Biofertilizers: Investigating the plant growth-promoting ability and mode of action of a novel PGPR using biochar as an inoculum carrier

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Abstract

The rise in the World's population has forced us to employ hasty plant growth-promoting products such as chemical fertilizers and agrochemicals, to meet up with the rising demands of food production. These products have had detrimental effects on the environment. To reduce the harmful effects of these synthetic products, scientists are now exploring more sustainable alternatives such as biofertilizers. This project evaluates the effectiveness of a novel plant growth-promoting bacteria *Fictibacillus enclensis* on plant growth.

This study has shown that *F. enclensis* promotes plant growth in peppers, barley, carrots. It also led to an increase in nitrogen content of bush beans. *Fictibacillus enclensis* has been shown to affect the barley root system by shortening the root length, while encouraging root hair growth. *Fictibacillus enclensis* was not found to produce the protein ACC deaminase and has little to no synergistic effect on plant growth when coupled with *Stenotrophomonas rhizophila*.

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PGPR	Plant growth promoting rhizobacteria
PGPB	Plant growth promoting bacteria
NB	Nutrient broth
BC	Biochar
ACC	1-amino-cyclopropane-carboxylic acid
F.encl	*Fictibacillus enclensis*
P.rho	*Pseudomonas rhodesiae*
S.rhiz	*Stenotrophomonas rhizophila*
EFF	Environmentally friendly fertilizer
GHG	Green house gas
VOC	Volatile organic compound
ROS	Reactive oxygen species
IAA	Indole acetic acid
GA	Gibberellic acid
CK	Cytokinins
NF	Nod factor
SBP	Siderophore binding protein
CEC	Cation exchange capacity
CHN	Carbon hydrogen nitrogen

1 Introduction

1.1 Sustainability and Food security

The term sustainability is often mistaken to pertain only to environmental sustainability, but the concerns of social equity and economic development are also included in the definition. According to the Brundtland Commission, the term sustainable development or sustainability is defined as " development that meets the needs of the present without compromising the ability of future generations to meet their own needs" (Ramsden 2010). Some factors responsible for or driving sustainability include population growth, climate change, resource allocation, environmental damage, energy usage, and socio-economic inequality (Roosa 2008). Of these factors, population growth is the major driver increasing the demand for all other resources. The world's population is expected to increase by 2 billion people in the next 30 years amounting to 11.2 billion by the year 2100 (Compant et al. 2005). Population growth has a major impact on environmental sustainability, and more specifically on food security. As defined by the Food and Agriculture Organization of the United Nations (FAO), food security not only incorporates the availability and access to sufficient quantities of an appropriate quality of food, but it also encompasses the utilization and stability of this food supply ("Food and Agriculture Organization of the United Nations" 2013). The Agricultural industry plays a vital role in the global supply of food, both directly and indirectly.

1.1.1 Agriculture

The Canadian agricultural industry is a major contributor to the Canadian Economy. According to Agriculture and Agri-Food Canada, primary agriculture, which is defined as work that is

performed within the boundaries of a farm, nursery, or greenhouse, contributed $39.8 billion in 2020, amounting to 2.1% of Canada's gross domestic product ("Overview of Canada's Agriculture and Agri-Food Sector - Agriculture.Canada.Ca," n.d.). Some of the top crops grown and exported in Canada are wheat, canola, soybeans, and potatoes, generating billions of dollars annually. There are 193,492 farms covering 68.9 million hectares of Canadian land (6.9% of Canada's land area) ("Overview of Canada's Agriculture and Agri-Food Sector - Agriculture.Canada.Ca," n.d.). In poorer countries, such as Liberia and Somalia, agriculture accounts for 40-60% of the country's GDP and 80 % of employment. It is no surprise that the agricultural industry is such a huge contributor to a country's economic well-being (*World Development Report 2008: Agriculture for Development* 2007).

As the world population continues to increase, the demand for a secure and healthy food supply will also increase resulting in an even greater threat to food security, as agricultural lands become limited or overused. Compared to traditional farming practices, intensive farming (as most of farming practices today) rely heavily on synthetic fertilizers, pesticides and machinery to increase industrial agro-outputs (Sun, DAI, and Yu 2017; Thirkell et al. 2017). According to the World Development Report, (*World Development Report 2008: Agriculture for Development* 2007), the rate of agricultural productivity growth is declining from ~ 2% per year during the green revolution to ~1% in recent years. Agricultural productivity depends primarily on two major factors: nutrient acquisition and stress resistance. One key driver of decreased agricultural productivity is soil degradation. Soil degradation refers to the decline in soil quality, induced primarily by human activity (Bindraban et al. 2012). Soil erosion, fertility loss, acidification,

salinity and carbon decline and compaction are all recognized as threats to agricultural productivity and food security (McBratney, Field, and Koch 2014). Soil erosion is just one of many threats to the agricultural industry; water pollution and air pollution are also major contributors to the destabilization of the environment. To curtail the effects of intense cultivation on the quality of the soil, water and air, several sustainable strategies must be implemented. Synthetic or chemical fertilizers are another key player known for its contribution to increased crop productivity and yields worldwide, but its long-term effects on soil air and water cannot be ignored.

1.1.2 Chemical fertilizers

Chemical fertilizers have been used for thousands of years when farmers used manure and other minerals to enhance the productivity of crops. Fertilization of soil increases soil fertility and crop productivity by providing the necessary nutrients required for plant growth (i.e., Potassium, Nitrogen, Phosphorus). However, the misuse of inorganic fertilizers is known to have adverse environmental effects such as eutrophication, accumulation of heavy metals and greenhouse gas emissions to name a few (Savci 2012). Irrigation runoff from crop fields leads to the excessive transport of nutrients into offsite environments, specifically waterways (Lentz and Lehrsch 2010), leading to a phenomenon known as eutrophication. Eutrophication occurs when lakes, rivers and coastal waters become rich in plant nutrients, mainly nitrogen and phosphorus, leading to an overgrowth in plant biomass such as algae (Nyenje et al. 2010). According to an issue of the South African Water Research Commission (2008), 54% of lakes and reservoirs in Asia, 53% in Europe, 48% in North America and 28% in Africa are impaired by eutrophication (Nyenje et

al. 2010). A study by (Lentz and Lehrsch 2010)found that the quantity of nutrients and timing of application significantly affected the extent of nutrient loss during irrigation runoff. When compared to manure, they found that inorganic fertilizer leads to higher nutrient runoff from a furrow-irrigated field in a two-year period. The major effects of eutrophication are extensive microalgal growth leading to decreased water clarity and destruction of benthic habitat by way of increased opacity and, hence, decreased sunlight penetration (Ansari and Gill 2013). Additionally, eutrophication is also known to cause bottom water hypoxia. This is the production of CO_2 from decomposed organic matter, leading to water acidification, all of which negatively impacts the ecology of water systems (Ansari and Gill 2013; Nyenje et al. 2010).

Chemical fertilizers are also known to accumulate and transfer heavy metals such as zinc, copper, arsenic and cadmium to plants, water bodies, and throughout the food chain (Zhao and Wang 2010; Spångberg et al. 2011). A study by Zhao and Wang (2010) tested the mercury levels in 10 different chemical fertilizers and found that calcium superphosphate fertilizers contained the most mercury among the 10 tested, with NPK (nitrogen, phosphorous, potassium) compound fertilizers coming in second. The authors also found that corn shoot biomass was gravely affected by mercury in the soil (lower shoot biomass). Another study tested the heavy metal/metalloid accumulation in wheat samples for chemical fertilizers and organic fertilizers and found that all metals (e.g., Zn, Co, Fe, Cd, Pb, Cu, Cr) were higher in the chemical fertilizer treatments compared to the organic fertilizer treatments (Ugulu et al. 2020).

Another concern for the overuse of synthetic fertilizers is their effects on air quality. Chemical fertilizer application is the largest source of agricultural greenhouse gas (GHG) emissions (Yang et al. 2015; Gong et al. 2012). In 2005 it was estimated that 10-12% of total global anthropogenic emissions stemmed directly from agricultural GHG emission (Yang et al. 2015). Not only are greenhouse gases produced during the manufacturing and transportation of chemical fertilizers, but they are also produced by chemical fertilizers in the soil. Methane, nitrous oxide and carbon dioxide are the three main GHG's associated with agriculture (Wang et al. 2017; Yang et al. 2015). A study by Wang et al. (2017), compared the quantities of greenhouse gas emission from different fertilizer types to identify the fertilizer with the lowest emission. This study was conducted in China using available data from 1993 to 2007 and found that fertilizers with ammonium hydroxide and calcium magnesium phosphate had the highest emission factor (Z. Wang et al. 2017; Bennetzen, Smith, and Porter 2016). As we know, GHGs contribute to the depletion of the earth's ozone layer, resulting in global warming, smog and air.

All of the aforementioned examples further emphasize the need for more environmentally friendly and sustainable forms of agricultural practices.

1.1.3 Sustainable agriculture

Considering all of the detrimental effects of the prolonged use of chemical fertilizers, more sustainable, and environmentally friendly alternatives to synthetic fertilizers has emerged. Sustainable agriculture entails farming in such a way that meets society's present food and textile needs without compromising the ability of future generations to meet those needs as well (Mir,

Shameem, and Parray 2019; Igiehon and Babalola 2017). Three eco-friendly alternatives to chemical fertilizers are organic fertilizers, which include 1) manure, peat, compost, seaweed etc.; 2) slow-release environmentally friendly fertilizers; and 3) biofertilizers.

Organic fertilizers are derived from dead or decaying plant and animal matter. They contain a significant amount of micro and macro-nutrients boosting soil fertility, improving the water holding capacity of the soil, and cation exchange capacity, ultimately leading to increased crop yield (Rostaei et al. 2018). Organic fertilizer applications make it difficult to over-fertilize plants since they are slow-release fertilizers. There is no risk of toxic chemical build-up as compared to chemical fertilizers. There are several studies that illustrate the ability of organic fertilizers to increase soil biodiversity and crop nutritive value, as well as to improve the microbial community and increase crop yield (Lazcano et al. 2013; Y. Zhao et al. 2009; Cao et al. 2011; Bengtsson, Ahnstrom, and Weibull 2005). In a study by Liu et al. 2016, a meta-analysis was conducted to investigate the ability of soil treated with organic fertilizer, to increase nematode biodiversity. It was found that organic fertilizers, which were more carbon-rich, increased species richness and nematode abundance when compared to inorganic fertilizer (greater nitrogen input from inorganic fertilizer lead to reduced species richness) (Liu et al. 2016). Lazcano et al. 2013 showed that in the short-term, organic fertilizers can increase the soil microbial community as opposed to inorganic fertilizers, which decrease soil microbial diversity both in the long and short term. This increase in soil biodiversity is attributed to the ability of organic fertilizers to increase soil respiration, soil water retention, microbial biomass, and soil porosity and nutrient content (Zhao et al. 2009). Additionally, organic fertilizers are known to

increase crop nutritive value. In a study by Rostaei et al. 2018, the chemical compositions and antioxidant activity of dill essential oil were evaluated in sole crop and dill-soybean intercropping systems treated with organic manure and chemical fertilizers. It was found that dill-soybean intercropping and application of organic manure enhanced the content of α-phellandrene and p-cymene, both useful for industrial use (Rostaei et al. 2018). The aerial yield of the essential oil of dill improved, as well as the chemical compositions and antioxidant activity, when organic manure was added versus chemical fertilizer (Rostaei et al. 2018). As promising as organic fertilizers may seem, they contain very low nutrient content and, therefore, more is required to provide plants with the required nutrient quantity; this makes it difficult to sustain large-scale agriculture without the use of inorganic fertilizers (Roba 2018). Additionally, organic fertilizers can be potentially pathogenic as they are derived from animal feces or decaying plant or animal organic matter that may have been contaminated with pathogens (Roba 2018). Weithmann et al. 2018 found that organic fertilizers from biowaste fermentation contribute to microplastic contamination in the environment. All previously mentioned disadvantages lead to the conclusion that although organic fertilizers are a better alternative to chemical fertilizers, more can be done in terms of their safety and practicality. This leads to another environmentally friendly alternative to chemical fertilizers, that releases nutrients into the soil in a slow, controlled manner. These are environmentally friendly fertilizers (EFFs).

EFFs were developed to meet the increasing demand for food production without jeopardizing the environment. EFFs are able to reduce soil pollution by retarding and controlling the release of nutrients in the soil preventing oversaturation usually caused by chemical fertilizers. These are

fertilizers coated with biodegradable or environmentally friendly materials such as chitosan, sodium alginate, and biochar (Chen et al. 2018). EFF's coating 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 (Skrzypczak et al. 2019; 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; Izydorczyk et al. 2020).

