EFFECTS OF SILICON AND BENEFICIAL BACTERIA ON SHEATH BLIGHT OF RICE AND THE MICROBIAL COMMUNITY OF RICE RHIZOSPHERE

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EFFECTS OF SILICON AND BENEFICIAL BACTERIA ON SHEATH BLIGHT OF RICE AND THE MICROBIAL COMMUNITY OF RICE RHIZOSPHERE

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfilment of the requirements for the degree of Master of Science

in

The Department of Plant Pathology and Crop Physiology

by

Jhonson Leonard
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ABSTRACT

Sheath blight (ShB), caused by *Rhizoctonia solani* Kühn, is an economically important soil-borne disease of rice (*Oryza sativa*) that can cause up to 50% yield losses. Application of fungicides is the most effective and commonly used method for managing sheath blight. However, the pathogen can develop new resistant strains upon continuous exposure to a fungicide. Moreover, the use of inorganic fungicides is not eco-friendly. The utilization of biological control agents (BCAs) can be a more sustainable and safe means of managing ShB. To develop alternate strategies for ShB management, I am exploring the effect of the silicon (Si) fertilization in reducing sheath blight disease in combination with BCAs. Bacteria were isolated from the rhizosphere of healthy rice plants and screened by dual-culture for their antifungal activity against *R. solani*. Among 534 isolates 24 antagonists were identified to *Bacillus spp* based on their 16S rDNA sequence. Two isolates were evaluated for their synergistic activity with silicon against ShB in a greenhouse setting. The combined application of silica and BCAs reduced the development of ShB in rice plants compared to non-treated control and plants that received a single treatment suggesting that simultaneous use of the two materials could effectively manage ShB. I also evaluated the effects of *R. solani* inoculation and BCAs application on the bacterial communities of rice rhizosphere in the field at the LSU AgCenter H. Rouse Caffey Rice Research Station. The genomic DNAs of the microbial communities of the rice rhizosphere of Si-treated and non-Si-treated plants were extracted. The 16S rDNA regions of the extracted DNA were amplified and sequenced. The beta diversity analysis performed with Qiime2 revealed that the microbiome profile of rhizosphere of the inoculated plants was significantly different from non-inoculated ones. Although silica application reduced development, it did not affect much the microbial profile on the rice rhizosphere.
CHAPTER 1. LITERATURE REVIEW

1.1. Sheath Blight of rice

Sheath blight (ShB) is an important rice (*Oryza sativa*) disease causing tremendous losses in almost every region of the world where rice is cultivated. In the absence of a measure of control, the yield losses vary between 10-30% (Xie 2008) and can even reach 50% when the environmental conditions are favorable (Marchetti 1991).

Sheath blight of rice is caused by the fungal pathogen *Rhizoctonia solani* Kühn the imperfect stage of *Thanatephorus cucumeris* (A.B.Frank) Donk. *R. solani* can infect many other plant species including barley (*Hordeum vulgare*), lettuce (*Lactuca sativa*) tomato (*Solanum lycopersicum*), sorghum (*Sorghum bicolor*), and maize (*Zea mays*) (Zhang et al., 2009).

Rice infection by *R. solani* mostly occurs during the late tillering stage when the floating sclerotia encounter the rice culms at the water level. The symptoms begin to appear above the water line as circular, oblong or ellipsoidal, green-gray water-soaked spots of approximately 1 cm long (Rush & Lee, 1992). The disease is more severe in an environment with high humidity (~ 95%) low sunlight and high temperature (28-32°C); such conditions amplify the horizontal (between plants) and vertical (in the same plant) spreading of the running hyphae (Rush & Lee, 1992). The lesions might spread on the entire sheaths of the plant, which can cause the canopy to open and facilitate the penetration of sunlight and the drop down of the air humidity. Under those microclimate conditions, the fungal hyphae start producing the sclerotia that overwinter in the soil or plant residues. The yield losses are results of poorly filled grains caused by massive infection at the growing stage, lodging or death of the culms (Wrather & Sweets, 2009).
To effectively manage ShB of rice, one can use several methods including cultural practices, planting of resistant rice varieties, application of pesticides and biological control. Managements by cultural practices comprise any control method aiming to create an unsuitable environment for the development of the pathogen. Among those methods include adequate plant spacing, crop rotation, and management of nitrogen (N) fertilizer. For the spacing, a canopy density of 160 to 214 plants per square meter is optimal for the control of the disease (Wrather & Sweets, 2009). Wrather et al. (2009) also mentioned that a timely N application of 30 pounds during the internode elongation stage can avoid the development of the disease. Cultural methods for management of ShB do not suppress the disease, given that they are not affecting the pathogen directly; instead, they can be used to alleviate disease severity as support to other management methods (Groth, 1996).

There is no rice variety entirely resistant to ShB disease. A few varieties showing partial resistance include YSBR1, Tetep, Teqing, and Jasmine 85 (Jia et al., 2012; Pinson et al., 2008). Partial resistant to ShB was correlated with the properties of plants to reduce fungal growth by producing phenolic compounds (Kosuge, 1969), and to prevent fungal penetration by secreting a wax layer (Hassan 2015; Marshall, 1980). The attempts to build resistant rice varieties again R. solani are very challenging because of the lack of knowledge about the loci involved in the resistant traits.

Although the pathogen can develop resistance to fungicides, chemical methods are currently the more effective available mean for ShB management (Su et al., 2012). For example, Azoxystrobin fungicides can offer a control efficiency at 71% regardless of their application alone or with other fungicides (Uppala & Xin-Gen, 2018). Given the risk of emergence of
resistant *R. solani* isolates, chemical control must be utilized when resistance plant variety is not available, or the other control methods fail.

1.2. Silicon and its benefits for plants

Silicon (Si) is the second most abundant element in the earth’s crust after oxygen. It is mostly present in forms that cannot be absorbed by plants. Silicon can be absorbed by plants only when it is in the form of monosilicic acid [Si(OH)]₄ (Raven, 1983). The Si content of the soil varies between 0.1 and 0.6 mM (Epstein, 1994; Raven, 1983). When the concentration exceeds 2 mM under 25°C, the Si is no longer available for the plants because Si(OH)₄ polymerizes into SiO₃ gel (Ma et al., 2001). Although the element has numerous benefits for diverse plant species in various environmental circumstances, it is not considered as essential for plant growth and development.

1.2.1. Classification of plants based on their silicon assimilation

Plant species have been classified into three groups based on their capacity to assimilate Si and their Si content. In 1990, Takahashi discriminated plants in active, passive, and rejective accumulators (Yan et al., 2018). This classification is following the first distinction established by Jones & Handreck in 1967 categorizing plant species into high-, intermediate-, and low-accumulators (Ma et al., 2006). The active accumulators have a Si content that varies between 1.5 and 10% and includes monocots, such as rice (*Oryza sativa*) sorghum (*Sorghum bicolor*) and wheat (*Triticum aetivum*). The passive accumulators include dryland grasses and have a Si content between 0.2 to 1.5%. The rejective accumulators have a Si content less than 0.2 % and include most dicot plant species.
The recent finding of new channels that facilitate the selection of Si by plant roots has allowed a better understanding of uptake of Si by plants and a refinement of the classification on a molecular basis (Coskun et al., 2019). Influx channels named Lsi1 allow Si(OH)$_4$ to enter the roots and channels named Lsi2 allow the element to penetrate the xylem (Ma et al., 2006). Lsi1 channels belong to a class of Aquaporin (AQPs) composed of six trans-membrane (TM) domains and two and half TM helices. This channel has a pore that allow the transit of some molecules from the liquid phase of the soil to the plants (Murata et al., 2000). The two half TM helices form a narrow region that contains two NPA (asparagine-proline-alanine) domains. Those Si influx transporters (Lsi1 and Lsi2) cluster within the Nodulin 26-like intrinsic protein III (NIP-III) subgroup of the aquaporins family. According to the presence of the NIP-III proteins, the structural and the number of amino acids that separate the two NAP domains, Coskun proposed to classify the plants in Si accumulator and Si non-accumulator. In the Si accumulator, the NAP domains are separated by 108 AAs (Deshmukh et al., 2015). This classification was in accordance with the classification that was made based on the Si content in plant since the plants that have a high Si content prosessed the molecular features describe above.

1.2.2. Benefits of silicon for plants

Silicon benefits more the high accumulators than the low accumulators. It is beneficial for various crops, including monocots such as rice (*Oryzae sativa*) (Shi et al., 2016) barley (*Hordeum vulgare* subsp. *vulgare* L.), sugarcane (*Saccharum officinarum* L.) (Meyer & Keeping, 2000), and wheat (*Triticum aestivum* L.) and broadleaf plants such as cucumber (*Cucumis sativus* L.) (Adatia et al., 1986; Pavlovic et al., 2013), *Arabidopsis thaliana* (Fauteux et al., 2006; Li et al., 2008) and strawberry (*Fragaria sp.*) (Kanto et al., 2004; Miyake & Takahashi, 1986).
The benefits of Si for plants include enhancement of resistance to biotic and abiotic stress and improvement of plant growth and yield (Fauteux et al., 2006; Li et al., 2008; Shi et al., 2016).

1.2.3. Resistance to abiotic stress

Abiotic stress, such as drought, salinity and metal contamination, constitute a major challenge to crop production. It has been shown that Si can help to alleviate drought, salinity stress, and toxicity to heavy metal. Silicon-induced tolerance to abiotic stress can be classified as biologically passive processes, such as the structural modification of cells, or as biologically active processes occurring at the transcriptomic level (Etesami & Jeong, 2018).

