SEED-ASSOCIATED BACTERIAL ENDOPHYTES FROM TURF GRASSES PROMOTE SEEDLING GROWTH AND DEFEND PLANTS FROM DISEASE

By

QIANG CHEN

A dissertation submitted to the

School of Graduate Studies

Rutgers, The State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

Graduate Program in Plant Biology

Written under the direction of

James F. White, Jr.

And approved by

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New Brunswick, New Jersey

May 2020

ABSTRACT OF THE DISSERTATION

Seed-Associated Bacterial Endophytes from Turf Grasses

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Animals and plants harbor microbiomes providing benefits to hosts. In plants, both prokaryotic and eukaryotic microbiomes have been universally found and shown to promote plant growth, enhance disease resistance and abiotic stress tolerance. Fungal endophytes from grasses have been well characterized, but bacterial endophytes were rarely studied. This dissertation will present research that was conducted to test the hypothesis that cool-season turfgrass seeds bear bacterial endophytes providing beneficial effects on host, such as seed germination, growth promotion, and antifungal effects. Bacteria were isolated from seeds of different turfgrass species. Among them, *Bacillus amyloliquefaciens*, *Bacillus pumilus*, and *Pantoea agglomerans* were demonstrated to promote seed germination and seedling growth, and alter root architecture on host. *B. amyloliquefaciens* strains were shown to produce antifungal lipopeptides that suppress the growth of several fungal pathogens. Metagenomic analysis on the bacterial community associated with turfgrass seeds from low and high moisture climate revealed that moisture level influenced the community structure of the bacteria on/in turf seeds. The abundance of several bacterial groups at different taxonomy ranks on/in the seeds

was either positively or negatively correlated with the seed germination rate. Overall, data supported that bacterial endophytes inhabiting turfgrass seeds benefited host by promoting seed germination and seedling development, as well as providing antifungal compounds. Moreover, moisture level was found to affect the structure of bacterial community on/in turfgrass seed, which further influenced the seed germination.

DEDICATION

To my maternal grandmother, Xiuyun, Hu, and in memory of my maternal grandfather, Shenggui Jiang, my paternal grandparents, Jitai Chen and Yafen Wang.

ACKNOWLEDGEMENTS

I would like to thank everyone in White Lab at Rutgers University for their help in the past few years. I would particularly like to thank Dr. James F. White for providing large amounts of help in guiding my research, and being exceptionally flexible in my PhD career. I thank the Plant Biology program for accepting me into the program, and providing assistantship. I also would like to thank my parents for your years of love and sacrifices for me to finish my prolonged education. At last, I thank my girlfriend, Chengyi Dan, for your love and support.

It is acknowledged here that much of the research in Chapter 4 of this dissertation was published with some additions as:

Chen, Q., Meyer, W. A., Zhang, Q., & White, J. F. (2020). 16S rRNA metagenomic analysis of the bacterial community associated with turf grass seeds from low moisture and high moisture climates. *PeerJ, 8,* e8417. DOI: 10.7717/peerj.8417

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INTRODUCTION

This dissertation includes four chapters that review, discuss, and provide data in support of the hypothesis that turfgrass seeds harbor bacterial endophytes with beneficial properties. The research in this dissertation mainly focuses on the growth promotion and antifungal effects of the bacterial endophytes isolated from cool-season turfgrass seeds, and the metagenomic analysis of the bacterial community associated with turfgrass seeds.

Chapter 1 is an introductory chapter that summarizes the diversity and functions of plant endophytes from previous studies. This chapter also reviews the endophytes associated with turfgrasses and their effects on the host.

Chapter 2 reports on the isolation and identification of bacterial endophytes from turfgrass seeds, as well as their promotional effects on seed germination and seedling development.

Chapter 3 presents research on the antifungal effects and lipopeptides production of *Bacillus amyloliquefaciens* strains isolated from cool-season turfgrass seeds.

Chapter 4 presents a metagenomic analysis on the bacterial community associated with turfgrass seeds and how moisture level may affect the structure of the bacterial community.

Chapter 1 Diversity and Functions of Endophytes

1.1 Endophytes

1.1.1 Definition of Endophytes

In Merriam-Webster's dictionary, "endophyte" is defined as "an organism (such as bacterium or fungus) living within a plant". The word "endophyte" is composed of two parts, "endo-" derived from Greek word "endon" meaning "within", and "-phyte" derived from Greek word "phyton" meaning "plant". The history of endophyte study was previously reviewed by Hardoim *et al.* in 2015. As a summary, endophytes were first described as a group of partly parasitic fungi living in plants by the German botanist Heinrich Friedrich Link (he used "Entophytae") (Link, 1809). But back to the 19th century, people believed that healthy plants should be sterile as Pasteur and other well-known scientists claimed that plants are free of microbes (Compant *et al.*, 2012). However, in 1887, Galippe was the first scientist to report the isolation of different microorganisms from the interior tissues of various vegetable plants (Galippe, 1887). Since then, scientists began to report the presence of microorganisms living inside healthy plants (Hiltner, 1904).

Though both bacteria and fungi have been isolated from healthy plants, endophytes for a long time referred only to fungi which invade the stems and leaves of plants without causing any disease symptoms (Samish *et al.*, 1963, Mundt & Hinkle, 1976, Carroll, 1988). In 1995, Wilson defined endophytes as "fungi or bacteria which, for all or part of their life cycle, invade the tissues of living plants and cause unapparent and asymptomatic infections entirely within plant tissues but cause no symptoms of disease"

(Wilson, 1995). Two years later, Hallmann gave a practical statement that endophytes "can be isolated from surface-disinfested plant tissue or extracted from within the plant, and that do not visibly harm the plant" (Hallmann *et al.*, 1997). Nowadays, with the discovery that plants host other types of microbial endophytes like algae and protozoa (Trémouillaux-Guiller *et al.*, 2002, Müller & Döring, 2009), the definition of endophytes is widely accepted as all organisms that spend at least part of their life cycle inside plants without causing disease symptoms (Hardoim *et al.*, 2015).

1.1.2 Diversity of Endophytes

Prokaryotic Endophytes

Only a few prokaryotic endophytes were found to be archaea. So far, endophytic archaea have mainly been detected in coffee (Oliveira *et al.*, 2013), maize (Chelius & Triplett, 2001), *Phragmites* (Ma *et al.*, 2013), rice (Sun *et al.*, 2008), and sugarcane (Shi *et al.*, 2015). They were assigned to three phyla, Euryarchaeota, Thaumarchaeota, and Crenarchaeota. In Shi's study with next-generation high-throughput sequencing, the dominant archaeal phylum was related to Euryarchaeota (79.33%) (Shi *et al.*, 2015). A similar result was also reported in Oliveira's study on coffee that 80.8% of the endophytic archaea fell into phyla Euryarchaeota (Oliveira *et al.*, 2013). Also, a review article showed 23 of 29 endophytic archaea sequences in PubMed database (as of Mar. 2014) were reported to be Euryarchaeota (Hardoim *et al.*, 2015). Overall, most endophytic archaea belong to phylum Euryarchaeota, but compose only a small group of prokaryotic endophytes.

Most prokaryotic endophytes are bacteria. They mainly belong to three phyla, Actinobacteira, Firmicutes, and Proteobacteria (Hardoim *et al.*, 2015). According to previous studies and reviews, Gamma-Proteobacteria is the class containing the largest number of bacterial endophytes (Hardoim *et al.*, 2015). Most endophytic Gamma-Proteobacteria are mainly from several genera: *Acinetobacter*, *Enterobacter*, *Pantoea*, and *Pseudomonas* (Table 1). Notably, those genera also contain species known as phytopathogens (Bull *et al.*, 2010, Bull *et al.*, 2012, Bull *et al.*, 2014). For example, *Pseudomonas* genus also has the pathogen *Pseudonomas syringae* which causes disease in a wide range of plant species (Morris *et al.*, 2008). But several *Pseudomonas fluorescens* strains were reported to suppress fungal diseases (Duijff *et al.*, 1997, Daulagala & Allan, 2003, Suzuki *et al.*, 2003, Prieto & Mercado-Blanco, 2008). Similarly, *Acinetobacter*, *Enterobacter*, and *Pantoea* consist of bacteria described as pathogens along with bacteria that are beneficial to host plants (Bull *et al.*, 2010, Hardoim *et al.*, 2013, Sheibani-Tezerji *et al.*, 2015). Thus, the relationships of these genera of bacteria with their host plants range from pathogenesis to mutualism. Alpha-Proteobacteria is another Gram-negative bacteria class with a large number of bacterial endophytes. Most of them belong to the four genera *Rhizobium*, *Methylobacterium*, *Sphingomonas*, and *Bradyrhizobium* (Table 1.1). Among them, *Rhizobium* and *Bradyrhizobium* are well-known for their nitrogen-fixing symbiosis with legumes. Among endophytic Beta-Proteobacteria genera, *Burkholderia* is a genus with strains that can colonize different hosts and environments.

Endophytic Gram-positive bacteria fall into two phyla, Actinobacteria and Firmicutes. Phylum Actinobacteria contains varied endophytes from more than 100

genera, including *Streptomyces*, *Microbacterium*, *Mycobacterium*, *Arthrobacter*, and *Curtobacterium* (Table 1.1). *Streptomyces* species are known for the ability of producing antibiotic metabolites. Most of Firmicutes endophytes are in genera *Bacillus*, *Paenibacillus*, and *Staphylococcus* (Table 1.1). *Bacillus thuringiensis* is the most well-known for its production of insecticidal proteins.

In summary, most prokaryotic endophytes can be assigned to five phyla, but many genera. Their functions depend on the hosts they inhabit and the environment condition they are in.

Table 1.1 Prokaryotic endophytes discovered in plants.

Prokaryotic Endophytes	Host	References
Actinobacteria		
Corynebacterium flavescens	Rice	Bacilio-Jiménez *et al.*, 2001
Microbacterium sp.	Rape	Sheng *et al.*, 2008
Microbacterium sp.	*Pogonatherum paniceum*	Quambusch *et al.,* 2014
Micrococcus sp.	Rice	Okunishi *et al.*, 2005
Firmicutes		
Bacillus amyloliquefaciens	Tomato	Tian *et al.*, 2017
Bacillus anthracis	Tomato	Tian *et al.*, 2017
Bacillus aryabhattai	Tomato	Tian *et al.*, 2017
Bacillus bingmayongensis	Tomato	Tian *et al.*, 2017
Bacillus cereus	Tomato	Tian *et al.*, 2017
Bacillus megaterium	Maize, Alfalfa	Liu *et al.*, 2006, Stajković *et al.*, 2009
Bacillus methylotrophicus	Tomato	Tian *et al.*, 2017

Prokaryotic Endophytes	Host	References
Firmicutes		
Bacillus pumilus	Rice, Tomato	Bacilio-Jiménez *et al.*, 2001, Tian *et al.*, 2017
Bacillus sp.	Maize	Riggs *et al.*, 2001
Bacillus subtilis	Mulberry, Tomato, Banana	Ji *et al.*, 2008, Tian *et al.*, 2017, Souza *et al.*, 2014
Bacillus thuringiensis	Banana	Souza *et al.*, 2014
Staphylococcus epidermidis	Tomato	Tian *et al.*, 2017
Staphylococcus saprophyticus	Carrot	Surette *et al.*, 2003
Staphylococcus sp.	Tomato	Tian *et al.*, 2017
Alpha-Proteobacteria		
Acetobacter diazotrophicus	Sugarcane	Dong *et al.*, 1994
Azospirillum sp.	Maize	Riggs *et al.*, 2001
Gluconacetobacter diazotrophicus	Sugarcane, Maize	Riggs *et al.*, 2001, Rouws *et al.*, 2010
Ochrobactrum sp.	Rice	Verma *et al.*, 2004
Rhizobium pusense	Tomato	Tian *et al.*, 2017
Rhizobium sp.	Poaceae family	Patel & Archana, 2017
Beta-Proteobacteria		
Achromobacter sp.	Poaceae family	Patel & Archana, 2017
Azoarcus sp.	Kallar grass	Hurek *et al.*, 1994, Reinhold-Hurek *et al.*, 2006
Burkholderia cepacia	Maize, Tomato, Yellow lupine	Riggs *et al.*, 2001, Tian *et al.*, 2017, Barac *et al.*, 2004
Burkholderia phytofirmans	Onion	Compant *et al.*, 2005, Naveed *et al.*, 2014
Burkholderia vietnamiensis	Poplar, Rice, Sugarcane	Govindarajan *et al.*, 2006, Govindarajan *et al.*, 2008, Xin *et al.*, 2009

Prokaryotic Endophytes	Host	References
Beta-Proteobacteria		
Herbaspirillum seropediacae	Maize, Rice, Sorghum	Riggs *et al.*, 2001, James *et al.*, 2002, Brusamarello-Santos *et al.*, 2017
Herbaspirillum sp.	Rice	Elbeltagy *et al.*, 2001
Ralstonia sp.	Poaceae family	Patel & Archana, 2017
Gamma-Proteobacteria		
Acinetobacter sp.	Poaceae family	Patel & Archana, 2017
Citrobacter sp.	Banana	Martínez *et al.*, 2003
Enterobacter asburiae	Sweet potato	Asis & Adachi, 2004
Enterobacter ludwigii	Tomato	Tian *et al.*, 2017
Enterobacter sp.	Maize, Hybrid poplar, Tomato	Riggs *et al.*, 2001, Naveed *et al.*, 2014, Tian *et al.*, 2017
Erwinia sp.	Soybean	Kuklinsky-Sobral *et al.*, 2004
Klebsiella pneumonia	Maize	Dong *et al.*, 2003, Iniguez *et al.*, 2004
Klebsiella sp.	Maize	Riggs *et al.*, 2001
Pantoea agglomerans	Maize, Rice	Riggs *et al.*, 2001, Verma *et al.*, 2001
Pantoea sp.	Rice, Soybean	Verma *et al.*, 2004 Kuklinsky-Sobral *et al.*, 2004
Pseudomonas fluorescences	Silvergrass, Rape, Black nightshade, Wheat	Duijff *et al.*, 1997, Long *et al.*, 2008, Sheng *et al.*, 2008, Oteino *et al.*, 2015
Pseudomonas guariconensis	Tomato	Tian *et al.*, 2017
Pseudomonas mohnii	Tomato	Tian *et al.*, 2017
Pseudomonas plecoglossicida	Tomato	Tian *et al.*, 2017
Pseudomonas putida	Hybrid poplar, Potato, Poplar	Germaine *et al.*, 2006, Andreote *et al.*, 2009, Khan *et al.*, 2014

Prokaryotic Endophytes	Host	References
Gamma-Proteobacteria		
Pseudomonas sp.	Black pepper	Aravind *et al.*, 2009
Pseudomonas thivervalensis	Black nightshade	Long *et al.*, 2008
Rahnella aquatilis	Sweet potato	Khan & Doty, 2009
Serratia marcescens	Rice	Gyaneshwar *et al.*, 2001
Stenotrophomonas rhizophila	Tomato	Tian *et al.*, 2017

Eukaryotic Endophytes

Though some alga and protozoa species have been reported as endophytes (Trémouillaux-Guiller *et al.*, 2002, Müller & Döring, 2009), most eukaryotic endophytes are identified to be fungi that belong to three phyla, Ascomycota, Basidiomycota, and Glomeromycota (Table 1.2). There are also some eukaryotic endophytes remaining unidentified. Most Ascomycota endophytes belong to the class Dothideomycetes (Table 1.2). Notably, Dothideomycetes also possesses many necrotrophic plant pathogens that produce host-specific toxins (Stergiopoulos *et al.*, 2012). Another class, Sordariomycetes, is also composed of both endophytic and pathogenic members (Table 1.2). Endophytic examples are *Balansia*, *Epichloë* , *Nemania*, and *Xylaria*. Among them, genus *Epichloë* is well-known for forming endophytic symbiosis with grasses (White & Cole, 1985, White, 1987, Clay, 1989, Meyer *et al.*, 2012). Sordariomycetes also includes some phytopathogens such as *Fusarium* and *Verticillium* species (Table 1.2).

In Basidiomycota, a large number of endophytes belong to the class Agaricomycetes (Table 1.2). Some *Sebacinales* endophytes in this class form the

beneficial ectomycorrhizal symbiosis with the roots of a wide range of plants like families Orchidaceae and Ericaceae (Weiss *et al.*, 2004). Other endophytes-containing classes include Atractiellomycetes, Microbotryomycetes, and Tremellomycetes (Table 1.2).

The phylum Glomeromycota has only one class, Glomeromycetes (Table 1.2). The endophytes in Glomeromycetes are well-known as arbuscular mycorrhizal fungi that form arbuscular mycorrhizas (AMs) associated with roots or thalli of land plants. AM is a widespread terrestrial symbiosis between land plants and Glomeromycota fungi. AM fungi are ecologically important because they help plant hosts absorb nutrients like nitrogen, sulfur, phosphates and micronutrients from soil.

In both bacterial and fungal endophytes, there are many taxa containing endophytes and phytopathogens, as well as some strains with unknown effects on plants host. It suggests that the functions of endophytes, both bacteria and fungi, should not be linked to their taxa.

Table 1.2 Eukaryotic endophytes discovered in plants.

Eukaryotic Endophytes	Host	References
Ascomycota		
Acremonium alternatum	Chinese yew	Larran *et al.*, 2001, Liu *et al.*, 2009
Alternaria alternata	Chinese yew, Camphor tree, Tomato, Wheat	Larran *et al.*, 2001 & 2002, Liu *et al.*, 2009, He *et al.*, 2012
Aspergillus flavus	Camphor tree	He *et al.*, 2012
Aspergillus niger	Camphor tree	He *et al.*, 2012
Botryosphaeria obtusa	Chinese yew	Liu *et al.*, 2009

Eukaryotic Endophytes	Host	References
Ascomycota		
Cladosporium cladosporioides	Barbary fig, Camphor tree	Bezerra *et al.*, 2012, He *et al.*, 2012
Cladosporium herbarum	Wheat	Larran *et al.*, 2002
Cladosporium sphaerospermum	Barbary fig	Bezerra *et al.*, 2012
Colletotrichum gloeosporioides	Camphor tree, Tomato	Larran *et al.*, 2001, He *et al.*, 2012
Diaporthe eres	Chinese yew	Liu *et al.*, 2009
Epichloe typhina	Grasses	White Jr, 1987
Epicoccum nigrum	Wheat	Larran *et al.*, 2002
Fusarium solani	Chinese yew	Liu *et al.*, 2009
Glomerella cingulata	Camphor tree	He *et al.*, 2012
Paraconiothyrium brasiliense	Chinese yew	Liu *et al.*, 2009
Basidiomycota		
Coprinellus sp.	Mountain cocoa	Thomas *et al.*, 2008
Ganoderma sp.	Mountain cocoa	Thomas *et al.*, 2008
Grammothele lineata	Jute mellow	Das *et al.*, 2017
Lentinus sp.	Mountain cocoa	Thomas *et al.*, 2008
Polyporaceae species	Mountain cocoa	Thomas *et al.*, 2008
Pycnoporus sanguineus	Oil palm	Rungjindamai *et al.*, 2008
Rhodotorula rubra	Wheat	Larran *et al.*, 2002
Schizophyllum commune	Oil palm	Rungjindamai *et al.*, 2008
Trametes elegans	Oil palm	Rungjindamai *et al.*, 2008
Zygomycota		
Rhizopus oryzae	Chinese yew	Liu *et al.*, 2009
Glomeromycota		
Gigaspora margarita	Carrot	Bécard & Fortin, 1988
Glomus caledonium	Carrot	Karandashov *et al.*, 2000
Glomus fasciculatum	Carrot	Declerck *et al.*, 1998
Glomus intraradices	Tomato, Carrot	Bago *et al.*, 1996, Declerck *et al.*, 1998

Eukaryotic Endophytes	Host	References
Glomeromycota		
Glomus mosseae	Red Clover	Mosse & Hepper, 1975
Glomus versiforme	Tomato	Bago *et al.*, 1996
Others		
Coccomyxa sp.	Ginkgo	Trémouillaux-Guiller *et al.*, 2002
Protozoa	Five-leaf aralia	Müller & Döring, 2009

1.1.3 Functions of Endophytes

Based on the definition of endophytes, not all endophytes provide beneficial effects to plant hosts. Those endophytes that have no obvious impact on plant but rely on the host for living are called commensal endophytes. Other endophytes benefit host in many ways, such as protection against pathogens and herbivores, providing nutrients, and stimulating plant growth. Under certain conditions, endophytes with no effects can turn to either beneficial or pathogenic. For instance, endophytic fungus in maize, *Fusarium verticillioides,* can be either symptomless or pathogens (Bacon *et al.*, 2008). The alteration depends on biotic factors such as plant genetics as well as abiotic factor like temperature and moisture (Bacon *et al.*, 2008).

Growth Stimulation

Endophytes promote plant growth via many ways (Clay, 1988, Long *et al.*, 2008). The best-described mechanism is probably that endophytes produce phytohormones (Shi *et al.*, 2009, Khan *et al.*, 2012). The production of auxin has been reported in many studies (Bastián *et al.*, 1998, Suzuki *et al.*, 2003, Shi *et al.*, 2009, Merzaeva & Shirokikh,

2010, Khan *et al.*, 2012). In Suzuki's study, it is suggested that indole-3-acetic acid (IAA) production by *Pseudomonas fluorescens* HP72 increased the plant colonization by endophytes but constructed short root systems as a drawback (Suzuki *et al.*, 2003). The production of cytokinin and gibberellins was also observed (Bastián *et al.*, 1998, Vadassery *et al.*, 2008, Khan *et al.*, 2012). The study on *Piriformospora indica* showed that the deletion of cytokinin biosynthesis genes lead to the loss of plant growth promoting effects (Vadassery *et al.*, 2008).

Another way of stimulating plant growth by endophytes is suggested to be related to photosynthesis. *Burkholderia phytofirmans* strain PsJN increased the CO_2 assimilation, transpiration rate, chlorophyll content, and photosynthetic rate on wheat with and without drought stress (Naveed *et al.*, 2014). Another study revealed that *Neotyphodium lolii* increased CO_2 fixation but not light interception and photochemistry (Spiering *et al.*, 2006).

Besides plant hormones production and interference in photosynthesis process, there are also other mechanisms involved. For example, endophytes can produce volatile compounds, such as 3-hydroxy-2-butanone and 2,3-butanediol, to promote plant growth (Ryu *et al.*, 2003, Ryu *et al.*, 2005,). Polyamines produced by two *Azospirillum brasilense* strains affect plant growth (Perrig *et al.*, 2007).

Nitrogen Fixation

Nitrogen fixation is a well-studied mechanism of plant-microbes interaction. *Gluconacetobacter diazotrophicus* is an endophytic bacterium originally isolated from sugarcane that fixes nitrogen (Dong *et al.*, 1994). Other *G. diazotrophicus* strains were

also found in rice (Meneses *et al.*, 2017) and pine needles (Carrell & Frank, 2014). It suggests that *G. diazotrophicus* strains may be able to fix nitrogen in a wide range of plant host. Therefore, this bacteria species can potentially be used as nitrogen resources in agriculture. Another nitrogen-fixing endophytic bacterium is *Paenibacillus* sp. strain P22, which was isolated from poplar plants (Scherling *et al.*, 2009). Nitrogen fixation ability is important for plant fitness, especially under low nitrogen environment.

Protection Against Abiotic Stress

Some endophytes are able to protect host plants against abiotic stress like drought, salinity and cold. Fungal endophytes, asexual *Epichloë* spp., formerly *Neotyphodium* spp., from grasses were shown to increase drought tolerance, and protect plants from water stress and nitrogen deficiency (Meyer *et al.*, 2012). Another fungal endophyte, *Piriformospora indica*, was found to enhance the salt tolerance in barley by up-regulating antioxidant activities in roots (Baltruschat *et al.*, 2008). Another study on *P. indica* indicated that this endophyte could also protect Chinese cabbage against drought stress by increasing the activities of antioxidant enzymes (Sun *et al.*, 2010). The well-studied bacterial endophyte *Burkholderia phytofirmans* strain PsJN was shown to enhance the drought tolerance in wheat and maize as well as increasing the chilling tolerance of grapevine (Barka *et al.*, 2006, Naveed *et al.*, 2014, Naveed *et al.*, 2014).

Protection Against Biotic Stress

Endophytes protect plant hosts against biotic stress mainly in two ways, inducing systemic resistance and producing antibiotic compounds. Induced systemic resistance

(ISR) is a type of plant defenses against pathogens induced by plant growth-promoting bacteria (PGPB). Unlike systemic acquired resistance (SAR) which involves the pathways regulated by salicylic acids, ISR depends on the pathways regulated by jasmonic acid and ethylene (Vallad & Goodman, 2004). Some *Pseudomonas* and *Bacillus* strains were identified to be the most common bacteria that produced elicitors triggering ISR (Kloepper *et al.*, 2004, Kloepper & Ryu, 2006). Elicitors include but are not limited to flagellins, lipopolysaccharides, volatiles and antibiotics (Heil & Bostock, 2002, Vallad & Goodman, 2004, Kloepper & Ryu, 2006). *Bacillus pumilus* strain SE34 and *Pseudomonas fluorescens* strain 89B61 were shown to elicit ISR against late blight on tomato (Yan *et al.*, 2002). Though the definition of ISR limits the elicitors to bacteria, some fungal endophytes were also reported to induce the systemic resistance (Vu *et al.*, 2006, Bae *et al.*, 2011). For example, *Fusarium oxysporum* was shown to induce systemic resistance on banana against burrowing nematode, *Radopholus similis* (Vu *et al.*, 2006).

Besides inducing systemic resistance, bacterial endophytes can also release antimicrobial compounds to protect hosts against pathogens. *Bacillus amyloliquefaciens* strain Bg-C31 was shown to protect *Capsicum* from bacterial wilt by secreting an antagonistic protein (Hu *et al.*, 2010). Another endophytic bacterial strain, *Bacillus mojavensis* strain RRC101, produced surfactin, an antifungal compound which is toxic to *Fusarium verticillioides*, a fungal pathogen on maize (Snook *et al.*, 2009). Surfactin is one type of lipopeptide, which has been found to be a versatile weapon to control plant disease (Ongena & Jacques, 2008).

Fungal endophytes are able to protect hosts by producing compounds that are antimicrobial or inhibitory to herbivores (White & Torres, 2010). These antagonistic compounds include alkaloids, steroids, peptides, flavonoids, etc. The best studied compounds are probably alkaloids produced by certain grass endophytes. Tremorgenic neurotoxins have been reported to be produced in endophyte-infected ryegrass and cause ryegrass staggers disorder of many herbivorous animals, e.g. sheep, cattle and horse (Fletcher & Harvey, 1981, Gallagher *et al.*, 1984).

1.2 Association of Turfgrass and Endophytes

1.2.1 Colonization of Fungal Endophytes on Turfgrass

Many grass species form mutualistic symbiosis with fungal endophytes from Clavicipitaceae family (White Jr, 1987, Clay, 1988). Grass-associated fungal endophytes mainly belong to five genera: *Atkinsonella*, *Balansia*, *Balansiopsis*, *Epichloë*, and *Myriogenospora* (Clay, 1988). Among them, *Epichloë* (formerly, anamorphs were in genus *Neotyphodium*) is probably the best-studied genus associated with turfgrass (Meyer *et al.*, 2012). Genus *Epichloë* includes beneficial endophytes as well as pathogens. Some *Epichloë* species completely lost the sexual life cycle, cause no symptom on host grasses, and disperse with grass seeds. For other cases, the fungal endophytes may cause choke disease by forming stromata and suppressing inflorescence development of host (Meyer *et al.*, 2012).

1.2.2 Function of Fungal Endophytes

Herbivore Deterrence

The mutualistic symbiosis formed between grasses and fungi provides defensive effects to host by producing alkaloids (White & Torres, 2010, Meyer *et al.*, 2012, Saikkonen *et al.*, 2013). Those biologically active alkaloids include ergot alkaloids, lolines, peramines and lolitrems, which can be toxic to insects or mammals (Rodriguez *et al.*, 2009). Lolitrems and ergot alkaloids exhibit higher toxicity to mammals than lolines and peramines. However, lolines and peramines are more toxic to insects. Lolines and peramines can also reduce the the barley yellow dwarf viruses (BYDVs) disease on hosts by deterring the consumption of sucking insects (Mahmood *et al.*, 1993, Lehtonen *et al.*, 2006).

Disease Resistance

Fungal endophytes in grasses also provide the grass disease resistance to hosts (Kuldau & Bacon, 2008). *In vitro* studies have shown that some fungal endophytes suppressed grass pathogens by producing antibiotics and degradative enzymes (White & Cole, 1985, Siegel & Latch, 1991). For example, fungitoxic phenolic compounds produced by *Epichloë typhina* were shown to be defensive in diseased timothy plant *Phleum pratense* (Koshino *et al.*, 1988). Compared to endophyte-free cultivars of Blue fescue, Chewings fescue, hard fescue, and strong creeping red fescue, related cultivars infected with fungal endophytes showed suppression of dollar spot caused by *Sclerotinia homoeocarpa* (Clarke *et al.*, 2006). In Clarke's study, the proposed mechanism of disease

resistance in endophytes-infected plants was due to the production of alkaloids, phytoalexins, or some novel compounds by endophytes.

Abiotic Stress Tolerance

Drought tolerance has been demonstrated in several studies (Bush *et al.*, 1997, Lewis *et al.*, 1997, Lewis, 2004). In Bush's study, lolines produced by fungal endophytes have been found to alter osmotic potential, thus providing tolerance to drought stress. Bacterial endophytes also showed the stress tolerance effects on hosts. Four bacterial strains in genera *Sphingomonas*, *Pantoea*, *Bacillus* and *Enterobacter*, isolated from elephant grass (*Pennisetum purpureum*) decreased osmotic stress caused by increased salinity levles (Li *et al.*, 2016).

In summary, all plants harbor endophytes that play important roles in plant development and fitness. These endophytes can potentially be utilized as biostimulants in agricultural industry.

1.3 References

Andreote, F. D., de Araujo, W. L., de Azevedo, J. L., van Elsas, J. D., da Rocha, U. N., & van Overbeek, L. S. (2009). Endophytic colonization of potato (*Solanum tuberosum* L.) by a novel competent bacterial endophyte, *Pseudomonas putida* strain P9, and its effect on associated bacterial communities. *Applied and Environmental Microbiology, 75*(11), 3396-3406.

Aravind, R., Kumar, A., Eapen, S., & Ramana, K. (2009). Endophytic bacterial flora in root and stem tissues of black pepper (*Piper nigrum* L.) genotype: isolation, identification and evaluation against *Phytophthora capsici. Letters in Applied Microbiology, 48*(1), 58-64.

Asis, C., & Adachi, K. (2004). Isolation of endophytic diazotroph *Pantoea agglomerans* and nondiazotroph *Enterobacter asburiae* from sweetpotato stem in Japan. *Letters in Applied Microbiology, 38*(1), 19-23.

Bacilio-Jiménez, M., Aguilar-Flores, S., del Valle, M. V., Pérez, A., Zepeda, A., & Zenteno, E. (2001). Endophytic bacteria in rice seeds inhibit early colonization of roots by *Azospirillum brasilense*. *Soil Biology and Biochemistry, 33*(2), 167-172.

Bacon, C., Glenn, A., & Yates, I. (2008). *Fusarium verticillioides*: managing the endophytic association with maize for reduced fumonisins accumulation. *Toxin Reviews, 27*(3-4), 411-446.

Bae, H., Roberts, D. P., Lim, H.-S., Strem, M. D., Park, S.-C., Ryu, C.-M., Melnick, R. L., & Bailey, B. A. (2011). Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms. *Molecular Plant-Microbe Interactions, 24*(3), 336-351.

Bago, B., Vierheilig, H., Piché, Y., & Azcon-Aguilar, C. (1996). Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. *New Phytologist, 133*(2), 273-280.

Baltruschat, H., Fodor, J., Harrach, B. D., Niemczyk, E., Barna, B., Gullner, G., Janeczko, A., Kogel, K.-H., Schäfer, P., & Schwarczinger, I. (2008). Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New Phytologist, 180*(2), 501-510.

Barac, T., Taghavi, S., Borremans, B., Provoost, A., Oeyen, L., Colpaert, J. V., Vangronsveld, J., & Van Der Lelie, D. (2004). Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. *Nature Biotechnology, 22*(5), 583.

Barka, E. A., Nowak, J., & Clément, C. (2006). Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. *Applied and Environmental Microbiology, 72*(11), 7246-7252.

Bastián, F., Cohen, A., Piccoli, P., Luna, V., Bottini, R., & Baraldi, R. (1998). Production of indole-3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media. *Plant Growth Regulation, 24*(1), 7-11.

Bécard, G., & Fortin, J. A. (1988). Early events of vesicular–arbuscular mycorrhiza formation on Ri T-DNA transformed roots. *New Phytologist, 108*(2), 211-218.

