Characterization of volatile organic compounds from potential plant growth-promoting bacteria

By

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ABSTRACT

Microorganisms play important roles in plant growth and development. The use of biofertilizers for crop production is not only beneficial to the plants but can serve as a form of sustainable agriculture. Volatile Organic Compounds (VOC's) are metabolites produced by microorganisms known to play a role in plant growth and defense. In this study, the VOC's produced by bacteria isolated from the shoots of grape vines were used to test the growth enhancing ability of the bacteria.

Four bacterial isolates were characterized for their ability to enhance soybean growth using the volatile organic compounds produced. All of the four isolates showed no plant growth promoting abilities. The four bacteria were identified to be *Stenotrophomonas rhizophila, Bacillus proteolyticus, Paenibacillus alvei* and unknown. Only S*enotrophomonas rhizophila* was identified with certainty.

The volatile profile of each bacterium was analyzed, and various compounds known to be produced by soil bacteria, in addition to some novel compounds were identified.

An additional experiment was conducted by placing the *S. rhizophila* bacteria directly into the soil along with biochar. This experiment was not completely conducted due to the disruption caused by the COVID-19 shutdown. Based purely on observation, more extensive plant growth was present for the *S. rhizophila* treatment.

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1.0 INTRODUCTION

1.1. Agriculture

Agriculture is essential for ensuring food security for the world's growing population (Jones et al. 2016). After the second world war, increasing food production was paramount for alleviating poverty and improving human nutrition (Jones et al. 2016). Food crops such as rice, wheat and maize provide nearly two-thirds of the global dietary energy intake (Jones et al. 2016). Agriculture is not only important for food security, providing clothing and shelter, but many countries also depend on agriculture as a main source of revenue (e.g. South American countries such as Peru) (Tello 2010). Agriculture is one of the highlights of human history, and its emergence led to the rise of sedentary human civilization (Foley et al. 2005).

As a result of the world's growing population and increased demand for food production, agriculture, particularly the planting of crops has had adverse environmental effects. About 40% of the Earth's ice-free surface area is agricultural croplands or pasture (Wilson et al. 2009). In addition to converting most forest lands and wetlands into croplands and the changing of natural ecosystems, crop production also has impacted nearby water sources (Foley et al. 2005). Based on the current scenario of rapid population growth and the need to effectively maintain food production at a level that does not over or under produce food, achieving efficient and productive agricultural land use while conserving biodiversity is imperative (Tscharntke et al. 2012).

1.2 Crop requirements

In addition to the need of soil (land space) for crop production, carbon dioxide, sunlight, water and nutrients are also required for plant growth (Gavito et al. 2001). The primary nutrients required for plant growth are nitrogen (N), phosphorus (P), and potassium(K) (Tavallali et al. 2018). The nutrient required in greatest amounts by plants is nitrogen and its availability or lack of availability limits crop yield, growth and development, and primary production (Gutiérrez 2012). Plant roots mainly absorb nitrogen from the ground in the form of nitrate (NO_3^-) and ammonium (NH_4^+), where nitrogen is involved in the synthesis of amino acids, proteins, DNA and organic compounds required for plant growth (Xuan et al. 2017). Phosphates are utilized in most plant processes; it is present as a constituent of nucleic acids (DNA and RNA), phospholipids and ATP (Smith et al. 2003). It is also present as a metabolite involved in protein activation, energy transfer and regulation of metabolic processes (Smith et al. 2003). The primary source of phosphate for plants is in the form of inorganic phosphate (P_i) or phosphate ions (PO_4^{3-}) (Smith et al. 2003). Plants with phosphorus deficiency have stunted growth, underdeveloped root systems and inhibition of flowering (Smith et al. 2003). Potassium is another important nutrient required for plant growth and is present in the form of potassium cations (K^+) (Wang et al. 2013). These potassium cations are abundant in living plant cells and play an important role in enzyme activation, membrane transport, anion neutralization, and osmoregulation (Wang et al. 2013). Potassium significantly affects many physiological processes in plants, namely, photosynthesis and transport/translocation of assimilation products (Wang et al. 2013). Potassium deficiency usually leads to curling of leaf tips, undeveloped roots systems, yellowing of leaves and reduced seed and fruit development (Wang et al. 2013). The ratio of nitrogen, phosphorus and potassium needed for optimal plant growth and development is plant

specific. These essential nutrients for plant growth are not always readily available in soil, especially after planting and replanting crops. Chemical fertilizers were introduced to address soil nutrient availability and increase crop productivity.

1.3 Fertilizers

Fertilizer use predates the 21st century by thousands of years, when farmers used manure and other minerals to enhance the productivity of crops (Russel et al. 1977). The first fertilizer produced by chemical processes was in the 19th century and was a superphosphate (Russel et al. 1977). Fertilization of soil increases soil fertility and crop productivity (Savci 2012). As a result of fertilizer availability and use, global food production has significantly increased (Ning et al. 2017). Inorganic fertilizers contain nitrate, ammonium, phosphates and potassium salts, and also contain a large amount of heavy metals (silver, cadmium, lead, copper, nickel) (Savci 2012). The excessive use of chemical fertilizers has adverse effects on both the terrestrial and aquatic environments. One such effect is eutrophication, which is caused by the excess input of nutrients via leaching and run off into rivers, lakes, estuaries and oceans, leading to increased growth of algae and aquatic weeds (Somura et al. 2012). This increased algal growth is problematic to water ways because it causes decreased sunlight penetration through the water surface and anoxic or hypoxic conditions resulting in the death of plant and animal species, water pollution and increased bacterial content. (Somura et al. 2012; Manuel 2014). In addition to eutrophication and its effects, excessive chemical fertilizer use also increases the number of invasive species due to access to excess nutrients that would not otherwise be available (Wersal et al. 2011). An example of an invasive species that thrives on nutrient rich environments is the *Myriophyllum aquaticum*, which is an aquatic weed that typically invades shallow wetlands, ponds, edges of

lakes etc. (Wersal et al. 2011). Other effects of chemical fertilizer use are increased soil acidification, and unbalanced mineral composition in soil and plants (Shorette 2012; Ning et al. 2017). The rhizosphere microbial community is also affected by practices such as chemical fertilizer application and the use of pesticides (Sawyer et al. 2019). Excess nitrogen can directly alter root exudate quantity and composition and increase the nitrates and ammonia available for microbes (Sawyer et al. 2019). This has been shown to affect microbial community structure, abundance and function (Sawyer et al. 2019). There is an increase in demand for agricultural practices and production to become more sustainable as a result of the negative impacts of chemical fertilizer use (Ning et al. 2017).

1.4 Sustainable Agriculture

Due to the negative impacts chemical fertilizers have imposed on the environment, alternative solutions that increase crop yield and productivity, and are environmentally friendly, have evolved. One such alternative is the use of organic fertilizers. Organic fertilizers are derived from both animal and plant matter and waste (Ning et al. 2017). They contain a high organic matter content and rich nutrient elements, leading to enhanced soil physical properties by improving aggregate stability and decreasing soil bulk density (Ning et al. 2017). Organic fertilizers also improve soil biological and biochemical properties and improve the soil microbial community (Ning et al. 2017). On the downside, using organic fertilizers alone may not meet the nutrient requirement for crops, as it releases nutrients slowly (Ning et al. 2017). Organic fertilizers can also be a source of environmental pollution, due to presence of heavy metals and even microbial pathogens, if they are not used properly (Ning et al. 2017). They may also contribute to increased greenhouse gas emission by releasing methane and carbon dioxide when applied to soil (Ning et al. 2017).

Another alternative to chemical fertilizers is the use of environmentally friendly fertilizers (EFF). These are fertilizers coated with biodegradable or environmentally friendly material such as, chitosan or sodium alginate (Chen et al. 2018). The coating on EFFs can prevent urea exposure in water and soil by serving as a physical barrier, leading to the reduction in urea hydrolysis and decreased nitrogen dioxide emission (Chen et al. 2018). They can also increase soil organic matter content and buffer soil acidity or alkalinity using its hydrogel coat (Chen et al. 2018). EFFs are more effective than chemical fertilizers because they improve nutrient efficiency and minimize leaching and volatilization losses of fertilizers, leading to reduced environmental hazards (Chen et al. 2018). The limitation to this alternative is the materials used for coating. Chitosan, used as a coating, may cause environmental pollution due to the emission of organic solvents (acetone) (Chen et al. 2018).

Lastly, the use of biofertilizers also seem promising in replacing chemical fertilizer use (Igiehon et al. 2017). Biofertilization is the process of boosting the abundance of microorganisms in the plant rhizosphere (Igiehon et al. 2017). Microorganisms such as arbuscular mycorrhizal fungi are important in helping plants obtain sufficient phosphorous from soil (Igiehon et al. 2017). Biofertilizers can be composed of several microbial strains that benefit plant uptake of nutrients by their interaction in the rhizosphere (Thomas et al. 2019). Important known mechanisms exhibited by plant-growth promoting rhizobacteria (PGPR) are atmospheric nitrogen fixation, phosphorus solubilization, enhancement of nutrient uptake or production of plant growth promoting hormones (Thomas et al. 2019). Rhizosphere microorganisms have emerged as a promising way to enhance plant growth and potentially address the problems faced by the use of chemical fertilizers.

1.5 The Rhizosphere

The rhizosphere is defined as the narrow bulk area surrounding plant roots, where plants take up the most nutrients and where numerous microorganisms are found (Mendes et al. 2013; Dotaniya and Meena 2015). It is considered one of the most complex ecosystems on earth and is characterized by the intense association of plant roots with microbial activity (Mendes et al. 2013). These microorganisms utilize root exudates, such as, water, polysaccharides, and primary and secondary metabolites (Fincheira and Quiroz 2018). The micro-organism diversity is higher near the rhizoplane and decreases with increasing distance from the rhizoplane (Dotaniya and Meena 2015). In addition, the number of microbial genes in the rhizosphere far outnumbers the plant genes; this is indicative of how important of a role the microbes play in plant life (Mendes et al. 2013). Microorganisms also exert influence on plants, known to have profound effects on seed germination, seedling vigor, plant growth and development, nutrition, diseases and productivity (Mendes et al. 2013; Dotaniya and Meena 2015). Thus, the plant-microbial relationship is thought to be commensalic or mutualistic (Dotaniya and Meena 2015; Jiang et al. 2017). The collective communities of plant associated microorganisms are known as the plant microbiome (Mendes et al. 2013). Plants can be viewed as the superorganisms that rely on its microbiome for specific functions and traits. In return, plants release their rhizodeposits (e.g. exudates, border cells) into the surroundings, thereby feeding the microbial community and influencing their composition and activity (Figure 1) (Mendes et al. 2013; Jiang et al. 2017).

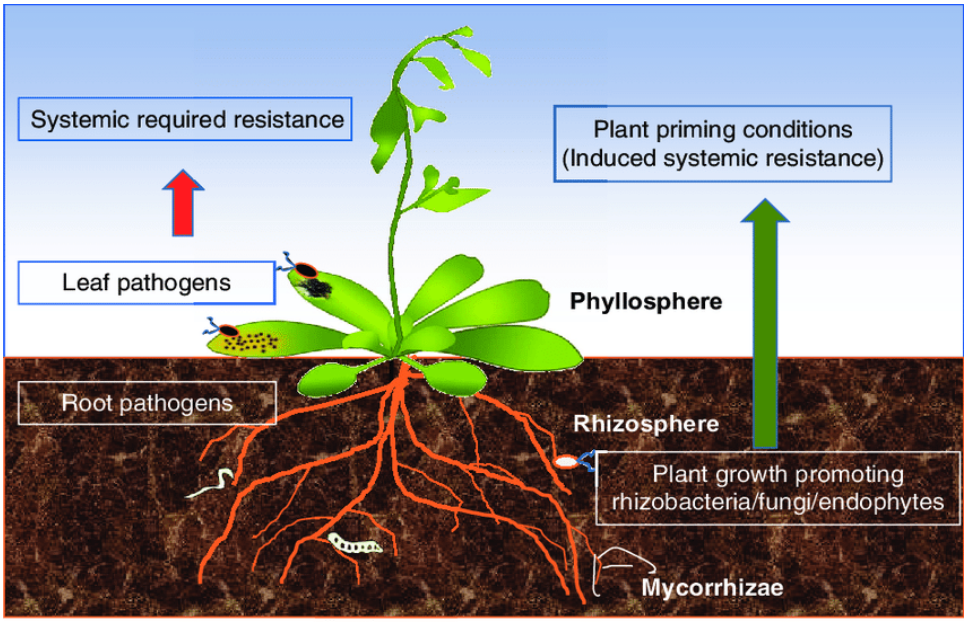

Figure 1. The rhizosphere community and its interaction with the plant (Liu et al. 2017)

Microorganisms secrete nonvolatile metabolites that induce plant growth through both direct and indirect pathways. Direct pathways involve the release of phytohormones (auxin, ethylene, and cytokines) and other organic substances which contribute to growth stimulation and nutrient availability (Goswami et al. 2016; Gupta et al. 2017). Indirect pathways involve substances that help prevent pathogenic attack through the production of antibiotics, hydrolytic enzymes, and hydrogen cyanide (Goswami et al. 2016). In addition to nonvolatile metabolites, microorganisms also release volatile metabolites that affect plant growth and overall health (Fincheira and Quiroz 2018). A new mechanism mediated by volatile organic compounds (VOCs) was first reported by Ryu et al. 2003; they studied the effects of volatiles released by *Bacillus subtilis* on the growth of *Arabidopsis thaliana.* This was the first evidence that VOCs can modulate growth, stress, nutrition, and health processes in plants (Fincheira and Quiroz 2018).