Slow-release and control-release fertilizers are two types of precision fertilizers available today. Slow-release fertilizers, as the name suggests, release nutrients in a slow manner but are not synchronized with the plants' needs for nutrients, whereas control-release fertilizers release nutrients in a gradual manner adjusted for the plants' growth stage and kinetics of the demand (Skrzypczak et al. 2019). Although environmentally friendly fertilizers seem very promising, there are a few downsides to applying this fertilization method. Firstly, in terms of cost, controlled and slow-release fertilizers cost more to manufacture than conventional chemical fertilizers. Secondly, Sulphur coated urea leads to lower soil pH creating an acidic environment that may lead to decrease in biodiversity. Lastly, in control release fertilizers, nutrients may not be released as predicted, due to low temperatures, flooded or droughted soil and/or poor microbe activity (Zulfiqar et al. 2019).

Although EFFs seem to address the issue of leaching excess nutrients into the environment, there are still limitations to their ability to fulfill this task efficiently. Whilst EFFs are limited to direct

plant growth promotion (by increasing nutrient concentration), another chemical fertilizer alternative addresses both direct and indirect plant growth promotion (through plant protection) in addition to addressing the issues of nutrient leaching. And these are biofertilizers.

1.2 Biofertilizers

Biofertilizers are defined as a substance containing living microorganisms which promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey 2003). Biofertilizers can be composed of several microbial strains that benefit the plant's uptake of nutrients by their interaction in the rhizosphere (narrow region of soil surrounding plant roots) or plant interior. Rhizobacteria may promote plant growth directly via resource acquisition or modulating plant hormone levels, or indirectly by decreasing inhibitory effects of pathogenic agents and pests by acting as a biocontrol agent (Glick 2012). Some direct promotion of growth by bacteria include: biological nitrogen fixation, where bacteria such as *Acetobacter diazotrophicus* increase nitrogen availability in the soil; synthesis of siderophores, which are iron-chelating compounds; solubilization of minerals such as phosphates; and synthesis of plant hormones such as auxins, cytokinins and gibberellins (Alizadeh 2011). Some common commercialized plant growth-promoting bacteria are *Agrobacterium radiobacter, Azospirillum brasilense, Azotobacter chroococcum, Bacillus fimus and Pseudomonas chlororaphis* (Glick 2012). The benefits of using biofertilizers are that they are a cheap source of nutrients, they protect the plant from both biotic and abiotic stress, and they are excellent suppliers of micro chemicals and micronutrients (Mahanty et al. 2017). They also supply organic matter, secrete

growth hormones, and counteract the negative impact of chemical fertilizers (Mahanty et al. 2017).

1.2.1 Plant growth-promoting bacteria

There is a complex community of microorganisms interacting with plants growing under field conditions. Most plant parts (roots, stems, leaves, flowers) are inhabited by a group of microorganisms that affect plant health. These groups of microorganisms are called the phytomicrobiome, whereas microorganisms plus the plant are referred to as the holobiont (Chouhan et al. 2021). There is a distinct phytomicrobiome associated with each part of the plant. For example, the rhizomicrobiome encompasses root-associated microbes and their genes, the phyllomicrobiome encompasses shoot-associated microbes and their genes, and the endomicrobiome encompasses inner plant tissue associated microbes and their genes (Chouhan et al. 2021; Backer et al. 2018). Plant growth-promoting rhizobacteria (PGPR) are a group of soil bacteria that stimulate the growth of the host plant by forming and associating with plant roots in the soil (Vessey 2003). Unlike Rhizobia, which form a symbiotic relationship with host plants (in nodules), PGPR are free-living found near or within the roots (Glick 1999). PGPR are the most widely studied group of plant growth-promoting bacteria (Compant et al. 2005; Dey et al. 2004). As seen in Figure 1 below, PGPR inhabits soil interfaces close to root surfaces, the rhizosphere and root interior establishing endophytic populations. Many of those endophytic bacteria are able to cross from the root cortex into the vascular system and thrive in the stem, leaves and other organs as endophytes (Backer et al. 2018b; Etesami and Maheshwari 2018; Compant et al. 2005). Rhizobium is a very important PGPR that has the ability to develop symbiotic

relationships with its specific host plant, leading to increased growth yield by biologically fixing atmospheric nitrogen (Miransari 2016). Legumes such as soybeans, peas and clover have a mutualistic relationship with bacteria housed in the root nodules (rhizobia) fixing nitrogen to receive carbon generated by photosynthesis from the plant (Heath et al. 2020; Miransari 2016). The exploitation of microorganisms for use in agriculture is one of the fastest-growing industries globally, increasing at a rate of 17% per annum. This growth is a result of public demand for less chemically treated food products and increased environmental awareness ("Article: EU Commits to 50% Cut in Agchem Use by 2030 | IHS Markit," n.d.; Chouhan et al. 2021).

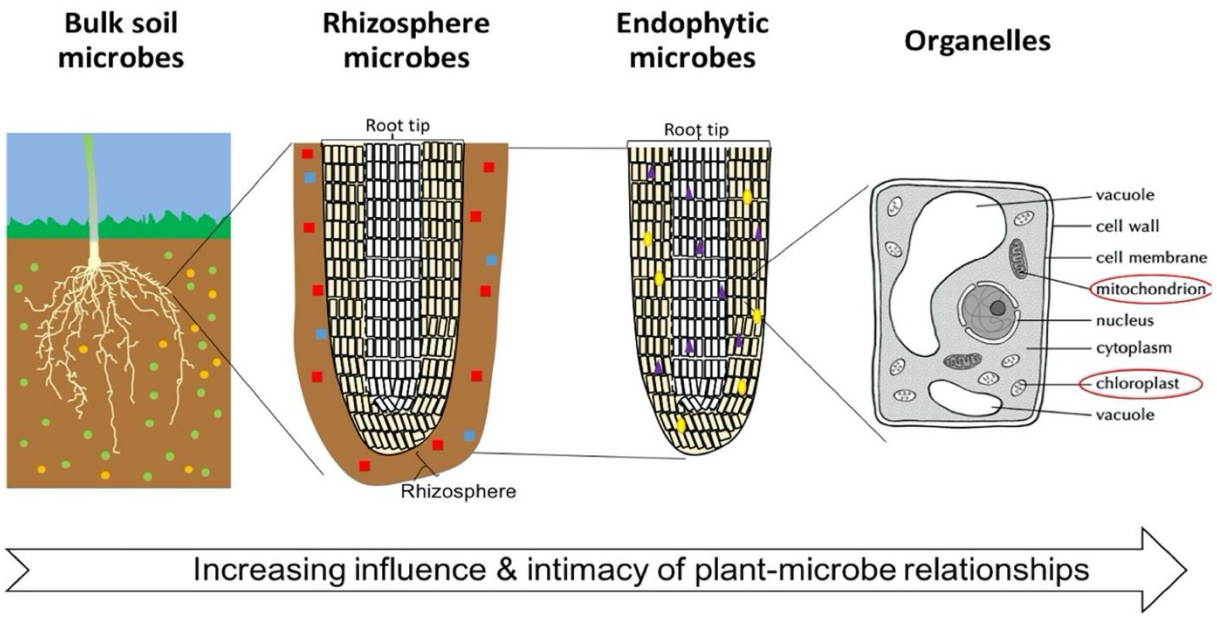

Figure 1. The relationship and degree of intimacy between microbes and plant roots in the bulk soil, rhizosphere and endosphere (Backer et al. 2018a). Accessed on: 28.04. 2022.This figure was reproduced with permission from Donald L. Smith (Author) ***Creative Commons Attribution License (CC BY)***.

A study by Rangel de Souza et al. 2016 examined the effects of the plant growth-promoting bacteria *Gluconacetobacter diazotrophicus* on *Arabidopsis thaliana* tissue and focused primarily

on plant physiology, growth, and activation of the plant immune system. They found that the bacteria promoted plant growth 50 days after inoculation. Plants that were inoculated showed higher canopy photosynthesis and lower plant transpiration, resulting in increased water-use efficiency. In addition, plant defences were activated as a result of the association between the plant and bacteria (Rangel de Souza et al. 2016). Gowtham et al. 2018 screened PGPR for the ability to induce resistance in chilli (*Capsicum annum L*.) against anthracnose disease which is a fungal disease that is presented as dark spots and sunken necrotic ring-like tissue on chilli peppers. They found that there was significant disease protection against anthracnose disease when plants were treated with *B. amyloliquefaciens* followed by *B. cepacia* and *P. rettgeri*, under greenhouse conditions (Gowtham et al. 2018). Salinity is a major limitation to the growth and yield of agricultural plants. Salinity stress induces various molecular and biochemical effects via the production of reactive oxygen species (ROS) (Khan et al. 2019). At high concentrations, ROS can be extremely harmful to plants by causing chlorophyll degradation, cell membrane oxidation and cell death. In this study by Khan et al. 2019, 126 isolates were acquired from different plants inhabiting sand dunes at Pohang beach, South Korea. Five (5) out of the 126 strains (*Arthrobacter woluwensis*, *Microbacterium oxydans*, *A. aurescens*, *Bacillus megaterium*, and *Bacillus aryabhattai* (AK5)) showed high tolerance to salt stress after inoculating them in normal and saline soil (NaCl; 200 mM) and growing soybeans. They also demonstrated plant growth promotion traits (production of indole-3-acetic acid (IAA), gibberellin (GA), and siderophores and increased phosphate solubilization). It was also found that the salt-tolerant gene *GmST1* was expressed in all the salt-tolerant bacteria (Khan et al. 2019). PGPB are

beneficial to plants in many ways and employ various mechanisms or modes of action that aid in this function.

1.2.2 Direct plant growth promotion

As highlighted previously, there are several ways in which PGPRs/PGPBs can enhance or promote plant growth. Here we will examine the direct mechanisms in which the bacteria host plant relationship can lead to the release or production of beneficial compounds that aid in the enhancement of plant growth and crop production. Direct mechanisms include processes which improve nutrient acquisition by the host plant and the production of phytohormones (auxins, gibberellin, cytokinin, and abscisic acid) (Munusamy 2021).

1.2.2.1 Nutrient Acquisition

Bacteria aid plants in acquiring nutrients by various means such as phosphate solubilization, potassium solubilization, nitrogen fixation and sequestering of iron by siderophores. Plant growth-promoting bacteria may employ one or more of the mechanisms listed above.

Nitrogen (N) plays a critical role in plant growth and productivity. As previously mentioned, Rhizobia bacteria engage in a mutualistic relationship with leguminous host plants leading to the release of nitrates and ammonia for plant consumption. Root nodule formation is initiated by flavonoids secreted from plant roots, responsible for the detection of rhizobia strains for colonization. Rhizobia releases signalling molecules called Nod factors (NF’s) and these molecules are recognized by LysM receptors present on the plasma membrane of the plant

leading to nodule formation and nitrogen fixation (Madsen et al. 2003; Chouhan et al. 2021). Like symbiotic microbes, non-symbiotic or endophytic microbes are also able to fix nitrogen in a similar manner.

Phosphorous (P) is another macronutrient that plays an important role in plant growth. Plants are unable to use P in its inorganic form; instead, it is absorbed in the form of orthophosphates ($H_2PO_4^-$ or HPO_4^{2-}) (Shin et al. 2004). Liebig's 'Law of the Minimum' states that plant growth is not dictated by the total available resources in the soil, but by the nutrient that is present in the least quantitative value relative to its demands for plant growth (Sharma, Sharma, and Javed 2022). According to Leibig's law, after N, P is the other most limiting nutrient for plant growth. Due to the low concentration of these phosphate ions (micromolar range), plants have adapted responses (morphological, physiological and biochemical) to enhance P acquisition with modifications in root growth and architecture (root-induced changes) as well as secretion of enzymes such as RNAses and phosphatases (Shin et al. 2004). These root-induced changes involve the acidification of the rhizosphere via proton release; exudation of carboxylate to mobilize P by chelation of cations bound to phosphate and ligand exchange; and enzyme-catalyzed hydrolysis to mobilize phosphates (Chen et al. 2006; Brito et al. 2020). P-solubilizing PGPR can increase the acquisition of P by plants through the acidification of soil, the release of enzymes and the production of carboxylates such as oxalate and citrate (Shen et al. 2011; Brito et al. 2020). P-solubilizing microorganisms account for 1% to 50 % of the P-solubilizing potential (Chen et al. 2006). Phosphorous plays a major role in cell structure and development, cell

division and energy transfer. It is a major component of nucleic acids, membrane lipids and phosphorylated intermediates (e.g. ATP) for energy metabolism (Khan et al. 2007).

