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Exploring Interactions of Phyllosphere Epiphytes with Plant Pathogenic Bacteria Pseudomonas and Xanthomonas on Tomato

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EXPLORING INTERACTIONS OF PHYLLOSPHERE EPIPHYTES WITH PLANT PATHOGENIC BACTERIA *PSEUDOMONAS* AND *XANTHOMONAS* ON TOMATO

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ABSTRACT

Recent studies have indicated the importance of resident microflora of plants in contributing towards overall plant health. Among difference components of the plant microbiome, *Methylobacterium* and *Sphingomonas* have been recognized as common residents of the phyllosphere for many host plants, however their role in disease control needs to be further investigated. The purpose of this study was to conduct experiments investigating the effectiveness of phyllosphere *Methylobacterium* and *Sphingomonas* isolated from red clover against common tomato phyllosphere bacterial pathogens, *Pseudomonas syringae* pv. tomato and *Xanthomonas perforans*. Additionally, this study uses *X. perforans* wild-type and *X. perforans* type VI secretion system (T6SS) mutant strains to observe the role of type VI secretion system in infection with a potential biocontrol agent. To explain interactions among the phyllosphere residents, nutritional similarity, motility and direct inhibition were observed *in vitro*. Based off the literature, it was hypothesized that *Sphingomonas* would prove to be a potential biocontrol agent against *P. syringae* pv. tomato, *X. perforans* wild-type and T6SS mutant. Methylobacteria would not prove to be a potential biocontrol agent against *P. syrinage* pv. tomato nor *X. perforans* wild-type and mutant. Lastly, it was hypothesized that *X. perforans* wild-type would be more virulent than the *X. perforans* T6SS when competing with a potential biocontrol agent (red clover commensal).

In planta experiments under growth chamber conditions indicated no significant change in disease with seeds that were soaked in a mix of red clover commensals when dipped in phyllosphere pathogens *P. syringae* pv. tomato and *X. perforans* wild-type and T6SS mutant. *P. syringae* pv. tomato was unable to infect control tomato plants in trial two under growth chamber

conditions, and was not tested further *in planta*. *In vitro* testing indicated a red clover phyllosphere commensal, *S. taxi* 55669, inhibited *X. perforans* wild-type and mutant colonies on R2a plates. Therefore, *S. taxi* 55669 was studied for disease protection further in the greenhouse with seedling dip experiments. To assess the effectiveness of *S. taxi* 55669 *in planta*, foliar disease percent and bacterial population counts were recorded on bacterial dipped seedlings co-inoculated with phyllopshere pathogens *X. perforans* wild-type and T6SS mutant. It is not recommended that *S. taxi* 55669 serve as a potential biocontrol for *P. syringae* pv. tomato 99B799 based off the neutral effect *S. taxi* 55669 had with *in vitro* testing. Methylobacteria observed in this study, did not show any benefits against disease against *P. syringae* pv. tomato and *X. perforans* wild-type and T6SS mutant. However, the results from this study indicate *S. taxi* 55669 should be studied further for plant health, and has potential as a biocontrol against *X. perforans*. *X. perforans* T6SS mutant was found to be less virulent in the presence of *S. taxi* 55669, than *X. perforans* wild-type. Based off the high NOI and decrease in foliar disease, this study shows *S. taxi* 5669 has potential as a biocontrol for *X. perforans*. The decrease in motility and bacterial populations of *X. perforans* T6SS mutant when in the presence of *S. taxi* 55669 highlights the importance of *icmF3* in motility and ability to attack resident phyllosphere bacteria. The lack of differentiation between direct inhibition on R2a plates of *X. perforans* wild-type and T6SS mutant in the presence of *S. taxi* 55669 indicates a part of the T6SS mutant system may still be functional.

INDEX WORDS: Plant Pathology, Plant Microbiology, Biological Control, *Methylobacterium*, *Sphingomonas.*

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INTRODUCTION

Since there are limited options for prevention and treatment of bacterial infections in plants, it may be that a way to prevent a bacterial infection is a healthy microbial defense system. Much like the animal microbiota, plants may be protected from disease by their microbiome (Schlaepii and Bulgarelli, 2015). When a plant is placed into the soil, it is placed into a microbial ecosystem. Soil is teeming with microscopic life: bacteria, fungi, protozoa, nematodes and algae. Of those microbes, bacteria are by far the most common. Bacteria may constitute 95% of soil microbes (Glick, 2012). Bacteria surrounding the root system are referred to as the rhizosphere, and bacteria on the root system are on the rhizoplane. The above ground environments are called the phyllosphere. The phyllosphere is subdivided into different microbial communities of the caulosphere (stems), phylloplane (leaves), anthosphere (flowers), and carposphere (fruits). Microbial communities located in the tissues (endophytes) are different than microbial communities on the plant surfaces (epiphytes). While bacteria have been isolated from plant tissues, most of the bacteria in the phyllosphere have been found to colonize the leaves. Epiphytic bacterial populations differ among and within plants of the same species (Hirano and Upper, 2000), over short time scales and growing season (Ercolani, 1991; Thompson et al.1993). Many of the bacteria colonizing plants are currently unculturable and are speculated to contribute to the fluctuations of the physiochemical environment of the phyllosphere over short time scales (Lindow and Leveau, 2002).

Bacteria may affect plants in one of the three ways: beneficial, harmful, or neutral. The number and type of bacteria that are found in different soils are influenced by many factors

including: the soil conditions, temperature, moisture, mineral concentration, and number and types of plants found in the soils. To cause disease, pathogenic bacteria must find a suitable host plant, pass through the external protective layers of the host, and gain access to the nutrients that it requires for its own growth and development. The effect that any specific bacterium has on the plant may change as the environmental conditions change. In addition, it is possible that a beneficial bacterium that is beneficial to one plant, may not be beneficial to another plant. For example, *Pseudomonas fluorescens* stimulated root development in blackcurrant plants while inhibiting root development in cherry (Dubeikovsky et al. 1993).

I. PLANT BACTERIA

1.0 Plant Growth Promoting Bacteria (PGPB)

There is still a limited understanding of PGPB-plant interactions, but a number of these bacteria are currently being used as adjuncts to agricultural practice. Commercial PGPB strains include at least 27 different PGPBs such as *Agrobacterium radiobacter* and *Azospirillum brasilense*. Before more widespread use of these PGPBs are available, Glick recommends a number of issues should be addressed: "I) Determination of those traits that are most important for efficacious functioning and subsequent selection of PGPB strains with appropriate biological activities. II) consistency among regulatory agencies in different countries regarding what strains can be released to the environment, and under what conditions genetically engineered strains are suitable for environmental use. III) A better understanding of the advantages and disadvantages of using rhizospheric versus endophytic bacteria. IV) Selection of PGPB strains that function optimally under specific environmental conditions (e.g., those that work well in warm and sandy soils versus organisms better adapted to cool and wet environments). V) Development of more effective means of applying PGPB to plants in various settings (e.g., in the field versus in the greenhouse). VI) a better understanding of the potential interactions between PGPB and mycorrhizae and other soil fungi," (Glick, 2012). The points illustrated are important to understanding how we can future study on PGPB can make progress getting these bacteria on the market.

Plant growth promoting bacteria include those that are free living, form specific symbiotic relationships with plants, bacterial endophytes that can colonize some or a portion of a

plant's interior tissues, and cyanobacteria. What is known about PGPB and how they interact with plants is that they can interact in one or more of the following ways: modulating the phytohoromone levels (produce cytokinins and gibberellins, effect indoleacetic acid production (IAA), alter ethylene levels), produce antibiotics and lytic enzymes, siderophores (preventing pathogens from acquiring iron), contribute to ecological competition, trigger induced systemic resistance, modulate the effects of environmental stress, and some can even secrete an antifreeze compound (Glick, 2012).

A genus of PGPB that has potential for crop application, *Methylobacterium,* is a well-known PGPB, but has yet to be studied enough to become commercially available (Glick, 2012). Methylobacteria are called pink-pigmented facultative methylotrophs, (PPFMs) because of the characteristic pink pigmentation of the colonies due to carotenoid synthesis. The exact evolutionary advantage of the pink pigmentation is not known, but it is possible that it protects from UV radiation (Manuella et al. 2016). The significance of this study is to better understand the role of beneficial Methylobacteria in the red clover phyllosphere and to investigate whether it can provide protection against *Pseudomonas syringae*.

Red Clover Associated Bacteria: Methylobacteria and *Sphingomonas*

1.1 *Methylobacterium* spp.

There are a large number of potential bacterial pathogens of plants but most of the interactions are asymptomatic due to a complex plant defense system and the stability of the microbial community (Dourado et al. 2016). *Methylobacterium* have been shown to induce systemic resistance (ISR, Induced Systemic Resistance) (Nigris, et al. 2013). In a study looking at *Methylobacterium* sp. IMBG290 inoculated potato plants that were challenged with

Pectobacterium atrosepticum, the *Methylobacterium* were shown to induce the plant antioxidant system at low density, but at high density the positive effect was not observed (Ardanov et al. 2011). *Methylobacterium* sp. IMBG290 have been studied for their biocontrol methods in potatoes against *Phytophthora infestans,* and *Pseudomonas syringae (*Ardanov et al. 2012). The biocontrol potential of using the microflora of red clover including *Methylobacterium* and *Sphingomonas*, for control of *Ralstonia* in potatoes (Poorniammal et al. 2009).

As of 2015, there were 51 reported species of Methylobacteria and they can be found in both the plant rhizosphere and the phyllosphere (Dourado et al. 2015). In addition, members of the *Methylobacterium* genus occupy soil, water, and grains (Madhaiya et al. 2012; Tani et al. 2012; Wellner et al. 2012). *Methylobacterium* can be opportunistic pathogens in humans as well (Sanders et al. 2000). *Methylobacterium* is in association with more than 70 species of plants (Dourado et al. 2015). They have been shown to colonize of a variety of host plants such as cotton, (Madhaiya et al. 2012), peanut (Madhaiyan et al. 2006), citrus (Araújo et al. 2002), eucalyptus (Andreote et al. 2009), sunn hemp (Sy et al. 2001), tobacco (Andreote et al. 2006), strawberry (Abanda-Nkpwatt et al. 2006), rice (Knief et al. 2012), tomato (Murugaiyan et. Al 2017), and red clover (Omer et al. 2004). The location and variety of Methylobacteria vary with the host plant; in some plants the Methylobacteria are more numerous in the phyllosphere rather than the rhizosphere and there are even multiple species of Methylobacteria that have been shown to colonize a plant host (Araújo et al. 2002, Andreote et al. 2009).

Methylobacteria are able to use C1 carbon compounds (methanol, formaldehyde) as a sole carbon source, or they can use multicarbon compounds (acetate, ethanol etc.). The ability to oxidize methanol using methanol dehydrogenase enzyme (MDH), (Dourado et al. 2015) is a feature all Methylobacteria have in common. Using methanol provides an advantage for plant

colonization (Sy et al. 2005). Plants secrete methanol from their stomata, which these bacteria can use. The metabolism of Methylobacteria allows them to colonize a variety of environments, one of which is the plant stomata. Depending on the species, Methylobacteria could provide ecological competition with plant pathogens by forming biofilms and growing near plant openings such as the stomata that pathogenic bacteria could use to enter the plant (Dourado et al. 2015). Biofilms allow for protection against desiccation and provide ecological competition with pathogens (Schluter, et al. 2015). *Methylobacterium* strains have been shown to produce AHL molecules (N-acyl-homoserine lactones) and other autoinducers as well; these molecules are responsible for cell-cell communication and increase with cell density (Pomini et al. 2009).

In addition, Methylobacteria have been known contribute to plant growth and development by providing nitrogen and phosphorus to the plant. Some species of *Methylobacterium* are involved in nitrogen fixation and nodule formation (Sy et al. 2001, Menna et al. 2006). Nitrogen fixation is beneficial to the plant by converting atmospheric nitrogen (N_2), to an absorbable nitrogen source for the plant such ammonia (NH_4^-); plants use nitrogen in NH4+ and NO_3- forms.

Another limiting nutrient for plants is phosphorus. The concentration of soluble phosphorus in the soil is usually low. *Methylobacterium* are beneficial to the plant by being able to solubilize phosphorus (Agafonova et al. 2013). Some strains of *Methylobacterium* provide additional growth benefits to plants by improving the cycling of nutrients such as siderophore production. This increases the iron supply to the plant and reduces heavy metal toxicity (Idris et al. 2004). Siderophores are low-molecular-mass compounds with a high affinity for iron that are produced by the bacteria to solubilize iron to promote its efficient uptake. In the genus

Methylobacterium, the iron uptake genes *iucA* and *iucC* have been described in 35 strains (Tani et al. 2012).

Methylobacteria may also aid in plant growth by producing phytohormones such as cytokinin and auxin that help promote plant growth (by promoting cell division and elongation, respectively) (Schaeuer, et al. 2011).

1.2 *Sphingomonas* spp.

Bacteria in the genus *Sphingomonas* are aerobic and yellow pigmenting belonging to the alpha-proteobacteria (Yabuucho et al. 1990). This genus encompasses over 55 known species with broad characteristics such as the ability to degrade aromatic and xenobiotic compounds and some occur as nitrogen-fixing bacteria. *Sphingomonas*, like *Methylobacterium*, can be isolated from a variety of sources such as water, air, dust and soil. Some soil isolated *Sphingomonas* have been reported to produce plant-growth stimulating factors (Adhikari et al. 2001, Enya et al. 2007, Tsavkelova et al. 2007). *Sphingomonas* bacteria are abundant in the phyllosphere of clover and soybean plants (Delmotte et al. 2009). Lastly, it is known that *Sphingomonas*, like *Methylobacterium*, can be opportunistic pathogens to humans (Yabuuchi and Kosako, 2005). *Sphingomonas* have not been studied as extensively as *Methylobacterium* for plant beneficial attributes and specificity of host plant interaction. However, various strains of Sphingomonas were evaluated for their protection against *P. syringae* pv. *tomato DC3000* in *Arabidopsis thaliana* (Innerebner et al. 2011). It was concluded that plants inoculated with various strains of both rhizospheric and epiphytic *Sphingomonas* bacteria suppressed disease and diminished pathogen growth when compared to *Methylobacterium* inoculated and sterile control groups. Protection was not seen for all isolates of *Sphingomonas*, just most of the *Sphingomonas* known

to colonize plants that were selected for the study. The *Sphingomonas* isolated from air, dust or water did not show protection for the plants. Carbon substrate utilization profiles of pathogens and the tested *Sphingomonas* and *Methylobacterium* were analyzed, (via Biolog plates), to reveal a nutritional niche overlap between pathogen and the plant-protective strains of *Sphingomonas* (Innerebner et al. 2011). Previous studies indicate that a bacterial strain with higher degree of similarity to a plant pathogen is likely to be a better antagonist (Wilson and Lindow, 1994).

1.3 Biological Control

An understanding in microbial ecology plays a vital role in the success of a biological control agent. The aim of biological controls are often the total elimination of a pathogenic population. To achieve this, scientists often manipulate the antagonistic properties of organisms deemed beneficial. However, reducing the interactions among the beneficial organism and the other vast majority of organisms present will lead to an oversimplification of hierarchy with the community. Therefore, the likelihood of success for the biological control agent is increased if it possesses other attributes in parallel with its antagonistic properties, that make it ecologically fit to reduce a specific pathogen population (Lindow et al., 2004).

Biological controls are based on three major concepts: competition, parasitism, and antibiosis (Lindow et al., 2002). Recent success with biological controls of plant pathogens also shows inducing systemic resistance in plants to be a characteristic of successful biological control agents. It has been seen competition has been successful with necrotrophic fungi (Kessel, 1999). This work stated that by giving early access to a food source to a strong saprophyte sharing the same substrate as the pathogen, it was possible to exclude the pathogen from the necrotic tissues. Parasitism was originally thought of as a powerful mechanism of

control, but now thought of as more successful when combined with initial competition. Antibiosis is when the biological control agent inhibits or deters the pathogen growth via metabolites produced by the biological control agent which may be antibiotics, toxins, bacteriocins etc. (Lindow et al., 2004). Lastly, systemic resistance is part of the plant immune system, and inducing these processes can allow the plant a fighting chance against the pathogen(s). Inducing systemic resistance is successful with a variety of root-associated mutualists, such as *Pseudomonas, Bacillus, Trichoderma,* and mycorrhiza (Pieterse et al., 2014).

II. PLANT BACTERIAL PATHOGENS

2.0 Bacterial Pathogenesis Introduction

Bacterial pathogens can colonize the leaves, roots and xylem of plants. They tend to enter plants either through wounds or natural openings, and then colonize the intercellular space and/or the xylem. Unlike fungi, bacteria are not able to directly penetrate the cuticle of plants (Dickinson, 2003). The first step to bacterial infection is recognition of the plant exudates/root exudates by the bacterium. These exudates are composed mainly of sugars, amino acids, organic acids and flavonoids. These are meant to attract specific and beneficial organisms (although pathogenic bacteria can recognize them if the exudates are coming from their host plant) (LeFevre et al. 2013; Hardoim et al. 2008). After recognition, the bacteria have to have some way of attaching and colonizing the area. This is done by pili and extracellular polysaccharides (Dourado et al. 2015). Most molecular work into infection and pathogenicity has been performed on phyllopsphere pathogens *Pseudomonas*, *Agrobacterium*, *Xanthomonas*, and *Erwinia*, as well as rhizosphere pathogens *Pantoea*, and *Ralstonia* species (Dickinson, 2003). *Pseudomonas* and *Xanthomonas* are described later in this introduction.

Bacteria stimulate gene expression (in the bacterial cells) in response to host factors. They colonize the plant, and need to obtain nutrients for their own growth and replication, and at the same time avoid or suppress the plant defense mechanisms. After colonization on the plant, quorum sensing (QS) is a common communication mechanism used by bacteria that enables them to sense population density and respond by the regulation of expression of particular genes (Dickinson, 2003).

Bacteria can work as a multicellular organism due the QS system once the bacterial population growth and the extracellular concentration of autoinducers reach an optimal level that regulate the transcription of different genes that could be related to the secretion system, biofilm formation, exchanges of DNA and others (Zhu et al. 2008).

In addition to cell-cell communication, some pathogenic bacteria produce plant cell-wall-degrading enzymes and/or alter production in plant hormones to their advantage. *Erwinia cartovora* produces an enzyme that can cause damage to plants by softening and macerating the plant tissue. Some bacteria can alter plant hormones, such as those that cause proliferation of plant tissue, (such as *P. syringae*, *A. tumefaciens*, *Pantoea herbicola*, *Gypsophila paniculata*, and *Rhodococcus fascians*. In the gall-forming *Pseudomonas*, IAA production is altered by the bacteria's indole-3-acetamide (IAM) pathway. This causes uncontrolled plant cell proliferation, producing galls (Dickinson, 2003).