Bezerra, J., Santos, M., Svedese, V., Lima, D., Fernandes, M., Paiva, L., & Souza-Motta, C. (2012). Richness of endophytic fungi isolated from *Opuntia ficus-indica* Mill.(Cactaceae) and preliminary screening for enzyme production. *World Journal of Microbiology and Biotechnology, 28*(5), 1989-1995.

Brusamarello-Santos, L. C., Gilard, F., Brulé, L., Quilleré, I., Gourion, B., Ratet, P., de Souza, E. M., Lea, P. J., & Hirel, B. (2017). Metabolic profiling of two maize (*Zea mays* L.) inbred lines inoculated with the nitrogen fixing plant-interacting

bacteria *Herbaspirillum seropedicae* and *Azospirillum brasilense*. *PLoS One, 12*(3), e0174576. doi:10.1371/journal.pone.0174576

Bull, C. T., Coutinho, T. A., Denny, T. P., Firrao, G., Fischer-Le Saux, M., Li, X., Saddle, G. S., Scortichini, M., Stead, D. E., & Takikawa, Y. (2014). List of new names of plant pathogenic bacteria (2011-2012). *Journal of Plant Pathology, 96*(2), 223-226.

Bull, C. T., de Boer, S. H., Denny, T. P., Firrao, G., Fischer-Le Saux, M., Saddler, G. S., Scortichini, M., Stead, D. E., & Takikawa, Y. (2012). List of new names of plant pathogenic bacteria (2008-2010). *Journal of Plant Pathology, 94*(1), 21-27.

Bull, C. T., de Boer, S. H., Denny, T. P., Firrao, G., Fischer-Le Saux, M., Saddler, G. S., Scortichini, M., Stead, D. E., & Takikawa, Y. (2010). Comprehensive list of names of plant pathogenic bacteria, 1980-(2007). *Journal of Plant Pathology*, 551-592.

Bush, L. P., Wilkinson, H. H., & Schardl, C. L. (1997). Bioprotective alkaloids of grass-fungal endophyte symbioses. *Plant Physiology, 114*(1), 1.

Carrell, A. A., & Frank, A. C. (2014). *Pinus flexilis* and *Picea engelmannii* share a simple and consistent needle endophyte microbiota with a potential role in nitrogen fixation. *Frontiers in Microbiology, 5*, 333.

Carroll, G. (1988). Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology, 69*(1), 2-9.

Chelius, M. K., & Triplett, E. W. (2001). The diversity of archaea and bacteria in association with the roots of *Zea mays* L. *Microbial Ecology, 41*(3), 252-263.

Clarke, B. B., White, J. F., Hurley, R. H., Torres, M. S., Sun, S., & Huff, D. R. (2006). Endophyte-mediated suppression of dollar spot disease in fine fescues. *Plant Disease, 90*(8), 994-998.

Clay, K. (1988). Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology, 69*(1), 10-16.

Clay, K. (1989). Clavicipitaceous endophytes of grasses: their potential as biocontrol agents. *Mycological Research, 92*(1), 1-12.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clement, C., & Ait Barka, E. (2005). Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Applied and Environmental Microbiology, 71*(4), 1685-1693.

Compant, S., Sessitsch, A., & Mathieu, F. (2012). The 125th anniversary of the first postulation of the soil origin of endophytic bacteria–a tribute to MLV Galippe. *Plant and Soil, 356*(1-2), 299-301.

Das, A., Rahman, M. I., Ferdous, A. S., Rahman, M. M., Nahar, N., Uddin, M. A., Islam, M. R., & Khan, H. (2017). An endophytic Basidiomycete, *Grammothele lineata*, isolated from *Corchorus olitorius*, produces paclitaxel that shows cytotoxicity. *PLoS One, 12*(6), e0178612.

Daulagala, P. W. H. K. P., & Allan, E. J. (2003). L-form bacteria of *Pseudomonas syringae* pv. *phaseolicola* induce chitinases and enhance resistance to *Botrytis cinerea* infection in Chinese cabbage. *Physiological and Molecular Plant Pathology, 62*(5), 253-263.

Declerck, S., Strullu, D. G., & Plenchette, C. (1998). Monoxenic culture of the intraradical forms of *Glomus* sp. isolated from a tropical ecosystem: a proposed methodology for germplasm collection. *Mycologia*, 579-585.

Dong, Y., Iniguez, A. L., Ahmer, B. M. M., & Triplett, E. W. (2003). Kinetics and strain specificity of rhizosphere and endophytic colonization by enteric bacteria on seedlings of *Medicago sativa* and *Medicago truncatula*. *Applied and Environmental Microbiology, 69*(3), 1783-1790.

Dong, Z., Canny, M. J., McCully, M. E., Roboredo, M. R., Cabadilla, C. F., Ortega, E., & Rodes, R. (1994). A nitrogen-fixing endophyte of sugarcane stems (a new role for the apoplast). *Plant Physiology, 105*(4), 1139-1147.

Doty, S. L., Freeman, J. L., Cohu, C. M., Burken, J. G., Firrincieli, A., Simon, A., Khan, Z., Isebrands, J. G., Lukas, J., & Blaylock, M. J. (2017). Enhanced degradation of TCE on a superfund site using endophyte-assisted poplar tree phytoremediation. *Environmental Science & Technology, 51*(17), 10050-10058.

Duijff, B. J., Gianinazzi-Pearson, V., & Lemanceau, P. (1997). Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol *Pseudomonas fluorescens* strain WCS417r. *The New Phytologist, 135*(2), 325-334.

Elbeltagy, A., Nishioka, K., Sato, T., Suzuki, H., Ye, B., Hamada, T., Isawa, T., Mitsui, H., & Minamisawa, K. (2001). Endophytic colonization and in planta nitrogen fixation by a *Herbaspirillum* sp. isolated from wild rice species. *Applied and Evnironmental Microbiology, 67*(11), 5285-5293. doi:10.1128/AEM.67.11.5285-5293.2001

Fletcher, L., & Harvey, I. (1981). An association of a *Lolium* endophyte with ryegrass staggers. *New Zealand veterinary journal, 29*(10), 185-186.

Galippe, V. (1887). Note sur la présence de micro-organismes dans les tissus végétaux. *Comptes Rendus Hebdomadaires des Séances et Mémoires de la Société de Biologie et des ses Filiales et Associées, 39*, 410-416.

Gallagher, R. T., Hawkes, A. D., Steyn, P. S., & Vleggaar, R. (1984). Tremorgenic neurotoxins from perennial ryegrass causing ryegrass staggers disorder of livestock: structure elucidation of lolitrem B. *Journal of the Chemical Society, chemical communications*(9), 614-616.

Germaine, K. J., Liu, X., Cabellos, G. G., Hogan, J. P., Ryan, D., & Dowling, D. N. (2006). Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. *FEMS Microbiology Ecology, 57*(2), 302-310.

Govindarajan, M., Balandreau, J., Kwon, S. W., Weon, H. Y., & Lakshminarasimhan, C. (2008). Effects of the inoculation of *Burkholderia vietnamensis* and related

endophytic diazotrophic bacteria on grain yield of rice. *Microbial Ecology, 55*(1), 21-37.

Govindarajan, M., Balandreau, J., Muthukumarasamy, R., Revathi, G., & Lakshminarasimhan, C. (2006). Improved yield of micropropagated sugarcane following inoculation by endophytic *Burkholderia vietnamiensis*. *Plant and Soil, 280*(1-2), 239-252.

Gyaneshwar, P., James, E. K., Mathan, N., Reddy, P. M., Reinhold-Hurek, B., & Ladha, J. K. (2001). Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. *Journal of Bacteriology, 183*(8), 2634-2645.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W., & Kloepper, J. (1997). Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology, 43*(10), 895-914.

Hardoim, P. R., Nazir, R., Sessitsch, A., Elhottová, D., Korenblum, E., van Overbeek, L. S., & van Elsas, J. D. (2013). The new species *Enterobacter oryziphilus* sp. nov. and *Enterobacter oryzendophyticus* sp. nov. are key inhabitants of the endosphere of rice. *BMC Microbiology, 13*(1), 1.

Hardoim, P. R., van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews, 79*(3), 293-320.

He, X., Han, G., Lin, Y., Tian, X., Xiang, C., Tian, Q., Wang, F., & He, Z. (2012). Diversity and decomposition potential of endophytes in leaves of a *Cinnamomum camphora* plantation in China. *Ecological Research, 27*(2), 273-284.

Heil, M., & Bostock, R. M. (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. *Annals of Botany, 89*(5), 503-512.

Hiltner, L. t. (1904). Ober neuter erfahrungen und probleme auf dem gebiete der bodenbakteriologie unter besonderer berucksichtigung der grundungung und brache. *Arbeiten der Deutshen Landwirschafthchen Gesellschaft, 98*, 59-78.

Hu, H. Q., Li, X. S., & He, H. (2010). Characterization of an antimicrobial material from a newly isolated *Bacillus amyloliquefaciens* from mangrove for biocontrol of Capsicum bacterial wilt. *Biological Control, 54*(3), 359-365.

Hurek, T., Reinhold-Hurek, B., Van Montagu, M., & Kellenberger, E. (1994). Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. *Journal of Bacteriology, 176*(7), 1913-1923.

Iniguez, A. L., Dong, Y., & Triplett, E. W. (2004). Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342. *Molecular Plant-Microbe Interactions, 17*(10), 1078-1085.

James, E. K., Gyaneshwar, P., Mathan, N., Barraquio, W. L., Reddy, P. M., Iannetta, P. P., . . . Ladha, J. K. (2002). Infection and colonization of rice seedlings by the plant growth-promoting bacterium Herbaspirillum seropedicae Z67. *Molecular Plant-Microbe Interactions, 15*(9), 894-906.

Ji, X., Lu, G., Gai, Y., Zheng, C., & Mu, Z. (2008). Biological control against bacterial wilt and colonization of mulberry by an endophytic Bacillus subtilis strain. *FEMS Microbiology Ecology, 65*(3), 565-573.

Johnston-Monje, D., & Raizada, M. N. (2011). Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS One, 6*(6), e20396.

Karandashov, V., Kuzovkina, I., Hawkins, H.-J., & George, E. (2000). Growth and sporulation of the arbuscular mycorrhizal fungus *Glomus caledonium* in dual culture with transformed carrot roots. *Mycorrhiza, 10*(1), 23-28.

Khan, A. L., Hamayun, M., Kang, S.-M., Kim, Y.-H., Jung, H.-Y., Lee, J.-H., & Lee, I.-J. (2012). Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. *BMC Microbiology, 12*(1), 3.

Khan, Z., & Doty, S. L. (2009). Characterization of bacterial endophytes of sweet potato plants. *Plant and Soil, 322*(1-2), 197-207.

Khan, Z., Roman, D., Kintz, T., delas Alas, M., Yap, R., & Doty, S. (2014). Degradation, phytoprotection and phytoremediation of phenanthrene by endophyte *Pseudomonas putida*, PD1. *Environmental Science & Technology, 48*(20), 12221-12228. doi:10.1021/es503880t

Kloepper, J. W., & Ryu, C.-M. (2006). Bacterial endophytes as elicitors of induced systemic resistance. In *Microbial root endophytes* (pp. 33-52): Springer.

Kloepper, J. W., Ryu, C.-M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology, 94*(11), 1259-1266.

Koshino, H., Terada, S. I., Yoshihara, T., Sakamura, S., Shimanuki, T., Sato, T., & Tajimi, A. (1988). Three phenolic acid derivatives from stromata of *Epichloë typhina* on *Phleum pratense*. *Phytochemistry, 27*(5), 1333-1338.

Kuklinsky-Sobral, J., Araújo, W. L., Mendes, R., Geraldi, I. O., Pizzirani-Kleiner, A. A., & Azevedo, J. L. (2004). Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environmental Microbiology, 6*(12), 1244-1251.

Kuldau, G., & Bacon, C. (2008). Clavicipitaceous endophytes: Their ability to enhance resistance of grasses to multiple stresses. *Biological Control, 46*(1), 57-71.

Larran, S., Monaco, C., & Alippi, H. (2001). Endophytic fungi in leaves of *Lycopersicon esculentum* Mill. *World Journal of Microbiology and Biotechnology, 17*(2), 181-184.

Larran, S., Perello, A., Simon, M., & Moreno, V. (2002). Isolation and analysis of endophytic microorganisms in wheat (*Triticum aestivum* L.) leaves. *World Journal of Microbiology and Biotechnology, 18*(7), 683-686.

Lehtonen, P. T., Helander, M., Siddiqui, S. A., Lehto, K., & Saikkonen, K. (2006). Endophytic fungus decreases plant virus infections in meadow ryegrass (*Lolium pratense*). *Biology Letters, 2*(4), 620-623.

Lewis, G. (2004). Effects of biotic and abiotic stress on the growth of three genotypes of *Lolium perenne* with and without infection by the fungal endophyte *Neotyphodium lolii*. *Annals of Applied Biology, 144*(1), 53-63.

Lewis, G., Ravel, C., Naffaa, W., Astier, C., & Charmet, G. (1997). Occurrence of *Acremonium* endophytes in wild populations of *Lolium* spp. in European countries and a relationship between level of infection and climate in France. *Annals of Applied Biology, 130*(2), 227-238.

Li, X., Geng, X., Xie, R., Fu, L., Jiang, J., Gao, L., & Sun, J. (2016). The endophytic bacteria isolated from elephant grass (*Pennisetum purpureum* Schumach) promote plant growth and enhance salt tolerance of Hybrid Pennisetum. *Biotechnology for Biofuels, 9*(1), 190.

Link, H. F. (1809). *Observationes in ordines plantarum naturales: Dissertatio Ima complectens anandrarum ordines epiphytas, mucedines gastromycos et fungos*.

Liu, K., Ding, X., Deng, B., & Chen, W. (2009). Isolation and characterization of endophytic taxol-producing fungi from *Taxus chinensis*. *Journal of Industrial Microbiology & Biotechnology, 36*(9), 1171.

Liu, X., Zhao, H., & Chen, S. (2006). Colonization of maize and rice plants by strain *Bacillus megaterium* C4. *Current Microbiology, 52*(3), 186-190.

Long, H. H., Schmidt, D. D., & Baldwin, I. T. (2008). Native bacterial endophytes promote host growth in a species-specific manner; phytohormone manipulations do not result in common growth responses. *PLoS One, 3*(7), e2702.

Ma, B., Lv, X., Warren, A., & Gong, J. (2013). Shifts in diversity and community structure of endophytic bacteria and archaea across root, stem and leaf tissues in the common reed, *Phragmites australis*, along a salinity gradient in a marine tidal wetland of northern China. *Antonie Van Leeuwenhoek, 104*(5), 759-768.

Mahmood, T., Gergerich, R., Milus, E., West, C., & D'arcy, C. (1993). Barley yellow dwarf viruses in wheat, endophyte-infected and endophyte-free tall fescue, and other hosts in Arkansas. *Plant Disease, 77*(3), 225-228.

Martínez, L., Caballero-Mellado, J., Orozco, J., & Martínez-Romero, E. (2003). Diazotrophic bacteria associated with banana (*Musa* spp.). *Plant and Soil, 257*(1), 35-47.

Meneses, C., Gonçalves, T., Alquéres, S., Rouws, L., Serrato, R., Vidal, M., & Baldani, J. I. (2017). *Gluconacetobacter diazotrophicus* exopolysaccharide protects bacterial cells against oxidative stress in vitro and during rice plant colonization. *Plant and Soil, 416*(1-2), 133-147.

Merzaeva, O. V., & Shirokikh, I. G. (2010). The production of auxins by the endophytic bacteria of winter rye. *Applied Biochemistry and Microbiology, 46*(1), 44-50.

Meyer, W. A., Torres, M. S., & White, J. F. (2012). Biology and applications of fungal endophytes in turfgrasses. *Turfgrass: Biology, use and management. John Stier, Brian Horgan and Stacy Bonos, American Society of Agronomy*, 713-731.

Morris, C. E., Sands, D. C., Vinatzer, B. A., Glaux, C., Guilbaud, C., Buffiere, A., Yan, S., Dominguez, H., & Thompson, B. M. (2008). The life history of the plant pathogen *Pseudomonas syringae* is linked to the water cycle. *The ISME journal, 2*(3), 321.

Mosse, B., & Hepper, C. (1975). Vesicular-arbuscular mycorrhizal infections in root organ cultures. *Physiological Plant Pathology, 5*(3), 215-223.

Müller, P., & Döring, M. (2009). Isothermal DNA amplification facilitates the identification of a broad spectrum of bacteria, fungi and protozoa in *Eleutherococcus* sp. plant tissue cultures. *Plant Cell, Tissue and Organ Culture (PCTOC), 98*(1), 35-45.

Mundt, J. O., & Hinkle, N. F. (1976). Bacteria within ovules and seeds. *Applied and Environmental Microbiology, 32*(5), 694-698.

Naveed, M., Hussain, M. B., Zahir, Z. A., Mitter, B., & Sessitsch, A. (2014). Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. *Plant Growth Regulation, 73*(2), 121-131.

Naveed, M., Mitter, B., Reichenauer, T. G., Wieczorek, K., & Sessitsch, A. (2014). Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. *Environmental and Experimental Botany, 97*, 30-39.

Okunishi S., Sako K., Mano H., Imamura A., & Morisaki H. (2005). Bacterial flora of endophytes in the maturing seed of cultivated rice (*Oryza sativa*). *Microbes and Environments, 20*(3), 168-177.

Oliveira, M. N., Santos, T. M., Vale, H. M., Delvaux, J. C., Cordero, A. P., Ferreira, A. B., Miguel, P. S., Tótola, M. R., Costa, M. D., Moraes, C. A., & Borges, A. C. (2013). Endophytic microbial diversity in coffee cherries of *Coffea arabica* from southeastern Brazil. *Canadian Journal of Microbiology, 59*(4), 221-230.

Ongena, M., & Jacques, P. (2008). *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends in Microbiology, 16*(3), 115-125.

Oteino, N., Lally, R. D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K. J., & Dowling, D. N. (2015). Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Frontiers in Microbiology, 6*, 745.

Patel, J. K., & Archana, G. (2017). Diverse culturable diazotrophic endophytic bacteria from Poaceae plants show cross-colonization and plant growth promotion in wheat. *Plant and Soil, 417*(1-2), 99-116.

Perrig, D., Boiero, M., Masciarelli, O., Penna, C., Ruiz, O., Cassán, F., & Luna, M. (2007). Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. *Applied Microbiology and Biotechnology, 75*(5), 1143-1150.

Prieto, P., & Mercado-Blanco, J. (2008). Endophytic colonization of olive roots by the biocontrol strain *Pseudomonas fluorescens* PICF7. *FEMS Microbiology Ecology, 64*(2), 297-306.

Quambusch, M., Pirttilä, A. M., Tejesvi, M. V., Winkelmann, T., & Bartsch, M. (2014). Endophytic bacteria in plant tissue culture: differences between easy-and difficult-to-propagate *Prunus avium* genotypes. *Tree Physiology, 34*(5), 524-533.

Reinhold-Hurek, B., Maes, T., Gemmer, S., Van Montagu, M., & Hurek, T. (2006). An endoglucanase is involved in infection of rice roots by the not-cellulose-metabolizing endophyte *Azoarcus* sp. strain BH72. *Molecular Plant-Microbe Interactions, 19*(2), 181-188.

Riggs, P. J., Chelius, M. K., Iniguez, A. L., Kaeppler, S. M., & Triplett, E. W. (2001). Enhanced maize productivity by inoculation with diazotrophic bacteria. *Functional Plant Biology, 28*(9), 829-836.

Rodriguez, R. J., White, J. F., Jr., Arnold, A. E., & Redman, R. S. (2009). Fungal endophytes: diversity and functional roles. *New Phytol, 182*(2), 314-330.

Rouws, L. F., Meneses, C. H., Guedes, H. V., Vidal, M. S., Baldani, J. I., & Schwab, S. (2010). Monitoring the colonization of sugarcane and rice plants by the endophytic diazotrophic bacterium *Gluconacetobacter diazotrophicus* marked with *gfp* and *gusA* reporter genes. *Letters in Applied Microbiology, 51*(3), 325-330.

Rungjindamai, N., Pinruan, U., Choeyklin, R., Hattori, T., & Jones, E. (2008). Molecular characterization of basidiomycetous endophytes isolated from leaves, rachis and petioles of the oil palm, *Elaeis guineensis*, in Thailand. *Fungal Diversity, 33*, 139-161.

Ryu, C.-M., Farag, M. A., Hu, C.-H., Reddy, M. S., Wei, H.-X., Paré, P. W., & Kloepper, J. W. (2003). Bacterial volatiles promote growth in *Arabidopsis*. *Proceedings of the National Academy of Sciences, 100*(8), 4927-4932.

Ryu, C.-M., Hu, C.-H., Locy, R. D., & Kloepper, J. W. (2005). Study of mechanisms for plant growth promotion elicited by rhizobacteria in *Arabidopsis thaliana*. *Plant and Soil, 268*(1), 285-292. doi:10.1007/s11104-004-0301-9

Saikkonen, K., Gundel, P. E., & Helander, M. (2013). Chemical ecology mediated by fungal endophytes in grasses. *Journal of Chemical Ecology, 39*(7), 962-968. doi:10.1007/s10886-013-0310-3

Samish, Z., Etinger-Tulczynska, R., & Bick, M. (1963). The microflora within the tissue of fruits and vegetables. *Journal of Food Science, 28*(3), 259-266.

Scherling, C., Ulrich, K., Ewald, D., & Weckwerth, W. (2009). A metabolic signature of the beneficial interaction of the endophyte *Paenibacillus* sp. Isolate and in vitro–grown poplar plants revealed by metabolomics. *Molecular Plant-Microbe Interactions, 22*(8), 1032-1037.

Sheibani-Tezerji, R., Naveed, M., Jehl, M. A., Sessitsch, A., Rattei, T., & Mitter, B. (2015). The genomes of closely related *Pantoea ananatis* maize seed endophytes having different effects on the host plant differ in secretion system genes and mobile genetic elements. *Frontiers in Microbiology, 6*, 440.

Sheng, X. F., Xia, J. J., Jiang, C. Y., He, L. Y., & Qian, M. (2008). Characterization of heavy metal-resistant endophytic bacteria from rape (*Brassica napus*) roots and their potential in promoting the growth and lead accumulation of rape. *Environmental Pollution, 156*(3), 1164-1170.

Shi, Y., Lou, K., & Li, C. (2009). Promotion of plant growth by phytohormone-producing endophytic microbes of sugar beet. *Biology and Fertility of Soils, 45*(6), 645-653.

Shi, Y., TaPa, M., Li, C., Yang, H., Zhang, T., Gao, Y., Sun, J., Zeng, J., Lin, Q., Cao, Z., OuTi, K., Li, Y., & Lou K. (2015). Diversity and space–time dynamics of endophytic archaea from sugar beet in the north slope of Tianshan Mountain revealed by 454 pyrosequencing and T-RFLP. *World Journal of Microbiology and Biotechnology, 31*(7), 1031-1039.

Siegel, M. R., & Latch, G. C. (1991). Expression of antifungal activity in agar culture by isolates of grass endophytes. *Mycologia*, 529-537.

Snook, M. E., Mitchell, T., Hinton, D. M., & Bacon, C. W. (2009). Isolation and characterization of Leu^7-surfactin from the endophytic bacterium *Bacillus mojavensis* RRC 101, a biocontrol agent for *Fusarium verticillioides*. *Journal of Agricultural and Food Chemistry, 57*(10), 4287-4292.

Souza, A., Cruz, J., Sousa, N., Procópio, A., & Silva, G. (2014). Endophytic bacteria from banana cultivars and their antifungal activity. *Genetics and Molecular Research, 13*(4), 8661-8670.

Spiering, M. J., Greer, D. H., & Schmid, J. (2006). Effects of the fungal endophyte, *Neotyphodium lolii*, on net photosynthesis and growth rates of perennial ryegrass (*Lolium perenne*) are independent of in planta endophyte concentration. *Annals of Botany, 98*(2), 379-387.

Stajković, O., de Meyer, S., Miličić, B., & Willems, A. (2009). Isolation and characterization of endophytic non-rhizobial bacteria from root nodules of alfalfa (*Medicago sativa* L.). *Botanica Serbica, 33*(1), 107-114.

Stergiopoulos, I., Collemare, J., Mehrabi, R., & De Wit, P. J. (2012). Phytotoxic secondary metabolites and peptides produced by plant pathogenic Dothideomycete fungi. *FEMS Microbiology Reviews, 37*(1), 67-93.

Sun, C., Johnson, J. M., Cai, D., Sherameti, I., Oelmüller, R., & Lou, B. (2010). *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *Journal of Plant Physiology, 167*(12), 1009-1017.

Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X., & Song, W. (2008). Endophytic bacterial diversity in rice (Oryza sativa L.) roots estimated by 16S rDNA sequence analysis. *Microbial Ecology, 55*(3), 415-424. doi:10.1007/s00248-007-9287-1

Surette, M. A., Sturz, A. V., Lada, R. R., & Nowak, J. (2003). Bacterial endophytes in processing carrots (*Daucus carota* L. var. sativus): their localization, population density, biodiversity and their effects on plant growth. *Plant and Soil, 253*(2), 381-390.

Suzuki, S., He, Y., & Oyaizu, H. (2003). Indole-3-acetic acid production in *Pseudomonas fluorescens* HP72 and its association with suppression of creeping bentgrass brown patch. *Current Microbiology, 47*(2), 138-143.

Thomas, S. E., Crozier, J., Aime, M. C., Evans, H. C., & Holmes, K. A. (2008). Molecular characterisation of fungal endophytic morphospecies associated with the indigenous forest tree, *Theobroma gileri*, in Ecuador. *Mycological Research, 112*(7), 852-860.

Tian, B., Zhang, C., Ye, Y., Wen, J., Wu, Y., Wang, H., Li, H., Cai, S., Cai, W., Cheng, Z., Lei, S., Ma, R., Lu, C., Cao, Y., Xu, X., & Zhang, K. (2017). Beneficial traits of bacterial endophytes belonging to the core communities of the tomato root microbiome. *Agriculture, Ecosystems & Environment, 247*, 149-156.

Trémouillaux-Guiller, J., Rohr, T., Rohr, R., & Huss, V. A. (2002). Discovery of an endophytic alga in *Ginkgo biloba*. *American Journal of Botany, 89*(5), 727-733.

Vadassery, J., Ritter, C., Venus, Y., Camehl, I., Varma, A., Shahollari, B., Novák, O., Strnad, M., Ludwig-Müller, J., & Oelmüller, R. (2008). The role of auxins and cytokinins in the mutualistic interaction between *Arabidopsis* and *Piriformospora indica*. *Molecular Plant-Microbe Interactions, 21*(10), 1371-1383.

Vallad, G. E., & Goodman, R. M. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Science, 44*(6), 1920-1934.

Verma, S. C., Ladha, J. K., & Tripathi, A. K. (2001). Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. *Journal of Biotechnology, 91*(2-3), 127-141.

Verma, S. C., Singh, A., Chowdhury, S. P., & Tripathi, A. K. (2004). Endophytic colonization ability of two deep-water rice endophytes, *Pantoea* sp. and *Ochrobactrum* sp. using green fluorescent protein reporter. *Biotechnology Letters, 26*(5), 425-429.

Vu, T., Hauschild, R., & Sikora, R. A. (2006). *Fusarium oxysporum* endophytes induced systemic resistance against *Radopholus similis* on banana. *Nematology, 8*(6), 847-852.

Weiss, M., Selosse, M.-A., Rexer, K.-H., Urban, A., & Oberwinkler, F. (2004). *Sebacinales*: a hitherto overlooked cosm of heterobasidiomycetes with a broad mycorrhizal potential. *Mycological Research, 108*(9), 1003-1010.

White, J. F., & Cole, G. T. (1985). Endophyte-host associations in forage grasses. III. In vitro inhibition of fungi by *Acremonium coenophialum*. *Mycologia, 77*(3), 487-489.

White, J. F., & Torres, M. S. (2010). Is plant endophyte-mediated defensive mutualism the result of oxidative stress protection? *Physiol Plant, 138*(4), 440-446.

White J. F. (1987). Widespread distribution of endophytes in the Poaceae. *Plant Disease, 71*(4), 340-342.

Wilson, D. (1995). Endophyte: the evolution of a term, and clarification of its use and definition. *Oikos*, 274-276.

Xin, G., Zhang, G., Kang, J. W., Staley, J. T., & Doty, S. L. (2009). A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. *Biology and Fertility of Soils, 45*(6), 669-674. doi:10.1007/s00374-009-0377-8

Yan, Z., Reddy, M., Ryu, C.-M., McInroy, J. A., Wilson, M., & Kloepper, J. W. (2002). Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. *Phytopathology, 92*(12), 1329-1333.

Chapter 2 Seed-Associated Bacterial Endophytes Promoted Seed Germination and Seedling Development of Cool Season Turf Grasses

2.1 Introduction

Bacterial endophytes have been found in various plants (Hallmann *et al.*, 1997, Garbeva *et al.*, 2001, Compant *et al.*, 2005, Souza *et al.*, 2014). For example, *Bacillus* spp. were discoverd in tomato, rice, maize (Bacilio-Jiménez *et al.*, 2001, Riggs *et al.*, 2001, Tian *et al.*, 2017). *Burkhoderia* spp. have been isolated from several plants, such as tomato, onion, and sugarcan (Compant *et al.*, 2005, Govindarajan *et al.*, 2006, Tian *et al.*, 2017). Some bacterial endophytes exhibited beneficial effects on plant hosts. For example, *Burkholderia* sp. strain PsJN was demonstrated to induce host defense reaction (Compant *et al.*, 2005).

Some endophytic bacteria have been found to benefit the seedlings of grasses in the family Poaceae. *Corynebacterium flavescens*, *Bacillus pumilus* and *Herbaspirillum seropedicae* strain Z67 were found to colonize rice seedlings and play a role in rice root colonization (Bacilio-Jiménez *et al.*, 2001, James *et al.*, 2002). *H. seropedicae* was also reported to regulate the gene expression of maize seedlings in early growth stage (do Amaral *et al.*, 2014). One of the genes is involved in gibberellin synthesis pathway which indicated this bacterium might affect the seed germination of maize. Another endophyte from *Bacillus* genus, *B. mojavensis,* reduced the stalk lesions caused on *Fusarium verticillioides* on maize seedlings, which helped the establishment of seeds (Bacon & Hinton, 2011).

The purpose of this study was to examine the endophytic bacteria associated with cool-season turfgrass seeds and evaluate their effects on seed germination and seedling growth. The results showed that endophytic bacteria strains *Bacillus amyloliquefaciens* strain SF2, *B. pumilus* strain SF3, and *Pantoea agglomerans* strain TF promoted seed germination and seedling growth with and without salinity stress.

2.2 Materials and Methods

2.2.1 Bacteria Isolation

Seeds of eight turf grass species, *Poa pratensis* (Kentucky bluegrass), *Lolium arundinacea* (tall fescue), *Lolium perenne* (perennial ryegrass), *Festuca ovina* (sheep fescue), *Festuca rubra* ssp. *Litoralis* (Slender creeping red fescue), *Festuca rubra* ssp. *rubra* (strong creeping red fescue), *Festuca brevipila* (hard fescue), *Festuca rubra* ssp. *commutate* (chewings fescue), were used for the endophytic bacteria isolation. Grass seeds were surface disinfected in 3% sodium hypochlorite (NaOCl) solution, shaking for 30 minutes at 150 rpm/min. The the disinfected seeds were rinsed with sterilized distilled water for 3 times, 30 seconds each time. Then the sterilized seeds were put on potato dextrose agar (PDA) media and 10% trypticase soy agar (TSA) media for the bacteria isolation. The isolated bacteria were were routinely grown at 28^{o}C on PDA agar and stored at -80^{o}C in PDB with 25% glycerol

2.2.2 Bacteria Identification

The extraction of bacterial genome DNA was performed with GenElute™ Bacterial Genomic DNA kit (Sigma Chemical Company, St. Louis, MO, USA) following the instructional protocol. 16S rRNA region was amplified using bacterial DNA as template with universial primers, 16S-27F (5'- AGAGTTTGATCMTGGCTCAG-3') and 16S-1525R (5'- AAGGAGGTGWTCCARCC-3') (M=A/C, W=A/T, R=A/G) (Lane, 1991). PCR amplifications were conducted using Invitrogen™ PCR SuperMix (Thermo Fisher Scientific Inc., Waltham, MA, USA) with the following thermocycles: initial denaturation at 94^{o}C for 5 min followed by 30 cycles (denaturation at 94^{o}C for 30s, annealing at 62^{o}C or 55^{o}C for 30s, and extension at 72^{o}C for 60s) and the final extension at 72^{o}C for 8 min. PCR products were verified with 1% agarose gel stained with SYBR™ Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA) and purified with QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The purified PCR products were sent to GENEWIZ Inc. (South Plainfield, NJ, USA) for Sanger sequencing, and the result sequences were compared with existent sequences in GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) using web-based *blastn* (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

2.2.3 Inoculation of Bacteria Isolates on Grasses

Seeds of tall fescue, Kentucky bluegrass and perennial ryegrass were disinfected in 3% NaOCl solution for 30 minutes and rinsed with sterilized distilled water for 30 seconds each time, 3 times. Two *Bacillus* strains isolated from sheep fescue and one *Pantoea* strain isolated from tall fescue were cultured in PDB at room temperature for 12

hours. Then, bacteria cells were collected by centrifugation at 3000rpm for 5min. The collected bacteria cells were water diluted to OD_{600} = 1.0 (about 4 x 10^8cells/ml). Both sterilized and unsterilized seeds of tall fescue, Kentucky bluegrass and perennial ryegrass were dipped into bacterial suspension for 5 min and then placed on 1.5% agar, 1.5% agar with 100mM NaCl and 1.5% agar with 200mM NaCl. For Kentucky bluegrass, after 7 days, 10 days, 14 days and 17 days growth on the media, the rate of germination was calculated. For tall fescue and perennial ryegrass, the seeds germination rate was counted every 24 hours from the third day to the seventh day.