Minerals such as iron (Fe) and zinc (Zn) are also necessary for plant growth and crop production. Fe acts as a catalyst in enzymatic processes, oxygen metabolism, electron transfer and DNA and RNA synthesis, making it an essential element in the growth of most living microorganisms (Ahmed and Holmström 2014). Fe is also essential in the formation of biofilm (allows microbes to attach to surfaces and is important in microbial survival), due to its regulation of surface motility and stability of polysaccharide matrix in microorganisms (Weinberg 2004). Like P, Fe is abundant in soils but unavailable to plants. This is where microbes come in; many bacterial strains can produce secondary metabolites called siderophores. Siderophores are low molecular weight ferric-iron-specific chelating agents, responsible for sequestering Fe, and forming soluble Fe^{3+} complexes, that are actively taken up by bacteria and plants via specific receptors (Kramer, Özkaya, and Kümmerli 2020; Neubauer et al. 2000). Siderophores are usually produced by plants and microorganisms under low iron conditions. Siderophore-mediated Fe transport systems vary between gram-positive and gram-negative bacteria (Fukushima, Allred, and Raymond 2014). Gram-positive bacteria have one cell membrane and a thick outer cell wall, employing siderophore-binding proteins (SBP's) anchored on their membrane to bind to siderophore-Fe complexes, whereas gram-negative bacteria have two cytoplasmic membranes and a thick cell wall, employing transmembrane outer membrane transporters to bind siderophore-Fe complexes (Fukushima, Allred, and Raymond 2014).

1.2.2.2 Plant growth regulators

Aside from increasing the availability of nutrients to plants, PGPRs can alter root architecture and promote plant growth by producing phytohormones that help increase the number of root hairs and lateral roots through increased nutrient and water uptake (Gupta and Sharma 2021).

Auxins are a group of hormones that play a role in regulating plant growth and development. They are essential to coordinating cell division and aiding in root and shoot development. Auxins perform several roles including: affecting plant cell division and differentiation; stimulating seed development as well as xylem and root development; initiating lateral adventitious root formation; mediating light, gravity and fluorescence responses; and playing a role in pigment formation, biosynthesis of metabolites and resistance to stressful conditions (Costacurta and Vanderleyden 1995; Bhattacharyya et al. 2012). Indole Acetic Acid (IAA) is one of the most common naturally occurring auxins found in plants regulated through tryptophan-dependent and tryptophan independent pathways (Somers, Vanderleyden, and Srinivasan 2004; Spaepen and Vanderleyden 2011).

Other phytohormones produced by bacteria are cytokinins (CKs) and gibberellins/ gibberellic acid (GA). Cytokinins are known to be produced by bacteria belonging to the *Athrobacter*, *Azospirillum* and *Bacillus* species, with important roles in apical dominance and regulating morphology and physiology of plants (Naseem, Kaltdorf, and Dandekar 2015; Riefler et al. 2006). Gibberellins belong to a large family of plant hormones, known to elicit a wide range of

plant responses such as seed germination, flower induction and pollen maturation (Ueguchi-Tanaka et al. 2007; Fahad et al. 2015).

Ethylene is a gaseous hormone that regulates plant growth and development and is active at extremely low concentrations (0.05 mL/L) (Backer et al. 2018). Ethylene is produced from two precursors: S-adenosyl-L-methionine (AdoMet) and 1- aminocyclopropane-1-carboxylate deaminase (ACC) (Patrick et al. 2006). It is commonly known to be produced during fruit ripening, flower opening and leaf senescence. Ethylene is also considered a stress hormone, due to its ability to activate signalling pathways to protect plants from deleterious stressors (Saleem et al. 2007). Under strenuous conditions, an increase in ethylene concentration may increase plant tolerance to stress or worsen the stress-response symptoms of the plant. Further discussion on ethylene regulation as an indirect plant growth-promoting mechanism will be explained.

1.2.3 Indirect plant growth promotion

Mechanisms involving plant protection from biotic and abiotic stress namely pathogens, pests, low pH, salinity stress as well as drought resistance, result in the indirect promotion of plant growth.

When pathogens accumulate in the surrounding soil of plants, a phenomenon known as negative soil feedback occurs, which decreases the performance of plants and leads to the promotion of plant biodiversity by selecting for successful dominant plant species (Berendsen et al. 2018). Plants are known to recruit beneficial microbes via root exudates and stimulate protective

biochemical responses in those microbes to overcome disease and other environmental pressures. This is known as induced systemic resistance (Chouhan et al. 2021; Berendsen et al. 2018). A study involving the inoculation of *Arabidopsi*s leaves with a mildew pathogen, *Hyaloperonospora arabidopsidis*, resulted in the recruitment of microbial taxa such as *Stenotrophomonas* spp., *Microbacterium* spp., *and Xanthomonas* spp. (Bakker et al. 2018; Berendsen et al. 2018). Microorganisms are extremely important in plant defence and overall plant health.

Volatile organic compounds (VOCs), which are low molecular weight, low boiling point and high vapour pressure lipophilic compounds derived from microbial metabolic pathways or plants, act as signalling molecules that can improve stress tolerance and stimulate plant growth (Raza et al. 2016; Ryu et al. 2003). Schulz-Bohem et al. 2018 found that the profiles of VOCs produced in healthy plant (*Carex arenaria*) roots vs plants infected by *Fusarium culmorum* were different, leading to the conclusion that the VOCs produced are related to the recruitment of beneficial microbes due to its high translocation capacity. Another study by Zhou et al. 2016 found that the bacteria *B. megaterium* secretes polyamine, an important compound involved in plant protection. In this same experiment, *B. megaterium* also secreted spermidine and induced polyamine production in *Arabidopsis*, which resulted in altered root architecture, increased biomass and elevated photosynthetic capacity of the plant. VOCs are known to act as signalling molecules to increase plant production of various phytohormones responsible for stress resistance (García-Gómez et al. 2019; Fincheira and Quiroz 2018).

Another indirect pathway, responsible for plant growth promotion and protection, is the production of ACC deaminase (ACCD) by microbes in response to increased ethylene production by plants under stress. When ethylene is produced above its threshold level, it becomes “stress ethylene”, which leads to stunted root growth and unfavourable growth parameters that result in hindered plant growth and development (Gupta and Pandey 2019). ACCD breaks down ACC, which is an immediate precursor of ethylene, to α-ketobutyrate and ammonia leading to the decrease in ethylene levels as seen in Figure 2. (Singh et al. 2015). The main way ACC deaminase catalyzes the reaction with ACC is by opening the cyclopropane ring of ACC by using nucleophilic addition and elimination (Singh et al. 2015). *Pseudomonas* spp. has been shown to produce ACC deaminase when *Pisum sativum* (host plant) is undergoing drought stress; as well as *P. putida* when host plant *Vigna radiata* is undergoing salinity stress and *B. phytofirmans PsJN* when host plant *Vitis vinifera* experiences low temperatures (Arshad, Saharoona, and Mahmood 2008; Mayak, Tirosh, and Glick 1999; Ait Barka, Nowak, and Clément 2006). ACC deaminase has been found to be present in three domains – eukaryote, bacteria and archaea – but is mainly produced in species of bacteria (both gram-positive and gram-negative) and some fungi (Nascimento et al. 2014). The presence of ACCD can be detected on the molecular level by amplifying and sequencing the *AcdS* gene, a structural gene encoding ACC deaminase (Nascimento et al. 2014).

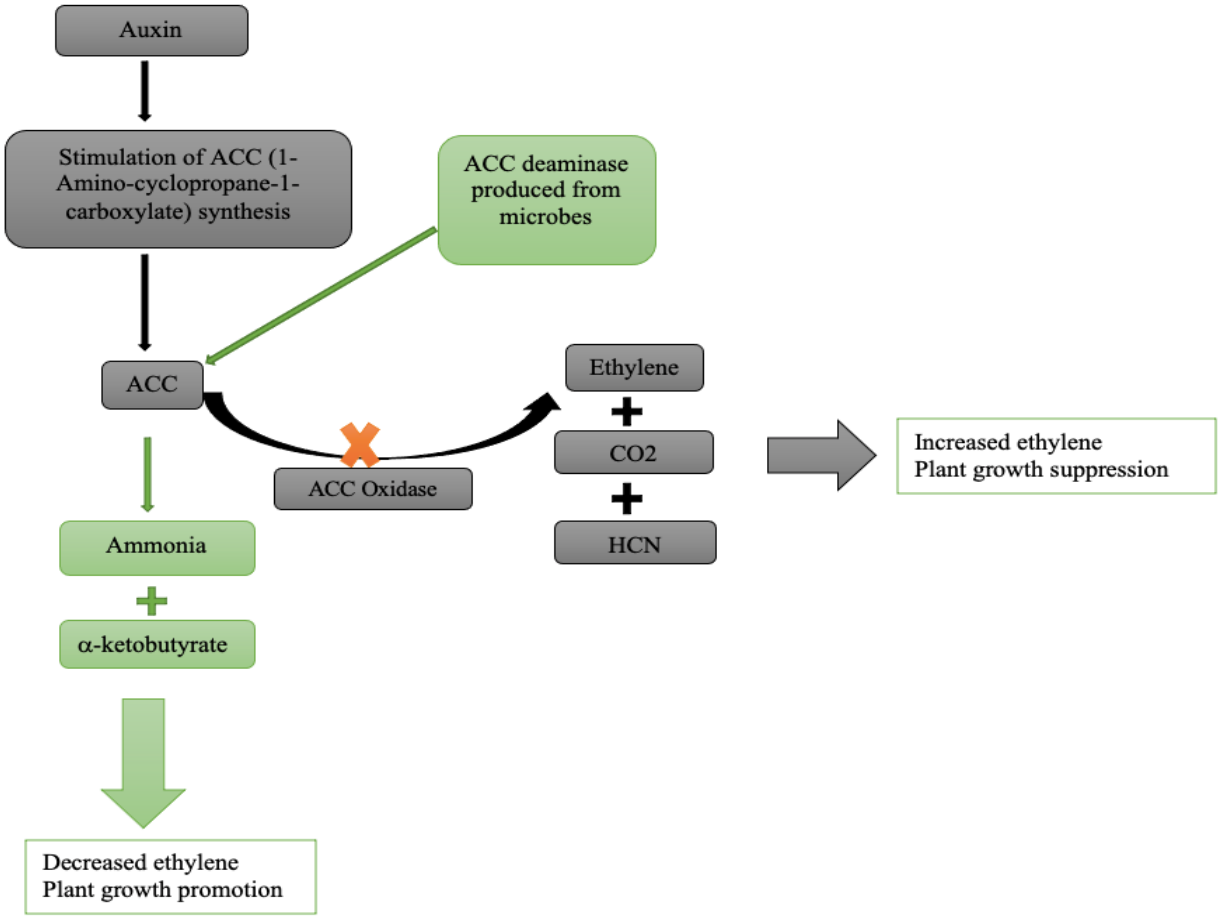

Figure 2. An illustration of ethylene production and ACC deaminase activity. In the grey path, ACC is oxidized to form ethylene and other by-products. In the green path, ACC deaminase breaks down ACC into ammonia and α-ketobutyrate, thereby decreasing ethylene concentration and promoting plant growth.

1.2.4 Bacteria Co-cultures

Microorganisms are known to coexist in complex networks in the rhizosphere. The effects of one microorganism on another may elicit responses through the release of metabolites as a result of resource competition, parasitism etc. In terms of positive outcomes or synergistic effects of co-cultures on the plant, there are four types of interactions: commensalism, cooperation, mutualism and syntrophy. Commensalism entails the increase in fitness in one partner, for example in a pair

of two species, without the other being affected; cooperation is the improved fitness in both partners with the same phenotype as a result of the interaction with one another; mutualism is increased fitness of both partners that do not benefit from the same resources, and syntrophy is where one partner benefits from the metabolites produced by the other and the benefitting partner removes inhibition induced by these metabolites for the producing partner (Canon et al. 2020).

Co-inoculation studies have proven to provide positive results for PGPR studies as compared to only testing a single bacteria/fungal species. In a co-inoculation study it was found that fluorescent *Pseudomonas* strains, *Rhizobia* and their combinations (co-inoculations) were used to examine plant growth promotion and fungal pathogen control. Both individual bacteria and combinations were positively correlated with each other with respect to shoot height, root length and dry weights, but were negatively correlated with germination (Dileep Kumar, Berggren, and Mårtensson 2001). Co-inoculated plants showed the best plant development overall for this study. In a 2017 study by Korir et al., the effect of co-inoculation of rhizobium and PGPR, on nodulation and growth of common bean *Phaseolus vulgaris L.*, in a low phosphorous-containing soil was examined. Co-inoculation of *Rhizobium* with *B. megaterium* and *P. polymyxa* enhanced the shoot and root dry weights more than treatments inoculated with only *Rhizobium*. The researchers suggested that this may have been due to the enhancement of plant growth by several mechanisms including the production of growth-promoting substances and solubilization of phosphorus (Korir et al. 2017). There are examples where co-inoculation increased growth and yield compared to single inoculation by providing plants with increased nutrient uptake. Co-inoculation studies can be even more advantageous to plants than single inoculation studies,

especially since the microbial community in soil and on plants is extremely diverse and complex. Although it may be difficult to predict bacterial interaction, due to increased resource competition as the number of species increases, it is worth investigating.