2.1 *Pseudomonas syringae*

Pseudomonas syringae are phyllosphere pathogens and infect plant through stomata. They maintain apoplastic infections and produce leaf spots, speck, and blight. They have infect a wide range of host plants including: apple, bean, pea, beetroot, stone fruit, barley, wheat, clover and horse chestnut trees (Hirano and Upper, 1995). It is known to have over 60 pathovars with specific host plant interactions, and is particularly damaging during frost because *Pseudomonas* produces a protein that nucleases ice formation on the plant. The ice will then puncture the plant cells causing damage that allow infection (Hirano and Upper, 1995).

Pseudomonas syringae are known to produce several toxins including: coronatine, syringomycin, syringopeptin, tabtoxin and phaseolotoxin. These toxins have a variety of

functions and can be pathovar specific. Coronatine causes the plant stomata to reopen after plant pathogen interactions force them to close (Melotto, 2008). Syringomycin and syringopeptin can produce pores in plant cell wall (eventually causing cell lysis), and at high concentrations, it can dissolve the plant cell (Scholz-Schroeder, 2016 and Dickinson, 2004). Tabtoxin causes chlorosis (damage to chlorophyll). Phaseolotoxin causes chlorotic signs on the plant. The toxin inhibits ornithine carbamoyl-transferase (OCTase), the enzyme involved in arginine biosynthesis, converting ornithine and carbamoyl phosphate to citrulline (Stacy and Keen, 1996). A study in 1998 showed that the production of toxins is widespread among this genus of bacteria and that some can produce more than one (Volsch and Weingart, 1998). For a plant to fight a non-host specific toxin it must produce an insensitive target protein or production of enzymes that can modify the protein rendering it inactive.

In addition to toxins, *Pseudomonas* has been shown to produce extracellular polysaccharides. Extracellular polysaccharides are large polymers that are important for many phytopathogenic bacteria. They function as both capsules around the bacteria and as fluidal slime release by the bacteria. Thus, they provide a barrier against desiccation and a defense against toxic plant compounds and induced host defense (Fett and Dunn, 1989).

The current treatment for *Pseudomonas* infection is antibiotics or a combination of antibiotics and bactericides such as copper supplements. Treatment depends on the season, type of plant and maturity of plant. Common antibiotics for *Pseudomonas* infection of citrus plants include streptomycin supplemented with copper compounds (Kennelly et al. 2007). *P. syringae* pv. tomato has a relatively wide host range including common bean (*Phaseolus vulgaris*), tepary bean (*P. acutifolius*), lima bean (*P. lunatus*), pigeon pea (*Cajanus cajan*), butterfly peas (*Centrosema* spp.), tick clover (*Desmodium* spp.), soybean (*Glycine max*), hyacinth bean (*Lablab*

purpureus), lentil (*Lens culinaris*), purple bush bean (*Macroptilium atropurpureum*), perennial soybean (*Neonotonia wightii*), jicama (*Pachyrhizus erosus*), pea (*Pisum sativum*), kudzu (*Pueraria lobata*), adzuki bean (*Vigna angularis*), mung bean (*V. radiata),* and black eyed pea (*Vigna unguiculata*) (Birch et al. 1981; Hunter and Taylor 2006; Patel and Walker 1965; Taylor et al. 1996).

2.2 *Xanthomonas perforans*

X. perforans is the causative agent of bacterial spot on tomato and pepper. While bacterial spot is found all over the world, it is particularly a problem for the southeastern US, because of high humidity, high temperatures and high rainfall. It was previously called *X. campestris* pv. *vesicatoria,* and grouped together with *X. euvesicatoria*, *X. vesicatoria*, and *X. gardneri*. These have all been found to be distinct species (Strayer-Scherer et al. 2011). *Xanthomonas* are gram-negative rods and are motile. In contrast to *P. syringae* pv. tomato, these organisms have xanthomonadin pigment. They grow relatively fast, compared to *Sphingomonas* and Methylobacteria.

Bacterial spot can be found on all above ground plant parts (including fruit) showing brown-black lesions. These spots usually don't appear larger than 3mm in diameter. General yellowing and decreased plant vitality may occur. The symptoms usually appear within 3-5 days post infection. Copper-mancozeb mixtures have been standard for controlling the spread of bacterial spot. Bacteriophages have been used to treat bacterial spot, but their effectiveness has been inconsistent in the field (Strayer-Scherer et al. 2011).

2.3 Type VI Secretion System (T6SS)

Currently, six secretion systems in are known bacteria (Costa et al., 2015). These secretion systems can either be secretory protein independent (type VI and type III) or dependent. In T6SS, cell touching induces synthesis and firing of the T6SS, which causes the other cell of the same species, in the cell matrix, to assemble and fire its own T6SS (Cost et al., 2015). T6SS allows bacteria directly into prey cell membranes or cytoplasm (Gallique et al., 2017). The detailed components of the T6SS are still uncharacterized. The intracellular multiplication factor (*IcmF*) protein is conserved for bacterial pathogens that use T6SS (Lin et al., 2015). It has been shown *IcmF3* mutant in Pseudomonas is defective in motility and defective for production of pyoverdine (Lin et al., 2015), thereby decreasing it's virulence. This current study observes how virulence is impacted with a *IcmF3* mutant in *X. perforans* when in the presence of a biological control agent.

2.4 Conclusion

This study focuses on the use of *Methylobacterium* and *Sphingomonas* for protection against bacterial infection in tomato plants. There are no studies showing testing of *Sphingomonas* against *P. syringae* in tomato plants. An additional interest for this study was to observe if the same beneficial results occur with *Pseudomas syringae* pv. *tomato* 99B799, *Xanthomonas perforans* wild-type and a type VI secretion system mutant of *X. perforans*.

III. EXPLORING INTERACTIONS OF PHYLLOSPHERE EPIPHYTES WITH PLANT PATHOGENIC BACTERIA PSEUDOMONAS AND XANTHOMONAS ON TOMATO

3.0 Introduction

Sphingomonas was chosen for this study in particular because of the growing interest of Sphingomonas as a plant growth promoter. The information for biocontrol effectiveness of *Sphingomonas* is lacking, and this study aims to contribute to a better understanding of what pathogens *Sphingomonas* may be effectively used against. *Methylobacterium* has been studied previously as a plant growth promoter and induce systemic resistance in some plants (Sy et al. 2001, Menna et al. 2006; Nigris, et al. 2013). However, *Methylobacterium* from red clover has not been challenged against *X. perforans* and *P. syringae* pv. tomato on tomato. Red clover was chosen because previous literature stated *Methylobacterium* could be found on red clover (Omer et al. 2004), and clover is relatively inexpensive, and used as a common cover crop. As stated before in chapter II., pathogen interactions can be incribedly specific. A phyllosphere biocontrol is more likely to be successful if it is challenged with a phyllosphere pathogen. Therefore, this study uses common tomato phyllosphere pathogens *X. peforans* and *P. syringae* pv. tomato. Lastly, recent literature highlighted the importance of Sphingomonas and Methylobacterium specifically, when tested against *P. syringae* pv. tomato and *X. campestris* on *Arabidopsis* (Innerebner et al. 2011).

3.1 Goals

This study has four main goals and the following objectives (a-f) were put into place to achieve those goals:

I. Isolate red clover phyllosphere residents: *Sphingomonas* and *Methylobacterium*

II. Explore possible reasons for any decrease in disease *in planta.*

a) Test effect of biological control agent on motility of pathogens using a motitlity assay.

b) Observe any changes in colony morphology when pathogens are plated with biological control agent.

c) Compare nutritional similarity using niche overlap indices (NOI) via BIOLOG GN3 plates.

III. Observe any decrease in disease *in planta* of Methylobacteria and Sphingomonas treated tomato plants inoculated with *P. syringae* pv. tomato and *X. perforans*.

d) Conduct seed-to-seedling experiments using red clover commensal soaked seeds and challenge with phyllosphere pathogens at two weeks old under growth chamber conditions.

e) Conduct bacterial dip experiments using four week old plants dipped in *S. taxi* 55669 and challenge with *Xanthomonas* under greenhouse conditions. Record bacterial counts and foliar disease percent.

IV. Record any difference in virulence between *X. perforans* wild-type and *X. perforans* Type VI secretion system mutant against potential biological control agent.

f) Seedling dip experiments using four week old plants dipped in *S. taxi* 55669 and challenge with *Xanthomonas* under greenhouse conditions. Record bacterial counts and foliar disease percent.

V. Support or reject the initial hypothesis:

Sphingomonas would prove to be a potential biocontrol agent against *P. syringae* pv. tomato and *X. perforans*, and Methylobacteria would not prove to be a potential biocontrol agent against *P. syringae* pv. tomato and *X. peforans*.

Materials and Methods

3.2 Initial Preparation and Bacterial Isolation

Bacterial Strains

Pseudomonas syringae pv. *tomato* 99B799 was provided by the Kloepper lab at Auburn University (AU), in Auburn, AL. *X. perforans* wild type and mutant strains were provided by the Potnis lab at AU. Wild-type refers to *X.perforans* that was isolated from a tomato field (in a previous study in the Potnis lab), and T6SS refers to a mutant created (by the Potnis lab) to study the effect of T6SS gene clusters on pathogenesis on tomato plants. The Potnis lab created a knockout mutant of *X. perforans* carrying an in-frame deletion in the core T6SS-III gene, *icmF3*. The *Methylobacterium* and *Sphingomonas* strains were isolated by grinding the plant leaves from red clover grown outdoors in a small vegetable garden in Columbus, GA in March-April 2017. Additional *Sphingomonas* isolates were obtained from tomato (Fla. 8000), grown in the same garden in April-May 2019. The tissue (10g) was placed into a sterile jar and washed with sterile water to remove adhering soil off, homogenized and diluted in buffer and the material distributed on plates. To obtain *Sphingomonas*, yellow and orange pigmented colonies were grown on

individual R2a plates. These colonies were picked off using sterile toothpicks and placed onto R2a plates containing Streptomycin (20mg/mL). To isolate *Methylobacterium,* slow growing, pink colonies were isolated and grown on R2a agar streak plates without Streptomycin. Confirmation of ability of the bacteria to use methanol was tested with mineral salts medium supplemented with methanol. To confirm the identity of presumptive *Methylobacterium* and *Sphingomonas* strains, samples of *Methylobacterium* were amplified using PCR and sequenced by Eurofins. To obtain a visual of native *Methylobacterium* on the tomato leaf surface, the tissue was plated on mineral salts agar, and incubated in a closed container at 31°C with methanol provided in the vapor phase.

3.3 Preparation of Bacterial Inocula

All bacterial strains were cultured on R2a plates for 48h at 31°C. Bacterial cells were scraped from the plate and suspended in sterile 0.01M $MgSO_4$. The cell suspensions were adjusted to an appropriate volume using a standard curve of optical density to obtain $1X10^8$ CFU/mL.

3.4 *In Vitro* Interactions: Colony Morphology on R2a and Motility Assay

Colony Morphology Observations on R2a

Bacterial suspensions (~$1X10^8$ CFU/mL) of the clover isolates and the pathogens were plated 0.5cm away from each other onto R2a plates to observe interactions. To observe *P. syringae* pv. tomato interactions, bacterial suspensions were plated at a 0.25cm distance from *P. syrinage* pv. tomato due to neutral interaction at 0.5cm for all strains tested. The plates were divided into quadrants to make four replicates per plate. Morphology was observed for one week at 22-25˚C and compared to control plates. Change in plated morphology from control plating

indicates interaction. Depending on severity, the result could be interpreted as inhibition or neutral.

Motility Assay

Bacterial suspensions of $1X10^8$ CFU/mL of the red clover isolates and pathogens were plated onto soft agar (0.3% agar) with nutrient broth plates to observe interactions. In the middle of the plate, 5uL of bacteria were aliquoted 1cm away from another bacteria sample. A decrease, increase or neutral effect on motility was recorded for each treatment daily over three days 22-25˚C and compared to control plates. This response was quantified by obtaining four points surrounding the swarming *X. perforans* on the plate. These four points are averaged and statistically analyzed for any significance. There were three plates per treatment.

3.5 Seed-to-Seedling: Growth Chamber Conditions

Preparation of Bacterial Suspension

Tomato seeds were soaked with $1x10^6$ CFU/mL cell suspensions of red clover isolates for two hours. Control seeds were soaked in sterile $MgSO_4$. The treatments were organized as shown in Table 1.

Table. 1 Seed to Seedling Treatments					
	Bacteria				
Treatment	*X. perforans WT*	*P. syringae* pv. tomato	*Methylobacterium*	*Sphingomonas*	$MgSO_4$
1					X
2				X	
3			X		
4		X			
5		X	X		
6		X		X	
7			X	X	
8		X	X	X	
9	X				
10	X		X		
11	X			X	
12	X		X	X	

X represents treatment of seeds with specific bacterium seedlings bacteria that were inoculated onto each treatment. Each treatment (1-12) correlates to air dried seeds (24 hrs) planted into pots (3 seeds/pot) with sterilized soil. Tomato seeds (Fla. 8000) were planted in sterilized soil and grown under greenhouse conditions with three seeds/pot and eight pots/treatment. The plants were grown in a chamber with 12 hour day/12 hour night photoperiods at 28°C under humid conditions. At two weeks old, each plant was dipped in a pathogen with the exception of the control groups. Each plant was evaluated for percent of leaf covered with disease at day 7 and 14 post infection of pathogen. The experiment was done twice.

3.6 Bacteria Dip Under Greenhouse Conditions

Tomato seeds (Fla. 8000) were planted in sterilized soil and grown under greenhouse conditions with one plant/pot and five pots/treatment. *S. taxi* 55669 was dipped on the plants at $1X10^6$CFU/mL. *X. perforans* WT and MUT were dipped on the plants at $1X10^6$ CFU/mL approximately 24 hrs after *S. taxi* 55669 and kept at high humidity. Disease was allowed to progress under regular greenhouse conditions. Table 2 represents the organization of experimental set up treatments of seedling dip under greenhouse conditions. Each "X" represents eight pots of three week old tomato plants that have been dipped in the (~$1x10^6$ CFU/mL) bacterial suspensions or $MgSO_4$.

Table. 2 Seedling Dip Treatments: Five pots/treatment				
	Bacteria			
Treatment	*X. perforans* wild-type	*X. perforans* T6SS mutant	*Sphingomonas*	$MgSO_4$
1				X
2			X	
3		X		
4	X			
5	X		X	
6		X	X	

Table 2 Disease percent on foliage measured on day 21 post infection. Bacterial counts were observed via spiral counter and selective media. R2a supplemented with Rifampin (50mg/mL) served as selective medium for obtaining *X. perforans* wild-type, and R2a supplemented with Nalidixic acid (50mg/mL) served as the selective medium for *X. perforans* T6SS mutant. To select for *Sphingomonas*, R2a was supplemented with Streptomycin (20mg/mL). Control plates were R2a with no supplements, therefore, the control bacterial counts were the total bacterial population when no *Xanthomonas* or other bacteria were added. Microflora ($28cm^2$) was taken of each treatment and homogenized with 0.01M of $MgSO_4$. The experiment was repeated twice.

3.7 Carbon Source Profiling

Previous studies (Innerebner, et al. 2011), have used BIOLOG plates to assess the carbon sources usages of potential plant growth promoting bacteria against the pathogen and other red clover isolates (and possibly tomato isolates). Using protocols outlined by the manufacturer, BIOLOG plates were used according to manufacturer's instructions to determine if there is nutritional similarity between the red clover/tomato isolate and pathogen. Their niche overlap indices (NOI) were calculated by using the formula of Wilson and Lindow (1994).

NOI=

No. of carbon sources used by biocontrol agent and target pathogen

Total no. of carbon sources utilized by the target pathogen

3.8 Statistical Analysis

JMP 15 software was used on all data collected, and a one-way ANOVA was performed on data obtained from the motility assay, pathogen and *Sphingomas* populations, and foliage disease percent obtained. Tukey's test was performed on post-ANOVA results.

IV. RESULTS

4.0 Isolation of Red Clover Phyllosphere Associated Methylobacteria and *Sphingomonas*

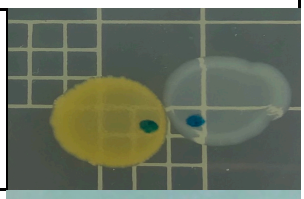
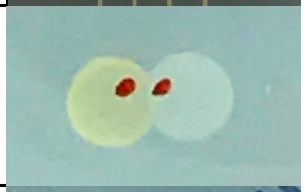
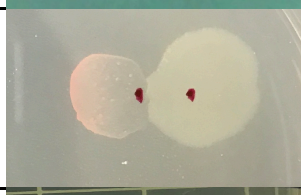
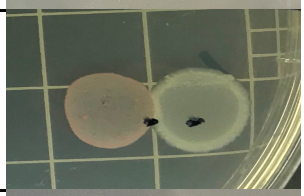
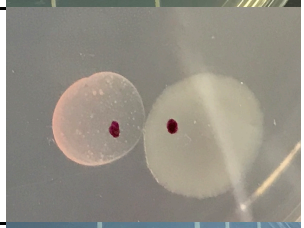
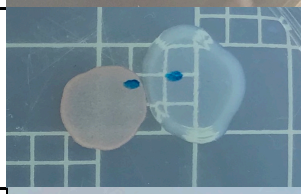
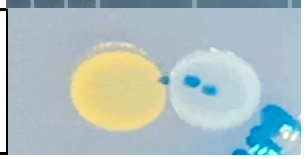
BLAST analysis of the pure cultures of three week old red clover tissue (stems and leaves): 97.25% certainty *Sphingomonas adhaesiva* NBRC 15099, 97.85% *Sphingomonas mucosissima* DSM 17494, and 99.09% *Sphingomonas taxi* ATCC 55669. The three *Methylobacterium* found were as follows: 97.92% *Methylobacterium* sp. Ap11E, 96.06% *Methylobacterium* sp. AN12, and 99.7% *Methylobacterium* sp. AN17. The following observed from the NCBI BLAST of the pure cultures of three week old tomato tissue (stems and leaves): 97.17% certainty *Sphingomonas azotifigens* NBRC 15497, and 96.56% *S. melonis*.

4.1 *In Vitro* Interactions: Motility Assay and Colony Morphology on R2a

Colony Morphology Observations on R2a

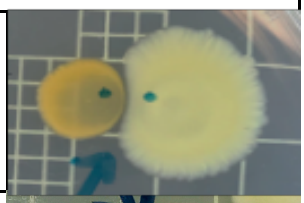
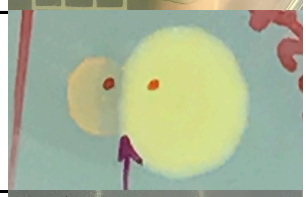
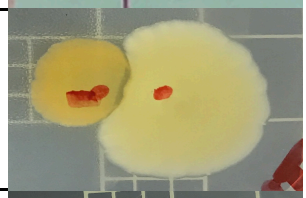
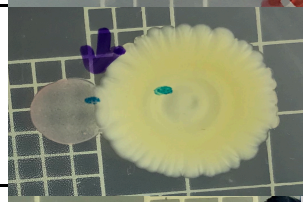
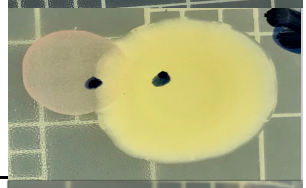
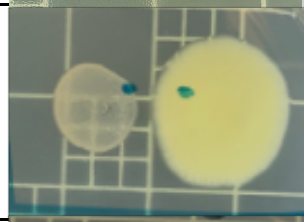
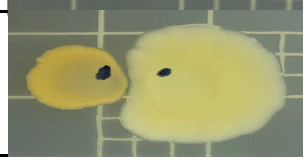
Table 3 shows the interaction of red clover isolated bacteria against the pathogen *P. syringae* pv. tomato on R2a plates. Most interactions observed were neutral, and were plated 0.25cm apart as previous lab work with 0.5cm showed neutral interaction for all. Colony morphologies are shown in Appendix A.