Silicon can help plants fighting against drought by preventing water losses through the production of a silica cuticle double layer under the epidermis and the modification of the stomatal conductance of the leaves (Gong et al., 2003). Silicon can also promote root elongation (Hattori et al., 2005) and conductance (Shi et al., 2016) to help the plant adjusting the loss-absorption water equilibrium so it can resist drought stress. Silicon has been found to alleviate salt stress by preventing the accumulation of NaCl and the translocation of toxic ions from roots to shoots (Liu et al., 2015). Similarly, Yeo et al. (1999) showed that the accumulation of SiO$_3$ in endodermis and exoderm cells led to the alleviation of NaCl toxicity. Under salinity stress, potassium uptake increased because Si nutrition can help the plant maintain the K/Na by stabilizing the proton pump activity of the root tip (Xu et al., 2015).

Silicon also alleviates the toxicity of Manganese (Mn) (Williams et al., 1957), Aluminum (Al) (Britez et al., 2002), and cadmium (Cd) (Hasanuzzaman et al., 2017).
study by Hasanuzzaman et al. (2017) has shown an improvement of the defense mechanism against Cd stress in plant supplemented with Si. This Si-induced defense is manifested by the reduction of reactive oxygen species (ROS) in Si-treated plants as a result of the augmentation of antioxidant compounds associated with an increase in ascorbate-glutathione and the velocity of glyoxalase pathways (Hasanuzzaman et al., 2017). Although there is few evidence of the direct involvement of Si in the cellular metabolic processes that would explain this systematical resistance to abiotic stress, many studies have shown that supplying plants with Si under diverse stress environment results in the transcriptional regulation of genes involved in water transport and stress related pathways, such as phenylpropanoid pathway, jasmonic acid pathway, and abscisic acid dependent or independent regulatory pathway (Manivannan et al., 2017).

1.2.4. **Silicon can help managing plant diseases**

The application of additional Si decreases disease severity in many plant-pathogen systems. It was shown that Si reduces the number of sporulating lesion of *M. grisea* per leaf area (Seebold et al., 2001). Epidermis cells of barley plants treated with Si resisted to the penetration by the hyphae of the powdery mildew pathogen (Carver et al., 1987).

In other fungal diseases, Si-treated plants showed a reduced number of fungal pathogen colonies. For example, the number of *Podosphaera fuliginea* colonies was reduced by about 43-94% on cucumber leaves (Menzies, 1991). Similarly, the application of Si led to the reduction of the number of mildew (*Uncinula necator*) colonies on grape (*vitis vinifera*) (Bowen et al., 1992). Blast lesion length on rice plants treated with Si was reduced by 40-80% (Seebold et al., 2001). The lesion extension and the progress curve of sheath blight disease of rice decreased in Si treated plants (Rodrigues et al., 2003).
1.2.5. Mechanism of induction of plant disease resistance

Many hypotheses have arisen to explain the mechanism by which Si confers resistant to plant diseases. The first proposed mechanism is the physical barrier hypothesis stating that Si protects plants by reinforcing the cuticle and cell walls of the host plant and by forming silicate papillae that slow down the progression of pathogenic structures (Kim et al., 2002). Silicon accumulating in the cell wall of the host plant to form a silica-double layer makes the penetration of pathogens difficult (Kim et al., 2002). This hypothesis was suggested in many plant-pathogen systems including powdery mildew in grape (Bowen et al., 1992), ShB of rice (Schurt et al., 2012) and rice blast (Hayasaka et al., 2008).

However, the mechanical enhancement of the plants is not sufficient to explain Si-induced plant defense against pathogens. Silicon may play an active prophylactic role in plants. Phenolic-like compounds were found at a high level in Si-treated plant upon infection of pathogens (Rodrigues & Datnoff, 2005). The invading hyphae of the rice blast pathogen were surrounded with amorphous material in Si-treated plant leaves (Rodrigues et al., 2003). Rodrigues et al. (2004) found that Si-treated plants produce higher levels of phytoalexins than non-treated plants. Silicon also leads to the differential regulation of several transcriptional factors related to plant defense in rice plants infected with pathogens (Rodrigues et al., 2005).

1.2.6. Silicon sources for plant fertilization

The amount of Si that is available in the soil is influenced not only by soil type, environmental factors, and soil pH but also the genotype of the cultivated crop. High Si accumulator crops, such as sugarcane and rice, can remove significant amount of Si from
the soil (Meyer and Keeping, 2001; Blecker et al., 2006). To compensate the export of Si by plants, fertilizer can be applied to raise the amount of Si(OH)₄ in the soil.

Many researchers use calcium silicate in the form of wollastonite. This compound contains a high level of Si (up to 50% of SiO₃). However, the intensive labor and the high cost that is required to produce wollastonite often limit its use in a large scale. Other silica fertilizers that are commonly used in research are the silica slags, such as iron/steel slag, electric furnace slag and blast furnace slag containing 5.4%, 21.1% and 17.3% of Si, respectively. Biochar and rice hull are among the plant-based silica fertilizers (Houben et al., 2014; Sun & Gong, 2001).

1.3. The soil microbiome: its dynamism, benefits for plants and analysis

The soil microbial community is extremely diverse; 1 gram of soil can contain a 10,000 – 50,000 species of microbes (Øvreås et al. 1998; Schloss & Handelsman, 2006). Soil properties such as texture (Girvan et al., 2003), N content (Frey et al., 2004), P content (Frey et al., 2004) and soil pH (Lauber et al., 2009; Rousk et al., 2010), largely influence the microbiome community. Among those factors, the pH is the one that affects the most the soil composition and diversity of microorganism. Bacterial taxa can grow in a narrow range of soil pH; thus, a slight change in the pH can cause a significant shift in the bacterial composition (Rousk et al., 2010). Phosphorus content, altitude, and the ratio of cations in the soil (e.g. Ca²⁺, Mg²⁺, and Al³⁺) also influence the soil microbial community (Faoro et al., 2010). However, the evidence of those factors influencing the microbial composition does not completely lessen the importance of the pH as a driver of the soil microbial composition since they can also alter the soil pH. Probably, the change in the microbial composition of the soil is the result of the synergistic interaction of diverse soil factors.
The species of plants can also influence the soil microbiome composition. The root exudates can serve as a substrate for some microorganisms in the rhizosphere while containing antimicrobial compounds to other organisms (Lakshmanan et al., 2014; Dakora et al., 2002). Since genotypically diverse plants secrete different exudate, they also have different shapes of soil microbial community. Indeed, the microbiome community associated with their hosts are considered as an extension of their hosts’ genomes (Turner et al., 2013). The application of root exudate can lead to changes in the microbiome, similar to those observed for plants growing in the same soil.

1.3.1. Role of soil microbes in plant health and productivity

Plant health, productivity, and capacity to accumulate nutrients increase with the soil microbial diversity. Some evidences showed that plant productivity depend more on the functional diversity than taxonomic diversity. (Maherali & Klironomos, 2007). The reduction of the microbial community diversity does not affect the health and productivity of plants much, suggesting that benefits are associated with the functional diversity of the microbiome rather than the taxonomic diversity. The microbiomes of different taxa can perform the same functional role (Maherali & Klironomos, 2007).

In addition to increasing plant productivity, the microbiome profile of a soil determines its ability to suppress plant diseases. Many isolated microbial species have been shown to have the ability to suppress diseases via the secretion of antimicrobial compounds that is detrimental to plant pathogens. Examples of this include fluorescent pseudomonads that produce the antibiotic 2, 4-DAPG which offer protection against soil born disease (Raaijmakers & Weller, 1998), and B. subtilis that release in the rhizosphere compounds, such as surfactin and iturin that are linked to plant resistance to diseases.
(Waewthongrak et al., 2014). Although many researches have been focused on disease suppressive ability of a particular taxon, it is believed that the soil ability to suppress disease is driven by the whole soil microbiome composition (Mendes et al., 2011).

1.3.2. Methods of microbiome analysis

The development of sequencing technology has allowed a more detailed and comprehensive study of the microbial community of different ecosystems. Before the application of metagenomics technics, scientist relied on the properties of the microorganism to be isolated and cultured in specific media for their taxonomic identification and characterization. Those traditional methods could only allow studying a small portion of the organism colonizing based on their morphological appearance, and the physiological and nutritional requirements for each specimen. New molecular tools had permitted to investigate a large amount of the microorganisms present in a sample.

The metagenomic approach relies on the sequencing of one or more short DNA fragments of normalized regions of the microbial genome that contain significant genetic variability at the species level. The 16s rRNA gene has been successfully used in the identification of bacterial community sequences (Wang & Qian, 2009). It is highly conserved across bacteria and possesses hypervariable regions that allow its utilization in the identification of individual bacteria. For the study of fungal microbiome communities, two genes are commonly used: the internal transcribed spacer (ITS) region and portions of the 18S rRNA (Anderson et al., 2003). However, it was recently found that the ITS offers a more accurate analysis than that of the 18s rRNA (Liu et al., 2018).

The workflow involves five steps: (i) collection of samples ii) DNA extraction; iii) targeted amplification; iv) sequencing; and v) data analysis (Abdelfattah et al., 2018). The
workflow starts with the sampling which process should be handled with care since it can be suggested to error due to contamination. Contamination occurring during the sampling, transportation and DNA extraction can significantly affect the output obtained for the samples since any DNA mistakenly included will be amplified (Salter et al., 2014). The bioinformatic analysis of the sequence data can be summarized in two central parts: the generation of Operational Taxonomic Units (OTUs) and the downstream analysis such as alpha and beta diversity assessment. The OTUs are subsequently compared to a database of identified sequence so they can be assigned a taxa name. During the second step, the OTU table is used for diverse statistical analysis that will be described later in this review. Among the more common bioinformatics software that can be used to analyze the sequence data, figure R, QIIME (Caporaso et al., 2010), MOTHUR (Schloss et al., 2009)

Independently of the software used, the generation of OTUs start by the demultiplexing of the sequence. In this step, the samples that were pulled during the PCR are separated back the original biological or experimental samples. Afterward, the sequences are filtered to discard low-quality reads. To pick the OTUs, the reads are aligned to a taxonomic database like greengenes or Sylva and reads that have 97% of similarity are represented by a single OTU (McDonald et al., 2012).

