under field conditions is the ultimate target. Production of the required amount of SynComs for large-scale application is also problematic as it would require additional technologies like bioreactors. Determining the effective method of application, whether it is liquid application or seed coating, is another hurdle to be overcome. Extensive field studies with a range of climatic conditions are required to ensure the activity of the applied inoculants.

The development of an effective SynCom is a novel opportunity to improve sustainable food production. It is clear from the literature that microbes can positively affect plant health and productivity. However, the complexity of dealing with multiple

microorganisms that interact with field crops in the real-world climate is challenging. It has been previously pointed out that these technologies would have to be transformative to growers for them to be adopted (Oresnik et al., 2016). In the short term, the SynCom approach is an opportunity to delve into the intricacies of plant-microbe interactions as well as microbial ecology. These advances are crucial to better understanding how microbes can be manipulated to deliver desired traits to plants. The complex SynComs constructed are clearly important for academic understanding but are not a pragmatic solution. The lessons that will be learned from these approaches, however, can provide valuable information to either produce SynComs that contain fewer microbes or to develop SynComs that can work synergistically with the native microbial communities already present in the field. The utilization of this technology will require a long-term multidisciplinary approach that includes microbiologists, plant biologists, agronomists, and fermentation specialists to facilitate the delivery of a working system.

Therefore, the present study was mainly focused on understanding soil bacterial communities in local Manitoba soils in order to identify the core microbial communities. By identifying core microbial communities, researchers can focus on specific taxa or functional groups that may have beneficial effects on soil fertility, nutrient cycling, or plant productivity. This foundational information helps guide the development of targeted strategies, such as SynComs, for improving agricultural practices and ecosystem sustainability.

Thesis goals and hypothesis

The overall goal of this thesis is to investigate bacterial communities present in field experiments having different soybean rotation treatments. Understanding bacterial diversity and composition under different crop rotations, in different years, and across each growing season could lead to the identification of major factors that influence bacterial dynamics in a crop-associated environment. There are three major goals in this thesis.

The first goal is to study the effect of crop rotation on bacterial diversity and community composition of soils associated with soybeans. Next to wheat and canola, soybean (*Glycine max*) is Manitoba's third most seeded crop (Manitoba government, 2023). Acreage under soybean cultivation in Manitoba has drastically increased over the past decade with the arrival of early-maturing and glyphosate-resistant varieties (Weerasekara, 2022). Since soybeans are a relatively new crop to the province, rotation studies are being conducted to optimize their productivity while maintaining soil health. Therefore, exploring bacterial communities under a soybean crop rotation system is an opportunity to elucidate the effect this has on bacterial communities.

The second goal of the thesis is to construct an archive of typical bacteria found in Manitoba soils. Building a microbial culture collection is a crucial step in the formulation of a SynCom with predictable plant traits. Further, culture collection is essential to study the structural and functional properties of bacteria through in-depth analysis.

The third goal of the thesis is to characterize phosphate-solubilizing bacteria isolated from local Manitoba soils. Phosphorus (P) is the second most limiting nutrient for plant growth in terrestrial ecosystems. The phenotypic trait of phosphate solubilization is often correlated with bacteria that confer plant growth-promoting traits. The isolation of such

organisms could contribute to the development of non-rhizobial inocula or their integration into synthetic microbial communities (SynComs) designed for agricultural applications. These PSBs could serve as key members within SynComs, complementing other functional groups (e.g., nitrogen fixers, biocontrol agents) to create a balanced and synergistic microbial consortium. Alternatively, standalone inoculant strategies could leverage these organisms to target P mobilization directly, enhancing crop growth in nutrient-limited soils.

Chapter 2: The effect of soybean in crop rotations on the composition of soil bacterial communities

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This work was planned and carried out by Ambihai Shayanthan in collaboration with Patricia Ann C. Ordoñez. PO assisted in sample collection, preparation and analysis.

2.1 Abstract

Within the last decade, the prevalence of soybean (*Glycine max* L.) in Manitoba has increased to the point that it is the third most seeded crop, with only wheat and canola being more significant. Since it is a relatively new crop to the province, rotation studies are taking place to optimize its productivity while maintaining soil health. Although there is evidence that plants can influence soil microbial communities, more needs to be understood about how crop rotation regimes influence microbial diversity and community structure over a prolonged period. This study examined the pattern of bacterial communities in two different sites having soybean in rotation for eight years to study the impact of crop rotation on bacterial diversity and to identify factors that may influence bacterial communities, using 16S rRNA gene sequencing targeting the V4 region. Soil samples were collected from two field experiment locations in Manitoba (Carman and Kelburn) from 2017 to 2021 with four crop rotations. These rotations were continuous soybean (CS), soybean-canola (SCa), soybean-corn (SCo), and soybean-wheat-canola-corn (SWCC). Analysis of the before-planting (BP) and after-harvest (R8) samples indicated that the bacterial diversity was primarily influenced by soil type, followed by the growing season and crop rotation. Regardless of the environmental variability, crop rotation treatments caused short-term changes in the diversity and composition of bacterial communities. Network analysis indicated that core OTUs across crop rotation treatments displayed both positive and negative interactions. These data suggest that crop rotations alter the diversity and composition of soil bacterial communities.

2.2 Introduction

Soybean (*Glycine max* L.) is a legume plant that is a rich source of oil and protein. It is well-known for its symbiotic relationship with the bacteria *Bradyrhizobium japonicum*, which supplies the plant with reduced nitrogen. Soybean is one of the largest export crops in Canada, with an overall production of around 6700 million kg in 2023 (Statistics Canada, 2023). Soybean cultivation has become popular in the eastern Canadian prairies due to its agronomic traits, such as the availability of short-season cultivars, herbicide resistance traits, and tolerance to wet growing conditions relative to other commonly grown crops in the region (Weerasekara, 2022). The acreage under soybean cultivation has drastically increased in Manitoba, and the province has become the second-largest producer, contributing nearly 20% of the national production in the last year (Statistics Canada, 2023). Since soybean is a relatively new crop in Manitoba, exploring the opportunities that could optimize soybean production is necessary.

Microbes are vital in soil ecosystem functioning, as they help with nutrient cycling, organic matter degradation, and mineral weathering (Turner et al., 2017). They also improve the fitness of plants through growth-promoting traits such as nutrient acquisition, abiotic stress tolerance, and protection against pathogen infection (Bakker et al., 2018; Oyserman et al., 2018; Pérez-Jaramillo et al., 2019). It has been reported that plants shape the microbiome of the rhizosphere through the secretion of exudates (Schmidt et al., 2019). Further, plants recruit a specific subset of microbes, the core microbiome, regardless of environmental variations such as climatic conditions, soil types, and seasonal changes (Shayanthan et al., 2022; Toju et al., 2020; Walters et al., 2018). Agronomic practices, such as fertilization and crop rotation, influence microbial communities and functions. Several studies reported that cropping sequences within the crop rotation can alter soil microbial communities and improve agronomic performance

(Bolaji et al., 2021; Chamberlain et al., 2020; D’acunto et al., 2018). Thus, it is necessary to understand the pattern of soil microbial communities and their functions in soils under different cropping systems, as they are integral and dynamic parts of soils.

Crop rotation is defined as growing different crops on the same land during successive cycles. Crop rotation can effectively increase crop yield and economic benefits by reducing insect pests, weeds, and pathogens (Bowles et al., 2022). Crop rotations have a significant role in the changes in microbial diversity, community composition, and networks (Jiang et al., 2016). Crop rotation also affects the soil's bacterial diversity and metabolic activity, where the most diverse rotation has shown the most varied and active soil microbial communities (D’acunto et al., 2018). Crop rotation sequences also directly influence the composition of microbial communities associated with soil and plants (Neupane et al., 2021; Zhang et al., 2019). The impact of cropping sequences on bacterial communities varies depending on the specific organisms involved. For example, a higher number of bacterial species was observed in a soybean-corn-corn rotation, while fungal species were higher in a corn-soybean-soybean rotation (Bolaji et al., 2021). Yang et al. (2021) reported that pulse frequency in crop rotation had minimal impact on the alpha diversity of the bacterial community but significantly altered the composition of both bacterial and fungal communities in soils during a long-term pulse intensification field experiment in the Canadian Prairies. Meanwhile, the inclusion of legumes in rotation has been reported to show no consistent effect on microbial diversity or richness. Thus, the overall impact of crop rotation on microbial diversity under different environmental conditions has been unclear.

Since the diversity of crop rotation in Manitoba increased with the introduction of soybean, it is necessary to understand the effect of soybeans in crop rotation on the composition

of microbial communities. In Manitoba, crop sequence was reported to affect bacterial diversity and richness in bulk soil and soybean rhizosphere soil while not affecting fungal diversity (Bolaji et al., 2021). Since differences among crop rotations have emerged slowly with time, it is important to understand the long-term impact of crop rotation on microbial communities (Mohr, 2018). The long-term effect of including soybean in crop rotations with canola, corn, or wheat on soil bacterial communities is not well understood in Manitoba. This study was conducted with the following objectives.

1) To study the influence of soil type and growing seasons on the diversity of soil bacterial communities, as part of understanding how these factors shape microbial dynamics in agricultural systems.
2) To compare the impact of continuous soybean cultivation versus crop rotation with soybean, canola, corn, and wheat on the diversity and composition of soil bacterial communities in Manitoba soils.
3) To identify core bacterial communities and their topological roles in ecological networks among crop rotation treatments.

2.3 Materials and Methods

2.3.1 Soil and site location

Soil samples were collected from the University of Manitoba Ian N. Morrison Research Farm near Carman, Manitoba (49.492261 N, 98.042497 W) and Richardson International's Kelburn Farm near St. Adolphe, Manitoba (49.694081 N, 97.122981 W) where crop rotation studies were conducted from 2014 to 2021. For this study, soil samples were collected from 2017 to 2021. According to the Canadian soil classification system, the soil at Carman belonged to

Gleyed Black Chernozem of the Rignold series. In contrast, soil at Kelburn are Orthic Dark Grey Black Chernozem of the St. Nobert Series (Mills & Haluschak, 1993). Selected soil properties and the previous crop history of the first and last year of sample collection at each location are given in Table 3.

2.3.2 Experimental design and agronomic practices

Four crop sequence treatments were tested at each site: 1) continuous soybean (CS), 2) soybean-canola (SCa), 3) soybean-corn (SCo), and 4) soybean-wheat-canola-corn (SWCC) (Table 4). The experiment was designed in a randomized complete block design (RCBD) with four replicates with a plot size of 8 m × 10 m. In Carman, tillage was done two times per season: after crop harvesting (fall) and before spring planting (spring) using a disk cultivator with cultipackers. In Kelburn, tillage was performed only in the fall using a disk cultivator, and no tillage was conducted in the spring due to the clay soil texture.

Soybean (cv. 24-10 RY), canola (cv. 73-75 RR), corn (cv. DKC 26-28 RIB), and wheat (cv. Carberry) were sown with a row spacing of 38, 19, 76, and 19 cm, respectively. Fertilizers were applied to both field experiments based on spring soil test recommendations, and the fertilizer rate was determined by each crop in the rotation sequence. For soybean, fertilizers were not applied, and seeds were inoculated with *Bradyrhizobium japonicum* (Cell-Tech liquid, Bayer Crop Science, Canada) each year before planting. Herbicides at recommended rates were used to control weeds throughout the study. A full description of the agronomic management for this experiment is available in Weerasekara (2022). After physiological maturity, crops were harvested to determine yield.

2.3.3 Sample collection

Soil samples were collected from 2017 to 2021. Ten 2 × 9 cm soil cores were collected and homogenized from each plot to form one sample per plot. This was repeated four times during the season: before planting (BP), at emergence (VE), at beginning seed fill (R5), and after-harvest (R8). The R8 samples were collected following crop harvesting. Samples were stored at 4°C, and subsamples were frozen at -80°C within the same day of sampling. At the BP stage, each plot was also sampled four times using a Dutch auger from 0 – 15 cm. These were homogenized to form one sample per plot and were sent for soil nutrient analysis to Agvise Laboratories (Northwood, USA). Samples collected before planting (BP) and at full maturity (R8) were considered for this study. The BP samples reflect the effects of crop rotation from the previous year (between growing seasons) as well as weather conditions, while the R8 samples represent the effects of crop rotation during the current growing season.

2.3.4 Nutrient analyses

The soil samples were sent to a commercial lab to analyze soil properties, such as pH, nitrate-N (NO_3^-), phosphorus, and organic matter content. Soil pH was measured using a 1:1 soil: water solution (Watson & Brown, 2015). Nitrate-N was extracted from the soil using 0.2 M KCl, and the cadmium reduction method was used to determine the result (Gelderman & Beegle, 2015). Available phosphorus (P) was determined by the Olsen method (Olsen et al., 1954) as described by Frank et al. (2015). Soil organic matter content was measured by weight loss on ignition (Combs & Nathan, 2015).

2.3.5 DNA extraction

DNA was extracted from frozen soil using Qiagen’s DNeasyPowerSoil Kit and protocol (Qiagen, Hilden, Germany). Briefly, 0.25 g of soil sample was added to the PowerBead tube with

Table 3. Selected soil properties of field experiment sites obtained from the analysis of spring soil sampling

Properties	**Years [a]**				
	2017	**2018**	**2019**	**2020**	**2021**
Carman					
Soil pH	5.07	4.86	5.07	5.33	4.91
Soil texture	←		sandy clay loam		→
Soil organic matter (%)	3.50	3.65	3.48	3.39	3.65
Nitrate-N (kg/ha)	31.66	23.40	29.76	16.88	36.78
Olsen-P (ppm)	13.13	13.88	18.94	15.94	17.94
Kelburn					
Soil pH	6.78	6.77	7.08	6.99	6.96
Soil texture	←		clay		→
Soil organic matter (%)	8.23	8.16	8.40	8.13	8.10
Nitrate-N (kg/ha)	54.80	53.67	45.82	33.56	34.96
Olsen-P (ppm)	12.06	12.00	12.81	15.19	16.63

[a] The values provided are averages of all plots sampled during each year.

Table 4. Crops grown in crop rotation treatments at the Carman and Kelburn sites during the sampling period

Year[a]	Continuous Soybean (CS)	Soybean-Canola (SCa)	Soybean-Corn (SCo)	Soybean-Wheat-Canola-Corn (SWCC)
2014	Soybean	Canola	Corn	Wheat
2015	Soybean	Soybean	Soybean	Canola
2016	Soybean	Canola	Corn	Corn
2017	Soybean	Soybean	Soybean	Soybean
2018	Soybean	Canola	Corn	Wheat
2019	Soybean	Soybean	Soybean	Canola
2020	Soybean	Canola	Corn	Corn
2021	Soybean	Soybean	Soybean	Soybean

[a] Crop rotation studies at these sites began in 2014, providing three years of rotation history prior to the 2017 sampling year. The crop sequence was identical at both sites.

a buffer and vortexed for homogenization and lysis. Using the supplied reagents, non-DNA material was removed by precipitation, and the supernatant was collected. Subsequently, a high-concentration salt solution was added to the supernatant to bind the DNA to a silica membrane. An ethanol-based wash solution was used to clean the bound DNA. Finally, bound DNA was eluted using an elution buffer that lacks salt and used for downstream analysis. The concentration of DNA was measured spectrophotometrically (NanoDrop Lite, Thermo Fisher Scientific, Waltham, USA) at 260 nm wavelength; additionally, a 260:280 absorbance ratio was determined. A volume of 20 µL of each extract was sent to Metagenom Bio Life Science Inc (Ontario) for 16S sequencing using an Illumina MiSeq. Primers 515F (Parada et al., 2016) – 806R (Apprill et al., 2015) primer set and procedures recommended by the Earth Microbiome Project (https://earthmicrobiome.org/protocols-and-standards/16s/) were used to target the V4 region of the 16S rRNA gene.

2.3.6 Library preparation and Sequencing

Library preparation was carried out as previously described by Ordoñez (2020). Briefly, each reaction was carried out in triplicate in a total volume of 25 µL. Each reaction mixture contained 2.5 µL of 10 × standard Taq buffer, 0.5 µL of 10 mM dNTP, 0.2 µL of BSA (20 mg/ml), 5.0 µL of 1 µM forward primer (515F; 5'-GTGCCAGCMGCCGCGG-3'), 5.0 µL of 1 µM reverse primer (806R; 5'-GGACTACNVGGGTWTCTAAT-3'), 2.0 µL DNA, 0.2 µL of Taq DNA polymerase (5 units/µL) and 9.6 µL of PCR water. DNA was denatured at 95°C for 5 min, followed by 35 cycles of 95 °C for 30 sec, 30°C for 30 sec, and 72°C for 50 sec, and then extended at 72°C for 10 min. Triplicate PCR products were pooled and resolved using a 2% TAE agarose gel. PCR products were pooled, gel purified and quantified using Qubit dsDNA HS

Assay Kit (Thermo Fisher Scientific, Waltham, USA). Indexed library DNA was sequenced with MiSeq Reagent Kit v2 (2 x 250 cycles). FASTQ files were generated for taxonomic analysis.

2.3.7 Bioinformatics and amplicon processing

16S rRNA amplicon data (FASTQ files) of the crop rotation study (2017 - 2021) was analyzed using the Mothur software package, version v.1.44.3 (Schloss et al., 2009), and a version of the MiSeq SOP (Kozich et al., 2013). Paired-end reads were assembled into contigs and filtered to remove contigs larger than 300 bp, that contained ambiguous base calls or had homopolymers > 8. Reference-based operational taxonomic unit (OTU) clustering was performed at a 97% similarity percentage. The reference database (V4-specific SILVA 132 database, http://www.arb-silva.de) was used to align and filter the contigs. Chimeras were identified by VSEARCH (Rognes et al., 2016) and removed. The Ribosomal Database Project (RDP) reference database that provides the classification of the sequences, with an 80% confidence score (Cole et al., 2014), was used to remove other sequences such as mitochondria, archaea, and Eukaryotes.

2.3.8 Statistical analyses

The processed OTU table, taxonomic affiliations, and metadata were stored as Phyloseq objects in R (McMurdie & Holmes, 2013), and further analyses were done using the packages stored in R software V.4.1.2 (https://www.r-project.org/).

2.3.8.1 Diversity indices

Phyloseq objects were rarefied to the minimal sample depth. Alpha diversity analysis was done using Shannon indexes (Shannon, 1948), and the Kruskal-Wallis test was used to determine whether there were significant differences in alpha diversity among the tested groups. Beta diversity was calculated based on the Bray-Curtis dissimilarities using the distance function of

the phyloseq package. The dissimilarity matrix was given for a Principal Coordinate Analysis (PCoA) and for the Permutational Multivariate Analysis of Variance (PERMANOVA; p value ≤ 0.01), using the adonis function of the vegan package (v.2.5-6) (Oksanen et al., 2019) for R. The dissimilarities were also used for the hierarchical clustering of samples. The phyla and genera with the relative abundance greater than 1% in each crop rotational treatment were identified using the phyloseq package in R, then plotted using ggplot2.

2.3.8.2 Linear discriminant analysis Effect Size (LEfSe) analysis

Linear discriminant analysis effect size (LEfSe) analysis was performed to identify significantly important microbial taxa across treatments (Segata et al., 2011). The R packages tidyverse (Wickham et al., 2019), microbiomeMarker (Yang et al., 2022), and knitr (Xie 2024) were used for this analysis. Only those features with logarithmic LDA score (effect size) analysis scores above the threshold score of 4.0 were called as differentially abundant.

2.3.8.3 Core microbiome

The core OTUs among different treatments were identified based on prevalence and relative abundance cut-off values at 90 % and 0.01 %, respectively. The R packages microbiome (Lahti et al., 2017), eulerr (Larsson, 2023), and microbiomeutilities (Shetty and Lahti, 2022) were used to identify the core OTUs, and the package VennDiagram (Chen and Boutros, 2011) was used to visualize the results as a Venn diagram.

2.3.8.4 Taxa co-occurrence networks

The co-occurrence networks of bacterial genera were estimated using the package NetCoMi (Peschel et al., 2021) in R Studio. Networks were constructed using Pearson's correlation coefficient as an association measure. The normalization method was the centered log-ratio (clr) transformation since Pearson correlations may lead to compositional effects when

applied to sequencing data (Matchado et al., 2021). A threshold of 0.3 is used for sparsification so that only OTUs with an absolute correlation greater than or equal to 0.3 are connected. The “unsigned” transformation was used so that the edge weight between strongly correlated taxa was high, regardless of the sign. Hence, a correlation of -1 and 1 would lead to an edge weight of 1.

2.4 Results

2.4.1 Bacterial diversities under different environmental conditions.

2.4.1.1 Soil types influence bacterial diversity.

It was hypothesized that different soil types influence the diversity of bacteria. To test this hypothesis, soil samples from field experiments with contrasting soil properties at Carman and Kelburn were analyzed.

Sequencing of the V4 region of the 16S rRNA genes yielded in a total of 5,644,389 and 5,105,074 assembled raw contigs at the BP and R8 sampling points in Carman and Kelburn, respectively in BP and R8 sampling points. Removal of low-quality and chimeric sequences yielded 3,920,141 and 3,580,565 high-quality sequences with an average length of 300 base pairs in Carman and Kelburn, respectively.

Both alpha and beta diversity were investigated using soil samples collected at the BP stage from 2017 to 2021. Alpha diversity measures the number of taxa (richness) or the relative abundance of those taxa (evenness) within a single sample or set of replicates. In this study, the Shannon index was used to measure both richness and evenness, and pairwise comparison was done using the Wilcoxon rank sum test. Analysis of alpha diversity indicates that there were significant differences between sites in terms of bacterial diversity (Wilcoxon rank sum test; $p < 2e^{-16}$) (Figure 3A). In general, Kelburn has a higher bacterial diversity than Carman. Beta diversity analysis calculates the similarity or distance between microbial communities in relation to environmental variation. In this study, beta diversity was calculated by Principal Coordinate Analysis (PCoA) using Bray-Curtis dissimilarities (Figure 3B). PCoA analysis indicates that there were distinct bacterial communities between sites, explaining 54.2% variation.

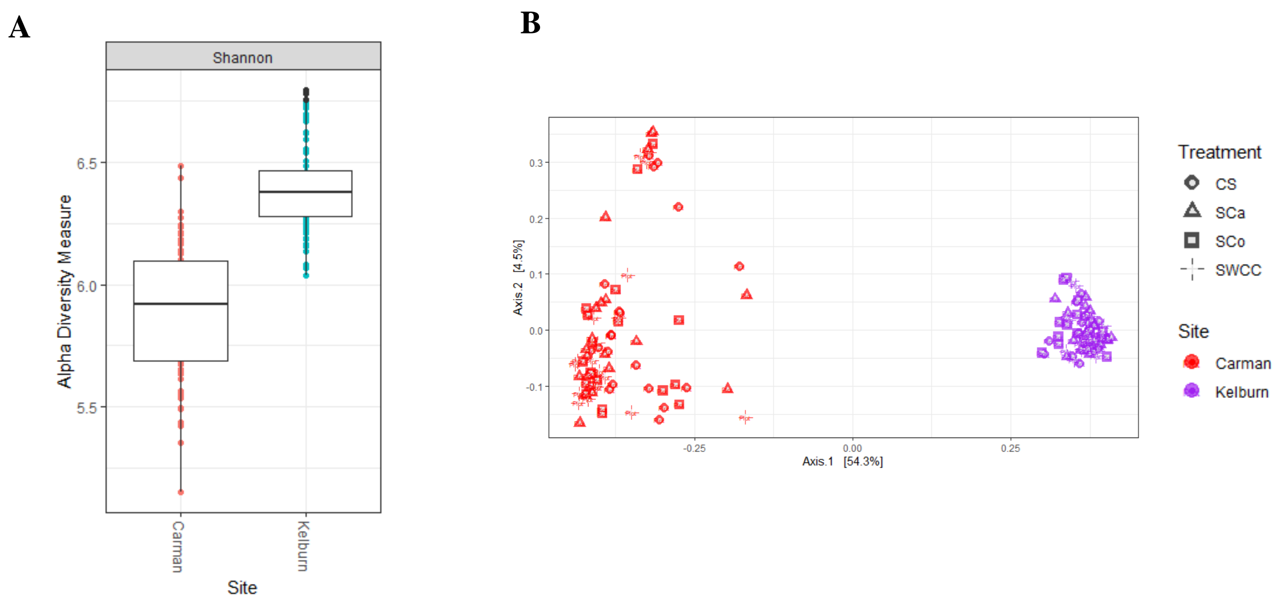


Figure 3. Comparison of bacterial diversity between Carman and Kelburn sites. Analysis of all the Before Planting (BP) samples collected from the Carman and Kelburn sites between 2017 and 2021 were used to determine the Alpha diversity (A), using the Shannon Index (Wilcoxon rank sum test; $p < 2e^{-16}$), and Beta diversity (B) by PCoA using Bray-Curtis dissimilarities (PERMANOVA; $p < 1e^{-04}$). In panel B, red and purple represent Carman and Kelburn, respectively. Shapes indicate crop sequence treatments, circle: CS (Continuous soybean), triangle: SCa (Soybean-Canola), square: SCo (Soybean-Corn), and cross: SWCC (Soybean-Wheat-Canola-Corn).

Taken together, bacterial diversities were significantly influenced by soil types at each experimental location.

2.4.1.2 Growing seasons influence the diversity of bacterial communities.

The diversity of bacterial communities is influenced by growing seasons, as environmental changes during previous seasons affect microbial composition and activity (Hu et al., 2019). To examine this impact, bacterial diversities were plotted over five years from 2017 to 2021. BP samples were used because they reflect the effects of the previous growing season, including effect of previous cropping and overwintering, ensuring that the observed effects were primarily due to weather conditions rather than temporary changes caused by crop rotation treatments. A comparison of alpha diversity between growing seasons in Carman and Kelburn is shown in Figure 4. The data show that the growing season significantly influenced bacterial diversity in both sites. However, the effects of the growing season were different between sites. Thus, the influence of soil type could be a reason for differences in the alpha diversity. In Carman, there was no significant change in the first three years, and a significant increase was observed in the following years (Figure 4A). Meanwhile, in Kelburn, alpha diversity significantly increased until 2019 and gradually declined (Figure 4B).

Beta diversity analyses between growing seasons in Carman and Kelburn are shown in Figure 5. The results show that the growing seasons significantly influenced the diversity of bacterial communities. Like alpha diversity, the effect of the growing season was not similar between sites. However, distinct clustering was observed in 2021 at both sites compared to other years, which could be an effect of a short winter. These results restate the influence of soil types on bacterial diversities. Overall, soil type is the primary factor that influences bacterial diversity.

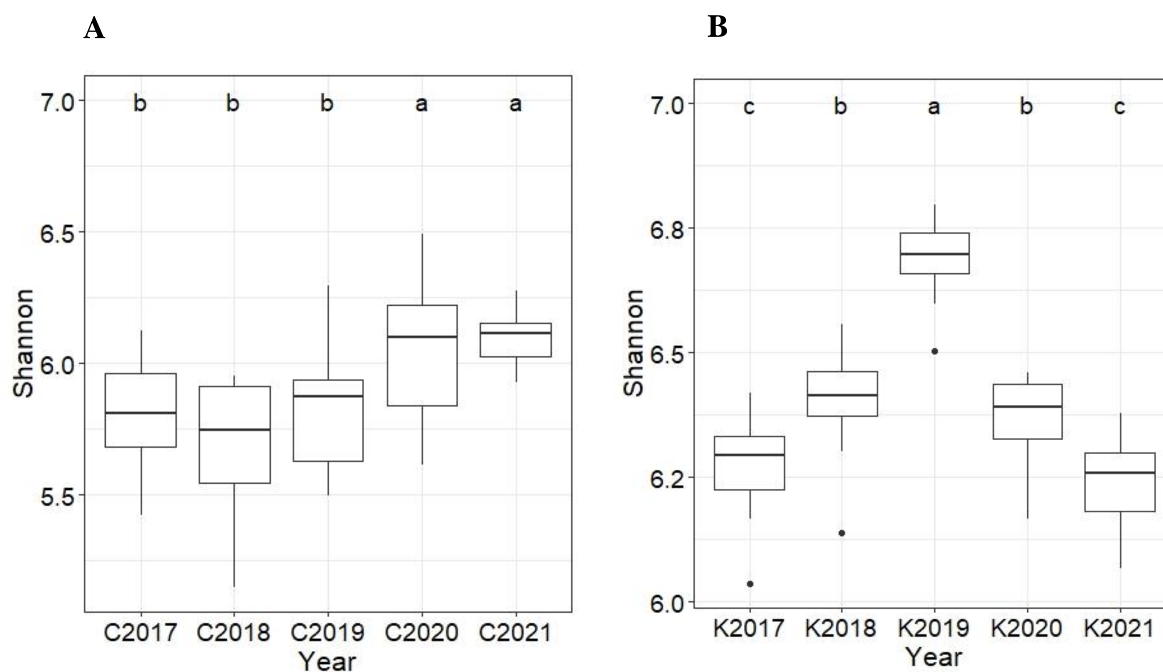


Figure 4. Comparison of alpha diversity between growing seasons based on Shannon Index

A) Carman and B) Kelburn

Alpha diversity was assessed using before planting (BP) samples collected from 2017 to 2021. The crop sequence treatments included Continuous Soybean (CS), Soybean-Canola (SCa), Soybean-Corn (SCo), and Soybean-Wheat-Canola-Corn (SWCC). Years were differentiated between sites by labeling with the first letter of the site: C2017 to C2021 for Carman and K2017 to K2021 for Kelburn. Boxplots with the same letters are not significantly different according to the Tukey HSD test at $p = 0.05$, a post-hoc analysis following a one-way ANOVA.

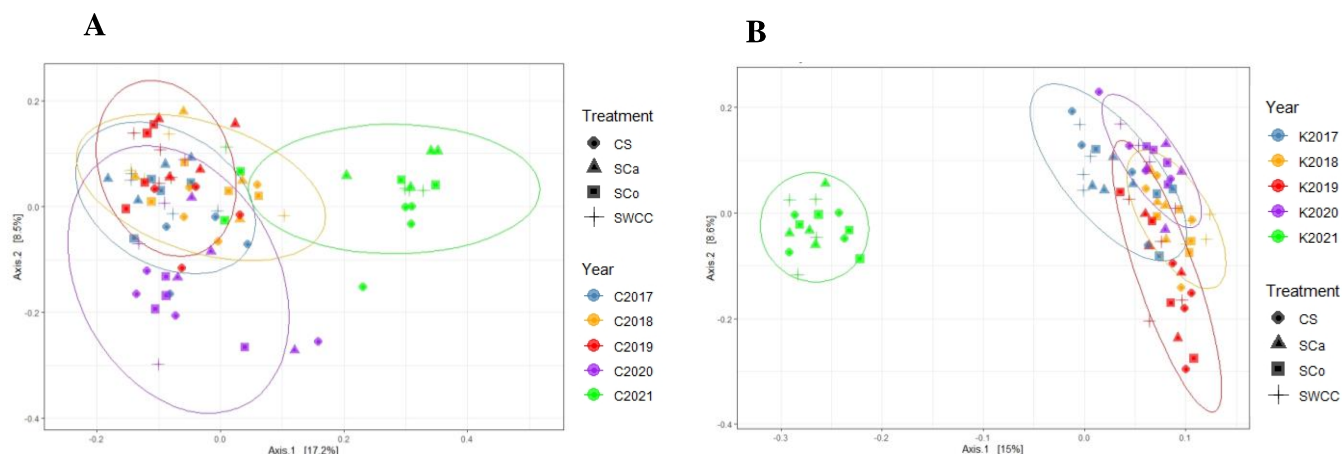


Figure 5. PCoA between growing seasons based on Bray-Curtis dissimilarity using all BP samples

A) Carman and B) Kelburn

The PCoA plots represent samples from different years, with colors indicating the following years: Blue for 2017, Orange for 2018, Red for 2019, Purple for 2020, and Green for 2021. Years are differentiated between sites by labeling with the first letter of the site (C2017 to C2021 for Carman and K2017 to K2021 for Kelburn). Shapes represent different crop sequence treatments: circle for Continuous Soybean (CS), triangle for Soybean-Canola (SCa), square for Soybean-Corn (SCo), and cross for Soybean-Wheat-Canola-Corn (SWCC). Each ellipse represents the 95% confidence interval around the group centroids. Statistical significance is indicated as follows: Carman (PERMANOVA; Year $p < 0.0001$; Treatment $p < 0.0001$) and Kelburn (PERMANOVA; Year $p < 0.0001$; Treatment NS).

The bacterial diversities are driven by weather, regardless of the soil variation during severe weather events.

2.4.2 The effect of crop rotation on the diversities of bacterial communities

Crop rotation affects soil microbial diversity (Sun et al., 2023). To investigate the impact of crop rotation on bacterial community diversity, R8 samples were analyzed. It is assumed that root exudates influence the soil, and the bacterial communities present during this stage reflect the effects of plant growth at the end of the growing season. The comparisons between the alpha diversity of crop rotation treatments in both field experiments indicate that the rotation treatments did not cause significant changes in the alpha diversity in both field experiment sites at R8 (Figure 6). Average values are shown as similar trends were observed throughout the sampling period (Appendix A).

Beta diversity was also analyzed, and each crop rotation treatment was compared to the continuous soybean treatment to visualize the differences. Overall, there were changes in the beta diversity that were dependent on the site (Figure 7). There was a significant clustering (PERMANOVA, $p < 0.05$) of populations between crop rotation treatments at Carman. Even though treatment had a significant effect ($p < 0.0001$), its effect size was small ($R^2 = 0.068$) compared to the larger impact of the growing seasons ($R^2 = 0.278$, $p < 0.0001$). No significant differences were observed among treatments at Kelburn.