1.6 Microbial volatile organic compounds

1.6.1 Bacterial volatiles and plant growth

Volatile organic compounds (VOC) are lipophilic compounds derived from microbial pathways (Park et al. 2015). They have low molecular weight, low boiling point, and high vapor pressure; this allows them to act as signaling molecules over short distances (Park et al. 2015). VOCs produced by plant-growth promoting rhizobacteria (PGPR) play major roles in plant growth promotion and resistance to pathogens or abiotic stress (Hashna et al. 2014; Raza et al. 2016). There are a large number of VOCs present under aerobic and anaerobic conditions produced by the microbiome in the soil (Syed-Ab-Rahman 2019). Bacterial VOC's can be grouped into aldehydes, ketones, alkyls, alcohols, alkenes, esters, alkynes, acids, ethers, heterocyclic and phenolic compounds (Kai et al. 2009). Examples of bacteria genera producing VOC's that promote plant growth are *Bacillus*, *Azotobacter, Rhizobium*, *Pseudomonas*, *Serratia*, *Arthrobacter* and *Stenotrophomonas* (Hashna et al. 2014; Goswami et al. 2016). Compounds produced by these bacteria that are known to promote plant growth are, Acetoin and 2,3-butanediol to name a few (Hashna et al. 2014).

About 2000 microbial VOC's emitted by 1000 microorganisms are registered on the microbial volatile database; 50 of the compounds are known to induce plant growth changes, plant physiology and defense (Lemfack et al 2018). Bacterial volatiles have been classified, and there are 75 fatty acid derivatives, 50 aromatic compounds, 74 nitrogen-containing compounds, 30 sulfur compounds, 96 terpenoids, 18 halogenated, selenium, tellurium and other metalloid compounds (Kai et al. 2009). Research has shown that exposure of plants to complex mixtures of VOC's released by PGPR is more effective in inducing growth promotion versus discrete or a simple mixture of VOC's (Cordovez et al 2017).

1.6.2 Signaling and metabolic changes

Although the direct physical involvement of soil microbiome and plants has resulted in the mutualistic relationship between the two, the role volatiles play as signaling molecules may be just as important in this mutualistic relationship. Volatiles are known to be used by plants as warning or attraction signals, especially in plant-plant and plant-insects communication (Bailly and Weisskopf 2012; Weston et al. 2012). As expressed by Bailly and Weisskopf 2012, this suggests that recognition mechanisms already exist for volatile detection. Another reason why the role of volatiles as signaling molecules is important is that the rhizosphere environment is conducive for volatile-mediated communication; partners are spatially close to one another and volatiles are better able to accumulate and reach the threshold of concentration needed for activity (Bailly and Weisskopf 2012). Plant growth stimulation may be a result of PGPR synthesizing and secreting canonical phytohormones auxins, cytokinins, indole-3-acetic acid and gibberellins or the decrease in ethylene level via 1-aminocyclopropane-1-carboxylate deaminase (Bailly and Weisskopf 2012; Ryu et al. 2003). Bacteria has also been known to increase N, P and iron (Fe) soil availability. For example, phosphate-solubilizing bacteria are known to solubilize mineral-bound insoluble phosphate, therefore making it more easily accessible (Bailly and Weisskopf 2012).

In regard to ethylene biosynthesis, specific genes *(CHIB*, *ERF1* and *GST1*) have been shown to respond to bacterial volatiles at the transcriptional level (Kwon et al. 2010). Other plant hormones, such as Jasmonate, which is released as a defense mechanism by plants when experiencing biotic and abiotic stress, may also be influenced by microbial volatiles in terms of signalling pathway activation (Kazan and Manners 2008). Plants are also known to attract beneficial microbes by emitting their own VOC's. Although VOC's released by the plant only

represent 1% of root metabolites, microbes can form biofilms on root and leaf surfaces, thereby protecting the plant from pathogen and herbivore invasions (Liu & Brettell 2019).

There are gaps in the literature concerning specific mechanisms and pathways involved in mVOC - plant communication and how they affect plant growth and defense. It is important that further studies be conducted to contribute to the understanding of these complex mixtures of volatiles and how they interact with plants. This study is looking into finding novel bacteria (isolated from grape vine stems) that have plant growth enhancing abilities, with a view to analyzing the VOCs they produce.

1.7 Objectives

The objective of the study is to identify novel bacteria with plant growth enhancing properties and analyze the organic volatiles produced. Out of a library of 500 bacterial isolates previously collected by colleagues of mine, seventeen microbial isolates were screened, and four were selected for larger scale testing. Plant growth promotion was determined by measuring average shoot height, shoot dry mass and root dry mass after growing soybeans on a growth shelf for fourteen days. Bacteria were grown on a nutrient agar growth medium and VOCs were extracted via solid phase microextraction; gas chromatography mass spectrometry was then used to analyze and identify volatiles. The four unidentified bacterial isolates were identified using Polymerase chain reaction (PCR) of the 16S ribosomal RNA gene and sequenced using sanger sequencing. The bacteria was imaged using a iodine-glycerol stain.

In addition to studying the effects of VOC's emitted by bacteria on plant growth, the growth enhancing ability of one of the identified bacteria species known as *Strenotrophomonas rhizophila* will also be observed by directly inoculating the bacteria in biochar and placing it in the soil.

Future research may include directly extracting the volatiles produced by bacteria – while the bacteria and the plant are in communication – for analysis. Also, it would be interesting to explore the communication mechanism between the plant and bacteria and explore plant pest resistant properties induced by bacteria. This type of research may aid in the shift from chemical fertilizers and its detrimental effects, to something more environmentally sustainable.

2.0 Materials and methods

2.1 Overview

2.11 Bacterial screening

Seventeen bacterial isolates from grape vines (isolated June -August 2018, by Kaitlyn Blatt-Janmaat and Brandon Logan) were screened for growth promoting properties using test plants: Roma tomato (*Salonum lycoperscium*) and Champion soybean (*Glycine max*). The growth period for each session ranged from 23 days to 36 days; after the sessions, the shoot length and plant dry mass was collected. For screening, 2 tomato seeds and 2 soybean seeds were separately placed in treatment pots. Most sessions tested 5 bacteria along with 1 control (only soil) for each plant. Four bacterial isolates from the seventeen screened were selected for large scale testing. These four bacterial isolates demonstrated the most plant growth per session for both tomatoes and soybeans when compared to the control. The bacteria with the greatest average shoot length and mass compared to the control for both soybeans and tomatoes were selected. This was not tested for statistical significance. Growth of bacteria, seed sterilization and setup/growth conditions were done similarly to that described in 2.2, 2.3 and 2.4 with variations in the sample size.

2.12 Larger scale testing

For more statistically significant data, 20 soybean seeds were planted for each of the 4 bacteria, the control (just soil) and the nutrient agar control (agar in a plate). Dry root mass, shoot mass and shoot length were measured for each treatment. Identification of the four bacteria was done using gram staining and polymerase chain reaction (PCR) of the 16S rRNA gene in conjunction with Sanger sequencing. The volatile organic compounds produced by each bacterium were analyzed using solid phase microextraction (SPME) in conjunction with Gas Chromatography-Mass Spectrometry (GC-MS).

2.2 Nutrient agar for the growth of bacteria

Nutrient agar for bacteria growth was made by adding 5g of sodium chloride, 5g of tryptone, 3g of yeast extract and 15g of agar to l L of deionized water in a 1000 mL glass bottle. The mixture was then sterilized in a Gentinge Vacuum Sterilizer at 121°C for 30 mins. 10 mL of the agar solution was poured into 30 small petri dishes (60 mm in diameter and 15 mm in height) and left to harden and cool. 50 μL of bacteria from bacterial frozen stocks (700 μL of nutrient broth and 300 μL of 50% glycerol), with a pile of bacteria collected from plates with an inoculation loop) was pipetted onto 10 plates of the dried agar for each bacterium and spread with an inoculation loop, parafilmed, and left to grow for 5 days at room temperature (21°C). The nutrient broth was made with 1.5g of sodium chloride, 1.5g of tryptone, 0.75g of yeast extract to 250 mL of deionized water in a 500 mL glass bottle

2.3 Seed sterilization

84 seeds were sterilized by submerging them in 10% sodium hypochlorite in a beaker for 2 minutes, rinsing them with deionized water, and then draining and rinsing them with 70% ethanol for 30 seconds. The ethanol was then drained, and seeds were rinsed for the final time with deionized water. To make sure seeds were microbe free, two seeds were placed on growth medium (nutrient agar) and left to grow for a day. If there were any signs of bacterial growth, all seeds were sterilized again.

2.4 Set up and growth conditions

After seed sterilization, all treatments were set up appropriately as per on table 1 and figure 2.1.

2.41 Session 1

80 red solo cups were sprayed with 70% ethanol inside and out and left to dry. The experimental set up is a modified version of that used by Tahir et al. and is depicted in figure 2.1. The bases of 40 solo cups were cut (about 3.3 cm) and covered with a ~ 6 cm x 6cm square of cheese cloth (secured with labeling tape). 400mL of sterilized (sterilized in a Gentinge Vacuum Sterilizer (model 533Ls) at 121°C for 30 mins) potting soil was placed in each solo cup (40) with a cheese cloth base, see figure 2. In order to locate where the seeds were planted, wooden skewers were cut about 3-4 cm in length and inserted about 2 cm apart. One seed was placed to the front of each skewer at about 1 cm deep. 10 pots were set up for the control, nutrient agar control, bacteria 1 and bacteria 2. To allow the volatiles to escape, without the risk of the media drying out, the lids were placed over each dish at the bottom of the solo cups (40 with base). The pots

with soil and seeds were inserted into the pots with the bacteria (or agar or control) and parafilmed around the top edge to prevent the escape of volatiles. There was about 1.8 cm of space between the top of the petri dish and the bottom of the cheese cloth. The pots were placed on a growth shelf and exposed to a 12-hour light cycle from 9:00 am to 9:00 pm, with the 3000 K LED lights positioned 21" above the top of the pots. Both the bottom and top of the growth shelf contained 5 pots of each treatment; this was done to eliminate the differences in environmental exposure (see figure 2.1). The temperature in the lab was at a constant 22°C +/- 1°C. The seeds were watered with 2 mL of water daily at the same time of day and left to grow for 15 days. This was also done for session 2 with bacteria 3 and 4.

Table 1.0 Summary of conditions for each treatment group.

Treatment	Condition
Control	Only potting soil
Nutrient Agar control	Potting soil + nutrient agar in plates with no bacterial
Bacteria 1	Potting soil + nutrient agar in plates with bacteria 1
Bacteria 2	Potting soil + nutrient agar in plates with bacteria 2
Bacteria 3	Potting soil + nutrient agar in plates with bacteria 3
Bacteria 4	Potting soil + nutrient agar in plates with no bacteria 4

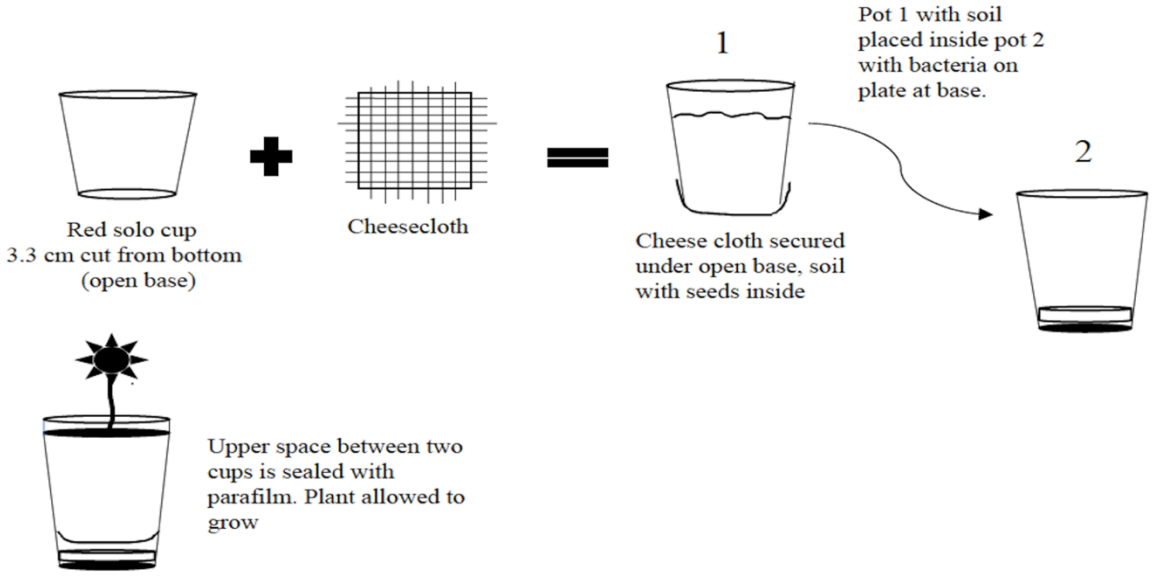

Figure 2.1 Set up for observing growth promoting properties for each bacterial isolate on soybeans plants. After the base of the cut solo cup was covered with the cheesecloth, the cup was filled with soil and inserted into another solo cup with bacteria at its base.