The principal statistical analysis that can be performed with the OUT table is the characterization of the alpha and beta diversity, which are the diversity within and between the samples, respectively. The indexes for alpha diversity can refer to the richness, the diversity, and the evenness of the community. The richness represents how many species are present, the evenness, how even are each species proportion relative to
each other. When one or few species dominate a community, it is considered to have low evenness, whereas when each species are more or less equally represented it is deemed to have high evenness. The metrics used to measure the alpha diversity include Chao 1 index, Shannon (or Shannon-Wiener) index and Simpson's index (Xia et al., 2018).

Beta diversity is a measure of the change in the species composition of different biological samples. It evaluates how the samples are similar. There are two main categories of similarity measures: binary similarity coefficients and quantitative similarity coefficients. When only presence/absence measurement data is available for species in a community, the binary similarity coefficients are used. When some relative abundance measures are also available for each species, the quantitative similarity coefficients will be applied. Beta diversity is calculated using a measure of similarity or dissimilarity distance to represent the relationships of the samples (Lozupone & Knight, 2008).

References


CHAPTER 2.  ISOLATION OF RICE RHIZOSPHERE BACTERIA AND THEIR COMBINED APPLICATION WITH SILICON TO CONTROL SHEATH BLIGHT OF RICE

2.1. Introduction

Rice (Oryza sativa L.) is an essential crop supplying around 21% of the calories consumed by human population (Nayar, 2014). Similar to many places where rice is grown, the rice industry in Louisiana is under the threat of many diseases, including sheath blight caused by Rhizoctonia solani (Su et al., 2012; Groth, 1991). When the conditions are favorable, this disease can cause up to 50% yield losses (Marchetti, 1991; Rush & Lee, 1992). There are limited number of options to control sheath blight since there is no variety utterly resistant to the disease (Li et al., 1995). The most used and efficient method of management is the application of fungicide (Su et al., 2012). However, this practice is not environmentally and economically sustainable (Gerhardson, 2002).

The inoculation of bacterial antagonists to the sheath blight pathogen or the application of compounds derived from those microorganisms is a promising alternative management practice for the disease (Bashar et al., 1970; Shrestha et al., 2016). Bacterial strains isolated from diverse parts of rice plants have been shown to negatively affect sheath blight in vivo and in vitro (Kanjananamaneesathian et al., 1998). Free-living rhizobacteria and endophytic bacteria help controlling plant diseases by parasitizing and competing with the pathogens, and via the production of inhibitory allelochemicals (Cazorla et al., 2007; Compant et al., 2005; Niranjan et al., 2006). Biological control
agents (BCAs) can also induce systemic resistance (ISR) in host plants (Bloemberg et al., 2001; Haas & Keel, 2003; Ryu et al., 2004).

The application of biological control agents has allowed to obtain effective control for sheath blight with a reducing input of chemical fungicides (Kiewnick et al., 2001; Someya et al., 2007). Combined application of fungicides and BCAs has significantly enhanced disease control compare to treatments of BCAs alone (Buck, 2004; Francés et al., 2002). The efficacy of fungicides in controlling sheath blight has been found to increase when applied with biofungicides (Boukaew et al., 2013).

Silicon (Si) was also reported to offer protection to rice plant against *R. solani*. The severity of sheath blight negatively correlates with the increase of the rate of silica fertilizer application in Si depleted soil (Rodrigues & Datnoff, 2005; Rodrigues et al., 2003). The propensity to alleviate abiotic and biotic stress in plants is very similar for Si and beneficial bacteria (Etesami, 2018). Both Si and bacteria can alleviate salinity and drought stress, improve nutrient uptake, and increase the resistance against pathogens (Damodaran et al., 2014; Etesami & Beattie, 2017; Etesami & Jeong, 2018). However, only one study, conducted by Mahmood et al. (2016), has explored the potential of the combined application of the two elements. They found that the application of rhizobacteria along with Si mitigates the effects of salinity on mungbean by regulating osmolytes, reducing lignification, improving mineral uptake, and decreasing tissue Na⁺ content. This group is the pioneer in studying the combined benefit of Si and rhizobacteria. This study was conducted to find bacterial antagonists in rice rhizosphere against the sheath blight pathogen and evaluate their synergetic effect with Si on the severity of the disease.
2.2. Materials and Methods

2.1.1. Isolation of bacteria from rice rhizosphere

Rice plants were collected from the H. Rouse Caffey Rice Research Station at Crowley, LA, as a source for isolation of rhizosphere bacteria. Three grams of soil was mixed with 15 mL of 10 mM of MgCl$_2$ in a 250 mL Erlenmeyer flask. The mixture was shaken at 190 rpm and 37°C for 60 minutes. To collect bacterial colonies, the solution was spread on Luria-Bertani (LB) agar plates of the following composition: 1% tryptone, 0.5% yeast extract, 1% NaCl2, and 1.8% agar. The LB agar medium was amended with cycloheximide at a concentration of 40 μg mL$^{-1}$ to prevent the growth of fungal colonies. After incubation for 24 h at 27°C, bacterial colonies of different morphological characteristics were picked with a toothpick and transferred to fresh LB agar plates for a subsequent incubation for 24 h. A code was assigned to each bacterial colony.

2.2.1. Screening of the isolated bacteria for their antagonistic activity against R. solani

To investigate which of the picked bacterial colonies can inhibit the growth of $R$. solani, they were grown simultaneously on a potato dextrose agar (PDA). Six different bacterial colonies were cultured with a mycelial plug (5 mm in diameter) from one week-grown $R$. solani and incubated for 72 h at 25 °C. The colonies showing antagonistic activity were selected for a second test, for which they were cultured overnight at 37 °C and 190 rpm in 1.5 mL of LB liquid media. After incubation, the bacteria cultures were washed twice and resuspended in 100 μL LB liquid medium for each culture. Subsequently, 10 μL of each bacterial suspension were dropped in three different locations on a PDA plate containing a plug of one week-grown $R$. solani at the center. After 72 h of incubation at 25 °C, the inhibition zones area was measured using a caliper.
The bacteria that suppressed the growth of *R. solani* were kept in sterile 15% glycerol solution at -20 °C.

### 2.2.2. Identification of the antagonistic bacteria

Bacterial isolates stored at -20°C were pre-cultured on LB agar for 20 h and grown in LB liquid culture for 24 h. The LB agar plates containing the bacteria were kept for Gram tests. The KOH test was used to differentiate gram-positive from gram-negative bacteria, for which 50 μL of 3% KOH solution was dropped on a microscope slide and the bacterial cells picked from the agar plate were suspended by rapid stirring in the KOH solution (Suslow et al. 1982). The toothpick was repetitively raised and lowered on the solution to see if a viscous and mucoid string is formed, a typical feature of Gram-negative bacteria in the KOH solution.

The bacterial genomic DNA was extracted using the Gen Elute bacterial genomic DNA kit (Sigma) following the protocol of the manufacturer. The 16S-rDNA regions of the extracted bacterial DNA were amplified by PCR using the two universal primers (Forward - 5’CCC GGG ATC CAA GCT TAA GGA GGT GAT CCA GCC 3’; Reverse - 5’CCG AAT TCG ACA ACA GAG TTT GAT CAT GGC TCA G3’) (Bukin et al., 2019). The amplified genetic materials were purified using the PCR purification kit. The sequences thereby obtained were compared with sequences from the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST).

### 2.1.2. Evaluation of the combine effects of silicon fertilizers and two bacterial strains

#### 2.2.2.1. Medium, plant materials and Si application

For the greenhouse experiment, the soil provided by the LSU greenhouse was used. Different ratios of soil and sand were measured and the combination that had the lowest level of
available Si was chosen (47 μg g⁻¹) (1:1, sand:soil). The rice variety Bengal was grown for 47 days in soil amended with wollastonite and in non-amended soil. The wollastonite was mixed to the soil mixture at a rate of 560 Kg Si ha⁻¹ of soil. The fertilizer was applied in the growing medium one day before planting by mixing it thoroughly until homogeneous growing media were obtained. The pots containing soil amended with wollastonite were kept separated from non-amended plots.

The experiment consisted of six treatments: plants grown in the Si-treated soil and sprayed with one of the two bacterial suspensions or MgCl₂ only, and plant grown in non-amended soil and sprayed with one of the two bacterial suspensions or MgCl₂ only. Each treatment was repeated six times.

2.2.2.2. Treatment of rice plants with rice associated bacteria (RABs).

Overnight cultures of RAB grown on LB agar were resuspended in a solution of MgCl₂ and 0.02 % of silwet, and bacterial suspension of ca. 5 X 10⁷ CFU mL⁻¹ (OD600 = 0.1) of each RAB was sprayed 24 h before the inoculation with R. solani until it drips at the late tillering stage (~ 47 days old). The silwet was added to facilitate the adhesion of the bacterial suspension to the plant surface.