The beta diversity analyses suggest that crop rotation treatments influenced soil bacterial communities, with differences between experimental sites. To determine whether this effect was long-lasting between seasons, PCoA was plotted using data from three consecutive sampling points. For example, Figure 8A was obtained by plotting BP and R8 samples from 2017 and BP samples from 2018. The PCoA comparisons indicate that within the growing season, there is a

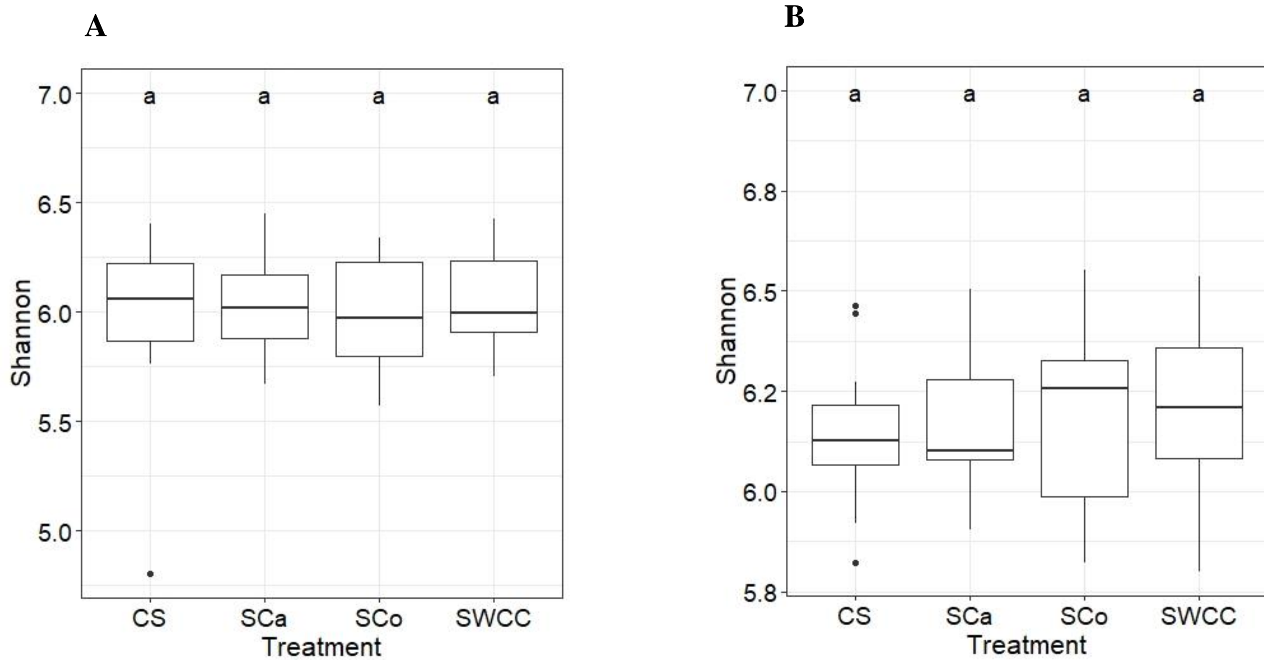


Figure 6. Comparison of alpha diversity between rotation treatments using Shannon Index

A) Carman and B) Kelburn

These figures present alpha diversity assessed using R8 samples from Carman and Kelburn, collected between 2017 and 2021. In 2018, Kelburn R8 samples were not collected due to issues with the initial planting. The rotation treatments include Continuous Soybean (CS), Soybean-Canola (SCa), Soybean-Corn (SCo), and Soybean-Wheat-Canola-Corn (SWCC). Boxplots with the same letters indicate no significant differences, as determined by the Tukey HSD test at p = 0.05, a post-hoc analysis following a one-way ANOVA.

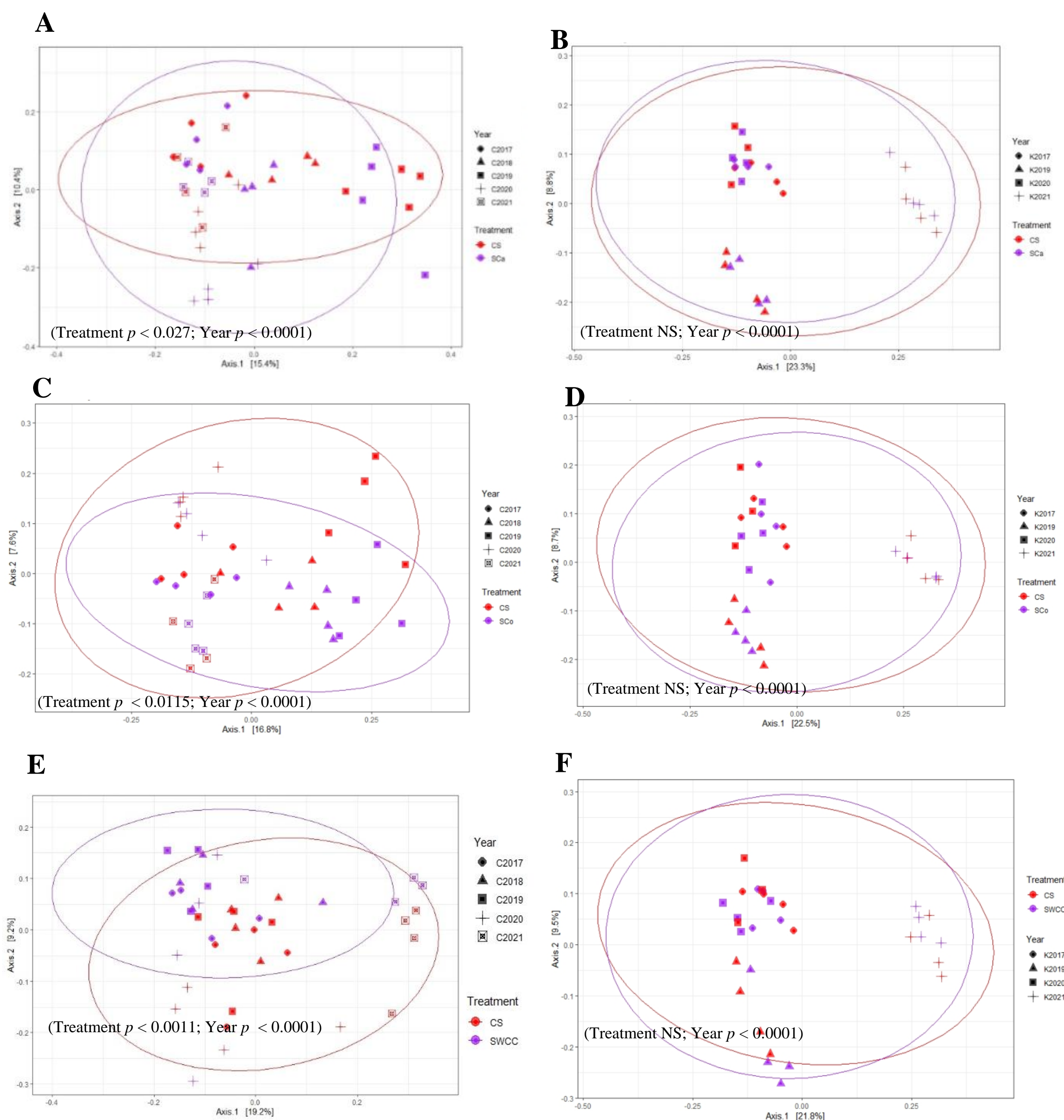
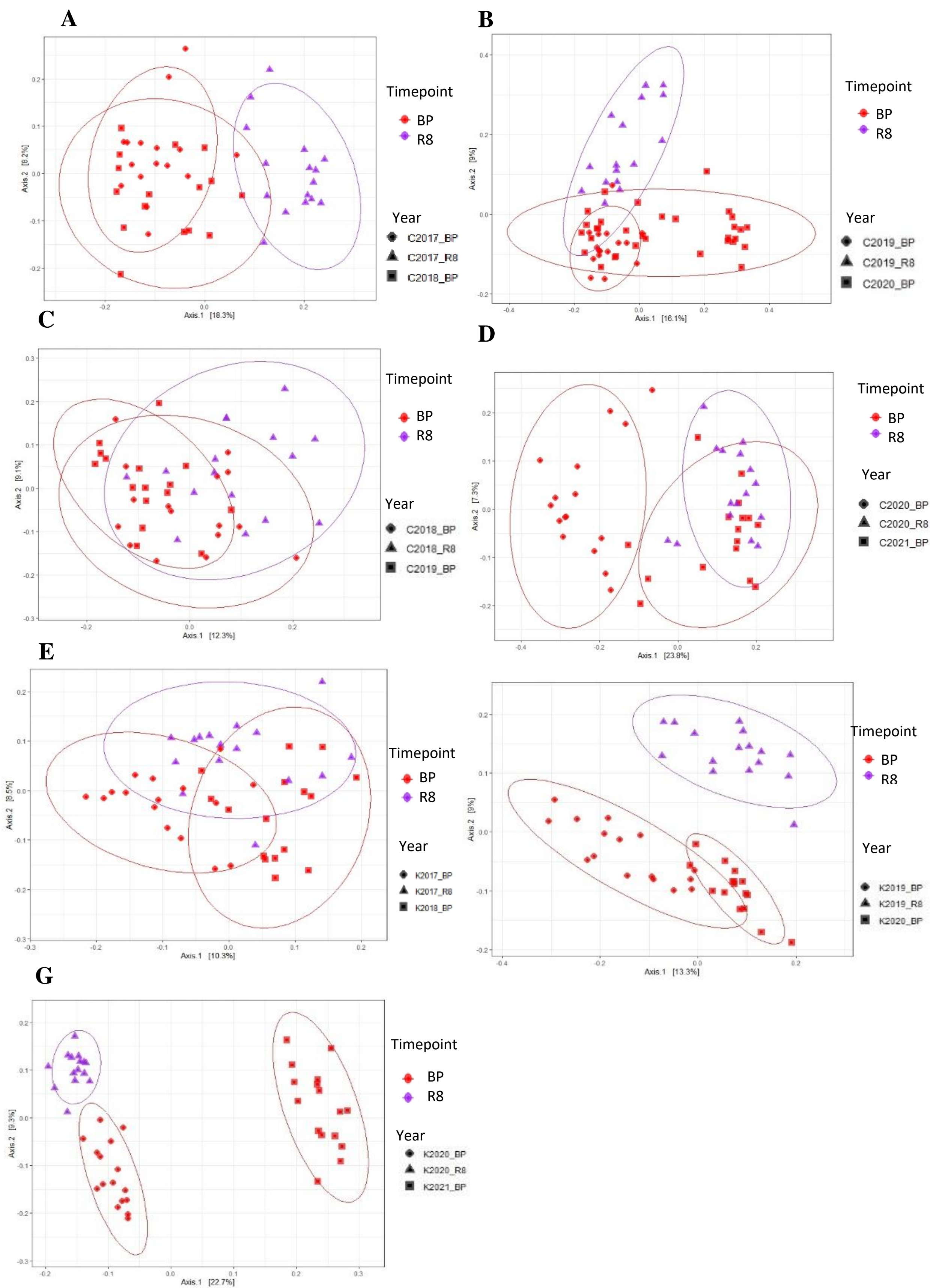


Figure 7. PCoA comparisons of treatments based on Bray-Curtis dissimilarities using R8 samples**.**

A & B) Continuous Soybean (CS) and Soy-Canola (SCa) Treatments: A) Carman and B) Kelburn. C & D) Continuous Soybean (CS) and Soy-Corn (SCo) Treatments: C) Carman and D) Kelburn. E & F) Continuous Soybean (CS) and Soy-Wheat-Canola-Corn (SWCC) Treatments: E) Carman and F) Kelburn. Different colors represent treatments, while different shapes indicate years at both sites. Each ellipse represents the 95% confidence interval around the group centroids. Years are differentiated between sites by labeling with the first letter of the site (C2017 to C2021 for Carman and K2017 to K2021 for Kelburn). K2021 was not plotted as samples were not collected due to issues with the initial planting. NS means non-significant based on PERMANOVA.


A
Timepoint
BP
R8
Year
C2017_BP
C2017_R8
C2018_BP
Axis.1 [18.3%]
Axis.2 [8.2%]
B
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BP
R8
Year
C2019_BP
C2019_R8
C2020_BP
Axis.1 [16.1%]
Axis.2 [9%]
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Year
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Figure 8. The legacy effect of changes in bacterial communities due to rotation treatments PCoA was performed using three consecutive sampling points: A-D) Carman and E-G) Kelburn.

A) Comparison between 2017 BP, 2017 R8, and 2018 BP.
B) Comparison between 2018 BP, 2018 R8, and 2019 BP.
C) Comparison between 2019 BP, 2019 R8, and 2020 BP.
D) Comparison between 2020 BP, 2020 R8, and 2021 BP.

E-G) Corresponding comparisons for Kelburn:
E) Comparison between 2017 BP, 2017 R8, and 2018 BP.
F) Comparison between 2019 BP, 2019 R8, and 2020 BP.
G) Comparison between 2020 BP, 2020 R8, and 2021 BP.
Different colors indicate time points (red for BP and purple for R8), while shapes differentiate the specific years. Years are distinguished by the first letter of the site (C2017 to C2021 for Carman and K2017 to K2021 for Kelburn). The comparison between 2018 BP, 2018 R8, and 2019 BP was not plotted as samples were not collected due to issues with the initial planting. Each ellipse represents the 95% confidence interval around the group centroids.

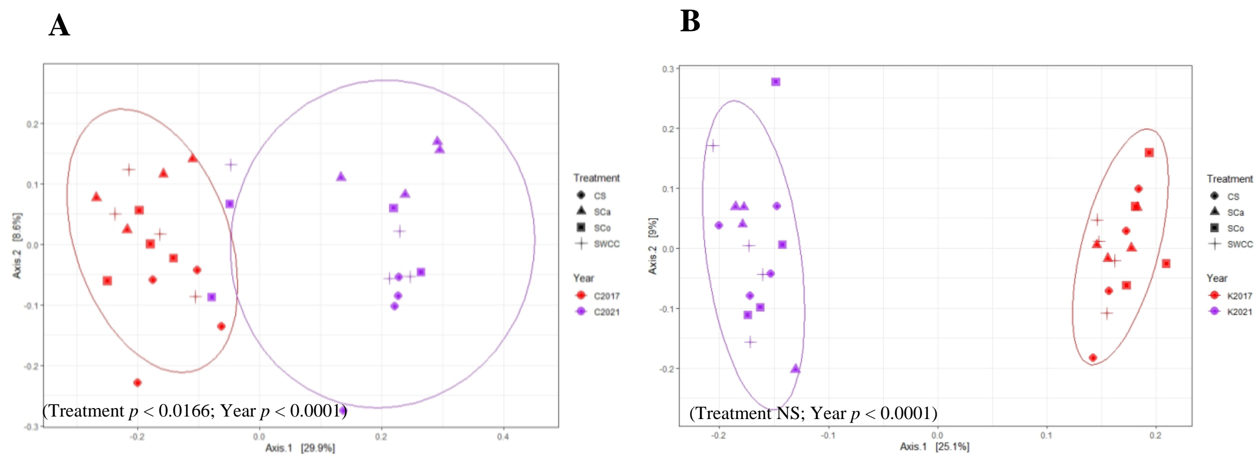


Figure 9. Long-term effects of crop rotation treatments on bacterial communities

A) Carman and B) Kelburn

PCoA was performed using BP samples from 2017 and 2021 for Carman and Kelburn. Different colors represent years (red for 2017 and purple for 2021), while shapes indicate treatments: circle for Continuous Soybean (CS), triangle for Soybean-Canola (SCa), square for Soybean-Corn (SCo), and cross for Soybean-Wheat-Canola-Corn (SWCC). Each ellipse represents the 95% confidence interval around the group centroids. NS – non significance based on PERMANOVA.

significant shift in bacterial communities from BP to R8 in both field experiments (Figure 8). However, the community tended to move back to its initial stage (BP) the following season, except for the Kelburn 2021 BP samples, where the cluster shifted differently. This could be due to the shorter winter that year. Therefore, crop rotation causes temporary shifts in bacterial communities that can be overwhelmed by environmental factors like overwintering.

To assess how crop rotation treatments affected soil bacterial communities over the five-year study period, we compared the BP samples from 2017 and 2021 as both years followed the same three-year crop rotation cycle. Beta diversity was captured by the first two axes of the ordination plot, explaining 25-30% of the total variation observed in microbial community composition across treatments and years (Figure 9). Crop rotation treatments significantly impacted bacterial communities at Carman, but had no significant effect at Kelburn. Shifts in bacterial communities were primarily driven by weather events. At both sites, the BP clusters of 2020 and 2021 were distinct from those of the previous years (Figure 8). These unusual weather events, including an early snowstorm in 2019, a short winter, and a drought in 2021, likely contributed to these shifts in community composition.

2.4.3 Influence of crop rotation on bacterial community composition

2.4.3.1 Effect of crop rotation within the growing seasons

Crop rotations lead to significant differences in the composition of microbial communities (Jiang et al., 2016). The composition of the communities was investigated to see if there was an impact of growing soybeans continuously as compared to the other crop rotation treatments on the composition of soil bacteria at both phylum and genus levels (Figure 10). All R8 samples from 2017 to 2021 were examined to assess overall changes in soil composition during the growing season, as we assumed this stage would provide an ideal representation of the

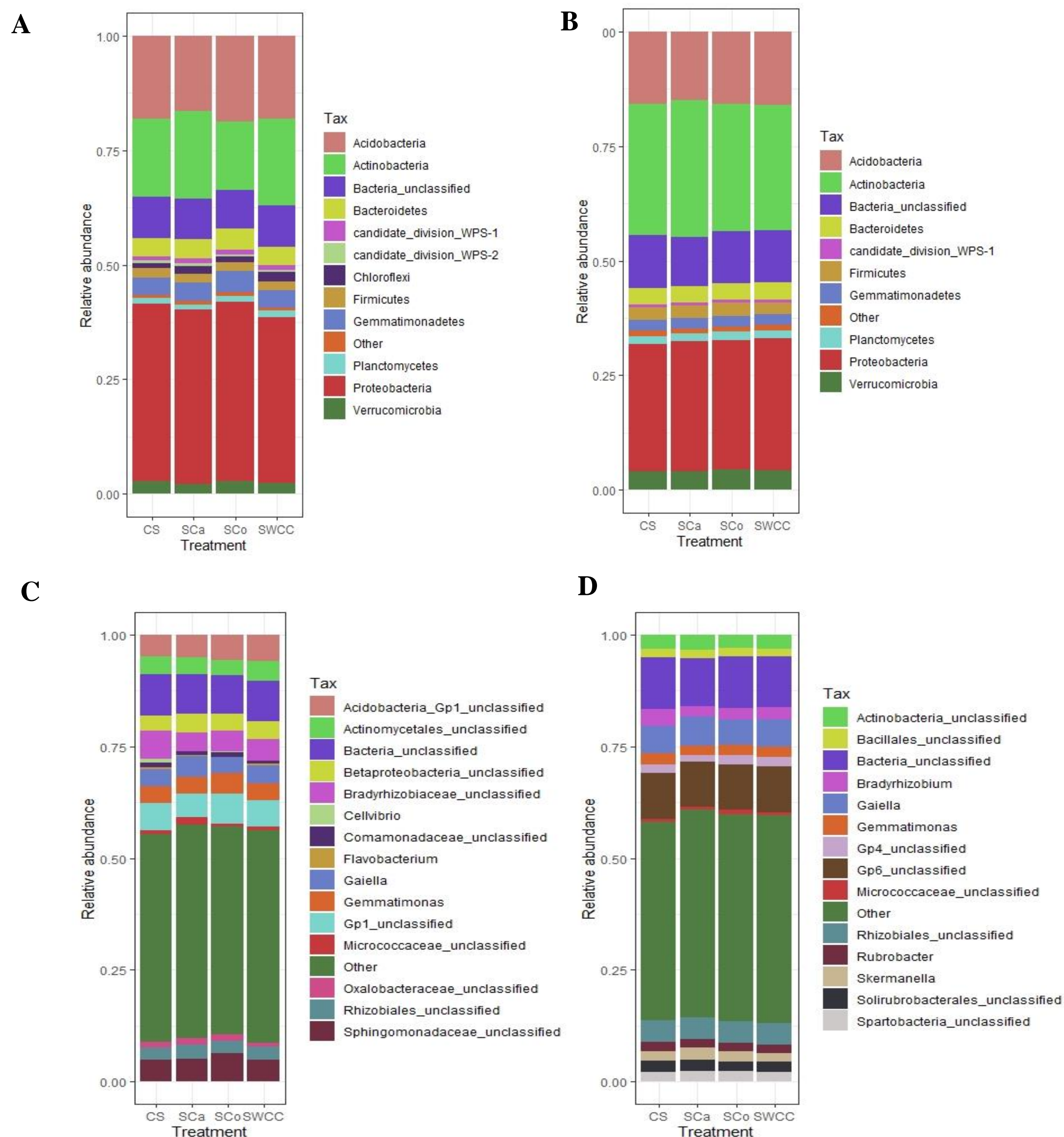


Figure 10. Relative abundance of bacteria across rotation treatments within the growing seasons Panels A and C represent data from the Carman site, while panels B and D represent data from the Kelburn site. The graphs were generated by analyzing R8 samples collected from both locations between 2017 and 2021. Each bar represents an average over five years with four biological replicates per year. Treatments were CS (Continuous soybean), SCa (Soybean-Canola), SCo (Soybean-Corn), and SWCC (Soybean-Wheat-Canola-Corn).

plant's influence on the soil environment. At the phylum level, there were few differences in the composition among treatments in both field experiment sites. In both field experiments, the dominant phyla were Proteobacteria, Actinobacteria, and Acidobacteria (Figure 10 A & B). However, genus-level profiling indicates that the relative abundance of genera varied with crop rotation treatments, while the major genera remained consistent. (Figure 10 C & D).

In Carman, the most abundant genera were *Acidobacteria_GP1_unclassified, Sphingomonadaceae_unclassified, Bradyrhizobiacae_unclassified, Gemmatimonas, Gaiella,* and *Betaproteobacteria_unclassified*. In Kelburn major genera were *GP6_unclassified, GP4_unclassified, Actinobacteria_unclassified, Pseudonocardia, Gaiella,* and *Rhizobiales_unclassified*. At Carman experiment site, a higher *Bradyrhizobiaceae_unclassified* population was observed in the continuous soybean treatment, and the lowest population was recorded in SCa treatment. Further, the *GP1_unclassified* population was also lower in the SCa rotations than all other treatments in Carman. However, *Gaiella* was higher in the SCa rotation than in other rotation treatments (Figure 10C).

In Kelburn, the relative abundance of each genus varied slightly. Thus, crop rotation treatments changed the relative abundance of bacterial communities at the genus level while the composition of major genera remained stable. It is also important to note that there was a substantial proportion of *Bacteria_unclassified* in both field experiments. Thus, it emphasizes the importance of culturing and identifying bacteria to interpret the results obtained from the culture-independent studies.

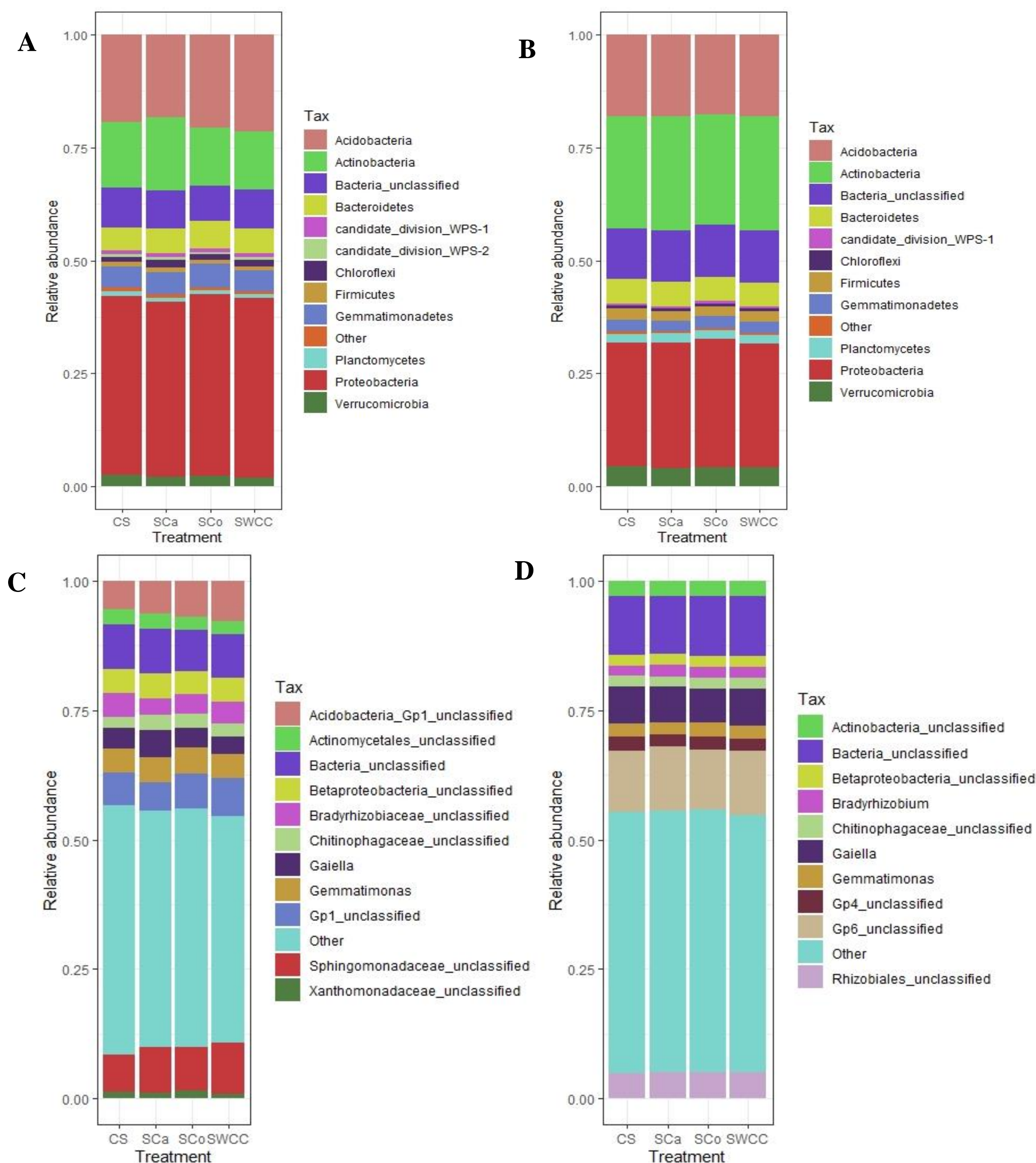


Figure 11. Comparative analysis of bacterial relative abundance across crop rotation treatments between growing seasons

Panels A and C present data from the Carman site, while panels B and D show data from the Kelburn site. These graphs are based on BP samples collected between 2017 and 2021. Each bar represents an average of five years, with four biological replicates per year. The color codes are specific to each site to reflect differences in genus composition. The crop rotation treatments analyzed include CS (Continuous soybean), SCa (Soybean-Canola), SCo (Soybean-Corn), and SWCC (Soybean-Wheat-Canola-Corn).

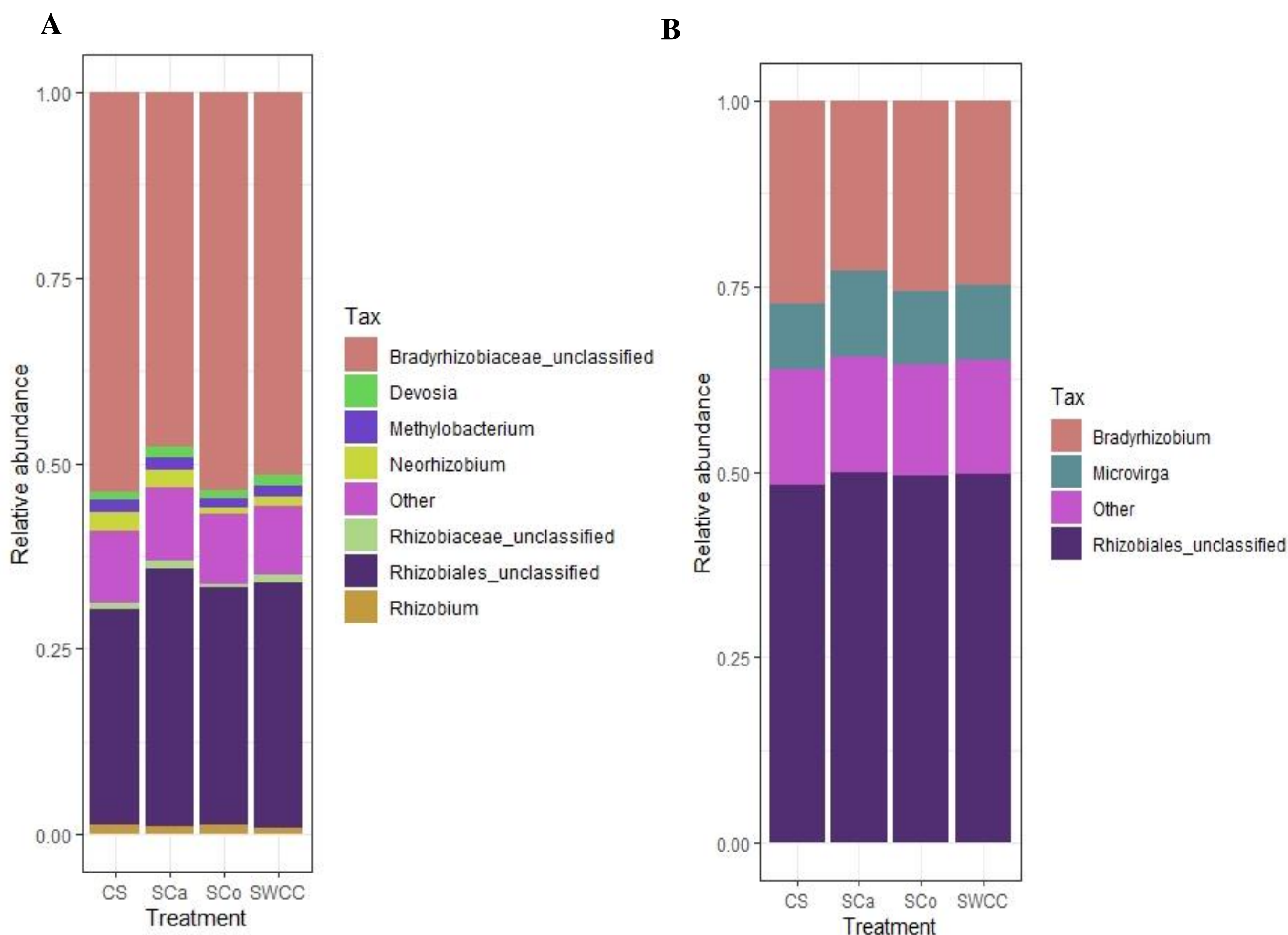


Figure 12. Comparison of *Bradyrhizobium* population among crop rotation treatments

A) Carman and B) Kelburn

These graphs are based on R8 samples collected from both locations between 2017 and 2021. Each bar represents an average over five years, with four biological replicates per year. The crop rotation treatments were CS (Continuous soybean), SCa (Soybean-Canola), SCo (Soybean-Corn), and SWCC (Soybean-Wheat-Canola-Corn).

2.4.3.2 Effect of crop rotation between the growing seasons

To compare bacterial abundance among treatments across growing seasons, all BP samples from 2017 to 2021 were included. It is assumed that the analysis of BP samples represents the changes that occurred between the seasons. Like what was seen within a growing season, the composition of phyla was similar among treatments in each field experiment (Figure 11). The dominant phyla were Proteobacteria, Acidobacteria, Actinobacteria, and Bacteroides, and a substantial proportion of Bacteria_ unclassified in both sites.

At the genus level, *Acidobacteria_GP1_unclassified, Actinomycetales_unclassified, Bradyrhizobiaceae_unclassified, Gaiella,* and *Sphingomonadaceae_unclassified* were dominant in Carman. In Kelburn, the major genera were GP6_unclassified, *Gaiella,* and *Rhizobiales_unclassified*. In both experimental sites, lower *Bradyrhizobiaceae_unclassified* population was observed in the soybean-canola treatment. However, *Gaiella* and *Betaproteobacteria_unclassified* populations were higher in the soybean-canola rotation (Figure 11). The results also show that the relative abundance of the bacterial genera among the crop rotation treatments was stable at Kelburn site. Collectively the data suggest that the differences between the two sites are not dependent on the crop rotation treatments, but other factors that may include, but are not limited to, soil and environmental effects.

2.4.3.3 Changes in the Bradyrhizobiaceae population

The density of soybean plants has been shown to affect the population of *B. japonicum* (Yudistira et al., 2021). It was hypothesized that the CS plots would have a higher *Bradyrhizobiaceae* population because these plots were continuously inoculated with *Bradyrhizobium japonicum* over eight years, and that the host plant could play role in

maintaining the population. To test this, the relative abundance of Rhizobiales, the order to which *Bradyrhizobiaceae* belongs, was analyzed and plotted against the crop rotation treatments using R8 samples collected from 2017 to 2021. The results show that the *Bradyrhizobiaceae* were the most abundant genus among all crop rotation treatments in Carman, accounting for more than 50% of the Rhizobiales population (Figure 12). In Kelburn, the *Bradyrhizobiaceae* was the second most abundant genus, accounting more than 25% of the Rhizobiales. We note that most of the population in Kelburn was labeled as *Rhizobiales-unclassified*. We observed that the CS treatment had a higher *Bradyrhizobiaceae* population, followed by SCo and SWCC. Of note, at both sites, the lowest population was observed in the SCa rotation, even though the plots were inoculated with *B. japonicum* on alternating years during the study period.

2.4.3.4 Differential abundance taxa between treatments

LEfSe (Linear discriminant analysis Effect Size) analysis was conducted to investigate the primary taxa that differ significantly due to crop rotation treatments. LEfSe is a bioinformatics tool used to identify biomarker features in high-dimensional datasets. It combines statistical testing (Kruskal-Wallis test) to detect significantly different features across classes with linear discriminant analysis to estimate the effect size of these features. This two-step process enables the identification of specific taxa that contribute to differences in microbial communities, helping to understand their ecological significance. For this analysis, a subset of the dataset was initially based on each crop rotation treatment, and then differentially abundant taxa between BP and R8 time points were identified. All BP and R8 samples from 2017 to 2021 were used.

The list of differentially abundant taxa between the time points for each treatment is provided in Tables 5 and 6. *Sphingomonadaceae* was enriched at the BP stage in all treatments in

Table 5. Differential abundance taxa between treatments in Carman

Treatment	Differentially abundant taxa	Enriched group[a]	ef_lda[b]	*p* value[c]
CS	*Sphingomonadaceae*	BP	4.372	0.0114
CS	*Actinomycetales_unclassified*	R8	4.025	0.0294
SCa	*Sphingomonadaceae*	BP	4.497	0.0027
SCa	*Bradyrhizobiaceae_unclassified*	R8	4.030	0.0051
SCo	*Sphingomonadaceae*	BP	4.957	0.0326
SCo	*Rhizomicrobium*	BP	4.602	0.0043
SWCC	*Sphingomonadaceae*	BP	4.692	0.0001
SWCC	*Acidobacteria_Gp1_unclassified*	BP	4.268	0.0304
SWCC	*Gemmatimonadaceae*	BP	4.021	0.0265
SWCC	*Rhizomicrobium*	BP	4.012	0.0027
SWCC	*Actinomycetales_unclassified*	R8	4.199	0.0007

[a] Indicates the group that has a higher relative abundance of specific taxa.

[b] **ef**fect size_ **l**inear **d**iscriminant **a**nalysis score generated using LEfSe.

[c] These values were generated by the Kruskal-Wallis test

Table 6. Differential abundance taxa between treatments in Kelburn

Treatment	Differentially abundant taxa	Enriched group[a]	ef_lda[b]	*p* value[c]
CS	*Chitinophagaceae*	BP	4.227	0.0188
SCa	Bacteria_unclassified	BP	4.320	0.0385
	Gp4_unclassified	BP	4.123	0.0304
	Pseudonocardiaceae	R8	4.425	0.0219
	Solirubrobacteraceae	R8	4.236	0.0109
SCo	No markers detected[d]			
SWCC	*Spartobacteria_unclassified*	BP	4.020	0.0356
	Pseudonocardiaceae	R8	4.340	0.0356
	Solirubrobacteraceae	R8	4.093	0.0356

[a] Indicates the group that has a higher relative abundance of specific taxa.

[b] **ef**fect size_ **l**inear **d**iscriminant **a**nalysis score generated using LEfSe.

[c] These values were generated by the Kruskal-Wallis test

[d] No differentially abundant taxa were detected between the tested groups.