2.2.4 WinRhizo Analysis of Root Architecture

Magenta vessels containing 25 grams of soil were sterilized by autoclaving at 121^{o}C for 60 minutes, five times, once every 24 hours. Suspensions containing 10^8 cells/ml of *B. amyloliquefaciens* strain SF2, *B. pumilus* strain SF3, *P. agglomerans* strain TF, and *E. coli* (negative control) were prepared in sterilized distilled water. Seeds of perennial ryegrass and tall fescue were sterilized with 4% NaOCl solution for 30 minutes and rinsed with sterilized distilled water for 5 times, 30 seconds each time. Sterilized grass seeds were then soaked in bacterial suspensions or sterilized distilled water (water control) for 1 hour, placed in sterilized magenta vessels with soil, and kept in a growth chamber at 28^{o}C with a 16h/8h light/dark cycle. After 35 days, seedlings were collected by carefully removing soil and washing off dirt from roots. Roots were separated from shoots and imaged on a light box with ruler. Root images were processed with WinRhizo in Dr. Huang's lab, which was used to measure average diameter(cm), total root length(cm), total root surface area(cm^2), total root volume (cm^3).

2.2.5 Root Dry Weights of Seedlings Under Pathogenic Stress

Magenta vessels containing 25 grams of soil were sterilized by autoclaving at 121°C for 60 minutes, five times, once every 24 hours. Suspensions containing 10^8 cells/ml of *B. amyloliquefaciens* strain SF2, *B. pumilus* strain SF3 and *P. agglomerans* strain TF were prepared in sterilized distilled water. Seeds of perennial ryegrass and tall fescue were sterilized with 4% NaOCl solution for 30 minutes and rinsed with sterilized distilled water for 5 times, 30 seconds each time. Sterilized grass seeds were then soaked in bacteria suspension as well as sterilized distilled water as control for 1 hour and placed in sterilized magenta vessels with soil. All boxes were further inoculated with turf pathogens, *Sclerotinia homoeocarpa*, and kept in growth chamber at 28°C with a 16h/8h light/dark cycle. After 30 days, grass roots were collected by gently rinsing the root with tap water to carefully remove soil. Clean seedling roots were dried in an incubator at 60°C for 72 hours and were weighted with analytical balance.

2.2.6 Seed Germination Test Under Salt Stress and Non-Stressed Conditions

Suspensions containing 10^8 cells/ml of *B. amyloliquefaciens* strain SF2, *B. pumilus* strain SF3 and *P. agglomerans* strain TF were prepared in sterilized distilled water. Seeds of Kentucky bluegrass, perennial ryegrass and tall fescue were sterilized with 4% NaOCl solution for 30 minutes and rinsed with sterilized distilled water for 5 times, 30 seconds each time. Sterilized and non-sterilized seeds were soaked in bacteria suspension as well as sterilized distilled water as control for 1 hour, separately. All treated seeds were then placed in petri dishes with 1.5% agar, 1.5% agar with 100mM

NaCl, and 1.5% agar with 200mM NaCl. All vessels were kept in growth chamber at 28^{o}C with a 16h/8h light/dark cycle. Seed germination was observed every 24 hours until no more seed germinated.

2.2.7 Microscopy Observation

To visualize bacteria on seedling roots, 7-day-old tall fescue seedlings and 10-day-old Kentucky bluegrass seedlings grown on 1.5% agar plates were stained for 10 hours by flooding agar plates with 5 ml of a solution of 100 mM potassium phosphate buffer, pH 6.9, 2.5 mM 3,3'-Diaminobenzidine tetrachloride (DAB), and 5 purpurogallin units/ml of horseradish peroxidase (Type VI, Sigma Chemical Company, St. Louis, MO). Seedling roots were then excised, placed on a slide containing aniline blue/lactophenol stain (aniline blue dye 0.05g, phenol crystals 20g, glycerol 40ml, lactic acid 20ml, H_2O 20ml), and examined by using bright field microscopy. Then seedling roots were observed with 100X, 200X, and 400X.

2.3 Results

2.3.1 Observation, Isolation, and Identification of Endophytic Bacteria in Cool-Season Turfgrasses

By staining the seedlings of tall fescue with DAB, we observed bacteria colonizing the root hairs by using light microscope (Figure 2.1). Then, we performed bacteria isolation with different nutrient media. Totally, thirteen bacteria strains were isolated from the seeds of seven cool-season turf grass species (Table 2.1). Most of the

isolated bacteria belonged to species *B. amyloliquefaciens* and *B. pumilus*. The only *P. agglomerans* strain was isolated from tall fescue.

2.3.2 Colonization of Isolated Bacteria on Turfgrass Roots

First, the compatibility of isolated bacterial endophytes with grass seedlings were observed with light microscope. As seen in Figure 2.2, *B. amyloliquefaciens* strain SF2, *B. pumilus* strain SF3, and *P. agglomerans* strain TF showed their colonization around root hairs (B, C, D). But in the control without bacterial inoculation, no bacterial cells were found.

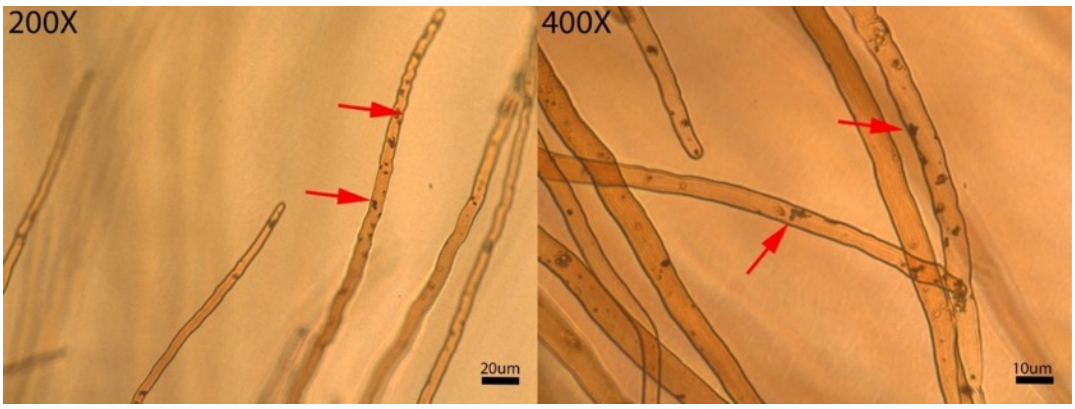

Figure 2.1 Photo of bacterial endophytes in root hairs of sterilized tall fescue seedlings.

Table 2.1 Seed-transmitted bacteria endophytes isolated from cool-season turf grass species.

Species	Isolates	16S rRNA GenBank Number
Festuca rubra ssp. *commutata*	*Bacillus pumilus* strain CF2	MN272306.1
Festuca rubra ssp. *rubra*	*Bacillus amyloliquefaciens* strain CRF2	MN272308.1
	Bacillus pumilus strain CRF1	MN272307.1
Festuca brevipila	*Bacillus amyloliquefaciens* strain HF3	MN272312.1
	Bacillus pumilus strain HF1	MN272310.1
Poa pratensis	*Bacillus amyloliquefaciens* strain KB2	MN272314.1
	Bacillus pumilus strain KB1	MN272313.1
Lolium perenne	*Bacillus amyloliquefaciens* strain PR1	MN272320.1
Festuca rubra ssp. *litoralis*	*Bacillus amyloliquefaciens* strain SCF2	MN272319.1
	Bacillus pumilus strain SCF1	MN272318.1
Festuca ovina	*Bacillus amyloliquefaciens* strain SF2	MN272316.1
	Bacillus pumilus strain SF3	MN272317.1
Lolium arundinacea	*Pantoea agglomerans* strain TF	MT270698.1

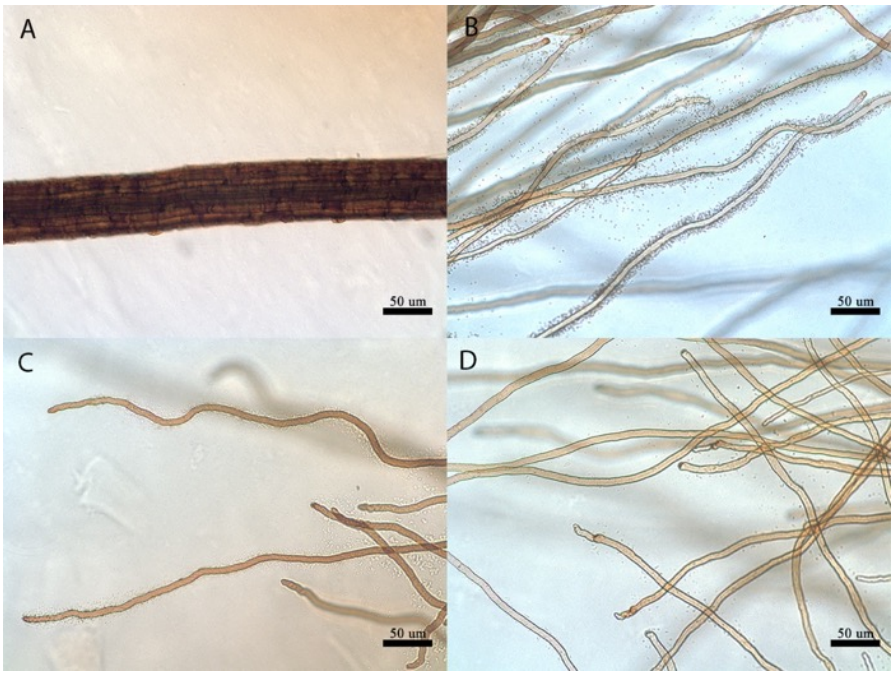

Figure 2.2 Colonization of bacterial endophytes on Kentucky bluegrass root hairs. A. Control, no inoculation; B. inoculated with *B. amyloliquefaciens* strain SF2; C. inoculated with *B. pumilus* strain SF3; D. inoculated with *P. agglomerans* strain TF.

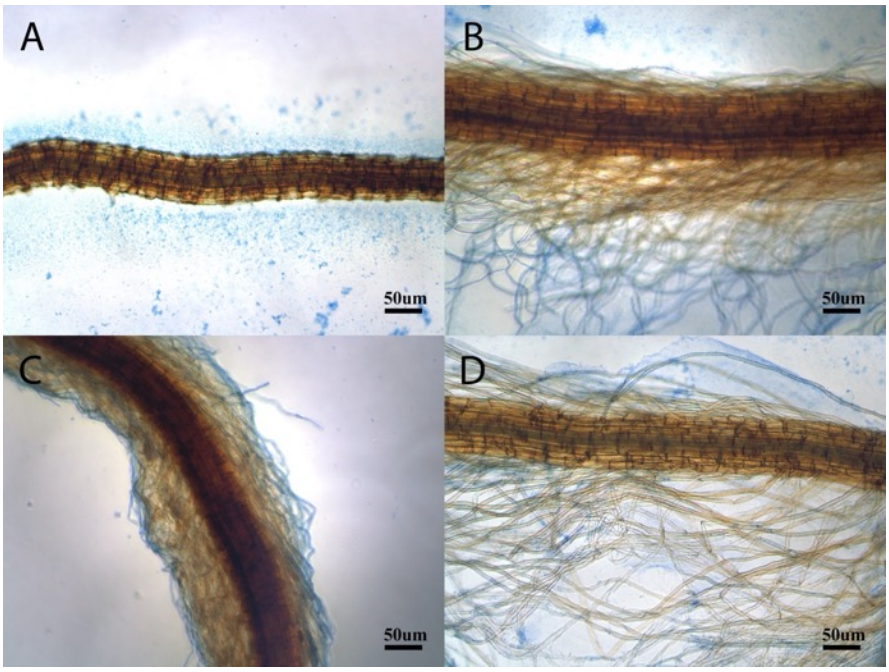

Figure 2.3 Stimulation of root hair growth on Kentucky bluegrass by bacterial endophytes (100X). A. Control, no inoculation; B. inoculated with *B. amyloliquefaciens* strain SF2; C. inoculated with *B. pumilus* strain SF3; D. inoculated with *P. agglomerans* strain TF.

Table 2.2 Root architecture analysis of tall fescue. † shows a significant difference from control based on Dunnett's multiple comparison test at critical value of 0.05.

	Average Diameter (μm)	Forks	Total Root Length (mm)	Total Surface Area (mm2)	Total Root Volume (mm3)	Tips
Control	114.36 ± 12.56	19.33 ± 10.19	5732.25 ± 1951.03	205929.80 ± 73773.58	595948.57 ± 243267.34	16.65 ± 6.20
B. amyloliquefaciens strain SF2	111.22 ± 14.26	26.26 ± 20.34	7895.39 ± 2560.18†	275580.03 ± 91403.21†	776764.64 ± 283804.49†	19.97 ± 11.29
B. pumilus strain SF3	119.16 ± 17.74	22.49 ± 12.44	6579.58 ± 2297.89	244477.79 ± 84850.63	740231.81 ± 311832.48	17.54 ± 7.33
P. agglomerans strain TF	139.82 ± 23.90†	38.26 ± 35.96†	6418.04 ± 3238.65	279476.08 ± 142718.46†	993199.31 ± 549704.44†	27.91 ± 17.20†
E. coli	113.44 ± 17.34	27.00 ± 21.54	6123.02 ± 1648.34	217943.22 ± 66759.50	630672.19 ± 235084.43	22.22 ± 18.28
ANOVA p-value	3.8265E-09	3.8893E-03	7.6046E-04	7.0033E-04	1.3474E-05	3.2560E-03

Table 2.3 Root architecture analysis of perennial ryegrass. † shows a significant difference from control based on Dunnett's multiple comparison test at critical value of 0.05.

	Average Diameter (μm)	Forks	Total Root Length (mm)	Total Surface Area (mm2)	Total Root Volume (mm3)	Tips
Control	102.96 ± 8.70	59.22 ± 30.10	12216.67 ± 3947.75	393293.81 ± 126744.16	1014289.00 ± 346479.40	44.53 ± 19.47
B. amyloliquefaciens strain SF2	103.10 ± 12.49	61.40 ± 27.12	14095.95 ± 3456.46†	453835.79 ± 113074.29†	1177926.92 ± 333035.34†	49.04 ± 21.27
B. pumilus strain SF3	103.34 ± 9.61	71.53 ± 32.05	12980.09 ± 2867.08	419434.26 ± 96359.76	1088014.87 ± 303404.99	45.74 ± 20.90
P. agglomerans strain TF	105.02 ± 9.00	53.39 ± 36.72	11723.65 ± 3044.03	386833.03 ± 106569.27	1022480.47 ± 319204.09	40.67 ± 25.88
E. coli	106.24 ± 9.58	52.29 ± 33.25	10152.05 ± 3439.55	336565.76 ± 112215.28	893643.37 ± 309372.23	48.74 ± 35.68
ANOVA p-value	0.6230	0.2689	5.6944E-04	2.7647E-03	0.0191	0.8084

2.3.3 Growth Promotion of Root Hairs by Isolated Bacterial Endophytes

By applying isolated bacterial endophytes, *B. amyloliquefaciens* strain SF2, *B. pumilus* strain SF3, and *P. agglomerans* strain TF, Kentucky bluegrass seedlings showed much better root hair growth than the control (Figure 2.3).

2.3.4 Root Architecture Analysis

To further detect the promotion effects of bacterial endophytes on host root growth, we analyzed the root architecture of tall fescue and perennial ryegrass inoculated with different bacterial endophytes. In the test with tall fescue as the host, *P. agglomerans* strain TF increased the average diameter, total root length, total surface area, and total root volume by 22.26%, 11.96%, 35.71%, and 66.66%, respectively (Table 2.2). But *B. amyloliquefaciens* strain SF2 increased total root length, total surface area and total root volume without changing the average diameter very much.

On perennial ryegrass, *B. amyloliquefaciens* strain SF2 promoted the root growth by increasing total root length, total surface area and total root volume. However, *B. pumilus* strain SF3 and *P. agglomerans* strain TF did not show significant growth promotion effects on the host seedlings (Table 2.3).

2.3.5 Seed Germination Without Stress Treatment

In the seed germination test, all three strains increased the germination rate of both sterilized and unsterilized Kentucky bluegrass seeds on 1.5% agar (Figure 2.4, Figure 2.5 and Figure 2.6). Also, *B. amyloliquefaciens* strain SF2, *B. pumilus* strain SF3 and *P. agglomerans* strain TF promoted the seed germination on sterilized seeds of

perennial ryegrass (Figure 2.7). In the test with tall fescue seeds, the three bacteria strains promoted the seed germination at 5 days. However, bacteria-treated seeds showed close seed germination rates as the control (Figure 2.8).

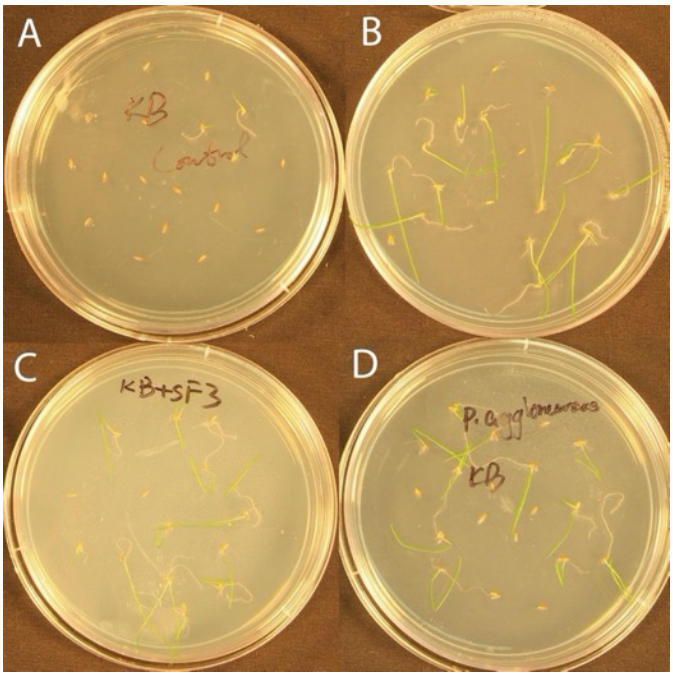

Figure 2.4 Growth promotion effects of bacterial endophytes on Kentucky bluegrass on 1.5% agar at 10 days. A. Control, no inoculation; B. inoculated with *B. amyloliquefaciens* strain SF2; C. inoculated with *B. pumilus* strain SF3; D. inoculated with *P. agglomerans* strain TF.

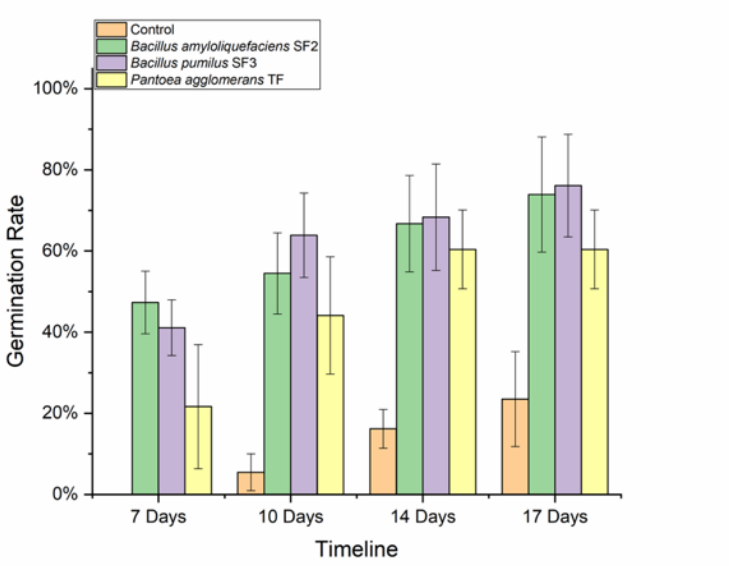

Figure 2.5 Seed germination rate of sterilized Kentucky bluegrass seeds on 1.5% agar. Seed germination rate was collected at 7days, 10days, 14days and 17days. Four replica plates were conducted, and error bars represent the standard deviation.

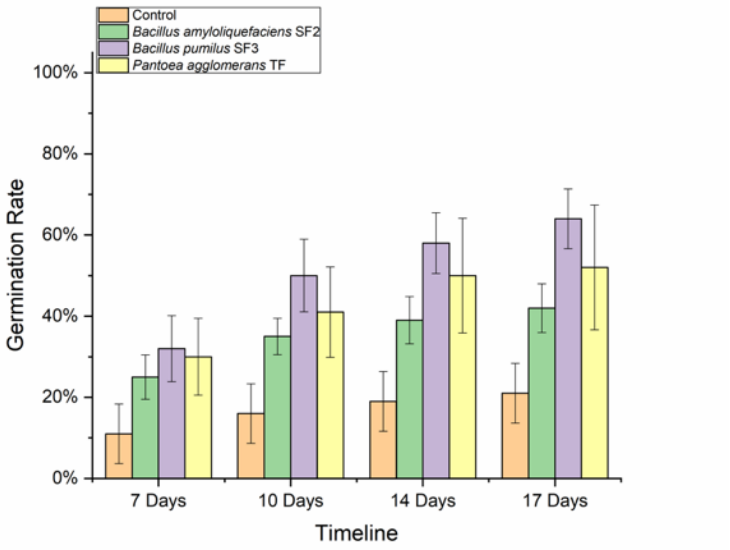

Figure 2.6 Seed germination rate of unsterilized Kentucky bluegrass seeds on 1.5% agar. Seed germination rate was collected at 7days, 10days, 14days and 17days. Four replica plates were conducted, and error bars represent the standard deviation.

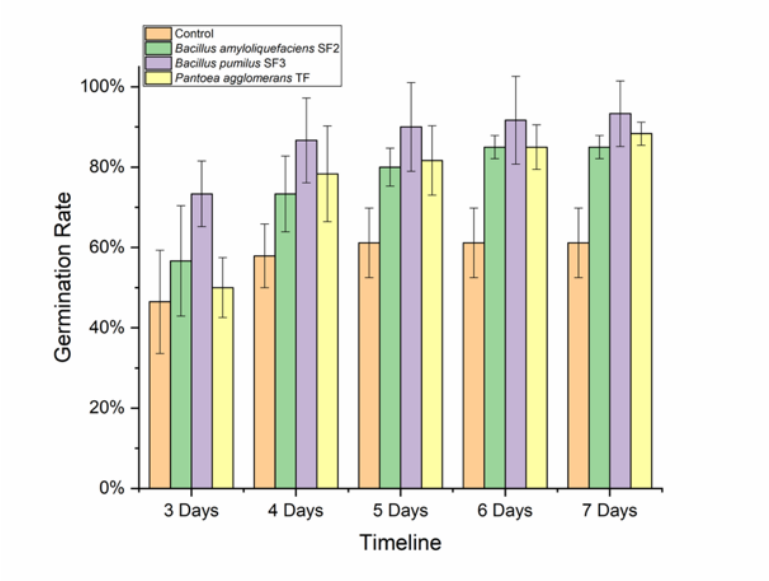

Figure 2.7 Seed germination rate of sterilized perennial ryegrass seeds on 1.5% agar. Seed germination rate was collected from 3days to 7days. Three replica plates were conducted, and error bars represent the standard deviation.

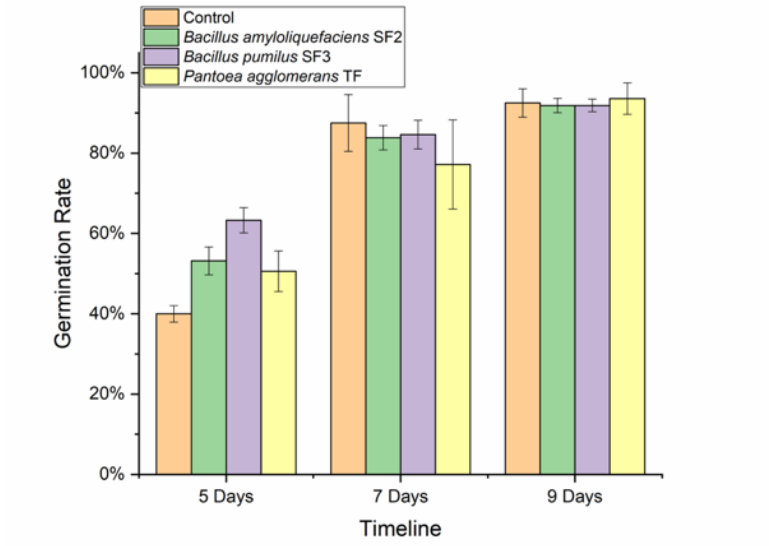

Figure 2.8 Seed germination rate of sterilized tall fescue seeds on 1.5% agar. Seed germination rate was collected at 5days, 7days, and 9days. Three replica plates were conducted, and error bars represent the standard deviation.

2.3.6 Seed Germination Under Salt Stress

Then, we conducted the similar seed germination test under salt stress. All three isolated bacteria strains *B. amyloliquefaciens* strain SF2, *B. pumilus* strain SF3 and *P. agglomerans* strain TF promoted the germination of perennial ryegrass seed with salt treatment of 100mM NaCl and 200mM NaCl (Figure 2.9 and Figure 2.10). They also increased the seed germination rate of Kentucky bluegrass on 1.5% agar with 100mM NaCl treatment (Figure 2.11).

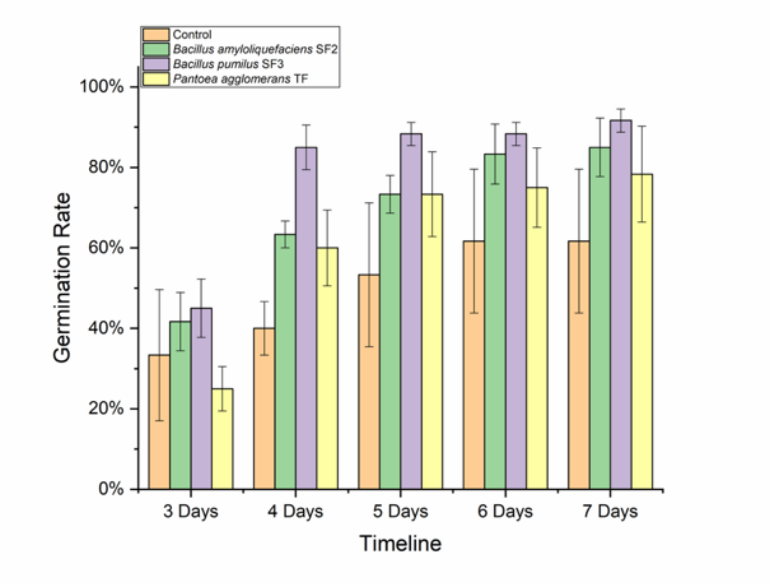

Figure 2.9 Seed germination rate of sterilized perennial ryegrass seeds on 1.5% agar with 100mM NaCl. Seed germination rate was collected from 3days to 7days. Three replica plates were conducted, and error bars represent the standard deviation.

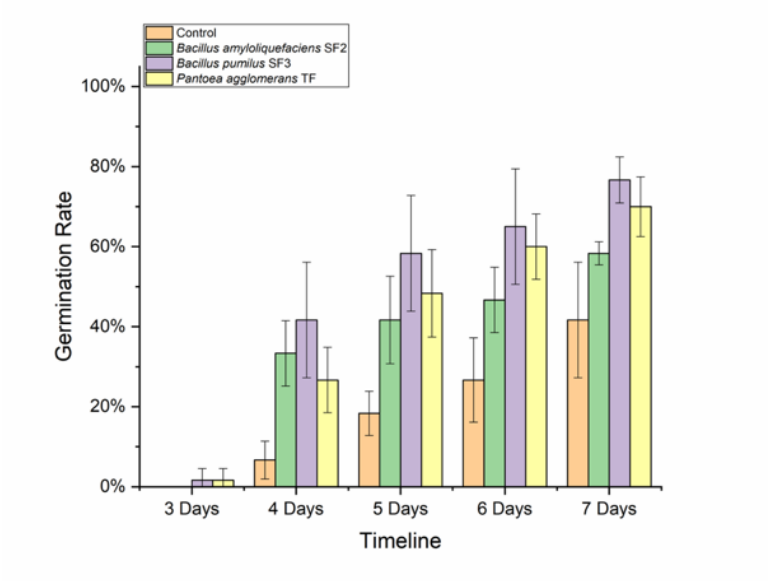

Figure 2.10 Seed germination rate of sterilized perennial ryegrass seeds on 1.5% agar with 200mM NaCl. Seed germination rate was collected from 3days to 7days. Three replica plates were conducted, and error bars represent the standard deviation.

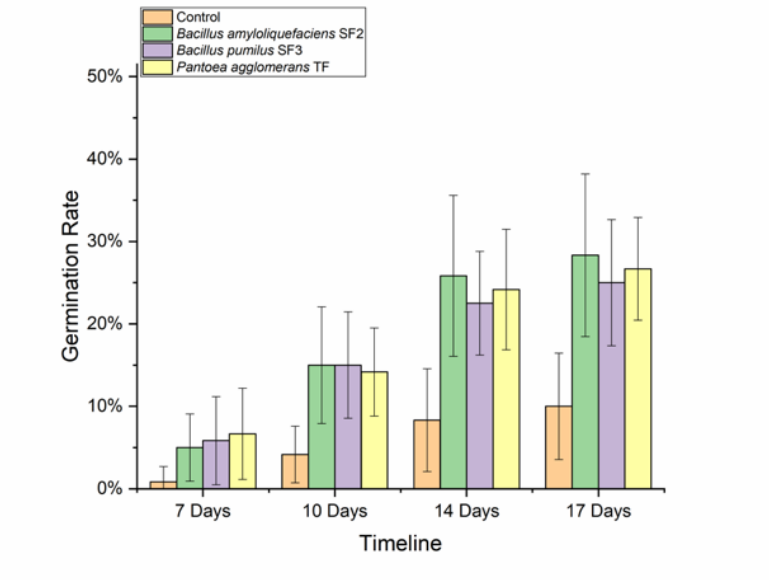

Figure 2.11 Seed germination rate of sterilized Kentucky bluegrass seeds on 1.5% agar with 100mM NaCl. Seed germination rate was collected at 7days, 10days, 14days and 17days. Three replica plates were conducted, and error bars represent the standard deviation.

2.3.7 Root Growth of Perennial Ryegrass and Tall Fescue Seedlings Under Pathogenic Stress

With the treatment of the three strains, *B. amyloliquefaciens* strain SF2, *B. pumilus* strain SF3 and *P. agglomerans* strain TF, both perennial ryegrass and tall fescue showed greater root dry weights than control (Figure 2.12 and Figure 2.13). After performing ANOVA test, the assay on tall fescue is significant (p-value = 0.0106, F = 4.1355) but the one on perennial ryegrass is not significant enough (p-value = 0.0539, F = 2.7667). Dunnett's multiple comparison test indicated that on tall fescue, *P. agglomerans* strain TF increased the root dry weights by at least 3.5mg (critical value at 0.05).

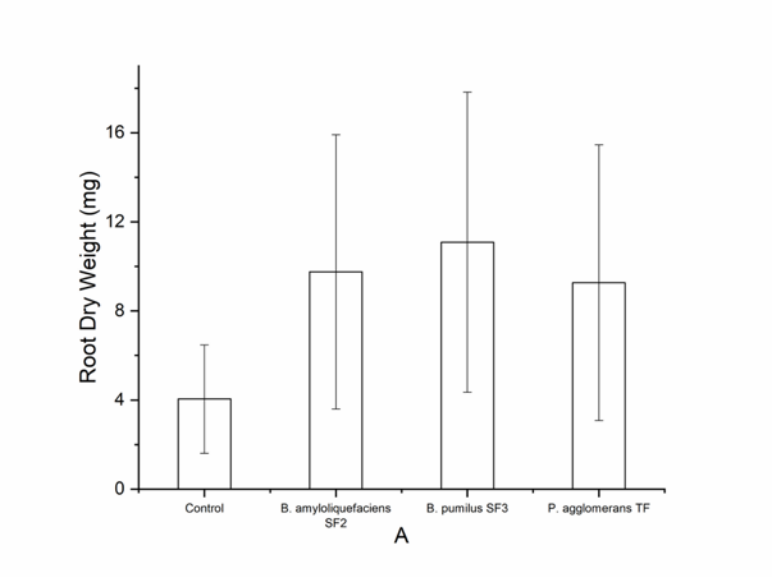

Figure 2.12 Root dry weight of perennial ryegrass seedlings inoculated with *Sclerotinia homoeocarpa* and endophytic bacteria at 30days. Nine replica seedlings were weighted, and error bars represent the standard deviation.