2.2.2.3. Inoculation of rice plants with R. solani

Mycelial plugs (1 cm in diameter) from a fresh R. solani culture, which was grown four days on potato dextrose agar, were used to inoculate rice plants at the lower sheath of the main tiller. Each mycelial plug was attached to the plant tissue and wrapped with a piece of aluminum foil. To evaluate sheath blight in the rice plants, the lesion length for each inoculated sheath was recorded using a caliper. The total lesion length was the sum of the lesion length of leaves in a culm. Six replications were used for each treatment.
2.2.3. **Extraction of silica from rice plant**

The analysis of Si content in the plant was conducted in the laboratory of Dr. Tubaña. Plant samples were dried, ground, and digested. Plant sample digestion was carried out following the Oven-Induced Digestion procedure (Kraska and Breitenbeck, 2010). One hundred mg of finely ground samples was placed into a 50 mL polyethylene screw-cap centrifuge tube. The plant tissue was digested by heating them with 4 mL of 50 % NaOH at 90°C for 4 hd. After the digestion, 1 mL of 5 mM ammonium fluoride (NH₄F) was added and the volume was adjusted to 50 mL with deionized water. The concentration of Si in the digested plant was measured, using the molybdenum blue colorimetric procedure. Two mL of the digest was swirled for about 10 sec with 10 mL of 20% acetic acid. Then 4 mL of 0.26 M ammonium molybdate was added. After 5 min, 2 mL of 8-Anilinonaphthalene-1-sulfonic acid ammoniate was added as a reducing agent, and the final volume was adjusted to 30 mL of 20% acetic acid. The resultant solution was kept at room temperature for 30 min, after which they were vigorously shaken before taking absorbance reading using a UV spectrophotometer (Hach DR 5000) set at 630 nm. Standard series of 0.4, 0.8, 1.6, 3.2, 4.8 and 6.4 mg L⁻¹ were also prepared by pipetting 0.5, 1, 2, 4, 6, and 8 mL of 24 mg L⁻¹ Si and treated in a same manner as the digested plant samples. The standards were used to generate a linear equation between the concentrations of Si in solution and their corresponding spectrophotometer readings. The equation obtained from the standard curve was used to calculate the Si content of each sample from their absorbance reading.

2.2.4. **Field experiment and treatments**

The field experiment was conducted at the H. Rouse Caffey Rice Research Station of LSU AgCenter. The site has been the subject of many experimentations on rice during which it
experienced alternate flooding and draining. The experiment was conducted from April to August 2018 using rice varieties Bengal and CL111. The field was divided into two sections one of which was amended with silica slag fertilizer, an industrial by-product derived from iron production that contains 5.4% of Si, at a rate of 500 kg Si ha\(^{-1}\). The Si fertilizer was applied manually.

Each section was divided into four blocks containing randomized plots for each of those treatments: Si amended and non-amended plots, plot inoculated, and non-inoculated with \(R.\ solani\). The silicate slag fertilizer was manually applied and incorporate to 3 to 5 cm deep to the soil.

### 2.2.5. Inoculation with \(R.\ solani\)

Inoculum of \(R.\ solani\) was prepared following a protocol adopted in Dr. Ham lab in the Department of Plant Pathology and Crop Physiology at LSU. Rice grain and husk were mixed according to a 2:1 ratio up to 1400 mL in flasks and 500 mL of water was added. The flasks were covered with cotton and aluminum foil and autoclaved. The culture of \(R.\ solani\) on potato dextrose agar (PDA) was added to the autoclaved mixture, which was subsequently kept at room temperature for ten days for the growth of the fungus. The prepared inoculum was combined with fresh husk/grain mixture to increase the volume of the inoculum to be used in the field. The inoculum was applied to rice plants at the tillering stage by hand sprinkling.

### 2.2.6. Statistical analysis.

The inhibition areas of \(R.\ solani\) growth caused by the bacteria were compared using the Tukey’s test in SAS. The interaction effect of Si and bacteria strains
applied was evaluated by the two-way ANOVA and the comparison between the treatment groups using the Tukey's HSD.

2.3. Results

2.3.1. Antagonistic activity of the rice associated bacteria

Five hundred and thirty-four bacterial isolates were isolated from the rhizosphere of the rice plants. Among those isolates, 49 isolates could inhibit the growth of *R. solani*. Twenty-four of the forty-nine bacterial isolates showed an inhibition zone larger than 1 cm$^2$. The Ryu’s 3% KOH test revealed that most of the isolate were Gram positive. The 16S ribosomal RNA (rRNA) of those isolates were aligned to the sequences of the NCBI database. The BLAST searches revealed that all the bacteria were closed to species belonging to the genus *Bacillus*. 14 of the 24 identified bacteria were *Bacillus subtilis* and *Bacillus amyloliquefaciens* (Table 2.1).

![Figure 2.1. Graph depicting antifungal activities of RRBs against *R. solani* evaluated by the area of the inhibition zone (cm$^2$). Based on Tukey’s test, the significant difference with P <0.0001 of bacteria that have a larger zone of inhibition are indicated by the letters above the columns. The error bars indicate the standard deviation of three replications.](image)
The two isolates RRB_5s2 and RRB_7s2 showed the largest inhibition area against *R. solani* and was found to be closest to *Bacillus thuringiensis* and *Bacillus methyloptrophicus*, respectively (Figure 2.1).

Figure 2.2. Selected plates of dual culture showing antifungal activities of RABs against *R. solani*. 
Table 2.1. Closest bacterial species from the NCBI database to the bacteria isolated from rice rhizosphere.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Closest Organism</th>
<th>Identity</th>
<th>Access code</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRB_5s2</td>
<td><em>Bacillus thuringiensis</em></td>
<td>94%</td>
<td>MF796624.1</td>
</tr>
<tr>
<td>RRB_19s2</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>96%</td>
<td>KY680541.1</td>
</tr>
<tr>
<td>RRB_7s2</td>
<td><em>Bacillus methylotrophicus</em></td>
<td>97%</td>
<td>KC171991.1</td>
</tr>
<tr>
<td>RRB_11s2</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>93%</td>
<td>CP018152.1</td>
</tr>
<tr>
<td>RRB_18s2</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>91%</td>
<td>MH001393.1</td>
</tr>
<tr>
<td>RRB_10s1</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>97%</td>
<td>CP030097.1</td>
</tr>
<tr>
<td>RRB_14s2</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>94%</td>
<td>KY680541.1</td>
</tr>
<tr>
<td>RRB_6s2</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>95%</td>
<td>MG252263.1</td>
</tr>
<tr>
<td>RRB_21s2</td>
<td><em>Bacillus subtilis</em></td>
<td>89%</td>
<td>JF343143.1</td>
</tr>
<tr>
<td>RRB_9s2</td>
<td><em>Bacillus axarquensis</em></td>
<td>95%</td>
<td>JF414764.1</td>
</tr>
<tr>
<td>RRB_20s2</td>
<td><em>Bacillus subtilis</em></td>
<td>93%</td>
<td>JF343143.1</td>
</tr>
<tr>
<td>RRB_31s1</td>
<td><em>Bacillus velezensis</em></td>
<td>91%</td>
<td>CP003838.1</td>
</tr>
<tr>
<td>RRB_30s1</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>97%</td>
<td>HE610819.1</td>
</tr>
<tr>
<td>RRB_27s2</td>
<td><em>Bacillus siamensis</em></td>
<td>97%</td>
<td>KY807043.1</td>
</tr>
<tr>
<td>RRB_25s2</td>
<td><em>Bacillus siamensis</em></td>
<td>98%</td>
<td>KY807043.1</td>
</tr>
<tr>
<td>RRB_29s1</td>
<td><em>Bacillus subtilis</em></td>
<td>96%</td>
<td>CP007165.1</td>
</tr>
<tr>
<td>RRB_24s1</td>
<td><em>Bacillus subtilis</em></td>
<td>97%</td>
<td>MH488974.1</td>
</tr>
<tr>
<td>RRB_28s1</td>
<td><em>Bacillus methylotrophicus</em></td>
<td>96%</td>
<td>KJ567098.2</td>
</tr>
<tr>
<td>RRB_32s1</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>94%</td>
<td>JQ798394.1</td>
</tr>
<tr>
<td>RRB_34s1</td>
<td><em>Bacillus velezensis</em></td>
<td>95%</td>
<td>CP016371.1</td>
</tr>
<tr>
<td>RRB_35s1</td>
<td><em>Bacillus velezensis</em></td>
<td>97%</td>
<td>KU146562.1</td>
</tr>
<tr>
<td>RRB_12s2</td>
<td><em>Bacillus amyloliquefaciens</em></td>
<td>94%</td>
<td>KM658261.1</td>
</tr>
<tr>
<td>RRB_15s1</td>
<td><em>Bacillus subtilis</em></td>
<td>90%</td>
<td>JF343143.1</td>
</tr>
</tbody>
</table>
2.3.2. The antagonist activities of the bacterial strains changed after a year of storage in glycerol.

After a year of storage, most of the bacteria showed a reduced antagonistic activity against *R. solani*. The bacteria that initially showed a high inhibition activity were reduced more in their activity than the ones that showed a lower activity. The inhibition areas produced by RRB_5s2, RRB_7s2, and RRB_8s2 were reduced by 68%, 62.74%, and 66.99%, respectively (Figure 2.3). In contrast, the inhibition zones of the bacteria that already had a low antagonistic activity either decreased slightly or increased. The inhibition area produced by RRB_19s2, RRB_20s2, RRB_21s2 and RRB_27s2 decreased by 9.54%, 25.13%, 12.24% and 4.79%, respectively (Figure 2.3). For the bacteria showing an increased antagonistic activity figured RRB_30s1, RRB_31s1, and RRB_32s1 with an increase of 33.65, 39.25 and 39.76, respectively (Figure 2.3).