Carman (Table 5). *Actinomycetales_unclassified* was enriched in CS and SWCC treatments at the R8 stage, while *Bradyrhizobiaceae* was found in SCa treatment. In Kelburn, there were no differentially abundant taxa in the CS rotation at the R8 stage or in the SCo rotation at both the BP and R8 stages (Table 6). *Pseudonocardiaceae* and *Solirubrobacteraceae* were enriched at the R8 stage in both SCa and SWCC treatments.

2.4.4 Bacterial interaction

2.4.4.1 Core microbiome between different crop rotations

Generally, core microbiomes are measured as the microbial taxa shared among two or more samples from a particular host or environment. The core microbiome lists potentially ecologically relevant taxa that can be prioritized for targeted culturing and omics study. In this study, core microbiome analysis was conducted to determine the shared OTUs between treatments in each field experiment using the R8 samples, and the results were illustrated as Venn diagrams. Among the core OTUs in each treatment, 43 core OTUs were shared between treatments in Carman, while 48 core OTUs were shared with Kelburn. The remaining OTUs, unique to each treatment will be referred to as 'unique core' in the following sections (Figure 13). The results revealed a significant overlap of OTUs between rotations within each site, accounting for 62.3% and 68.1% in Carman and Kelburn, respectively. Only 3 OTUs were common between sites, namely *Bradyrhizobiaceae_unclassified, Micrococcaceae_unclassified*, and *Rhizobiales_unclassified* (Table 7). Moreover, unique core OTUs were identified in each treatment across both field experiment sites, but these were not comparable between the sites (Table 8).

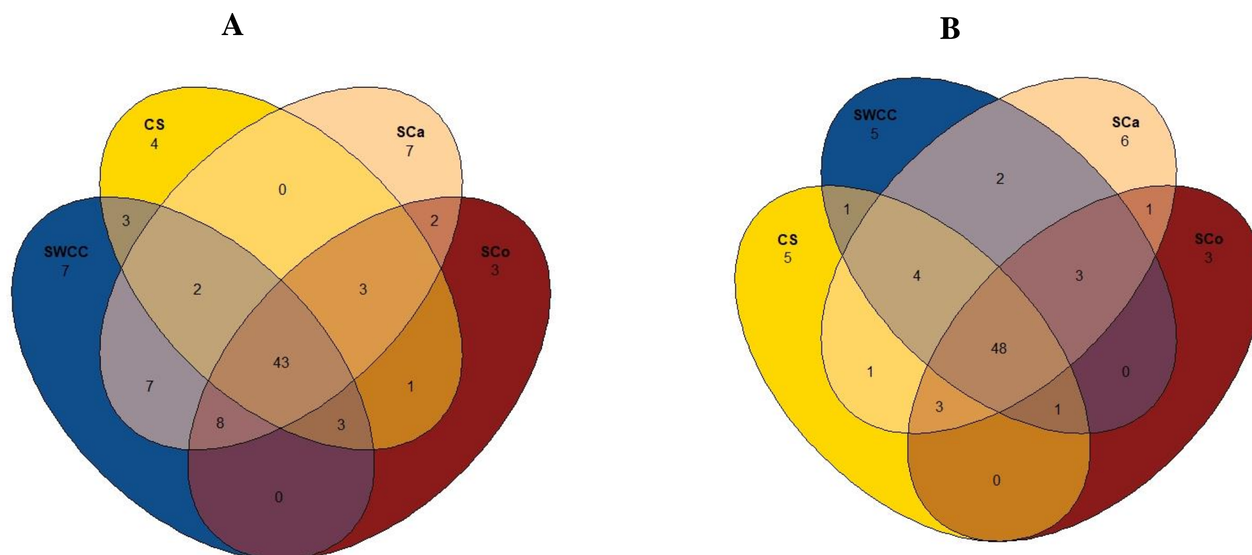


Figure 13. Core microbiome composition across crop rotation treatments in Carman and Kelburn

A) Carman and B) Kelburn

All R8 samples collected from 2017 to 2021 were included in the analysis. Core OTUs for each treatment were identified using a prevalence threshold of 90% and a relative abundance cutoff of 0.01%. The intersection of core OTUs across all treatments represents the core OTUs of Carman and Kelburn. The treatments included CS (Continuous Soybean), SCa (Soybean-Canola), SCo (Soybean-Corn), and SWCC (Soybean-Wheat-Canola-Corn).

Table 7: List of core OTUs shared between crop rotation treatments at Carman and Kelburn

Carman	Kelburn
OTU000001:Bradyrhizobiaceae_unclassified	*OTU000001:Bradyrhizobiaceae_unclassified*
OTU000002:Sphingomonadaceae_unclassified	*OTU000004:Spartobacteria_unclassified*
OTU000003:Betaproteobacteria_unclassified	*OTU000006:Rhizobiales_unclassified*
OTU000005:Gemmatimonas	*OTU000009:Solirubrobacterales_unclassified*
OTU000007:Acidobacteria_Gp1_unclassified	*OTU000010:Ramlibacter*
OTU000008:Gp1_unclassified	*OTU000011:Skermanella*
OTU000012:Micrococcaceae_unclassified	*OTU000012:Micrococcaceae_unclassified*
OTU000017:Gp1_unclassified	*OTU000013:Bacteria_unclassified*
OTU000018:Rhizobiales_unclassified	*OTU000014:Bacillales_unclassified*
OTU000020:Gp1_unclassified	*OTU000015:Gp6_unclassified*
OTU000021:Sphingomonadaceae_unclassified	*OTU000016:Bacillales_unclassified*
OTU000027:Gaiella	*OTU000018:Rhizobiales_unclassified*
OTU000032:Gaiella	*OTU000019:Gp6_unclassified*
OTU000033:Gaiella	*OTU000022:Rubrobacter*
OTU000034:Acidobacteria_Gp1_unclassified	*OTU000024:Solirubrobacter*
OTU000040:Ktedonobacter	*OTU000025:Gp6_unclassified*
OTU000045:Rhizomicrobium_unclassified	*OTU000026:Solirubrobacter*
OTU000047:Acidobacteria_Gp1_unclassified	*OTU000029:Gp6_unclassified*
OTU000050:Gaiella	*OTU000030:Gp6_unclassified*
OTU000053:Jatrophihabitans	*OTU000031:Rhizobiales_unclassified*
OTU000060:Actinomycetales_unclassified	*OTU000035:Intrasporangiaceae_unclassified*
OTU000061:Acidobacteria_Gp1_unclassified	*OTU000036:Gaiella*
OTU000062:Betaproteobacteria_unclassified	*OTU000041:Gp4_unclassified*
OTU000064:Conexibacter	*OTU000043:Pseudonocardia*
OTU000065:Chitinophagaceae_unclassified	*OTU000044:Pseudonocardia*
OTU000066:Nocardioidaceae_unclassified	*OTU000048:Micromonosporaceae_unclassified*
OTU000070:Bacteria_unclassified	*OTU000055:Gp16_unclassified*
OTU000072:Actinomycetales_unclassified	*OTU000056:Gp6_unclassified*
OTU000073:Acidobacteria_Gp1_unclassified	*OTU000057:Bacteria_unclassified*
OTU000074:Gp1_unclassified	*OTU000058:Gp6_unclassified*
OTU000077:Alphaproteobacteria_unclassified	*OTU000059:Gp16_unclassified*
OTU000078:Actinomycetales_unclassified	*OTU000063:Gp6_unclassified*
OTU000079:Acidobacteria_Gp1_unclassified	*OTU000067:Gp6_unclassified*
OTU000080:Acidobacteria_Gp1_unclassified	*OTU000068:Povalibacter*
OTU000081:Gp3_unclassified	*OTU000068:Actinobacteria_unclassified*
OTU000088:Actinomycetales_unclassified	*OTU000071:Bacteria_unclassified*
OTU000092:Rhizobiales_unclassified	*OTU000082:Gaiella*
OTU000093:Acetobacteraceae_unclassified	*OTU000095:Gaiella*
OTU000122:Gaiella	*OTU000096: Microvirga*
OTU000098:Ktedonobacter	*OTU000097:Gaiella*
OTU000145:Gp1_unclassified	*OTU000100:Gp3_unclassified*
OTU000179:Gemmatimonas	*OTU000105:Actinobacteria_unclassified*
OTU000186:Burkholderia	*OTU000109:Gp6_unclassified*
	OTU000126:Microvirga
	OTU000127:Rubrobacter
	OTU000138:Bacteria_unclassified
	OTU000154:Skermanella
	OTU000197:Gaiella

Table 8: List of unique core OTUs between crop rotation treatments at Carman and Kelburn

Carman	Kelburn
CS	**CS**
OTU000086:Betaproteobacteria_unclassified	*OTU000089:Sphingomonas*
OTU000201:Acidobacteria_Gp3_unclassified	*OTU000091:Gemmatimonas*
OTU000243:Bacteria_unclassified	*OTU000181:Spartobacteria_unclassified*
OTU000266:Actinomycetales_unclassified	*OTU000185:Gp6_unclassified*
OTU000086:Betaproteobacteria_unclassified	*OTU000199:Actinobacteria_unclassified*
SCa	**SCa**
OTU000016:Bacillales_unclassified	*OTU000038:Planococcaceae_unclassified*
OTU000023:Planococcaceae_unclassified	*OTU000099:Opitutus*
OTU000083:Bacteria_unclassified	*OTU000160:Gaiella*
OTU000146:Acetobacteraceae_unclassified	*OTU000174:Gp6_unclassified*
OTU000184:candidate_division_WPS-2_unclassified	*OTU000228:Skermanella*
OTU000211:Bacteria_unclassified	*OTU000263:Reyranella_unclassified*
OTU000233:Gammaproteobacteria_unclassified	
OTU000146:Acetobacteraceae_unclassified	
SCo	**SCo**
OTU000051:Burkholderiales_unclassified	*OTU000028:Massilia*
OTU000102:Alphaproteobacteria_unclassified	*OTU000042:Gp6_unclassified*
OTU000218:Nakamurella	*OTU000049:Rhizobiales_unclassified*
SWCC	**SWCC**
OTU000028:Massilia	*OTU000051:Burkholderiales_unclassified*
OTU000148:Bacteria_unclassified	*OTU000101:Gp6_unclassified*
OTU000156:Streptacidiphilus	*OTU000204:Gp4_unclassified*
OTU000188:Actinomycetales_unclassified	*OTU000226:Bacteria_unclassified*
OTU000189:Acidobacteria_Gp3_unclassified	*OTU000312:Gaiella*
OTU000210:Reyranella_unclassified	
OTU000406:Ktedonobacter	

Crop rotation treatments were CS (Continuous Soybean), SCa (Soybean-Canola), SCo (Soybean-Corn), and SWCC (Soybean-Wheat-Canola-Corn).

2.4.4.2 Comparison of bacterial associations among rotation treatments through network analysis.

Understanding the complex interplay of microbial communities in their natural habitat is essential, and network analysis is the most common exploratory data analysis approach to estimate this. In this study, NetComi was used for network analysis using Pearson correlation. NetComi constructs a genus-level network by first calculating Pearson correlation coefficients between the relative abundances of genera across samples. These coefficients quantify the strength of relationships between genera in terms of relative abundance across samples. The resulting correlation matrix is used to create a network, where nodes represent genera and edges denote significant correlations. Community detection algorithms then identify clusters within this network, revealing interactions and ecological relationships among genera.

Since it has been shown that the rhizosphere microbiome changes as a plant grows (Sugiyama et al., 2014), the samples at R8 might best reflect the culmination of the plant interaction with the soil bacteria. R8 samples were analyzed separately to construct networks based on the top 25 most abundant OTUs from each treatment. Overall, positive interactions were stronger than negative ones, and bacterial interactions varied across treatments at both sites (Figure 14 & 15). In Carman, *Bradyrhizobiaceae_unclassified, GP1_ unclassified, Sphingomonadaceae_unclassified,* and *Gaiella* were abundant among treatments, and the interaction between them was not similar between treatments (Figure 14). *Bradyrhizobiaceae_unclassified* and *Actinomycetale_unclassified* had strong positive interaction in all treatments except SCa. The genus *Ktedonobacter* was in low abundance and had highly connected edges in all treatments. *GP1_ unclassified* was the hub species in CS and SCa treatments.

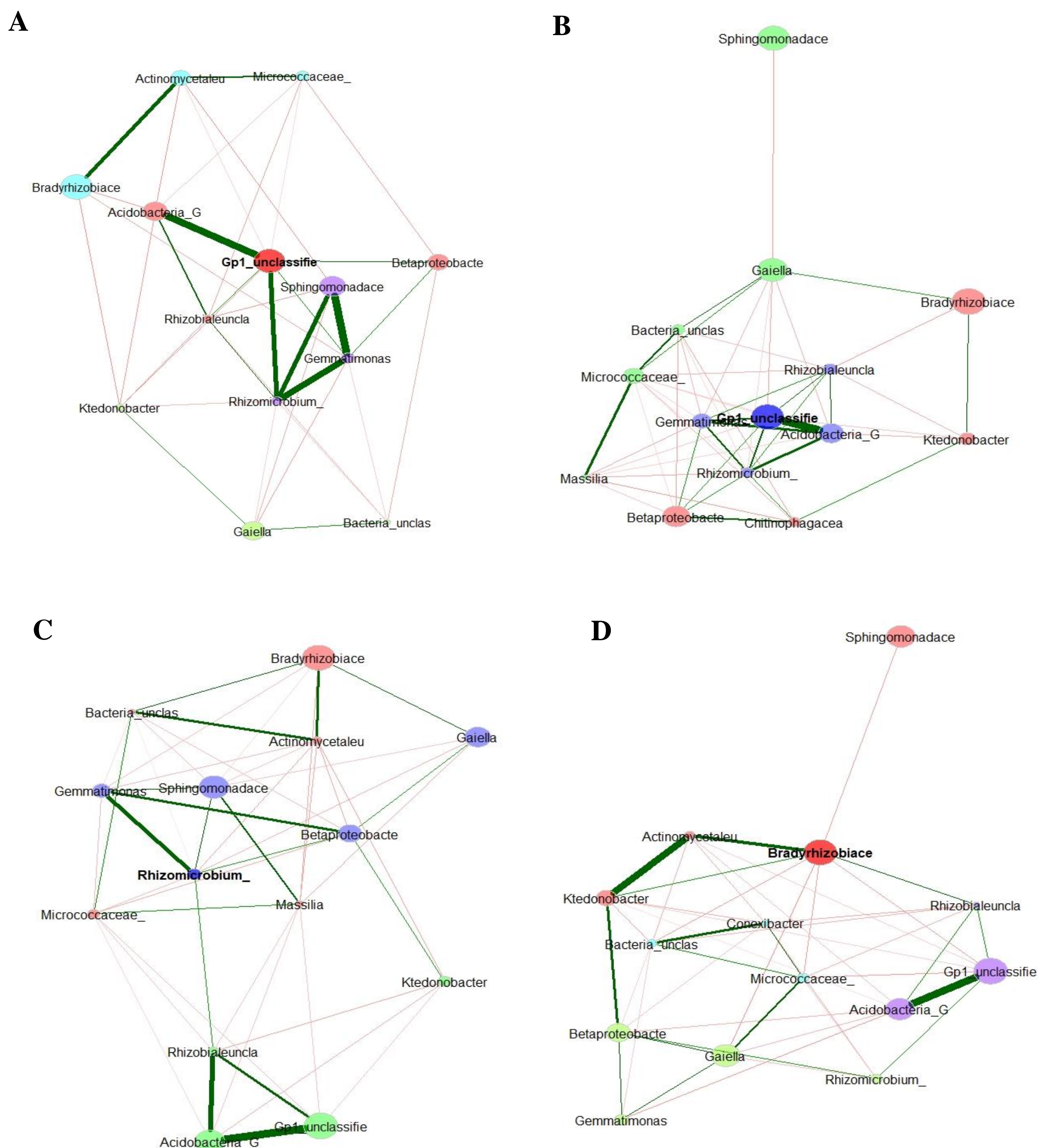


Figure 14. Genus-level co-occurrence networks for microbial communities under different treatments in Carman

Panels A through D represent different crop rotation treatments: A) CS (Continuous soybean), B) SCa (Soybean-Canola), C) SCo (Soybean-Corn), and D) SWCC (Soybean-Wheat-Canola-Corn). R8 samples collected from 2017 to 2021 were used in this analysis. Nodes represent bacterial genera, with node size proportional to their relative abundance. Edges indicate significant Pearson correlations ($|r| \geq 0.3$, $p < 0.05$), with positive correlations shown in blue and negative correlations in red with edge width reflecting the strength of these correlations. Hubs, identified by bold text, signify highly connected nodes. Node color reflects modularity classes, highlighting treatment-specific community structures. Note that color codes vary between treatments. Network metrics, including edge density, clustering coefficient, and modularity, are annotated for each treatment.

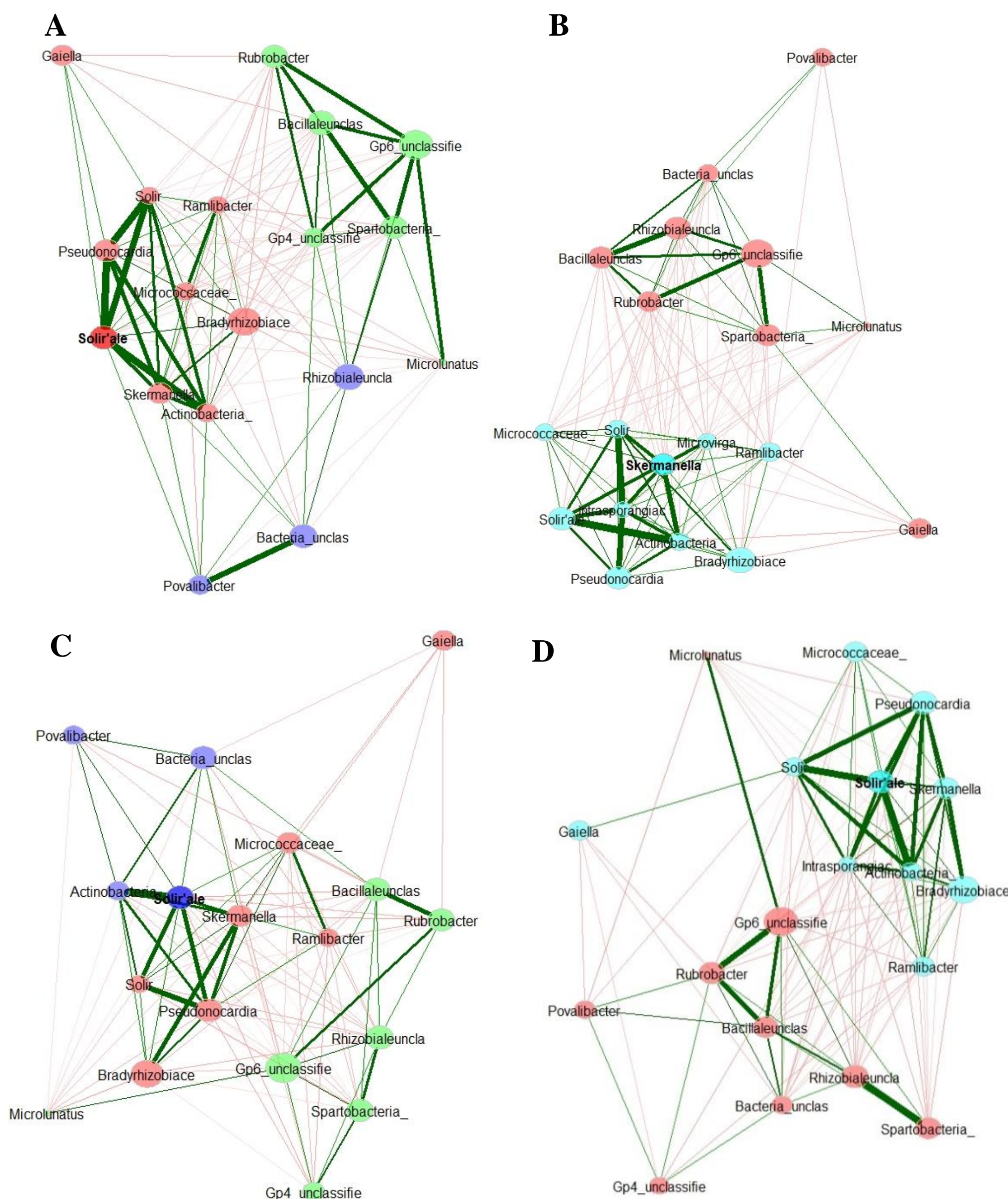


Figure 15. Genus-level co-occurrence networks for microbial communities under different treatments in Kelburn

Panels A through D represent different crop rotation treatments: A) CS (Continuous soybean), B) SCa (Soybean-Canola), C) SCo (Soybean-Corn), and D) SWCC (Soybean-Wheat-Canola-Corn). R8 samples collected from 2017 to 2021 were used in this analysis. Nodes represent bacterial genera, with node size proportional to their relative abundance. Edges indicate significant Pearson correlations ($|r| \geq 0.3$, $p < 0.05$), with positive correlations shown in blue and negative correlations in red with edge width reflecting the strength of these correlations. Hubs, identified by bold text, signify highly connected nodes. Node color reflects modularity classes, highlighting treatment-specific community structures. Note that color codes vary between treatments. Network metrics, including edge density, clustering coefficient, and modularity, are annotated for each treatment.

In Kelburn, there was a strong positive correlation within clusters and a weak negative association between clusters (Figure 15). The genus *Solirubrobacter* was hub in all treatments except SCa. In this treatment, the genus *Skermanella* was hub. In both sites, most of the core OTUs previously identified (Table 7) were present within the networks constructed using the top 25 tax.

Network parameters at Carman display a more structured microbial community with 4 clusters in CS and SWCC treatments and 3 in SCa and SCo (Table 9). Average path lengths range from 1.180 to 1.217, indicating a complex interaction network. The clustering coefficient varies, peaking at 0.633 for SCa, while modularity values are positive, particularly 0.106 for SCo, signifying community cohesion. The edge density is highest in SCa (0.538), and the positive edge percentage reaches 44.90% in SCa, reflecting beneficial interactions among taxa.

At Kelburn, the network features a less defined microbial community structure compared to Carman, with 3 clusters in CS and SCo and 2 in SCa and SWCC. The average path lengths are consistently low (1.006 to 1.037), suggesting efficient connectivity. Clustering coefficients are high, especially 0.854 for SCa, but all treatments exhibit negative modularity, indicating a lack of strong community structure. Edge density is highest in SCa (0.76), and the positive edge percentage reaches 48.25% in CS, suggesting many beneficial interactions despite the absence of clear clustering.

Overall, networks of Carman show structured microbial communities with positive modularity and varied clusters, while networks of Kelburn lack strong community structure, exhibits negative modularity, and has efficient connectivity with tight clustering but fewer distinct groups.

Table 9: Comparison of network metrics between rotation treatments

Metrics	Treatments			
	CS	**SCa**	**SCo**	**SWCC**
Carman				
Number of clusters[a]	4	3	3	4
Average path length[b]	1.217	1.199	1.18	1.204
Clustering coefficient[c]	0.479	0.633	0.597	0.518
Modularity[d]	0.034	0.006	0.106	0.098
Edge density[e]	0.474	0.538	0.505	0.472
Positive edge percentage[f]	40.54	44.90	39.13	39.53
Kelburn				
Number of clusters[a]	3	2	3	2
Average path length[b]	1.006	1.037	1.037	1.033
Clustering coefficient[c]	0.814	0.854	0.782	0.816
Modularity[d]	-0.028	-0.028	-0.038	-0.053
Edge density[e]	0.745	0.76	0.673	0.69
Positive edge percentage[f]	48.25	47.69	40.78	44.5

[a] Count of identified communities.

[b] Mean shortest distance between nodes.

[c] Proportion of connected neighbors for a node.

[d] Degree of community structure relative to random networks.

[e] Ratio of existing edges to possible edges.

[f] Proportion of positive correlations among all edges.

2.5 Discussion

Despite the important role crop rotations play in sustainable agriculture, the analysis of their effect on the dynamics of soil microbial communities still needs to be understood. This study was conducted to explore the impact of crop rotation treatments on the diversity, composition, and interactions of soil bacterial communities. Since several factors influence soil bacterial communities, this study involved sampling over five years from a long-term experiment to verify the impact of environmental factors on treatment effects.

In this study, we found that bacterial diversity significantly differed between the studied sites and growing seasons. While the effects of rotation treatments on bacterial richness and evenness were not significant, soil bacterial communities were influenced by crop rotation treatments, exhibiting variations across different experimental sites. This suggests that the impact of crop rotation on microbial diversity may vary depending on environmental conditions and site-specific factors. Peiffer et al. (2013) reported that field environments were the primary drivers of microbial diversity in a large-scale longitudinal study involving 27 maize inbred lines planted across three fields. Although factors like maize inbreds and technical variations were present, the environmental conditions of each field had the most significant impact on shaping microbial diversity. Huo et al. (2023) found that the experimental site was the primary driver of bacterial richness and evenness. They also observed significant effects on bacterial community composition in response to the interaction between sites, rotational sequence, and year of study. Bolaji et al. (2021) reported that microbial community diversity was not influenced by crop sequences using 2-year crop sequences of corn-soybean, canola-soybean, and soybean-soybean. Sun et al. (2023) reported that while cropping sequences influenced the diversity and composition of the soil microbial community, their effects on bacterial diversity were not

statistically significant. Chamberlain et al. (2020) investigated soil bacterial communities associated with a long-term crop rotation study established in 2002 and sampled in 2017, which included continuous corn, continuous soybean, and annually rotated corn-soybean treatments. They reported that the richness and diversity of bacterial communities did not differ among the crop rotation treatments.

The dominant taxa of soil bacterial communities can vary with different crop rotation, fertilizer management, and crop physiological age (Li et al., 2022). It has been reported that crop rotation treatments significantly influenced the soil microbial communities in a study conducted over 7 years involving potato, oat, and forage maize (Li et al., 2023). Thus, studying the compositional changes of bacterial communities will provide insight into how rotation treatments affect them. In both sites, Proteobacteria, Actinobacteria, and Acidobacteria were the most abundant phyla among the crop rotation treatments at the end of the study. This result is similar to the previous findings related with soybean of Sugiyama et al., (2014), Bolaji et al., (2021), Liu et al., (2020), and Li et al., (2022), indicating these are the dominant phyla in crop rotation systems with soybean. Proteobacteria, Actinobacteria, and Acidobacteria dominate the rhizosphere due to their ability to utilize root exudates, diverse metabolic capabilities, significant ecological roles (e.g., nutrient cycling, pathogen suppression), and adaptation to soil conditions (Buée et al., 2009; Mendes et al., 2013; Zuo et al., 2021). It has also been reported that there were little phylogenic differences at higher taxonomic orders in the agricultural field experiments (Chamberlain et al., 2020; Fierer, 2017; Mendes et al., 2015).

Despite the lack of changes in the composition of bacteria at the phyla level, compositional shifts were observed at the genus level in response to the crop rotation treatments. Major changes at the genus level were mainly observed in the SCa treatment compared to other

treatments during the growing season, where the lowest abundance of *Bradyrhizobium_unclassified* was noted compared with other rotation treatments. In contrast, Bolaji et al., 2021 reported that a canola-soybean rotation had a bacterial community composition like continuous soybean cropping based on their two-year study. It is of note that in the current study, the sampling happened after the rotation treatments had run for 4 (2017) and 8 (2021) years and specifically compared the Rhizobiales community (Figure 12).

In both sites, the *Bradyrhizobiaceae_unclassified* population was higher in CS treatments compared to other treatments. The continuous inoculation of *Bradyrhizobium japonicum* to the CS plots over eight years could be the reason for this observation. The genus composition was unique between the sites within and between growing seasons, suggesting the effect of soil properties. For example, *Acidobacteria_GP1_unclassified* was enriched in Carman, while *GP6_unclassified* was higher in Kelburn. Carman soils are acidic and low in organic matter, while Kelburn soils have a neutral pH and high organic matter content (Table 1). It has been reported that the relative abundance of Acidobacteria varies with soil carbon content and soil pH (Kim et al., 2021; Liu et al., 2016).

Sphingomonadaceae_unclassified and *Gaiella* were higher in Carman and genera *Sphingomonadaceae* and *Gaiella* were reported to have antagonistic effects on plant pathogenic fungi (Li et al., 2022). However, their relative abundance was lower in CS treatments than other crop rotation treatments, indicating that continuous cropping may increase the likelihood of plant pathogens. *Sphingomonadaceae* includes many soil-dwelling bacteria that can survive in low-nutrient environments and use a variety of carbon sources (Chamberlain et al., 2020). Similarly, *Gaiella, Solirubrobacterales_unclassified,* and *Rubrobacter* were abundant in Kelburn, and those genera have antibiotic properties (Riahi et al., 2022; Rosenberg & Zilber-Rosenberg, 2016).

Unlike Carman, there were no changes in the relative abundance of these genera in the continuous soybean (CS) treatment. Thus, the composition of bacterial genera may reflect the influence of plant species on the selection of bacteria in root-associated soils and is dependent on soil types.

Plants are believed to actively recruit core microbiomes to maintain health and enhance productivity, ensuring persistence and functional benefits that support growth and defense mechanisms in various environmental conditions (Hamonts et al., 2018). Several studies reported the presence of common core microbiomes in crops (reviewed in Shayanthan, Ordoñez, and Oresnik 2022). These findings suggest that they could be further investigated for specific purposes in synthetic biology. This study's core OTUs among treatments accounted for nearly two-thirds of the identified taxa across different treatments. Similarly, core microbiome analysis in soybean fields from two Canadian provinces, Ontario and Quebec, revealed that almost 70% of the most dominant bacterial taxa were shared between the two regions. These were *Bradyrhizobium, Acidimicrobiales, Gaiellales, Chloroflexi S085, Chloroflexi TK10, Gemmatimonadaceae, Nitrospira, Rhodobiaceae, Sphingomonas, Comamonadaceae, Nitrosomonadaceae,* and *Incertae Sedis Acidibacter* (Trépanier, 2019). Among the core microbiome, few genera, *Bradyrhizobium* and *Gaiellales* are the shared taxa between the previous study and our current study. This could be due to the use of a *B. japonicum* inoculum in both studies.

LEfSe analysis also indicates that few taxa were differently abundant between the crop rotation treatments, and those were not similar between sites. This could be due to the functional redundancy of microbes, which facilitates the host plant's fitness under environmental variation, as multiple taxa can perform a specific biochemical reaction. Banerjee et al. (2016) reported that

adding different nutrient sources to soil altered the composition of the microbial community, yet the decomposition rate remained unaffected. The experiment involved microcosm setups, where microbial interactions were monitored over 50 days to assess functional redundancy and identify keystone taxa. This suggests that despite shifts in community structure, critical functions like organic matter decomposition can be maintained by different microbial assemblages, highlighting the resilience of soil microbial communities.

Network analysis revealed potential interactions among bacterial communities across different crop rotation treatments, with various network parameters such as nodes, edges, and degrees showing variation in response to these treatments. For instance, modularity was higher in the SWCC and SCo rotations and lower in the SCa rotation. The microbial networks in Carman exhibited more structured and well-defined communities, while Kelburn displayed a more random interaction pattern, despite having a higher edge density and a greater percentage of positive interactions. Kong et al. (2023) reported that higher network complexity and robustness in a continuous leguminous alfalfa system correlated with increased bacterial diversity. Huo et al. (2023) also found that co-occurrence networks varied with rotation treatments and years, exhibiting greater complexity in diverse rotations. Crop rotation influenced the complexity of bacterial networks in the soybean rhizosphere, based on field experiments conducted in China in 2019 and sampled in 2020 (Chen et al., 2022a). Li et al. (2023) observed significant differences in network topological properties and changes in keystone taxa within bacterial and fungal communities due to crop rotation. Moreover, Yang et al. (2023) demonstrated that soil microbiome network complexity had a more substantial impact on multifunctionality than microbiome diversity and community composition. These findings emphasize the role of crop

rotation in shaping the complexity and functionality of soil microbial networks, underscoring its importance in promoting sustainable agricultural practices.

While bacterial diversity and composition showed changes due to crop rotation, we observed that the main drivers were soil type and growing season. This indicates that crop rotation alone may not significantly improve long-term multifunctionality in soil bacterial communities. Environmental factors like soil type and seasonal variations may mask the effects of crop rotations on microbial diversity. Although crop rotations did modify bacterial composition, their influence on ecosystem functionality is more complex and requires further exploration of functional traits. The findings highlight the need for refined perspectives on microbial responses to crop management, especially in regions like Manitoba, where soybean is a relatively new crop. Network analysis revealed core operational taxonomic units (OTUs) with positive and negative correlations, providing insights into microbial interactions and showcasing functional redundancy within bacterial communities. In summary, our study showed that the direct impact of crop rotation on microbial diversity might be limited in the short term. It also provides essential insights into soil microbiome resilience and functionality, suggesting areas for future research that may lead to improved agricultural practices.

Chapter 3: Isolation and characterization of soil bacterial communities from agricultural soils in Manitoba

Authors: Ambihai Shayanthan and Ivan J. Oresnik. This study was planned and executed by Ambihai Shayanthan.

3.1 Introduction

Bacteria are the most abundant microorganisms, and global bacterial diversity ranges between 10^7 and 10^9 bacteria per gram of soil. Although microbiology has traditionally relied on the culturing of microorganisms, only a tiny fraction of known bacterial species (~12,000) have been described so far (https://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date.html).

Advancements in high-throughput sequencing and bioinformatic tools favor culture-independent characterization, which has allowed the exploration of the total diversity of bacterial communities without the need for culturing. However, these 'omics approaches are not sustainable, as functional predictions are based on the availability of well-annotated genomes of the cultured representatives (Choi et al., 2017; Kim et al., 2020). Even though single-cell genomics can be valuable in describing as yet-uncultured bacterial lineages, it is not adequate to decipher the pan-metabolic capability of an entire lineage (Youssef et al., 2011). For example, it has been estimated that more than 50% of the detected genes from candidate bacterial phyla have no predicted functions, and more than 85% cannot be assigned to KEGG metabolic pathways (Marcy et al., 2007). These databases have mainly been built from research focused on human health and biotechnology, which can lead to misleading annotations of genes from soil microbiomes (Choi et al., 2017).

Soil is an extensive bacterial diversity reservoir consisting of over ten phyla (Banerjee & van der Heijden, 2023; Wang et al., 2024). Despite the continuous increase in the deposition of 16S rRNA gene sequences into GenBank, the diversity of successfully cultivated species is lacking, even with the inclusion of recently developed novel cell isolation technologies (Choi et

al., 2017; Kim et al., 2020; Thrash, 2019). Several of these lineages have no known cultivated isolates (Lloyd et al., 2018).

Building a microbial culture collection is crucial in formulating a SynCom (de Souza et al., 2020; Finkel et al., 2017; Shayanthan et al., 2022). Culture libraries have immense potential for downstream analysis. They could serve as a source for further exploration, including diverse areas such as understanding bacterial genome evolution and uncovering functional potential.

This chapter was designed to initiate a culture collection using bacterial strains present in Manitoba soils. It primarily focused on isolating bacteria using traditional techniques and different media to identify culturable species under normal growing conditions. This initial step helps identify cultivable strains within the diversity revealed by 16S rRNA gene analysis. By doing so, it provides a more complete understanding of both easily culturable and difficult-to-culture microorganisms, highlighting the need for new techniques to capture a broader range of microbes. It also has the potential to pave the way for targeted culturing and characterization in the future.