Table 3. Interaction on R2a with *P. syringae* pv. tomato 99B799. Morphology observed for one week at 22-25˚C .

Commensal	Result	
Sphingomonas mix	Neutral	
S. adhaesiva NBRC 15099	Neutral	
S. mucosissima DSM 17494	Neutral	
S. taxi ATCC 55669	Neutral	
Methylobacterium sp. Ap11E	Inhibited by pathogen	
Methylobacterium sp. AN12	Neutral	
Methylobacterium sp. AN17	Slightly inhibited by pathogen	
Methylobacteria mix	Neutral	
Methylobacteria and *Sphingomonas* mix	Neutral	

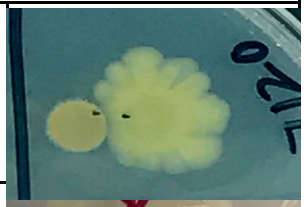
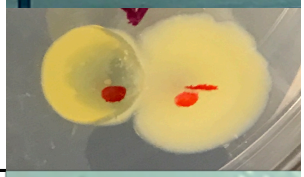
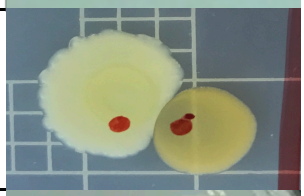
Growth of *Methylobacterium* sp. AN12 was inhibited by *P. syringae* pv. *tomato* colony. Slight inhibition of growth was seen in *Methylobacterium* sp. AN17 but not in the Methylobacteria mix. None of the *Sphingomonas* were inhibited by *P. syringae* pv. tomato nor did they inhibit growth of *P. syringae* pv. tomato. Table 3 shows interaction of red clover bacteria with *X. perforans* wild-type. Most *Sphingomonas* were inhibited by *X. perforans* except *S. taxi* 55669.

Table 4. Interaction on R2a with *X. perforans* T6SS mutant . Morphology observed for one week at 22-25˚C .

Commensal	Result	
Sphingomonas mix	Inhibited pathogen	
S. adhaesiva NBRC 15099	Inhibited by pathogen	
S. mucosissima DSM 17494	Inhibited by pathogen	
S. taxi ATCC 55669	Inhibited pathogen	
Methylobacterium sp. Ap11E	Neutral	
Methylobacterium sp. AN12	Neutral	
Methylobacterium sp. AN17	Neutral	
Methylobacteria mix	Neutral	
Methylobacteria and *Sphingomonas* mix	Inbition of pathogen	

Most *Sphingomonas* were inhibited by *X. perforans* except *S. taxi* 55669, and the Sphingomonas mix culture. Due to this fact, *S. taxi* 55669 was used in the greenhouse study and not the other *Sphingomonas* cultures. All Methyobacteria were neutral in response to *X. peforans* wild-type and mutant as shown in Table 4. and Table 5.

Table 5. Interaction on R2a with *X. perforans* wild-type. Morphology observed for one week at 22-25˚C

Commensal	Result	
Sphingomonas mix	Inhibited pathogen	
S. adhaesiva NBRC 15099	Inhibited by pathogen	
S. mucosissima DSM 17494	Inhibited by pathogen	
S. taxi ATCC 55669	Inhibited pathogen	
Methylobacterium sp. Ap11E	Neutral	
Methylobacterium sp. AN12	Neutral	
Methylobacterium sp. AN17	Neutral	
Methylobacteria mix	Neutral	
Methylobacteria and *Sphingomonas* mix	Inhibition of pathogen	

X. perforans T6SS showed the same responses as *X. perforans* wild-type on R2a plating with red clover commenals.

Motility Assay

Motility can be seen as an irregular growth protruding from pathogen colony. Preliminary testing revealed no visible signs of change in motility in soft agar after three days, and therefore data was collected for only the first three days. Day 2 treatments were not significantly different from control plate for *X. perforans* wild-type motility assay ($P>0.05$, $P=0.1176$). Day 3 treatments were not significantly different from control plate for *X. perforans* wild-type motility assay. For both day 2 and day 3 diameters, *Sphingomonas* and Methylobacteria treatments appeared to decrease motility diameters of *X. perforans* wild-type, but this was not significantly different from the control treatment. *X. perforans* T6SS Day 2 none of the treatments were significantly different than the control ($P>0.05$). Figure 2. shows most treatments were significantly decreased in average diameter for day 3 of swimming of motility when compared to the control *X. perforans* T6SS mutant except for plates with *S. mucoissima* DSM 17494, *S. adhaesiva* NBRC 15099, and *Methylobacterium* mix. The following plates were significantly different than the control *X. perforans* T6SS plate: *X. perforans* T6SS plated with *Sphingomonas* with Methylobacteria mix ($P=0.0212$), *Methylobacterium* sp. IIE ($P=0.0322$), *S. taxi* 55669 ($P=0.0010$), *Sphingomonas* mix ($P= 0.0016$), *Methylobacterium* sp.AN12 ($P=0.0025$), and *Methylobacteirum adhaesivum* ($P=0.0025$).

4.2 Seed-to-seedling: Growth Chamber Conditions

Disease was observed for all plants except the control inoculated with sterile buffer. Using a disease scoring method, there was not a significant difference observed between the treatments. Therefore, it was not necessary to sample the microflora to obtain bacterial population counts. There was no visible signs of disease caused by red clover commensal bacteria on tomato. The treatment that varied the most was treated with six red clover associated bacteria and *X. perforans*, but it was not significantly different from the other treatments. As Figure 3. shows, *P. syringae* pv. tomato 99B799 was not able to cause over 5% foliage infection, and eventually did not cause any infection in trial 2. Therefore, the treatments were not

significantly different for *P. syringae* pv. tomat*o* 99B799 treated plants and it's use was discontinued from the study for disease assays.

4.3 Plant Dip (*Xanthomonas* Under Greenhouse Conditions)

Bacteria Populations and Disease Scores (%)

Figure 4 shows *X. peforans* bacterial populations did not significantly decrease for *X. perforans* wild-type when seedlings were pretreated with *S. taxi* 55669 (P>0.05) for both trials. However, bacterial populations did significantly decrease for *X. perforans* T6SS mutant (P<0.0001) when compared to *X. perforans* mutant control and *X. perforans* wild-type control for both trials (Figure 4. and Figure 5.). The populations of *X. perforans* mutant and *X. perforans* wild-type controls were significantly different in the first trial (P=0.025), but not in the second trial. For both trials, disease was significantly reduced for the wild-type treatments with *S. taxi* 55669 (P<0.0001trial 1, P=0.0006 trial 2) as shown in Figure 6. In trial 1, mutant treatments with *S. taxi* 55669 decreased in disease percent (P<0.0001trial 1) but in trial 2, this effect was not found (P=0.1671). There was a decrease in disease for the mutant treated plants, as shown in Figure 6., but this decrease was not significant. The results varied as the error bars dictate, and there were five replicates per treatment.

4.4 Carbon Sources Profiling

Carbon source utilization was obtained for *S. taxi* 55669. Substrate utilization tables are recorded in Appendix E. Of the samples tested (Table 6), *S. taxi* 55669 used more of the same sources to *X. perforans* wild-type.

Table 6.

Bacteria	NOI
P. syrinage pv. tomato 99B799	0.7241
S. adhaesiva NBRC 15099	0.7068
S. azotifigens NBRC 15497	0.6896
S. mucoissima DSM 17494	0.431
S. taxi 55669	0.7758
Methylobacterium sp. ApIIE	0.6071

In Table 6., *S. adhaesiva* NBRC 15099 had the second largest NOI, and *S. mucoissima* DSM 17494 had the smallest NOI.

V DISCUSSION

5.0 *Methylobacteria* Ineffective for Both *Pseudomonas* and *Xathomonas* Inhibition *in Vitro*

As indicated by Table 3, the results of the interaction between red clover and *P. syringae* are as expected, as previous literature states that Methylobacteria stimulate the immune system of plants, but would not be ecologically competitive with a pathogen. Being slow growers, most *Methylobacterium*, are expected to be inhibited by the pathogens *in vitro* (Innerebner et al., 2011). It is interesting to see the mixture of *Methylobacterium* has a neutral interaction with the pathogen, which points to *Methylobacterium* sp. AN12 being the major component of the observed neutral response. All Methylobacteria were neutral in response to *X. peforans* wild-type, which was not expected. *X. perforans* grows much faster than Methylobacteria, and it was expected to consume more nutrients around the colonies of Methylobacteria. However, this response was not observed, indicating *P. syringae* pv. *tomato* may be secreting a compound that *X. peforans* is not. This was seen with *X. perforans* T6SS mutant as well as shown in Table 5.

5.1 *S. taxi* 55669 *in Vitro* Inhibits *X. perforans* but Neutral with *P. syringae* pv. tomato

Previous studies have shown that *Sphingomonas* have a similar nutrient profile against *P. syringae* pv. *tomato* and therefore could inhibit the pathogen *in vitro* (Innerebner et al., 2011). It was not expected that *Sphingomonas* would have a neutral interaction against *P. syringae* pv. *tomato* as shown in Table 3 based on previous literature. This same work, showed *Sphingomonas* was ineffective against *X. campestris*. This current work is the first example of *Sphingomonas* inhibiting a *Xanthomonas*. Tables 3 and 4 show most *Sphingomonas* agree with previous literature and were inhibited by *X. perforans* except *S. taxi* 55669. Thus, this strain was selected for additional study in the greenhouse for use against *X. perforans* wild-type and type VI secretion system mutant on seedlings. It is notable that the *Sphingomonas* mixture still inhibited *X perforans* wild-type and T6SS mutant. Methylobacteria mix with *Sphingomonas* mix also inhibited both *Xanthomonas* pathogens. Thus, indicating no negative relationships amongst the red clover commensals that could deter *S. taxi* 55669 from inhibiting the pathogens.

5.2 Swimming Motility Decreased in T6SS Mutant

Common practice is to have at least three motility assay plates per treatment. However, with the error bars (Figure 2), showing large variation in the data, this study would have benefitted from the addition of further replicates. There was not a significant difference with *X. perforans* wild-type plates for day 2 or day 3, but there was a significant decrease in motility diameters with day 3 of *X. perforans* mutant. This supports the current literature on the role of type VI secretion systems and decreased motility with varying virulence (Kamber et al., 2017). It is worth noting that each *Methylobacterium* isolate tested separately produced a significant decrease in motility diameters when compared to the control, but the *Methylobacterium* mix did not produce significantly decreased diameters. Additionally, shown in Figure 2 not all *Sphingomonas* decreased diameters significantly when compared to the control. *S. taxi* 55669 was the only *Sphingomonas* that decreased diameters of *X. perforans* T6SS.

5.3 *Methylobacteria* and *Sphingomonas* Mixtures Ineffective *In Vivo* Under Growth Chamber Conditions for Both Trials

As shown in Figure 3 there was not a significant difference observed between the treatments in the growth chamber study. It can be concluded that the initial inoculum at $1x10^6$ CFU/mL of these bacteria on seed does not protect against the pathogens on tomato under growth chamber conditions (28°C with high humidity). Future work could test the seed dip under greenhouse conditions, and determine if greenhouse conditions allow for significant disease protection against *X. perforans,* as there was no significant difference among *Sphingomonas* treated plants under growth chamber conditions ($P>0.05$). *P. syringae* pv. tomato was able to cause disease, but in low numbers. Figure 3. illustrates *P. syringae* pv. tomato was not able to cause disease as it's characteristic halo bacterial spot was not present. When grown in lab culture, over time, cultures may lose their virulence. It is not known how many times this strain has been passaged in culture. The rest of the study focused on *X. perforans*.

5.4 Significant Decrease in Bacterial Populations of *X. perforans* Mutant When Treated with *S. taxi* 55669

It was anticipated that a low *X. perforans* population would correlate to a high population in *S. taxi* 55669, along with low foliar disease percent. However, *S. taxi* 55669 was not isolated from the tomato phyllosphere even when there was a significant decrease in the population of *X. perforans* T6SS mutant (Figure 4) when treated with *S. taxi* 55669. Selective media (R2a with 20ug/mL Streptomycin), was prepared for *S. taxi* 55669 isolation from tomato, and a culture of *S. taxi* 55669 was achieved from the initial inoculum of the tomato plants to verify the culture of *S. taxi* 55669 was viable. However, *S. taxi* 55669 was not isolated from either trials 1 or 2 from the selective media post pathogen inoculation. These results indicate this strain is not a natural colonizer of tomato, and has low epiphytic fitness for tomato, and much like Salmonella infections of tomato, the populations would decrease over time (Potnis et al., 2014). However, recent research has indicated the importance of *Sphingomonas* in field plants (Newberry et al., 2020). When *S. phyllosphaerae*, S. *paucimobilis*, and *S. parapaucimobilis* was present, *X. perforans* was not found. Newberry et al., also found *S. taxi* was a natural colonizer of weeds around tomato and pepper. Thus, further demonstrating *Sphingomonas* have been found on tomato, and it is possible *S. taxi* can survive on tomato, but it is not a natural colonizer, and its populations would eventually decrease.

5.5 Significant Decrease in Foliar Disease for Wild-Type but not Bacterial Populations

The decrease in motility observed in the motility assay supports the decrease in bacteria population and decrease bacterial spot found on *X. perforans* mutant treated plants (Figure 3, Figure 4). The percent of foliage disease present was found to be significantly decreased for both *X. perforans* wild-type and *X. perforans* mutant in trial one (Figure 6). The dramatic decrease in disease percent found for *X. perforans* mutant in trial 1 could be due to decrease in T6SS function, along with nutritional competition with *S. taxi* 55669. This effect was not seen in trial 2, and it is speculated that this was due to higher temperatures and humidity in the greenhouse at the time of the experiment. *S. taxi* 55669 which may be inducing systemic resistance in the plant allowing it to decrease the presence of the bacterial population such as other biocontrol agents. As stated in the introduction of Chapter II, cell contact is necessary for T6SS to work, and both organisms need to have T6SS (Lindow et al., 2002). It is well known that *Xanthomonas* uses

T6SS as indicated by numerous studies including work showing the distribution of T6SS (Boyer et al., 2009). *Sphingomonas* was not known to have a T6SS until 2020 (Luo et al., 2020). In T6SS bacteria, toxins are fired into the competing bacterial cell after cell-to-cell contact, thereby killing the other cell and decreasing bacterial population numbers and foliar disease. The difference in decrease in foliar disease and bacterial populations of *S. taxi* 55669 against *X. perforans* wild-type and *X. perforans* T6SS mutant could be attributed to a decrease of T6SS function by knocking out *icmF3* (a part of the T6SS in *Xanthomonas*). *IcmF3* is only part of the T6SS, but if this system is not working properly, *X. perforans* would not fight off a phyllosphere resident as well as it normally could. Therefore, this study shows having a T6SS with functioning *icmF3* is advantageous for *X. perforans* in ability to fight off resident microflora and cause disease.

5.6 Nutritional Similarity

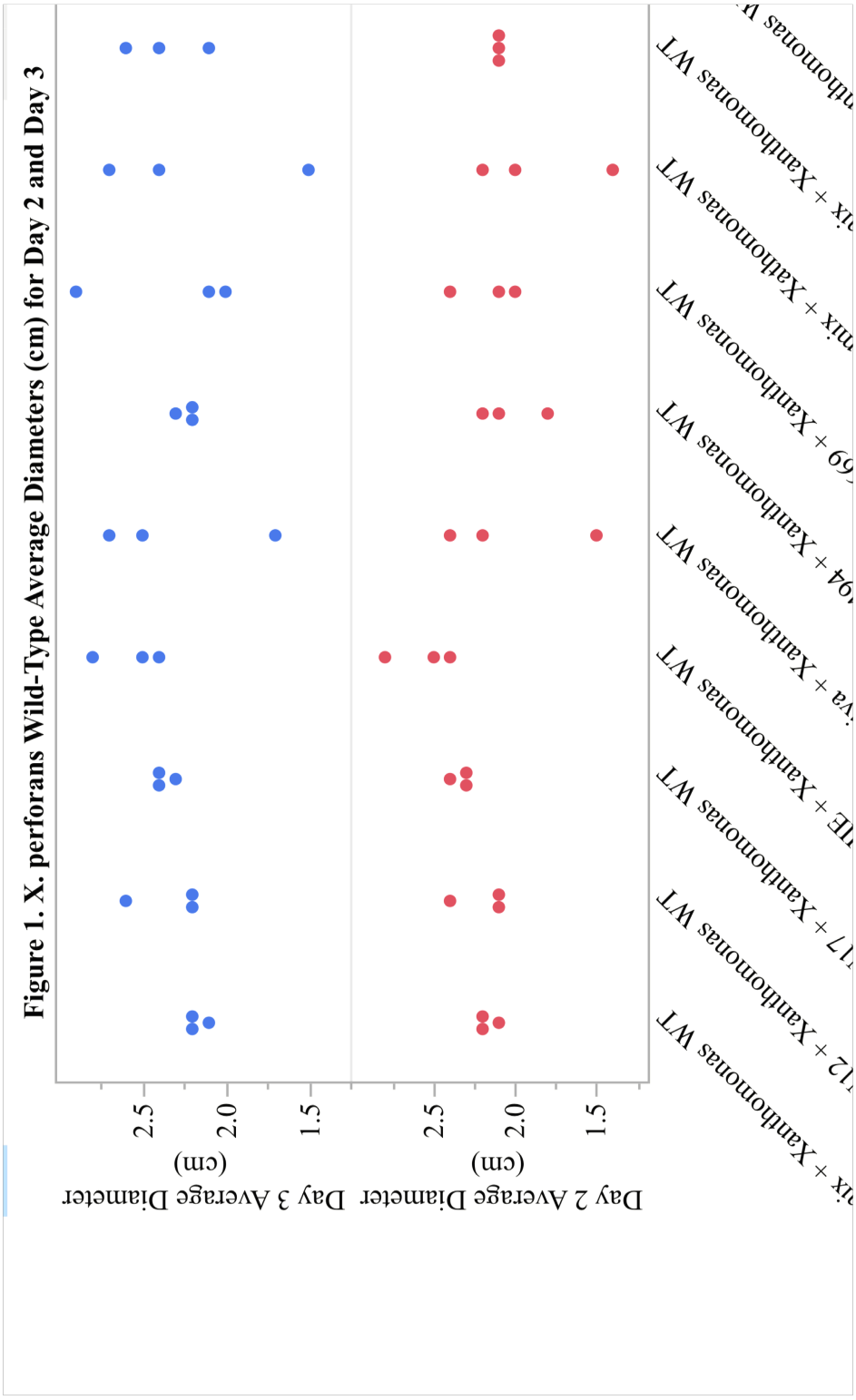
S. taxi 55669 had more similarity in carbon source usage with *X. perforans* wild-type (Table 6) than the other bacteria tested. Thus, the decrease of disease could be due to nutritional competition between the pathogen and clover bacteria. *S. adhaesiva* NBRC 15099 had the second largest NOI, and *S. mucoissima* DSM 17494 had the smallest NOI. In previous work, the findings agree with the results from this study on low carbon source utilization similarity and lack of protection from disease by *Methylobacterium* (Innerebner et al., 2011). However, Innerebner et al., did not test *P. syringae* pv. tomato DC3000 on tomato itself, rather, they tested on Arabidopsis, and this work did not test *P. syringae* pv. tomato 99B799. Lastly, this study did not test under greenhouse or growth chamber conditions, and their *Sphingomonas* did not provide protection against *X. campestris*, while this current work indicates a reduction in disease for *X. perforans* (Innerebner et al., 2011). Being given the chance to colonize tomato first, *S. taxi* 55669 may have gained an advantage in acquiring nutrients and competing with *X. perforans* populations. Previous research has indicated nutritional similarity as one way to decrease pathogen populations (Lindow et al., 2002). As stated in Chapter I, a biological control may not be effective due solely to nutritional similarity to the pathogen, and a biological control has a higher chance of being effective in field conditions, when combined with other modes of action to compete with pathogens. Other modes of action to compete with pathogen population

could include but are not limited to stimulating the immune system, decreasing pathogen motility, secreting toxin production via direct contact such as in T6SS etc. (Lindow et al., 2002).