![Figure 2.3. Graph depicting changes in the antifungal activities of RRBs against *R. solani* evaluated by the area of the inhibition zone (cm²).](image)

2.3.3. Effect of the combined application of silicon and two antagonistic bacterial strains

The application of Si and bacterial strains reduced the total lesion length of sheath blight (Figure 4). In the plants treated with silicon, RRB-7s2 yielded to a reduction of 27
% while RRB_5s2 showed a reduction of 17.55 % in lesion length compared with the non-spayed plants (Figure 2.4). Among the plants without Si treatment, the RRB-7s2 reduced the lesion length by 28.46% and RRB-5s2 by 30.63% (Figure 2.4). The two-way ANOVA revealed that the interaction between Si and bacterial application, and the main effect of Si was not significant. Only the main effect of the bacterial strains was significant (P<0.05). However, plant grown in Si-amended soil tend to show lower lesion length than the plants grown in non-amended soil; the reduction was 28.31%, 8.79% and 52.25%, in plant treated with RRB_7s2, RRR_5s2, only MgCl₂ respectively (Figure 2.4).

![Graph](image)

**Figure 2.4.** Combined effects of silica fertilizer and RRBs on sheath blight lesion. The letter above each column indicates statistically significant difference based on Tukey’s test (P < 0.05).
2.3.4. The silicon content in the plant.

The plant was digested to evaluate their $\text{H}_4\text{SiO}_4$ content by spectrophotometry. The Si content in a plant grown in soil amended with wollastonite was not significantly different from that of plant grown in soil without amendment, albeit they contained less Si (Figure 2.6).
2.1.3. **The effect of silicon on effect of silicon on ShB on the field**

The effect of Si on sheath blight was evaluated in the field with the silica fertilizer in the form of silica slag (Figure 2.7). The test was performed with two varieties: Bengal and CL111. The severity of sheath blight was evaluated using the scale proposed by IRRI (Table 2.2). The Bengal variety grown in plots amended with the silica slag exhibited a significantly (P>0.05) lower value of the severity of sheath blight than a plant grown in the plot without silica amendment. Although the CL111 also shown a lower severity of sheath blight when grown in soil amended with silica slag, the reduction was not significant compared with the plot without amendment.

![Figure 2.7](image.png)  
Figure 2.7. Effect of silica slag fertilizer on the sheath blight severity of Bengal and CL111 in field trial The letter above each column indicates statistically significant difference based on Tukey’s test (P < 0.05).
Table 2.2. Scoring system for the evaluation of sheath blight severity in the field.

<table>
<thead>
<tr>
<th>Scores</th>
<th>Relative lesion height</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No infection observed</td>
</tr>
<tr>
<td>1</td>
<td>&lt;20%</td>
</tr>
<tr>
<td>3</td>
<td>20-30%</td>
</tr>
<tr>
<td>5</td>
<td>31-45%</td>
</tr>
<tr>
<td>7</td>
<td>46-65%</td>
</tr>
<tr>
<td>9</td>
<td>&gt;65%</td>
</tr>
</tbody>
</table>

2.4. Discussion

In this study, 24 bacterial isolated from the rice rhizosphere showed various levels of antifungal activity against *R.solani*. The majority of those strain showed a reduced activity after storage for a year in glycerol at -20°C. Most of the isolates were *Bacillus amyloliquefaciens* and *Bacillus subtilis* based of the closest similarity of their 16S rDNA sequences to the sequences in the NCBI database. Other species such as: *Bacillus siamensis*, *Bacillus velezensis* and *Bacillus thuringiensis* were also found. The percentage of similarity varied between 89 and 98%, which suggests that the quality of the 16S rDNA data was not enough to have a higher similarity percentage. In a recent study by Shrestha et al. (2016), 100% query coverage was obtained by combining the 16S rDNA with the *gyrB* gene. However, the results from this study concord with the hereby study since the majority of the antagonist bacteria against the sheath blight pathogen belonged to *Bacillus subtilis* and *Bacillus amyloliquefaciens*. The percentage of *Bacillus amyloliquefaciens* and *Bacillus subtilis* strains among the bacterial isolates suggest that those two species could be the most habitual culturable antagonistic bacteria associated with rice rhizosphere.

These results suggest that the rhizosphere of healthy rice could represent an accessible and useful source for isolation of *Bacillus* sp. with promising antagonistic ability. The plant rhizosphere is the recipient of nutrients actively secreted by the plant.
root and mucilage (Dennis et al., 2010; Turner et al., 2013). The rhizosphere contains abundant microorganisms with strong competition and colonization ability. *Bacillus* sp. are widely used as biocontrol agents against various phytopathogens (Bashar et al., 1970; Khedher et al., 2015; Beneduzi et al., 2012). Recent studies include the control of cotton damping-off disease caused by *Rhizoctonia solani* (Guo et al., 2014), black leaf streak of banana caused by *Mycosphaerella fijiensis* (Gutierrez et al., 2015), late blight of tomato caused by *Phytophthora infestans* (Chithrashree et al., 2011), citrus canker caused by *Xanthomonas axonopodis pv. citri* (Huang et al., 2012), bacterial blight of rice caused by *Xanthomonas oryzae pv. oryzae* (Chithrashree et al., 2011).

The direct antagonistic activity depending on the antibiotic production of the pathogen is a critical factor in the biocontrol mechanism (Cawoy et al., 2015). *Bacillus amyloliquefaciens* and *Bacillus subtilis* produce cyclic lipopeptides (LPs) belonging to surfactin, iturin and fengycin families, which are known to be responsible for the antimicrobial activity (Fiddaman et al., 2013; Maget-Dana et al., 1992; Ongena et al., 2007; Romero et al., 2007).

The difference in the microbial activity observed among bacterial isolates might be attributed to the difference in the production of antibiotic compounds. The study by Cawoy et al. (2015) showed that that the inhibition zone of *Bacillus amyloliquefaciens* and *Bacillus subtilis* varies with their ability to produce surfactin (S), iturin (I) and fengycin (F) or two of these compounds. In that study, they used strains having different production signatures of LPs. Some of the strains were a producer of the three LP families; others produced only two of the compounds. The antagonism of those strains was measured against four phytopathogens: *Cladosporium cucumerinum, Botrytis cinereal, Fusarium oxysporum,* and *Pythium aphanidermatum.* They found that the strains that produced all three LPs (I+F+S) were the most
efficient in fungal pathogen inhibition and the presence of iturin was the main component involved in high antagonistic activities. When only two LP type were present, the inhibition area varied with the species of phytopathogens. The antagonism against *F. oxyporum* depended on the presence of iturin and fengycin, whereas the inhibition of *C. cucumericum* relied on the surfactin and fengycin production.

It can be speculated that the different antagonist capacity observed after the long-term storage might be due to a decrease or the complete cessation in the production of one of the LP families. The increase in the antimicrobial activity might be due to the loss of the ability of the strain to produce a compound that plays a negative role for the antagonistic activity. This phenomenon was observed in the direct antagonism activity of *Bacillus* sp. against *Botrytis* sp. Mutants that could not produce surfactin, the LP family irrelevant for antagonism activity against *Botrytis*, showed the same or a larger inhibition area than the wild-type (Cawoy et al., 2015). Thus, further investigation is needed to characterize the LP compounds produced by the bacterial strains of this study for their antagonism against *R. solani*. The antagonistic effect might also be caused by other compounds such as bacilysin, mycosubtilin and siderophore (Beneduzi et al., 2012; Raaijmakers et al. 2002). The evaluation of those materials might be important to understand the antagonistic effect of the bacterial strains of this study.

Despite the low representation of *Bacillus velezensis* among the isolates and the low similarity percentage obtained, this species might a potential antagonist against *R. solani* because of its involvement in the control of other fungal pathogens (Myo et al., 2019). First described in 2005, various strains of *Bacillus velezensis* were subject of many studies for their potential as biopesticide (L. Huang et al., 2017). Strains of *Bacillus*
*velezensis* showed biocontrol activity against a wide range of fungal and bacterial pathogens by the production of volatile organic compounds and promoted plant growth via the production of indole-3-acetic acid (IAA) and siderophore. Moreover, it is closely related to *B. methylotrophic* and *B. amyloliquefaciens*, two *Bacillus* species that are well known to be potent biological control agents (Myo et al., 2019).

The suppressive effect of bacterial strains can also be attributed to the induction of systemic resistance in the plants (Chithrashree et al., 2011). Induced Systemic Resistance (ISR) in plants is an increase of the entire plant defensive capacity against various plant pathogen upon the local penetration induced by beneficial microbes (Pieterse et al., 2014). This phenomenon had been initially demonstrated in *Pseudomonas* spp. and other Gram-negative bacteria; later it was shown that Gram-positive bacteria including *Bacillus* sp., particularly *B. amyloliquefaciens* and *B. subtilis* elicit ISR in Arabidopsis, several vegetables, tobacco, and tropical crops (Kloepper et al., 2004). The ISR in plants induced by *B. bacillis* is probably triggered by surfactin, microbial volatile organic compounds, and other unidentified secondary metabolites. The plant defense is also induced by the bacteria by affecting the jasmonic acid pathway and/or the ethylene signaling pathway (Ryu et al., 2003).

The application of bacteria and Si reduced the severity of ShB. Regardless of the bacterial application, plants grown in soil amended with Si showed shorter lesion size than plants grown in non-amended soil. For the same level of Si, plants treated with bacteria showed shorter lesions. However, two-way ANOVA revealed that there is no interaction between Si and bacterial treatments, and only the reduction induced by the bacterial strains as a leading factor was significant. The effect of Si might be hidden by the effect of bacterial application and the variation among the different repetition since the percentage of reduction of the lesion length is
high is silica-treated plant except the plants treated with RRB-7s2: where 28.31%, 8.79% and 52.25% reduction in the plants treated with RRB-5s2, RRR-7s2, and MgCl2 only, respectively. Silicon application has been reported to affect the intensity of ShB and other rice diseases negatively (Rodrigues & Datnoff, 2005; Rodrigues et al., 2003). Rodrigues et al. (2003) reported a reduction of the relative ShB lesion length of 24% in plants treated with Si. Datnoff et al. (1991) reported a reduction of 31% and 15% for panicle blast and brown spot, respectively, in plants grown in soil amended with calcium silica slag (Rodrigues & Datnoff, 2005).