3.2 Materials and Methods

3.2.1 Isolation of bacteria from soil

Serial dilution was used to isolate bacteria from soil samples collected during 2020 (BP and VE) and 2021 (BP), which were stored at 4°C. Briefly, 1g of soil was added to 9 ml water and shaken for 30 minutes. Then, samples were diluted up to 10^{-5}. Finally, 1 ml of aliquots from 10^{-3}, 10^{-4}, and 10^{-5} were transferred to nutrient agar plates (3g yeast extract, 5g peptone, and 15g agar per liter) having the antifungal compound cycloheximide (100 mg/L). Plates were kept at 28° C and checked daily for visible colonies for a week. Colonies were selected based on their

appearance and re-streaked. After three rounds of single-colony purification, cultures were grown overnight in TY, mixed with 8% DMSO solution (24% vol/vol DMSO in TY) at a 2:1 ratio, and stored at -80°C.

3.2.2 Isolation of bacteria from root and nodule

A growth chamber study was carried out to isolate bacteria from soil, root, and nodule samples. Soybean plants were grown for four weeks in Leonard jar assemblies under nitrogen-free conditions in the growth chamber (Oresnik et al., 1994). Plants were inoculated with a soil slurry (10 g soil in a 50 ml sterile dH_2O) prepared from soil samples collected during the crop rotation study. After four weeks, the plants were harvested. Rhizosphere soil samples, which consist of soil adhering to the roots, were collected by uprooting the plants and gently shaking the roots by hand to obtain approximately 1 g of soil. This was then suspended in sterile dH_2O and serially diluted (Barillot et al., 2013). Distinct colonies were purified and stored as described above.

Roots were washed, dried between sheets of tissue, and surface sterilized using 70% ethanol for one minute, 20% household bleach (approximately 2.2% NaOCl) (5 min), and thiosulphate Ringer solution (5 min) to isolate endo-rhizosphere bacteria (Cavaglieri et al., 2009). Then, 1 g of roots was macerated with phosphate-buffered saline (PBS) using a mortar and pestle. PBS was prepared with 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, and 0.24 g of KH_2PO_4 per liter, and the pH was adjusted to 7.4. The homogenates were serially diluted and plated onto the nutrient agar plates containing cycloheximide. Well-separated colonies were picked, and subsequently, single colony purified.

Table 10. Compositions of media used for the culturing

Components	Medium type (g/L distilled water (DW))			
	Nutrient agar (NA)	R2A	GYM Streptomyces (GYM)	Rhizobium medium (Rhi)
Glucose		0.5	4	
Yeast extract	3	0.5	4	1
Peptone	5			
Proteose Peptone		0.5		
K_2HPO_4		0.3		
Malt extract			10	
Mannitol				10
$MgSO_4 \times 7\ H_2O$		0.05		
Na-pyruvate		0.3		
$CaCO_3$			2	
Casamino acids		0.5		
Soil extract*				200 mL
Soluble starch		0.5		
Agar	15	15	12	15

(* Air-dried soil 80g, Na_2CO_3 0.2 g, and DW 200mL. Soil suspension was autoclaved for one hour at 121°C and supernatant was used)

To isolate bacteria from nodules, the nodules were carefully separated from the roots and surface sterilized with 1% bleach, washed with sterile distilled water. The nodules were crushed in a microcentrifuge tube containing 100 µL sterile water, and then centrifuged at low-speed spin 1000rpm for 2 minutes. The resulting supernatant was streaked onto nutrient agar plates and incubated at 28° C for 3-4 days. Well-isolated colonies were selected, purified, and frozen at -80° C.

3.2.3 Effect of different media on bacterial isolation

To compare the effect of different media on the isolation of culturable bacteria, soil bacteria were cultured on four different culture media, namely nutrient agar (NA), R2A, GYM Streptomyces (GYM), and Rhizobium medium (Rhi). The composition of each medium is given in Table 10. This analysis used soil samples stored at 4°C from the before planting (BP) in 2021. Soil sampling and preservation methods were described in Chapter 2. Duplicates of the composite samples were then subjected to serial dilution and plating, following the procedure described above. Well-isolated colonies displaying distinct visual differences were selected from each medium, restreaked, and purified.

3.2.4 DNA extraction and analysis

Bacterial DNA was extracted using Qiagen's DNeasy PowerSoil Kit and protocol (Qiagen, Hilden, Germany) as described in Chapter 2. Selected bacteria were grown on agar plates, and a few colonies were dispersed in 100 µl sterilized dH_2O. This suspension was used for DNA extraction. The V4 region of the rRNA gene was amplified using the 515F (5'-GTGCCAGCMGCCGCGG-3') – 806R (5'-GGACTACNVGGGTWTCTAAT-3') primer pair. Each reaction mixture contained 5.0 µL of 1 µM each forward and reverse primers, 0.5 µL of 10 mM dNTPs, 5 µL of 10 × standard Taq buffer, 0.5 µL of Taq DNA polymerase, 2.0 µL DNA,

and 27.5 µL of PCR water. DNA was denatured at 95°C for 5 min, followed by 30 cycles of 95 °C for 30 sec, 43 °C for 30 sec, and 72°C for 1 sec, and then extended at 72°C for 5 min. The PCR products were sent to Centre de expertise et de services Génome Québec for Sanger sequencing. The resulting FASTA files of the forward and reverse sequences were then used as input files to construct a contig using CAP3 (Huang & Madan, 1999). The resultant contig was BLASTed using NCBI to find the closest species.

3.3 Results and Discussion

3.3.1 Overview of the culture library and its economic importance

Culturing yielded 265 bacterial isolates, including 247 from soils, 13 from soybean roots, and 5 from nodules. Among the soil isolates, 3 were lost as they did not grow after freezing, resulting in 262 isolates (Appendix C). Of these, 168 out of 262 isolates were identified through comparative analysis of partial 16S rRNA gene sequences (Table 11).

Based on the results, Firmicutes (36%) was the dominant phylum in the culture library, followed by Proteobacteria (32%) and Actinobacteria (27%) (Figure 16A). It has been reported that Actinobacteria, Firmicutes, and Proteobacteria were abundant in the root-associated soils of the pea (*Pisum sativum* L.) cultivar, based on the 16S rRNA gene sequencing identification of isolates (Sherpa et al., 2021).

Among Actinomycetes, *Streptomyces, Arthrobacter*, and *Pseudarthrobacter* were dominant (Figure 16B). Those genera had many beneficial properties, such as pesticide degradation, plant growth-promoting activities like biological nitrogen fixation, phosphorus, and potassium solubilization, biocontrol of insect pests and plant pathogens, and crop improvement (Issifu et al., 2022; Mawang et al., 2021; Olanrewaju & Babalola, 2019).

Table 11. Tentative identification of the bacterial strains based on sequencing of V4 region of 16S rRNA gene

Isolate name	Genus based on NCBI	Coverage %[a]	E-value [b]	% identity [c]	# of Base pairs [d]
SI01	*Paraburkholderia*	100	3.00E-76	90	99
SI02	*Paraburkholderia*	98	2.00E-65	99	136
SI03	*Paraburkholderia*	100	1.00E-145	99	280
SI04	*Paraburkholderia*	91	3.00E-59	96	145
SI05	*Burkholderia*	100	1.00E-90	97	194
SI06	*Paraburkholderia*	100	8.00E-60	99	126
SI07	*Paraburkholderia*	100	2.00E-98	100	193
SI08	*Paraburkholderia*	100	5.00E-62	98	134
SI09	*Paraburkholderia*	100	2.00E-55	100	115
SI10	*Stenotrophomonas*	99	1.00E-145	100	280
SI11	*Stenotrophomonas*	99	1.00E-145	100	280
SI12	*Chryseobacterium*	100	4.00E-146	99	282
SI13	*Bacillus*	100	1.00E-145	99	281
SI14	*Chryseobacterium*	100	9.00E-54	98	117
SI15	*Pseudarthrobacter*	99	4.00E-146	99	283
SI16	*Arthrobacter*	99	1.00E-145	100	280
SI17	*Pseudomonas*	100	2.00E-98	100	193
SI18	*Stenotrophomonas*	100	2.00E-98	100	193
SI19	*Novosphingobium*	99	1.00E-141	99	276
SI20	*Pseudomonas*	99	1.00E-145	100	280
SI21	*Stenotrophomonas*	100	1.00E-99	100	195
SI22	*Pseudarthrobacter*	99	1.00E-145	100	280
SI23	*Pseudomonas*	99	1.00E-145	100	280
SI24	*Brevundimonas*	99	1.00E-145	100	280
SI25	*Pseudoroseomonas*	99	1.00E-145	100	280
SI26I	*Stenotrophomonas*	99	1.00E-145	100	280
SI26 II	*Pseudarthrobacter*	99	4.00E-146	100	283
SI27	*Pseudomonas*	100	4.00E-146	100	282
SI28	*Bacillus*	98	4.00E-99	100	197
SI29	*Pseudarthrobacter*	99	4.00E-146	100	281
SI32	*Paenibacillus*	86	8.00E-97	99	222
SI33	*Bacillus*	100	3.00E-96	100	222
SI34	*Neobacillus*	85	3.00E-96	100	222
SI38	*Mucilaginibacter*	100	3.00E-53	99	114
SI39	*Paenibacillus*	99	4.00E-94	97	196

Isolate name	Genus based on NCBI	Coverage %[a]	E-value [b]	% identity [c]	# of Base pairs [d]
SI40	*Clavibacter*	100	6.00E-50	100	105
SI42	*Bacillus*	99	1.00E-145	100	280
SI43	*Pseudomonas*	100	3.00E-95	100	187
SI44	*Stenotrophomonas*	98	1.00E-99	100	198
SI45	*Janthinobacterium*	98	2.00E-99	100	198
SI46	*Rothia*	99	6.00E-144	99	280
SI47	*Rothia*	99	5.00E-145	100	279
SI48	*Peribacillus*	99	1.00E-145	100	280
SI49	*Pseudomonas*	98	1.00E-100	100	199
SI50	*Priestia*	94	4.00E-95	98	204
SI51	*Pseudarthrobacter*	99	1.00E-145	100	280
SI52	*Rhodococcus*	99	1.00E-145	100	280
SI55	*Collimonas*	100	1.00E-94	98	195
SI59	*Flavobacterium*	100	4.00E-57	99	121
SI60	*Paenibacillus*	100	2.00E-98	100	193
SI61	*Bacillus*	97	2.00E-55	100	119
SI62	*Pseudomonas*	100	6.00E-61	100	125
SI63	*Bacillus*	100	2.00E-93	98	192
SI64	*Stenotrophomonas*	100	2.00E-93	97	196
SI65	*Chryseobacterium*	100	1.00E-58	100	121
SI67	*Streptomyces*	99	1.00E-145	100	280
SI68	*Metabacillus*	100	3.00E-95	98	197
SI69	*Pseudomonas*	100	7.00E-92	97	193
SI70	*Microbacterium*	99	6.00E-144	99	280
SI71	*Peribacillus*	96	1.00E-100	100	204
SI72	*Bacillus*	99	1.00E-145	100	280
SI73	*Bacillus*	99	1.00E-145	100	280
SI74	*Rhodococcus*	99	6.00E-144	100	277
SI75	*Peribacillus*	99	2.00E-144	100	278
SI77	*Peribacillus*	99	3.00E-101	100	200
SI78	*Bacillus*	99	1.00E-145	100	280
SI79	*Peribacillus*	97	1.00E-99	100	199
SI80	*Bacillus*	100	7.00E-102	100	199
SI81	*Bacillus*	99	5.00E-145	100	279
SI82	*Bacillus*	99	1.00E-145	100	280
SI83	*Bacillus*	99	1.00E-145	100	282
SI84	*Peribacillus*	99	6.00E-144	99	280
SI85	*Streptomyces*	99	5.00E-145	100	279
SI86	*Bacillus*	99	1.00E-145	100	280

Isolate name	Genus based on NCBI	Coverage %[a]	E-value [b]	% identity [c]	# of Base pairs [d]
SI87	*Bacillus*	99	5.00E-145	100	279
SI88	*Chryseobacterium*	100	1.00E-141	99	282
SI90	*Peribacillus*	100	4.00E-146	99	282
SI91	*Rossellomorea*	99	1.00E-145	100	280
SI92	*Peribacillus*	99	1.00E-145	100	280
SI93	*Bacillus*	100	4.00E-110	100	214
SI94	*Bacillus*	99	1.00E-145	100	280
SI95	*Bacillus*	99	2.00E-134	100	280
SI98	*Streptomyces*	99	1.00E-145	100	280
SI100	*Bacillus*	100	5.00E-62	97	127
SI101	*Streptomyces*	99	1.00E-145	100	280
SI102	*Lysobacter*	100	2.00E-144	99	279
SI104	*Staphylococcus*	99	1.00E-145	100	280
SI108	*Bacillus*	99	1.00E-145	100	280
SI109	*Bacillus*	99	1.00E-145	100	280
SI110	*Bacillus*	99	1.00E-145	100	280
SI111	*Bacillus*	99	4.00E-146	99	283
SI112	*Bacillus*	99	1.00E-145	100	280
SI116	*Bacillus*	99	1.00E-145	100	280
SI117	*Paenarthrobacter*	99	1.00E-145	100	280
SI119	*Pseudomonas*	100	1.00E-146	99	283
SI120	*Bacillus*	99	1.00E-145	100	280
SI121	*Streptomyces*	99	1.00E-145	100	280
SI122	*Pseudomonas*	99	1.00E-145	100	280
SI123	*Enterobacter*	99	2.00E-144	100	278
SI124	*Priestia*	99	1.00E-145	100	280
SI125	*Priestia*	99	2.00E-113	100	222
SI127	*Peribacillus*	93	5.00E-99	100	208
SI128	*Pseudomonas*	100	1.00E-57	96	119
SI129	*Bacillus*	100	3.00E-100	100	196
SI130	*Bacillus*	99	1.00E-145	100	280
SI131	*Bacillus*	100	3.00E-100	100	196
SI132	*Neobacillus*	100	2.00E-98	100	204
SI139	*Arthrobacter*	100	3.00E-53	99	115
SI140	*Pseudomonas*	100	4.00E-57	100	119
SI141	*Stenotrophomonas*	100	1.00E-90	96	196
SI142	*Pseudomonas*	100	1.00E-58	97	127
SI144	*Microbacterium*	98	1.00E-83	98	178
SI167	*Streptomyces*	99	1.00E-145	100	280

Isolate name	Genus based on NCBI	Coverage %[a]	E-value [b]	% identity [c]	# of Base pairs [d]
SI170	*Microbacterium*	99	6.00E-144	99	280
SI172	*Peribacillus*	100	4.00E-146	99	282
SI174	*Rhodococcus*	99	6.00E-144	100	277
SI175	*Bacillus*	99	2.00E-144	100	278
SI176	*Rhizobium*	99	6.00E-144	99	279
SI177	*Pseudoduganella*	99	3.00E-142	99	280
SI180	*Pseudomonas*	100	2.00E-139	100	267
SI181	*Bacillus*	99	2.00E-143	99	279
SI182	*Streptomyces*	99	4.00E-146	99	283
SI183	*Bacillus*	99	1.00E-145	99	282
SI184	*Microbacterium*	99	2.00E-144	99	283
SI185	*Streptomyces*	99	5.00E-145	100	279
SI187	*Bacillus*	99	5.00E-145	100	279
SI188	*Streptomyces*	99	6.00E-144	100	277
SI189	*Priestia*	99	8.00E-143	100	275
SI191	*Streptomyces*	99	6.00E-144	100	277
SI193	*Peribacillus*	100	2.00E-138	100	265
SI194	*Pedobacter*	100	8.00E-143	99	279
SI195	*Arthrobacter*	99	6.00E-144	100	277
SI196	*Kocuria*	99	4.00E-146	99	283
SI197	*Arthrobacter*	99	6.00E-144	100	277
SI199	*Arthrobacter*	99	6.00E-144	100	277
SI202	*Bacillus*	99	5.00E-145	100	279
SI203	*Pedobacter*	99	1.00E-145	99	282
SI206	*Streptomyces*	99	2.00E-144	99	280
SI208	*Streptomyces*	99	2.00E-144	99	280
SI209	*Streptomyces*	99	1.00E-145	100	280
SI210	*Streptomyces*	99	2.00E-144	100	280
SI212	*Stenotrophomonas*	100	2.00E-139	100	267
SI213	*Bacillus*	100	6.00E-73	100	*207*
SI214	*Arthrobacter*	99	6.00E-144	100	277
SI217	*Priestia*	99	6.00E-144	100	277
SI220	*Bacillus*	100	6.00E-139	100	269
SI222	*Hymenobacter*	99	2.00E-144	100	280
SI223	*Stenotrophomonas*	100	1.00E-140	100	269
SI226	*Pseudomonas*	99	1.00E-145	100	280
SI227	*Arthrobacter agilis*	100	2.00E-139	100	267
SI229	*Streptomyces*	100	2.00E-143	100	277
SI230	*Pseudomonas*	100	4.00E-146	100	282

Isolate name	**Genus based on NCBI**	**Coverage %[a]**	**E-value [b]**	**% identity [c]**	**# of Base pairs [d]**
SI242	*Kocuria*	99	1.00E-145	100	282
SI243	*Streptosporangium*	99	4.00E-146	100	283
SI244	*Streptomyces*	99	4.00E-146	100	283
SI245	*Pseudoroseomonas*	99	1.00E-145	100	280
NI01	*Mycolicibacterium*	99	4.00E-146	100	283
NI02	*Novosphingobium*	99	4.00E-145	100	280
NI03	*Shinella*	99	1.00E-145	100	280
NI04	*Sphingomonas*	99	1.00E-145	100	280
NI05	*Bradyrhizobium*	99	1.00E-145	100	280
RI01	*Sphingomonas*	99	5.00E-145	100	279
RI03	*Dermacoccus*	99	6.00E-146	100	277
RI04	*Pseudomonas*	100	5.00E-140	100	268
RI05	*Rhizobium*	99	1.00E-145	100	280
RI07	*Pseudomonas*	99	1.00E-145	100	280
RI08	*Pseudomonas*	98	1.00E-145	100	283
RI12	*Staphylococcus*	99	1.00E-145	100	280

[a] coverage - percentage of query sequence.

[b] E value was obtained from expected value calculation by NCBI blast.

[c] Percent identity is the percentage of query that matches the target.

[d] Number of base pairs blasted.

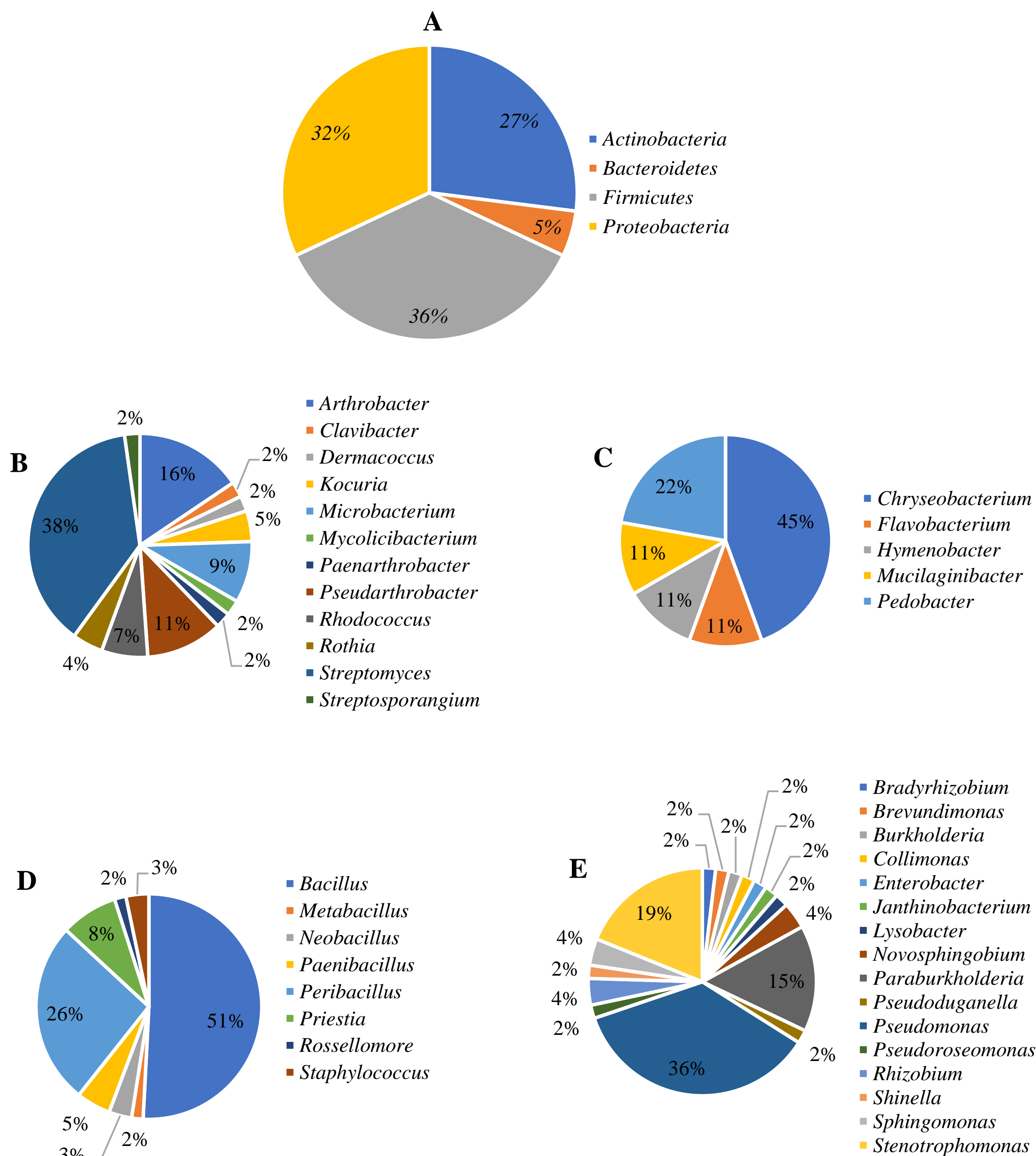


Figure 16. Taxonomic composition of the culture library, for those strains for which a partial 16S rRNA gene sequence was obtained

A) Distribution of different phyla, B) Actinobacteria, C) Bacteroidetes, D) Firmicutes, and E) Proteobacteria. Values are presented as a percentage of the total bacterial isolates in each category for visualization purposes. The total number of isolates in panels A-E are 168, 45, 9, 61, and 53, respectively.

Even though most of the isolated Actinomycetes were beneficial, some pathogenic bacteria, such as *Clavibacter, Kocuria*, and *Rothia*, were also found. The genus *Clavibacter* includes bacterial plant pathogens (Osdaghi et al., 2022), while *Kocuria* and *Rothia* mainly consist of opportunistic pathogens in humans in immunocompetent individuals (Biswal et al., 2023; Fatahi-Bafghi, 2021).

Only nine strains belonged to the phylum Bacteroidetes, including four bacterial isolates from the genus *Chryseobacterium*, two from *Pedobacter*, and the rest from *Flavobacterium*, *Hymenobacter*, and *Mucilanginibacter*. (Figure 16C). Among Bacteroides, genera *Chryseobacterium* and *Flavobacterium* were reported to be the most abundant rhizobacteria in the rhizospheres of cucumber, sweet potato, tomato, and wheat (Nishioka et al., 2016). Further, these genera also possess PGP capabilities, like solubilization of P and production of IAA and ACC deaminase (Nishioka et al., 2016). The genus *Pedobacter* has recently become associated with its ability to promote plant growth and its potential to produce antimicrobial substances.

The genus *Pedobacter* is also recognized as an environmental superbug due to its ability to withstand a wide range of known antibiotics. Certain *Pedobacter* spp. exhibits antimicrobial properties, although the characterization of their bioactive compounds is presently confined to antifungal chitinase and non-ribosomal peptides referred to as "(iso)pedopeptins" (covas et al., 2023).

The genus *Bacillus* primarily dominated the phylum Firmicute*s*, followed by *Peribacillus* (Figure 16D). *Bacillus* strains were abundant among culturable bacteria in the cacao rhizosphere and *Zea mays* rhizosphere (Crisostomo-Panuera et al., 2024). This suggests that members of the Firmicutes phylum are commonly found in the rhizosphere of significant crops. The genus *Bacillus* includes highly versatile microorganisms capable of producing diverse products and

thriving in various agro-industrial wastes and harsh environments. They yield eco-friendly and beneficial products ranging from foods to biocides (Herrmann et al., 2024). The genera *Peribacillus, Neobacillus,* and *Metabacillus* were recently reclassified from the genus *Bacillus* (Patel & Gupta, 2020). *Peribacillus simplex*, belonging to the genus *Peribacillus*, has been reported to possess several beneficial functions, such as PGP abilities, biocontrol activity against a wide range of harmful plant pests, and potential bioremediation agent for heavy metals and pesticide residues (Manetsberger et al., 2023).

Pseudomonas was the most abundant genus in Proteobacteria, followed by *Stenotrophomonas* and *Paraburkholderia* (Figure 16E). It is important to note that some species within *Pseudomonas*, such as *Pseudomonas syringae*, are known plant pathogens (Baltrus et al., 2017). The genus *Pseudomonas* is widely used as a crop inoculant due to its ability to enhance plant growth in various ways, such as nutrient uptake, ACC deaminase activity, and antioxidant activities (Mehmood et al., 2023). *Stenotrophomonas* is also recognized as a potential plant growth-promoting rhizobacteria (PGPR) because of its capability to solubilize phosphate, produce siderophores, and synthesize phytohormones. Additionally, *Stenotrophomonas* acts as a microbial biocontrol agent by exhibiting antagonistic activity against phytopathogens and insect pests (Kumar et al., 2023). *Paraburkholderia*, also known as a PGPR, possesses several plant growth-promoting mechanisms ranging from biological nitrogen fixation to antifungal activity (Vio et al., 2020).

3.3.2 Impact of different media on bacterial isolation

A total of 78 bacterial isolates were obtained from all media. Of these, 40 were isolated from GYM and Rhi media, 38 from R2A, and 25 from NA. Six of these isolates were common across the different media, with the remaining isolates being unique to each medium (Figure 17).

The six common strains belong to the following genera: *Rhodococcus, Bacillus, Microbacterium, Streptomyces*, and *Pedobacter*. The genera present in the unique isolates from each medium are as follows:

GYM: *Streptomyces, Pseudoduganella, Bacillus*, and *Pseudomonas*.

NA: *Stenotrophomonas, Kocuria, Streptosporangium*, and *Streptomyces*.

R2A: *Streptomyces, Pedobacter*, and *Streptomyces*.

Rhi: *Bacillus, Arthrobacter,* and *Streptomyces*.

This result suggests that no single culture medium can capture all cultivable bacteria, as indicated by the variation in bacterial genera isolated across different media. Specifically, the unique isolates from each medium show distinct bacterial communities, with only a small number of isolates being common across media. This highlights the importance of using multiple media to increase species diversity and capture a broader range of cultivable bacteria. As the growth requirements of each bacterium vary, a single culture medium cannot be used to exploit all the bacteria present in a particular environment (Al-blooshi et al., 2021; Bonnet et al., 2020). Since these comparisons were based on visual appearance, there might be similarities in the unique strains, as some bacteria exhibit different growth characteristics under various growing conditions. These strains will need to be sequenced or better characterized to resolve differences.

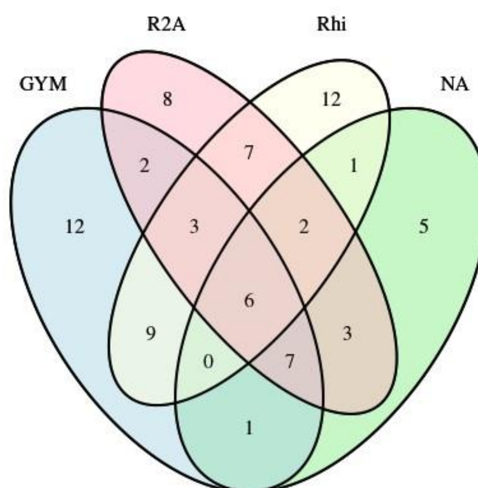


Figure 17. Venn diagram illustrating the overlap and unique genera of bacterial isolates across different culture media

The diagram shows the number of genera unique to each medium and those shared between media. Each oval represents the isolates specific to a particular medium, with the overlapping areas indicating the isolates shared between two or more media. Nutrient agar (NA), R2A, GYM Streptomyces (GYM), and Rhizobium medium (Rhi).

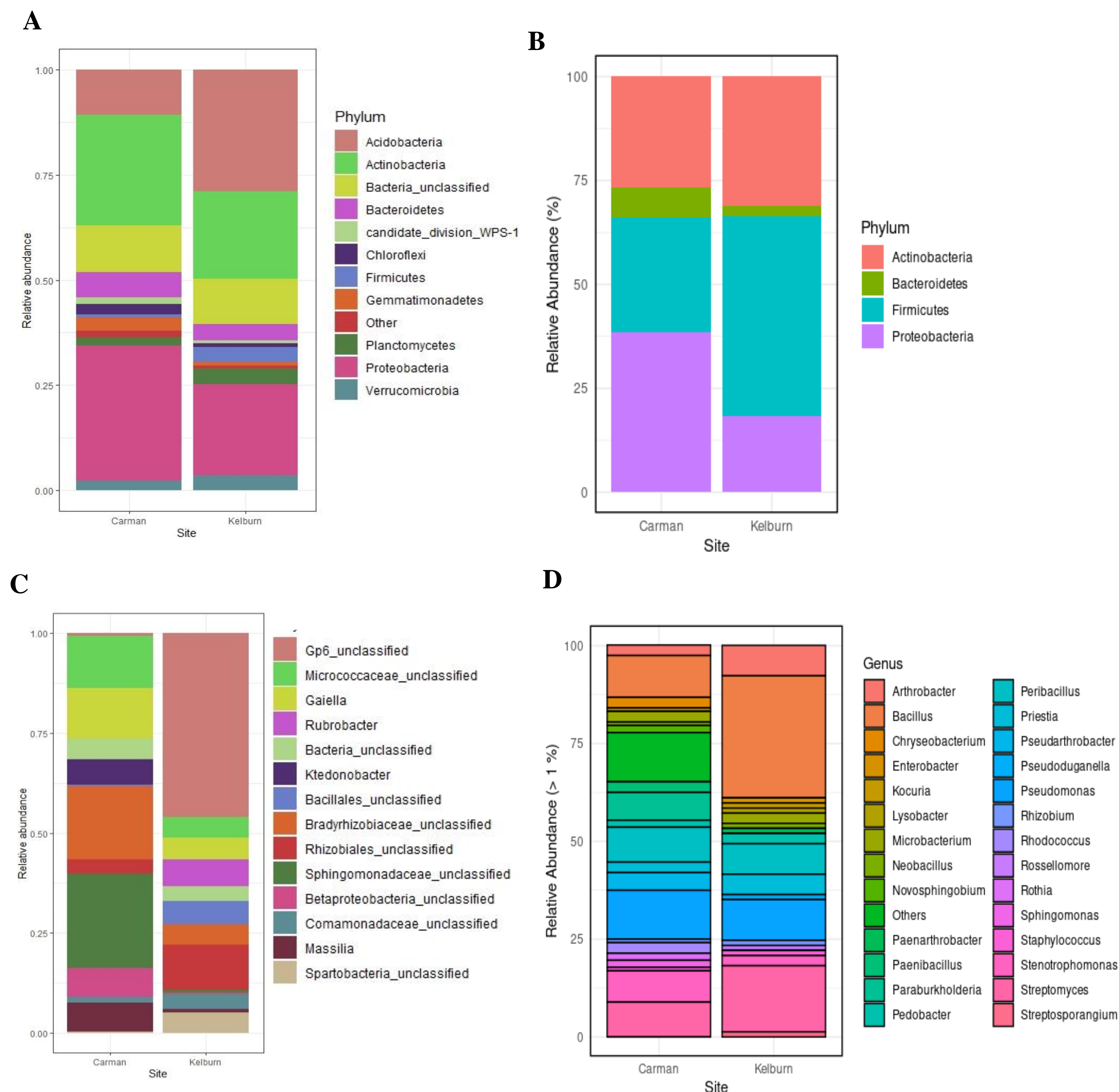


Figure 18. Comparison of total bacterial communities vs. culturable bacteria

Panels A and C show the phylum and genus composition of bacterial communities as determined by 16S rRNA gene sequencing, while panels B and D display the phylum and genus composition obtained through culturing methods. The graphs compare culture-independent (sequencing) and culture-dependent (culturing) analyses of BP samples from Carman and Kelburn in 2021. Panels A and C represent the relative abundance of the top 25 taxa, whereas panels B and D illustrate the relative abundance as a percentage of the total bacterial strains identified through sequencing and culturing. Less abundant taxa are grouped under "Others."

3.3.3 Community composition between culture-dependent and independent characterization

To compare the composition of bacterial communities between culturing and culture-independent characterization, samples collected in the previous chapter from the 2021 BP stage were analyzed using both approaches. The results of the 16S rRNA gene sequencing revealed that the most dominant phyla were Proteobacteria, Actinobacteria, Acidobacteria, and a substantial proportion of unclassified bacteria in both sites (Figure 18A). In addition, Bacteroidetes, Firmicutes, Planctomycetes, Verrucomicrobia, and Gemmatimonadetes were also observed to a lesser extent. However, culturing yielded only four bacterial phyla, namely Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes, in both sites (Figure 18B). This is not surprising since these phyla include many fast-growing bacteria.

The genus composition obtained from the sequencing varied greatly among sites (Figure 18C). The most abundant genera observed in Carman experimental site were *Sphingomonas_unclassified, Gaiella, Bradyrhizobium_unclassified, Micrococcus_unclassified,* and *Betaproteobacteria_unclassified*. Meanwhile *GP6_unclassified, Rhizobiales_unclassified, Bradirhizobium_unclassified, Rubrobacter, and Bacillale_unclassified*. Meanwhile, the genus composition obtained via culturing indicates that the key genera in Carman were *Bacillus, Pseudomonas, Stenotrophomonas, Streptomyces*, and *Peribacillus* (Figure 18D).