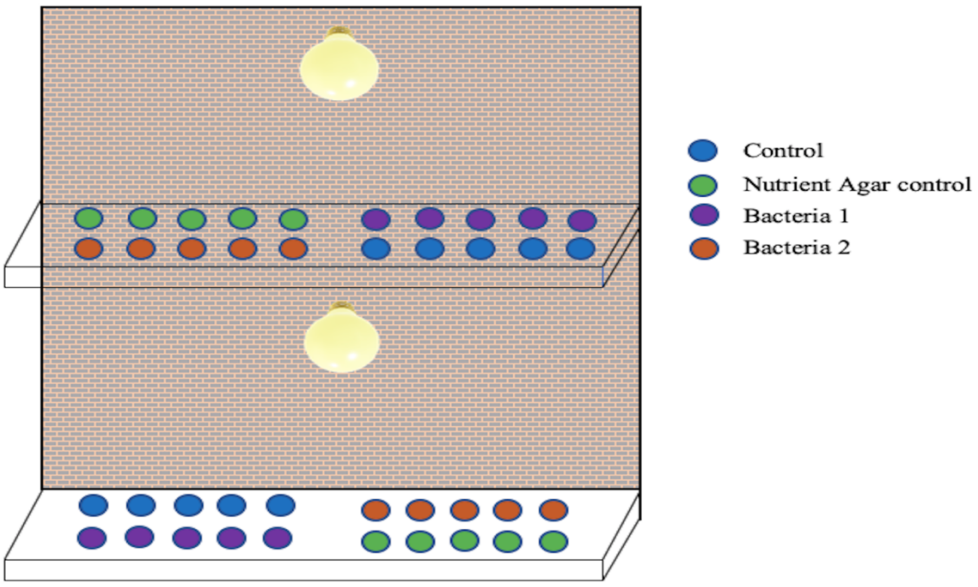

Figure 2.2 Arrangement of pots based on treatments on a growth shelf. Each treatment was present on both levels of the shelf and exposed to similar light intensities (positioned at opposite corners)

2.5 Experiment with Biochar

One out of the four bacteria (*Stenotrophomonas rhizophila*) was used to test its growth enhancing abilities when delivered via biochar. Rather than the bacterial VOC's interacting with the plant, biochar inoculated with bacteria was placed directly into the soil. This procedure was modified from Xiong et al 2017. Nutrient broth (50%) was first prepared by adding 0.75 grams of yeast extract, 1.25g of sodium chloride, 1.25 grams of tryptone and 500 ml of deionized water in a 1L glass bottle. The nutrient broth solution along with 600 mL of coarse biochar (separate flask) was sterilized for 30 mins in a Gentinge Vacuum Sterilizer (model 533Ls) at 121°C. 400 mL of solution was left to cool to room temperature (21°C). 200 mL of nutrient broth solution was poured into two sterile Erlenmeyer flasks. From frozen stocks, 50 μL of bacterial solution was pipetted into one if the nutrient broth solutions; the solution was swirled for 10s. Sterilized biochar (200mL) was added to the nutrient broth and bacteria mixture and placed in a 30°C Amerex Instruments Gyromax 737 incubator with shaking at 213 rpm for 24 hours. In the second Erlenmeyer flask containing nutrient broth, 200 mL of biochar was placed and let to sit for 24 hours. The treatments were set up as shown in table 1.1.

Table 1.1 Treatments and conditions for bacteria in soil experiment.

Treatment	Condition
Control	Only potting soil
Biochar control	Potting soil + biochar
Nutrient broth &Biochar control	Potting soil + biochar+ nutrient broth
Stenotrophomonas	Potting soil + biochar + nutrient broth + bacteria

Sterilized potting soil (400 mL) was placed into all 40 sterilized red solo cups. 20 mL of biochar was placed in the 10 biochar control pots and mixed; 20mL of biochar from the biochar and

nutrient broth solution was placed into another 10 solo cups and mixed; 20 mL of the biochar mixed with bacteria and nutrient broth treatment was placed into 10 red solo cups and mixed thoroughly. Two soybean seeds were placed at about 2 cm apart and 1 cm deep into the soil. The seeds were watered with 2 cm of water for the first week, then inconsistently (due to inability to access building) until the soil was damp for the remaining 19 days and left to grow for 26 days on a growth shelf at room temperature (as previously explained).

2.6 Drying and weighing

After growing for 15 days the soybean plants were uprooted and the soil on the roots was rinsed out. The plant was then cut where the root furthest up the shoot stopped, separating the roots and the shoot. The shoot was measured from its base to the point where it began branching for leaf development (not seed leaf). The root was measured from the tip of the longest root to where it was cut at the stem. Both plant pieces were then wrapped in tissue paper and placed in a convection oven for 15 minutes at 55°C, then placed in a desiccator for two days to completely dry out. The dry masses of the roots and shoots were found using an analytical scale. This was done for session 2 with bacteria 3 and 4.

2.7 Bacteria staining

Bacteria was visualized using an iodine-glycerol method used by Vignesh et al. 2008). After growing the four selected bacteria strains on nutrient agar medium for three days, a small pile of bacteria was smudged on a glass slide using an inoculation loop. A cover slip was then placed on top of the bacteria and small drops of iodine glycerol solution was pipetted using a disposable glass pipette on to the glass slide until there was enough solution beneath the cover

slip to completely cover the bacteria. Images of each bacteria were taken using a microscope camera and light microscope Olympus model:CX41RF (Japan).

2.8 DNA Extraction

Bacteria was grown on nutrient agar media for 3 days before being extracted. Prior to the extraction process, the cryogenic vials used were rinsed with 70% ethanol to remove residual cellular contents; about 1 mL of 70% ethanol was pipetted using a disposable glass pipette, into four 2 mL cryogenic vials containing 0.5 mm (diameter) glass beads. The cryogenic vials were then vortexed and drained into a waste beaker. 750 µL of 70% ethanol was pipetted into four cryogenic vials. An inoculation loop filled with bacteria was placed into cryogenic vials containing 70% ethanol and glass beads. Vials with bacteria and 70% ethanol were then vortexed for 2 minutes to ensure that all cells were broken, and DNA was extracted.

2.9 Polymerase chain reaction (PCR)

The Master Mix (with GoTaq green), Primers, and Nuclease-Free Water were thawed and vortexed at room temperature. In a freezer box, 50µL PCR samples in strip tubes were prepared by adding 19uL Nuclease-free Water, 25µL Master Mix, 2µL of 20µM Forward 16S rRNA Primer, 2µL of 20µM 16S rRNA Reverse Primer (table 1.2), and 2uL template DNA. The solution was mixed by gently tapping the tube. A negative control sample was prepared by adding all ingredients except template DNA to test reagents for contamination. PCR Tubes were spun for 1min at 1000 RCF on the Eppendorf Centrifuge 5430R.The samples were then placed into the BIO-RAD C1000 TouchTM thermocycler and were set to Sit Method (conditions as outlined in Table 1.3); the sample volume was set to 50uL per tube, and lid temperature was set to 105ºC to prevent evaporation (Promega, 2018).

Table 1.2 Universal 16S rRNA primer sequences used in PCR.

Primer Name	Sequence (5'-3')	Product size (bp)	References
fD1	AGAGTTTGATCCTGGCTCAG	1500	Marin et al. 2012
rP2	ACGGCTACCTTGTTACGACTT	1500	Marin et al. 2012

Table 1.3 BIO-RAD C1000 TouchTM thermocycler conditions (Sit method).

Step	Temperature °C	Time (min)	Cycles
Initial Denaturation	95	2	1
Annealing	52	0.5	
Extension	73	2	33
Denaturation	95	0.5	
Final Extension	73	5	1
Storage	12	Indefinite	1

3.0 Gel Electrophoresis

The samples were visualized using agarose gel electrophoresis. A 200 mL bottle of 1.5% Agarose gel with 6uL of ethidium bromide was made and microwaved at intervals of 1 minute until the Agarose was fully dissolved. When the Agarose was cool enough to comfortably touch with gloved hands, it was poured into a gel mold (until the level of the gel was just below top of the comb teeth ~ 50 mL) with the appropriate comb placed at the end of the tray. The agar was firm to touch after approximately 20 min. The comb was gently removed, and the vice was loosened. The tray and gel were transferred into the appropriate electrophoresis chamber. The chamber was then filled with 0.5x TBE buffer until the gel was covered.

5uL of DNA Ladder was placed in the first well. Remaining wells were loaded with 5uL of samples. The lid was placed, and the apparatus was set to 100V for approximately 1 hour. The gel was gently transferred from the tray to the transfer plate when the electrophoresis run was complete, then from the plate into the BIO-RAD Gel Doc ™ XRT Imaging System. The gel was straightened to line it up on the computer screen, and images of the gel were taken.

Samples with DNA present were package and mailed to Genome Quebec for sequencing. The samples were prepared for mailing, by adding 20 uL of each selected DNA sample into PCR tubes; 5 uL of the forward primer successfully used on that sample and 5 uL of nuclease free water were added to a separate set of tubes. The tubes were then affixed to a cold pack using parafilm and tape, packaged and then mailed. The Basic Alignment Search tool (BLAST) (16S ribosomal RNA sequencing) was used to identify bacteria using sequences provided by Genome Quebec.

3.1 SPME- Bacteria headspace sampling

The volatiles were extracted using a modified method from Quiroz et al. Headspace sampling of strains was conducted in 20mL vials filled with 10mL of nutrient agar. Vials were sterilized before use with 95% ethanol followed by 15 minutes at 121ºC in an autoclave. 10mL of agar was added to the vial and inoculated with 10µL of the bacterial isolate from frozen stock. The vials were sealed with PTFE Mininert® vial caps and allowed to incubate at room temperature for 5 days.

A 50/30µm CAR/DVB/PDMS SPME fiber was utilized for headspace sampling. After the growth period, vials were checked for contamination, transferred to a jacketed beaker and

allowed to equilibrate at 35ºC for 10 minutes. The fiber was injected into the vial and the headspace was sampled for 10 minutes. Each sample was analyzed in triplicate.

3.2 Gas Chromatography- Mass spectrometry (GC-MS)

The volatiles extracted were analyzed using a modified method from Quiroz et al. GC-MS analysis of all volatile compounds was conducted on a Varian 3800 Gas Chromatography apparatus coupled to a Varian 2000 Ion Trap Mass Spectrometer. A VF-5MS capillary column (Varian, CP8944, 30m x 0.25 mm, 0.25 μm) was utilized to separate the biological samples. The oven temperature was initially set at 35°C, held for 2 minutes, increased to 260°C at 15°C/ min, and finally held for 2 minutes. Helium (99.9%) was used as the carrier gas and set at a constant flow rate of 1 mL/min. The fibers were manually injected and desorbed in the injection port at 260°C for 3 minutes. The transfer line and ion source were maintained at 250 °C. Potential peaks of interest were selected using the quantification function of the Varian MS workstation software with (DEFAULT PARAMETERS). The base peak of each potential hit was extracted and compared across the replicates completed for each bacterial strain. Hits present in two or more replicates were considered valid. Once potential hits were established, the base peak of the sample was compared to the base peak of both the fiber and the media blank to ensure that hits were not a result of the matrix. The remaining hits were classified as peaks of interest. The retention indices were calculated with reference to a C8-C20 alkane standard series (Sigma-Aldrich) and tentative assignments were made based on retention index similarity and comparison with the National Institute for Standards and Technology (NIST) 2017 Mass Spectral Library. To tentatively assign the compound, a R. match value of >650 was required if the retention index was within 10 while a R. match value of >750 was required if the retention index was not listed in literature or differed

by more than 10. In some instances, a standard was injected to confirm the identity of particular compounds.

3.3 Statistical Analysis

Data was statistically analyzed using SYSTAT version 13.2 and Minitab version 17.2.1. In order to pool the control and nutrient agar results from session 1 and 2, a two-way ANOVA was conducted (after passing a Ryan-Joiner normality test and a Levene's test for homogeneity). After concluding that there was no interaction and no significant difference when comparing the control data for session 1 versus session 2 and comparing the nutrient agar control data in session 1 versus session 2, the data was pooled as one set of control data and nutrient agar control data. The data for the each of the three bacteria was tested for normality and homoscedasticity. Only two of the three response variables measured (root mass and shoot mass) were normally distributed, therefore a Kruskal-Wallis test was conducted to test for significant differences in shoot height, shoot mass and root mass among all treatments.

4.0 RESULTS

4.1 Bacteria Screening

The plant dry mass and shoot height for both soybean and tomato plants were recorded for a total of 17 bacterial isolates (figure 3.1 -3.8). Bacteria selected from this screening process are indicated on the graphs (figure 3.1 -3.8) by a black star; LALA04B2(from trial 4), LALA03B1 (from trial 1), LA?05B1 (from trial 3) and LALA06B2 (from trial 4) were all selected for larger scale testing.

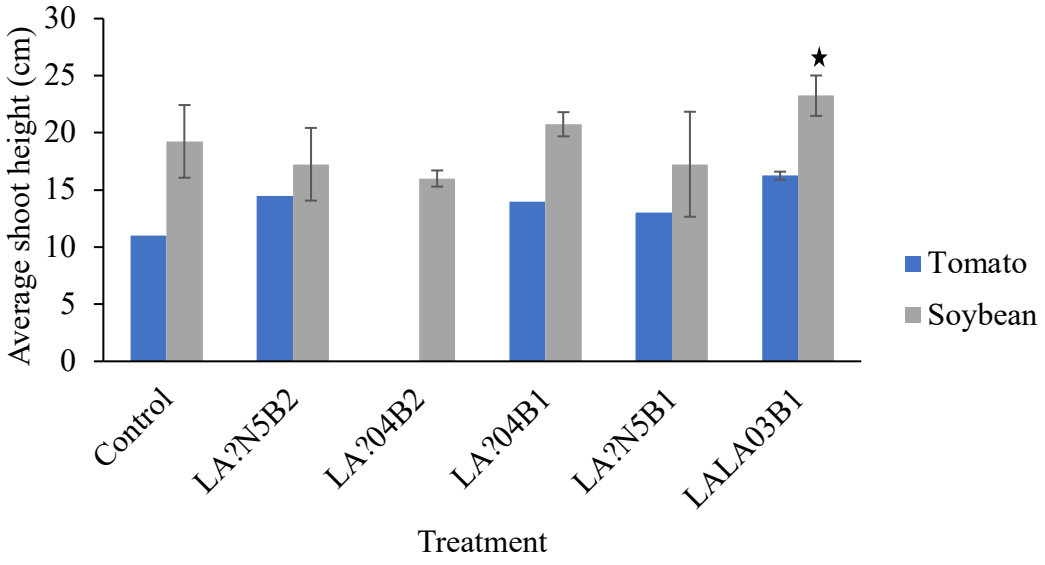

Figure 3.1 Average shoot height and standard deviation of tomato plants (*Salonum lycoperscium*) and soybean plants (*Glycine max*) for varying treatments; trial 1. Tomato and soybean plants grew for 34 and 32 days respectively.