The non-significance of Si as main factor might be due to the lack of continuous application of fertilizer. A similar observation was made by Heine et al. (2006) on the infection of tomato by *Pythium aphanidermatum*. The single application of monosilicylic acid on tomato plant did not affect the spread of *Pythium aphanidermatum* whereas continuous Si supply significantly inhibited the spread of the pathogen (Heine et al., 2006). Assuming that the prophylactic properties of Si derived from silicified cells and silica bodies preventing the fungal to penetrate and spread inside the plant, the variation observed among the Si-treated plants could be attributed to the irregularity of those structures. Indeed, many studies reported that the repartition of silicified cells, in which Si was deposited and polymerized, and silica bodies are not uniform. Consequently, some areas of the leaves are left unprotected from the penetration of pathogens, and their structure colonize those areas with more facility (Kim et al., 2002; Ma & Yamaji, 2006; Motomura, 2004).
2.5. Conclusions

This study aimed to investigate the synergetic effect of bacterial antagonists and Si on sheath blight disease of rice. Bacteria were isolated from healthy rice plants and screened in duel culture for their ability to suppress *R. solani* growth. The analysis of the 16S rDNA of the isolates that suppressed the growth of *R. solani* revealed that they were closest to *Bacillus* spp with a majority of *Bacillus amyloliquefaciens*. Like previous research, this study suggests that the rhizosphere can be a source for bacterial biological control agents that are antagonistic against *R. solani*. The antagonistic activities of the bacteria were different among isolates and changed after a year of storage in glycerol. Most of the isolates were less active in the second year than when they were freshly isolated. Changes in the ability of bacterial spp. to antagonize plant fungal pathogens have been attributed to changes in the secretion patterns of antifungal compounds such as iturin surfactin and fengycin. I recommend comparing the amount of those compounds secreted among the bacteria isolated in this study. The synergetic effect of the beneficial bacteria and Si was not significant, even though sheath blight lesions were less severe in plants grown in Si amended soil treated with bacteria than in plant that received other treatments.

References


CHAPTER 3. EVALUATION OF THE EFFECT OF *RHIZOCTONIA SOLANI* AND SILICON ON THE MICROBIOME STRUCTURE OF THE RICH RHIZOSPHERE

3.1. Introduction

The soil represents one of the richest and most diverse microbial ecosystems on earth (Badri et al., 2009; Hütsch et al., 2002). However, most of the diversity and contrasts observed between different soil microbiome is due to a few taxonomic groups including Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria, Bacteroidetes, and Firmicutes (Fierer et al., 2009). The microorganisms associated with plants can be found in the rhizosphere, in the plant tissue or on the surface of the above-ground plant parts. This assembly of microbes can significantly impact plant growth and development. (Vorholt, 2012).

Indeed, Rhizosphere microbes and plants mutually benefit each other. In addition to their beneficial effect on plant health, microbes can help plants to grow and function more effectively by improving water retention or nutrient uptake and utilization (Olanrewaju et al., 2019). Plants, for their part, provide carbon metabolites that can act as a food and energy source for the microbes. However, some microorganisms are pathogenic and therefore can negatively affect the growth and development of plants (Schirawski et al., 2018).

The bacterial microbiota composition and structure of the rhizosphere can be influenced by a combination of edaphic and plant host-derived factors (Bulgarelli et al., 2013). Host plants select distinctive rhizosphere microbiota from soil biomes by rhizodeposition (Hütsch et al., 2002; Nguyen, 2003). Rhizodeposition refers to the secretion into the rhizosphere of a wide range of compounds and release of specialized cells, called border cells. The most known bacteria are organotroph, meaning they derive their nutrient from organic compounds of which accessibility and availability are limited in the soil (Demoling et al., 2007). The secreted
compounds include organic acid ions, inorganic ions, phyto-siderophore, sugar
polysaccharides mucilage, amino acids, and vitamins (Dakora et al., 2002).

The compounds in root exudates, which vary substantially depending on the
species age (Pausch et al., 2013; Pausch & Kuzyakov, 2018) and health status of plants,
are necessary signals for microbes as they can either attract the microbes to or repulse
them from the plant roots (Lakshmananet al., 2014). Therefore, changes in the root
exudate can influence the composition of rhizosphere microbiome. A preview research
showed that plants could select beneficial microbes upon the attack of a pathogen
(Berendsen et al., 2012). Although the processes involved in plant-microbiome
interactions as a consequence of exudate secretion are not yet well understood, it is
believed that changes in the exudate composition account for this recruitment (Hu et al.,
2018).

The increasing production of iron slag fertilizer has drawn attention to the benefits
of slag-based silicate fertilizer for plants (Wang et al., 2015). It has been shown that crop
productivity is improved, and soil acidification and metal contamination is alleviated
owing to the application of slag-based silicate fertilizers (Annunziata & Coll, 2012;
Wang et al., 2015). Continuous cultivation of rice (Oryza sativa) field depletes the Si
content and thus negatively impacts soil quality and yields. Therefore, silica fertilizer
application is necessary for the sustainability of rice cultivation. It has been postulated
that benefits of silicon (Si) fertilizer amendment for plants rely on modifications in the
microbial community and functions that cause changes in biogeochemical cycles of N
and C, and organic matter decomposition (Das et al., 2019).
The objectives of this study were to investigate if the amendment of rice plants with Si and their inoculation with \(\text{R. solani}\) cause change in the composition and diversity of the associated rhizosphere microbiome populations in field conditions. I hypothesized that the inoculation of rice plant with \(\text{R. solani}\) and the application of Si fertilizer could shift the microbial structure of rice rhizosphere.

3.2. Materials and Methods

3.2.1. Field experiment and treatments

The field experiment was conducted at the H. Rouse Caffey Rice Research Station of LSU AgCenter. The site has been the subject of many experimentations on rice during which it experienced alternate flooding and draining. The experiment was conducted from April to August 2018 on the rice varieties Bengal and CL111. The field was divided into two sections one of which was amended with silica slag fertilizer, an industrial by-product derived from iron production that contains 5.4% of Si, at a rate of 500 kg ha\(^{-1}\). The Si fertilizer was applied manually. Each section was divided into four blocks containing randomized plots for each of those treatments: Si amended and non-amended plots, plot inoculated, and non-inoculated with \(\text{R. solani}\). The silicate slag fertilizer was manually applied and incorporated to 3 to 5 cm deep to the soil.

3.2.2. Inoculation with \(\text{R. solani}\) and collection of soil samples

Before the inoculation with \(\text{R. solani}\), sampling was conducted at the tillering growth stage and the entire culms of four plants were randomly taken from each plot for the extraction of the microbiome rhizosphere DNA. The spots where the plants were collected were marked with flags. After this sampling, half of the plots were inoculated with \(\text{R. solani}\). Five weeks after
the inoculation, second collection was made where I chose plants that were close to the flags in order to limit variation that might occur because of position in the field. In the inoculated plants I collected plants that were heavily infected. The DNA of the microbiomes of the rhizosphere were also extracted. The analysis of the sequence data was presented only for the sample collected in the field after inoculation.

Inoculum of *R. solani* was prepared following a protocol adopted in Dr. Ham lab in the Department of Plant Pathology and Crop Physiology at LSU. Rice grain and husk were mixed according to a 2:1 ratio up to 1400 mL in flasks and 500 mL of water was added. The flasks were covered with cotton and aluminum foil and autoclaved. Sixteen cm2 PDA plug containing 7 day-old *R. solani* was added to the autoclaved mixture, which was subsequently kept at room temperature for ten days for the growth of the fungus. The prepared inoculum was combined with fresh husk/grain mixture to increase the volume of the inoculum to be used in the field. The inoculum was applied to rice plants at the tillering stage by hand sprinkling.

3.2.3. Evaluation of sheath blight severity

For the evaluation of sheath blight, I used the standard evaluation system adopted by the International Rice Research Institute. Fifteen rice plants were randomly selected from each plot. A score was attributed to each observation based on the proportion of the stem showing sheath blight symptom following the values presented in (Table 3.1).

3.2.4. DNA extraction and sequencing

Deoxyribonucleic acid was extracted from 0.24 g of moist and homogenized soil from the rhizosphere using the PowerSoil DNA isolation kit and following the manufacturer’s protocol (Qiagen).
Table 3.1. Scores used for field evaluation of sheath blight disease of rice in relation to the proportion of symptoms observed.

<table>
<thead>
<tr>
<th>Score</th>
<th>Lesions %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No infection observed</td>
</tr>
<tr>
<td>1</td>
<td>Lesions limited to lower 20% of the plant height</td>
</tr>
<tr>
<td>3</td>
<td>20-30%</td>
</tr>
<tr>
<td>5</td>
<td>31-45%</td>
</tr>
</tbody>
</table>

The DNA quality was assessed by Nanodrop spectrophotometry. Aliquots of the five best DNA samples for each treatment groups were submitted to the Biocomplexity Institute of Virginia Tech (BIVT) for 16S rDNA amplification library preparation and Illumina Mi-Seq sequencing. The BIVT used the standard method for soil microbiome analysis that is described in the following paragraph (http://www.earthmicrobiome.org/).