Bacillus, *Streptomyces, Pseudomonas*, *Arthrobacter*, and *Peribacillus* were the major genera in Kelburn. The comparison of the phylum and genus between both methods indicates a gap between total bacterial communities derived from culture-independent and culture-dependent communities. Core microbiome comparisons between both methods imply that more than half of the genera were common between sites in each method (Figure 19). However, among the 46 core genera obtained through sequencing, only two are core genera in the Venn

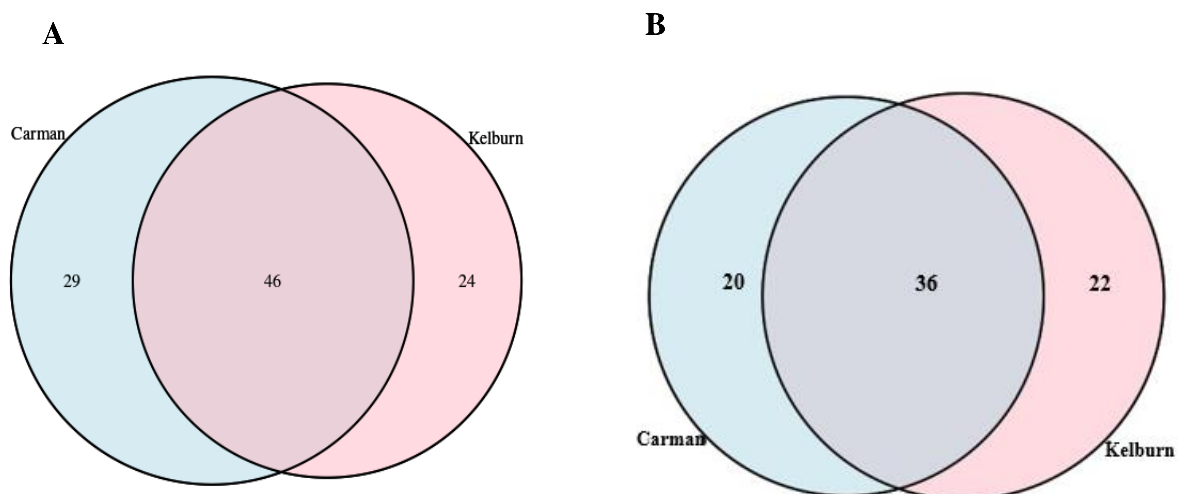


Figure 19. Comparison of core microbiomes at genus level

A) Culture-independent and B) Culture-dependent

Figure A presents the core microbiome based on 16S rRNA gene sequencing analysis of BP 2021 samples (culture-independent approach) collected from Carman and Kelburn experimental sites, while B shows the core microbiome derived from the total number of bacterial strains identified through culturing at each site (culture-dependent approach).

Table 12. List of core and unique OTUs between sites based on 16S rRNA gene sequencing

Common	
Acetobacteraceae_unclassified	*Intrasporangiaceae_unclassified*
Acidimicrobiales_unclassified	*Microbacteriaceae_unclassified*
Acidobacteria_Gp3_unclassified	*Micrococcaceae_unclassified*
Actinomycetales_unclassified	*Micromonosporaceae_unclassified*
Alphaproteobacteria_unclassified	*Mycobacterium*
Bacillales_unclassified	*Myxococcales_unclassified*
Bacteria_unclassified	*Nocardioides*
Bacteria_unclassified	*Opitutus*
Bacteria_unclassified	*Oxalobacteraceae_unclassified*
Betaproteobacteria_unclassified	*Planctomycetaceae_unclassified*
Blastococcus	*Proteobacteria_unclassified*
Bradyrhizobiaceae_unclassified	*Reyranella_unclassified*
Burkholderiales_unclassified	*Rhizobiales_unclassified*
candidate_division_WPS-1_unclassified	*Rhodospirillales_unclassified*
Chitinophagaceae_unclassified	*Solirubrobacter*
Comamonadaceae_unclassified	*Spartobacteria_unclassified*
Deltaproteobacteria_unclassified	*Sphingomonadaceae_unclassified*
Gaiella	*Sphingomonas*
Gammaproteobacteria_unclassified	*Streptomycetaceae_unclassified*
Gemmata	*Subdivision3_unclassified*
Gemmatimonas	*Verrucomicrobia_unclassified*
Gp16_unclassified	*Xanthomonadaceae_unclassified*
Gp3_unclassified	
Gp6_unclassified	
Unique Carman	**Unique Kelburn**
Acidobacteria_Gp1_unclassified	*Acidobacteria_Gp4_unclassified*
Armatimonas/Armatimonadetes_gp1	*Chloroflexi_unclassified*
Burkholderia	*Flavisolibacter*
candidate_division_WPS-2_unclassified	
Candidatus_Solibacter_unclassified	*Gp4_unclassified*
Chthonomonas/Armatimonadetes_gp3	*Gp5_unclassified*
Conexibacter	*Gp7_unclassified*
Gp1_unclassified	*Hyphomicrobiaceae_unclassified*
Gp2_unclassified	*Kribbella*

Unique Carman	Unique Kelburn
Granulicella_unclassified	*Methylobacteriaceae_unclassified*
Hymenobacter	*Microvirga*
Jatrophihabitans	*Nitrospira*
Ktedonobacter	*Pedomicrobium*
Marmoricola	*Pirellula*
Massilia	*Planococcaceae_unclassified*
Modestobacter	*Polyangiaceae_unclassified*
Mucilaginibacter	*Povalibacter*
Nakamurella	*Pseudonocardia*
Nocardioidaceae_unclassified	*Rubrobacter*
Pedobacter	*Sinobacteraceae_unclassified*
Phenylobacterium	*Skermanella*
Phycicoccus	*Solirubrobacterales_unclassified*
Pseudomonas	*Sphaerobacteraceae_unclassified*
Rhizomicrobium_unclassified	*Zavarzinella*
Rhodanobacter	
Rhodospirillaceae_unclassified	
Segetibacter	
Singulisphaera	
Sphingobacteriaceae_unclassified	

Table 13. List of core and unique genera between sites based on culturing

Common	Unique Carman	Unique Kelburn
Arthrobacter	*Bacillus*	*Arthrobacter*
Bacillus	*Streptomyces*	*Bacillus*
Kocuria	*Microbacterium*	*Pedobacter*
Microbacterium	*Rhodococcus*	*Pseudoduganella*
Pedobacter	*Hymenobacter*	*Pseudomonas*
Peribacillus	*Stenotrophomonas*	*Streptomyces*
Priestia		*Streptosporangium*
Pseudomonas		
Rhizobium		
Stenotrophomonas		
Streptomyces		

diagram obtained from culturing (Tables 12 & 13). This observation suggests that targeted culturing should be prioritized, focusing on species that appeared in the culture-independent analysis but were not cultured. This approach could help create a more comprehensive culture collection.

Chapter 4: Isolation of non-symbiotic phosphate solubilizing *Paraburkholderia strydomiana*

Authors: Ambihai Shayanthan, Anna Motnenko, and Ivan J. Oresnik. This work was planned and carried out by Ambihai Shayanthan. Phylogenetic analysis was done by Anna Motnenko.

4.1 Abstract

Phosphorus (P) is a major nutrient required for plant growth and is often present as insoluble forms in soils. Even though P fertilization satisfies immediate plant growth, it is easily converted to insoluble forms through various soil processes. This results in poor P use efficiency, leading to over-fertilization, which is economically and environmentally costly. Using phosphate-solubilizing bacteria (PSB) would be a cost-effective way to improve plant growth while minimizing environmental hazards, as they can solubilize insoluble P. In this study, phosphate solubilizing bacteria strains were isolated from agricultural soils in Manitoba based on their growth on Pikovskaya's agar (PVK) medium. A quantitative P solubilization assay was done to ensure their ability, followed by germination assays to determine their plant growth-promoting potential. Finally, whole genome sequencing was used to explore their genetic potential. Quantitative assays confirmed that the selected bacterial strains can solubilize insoluble $Ca_3(PO_4)_2$, and their capacity ranged between 95-144 (mg/dL). Results of a Vigor Index assay revealed that the tested strains influenced seedling growth either positively or negatively, and the Vigor Index was driven by the germination rate. Prominent growth promotion was observed in soybeans compared to canola and wheat. Based on the whole genome sequencing, the isolates belonged to *Paraburkholderia strydomiana, Paraburkholderia graminis, and Burkholderia ambifaria*. All the *P. strydomiana* strains showed positive PGP traits in the tested seedlings, while *P. graminis,* and *B. ambifaria* had a negative effect on soybean seedlings. Collectively, the isolated bacterial strains can potentially be used as PSB, but further studies are needed to confirm this.

4.2 Introduction

Among macronutrients, phosphorus (P) is the second most limiting nutrient for the growth and development of plants. It plays a vital role in plant metabolic processes, such as respiration, cell division, tissue development, biosynthesis of macromolecules, and photosynthesis (Rawat et al., 2021). However, the amount of total P in soil is low compared to other nutrients, and among the total P, only 0.1% is available P (P_i) (Zhu et al., 2016; Zou et al., 1992) as plants uptake P mainly in the form of phosphate anions, either HPO_4^{2-} and $H_2PO_4^{-}$. The amount of P_i critically determines the success of plants in natural and agricultural ecosystems (Cheng et al., 2023). Consequently, the demand for phosphatic fertilizers has increased to maintain plant productivity. However, P_i can easily be unavailable due to immobilization either by combining with aluminum or iron in acid soils or binding to calcium in alkaline-calcareous soils. The accumulation of immobilized P in soils and potential transfer to water bodies ultimately leads to environmental eutrophication (Gatiboni et al., 2020). Therefore, it is essential to explore ways to improve the bioavailability of immobilized P in agricultural soils as a strategy to mitigate the overuse of P fertilizers (Yu et al., 2019).

Soil microorganisms have the potential to scavenge nutrients from natural reservoirs, such as solubilizing insoluble P, thereby enhancing the bioavailability of essential nutrients for plants (Philippot et al., 2023). Among soil microbes, bacteria are abundant in soils, and their population is higher in the plant rhizosphere compared to bulk soil (Banerjee & van der Heijden, 2023). The application of PSB could be one of the promising management strategies to improve P use efficiency as they can convert insoluble P into soluble forms (Li et al., 2020; Yu et al., 2022). PSB isolates have been readily obtained from various environments ranging from rhizosphere soils and agricultural soils to sewage sludges (Song et al., 2021).

Several mechanisms are involved in phosphate solubilization by PSB. These include the exudation of organic acids, protons, or siderophores, the excretion of extracellular enzymes, and substrate degradation through mineralization (Cheng et al., 2023; Gatiboni et al., 2020; Rawat et al., 2021). Many bacterial genera are capable of phosphate solubilization, including *Pseudomonas, Enterobacter, Bacillus, Serratia, Pantoea, Rhizobium, Arthrobacter, and Burkholderia* (Janati et al., 2023; Rawat et al., 2021; Song et al., 2021). *Pseudomonas* is well known for its ability to solubilize phosphate and its plant growth-promoting abilities (Mehmood et al., 2023). Recently, the genus *Paraburkholderia* has been reported to show P solubilization ability. *Paraburkholderia* is a Gram-negative, non-spore-forming, rod-shaped bacteria with a G+C content of 58.9–65.0 mol% (Beukes et al., 2019). This new genus includes most of the plant beneficial and environmental species initially classified as *Burkholderia*, which contains several pathogenic species (Vio et al., 2020).

Additionally, PSB isolates were reported to have other multifunctional traits that affect plant health including growth promotion, nitrogen fixation, and stress tolerance. Biswas et al., (2018) reported that three PSB strains, *Bacillus megaterium* (MF 589715), *Staphylococcus haemolyticus* (MF 589716), and *Bacillus licheniformis* (MF 589720), could solubilize P, increase resistance to the metals Cu and Zn at significant concentrations, and improve growth promotion. In another study, three PSB strains isolated from rice rhizosphere showed P solubilization, indole acetic acid (IAA) production, antagonistic effect against bacterial leaf blight disease, and improved rice growth under P deficit soils (Rasul et al., 2019). Screening of PSB from soybean rhizosphere under a maize–soybean intercropping system revealed that isolated PSB had the potential to increase P availability by secreting several organic acids, produce IAA and siderophores, and improve germination and growth of maize seedlings (Song et al., 2021). It has

also been reported that PSB can lower the leaching losses of P via the improvement of plant absorption and use of soil P (Yu et al., 2022). PSB can reduce soil P, which has accumulated in the soil over time from past fertilization or natural processes, by increasing the fertilizer use efficiency and improving the long-term efficiency of agricultural production (Cheng et al., 2023).

Improved crop production due to PSB application has also been reported in other agricultural crops, including maize (Yu et al., 2022), wheat (Liu et al., 2019b), soybean (Afzal et al., 2010), barley (Chouyia et al., 2020), and common bean (Souza et al., 2023). However, the performance of PSB can be influenced by several factors, including interactions with other microorganisms, agronomic activities, and various environmental factors such as temperature, precipitation, soil nutrient status, and soil texture (Janati et al., 2023). The occurrence, abundance, diversity, and bioactivity of PSB vary in different soils. Climatic conditions significantly influence P solubilizing ability, impacting the composition of the studied sites' PSB communities (Janati et al., 2023). Therefore, screening PSB from local agricultural soils is necessary to ensure they can withstand the indigenous microflora and contribute to sustainable agricultural production. This study was aimed to 1) isolate PSB from Manitoba soils, 2) quantify their phosphate solubilizing ability and their plant growth promotion potential, and 3) evaluate the overall genetic uniqueness of the isolated strains relative to their closest known relatives.

4.3 Materials and Methods

4.3.1 Culture conditions and isolation of phosphate solubilizing bacteria

Bacterial strains were generally grown at 28°C using a Tryptone Yeast Extract (TY) medium, which contained 5 g Tryptone and 3 g yeast extract per litre of water (Malviya, 2013). To isolate phosphate-solubilizing bacteria from soil samples, 5 g of soil was mixed with 50 ml of

sterile water. This was serially diluted, and 0.1 ml of relevant dilutions were plated onto Pikovskaya (PVK) medium (Nautiyal, 1999) to visualize phosphate solubilization. This PVK contained (per litre): 10 g glucose, 5 g $Ca_3(PO_4)_2$ 5 g $(NH_4)_2SO_4$, 0.5 g NaCl, 0.2 g $MgSO_4$, 0.2 g KCl, 0.2 g yeast extract, 0.5 g $MnSO_4$, and 0.002 g Fe SO_4 (Nautiyal, 1999). Plates were incubated at 28°C for one week and then examined for zones of clearing surrounding bacterial colonies. Colonies forming larger zones of clearing were selected and single colony purified three times before being used. Purified cultures were grown overnight in TY to late log phase and mixed with a freezing solution (TY containing 24% vol/vol DMSO) such that the final concentration of DMSO was 8%. These were stored as frozen permanents at -80°C.

4.3.2 DNA extraction and amplification of the V4 region of the rRNA gene

To extract bacterial genomic DNA from isolated strains, two to three freshly grown colonies were picked from an agar plate and resuspended in 0.1 ml double-distilled H_2O. 50 µL of this resuspension was extracted using Qiagen’s DNeasy PowerSoil kit using the manufacturer’s protocol. Initial identification of bacterial isolates was done by sequencing the V4 region of the 16S rRNA gene. Briefly, the V4 region was amplified using the 515F; 5'-GTGCCAGCMGCCGCGG-3' (Parada et al., 2016) – 806R; 5'-GGACTACNVGGGTWTCTAAT-3' (Apprill et al., 2015) primer pair. The PCR products were gel-isolated and sent to the Centre d’expertise et de services Génome Québec for Sanger sequencing, along with both forward and reverse primers. The resultant sequence was BLASTed against the NCBI nucleotide database to determine a putative identification.

4.3.3 Quantification of phosphate solubilization

To quantitate phosphate solubilization by bacterial cultures a QuantiChrom™ Phosphate Assay Kit was utilized. Briefly, three independent colonies were grown overnight in 5 mL TY

broth cultures. The optical densities (OD_{600}) of the cultures were then normalized to 1.0. Then, 1 mL was added to 5 mL of PVK broth. A control was included where 1 mL ddH_2O water was added instead of a bacterial culture. The tubes were incubated on a rotary incubator at 28°C for three days. The cultures were then pelleted at 10,000 rpm for 10 minutes, and the supernatants were used. To assay, 50 µL of a provided blank control (0 P_i), a 0.28 mg dL^{-1} P_i standard, the broth control, and the samples were transferred to a clear bottom 96-well plate. To this, 100 µL of the provided reagent (which contained Maclchite Green and molybdate for color development) was added to each well. The contents were mixed well and incubated for 30 min at room temperature. Color development was assayed at 620 nm using a plate reader. Phosphate concentration was calculated as follows:

Phosphate concentration = $(OD_{Sample} - OD_{Blank})/(OD_{Standard} - OD_{Blank}) \times 0.28$ (mg/dL)

OD_{Blank}, $OD_{Standard}$, and OD_{Sample} are the OD_{620nm} values of the 0 P_i Blank, the 0.28 mg dL^{-1} P_i Standard, and the Sample, respectively. Conversions: 1 mg/dL P_i equals 105.3 µM, 0.001%, or 10 ppm.

4.3.4 Assessment of plant growth promoting ability

Plant growth-promoting activity was determined using a vigor index (Abdul-Baki & Anderson, 1973). Briefly, this index measures growth promotion using the mean root length, mean shoot length, and seed germination.

Vigor Index (VI) = (Mean shoot length (cm) + mean root length (cm)) × (% germination)

To carry out these assays, seeds were first surface sterilized for 20 minutes using a 1% hypochlorite solution. Subsequently, they were washed with approximately 10 volumes of sterile ddH_2O over a period of approximately 30 minutes. Bacteria being tested were first grown in 5 mL broth culture of TY and grown overnight. The overnight culture was then used to inoculate a

100 mL broth culture of TY. The OD_{600} of these cultures was adjusted to an optical density of 1. A volume of 20 ml of the adjusted cultures was used to soak the surface sterilized seeds. Seeds were treated with 20 mL of the bacterial culture for 30 minutes. Control seeds were soaked in 20 mL of sterilized water ddH_2O. Finally, the seeds were individually planted using sterile forceps into seed-starting trays (72-cell seed starter tray, each cell 1.5 inches in diameter and depth; tray size: 21.4 inches × 11.4 inches × 2.1 inches). Each cell was filled with a sterile sand/vermiculite (1:1) mixture. These were subsequently covered with plastic cling film to reduce the drying of the seedlings. A total of 24 seeds were planted for each treatment. Seedlings were harvested 15 days after planting, and root and shoot lengths were measured. Significance was determined using a two-way ANOVA using Dunnett's test for post hoc analysis.

4.3.5 Whole genome sequencing

Whole genome sequencing was carried out as previously described using a MinION sequencer (Hawkins et al., 2022). To extract genomic DNA, bacteria were grown overnight in TY broth, and genomic DNA was extracted using the PureLink Genomic DNA mini kit (Invitrogen), library preparation was performed using SQK-LSK-109 and EXP-NBD-104 kits following the manufacturer's instructions (Oxford Nanopore Technologies, Oxford, UK). Sequencing was carried out using a Nanopore MinION Mk1B system with R10.3 flow cells. Sequencing was stopped once there was enough data to achieve approximately 100x coverage across all genomes. Sequencing reads were then base-called using Guppy-GPU (Wick et al., 2019). The generated FASTQ files were subjected to quality control and adaptor trimming. Adaptor trimming was performed using BBduk (Bushnell et al., 2017). *De novo* genome assembly was achieved using Flye, followed by three rounds of polishing using minimap2 (Kolmogorov et al., 2019; H. Li, 2018). Genome completion was estimated using CheckM

(Parks et al., 2015) and found to be above 99%, with an estimated 1% contamination in all assemblies. Default parameters were used for all software in the analysis.

4.3.6 Phylogenetic analysis

Phylogenetic analysis was carried out using programs and packages available on the Type Strain Genome Server (TYGS), found at https://tygs.dsmz.de/background/show, as well as KBASE, found at https://www.kbase.us/ and maintained by the United States Department of Energy. Assembled genomes were submitted to the Type Strain Genome Server (TYGS) to obtain DNA-DNA Hybridization (dDDH) values as well as G+C content (Meier-Kolthoff et al., 2021). Phylogenetic trees were also generated on the TYGS server using the Genome BLAST Distance Phylogeny approach (GBDP) under the algorithm 'coverage' and distance formula d5.n (Meier-Kolthoff et al., 2013). Average Nucleotide Identity (ANI) values were calculated using fastANI on KBase (Jain et al., 2018). ANIclustermap (Shimoyama 2022, found at https://github.com/moshi4/ANIclustermap) was used to visualize ANI data. Mauve alignments were used to determine regions of homology between bacterial genomes (Darling et al., 2004).

4.4 Results

4.4.1 Isolation of phosphate-solubilizing bacteria

Phosphate solubilizing bacteria are often correlated with strains that have plant growth-promoting characteristics (Shen et al., 2021). Many of the phosphate-solubilizing bacteria that have previously been isolated have been from the rhizosphere of several crop plants. These tended to be more metabolically active than those isolated from non-rhizosphere sources (Gyaneshwar et al., 2002). We had access to soil samples collected in previous chapter 2 from 2018 which were were taken adjacent to the roots from a rotation study that included soybean,

canola, corn, and wheat. Since there was a diversity of crops sampled, it was reasoned that this could increase the probability of isolating diverse species of phosphate-solubilizing bacteria.

After soils from each crop were initially plated onto PVK agar, 20 putative phosphate solubilizing bacteria were found. Following single colony purification and retesting for phosphate solubilizing activity, 9 strains consistently produced larger zones of clearing and were selected for further analysis. These were labeled as SI01-SI09. Based on the sequencing results of the V4 regions of the isolates (Appendix 1), as well as the sample/plate from which each of the strains was initially isolated, as well as growth characteristics, it was deemed likely that SI02 and SI03 were likely siblings, so SI03 was not further characterized. This left 8 isolates capable of solubilizing insoluble phosphate on PVK agar (Figure 20A, Figure 20B). SI01 and SI09 were isolated from the corn plot, SI02-SI05 and SI07 from the soybean plot, and SI06 and SI08 from the canola and wheat plots, respectively. Based on the V4 sequences, except for SI05 and SI06, the strains were tentatively identified as *Paraburkholderia strydomiana*. SI05 and SI06 were tentatively identified *as Burkholderia ambifaria* and *Paraburkholderia graminis,* respectively.

To quantify their ability, these strains were grown overnight in TY, subcultured into PVK broth for three days, and the supernatants were analyzed for soluble P_i (Figure 20C). The results show that in all cases, the isolates could solubilize between 95-144 mg/dL phosphate, in contrast to the 100 mg/L normally found in PVK medium. The quantification also corroborated the visual observation that each of the strains looked similar on PVK agar.

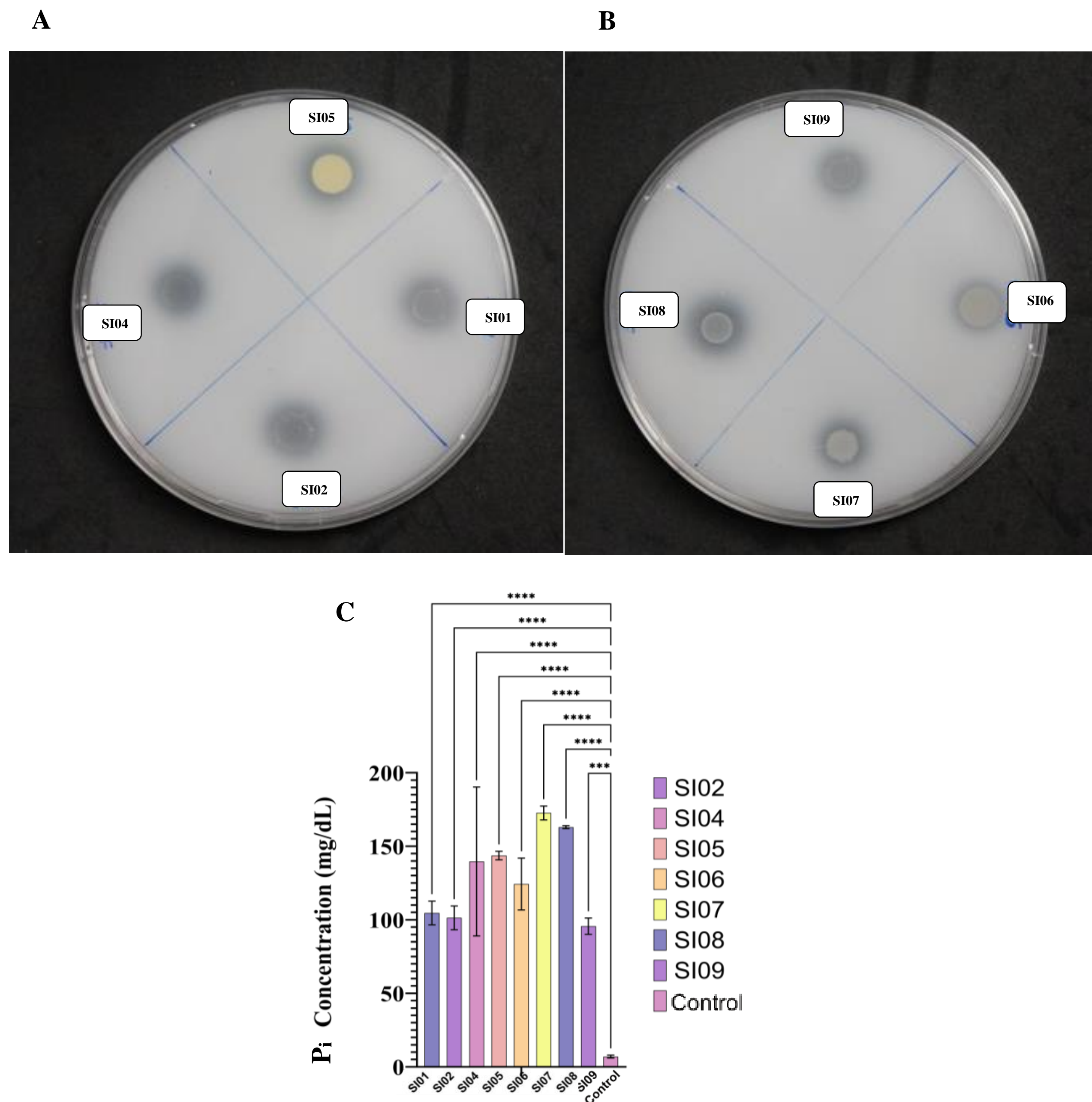


Figure 20. Effect of bacterial strains on phosphate solubilization.

A & B. Phenotypic assessment of isolated strains and C. Quantification of phosphate solubilization ability. The data are presented as the average ± standard deviation (n=3). Significance was determined with a one-way ANOVA using Dunnett's post hoc test. ****, p <0.0001; ***, p=0.0001.

4.4.2 Plant growth enhancement through PSB inoculation

Seed germination and seedling emergence are critical steps in plant development. These early traits are often correlated with better plant health at later stages of growth (Gardarin et al., 2016). The seedling vigor index (VI) helps quantify seedling growth. It is calculated by multiplying the germination percentage by the average seedling length of shoots and roots (Sharma, 2018). To determine if the isolated strains had plant growth-promoting traits, they were used to treat surface sterilized seeds of the plants used in the rotation study, specifically soybean, canola, and wheat. Due to inconsistent growth under *in vitro* conditions, strains SI01 and SI08 were excluded from these assays.

When soybean seeds were inoculated with the phosphate solubilizing strains, it appeared that strains treated with SI02, SI04, SI07, and SI09 looked better visually than the control plants that were treated with water. Seeds treated with SI05 and SI06 appeared to look smaller and negatively impacted compared to the control plants (Figure 21A). When the root and shoot length were measured, and the percentage of germinated seedlings was used to calculate the vigor index, it showed that treating the soybean seeds with these strains led to significant differences (Figure 22A). for soybean, seeds treated with SI02, SI04, SI07, and SI09 increased the vigor index, whereas SI05 and SI06 decreased the vigor index (Figure 22A). Looking through the individual measures that were used to determine the vigor index, it was noted that changes that were seen in the soybean seedlings were related to the percentage of seeds that germinated in the presence of the treatments (Figure 23A). We note that all the strains that affected the germination rate on soybean were *P. strydomiana*.

A

B

C

Figure 21. Effect of PSB inoculation on seedling growth

A. Soybean, B. Canola, and C. Wheat. Representative plants

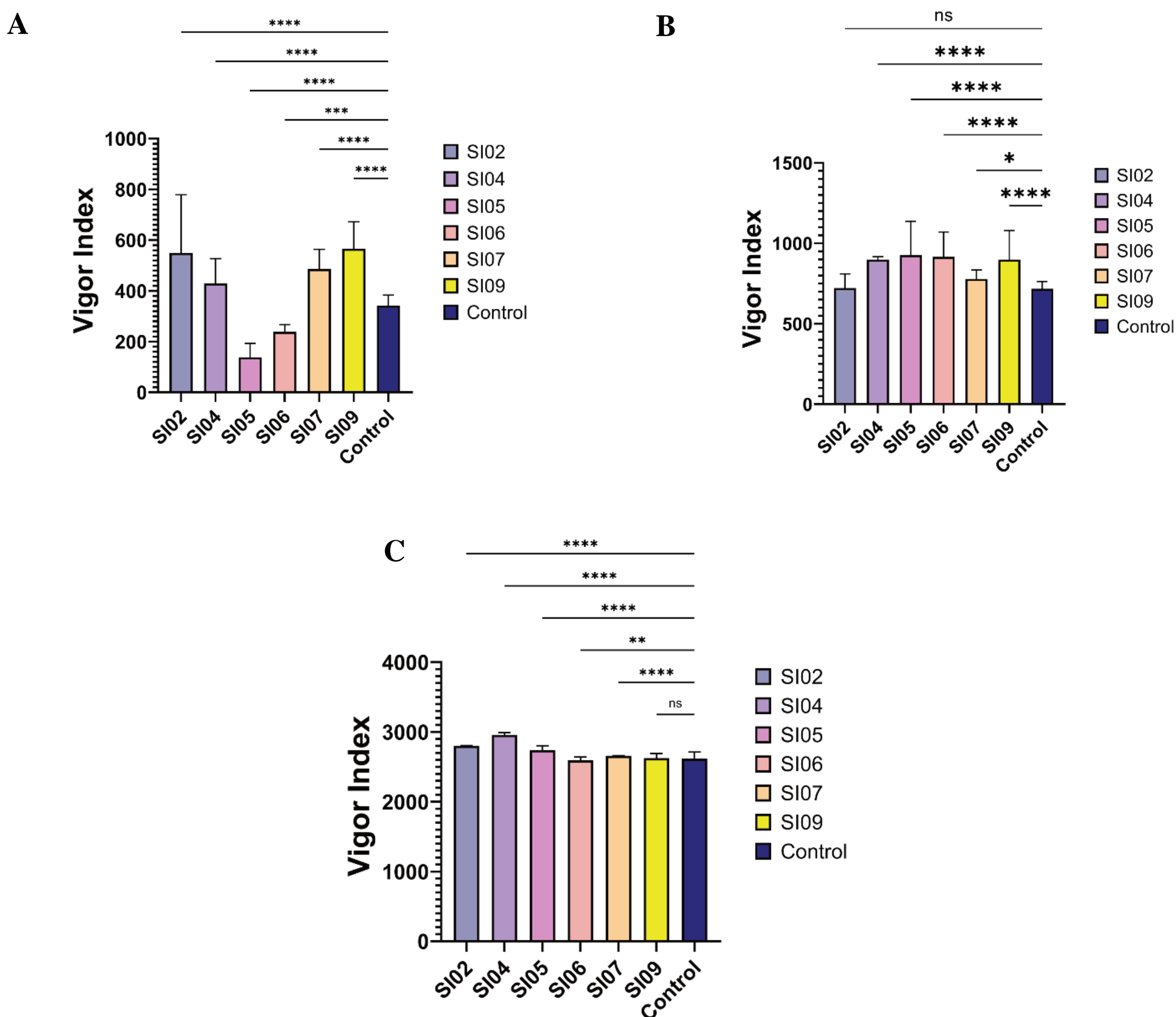


Figure 22. Vigor Index A) Soybean, B) Canola and C) Wheat

The data represents two independent trials consisting of at least 24 seeds/trial. Significance was determined using a two-way ANOVA using Dunnett's post hoc test. Vigor index is calculated based on the shoot and root length multiplied by the percent germination. Significance is denoted as *, p= 0.026; **, p=0.008;***, p=0.0001; ****, p<0.000001.

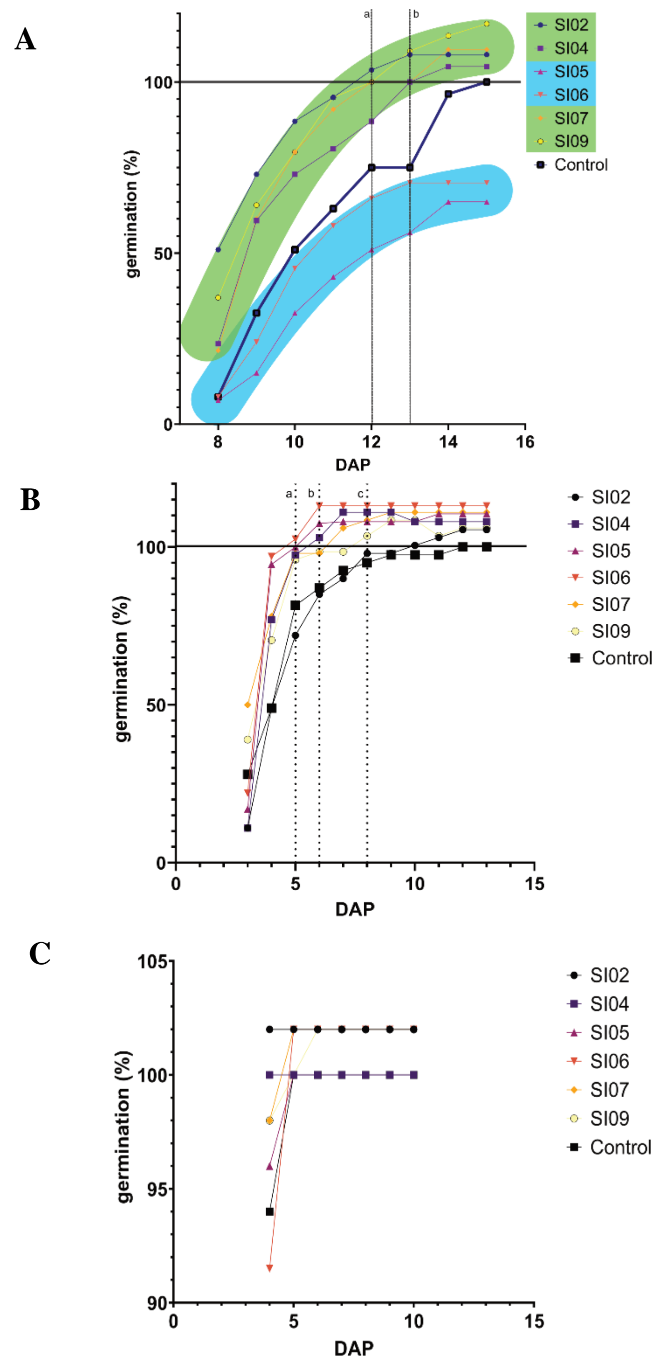


Figure 23. Germination percentage of seedlings A) Soybean, B) Canola and C) Wheat.

Germination rate of uninoculated control was considered 100% and compared the inoculated treatments relative to control. Lettering on the graphs indicates points where strains reached 100% germination compared to the control. For visualization purposes, higher germination is shaded in green, while blue shading indicates a lower germination percentage. DAP- Days after planting.

When canola and wheat seeds were treated with the phosphate solubilizing strains, all except SI02 appeared to have longer roots (Figure 21B & 21C). Once the seedlings were measured, the calculated vigor index was also shown to be significantly different (Figure 22B & 22C).

4.4.3 Genome sequencing

P. strydomiana strains have previously been isolated as nitrogen-fixing nodules from *Hypocalyptus sophoroides* in South Africa (Beukes et al., 2019). In addition, *Paraburkholderia* are strongly associated with having PGP traits. Since the isolates in this study were not obtained from nodules but were instead selected for their ability to solubilize phosphate, it was of interest to sequence these isolates to determine if their genome sequence might offer clues as to their abilities.