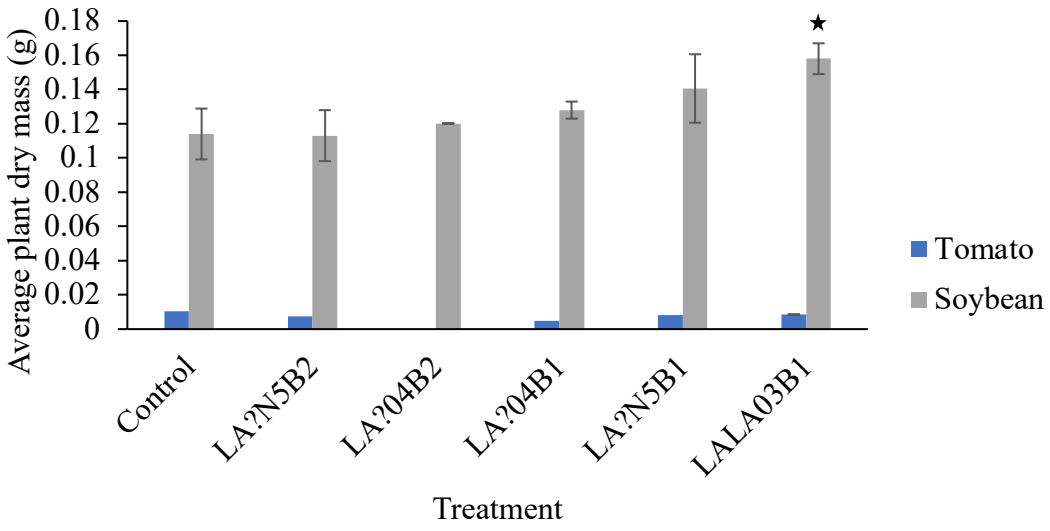

Figure 3.2 Average plant dry mass (g) and standard deviation of tomato plants (*Salonum lycoperscium*) and soybean plants (*Glycine max*) for varying treatments; trial 1. Tomato and soybean plants grew for 34 and 32 days respectively.

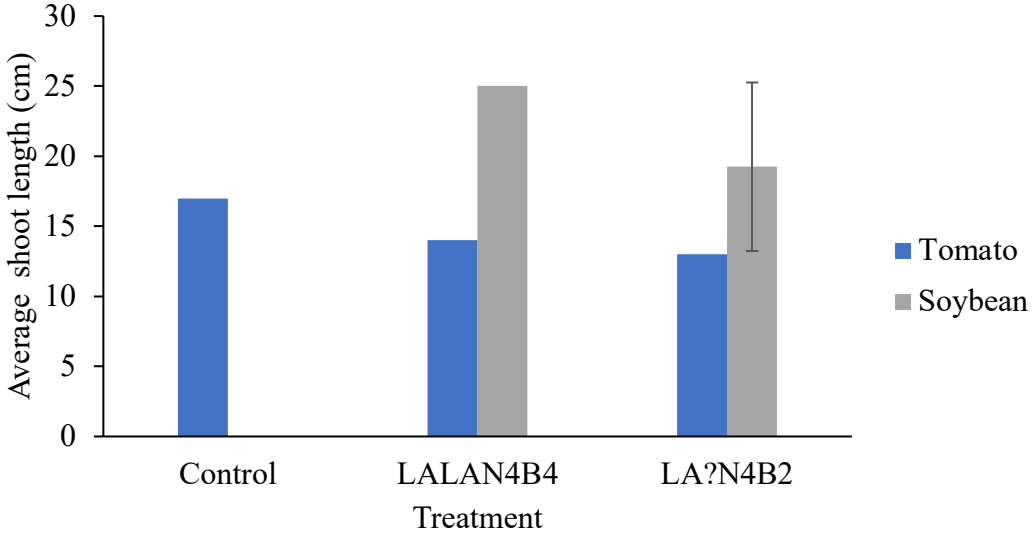

Figure 3.3 Average shoot height and standard deviation of tomato plants (*Salonum lycoperscium*) and soybean plants (*Glycine max*) for varying treatments; trial 2. Tomato and soybean plants grew for 23 days.

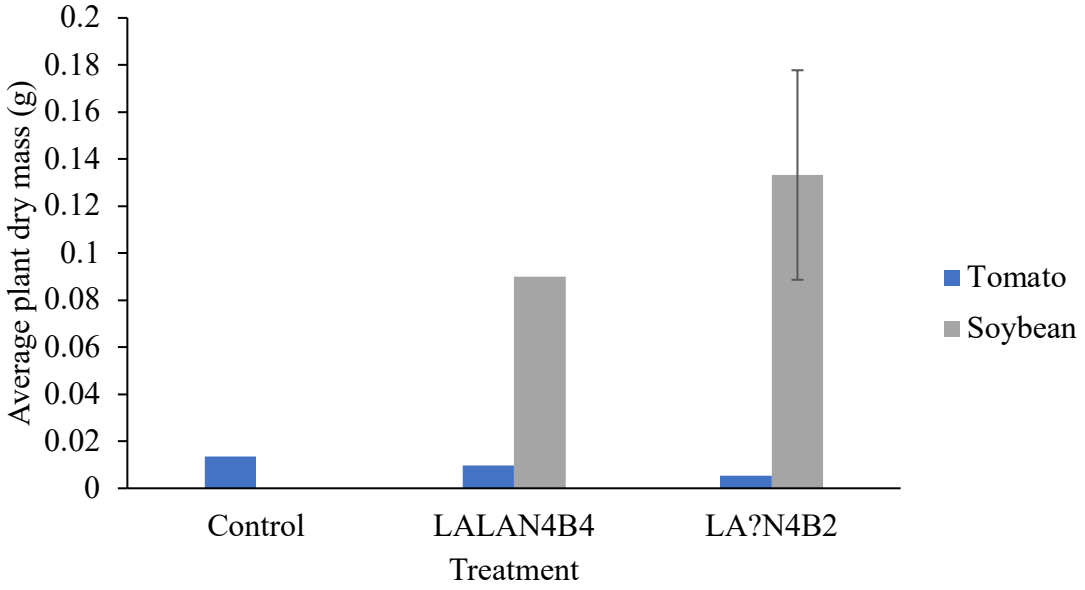

Figure 3.4 Average plant dry mass (g) and standard deviation of tomato plants (*Salonum lycoperscium*) and soybean plants (*Glycine max*) for varying treatments; trial 2.Tomato and soybean plants grew for 23 days.

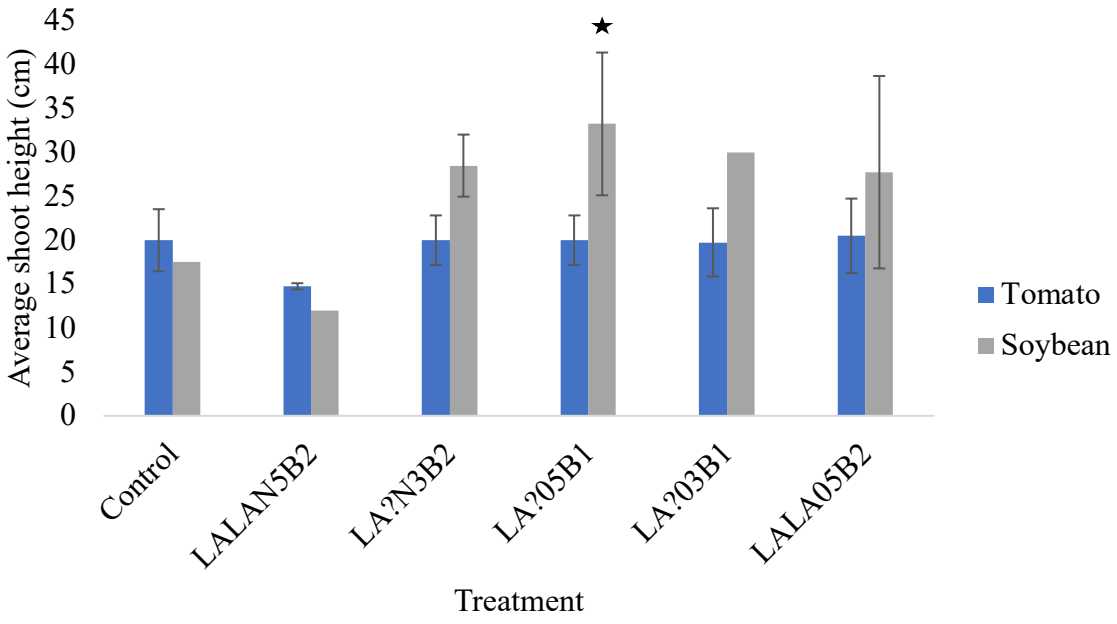

Figure 3.5 Average shoot height and standard deviation of tomato plants (*Salonum lycoperscium*) and soybean plants (*Glycine max*) for varying treatments; trial 3. Tomato and soybean plants grew for 21 and 23 days respectively.

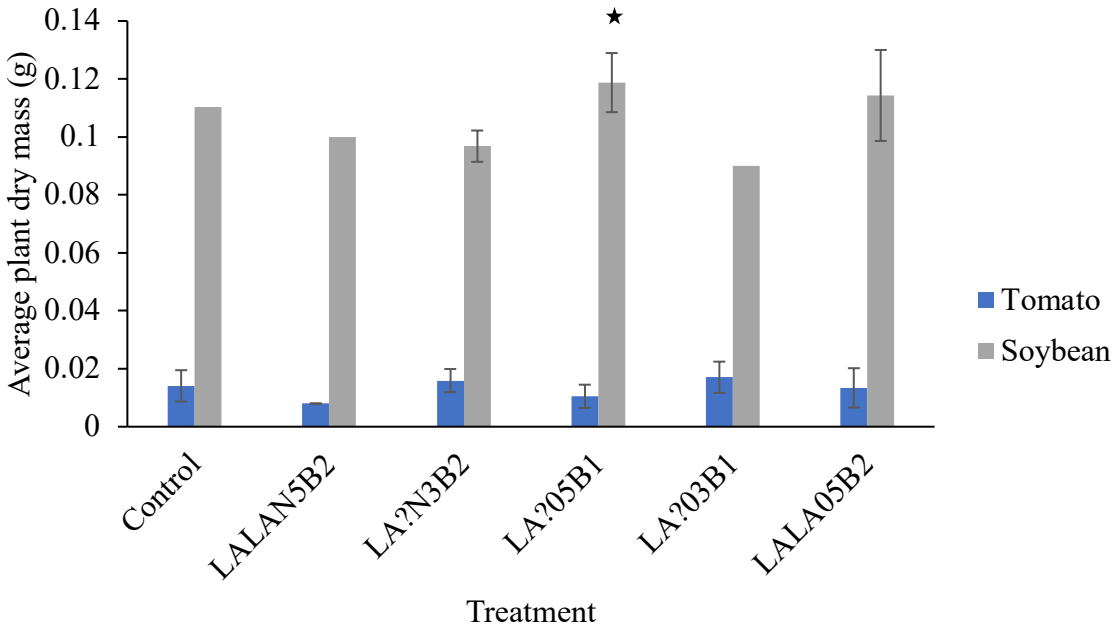

Figure 3.6 Average plant dry mass (g) and standard deviation of tomato plants (*Salonum lycoperscium*) and soybean plants (*Glycine max*) for varying treatments; trial 3. Tomato and soybean plants grew for 21 and 23 days respectively.

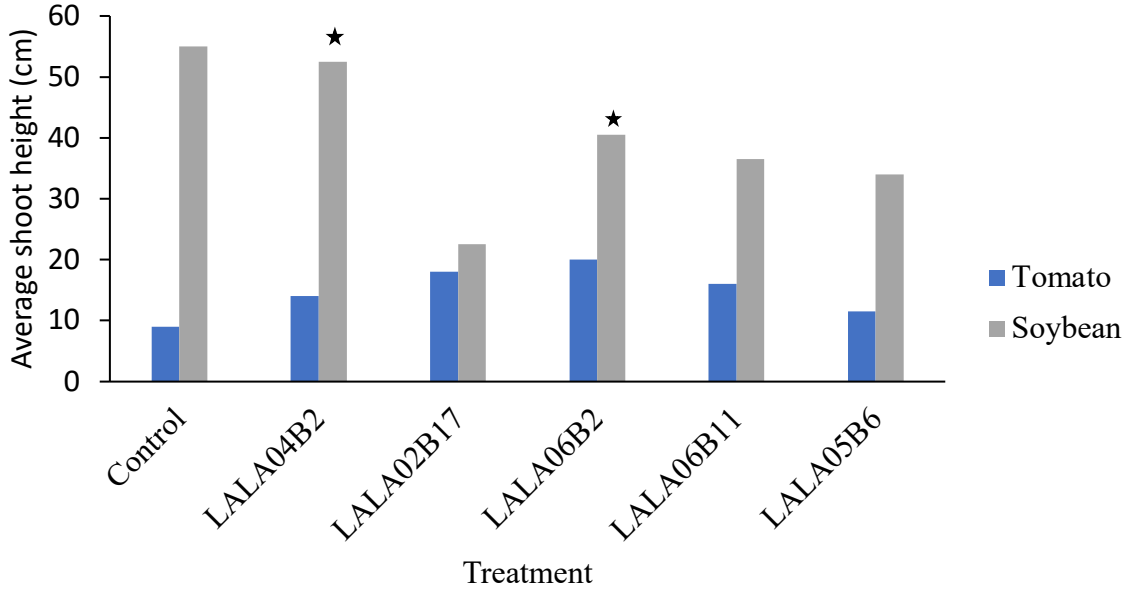

Figure 3.7 Average shoot height and standard deviation of tomato plants (*Salonum lycoperscium*) and soybean plants (*Glycine max*) for varying treatments; trial 4. Tomato and soybean plants grew for 27 and 30 days respectively.