The 16S rDNA was amplified using primers 515F 5’-GTGYCAGCMGCCGCGGTAA-3’ (Parada et al. 2016) and 926R 5’-CCGYCAATTYMTTTRAGTTT-3’ (Quince et al., 2011), which cover the V4-V5 region of the 16Sr DNA gene and produce amplicons of 400 to 500 bp. Thermocycling consisted of an initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 45 s, 50°C for 60 s and 72°C for 90 s. And the final extension time was implemented at 72°C for 10 min. The triplicate PCR reactions for each sample were pooled into a single volume (75 µL) without combining amplicons from different samples. Amplicons from each sample were run on an agarose gel to verify the presence of PCR products. An equal amount of amplicon from each sample (240 ng) was combined into a single, sterile tube. The amplicon pools were purified and sequenced by the Illumina MiSeq at 2 × 150 bp by using paired-end reads.
3.2.5. **Analysis of 16S rRNA amplicon sequencing using QIIME2**

Five randomly selected 16s rDNA amplicon libraries were examined using FastQC v0.11.8. Paired read files from each library were imported with a metadata file into a gzi artifact, as described in the QIIME2 online tutorial (https://docs.qiime2.org/2019.4/tutorials/). Subsequently, the sequences were trimmed and denoised. The plugin dada2 implemented in QIIME2 was used to identify and filter the chimeric sequences. The forward and reverse primer sequences were trimmed (--p-trim-left-f 17 --p-trim-left-r 21) and the truncation length parameters were set to 300 bp for both forward and reverse sequences. After chimera removal and Operational Taxonomic Units (OTUs) picking, the sequence features were aligned using the Mafft plug-in and an unrooted phylogenic tree was generated using the Fasttree plug-in with default parameters. The taxonomy of the DADA2-inferred sequences was determined based on their pairwise identity to sequences in the SILVA reference database.

3.2.6. **Analysis of alpha and beta diversity**

The variation of the microbial community within and among treatment groups was accessed by describing the alpha and beta diversities. The alpha diversity was implemented in QIIME2 and was evaluated using observed OTUs, Shannon's index, and Pielou's Evenness index(Xia et al, 2018). Kruskal-Wallace ANOVA (KWA) was used to determine differences between indices of alpha diversity. Beta diversity was analyzed using: the Bray-Curtis similarity assessing the presence/absence ratio and the abundance of OTUs, the Jaccard distance focusing on the shared OTUs among the samples and the Unwheited Unifrac distance evaluating the presence/absence ratio irrespectively of the abundance. The above-mentioned beta diversity measurements were analyzed using
Kruskal–Wallis H test and Permutational multivariate analysis of variance (PERMANOVA) in QIIME2, and the principal coordinate analysis (PCoA) was visualized in a two-dimensional EMperor Plot.

3.2.7. **Assessment of the correlation of the soil pH, total N and C to phylogenetic diversity**

Soil samples collected from the rice rhizosphere were submitted to the Soil Testing and Plant Analysis Laboratory for a routine test including the evaluation of the pH and total nitrogen and carbon. Soil pH (1:1 soil:water) testing was performed as described by Mclean (1982). Total carbon and total nitrogen were determined using dry combustion. Fours replicate samples from each treatment were analyzed. I first assessed if those parameters were showing significantly different values respective to the treatments by an ANOVA. Afterward, their correlation to the faith phylogenetic was performed using QIIME2 by the spearman correlation.

3.3. **Results**

3.3.1. **General analyses of the sequence data**

An OTU table was produced using the QIIME2 pipeline. The table was extracted from QIIME2 in order to perform a part of the downstream analysis on Excel and Explicit. In total, the data contained 1047 unique OTUs with a total of 368,583 reads per sample with a standard deviation of 18,429. Table 3.2 shows the average number of OTUs and reads from the sample grouped according to the treatments. The rarefaction curves reach complete saturation for all the sample indicating that no additional OTUs would be detected with additional sequencing (Figure 3.1).
Table 3.2. Number of operational taxonomic unit per read from the rice rhizosphere of rice plants treated with silicate fertilizer and inoculated with \textit{R. solani}.

<table>
<thead>
<tr>
<th>Table 3.2. Number of operational taxonomic unit per read from the rice rhizosphere of rice plants treated with silicate fertilizer and inoculated with \textit{R. solani}.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatments</strong></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>\textit{R. solani} only</td>
</tr>
<tr>
<td>Silica slag only</td>
</tr>
<tr>
<td>Silica slag + \textit{R. solani}</td>
</tr>
<tr>
<td><strong>Average</strong></td>
</tr>
<tr>
<td>206.2</td>
</tr>
<tr>
<td>245.4</td>
</tr>
<tr>
<td>333.8</td>
</tr>
<tr>
<td>313.2</td>
</tr>
</tbody>
</table>

Figure 3.1. Rarefaction curves representing observed operational taxonomic units (OTUs) per number of sequences per sample described per curve of different colors.

3.3.2. Bacterial Community Composition

The analysis at the phylum level shows that the rhizosphere of the rice plant was predominantly colonized by Proteobacteria (25.42% of the total reads), Chloroflexi (20.28%) and Bacteroidetes (13.82%) (Figure 3.2). The fourth, fifth, and sixth most abundant taxonomic group were the Cyanobacteria (11%), Acidobacteria (10.4%), Verrucomicrobacteria (4.28%) and Firmicutes (2.82%), respectively (Figure 3.2). The
abundance of the taxa changed depending on the type of treatment received by the plants. For the inoculated plants, Chloroflexi were the most abundant phylum, while in the non-inoculated ones the Proteobacteria were the most abundant. The relative abundance of the Bacteroidetes was low in the control (Si-,R-) where they represented 13.12 % of the reads (Figure 3.2)

![Figure 3.2. Rice rhizosphere microbiome composition of each treatment groups at the phylum level represented by the relative abundance of sequence reads for the twenty more abundant phyla. The letter Si represents application of silica slag and the letter R represents inoculation with *R. solani*. – and + signs represent absence and presence, respectively.](image)

The highest abundance of the Bacteroidetes was found in the Si-treated and inoculated plant. The proteobacteria were more abundant in the rhizosphere of non-inoculated plants than the inoculated ones whether they were treated with Si or not. The highest abundance of cyanobacteria was also found in the control and represented 25.38 % of the reads. In the treated plants it represented 5.64%, 6.54 % and 7.26% for non-inoculated Si-treated, inoculated Si-treated and non-inoculated Si-treated plants, respectively (Figure 3.2). The representativity of the Firmicutes and Ignavibacteriae were higher in the Rhizosphere bacteria of the inoculated
plant than in that of non-inoculated plants. The analysis at the genus level showed that Flavisolibacter were the more abundant genes in the Bacteroidetes, Bryobacter in Acidobacter, Geobacter in Proteobacter, Anaerolinea in Chloroflexix, and Bacillus in Firmicutes (Figure 3.3).

Figure 3.3. Rice rhizosphere microbiome composition of each treatment groups at the genus level represented by the relative abundance of sequence reads for the first 31 most abundant genera. The letter Si represents application of silica slag and the letter R represents inoculation with R. solani. – and + signs represent absence and presence, respectively.

3.3.3. Alpha diversity

The alpha diversity analysis was performed for species richness and evenness within each treatment (Figures 3.4 & 3.5). The species richness was evaluated by the Shannon-Wiener Diversity Index, and the evenness was evaluated by the Pielou’s Evenness Index. The bacterial community of the silica treated plant was significantly (P=0.01) more diverse than that of the non-Si-treated plants for the Shannon diversity
The higher Shannon index indicated that the rhizosphere of the Si-treated plants has more distinct species than that of non-Si-treated ones.

![Graph showing Evenness and Diversity](image)

Figure 3.4. Evenness (a) and diversity (b) in the microbial communities of the rice rhizospheres as affected by silicate fertilizer amendment, which were evaluated by Shannon diversity index (P=0.01) and Pielou’s Evenness index (P=0.59), respectively. Asterisks indicate that the treatment group was significantly different for the Shannon index (P < 0.05). No significant differences were for the evenness in microbial community (P > 0.05).

Pielou's Evenness index shows no significant difference (p= 0.59) between the treatments (Figure 3.4). The distribution of the species abundance was the same within the rhizosphere bacterial community of Si-treated and non-Si-treated plants.

The rhizosphere bacterial community of plant inoculated with *R. solani* was not significantly different from that of non-inoculated plants regarding the Shannon index (P = 0.15).
(Figure 3.5). This suggests that the same species richness was found within the Rhizosphere of non-inoculated plant and inoculated plants. However, the evenness evaluated by the Pielou's Evenness indicated that, in the rhizosphere of the inoculated plants, the species were distributed differently (P= 0.03) within each treatment groups).

Figure 3.5. Evenness (a) and diversity (b) in the microbial communities of rice rhizospheres as affected by inoculation with *R. solani*, which were evaluated by Shannon diversity index (P=0.15) and Pielou’s Evenness index (P=0.03), respectively. The treatment groups were significantly different for the Pielou’s Evenness (P < 0.05).
Figure 3.6. Comparison of the Shannon and Simpson index for non-inoculated and inoculated plants.

Figure 3.7. Comparison of the Shannon and Simpson index for plants grown in Si-amended and Non-amended soils.
3.3.4. Beta diversity

Measures of similarity/dissimilarity among the rhizosphere bacterial community were visualized using Principal Coordinates Analysis (PCoA). The first two principal components (PC1 & PC2) derived from Bray-Curtis dissimilarity and unweighted and weighted Unifrac distances showed clear separation of the communities in the rhizosphere of inoculated plants from non-inoculated plants (Figure 3.8), only data from the unweighted Unifrac distances is shown. The significance of these clusters patterns was statistically confirmed using PERMANOVA (Table 3.3) For all three distance measures (Bray-Curtis, Jaccard, and unweighted UniFrac), the separation of the microbiome communities of the rhizosphere of inoculated plants and non-inoculated were significantly different (P =0.001)

Figure 3.8. Two-dimensional principal coordinates analysis (PCoA) plots of 16S rRNA diversity from rhizosphere soil samples based on the Bray Curtis distance. Dark triangles represent samples from inoculated plants and the dark spheres represent those from plants grown in the non-amended soil. PERMANOVA test indicates that microbial communities are significantly different based on site (P = 0.0001).
Figure 3.9. Two-dimensional principal coordinates analysis (PCoA) plots of 16S rRNA diversity from rhizosphere soil samples based on the Bray Curtis distance. Gray triangles represent samples from plants grown in Si-amended soil and the dark spheres represent those from plants grown in non-amended soil. PERMANOVA test indicates that microbial communities are significantly different based on site ($P = 0.106$).