The method of application of treatments to plants of single inoculants or co-inoculants may significantly affect the overall results produced by each experiment. There are various ways in which bacteria can be added to soil and choosing the right method is as pertinent to crop growth as selecting the right bacterial strain or combination of bacterial strains.

1.3 Biochar as an inoculum carrier

The four main types of formulations for biofertilizer delivery used thus far are liquid, peat, powders and freeze-dried, all of which have their limitations based on the target crop, cost, ease of use and environmental constraints (Herrmann and Lesueur 2013).

Biochar is another bacterial inoculum carrier that could potentially increase the effectiveness of biofertilizer application. Biochar is a porous organic material produced through pyrolysis of biomass (Shaaban et al. 2018). As seen in Figure 3, both coarse and fine biochar have a high surface area, due to their porous nature, and contain microscopic pockets in which bacteria are able to survive and multiply.

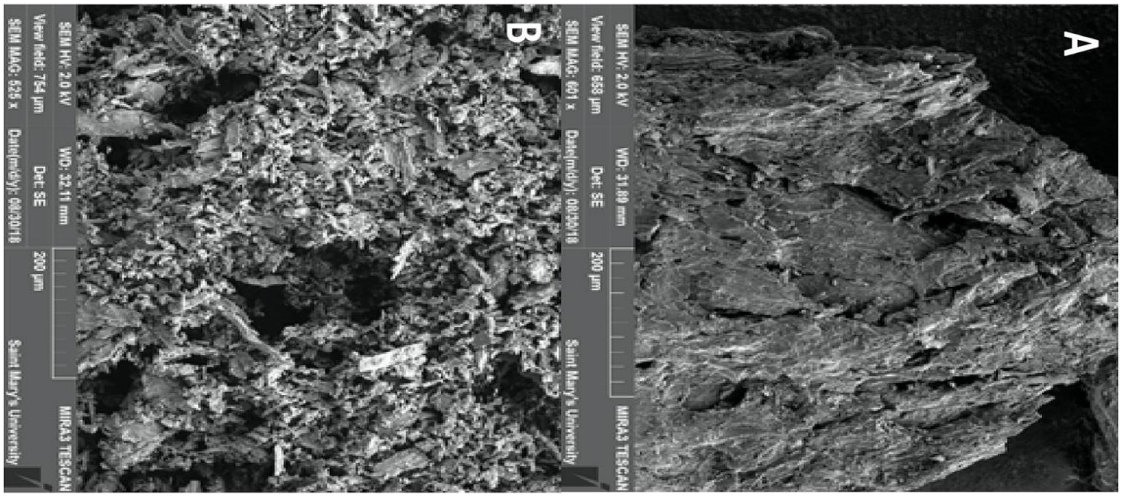

Figure 3. SEM of coarse (A) versus fine biochar (B) (K. L. Blatt-Janmaat, MacQuarrie, and Sit 2020).

Biochar can also function as a soil amendment, providing opportunities to store carbon in the soil for a long period of time, and affects several soil properties such as electrical conductivity (EC), pH, cation exchange capacity (CEC), nutrient levels, porosity, water retention capacity and bulk density (Shaaban et al. 2018; Weber and Quicker 2018).

Heavy metals present in soil can be detrimental to not only humans but to animal life as well. Unfortunately, heavy metals such as lead, copper and cadmium are naturally occurring in soils but may be enhanced due to mining and other anthropogenic activities. Puga et al. 2015 aimed to evaluate whether biochar could reduce the mobility of heavy metals and improve plant growth. It was found that the application of biochar to a metal-contaminated mine soil decreased the content and uptake of heavy metals such as cadmium, lead and zinc whilst increasing the concentrations of nutrients, such as phosphorus (Puga et al. 2015).

Biochar is also able to influence the root-associated bacterial community to benefit the plant (Kolton et al. 2011). They assessed the effect of the addition of biochar on the bacterial community present in the rhizosphere of mature sweet pepper (*Capsicum annuum* L.) plants and found a difference in the root-associated microbial community structure of sweet pepper plants grown in soil with biochar vs. soil without biochar, further confirming the difference that biochar makes as an inoculum carrier. It is well documented that biochar has the ability to provide favourable conditions for the proliferation of microbes which in turn produce beneficial metabolites to support plant growth and health (Egamberdieva et al. 2016). Ma et al. 2022 conducted an experiment comparing three types of biochar with rhizobacteria on the growth of lettuce, enzyme activity and total microbial activity. They found that when comparing plant dry mass specifically, all plants amended with biochar alone had significantly higher dry masses compared to control treatments. However, when comparing soil containing biochar inoculated with bacteria, there was a preference for the type of biochar based on the bacteria used. All treatments containing biochar and bacteria had significantly higher soil enzyme activity compared to control treatments. These results indicate that although biochar alone can improve plant growth, the biochar bacteria relationship is very specific and may dictate the overall effect on plant growth. Biochar has proven to show great promise as a stand-alone treatment, contributing to soil fertility, and in conjunction with microorganisms to house and aid in the transportation of bacteria.

1.4 Objectives

As a contribution towards the efforts to curtail the imminent threat to food security resulting from the detrimental effects of modern farming practices and to adopt more sustainable agricultural practices, this project aims to further investigate the plant growth-promoting abilities of a novel PGPR *Fictibacillus enclensis*, with hopes to develop it into a commercially available biofertilizer. The overarching goal of this research is to explore the effectiveness of *F. enclensis* as a plant growth promoter and to investigate its ability to produce ACC deaminase as a mechanistic characteristic of its plant growth-promoting abilities.

The effectiveness of *F. enclensis* as a plant growth promoter will be determined by measuring several growth parameters of different plants using biochar as the inoculum carrier of choice, of the bacterial treatments, and comparing these treatments to that of chemical fertilizer treatments and control treatments. The bacteria *Stenotrophomonas rhizophila* was selected as a co-culture strain of choice in conjunction with *F. enclensis*. *Stenotrophomonas rhizophila* was isolated on the stems of grapevines by previous lab members and is known for its plant-growth-promoting properties, as well as its plant defence abilities (Berendsen et al. 2018). The co-culture studies aim to investigate the potential synergistic effect on plant growth when combining *F. enclensis* and *S. rhizophila* strains into one treatment. The biocontrol abilities of *F. enclensis* will be evaluated by comparing the number of pests present on plants before and after treatments. The ability to produce ACC deaminase as a mode of action to promote plant growth will also be investigated, both qualitatively and quantitatively for all bacteria and bacteria co-cultures involved in this study.

2 Materials and methods

The bacteria tested for plant growth promotion in these experiments are *F. enclensis* and *S. rhizophila. Fictibacills enclensis* was isolated on a piece of biochar at Saint Mary's University (Halifax, NS, Canada) by Kaitlyn Blatt-Janmaat (K. Blatt-Janmaat 2018) and identified using 16s rRNA sequencing. This bacteria was considered a novel potential plant growth-promoting rhizobacteria due to its genus *Ficitibacillus* being a plant growth-promoting endophyte, known for its siderophore production and nutrient solubilization (Dastager et al. 2013; Borah et al. 2021). *Stenotrophomonas rhizophila* was isolated by Kaitlyn Blatt-Janmaat and Brandon Logan from the stem of grapevines at L'Acadie Vineyards, in Wolfville (NS, Canada). *Pseudomonas rhodesiae* was used as a positive control since it is a known and commercially available PGPR (purchased from ATCC®). All media components were purchased from Fisher Scientific and chemical reagents were purchased from Sigma-Aldrich.

2.1 Bacteria quantification and qualification

All bacteria used in this project were quantified and a standard curve of concentration vs optical density (OD 600 nm) was constructed for future reference. The growth of each bacterium was measured over three days and a graph of concentration vs time was computed for future reference. To determine whether *F. enclensis* and *S. rhizophila* had a positive relationship, co-inoculation studies were conducted prior to inoculation in plants.

2.1.1 Quantification of bacteria

This method was taken from (Brown 2011) Biotechnology: A Laboratory Skills Course, Bio-Rad Explorer's textbook. All sterile solutions/solids/ instruments were made sterile using a Getinge Vacuum Steam Sterilizer (Model 533Ls, Göteborg, Sweden) at 121°C for 30 minutes unless stated otherwise. A series of 5 volumes from a bacterial stock solution was pipetted into five 15 mL centrifuge tubes containing 10 mL of nutrient broth (5 g tryptone, 5 g sodium chloride, 3 g yeast extract, 1 L of water) creating five unknown concentrations for each bacteria quantified. The optical density (OD) at 600 nm of all unknown concentrations was measured in triplicate for each and recorded. Each unknown concentration was serially diluted using the following method. Seven sterile microcentrifuge tubes and seven nutrient agar plates (5 g tryptone, 5 g sodium chloride, 3 g yeast extract, 15g agar, and 1 L water) were labelled with the appropriate dilution factors (10^1, 10^2,10^3, 10^4, 10^5, 10^6, 10^7). Using a 1000 µL micropipette, 900 µL of nutrient broth (5 g tryptone, 5 g sodium chloride, 3 g yeast extract, 1 L water) was pipetted into each 2.5 mL microcentrifuge tube. Using a 20-200 µL micropipette, 100 µL of bacteria stock was placed in the microcentrifuge tube labelled 10^1 and vortexed (Fischer Scientific, Waltham Massachusetts, USA, model NO: 945404). One hundred (100) µL of the solution was removed from the 10^1 centrifuge tube and placed in the 10^2 centrifuge tube. Each bacterial culture was serially diluted until the tube labelled 10^7 was used. One hundred (100) µL of solution from each microcentrifuge tube was micro pipetted onto its corresponding nutrient agar plate and spread thoroughly with an inoculation loop. The bacteria cultures were left to grow in an Amerex Instruments Gyromax (Concord, CA, USA) 737 incubator at 30 °C for 24 hours. After 24 hours the number of colonies on plates containing between 30 to 300 colonies was counted and

recorded. The colony-forming units (CFU) per mL were calculated using the formula: CFU/mL=CFU*dilution factor*1/aliquot. A standard curve of OD_{600} versus CFU/mL was created for the bacteria quantified. This quantification was done for *F. enclensis, S. rhizophila* and a commercialized PGPR *P. rhodesiae*.

2.1.2 Bacteria growth measurements

To better facilitate the application of a more accurate quantity of the bacteria inoculated with plants, the growth rate of each bacterium being studied was determined. One hundred (100) mL of each bacteria, which is approximately $8.1x10^{7}$ CFU/mL of *F. enclensis,* $1.7x\ 10^{8}$ CFU/mL *P. rhodesiae* and $2.2x10^{8}$ CFU /mL *S. rhizophila*, was pipetted into a 15 mL centrifuge tube containing 10 mL of nutrient broth. The bacteria were left to grow for 24, 48 and 72 hours in an Amerex Instruments Gyromax 737 incubator (Concord, CA, USA) at 30 °C, 100 rpm. After each day the optical density at 600 nm was measured and recorded. Each OD measurement was made in triplicate for each treatment and the average of the three was calculated. The CFU/mL was calculated using the standard curve and a graph of CFU versus time was plotted for each bacterium.

2.1.3 Bacteria co-culture

After selecting *S. rhizophila* as a co-inoculant with a potential synergistic effect on plant growth promotion, a *S. rhizophila* and *F. enclensis* co-culture experiment was conducted to determine whether there were any contact-dependent or contact-independent inhibitory effects when grown together. A sterile 12-well cell culture plate was filled with 3 mL of nutrient agar (5 g tryptone, 5 g sodium chloride, 3 g yeast extract, 15g agar, and 1 L water) in each well. There were four

treatment groups (control, *F. enclensis, S. rhizophila, and F. enclensis + S. rhizophila*) each done in triplicate. Twenty-five (25) μL *F. enclensis* and *S. rhizophila*, was placed on their corresponding plates and spread with an inoculation loop; 12.5 μL of *F. enclensis* was spread on one half of a culture plate and 12.5 μL of *S. rhizophila* was spread on the other half of the same culture plate for the co-inoculated group. The bacteria were left to grow for 48 hours in an Amerex Instruments Gyromax 737 incubator at 30 °C, and growth was monitored each day.

2.2 Plant growth bioassay

The plant growth-promoting abilities of *F. enclensis* and the *F. enclensis + S. rhizophila* co-culture were determined through several experiments using different crops and measuring multiple parameters.