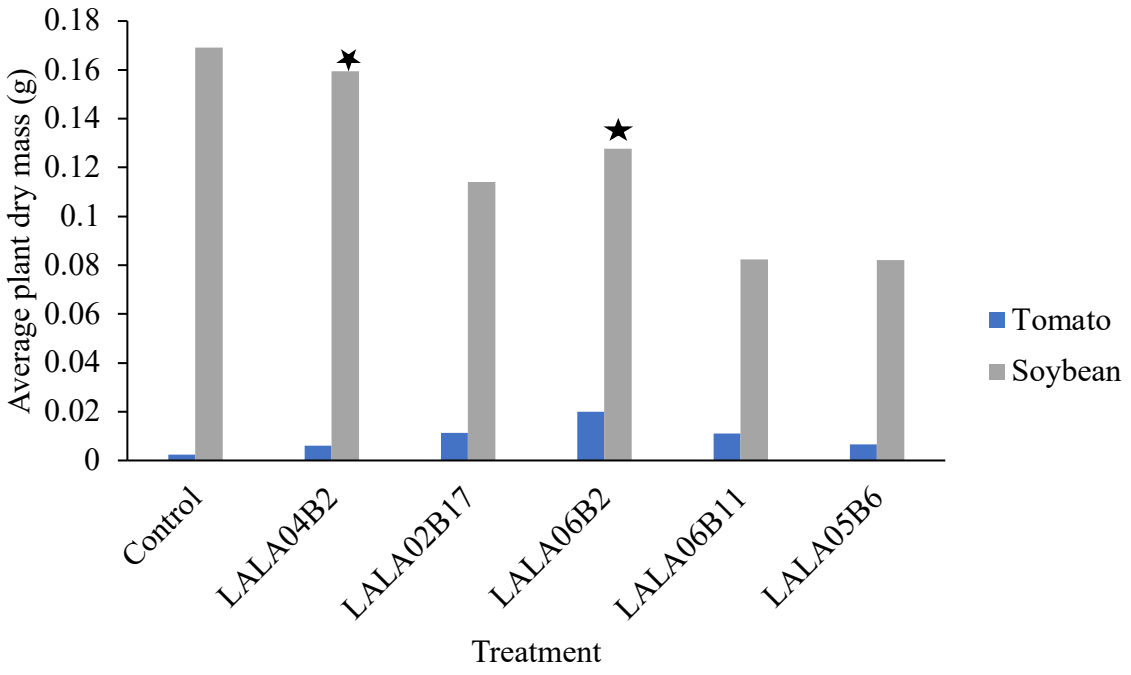

Figure 3.8 Average plant dry mass (g) and standard deviation of tomato plants (*Salonum lycoperscium*) and soybean plants (*Glycine max*) for varying treatments; trial 4. Tomato and soybean plants grew for 27 and 30 days respectively.

4.2 Visualizing selected bacteria

As seen in Figure 5.0, selected bacteria grow different from one another with distinctive forms, opacity, pigment and surface appearance. Figures 6.1 to 6.4 shows images of bacterial cells stained with iodine and imaged on a light microscope.

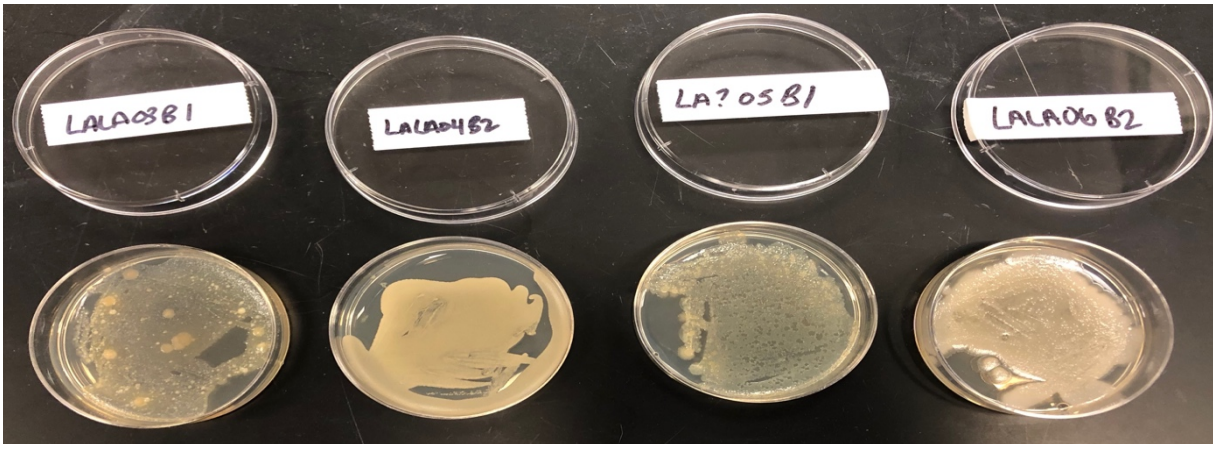

Figure 3.9 The four selected bacteria growing on nutrient agar plates. LALAO4B2 is opaquer and smoother in appearance versus LALAO3B, LA? O5B1 and LALAO6B2.

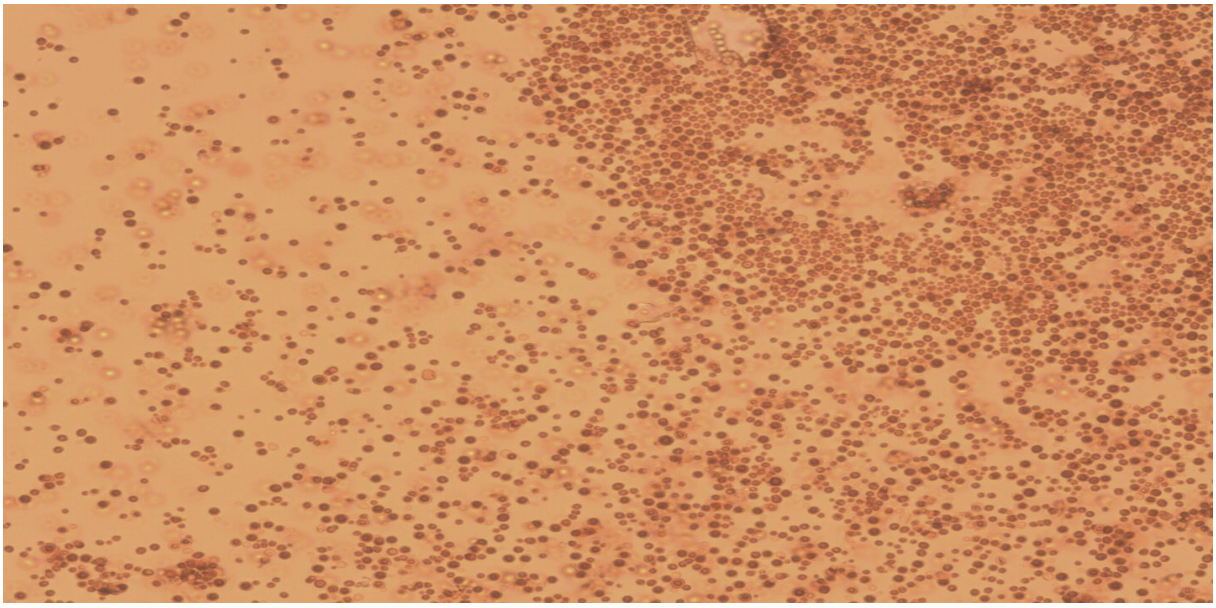

Figure 4.0 Image of LALAO4B2 MGX400 stained with iodine, visualized using a Light microscope. Cells are very circular in shape and very visible.

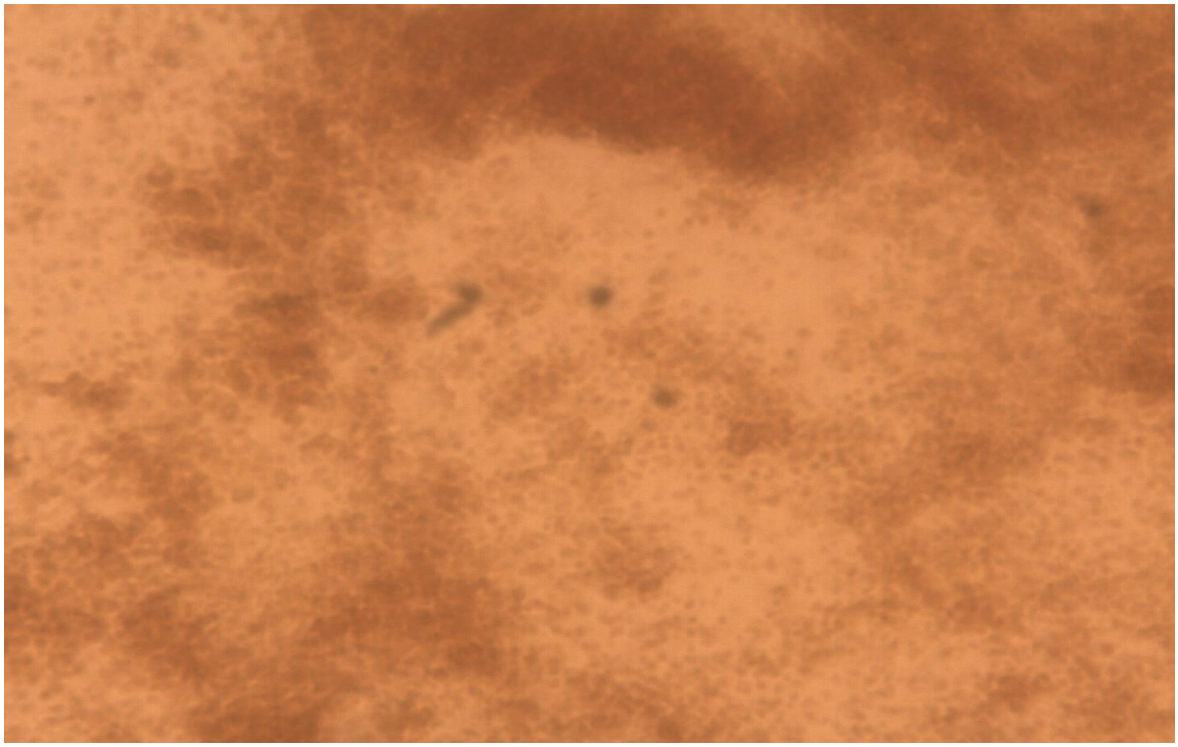

Figure 4.1 LALAO3B1 MGX1000 stained with iodine visualized using a light microscope. Cells are very small with little to low visibility of individual cells.

Figure 4.2 *Stenotrophomonas rhizophila* (LA? O5B1) MG x400 stained with iodine visualized using a light microscope. Cells appear to be very minute and circular in shape.

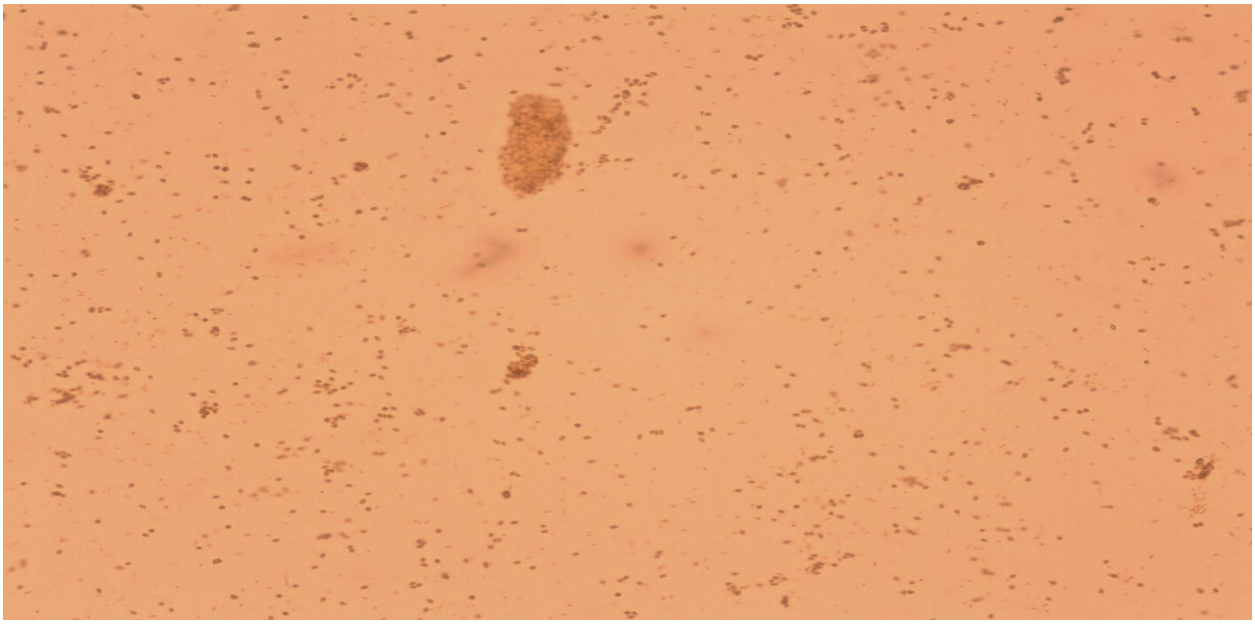

Figure 4.3 LALAO6B2 MG X400 stained with iodine visualized using a light microscope. Cells are circular in shape and very small.