There is no remarkable difference between the communities in regards silicate slag amendment. Bacterial communities of the rhizosphere of Si-treated plants were not significantly different from those of plants that did not grow in soil amended with silicate slag fertilizer based on the Bray-Curtis distance ($P = 0.106$) (Table 3.4).

Table 3.3. Results of Permutational Multivariate Analysis of Variance (PERMANOVA) based on Bray–Curtis, Jaccard and Unweighted-Unifrac dissimilarities using abundance data of rice rhizosphere bacterial communities of inoculated plant vs non-inoculated plants.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Bray–Curtis</th>
<th>Jaccard</th>
<th>Unweighted-unifrac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permutation N</td>
<td>999</td>
<td>999</td>
<td>999</td>
</tr>
<tr>
<td>Pseudo-F</td>
<td>3.956259</td>
<td>2.106457</td>
<td>2.805245</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>q-value</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 3.4. Results of Permutational Multivariate Analysis of Variance (PERMANOVA) based on Bray–Curtis, Jaccard and Unweighted-Unifrac dissimilarities using abundance data of rice rhizosphere bacterial communities of Si treated plants vs non-Si treated plants.

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Bray–Curtis</th>
<th>Jaccard</th>
<th>Unweighted-unifrac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Permutation N</td>
<td>999</td>
<td>999</td>
<td>999</td>
</tr>
<tr>
<td>Pseudo-F</td>
<td>1.39775</td>
<td>1.267655</td>
<td>1.402963</td>
</tr>
<tr>
<td>p-value</td>
<td>0.106</td>
<td>0.03</td>
<td>0.047</td>
</tr>
<tr>
<td>q-value</td>
<td>0.106</td>
<td>0.03</td>
<td>0.047</td>
</tr>
</tbody>
</table>

3.3.5. Correlation of the soil pH, total N, and total C to the phylogenetic diversity.

Only the pH was significantly (P=0.006) affected by the treatments (Table 3.5). The total N and the total C did not differ in the rhizosphere of the samples. The environment fitting analysis did not show any correlation between the soil parameters pH (Figure 3.10), N or C and the phylogenetic diversity of bacterial community. This result suggests that the treatment groups drive changes in the microbiome community by a mechanism independent from those parameters.

Table 3.5. pH, total carbon (C), total nitrogen (N) and C/N ratio of the rice rhizosphere soils of treatment groups (P-value < 0.05).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>pH</th>
<th>N %</th>
<th>C %</th>
<th>C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si-R-</td>
<td>7.6 ± 0.10</td>
<td>0.1068 ± 0.0036</td>
<td>0.8756 ± 0.0596</td>
<td>8.2178 ± 0.7938</td>
</tr>
<tr>
<td>Si-R+</td>
<td>7.47 ± 0.14</td>
<td>0.1022 ± 0.0114</td>
<td>0.8516 ± 0.0899</td>
<td>8.375 ± 0.8229</td>
</tr>
<tr>
<td>Si+R-</td>
<td>7.918 ± 0.15</td>
<td>0.1074 ± 0.0045</td>
<td>0.9284 ± 0.0705</td>
<td>8.6426 ± 0.5167</td>
</tr>
<tr>
<td>Si+R+</td>
<td>7.89 ± 0.31</td>
<td>0.1064 ± 0.0032</td>
<td>0.8708 ± 0.0520</td>
<td>8.182 ± 0.3563</td>
</tr>
<tr>
<td>Significance</td>
<td>Yes (P = 0.0067)</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>
3.4. Discussion

The purpose of this study was to investigate the effects of *R. solani* inoculation and silicate fertilizer amendment on the microbial community of rice rhizosphere. Metagenomic analysis of the 16S rDNA revealed clear differentiation of the rhizosphere bacterial community structure between inoculated and non-inoculated plants.

The microbial taxonomy analysis based on 16S rRNA gene suggested that Acidobacteria, Bacteroidetes, Proteobacteria, Chloroflexi, Firmicutes, Cyanobacteria, and Verrucomicrobia occupied approximately 88.12% of the bacterial communities of the rice rhizosphere. Specific distribution patterns were shown in some microbial taxa across treatments groups. Proteobacteria and Chloroflexi were the most abundant in all the sample except for the non-treated plants (SI-R-), in which Proteobacteria and Cyanobacteria were the most abundant ones. The phyla Proteobacteria and Chloroflexi were previously found to the most abundant bacterial phyla in rice soils (Liu et al., 2014; Rothenberg et al., 2016). The majority of the beneficial agents are found in the phyla Proteobacteria and Firmicutes.
(Chen et al., 2010; Ramakrishna et al., 2019). In this study, Firmicutes were relatively higher in the inoculated plants.

Some previous investigations have shown that pH, total C and total N are significantly correlated with the microbial community structure in paddy soil (Wang et al., 2015). However, in this study, neither the pH nor the total N and C was correlated with the microbial phylogetic diversity. I postulate that the response of the microbiota structure to the inoculation with *R. solani* is likely due to the secretion of compounds by the infected plant having no effect of the total C and N.

The study of the alpha diversity yielded opposite results when samples were discriminated based on the slag silicate fertilizer application and on inoculation with *R. solani*. The Shannon index of the rhizosphere community of plants grown indicated that Si-amended soil differed from that of plants grown in non-amended soil of the plants. But the Pielou's evenness index of the treatment groups was not different. The opposite pattern was obtained when comparing inoculated and non-inoculated plants. There was a significant decrease in the evenness for the inoculated plants. This result suggests the inoculation of plants with *R. solani* led to change in the relative representativity of major taxonomic features. Although the Shannon index takes into account of the relative abundance of the features, it gives more importance to less common categories, and it is more informative when applied to a random sample drawn from a community that the number of species is known (Pielou, 1966). In opposition to the Shannon index, the Pielou's evenness is not affected by small changes since it is more sensible to the more abundant features than the less common ones (Xia et al., 2018).
A study by Yang et al. (2017) that aimed to evaluate the relationship between soil microbiome and plant health, and to reveal which microbiome may play a role in inhibiting bacterial wilt revealed that wilt infection was closely related to the composition of soil microbial community. They found that communities were more diverse in the soil of healthy plants. Other studies also showed that high microbial diversity was positively correlated with plant health (Luan et al., 2015)(Bulluck & Ristaino, 2002).

For example, Luan et al. (2015) showed that healthy cotton had more diverse microbial communities than plants infected with Fusarium sp. In opposition to our study, the comparison in the above-mentioned study was established between healthy plants and naturally infected plants. The correlation reported in those studies suggests that the presence of some taxa group in the higher diverse soil microbial communities prevented the healthy plants from infection by pathogens. However, in this present study, it is suggested that the higher diversity observed in the infected plant is likely due to plant exudate secretion as a response to R. solani infection. Recent advancements in plant-microbe interactions research revealed that plants can recruit protective microorganism and enhance microbial activities upon pathogen attack (Berendsen et al., 2012).

Silicon fertilization did not influence the structure of the bacterial community of rice rhizosphere to a great extent. In a previous study by Das et al. (2019), a few major phyla were found to differ significantly when Si-fertilizer was applied to rice paddy (Alphaproteobacteria, Verrucomicrobia, and Betaproteobacteria). However, the author found significant changes for functional genes that are important for the assimilation and degradation of C by the micro-organisms in response to Si-fertilization. Although Si might not have great impact on the soil microbial structure, it might influence the functional role of the microbial community.
3.5. Conclusions

The objectives of this study were to investigate structural changes in the bacterial communities of the rhizosphere of rice plants grown in soil amended with Si and inoculated with *R. solani*. The metagenomic analysis of the bacterial community was carried out on Illumina MiSeq sequences of the 16S rRNA. The application of silicate fertilizer increased the diversity of the microbial community but did not provoke major shifts. On the other hand, inoculated plants showcased the same level of diversity as non-inoculated plants in terms of species presence/absence, but there were drastic shifts in the microbial composition among the more representative taxonomic features, probably resulting from a modification of the root exudates secretion. These changes could be seen by analyzing the beta diversity. The analysis of soil bacterial community’s beta diversity measured by the Bray-Curtis distance revealed differences based on the inoculation with *R. solani*. However, no noticeable differences were observed based on Si-treatment. Additional research is necessaire to understand the mechanism driving changes in rice rhizosphere microbiome originated from the inoculation with *R. solani*. Finally, Given the rising interest in the application of silicon to increase the yield of plants and to alleviate biotic and abiotic stress, it is important to know what are the potential beneficial or detrimental effect that an external source of silicon could have on the microbial communities of the soil and the plants.

References


Leonard Jhonson officially received his Bachelor of Science in Natural Resources and Environment at the State University of Haiti in 2015. He worked at the Ministry of Agriculture for a year doing field trial to test the effect of different fertilizer rates on rice growth and yield. Afterward, he worked at the Ministry of Environment of Haiti, where he was responsible for the management of a protected area. In 2017 he earned a scholarship from the USAID-AREA project and enrolled in the department of Plant Pathology and Crop Physiology at Louisiana State University where he worked under the direction of Dr. Jong Hyun Ham. In 2018, he presented his research at the International Congress of Plant Pathology. He hopes to receive a Master of Science degree in plant pathology in August 2019.