2.2.1 Inoculation of biochar

The biochar used in this experiment was acquired from Canadian AgriChar Inc. (Maple Ridge BC, Canada). It was prepared using the proprietary patented anaerobic pyrolysis technique (500 to 800 °C) used to convert biomass feedstock into a special biochar formula called CHAR+^{TM}. This procedure was modified from (Xiong et al. 2017) and formulations were made specific to the experiment and volume of soil used. Fifty (50) μL bacteria were plated onto sterile nutrient agar plates (5g tryptone, 5g sodium chloride, 3g yeast extract, 15g agar, 1L reverse osmosis water) from 25% v/v glycerol frozen stock and left to grow for 48 hrs in an Amerex Instruments Gyromax 737 incubator at 30°C, shaking at 100 rpm. After bacterial growth, 1 inoculation loop full of bacteria was added into a 15 mL centrifuge tube containing 10 mL of sterile nutrient broth solution (5g tryptone, 5g sodium chloride, 3g yeast extract, 1L water) and left to grow for 48 hrs

in an incubator at 30°C, shaking at 100 rpm, this was the working stock solution, containing 8.1x10 ^{7} CFU/mL of *F. enclensis*, 1.7x 10^{8} CFU/mL *P. rhodesiae*, and 2.2x10 ^{8} CFU /mL *S. rhizophila*. Equal parts of sterile biochar and inoculated nutrient broth (1% v/v of final nutrient broth volume is taken from bacterial stock solution) [1:1] v/v were combined and allowed to grow in an incubator at 30°C, shaking at 100 rpm for 48 hrs. This procedure was followed for all biochar inoculated bacterial strains used in this project.

2.2.2 Field trial- Barley

The barley field trial was conducted on a small plot of land at the Kentville Research and Development Centre (Agriculture and Agri-food Canada) (Kentville, NS, Canada) in June of 2021. The goal of this experiment was to test the effects of biochar and bacteria on plant growth. There were 64 plots, each with dimensions of 1m x 0.4 m x 0.05 m. Barley (*Hordeum vulgare*) was planted in a Latin square design based on treatment groups as seen in Figure 4. The treatment groups and their numerical assignment can be seen in Tables 1 and 2.

Table 1. Composition of each treatment group in the barley field trial.

Treatment	**Composition**
Control	Only soil
P+NB	*P. rhodesiae* + nutrient broth
P+NBBC	*P. rhodesiae* + nutrient broth + biochar
F+NB	*F. enclensis* + nutrient broth
F+NBBC	*F. enclensis* + nutrient broth + biochar
F+S+NB	*F. enclensis* + *S. rhizophila* + nutrient broth
F+S+NBBC	*F. enclensis* + *S. rhizophila* + nutrient broth + biochar
Fertilizer	Fertilizer (20-20-20 Miracle-Gro^{TM} Marysville Ohio,USA)

Table 2. Numerical assignment for each treatment for the barley field trial.

Treatment	Assigned #
C	1
P+NB	2
P+NBBC	3
F + NBB	4
F +NB BC	5
F+S + NB	6
F+S NBBC	7
Fertilizer	8

1	2	3	4	5	6	7	8
2	3	4	5	6	7	8	1
3	4	5	6	7	8	1	2
4	5	6	7	8	1	2	3
5	6	7	8	1	2	3	4
6	7	8	1	2	3	4	5
7	8	1	2	3	4	5	6
8	1	2	3	4	5	6	7

Figure 4.Latin square design depicting the arrangement of barley treatment groups in the field.

The ratio of nutrient broth/biochar to soil was 5% v/v. This amounted to 1 L of treatment per plot. The biochar inoculation method described above was followed apart from the bacteria growth conditions and the biochar addition time. The bacteria were introduced to nutrient broth only and were left to grow for 48 hours at room temperature (22 °C). On the day of planting, the sterile biochar was added to the nutrient broth ([1:1] v/v) and mixed thoroughly and left to sit for 30 minutes. The nutrient broth-only treatments contained 1 % v/v of grown in 1 L of nutrient broth at room temperature (22 °C) for 48 hours. *Ficitibacillus enclensis* + *S. rhizophila* co-

culture treatments are the result of 5 mL of *F. enclensis* (8.1×10^{7} CFU/mL) and 5 mL of *S. rhizophila* (2.2×10^{8} CFU /mL) each added to separate flasks of 500 mL nutrient broth incubated at room temperature (22 °C) for 48 hours and subsequently combined together. All treatments were applied to their corresponding plots and mixed into the soil. Twenty barley seeds (*Hordeum vulgare*) ordered from West Coast Seeds, Delta BC, Canada; 10 in each row, 2 rows per plot, were sown on each plot at 5 cm deep. One (1) liter of water was added to the control and fertilizer treatments after planting. Fertilizer (20-20-20 Miracle-Gro™, Marysville Ohio, USA) and bacteria treatments were added every two weeks after planting. The fertilizer was added as per dilution instructions from the manufacturer and the initial volume of nutrient broth added to the bacteria treated plots were added every two weeks. Bacteria treatments for reapplication contained only nutrient broth and bacteria (1L) and control treatments received only water. Application of bacteria and fertilizer occurred every two weeks until harvest. Water was also applied every two weeks or naturally by the rain. Barley was left to grow for approximately 2 months before harvesting. The stem height, spike length and spike dry mass were recorded and statistically analyzed.

2.2.3 Greenhouse trial- Peppers

This experiment was designed to test the effects of multiple treatment applications on plant growth. Pepper seeds (*Capsicum chinense)* were sterilized using 10 % v/v sodium hypochlorite for 10 minutes, 70 % v/v ethanol for 5 minutes and rinsed three times with sterile deionized water. The seeds were then pregerminated in red solo cups (5 seeds per cup) filled with 100 mL of sterilized Golfgreen® (Canadian Tire, Halifax, NS, Canada) potting soil (no NPK). Plants

were left on a growth shelf equipped with 3000 K LED lights, 21 inches above pots, and exposed to a 12-hour light cycle from 7:00 to 19:00 in the Sit lab (Saint Mary's University, Halifax, NS, Canada) for 21 days at 23 °C. The germinated plants were then transferred to pots containing each treatment in July 2021. The biochar inoculation procedure mentioned in *section 2.2.1* was followed for 2 L of soil in each pot (5 % v/v treatment volume to soil). To ensure a more homogenous mixture the bacteria/biochar mixture (1.4 L) was added to 28 L of sterilized Golfgreen® potting soil (no NPK), mixed thoroughly and then added to individual pots. There were 6 treatment groups and 7 pots per treatment seen in *Table 3* below. Pots were placed in a greenhouse at Saint Mary's University and left to grow for 60 days. Fertilizer (20-20-20 Miracle-Gro^{TM}) and bacteria treatments (same volume as the initial volume of nutrient broth used) for multiple application treatment groups were added every two weeks. After 81 days of growth, it was found that the plants were infested with aphids (*Aphidoidae)*. The pepper leaves were accidentally treated with 1% v/v acetic acid solution rather than 0.1% v/v acetic acid solution. Acetic acid treatment can be used to ward off aphids from plants. This resulted in the deterioration of the leaves and plant after 2 days. The average number of infected leaves, as well as the leaf width, was recorded for each treatment group. The total number of aphids present on each plant after treatment application was also averaged and statistically analyzed.

Table 3. Corresponding soil condition and bacteria application type for treatment groups.

Treatment	**Conditions**
Control	Only potting soil
***F.encl* x S**	Biochar + single application of *F. enclensis*
***F.encl* x M**	Biochar + multiple applications of *F. enclensis*
***P.rho* x S**	Biochar + single application of *P. rhodesiae*
***P.rho* x M**	Biochar+ multiple applications of *P. rhodesiae*
Fertilizer	Fertilizer + potting soil

2.2.4 Greenhouse/growth shelf trial- carrots

This experiment followed a similar procedure to the pepper trial. Carrots (*Daucus carota*) purchased from West Coast Seeds, were planted directly from seed into 2 L of potting soil Golfgreen®. Carrot seeds were sterilized using 10 % v/v sodium hypochlorite for 10 minutes, 70 % v/v ethanol for 5 minutes and rinsed three times with sterile deionized water. The seeds were placed in the soil in which peppers were previously planted (2 seeds per pot). The treatment groups followed that in *Table 3.* Fertilizer (20-20-20 Miracle-Gro^{TM}) and bacteria treatments (same volume as the initial volume of nutrient broth used) for multiple application treatment groups were added every two weeks. Plants were initially grown in the greenhouse at Saint Mary's University in October 2021 and transferred after 21 days to a growth shelf (equipped with 6500 K LED lights, 1.5 inches above pots, and exposed to a 12-hour light cycle from 7:00 to 19:00) in Dr. Zhongmin Dong's laboratory at Saint Mary University due to light limitations.

Carrots were left to grow for 100 additional days and harvested. The root length, shoot length, root dry and shoot dry mass were recorded for all plants and statistically analyzed.

2.2.5 Growth shelf – barley

The plant growth-promoting ability of *F. enclensis* and the *F. enclensis/S. rhizophila* co-culture was analyzed using barley. Biochar was inoculated for 500 mL of sterile Golfgreen® (no NPK) potting soil, as explained in the inoculation procedure in *section 2.2.1*. The inoculated soil was incorporated into red solo cups (purchased from the Atlantic Superstore, Halifax, NS). Barley seeds were surface sterilized using 10 % v/v sodium hypochlorite for 10 minutes, 70 % v/v ethanol for 5 minutes and rinsed three times with sterile deionized water. Seeds were planted directly into inoculated soil and left to grow for 14 days on a growth shelf equipped with 3000 K LED lights, 21 inches above pots, and exposed to a 12-hour light cycle from 7:00 to 19:00 in the Sit lab (Saint Mary's University) at 22 +/- 1 °C. There were 6 treatment groups with 5 pots per treatment and 4 seeds per pot. The composition of the treatment groups is outlined below in *Table 4.* For the co-culture treatment group, each bacterium was grown in equal parts separately and then combined to form a final volume of 10 mL before biochar inoculation. The shoot dry mass, as well as the CHN (carbon, hydrogen, nitrogen) composition of the shoot, was recorded and statistically analyzed.

Table 4. Composition of treatment groups.

Treatment	**Composition**
Control	Only potting soil
F. enclensis	Biochar+ *F. enclensis*

P. rhodesiae	Biochar + P. rhodesiae
S. rhizophila	Biochar + *S. rhizophila*
F+S	Biochar + ½ *F. enclensis* + ½ *S. rhizophila*
Fertilizer	Fertilizer + potting soil

2.2.5.1 Elemental analysis

Barley shoots were placed in paper bags and dried in a convection oven (VWR 1500E, Radnor Pennsylvania, USA) convection oven for 15 minutes at 55 °C. The dried leaves were stored at -20°C until analysis. All the shoots for each treatment were ground into a fine powder using a mortar and pestle. Approximately 4.0 mg of dried barley shoot was weighed and analyzed in triplicate using a Perkin Elmer 2400 Series II CHN Analyzer (Waltham, Massachusetts, United States). An average of the three measurements for each element per treatment was analyzed to observe the impact of bacterial inoculation on the uptake of carbon, nitrogen, and hydrogen.

2.2.6 Pepper/Barley- germination

As a preliminary test, the effect of bacteria treatment on germination rate was first noted in pepper plants. Three 3 replicates of germination tests were conducted on barley seeds to verify the effects of the treatment on germination rate.

2.2.6.1 Peppers

Pepper (*Capsicum chinense*) seeds were sterilized using 10 % v/v sodium hypochlorite for 10 minutes, 70 % ethanol for 5 minutes and rinsed three times with sterile deionized water. Six glass

Petri dishes (Fischer Scientific, Waltham Massachusetts, USA) 90 mm in diameter, were also sterilized using a Getinge Vacuum Steam Sterilizer (Model 533Ls, Göteborg Sweden) at 121°C for 30 minutes. Six Whatman filter papers (Fischer Scientific, Waltham Massachusetts, USA) (90 mm in diameter) were sterilized by soaking in 10% v/v sodium hypochlorite for 15 minutes, 70 % v/v ethanol for 10 minutes, rinsed twice in sterile deionized water and dried using a heat gun. The filter paper was added to the petri dish along with 1 mL of each treatment solution. There were six treatment groups: control (only water), *F. enclensis, P. rhodesiae, S. rhizophila,* F+S *(F. enclensis + S. rhizophila)* and fertilizer. One (1) mL from a 10 mL ($8.1x10^{7}$ CFU/mL of *F. enclensis*, $1.7x\ 10^{8}$ CFU/mL *P. rhodesiae* and $2.2x10^{8}$ CFU /mL *S. rhizophila*) bacteria stock solution was added to corresponding bacteria treatments. One (1) mL of water and 1 mL of fertilizer (diluted as per manufacturer's instructions) was also added to corresponding Petri dishes. Eleven (11) sterile pepper seeds were evenly distributed onto the filter paper and the Petri dishes were covered, sealed with parafilm and placed in a dark cupboard at room temperature for 7 days. The germination status of the seeds was observed every day and the number of germinated seeds per day was recorded.

2.2.6.2 Barley

The barley germination test was conducted in 500 mL of sterile Golfgreen® (no NPK) soil on a growth shelf in the Sit lab at Saint Mary's University. The bacteria and biochar inoculation method from *section 2.2.1* was followed for treatments mentioned in *Table 4.* There were 5 pots with 4 barley seeds each making a total of 20 seeds per treatment. After adding inoculated biochar all pots were watered with 5 mL of water. Pots were left to grow on a growth shelf

equipped with 3000 K LED lights, 21 inches above pots, and exposed to a 12-hour light cycle from 7:00 to 19:00 in the Sit lab (Saint Mary's University) at 22 +/- 1 °C. The number of germinated seeds on days 3 to 7 was counted and the germination rate was determined. This experiment was repeated three times.