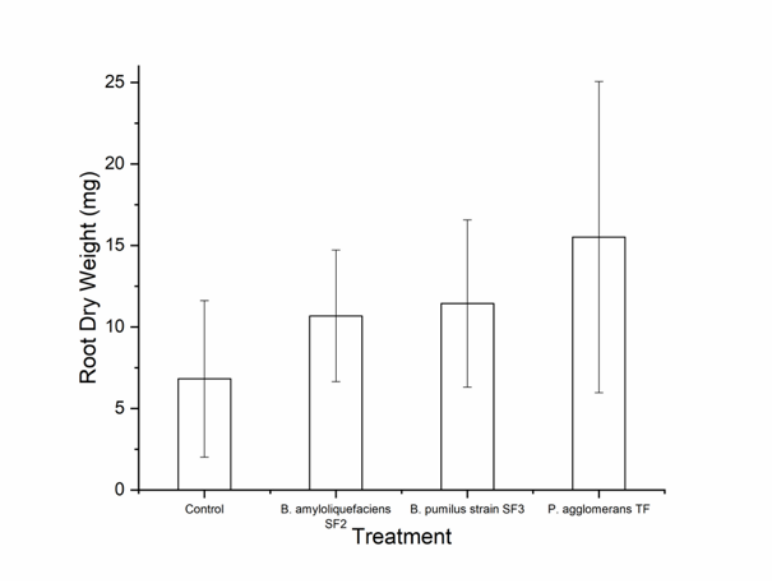

Figure 2.13 Root dry weight of tall fescue seedlings inoculated with *Sclerotinia homoeocarpa* and endophytic bacteria at 30days. Nine replica seedlings were weighted, and error bars represent the standard deviation.

2.4 Discussion

2.4.1 Bacterial Endophytes Colonized Cool-Season Turfgrass

From the observation and isolation assays, we found the colonization of bacterial endophytes on the seeds and seedlings of cool-season turfgrass (Figure 2.1 and Table 2.1). After being inoculated on the grass seed, the isolated bacteria tended to colonize the root hairs. Previous study showed the colonization of *Pseudomonas* spp. on olive root hairs (Mercado-Blanco & Prieto, 2012). Another bacterial endophyte, *H. seropedicae* was also found to colonize the roots of various Poaceae crops, such as maize, rice and wheat (Monteiro *et al.*, 2012). It suggested that bacterial endophytes not only survive in plants tissues but also live on the surface of plant roots as epiphytes.

2.4.2 Bacterial Endophytes Increased Turf Seed Germination Rate under Both Salt Stress and Non-Stress Conditions

When inoculated with isolated bacterial endophyte strains *B. amyloliquefaciens* strain SF2, *B. pumilus* strain SF3 and *P. agglomerans* strain TF, all three tested turf species exhibited better seed germination than the control without bacteria treatment under both salt stress and non-stress conditions (Figure 2.4 to Figure 2.11). In the test on turf seeds without stress treatment, both Kentucky bluegrass and perennial ryegrass showed higher germination rates when treated with bacterial isolates (Figure 2.4 to Figure 2.7). However, tall fescue seeds with no bacterial inoculation had similar germination rate with the groups with bacterial inoculation after 9 days (Figure 2.8). But after 5 days, treatments with isolated bacteria had higher germination rates than the control, which suggested endophytic bacteria promoted the seed germination by shortening the germination time (Figure 2.8). When the seeds were germinated with salt treatment, both Kentucky bluegrass and perennial ryegrass with bacterial inoculation showed better germination than the control (Figure 2.9 to Figure 2.11). Promotion of seed germination by endophytic bacteria was also reported by other researchers (Demissie *et al.*, 2013).

2.4.3 Promotion of Seedling Root Growth by Bacterial Endophytes

With the observation of promoting root hair growth by bacterial endophytes (Figure 2.3), we performed root architecture analysis by using WinRhizo. On tall fescue, all three isolated endophytic bacterial strains increased total root length, total root surface area, and total root volume (Table 2.2). But only *P. agglomerans* strain TF significantly

increased the average diameter (by 22.26%) compared with control. The roots of seedlings treated with *B. amyloliquefaciens* strain SF2 showed similar average diameter but much longer root length than the control. In the test with perennial ryegrass as the host plants, only *B. amyloliquefaciens* strain SF2 showed the promotion effects on root growth. Since we isolated *B. amyloliquefaciens* and *P. agglomerans* from perennial ryegrass and tall fescue, respectively (Table 2.1), it might suggest that bacterial endophytes had better mutualistic interaction with their nature hosts. In the analysis on both tall fescue and perennial ryegrass, *E. coli* was used as the negative control. Interestingly, it did show little growth promotion effects on tall fescue, but most likely inhibited the root growth of perennial ryegrass.

2.5 Conclusion

Cool-season turfgrass species harbor the bacteria endophytes that promote the seed germination the seedlings root growth. Those bacteria include *B. amyloliquefaciens, B. pumilus,* and *P. agglomerans.* The bacteria endophytes showed better growth promotion effects on their natural hosts.

2.6 References

Bacilio-Jiménez, M., Aguilar-Flores, S., del Valle, M. V., Pérez, A., Zepeda, A., & Zenteno, E. (2001). Endophytic bacteria in rice seeds inhibit early colonization of roots by *Azospirillum brasilense*. *Soil Biology and Biochemistry, 33*(2), 167-172.

Bacon, C. W., & Hinton, D. M. (2011). In planta reduction of maize seedling stalk lesions by the bacterial endophyte *Bacillus mojavensis*. *Canadian Journal of Microbiology, 57*(6), 485-492.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clement, C., & Ait Barka, E. (2005). Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Applied and Evnironmental Microbiology, 71*(4), 1685-1693.

Demissie, S., Muleta, D., & Berecha, G. (2013). Effect of phosphate solubilizing bacteria on seed germination and seedling growth of faba bean (*Vicia faba* L.). *International Journal of Agricultural Research, 8*(3), 123-136.

do Amaral, F. P., Bueno, J. C. F., Hermes, V. S., & Arisi, A. C. M. (2014). Gene expression analysis of maize seedlings (DKB240 variety) inoculated with plant growth promoting bacterium *Herbaspirillum seropedicae*. *Symbiosis, 62*(1), 41-50.

Garbeva, P., Overbeek, L. S., Vuurde, J. W., & Elsas, J. D. (2001). Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. *Microbial Ecology, 41*(4), 369-383.

Hallmann, J., Quadt-Hallmann, A., Mahaffee, W., & Kloepper, J. (1997). Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology, 43*(10), 895-914.

James, E. K., Gyaneshwar, P., Mathan, N., Barraquio, W. L., Reddy, P. M., Iannetta, P. P., Olivares, F. L., & Ladha, J. K. (2002). Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. *Molecular Plant-Microbe Interactions, 15*(9), 894-906.

Mercado-Blanco, J., & Prieto, P. (2012). Bacterial endophytes and root hairs. *Plant and Soil, 361*(1), 301-306.

Monteiro, R. A., Balsanelli, E., Wassem, R., Marin, A. M., Brusamarello-Santos, L. C. C., Schmidt, M. A., Tadra-Sfeir, M. Z., Pankievicz, V. C. S., Cruz, L. M., Chubatsu, L. S., Pedrosa, F. O., & Souza, E. M. (2012). *Herbaspirillum*-plant interactions: microscopical, histological and molecular aspects. *Plant and Soil, 356*(1-2), 175-196. doi:10.1007/s11104-012-1125-7

Souza, A., Cruz, J., Sousa, N., Procópio, A., & Silva, G. (2014). Endophytic bacteria from banana cultivars and their antifungal activity. *Genetics and Molecular Research, 13*(4), 8661-8670.

Chapter 3 The Turfgrass-Associated *Bacillus amyloliquefaciens* Strains Producing Fengycin, Iturin and Surfactin Inhibit the Growth of Turf Fungal Pathogens

3.1 Introduction

Lipopeptide is a category of biosurfactants, amphiphilic molecules that consist of a lipid connected to a peptide via ester and/or amide bonds. Lipopeptides are mainly produced by *Bacillus* spp., *Paenibacillus* spp., and *Pseudomonas* spp. (Ongena & Jacques, 2008, Raaijmakers *et al.*, 2010, Smyth *et al.*, 2010). Other bacteria species were also found to produce lipopeptides. For instance, grass endophyte *Herbaspirillum seropedicae* was found to produce serobactins (Rosconi *et al.*, 2013).

Lipopeptides are synthesized by non-ribosomal peptide synthetases (NRPSs) or hybrid polyketide synthases and non-ribosomal peptide synthetases (PKSs/NRPSs). NRPSs are large enzyme complexes composed of multiple modules and are functionally independent of mRNA. A module contains three domains, adenylation domain (A-domain), peptidyl carrier protein (PCP-domain) or thiolation domain (T-domain), and condensation domain (C-domain) (Martínez-Núñez & López, 2016).

In *Bacillus*, the most studied lipopeptides are characterized into three families, surfactin family, iturin family and fengycin family (Ongena & Jacques, 2008). *Bacillus* lipopeptides from all three families are composed of a cyclic peptide ring connected to a fatty acid (Ongena & Jacques, 2008). The grouping of different lipopeptides is based on the different numbers and structures of amino acids in the cyclic peptide chain. This gives them different biological activities and functions (Raaijmakers *et al.*, 2010).

All members from the surfactin family contain a heptapeptide with an LLDLLDL chiral sequence and a beta-hydroxy fatty acid of 13-15 carbons (Table 3.1), to form a lactone ring (Peypoux *et al.*, 1999). With the amphiphilic property, surfactins can adhere to lipid bilayers and disrupt cell membranes (Ongena & Jacques, 2008). This provides surfactins with antiviral, antimycoplasma, and antibacterial activities. Biofilm formation by *Bacillus* spp. on plant surfaces also depends on the production of surfactins.

In addition, surfactins can serve as the elicitors of induced systemic resistance (ISR), a type of plant defenses against pathogens, in plants (Ongena *et al.*, 2007). In a study on tobacco, surfactin was reported to induce the early defensive events like production of reactive oxygen species (ROS) as well as stimulate the defense enzymes phenylalanine ammonia-lyase and lipoxygenase (Jourdan *et al.*, 2009).

Lipopeptides from iturin family consist of heptapeptides with an LDDLLDL chiral sequence and a beta-hydroxy fatty acid of 14-17 carbons (Table 3.1), including ituirin A and C, bacillomycin D, F, L and LC, and mycosubtilin. They exhibited strong antifungal activities but limited antibacterial effects (Moyne *et al.*, 2001, Hiradate *et al.*, 2002, Yu *et al.*, 2002). This is different from surfactins which display antibacterial activities but no remarkable fungitoxicity. It was suggested to be caused by the different underlaying mechanisms (Ongena & Jacques, 2008).

The third family of lipopeptides produced by *Bacillus* spp. is fengycin, which contains fengycins A and B, and plipastatins A and B. They contain a decapeptides with an LDDDLDLLL or LDLDLDLLDL chiral sequence linked to a beta-hydroxy fatty acid of 14-18 carbons (Table 3.1). The carbon chain in fengycin family can be either saturated

or unsaturated. Compared to surfactin and iturin families, fengycin family is less well known but they can also interact with lipid layers and retain fungal toxicity.
Studies suggested that lipopeptides acted in a synergistic way (Romero *et al.*, 2007, Alvarez *et al.*, 2012). For example, *Bacillus subtilis* that produced iturin and fengycin showed antagonistic effect against *Podosphaera fusca* (Romero *et al.*, 2007).

Table 3.1 Characteristics of *Bacillus* lipopeptides in surfactin, iturin, and fengycin.

Lipopeptides family	Surfactin	Iturin	Fengycin
Peptides	Heptapeptide	Heptapeptide	Decapeptides
Chiral sequence	LLDLLDL	LDDLLDL	LDDDLDLLL or LDLDLDLLDL
Fatty acid	13-15 carbons	14-17 carbons	14-18 carbons

3.2 Materials and Methods

3.2.1 Microorganisms and Growth Conditions

Eight *Bacillus amyloliquefaciens* strains, one *Bacillus pumilus* strain and six pathogenic fungi were used in this study. All *Bacillus* strains were isolated from cool-season grass seeds as described in Chapter 1. *B. amyloliquefaciens* strains were grown in LB broth (Sigma-Aldrich Chemical, St. Louis, MO) at 28ºC and 150 rev/min.

Four turfgrass-pathogenic fungi, *Clarireedia homoeocarpa* (former name *Sclerotinia homoeocarpa*), *Colletotrichum cereale*, *Fusarium* spp., and *Rhizoctonia solani*, as well as two other plant pathogens (*Alternaria* spp. and *Neofusicoccum australe*) in the antagonistic tests were kindly supplied by Dr. Clarke's lab at Department of Plant

Biology, Rutgers. All pathogens were routinely grown on potato dextrose agar (PDA) at 25^{o}C.

3.2.2 Effect of *B. amyloliquefaciens* Strains on Fungal Pathogens

The antifungal effects of bacteria isolated from sterilized cool-season turfgrass seeds was evaluated by co-inoculating bacteria and fungi on PDA and measuring the distance between bacteria and fungi. The *Bacillus* strains were inoculated on PDA by streaking in a manner that would create the three separate areas. The fungi were inoculated in the center of the three areas. The co-cultures were incubated at 28°C and observed after 72 hours. The inhibitory zones were measured as the distance between the streaking bacteria and the nearest edge of growing fungi.

3.2.3 Extraction of Lipopeptides from *B. amyloliquefaciens*

Lipopeptides were isolated by the acid precipitation method (Smyth *et al.*, 2010). *B. amyloliquefaciens* strains were cultured in 1L LB broth in 2L flask at 28°C for 24 hours by shaking at the speed of 150 rev/min. The culture was then centrifuged to remove the cells at 13000g for 15min at 4^{o}C. The collected supernatant was acidified with 5N HCl until the solution reached pH 2.0, and then incubated at 4^{o}C for 16 hours to allow the formation of precipitate. After centrifuging the solution at 13000g for 15min at 4^{o}C, the supernatant was discarded, and the pellet of precipitate was collected and dissolved with methanol by stirring continuously for 4 hours. The methanol solution was then filtered to remove the remaining material. The filtered solution was concentrated by rotary

evaporator at 45°C. The concentrated extraction product was then re-dissolved in methanol.

3.2.4 Mass Spectrometry Analysis

All isolated products were diluted in methanol to a concentration of 100µg/µl, followed by a ten-fold dilution with 2.5mg/ml of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 0.1% trifluoroacetic acid for a final concentration of 10µg/µl. Data was acquired at reflector positive mode from 800 to 4000 m/z on an ABI-MDS SCIEX 4800 MALDI-TOF/TOF™ mass spectrometer analyzer (Applied Biosystems, Froster City, CA). The MALDI-TOF analysis was conducted at the Center for Integrative Proteomics Research (Rutgers University, Piscataway, NJ). The masses detected were used to identify the components of lipopeptides extracted from *B. amyloliquefaciens* strains.

3.2.5 Detection of Non-Ribosomal Peptide Synthetase Genes

The extraction of bacterial genome DNA was performed with GenElute™ Bacterial Genomic DNA kit (Sigma Chemical Company, St. Louis, MO, USA) following the instructional protocol. *bamC*, *fenD*, *ituC*, and *sfp* primers (Table 3.2) were used to detect the NRPS genes for bacillomycin, fengycin, iturin and surfactin, respectively. PCR amplifications were conducted using Invitrogen™ PCR SuperMix (Thermo Fisher Scientific Inc., Waltham, MA, USA) with the following thermocycles: initial denaturation at 94ºC for 5 min followed by 30 cycles (denaturation at 94ºC for 30s, annealing at 62ºC or 55ºC for 30s, and extension at 72ºC for 60s) and the final extension

at 72°C for 8 min. PCR products were visualized with 1% agarose gel stained with SYBR™ Safe DNA Gel Stain (Invitrogen, Carlsbad, CA, USA) and purified with QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The purified PCR products were sent to GENEWIZ Inc. (South Plainfield, NJ, USA) for Sanger sequencing. The result sequences were submitted to GenBank and compared with existent sequences in GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) using web-based *blast* (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Table 3.2 Primer sequences employed to identify the genes involved in lipopeptides production.

Lipopeptides synthetase	Primers	Sequences	References
Iturin	ituC-F	CCCCCTCGGTCAAGTGAATA	Tabbene *et al.*, 2011
	ituC-R	TTGGTTAAGCCCTGATGCTC	
Bacillomycin	bamC-F	AGTAAATGAACGCGCCAATC	
	bamC-R	CCCTCTCCTGCCACATAGAG	
Surfactin	sfp-F	ATGAAGATTTACGGAATTTA	
	sfp-R	TTATAAAAGCTCTTCGTACG	
Fengycin	fenD-F	CCTGCAGAAGGAGAAGTGAAG	Joshi & McSpadden Gardener, 2006
	fenD-R	TGCTCATCGTCTTCCGTTTC	

3.3 Results

3.3.1 Inhibitory Effects of *B. amyloliquefaciens* Strains on Pathogenic Fungi

The inhibitory effects of *B. amyloliquefaciens* strains on fungal pathogens were evaluated under growth chamber conditions. All eight *B. amyloliquefaciens* strains inhibited the growth fungal pathogens, *Alternaria* spp., *Clarireedia homoeocarpa*, *Colletotrichum cereal*, *Fusarium* spp., *Neofusicoccum austral*, and *Rhizoctonia solani*

(Figure 3.1 – Figure 3.6). However, as the negative control, *B. pumilus* strain SF3 didn't show any growth inhibitory effects on the fungi (Figure 3.1 – Figure 3.6). All eight strains showed similar inhibitory effects on fungal pathogens, *Alternaria* spp., *Colletotrichum cereale*, and *Fusarium* spp. (Figure 3.7). However, the inhibitory effects of *B. amyloliquefaciens* strain KB2 on *Clarireedia homoeocarpa*, *Neofusicoccum australe*, and *Rhizoctonia solani* were not as extensive as the other seven strains (Figure 3.7). *B. amyloliquefaciens* strains created the inhibitory zones of 16-20mm on *Colletotrichum cereal*, but 2-7mm on *Clarireedia homoeocarpa* and 3-8mm on *Rhizoctonia solani* (Figure 3.7 and).

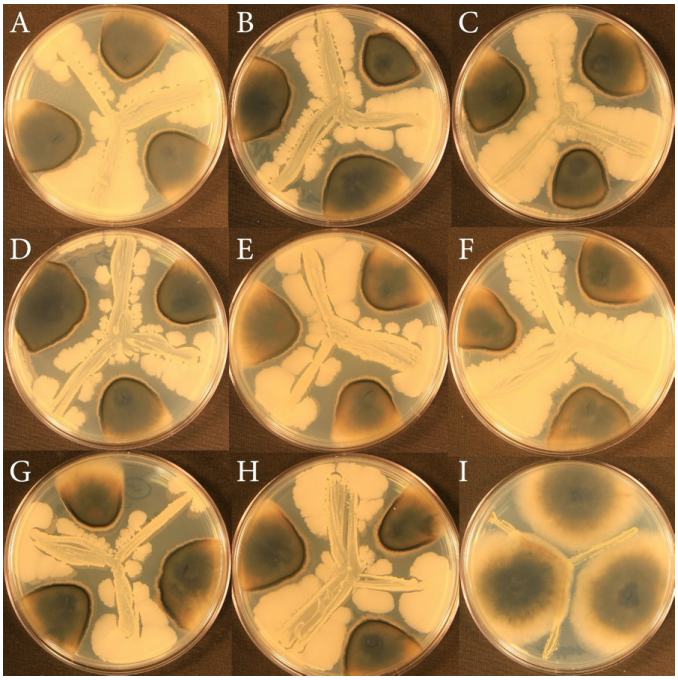

Figure 3.1 Inhibition of *Alternaria* spp. growth by *B. amyloliquefaciens* strains (A – H). *B. pumilus* strain SF3 (I) was used as negative control. *B. amyloliquefaciens* strains: A: CRF2; B: HF3; C: KB2; D: KB5; E: PR1; F: PR2; G: SCF2; H: SF2.

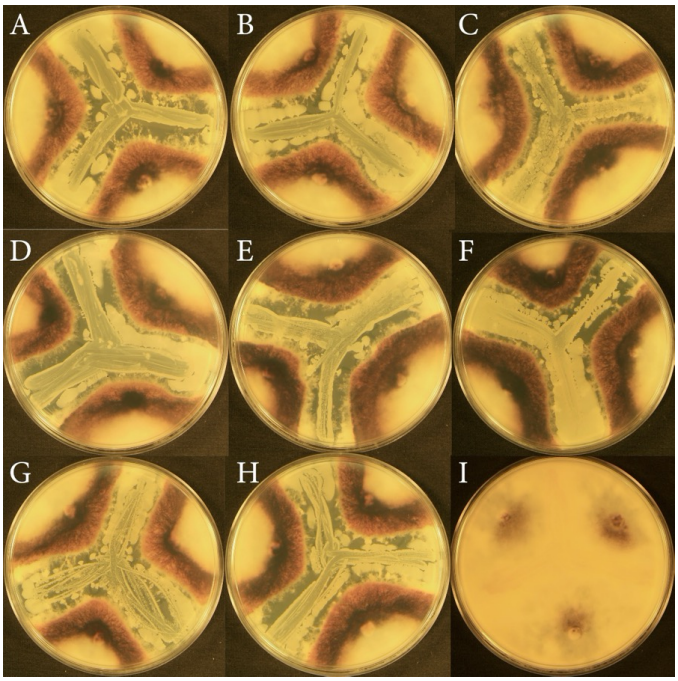

Figure 3.2 Inhibition of *Clarireedia homoeocarpa* growth by *B. amyloliquefaciens* strains (A – H). *B. pumilus* strain SF3 (I) was used as negative control. *B. amyloliquefaciens* strains: A: CRF2; B: HF3; C: KB2; D: KB5; E: PR1; F: PR2; G: SCF2; H: SF2.

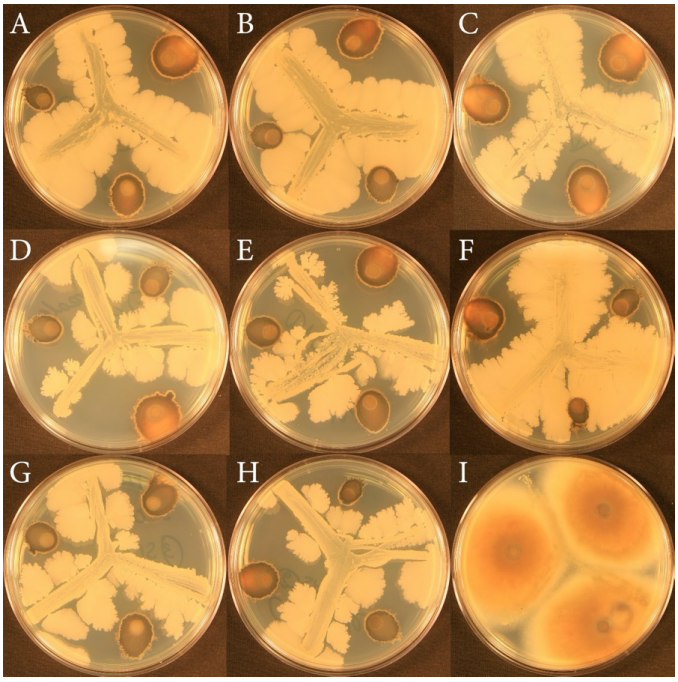

Figure 3.3 Inhibition of *Colletotrichum cereale* growth by *B. amyloliquefaciens* strains (A – H). *B. pumilus* strain SF3 (I) was used as negative control. *B. amyloliquefaciens* strains: A: CRF2; B: HF3; C: KB2; D: KB5; E: PR1; F: PR2; G: SCF2; H: SF2.

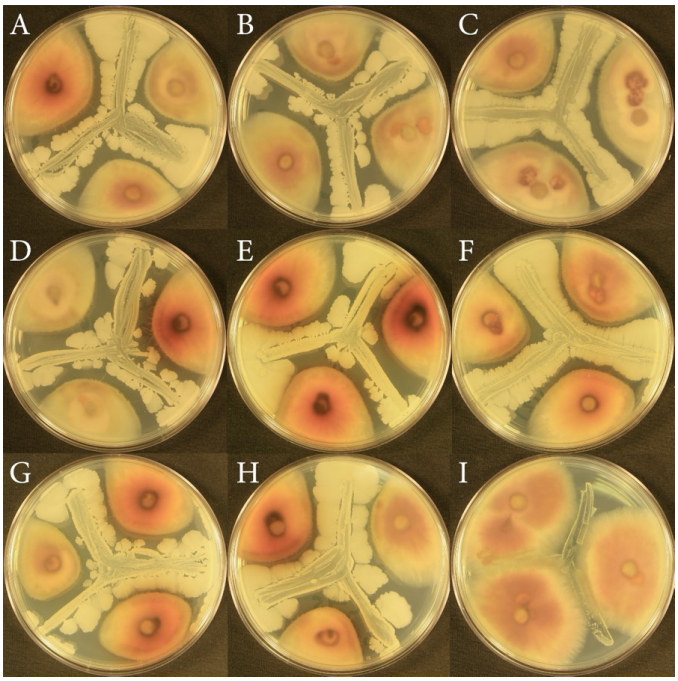

Figure 3.4 Inhibition of *Fusarium* spp. growth by *B. amyloliquefaciens* strains (A – H). *B. pumilus* strain SF3 (I) was used as negative control. *B. amyloliquefaciens* strains: A: CRF2; B: HF3; C: KB2; D: KB5; E: PR1; F: PR2; G: SCF2; H: SF2.

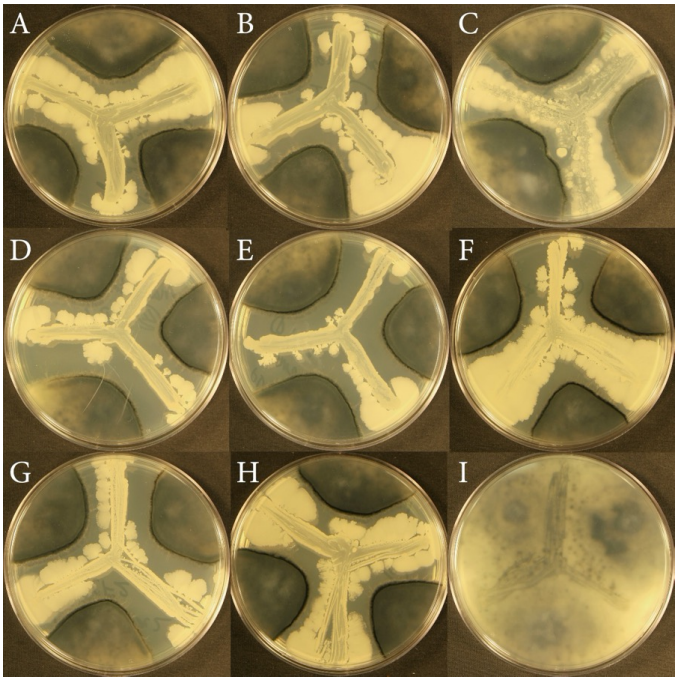

Figure 3.5 Inhibition of *Neofusicoccum austral* growth by *B. amyloliquefaciens* strains (A – H). *B. pumilus* strain SF3 (I) was used as negative control. *B. amyloliquefaciens* strains: A: CRF2; B: HF3; C: KB2; D: KB5; E: PR1; F: PR2; G: SCF2; H: SF2.

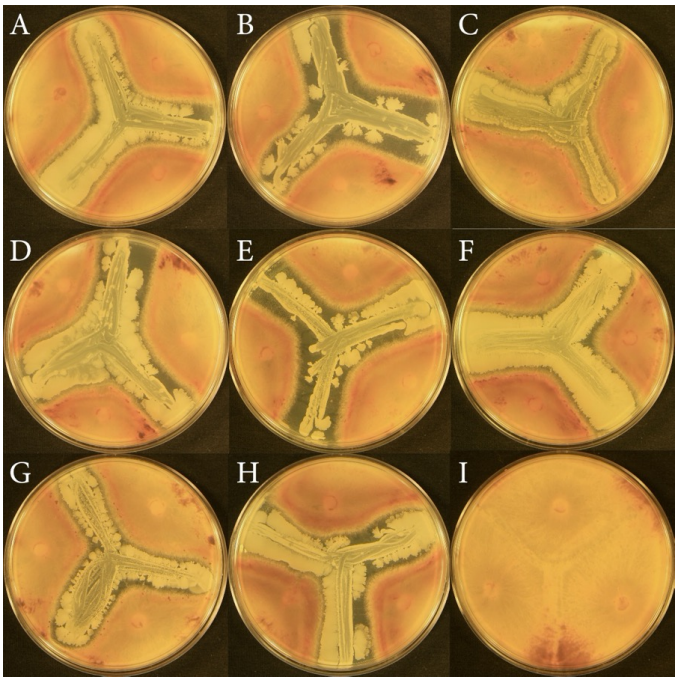

Figure 3.6 Inhibition of *Rhizoctonia solani* growth by *B. amyloliquefaciens* strains (A – H). *B. pumulus* strain SF3 (I) was used as negative control. *B. amyloliquefaciens* strains: A: CRF2; B: HF3; C: KB2; D: KB5; E: PR1; F: PR2; G: SCF2; H: SF2.

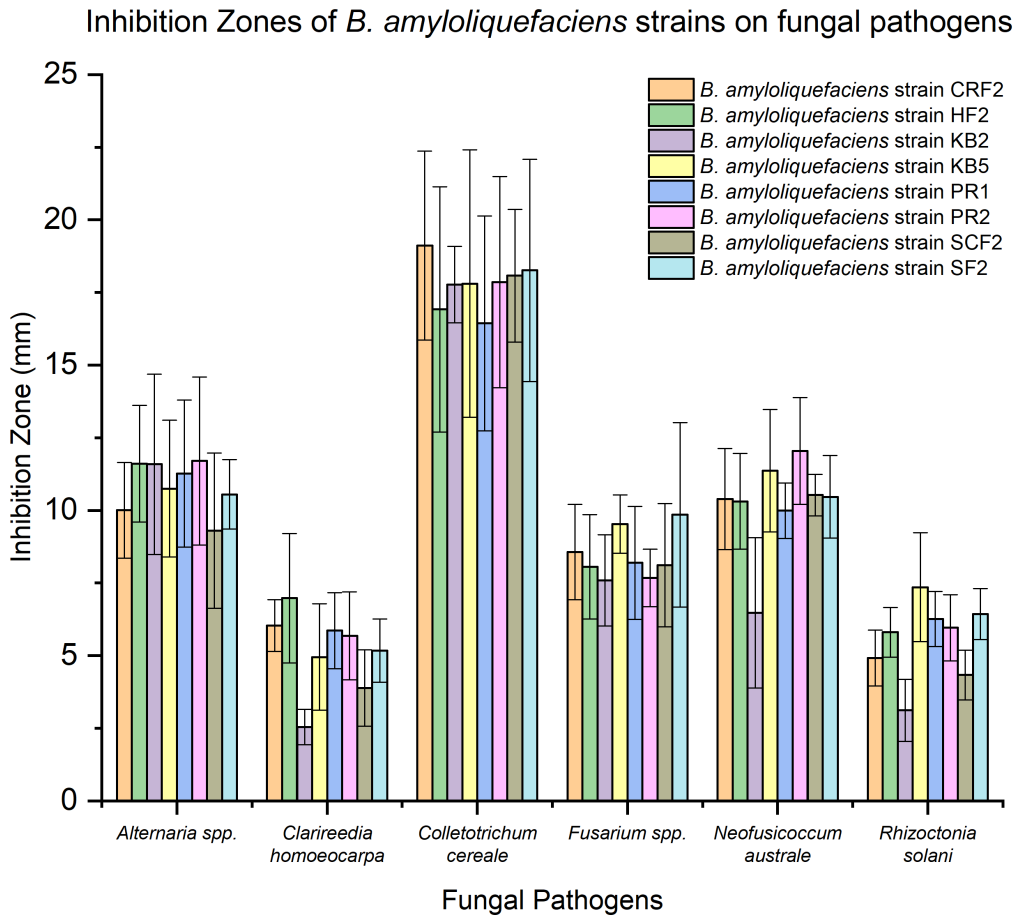

Figure 3.7 Bar chart of inhibition zones of *B. amyloliquefaciens* on fungal pathogens. Three replica plates were measured, and for each plate, six measurements were taken. Error bars represent the standard deviation of measuremants. Negative control *B. pumilus* strain SF3 had zero inhibitory zone.