VI CONCLUSION

It is not known how the plant immune system is triggered by *S. taxi* 55669. Current literature shows that *S. taxi* has potential as a plant growth promoter (Eevers et al., 2015). It is possible *S. taxi* 55669 could be stimulating the plant immune system, and one way microbes do that is by inducing systemic resistance (ISR) in the plant. ISR primes the whole plant body for enhanced defense against a broad range of pathogens and insect herbivores (Pieterse et al., 2014). While most microbes that have been studied for ISR are found in the rhizosphere, there are some phyllophere plant growth promoting bacteria such as *Rhodopseudomonas palustris* that stimulate ISR (Su et al., 2019).

Although this study did not see any protective effects of Methylobacteria on tomato, and it was hypothesized Methylobacteria would not be effective based on the literature of Methylobacteria against *Xanthomonas campestris*, recent research indicates the presence of Methylobacteria under field conditions has a negative correlation with *X. perforans* (Newberry et al., 2020). Newberry et al., isolated strains that were not the same as the strains isolated from red clover. However, future work can assess the ability of those strains of Methylobacteria (*M. extorquens*, and *M. populi*), against *X. perforans* populations. The results obtained from this red clover commensal study support the hypothesis indicating a *Sphingomonas* from red clover (*S. taxi* 55669) was effective against *X. perforans*, and reject the hypothesis that a *Sphingomonas* from red clover would be an effective biocontrol against *P. syringae* pv. tomato 99B799. Additionally, this work supports the hypothesis that Methylobacteria found on red clover would be ineffective against *P. syringae* pv. tomato 99B799 and *X. perforans*. Additional time for this study would have allowed experiments such as seed-to-seedling of *S. taxi* 55669 against a bacterial dip of *X. perforans* seeding. Future work can assess when *S. taxi* 55669 starts to decrease in population on tomato, and if plant age plays a role in the ability to colonize. Lastly, due to advances in DNA sequencing, it would be interesting to see the dysbiosis in the microbiota of the phyllosphere due to *X. perforans* infection, and *S. taxi* 55669 treatment. Further testing would be necessary to confirm its potential as a plant growth promoter and biological control agent.



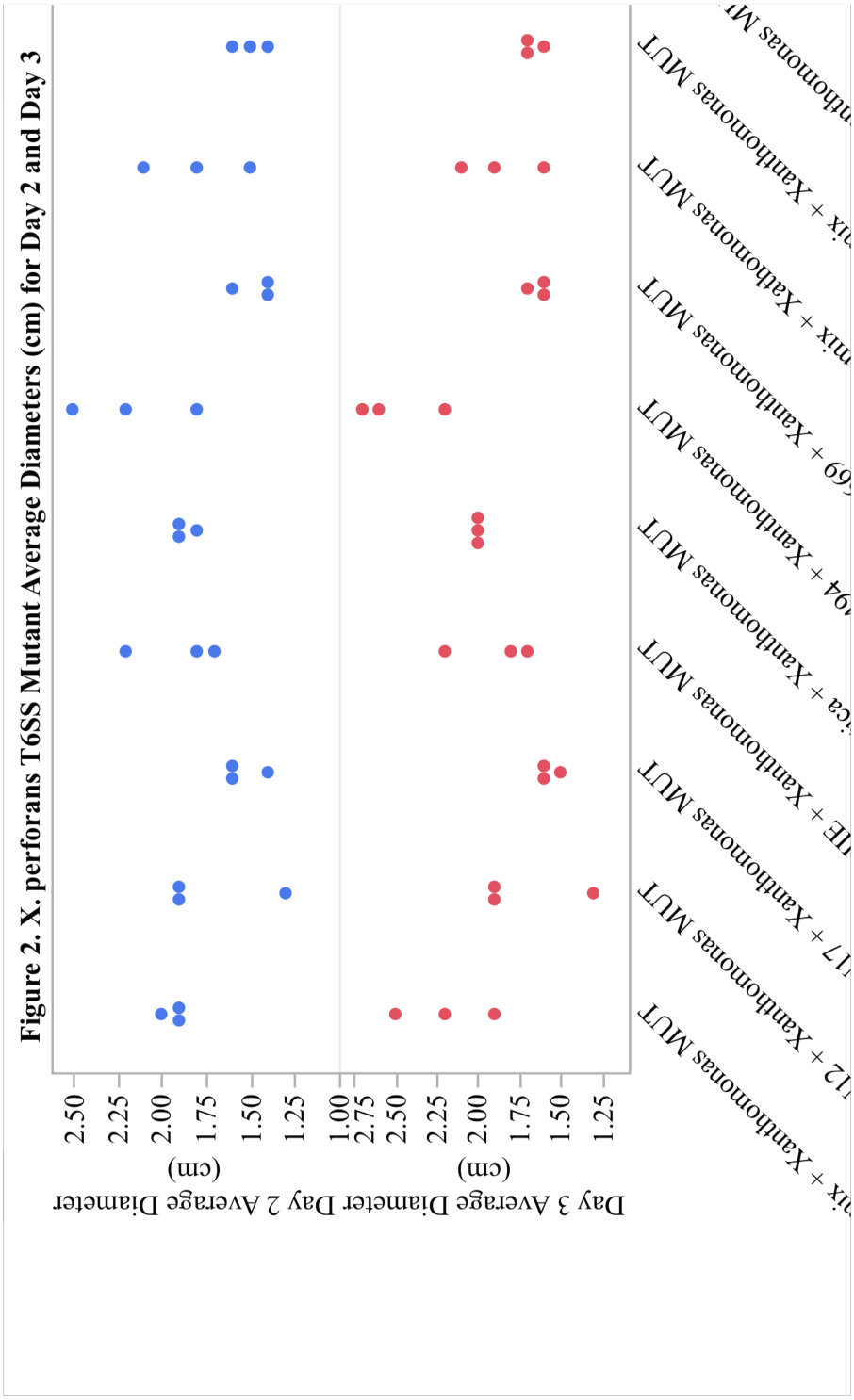


Figure 2. X. perforans T6SS Mutant Average Diameters (cm) for Day 2 and Day 3

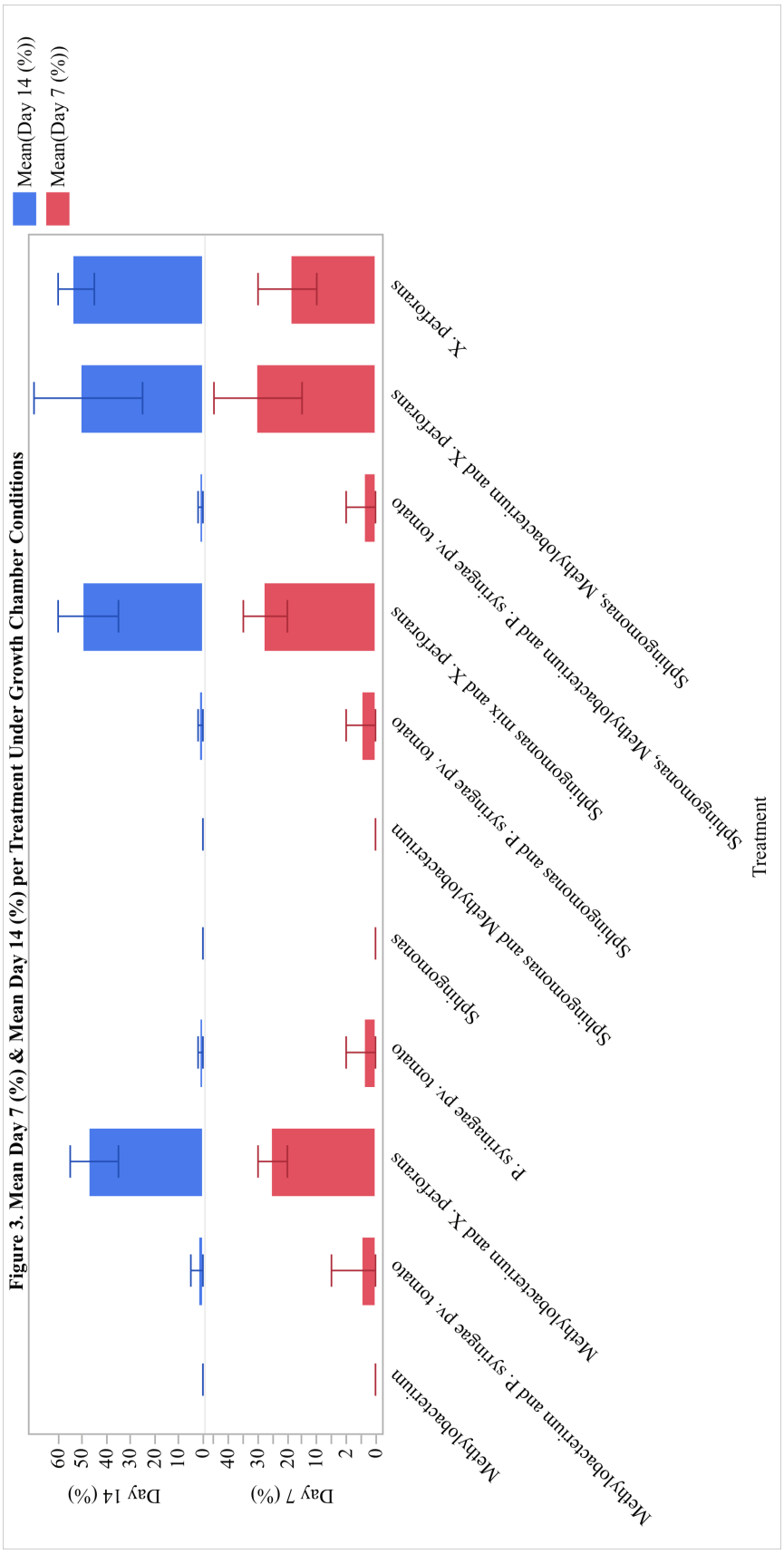


Figure 3. Mean Day 7 (%) & Mean Day 14 (%) per Treatment Under Growth Chamber Conditions

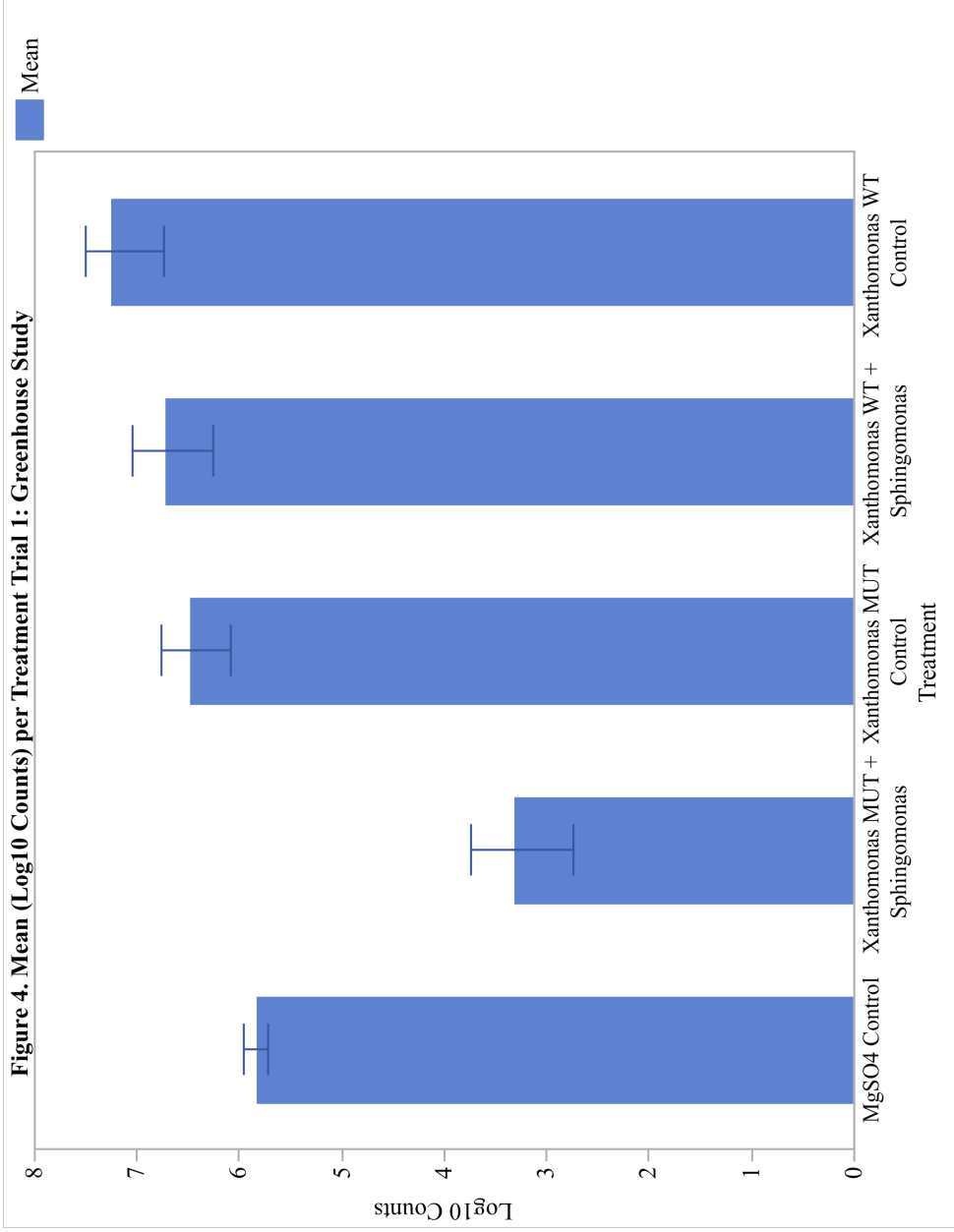


Figure 4. Bacterial counts of *X. perforans* taken on day 21 post inoculation of *X. perforans*. $MgSO_4$ control counts were off overall bacterial population. No *Xanthomonas* found on $MgSO_4$ control plates.

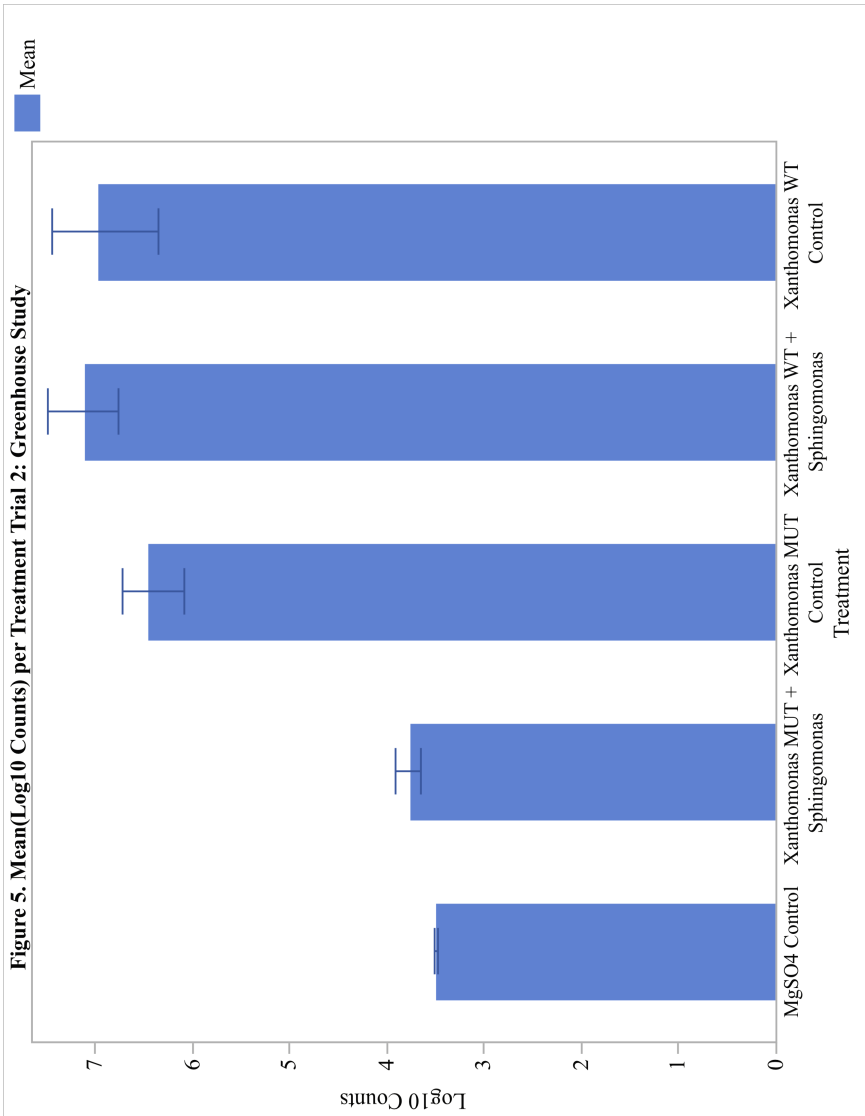


Figure 5. Bacterial counts of *X. perforans* taken on day 21 post inoculation of *X. perforans*. $MgSO_4$ control counts were off overall bacterial population. No *Xanthomonas* found on $MgSO_4$ control plates.