2.2.7 Barley- root growth analysis

After observing the effects of the bacteria treatments on root emergence, a root growth analysis was conducted on barley seeds to measure the effects of each treatment on root growth. Seed germination pouches purchased from CYG™, Roseville, MN, USA (18 cm high, 16.5 cm wide) were sterilized using a Getinge Vacuum Steam Sterilizer (Model 533Ls) at 121°C for 30 minutes. Barley seeds were sterilized by soaking in 10% v/v sodium hypochlorite for 10 minutes, 70 % v/v ethanol for 5 minutes, and rinsed three times in sterile deionized water. Sterile seeds were placed in seed pouches containing 5 mL of sterile deionized water. Each pouch contained 5 seeds, 4 pouches per treatment, amounting to a total of 20 seeds per treatment. Treatments groups were as outlined in *Table 1.4.* Seeds were left to germinate for 24 hours (root formation) on a growth shelf equipped with 3000 K LED lights, 21 inches above pots, and exposed to a 12-hour light cycle from 7:00 to 19:00 in the Sit lab (Saint Mary's University) at 22 +/- 1 °C. After 24 hours, 10 mL of each treatment was applied to the seed pouches and left to grow for an additional three days. Fertilizer treatment was diluted as per the manufacturer's instructions and bacteria treatments contained approximately $8.1x10^7$ CFU/mL of *F. enclensis*, $1.7x\ 10^8$ CFU/mL *P. rhodesiae* and $2.2x10^8$ CFU /mL *S. rhizophila*. The root length was measured using a 30 cm

ruler for three days and the shoot length was measured on the third and fifth days. This experiment was repeated twice.

2.2.8 Growth shelf- Bush beans

The effects of *F. enclensis* and the *F. enclensis/S. rhizophila* co-culture on plant growth was also studied on bush beans (*Phaseolus vulgaris*). Biochar was inoculated based on the inoculation procedure in section *2.2.1* and added to 500 mL of sterile Golfgreen® (no NPK) potting soil. Bush bean seeds were surface sterilized using 10 % v/v sodium hypochlorite for 5 minutes, 70 % v/v ethanol for 2 minutes and rinsed three times with sterile deionized water. Seeds were planted directly into inoculated soil and left to grow for 96 days. There were 6 treatment groups with five pots per treatment and 2 seeds per pot. Plants were watered every 3-4 days. The composition of the treatment groups is outlined in *Table 4* above. The leaf surface area, bean dry mass and CHN composition of the bean for each treatment group were measured and analyzed.

2.2.8.1 Elemental Analysis

Bush beans were placed in paper bags and dried in a VWR 1500E convection oven for 20 minutes at 55 °C. The dried beans were stored at -20°C until further analysis. The beans were then ground into a fine powder using a mortar and pestle and transferred into a glass vial. Approximately 4.0 mg of dried bush beans were weighed and analyzed in triplicate using a Perkin Elmer 2400 Series II CHN Analyzer. The average CHN composition of each treatment was determined and used for statistical analysis.

2.3 Analysis of ACC deaminase production

ACC deaminase is produced by PGPR in response to a rise in ethylene production by plants due to plant stress. The quantification and qualification of ACC deaminase production were analyzed on *F. enclensis, S. rhizophila, P. rhodesiae* and the *F. enclensis* and *S. rhizophila co-culture,* to determine their ability to stimulate plant growth via this pathway. This procedure was taken from (Penrose and Glick 2008; Saini and Khanna 2013).

There are several techniques employed to test the ability of bacterium to produce ACC deaminase namely 1) PCR and identifying the *AcdS* gene present in the bacterium; 2) the use of a colorimetric ninhydrin assay; and lastly 3) the method employed in this study, the colorimetric 2,4- dinitrophenylhydrazine assay of the product α-ketobutyrate. The method of quantifying ACC deaminase using the colorimetric 2,4- dinitrophenylhydrazine assay of the product α-ketobutyrate, was developed by Penrose and Glick (2008), where the bacteria is first assessed for the ability to synthesize ACC on DF (Dworkin Forster) minimal media plates, where the bacteria is grown on plates with no nitrogen source and is forced to break down ACC into α-ketobutyrate and ammonia, in order to use ammonia as its sole Nitrogen source.

To measure the activity of ACC deaminase, a standard curve on known α-ketobutyrate, concentration is made by measuring the absorbance on the solutions at 540 nm. 2,4-dinitrophenyl hydrazine is added to α-ketobutyrate to derivatize α- ketobutyrate as a phenylhydrazone. The colour of the phenylhydrazone is then developed by mixing the solution with NaOH, the absorbance of this colour change is measured at 540 nm to provide the lowest

detection limits for this compound. Prior to measuring ACC deaminase activity, the bacteria must be in a condition that induces ACC deaminase. This ACC deaminase induction is achieved by first growing the bacteria in rich media and then growing it in minimal media with on ACC encouraging the breakdown of ACC for survival. The supernatant containing α-ketobutyrate is assayed in a similar fashion to the standard curve and measured at 540 nm (Penrose and Glick 2008).

2.3.1 ACC deaminase qualification

In place of the Dworkin and Foster minimal medium, Norris Glucose Nitrogen Free medium was used in conjunction with ACC (Fischer Scientific) and $(NH_4)_2SO_4$.

The minimal media with $(NH_4)_2SO_4$ as a sole source of nitrogen was prepared by adding 12.5g of nitrogen-free medium powder and 2.0 g of $(NH_4)_2SO_4$ to 1 L of water in a glass bottle. Another bottle of minimal media was prepared with nitrogen-free medium powder but without $(NH_4)_2SO_4$. Both bottles of solution were autoclaved for 15 minutes in a Getinge Vacuum Steam Sterilizer (Model 533Ls) at 121°C. A 0.5 M solution of ACC was made, filtered through a 0.2 μm membrane (Fischer Scientific, Waltham Massachusetts, USA) and the filtrate was stored at -20 °C for later use.

Each bacterium was grown in 10 mL of nutrient broth medium (5 g tryptone, 5 g sodium chloride, 3 g yeast extract, and 1 L of water) for 24 hours at 200 rpm at 30°C. The cell pellets were then collected by centrifugation at 8000 rpm for 5 minutes. Pellets were then washed twice

with sterile deionized water and resuspended in 1 mL of sterile deionized water. Each bacterium in distilled water was spot inoculated on Petri plates containing minimal media made with 1.5% W/v agar and supplemented with 3mM of ACC (60 mL of ACC spread across 10 mL of agar and left to dry), and left to grow for 3 days at 30 °C. The growth of the bacteria on ACC supplemented plates was compared to a positive control (plates with only $(NH_4)_2SO_4$ as the sole nitrogen source) and negative control (minimal medium without ACC or $(NH_4)_2SO_4$). Plates with significant bacterial growth were determined to be ACC deaminase producers.

2.3.2 ACC deaminase quantification

The activity of ACC deaminase was assayed according to the method by (Honma and Shimomura 1978). This method measured the amount of α-ketobutyrate produced when ACC deaminase cleaves ACC. The bacteria cells were first placed in growth conditions that favoured ACC deaminase induction, then the number of μmoles of α-ketobutyrate produced from the reaction was determined by comparing the absorbance at 540 nm of a bacterial sample to an α-ketobutyrate standard curve of range between 0.1 and 1.0 μmols.

2.3.2.1 α-Ketobutyrate Standard curve

A stock solution of 100 mM of α-ketobutyrate (Sigma-Aldrich, Burlington MA, USA) was prepared using 0.1 M Tris-HCl (Sigma-Aldrich) pH 8.5 and stored at 4 °C. This stock solution was further diluted to a 10 mM solution. 200 μL of 6 known α-ketobutyrate concentrations were prepared and transferred to a glass test tube (100 mm x 13 mm). Readings for each point were done in duplicate and the average of the two was used to create the standard curve. 300 μL of

2,4- dinitrophenyl-hydrazine reagent (0.2%, in 2 N HCl Sigma Aldrich) was added to each test tube and vortexed. This mixture was then left in an incubator at 30 °C for 30 minutes. To develop the colour of the newly formed phenylhydrazone, 2.0 mL of 2 N NaOH was added, vortexed and the absorbance was measured at 540 nm.

2.3.2.2 Induction of ACC deaminase activity

5 µl of each bacterium (8.1x10 ^{7} CFU/mL of *F. enclensis*, 1.7x 10^{8} CFU/mL *P. rhodesiae* and 2.2x10^{8} CFU /mL *S. rhizophila*) was grown (mid to late log phase) in 15 mL of nutrient broth medium. There were two culture tubes containing 5 µL of each bacterium in 15 mL of nutrient broth. The culture tubes were incubated overnight in a shaking water bath at 30 °C and 200 rpm. The tubes were combined and harvested by centrifugation (Centurion Scientific, model: K1015, SS34 rotor) at 8000xg at 4°C for 10 min. The supernatant was removed, and the pellets were washed with 5 mL of minimal medium and centrifuged again for 10 mins at 8000xg, at 4° C. The cells were then resuspended in 7.5 mL of minimal medium in a fresh culture tube. Prior to incubation, the 0.5 M ACC solution was thawed and 45 µL was added to the cell suspension (amounting to a final ACC concentration of 3.0 mM). The bacteria were left for 24 hrs in a shaking water bath at 30 °C, 200 rpm. After overnight growth, the bacteria were centrifuged at 8000xg for 10 mins, 4°C and the cells were washed by suspending the pellet in 5.0 mL of 0.1 M Tris-HCl, pH 7.6. The cells were then washed twice by adding 5.0 mL of 0.1 M Tris- HCl pH 7.6, centrifuging for 10 mins at 8000xg, 4°C and discarding the supernatant. The pelleted cells were stored at -20 °C for measurement of ACC deaminase activity.

2.3.2.3 Measurement of ACC deaminase activity

The pelleted cells from section 2.3.2.2 were each suspended in 1 mL of 0.1 M Tris-HCl pH 7.6 and transferred to a 1.5 mL microcentrifuge tube. The contents were then centrifuged for 5 mins at 16,000xg in a Centurion Scientific, model K1015 centrifuge. The supernatant was removed with a fine tip transfer pipette and the pellet is resuspended in 600 μL of 0.1 M Tris HCl pH 8.5. Thirty (30) μl of toluene was then added to the cell suspension and vortexed for 30 s. Similarly, to the measurements for the standard curve, all measurements were carried out in duplicate and the average of the two was used for statistical analysis. Two hundred μL of toluenized cells were placed in a new 1.5 mL microcentrifuge tube with 20 μl of 0.5 M ACC added to the suspension and then vortexed. Cells were then incubated at 30 °C for 15 minutes. One (1) mL of 0.56 N HCl was added to the suspension and centrifuged for 6 mins at 16,000xg. One (1) mL of the supernatant was added to 800 μl of 0.56 N HCl in a glass test tube (100 x 13 mm) and vortexed. Three hundred μl of 2,4-dinitrophenylhydrazine (0.2% 2,4-dinitrophenylhydrazine in 2 N HCl) was added to the mixture, vortexed and incubated for 30 mins at 30 °C. Two mL of 2 N NaOH was added to this suspension, mixed, and measured at 540 nm. The absorbance value of the reagents plus ACC only was used as a reference or blank for the spectrophotometric readings (after incubatory periods). This reading resulted in the absorbance value of only the bacteria extract. To obtain the contribution of the extract, the absorbance value of the bacterial extracts and assay reagents without ACC is measured and subtracted from the previous value. This value was then used (standard curve) to calculate the amount of α-ketobutyrate generated from the deamination of ACC.

2.4 Statistical Analysis

All data was statically analyzed using RStudio version 2022.02.0. Sample sizes of 18 and above were first tested for normality using a Shapiro Wilk's test and equality of variances using a Levene's Test. For data testing positive for normality and equality of variances, a one-way ANOVA followed by a Tukey's post hoc test was conducted. For smaller samples sizes (below 18) the data was tested for significant differences using the non-parametric Kruskal-Wallis test followed by Dunn's post hoc test.

3 Results and Discussion

3.1 Bacteria quantification and qualification

All bacteria used in this study were first quantified to properly assess the effect of treatment groups on plants and the parameters being studied. The quantification of viable bacteria plays a crucial role in biofertilizer formulations and other biocontrol applications. *Figure 5*, 6 and *7* represents the standard curve of *F. enclensis*, *P. rhodesiae* and *S. rhizophila* respectively. Growth measurements were conducted over a period of 72 hours to observe the growth curve for each bacterium (*Figure 8*). *Figure 9* depicts *F. enclensis* grown separately and the *F. enclensis*, *S.rhziphila* co-culture. The co-culture study confirmed that the two bacteria were able to grow and thrive in the same environment. This quantification and qualification information was used to design a study that would more accurately assess and answer the desired objectives.