3.3.2 Analysis of Lipopeptides Produced by *B. amyloliquefaciens* Strains

MALDI-TOF mass spectrometry was employed to analyze the lipopeptide production of *B. amyloliquefaciens* strains. By comparing the mass data observed in our study with the mass numbers reported for the lipopeptides in previous studies (Vater *et al.*, 2002, Koumoutsi *et al.*, 2004), the major lipopeptides produced by the isolated *B. amyloliquefaciens* strains were surfactin, fengycin and iturin (Figure 3.8 – Figure 3.15 and Table 3.3). Surfactin (C13 – C17) was found across all eight strains. However,

surfactin was less abundant in strains HF3 and PR2 then the other strains. Fengycin (C14 – C17) was also detected across all eight strains. Strain KB2 had less abundant fengycin than other strains. Comparing to surfactin and fengycin, iturin was less abundant and was only detected in only 5 strains.

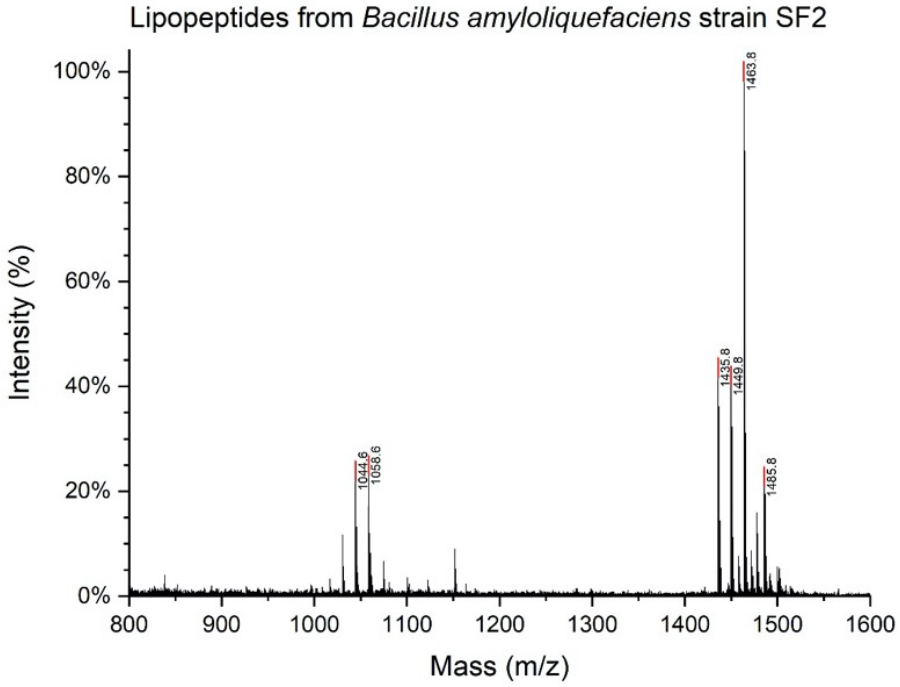

Figure 3.8 MALDI-TOF mass spectrometric analysis of cyclic lipopeptides of *Bacillus amyloliquefaciens* strain CRF2.

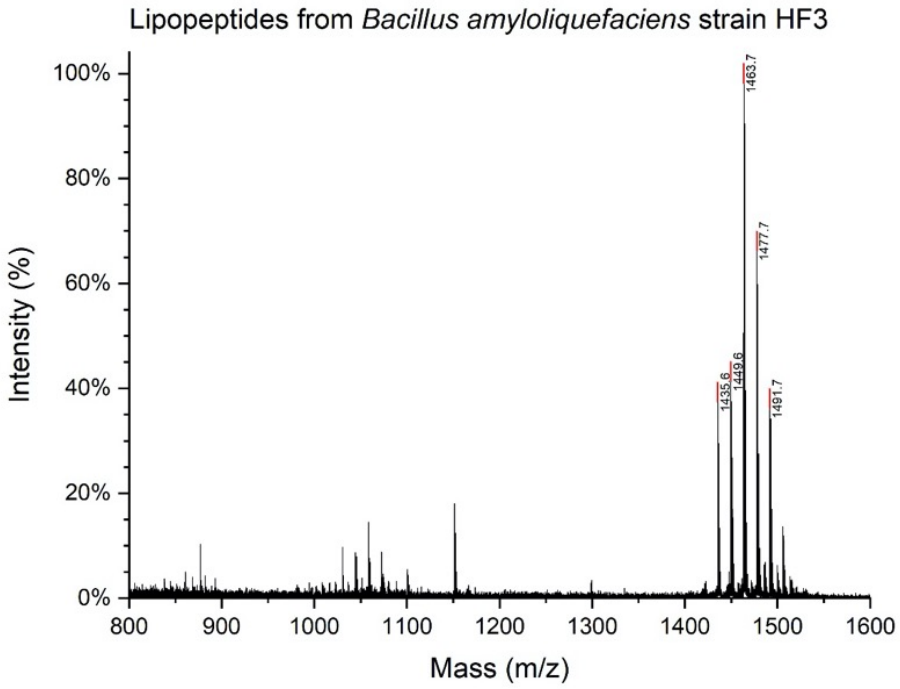

Figure 3.9 MALDI-TOF mass spectrometric analysis of cyclic lipopeptides of *Bacillus amyloliquefaciens* strain HF3.

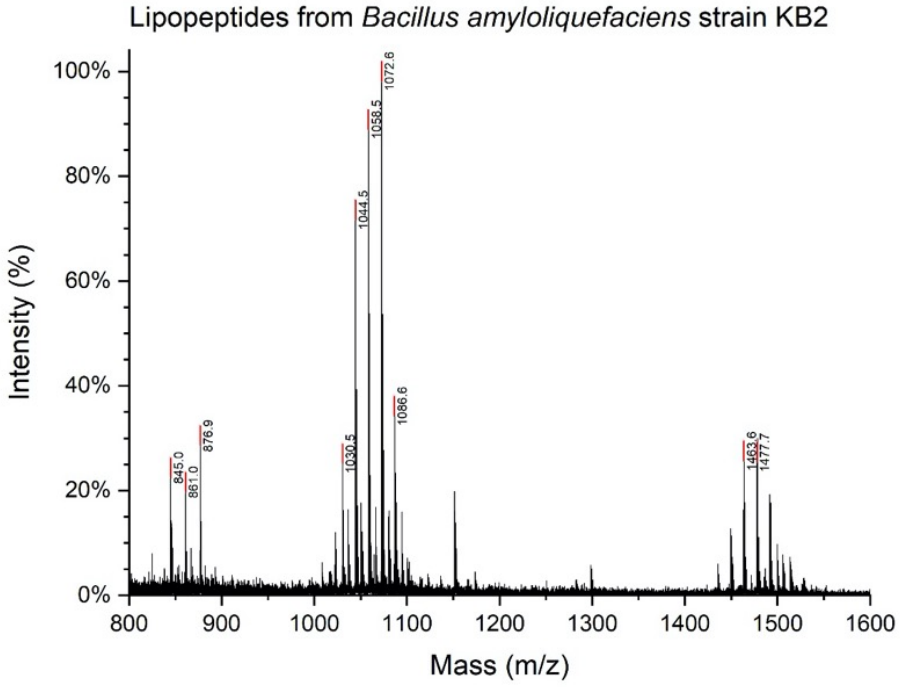

Figure 3.10 MALDI-TOF mass spectrometric analysis of cyclic lipopeptides of *Bacillus amyloliquefaciens* strain KB2.

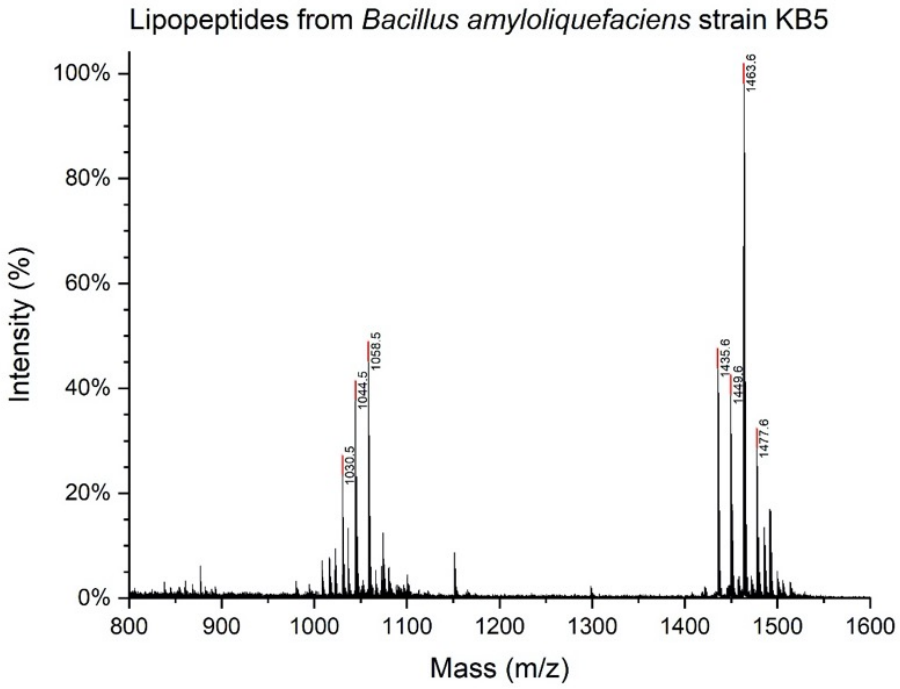

Figure 3.11 MALDI-TOF mass spectrometric analysis of cyclic lipopeptides of *Bacillus amyloliquefaciens* strain KB5.

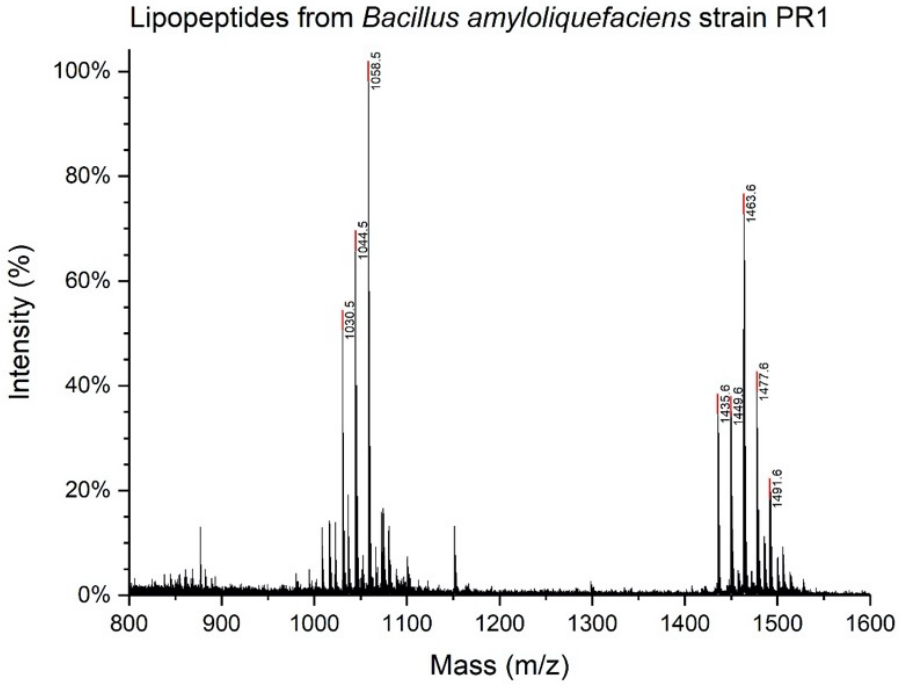

Figure 3.12 MALDI-TOF mass spectrometric analysis of cyclic lipopeptides of *Bacillus amyloliquefaciens* strain PR1.

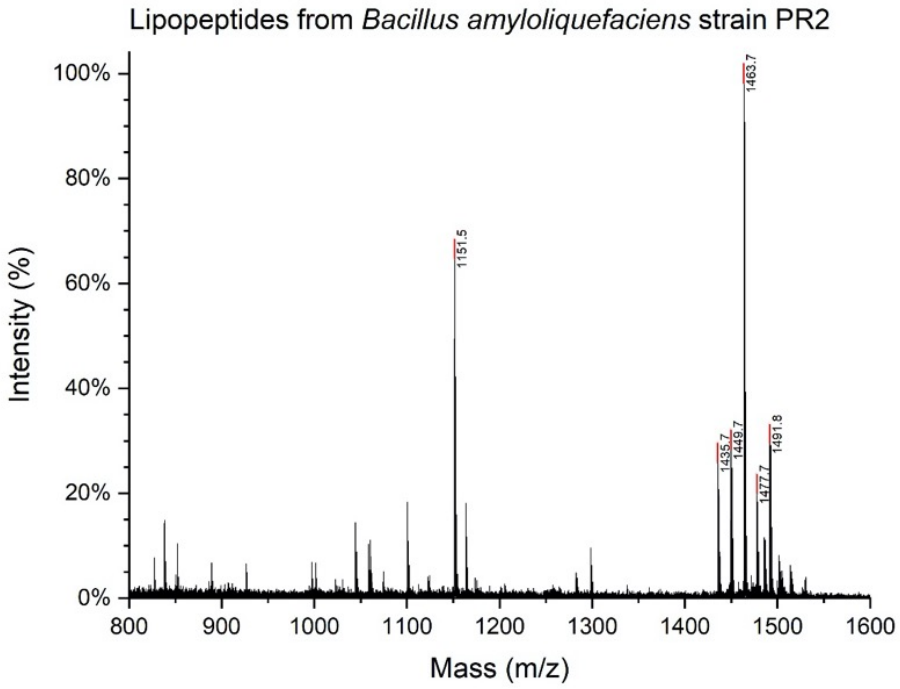

Figure 3.13 MALDI-TOF mass spectrometric analysis of cyclic lipopeptides of *Bacillus amyloliquefaciens* strain PR2.

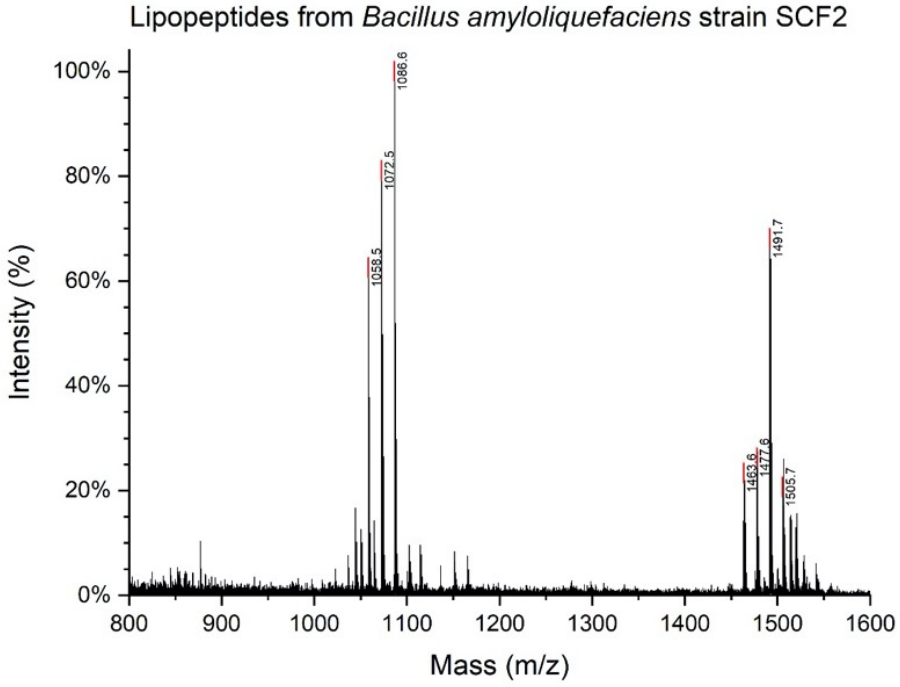

Figure 3.14 MALDI-TOF mass spectrometric analysis of cyclic lipopeptides of *Bacillus amyloliquefaciens* strain SCF2.

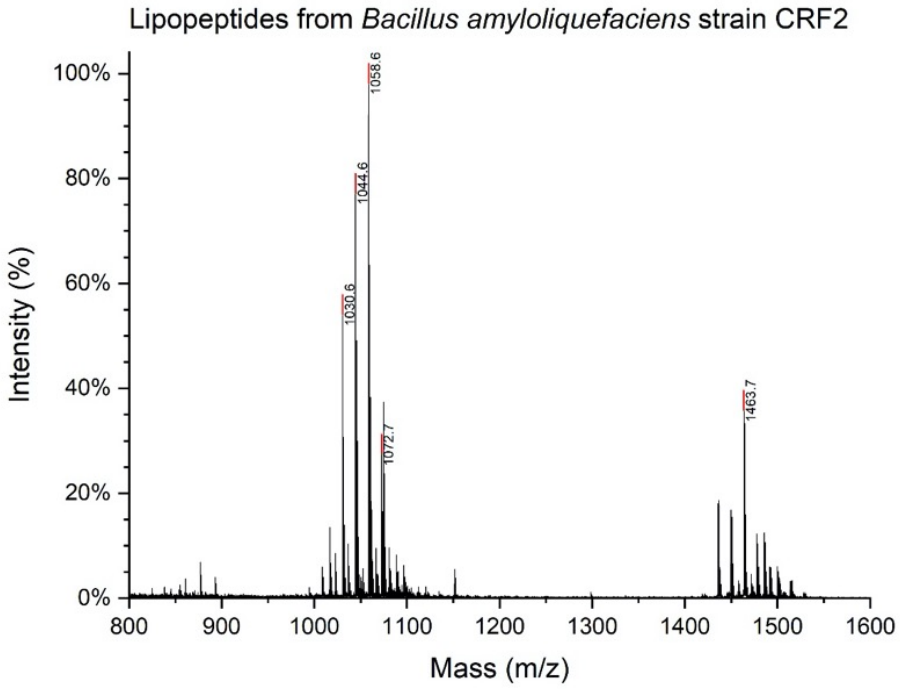

Figure 3.15 MALDI-TOF mass spectrometric analysis of cyclic lipopeptides of *Bacillus amyloliquefaciens* strain SF2.

3.3.3 PCR Detection of Non-Ribosomal Lipopeptide Synthetases

The *fenD*, *ituC*, and *sfp* genes responsible for the synthesis of fengycin, iturin and surfactin, respectively, were detected in all strains. However, *bamC* were not detected in any *B. amyloliquefaciens* strains in our study. The *ituC* sequences of all eight strains showed high identity (> 99%) to *ituC* gene of *Bacillus subtilis* and *B. amyloliquefaciens* in the GenBank database (Table 3.4). Also, the *sfp* fragments of all our strains had a high identity (> 99%) to the *sfp* gene of *B. subtilis* and *B. amyloliquefaciens* in the GenBank database. However, the *fenD* genes of different strains showed some variation to each other and a slightly lower identity (97% - 99%) to *fenD* genes of *B. subtilis* and *B. amyloliquefaciens* in the GenBank database comparing to *ituC* and *sfp* genes.

Table 3.3 Assignments and relatively intensity of all mass peaks obtained by MALDI-TOF mass spectrometry from all *B. amyloliquefaciens* strains.

Lipopeptides Family	Main mass peaks(m/z)	Assignment	Relative intensity (%) of *B. amyloliquefaciens* strains							
			CRF2	HF3	KB2	KB5	PR1	PR2	SCF2	SF2
Surfactin	1016.5	Val-7-C13-Surfactin [M+Na]+	13.53			7.81	14.25			
Surfactin	1030.5	Leu/Ile-7-C13-Surfactin [M+Na]+	55.90	9.74	26.99	25.21	52.46			11.74
Surfactin	1044.5	Leu/Ile-7-C14-Surfactin [M+Na]+	79.04	8.74	73.52	39.53	67.68	14.47	16.72	23.84
Surfactin	1058.5	Leu/Ile-7-C15-Surfactin [M+Na]+	100.00	14.55	90.82	47.00	100.00		62.55	24.87
Surfactin	1072.5	Leu/Ile-7-C16-Surfactin [M+Na]+	29.25	8.82	100.00		15.94		81.05	
Surfactin	1086.6	Leu/Ile-7-C17-Surfactin [M+Na]+			36.07				100.00	
Surfactin	1074.5	Leu/Ile-7-C15-Surfactin [M+K]+	37.41			12.47				
Iturin	1008.5	C12-Iturin [M+Na]+	6.07			7.18	12.92			
Iturin	1022.5	C13-Iturin [M+Na]+	8.59		11.99	9.46	13.96		5.04	
Iturin	1036.5	C14-Iturin [M+Na]+	10.38		16.39	13.32	19.12		7.53	
Iturin	1050.5	C15-Iturin [M+Na]+			17.70				12.62	
Iturin	1064.6	C16-Iturin [M+Na]+							14.31	
Iturin	1052.6	C14-Iturin [M+K]+	5.66				7.67			
Iturin	1066.5	C15-Iturin [M+K]+	9.56		16.83	5.28	9.17			
Iturin	1080.5	C16-Iturin [M+K]+	9.66		16.17	5.92	13.22			
Iturin	1094.5	C17-Iturin [M+K]+			15.92					
Fengycin	1435.6	Ala-6-C14-Fengycin [M+H]+	18.01	39.22		45.66	36.45	27.62		43.49
Fengycin	1449.6	Ala-6-C15-Fengycin [M+H]+	16.83	43.13	12.71	40.59	36.01	30.07		41.93
Fengycin	1463.6	Ala-6-C16-Fengycin [M+H]+	37.63	100.00	27.50	100.00	74.72	100.00	23.32	100.00
Fengycin	1477.7	Ala-6-C17-Fengycin [M+H]+	12.23	68.04	27.25	30.36	40.71	21.65	26.21	15.95
Fengycin	1485.6	Ala-6-C16-Fengycin [M+Na]+	12.52	6.41		13.51	11.24			22.64
Fengycin	1499.6	Ala-6-C17-Fengycin [M+Na]+	6.03	6.26		5.17	7.11			
Fengycin	1491.7	Val-6-C16-Fengycin [M+H]+	6.03	37.97	19.34	16.98	20.30	31.20	68.08	
Fengycin	1505.7	Val-6-C17-Fengycin [M+H]+		13.65			9.26		20.64	

Table 3.4 GenBank accession number of non-ribosomal lipopeptide synthetase genes.

B. amyloliquefaciens strains	*fenD*	*ituC*	*sfp*
CRF2	MT125621	MT125629	MT125630
HF3	MT125619	MT125627	MT125631
KB2	MT125618	MT125626	MT125632
KB5	MT125617	MT125625	MT125633
PR1	MT125620	MT125628	MT125634
PR2	MT125616	MT125624	MT125635
SCF2	MT125615	MT125623	MT125636
SF2	MT125614	MT125622	MT125637

3.4 Discussion

3.4.1 Antagonistic Effects of *B. Amyloliquefaciens* Strains on Fungal Pathogens

All eight *B. amyloliquefaciens* strains exhibited inhibitory effects against the six fungal pathogens in our study. They had the best antagonistic effects against *Colletotrichum cereale,* which causes anthracnose on turfgrass (Table 3.2 and Figure 3.7). This result corresponds to a former study that showed *B. amyloliquefaciens* C2LP had better inhibitory effects on *Colletotrichum* species than *Rhizoctonia* (Dang *et al.*, 2019). When comparing the effects of different strains on pathogens, strains KB2 showed less effects on *Clarireedia homoeocarpa*, *Neofusicoccum australe*, and *Rhizoctonia solani*. *C. homoeocarpa* and *R. solani* are the pathogens responsible for turfgrass disease dollar spot and brown patch, respectively. As all *B. amyloliquefaciens* strains in our study inhibited the growth of turf pathogens *C. homoeocarpa, C. cereale* and *R. solani,* we could employ them as seed coatings or formulate them into biopesticides to control the corresponded turf disease. Also, the *B. amyloliqufaiens* strains in this test showed inhibitory effects on *Alternaria* spp., *Fusarium* spp., *N. austral*, and *R. solani* that could

cause various diseases on other plants, these strains could potentially be used to suppress fungal disease on other plant.

3.4.2 Lipopeptides Production of *B. amyloliquefaciens* Strains

All eight strains can produce fengycin and surfactin. However, strains HF3, PR2 and SF2 didn't show any iturin production (Table 3.3). In strains CRF2, KB2, PR1 and SCF2, surfactin level was higher than fengycin level and iturin level. In strains HF3, KB5, PR2, and SF2, fengycin level was higher than surfactin level and iturin level.

The PCR detection of lipopeptides synthetase genes showed fengycin, iturin, and surfactin synthetases existed in all eight strains. But bacillomycin synthetase were not found in any strains. This is the same as MALDI-TOF lipopeptides analysis except for iturin. The *ituC* gene was detected in all strains but the production of iturins were not found in strains HF3, PR2 and SF2. This suggests that *ituC* might not be sufficient for iturin production. Further research is necessary to explore other genes or factors influencing iturin production.

Strain KB2 which produced less fengycin than all other strains showed less inhibitory effects on *C. homoeocarpa, C. cereale* and *R. solani.* This indicated that fengycin family lipopeptides might be responsible for the biocontrol of those pathogens.

3.5 Conclusion

The *B. amyloliquefaciens* strains associated with cool-season turfgrass seeds inhibited the growth of fungal pathogens, including turf pathogens *C. homoeocarpa, C.*

cereale and *R. solani*. They also produced antifungal lipopeptides fengycin, iturin and surfactin with some variability in production among the different strains.

3.6 References

Dang Y., Zhao F., Liu X., Fan X., Huang R., Gao W., Wang S., & Yang C. (2019). Enhanced production of antifungal lipopeptide iturin A by *Bacillus amyloliquefaciens* LL3 through metabolic engineering and culture conditions optimization. *Microbial Cell Factories, 18*(1), 68.

Hiradate S., Yoshida S., Sugie H., Yada H., & Fujii Y. (2002). Mulberry anthracnose antagonists (iturins) produced by *Bacillus amyloliquefaciens* RC-2. *Phytochemistry, 61*(6), 693-698.

Joshi R., & McSpadden Gardener B. B. (2006). Identification and characterization of novel genetic markers associated with biological control activities in *Bacillus subtilis*. *Phytopathology 96*(2), 145-154.

Jourdan E., Henry G., Duby F., Dommes J., Barthelemy J.-P., Thonart P., & Ongena M. (2009). Insights into the defense-related events occurring in plant cells following perception of surfactin-type lipopeptide from *Bacillus subtilis*. *Molecular Plant-Microbe Interactions, 22*(4), 456-468.

Martínez-Núñez M. A., & y López V. E. L. (2016). Nonribosomal peptides synthetases and their applications in industry. *Sustainable Chemical Processes, 4*(1), 13.

Moyne A.L., Shelby R., Cleveland T.E., & Tuzun S. (2001). Bacillomycin D: an iturin with antifungal activity against *Aspergillus flavus*. *Journal of Applied Microbiology 90*(4), 622-629.

Ongena M., & Jacques P. (2008). *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. *Trends in Microbiology, 16*(3), 115-125.

Ongena M., Jourdan E., Adam A., Paquot M., Brans A., Joris B., Arpigny J. L., & Thonart P. (2007). Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environmental Microbiology, 9*(4), 1084-1090.

Peypoux F., Bonmatin J. M., & Wallach J. (1999). Recent trends in the biochemistry of surfactin. *Applied Microbiology and Biotechnology, 51*(5), 553-563.

Raaijmakers J. M., de Bruijn I., Nybroe O., & Ongena M. (2010). Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiology Reviews, 34*(6), 1037-1062.

Romero D., de Vicente A., Rakotoaly R. H., Dufour S. E., Veening J.-W., Arrebola E., Cazorla F. M., Kuipers O. P., Paquot M., & Pérez-García A. (2007). The iturin and fengycin families of lipopeptides are key factors in antagonism of *Bacillus*

subtilis toward *Podosphaera fusca*. *Molecular Plant-Microbe Interactions, 20*(4), 430-440.

Rosconi F., Davyt D., Martinez V., Martinez M., Abin-Carriquiry J. A., Zane H., Butler A., de Souza E. M., & Fabiano E. (2013). Identification and structural characterization of serobactins, a suite of lipopeptide siderophores produced by the grass endophyte *Herbaspirillum seropedicae*. *Environmental Microbiology, 15*(3), 916-927.

Smyth T. J. P., Perfumo A., McClean S., Marchant R., & Banat I. M. (2010). Isolation and Analysis of Lipopeptides and High Molecular Weight Biosurfactants. In *Hand book of Hydrocharbon and Lipid Microbiology*, 3687-3704.

Tabbene O., Kalai L., Ben Slimene I., Karkouch I., Elkahoui S., Gharbi A., Cosette P., Mangoni M. L., Jouenne T., & Limam F. (2011). Anti-candida effect of bacillomycin D-like lipopeptides from *Bacillus subtilis* B38. *FEMS Microbiology Letter, 316*(2), 108-114.

Yu G. Y., Sinclair J. B., Hartman G. L., & Bertagnolli B. L. (2002). Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctonia solani*. *Soil Biology and Biochemistry, 34*(7), 955-963.

Chapter 4 16S rRNA Metagenomic Analysis of the Bacterial Community Associated with Turf Grass Seeds from Low Moisture and High Moisture Climates

4.1 Introduction

Plants bear numerous microbes that influence their nutrition, development, stress responses and phenotypes (Henning *et al.*, 2016, White *et al.*, 2014, White *et al.*, 2018). Plant-associated microbes generally come from the surrounding environment; however, Johnston-Monje and Rhizada (2011) showed that corn seeds vectored a diverse array of microbes. Further, Johnston-Monje *et al.* (2016) found that the rhizospheres of corn seedlings were composed of microbes that originated both from seeds and bacteria recruited from soils. These seed-vectored bacteria can influence germination and share a mutualistic association with the host seedlings (Cruz *et al.*, 2014, Shaid & Thomas, 2019, Somova *et al.*, 2001, Zhu *et al.*, 2017). Without seed-vectored bacteria, seedlings may lose gravitropic response of roots, fail to develop root hairs, and are more susceptible to soil-borne pathogens (Verma *et al.*, 2017, 2018).

Some turf grasses possess fungal endophytes of ascomycete genus *Epichloë* that provide resistance to pathogens and insects, and increase abiotic stress tolerance in the host (Bultman & Bell, 2003, Clay, 1990, Meyer *et al.*, 2012, White, 1987). Turf breeders have long been employing these environmentally safe endophytes to enhance turfgrass performance and stress tolerance (Meyer *et al.*, 2012). Another important microbe resource, the seed-transmitted bacterial communities of turf grasses, are yet to be fully explored. Many of these bacteria are vectored on the surfaces of seeds and embedded within dried plant tissues (paleas and lemmas) that adhere tightly to seed surfaces (White

et al., 2019). During seed germination some of these seed-surface microbes are activated and they externally and internally colonize seedling roots at the root tip meristems, becoming intercellular and intracellular endophytes in the emergent seedling roots (Verma *et al.*, 2017, 2018, White *et al.*, 2018). In this study, we employed Illumina HTS and 16S metagenomic analysis to investigate the bacterial community associated with cool-season turfgrass seeds produced in low moisture (LM) and high moisture (HM) climates. We also evaluated the potential influence of the bacterial community on seed germination rates and seedling growth rates. The results showed that HM seeds vectored a denser and more diverse bacterial community than LM seeds. Also, bacterial groups at different taxonomic ranks correlated with the seed germination rate and time.

4.2 Materials & Methods

4.2.1 Total DNA Extraction from Seeds of Cool-Season Turfgrasses

Seeds of twenty-seven cool-season turf cultivars were obtained from DLF Pickseed USA (Table 4.1). All varieties were produced from 2011 to 2015 at either Store Hedinge, Denmark or Les Alleuds, France. Based on the precipitation data collected from The National Oceanic and Atmospheric Administration (NOAA), the seeds were classified into LM seeds (annual precipitation < 750 mm) and HM seeds (annual precipitation >750 mm). With this classification, five samples were classified as LM seeds while twenty-two samples as HM seeds. 100mg of seeds of each turf cultivar were weighed out and washed with sterilized distilled water for 3 times, 30 seconds each time to remove the soil. The cleaned seeds were then ground into powder with a sterilized mortar and pestle for total DNA extraction. The DNA extraction was conducted with

DNeasy® PowerSoil® Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. This PowerSoil® Kit was chosen due to its versatility with diverse sample types. The concentration of extracted DNA was measured with The NanoDrop® ND-1000 Spectrophotometer and normalized to 5ng/µl for the library preparation.

Table 4.1 Information of seed resources. For each seed sample, sample number, species, variety, production year, production location, and the annual rainfall are reported.