Whole genome sequencing using the MinION yielded greater than 35× in depth per sample (Table 14). As expected, six of the eight isolates belonged to the species *P. strydomiana* (SI01, SI02, SI04, SI07, SI08, and SI09), and the other two were *P. graminis* (SI06) and *B. ambifaria* (SI05).

The genome assembly of *B. ambifaria* (SI05) showed 5 contigs with a total size of 8.076 Mbp, an N50 of 3.02 Mbp, a GC content of 66.3 %, and coverage of 75×. Using the Rapid Annotation Subsystem Technology (RAST), this genome was predicted to contain 7130 protein-coding genes with 81 tRNA genes. The genome assembly of *P. graminis* (SI06) resulted in a total size of 7.243 Mbp with 3 contigs, an N50 of 4.256 Mbp, a GC content of 60.4%, and a coverage of 50×. It comprises 6385 protein-coding genes and 69 tRNA genes. The genome assemblies of *P. strydomiana* (SI01, SI02, SI04, SI07, SI08, and SI09) had 3 contigs, except for

Table 14. Genomic features of isolated PSB strains

Genome	SI01	SI02	SI04	SI05	SI06	SI07	SI08	SI09
Coverage	35×	35×	118×	75×	50×	72×	52×	74×
N50 assembly	3,953,818	4,325,818	4,325,843	3,551,557	4,255,561	3,974,054	3,953,781	3,953,764
Total length (bp)	7,790,703	7,767,803	7,765,181	8,076,343	7,242,740	7,849,785	7,790,637	7,790,621
Contig #	3	3	3	4	3	9*	3	3
Contig length (bp)	3,953,818	4,325,818	4,325,843	3,551,557	4,255,561	3,974,054	3,953,781	3,953,764
	3,305,972	3,195,775	3,194,468	3,014,549	2,861,885	2,962,017	3,305,954	3,305,952
	530,913	246,210	244,870	1,268,256	125,294	454,603	530,902	530,905
				241,981		330,751		
						104,768		
						7,966		
						7,138		
						5,457		
						3,031		
GC	61.7%	61.8%	61.8%	66.3%	62.8%	61.8%	61.7%	61.7%
Protein coding (CD)	7161	6929	6915	7130	6385	7226	7057	7065
rRNA loci	12	14	14	18	12	12	12	12
tRNA genes	65	65	65	81	69	68	66	66

* 9 contigs can be scaffolded to 3 when aligned with SI04

SI07, which had 9 contigs. The total size ranged between 7.2 and 7.791 Mbp, the N50 ranged from 3.9 to 4.33 Mbp, the GC content ranged from 58.2% to 61.8%, and the coverage ranged from 35× to 118×. The protein-coding and tRNA genes ranged between 6915 – 7226 and 65-68, respectively.

4.4.4 Genomic features of PSB strains

The assembled genomes were used to calculate each isolate's pair-wise ANI and dDDH values and its closest known relatives using TYGS. The nearest related species for each query is given in Table 15. The ANI value of each strain was greater than the 95% threshold value compared with the type strain (Table 15). Consistent with this, the dDDH values were above 70% threshold compared to the type strain. The reference genome sequences of the three type strains were obtained from NCBI and used for an all-against-all ANI analysis. The ANI visualization revealed the presence of subgroups among *P. strydomiana* strains (Figure 24A). Phylogenetic analysis was done to determine the closely related species for the PSB strains using a Genome Blast Distance Phylogeny approach, and the resultant phylogenetic tree corroborated the ANI analysis (Figure 24B). It is clear from the data that although SI01, SI02, SI04, SI07, SI08, and SI09 were all identified as being *P. strydomiana*, these appear to represent three distinct strains (Figure 24).

Except for SI07, all the *P. strydomiana* strains assembled into three contigs (Figure 25). We note if the other assemblies were used as a scaffold, SI07 could also be assembled into three contigs. The length of the largest replicon (Contig 1) was between 3.8 - 4.3 Mb. The second largest replicon (Contig 2) varied from 3.2 - 3.3 Mb. The final replicon (Contig 3) showed the most variability with strains SI02 and SI04 having a replicon of approximately 0.240 Mb, whereas SI01, SI08, and SI09 had a replicon of approximately 0.5 Mb. Mauve alignments clearly

Table 15. Genome comparison with type strains

Query strain	Nearest related species	Percent G+C	ANI	dDDH (d0, in %)
SI01	*Paraburkholderia strydomiana* WK1.1fT	61.7	97.7	71.3
SI02	*Paraburkholderia strydomiana* WK1.1fT	61.8	97.8	72.7
SI04	*Paraburkholderia strydomiana* WK1.1fT	61.8	97.7	72.7
SI05	*Burkholderia ambifaria* AMMD	66.3	97.6	85.6
SI06	*Paraburkholderia graminis* C4D1M	62.8	98.4	82.6
SI07	*Paraburkholderia strydomiana* WK1.1fT	61.8	97.6	70
SI08	*Paraburkholderia strydomiana* WK1.1fT	61.7	97.7	71.3
SI09	*Paraburkholderia strydomiana* WK1.1fT	61.7	97.7	71.3

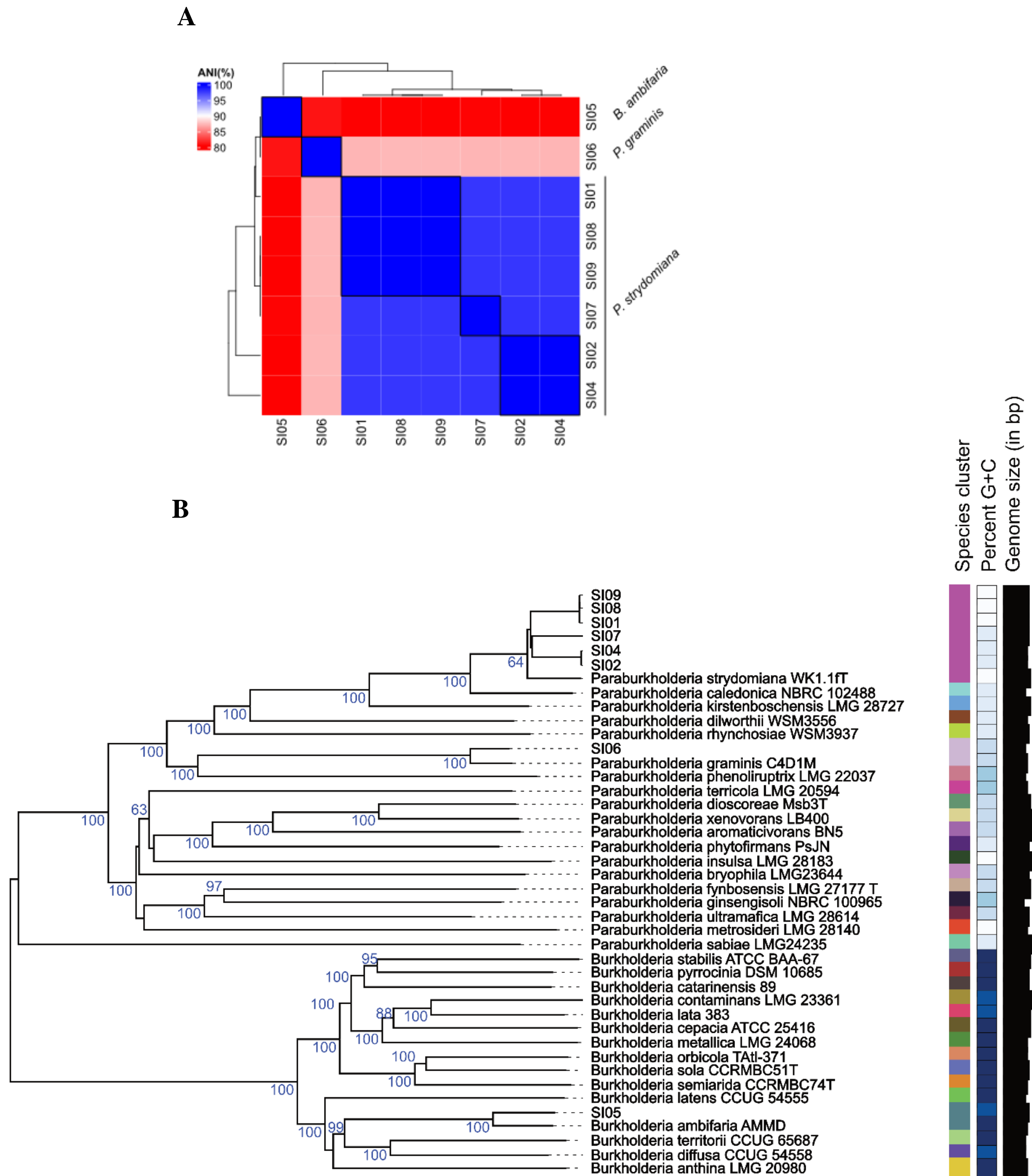
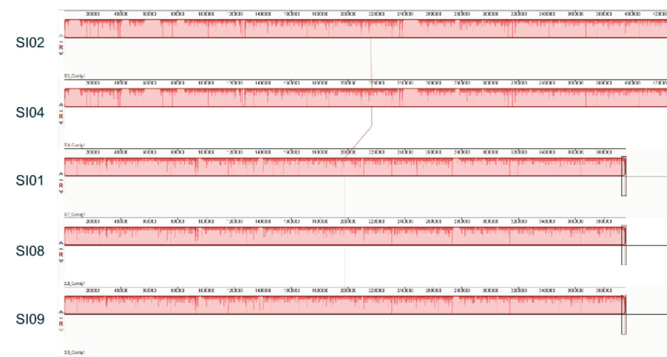


Figure 24. Identification of PSB strains based on phylogenomic analysis.

A. Heatmap of ANI percentage identities for the PSB strains. Cells corresponding to >95% ANI were shown in dark blue. B. Phylogenetic tree constructed using Genome BLAST Distance Phylogeny approach (GBDP) under the algorithm 'coverage' and distance formula d5. The Adjoining color bars represent species cluster, percent G+C, and genome size.



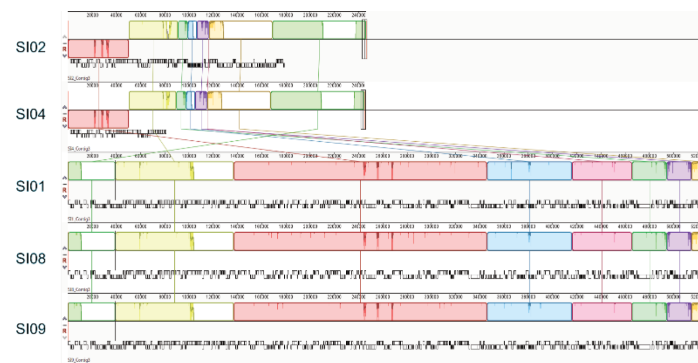


Figure 25. Mauve alignment of the *P. strydomiana* strains

show high concordance between strains for Contigs 1 and 2, whereas there is a greater degree of variability between strains in Contig 3.

Although many PGP bacteria have been reported (Abou Jaoudé et al., 2024; Khoso et al., 2024), very little is known about the molecular basis for their ability to promote plant growth. Several genes are correlated with plant growth promotion (Bruto et al., 2014). These include pyrroloquinoline quinone encoding genes (*pqq*), which work in concert with periplasmic dehydrogenases facilitating redox reactions (Rawat et al., 2021); the presence of *nifHDK,* which encodes the structural nitrogenase components (Koirala & Brözel, 2021); *acdS,* which encodes 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase which degrades ACC which is an ethylene precursor (Bouffaud et al., 2018); the genes *hcnABC* which encode enzymes that produce hydrogen cyanide which has been postulated to function as a biocontrol factor (Da Cruz Nizer et al., 2023); *phlABCD* which encode genes necessary for the biosynthesis of 2, 4-diacetylphloroglucinol which is implicated in playing a role in disease suppressive soils (Almario et al., 2017); as well as acetoin and 2, 3-butandiol synthesis, which is encoded by *budABC* and has been shown to affect stomatal activity (Biswas et al., 2012). To determine if any of these might be present, the genomes of each of the strains were queried for their presence. The results show that all the strains contained *pqqBCDE*, *hcnABC*, and *acdS*. The strains appeared to contain two genes necessary for the synthesis of acetoin/2,3-butanediol but were missing the initial step of the pathway. The strains did not contain genes indicative of nitrogenase, or 2,4-diacetylphlorogucinol (Table 16).

Table 16. Presence of genes associated with plant growth promotion

Gene function	Gene	SI01	SI02	SI04	SI05	SI06	SI07	SI08	SI09
Phosphate solubilization	*pqqB*								
	pqqC								
	pqqD								
	pqqE								
	pqqF								
	pqqG								
2,4-Diacetylphloroglucinol synthesis	*phlA*								
	phlB								
	phlC								
	phlD								
Hydrogencyanide synthesis	*hcnA*								
	hcnB								
	hcnC								
Acetoine/2,3-butanediol synthesis	*budA*								
	budB								
	budC								
Nitric oxide synthesis	*nirK*								
Auxin synthesis	*ipdC*								
ACC deamination	*acdS*								
Nitrogen fixation	*nifD*								
	nifH								
	nifK								

Presence (green) or absence (tan) of common PGPR genes. To identify genes within the sequenced genomes, a known protein closely related to the strains was found in Uniprot and used to do BLASTP queries of each genome. The presence or absence was based on comparing the best BLASTP hit using the following cut-offs: % Identity, >26%; % coverage, >77%; and Expect value, > e^{-8}. The Uniprot queries that were used are as follows*: pqqB, B. cenocepacia* YG-3, A0A3Q9F9H9; *pqqC, B. cenocepacia* ATCC BAA-245, B4EHL3; *pqqD, B. cepacia strain* GG4, A0A9W3PBQ3; *pqqE, B. cenocepacia*, A0A8I1B1M; *pqqF, P. putida* ATCC 47054, Q88QV3; *pqqG, B. plantarii*, A0A0B6S5K6; *phlA, Pseudomonas sp.* CM1A2, A4GS14; *phlB, Pseudomonas sp.* CM1A2, A4GS28; *phlC, Pseudomonas sp.* CM1A2, A4GS41; *phlD, P. fluorescens*, ATCC BAA-477, Q4K418; *hcnA, P. aeruginosa* ATCC 15692, G3XD67; *hcnB, P. aeruginosa* ATCC 15692, Q9I1S2; *hcnC, P. aeruginosa* ATCC *15692*, G3XD12; *budA, Klebsiella pneumoniae*, A0A0J4RJK0; *budB, K.pneumoniae*, P27696; *budC, Paraburkholderia graminis*, B1G329; *nirK, Sinorhizobium meliloti* 1021, Q92Z29; *ipdC, Enterobacter cloacae*, P23234; *acdS, P. fluorescens*, Q51813; *nifD, P. xenovorans* LB400, Q13N41; *nifH, P. xenovorans*, A6Y965; and *nifK, P. xenovorans* LB400, Q13N42.

4.5 Discussion

In this study, phosphate solubilizing strains were isolated from agricultural fields in Manitoba and tested for their PGP traits. The isolated strains belonged to three different gram-negative Betaproteobacteria species: *P. graminis*, *B. ambifaria,* and *P. strydomiana. P. graminis* is a common rhizobacterium initially isolated from the rhizosphere of corn and wheat. Its plant growth promotion mechanism is unknown (Ndlovu et al., 2013). It has been reported to be present in the root nodules of *Acacia pycnantha* without any nodulation capacity or nitrogen fixation ability (De Meyer et al., 2018). Onofre-Lemus et al., (2009) reported that *P. graminis* exhibited ACC (1-aminocyclopropane-1-carboxylate) deaminase activity. Strains *of B. ambifaria* have been isolated as plant growth-promoting bacteria with a wide range of phenotypes that include it being capable of endophytic growth (An et al., 2022), biocontrol of *Fusarium* (An et al. 2022), promoting plant growth by increasing nitrogen uptake (Parra-Cota et al., 2014), or by altering the plant microflora (Ciccillo et al., 2002). Whereas *P. strydomiana* has been more recently isolated from South African legumes, where it was shown to be able to form nitrogen-fixing root nodules (Beukes et al., 2019).

The direct oxidation of aldo-sugar is the predominant phosphate solubilization mechanism in many gram-negative bacteria (Alaylar et al., 2019; Rawat et al., 2021; Wan et al., 2020; Wang et al., 2022). This oxidation is mediated by a membrane-bound glucose dehydrogenase that requires pyrroloquinoline quinone (PQQ) as a cofactor. PQQ is a small active redox cofactor encoded by the *pqq* operon, consisting of the core genes *pqqABCDEF* responsible for dehydrogenase activity and phosphate solubilization. Although the *pqq* operon can be made up of 6 genes, it has been shown that *pqqA*, *pqqC*, *pqqD* and *pqqE* are essential for phosphate solubilizing activity (Shen et al., 2012). All the strains analyzed contained *pqqBCDE*

within a single operon but not *pqqF* or *pqqA*. The gene *pqqA* encodes a small peptide of approximately twenty amino acids and is often missed or misannotated within genome assemblies, so it was not systematically analyzed. We note that the *Burkholderia ambifaria* (SI05), the *pqqA* gene, is found in an operon with the rest of the *pqq* genes. Since PqqA is essential for function, we assume the other strains carry this component. However, although BLASTP analysis using PqqA from *Pseudomonas florescens* could detect *pqqA*-like genes, they were often not within a genetic context to suggest they were bonafide hits and would need functional characterization to confirm or refute these candidates.

The germination assays indicated that isolated PSB strains can improve the seedling vigor index on tested crops by increasing the germination rate compared to shoot and root length. The inoculation of *P. graminis* promoted the growth of broccoli (Jeon et al., 2021) and improved tomato seedling regrowth during chilling stress (Caradonia et al., 2019). Several studies have reported that *B. ambifaria* enhances plant growth in a variety of crops, including wheat (An et al., 2022), maize (Batista et al., 2018; Ciccillo et al., 2002), *Anoectochilus roxburghii* (Wang et al., 2022), *Amaranthus cruentus,* and *A. hypochondriacus* (Parra-Cota et al., 2014) and soybean (Batista et al., 2018). In contrast, both *P. graminis* and *B. ambifaria* exhibited significantly lower growth in soybeans in our study.

The previous isolation of *P. strydomiana* strains (*Paraburkholderia strydomiana* WK1.1fT) was from the nodules of the *Hypocalyptus sophoroides*. *P. strydomiana* strains' nodulation ability has been proven on either cowpea (*Vigna unguiculata*) or siratro (*Macroptilium atropurpureum*) (Beukes et al., 2019). *P. strydomiana* is closely related to other nodulating species, such as *P. kirstenboschensis, P. dilworthii,* and *P. rhynchosiae*. Our *P. strydomiana* strains were isolated as free-living phosphate-solubilizing bacteria. The previous

genome sequence for this species was deposited as a draft genome consisting of 259 contigs with an estimated genome size of 8,397,958 bp (Beukes et al., 2019). Our data show that we have isolated three distinct strains with a genome consisting of 3 replicons with genome sizes between 7,767,803 and 7,849,785 bp (Table 14). We found that if we used contigs from the draft genome of the type strain WK1.1f and used our *P. strydomiana* genomes as scaffolds, we could reduce the genome of the type strain to three replicons. Notably, whereas the original *P. strydomiana* strains could interact symbiotically, our strains did not have genes associated with nitrogen fixation.

P. strydomiana is a relatively newly described species only described as a symbiotic organism. This study identifies three distinct bacterial species isolated for their phosphate-solubilizing ability, which were further demonstrated to promote plant growth during the initial stages of development. Coupled with full genome sequencing, this will allow for a more in-depth biological investigation of these organisms to elucidate mechanisms of plant growth promotion.

Chapter 5: Conclusions

5.1 Conclusions from the current work

This thesis aimed to characterize soil bacterial communities in experiments with different soybean crop rotations practices. In Chapter 2, we used a culture-independent method, 16S rRNA gene-based community analysis, to explore the diversity of soil bacterial communities associated with various soybean rotation practices in Manitoba. We created a culture library of soil bacteria present in two Manitoba agricultural soils. Chapter 4 describes the impact of nine PSB strains isolated from Manitoba soils on nutrient availability and plant growth promotion. Overall, this study investigated the combined effects of crop rotation, soil type, and growing season on soil bacterial communities by analyzing shifts in microbial diversity and composition. Additionally, we established an archive of bacteria from Manitoba soils cultured under normal conditions and employed targeted techniques to isolate and enhance phosphate-solubilizing bacteria. These findings provide valuable insights into the influence of agricultural practices on microbial dynamics.

In Chapter 2, we have shown that the diversity of soil bacterial communities is primarily influenced by soil type, followed by growing season, and then crop rotation treatments. This is unsurprising, as soil is a complex environment with diverse microhabitats. Even though these habitats are only micrometers to millimeters apart, soil properties can vary widely (Fierer, 2017). This variation is caused by the soil formation process, which is primarily influenced by climate, organisms, topography, parent material, and time. Further, within a soil profile, environmental conditions can vary widely, such as rhizosphere, water flow paths, and animal burrows that lead to distinct microbial communities (Fierer, 2017). We collected and composited nine cores from each plot to minimize this effect, repeating the sampling across five consecutive seasons. Thus, the heterogeneous nature of soil exerts a significant influence on microbial dynamics.

Among soil properties, soil pH is a critical driver affecting the structure and diversity of the soil microbiome (Philippot et al., 2023). We observed distinct bacterial communities between the Carman and Kelburn sites, with soil pH ranging from 4.8 to 5.3 in Carman and 6.7 to 7.1 in Kelburn. Acidobacteria generally show distinct responses to soil pH, and their composition varies accordingly. Liu et al., (2016) reported that soil pH influences OTU richness, phylogenetic diversity, and composition of Acidobacteria populations, with GP1 dominating in acidic pH and GP6 in neutral pH. Consistent with this, in Carman, *Acidobacteria_GP1_unclassified* was one of the dominant genera, while *GP6_unclassified* was dominant in Kelburn and we also noticed higher bacterial diversity in Kelburn than in Carman.

Soil texture is the second most important factor influencing the soil microbial communities, and its effect is highly taxon-dependent (Xia et al., 2020). For example, members of Actinobacteria and Chloroflexi were positively correlated with finer textures in Bermudagrass ecosystems, while Acidobacteria and Betaproteobacteria were positively associated with sand content (Xia et al., 2020). Similarly, we observed a higher relative abundance of Acidobacteria and Betaproteobacteria, along with a lower abundance of Actinobacteria in Carman compared to Kelburn (Figure 10A & B). Carman soils have a sandy clay texture, while Kelburn soils have a clay texture. Soil texture influences the soil's ability to retain water and organic matter, which are critical factors shaping the microclimate experienced by soil microbes.

Seasonal changes, often linked to fluctuations in climate and plant growth, can cause variations in soil properties, thereby influencing shifts in microbial diversity (Shen et al., 2021). Moisture and temperature are two important factors that affect various environmental processes, such as nutrient availability, the diffusion of solvents and gases, soil pH, the migration of microorganisms, and the mineralization rate, leading to changes in microbial dynamics. Soil

microbial communities are dynamic, with taxa abundances changing rapidly, yet highly resilient, enduring perturbations over time (Tecon & Or, 2017). Thus, environmental fluctuations may lead to a taxonomic legacy in the soil microbiome composition (Meisner et al., 2021). Consistent with previous findings, we observed that the composition of bacterial communities remained stable between growing seasons, despite temporary changes occurring within each season.

The distinct genus compositions observed between sites at the R8 stage suggest that plants selectively shape their surrounding microbiomes. Plants recruit taxa that do not necessarily need to be the same but can perform similar functions to support plant growth (Lemanceau et al., 2017b). Soil microbial communities are diverse but functionally similar due to redundancy (Chen et al., 2021). Many taxonomically distinct microorganisms can encode the same energy-yielding metabolic functions. Taxa encoding these functions can vary widely across space and time, likely due to ecological drift among equivalent organisms (Louca et al., 2018). While microbial taxonomic compositions vary significantly across climate zones, functional potentials remain stable (Chen et al., 2022b). We also observed that the genera composition remained constant at each field experiment site, both within and between growing seasons, despite changes in the relative abundance. Climate and soil properties influence taxonomic compositions more than functional potentials (Chen et al., 2022b). Furthermore, OTUs with more shared functions exhibited stronger positive correlations, underscoring the importance of metabolic niche effects (Chen et al., 2022b).

The results of core microbiome analysis also emphasize the role of plants in recruiting and shaping their surrounding microbial communities. Additionally, the network analysis indicated that most core OTUs are interconnected within the microbial network, exhibiting positive or negative correlations between different genera. Understanding these interactions

within the core microbiome can ultimately inform the design of more effective synthetic communities, enhancing their stability and functionality in various ecological contexts. Furthermore, insights into the recruitment and network interactions of core OTUs would provide a blueprint for constructing SynComs that effectively mimic natural systems. This knowledge can be used to enhance plant growth, health, and resilience by engineering microbial communities that foster beneficial interactions and suppress harmful ones. Integrating knowledge gained from natural microbiomes into synthetic biology holds promise for advancing agricultural practices and developing sustainable solutions for crop management.

While SynComs show significant potential for enhancing agricultural productivity and sustainability, several challenges must be addressed to realize their practical feasibility. Overcoming technical, regulatory, and adoption barriers will require interdisciplinary research, collaboration between scientists, industry, and policymakers, and long-term field trials. SynComs could become a valuable tool for modern, sustainable agriculture with the right strategies.

Our study utilized 16S rRNA gene sequenced-based microbial community analysis to investigate changes in soil bacterial diversity within a soybean crop rotation system. While 16S rRNA gene sequencing provides limited insights into the functional roles of soil bacteria, it remains a valuable tool for assessing overall bacterial diversity and its variations across different conditions, such as weather, soil types, and disease status. Its cost-effectiveness, with expenses per sample ranging from $50 to $150 compared to $1,000 to $3,000 for metagenomic and metatranscriptomic analyses, makes it a practical choice for large-scale studies. The ability of 16S marker gene surveys to identify common patterns and trends is beneficial for guiding more targeted and functional analyses, ultimately optimizing research expenditures and enhancing the understanding of soil microbial dynamics in agricultural systems.

Even though omics technologies, including metagenomics, transcriptomics, and proteomics, provide extensive data on microbial communities, we are still left with missing pieces, as many bacteria often remain unclassified. This is primarily due to the incompleteness of current reference databases, which may need more sequences for many novel or less-studied microorganisms. Omics studies can uncover novel or rare bacterial taxa that are not well-represented in existing databases. For example, the current genomic databases mainly focus on human health and biotechnology research, which do not accurately represent soil microbiomes. As a result, many bacterial sequences identified in omics studies cannot be accurately classified, leading to gaps in our understanding. These unclassified bacteria may possess unique or previously unknown biological functions, making their identification crucial for understanding ecosystem dynamics and microbial interactions.

Additionally, the high complexity of microbial genomes and the variability in gene functions contribute to the difficulty of classifying these bacteria. Omics data often include genes with unknown functions or novel gene combinations that do not match known references. The high-throughput nature of omics technologies also means that even minor errors in sequencing or computational analysis can lead to significant issues in classification. Therefore, addressing these missing puzzle pieces requires improvements in reference databases, enhanced computational tools, and better methods for culturing and characterizing microorganisms. Collaborative efforts to expand genomic resources and refine classification algorithms are essential for filling these gaps and achieving a more complete understanding of microbial ecosystems.

In the third chapter, a bacterial culture library was preserved from local Manitoba soils. Cultivating and analyzing microbial communities offers essential insights into their structure and dynamics, emphasizing the importance of culture-based methods in environmental microbiology.

This study enhances our understanding of microbial composition and dynamics within the ecosystem studied.

The 16S rRNA gene sequencing identified a wide range of phyla, including Proteobacteria, Actinobacteria, Acidobacteria, and a substantial proportion of unclassified bacteria, along with Bacteroidetes, Firmicutes, Planctomycetes, Verrucomicrobia, and Gemmatimonadetes. In contrast, culturing methods yielded only four phyla: Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes, likely due to the predominance of fast-growing bacteria in these groups. These findings emphasize the need for improved culturing techniques, as traditional methods often fail to capture the full spectrum of microbial communities.

Improved culturing techniques are essential for advancing our understanding of microbial diversity and function, as microbiome databases rely on well-annotated genomes. By diversifying culture media, extending incubation periods, and optimizing growth conditions, we can isolate a broader range of microbes, thereby enhancing our knowledge of their ecological roles and interactions. Additionally, improved culturing facilitates the study of microbial physiology, genetics, and potential applications in biotechnology, agriculture, and medicine. Ultimately, refining culturing techniques bridges the gap between culture-dependent and culture-independent methods, providing a more comprehensive view of microbial ecosystems.

The genus composition obtained from sequencing revealed significant variability among sites, with distinct dominant genera in Carman and Kelburn. Conversely, culturing identified *Bacillus, Pseudomonas*, and *Streptomyces* as key genera in both locations. This comparison indicates a notable gap between the bacterial communities identified through culture-independent and culture-dependent methods, emphasizing the need for integrated approaches to understand microbial ecosystems fully.

Comparisons of the core microbiome using sequencing and culturing methods show that over half of the genera were consistent across different sites within each technique. However, only two of the 46 core genera identified through sequencing were also found in the core genera obtained via culturing. This discrepancy suggests the need for targeted culturing, focusing on the missing species, to develop a more comprehensive culture collection.

The consistency of the core microbiome across sites, which was revealed through sequencing and culturing methods, highlights its potential in SynCom formulation. Incorporating core microbes could improve crop productivity and soil health by promoting nutrient uptake and disease suppression, thereby reducing reliance on chemical inputs. Adapting a SynCom with core microbes allows customized solutions to specific environmental challenges, ensuring optimized performance and predictability (Toju et al., 2020). The core microbiome approach could reduce the uncertainty and risks associated with introducing a SynCom into a natural environment, thereby increasing the likelihood of its sustainability. Moreover, understanding the roles and interactions of core microbes can drive fundamental research and innovation in microbial ecology, synthetic biology, and biotechnology, leading to novel applications and technologies.

Chapter 4 revealed that the isolated PSB strains can potentially increase phosphorus solubilization and promote plant growth. Since the isolated *P. strydomiana* strains can solubilize insoluble phosphorus and promote soybean growth, they open a new direction for soybean cultivation. These strains could be co-inoculated with *B. japonicum*, and this combination would provide essential nutrients for plants, creating a mutually beneficial scenario. *B. japonicum* enhances nitrogen fixation by converting atmospheric nitrogen into a form for plant use, while *P. strydomiana* solubilizes insoluble phosphates, increasing phosphorus availability. The synergy between these bacteria can improve plant nutrition, supporting robust growth and higher crop

yields. By supplying essential nutrients, co-inoculation reduces the need for chemical fertilizers, thereby lowering agricultural costs and minimizing environmental impact.

Although co-inoculation of *B. japonicum* and *P. strydomiana* could offer promising benefits for soybean cultivation, careful study of their compatibility and efficiency, consideration of environmental conditions, and economic viability is required to ensure widespread adoption and success. Variability in field conditions, such as soil pH and moisture, can also affect the efficacy of these microbes, leading to inconsistent results. Economic considerations are crucial, as the benefits of increased yields and reduced fertilizer costs must outweigh the expenses associated with microbial inoculants for farmers to adopt this practice widely. Therefore, the cost of producing, applying, and maintaining microbial inoculants must be considered. Furthermore, regulatory and safety concerns regarding introducing non-native microbial species must be addressed. By tailoring co-inoculation strategies, maximizing their benefits across diverse agricultural systems is possible.

5.2 Future work

Exploring bacterial diversity under different crops

In the first chapter, we demonstrated how crop rotation treatments influence the diversity and composition of bacterial communities. However, the effects of crop type and physiological age on soil bacterial communities remain underexplored. Understanding microbial diversity across different crops is essential for formulating effective SynComs, as each crop recruits distinct microbial communities to adapt to its specific ecological niche. This knowledge allows for the targeted selection of microbial strains in SynCom formulations, ensuring that these communities perform desired functions specific to each

crop. Additionally, understanding this diversity helps to predict how SynComs will interact with native microbial communities in the field, emphasizing the need for SynComs to complement rather than disrupt existing beneficial relationships.

The current study aimed to address these gaps by 1) examining bacterial diversity, community composition, and species interactions in soils associated with soybean, canola, and corn; 2) investigating how plant growth stages affect bacterial communities in these soils; and 3) identifying critical ecological drivers of bacterial diversity and community structure. By surveying bacterial communities under multiple crops and physiological conditions, this research will provide detailed comparisons of how bacterial populations change with growth stages. The findings will guide the selection of bacteria for future SynCom studies, identifying those consistently associated with different crops and contributing to beneficial plant-microbe interactions.

Exploring microbial dynamics in crop rotation systems

Future research should focus on several key areas to enhance understanding of microbial dynamics in crop rotations. First, studies should explore microbial functional traits and enzyme activities, assessing abilities like nitrogen fixation and organic matter degradation to better understand soil functions. Second, longer-term studies could reveal more significant effects on microbial communities over time. In microbial ecology, long-term effects often refer to changes that occur over decades or multiple crop cycles, where deeper ecological shifts in soil structure, microbial networks, and ecosystem functions are more pronounced and persistent. Incorporating additional soil health metrics, such as enzyme activities, organic carbon content, and microbial respiration rates, will provide a comprehensive view of soil

health. Lastly, investigating more complex crop rotations or cover crops may stimulate beneficial microbial species better than simpler systems.

Assessing agronomic impacts on soil microbial communities and resilience

Longitudinal studies that assess microbial diversity, composition, and functionality across different agronomic practices, such as crop rotation, cover cropping, and varying fertilization regimes, are essential to understanding their impact on soil microbial communities. Incorporating advanced molecular techniques, including metagenomics and metabolomics, is necessary to comprehend microbial responses to these practices and their roles in nutrient cycling and soil health. Exploring interactions between microbial communities and plant roots under different management strategies is important to elucidate mechanisms that enhance plant resilience to stressors like drought, disease, and salinity. Experimental designs should be integrated with field trials and controlled environments to validate findings. Finally, modeling approaches incorporating microbial dynamics into agronomic decision-making frameworks could provide insights into sustainable practices that optimize soil health and agricultural productivity.

Deciphering the functional potential of bacterial communities

This study used 16S rRNA gene analysis to examine the bacterial communities under different crop rotation treatments. This technique allowed us to understand the overall bacterial diversity, enabling the identification of specific taxa within complex communities. However, the long-term objective is to incorporate this knowledge to develop SynComs with desired functionalities. Future research should focus on enhancing the predictive power of

16S rRNA gene sequence data to inform the design of SynComs with specific ecological or functional goals, such as improving soil fertility or enhancing plant growth.

To achieve this, it is essential to integrate the 16S rRNA data with metagenomic, metatranscriptomic, and metabolomic analyses. This multi-omics approach can provide a more comprehensive view of the functional roles of microbial taxa identified through 16S rRNA gene sequencing. By correlating taxonomic data with functional traits, key microbial taxa and their interactions can be identified, facilitating the selection of compatible strains for SynCom formulation. Given the vast data generated by omics approaches, advancements in bioinformatics tools are crucial for accurately predicting microbial functions. Developing algorithms that connect specific taxa with their metabolic capabilities will improve our ability to design effective SynComs for targeted environments. While advancing database integration by incorporating multi-omics approach is crucial, it is equally important to address the biological complexity of microbial interactions and their functional outcomes in real-world conditions. Progressive optimization of SynComs in diverse environmental settings will be essential to address biological complexities and enhance their functional effectiveness.