3.1.1 Bacteria quantification

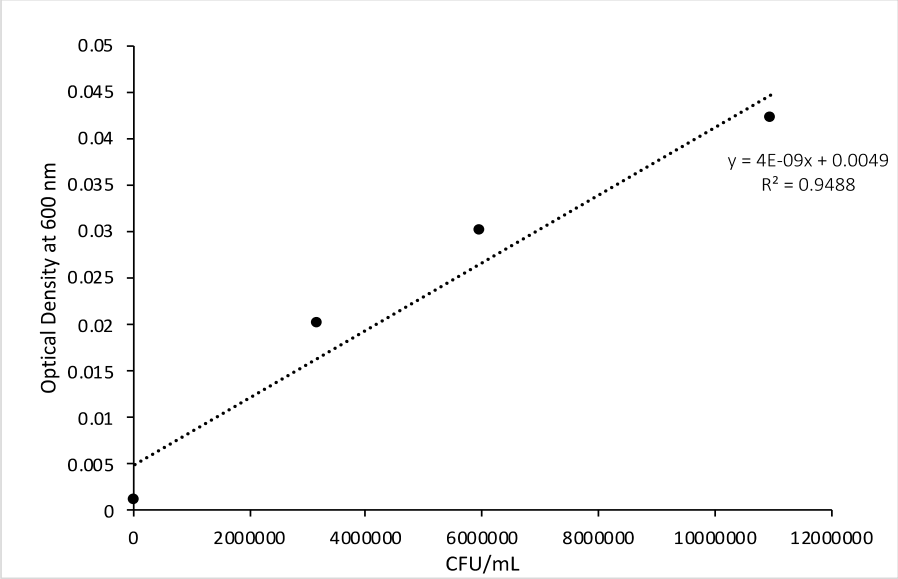

Figure 5. Standard curve of colony-forming units (CFU) per mL vs optical density measured at 600 nm for the bacterium F. enclensis.

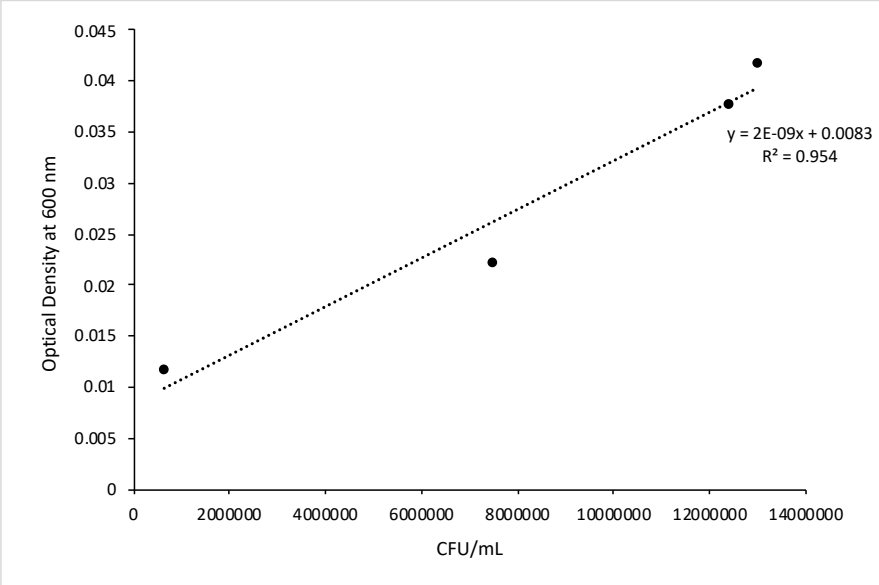

Figure 6. Standard curve of colony-forming units (CFU) per mL vs optical density measured at 600 nm for the bacterium P. rhodesiae.

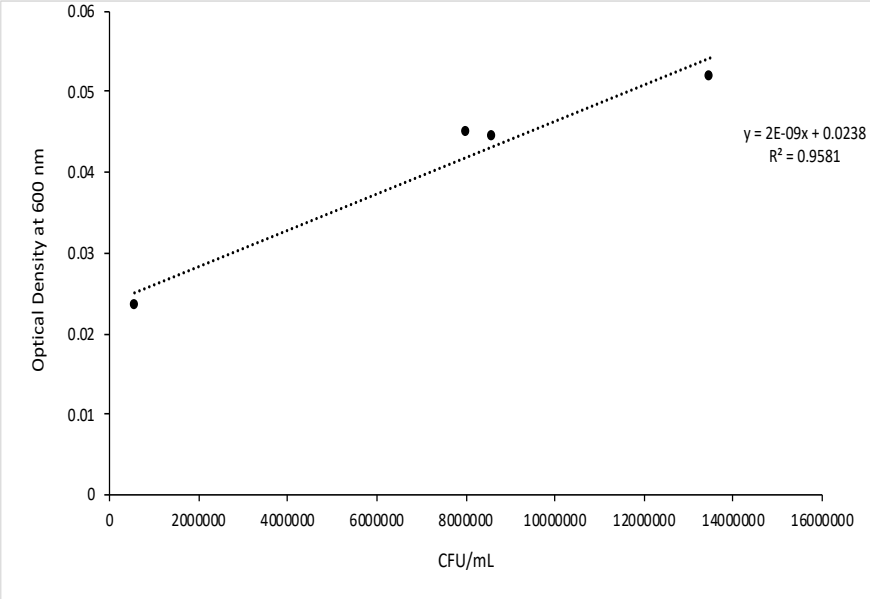

Figure 7.Standard curve of colony-forming units (CFU) per mL vs optical density measured at 600 nm for the bacterium S. rhizophila.

Both colony counting and the spectrophotometric method of optical density (OD) measurement were utilized to quantify the bacteria. Counting colony-forming units excludes damaged or

inactive cells and only detects bacteria that can grow on the specific solid media used (nutrient agar in this case) (Pan et al. 2014). Although this method is not the most accurate quantification method, when paired with spectrophotometry, it results in a good enough estimation of the bacterial cells in the solution. Measuring the optical density of the culture measures its turbidity which is determined by both live and dead cells (Schacht et al. 2012). With the same culture procedure repeated for each experiment, this ensures that roughly equal quantities of bacteria (CFU/mL) are present per treatment.

Pseudomonas rhodesiae and *S. rhizophila* had a very similar number of CFU/mL, higher than that of *F. enclensis*. This observation also correlated positively with the turbidity of the culture solutions. All three bacteria had CFU in the 10^7 CFU/mL range which indicated very similar growth rates and growth curves.

3.1.2 Bacteria growth measurements

All bacteria follow the same growth curve pattern when cultured in fresh medium where there are four phases: the lag phase, the log phase, the stationary phase and death phase (Lin et al. 2011; Wang et al. 2015). The log phase is the phase of most interest because this is where the exponential growth of bacteria takes place and where most cells are viable. By observing the growth curve of *F. enclensis*, *P. rhodesiae* and *S. rhizophila*, it is easy to identify which bacterium grows the fastest and at what point during its growth is it most suitable to inoculate with biochar, soil or a plant. The stationary phase or plateau of the growth curve depicts a point where the number of live cells is equal to the number of dead cells as a result of the nutrient depletion (Wang et al. 2015). Harvesting cells during or after the stationary phase runs the risk of

having a significant number of dead cells within the biofertilizer cocktail and may lead to poor results or less effectiveness.

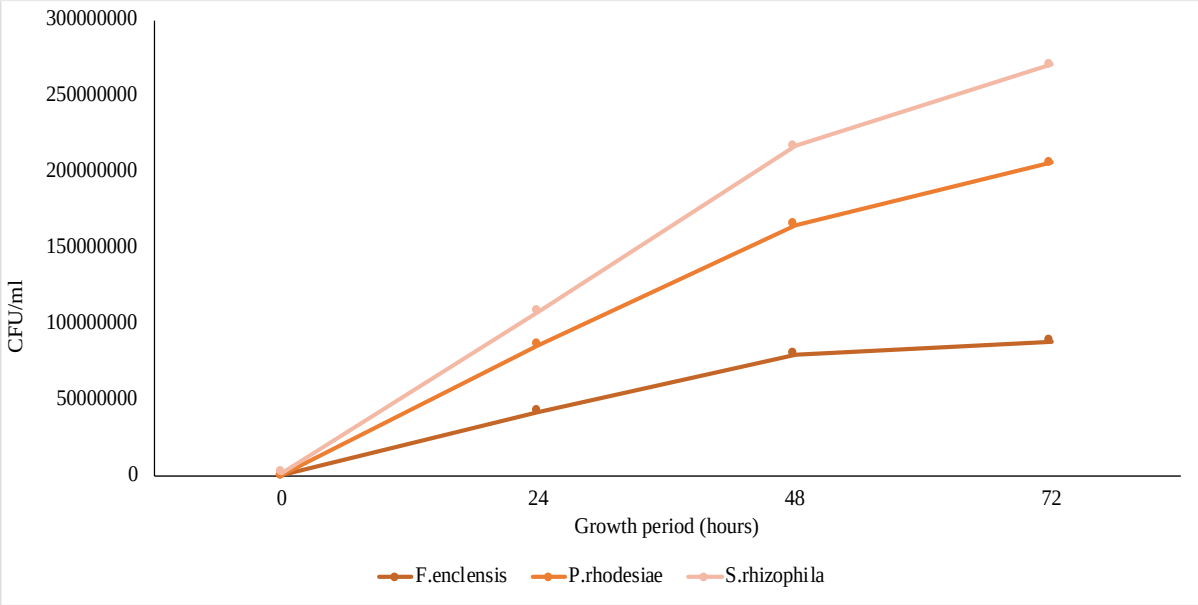

Figure 8. Growth rate of F. enclensis, P. rhodesiae and S. rhizophila expressed as colony-forming units per mL versus time. Each reading was done in triplicate and the average of the three was used in this graph.

As seen in this figure (*Figure 8*) *S. rhizophila* appears to grow much faster and has more CFU/mL than *P. rhodesiae* and *F. enclensis. Fictibacillus enclensis* was the slowest growing of the three bacteria within the first 72 hours. It was observed that *F. enclensis* grew much faster when cultured from agar plates as opposed to directly from frozen stocks. It was determined from this figure that inoculating biochar with the bacteria would be more effective during the mid to late log phase, which coincided with no more than 48 hours of growth. This ensured that a higher concentration of active cells was transferred to biochar and plants. Bacteria in soil are exposed to adverse conditions such as temperature changes, lack of nutrient availability and

moisture, soil acidity and inhibition by other microorganisms, and so it is paramount that the inoculant can survive most of these conditions to have the desired effect.

3.1.3 Bacteria co-culture

Co-inoculation studies on PGPRs and biocontrol agents are the new innovative approach to plant health management and crop yield improvement (Marimuthu et al. 2002; Orozco-Mosqueda et al. 2018) Single strains as inoculants often result in inconsistent efficiencies in the agricultural field (Felici et al. 2008). This may result from contrasting soil environments exposed to adverse abiotic and biotic conditions. One single, microbial agent is unlikely to be active in all soil environments (Bagheri et al. 2022). Application of binary or multiple microbial mixtures would mimic the natural environment and hence broaden the spectrum of biocontrol activity and would enhance the efficacy of plant growth promotion in crops (Raupach and Kloepper 1998; Marimuthu et al. 2002).

a) b)

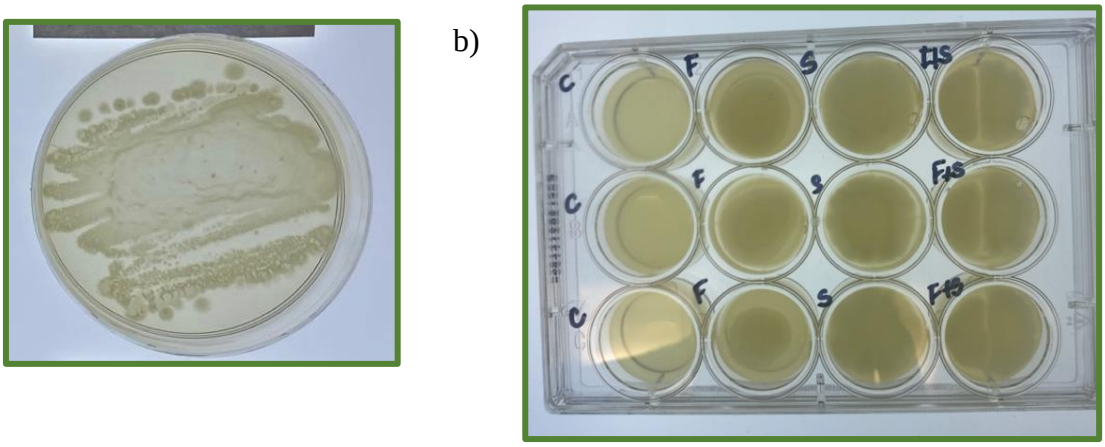

Figure 9. F. enclensis (a) and F. enclensis, S. rhizophila co-culture experiment (b). For the Co-culture experiment, from left to right: control, F. enclensis only, S. rhizophila only, F. enclensis and S. rhizophila co-culture.