Sample Number	Species	Variety	Year	Location	Annual Rainfall (mm)
V01	*Festuca rubra*	Caldri	2011	Store Hedinge, Denmark	799.08
V02	*Festuca rubra*	Blenheim	2011	Store Hedinge, Denmark	799.08
V03	*Festuca rubra*	FRC 1310	2011	Store Hedinge, Denmark	799.08
V04	*Festuca rubra*	Troville	2011	Store Hedinge, Denmark	799.08
V05	*Festuca rubra*	Beudin	2011	Store Hedinge, Denmark	799.08
V06	*Festuca rubra*	Cezanne	2011	Store Hedinge, Denmark	799.08
V07	*Lolium arundinacea*	Braveheart	2011	Store Hedinge, Denmark	799.08
V08	*Lolium arundinacea*	Greenbrooks	2011	Store Hedinge, Denmark	799.08
V09	*Lolium perenne*	Mercitwo	2014	Store Hedinge, Denmark	850.39
V10	*Lolium perenne*	Bizet 1	2014	Store Hedinge, Denmark	850.39
V11	*Lolium perenne*	Galleon	2015	Store Hedinge, Denmark	738.38

Sample Number	Species	Variety	Year	Location	Annual Rainfall (mm)
V12	*Lolium perenne*	Clementine	2013	Store Hedinge, Denmark	550.93
V13	*Lolium perenne*	Melbourne	2014	Store Hedinge, Denmark	850.39
V14	*Lolium perenne*	Chardin	2011	Store Hedinge, Denmark	799.08
V15	*Lolium perenne*	Fabian	2014	Store Hedinge, Denmark	850.39
V16	*Lolium perenne*	Chardin	2014	Store Hedinge, Denmark	850.39
V17	*Lolium perenne*	Columbine	2011	Store Hedinge, Denmark	799.08
V18	*Lolium perenne*	Tetragreen	2014	Les Alleuds, France	786.38
V19	*Lolium perenne*	DLF LPG 3022	2011	Store Hedinge, Denmark	799.08
V20	*Lolium perenne*	Fabian	2013	Les Alleuds, France	786.64
V21	*Lolium perenne*	Tetratop	2015	Les Alleuds, France	633.48
V22	*Lolium perenne*	Tetradry	2013	Les Alleuds, France	786.64
V23	*Lolium perenne*	Tetrastar	2014	Les Alleuds, France	786.38
V24	*Lolium perenne*	Tetragreen	2011	Les Alleuds, France	587.5
V25	*Lolium perenne*	Essence	2012	Store Hedinge, Denmark	636.02
V26	*Lolium perenne*	Hamlet HZ 2NF1	2012	Store Hedinge, Denmark	636.02
V27	*Lolium perenne*	Berlioz 1	2011	Store Hedinge, Denmark	799.08

4.2.2 Library Preparation and Sequencing

The preparation of DNA libraries for each sample followed the Illumina guidelines. By using 12.5ng of the normalized DNA from turf seeds as the template, V3-V4 hypervariable regions of bacterial 16S rRNA gene were amplified with the primer pair , S-D-Bact-0341-b-S-17 (5'-CCTACGGGNGGCWGCAG-3') and S-D-Bact-0785-a-A-21 (5'-GACTACHVGGGTATCTAATCC-3') fused with Illumina overhang forward adapters (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3') and reverse adapter (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'), respectively (Klindworth *et al.*, 2013). PCR clean-ups were conducted to purify the 16S V3-V4 amplicons away from free primers and primer dimers. Nextera XT index primers were then used for the index PCR and PCR clean-ups were performed again to generate the final library. The generated 16S V3-V4 region library was paired-end sequenced (2 x 300bp) on an Illumina MiSeq platform in the Genome Cooperative Sequencing Facility, School of Environmental and Biological Sciences at Rutgers.

4.2.3 Bacterial Community Structure Analysis

The collected sequencing data in FASTQ format was processed and analyzed with the QIIME2 software suite (Caporaso *et al.*, 2010). The raw Illumina reads were imported into QIIME2 with "Casava 1.8 paired-end demultiplexed fastq" method, and then denoised and filtered with *dada2* pipeline to remove noisy and chimeric sequences, construct denoised paired-end sequences, and dereplicate them (Callahan *et al.*, 2016). *de novo* clustering was then carried out with *VSEARCH* plugin at 99% identity to generate Operational Taxonomic Units (OTUs) (Rognes *et al.*, 2016). The taxonomy assignment

of OTUs was performed by using *feature-classifier* against the SILVA 1.28 database (Released September 29, 2016). After removing mitochondria and chloroplast sequences, the filtered data were aligned with *mafft* program and *fasttree* method to generate rooted and unrooted phylogenetic trees (Price *et al.*, 2010). All core metrics used in alpha and beta diversity analysis were computed based on the rooted phylogenetic tree. Alpha diversity (intra group diversity) was calculated with the observed OTUs and Faith's Phylogenetic Diversity (Faith, 1992) at the sample depth of 1000 reads to normalize the variance and this excluded 4 samples (3 HM samples and 1 LM sample), leaving 4 LM samples and 19 HM samples. The Kruskal-Wallis (pairwise) test was utilized to assess the statistically significant differences in alpha diversity among samples. Beta diversity was performed with both qualitative (Jaccard and unweighted UniFrac) and quantitative (Bray-Curtis and weighted UniFrac) distance metrics at sample depth of 1000 reads. In this process, QIIME2 *diversity* plugin was employed. Statistically significant differences in beta diversity among different groups was evaluated by permutation-based ANOVA (PerMANOVA) test (Anderson, 2005) with 999 permutations (beta-group-significance command in *diversity* plugin). Principal coordinates analysis plots (PCoA) were generated by *Emperor* tool of QIIME2 to explore the bacterial community structure. The bar plots showing taxonomy levels were generated by QIIME2 *taxa* plugin. The analysis workflow is shown in Figure 4.1.

The venn diagram was generated with a web-based tool (http://bioinformatics.psb.ugent.be/webtools/Venn) to calculate the intersection(s) of the list of elements that in this study was represented by the list of genera of bacteria found in

each climate condition and species. The graphical output is in the form of a Venn/Euler diagram.

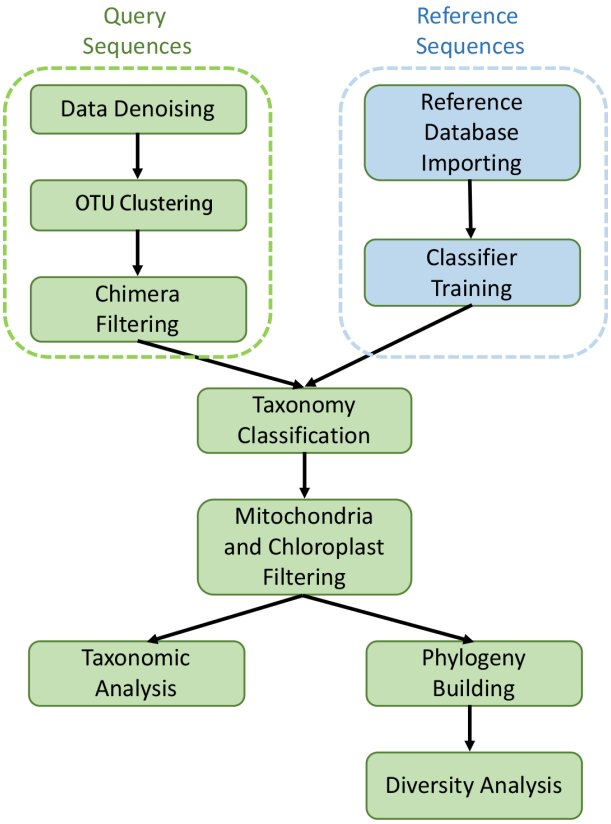

Figure 4.1 Graphical workflow of metagenomic analysis in our study.

4.2.4 Seed germination test

Seeds of 19 cool-season cultivars were placed in Petri dishes containing 25ml 1.5% agar. All Petri dishes were kept in a growth chamber at 28°C. Seed germination was observed every 24 hours until no more seed germinated. Seed germination rates and time for each sample were calculated and Pearson's correlation analysis was performed and visualized with python-based libraries *SciPy* (V0.19.1), *pandas* (V0.22.0), *seaborn* (V0.9.0) and *matplotlib* (V2.2.3).

4.3 Results

4.3.1 Sequence Analysis

In total, 7,405,226 sequences (about 274,368 sequences per sample) were generated by Illumina MiSeq sequencing and imported into QIIME2 pipeline suite for analysis. After being denoised and dereplicated with *dada2* pipeline, the remaining high-quality sequences were clustered into 310 OTUs that had an average length of 427 bp, ranging from 267 bp to 440 bp. After the removal of mitochondrial and chloroplast genomes, a total of 247 OTUs were used to represent the bacterial profile of turf seeds samples (Appendix 2).

4.3.2 Diversity of Bacteria Associated with Turf Seeds from LM and HM Climates

The bacterial community associated with turf seed samples was composed of 5 phyla, 8 classes, 21 orders, 37 families and 69 genera. The bacterial community vectored by seeds produced in HM climates covered all discovered taxonomies, with 6644 sequences/sample. However, seeds from LM climate only hosted part of them, 4 phyla, 8 classes, 9 orders, 10 families and 15 genera, with 2821 sequences/sample.

Regardless of the climate and turf species, bacterial communities at phylum level were dominated by Proteobacteria (51%) and Bacteroidetes (40%). Proteobacteria took 89% and 50% of the bacterial community on LM and HM climates seeds, respectively. Bacteroidetes was abundant in HM climate seeds (39%) but not LM climate seeds (2%). Actinobacteria (6%) and Firmicutes (3%) also comprised a portion of the bacterial community and exhibited no significant difference between the two climate types.

Both HM and LM seeds shared some of the most abundant bacterial classes, i.e. Actinobacteria, Bacteroidia, Bacilli, Alphaproteobacteria and Gammaproteobacteria (Figure 4.2). Compared to LM seeds, seeds from HM environment was richer in terms of Faith's Phylogenetic diversity (Figure 4.3 & Table 4.2).

At class level, Actinobacteria, Bacteroidia and Bacilli had the same portion as phyla Actinobacteria, Bacteroidetes and Firmicutes, respectively (Table 4.3). Alphaproteobacteria and Gammaproteobacteria were the two classes within phylum Proteobacteria. Alphaproteobacteria took 6% and 18% of the bacterial community of LM and HM climate seeds, respectively. Gammaproteobacteria was 83% and 32% for LM and HM climate, respectively.

At genus level, LM seeds harbored a significantly higher percentage of *Massilia* ($p = 0.013$), *Pantoea* ($p = 0.060$), and *Pseudomonas* ($p = 0.045$) compared to HM seeds (Table 4.3). In contrast, HM seeds harbored more of *Flavobacterium* ($p < 0.001$), *Chryseobacterium* ($p < 0.001$), *Pedobacter* ($p < 0.001$), *Sphingomonas* ($p = 0.035$), and *Erwinia* ($p = 0.122$) (Table 4.3).

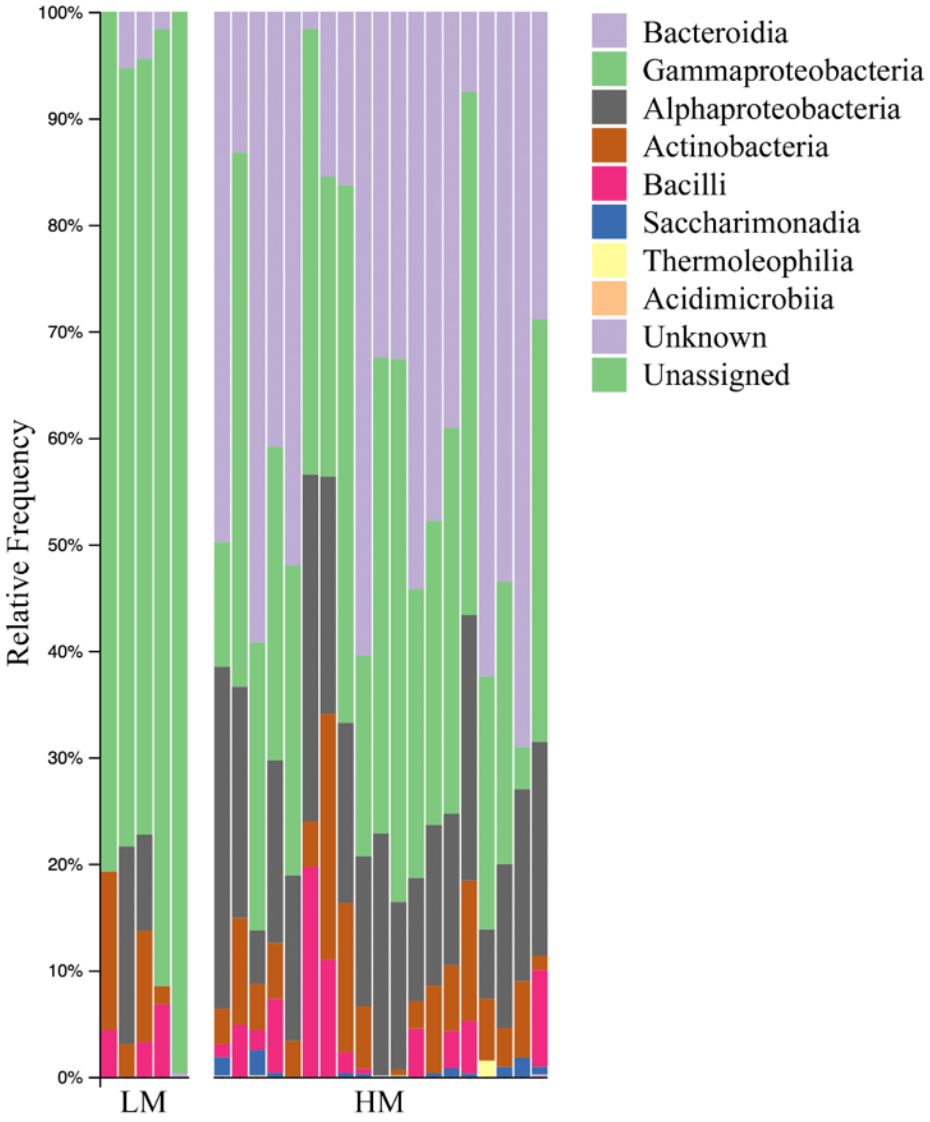

Figure 4.2 Bar plot analysis illustrating the relative abundance and distribution of the OTUs assigned to class-level taxonomy. LM: low moisture; HM: high moisture.

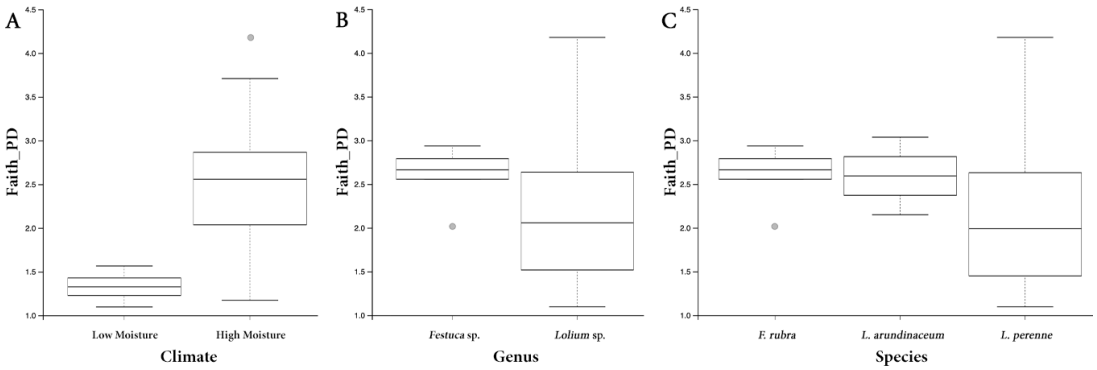

Figure 4.3 Box plots depicting the Faith's Phylogenetic Diversity for different climate conditions (A), different genera (B), and different species (C).

Table 4.2 Kruskal-Wallis pairwise test with measure of Faith PD metric as response variable.

	Group 1	Group 2	p-value
Climate	LM (n=4)	HM (n=19)	0.0058
Genus	*Festuca* (n=6)	*Lolium* (n=17)	0.2076
Species	*Festuca rubra* (n=6)	*Lolium arundinacea* (n=2)	0.7389
	Festuca rubra (n=6)	Lolium perenne (n=15)	0.1391
	Lolium arundinacea (n=2)	*Lolium perenne* (n=15)	0.2967

The bacterial community structure of LM seeds was different from HM seeds (Figure 4.4). LM seeds did not bear any unique bacteria genus that was not present in HM seeds (Figure 4.5A & Appendix 3.1). However, HM seeds harbored 55 genera that LM seeds didn't. Seeds of the three turf species shared 4 phyla and 27 genera, including five genera that were uncultured or unknown species from either Bacterioidetes or Patescibacteria (Figure 4.5B, Appendix 3.2 and Table 4.5).

Table 4.3 Composition of bacterial community from LM and HM climate seeds.

Taxa level	Taxa name	Ave. Percentage		
		LM	HM	Combined
Phylum	Actinobacteria **	6%	7%	6%
	Bacteroidetes †	2%	39%	40%
	Firmicutes **	3%	4%	3%
	Patescibacteria	—	<1%	<1%
	Proteobacteria †	89%	50%	51%
Class	Actinobacteria **	6%	6 %	6%
	Bacteroidia	2%	39%	40%
	Bacilli **	3%	4%	3%
	Saccharimonadia	—	<1%	<1%
	Alphaproteobacteria †	6%	18%	15%
	Gammaproteobacteria †	83%	32%	36%
Order	Micrococcales **	6%	5%	5%
	Cytophagales	<1%	2%	2%
	Flavobacteriales †	<1%	11%	9%
	Sphingobacteriales †	1%	26%	21%
	Bacillales **	3%	4%	4%
	Saccharimonadales	—	<1%	<1%
	Rhizobiales	—	3%	2%
	Sphingomonadales †	6%	15%	13%
	Betaproteobacteriales †	30%	13%	17%
	Enterobacteriales †	24%	12%	15%
	Pseudomonadales †	29%	6%	11%
Family	Microbacteriaceae **	6%	5%	5%
	Hymenobacteraceae	<1%	1%	1%
	Flavobacteriaceae †	<1%	3%	3%
	Weeksellaceae †	<1%	7%	6%
	Sphingobacteriaceae †	1%	26%	21%
	Paenibacillaceae **	3%	4%	4%
	Rhizobiaceae †	—	2%	1%
	Sphingomonadaceae †	6%	15%	13%
	Burkholderiaceae †	30%	13%	17%
	Enterobacteriaceae †	24%	12%	15%
	Pseudomonadaceae †	29%	6%	11%
Genus	*Curtobacterium* **	2%	2%	2%
	Hymenobacter	<1%	1%	1%

Taxa level	Taxa name	Ave. Percentage		
		LM	HM	Combined
	Flavobacterium †	<1%	3%	3%
	Chryseobacterium †	<1%	7%	6%
	Mucilaginibacter	—	<1%	<1%
	Pedobacter †	1%	25%	20%
	Paenibacillus **	3%	4%	4%
	*Rhizobium**	—	2%	1%
	Sphingomonas †	6%	15%	13%
	Duganella †	5 %	3%	3%
	Massilia †	25%	9%	12%
	Erwinia †	<1%	3%	3%
	Pantoea †	23%	9%	12%
	Pseudomonas †	29%	6%	11%
Unassigned		<1%	<1%	<1%

*: Rhizobium group also includes *Allorhizobium*, *Neorhizobium*, and *Pararhizobium*.
**: Bacterial groups without significant difference between LM and HM.
†: Bacterial groups with significant difference between LM and HM.

Table 4.4 PERMANOVA pairwise results considering as response variable Bray-Curtis dissimilarity matrix.

	Group 1	Group 2	Sample size	Permutations	pseudo-F	p-value	q-value
Climate	LM	HM	23	999	2.71867887	0.002	0.002
Genus	*Festuca*	*Lolium*	23	999	1.32100421	0.18	0.18
Species	*Festuca rubra*	*Lolium arundinacea*	8	999	1.37785001	0.101	0.1635
	Festuca rubra	*Lolium perenne*	21	999	1.52206514	0.109	0.1635
	Lolium arundinacea	*Lolium perenne*	17	999	1.54914181	0.204	0.204

Table 4.5 Seed-vectored bacterial genera shared by *Loium arudinacea*, *Lolium perenne*, and *Festuca rubra*.

Phylum	Genus
Actinobacteria	*Curtobacterium*
	Sanguibacter
Bacteroidetes	*Chryseobacterium*
	Dyadobacter
	Flavobacterium
	Mucilaginibacter
	Pedobacter
	Sphingobacterium
	Spirosoma
	uncultured Sphingobacteriaceae
	unknown Sphingobacteriaceae
Patescibacteria	uncultured bacterium
	uncultured *Sphingobium* sp.
	unknown Saccharimonadales
Proteobacteria	*Rhizobium**
	Aureimonas
	Brevundimonas
	Devosia
	Duganella
	Erwinia
	Massilia
	Novosphingobium
	Pantoea
	Pigmentiphaga
	Pseudomonas
	Sphingomonas
	Verticia

*: *Rhizobium* group also includes *Allorhizobium*, *Neorhizobium*, and *Pararhizobium*.

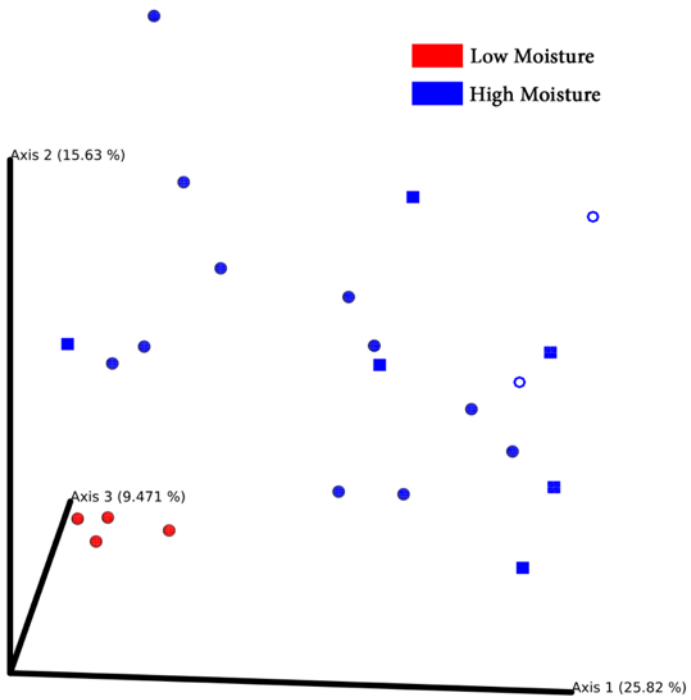

Figure 4.4 Principal Coordinates Analysis (PCoA) Emperor plots based on Bray-Cuitis diversity matrix. Samples are scattered concerning their bacterial community. Species were represented by different shapes: ring – *Lolium arudinacea*; sphere – *Lolium perenne*; square – *Festuca rubra*.

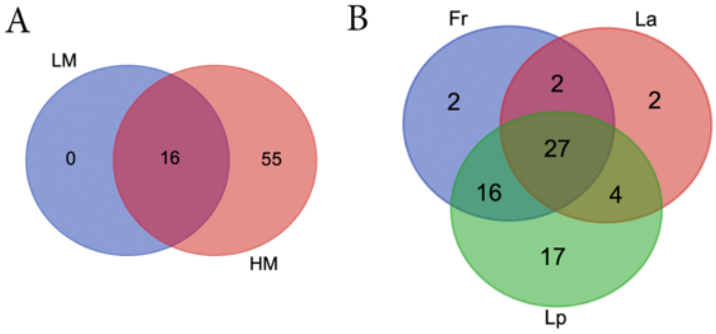

Figure 4.5 Venn diagrams showing the number of shared bacterial genera between different climate (A) and among different turf species (B). LM: low moisture; HM: high moisture; Fr: *Festuca rubra*; Lp: *Lolium perenne*; La: *Loium arudinacea*.

4.3.3 Correlation of Seed Germination and Bacterial Endophyte Composition Associated with Turf Seeds

Bacterial groups at different taxonomic ranks correlated with the seed germination rate and time (Figure 4.6 & Figure 4.7). Among the 5 phyla that we discovered through diversity analysis, Proteobacteria correlated positively with the seed germination rate ($p=0.028$) and negatively with the seed germination time ($p=0.016$). Phylum Actinobacteria also showed a negative correlation with the seed germination time ($p=0.040$) but not a significant correlation with germination rate ($p=0.120$). Another phylum, Firmicutes, showed correlation with germination ($p=0.109$) rate and germination time ($p=0.069$), but this was not statistically significant. However, the abundance of Bacteroidetes was negatively associated with the seed germination rate ($p=0.008$), and positively associated with the seed germination time ($p=0.002$).

At class level, Bacilli and Gammaproteobacteria were groups showing exactly the same correlation as phyla Firmicutes and Proteobacteria, respectively (Figure 4.6 & Figure 4.7). Also, the abundance of Bacteroidia and Gammaproteobacteria showed a similar correlation to phylum Bacteroidetes and Proteobacteria.

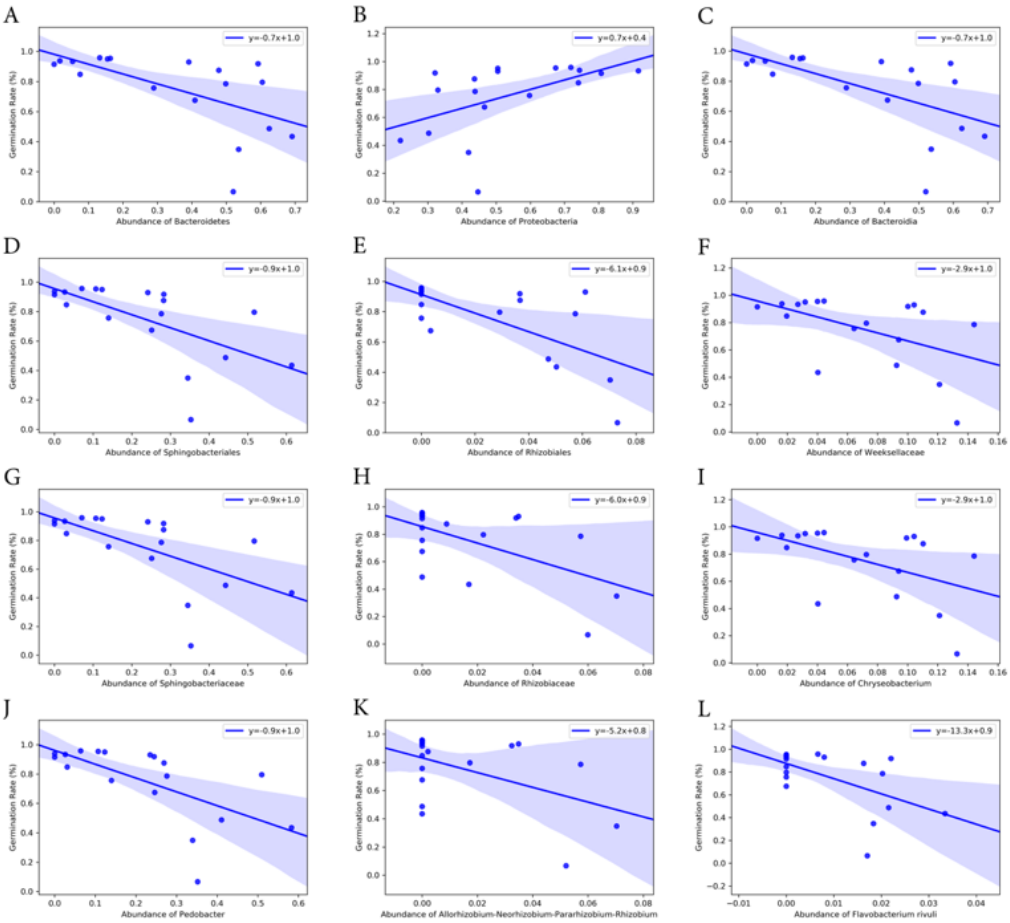

Figure 4.6 Correlation of seed germination rate with abundance of bacteria groups at different taxonomy levels. Phylum: A and B; Class: C; Order: D and E; Family: F – H; Genus: I – K; Species: L. Pearson's correlation analysis was performed and visualized with python-based libraries *SciPy*, *pandas*, *seaborn* and *matplotlib*. The abundance of bacterial groups at different taxonomy levels is on the X-axis, and the germination rate is on the Y-axis.

At family level, seed germination rate was positively related to the abundance of bacteria from families Microbacteriaceae (p=0.090), Paneibacillaceae (p=0.109) and Pseudomonadaceae (p=0.138), and negatively associated with the abundance of bacteria from Rhizobiaceae (p=0.014), Sphingobacteriaceae (p=0.005), and Weeksellaceae (p=0.033). As expected, seed germination time also correlated negatively with the abundance of these bacterial families. Seed germination time was positively associated

with Rhizobiaceae (p=0.004), Sphingobacteriaceae (p=0.002), and Weeksellaceae (p=0.008), but negatively with Microbacteriaceae (p=0.032) and Paneibacillaceae (0.069) and Pseudomonadaceae (p=0.049).

At genus level, the abundance of *Rhizobium, Chryseobacterium* and *Pedobacter* was negatively associated with germination rate (p-value 0.041, 0.033, and 0.004, respectively) but positively with germination time (p-value 0.015, 0.008, and 0.001, respectively). But the abundance of *Pseudomonas* was positively related with germination rate (p = 0.138) but negatively associated with the germination time (p = 0.049), although the correlation was not significant.

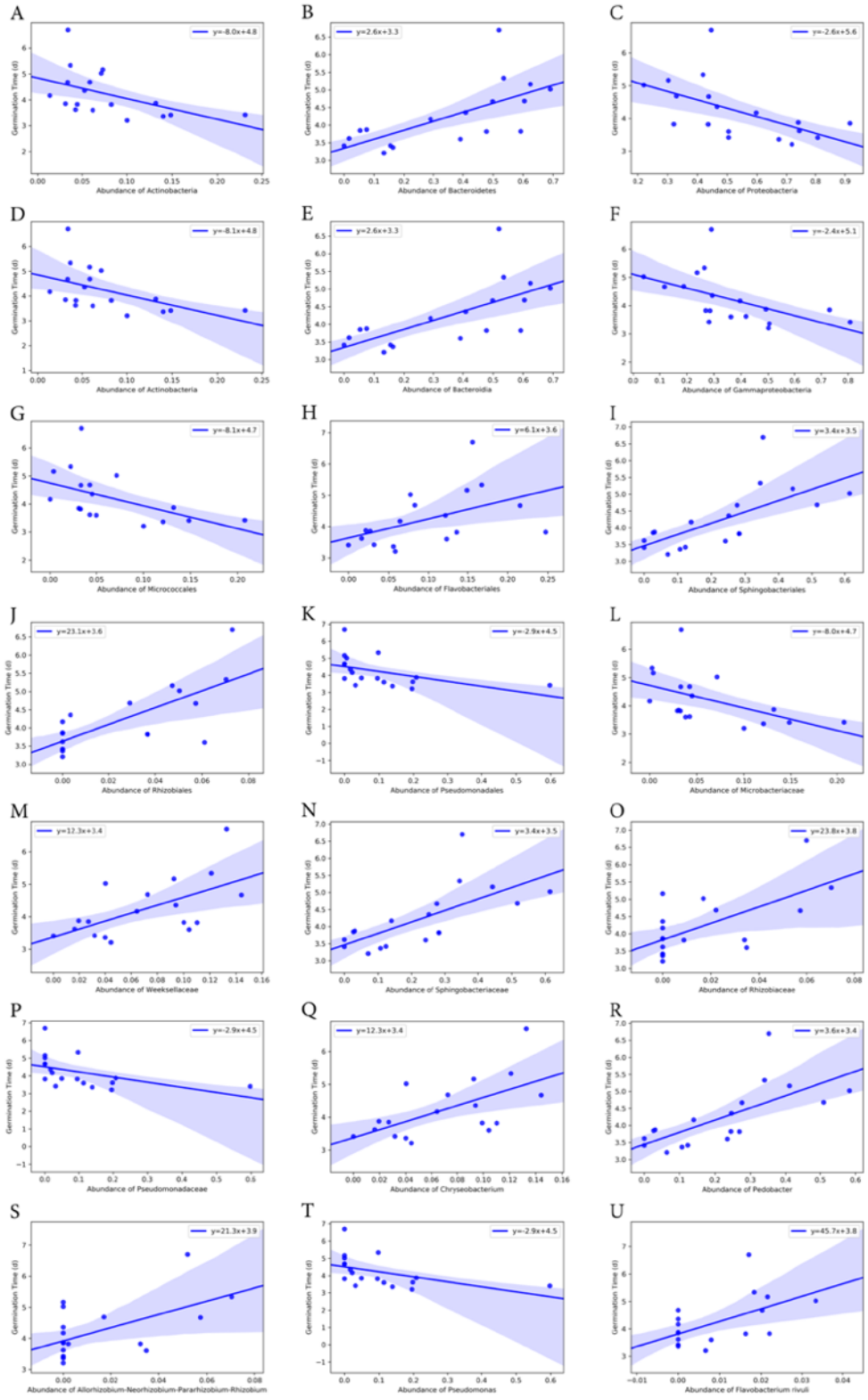

Figure 4.7 Correlation of average seed germination time with abundance of bacteria groups at different taxonomy levels. Phylum: A – C; Class: D – F; Order: G – K; Family: L – P; Genus: Q – T; Species: U. earson's correlation analysis was performed and

visualized with python-based libraries *SciPy*, *pandas*, *seaborn* and *matplotlib*. The abundance of bacterial groups at different taxonomy levels is on the X-axis, and the germination time is on the Y-axis.