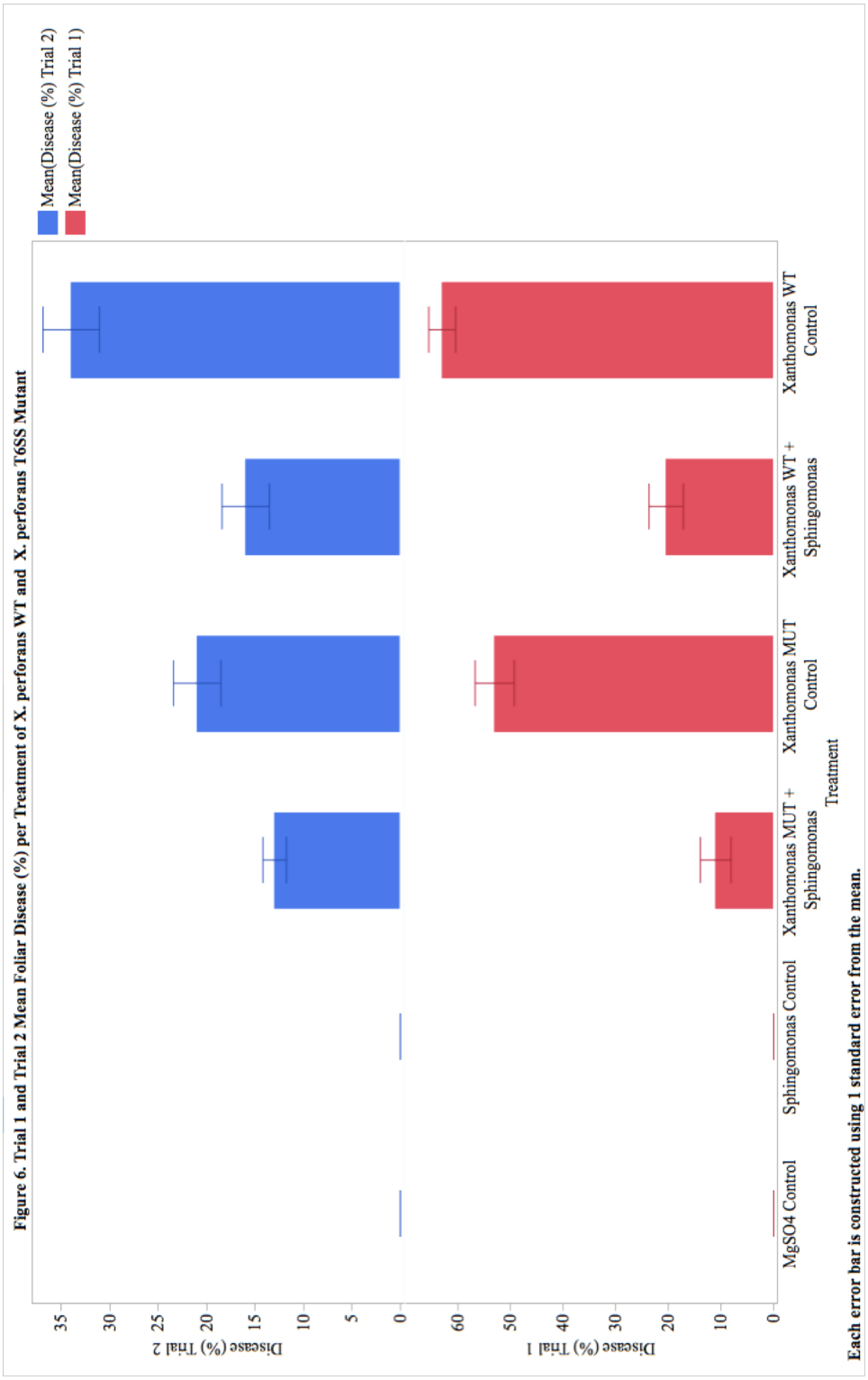


Figure 6. Trial 1 and Trial 2 Mean Foliar Disease (%) per Treatment of X. perforans WT and X. perforans T6SS Mutant

Each error bar is constructed using 1 standard error from the mean.

Figure 6. Foliar disease percent shown with standard error bars.

REFERENCES

Abanda-Nkpwatt, D., M. Müsch, J. Tschiersch, M. Boettner, and W. Schwab. Molecular Interaction Between *Methylobacterium extorquens* and Seedlings: Growth Promotion, Methanol Consumption, and Localization of the Methanol Emission Site. *J. Exp. Bot* 2006. Vol: 57 (15). pp. 4025-4032. Google Scholar. 3 Dec. 2016.

Adhikari, T. B., C. M. Joseph, G. P. Yang, D. A. Phillips, and L. M. Nelson. 2001. Evaluation of bacteria isolated from rice for plant growth promotion and biological control of seedling disease of rice. *Can J Microbiol.* Vol: 47. Pp. 916-924.

Andredote, F. D., P.T. Lacava, C.S. Gai *et al.* Model Plants for Studying the Interaction Between *Methylobacterium mesophilicum* and *Xylella fastidiosa*. *Can. J. Microbiol.* Vol: 52 (5). pp. 419-426. 2006. Google Scholar. 4 Dec. 2016.

Andreote, F.D., R. T. Carneiro, J. F. Salles et al. Culture-independent Assessment of Rhizobiales-related Alphaproteobacteria and the Diversity of *Methylobacterium* in the Rhizophere and Rhizoplane of Transgenic Eucalyptus. *Microb Ecol.* 2009. Vol: 57 (1). pp. 82-93. Google Scholar. 2 Dec. 2016.

Araújo, W.L., J. Marcon, W. Maccheroni Jr., J. D. van Elsas, J. W. van Vuurde, and J. L. Azevedo. Discovery of Endophytic Bacterial Populations and Their Interaction with Xylella fastidiosa in Citrus Plants. Appl. Environ. Microbiol. 2002. Vol: 68 (10). pp. 4906-4914. 2002. Google Scholar. 24 Nov. 2016.

Ardanov, P., A. Sessitsch, H. Häggman, N. Kozyrovska, and A. M. Pirttilä. *Methylobacterium*-Induced Endphyte Community Changes Correspond with Protection of Plants Against Pathogen Attack. 2012. *PLoS ONE*. Vol: 7 (10). Google Scholar. 14 Nov. 2016.

Ardanov, P., L. Ovacharenko, I. Zaets, N. Kozyrovska, and A. M. Pirttliä. Endophytic Bacteria Enhancing Growth and Disease Resistance of Potato (*Solanum tuberosum*). *Biological Control*. 2011. Eds 1: 56. pp. 43-49. Google Scholar. 5 Dec. 2016.

Balogh, B., J. B. Jones, F.B. Iriarte, and M.T. Momol. Phage Therapy for Plant Disease Control. *Curr Pharm Biotechnol.* 2010. Vol: 11 (1). pp. 48-57. https://www.ncbi.nlm.nih.gov/pubmed/20214607. 4 Dec. 2016.

Birch, R. G., Alvarez, A. M., and Patilm, S. S. 1981. A bacterial leaf spot caused in Yam bean by Pseudomonas syringae pv. phaseolicola. *Phytopathology.* Vol: 71. Pp. 1289-1293.

Bouza, L., Jacque, M., Sotta B., and Miginiac, E. Relations between auxin and cytokinin contents and *in vitro* rooting of tree Peony (*Paeonia suffruticosa* Andr.). *Plant Growth Regulation.* 1994. Vol: (15). pp. 69-73.

Boyer, F., Fichant, G., Berthod, J., Vanderbrouck, Y., Attree, I. Dissecting the Bacterial Type VI Secretion System by a Genome Wide *in Silico* Analysis: What Can be Learned From Available Microbial Genomic Resources? 2009. *BMC Genom*. Vol (10): pp. 104.

Clover Diseases. *University of Illinois College of Agriculture Cooperative Extensive Service and Vocational Agriculture Service.* 1982. https://archive.lib.msu.edu/DMC/extension_publications/e1692/e1692-1982.pdf. 5 Dec. 2016.

Costa, T., Felisberto-Rodrigues, C., Meri, A., Prevost, M., Redzij, A., Trokter, M., Wand aksman, G. Secretion Systems in Gram-Negative Bacteria: Structural and Mechanistic Insights. 2015. *Nature Rev Micro.* Vol (13): pp. 343-359.

Dickinson, M. Molecular Plant Pathology. BIOS Scientific Publishers. 2003.

Dianese, A., Ji, P., and Wilson, M. Nutritional Similarity Between Leaf-Associated Nonpathogenic Bacteria and the Pathogen is Not Predictive of Efficacy in Biological Control of Bacterial Spot of Tomato. 2003. *App Environ Microbiol.* Vol (69): pp. 3484-3491.

Delmotte, N., C. Knief, S. Chaffron, G. Innerebner, B. Roschitzki, R. Schlapbach, C. von Mering, and J. A. Vorholt. 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proc Natl Acad Sci USA.* Vol: 106. Pp. 16428-33.

Dourado, M.N., A. A. C. Neves, D. S. Santos, and W. L. Araújo. Biotechnological and Agronomical Potential of Endophytic Pink-Pigmented Methylotrophic *Methylobacterium*. *BioMed Res. Int.* 2015. http://dx.doi.org/10.1155/2015/909016. 24 Nov. 2016.

Drapcho, D. J., D. Sisterson, and Romesh Kumar. Nitrogen Fixation by Lightning activity in a Thunderstorm. *Atmospheric Environment.* Vol: 17 (4). pp. 729-734. 1983. http://www.sciencedirect.com/science/article/pii/0004698183904201. 5 Dec. 2016.

Dubeikovsky, A.N., E. A. Mordukhova, V. V. Kochetkov, F. Y. Polikarpova, and A. M Boronin. Growth Promotion of Blackcurrent Softwood Cuttings by Recombinant Strain *Pseudomonas fluorescens* BSP53a Synthesizing an Increased Amount of Indole-3-Acetic Acid. *Soil Biol. Biochem.*. 1993. Vol: 25 (5). pp. 1277-1281. Google Scholar. 4 Dec. 2016.

Eaton, A. How Insecticides Work. *University of New Hampshire Cooperative Extension.* 2016. https://extension.unh.edu/resources/files/resource000504_rep526.pdf. 4 Dec. 2016.

Enya, J., H. Shinohara, S. Yoshida, T. T. H. Negishi, K. Suyama, and S. Tsushima. 2007. Culturable leaf-associated bacteria on tomato plants and their potential as biological control agents. Microb Ecol. Vol: 53. Pp. 524-536.

Erolani, G.L. 1991. Distribution of epiphytic bacteria on olive leaves and the influence of leaf age and sampling time. Microb. Ecol. Vol: 21. pp. 35-38.

Fett, W. F., and M. F. Dunn. Exopolysacchardies Produced by Phytopathogenic *Pseudomonas syringae* Pathovars in Infected Leaves of Susceptible Hosts. *Plant. Physiol.* Vol: 80. pp. 5-9. 1989. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1055789/pdf/plntphys00635-0020.pdf. 5 Dec. 2016.

Fernandez-Sanz , AM, Rodicio, M.R., Gonzalez, A. J. 2016. *Pseudomonas syrinage* pv. *phaseolicola* isolated from weeds in bean crop fields. *Lett Appl Microbiol.* Vol: 62(4). Pp. 344-8.

Gallique, M., Bouteiller, M., and Merieau, A. The Type VI Secretion System: A Dynamic System for Bacterial Communication. 2017. *Frontiers*. https://doi.org/10.3389/fmicb.2017.01454.

Glick, B. Plant-Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica.* 2012. http://dx.doi.org/10.6064/2012/963401. 24 Nov. 2016.

Glick, B. R., D. M. Penrose, and L. Jiping. A Model for the Lowering of Plant Ethylene Concentrations by Plant Growth-Promoting Bacteria. *J. theor. Biol.* 1997. Vol: 190. pp. 63-68.

Green Manure. *Encyclopaedia Britannica*. 2016. https://www.britannica.com/topic/green-manure. 1 Dec. 2016.

Hardoim, P.R., L. S. van Overbeek, and J. D. van Elsas. Properties of Bacterial Endophytes and Their Proposed Rule in Plant Growth. *Trends Microbiol.* 2008. Vol: 16 (10). pp. 463-471. Google Scholar. 30 Nov. 2016.

Hassan, Eman and El-Meneisy Afaf. (2014). Biocontrol of Halo Blight of Bean Caused by Pseudomonas phaseolicola. *International Journal of Virology*. Vol: 10. Pp. 235-242.

Hirano, S. S., and C. D. Upper. 2000. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*---a pathogen, ice nucleus, and epiphyte. *Microbiol. Mol. Biol. Rev.* Vol: (64). pp. 624-653.

Hunter, P. J., and Taylor, J. D. 2006. Patterns of interaction between isolates of three pathovars of Pseudomonas syringae and accessions of a range of host and non-host legume species. *Plant Pathol*. Vol: 5. Pp. 46-53.

Idris, R., R. Trifonova, M. Puschenreiter, W. W. Wenzel, and A. Sessitsch. Bacterial Communities Associated with Flowering Plants of the Ni Hyperaccumulator *Thlaspi* geosingense. *Appl. Environ. Microbiol.* 2004. Vol: 70 (5). pp. 2667-2677.

Innerebner, G., Knief, C., and Vorholt, J. *Sphingomonas* strains protect *Arabidopsis thaliana* against leaf pathogenic *Pseudomonas syrinage* in a controlled model system. *Appl Environ Microbiol.* 2011. Vol: 77 (10). Pp. 3202-3210.

Ivanova, E., Doronina, N., Shepelyakovskaya, A., Laman, A., Brovko, F., Trotsenko, Yu. Faculative and Obligate Aerobic Methylobacteria Synthesize Cytokinins. *Mikrobiologiya.* 2000. Vol: 69. pp. 764-769. Mar. 2018.

Ivanova, E., Doronina N., Trotsenko, Y. Aerobic Methylobacteria Are capable of Synthesizing Auxins. *Mikrobiologiya.* 2001. Vol: 70. pp. 392-397. Mar. 2018.

Isleib, J, Diseases of Red Clover and Birdsfoot Trefoil. *Michigan State Extension*. http://msue.anr.msu.edu/news/diseases_of_red_clover_and_birdsfoot_trefoil. 2012. 30 Nov. 2016.

Jones, E., V. Salin, and G. W. Williams. Nisin and the Market for Commercial Bacteriocins. *TAMRC Consumer and Product Research Report.* http://ageconsearch.umn.edu/bitstream/90779/2/CP%2001%2005%20Nisin%20Report.pdf. 4 Dec. 2016.

Ji, P., and M. Wilson. Assessment of the Importance of Similarity in Carbon Source Utilization Profiles Between the Biological Control Agent and the Pathogen Control of Bacterial Speck of Tomato. 2002. Appl. Environ. Microbiol. Vol: 68. Pp. 4383-4389.

Kabbage, M., O. Yarden, M. B. Dickman. Pathogenic Attributes of *Sclerotinia sclerotiorum*: Switching From a Biotrophic to Nectrotrophic Lifestyle. *Plant Sci*. 2014. Vol: 233. pp. 53-60. http://www.sciencedirect.com/science/article/pii/S0168945214003057. 17 Nov. 2016.

Kamber, T., Pothier, J.F., Pelludat, C. *et al.* Role of the type VI secretion systems during disease interactions of *Erwinia amylovora* with its plant host. *BMC Genomics* **18,** 628 (2017). https://doi.org/10.1186/s12864-017-4010-1

Kennelly, M.M., F. M. Cazorla, A. Vicente, C. Ramos, and G. W. Sundin. *Pseudomonas syringae* Diseases of Fruit Trees. *APS.* 2007. http://apsjournals.apsnet.org/doi/pdf/10.1094/PD-91-0004. 5 Dec. 2016.

Kessel, G. Biological Control of *Botrytis* spp. By *Ulocladium atrum*, an Ecological Analysis. 1999. PhD Thesis, Wageningen University, The Netherlands. Pp. 155.

Knief, C., N. Delmotte, S. Chaffron, *et al*. Metaproteogenomic Analysis of Microbial Communities in the Phyllosphere and Rhizosphere of rice. *ISME J.* Vol: 6 (7). pp. 1378-1390. 2012. Google Scholar. 4 Dec. 2016.

Lacava, P.T., J. L. Azevedo, T.A. Miller, and J.S. Hartung. Interactions of *Xylella fastidiosa* and Endophytic Bacteria in Citrus: A Review. *Tree For. Sci. Biotech.*. pp. 40-48. 2009. https://naldc.nal.usda.gov/download/35047/PDF. 5 Dec. 2016.

Lacava, P.T., W. B. Li, W. L. Araújo, J. L. Azevedo, and J. S. Hartung. Rapid, Specific and Quantitative Assays for the Detection of the Endophytic Bacterium *Methylobacterium*

mesophilicum in Plants. *J. Microbiol. Methods.*. 2006. Vol: 65 (3). pp. 535-541. Google Scholar. 14 Nov. 2016.

LeFevere, G.H. R. M. Hozalski, and P. J. Novak. Root Exudate Enhanced Contaminant Desorption: An Abiotic Contribution to the Rhizosphere Effect. *ES&T.* Vol 47 (20). pp. 11545-11553. Google Scholar. 4 Dec. 2016.

Lin, J., Cheng, J., Chen, K., Guo, C., Zhang, W., Yang, X., Ding, W., Ma, L., Wang, Y., and Shen, X. The *IcmF3* Locus is Involved in Multiple Adaptation and Virulence Related Characteristics in *Pseudomonas aeruginosa* PAO1. 2015. *Front Cell Infect Microbiol*. DOI: 10.3389/fcimb.2015.00070. https://www.ncbi.nlm.nih.gov/pubmed/26484316. 5 May 2020.

Lindow, S.E., and J.H. Leveau. 2002. Phyllosphere Microbiology. Curr. Opin. Biotechnol. Vol: 13. pp. 238-243.

Link, V.H., and K. B. Johnson. White Mold. 2012. Plant Health Instructor. http://www.apsnet.org/edcenter/intropp/lessons/fungi/Ascomycetes/Pages/WhiteMold.aspx. 17 Nov. 2016.

Luo, Y., Zhou, M., Wang, F., Gao, J., Sheng, H., and An, L. Complete Genome Sequence of *Sphingomonas* sp. Cra20, a Drought Resistant and Growth Promoting Rhizobacteria. 2020. *Genomic*s. https://doi.org/10.1016.j.ygeno.2020.04.013. May 2020.

Madhaiyan, N., B. V. Suresh Reddy, R. Anandham, et al. Plant Growth-promoting Methylobacterium Induces Defense Responses in Groundnut (*Archia hypogaea*) Compared with Rot Pathogens. *Curr Microbiol.* 2006. Vol: 52 (4). pp. 270-276. Google Scholar. 6 Dec. 2016.

McGrath, M.T. What Are Fungicides. *The Plant Health Instructor.* 2004. http://www.apsnet.org/edcenter/intropp/topics/Pages/fungicides.aspx. 4 Dec. 2016.