4.3 Bacteria Identification

Three of the four bacteria tested were identified to be *Stenotrophomonas rhizophila, Bacillus Proteolyticus, Paenibacillus alvei.* Only the *Stenotrophomonas rhizophila* had a high percentage identity and query cover. The LALAO3B1 remains unidentified.

Table 2.0 Identity of three of the four bacteria isolated from L'Acadie Vineyards, Wolfville, NS.

Assigned Identity	Bacteria Identity	Percent Identity	Query cover
LA? O5B1	*Stenotrophomonas rhizophila*	98.89%	96%
LALAO6B2	*Bacillus Proteolyticus*	97.45%	52%
LALAO4B2	*Paenibacillus alvei*	98.77%	51%
LALAO3B1	unknown	unknown	unknown

4.4 Analysis of Volatile Organic Compounds

Ethyl 2-methylbutylrate (Butanoic acid, 2-methyl-, ethyl ester) was the most abundant organic volatile present in the volatile profile of *Stenotrophomonas rhizophila,* with a total of five organic compounds produced and identified. There were two abundant compounds present in the volatile profile of the LALAO4B2 bacteria identified to be 2-Tert-Butyl-3-methyloxirane (Oxirane, 2-(1,1-dimethylethyl)-3-methyl) and 2-nonanone. Several organic volatiles were identified in the profile of LALAO3B4, including 2-nonanone and various organic acids. Five organic volatiles were identified in the LALAO6B2 headspace, with 1,3-Pentadiene, (Z)- being the most abundant.

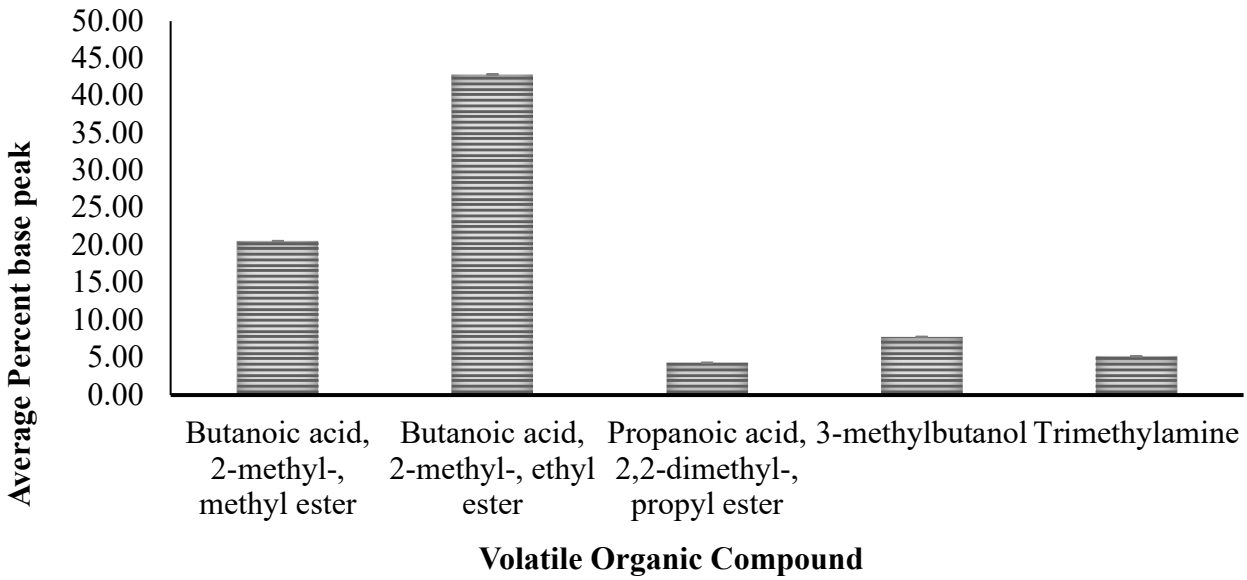

Figure 4.4 Volatile organic profile for *Stenotrophomonas rhizophila* showing organic compounds produced, its average percent abundance and standard error of the mean. Five organic volatiles were identified.

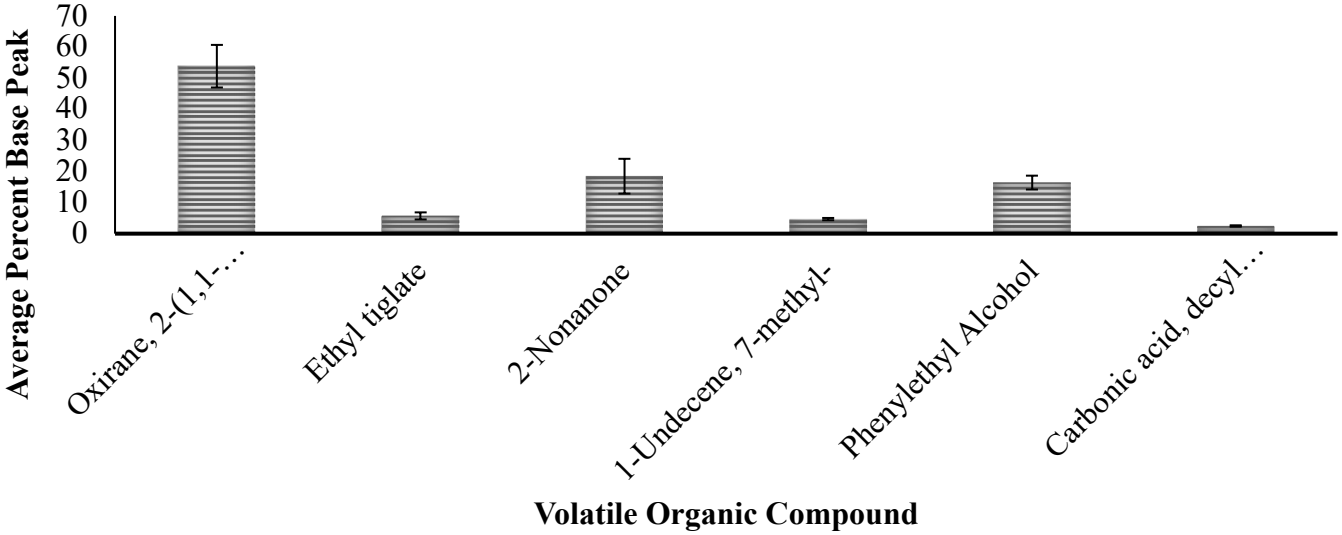

Figure 4.5 Volatile organic profile for LALAO4B2 showing organic compounds produced, its average percent abundance and standard error of the mean. Six organic compounds were identified.

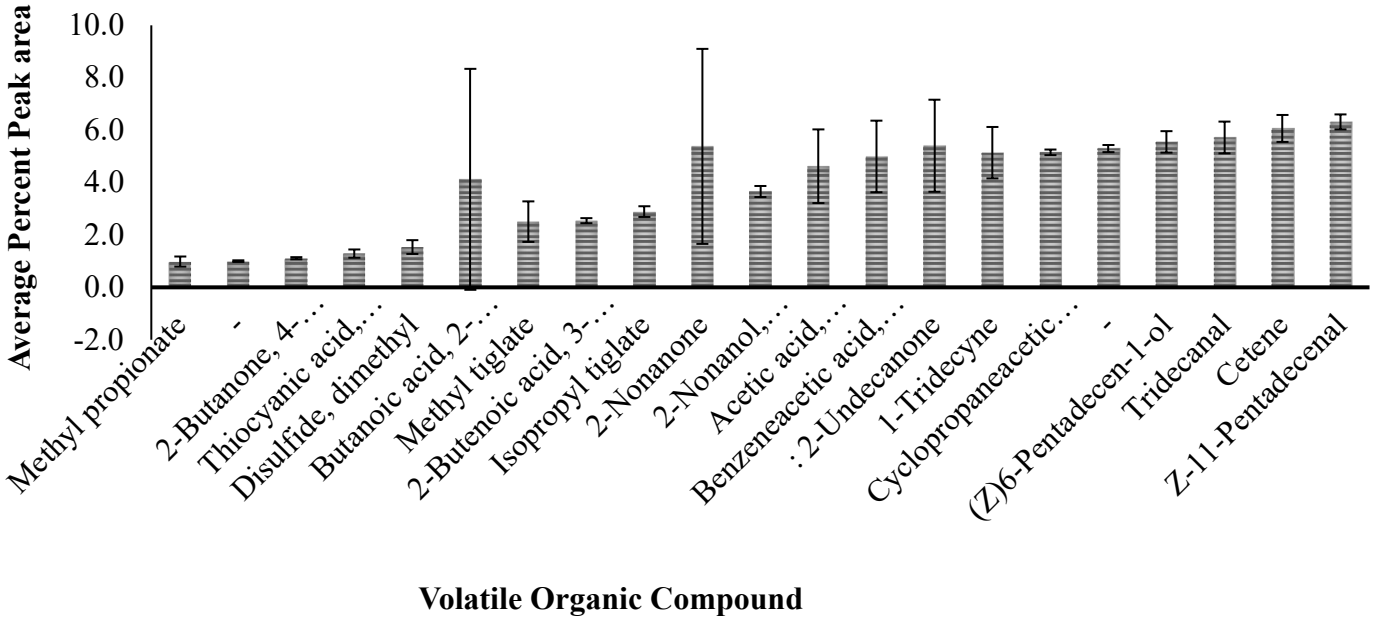

Figure 4.6 Volatile organic profile for LALAO3B1 showing organic compounds produced, its average percent abundance and standard error of the mean. There were 20 out of 21 compounds identified.

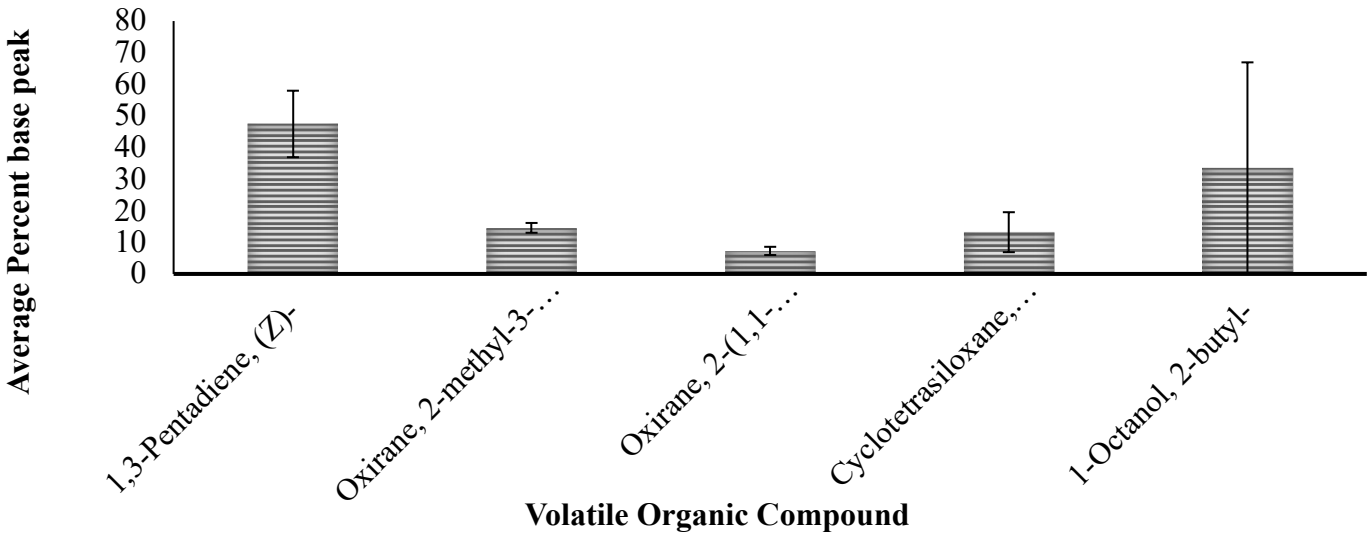

Figure 4.7 Volatile organic profile for LALAO6B2 showing organic compounds produced, its average percent abundance and standard error of the mean. There were five compounds identified.

4.5 Statistical analysis

4.51 Normality tests for two controls

In order for comparisons to be made among the two control treatments and four bacteria treatments, the control and nutrient agar control treatments should have no significant difference between the two sessions. A two-way ANOVA was first conducted to test whether the controls in both sessions had the same distribution. The control data for session 1 and 2 was pooled and used in a one-way ANOVA to test for significant differences in treatments.

Table 2.1 Ryan-Joiner normality test for control and nutrient agar control in session1 and 2.

	Control			Nutrient agar control		
Measure	N	RJ value	P-Value	N	RJ value	P-Value
Shoot length	23	0.958	0.068	23	0.98	> 0.1
Shoot mass	23	0.98	> 0.1	23	0.983	> 0.1
Root mass	23	0.968	> 0.1	23	0.982	> 0.1

4.52 Two-way Analysis of Variances

Table 2.2 Two-way ANOVA data on controls.