4.4 Discussion

4.4.1 A Complex Bacterial Community Associated with Turf Seeds

Compared to LM seeds, HM seeds harbored a more diverse bacterial community with many more bacterial cells (i.e., a higher bacterial load), as there were more sequences associated with HM seeds. The More Individuals Hypothesis, which predicts that communities with more individuals will have more species (Storch, Bohdalková & Okie, 2018), can explain the higher diversity associated with HM seeds. This result is similar to previous studies on soils indicating that moisture controls the structure and function of the soil microbial community (Brockett *et al.*, 2012, Griffiths *et al.*, 2003, Steven *et al.*, 2013). Water availability in soil controls bacterial composition (Zeglin *et al.*, 2011). Similarly, relatively high moisture will favor growth and replication of bacteria on seeds, while low moisture conditions will suppress development of the bacterial community associated with seeds.

Both LM and HM seeds vectored a large number of bacteria and shared some groups. For example, the abundance of *Curtobacterium* spp. was similar in both LM and HM seeds (LM 2%, HM 2%). *Curtobacterium* is a Gram-positive endophytic bacterial genus in rice seeds (*Oryza sativa*), field-grown tall fescue (*Lolium arundinacea*) and *Noccaea goesingensis* (de los Santos *et al.*, 2015, Mano *et al.*, 2006, Ruiz *et al.*, 2011). Some *Curtobacterium* strains provide host growth promotion and pathogen antagonistic effects (de los Santos *et al.*, 2015, Ruiz *et al.*, 2011). *Paenibacillus* was the only genus from phylum Firmicutes in both LM and HM seeds (LM 3%, HM 4%). *Paenibacillus*

have been isolated from many plants and shown to produce indole-3-acetic acid (IAA), solubilize phosphate, and inhibit the growth of phytopathogens (Aswathy *et al.*, 2013, Diaz Herrera *et al.*, 2016, Ruiz *et al.*, 2011, Rybakova *et al.*, 2015).

Two of the genera, *Mucilaginibacter* and *Rhizobium*, were found in HM seeds but not LM seeds. *Mucilaginibacter* spp. can promote plant growth and produce extracellular polysaccharides (An *et al.*, 2009, Lee *et al.*, 2013, Madhaiyan *et al.*, 2010, Mannisto *et al.*, 2010). *Rhizobium* together with *Allorhizobium*, *Neorhizobium*, and *Pararhizobium*, composed 2% of the bacterial community on HM seeds. These genera comprise well-studied bacteria that promote growth of plants and nodulate legumes to fix nitrogen (Gutierrez-Zamora & Martınez-Romero, 2001, Kiers *et al.*, 2003, Yanni *et al.*, 1997). At the phylum level, LM seeds hosted more abundant Gammaproteobacteria than HM seeds. Several genera within Gammaproteobacteria contributed to these results, i.e. *Duganella*, *Massilia*, *Pantoea*, and *Pseudomonas*. However, these bacteria were still found on a large portion of the HM seeds. *Duganella* spp. can suppress the growth of plant pathogens (Cretoiu *et al.*, 2013, Haack *et al.*, 2016). *Massilia* is a root-colonizing bacterial genus with the ability to degrade chitin (Adrangi *et al.*, 2010, Faramarzi *et al.*, 2009, Ofek *et al.*, 2012). *Pantoea* spp. promote plant growth and tolerance of environmental stresses (Chen *et al.*, 2017, Feng *et al.*, 2006, Ferreira *et al.*, 2008, Gond *et al.*, 2015b). *Pseudomonas* contains many endophytic bacterial strains that benefit hosts by producing IAA, producing biocontrol lipopeptides, and solubilizing phosphate (Oteino *et al.*, 2015, Prieto & Mercado-Blanco, 2008, Suzuki *et al.*, 2003).

Some bacterial genera were more abundant in HM seeds than LM seeds, including *Flavobacterium*, *Chryseobacterium*, *Pedobacter*, *Sphingomonas*, and *Erwinia*.

Most of the bacteria comprised a very small portion of the bacterial community of LM seeds, but *Sphingomonas* made up 6%. *Flavobacterium* sp. has been found to promote plant growth and provide biocontrol activity to the hosts (Kolton *et al.*, 2016, Soltani *et al.*, 2010). *Chryseobacterium* spp. were also shown to be plant growth promoting bacteria (Dardanelli *et al.*, 2009, Gutiérrez Mañero *et al.*, 2003). Although *Pedobacter* has not been found to promote growth of plants, it can induce the production of antimicrobial compounds by *Pseudomonas fluorescens* Pf0-1 (Garbeva *et al.*, 2011). *Sphingomonas* is an alphaproteobacterial genus containing strains that produce IAA and provide nutrients to hosts (Okunishi *et al.*, 2005, Ruiz *et al.*, 2011). *Erwinia* spp. have also been identified as endophytes in some plant species (Verma, 2019). However, genus *Erwinia* is well-known to contain many plant pathogenic species.

In total, the above genera together comprised 89% and 95% in HM and LM seeds, respectively. Some of the bacterial genera include plant pathogens, e.g. *Ewrinia* and *Pseudomonas*. However, most of the bacteria are known to contain mainly plant growth promoting rhizobacteria (PGPR), e.g. *Paenibacillus, Pseudomonas, Rhizobium, Pantoea*. The seed microbes are important because they stimulate seedling development, increase stress tolerance in seedlings and protect seedlings from disease (Verma *et al.*, 2017, 2018, White *et al.*, 2018, 2019). Thus, a more diverse bacterial community on HM seeds may provide hosts with more microbial resources to utilize for plant development and stress tolerance.

In the study, the samples of LM and HM seeds had unequal sizes (LM: 4, HM: 19), which could create a bias in our final result. However, HM seeds vectored a more diverse bacterial community with significantly more bacteria cells (Figure 4.3 & Table

4.2). Also, the different abundances of bacterial groups between LM and HM seeds were statistically significant.

4.4.2 The Bacterial Community Affected Seed Germination and Growth

Seeds from HM climates tended to show slower germination and reduced seedling growth rates. These seeds vectored a denser and more diverse community of bacteria, which may benefit seedlings but not without a cost. We hypothesize that the higher microbial load competes with seedlings, which slows germination and development of the host. This nutritional cost may result in slower seed germination and seedling development rates. Seed growers have observed that seed from high moisture climates seems to establish better compared to seed from low moisture climates (W. Meyer, Unpublished data). The widely accepted explanation is that in the HM seeds, the inhibitors are removed by rain that helps the HM seed to germinate earlier. But in the LM seeds, there are more inhibitors still in the seed to slow down germination. From our study, we proposed another hypotheis that seeds from high moisture climates vectored more microbes, which may produce more stimulators that benefit the establishment of seeds and seedlings. While, seeds with richer and denser microbiomes grow slower initially, they may be better protected from soil borne pathogens than seeds with less developed microbiomes.

Seeds that have formed in low moisture situations, or where the natural microbiome has otherwise been damaged, could be remediated through application of microbes in seed coatings (Pedrini *et al.*, 2017). Coating formulations with the correct

microbes at the optimal concentrations could result in better fitness of seeds and seedlings.

4.5 Conclusion

We surveyed the bacterial community associated with seeds of several species of cool-season turfgrasses and identified the dominant bacterial groups of the communities at different taxonomic levels. Regardless of the moisture level during seed production and species of seeds, the core bacterial community included many PGPB strains. Seeds produced in high moisture conditions maintained a denser and more diverse bacterial community than seeds produced in low moisture conditions. This seed microbiome may help seedlings tolerate stress but may also compete with seedlings for nutrients and slow early seedling growth.

4.6 References

Adrangi S., Faramarzi M. A., Shahverdi A. R., & Sepehrizadeh Z. (2010). Purification and characterization of two extracellular endochitinases from *Massilia timonae*. *Carbohydrate Research, 345*(3), 402-407.

An D. S., Yin C. R., Lee S. T., & Cho C. H. (2009). *Mucilaginibacter daejeonensis* sp. nov., isolated from dried rice straw. *International Journal of Systematic and Evolutionary Microbiology, 59*(5), 1122-1125.

Anderson M. J. (2005). PERMANOVA: a FORTRAN computer program for permutational multivariate analysis of variance. *Department of Statistics, University of Auckland, New Zealand* 24.

Aswathy A. J., Jasim B., Jyothis M., & Radhakrishnan E. K. (2013). Identification of two strains of *Paenibacillus* sp. as indole 3 acetic acid-producing rhizome-associated endophytic bacteria from *Curcuma longa*. *3 Biotech, 3*(3), 219-224.

Brady S. M., Sarkar S. F., Bonetta D., & McCourt P. (2003). The *ABSCISIC ACID INSENSITIVE 3 (ABI3)* gene is modulated by farnesylation and is involved in auxin signaling and lateral root development in *Arabidopsis*. *The Plant Journal, 34*(1), 67-75.

Brockett B. F. T., Prescott C. E., & Grayston S. J. (2012). Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biology and Biochemistry, 44*(1), 9-20.

Bultman T. L., & Bell G. D. (2003). Interaction between fungal endophytes and environmental stressors influences plant resistance to insects. *Oikos, 103*(1), 182-90.

Callahan B. J., McMurdie P. J., Rosen M. J., Han A. W., Johnson A. J., & Holmes S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*(7), 581–583.

Caporaso J. G., Kuczynski J., Stombaugh J., Bittinger K., Bushman F. D., Costello E. K., Fierer N., Peña A. G., Goodrich J. K., & Gordon J. I. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*(5), 335-336.

Chen C., Xin K., Liu H., Cheng J., Shen X., Wang Y., & Zhang L. (2017). *Pantoea alhagi*, a novel endophytic bacterium with ability to improve growth and drought tolerance in wheat. *Scientific Reports, 7*(1), 1-14.

Clay K. (1990). Fungal endophytes of grasses. *Annual Review of Ecology and Systematics, 21*(1), 275-297.

Cretoiu M. S., Korthals G. W., Visser J. H., & van Elsas J. D. (2013). Chitin amendment increases soil suppressiveness toward plant pathogens and modulates the actinobacterial and oxalobacteraceal communities in an experimental agricultural field. *Applied and Environmental Microbiology, 79*(17), 5291-5301.

Cruz R. S., Yañez-Ocampo G., & Wong-Villarreal A. (2014). Effect of nodulating bacteria on the seed germination of *Capsicum* spp. *African Journal of Microbiology Research, 8*(7), 659-663.

Dardanelli M. S., Manyani H., González-Barroso S., Rodríguez-Carvajal M. A., Gil-Serrano A. M., Espuny M. R., López-Baena F. J., Bellogín R. A., Megías M., & Ollero F. J. (2009). Effect of the presence of the plant growth promoting rhizobacterium (PGPR) *Chryseobacterium balustinum* Aur9 and salt stress in the pattern of flavonoids exuded by soybean roots. *Plant and Soil, 328*(1-2), 483-493.

Datta C., & Basu P. S. (2000). Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. *Microbiological Research, 155*(2), 123-127.

de los Santos M. C., Taulé C., Mareque C., Beracochea M., & Battistoni F. (2015). Identification and characterization of the part of the bacterial community associated with field-grown tall fescue (*Festuca arundinacea*) cv. SFRO Don Tomás in Uruguay. *Annals of Microbiology, 66*(1), 329-342.

Diaz Herrera S., Grossi C., Zawoznik M., & Groppa M. D. (2016). Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of *Fusarium graminearum*. *Microbiological Research, 186*, 37-43.

Faith D. P. (1992). Conservation evaluation and phylogenetic diversity. *Biological Conservation, 61*(1), 1-10.

Faramarzi M., Fazeli M., Yazdi M. T., Adrangi S., Al-Ahmadi K. J., Tasharrofi N., & Mohseni F. A. (2009). Optimization of cultural conditions for production of chitinase by a soil isolate of *Massilia timonae*. *Biotechnology, 8*(1), 93-99.

Feng Y., Shen D., & Song W. (2006). Rice endophyte *Pantoea agglomerans* YS19 promotes host plant growth and affects allocations of host photosynthates. *Journal of Applied Microbiology, 100*(5), 938-945.

Ferreira A., Quecine M. C., Lacava P. T., Oda S., Azevedo J. L., & Araujo W. L. (2008). Diversity of endophytic bacteria from *Eucalyptus* species seeds and colonization of seedlings by *Pantoea agglomerans*. *FEMS Microbiology Letters, 287*(1), 8-14.

Garbeva P., Silby M. W., Raaijmakers J. M., Levy S. B., & Boer W. (2011). Transcriptional and antagonistic responses of *Pseudomonas fluorescens* Pf0-1 to phylogenetically different bacterial competitors. *The ISME Journal, 5*(6), 973-985.

Gond S. K., Bergen M. S., Torres M. S., White J. F., & Kharwar R. N. (2015a). Effect of bacterial endophyte on expression of defense genes in Indian popcorn against *Fusarium moniliforme*. *Symbiosis, 66*(3), 133-140.

Gond S. K., Torres M. S., Bergen M. S., Helsel Z., & White J. F. (2015b). Induction of salt tolerance and up-regulation of aquaporin genes in tropical corn by rhizobacterium *Pantoea agglomerans*. *Letters in Applied Microbiology, 60*(4), 392-399.

Griffiths R. I., Whiteley A. S., O'Donnell A. G., & Bailey M. J. (2003). Physiological and community responses of established grassland bacterial populations to water stress. *Applied and Environmental Microbiology, 69*(12), 6961-6968.

Gutiérrez Mañero F. J., Probanza A., Ramos B., Colón Flores J. J., & Lucas García J. A. (2003). Effects of culture filtrates of rhizobacteria isolated from wild lupine on germination, growth, and biological nitrogen fixation of lupine seedlings. *Journal of Plant Nutrition, 26*(5), 1101-1115.

Gutierrez-Zamora M., & Martınez-Romero E. (2001). Natural endophytic association between *Rhizobium etli* and maize (*Zea mays* L.). *Journal of Biotechnology, 91*(2-3), 117-126.

Haack F. S., Poehlein A., Kroger C., Voigt C. A., Piepenbring M., Bode H. B., Daniel R., Schafer W., & Streit W. R. (2016). Molecular keys to the *Janthinobacterium* and *Duganella* spp. interaction with the plant pathogen *Fusarium graminearum*. *Frontiers in Microbiology, 7*, 1668.

Hardoim P. R., van Overbeek L. S., Berg G., Pirttila A. M., Compant S., Campisano A., Doring M., & Sessitsch A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews, 79*(3), 293-320.

Henning J. A., Weston D. J., Pelletier D. A., Timm C. M., Jawdy S. S., & Classen A. T. (2016). Root bacterial endophytes alter plant phenotype, but not physiology. *PeerJ, 4*, e2606.

Irizarry I., & White J. F. (2017). Application of bacteria from non-cultivated plants to promote growth, alter root architecture and alleviate salt stress of cotton. *Journal of Applied Microbiology, 122*(4), 1110-1120.

Johnston-Monje D., Lundberg D. S., Lazarovits G., Reis V. M., & Raizada M. N. (2016). Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. *Plant and Soil, 405*(1-2), 337-355.

Johnston-Monje D., & Raizada M. N. (2011). Conservation and diversity of seed associated endophyes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS ONE 6*(6).

Kiers E. T., Rousseau R. A., West S. A., & Denison R. F. (2003). Host sanctions and the legume–rhizobium mutualism. *Nature, 425*(6953), 78-81.

Klindworth A., Pruesse E., Schweer T., Peplies J., Quast C., Horn M., & Glöckner F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 41*(1), e1-e1.

Kolton M., Erlacher A., Berg G., & Cytryn E. (2016). The *Flavobacterium* genus in the plant holobiont: ecological, physiological, and applicative insights. In: Castro-Sowinski S., ed. *Microbial Models: From Environmental to Industrial Sustainability*: Springer, 189-207.

Lee H. R., Han S. I., Rhee K. H., & Whang K. S. (2013). *Mucilaginibacter herbaticus* sp. nov., isolated from the rhizosphere of the medicinal plant *Angelica sinensis*. *International Journal of Systematic and Evolutionary Microbiology, 63*(8), 2787-2793.

Liu P. P., Montgomery T. A., Fahlgren N., Kasschau K. D., Nonogaki H., & Carrington J. C. (2007). Repression of *AUXIN RESPONSE FACTOR10* by microRNA160 is critical for seed germination and post-germination stages. *The Plant Journal, 52*(1), 133-146.

Madhaiyan M., Poonguzhali S., Lee J. S., Senthilkumar M., Lee K. C., & Sundaram S. (2010). *Mucilaginibacter gossypii* sp. nov. and *Mucilaginibacter gossypiicola* sp. nov., plant-growth-promoting bacteria isolated from cotton rhizosphere soils. *International Journal of Systematic and Evolutionary Microbiology, 60*(10), 2451-2457.

Mannisto M. K., Tiirola M., McConnell J., & Haggblom M. M. (2010). *Mucilaginibacter frigoritolerans* sp. nov., *Mucilaginibacter lappiensis* sp. nov. and *Mucilaginibacter mallensis* sp. nov., isolated from soil and lichen samples. *International Journal of Systematic and Evolutionary Microbiology, 60*(12), 2849-2856.

Mano H., Tanaka F., Watanabe A., Kaga H., Okunishi S., & Morisaki H. (2006). Culturable surface and endophytic bacterial flora of the maturing seeds of rice

plants (*Oryza sativa*) cultivated in a paddy field. *Microbes and Environments, 21*(2), 86-100.

Meyer W. A., Torres M. S., & White J. F. (2012). Biology and applications of fungal endophytes in turfgrasses. In: Stier J., Horgan B., Bonos S., ed. *Turfgrass: Biology, Use and Management.* Madison: American Society of Agronomy, 713-731.

Miransari M., & Smith D. L. (2014). Plant hormones and seed germination. *Environmental and Experimental Botany, 99*, 110-121.

Ofek M., Hadar Y., & Minz D. (2012). Ecology of root colonizing Massilia (Oxalobacteraceae). *PLoS One, 7*(7).

Okunishi S., Sako K., Mano H., Imamura A., & Morisaki H. (2005). Bacterial flora of endophytes in the maturing seed of cultivated rice (*Oryza sativa*). *Microbes and Environments, 20*(3), 168-177.

Oteino N., Lally R. D., Kiwanuka S., Lloyd A., Ryan D., Germaine K. J., & Dowling D. N. (2015). Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Frontiers in Microbiology, 6*, 745.

Pace N. R., Stahl D. A., Lane D. J., & Olsen G. J. (1986). The analysis of natural microbial populations by ribosomal RNA sequences. In: Marshall K. C., ed. *Advances in Microbial Ecology*. Berlin: Springer, 1-55.

Pedrini S., Merritt D. J., Stevens J., & Dixon K. (2017). Seed coating: science or marketing spin? *Trends in Plant Science, 22*(2), 106-116.

Poinar H. N., Schwarz C., Qi J., Shapiro B., MacPhee R. D. E., Buigues B., Tikhonov A., Huson D. H., Tomsho L. P., & Auch A. (2006). Metagenomics to paleogenomics: large-scale sequencing of mammoth DNA. *Science, 311*(5759), 392-394.

Price M. N., Dehal P. S., & Arkin A. P. (2010). FastTree 2 – Approximately maximum-likelihood trees for large alignments. *PLoS ONE, 5*(3).

Prieto P., & Mercado-Blanco J. (2008). Endophytic colonization of olive roots by the biocontrol strain *Pseudomonas fluorescens* PICF7. *FEMS Microbiology Ecology, 64*(2), 297-306.

Riesenfeld C. S., Schloss P. D., & Handelsman J. (2004). Metagenomics: genomic analysis of microbial communities. *Annual Review of Genetics, 38*, 525-552.

Rognes T., Flouri T., Nichols B., Quince C., & Mahé F. (2016). VSEARCH: a versatile open source tool for metagenomics. *PeerJ, 4*, e2584.

Ruiz D., Agaras B., de Werra P., Wall L. G., & Valverde C. (2011). Characterization and screening of plant probiotic traits of bacteria isolated from rice seeds cultivated in Argentina. *Journal of Microbiology, 49*(6), 902-912.

Rybakova D., Cernava T., Köberl M., Liebminger S., Etemadi M., & Berg G. (2015). Endophytes-assisted biocontrol: novel insights in ecology and the mode of action of *Paenibacillus*. *Plant and Soil, 405*(1-2), 125-140.

Schmidt T. M., DeLong E. F., & Pace N. R. (1991). Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. *Journal of Bacteriology, 173*(14), 4371-4378.

Shaik S. P., & Thomas P. (2019). *In vitro* activation of seed-transmitted cultivation-recalcitrant endophytic bacteria in tomato and host–endophyte mutualism. *Microorganisms, 7*(5), 132.

Soltani A.-A., Khavazi K., Asadi-Rahmani H., Omidvari M., Abaszadeh Dahaji P., & Mirhoseyni H. (2010). Plant growth promoting characteristics in some *Flavobacterium* spp. isolated from soils of Iran. *Journal of Agricultural Science, 2*(4), 106.

Somova L., Pechurkin N., Sarangova A., & Pisman T. 2001. Effect of bacterial population density on germination wheat seeds and dynamics of simple artificial ecosystems. *Advances in Space Research, 27*(9), 1611-1615.

Steven B., Lionard M., Kuske C. R., & Vincent W. F. (2013). High bacterial diversity of biological soil crusts in water tracks over permafrost in the high arctic polar desert. *PLoS One, 8*(8).

Storch D., Bohdalková E., & Okie J. (2018). The more-individuals hypothesis revisited(), the role of community abundance in species richness regulation and the productivity-diversity relationship. *Ecology Letters, 21*(6), 920-937.

Suzuki S., He Y., & Oyaizu H. 2003. Indole-3-acetic acid production in *Pseudomonas fluorescens* HP72 and its association with suppression of creeping bentgrass brown patch. *Current Microbiology, 47*(2), 138-143.

Tsavkelov E. A., Cherdyntseva T. A., Botina S. G., & Netrusov A. I. (2007). Bacteria associated with orchid roots and microbial production of auxin. *Microbiological Research, 162*(1), 69-76.

Verma P. (2019). Seed endophytes in crop plants: metagenomic approaches to study the functional roles and interactions. In: Verma S. K, White, J. F., ed. *Seed Endophytes*. Berlin: Springer, 483-507.

Verma S., Kingsley K., Irizarry I., Bergen M., Kharwar R., & White J. F. (2017). Seed vectored endophytic bacteria modulate development of rice seedlings. *Journal of Applied Microbiology*, *122*(6), 1680-1691.

Verma S. K., Kingsley K., Bergen M., English C., Elmore M., Kharwar R. N., & White J. F. (2018). Bacterial endophytes from rice cut grass (*Leersia oryzoides* L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. *Plant Soil, 422*(1-2), 223–238.

White J. F., Kingsley K. L., Butterworth S., Brindisi L., Gatei J. W., Elmore M. T., Verma S. K., Yao X., & Kowalski K. P. (2019). Seed-vectored microbes: their roles in improving seedling fitness and competitor plant suppression. In: Verma S. K., White, J. F., ed. *Seed Endophytes*. Berlin: Springer,3-20.

White J. F., Kingsley K. L., Verma S. K., & Kowalski K. P. (2018). Rhizophagy cycle: An oxidative process in plants for nutrient extraction from symbiotic microbes. *Microorganisms, 6*(3), 95.

White J. F., Torres M. S., Johnson H., Irizarry I., & Tadych M. (2014). A functional view of plant microbiomes: endosymbiotic systems that enhance plant growth and survival. In: Verma V. C., Gange A. C., ed. *Advances in Endophytic Research*. Berlin: Springer, 425-439.

White Jr J. (1987). Widespread distribution of endophytes in the Poaceae. *Plant Disease, 71*(4), 340-342.

Yanni Y. G., Rizk R., Corich V., Squartini A., Ninke K., Philip-Hollingsworth S., Orgambide G., de Bruijn F., Stoltzfus J., & Buckley D. (1997). Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. In: Ladha, J. K., de Bruijn, F. J., Malik, K. A., ed. *Opportunities for Biological Nitrogen Fixation in Rice and Other Non-Legumes.* Berlin: Springer, 99-114.

Yuan C. L., Mou C. X., Wu W. L., & Guo Y. B. (2010). Effect of different fertilization treatments on indole-3-acetic acid producing bacteria in soil. *Journal of Soils and Sediments, 11*(2), 322-329.

Zeglin L. H., Dahm C. N., Barrett J. E., Gooseff M. N., Fitpatrick S. K., & Takacs-Vesbach C. D. (2011). Bacterial community structure along moisture gradients in the parafluvial sediments of two ephemeral desert streams. *Microbial Ecology, 61*(3), 543-556.

Zhu Y. L., She X. P., Wang J. S., & Lv H. Y. (2017). Endophytic bacterial effects on seed germination and mobilization of reserves in *Ammodendron biofolium*. *Pakistan Journal of Botany, 49*(5), 2029-2035.

APPENDICES

Appendix 1 Analysis Codes of Metagenomic Data with QIIME2

A1.1 Importing Illumina exported data into QIIME

```
qiime tools import \
    --type 'SampleData[PairedEndSequencesWithQuality]' \
    --input-path Original_Data \
    --input-format CasavaOneEightSingleLanePerSampleDirFmt \
    --output-path demux-paired-end.qza

qiime demux summarize \
    --i-data demux-paired-end.qza \
    --o-visualization demux-paired-end.qzv

qiime tools view demux-paired-end.qzv
```

Output artifacts:
demux-paired-end.qza (imported data)
Output visualizations:
demux-paired-end.qzv (visualization summary file)

A1.2 *dada2* denoising

```
qiime dada2 denoise-paired \
    --i-demultiplexed-seqs demux-paired-end.qza \
    --p-trim-left-f 13 \
    --p-trim-left-r 13 \
    --p-trunc-len-f 280 \
    --p-trunc-len-r 280 \
    --o-table 2_dada2_denoised/table-dada2.qza \
    --o-representative-sequences 2_dada2_denoised/rep-seqs-dada2.qza \
    --o-denoising-stats 2_dada2_denoised/denoising-stats-dada2.qza

qiime feature-table summarize \
    --i-table 2_dada2_denoised/table-dada2.qza \
    --o-visualization 2_dada2_denoised/table-dada2.qzv \
    --m-sample-metadata-file metadata.tsv

qiime feature-table tabulate-seqs \
    --i-data 2_dada2_denoised/rep-seqs-dada2.qza \
    --o-visualization 2_dada2_denoised/rep-seqs-dada2.qzv
```

*qiime metadata tabulate *
*--m-input-file 2_dada2_denoised/denoising-stats-dada2.qza *
--o-visualization 2_dada2_denoised/denoising-stats-dada2.qzv

Output artifacts:
table-dada2.qza
rep-seqs-dada2.qza
denoising-stats-dada2.qza
Output visualizations:
table-dada2.qzv
rep-seqs-dada2.qzv
denoising-stats-dada2.qzv

A1.3 *de novo* clustering

*qiime vsearch cluster-features-de-novo *
*--i-table 2_dada2_denoised/table-dada2.qza *
*--i-sequences 2_dada2_denoised/rep-seqs-dada2.qza *
*--p-perc-identity 0.99 *
*--o-clustered-table 3_de-novo_clustering/table-dn-99.qza *
--o-clustered-sequences 3_de-novo_clustering/rep-seqs-dn-99.qza

*qiime feature-table summarize *
*--i-table 3_de-novo_clustering/table-dn-99.qza *
*--o-visualization 3_de-novo_clustering/table-dn-99.qzv *
--m-sample-metadata-file metadata.tsv

*qiime feature-table tabulate-seqs *
*--i-data 3_de-novo_clustering/rep-seqs-dn-99.qza *
--o-visualization 3_de-novo_clustering/rep-seqs-dn-99.qzv

Output artifacts:
table-dn-99.qza
rep-seqs-dn-99.qza
Output visualizations:
table-dn-99.qzv
rep-seqs-dn-99.qzv

A1.4 Chimera filtering

*qiime vsearch uchime-denovo *
*--i-table 3_de-novo_clustering/table-dn-99.qza *
*--i-sequences 3_de-novo_clustering/rep-seqs-dn-99.qza *
--output-dir 4_uchime-dn-99

*qiime metadata tabulate *

```
    --m-input-file 4_uchime-dn-99/stats.qza \
    --o-visualization 4_uchime-dn-99/stats.qzv

qiime feature-table filter-features \
    --i-table 3_de-novo_clustering/table-dn-99.qza \
    --m-metadata-file 4b_uchime-dn-99/nonchimeras.qza \
    --o-filtered-table 4b_uchime-dn-99/table-nonchimeric-wo-borderline.qza

qiime feature-table filter-seqs \
    --i-data 3_de-novo_clustering/rep-seqs-dn-99.qza \
    --m-metadata-file 4b_uchime-dn-99/nonchimeras.qza \
    --o-filtered-data 4b_uchime-dn-99/rep-seqs-nonchimeric-wo-borderline.qza

qiime feature-table summarize \
    --i-table 4b_uchime-dn-99/table-nonchimeric-wo-borderline.qza \
    --o-visualization 4b_uchime-dn-99/table-nonchimeric-wo-borderline.qzv

qiime feature-table tabulate-seqs \
    --i-data 4b_uchime-dn-99/rep-seqs-nonchimeric-wo-borderline.qza
    --o-visualization 4b_uchime-dn-99/rep-seqs-nonchimeric-wo-borderline.qzv
```

Output artifacts:
4_uchime-dn-99/chimeras.qza
4_uchime-dn-99/nonchimeras.qza
4_uchime-dn-99/stats.qza
4_uchime-dn-99/table-nonchimeric-wo-borderline.qza
4_uchime-dn-99/rep-seqs-nonchimeric-wo-borderline.qza
Output visualizations:
4_uchime-dn-99/stats.qzv
4_uchime-dn-99/table-nonchimeric-wo-borderline.qzv
4_uchime-dn-99/rep-seqs-nonchimeric-wo-borderline.qzv

A1.5 Classifier training

```
qiime tools import \
    --type 'FeatureData[Sequence]' \
    --input-path silva_132_99_16s.fna
    --output-path silva_132_99_16s.qza

qiime tools import \
    --type "FeatureData[Taxonomy]" \
    --input-format HeaderlessTSVTaxonomyFormat \
    --input-path 99/consensus_taxonomy_7_levels.txt \
    --output-path taxonomy_99_consensus_7_levels.qza

qiime feature-classifier extract-reads \
```

*--i-sequences silva_132_99_16s.qza *
*--p-f-primer GCCTACGGGNGGCWGCAG *
*--p-r-primer GACTACHVGGGTATCTAATCC *
*--p-identity 0.99 *
--o-reads silva_132_99_16S_ref_seqs_99.qza

*qiime feature-classifier fit-classifier-naive-bayes *
*--i-reference-reads silva_132_99_16s_ref_seqs_99.qza *
*--i-reference-taxonomy taxonomy_99_consensus_7_levels.qza *
--o-classifier classifier_99_consensus_7_levels.qza

Output artifacts:
0_classifier/silva_132_99_16s.qza
0_classifier/taxonomy_99_consensus_7_levels.qza
0_classifier/silva_132_99_16s_ref_seqs_99.qza
0_classifier/classifier_99_consensus_7_levels.qza

A1.6 Taxonomy classification

*qiime feature-classifier classify-sklearn *
*--i-classifier 0_classifier/classifier_99_consensus_7_levels.qza *
*--i-reads 4_uchime-dn-99/rep-seqs-nonchimeric-wo-borderline.qza *
--o-classification 5_taxonomy/taxonomy-dn-99-nonchimeric-wo-borderline_99_consensus_7_levels.qza

*qiime metadata tabulate *
*--m-input-file 5_taxonomy/taxonomy-dn-99-nonchimeric-wo-borderline_99_consensus_7_levels.qza *
--o-visualization 5_taxonomy/taxonomy-dn-99-nonchimeric-wo-borderline_99_consensus_7_levels.qzv

Output artifacts:
5_taxonomy/taxonomy-dn-99-nonchimeric-wo-borderline_99_consensus_7_levels.qza
Output visualizations:
5_taxonomy/taxonomy-dn-99-nonchimeric-wo-borderline_99_consensus_7_levels.qzv