Meena, P., M. Hungria, F. G. Barcellos, E. V. Bangel, P. N. Hess, and E. Martínez-Romero. Molecular Phylogeny Based on the 16S rRNA gene of Elite Rhizobial Strains Used in Brazilian Commericial Inoculants. *Sys. Appl. Microbiol.* 2006. Vol: 29 (4). pp. 315-332. 2006. Google Scholar. 14 Nov. 2016.

Melotto, M., W. Underwood, H. S. Yang, 2008. Role of Stomata in Plant Innate Immunity and Foliar Bacterial Diseases. *Ann. Rev. Phytopathol.*. Vol: 46. pp. 101-122. Google Scholar. 30 Nov. 2016.

Murugaiyan, Senthilkumar & Ramasamy, Krishnamoorthy. 2017. Isolation and Characterization of Tomato Leaf Phyllosphere Methylobacterium and Their Effect on Plant Growth. International Journal of Current Microbiology and Applied Sciences. 6. 2121-2136. 10.20546/ijcmas.2017.611.250.

Nigris, S., E. Baldan, M. Zottini, A. Squartini, and B. Baldan. Is the Bacterial Endophyte Community, Living in Glera (*Vitis vinifera*) Plants, Active in Biocontrol. *Endophytes for Plant Protection: State of the Art.* C. Schnerider, C. Leifert, and F. Feldmann, Eds. pp. 12-16, Deutsche Phytomedizinische Gesellschaft, 2013. Google Scholar. 5 Dec. 2016.

Newberry, Bhandari, Kemble, Sikora, and Potnis. Genome-resolved Metagenomics to Study Co-occurrence Patterns and Intraspecific Heterogeneity Among Plant Pathogen Metapopulations. 2020. *Environ Microbiol*. DOI: 10.1111/1462-2920.14989. 11 May 2020.

Omer, Z.S., R. Tombolini, and B. Gerhardson. Plant Colonization by Pink-Pigmented Facultative Methylotropic Bacteria (PPFMs). *FEMS Microbial Ecol.* Vol 47 (3). pp. 319-326. 2004. Google Scholar. 6 Dec. 2016.

Pieterse, C., Zamioudis, C., Berendesen, R., Weller, D., Van, S., and Bakker, P. Induced Systemic Resistance by Beneficial Microbes. 2014. *Ann. Rev. Phytopathol*. Vol (52): pp. 347-375.

Poorniammal, R., S. P. Sundaram, and K. Kumutha. *In Vitro* Biocontrol Activity of *Methylobacterium extorquens* Against Fungal Pathogens. *Int. J. Plant Prot.* Vol: 2. pp. 59-62. 2009. Google Scholar. 5 Dec. 2016.

Potnis, et al. *Xanthomonas perforans* Colonizaton Influences *Salomonella enterica* in the Tomato Phyllosphere. 2014. *Appl Environ Microbiol.* Vol: 80(10). pp. 3173-3180.

Patel, P. N., and Walker, J. C. 1965. Resistance in Phaseolus to halo blight. *Phytopathology*. Vol: 55. Pp. 889-894.

Pieterse, C., Zamioudis, C., Berendsen, R., Weller, D., Van Wees, S., and Bakker, P. Induced Systemic Resistance by Beneficial Microbes. 2014. Ann. Rev. Phytopathol. Vol (52): 347-75.

Prosen, D., Hatziloukas, E., Schaad, N.W. and Panopoulos, N.J. 1993. Specific detection of *Pseudomonas syringae* pv. *phaseolicola* DNA in bean seed by polymerase chain reaction-based amplification of a phaseolotoxin gene region. *Phytopathology*. Vol: 83. Pp. 965–970.

Red Clover. *Trifolium pretense.* SARE. University of Maryland. 2012. 20 Feb. 2018.

Sanders, J.W., J. W. Martin, M. Hooke, and J. Hooke. *Methylobacterium mesophilicum* Infection: Case Report and Literature Review of an Unusual Opportunistic Pathogen. *Clin. Infect. Dis.* 2000. Vol: 30(6). pp. 936-938. Google Scholar. 24 Nov. 2016.

Schaeuer, S., and U. Kutschera. A Novel Growth-Promoting Microbe. *Methylobacterium funariae* sp. nov., isolated from the leaf surface of a common moss. *Plant Signal. Behav.* 2011. Vol: 6 (4). pp. 510-515. Google Scholar. 14 Nov. 2016.

Schlaepii, K., and D. Bulgarelii. The Plant Microbiome at Work. *Mol Plant Microbe Interact.* Vol: 3. pp. 212-217. 2015. https://www.ncbi.nlm.nih.gov/pubmed/25514681. 4 Dec. 2016.

Scholz-Schroeder, B.K., J.D. Soule, and D. C. Gross. The *sypA, sypS*, and *sypC* Synthetase Genes Encode Twenty-two Modules Involved in the Nonribosomal Peptide Synthesis of Syringopeptin by *Pseudomonas syringae* pv. syringae B301D. *Mol Plant Microbe Interact*. Vol: 16. pp. 271-80. 2003. Google Scholar. 30 Nov. 2016.

Schluter, J., C. D. Nadell, B. L. Bassler, K. R. Foster. Adhesion as a Weapon in Microbial Competition. *ISME J.* 2015. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4268496/. 5 Dec. 2016.

Simon, U.K., R. Bauer, and F. Oberwinkler. The Unique Cellular Interaction Between the Leaf Pathogen *Cymadothea trifolii* and *Trifolium repens. Mycologia*. 2004. Vol: 96 (6). pp. 1209-1217. http://www.mycologia.org/content/96/6/1209.full. 14 Nov. 2016.

Stacey, G., and N. T. Keen. *Plant Microbe Interactions*. 1996. https://books.google.com/books?id=s-slEOL88b0C&pg=PA48&lpg=PA48&dq=Phaseolotoxin+in+plants+what+does+it&source=bl&ots=vMQs9fresE&sig=98AkqrXR_QF7jE-_DWB0Kro-fKc&hl=en&sa=X&ved=0ahUKEwjiuKjWyMXQAhVDyyYKHUahBK4Q6AEIRzAI#v=onepage&q=Phaseolotoxin%20in%20plants%20what%20does%20it&f=false. 30 Nov. 2006.

Strayer-Scherer, A., Liao, Y., Abrahamian, P., Timilsina, S., Paret, M., Momol, T, Jones, J., and Vallad, G. Integrated Management of Bacterial Spot on Tomato in Florida. 2011. IFAS Extension. UF. 6 Aug. 2019.

Su, P., Zhang, D., Zhang, Z., Chen, A., Hamid, M., Li, C., Du, J., Cheng, J., Tan, X., Zhen, L., Zhen, L., Zhai, Z., Tang, W., Chen, J., Zhou, X., and Liu, Y. Characterization of *Rhodopseudomonas palustris* Population Dynamics on Tomato Phyllopshere and

Induction of Plant Resistance to Tobacco Mosaic Virus. 2019. *Society for Applied Microbiology*. https://sfamjournals.onlinelibrary.wiley.com/doi/10.1111/1751-7915.13486. 11 May 2020.

Subramania, S. and D. L. Smith. Bacteriocins From the Rhizosphere Microbiome-From an Agriculture Perspective. 2015. *Plant Sci*. http://journal.frontiersin.org/article/10.3389/fpls.2015.00909/full. 4 Dec. 2016.

Sy, A., A. C. Timmers, C. Knief, and J. A. Vorholt. Methylotrophic Metabolism is Advantageous for *Methylobacterium extorquens* During Colonization of *Medicago truncatula* Under Competitive Conditions. *App. Environ. Microbiol.* 2005. Vol: 71 (11). pp. 7245-7252. Google Scholar. 16 Nov. 2016.

Sy, A., E. Giraud, P. Jourand, *et al.* Methylotrophic *Methylobacterium* Bacteria Nodulate and Fix Nitrogen in Symbiosis with Legumes. *J. Bacteriol.*. 2001. Vol: 183 (1). pp. 214-220. Google Scholar. 4 Dec. 2016.

Stockwell, V.O., and B. Duffy. Use of Antibiotics in Plant Agriculture. *Rev Sci Tech.* Vol: 31 (1). pp. 2002. 199-210. https://www.ncbi.nlm.nih.gov/pubmed/22849276. 4 Dec. 2016.

Tani, A., Y. Takai, I. Suzukawa, M. Akita, H. Murase, and K. Kimbara. Practical Application of Methanol-mediated Mutualistic Symbiosis Between *Methylobacterium* Species and a Roof Greening Moss, *Racomitrium japonicum*. *PLoS ONE*. 2012. Vol: 7 (3). Article ID e33800. 5 Dec. 2016.

Taylor, J. D., Teverson, D. M., Allen, D. J., and Pastor-Corrales, M. A. 1996. Identification and origin of races of Pseudomonas syringae pv. phaseolicola from Africa and other bean growing areas. *Plant Pathol.* Vol: 45. Pp. 469-478.

Thompson, I. P., M.J. Bailey, R. J. Ellis, A.K. Lilley, P. J. McCormack, K. J. Purdy, and P.B. Rainey. 1995. Short-term community dynamics in the phyllosphere microbiology of field grown sugar beet. *FEMS Microbiol*. Ecol. Vol: 16. pp. 205-211.

Tian, Y., Zhao, Y., Wu., X., Liu, F., Hu, B., and Walcott, R. R. The Type VI Protein Secretion System Contributes to Biolfilm Formation and Seed-to-seedling Transmission of *A. eidovorax citrulli* on Melon. 2015. *Mol Plant Pathol.* Vol: 16(1). Pp. 38-47.

Tsavkelova, E. A., T. A. Cherdyntseva, S. G. Botina, and A. I. Netrusov. 2007. Bacteria associated with orchid roots and microbial production of auxin. Microbiol Res. Vol: 162. Pp. 69-76.

Völksch, B., and H. Weingart. Toxin Production by Pathovars of *Pseudomonas syringae* and Their Antagonistic Activities Against Epiphytic Microorganisms. *J Basic Microbiol.* 1998. https://www.ncbi.nlm.nih.gov/pubmed/9637014. 4 Nov. 2016.

Warren, and L.V. Gusta. *Biological Ice Nucleation and Its Applications.* pp. 41-61.

Wellner, S., N. Lodders, and P. Kämpfer. *Methylobacterium cerastii.* Isolated from the Leaf Surface of *Cerastium holosteoides*. *Int. J. Syst. Evol. Microbiol.* 2012. Vol: 62 (4). pp. 917-924. Google Scholar. 4 Dec. 2016.

Wilson, M., and Lindow, S. E. Coexistence among epiphytic bacterial populations mediated through nutritional resource partitioning. *Appl. Environ. Microbiol*. 1994. Vol (6): pp. 4468-4477. 4 Aug. 2019.

Wilson, M., and Lindow, S. E. Ecological Similarity and Coexistence of Epiphytic Ice-nucleating (Ice+) *Pseudomonas syringae* Strains and a Non-ice-nucleating (Ice-) Biological Control Agent. *Appl. Environ. Microbiol.* 1993. 60: 3128-3137.

Woojong, Y., S. Sundaram, K. Seshadri, K. Kiyoon, L. Gillseung, and S. Tongmin. Ethylene Emission and PR Protein Synthesis in ACC Deaminase Producing *Methylobacterium* spp. Inoculated Tomato Plants (*Lycopersicon esculentum* Mill.) Challenged with *Ralstonia solanacearum* Under Greenhouse Conditions. *Plant Physiol Biochem.* 2013. Vol: 67. pp. 95-104.

Why is Seed Health Important. *International Seed Federation.* 2015. http://www.worldseed.org/our-work/phytosanitary-matters/seed-health/. 4 Dec. 2016.

Yabuuchi, E., and Kosako Y., 2005. Sphingomonas. *Bergey's Manual of Systematic Bacteriology. The Alpha-, Beta-, Delta-, and Epsilonproteobacteria.* 2nd ed., Vol. 2, part C. Pp. 234-158. Springer, East Lansing.

Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T. and Yamamoto, H. 1990. Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas*. *Microbioland Immunol.. Vol:* 34. Pp. 99– 119.

Zhu, H. and S. J. Sun. Inhibition of Bacterial Quorum Sensing-regulated Behaviors by *Tremella fuciformis* Extract. *Curr Microbiol.* 2008. Vol: 57 (5). pp. 418-422.

APPENDICES

Appendix A
Colony Morphology Pictures

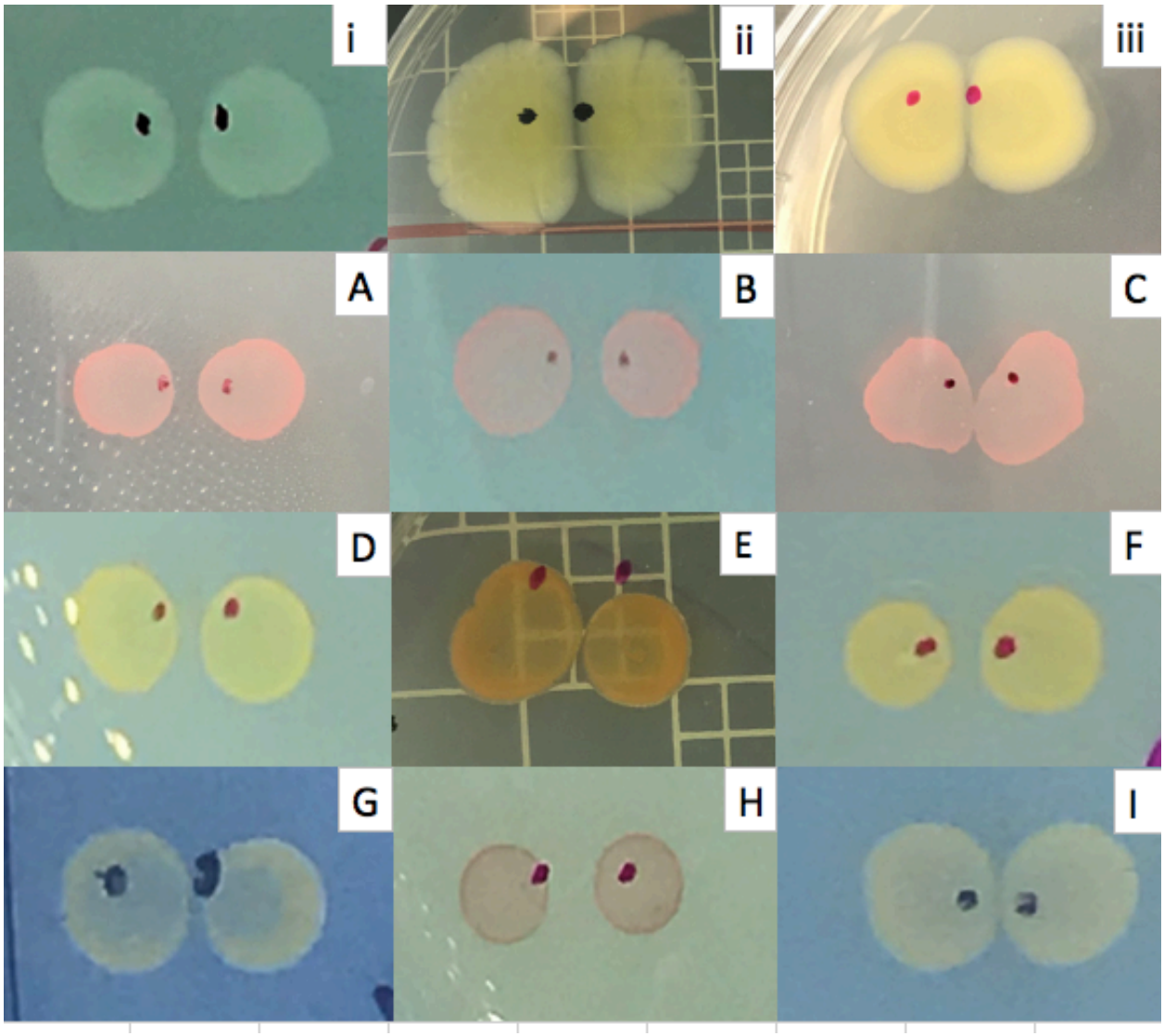


Figure 7. R2a Interactions: Control Photos. 1X10^8 CFU/mL bacterial suspension on R2a plate for controls. *P. syringae* pv. *tomato* 99B799 (i) interactions observed at 0.25cm, but the others were observed at 0.5cm. ii) *X. perforans* wild-type, (iii) *X. perforans* T6SS mutant, (A) *Methylobacterium* sp. AN12, (B) *Methylobacterium* sp. ApIIE, (C) *Methylobacterium* sp. AN17, (D) *S. adhaesiva* NBRC 15099, (E) *S. mucoissima* DSM 17494, (F) *S. taxi* 55669, (G) *Sphingomonas* mix (all three), (H) Methylobacteria mix (all three), and *Sphingomonas* mix with Methyobacteria mix (all six) on R2a plate. Interactions observed for one week at 22-25°C.

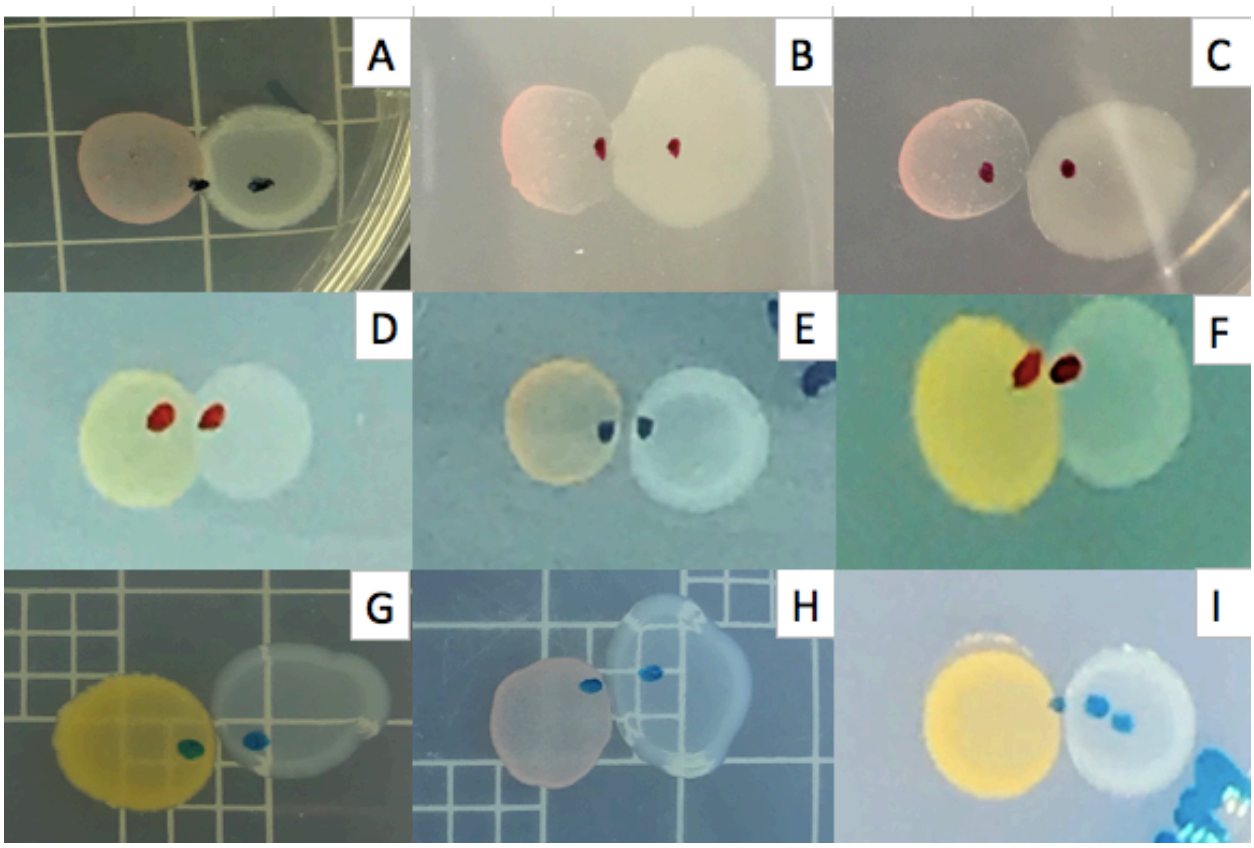


Figure 8. R2a interactions: *P. syringae* Photos. *P. syringae* pv. *tomato* 99B79 (right), plated 0.25cm apart from red clover associated bacteria. (A) *Methylobacterium* sp. AN12, (B) *Methylobacterium* sp. ApIIE, (C) *Methylobacterium* sp. AN17, (D) *S. adhaesiva* NBRC 15099, (E) *S. mucoissima* DSM 17494, (F) *S. taxi* 55669, (G) *Sphingomonas* mix (all three), (H) Methylobacteria mix (all three), and *Sphingomonas* mix with Methyobacteria mix (all six) on R2a plate. All bacterial suspensions were $1X10^8$ CFU/mL on R2a plates, and interactions observed for one week at 22-25°C.