The need for advanced bioinformatics and artificial intelligence (AI) tools

Developing advanced bioinformatics and AI tools is crucial to managing the complexity and volume of data generated by omics technologies. Omics studies produce vast amounts of data across genomics, transcriptomics, proteomics, and metabolomics, requiring sophisticated computational methods for effective management, integration, and analysis. Traditional bioinformatics tools often struggle with these large and complex datasets, creating data processing and interpretation bottlenecks. Therefore, investing in the advancement of

bioinformatics and AI tools is critical to unlocking the full potential of modern biological research and applications.

Furthermore, developing user-friendly and accessible bioinformatics and AI tools is essential for streamlining data analysis. AI tools can provide advanced techniques like pattern recognition, predictive modeling, and machine learning. For instance, AI can aid in predicting protein structures, understanding microbial community dynamics, and designing personalized medicine approaches. These tools facilitate multi-omics data integration, enabling a holistic view of biological systems and their functions.

Bridging Omics approaches and culturing

The integration of multi-omics data with culturing methods is essential for the development of SynComs. This combines predictive insights with experimental validation to create more accurate and effective microbial community designs. While multi-omics approaches can predict microbial taxa's potential functions and interactions, culturing provides a way to validate these predictions in a controlled environment. Culturing can provide real-world examples, confirm the functional roles of identified microbes, assess their growth patterns, and evaluate their performance, which omics data alone cannot fully capture.

A synergistic strategy is needed to bridge these two approaches effectively. For example, multi-omics data can guide the selection of microbial strains for culturing by identifying those with desirable traits or interactions. Conversely, insights from culturing experiments can refine omics predictions, improving the accuracy of functional annotations and interaction models. This integration ensures that SynComs are theoretically optimized and experimentally validated, leading to more effective and reliable microbial community designs.

Building SynComs: Advancing agriculture through microbial collections

Building a microbial culture collection is a critical step in developing SynComs with predictable plant traits. Next steps include high-throughput screening for beneficial traits like nutrient solubilization and phytohormone production, followed by functional genomics and metabolomics to understand underlying mechanisms. Promising strains can be assembled into SynComs with complementary functions and tested in greenhouse and field trials to evaluate their impact on plant growth and soil health. Performance data will guide repetitive assessments of SynCom composition to optimize outcomes across diverse environments. This pipeline transforms the culture collection into a resource for advancing sustainable agriculture and microbial ecology.

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Appendices

Appendix A

Comparison of Alpha diversity between treatment at R8 stage

Treatments [t]	2017	2018	2019	2020	2021
CS	5.978[a]	5.666 [a]	6.078 [a]	6.198 [a]	5.907 [a]
SCa	5.801[a]	5.867 [a]	5.912 [a]	6.134 [a]	6.072 [a]
SCo	5.797[a]	5.815 [a]	5.852 [a]	6.233 [a]	5.937 [a]
SWCC	5.802[a]	5.911 [a]	6.003 [a]	6.164 [a]	5.945[a]

Comparison of Alpha diversity between treatment at BP stage

Treatments [t]	2017	2018	2019	2020	2021
CS	5.910[a]	5.769 [a]	5.930 [a]	5.985 [a]	6.033 [a]
SCa	5.555 [b]	5.600 [ab]	5.714 [a]	6.024 [a]	5.936 [a]
SCo	5.666 [ab]	5.679 [ab]	5.579 [a]	5.947 [a]	6.004 [a]
SWCC	5.658 [ab]	5.342 [b]	5.590 [a]	5.694 [a]	5.995 [a]

[t] The rotation treatments include Continuous Soybean (CS), Soybean-Canola (SCa), Soybean-Corn (SCo), and Soybean-Wheat-Canola-Corn (SWCC). Boxplots with the same letters indicate no significant differences, as determined by the Tukey HSD test at $p = 0.05$ following a one-way ANOVA.

Appendix B

R2A MEDIUM

Yeast extract	0.50 g
Proteose Peptone	0.50 g
Casamino acids	0.50 g
Glucose	0.50 g
Soluble starch	0.50 g
Na-pyruvate	0.30 g
K_2HPO_4	0.30 g
$MgSO_4 \times 7\ H_2O$	0.05 g
Agar	15.00 g
Distilled water	1000.00 ml
pH	7.2

RHIZOBIUM MEDIUM

Yeast extract	1.0 g
Mannitol	10.0 g
Agar	15.0 g
Soil extract:	200.0 ml
Air-dried garden soil	80.0 g
Na_2CO_3	0.2 g
Distilled water	200.0 ml
Distilled water	800.0 ml
pH	7.2

GYM STREPTOMYCES MEDIUM

Glucose	4.0 g
Yeast extract	4.0 g
Malt extract	10.0 g
$CaCO_3$	2.0 g
Agar	12.0 g
Distilled water	1000.0 ml
pH	7.2

Appendix C

Associated metadata of the culture library

No	Isolate name	Extraction method	Site	Crop	Treatment	Time point	Year	Genus based on NCBI
1	SI01	SE	Carman	Corn	SCo	R1	2018	*Paraburkholderia*
2	SI02	SE	Carman	Soybean	CS	R1	2018	*Paraburkholderia*
3	SI03	SE	Carman	Soybean	CS	R1	2018	*Paraburkholderia*
4	SI04	SE	Carman	Soybean	CS	R1	2018	*Paraburkholderia*
5	SI05	SE	Carman	Soybean	CS	R1	2018	*Burkholderia*
6	SI06	SE	Carman	Canola	SCa	R1	2018	*Paraburkholderia*
7	SI07	SE	Carman	Soybean	CS	R1	2018	*Paraburkholderia*
8	SI08	SE	Carman	Wheat	SWCC	R1	2018	*Paraburkholderia*
9	SI09	SE	Carman	Corn	SCo	R1	2018	*Paraburkholderia*
10	SI10	SE	Carman	Soybean	CS	R1	2018	*Stenotrophomonas*
11	SI11	PE	Carman	Soybean	CS	R1	2018	*Stenotrophomonas*
12	SI12	PE	Carman	Soybean	CS	R1	2018	*Chryseobacterium*
13	SI13	PE	Carman	Soybean	CS	R1	2018	*Bacillus*
14	SI14	PE	Carman	Soybean	CS	R1	2018	*Chryseobacterium*
15	SI15	PE	Carman	Soybean	CS	R1	2018	*Pseudarthrobacter*
16	SI16	PE	Carman	Soybean	CS	R1	2018	*Arthrobacter*
17	SI17	PE	Carman	Soybean	CS	R1	2018	*Pseudomonas*
18	SI18	PE	Carman	Soybean	CS	R1	2018	*Stenotrophomonas*
19	SI19	PE	Carman	Soybean	CS	R1	2018	*Novosphingobium*
20	SI20	PE	Carman	Soybean	CS	R1	2018	*Pseudomonas*
21	SI21	PE	Carman	Soybean	CS	R1	2018	*Stenotrophomonas*
22	SI22	PE	Carman	Soybean	CS	R1	2018	*Pseudarthrobacter*
23	SI23	PE	Carman	Soybean	CS	R1	2018	*Pseudomonas*
24	SI24	PE	Carman	Soybean	CS	R1	2018	*Brevundimonas*
25	SI25	PE	Carman	Soybean	CS	R1	2018	*Pseudoroseomonas*
26	SI26I	PE	Carman	Soybean	CS	R1	2018	*Stenotrophomonas*
27	SI26 II	PE	Carman	Soybean	CS	R1	2018	*Pseudarthrobacter*
28	SI27	PE	Carman	Soybean	CS	R1	2018	*Pseudomonas*
29	SI28	PE	Carman	Soybean	CS	R1	2018	*Bacillus*
30	SI29	PE	Carman	Soybean	CS	R1	2018	*Pseudarthrobacter*
31	SI30	PE	Carman	Didn't grow				
32	SI31	PE	Carman	Didn't grow				
33	SI32	SE	Carman	Corn	SWCC	BP	2020	*Paenibacillus*
34	SI33	SE	Carman	Corn	SWCC	BP	2020	*Bacillus*
35	SI34	SE	Carman	Corn	SWCC	BP	2020	*Neobacillus*

No	Isolate name	Extraction Method	Site	Crop	Treatment	Time point	Year	Genus based on NCBI
36	SI35	SE	Carman	Corn	SWCC	BP	2020	*Not yet sequenced*
37	SI36	SE	Carman	Corn	SWCC	BP	2020	*Not yet sequenced*
38	SI37	SE	Carman	Didn't grow				
39	SI38	SE	Carman	Corn	SWCC	BP	2020	*Mucilaginibacter*
40	SI39	SE	Carman	Corn	SWCC	BP	2020	*Paenibacillus*
41	SI40	SE	Carman	Corn	SWCC	BP	2020	*Clavibacter*
42	SI41	SE	Carman	Corn	SWCC	BP	2020	*Not yet sequenced*
43	SI42	SE	Carman	Corn	SCo	BP	2020	*Bacillus*
44	SI43	SE	Carman	Corn	SCo	BP	2020	*Pseudomonas*
45	SI44	SE	Carman	Corn	SCo	BP	2020	*Stenotrophomonas*
46	SI45	SE	Carman	Corn	SCo	BP	2020	*Janthinobacterium*
47	SI46	SE	Carman	Corn	SWCC	BP	2020	*Rothia*
48	SI47	SE	Carman	Corn	SWCC	BP	2020	*Rothia*
49	SI48	SE	Carman	Corn	SWCC	BP	2020	*Peribacillus*
50	SI49	SE	Carman	Corn	SCo	BP	2020	*Pseudomonas*
51	SI50	SE	Carman	Corn	SCo	BP	2020	*Priestia*
52	SI51	SE	Carman	Corn	SCo	BP	2020	*Pseudarthrobacter*
53	SI52	SE	Carman	Corn	SCo	BP	2020	*Rhodococcus*
54	SI53	SE	Carman	Corn	SCo	BP	2020	*Not yet sequenced*
55	SI54	SE	Carman	Corn	SCo	BP	2020	*Not yet sequenced*
56	SI55	SE	Carman	Corn	SCo	BP	2020	*Collimonas*
57	SI56	SE	Carman	Corn	SCo	BP	2020	*Not yet sequenced*
58	SI57	SE	Carman	Corn	SCo	BP	2020	*Not yet sequenced*
59	SI58	SE	Carman	Corn	SCo	BP	2020	*Not yet sequenced*
60	SI59	SE	Carman	Canola	SCa	BP	2020	*Flavobacterium*
61	SI60	SE	Carman	Canola	SCa	BP	2020	*Paenibacillus*
62	SI61	SE	Carman	Soybean	CS	BP	2020	*Bacillus*
63	SI62	SE	Carman	Soybean	CS	BP	2020	*Pseudomonas*
64	SI63	SE	Carman	Soybean	CS	BP	2020	*Bacillus*
65	SI64	SE	Carman	Soybean	CS	BP	2020	*Stenotrophomonas*
66	SI65	SE	Carman	Soybean	CS	BP	2020	*Chryseobacterium*
67	SI66	SE	Carman	Soybean	CS	BP	2020	*Not yet sequenced*
68	SI67	SE	Carman	Canola	SCa	BP	2020	*Streptomyces*
69	SI68	SE	Carman	Canola	SCa	BP	2020	*Metabacillus*
70	SI69	SE	Carman	Canola	SCa	BP	2020	*Pseudomonas*
71	SI70	SE	Carman	Soybean	CS	BP	2020	*Microbacterium*
72	SI71	SE	Carman	Soybean	CS	BP	2020	*Peribacillus*
73	SI72	SE	Carman	Soybean	CS	BP	2020	*Bacillus*
74	SI73	SE	Carman	Soybean	CS	BP	2020	*Bacillus*

No	Isolate name	Extraction Method	Site	Crop	Treatment	Time point	Year	Genus based on NCBI
75	SI74	SE	Carman	Soybean	CS	BP	2020	*Rhodococcus*
76	SI75	SE	Carman	Soybean	CS	BP	2020	*Peribacillus*
77	SI76	SE	Carman	Soybean	CS	BP	2020	*Not yet sequenced*
78	SI77	SE	Carman	Soybean	CS	BP	2020	*Peribacillus*
79	SI78	SE	Carman	Soybean	CS	BP	2020	*Bacillus*
80	SI79	SE	Carman	Soybean	CS	BP	2020	*Peribacillus*
81	SI80	SE	Carman	Soybean	CS	BP	2020	*Bacillus*
82	SI81	SE	Carman	Soybean	CS	BP	2020	*Bacillus*
83	SI82	SE	Kelburn	Canola	SCa	BP	2020	*Bacillus*
84	SI83	SE	Kelburn	Canola	SCa	BP	2020	*Bacillus*
85	SI84	SE	Kelburn	Canola	SCa	BP	2020	*Peribacillus*
86	SI85	SE	Kelburn	Canola	SCa	BP	2020	*Streptomyces*
87	SI86	SE	Kelburn	Canola	SCa	BP	2020	*Bacillus*
88	SI87	SE	Kelburn	Corn	SCo	BP	2020	*Bacillus*
89	SI88	SE	Kelburn	Corn	SCo	BP	2020	*Chryseobacterium*
90	SI89	SE	Kelburn	Corn	SCo	BP	2020	*Not yet sequenced*
91	SI90	SE	Kelburn	Corn	SCo	BP	2020	*Peribacillus*
92	SI91	SE	Kelburn	Corn	SCo	BP	2020	*Rossellomorea*
93	SI92	SE	Kelburn	Corn	SCo	BP	2020	*Peribacillus*
94	SI93	SE	Kelburn	Corn	SCo	BP	2020	*Bacillus*
95	SI94	SE	Kelburn	Corn	SCo	BP	2020	*Bacillus*
96	SI95	SE	Kelburn	Corn	SCo	BP	2020	*Bacillus*
97	SI96	SE	Kelburn	Corn	SCo	BP	2020	*Not yet sequenced*
98	SI97	SE	Kelburn	Corn	SCo	BP	2020	*Not yet sequenced*
99	SI98	SE	Kelburn	Soybean	CS	BP	2020	*Streptomyces*
100	SI99	SE	Kelburn	Soybean	CS	BP	2020	*Not yet sequenced*
101	SI100	SE	Kelburn	Soybean	CS	BP	2020	*Bacillus*
102	SI101	SE	Kelburn	Soybean	CS	BP	2020	*Streptomyces*
103	SI102	SE	Kelburn	Soybean	CS	BP	2020	*Lysobacter*
104	SI103	SE	Kelburn	Soybean	CS	BP	2020	*Not yet sequenced*
105	SI104	SE	Kelburn	Soybean	CS	BP	2020	*Staphylococcus*
106	SI105	SE	Kelburn	Soybean	CS	BP	2020	*Not yet sequenced*
107	SI106	SE	Kelburn	Soybean	CS	BP	2020	*Not yet sequenced*
108	SI107	SE	Kelburn	Soybean	CS	BP	2020	*Not yet sequenced*
109	SI108	SE	Kelburn	Corn	SCo	BP	2020	*Bacillus*
110	SI109	SE	Kelburn	Corn	SCo	BP	2020	*Bacillus*
111	SI110	SE	Kelburn	Corn	SWCC	BP	2020	*Bacillus*
112	SI111	SE	Kelburn	Corn	SWCC	BP	2020	*Bacillus*

No	Isolate name	Extraction Method	Site	Crop	Treatment	Time point	Year	Genus based on NCBI
113	SI112	SE	Kelburn	Corn	SWCC	BP	2020	*Bacillus*
114	SI113	SE	Kelburn	Corn	SWCC	BP	2020	*Not yet sequenced*
115	SI114	SE	Kelburn	Corn	SWCC	BP	2020	*Not yet sequenced*
116	SI115	SE	Kelburn	Corn	SWCC	BP	2020	*Not yet sequenced*
117	SI116	SE	Kelburn	Corn	SWCC	BP	2020	*Bacillus*
118	SI117	SE	Kelburn	Corn	SWCC	BP	2020	*Paenarthrobacter*
119	SI118	SE	Kelburn	Corn	SWCC	BP	2020	*Not yet sequenced*
120	SI119	SE	Kelburn	Corn	SCo	BP	2020	*Pseudomonas*
121	SI120	SE	Kelburn	Corn	SCo	BP	2020	*Bacillus*
122	SI121	SE	Kelburn	Canola	SCa	BP	2020	*Streptomyces*
123	SI122	SE	Kelburn	Soybean	CS	VE	2020	*Pseudomonas*
124	SI123	SE	Kelburn	Soybean	CS	VE	2020	*Enterobacter*
125	SI124	SE	Kelburn	Soybean	CS	VE	2020	*Priestia*
126	SI125	SE	Kelburn	Soybean	CS	VE	2020	*Priestia*
127	SI126	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
128	SI127	SE	Kelburn	Soybean	CS	VE	2020	*Peribacillus*
129	SI128	SE	Kelburn	Soybean	CS	VE	2020	*Pseudomonas*
130	SI129	SE	Kelburn	Soybean	CS	VE	2020	*Bacillus*
131	SI130	SE	Kelburn	Soybean	CS	VE	2020	*Bacillus*
132	SI131	SE	Kelburn	Canola	SCa	VE	2020	*Bacillus*
133	SI132	SE	Kelburn	Soybean	CS	VE	2020	*Neobacillus*
134	SI133	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
135	SI134	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
136	SI135	SE	Kelburn	Corn	SWCC	VE	2020	*Not yet sequenced*
137	SI136	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
138	SI137	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
139	SI138	SE	Kelburn	Corn	SWCC	VE	2020	*Not yet sequenced*
140	SI139	SE	Kelburn	Soybean	CS	VE	2020	*Arthrobacter*
141	SI140	SE	Kelburn	Corn	SWCC	VE	2020	*Pseudomonas*
142	SI141	SE	Kelburn	Soybean	CS	VE	2020	*Stenotrophomonas*
143	SI142	SE	Kelburn	Soybean	CS	VE	2020	*Pseudomonas*
144	SI143	SE	Kelburn	Corn	SCo	VE	2020	*Not yet sequenced*
145	SI144	SE	Kelburn	Canola	SCa	VE	2020	*Microbacterium*
146	SI145	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
147	SI146	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
148	SI147	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
149	SI148	SE	Kelburn	Canola	SCa	VE	2020	*Not yet sequenced*
150	SI149	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*

No	Isolate name	Extraction Method	Site	Crop	Treatment	Time point	Year	Genus based on NCBI
151	SI150	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
152	SI151	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
153	SI152	SE	Kelburn	Corn	SCo	VE	2020	*Not yet sequenced*
154	SI153	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
155	SI154	SE	Kelburn	Canola	SCa	VE	2020	*Not yet sequenced*
156	SI155	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
157	SI156	SE	Kelburn	Corn	SCo	VE	2020	*Not yet sequenced*
158	SI157	SE	Kelburn	Corn	SCo	VE	2020	*Not yet sequenced*
159	SI158	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
160	SI159	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
161	SI160	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
162	SI161	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
163	SI162	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
164	SI163	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
165	SI164	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
166	SI165	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
167	SI166	SE	Kelburn	Soybean	CS	VE	2020	*Not yet sequenced*
168	SI167	SE	Kelburn	Soybean	CS	BP	2021	*Streptomyces*
169	SI168	SE	Kelburn	Soybean	SWCC	BP	2021	*Not yet sequenced*
170	SI169	SE	Kelburn	Soybean	SCa	BP	2021	*Streptomyces*
171	SI170	SE	Carman	Soybean	SCa	BP	2021	*Microbacterium*
172	SI171	SE	Kelburn	Soybean	SCa	BP	2021	*Not yet sequenced*
173	SI172	SE	Kelburn	Soybean	CS	BP	2021	*Peribacillus*
174	SI173	SE	Kelburn	Soybean	SWCC	BP	2021	*Not yet sequenced*
175	SI174	SE	Carman	Soybean	CS	BP	2021	*Rhodococcus*
176	SI175	SE	Carman	Soybean	SCa	BP	2021	*Bacillus*
177	SI176	SE	Carman	Soybean	SCa	BP	2021	*Rhizobium*
178	SI177	SE	Kelburn	Soybean	SCa	BP	2021	*Pseudoduganella*
179	SI178	SE	Carman	Soybean	CS	BP	2021	*Not yet sequenced*
180	SI179	SE	Kelburn	Soybean	CS	BP	2021	*Not yet sequenced*
181	SI180	SE	Carman	Soybean	CS	BP	2021	*Pseudomonas*
182	SI181	SE	Carman	Soybean	CS	BP	2021	*Bacillus*
183	SI182	SE	Carman	Soybean	CS	BP	2021	*Streptomyces*
184	SI183	SE	Carman	Soybean	CS	BP	2021	*Bacillus*
185	SI184	SE	Carman	Soybean	CS	BP	2021	*Microbacterium*
186	SI185	SE	Carman	Soybean	CS	BP	2021	*Streptomyces*
187	SI186	SE	Carman	Soybean	SWCC	BP	2021	*Not yet sequenced*
188	SI187	SE	Kelburn	Soybean	CS	BP	2021	*Bacillus*

No	Isolate name	Extraction Method	Site	Crop	Treatment	Time point	Year	Genus based on NCBI
189	SI188	SE	Carman	Soybean	CS	BP	2021	*Streptomyces*
190	SI189	SE	Carman	Soybean	SCo	BP	2021	*Priestia*
191	SI190	SE	Kelburn	Soybean	CS	BP	2021	*Not yet sequenced*
192	SI191	SE	Carman	Soybean	CS	BP	2021	*Streptomyces*
193	SI192	SE	Carman	Soybean	SCa	BP	2021	*Not yet sequenced*
194	SI193	SE	Carman	Soybean	CS	BP	2021	*Peribacillus*
195	SI194	SE	Carman	Soybean	CS	BP	2021	*Pedobacter*
196	SI195	SE	Kelburn	Soybean	CS	BP	2021	*Arthrobacter*
197	SI196	SE	Carman	Soybean	SCa	BP	2021	*Kocuria*
198	SI197	SE	Kelburn	Soybean	SCa	BP	2021	*Arthrobacter*
199	SI198	SE	Kelburn	Soybean	SCa	BP	2021	*Not yet sequenced*
200	SI199	SE	Carman	Soybean	SCo	BP	2021	*Arthrobacter*
201	SI200	SE	Kelburn	Soybean	CS	BP	2021	*Not yet sequenced*
202	SI201	SE	Kelburn	Soybean	SCa	BP	2021	*Not yet sequenced*
203	SI202	SE	Kelburn	Soybean	CS	BP	2021	*Bacillus*
204	SI203	SE	Kelburn	Soybean	CS	BP	2021	*Pedobacter*
205	SI204	SE	Carman	Soybean	CS	BP	2021	*Not yet sequenced*
206	SI205	SE	Carman	Soybean	SCo	BP	2021	*Not yet sequenced*
207	SI206	SE	Carman	Soybean	SCa	BP	2021	*Streptomyces*
208	SI207	SE	Carman	Soybean	SCa	BP	2021	*Not yet sequenced*
209	SI208	SE	Kelburn	Soybean	SWCC	BP	2021	*Streptomyces*
210	SI209	SE	Carman	Soybean	SCa	BP	2021	*Streptomyces*
211	SI210	SE	Carman	Soybean	CS	BP	2021	*Streptomyces*
212	SI211	SE	Carman	Soybean	CS	BP	2021	*Not yet sequenced*
213	SI212	SE	Carman	Soybean	SCo	BP	2021	*Stenotrophomonas*
214	SI213	SE	Carman	Soybean	SCa	BP	2021	*Bacillus*
215	SI214	SE	Carman	Soybean	CS	BP	2021	*Arthrobacter*
216	SI215	SE	Carman	Soybean	SCo	BP	2021	*Not yet sequenced*
217	SI216	SE	Carman	Soybean	SCa	BP	2021	*Not yet sequenced*
218	SI217	SE	Carman	Soybean	CS	BP	2021	*Priestia*
219	SI218	SE	Kelburn	Soybean	SCo	BP	2021	*Not yet sequenced*
220	SI219	SE	Carman	Soybean	CS	BP	2021	*Not yet sequenced*
221	SI220	SE	Carman	Soybean	CS	BP	2021	*Bacillus*
222	SI221	SE	Kelburn	Soybean	CS	BP	2021	*Not yet sequenced*
223	SI222	SE	Carman	Soybean	CS	BP	2021	*Hymenobacter*
224	SI223	SE	Carman	Soybean	CS	BP	2021	*Stenotrophomonas*
225	SI224	SE	Kelburn	Soybean	CS	BP	2021	*Not yet sequenced*
226	SI225	SE	Carman	Soybean	SWCC	BP	2021	*Not yet sequenced*

No	Isolate name	Extraction Method	Site	Crop	Treatment	Time point	Year	Genus based on NCBI
227	SI226	SE	Carman	Soybean	CS	BP	2021	*Pseudomonas*
228	SI227	SE	Carman	Soybean	SCo	BP	2021	*Arthrobacter agilis*
229	SI228	SE	Carman	Soybean	SWCC	BP	2021	*Not yet sequenced*
230	SI229	SE	Kelburn	Soybean	CS	BP	2021	*Streptomyces*
231	SI230	SE	Carman	Soybean	CS	BP	2021	*Pseudomonas*
232	SI231	SE	Carman	Soybean	SWCC	BP	2021	*Not yet sequenced*
233	SI232	SE	Carman	Soybean	SCa	BP	2021	*Not yet sequenced*
234	SI233	SE	Kelburn	Soybean	CS	BP	2021	*Not yet sequenced*
235	SI234	SE	Carman	Soybean	CS	BP	2021	*Not yet sequenced*
236	SI235	SE	Carman	Soybean	SWCC	BP	2021	*Not yet sequenced*
237	SI236	SE	Carman	Soybean	CS	BP	2021	*Not yet sequenced*
238	SI237	SE	Kelburn	Soybean	CS	BP	2021	*Not yet sequenced*
239	SI238	SE	Carman	Soybean	SCo	BP	2021	*Not yet sequenced*
240	SI239	SE	Carman	Soybean	SWCC	BP	2021	*Not yet sequenced*
241	SI240	SE	Carman	Soybean	SCo	BP	2021	*Not yet sequenced*
242	SI241	SE	Carman	Soybean	SWCC	BP	2021	*Not yet sequenced*
243	SI242	SE	Kelburn	Soybean	SWCC	BP	2021	*Kocuria*
244	SI243	SE	Kelburn	Soybean	SWCC	BP	2021	*Streptosporangium*
245	SI244	SE	Kelburn	Soybean	SWCC	BP	2021	*Streptomyces*
246	SI245	SE	Carman	Soybean	CS	BP	2021	*Not yet sequenced*
247	SI246	SE	Carman	Soybean	SWCC	BP	2021	*Not yet sequenced*
248	NI01	PE	Carman	Soybean	CS	R1	2018	*Mycolicibacterium*
249	NI02	PE	Carman	Soybean	CS	R1	2018	*Novosphingobium*
250	NI03	PE	Carman	Soybean	CS	R1	2018	*Shinella*
251	NI04	PE	Carman	Soybean	CS	R1	2018	*Sphingomonas*
252	NI05	PE	Carman	Soybean	CS	R1	2018	*Bradyrhizobium*
253	RI01	PE	Carman	Soybean	CS	R1	2018	*Sphingomonas*
254	RI02	PE	Carman	Soybean	CS	R1	2018	*Not yet sequenced*
255	RI03	PE	Carman	Soybean	CS	R1	2018	*Dermacoccus*
256	RI04	PE	Carman	Soybean	CS	R1	2018	*Pseudomonas*
257	RI05	PE	Carman	Soybean	CS	R1	2018	*Rhizobium*
258	RI06	PE	Carman	Soybean	CS	R1	2018	*Not yet sequenced*
259	RI07	PE	Carman	Soybean	CS	R1	2018	*Pseudomonas*
260	RI08	PE	Carman	Soybean	CS	R1	2018	*Pseudomonas*
261	RI09	PE	Carman	Soybean	CS	R1	2018	*Not yet sequenced*
262	RI10	PE	Carman	Soybean	CS	R1	2018	*Not yet sequenced*
263	RI11	PE	Carman	Soybean	CS	R1	2018	*Not yet sequenced*
264	RI12	PE	Carman	Soybean	CS	R1	2018	*Staphylococcus*
265	RI13	PE	Carman	Soybean	CS	R1	2018	*Not yet sequenced*

SI - soil isolate, NI - nodule isolate, RI – root isolate, SE- soil extraction, PE- pot extraction, CS - continuous soybean, SCa - soybean-canola, SCo - soybean-corn, SWCC - soybean-wheat-canola-corn), BP – before planting and R1 – Beginning bloom, 16S (V4)- 16S rRNA sequencing with targeted region V4. Not yet sequenced – bacterial strains preserved, but not sequenced yet. Partial sequences are given Appendix D.

Appendix D

Partial sequences obtained through 16S rRNA gene sequencing targeting the V4 region

> SI01_Paraburkholderia strydomiana
CTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGCAAGCCTGATCCAGCAATGCCGCGTGTGTGAA
GAAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAAACCTNNNNGGTTAATACNNNNNGGGGGATGACG
GTANNNNNNAGAATAAGCANNNNNCTAACTACGTGCCAGCAGCCGCGGTAA

> SI02_Paraburkholderia strydomiana_F
TGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAGCACTTTTGTCCGGAAAGAAAACCTCGTGGTTAATACCCG
TGGGGGATGACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAAT

> SI02_Paraburkholderia strydomiana_R
CCCACGGGTATTAACNNNNAGGTTTTCTTTCCGGNNNAAGNNNNNNNNNNNNNNNNGCCTTCTNNNACACGCGG
CATTGCTGNNNNNNNNNTGCGCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTA

> SI03_Paraburkholderia strydomiana
GTCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTT
CGCTAAGACAGATGTGAAATCCCCGGGCTTAACCTGGGAACTGCATTTGTGACTGGCGGGCTAGAGTATGGCAG
AGGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAGCCCC
CTGGGCCAATACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI04_Paraburkholderia strydomiana_F
GTGNGNNNAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAAACCTNNNNGGTTAATACCNNNNNNNGG
GATGACGGTANNNNNNAGAATAAGCNNNNNNCTAACTNNTGTGCCA

> SI04_Paraburkholderia strydomiana_R
GTCGTNTCNNCCGGGTATTAANCNGAGGTTTTCTTTCCGGACAAAAGTGCTTTACAACCCGAAGGCCTTCTTCN
ACACGCGGCATTGCTGGATCAGGCTTGCGCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTA

> SI05_Burkholderia ambifaria
TCCTACGGGAGGCAGCAGTGGGGATATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGTGTGAA
GAAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAATCCTTGGTTCTAATATAGCCGGGGGGGGGGGTA
CCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAA

> SI06_Paraburkholderia graminis_F
GTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAAACCGCCTGGCTAATATCCGGGCGGGATG
ACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAA

> SI06_Paraburkholderia graminis_R
GCCCGGATATTAGCNNGGCGGTTTTCTTTCCGGACAAAAGTGCTTTACAACCCGAAGGCCTTCTNNNACACGCG
GCATTGCTGGATCAGGCTTGCGCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTA

> SI07_Paraburkholderia strydomiana
TCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGCAAGCCTGATCCAGCAATGCCGCGTGTGTGAAG
AAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAAACCTCGTGGTTAATACCCGTGGGGGATGACGGTA
CCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI08_Paraburkholderia strydomiana_F
TGCCGCNNGTGTGAGAAGGCCTTCGGGTTGTAAAGCACTTTTGTCCGGAAAGAAAACCTCGTGGTTAATACCCG
TGGGGGATGACGGTACCGGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI08_Paraburkholderia strydomiana_R
GGGTANNNCCACGAGGTTTTCTTTCCGGNNNAAGTGCTTTACAACCCGAAGGCCTTCTNNNACACGCGGCATTG
CTGGATCAGGCTTGCGCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGT

> SI09_   Paraburkholderia strydomiana_F
CGCGTGTGNNNNNNGGCCTTCGGGTTGNNAGCACTTTTGTCCGGAAAGAAAACCTCGTGGTNNNACCCGTGGGG
GATGACGGNNNCGGAAGAATAAGCACCGGCTAACTACGTGCCNNCAGCCGC

>SI09_Paraburkholderia strydomiana_R
GAGGTTTTCTTTCCGGACAAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACACGCGGCATTGCTGGATCAGG
CTTGCGCCCATTGTCCAAAATTCCCCACTGCTGCCTCCCGT

> SI10_Stenotrophomonas lactitubi
GTCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTT
GTTTAAGTCTGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGAAACTGGACAACTAGAGTGTGGTAG
AGGGTAGCGGAATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTAC
CTGGACCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI11_Stenotrophomonas lactitubi
GTCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTT
GTTTAAGTCTGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGAAACTGGACAACTAGAGTGTGGTAG
AGGGTAGCGGAATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTAC
CTGGACCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI12_Chryseobacterium hispalense
GTGTCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTCCGTAGGCGG
ATGCGTAAGTCAGTGGTGAAATCTCACAGCTCAACTGTGAAACTGCCATTGATACTGCGTGTCTTGAGTGAGGT
TGAAGTAGCTGGAATAAGTAGTGTAGCGGTGAAATGCATAGATATTACTTAGAACACCAATTGCGAAGGCAGGT
TACTAAGTCTCAACTGACGCTGATGGACGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI13_Bacillus zanthoxyli
GTGTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGG
TTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGA
AGAGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCT
TTTTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC

> SI14_Chryseobacterium endophyticum_F
GGACGACGGCCCTATGGGTTGTAAACTTCTTTTGTACAGGGATAAACCTACCCTCGTGAGGGTAGCTGAAGGTA
CTGTAAATAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTA

> SI14_Chryseobacterium endophyticum_R
TCACGAGGGTAGGTNNNCCCTGTACAAAAGAAGTTTACAACCCATAGGGCCGTCGTCCTTCACGCGGGATGGCT
GGATCAGGCGCTAACCCATTGTCCAATATTCCTCACTGCTGCCTCCCGTAGGAGT

> SI15_Pseudarthrobacter psychrotolerans
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GTTTGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATG
TAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGG
TCTCTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC

> SI16_Arthrobacter celericrescens
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCTGTGAAAGACCGGGGCTCAACTCCGGTTCTGCAGTGGGTACGGGCAGACTAGAGTGATGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC

> SI17_Pseudomonas uvaldensis
TCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAG
AAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCCATTACCTAATACGTGATGGTTTTGACGTTA
CCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTA

> SI18_Stenotrophomonas cyclobalanopsidis
TCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAG
AAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCCGGCTAATACCTGGTTGGGATGACGGTA
CCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTA

> SI19_Novosphingobium silvae
GTCAGCAGCCGCGGTAATACGGAGGGAGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCGCGTAGGCGGCT
GCTCAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGGAACTGCCTTTGAAACTAGGTAGCTAGAATCTTGGAG
AGGTCAGTGGAATTCCGAGTGTAGAGGTGAAATCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGACTGAC
TGGACAAGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT

> SI20_Pseudomonas arenae
GTCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTT
TGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGACTGACTAGAGTATGGTAG
AGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCAC
CTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI21_Stenotrophomonas cyclobalanopsidis
TCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAG
AAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCCGGCTAATACCTGGTTGGGATGACGGTA
CCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAAT

> SI22_Pseudarthrobacter psychrotolerans
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC

> SI23_Pseudomonas alliivorans
GTCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTT
TGTTAAGTTGAATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTAGGGCAG
AGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCAC
CTGGGCTCATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI24_Brevundimonas huaxiensis
GTCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCTCGGAATTACTGGGCGTAAAGGGAGCGTAGGCGGAC
ATTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCTCGGAATTGCCTTTGATACTGGGTGTCTTGAGTATGAGAG
AGGTGTGTGGAACTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGACACA
CTGGCTCATTACTGACGCTGAGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI25_Pseudoroseomonas suffusca
GTCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTACTCGGAATTACTGGGCGTAAAGGGCGCGTAGGCGGCG
TTCCAAGTTAGGCGTGAAAGTCCTGGGCTCAACCTGGGAACTGCGCTTAAGACTGGAGTGCTAGAGGATGGAAG

AGGGTTGTGGAATTCCCAGTGTAGAGGTGAAATTCGTAGATATTGGGAAGAACACCGGTGGCGAAGGCGGCAAC
CTGGTCCATTTCTGACGCTGAGGCGCGATAGCGTGGGGAGCAAACAGGATTAGATACC

> SI26I_Stenotrophomonas lactitubi
GTCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTT
GTTTAAGTCTGTTGTGAAAGCCCTGGGCTCAACCTGGGAACTGCAGTGGAAACTGGACAACTAGAGTGTGGTAG
AGGGTAGCGGAATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTAC
CTGGACCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI26II_Pseudarthrobacter psychrotolerans
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GTTTGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATG
TAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGG
TCTCTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC

> SI27_Pseudomonas arenae
GTGTCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGG
TTTGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGACTGACTAGAGTATGGT
AGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACC
ACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI28_Bacillus zanthoxyli
CCTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAG
TGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAACTGCTCGTACCTTGA
CGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGT

> SI29_Pseudarthrobacter psychrotolerans
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACCC

> SI32_Paenibacillus liaoningensis
ACTTTCGTCCATTGCGGAAGATTCCCTNNTCTTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAG
TCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCTAA
GGAGAGTAACTGCTCTTTAGGTGACGGTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI33_Bacillus pumilus
ACTTTCGTCCATTGNNNAAGATTCCCTNNTNNNACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAG
TCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTG
CGAGAGTAACTGCTCGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI34_Neobacillus endophyticus
ACTTTCGTCCATTGTGGAAGATTCCCTNNTNNNACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAG
TCTGATGGAGCAACGCCGCGTGAGCGATGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTA
CCGGAGTAACTGCCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI38_Mucilaginibacter auburnensis_F
CCGCNNNNAGGAAGACGGCCCTACGGGTTNNNACTGCTTTTGCAGGGGAATAAACCTTGGTATGTATACCAAGC
TGAATGTACTCTGAGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTA

> SI38_Mucilaginibacter auburnensis_R
AGGTTTATTCCCCTGCAAAAGCAGTTTACAACCCGTAGGGCCGTCTTCCTGCACGCGGCATGGCTGGTTCAGAC
TTCCGTCCATTGACCAATATTCCTTACTGCTGCCTCCCGT

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> SI39_    Paenibacillus piscarius
TACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGCAAGCCTGACGGAGCAACGCCGCGTGAGTG
ATGAAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGTCCGGTANNGTAACNNNTGCCGGAGTGACG
GTACCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI40_Clavibacter sepedonicus_F
GGGATGACGGCCTTCGGGTTGTAAACCTCTTTTAGTAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAAAAGC
ACCGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI40_Clavibacter sepedonicus_R
AAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTTGCTGCATCAGGCTTTCGCCCATTGTGCAATA
TTCCCCACTGCTGCCTCCCGTAGGAGTAGGG

> SI42_Bacillus zanthoxyli
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
TTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI43_Pseudomonas fildesensis
GGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTC
TTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTTACCTAATACGTGATTGTTTTGACGTTACCGACA
GAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTA

> SI44_Stenotrophomonas cyclobalanopsidis
CCTACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGG
TGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATCCAGCCGGCTAATACCTGGTTGGGATGA
CGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTA

> SI45_Janthinobacterium rivuli
CCTACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGAG
TGAAGAAGGCCTTCGGGTTGTAAAGCTCTTTTGTCAGGGAAGAAACGGTGAGAGCTAATATCTCTTGCTAATGA
CGGTACCTGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI46_Rothia terrae
GTCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTTGTAGGCGGTT
TGTCGCGTCTGCTGTGAAAGCCCGGGGCTTAACCCCGGGTTTGCAGTGGGTACGGGCTAACTAGAGTGCAGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCTGTAACTGACGCTGAGAAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC

> SI47_Rothia terrae
GTCAGCAGCCGCGGTAATACGTAGGGCGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTTGTAGGCGGTT
TGTCGCGTCTGCTGTGAAAGCCCGGGGCTTAACTCCGGGTTTGCAGTGGGTACGGGCTAACTAGAGTGCAGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCTGTAACTGACGCTGAGAAGCGAAAGCATGGGGAGCGAACAGGATTAGATAC

> SI48_Peribacillus frigoritolerans
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
CCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC
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> SI49_Pseudomonas uvaldensis
CNACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGT
GAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCCATTACCTAATACGTGATGGTTTTGAC
GTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAAT

> SI50_Priestia veravalensis
AGATTCCCTANTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCG
CGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACAAGAGTAACTGCNNNTA
CCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI51_Pseudarthrobacter psychrotolerans
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC

> SI52_Rhodococcus qingshengii
GTCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTGTCCGGAATTACTGGGCGTAAAGAGTTCGTAGGCGGTT
TGTCGCGTCGTTTGTGAAAACCAGCAGCTCAACTGCTGGCTTGCAGGCGATACGGGCAGACTTGAGTACTGCAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCT
CTGGGCAGTAACTGACGCTGAGGAACGAAAGCGTGGGTAGCGAACAGGATTAGATACC

> SI55_Collimonas arenae
TCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAATGGGGGCAACCCTGATCCAGCAATGCCGCGTGAGTGAAG
AAGGCCTTCGGGTTGTAAAGCTCTTTTGTCAGGGAAGAAACGGTGAGTGCTAATACCACTTGCTAATGACGGTA
CCTGAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAAT

> SI59_Flavobacterium geliluteum_F
AGGATGACGGTCCTATGGATTGTAAACTGCTTTTATACGAGAAGAAACACTGCTTCGTGAAGTAGCTTGACGGT
ATCGTAAGAATAAGGATCGGCTAACTCCGTGCCAGCAGCCGCGGTAA

> SI59_Flavobacterium geliluteum_R
AGCTACTTCACGAANNNGTGTTTCTTCTCGTATAAAAGCAGTTTACAATCCATAGGACCGTCATCCTGCACGCG
GCATGGCTGGATCAGGCTTGCGCCCATTGTCCAATATTCCTCACTGCTGCCTCCCGTAGGA

> SI60_Paenibacillus chitinolyticus
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGCAAGTCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGTTTTCGGATCGTAAAGCTCTGTTGCCAGGGAAGAACGCCAAGGAGAGTAACTGCTCTTTGGGTGACGGTA
CCTGAGAAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI61_Bacillus wiedmannii
TTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCG
TCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGTAGG

> SI62_Pseudomonas silesiensis_F
GTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTTACCTAATACGTATCTGTTTTG
ACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTA

> SI62_Pseudomonas silesiensis_R
aacagatacgtattaggtaactgcccttcctNNNacttaaagtgctttacaatccgaagaccttcttcacacac
gcggcatggctggatcNNNctttcgcccattgtccaatattccccactgctgcctcccgtagga

> SI63_Bacillus aerius
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCTTGCACCTTGACGGTA
CCTAACCAGNNNGCCACGNCTAACTACGTGCCAGCAGCCGCGGT

> SI64_Stenotrophomonas rhizophila
TCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAG
AAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAAAGCAGTCGATTAATACTCNNNNTGTTCTGACGGT
ACCCAAAGAATAAGCACCGGCTANCTTCGTGCCAGCAGCCGCGGTAAT

> SI65_Chryseobacterium tagetis_F
GGACGACGGCCCTATGGGTTGTAAACTTCTTTTGTATAGGGATAAACCTTTCCACGTGTGGAAAGCTGAAGGTA
CTATACGAATAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAAT

> SI65_Chryseobacterium tagetis_R
CCCTATACAAAAGAAGTTTACAACCCATAGGGCCGTCGTCCTTCACGCGGGATGGCTGGATCAGGCTCTCACCC
ATTGTCCAATATTCCTCACTGCTGCCTCCCGTAGNA

> SI67_Streptomyces silvae
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI68_Metabacillus bambusae
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAACGNNN
NAAGGCCTTCGGGTCGTAAAGTTCTGTTGTTAGGGAAGAACAAGTACCAGAGTAACTGCTGGTACCTTGACGGT
ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA

> SI69_Pseudomonas fildesensis
TCCTACGGGAGGCAGNNNTGGGGAATATTGGACAATGGGCGAAAGNNNGATCCAGCCATGCCGCGTGTGTGAAG
AAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTTACCTAATACGTGATTGTTTTGACGTTA
CCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTA

> SI70_Microbacterium diaminobutyricum
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCTGTGAAAACTGGAGGCTCAACCTCCAGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAG
GGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCT
CTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGATTAGATACC

> SI71_Peribacillus loiseleuriae
ATTCCCTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCG
TGAACGAAGAAGGCCTTCGGGTCGTAAAGTTCTGTTGTTAGGGAAGAACAAGTACCAGAGTAACTGCTGGTACC
TTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT

> SI72_Bacillus wiedmannii
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
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AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT
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> SI73_Bacillus pumilus
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAG

AGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCT
CTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI74_Rhodococcus sovatensis
GTCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTACTGGGCGTAAAGAGTTCGTAGGCGGTT
TGTCGCGTCGTTTGTGAAAACCCGGGGCTCAACTTCGGGCTTGCAGGCGATACGGGCAGACTTGAGTGTTTCAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCT
CTGGGAAACAACTGACGCTGAGGAACGAAAGCGTGGGTAGCAAACAGGATTAGAT

> SI75_Peribacillus frigoritolerans
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
CCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATA

> SI77_Peribacillus loiseleuriae
CTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAAC
GAAGAAGGCCTTCGGGTCGTAAAGTTCTGTTGTTAGGGAAGAACAAGTACCAGAGTAACTGCTGGTACCTTGAC
GGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA

> SI78_Bacillus pumilus
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCT
CTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI79_Peribacillus loiseleuriae
CCCTACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGA
ACGAAGAAGGCCTTCGGGTCGTAAAGTTCTGTTGTTAGGGAAGAACAAGTACCAGAGTAACTGCTGGTACCTTG
ACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI80_Bacillus pumilus
GTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGA
GTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTC
TGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC

> SI81_Bacillus pumilus
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCT
CTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC

> SI82_Bacillus wiedmannii
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI83_Bacillus halotolerans
TGTGTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCG
GTTCCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGAC
TCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC

> SI84_Peribacillus frigoritolerans
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
CTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI85_Streptomyces hydrogenans
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGGTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC

> SI86_Bacillus wiedmannii
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI87_Bacillus wiedmannii
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
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CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC

> SI88_Chryseobacterium mulctrae
GTGTCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTCCGTAGGCGG
ATCCGTAAGTCAGTGGTGAAATCTCATAGCTTAACTATGAAACTGCCATTGATACTGCGGGTCTTGAGTAAAGT
AGAAGTGGCTGGAATAAGTAGTGTAGCGGTGAAATGCATAGATATTACTTAGAACACCAATTGCGAAGGCAGGT
CACTATGTTTTAACTGACGCTGATGGACGAAAGCGTGGGGAGCGAACAGGATTAGNTACC

> SI90_Peribacillus frigoritolerans
GTGTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGG
TTCCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGA
AGAGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACT
TTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI91_Rossellomorea arthrocnemi
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGATATTTGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI92_Peribacillus frigoritolerans
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
CCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI93_Bacillus stercoris
AGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAG
TGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAG
GCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI94_Bacillus stercoris
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCT
CTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI95_Bacillus zanthoxyli
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
TTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGG

> SI98_Streptomyces triticiradicis
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGGTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI100_Bacillus zanthoxyli_F
ACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGNNNACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAACTGC
TCGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI100_Bacillus zanthoxyli_R
TACTCTCGTACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCT
CCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGA

> SI101_Streptomyces huasconensis
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI102_Lysobacter antibioticus
GTGTCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGG
TTTGTTAAGTCTGATGTGAAAGCCCTGGGCTCAACCTGGGAATGGCATTGGAAACTGGCTTACTAGAGTGCGGT
AGAGGGTAGTGGAATTCCCGGTGTAGCAGTGAAATGCGTAGATATCGGGAGGAACATCTGTGGCGAAGGCGACT
ACCTGGACCAGCACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGAT

> SI104_Staphylococcus schweitzeri
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTT
TTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACC

> SI108_Bacillus halotolerans
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTT
CCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCT
CTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI109_Bacillus zanthoxyli
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTT
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AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
TTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI110_Bacillus zanthoxyli
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTT
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AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
TTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI111_Bacillus pumilus
TGTGTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCG
GTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAG
AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGAC
TCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI112_Bacillus zanthoxyli
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
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> SI116_Bacillus zanthoxyli
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
TTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI117_Paenarthrobacter ureafaciens
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCTGTGAAAGACCGGGGCTCAACTCCGGTTCTGCAGTGGGTACGGGCAGACTAGAGTGCAGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCTGTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC

> SI119_Pseudomonas kielensis
TGTGTCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTG
GTTTGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCAAAACTGACAAGCTAGAGTATGG
TAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGAC
CACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI120_Bacillus subtilis
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCT
CTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI121_Streptomyces aridus
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI122_Pseudomonas koreensis
GTCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTT
TGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTATGGTAG

AGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCAC
CTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI123_Enterobacter mori
GTCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTC
TGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTAGAGTCTTGTAG
AGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCC
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> SI124_Priestia megaterium
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
TTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI125_Priestia megaterium
AAAGCCGCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCGCTTTTCT
CTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGA
AACCGCCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGAC

> SI127_Peribacillus frigoritolerans
CGGAAGATTCCCTNCTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAAC
GCCGCGTGAACGAAGAAGGCCTTCGGGTCGTAAAGTTCTGTTGTTAGGGAAGAACAAGTACCAGAGTAACTGCT
GGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI128_Pseudomonas fildesensis_F
TGNAGAAGGTCTTCGGATTNNNAGCACTTTAAGTTGGGAGGAAGGGCAGTTACCTAATACGTAATTGTTTTGAC
GTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTA

> SI128_Pseudomonas fildesensis_R
CTGCCCTTCCTCCCAACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGG
CTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGA

> SI129_Bacillus cereus
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGT
ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT

> SI130_Bacillus anthracis
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI131_Bacillus cereus
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATG
AAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGT
ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT

> SI132_Neobacillus pocheonensis
TCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGCGATG
AAGGCCTTCGGGTCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTATCGGAGTAACTGCCGGTACCTTGACGGTA
CCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI139_Arthrobacter celericrescens_F
GGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGTAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGC
GCCGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI139_Arthrobacter celericrescens_R
TTCTTCCCTACTGAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCTGCATCAGGCTTGCGC
CCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGT

> SI140_Pseudomonas coronafaciens_F
AGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTTACCTAATACGTAAGTGTTTTGACGTTAC
CGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTA

> SI140_Pseudomonas coronafaciens_R
TTACGTATTAGGTAAATGCCCTTCCTNNNACTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGC
ATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGA

> SI141_Stenotrophomonas nematodicola
TCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAG
AAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAAAGCAGTCGGCTAATACNNNNNNTGTTCTGACGGT
ACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAAT

> SI142_Pseudomonas coronafaciens_F
GTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATTTACCTAATACGNNNGTGTTTTG
ACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAAT

> SI142_Pseudomonas coronafaciens_R
ATGCCCTTCCTCNNNCTTAAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGC
TTTCGCCCATTGTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGT

> SI144_Microbacterium thalassium
CCNACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAGCCTGATGCAGCAACGCCGCGTGAG
GGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAAGGAAGAAGCGAAAGTGACGGTACTTGCAGAAAAAGCG
CCGGCTAACTACGTGCCAGCAGCCGCGGTA

> SI167_Streptomyces caviscabies
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI169_Streptomyces zaomyceticus
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GCTTGTCACGTCGGGTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGG
TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA
TCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI170_Microbacterium binotii
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCTGTGAAAACTGGAGGCTCAACCTCCAGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAG
GGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGATCT
CTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGATTAGATACC

> SI172_Bacillus sp.
GTGTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGG
TTCCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGA
AGAGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACT
TTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI174_Rhodococcus fascians
GTCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTACTGGGCGTAAAGAGTTCGTAGGCGGTT
TGTCGCGTCGTTTGTGAAAACCCGGGGCTCAACTTCGGGCTTGCAGGCGATACGGGCAGACTTGAGTGTTTCAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGGTCT
CTGGGAAACAACTGACGCTGAGGAACGAAAGCGTGGGTAGCAAACAGGATTAGAT

> SI175_Bacillus sp.
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
CCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATA

> SI176_Rhizobium viscosum
TGTGTCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCG
GACATTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGGTGTCTTGAGTATGG
AAGAGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGC
TCACTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGA

> SI177_Pseudoduganella plicata
GTCAGCAGCCGCGGTAATACGTAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTT
TTGTAAGTCTGTTGTGAAATCCCCGGGCTCAACCTGGGAATGGCAATGGAGACTGCAAGGCTAGAGTTTGGCAG
AGGGGGGTAGAATTCCACGTGTAGCAGTGAAATGCGTAGAGATGTGGAGGAACACCGATGGCGAAGGCAGCCCC
CTGGGTCAAAACTGACGCTCATGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI180_Pseudomonas koreensis
GCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTG
GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTATGGTAGAGGGTGGTGG
AATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT
ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAT

> SI181_Bacillus subtilis
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAG
AGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCT
CTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC

> SI182_Streptomyces dioscori
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GTCTGTCGCGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCAGACTAGAGTGTGG
TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA
TCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI183_Bacillus halotolerans
TGTGTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCG
GTTCCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGAC
TCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC

> SI184_Microbacterium liquefaciens
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GTTTGTCGCGTCTGCTGTGAAATCCGGAGGCTCAACCTCCGGCCTGCAGTGGGTACGGGCAGACTAGAGTGCGG
TAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGA
TCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGATTAGATACC

> SI185_Streptomyces zaomyceticus
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGGTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATAC

> SI187_Bacillus wiedmannii
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC

> SI188_Streptomyces caviscabies
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGAT

> SI189_Priestia megaterium
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
TTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAG

> SI191_Streptomyces caviscabies
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGAT

> SI193_Peribacillus frigoritolerans
GCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTCCTTAAGTCT
GATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAAAGTGG
AATTCCAAGTGTAGCGGTGAAATGCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTA
ACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAG

> SI194_Pedobacter psychrotolerans
GTGTCAGCAGCCGCGGTAATACGGAGGATCCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCGG
CCTGTTAAGTCAGGGGTGAAAGACGGTAGCTCAACTATCGCAGTGCCCTTGATACTGATGGGCTTGAATGGACT
AGAGGTAGGCGGAATGAGACAAGTAGCGGTGAAATGCATAGATATGTCTCAGAACACCGATTGCGAAGGCAGCT
TACTATGGTTTAATTGACGCTGAGGCACGAAAGCGTGGGGATCAAACAGGATTAGAT

> SI195_Arthrobacter dokdonellae
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGAT

> SI196_Kocuria rhizophila
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GTTTGTCGCGTCTGCTGTGAAAGCCCGGGGCTTAACCCCGGGTGTGCAGTGGGTACGGGCAGACTTGAGTGCAG
TAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGG
TCTCTGGGCTGTTACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATACC

> SI197_Arthrobacter dokdonellae
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGAT

> SI199_Arthrobacter dokdonellae
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGAT

> SI202_Bacillus wiedmannii
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC

> SI203_Pedobacter ghigonis
TGTGTCAGCAGCCGCGGTAATACGGAGGATCCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCG
GCCTGTTAAGTCAGGGGTGAAAGACGGTAGCTCAACTATCGCAGTGCCCTTGATACTGATGGGCTTGAATGGAC
TAGAGGTAGGCGGAATGAGACAAGTAGCGGTGAAATGCATAGATATGTCTCAGAACACCGATTGCGAAGGCAGC
TTACTATGGTCTTATTGACGCTGAGGCACGAAAGCGTGGGGATCAAACAGGATTAGATAC

> SI206_Streptomyces hydrogenans
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GCTTGTCACGTCGGGTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGG
TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA
TCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGAT

> SI208_Streptomyces manipurensis
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GCTTGTCACGTCGGATGTGAAAGCCCGAGGCTTAACCTCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGG
TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA
TCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGAT

> SI209_Streptomyces aridus
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCT
TGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAG
GGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI210_Streptomyces aridus
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GCTTGTCACGTCGGATGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGG
TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA
TCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGAT

> SI212_Stenotrophomonas rhizophila
GCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAGTCT
GTTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGTGG
AATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCAAC
ACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGAT

> SI213_Bacillus cereus
GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGAGACTTGAGTGCA
GAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAAGGCGA
CTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGAT

> SI214_Arthrobacter dokdonellae
GTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTT
TGTCGCGTCTGCCGTGAAAGTCCGGGGCTCAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGATGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCATTAACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGAT

> SI217_Priestia megaterium
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTT
TCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAG
AGAAAAGCGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGGCTTT
TTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGAT

> SI220_Bacillus subtilis
CCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGT
CTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGT
GGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTG
TAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGAT

> SI222_Hymenobacter tenuis
TGTGTCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTGTCCGGATTTATTGGGTTTAAAGGGTGCGTAGGCG
GCCCGTTAAGTCCGGGGTGAAAGCCCACAGCTCAACTGTGGAACTGCCCTGGATACTGGCGGGCTTGAGTCCAG
ACGAGGTTGGCGGAATGGATGGTGTAGCGGTGAAATGCATAGATACCATCCAGAACCCCGATTGCGAAGGCAGC
TGACTAGGCTGGTACTGACGCTGAGGCACGAAAGCGTGGGGAGCGAACAGGATTAGAT

> SI223_Stenotrophomonas rhizophila
CCGCGGTAATACGAAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTTGTTTAAGT
CTGTTGTGAAAGCCCTGGGCTCAACCTGGGAATTGCAGTGGATACTGGGCGACTAGAGTGTGGTAGAGGGTAGT
GGAATTCCCGGTGTAGCAGTGAAATGCGTAGAGATCGGGAGGAACATCCATGGCGAAGGCAGCTACCTGGACCA
ACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGAT

> SI226_Pseudomonas koreensis
GTCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTT
TGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTATGGTAG
AGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCAC
CTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> SI227_Arthrobacter agilis
GCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCT
GCCGTGAAAGTCCGGGGCTTAACTCCGGATCTGCGGTGGGTACGGGCAGACTAGAGTGCAGTAGGGGAGACTGG
AATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCTCTGGGCTGTA
ACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGAT

> SI229_Streptomyces hydrogenans
GTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGG
CTTGTCACGTCGGGTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGGT
AGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGAT
CTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAG

> SI230_Pseudomonas koreensis
TGTGTCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTG
GTTTGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTATGG
TAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGAC
CACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC

> SI242_Kocuria rhizophila
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GTTTGTCGCGTCTGCTGTGAAAGCCCGGGGCTTAACCCCGGGTGTGCAGTGGGTACGGGCAGACTTGAGTGCAG
TAGGGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGG
TCTCTGGGCTGTTACTGACGCTGAGGAGCGAAAGCATGGGGAGCGAACAGGATTAGATAC

> SI243_Streptosporangium longisporum
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGTG
GCTTGTCACGTCGGGTGTGAAAGCTTGGGGCTTAACTCCAGGTCTGCATTCGATACGGGCTGGCTAGAGGTAGG
TAGGGGAGAACGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGT
TCTCTGGGCCTTACCTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> SI244_Streptomyces aureus
TGTGTCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCG
GCTTGTCACGTCGGTTGTGAAAGCCCGGGGCTTAACCCCGGGTCTGCAGTCGATACGGGCAGGCTAGAGTGTGG
TAGGGGAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGA
TCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> NI01_Mycolicibacterium nivoides
TGTGTCAGCAGCCGCGGTAATACGTAGGGTCCGAGCGTTGTCCGGAATTACTGGGCGTAAAGAGCTCGTAGGTG
GTTTGTCGCGTTGTTCGTGAAAACTCACAGCTTAACTGTGGGCGTGCGGGCGATACGGGCAGACTAGAGTACTG
CAGGGGAGACTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGG
TCTCTGGGCAGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACC

> NI02_Novosphingobium guangzhouense
GTCAGCAGCCGCGGTAATACGGAGGGAGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCGCGTAGGCGGTT
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AGGTCAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGACTGA
CTGGACAAGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> NI03_Shinella oryzae
GTCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGGT
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AGGTAAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTTA
CTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> NI04_Sphingomonas populi
GTCAGCAGCCGCGGTAATACGGAGGGAGCTAGCGTTATTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCT
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AGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTCA
CTGGACTGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> NI05_Bradyrhizobium australiense
GTCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCTCGGAATCACTGGGCGTAAAGGGTGCGTAGGCGGGT
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AGGTGAGTGGAACTGCGAGTGTAGAGGTGAAATTCGTAGATATTCGCAAGAACACCAGTGGCGAAGGCGGCTCA
CTGGCCCGATACTGACGCTGAGGCACGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> RI01_Sphingomonas mali
GTCAGCAGCCGCGGTAATACGGAGGGAGCTAGCGTTATTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCT
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AGGTGAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTCA
CTGGACTGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC

> RI03_Dermacoccus nishinomiyaensis
GTCAGCAGCCGCGGTAATACGTAGGGTGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGAGCTTGTAGGCGGTC
TGTCGCGTCTGCTGTGAAAGACCGGGGCTTAACTCCGGTTCTGCAGTGGGTACGGGCAGACTAGAGTGTGGTAG
GGGAGACTGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGATGGCGAAGGCAGGTCT
CTGGGCCATTACTGACGCTGAGAAGCGAAAGCATGGGGAGCGAACAGGATTAGAT

> RI04_Pseudomonas glycinis
GCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTG
GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTATGGTAGAGGGTGGTGG
AATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGAT
ACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA

> RI05_Rhizobium daejeonense
GTCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGAT
ATTTAAGTCAGGGGTGAAATCCCAGAGCTCAACTCTGGAACTGCCTTTGATACTGGGTATCTTGAGTATGGAAG
AGGTAAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTTA
CTGGTCCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> RI07_Pseudomonas alliivorans
GTCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTT
TGTTAAGTTGAATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTAGGGCAG
AGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCAC
CTGGGCTCATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> RI08_Pseudomonas alliivorans
TNNGTCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTG
GTTTGTTAAGTTGAATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTAGGG
CAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGAC
CACCTGGGCTCATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACC

> RI12_Staphylococcus schweitzeri
GTCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTT
TTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAG
AGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTT
CTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACC

Appendix E

Genes contributing to plant growth-promoting (PGP) potential – SI01

Gene	Identity	Coverage (%)	E-value
pqqB	58.90	99.00	2.54E-120
pqqC	76.79	93.00	7.35E-134
pqqD	50.67	80.00	1.07E-19
pqqE	71.03	94.00	0
pqqF	25.43	21.00	9.8
pqqG	25.89	33.00	1.58E-04
phlA	25.00	51.00	7.26E-04
phlB	29.11	66.00	0.42
phlC	37.29	36.00	8.82E-04
phlD	25.18	40.00	1.4
hcnA	38.55	79.00	6.69E-12
hcnB	33.76	98.00	9.46E-49
hcnC	26.57	77.00	1.23E-18
budB	33.94	96.00	1.34E-101
budC	75.67	100.00	1.50E-151
nirK	24.39	51.00	1.09E-05
ipdC	22.73	96.00	4.59E-11
acdS	82.84	100.00	0.00E+00
nifD	34.43	52.00	1.9
nifH	44.44	12.00	7.83E-05
nifK	30.00	11.00	1.5

Genes contributing to plant growth-promoting (PGP) potential – SI02

Gene	**Identity**	**Coverage**	**E-value**
pqqB	58.90	99.00	2.54E-120
pqqC	76.79	93.00	7.35E-134
pqqD	50.67	80.00	1.07E-19
pqqE	71.03	94.00	0
pqqF	25.43	21.00	9.8
pqqG	25.89	33.00	1.58E-04
phlA	25.00	51.00	7.26E-04
phlB	29.11	66.00	0.42
phlC	37.29	36.00	8.82E-04
phlD	25.18	40.00	1.4
hcnA	38.55	79.00	6.69E-12
hcnB	33.76	98.00	9.46E-49
hcnC	26.57	77.00	1.23E-18
budB	33.94	96.00	1.34E-101
budC	75.67	100.00	1.50E-151
nirK	24.39	51.00	1.09E-05
ipdC	22.73	96.00	4.59E-11
acdS	82.84	100.00	0.00E+00
nifD	34.43	52.00	1.9
nifH	44.44	12.00	7.83E-05
nifK	30.00	11.00	1.5

Genes contributing to plant growth-promoting (PGP) potential – SI04

Gene	**Identity**	**Coverage (%)**	**E-value**
pqqB	58.90	99.00	1.82E-120
pqqC	76.34	93.00	2.76E-133
pqqD	50.67	80.00	3.38E-20
pqqE	71.03	94.00	0
pqqF	30.77	7.00	2.7
pqqG	26.44	35.00	1.22E-04
phlA	25.00	51.00	7.31E-04
phlB			
phlC	37.29	0.36	0.001
phlD			
hcnA	38.55	0.79	6.52E-12
hcnB	33.76	0.98	1.67E-47
hcnC	26.27	77.00	3.64E-18
budA	31.03	0.11	5.6
budB	33.94	96.00	3.53E-101
budC	75.67	100.00	3.12E-151
nirK	24.88	51.00	3.30E-06
ipdC	22.73	96.00	6.67E-11
acdS	82.84	100.00	0
nifD	34.43	52.00	1.8
nifH	44.44	0.12	7.89E-05
nifK	30.00	11.00	1.5

Genes contributing to plant growth-promoting (PGP) potential – SI05

Gene	Identity	Coverage (%)	E-value
pqqB	82.08	100.00	0
pqqC	86.55	99.00	1.56E-157
pqqD	44.44	87.00	4.35E-18
pqqE	87.60	98.00	0
pqqF	23.68	13.00	7.4
pqqG	29.94	24.00	3.51E-07
phlA	27.12	36.00	0.33
phlB	27.27	0.28	1.9
phlC	28.07	0.35	0.16
phlD	23.22	0.89	5.84E-09
hcnA	46.67	70.00	7.85E-10
hcnB	35.08	0.99	6.56E-54
hcnC	25.53	86.00	2.21E-22
budA	29.88	32.00	3.2
budB	32.96	94.00	4.50E-97
budC	58.49	100.00	1.79E-106
nirK	24.47	58.00	5.44E-06
ipdC	23.33	88.00	2.78E-13
acdS	83.14	0.99	0
nifD	40.00	21.00	0.39
nifH	44.44	12.00	3.82E-04
nifK	25.61	16.00	0.31

Genes contributing to plant growth-promoting (PGP) potential- SI06

Gene	**Identity**	**Coverage (%)**	**E-value**
pqqB	58.90	99.00	8.15E-120
pqqC	76.34	93.00	8.60E-127
pqqD	48.86	91.00	3.06E-21
pqqE	69.84	96.00	0
pqqF	28.06	18.00	0.81
pqqG	28.02	35.00	3.12E-07
phlA	25.00	51.00	2.08E-04
phlB	33.85	0.51	2.4
phlC	37.29	0.36	0.001
phlD	22.83	0.89	2.20E-07
hcnA	38.09	80.00	2.55E-12
hcnB	34.91	0.98	7.26E-51
hcnC	31.34	76.00	6.56E-27
budA	22.12	43.00	1.3
budB	34.13	96.00	2.13E-103
budC	98.48	100.00	0.00E+00
nirK	24.39	51.00	4.64E-06
ipdC	24.12	97.00	2.49E-20
acdS	82.54	0.99	0
nifD	33.33	40.00	3.1
nifH	44.44	12.00	6.76E-05
nifK	35.85	10.00	0.43

Genes contributing to plant growth-promoting (PGP) potential - SI07

Gene	Identity	Coverage (%)	E-value
pqqB	59.22	99.00	1.96E-121
pqqC	76.79	93.00	3.86E-134
pqqD	52.00	80.00	1.95E-20
pqqE	71.03	94.00	0
pqqF	30.77	7.00	2.7
pqqG	25.89	33.00	1.51E-04
phlA	25.00	51.00	7.37E-04
phlB			
phlC	37.29	0.36	0.001
phlD	29.23	0.18	7.5
hcnA	37.35	0.79	1.71E-11
hcnB	33.76	98.00	7.95E-48
hcnC	26.27	0.77	3.77E-18
budA	31.03	11.00	5.6
budB	33.94	96.00	3.56E-101
budC	75.66	100.00	2.29E-151
nirK	24.88	51.00	3.70E-06
ipdC	23.90	102.00	2.38E-16
acdS	82.84	100.00	0
nifD	34.43	0.52	1.8
nifH	44.44	12.00	7.96E-05
nifK	30.00	11.00	1.5

Genes contributing to plant growth-promoting (PGP) potential - SI08

Gene	Identity	Coverage (%)	E-value
pqqB	58.90	99.00	2.15E-120
pqqC	76.79	93.00	7.34E-134
pqqD	50.67	80.00	1.07E-19
pqqE	71.03	94.00	0
pqqF	25.43	21.00	9.8
pqqG	25.89	33.00	1.58E-04
phlA	25.00	51.00	7.25E-04
phlB	29.11	0.66	0.42
phlC	37.29	0.36	8.81E-04
phlD	25.18	0.39	1.4
hcnA	37.80	78.00	1.37E-08
hcnB	33.76	0.98	9.46E-49
hcnC	26.57	77.00	1.23E-18
budA			
budB	33.94	96.00	1.34E-101
budC	75.66	100.00	1.50E-151
nirK	24.39	51.00	1.09E-05
ipdC	22.73	96.00	4.59E-11
acdS	82.84	100.00	0
nifD	34.43	0.52	1.9
nifH	44.44	12.00	7.82E-05
nifK	30.00	11.00	1.5

Genes contributing to plant growth-promoting (PGP) potential - SI09

Gene	Identity	Coverage (%)	E-value
pqqB	58.90	99.00	2.54E-120
pqqC	76.79	93.00	7.35E-134
pqqD	50.67	80.00	1.07E-19
pqqE	71.03	94.00	0
pqqF	25.43	21.00	9.8
pqqG	25.89	33.00	1.59E-04
phlA	25.00	51.00	7.26E-04
phlB	29.11	0.66	0.42
phlC	37.29	0.36	8.82E-04
phlD	25.17	0.39	1.4
hcnA	37.80	78.00	1.38E-08
hcnB	33.76	0.98	9.47E-49
hcnC	26.57	77.00	1.23E-18
budA			
budB	33.94	96.00	1.34E-101
budC	75.66	100.00	1.50E-151
nirK	24.39	51.00	1.10E-05
ipdC	22.73	96.00	4.59E-11
acdS	82.84	100.00	0
nifD	34.43	0.52	1.9
nifH	44.44	12.00	7.83E-05
nifK	30.00	11.00	1.5