Source	Type III SS	df	Mean Squares	F-ratio	p-Value
Control$	0.365	1	0.365	0.062	0.804
Part$	959.069	2	479.534	81.279	0.000
Part$*Control$	0.780	2	0.390	0.066	0.936
Error	371.689	63	5.900		

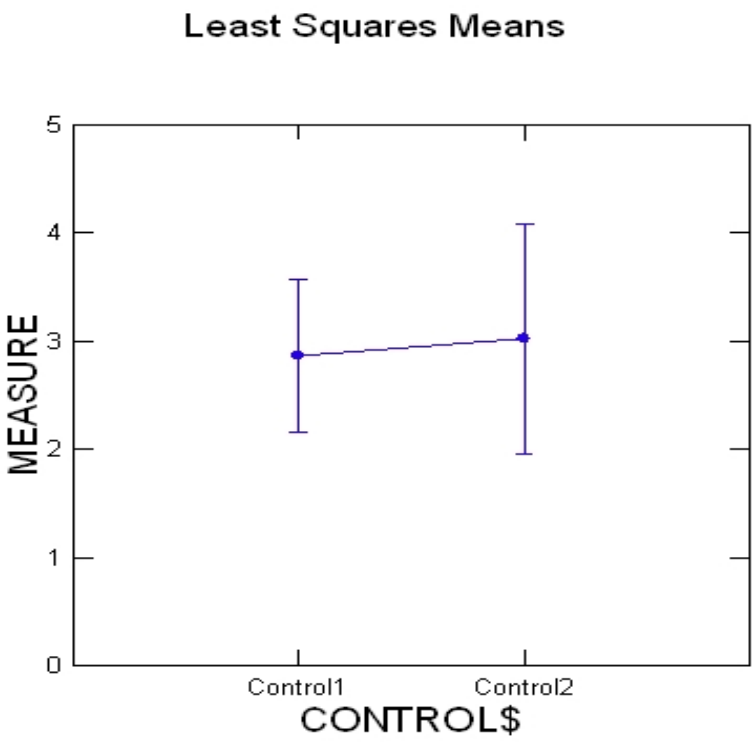

Figure 4.8 Showing the means of control 1 and 2 and the variation about the mean. Means were very similar. Means and variation about the mean are similar.

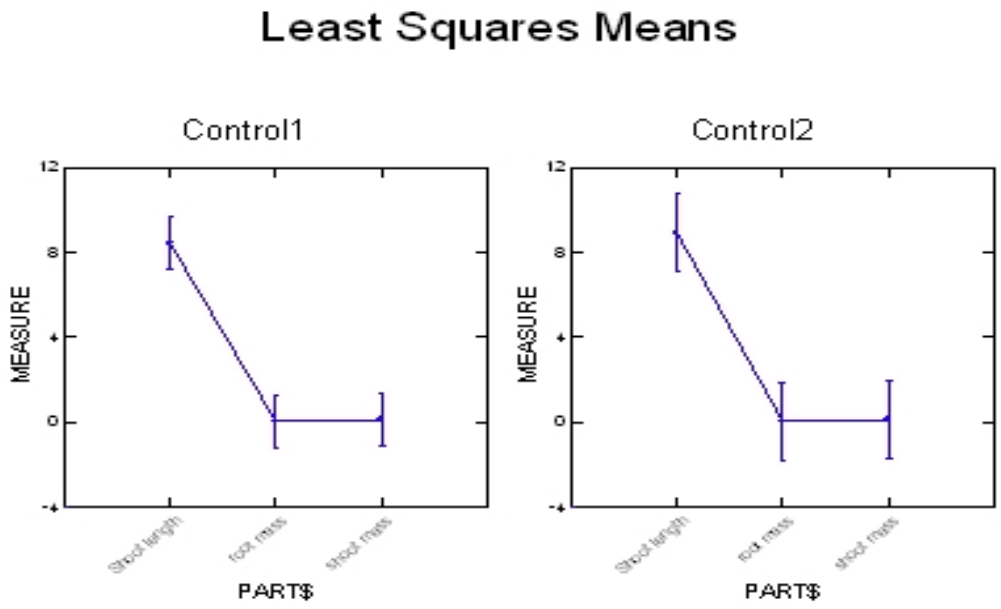

Figure 4.9 Comparing the means and variations about the mean for measured plant parts in control 1 and 2. There was no interaction when comparing control 1 and 2.

Table 2.3 Two-way ANOVA data on nutrient agar controls.

Source	Type III SS	df	Mean Squares	F-ratio	p-Value
Control$	2.148	1	2.148	0.247	0.621
Part$	1,230.958	2	615.479	70.704	0.000
Part$*Control$	3.718	2	1.859	0.214	0.808
Error	548.418	63	8.705		

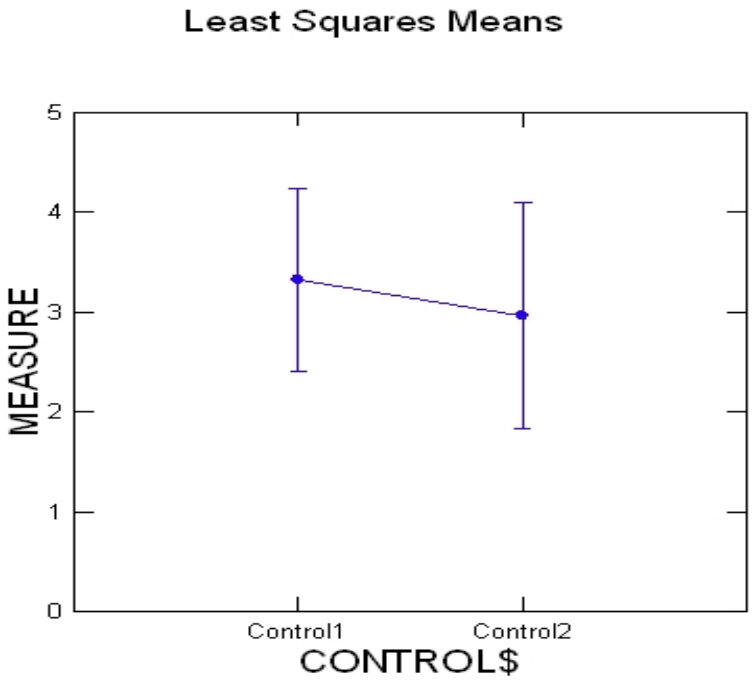

Figure 5.0 Showing the means of nutrient agar control 1 and 2 and the variation about the Mean. Means and variation about the mean are similar.

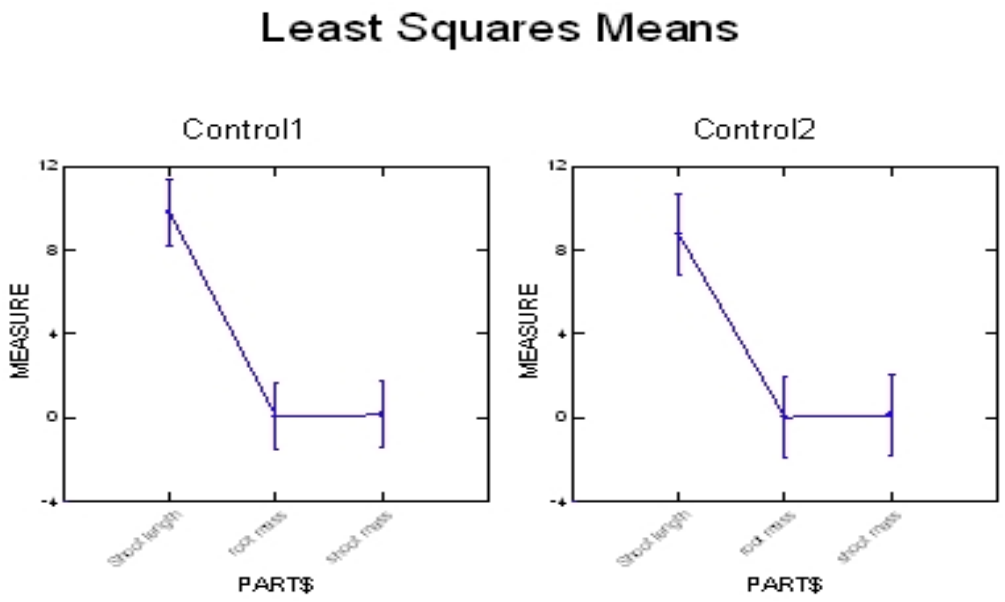

Figure 5.1 Comparing the means and variations about the mean for measured plant parts in nutrient agar control 1 and 2. There was no interaction between nutrient agar control 1 and 2.

4.53 Testing for variation among treatments

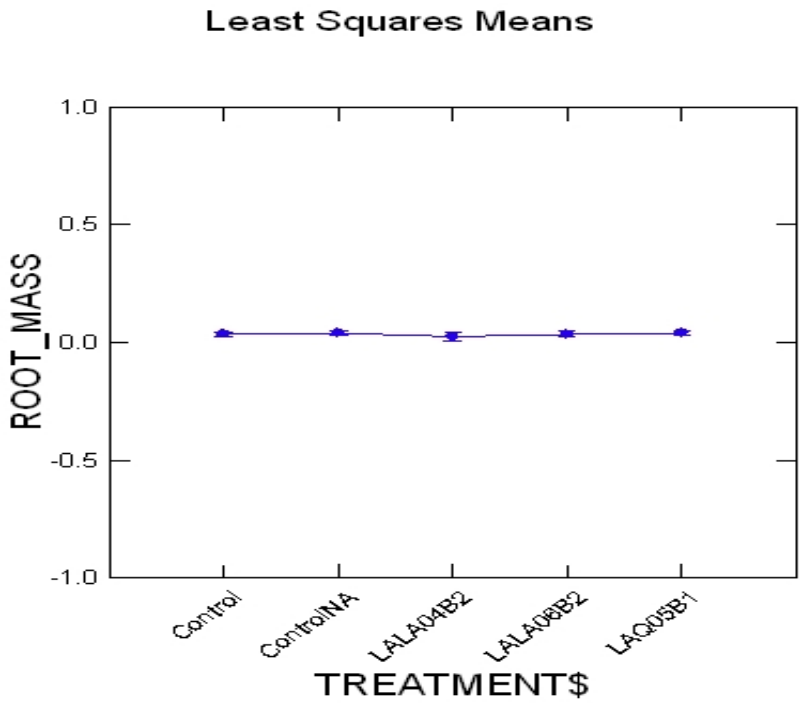

Figure 5.2 Showing the least square means of the treatments for root mass. Shapiro-Wilk normality test p-Value of 0.072, a Levene's test for homogeneity of variances with a p-Value of 0.308. Kruskal-Wallis Test Statistic: 2.417 The p-value is 0.660 assuming chi-square distribution with 4 df.

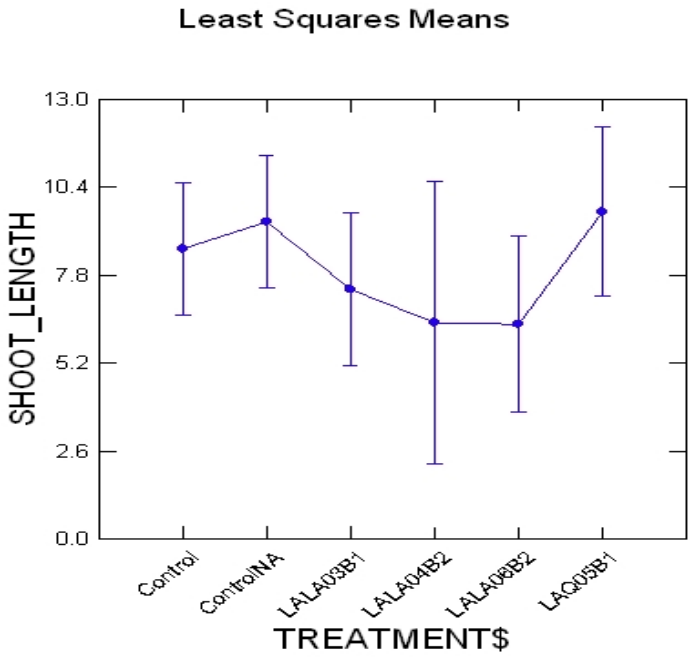

Figure 5.3 Showing the least square means of the treatments for shoot length. Shapiro-Wilk normality test p-Value of 0.026, a Levene's test for homogeneity of variances with a p-Value of 0.332. Kruskal-Wallis Test Statistic: 5.522 The p-value is 0.356 assuming chi-square distribution with 5 df.

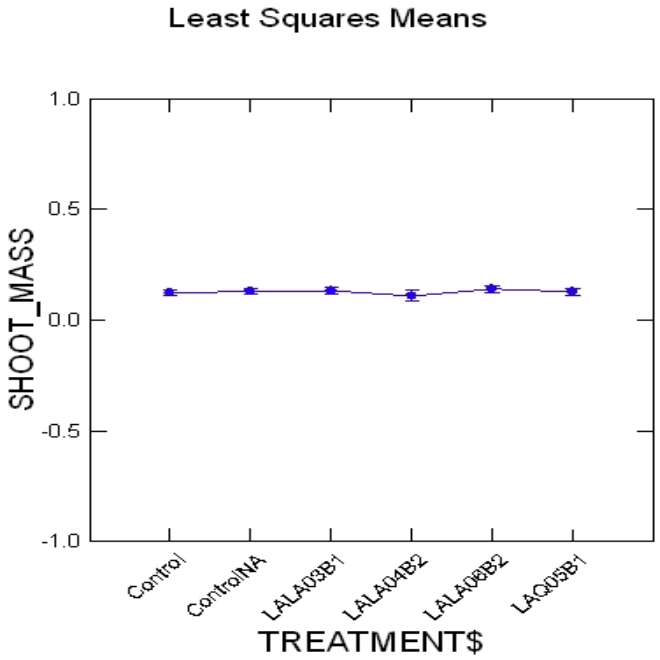

Figure 5.4 Showing the least square means of the treatments for shoot mass. Shapiro-Wilk normality test p-Value of 0.054, a Levene's test for homogeneity of variances with a p-Value of 0.404. Kruskal-Wallis Test Statistic: 7.681 The p-value is 0.1758 assuming chi-square distribution with 5 df.