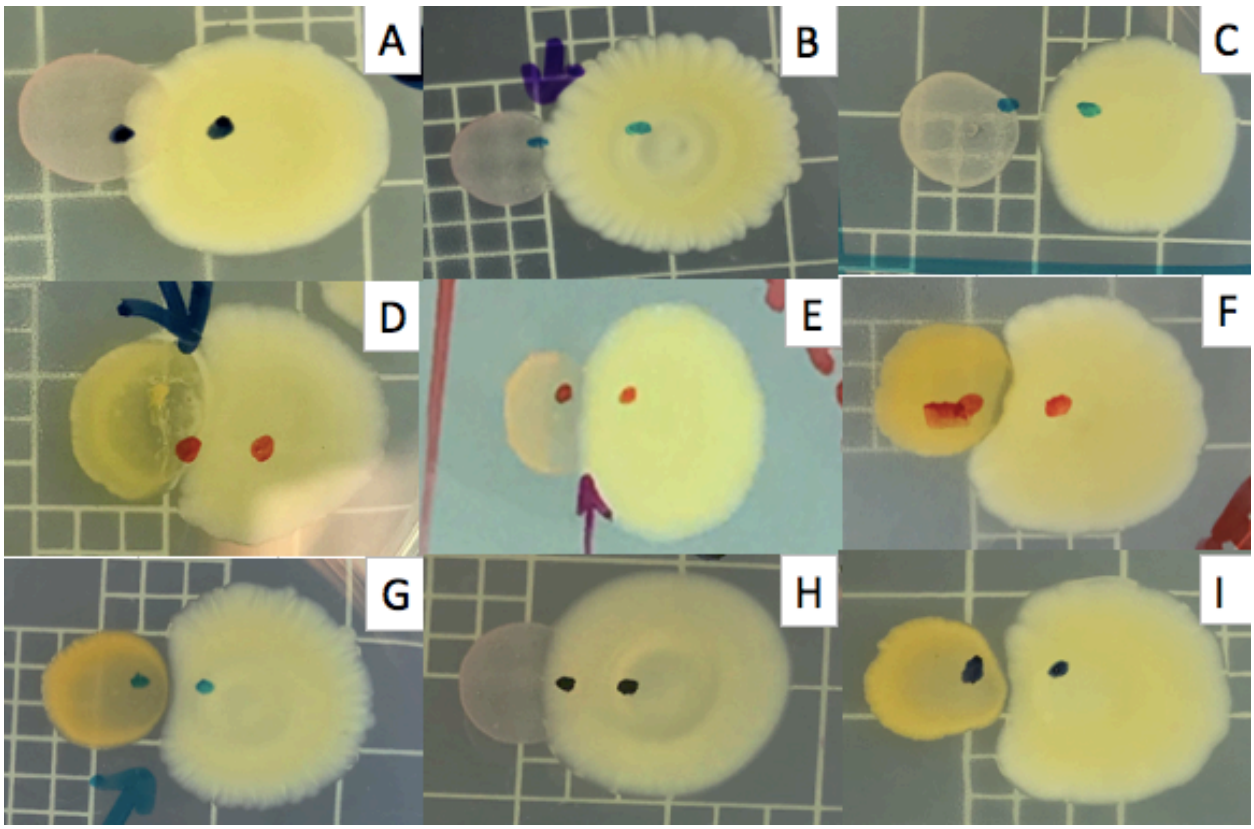


Figure 8. R2a Interactions: *X. perforans* Mutant Photos. *X. perforans* T6SS mutant (right), plated 0.5cm apart from red clover associated bacteria. (A) *Methylobacterium* sp. AN12, (B) *Methylobacterium* sp. ApIIE, (C) *Methylobacterium* sp. AN17, (D) *S. adhaesiva* NBRC 15099, (E) *S. mucoissima* DSM 17494, (F) *S. taxi* 55669, (G) *Sphingomonas* mix (all three), (H) Methylobacteria mix (all three), and *Sphingomonas* mix with Methyobacteria mix (all six) on R2a plate. All bacterial suspensions were $1X10^8$ CFU/mL on R2a plates, and interactions observed for one week at 22-25°C.

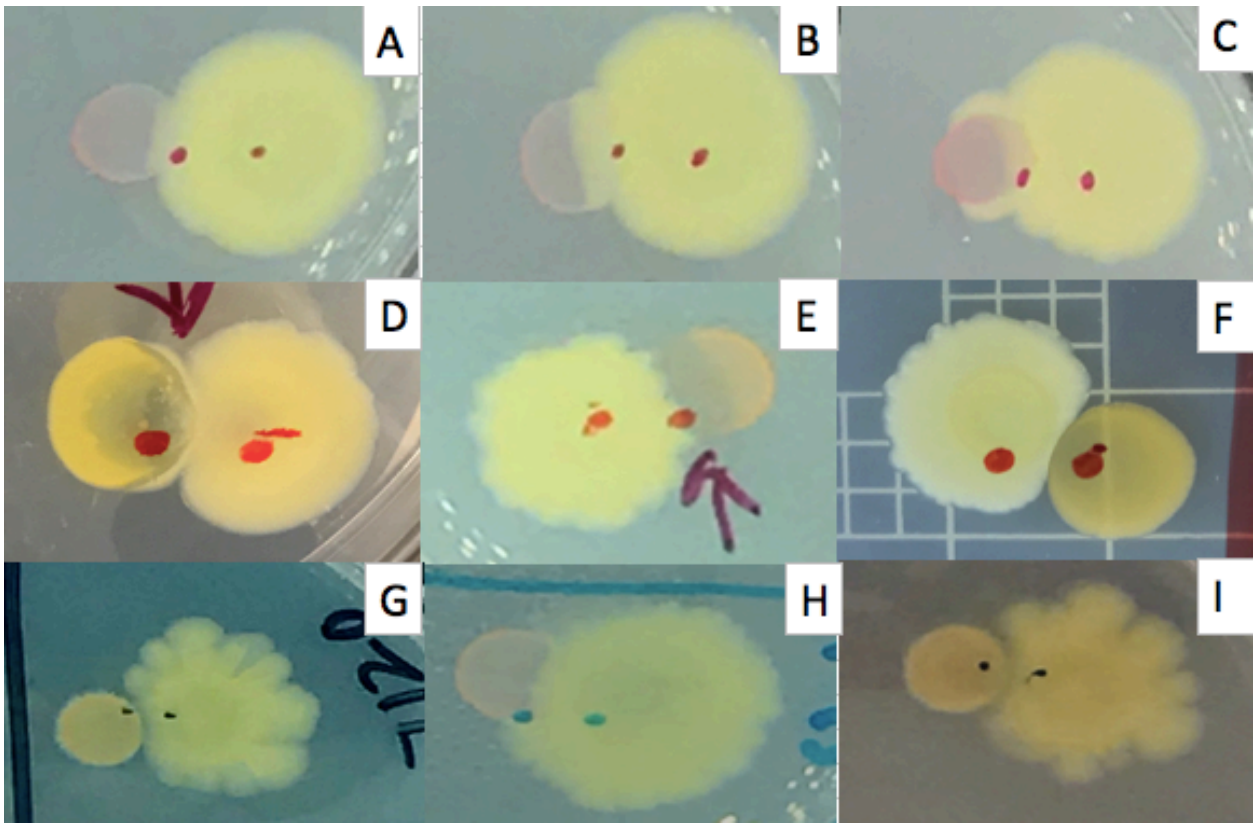


Figure 9. R2a Interactions: *X. perforans* Wild-Type Photos. *X. perforans* wild-type (right), plated 0.5cm apart from red clover associated bacteria. (A) *Methylobacterium* sp. AN12, (B) *Methylobacterium* sp. ApIIE, (C) *Methylobacterium adheasivum*, (D) *S. adhaesiva* NBRC 15099, (E) *S. mucoissima* DSM 17494, (F) *S. taxi* 55669, (G) *Sphingomonas* mix (all three), (H) Methylobacteria mix (all three), and *Sphingomonas* mix with Methyobacteria mix (all six) on R2a plate. All bacterial suspensions were $1X10^8$ CFU/mL on R2a plates, and interactions observed for one week at 22-25°C.

Appendix B

Table 7. Motility Assay Diameters

Treatment	Day 2 Average Diameter (cm)	Day 3 Average Diameter (cm)
	1.2	2.6
Xanthomonas MUT Control	2.3	2.5
Xanthomonas MUT Control	2.4	2.5
Xanthomonas MUT Control	1.6	1.6
Methylobacterium sp. AN17 + Xanthomonas MUT	1.4	1.5
Methylobacterium sp. AN17 + Xanthomonas MUT	1.6	1.6
Methylobacterium sp. AN17 + Xanthomonas MUT	1.3	1.3
Methylobacterium sp. AN12 + Xanthomonas MUT	1.9	1.9
Methylobacterium sp. AN12 + Xanthomonas MUT	1.9	1.9
Methylobacterium sp. AN12 + Xanthomonas MUT	2.2	2.2
Methylobacterium sp. IIE + Xanthomonas MUT	1.7	1.7
Methylobacterium sp. IIE + Xanthomonas MUT	1.8	1.8
Methylobacterium sp. IIE + Xanthomonas MUT	1.9	1.9
Methylobacteria mix + Xanthomonas MUT	1.9	2.5
Methylobacteria mix + Xanthomonas MUT	2	2.2
Methylobacteria mix + Xanthomonas MUT	1.8	2
S. endophytica + Xanthomonas MUT	1.9	2
S. endophytica + Xanthomonas MUT	1.9	2
S. endophytica + Xanthomonas MUT	1.8	2.2
S. mucosissima DSM 17494 + Xanthomonas MUT	2.2	2.7
S. mucosissima DSM 17494 + Xanthomonas MUT	2.5	2.6
S. mucosissima DSM 17494 + Xanthomonas MUT	1.4	1.7
S. taxi ATCC 55669 + Xanthomonas MUT	1.6	1.6
S. taxi ATCC 55669 + Xanthomonas MUT	1.4	1.6
S. taxi ATCC 55669 + Xanthomonas MUT	1.6	1.7
Sphingomonas mix + Xanthomonas MUT	1.5	1.7
Sphingomonas mix + Xanthomonas MUT	1.4	1.6
Sphingomonas mix + Xanthomonas MUT	1.5	1.6
Sphingomonas and Methylobacteria mix + Xathomonas MUT	2.1	2.1
Sphingomonas and Methylobacteria mix + Xathomonas MUT	1.8	1.9
Sphingomonas and Methylobacteria mix + Xanthomonas MUT	2.3	2.3
Xanthomonas WT Control	2.1	2.2

Appendix B

Treatment	Day 2 Average Diameter (cm)	Day 3 Average Diameter (cm)
Xanthomonas WT Control	2.1	2.2
Xanthomonas WT Control	2.4	2.4
Methylobacterium sp. AN17 + Xanthomonas WT	2.3	2.3
Methylobacterium sp. AN17 + Xanthomonas WT	2.3	2.4
Methylobacterium sp. AN12 + Xanthomonas WT	2.4	2.4
Methylobacterium sp. AN12 + Xanthomonas WT	2.4	2.6
Methylobacterium sp. AN12 + Xanthomonas WT	2.1	2.2
Methylobacterium sp. IIE + Xanthomonas WT	2.1	2.2
Methylobacterium sp. IIE + Xanthomonas WT	2.4	2.4
Methylobacterium sp. IIE + Xanthomonas WT	2.8	2.8
Methylobacteria mix + Xanthomonas WT	2.5	2.5
Methylobacteria mix + Xanthomonas WT	2.1	2.1
Methylobacteria mix + Xanthomonas WT	2.2	2.2
S. adhaesiva + Xanthomonas WT	2.2	2.2
S. adhaesiva + Xanthomonas WT	1.5	1.7
S. adhaesiva + Xanthomonas WT	2.2	2.7
S. mucosissima DSM 17494 + Xanthomonas WT	2.4	2.5
S. mucosissima DSM 17494 + Xanthomonas WT	1.8	2.2
S. mucosissima DSM 17494 + Xanthomonas WT	2.2	2.3
S. taxi ATCC 55669 + Xanthomonas WT	2.1	2.2
S. taxi ATCC 55669 + Xanthomonas WT	2.4	2.9
S. taxi ATCC 55669 + Xanthomonas WT	2	2
Sphingomonas mix + Xanthomonas WT	2.1	2.1
Sphingomonas mix + Xanthomonas WT	2.1	2.1
Sphingomonas mix + Xanthomonas WT	2.1	2.6
Sphingomonas and Methylobacteria mix + Xathomonas WT	2.1	2.4
Sphingomonas and Methylobacteria mix + Xathomonas WT	2.2	2.4
Sphingomonas and Methylobacteria mix + Xanthomonas WT	2	2.7
	1.4	1.5

Appendix C

Seed-to-seedling Disease Scores (%) Trials 1 and 2

Table 8. X. *perforans* Wild-Type Growth Chamber Disease Score (%) Trial 1 and 2		
Treatment	Day 7	Day 14
X. perforans	T1: 10, 25, 20 T2: 10, 30, 15	T1: 60, 45, 50 T2: 55, 55, 55
Sphingomonas and *X. perforans*	T1:30, 20, 35 T2: 20, 30, 30	T1: 60, 45, 35 T2: 55, 50, 50
Methylobacterium and *X. perforans*	T1: 20, 30, 30 T2: 25, 25, 20	T1: 50, 50, 35 T2: 55, 40, 50
Sphingomonas, *Methylobacterium* and *X. perforans*	T1: 15, 30, 40 T2: 15, 35, 45	T1: 60, 70, 25 T2: 50, 45, 50

Table 9. *P. syringae* pv. tomato 99B799 Growth Chamber Treatment Disease Score (%) Trial 1 and 2		
Treatment	Day 7	Day 14
P. syringae pv. *tomato*	T1: 1, 2, 1 T2: 0, 0, 0	T1: 1, 2, 1 T2: 0, 0, 0
Sphingomonas and *P. syringae* pv. *tomato*	T1: 2, 1, 2 T2: 0, 0, 0	T1: 2, 1, 2 T2: 0, 0, 0
Methylobacterium and *P. syringae* pv. *tomato*	T1: 1, 5, 1 T2: 0, 0, 0	T1: 1, 5, 1 T2: 0, 0, 0
Sphingomonas, *Methylobacterium* and *P. syringae* pv. *tomato*	T1: 2, 1, 1 T2: 0, 0, 0	T1: 2, 1, 1 T2: 0, 0, 0

Table 10. *P. syringae* pv. tomato 99B799 Growth Chamber Treatment Disease Score (%) Results: Trial 1 and 2.		
Treatment	Day 7	Day 14
P. syringae pv. *tomato*	0	0
Sphingomonas and *P. syringae* pv. *tomato*	0	0
Methylobacterium and *P. syringae* pv. *tomato*	0	0
Sphingomonas, *Methylobacterium* and *P. syringae* pv. *tomato*	0	0

Appendix D

Bacterial Counts	Colony Counts (CFU/cm^2)	LOG10
Xanthomonas MUT + Sphingomonas	3.40E+03	3.5314789
Xanthomonas MUT + Sphingomonas	5.50E+03	3.7403627
Xanthomonas MUT + Sphingomonas	5.50E+02	2.7403627
Xanthomonas MUT + Sphingomonas	1.65E+03	3.2174839
Xanthomonas MUT Control	3.74E+06	6.5728716
Xanthomonas MUT Control	1.22E+06	6.0863598
Xanthomonas MUT Control	5.80E+06	6.763428
Xanthomonas WT + Sphingomonas	5.69E+06	6.7551123
Xanthomonas WT + Sphingomonas	1.11E+07	7.045323
Xanthomonas WT + Sphingomonas	6.33E+06	6.8014037
Xanthomonas WT + Sphingomonas	1.81E+06	6.2576786
Xanthomonas WT Control	2.29E+07	7.3598355
Xanthomonas WT Control	2.14E+07	7.3304138
Xanthomonas WT Control	1.94E+07	7.2878017
Xanthomonas WT Control	5.48E+06	6.7387806
Xanthomonas WT Control	3.18E+07	7.5024271
MgSO4 Control	5.80E+05	5.763428
MgSO4 Control	9.10E+05	5.9590414
MgSO4 Control	6.33E+05	5.8014037
MgSO4 Control	5.27E+05	5.7218106
MgSO4 Control	7.49E+05	5.8744818

Appendix D

Bacterial Counts

Treatment: Trial 2	Colony Counts (CFU/cm^2)	LOG10
Xanthomonas MUT + Sphingomonas	5.50E+03	3.7403627
Xanthomonas MUT + Sphingomonas	4.50E+03	3.6532125
Xanthomonas MUT + Sphingomonas	5.00E+03	3.69897
Xanthomonas MUT + Sphingomonas	8.20E+03	3.9138139
Xanthomonas MUT Control	4.75E+06	6.6766936
Xanthomonas MUT Control	4.64E+06	6.666518
Xanthomonas MUT Control	5.27E+06	6.7218106
Xanthomonas MUT Control	1.22E+06	6.0863598
Xanthomonas MUT Control	1.22E+06	6.0863598
Xanthomonas WT + Sphingomonas	6.85E+06	6.8356906
Xanthomonas WT + Sphingomonas	5.80E+06	6.763428
Xanthomonas WT + Sphingomonas	2.04E+07	7.3096302
Xanthomonas WT + Sphingomonas	3.09E+07	7.4899585
Xanthomonas WT Control	2.69E+07	7.4297523
Xanthomonas WT Control	6.12E+06	6.7867514
Xanthomonas WT Control	2.79E+07	7.4456042
Xanthomonas WT Control	6.22E+06	6.7937904
Xanthomonas WT Control	2.25E+06	6.3521825
MgSO4 Control	3.00E+03	3.4771213
MgSO4 Control	3.25E+03	3.5118834
MgSO4 Control	3.05E+03	3.4842998
MgSO4 Control	3.00E+03	3.4771213