A1.7 Filtering out mitochondria and chloroplast

*qiime taxa filter-table *
*--i-table 4b_uchime-dn-99/table-nonchimeric-wo-borderline.qza *
*--i-taxonomy 5_taxonomy/taxonomy-dn-99-nonchimeric-wo-borderline_99_consensus_7_levels.qza *
*--p-exclude mitochondria,chloroplast,Mitochondria,Chloroplast *
--o-filtered-table 6_filtering_out_mitochondria_chloroplast/table-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qza

*qiime taxa filter-seqs *
*--i-sequences 4b_uchime-dn-99/rep-seqs-nonchimeric-wo-borderline.qza *
*--i-taxonomy 5_taxonomy/taxonomy-dn-99-nonchimeric-wo-borderline_99_consensus_7_levels.qza *
*--p-exclude mitochondria,chloroplast,Mitochondria,Chloroplast *
--o-filtered-sequences 6_filtering_out_mitochondria_chloroplast/rep-seqs-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qza

*qiime feature-table summarize *
*--i-table 6_filtering_out_mitochondria_chloroplast/table-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qza *
--o-visualization 6_filtering_out_mitochondria_chloroplast/table-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qzv

*qiime feature-table tabulate-seqs *
*--i-data 6_filtering_out_mitochondria_chloroplast/rep-seqs-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qza *
--o-visualization 6_filtering_out_mitochondria_chloroplast/rep-seqs-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qzv

Output artifacts:
6_filtering_out_mitochondria_chloroplast/table-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qza
6_filtering_out_mitochondria_chloroplast/rep-seqs-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qza
Output visualizations:
6_filtering_out_mitochondria_chloroplast/table-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qzv
6_filtering_out_mitochondria_chloroplast/rep-seqs-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qzv

A1.8 Generating phylogenetic tree

*qiime phylogeny align-to-tree-mafft-fasttree *
*--i-sequences 6_filtering_out_mitochondria_chloroplast/rep-seqs-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qza *
*--o-alignment 7_phylogeny/aligned-rep-seqs.qza *
*--o-masked-alignment 7_phylogeny/masked-aligned-rep-seqs.qza *
*--o-tree 7_phylogeny/unrooted-tree.qza *
--o-rooted-tree 7_phylogeny/rooted-tree.qza

Output artifacts:
7_phylogeny/aligned-rep-seqs.qza
7_phylogeny/masked-aligned-rep-seqs.qza
7_phylogeny/unrooted-tree.qza
7_phylogeny/rooted-tree.qza

A1.9 Taxonomy barplot

*qiime taxa barplot *
*--i-table 6_filtering_out_mitochondria_chloroplast/table-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qza *
*--i-taxonomy 5_taxonomy/taxonomy-dn-99-nonchimeric-wo-borderline_99_consensus_7_levels.qza *
*--m-metadata-file metadata.tsv *
--o-visualization 8_barplot/barplots-nonchimeric-wo-borderline-no-mitochondria-no-cloroplast.qzv

Output visualizations:
8_barplot/barplots-nonchimeric-wo-borderline-no-mitochondria-no-cloroplast.qzv

A1.10 Diversity analysis

*qiime diversity core-metrics-phylogenetic *
*--i-phylogeny 7_phylogeny/rooted-tree.qza *
*--i-table 6_filtering_out_mitochondria_chloroplast/table-nonchimeric-wo-borderline-no-mitochondria-no-chloroplast.qza *
*--p-sampling-depth 1000 *
*--m-metadata-file metadata.tsv *
--output-dir 9_diversity_analysis/core-metrics-results_1000

Output artifacts:
9_diversity_analysis/core-metrics-results_1000

A1.10.1 Alpha diversity

*qiime diversity alpha-group-significance *
*--i-alpha-diversity 9_diversity_analysis/core-metrics-results_1000/faith_pd_vector.qza *
*--m-metadata-file metadata.tsv *
--o-visualization 9_diversity_analysis/core-metrics-results_1000/faith_pd-group-significance.qzv

Output visualizations:
9_diversity_analysis/core-metrics-results_1000/faith_pd-group-significance.qzv

A1.10.2 Beta diversity

*qiime diversity beta-group-significance *
*--i-distance-matrix 9_diversity_analysis/core-metrics-results_1000/unweighted_unifrac_distance_matrix.qza *
*--m-metadata-file Metadata_QIIME2_v11.tsv *
*--m-metadata-column Climate *

*--o-visualization 9_diversity_analysis/core-metrics-results_1000/unweighted_unifrac-climate-significance.qzv *
--p-pairwise

*qiime diversity beta-group-significance *
*--i-distance-matrix 9_diversity_analysis/core-metrics-results_1000/unweighted_unifrac_distance_matrix.qza *
*--m-metadata-file Metadata_QIIME2_v10.tsv *
*--m-metadata-column Genus *
*--o-visualization 9_diversity_analysis/core-metrics-results_1000/unweighted_unifrac-genus-significance.qzv *
--p-pairwise

*qiime diversity beta-group-significance *
*--i-distance-matrix 9_diversity_analysis/core-metrics-results_1000/unweighted_unifrac_distance_matrix.qza *
*--m-metadata-file Metadata_QIIME2_v10.tsv *
*--m-metadata-column Species *
*--o-visualization 9_diversity_analysis/core-metrics-results_1000/unweighted_unifrac-species-significance.qzv *
--p-pairwise

Output visualizations:
9_diversity_analysis/core-metrics-results_1000/unweighted_unifrac-climate-significance.qzv
9_diversity_analysis/core-metrics-results_1000/unweighted_unifrac-genus-significance.qzv
9_diversity_analysis/core-metrics-results_1000/unweighted_unifrac-species-significance.qzv

Appendix 2 The assigned taxa and GenBank accession number of all generated OTUs.

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
TSCW_OTU_001	427	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Propionibacteriales;D_4__Nocardioidaceae; D_5__Aeromicrobium	0.999790685	MT110691
TSCW_OTU_002	25	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.99998422	MT110692
TSCW_OTU_004	1467	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae; D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.947012666	MT110693
TSCW_OTU_005	8	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae; D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.987928101	MT110694
TSCW_OTU_007	194	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Crocinitomicaceae; D_5__Fluviicola	0.999979208	MT110695
TSCW_OTU_008	22	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Mucilaginibacter;D_6__Mucilaginibacter sp. PAMC 26640	0.996618932	MT110696
TSCW_OTU_009	34	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter;D_6__Pedobacter sp. HME6451	0.978110605	MT110697
TSCW_OTU_010	556	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Mucilaginibacter	0.999997355	MT110698
TSCW_OTU_011	8	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Sphingobacterium;D_6__Sphingobacterium sp. 23D10-4-9	0.98305309	MT110699
TSCW_OTU_012	18	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Moheibacter	1	MT110700

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
TSCW_OTU_013	117	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999971755	MT110701
TSCW_OTU_014	560	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Sphingomonas	0.993261367	MT110702
TSCW_OTU_015	1060	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999269952	MT110703
TSCW_OTU_016	139	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium sp. JM-222	0.9432458	MT110704
TSCW_OTU_017	296	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Sphingobium	0.979555223	MT110705
TSCW_OTU_018	307	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium	0.999868067	MT110706
TSCW_OTU_019	72	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter	1	MT110707
TSCW_OTU_020	7780	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.994884157	MT110708
TSCW_OTU_021	35	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae; D_5__uncultured	0.989010981	MT110709
TSCW_OTU_022	16	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999999706	MT110710
TSCW_OTU_023	150	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter	1	MT110711
TSCW_OTU_024	27	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;	0.77741282	MT110712

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter;D_6__Hymenobacter arcticus		
TSCW_OTU_025	340	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Xylophilus;D_6__uncultured bacterium	0.901022532	MT110713
TSCW_OTU_026	122	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Pigmentiphaga	0.998285832	MT110714
TSCW_OTU_027	25	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium;D_6__Chryseobacterium sp. THG-DN3.6	0.992767543	MT110715
TSCW_OTU_028	180	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae	0.999999999	MT110716
TSCW_OTU_029	1175	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas	0.999999217	MT110717
TSCW_OTU_030	19	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.996915864	MT110718
TSCW_OTU_031	814	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Rhodanobacteraceae; D_5__Luteibacter;Ambiguous_taxa	0.960198168	MT110719
TSCW_OTU_032	180	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Kineosporiales;D_4__Kineosporiaceae	0.99995028	MT110720
TSCW_OTU_033	102	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium noncentrifugens	0.999748207	MT110721
TSCW_OTU_035	825	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999957105	MT110722
TSCW_OTU_036	57	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium hauense	0.950466831	MT110723

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
TSCW_OTU_037	1395	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas	0.999980861	MT110724
TSCW_OTU_038	17	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Moraxellaceae; D_5__Alkanindiges;D_6__uncultured bacterium	0.950171793	MT110725
TSCW_OTU_039	15	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae; D_5__Taibaiella	0.999999967	MT110726
TSCW_OTU_040	117	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Sphingomonas	0.999917973	MT110727
TSCW_OTU_041	559	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas	0.999982033	MT110728
TSCW_OTU_042	45	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae; D_5__Spirosoma;Ambiguous_taxa	0.954052278	MT110729
TSCW_OTU_043	72	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter;D_6__uncultured soil bacterium	0.800080658	MT110730
TSCW_OTU_044	79	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Frankiales;D_4__Geodermatophilaceae; D_5__Geodermatophilus;Ambiguous_taxa	0.833178647	MT110731
TSCW_OTU_045	255	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.999986215	MT110732
TSCW_OTU_046	291	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__unidentified	0.961775041	MT110733
TSCW_OTU_047	168	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae; D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.966728919	MT110734

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
TSCW_OTU_048	3701	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.999998107	MT110735
TSCW_OTU_049	211	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999978744	MT110736
TSCW_OTU_050	77	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999966519	MT110737
TSCW_OTU_051	280	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999937573	MT110738
TSCW_OTU_052	516	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium	0.99996928	MT110739
TSCW_OTU_053	87	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Mucilaginibacter	0.999997046	MT110740
TSCW_OTU_054	41	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas	0.999284099	MT110741
TSCW_OTU_055	43	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999833953	MT110742
TSCW_OTU_056	110	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli; D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus	0.999983509	MT110743
TSCW_OTU_057	1006	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium sp. NW20	0.996238099	MT110744
TSCW_OTU_058	2114	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999858653	MT110745
TSCW_OTU_060	473	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;	0.959367198	MT110746

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_5__Xylophilus;Ambiguous_taxa		
TSCW_OTU_061	144	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Corynebacteriales;D_4__Nocardiaceae; D_5__Rhodococcus;D_6__Rhodococcus corynebacterioides	0.911156984	MT110747
TSCW_OTU_063	13	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.999993897	MT110748
TSCW_OTU_064	124	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999922723	MT110749
TSCW_OTU_067	175	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae;D_5__Aureimonas	0.996587617	MT110750
TSCW_OTU_068	9843	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Massilia	0.973156583	MT110751
TSCW_OTU_069	43	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium sp. R-38295	0.996172784	MT110752
TSCW_OTU_070	376	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae; D_5__Pantoea	0.962647515	MT110753
TSCW_OTU_071	157	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium;D_6__Chryseobacterium sp.	0.915565233	MT110754
TSCW_OTU_072	69	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.99994638	MT110755
TSCW_OTU_073	2347	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli; D_3__Bacillales;D_4__Paenibacillaceae; D_5__Paenibacillus;D_6__Triticum aestivum (bread wheat)	0.843710904	MT110756
TSCW_OTU_074	62	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae; D_5__uncultured;D_6__uncultured bacterium	0.906334209	MT110757

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
TSCW_OTU_075	9	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter	1	MT110758
TSCW_OTU_076	19	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae; D_5__Fibrella	0.999999999	MT110759
TSCW_OTU_079	913	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.999999571	MT110760
TSCW_OTU_080	174	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae; D_5__Erwinia;D_6__Erwinia rhapontici	0.702007808	MT110761
TSCW_OTU_081	949	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium;D_6__Chryseobacterium soldanellicola	0.856756247	MT110762
TSCW_OTU_082	87	D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__Saccharimonadaceae; D_5__uncultured bacterium;D_6__uncultured bacterium	0.890495909	MT110763
TSCW_OTU_083	18	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter	1	MT110764
TSCW_OTU_084	5	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Cytophaga sp. JSC-P2-223-10	0.753868655	MT110765
TSCW_OTU_085	49	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae; D_5__Taibaiella;D_6__uncultured bacterium	0.77752243	MT110766
TSCW_OTU_086	1	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.886999737	MT110767
TSCW_OTU_087	24	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter;D_6__uncultured Sphingobacteriaceae bacterium	0.808592929	MT110768
TSCW_OTU_088	10	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;	0.999997598	MT110769

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium		
TSCW_OTU_089	7	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Acetobacterales;D_4__Acetobacteraceae; D_5__Roseomonas	0.999839942	MT110770
TSCW_OTU_090	152	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Mucilaginibacter	0.99999599	MT110771
TSCW_OTU_091	206	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999856241	MT110772
TSCW_OTU_092	549	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Xanthomonadaceae; D_5__Stenotrophomonas;D_6__Stenotrophomonas rhizophila	0.765822672	MT110773
TSCW_OTU_093	183	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999701446	MT110774
TSCW_OTU_094	222	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Sphingomonas	0.9537346	MT110775
TSCW_OTU_095	6	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__env.OPS 17	1	MT110776
TSCW_OTU_096	55	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999796552	MT110777
TSCW_OTU_097	11	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Methylophilaceae; D_5__Methylophilus	0.995764824	MT110778
TSCW_OTU_098	39	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Mucilaginibacter	0.988387795	MT110779
TSCW_OTU_099	123	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae;	0.999936451	MT110780

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_5__Pseudomonas		
TSCW_OTU_101	67	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Beijerinckiaceae; D_5__Methylobacterium	0.999999988	MT110781
TSCW_OTU_102	23	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae; D_5__Flavitalea;Ambiguous_taxa	0.708480675	MT110782
TSCW_OTU_103	13	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas	0.999853416	MT110783
TSCW_OTU_104	236	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Caulobacterales;D_4__Caulobacteraceae; D_5__Brevundimonas	0.999800062	MT110784
TSCW_OTU_105	23	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae	0.99999931	MT110785
TSCW_OTU_106	2251	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae; D_5__Erwinia	0.732930503	MT110786
TSCW_OTU_107	119	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter;D_6__Pedobacter sp. HME6451	0.985446796	MT110787
TSCW_OTU_108	838	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999982537	MT110788
TSCW_OTU_109	25	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Massilia	0.999592596	MT110789
TSCW_OTU_110	449	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Duganella;D_6__uncultured bacterium	0.821416245	MT110790
TSCW_OTU_111	31	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhodobacterales;D_4__Rhodobacteraceae	0.999999953	MT110791
TSCW_OTU_112	133	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;	0.999362866	MT110792

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium		
TSCW_OTU_113	4351	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999983255	MT110793
TSCW_OTU_114	226	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium sp. JM-222	0.970369258	MT110794
TSCW_OTU_115	64	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Xenophilus;Ambiguous_taxa	0.725072309	MT110795
TSCW_OTU_116	9	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999999715	MT110796
TSCW_OTU_117	216	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Massilia	0.999674309	MT110797
TSCW_OTU_118	1176	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999957754	MT110798
TSCW_OTU_119	67	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Sphingobacterium	0.978938204	MT110799
TSCW_OTU_120	126	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.946317939	MT110800
TSCW_OTU_122	88	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.973617333	MT110801
TSCW_OTU_123	1710	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter;D_6__Pedobacter ginsengiterrae	0.759269757	MT110802
TSCW_OTU_124	7429	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;	0.888457413	MT110803

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas;D_6__Pseudomonas graminis		
TSCW_OTU_125	648	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Kineosporiales;D_4__Kineosporiaceae;D_5__Kineococcus	0.999998385	MT110804
TSCW_OTU_126	20	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Caulobacterales;D_4__Caulobacteraceae; D_5__Brevundimonas	0.999995381	MT110805
TSCW_OTU_127	43	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Sphingomonas	0.770042947	MT110806
TSCW_OTU_128	114	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter;D_6__Amia calva (bowfin)	0.773289961	MT110807
TSCW_OTU_129	127	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae; D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.886220829	MT110808
TSCW_OTU_131	2592	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Massilia	0.998657108	MT110809
TSCW_OTU_132	29	D_0__Bacteria;D_1__Actinobacteria;D_2__Acidimicrobiia; D_3__Microtrichales;D_4__Iamiaceae; D_5__Iamia	0.999998486	MT110810
TSCW_OTU_133	453	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Sanguibacteraceae; D_5__Sanguibacter;Ambiguous_taxa	0.888814197	MT110811
TSCW_OTU_134	75	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999978021	MT110812
TSCW_OTU_135	57	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter;D_6__uncultured bacterium	0.818138357	MT110813
TSCW_OTU_137	29	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae	0.999941682	MT110814

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
TSCW_OTU_138	10	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.99994863	MT110815
TSCW_OTU_140	84	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Devosiaceae;D_5__Devosia	0.842829537	MT110816
TSCW_OTU_141	408	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Bacteroidetes bacterium PDD-58b-27	0.976259075	MT110817
TSCW_OTU_142	125	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae	0.999999427	MT110818
TSCW_OTU_143	1262	D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli; D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus	0.999973497	MT110819
TSCW_OTU_144	506	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae; D_5__Curtobacterium;Ambiguous_taxa	0.701538382	MT110820
TSCW_OTU_145	116	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Xanthomonadaceae; D_5__Xanthomonas	0.961112955	MT110821
TSCW_OTU_146	1045	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999965445	MT110822
TSCW_OTU_147	24	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Xylophilus;D_6__uncultured bacterium	0.727575591	MT110823
TSCW_OTU_148	148	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae; D_5__Pantoea	0.842443656	MT110824
TSCW_OTU_149	21	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Duganella	0.991286349	MT110825
TSCW_OTU_150	979	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium;D_6__Chryseobacterium sp. HP1I	0.798353484	MT110826

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
TSCW_OTU_151	18	D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__Saccharimonadaceae; D_5__uncultured bacterium;D_6__uncultured bacterium	0.955289837	MT110827
TSCW_OTU_152	85	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae; D_5__Serratia	0.883216837	MT110828
TSCW_OTU_153	552	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Duganella	0.950508002	MT110829
TSCW_OTU_154	615	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.999944699	MT110830
TSCW_OTU_155	352	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.999991026	MT110831
TSCW_OTU_156	79	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Sphingomonas	0.993085676	MT110832
TSCW_OTU_157	27	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium hauense	0.889291249	MT110833
TSCW_OTU_158	1	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Xanthomonadaceae; D_5__Stenotrophomonas	0.997725841	MT110834
TSCW_OTU_159	128	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Verticia;Ambiguous_taxa	0.876651746	MT110835
TSCW_OTU_160	660	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas;D_6__Pseudomonas viridiflava	0.872033162	MT110836
TSCW_OTU_161	222	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Corynebacteriales;D_4__Nocardiaceae;D_5__Rhodococcus	0.999999956	MT110837
TSCW_OTU_163	209	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;	0.932282191	MT110838

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_5__Frondihabitans;Ambiguous_taxa		
TSCW_OTU_164	24	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Caulobacterales;D_4__Caulobacteraceae; D_5__Brevundimonas;D_6__uncultured alpha proteobacterium	0.714576485	MT110839
TSCW_OTU_165	156	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Sphingomonas	0.997906569	MT110840
TSCW_OTU_166	75	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae;D_5__Dyadobacter	0.999999971	MT110841
TSCW_OTU_167	2447	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Duganella	0.952693182	MT110842
TSCW_OTU_168	8	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Sphingobacterium	0.99804239	MT110843
TSCW_OTU_169	22	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.995076911	MT110844
TSCW_OTU_170	1267	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Sphingomonas	0.941916584	MT110845
TSCW_OTU_171	38	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__metagenome	0.810761586	MT110846
TSCW_OTU_172	39	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.992088348	MT110847
TSCW_OTU_173	2472	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.99997569	MT110848
TSCW_OTU_174	12618	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Sphingomonas	0.999995608	MT110849

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
TSCW_OTU_175	2299	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae; D_5__Curtobacterium	0.848180499	MT110850
TSCW_OTU_176	49	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Micrococcaceae	0.999999527	MT110851
TSCW_OTU_177	20	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999991975	MT110852
TSCW_OTU_178	213	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Mucilaginibacter	0.999919616	MT110853
TSCW_OTU_179	53	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__uncultured	0.767137416	MT110854
TSCW_OTU_180	1947	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Massilia	0.994297343	MT110855
TSCW_OTU_181	201	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999973517	MT110856
TSCW_OTU_182	52	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Devosiaceae; D_5__Devosia	0.999255133	MT110857
TSCW_OTU_184	103	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter;D_6__uncultured bacterium	0.822710447	MT110858
TSCW_OTU_186	13	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas	0.999649985	MT110859
TSCW_OTU_187	9	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.999987035	MT110860
TSCW_OTU_188	332	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;	0.818052073	MT110861

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia		
TSCW_OTU_189	23	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae; D_5__Chryseobacterium	0.992973514	MT110862
TSCW_OTU_190	289	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium sp. CP_32	0.751783468	MT110863
TSCW_OTU_191	93	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Novosphingobium	0.942466933	MT110864
TSCW_OTU_192	54	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium hauense	0.977981202	MT110865
TSCW_OTU_193	25	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999377214	MT110866
TSCW_OTU_194	74	D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia; D_3__Solirubrobacterales;D_4__Solirubrobacteraceae; D_5__uncultured	0.999999926	MT110867
TSCW_OTU_195	22	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999952159	MT110868
TSCW_OTU_196	6	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Pigmentiphaga	0.999894711	MT110869
TSCW_OTU_197	312	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium sp. JM-222	0.961671417	MT110870
TSCW_OTU_198	35	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter	1	MT110871
TSCW_OTU_199	130	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;	0.999942179	MT110872

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_5__Pedobacter		
TSCW_OTU_200	822	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Pantoea	0.776512819	MT110873
TSCW_OTU_201	227	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae; D_5__Dyadobacter;D_6__uncultured bacterium	0.914341066	MT110874
TSCW_OTU_202	65	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999999653	MT110875
TSCW_OTU_203	353	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999452433	MT110876
TSCW_OTU_204	168	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter;D_6__Amia calva (bowfin)	0.709454339	MT110877
TSCW_OTU_205	236	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Verticia;Ambiguous_taxa	0.885124285	MT110878
TSCW_OTU_206	125	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Massilia	0.999856452	MT110879
TSCW_OTU_207	126	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Mucilaginibacter	0.999995593	MT110880
TSCW_OTU_208	137	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Xylophilus;D_6__uncultured bacterium	0.963105712	MT110881
TSCW_OTU_209	12	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Bacteroidetes bacterium PDD-58b-27	0.924646284	MT110882
TSCW_OTU_211	177	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.993888753	MT110883

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
TSCW_OTU_212	19	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae; D_5__Plantibacter;Ambiguous_taxa	0.72575486	MT110884
TSCW_OTU_213	11710	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae; D_5__Pantoea	0.882814416	MT110885
TSCW_OTU_214	159	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999876284	MT110886
TSCW_OTU_215	970	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.998664094	MT110887
TSCW_OTU_216	70	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Caulobacterales;D_4__Caulobacteraceae; D_5__Brevundimonas	0.999985458	MT110888
TSCW_OTU_218	6	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae	0.999995026	MT110889
TSCW_OTU_219	123	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter	1	MT110890
TSCW_OTU_220	303	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999735949	MT110891
TSCW_OTU_221	958	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae; D_5__Pantoea	0.747905784	MT110892
TSCW_OTU_222	788	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae; D_5__Rathayibacter;Ambiguous_taxa	0.94670912	MT110893
TSCW_OTU_223	1059	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999965594	MT110894
TSCW_OTU_224	2	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;	0.999818095	MT110895

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas		
TSCW_OTU_225	2438	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae	0.999861675	MT110896
TSCW_OTU_226	109	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae; D_5__Pseudoclavibacter;Ambiguous_taxa	0.801486176	MT110897
TSCW_OTU_227	83	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Herbaspirillum;D_6__uncultured beta proteobacterium	0.711316975	MT110898
TSCW_OTU_228	37	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Sphingobacterium;D_6__Sphingobacterium faecium	0.71612314	MT110899
TSCW_OTU_230	48	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae; D_5__Spirosoma	0.999999998	MT110900
TSCW_OTU_231	328	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.998958555	MT110901
TSCW_OTU_232	1	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.995677173	MT110902
TSCW_OTU_233	7584	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae; D_5__Pedobacter	0.999993831	MT110903
TSCW_OTU_234	376	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter;D_6__Triticum aestivum (bread wheat)	0.710004911	MT110904
TSCW_OTU_235	51	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae; D_5__Hymenobacter	1	MT110905
TSCW_OTU_236	350	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae;	1	MT110906

Feature ID	Frequency	Assigned Taxon	Confidence	GenBank#
		D_5__Hymenobacter		
TSCW_OTU_237	345	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae; D_5__Pseudomonas	0.999826971	MT110907
TSCW_OTU_238	1042	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae; D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium	0.988929099	MT110908
TSCW_OTU_239	92	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium	0.999968394	MT110909
TSCW_OTU_240	124	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Massilia	0.999472911	MT110910
TSCW_OTU_242	5	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Massilia	0.999124818	MT110911
TSCW_OTU_243	449	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae; D_5__Novosphingobium	0.719548176	MT110912
TSCW_OTU_244	1457	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae; D_5__Dyadobacter	0.999999987	MT110913
TSCW_OTU_245	804	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae; D_5__Variovorax	0.78635908	MT110914
TSCW_OTU_246	151	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Devosiaceae; D_5__Devosia	0.956402167	MT110915
TSCW_OTU_247	1615	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae; D_5__Flavobacterium;D_6__Flavobacterium rivuli	0.982971398	MT110916

Appendix 3 Venn Diagram Results

A3.1 Venn diagram results showing the shared bacterial genera between different climate. LM: low moisture; HM: high moisture.

Names	total	elements
HM LM	16	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingomonas
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__Pedobacter
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Duganella
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Massilia
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia
		D_0__Bacteria;__;__;__;__;__
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Curtobacterium
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Frondihabitans
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
		D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli; D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Pantoea
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae;
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae;D_5__Chryseobacterium
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Flavobacterium
HM	55	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Moraxellaceae;D_5__Alkanindiges
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Methylophilaceae;D_5__Methylophilus
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__uncultured Sphingobium sp.; D_5__uncultured Sphingobium sp.
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae;D_5__Flavitalea
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Novosphingobium
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Devosiaceae;D_5__Devosia

Names	total	elements
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Frankiales;D_4__Geodermatophilaceae;D_5__Geodermatophilus
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__uncultured
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Sanguibacteraceae;D_5__Sanguibacter
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Verticia
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Plantibacter
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae;D_5__Spirosoma
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Pseudoclavibacter
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Pigmentiphaga
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae;__
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Variovorax
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Xylophilus
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__Saccharimonadaceae;__
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Herbaspirillum
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae;D_5__Fibrella
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae;D_5__Aureimonas
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae;D_5__uncultured
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;__
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__Saccharimonadaceae;D_5__uncultured bacterium
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Acetobacterales;D_4__Acetobacteraceae;D_5__Roseomonas
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Serratia
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Crocinitomicaceae;D_5__Fluviicola
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Kineosporiales;D_4__Kineosporiaceae;__
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__marine metagenome;D_5__marine metagenome
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae;__
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;

Names	total	elements
		D_3__Rhizobiales;D_4__Rhizobiaceae;D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__env.OPS 17;
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__Sphingobacterium
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhodobacterales;D_4__Rhodobacteraceae;__
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__uncultured bacterium;D_5__uncultured bacterium
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae;D_5__Moheibacter
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Xanthomonadaceae;D_5__Xanthomonas
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Propionibacteriales;D_4__Nocardioidaceae;D_5__Aeromicrobium
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Micrococcaceae;__
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Corynebacteriales;D_4__Nocardiaceae;D_5__Rhodococcus
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae;D_5__Taibaiella
		D_0__Bacteria;D_1__Actinobacteria;D_2__Thermoleophilia; D_3__Solirubrobacterales;D_4__Solirubrobacteraceae;D_5__uncultured
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;__;__
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae;D_5__Dyadobacter
		Unassigned;__;__;__;__;__
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingobium
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Caulobacterales;D_4__Caulobacteraceae;D_5__Brevundimonas
		D_0__Bacteria;D_1__Actinobacteria;D_2__Acidimicrobiia; D_3__Microtrichales;D_4__Iamiaceae;D_5__Iamia
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Rhodanobacteraceae;D_5__Luteibacter
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__Mucilaginibacter
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Xanthomonadaceae;D_5__Stenotrophomonas
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Xenophilus
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Kineosporiales;D_4__Kineosporiaceae;D_5__Kineococcus
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Rathayibacter

A3.2 Venn diagram results showing the shared bacterial genera between different species. Fr: *Festuca rubra*; Lp: *Lolium perenne*; La: *Loium arudinacea.*

Names	Total	Elements
Fr La Lp	27	D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__uncultured Sphingobium sp.; D_5__uncultured Sphingobium sp.
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Novosphingobium
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Devosiaceae;D_5__Devosia
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingomonas
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__uncultured
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Sanguibacteraceae;D_5__Sanguibacter
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Verticia
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae;D_5__Spirosoma
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Pigmentiphaga
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__Pedobacter
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Duganella
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Pseudomonadaceae;D_5__Pseudomonas
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae;D_5__Aureimonas
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;__
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Massilia
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__Saccharimonadaceae; D_5__uncultured bacterium
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Erwinia
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae; D_5__Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Curtobacterium
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__Sphingobacterium
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Pantoea
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia;

Names	Total	Elements
		D_3__Saccharimonadales;__;__
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae;D_5__Dyadobacter
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Caulobacterales;D_4__Caulobacteraceae;D_5__Brevundimonas
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__Sphingobacteriaceae;D_5__Mucilaginibacter
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae;D_5__Chryseobacterium
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Flavobacteriaceae;D_5__Flavobacterium
Fr La	2	D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Pseudoclavibacter
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae;D_5__Sphingobium
Fr Lp	16	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Variovorax
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Xylophilus
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae;D_5__uncultured
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Serratia
		D_0__Bacteria;__;__;__;__;__
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Hymenobacteraceae;D_5__Hymenobacter
		D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli; D_3__Bacillales;D_4__Paenibacillaceae;D_5__Paenibacillus
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;__
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Xanthomonadaceae;D_5__Xanthomonas
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Propionibacteriales;D_4__Nocardioidaceae;D_5__Aeromicrobium
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Corynebacteriales;D_4__Nocardiaceae;D_5__Rhodococcus
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Enterobacteriales;D_4__Enterobacteriaceae;__
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Beijerinckiaceae;D_5__Methylobacterium
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Rhodanobacteraceae;D_5__Luteibacter
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Kineosporiales;D_4__Kineosporiaceae;D_5__Kineococcus
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Rathayibacter
La Lp	4	D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Sphingomonadales;D_4__Sphingomonadaceae;
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Crocinitomicaceae;D_5__Fluviicola

Names	Total	Elements
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Kineosporiales;D_4__Kineosporiaceae;
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae;D_5__Taibaiella
Fr	2	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Methylophilaceae;D_5__Methylophilus
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Plantibacter
La	2	D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Pseudomonadales;D_4__Moraxellaceae;D_5__Alkanindiges
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Frankiales;D_4__Geodermatophilaceae;D_5__Geodermatophilus
Lp	17	D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Chitinophagales;D_4__Chitinophagaceae;D_5__Flavitalea
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__Saccharimonadaceae;__
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Herbaspirillum
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Cytophagales;D_4__Spirosomaceae;D_5__Fibrella
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Acetobacterales;D_4__Acetobacteraceae;D_5__Roseomonas
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__marine metagenome; D_5__marine metagenome
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhizobiales;D_4__Rhizobiaceae;__
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Sphingobacteriales;D_4__env.OPS 17;
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Microbacteriaceae;D_5__Frondihabitans
		D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria; D_3__Rhodobacterales;D_4__Rhodobacteraceae;__
		D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia; D_3__Saccharimonadales;D_4__uncultured bacterium; D_5__uncultured bacterium
		D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia; D_3__Flavobacteriales;D_4__Weeksellaceae;D_5__Moheibacter
		D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria; D_3__Micrococcales;D_4__Micrococcaceae;__
		Unassigned;__;__;__;__;__
		D_0__Bacteria;D_1__Actinobacteria;D_2__Acidimicrobiia; D_3__Microtrichales;D_4__Iamiaceae;D_5__Iamia
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Xanthomonadales;D_4__Xanthomonadaceae;D_5__Stenotrophomonas
		D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria; D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Xenophilus