4.6 Stenotrophomonas and biochar

As a result of the school shut down due to COVID-19, I was unable to obtain shoot length, root mass and shoot mass from this experiment. Data obtained were only images of the plants and the number of plants which grew for each treatment.

Figure 5.5. Image of soybeans grown in the control (only potting soil) treatment for 26 days. 5 out of 10 soybeans geminated and grew.

Figure 5.6. Image of soybeans grown in the control (only potting soil) treatment for 26 days. 3 out of 10 soybean plants germinated and grew.

Figure 5.7. Image of soybeans grown in the Biochar control (potting soil and biochar) treatment for 26 days. 4 out of 10 soybean plants germinated and grew.

Figure 5.8. Image of soybeans grown in the Biochar control (potting soil and biochar) treatment for 26 days. 6 out of 10 soybean plants germinated and grew.

Figure 5.9. Image of soybeans grown in the Biochar and nutrient broth control (potting soil, biochar and nutrient broth) treatment for 26 days. 4 out of 10 soybean plants germinated and grew.

Figure 6.0. Image of soybeans grown in the Biochar and nutrient broth control (potting soil biochar and nutrient broth) treatment for 26 days. 3 out of 10 soybean plants germinated and grew.

Figure 6.1. Image of soybeans grown in the *Stenotrophomonas rhizophila* (potting soil biochar, nutrient broth and bacteria) treatment for 26 days. 4 out of 10 soybean plants germinated and grew.

Figure 6.2. Image of soybeans grown in the *Stenotrophomonas rhizophila* (potting soil biochar, nutrient broth and bacteria) treatment for 26 days. 3 out of 10 soybean plants germinated and grew.

5.0 DISCUSSION

5.1 Bacteria screening

When comparing bacteria from trial 1 (figure 3.1 and 3.2, LALAO3B1 has the highest average shoot length and shoot mass in both soybeans and tomatoes. This bacteria was therefore selected as one of the bacteria for large scale testing. In addition to LALAO3B1, LA?O5B1 which has been identified as the bacteria *Stenotrophomonas rhizophila,* also showed the highest average shoot length and plant mass (figure3.5 and 3.6) when compared with other treatments in that trial; specifically in the soybean plants. In trial 4, two bacterial isolates were selected LALAO4B2, LALAO6B2, *Paenibacillus alvei* and *Bacillus Proteolyticus* respectively; they both had the highest average plant mass and shoot length in soybean plants and *Bacillus Proteolyticus* had the highest average mass and shoot length in tomatoes. No bacteria was selected from trial 2 because bacteria had conflicting results when comparing average shoot length and shoot mass.

Overall soybean plants seemed to grow much faster than tomato plants and were therefore selected as the plant species for large scale testing.

5.2 Bacteria Identification and visualization

Paeibacillus alvei is depicted in figure 4.0, is an aerobic, gram positive bacterium which forms endospores (Kim et al. 2011). *P.alvei* is a known invader of the foulbrood of honeybees (Forsgren 2010). When comparing the stained bacteria in figure 4.0 and the bacteria found by researchers in figures 6.3 and 6.4, it is clear to see that they are not the same bacteria. This leads me to believe that the identification for LALAO4B2 is not *Paeibacillus alvei;* these bacteria are rod shaped compared to the circular like form in figure 4.0. In addition to the difference in

appearance, there was a 51% query cover which meant that only about half of the query sequence overlapped with the reference sequence, further affirming that the *Paeibacillus alvei* is the incorrect identification of the bacteria isolated and tested.

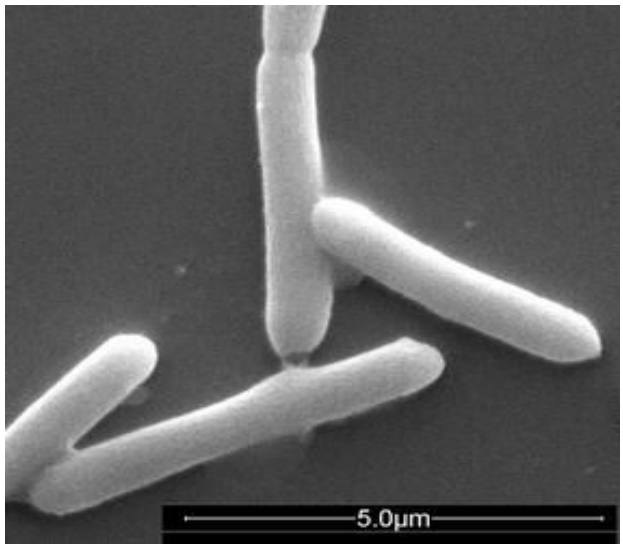

Figure 6.3 Scanning electron micrograph of *P.alvei* cells (Chevrot et al. 2013)

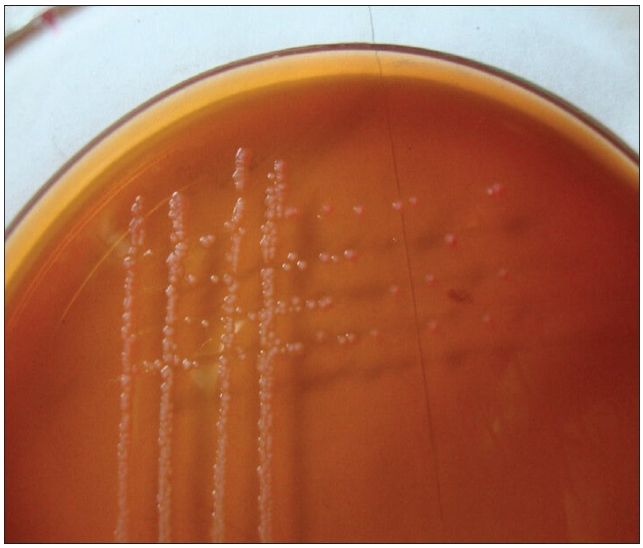

Figure 6.4*P.alvei* on growth medium (Padhi et al. 2013)

The microorganism LALAO3B1 was not identified using the extraction and identification method used. As seen in figure 4.1 at MGX 1000, the cells are barely visible. An improved extraction method should be implemented to correctly identify this microorganism.

The *Stenotrophomonas* species as seen in figure 4.2 and 3.9 are free-living, gram negative bacteria that are capable of fixing nitrogen, promoting plant growth and protecting plants from biotic and abiotic stress Park et al. 2005; Emgamberdieva et al. 2011; Berge et al. 2013). According to research done by Alavi et al., *S.rhizophila* produced spermidine and glucosylglyceral in response to the release of exudates by plants; those substances released are able to protect plant roots and other rhizosphere microbiome against abiotic stress. After

amplifying the 16S rRNA using PCR and submitting our samples to Genome Quebec for Sanger sequencing, a sequence with a 96% query cover and about 99% identity was found when searched in the NIST BLAST system; based on these results and the characteristic yellow transparent growth pattern (figure 3.9), there is very little doubt that the bacteria isolated and tested is *S. rhizophila.*

Figure 3.9 and 4.3 shows the bacteria identified as *Bacillus Proteolyticus*. *B. Proteolyticus* is a protease producing bacillus species associated with fresh water and marine fish (Bhaskar et al. 2007). A query cover of 52% was found when 16S rRNA sequences were input into the BLAST database. Based on these results, it is unclear whether or not the bacteria identified as *B. Proteolyticus* is what was isolated and tested.

5.3 Organic Volatile compounds produced

Based on Figure 6.5 the compound Ethyl 2-methylbutylrate (Butanoic acid, 2-methyl-, ethyl ester) produced by *S.rhizophila* is the most abundant volatile present based on its high percent peak area.

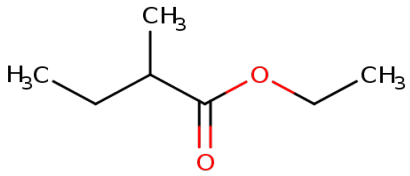

Figure 6.5 Structure of the ester Ethyl 2-methylbutylrate.

This compound was found as a compound produced by microorganisms when searched in the mVOC database. This compound is mostly produced by fungi and bacteria, specifically bacteria

such as *E.coli* and *S.aureus* (Tait et al. 2014). The remaining compounds were not found in the mVOC database.

Figure 4.5 shows the VOC's produced by LALAO4B2. The compound 2-Tert-Butyl-3-methyloxirane (figure 6.6) was the most abundant VOC detected. This compound was not found in the mVOC database.

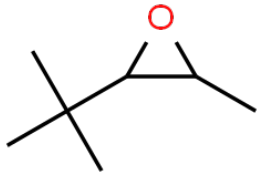

Figure 6.6 structure of 2-Tert-Butyl-3-methyloxirane

The compound 2-nonanone from figure 4.5 and figure 6.7 is produced by bacteria such *as B.simplex, B.substilis* and *B.weihenstephanensis* (Gu et al. 207). The biological function of this compound is that it reduces movement of *Panagrelleus redivivus* and *Bursaphelenchus xylophilus* (Gu et al. 2007).

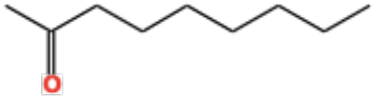

Figure 6.7 structure of 2-nonanone

All other compounds found in the volatile profile of LALAO4B2 were not found in the mVOC database.

In figure 4.6 the bacteria LALAO3B1 produces several compounds that are produced by bacteria. Specifically, Dimethyldisulfide, 2-nonanone, (Z)6-Pentadecen-1-ol, Ethyl 2-methylbutylrate.

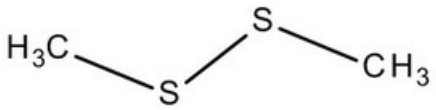

Figure 6.8 Structure of dimethyl disulfide

Dimethyl disulfide (figure 6.8) is produced by bacteria such as *Actinomycetes Spp* and *Aeromonas veronii,* and functions by having an antagonistic effect against sapstain fungi (Schultz and Dickschat 2007). (Z)6-Pentadecen-1-ol is produced by Streptomyces Alboflavus TD-1 and has inhibitory activity on mycelia growth (Wang et al. 2013).

The Volatile profile for LALAO6B2 depicted in figure 4.7 contains 2-Butyloctanol, which is known to be produced by Carnobacterium divergens 9P and Pseudomonas Fragi 25 (Ercolini et al. 2009). Figure 6.9 depicts the structure of 2-Butyloctanol. All other organic volatiles produced by LALAO6B2 are not present in the mVOC database.

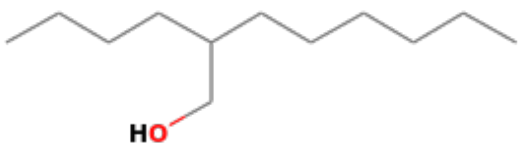

Figure 6.9 Structure of 2-Butyloctanol

5.4 Statistical Analysis

The control and nutrient agar control data was pooled (p= 0.804 and 0.621 respectively table 2.2-2.3; hence we failed to reject the null hypothesis that there is no difference in control and nutrient agar control data in session1 and 2) and used to compare the shoot length, mass and root mass to that of the four bacteria tested. There was no difference in root mass, shoot length and shoot mass in any of the treatments (figure 5.2,5.3, 5.4), where a p value of 0.660,0.356 and 0.404 was obtained respectively with α=0.05. Hence, we fail to reject the null hypothesis that all distributions are equal.

Plants seemed to have grown unhealthy (thin, weak stems) and this may have been the reason for the lack of statistical significance found within the data; there may have been other confounding variables such as sunlight or a fungus limiting the growth of the plant. In addition, due the lack of a positive control, it was difficult to determine if the results were insignificant due to the bacteria or the experimental design. A positive control should be included in any future studies.

5.5 Stenotrophomonas and biochar experiment

Although measurements were not taken for this experiment, the number of plants grown was recorded and a rough comparison of shoot length can be made. A total of 8, 10, 7 and 7 out of 20 soybeans seeds planted each grew for the control, biochar control, biochar and nutrient broth control and the *Stenotrophomonas rhizophila* treatments respectively. Although more seeds germinated from the biochar control, there was more extensive growth in the treatment containing the tested bacteria; all 7 plants were of similar length and development, compared to those of the control experiment where there were short, underdeveloped plants. Based on the

images in figures 5.5 to 6.2, all plants grown with biochar seemed to have greater shoot length than that of the control. Definitive conclusions cannot be made from this experiment due to the inability to test for statistical significance.

6.0 CONCLUSION

Only one bacterial isolate was successfully identified (*Stenotrophomonas rhizophila*), two of the four were identified without confidence (LALAO4B2 and LALAO6B2) and no identity was found for the LALAO3B1 isolate. The volatile profiles for all four bacteria were obtained; some compounds were found to be produced by other bacteria species. There was no significant difference in the root mass, shoot length and shoot mass in all six treatments.

There were many possible sources of error, one of which could be the concentration of volatiles present in the headspace between the petri dish and the soil. The concentration may have been too low to elicit any growth promotion. In addition, cotyledons present on shorter soybean plants resulted in a higher dry mass when compared to taller more developed plants without the seed leaf present. For further studies, a positive control (bacteria proven to have growth enhancing properties) should be present in order to be certain that the bacteria and not the experimental set up lead to the results obtained.

Although the experiment with the S*tenotrophomonas rhizophila* bacteria could not be successfully completed, it may be beneficial to repeat this experiment and test for statistical

significance. Based on observation, the bacteria treatment seemed to have more extensive soybean growth than any other treatment, this may lead to the potential characterization of S*tenotrophomonas rhizophila* as a plant-growth promoting rhizobacteria.

7.0 REFERENCES

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