Appendix D

Disease (%) Day 21 Trial 1 and 2 for Greenhouse Study

Trial	Treatment	(%) Disease Day 21	Scale
Trial 1	Xanthomonas WT + Sphingomonas	25	1
Trial 1	Xanthomonas WT + Sphingomonas	12	1
Trial 1	Xanthomonas WT + Sphingomonas	15	1
Trial 1	Xanthomonas WT + Sphingomonas	30	1
Trial 1	Xanthomonas WT + Sphingomonas	20	1
Trial 2	Xanthomonas WT + Sphingomonas	10	1
Trial 2	Xanthomonas WT + Sphingomonas	15	1
Trial 2	Xanthomonas WT + Sphingomonas	25	1
Trial 2	Xanthomonas WT + Sphingomonas	15	1
Trial 2	Xanthomonas WT + Sphingomonas	15	1
		(%) Disease Day 21	Scale
Trial 1	Xanthomonas WT Control	65	3
Trial 1	Xanthomonas WT Control	55	3
Trial 1	Xanthomonas WT Control	60	3
Trial 1	Xanthomonas WT Control	65	3
Trial 1	Xanthomonas WT Control	70	3
Trial 2	Xanthomonas WT Control	30	1
Trial 2	Xanthomonas WT Control	35	2
Trial 2	Xanthomonas WT Control	40	3

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	Xanthomonas WT Control	25	
Trial 2			
Trial 2	Xanthomonas WT Control	40	3

		(%) Disease Day 21	Scale
Trial 1	MgSo4 Control	0	0
Trial 1	MgSo4 Control	0	0
Trial 1	MgSo4 Control	0	0
Trial 1	MgSo4 Control	0	0
Trial 1	MgSo4 Control	0	0
Trial 2	MgSo4 Control	0	0
Trial 2	MgSo4 Control	0	0
Trial 2	MgSo4 Control	0	0
Trial 2	MgSo4 Control	0	0
Trial 2	MgSo4 Control	0	0

		(%) Disease Day 21	Scale
Trial 1	Xanthomonas MUT Control	60	3
Trial 1	Xanthomonas MUT Control	50	3
Trial 1	Xanthomonas MUT Control	55	3
Trial 1	Xanthomonas MUT Control	60	3
Trial 1	Xanthomonas MUT Control	40	2
Trial 2	Xanthomonas MUT Control	30	1
Trial 2	Xanthomonas MUT Control	25	1
Trial 2	Xanthomonas MUT Control	30	1
Trial 2	Xanthomonas MUT Control	20	1
Trial 2	Xanthomonas MUT Control	20	1

		(%) Disease Day 21	Scale
Trial 1	Xanthomonas MUT + Sphingomonas	5	1
Trial 1	Xanthomonas MUT + Sphingomonas	5	1
Trial 1	Xanthomonas MUT + Sphingomonas	15	1

Appendix D

Trial 1	Xanthomonas MUT + Sphingomonas	10	1
Trial 1	Xanthomonas MUT + Sphingomonas	20	1
Trial 2	Xanthomonas MUT +Sphingomonas	15	1
Trial 2	Xanthomonas MUT +Sphingomonas	15	1
Trial 2	Xanthomonas MUT +Sphingomonas	15	1
Trial 2	Xanthomonas MUT +Sphingomonas	10	1
Trial 2	Xanthomonas MUT + Sphingomonas	10	1

Appendix D

		(%) Disease Day 21	Scale
Trial 1	Sphingomonas Control	0	0
Trial 1	Sphingomonas Control	0	0
Trial 1	Sphingomonas Control	0	0
Trial 1	Sphingomonas Control	0	0
Trial 1	Sphingomonas Control	0	0
Trial 2	Sphingomonas Control	0	0
Trial 2	Sphingomonas Control	0	0
Trial 2	Sphingomonas Control	0	0
Trial 2	Sphingomonas Control	0	0
Trial 2	Sphingomonas Control	0	0

Appendix E

Carbon Utilization Profile

Nutritional Profile of *P. syringae* 99B799

Carbon Sources Used	Carbon Sources Not Used
Dextrin	D-Maltose
Gentibiose	D-Trehalose
D-Turanose	D-Cellobiose
α-D-Lactose	Sucrose
D-Melibiose	Stachynose
D-Salicin	D-Raffinose
N-Acetyl-D-Glucosamine	α-D-Glucose
N-Acetyl-β-D-Mannosamine	D-Sorbitol
N-Acetyl-D-Galactosamine	D-Aspartic Acid
N-Acetyl-Neuraminic Acid	D-Serine
β-Methyl-D-Glucoside	L-Galactonic Acid Lactone
D-Mannose	P-Hydroxy-Phenylacetic Acid
D-Fructose	Methyl Pyruvate
D-Galactose	Bromo-Succinic Acid
3-Methyl Glucose	ɣ-Amino-Butryic Acid
D-Fucose	α-Hydroxy-Butyric Acid
L-Fucose	α-Keto-Butyric Acid
L-Rhamnose	Propionic Acid
Inosine	Citric Acid
D-Mannitol	
D-Arabitol	
Myo-Insitol	
Glycerol	
D-Glucose-6-PO4	
D-Fructose-6-PO4	
D-Saccharic Acid	
Gelatin	
Glycyl-L-Proline	
L-Alanine	
L-Arginine	
L-Aspartic Acid	
L-Glutamic Acid	
L-Histidine	
L-Pyroglutamic Acid	
L-Serine	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *P. syringae* 99B799

Carbon Sources Used	**Carbon Sources Not Used**
Pectin	
D-Glucuronic Acid	
D-Galacturonic Acid	
D-Gluconic Acid	
Glucuronamide	
Mucic Acid	
Quinic Acid	
D-Lactic Acid Methyl Ester	
L-Lactic Acid	
α-Keto-Glutaric Acid	
D-Malic Acid	
L-Malic Acid	
Tween 40	
β-Hydroxy-D, L-Butryic Acid	
Acetoacetic Acid	
Acetic Acid	
Formic Acid	
Acetoacetic Acid	
Acetic Acid	
Formic Acid	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *X. perforans* wild-type

Carbon Sources Used	Carbon Sources Not Used
Dextrin	Stachynose
D-Maltose	β-Methyl-D-Glucoside
D-Trehalose	
D-Cellobiose	N-Acetyl-D-Galactosamine
Gentibiose	N-Acetyl-Neuraminic Acid
Sucrose	L-Fucose
D-Turanose	D-Sorbitol
D-Raffinose	Myo-Insitol
α-D-Lactose	D-Aspartic Acid
D-Melibiose	L-Pyroglutamic Acid
D-Salicin	P-Hydroxy-Phenylacetic Acid
α-D-Glucose	D-Lactic Acid Methyl Ester
N-Acetyl-β-D-Mannosamine	L-Lactic Acid
D-Mannose	ɣ-Amino-Butryic Acid
D-Fructose	
D-Galactose	
3-Methyl Glucose	
D-Fucose	
L-Rhamnose	
Inosine	
D-Arabitol	
Glycerol	
D-Glucose-6-PO_4	
D-Fructose-6-PO4	
D-Serine	
Gelatin	
Glycyl-L-Proline	
L-Alanine	
L-Arginine	
L-Aspartic Acid	
L-Glutamic Acid	
L-Histidine	
Pectin	
D-Galacturonic Acid	
L-Galactonic Acid Lactone	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *X. perforans* wild-type

Carbon Sources Used	Carbon Sources Not Used
D-Gluconic Acid	
Glucuronamide	
Mucic Acid	
Quinic Acid	
D-Saccharic Acid	
Methyl Pyruvate	
Citric Acid	
α-Keto-Glutaric Acid	
D-Malic Acid	
L-Malic Acid	
Bromo-Succinic Acid	
Tween 40	
α-Hydroxy-Butyric Acid	
β-Hydroxy-D, L-Butryic Acid	
α-Keto-Butyric Acid	
Acetoacetic Acid	
Propionic Acid	
Acetic Acid	
Formic Acid	
D-Glucuronic Acid	
D-Mannitol	
N-Acetyl-D-Glucosamine	
L-Serine	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *S. taxi* 55669

Carbon Sources Used	Carbon Sources Not Used
Dextrin	D-Turanose
D-Maltose	D-Salicin
D-Trehalose	N-Acetyl-β-D-Mannosamine
D-Cellobiose	N-Acetyl-D-Galactosamine
Gentibiose	N-Acetyl-Neuraminic Acid
Sucrose	Inosine
Stachynose	D-Mannitol
D-Raffinose	Glycerol
α-D-Lactose	D-Glucose-6-PO_4
D-Melibiose	D-Serine
β-Methyl-D-Glucoside	L-Pyroglutamic Acid
N-Acetyl-Neuraminic Acid	Quinic Acid
α-D-Glucose	Methyl Pyruvate
D-Mannose	Bromo-Succinic Acid
D-Fructose	
D-Galactose	
3-Methyl Glucose	
D-Fucose	
L-Fucose	
L-Rhamnose	
D-Sorbitol	
D-Arabitol	
D-Fructose-6-PO4	
D-Aspartic Acid	
Gelatin	
Glycyl-L-Proline	
L-Alanine	
L-Arginine	
L-Aspartic Acid	
L-Glutamic Acid	
L-Histidine	
L-Serine	
Pectin	
D-Galacturonic Acid	
L-Galactonic Acid Lactone	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *S. taxi* 55669

Carbon Sources Used	Carbon Sources Not Used
D-Glucuronic Acid	
Glucuronamide	
D-Saccharic Acid	
P-Hydroxy-Phenylacetic Acid	
D-Lactic Acid Methyl Ester	
Citric Acid	
α-Keto-Glutaric Acid	
Tween 40	
γ-Amino-Butryic Acid	
α-Hydroxy-Butyric Acid	
α-Keto-Butyric Acid	
Acetoacetic Acid	
Propionic Acid	
Acetic Acid	
β-Hydroxy-D, L-Butryic Acid	
N-Acetyl-β-D-Mannosamine	
Myo-inositol	
D-Aspartic Acid	
D-Gluconic Acid	
Mucic Acid	
L-Lactic Acid	
L-Malic Acid	
D-Malic Acid	
Formic Acid	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *S. azotifigens* NBRC 15497

Carbon Sources Used	Carbon Sources Not Used
Dextrin	Stachynose
D-Maltose	N-Acetyl-β-D-Mannosamine
D-Maltose	N-Acetyl-D-Galactosamine
D-Trehalose	N-Acetyl-Neuraminic Acid
D-Cellobiose	L-Rhamnose
Gentibiose	Inosine
Sucrose	D-Sorbitol
D-Turanose	D-Mannitol
D-Raffinose	D-Arabitol
α-D-Lactose	Myo-Insitol
D-Melibiose	Glycerol
β-Methyl-D-Glucoside	L-Arginine
D-Salicin	L-Histidine
N-Acetyl-D-Glucosamine	L-Pyroglutamic Acid
α-D-Glucose	D-Serine
N-Acetyl-β-D-Mannosamine	D-Gluconic Acid
D-Mannose	Mucic Acid
D-Fructose	Quinic Acid
D-Galactose	D-Saccharic Acid
3-Methyl Glucose	D-Lactic Acid Methyl Ester
D-Fucose	Citric Acid
L-Fucose	α-Keto-Glutaric Acid
D-Glucose-6-PO_4	D-Malic Acid
Gelatin	L-Malic Acid
Glycyl-L-Proline	24
L-Alanine	D-Fructose-6-PO4
L-Aspartic Acid	D-Aspartic Acid
L-Glutamic Acid	
Pectin	
D-Galacturonic Acid	
L-Galactonic Acid Lactone	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *S. azotifigens* NBRC 15497

Carbon Sources Used	Carbon Sources Not Used
D-Glucuronic Acid	
Glucuronamide	
P-Hydroxy-Phenylacetic Acid	
Methyl Pyruvate	
L-Lactic Acid	
Tween 40	
ɣ-Amino-Butryic Acid	
α-Hydroxy-Butyric Acid	
β-Hydroxy-D, L-Butryic Acid	
α-Keto-Butyric Acid	
Acetoacetic Acid	
Propionic Acid	
Acetic Acid	
Formic Acid	
L-Serine	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *S. mucoissima* DSM 17494

Carbon Sources Used	Carbon Sources Not Used
Dextrin	α-D-Lactose
D-Maltose	D-Melibiose
D-Trehalose	β-Methyl-D-Glucoside
D-Cellobiose	D-Salicin
Gentibiose	N-Acetyl-D-Glucosamine
Sucrose	N-Acetyl-β-D-Mannosamine
D-Turanose	N-Acetyl-D-Galactosamine
Stachynose	N-Acetyl-Neuraminic Acid
D-Raffinose	D-Mannose
α-D-Glucose	D-Fructose
D-Sorbitol	D-Galactose
Gelatin	3-Methyl Glucose
Glycyl-L-Proline	D-Fucose
L-Aspartic Acid	L-Fucose
L-Glutamic Acid	L-Rhamnose
Pectin	Inosine
D-Galacturonic Acid	D-Mannitol
Quinic Acid	D-Arabitol
D-Lactic Acid Methyl Ester	Myo-Insitol
α-Keto-Glutaric Acid	Glycerol
D-Malic Acid	D-Glucose-6-PO_4
L-Malic Acid	D-Fructose-6-PO4
Tween 40	D-Aspartic Acid
ɣ-Amino-Butryic Acid	L-Alanine
β-Hydroxy-D, L-Butryic Acid	L-Arginine
α-Keto-Glutaric Acid	L-Histidine
Acetoacetic Acid	L-Pyroglutamic Acid
Acetic Acid	L-Serine
	D-Glucuronic Acid
	L-Galactonic Acid Lactone
	D-Gluconic Acid

Appendix E

Carbon Utilization Profile

Nutritional Profile of *S. mucoissima* DSM 17494

Carbon Sources Used	Carbon Sources Not Used
	Glucuronamide
	Mucic Acid
	D-Succharic Acid
	P-Hydroxy-Phenylacetic Acid
	Methyl Pyruvate
	L-Lactic Acid
	Citric Acid
	Bromo-Succinic Acid
	α-Hydroxy-Butyric Acid
	Propionic Acid
	Formic Acid
	D-Serine

Appendix E

Carbon Utilization Profile

Nutritional Profile of *S. adhaesiva* NBRC 15099

Carbon Sources Used	Carbon Sources Not Used
Dextrin	D-Maltose
Gentibiose	D-Trehalose
D-Turanose	D-Cellobiose
β-Methyl-D-Glucoside	Sucrose
α-D-Lactose	Stachynose
D-Melibiose	D-Raffinose
D-Salicin	α-D-Glucose
N-Acetyl-D-Glucosamine	D-Sorbitol
N-Acetyl-β-D-Mannosamine	Myo-Insitol
N-Acetyl-D-Galactosamine	Glycerol
N-Acetyl-Neuraminic Acid	D-Aspartic Acid
N-Acetyl-D-Galactosamine	D-Serine
N-Acetyl-Neuraminic Acid	L-Galactonic Acid Lactone
D-Mannose	P-Hydroxy-Phenylacetic Acid
D-Fructose	Methyl Pyruvate
D-Galactose	Citric Acid
3-Methyl Glucose	D-Succharic Acid
D-Fucose	Bromo-Succinic Acid
L-Fucose	ɣ-Amino-Butryic Acid
L-Rhamnose	α-Hydroxy-Butyric Acid
Inosine	α-Keto-Butyric Acid
D-Mannitol	Propionic Acid
D-Arabitol	
D-Glucose-6-PO_4	
D-Fructose-6-PO4	
Gelatin	
Glycyl-L-Proline	
L-Alanine	
L-Arginine	
L-Aspartic Acid	
L-Glutamic Acid	
L-Histidine	
L-Pyroglutamic Acid	
L-Serine	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *S. adhaesiva* NBRC 15099

Carbon Sources Used	Carbon Sources Not Used
D-Galacturonic Acid	
D-Glucuronic Acid	
D-Gluconic Acid	
Glucuronamide	
Mucic Acid	
Quinic Acid	
D-Lactic Acid Methyl Ester	
L-Lactic Acid	
α-Keto-Glutaric Acid	
D-Malic Acid	
L-Malic Acid	
Tween 40	
β-Hydroxy-D, L-Butryic Acid	
Acetoacetic Acid	
Acetic Acid	
Formic Acid	

Appendix E

Carbon Utilization Profile

Nutritional Profile of *Methylobacterium* sp. ApIIE

Carbon Sources Used	Carbon Sources Not Used
Dextrin	Stachynose
D-Maltose	D-Raffinose
D-Trehalose	D-Melibiose
Gentibiose	N-Acetyl-D-Glucosamine
D-Cellobiose	N-Acetyl-β-D-Mannosamine
Sucrose	N-Acetyl-D-Galactosamine
D-Turanose	D-Mannose
α-D-Lactose	3-Methyl Glucose
β-Methyl-D-Glucoside	D-Fucose
D-Salicin	L-Fucose
N-Acetyl-Neuraminic Acid	D-Sorbitol
α-D-Glucose	D-Mannitol
D-Fructose	Myo-Insitol
D-Galactose	D-Serine
L-Rhamnose	Gelatin
Inosine	Glycyl-L-Proline
D-Arabitol	L-Alanine
Glycerol	L-Arginine
D-Glucose-6-PO_4	L-Histidine
D-Fructose-6-PO4	L-Pyroglutamic Acid
D-Aspartic Acid	L-Serine
L-Aspartic Acid	D-Galacturonic Acid
L-Glutamic Acid	L-Galactonic Acid Lactone
Pectin	D-Glucuronic Acid
D-Gluconic Acid	Glucuronamide
Mucic Acid	Quinic Acid
D-Saccharic Acid	P-Hydroxy-Phenylacetic Acid
Methyl Pyruvate	D-Lactic Acid Methyl Ester
L-Lactic Acid	ɣ-Amino-Butryic Acid
Citric Acid	α-Hydroxy-Butyric Acid
α-Keto-Glutaric Acid	β-Hydroxy-D, L-Butryic Acid
D-Malic Acid	α-Keto-Butyric Acid
L-Malic Acid	Acetoacetic Acid

Appendix E

Carbon Utilization Profile

Nutritional Profile of *Methylobacterium* sp. ApIIE

Carbon Sources Used	**Carbon Sources Not Used**
Bromo-Succinic Acid	
Tween 40	
Propionic Acid	
Acetic Acid	
Formic Acid	