Fictibacillus as mentioned before was isolated on a piece of biochar at Saint Mary's University b*y* (Blatt-Janmaat 2018) and is the bacteria of focus in this study. This bacterium was first discovered and named by (Dastager et al. 2013) from marine sediment. *F. enclensis* cells are aerobic, Gram-positive staining motile rods. They are about 0.45-0.46 × 3.0-3.2 µ in length (Dastager et al. 2013). They grow optimally at 30℃ on nutrient agar, and as seen in *Figure 9*, they are cream in colour and opaque. *S. rhizophila* was selected as the co-inoculant of choice due to its plant-growth-promoting and plant defence abilities (Berendsen et al. 2018). There were no contact-dependent or contact-independent inhibitory effects observed in the co-inoculation study (*Figure 9*). The *F. enclensis/S. rhizophila* cocktail was used in further bioassay studies to assess its potential synergistic effect on plant growth.

3.2 Plant growth bioassay

Fictibacillus enclensis and the *F. enclensis/S. rhizophila* co-culture were used in a bioassay to measure their effects on plant growth. The conditions for growth were established by Kaitlyn Blatt-Janmaat (2018) and modified slightly to achieve the desired effect. Barley, peppers, carrots, and bush beans were planted and allowed to develop over various growth periods to assess the effects *of F. enclensis*, the co-culture and biochar on overall plant growth and health.

3.2.1 Field trial- Barley

The barley field trial was designed to assess the plant growth-promoting ability of *F. enclensis* and the effectiveness of biochar as an inoculum carrier and soil amender. This study contained a negative control (only water and soil), a positive control (*P. rhodesiae* treatment) and the widely

utilized chemical fertilizer (20-20-20 Miracle-Gro^{TM}) treatment as another positive control. As previously mentioned, this study was conducted at the Kentville Research and Development Center in Kentville, Nova Scotia, From June to August.

Barley is one of the oldest cultivated crops growing in extremely wide geographic ranges from high mountain tops down to the seacoasts. Barley grows best in well-drained, fertile light clay soils and prefers cool dry growing areas (Newton et al. 2011). The basic structure of barley, as depicted in *Figure 10* are the roots, the stem, leaves, and spike (containing the kernel that could be awned or awnless) (Grant, Brennan, and Hoad 2020). The height of the barley stem, length of the spike and dry mass of the spike was used for statistical analysis.

Figure 10.Diagram depicting the stem and spike of the barley plant measured and used for statistical analysis.

Figure 11. Barley plant infested by barley flea beetles (Phyllotreta vittula).

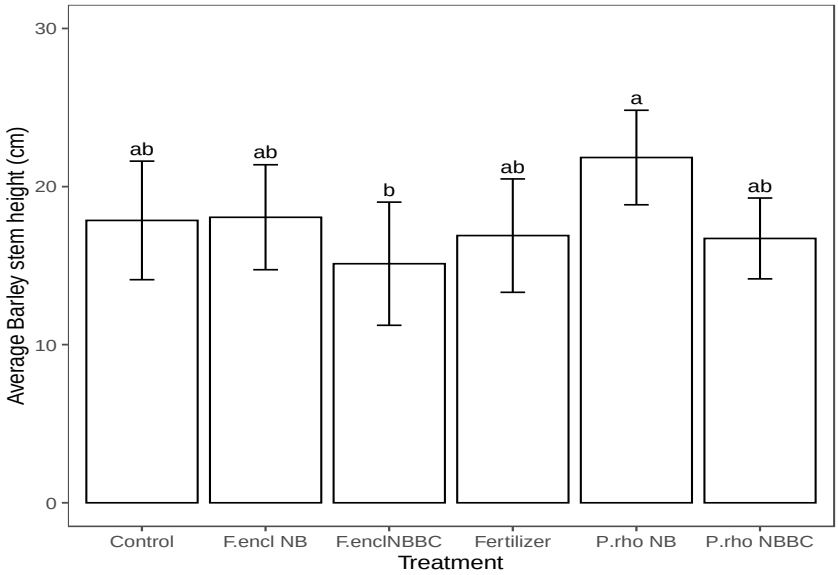

Figure 12. Average stem height of barley for each treatment group measured at the Kentville Research and Development Center, Kentville (Nova Scotia). Each bar represents the mean ± SD. A Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found significant differences between treatment groups; chi-squared = 16.796, df = 5, p-value = 0.004903, n=10. Letters denote significant differences between treatment groups. Bar "a" (P.rho nutrient broth) has the highest mean and is significantly different to bar "b". Bars with letters "ab" are not significantly different to both a and b and are not significantly different to each other.

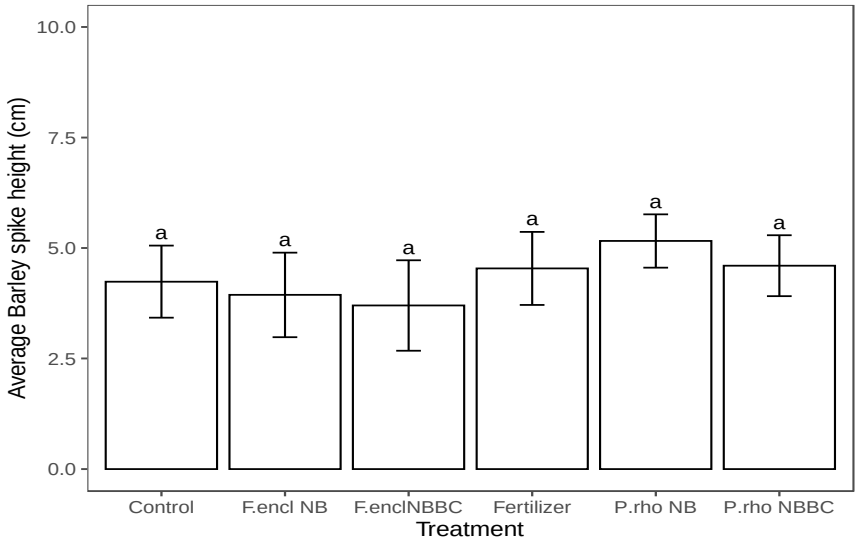

Figure 13. The average spike height of barley for each treatment group was measured at the Kentville Research and Development Center, Kentville Nova Scotia. Each bar represents the mean ± SD. A Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found no significant differences between treatment groups (chi-squared = 3.9812, Df = 5, p-value = 0.5521, n=10). Letters denote significant differences between treatment groups.

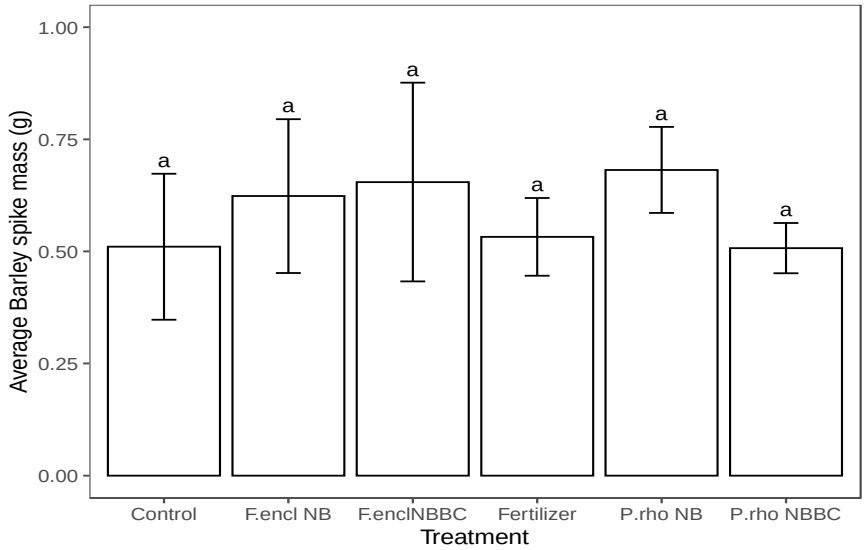

Figure 14. The average spike mass of barley for each treatment group was measured at the Kentville Research and Development Center, Kentville Nova Scotia. Each bar represents the mean ± SD. A Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found no significant differences between treatment groups (chi-squared = 9.6726, df = 5, p-value = 0.08506, n=10). Letters denote significant differences between control and treatment groups.

Although 1280 seeds were planted, only 70 seeds germinated and grew to a cultivatable stage. The factors most likely responsible for this result are the lack of irrigation and, as seen *in Figure 11,* an infestation of barley flea beetles after one month of growth. The leaves of all the plants across all treatment groups were severely damaged, resulting in decreased photosynthetic output, leading to a decline in overall plant health. The plants were watered manually every two weeks upon reapplication of treatments, and naturally by the rain. I was advised by a staff member that plants received sufficient water due to frequent precipitation but that did not seem to be the case as the soil was dry on reapplication days and plants were usually withered. Of the 70 plants collected the 10 highest measurements from each treatment was selected for statistical analysis. There was on 10 plants collected for the *F.enclensis* nutrient broth treatment. *Figure 12* shows no significant difference in stem height between the control, *F. enclensis* nutrient broth only treatment, the fertilizer treatment and the *P. rhodesiae* biochar treatment (chi-squared = 16.796, df = 5, p-value = 0.004903). All the aforementioned treatments had significantly different stem heights from *P. rhodesiae* nutrient-only treatment and the *F. enclensis* biochar treatment. These results are not in accordance with the prediction that treatments containing biochar may outperform treatments with only nutrient broth and bacteria, due to biochar's ability to house bacteria and aid in its transportation to plant roots and the added benefits of water retention and improved soil fertility (Agegnehu et al. 2016). There was no significant difference between treatment groups when comparing spike height and spike dry mass (chi-squared = 3.9812, Df = 5, p-value = 0.5521; chi-squared = 9.6726, df = 5, p-value = 0.08506). The inconclusive results obtained from this study could also be due to improper tillage. Only a few cm of topsoil was properly tilled, and although the soil was manually broken up before planting, the root system of

the barley may not have been exposed to the light clay soil it prefers, and hence the water and nutrition from that soil (Newton et al. 2011). All these factors pertinent to crop growth hindered the ability to accurately assess the effect of the treatment groups on barley growth.

3.2.2 Greenhouse trial- Peppers

This experiment conducted on Habanero pepper (*Capsicum chinense*) plants assessed the potential advantages of multiple bacteria applications versus a single application on plant growth. In addition, coincidentally, the biocontrol/pest control ability of the treatment groups was also assessed due to an infestation of aphids (*Aphidoidae).* Chilli peppers are a popular vegetable, consumed worldwide due to their sensory properties. There was an increase in Habanero chilli production of about 34.5 million tons in 2016. Countries such as China, Mexico, Spain and Indonesia are the largest producers of chilli peppers, and this results in a significant socio-economic and agricultural importance of these products to certain countries (Ramírez-Rivera et al. 2021). These peppers grow ideally in warm climates in soil abundant in phosphorus and calcium.

Figure 15. Image of pepper plant infested with Aphids (Aphidoidae).

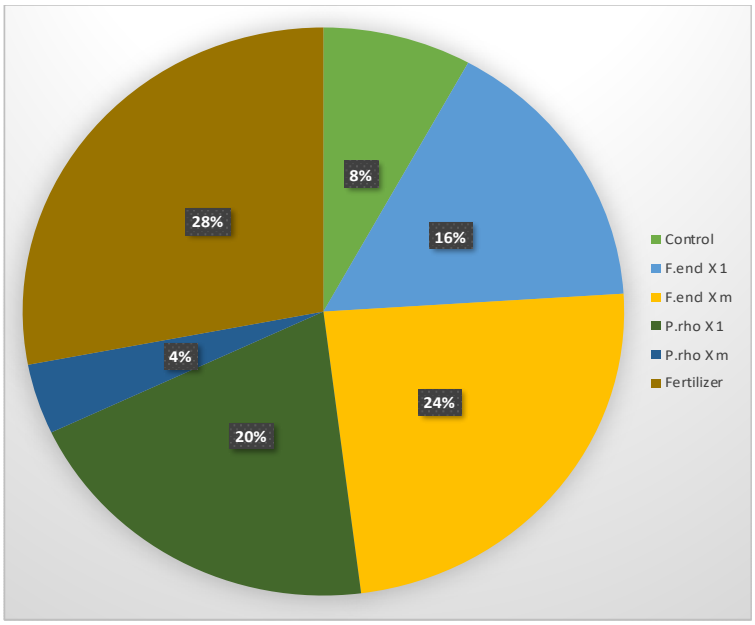

Figure 16. Percentage of damaged plant leaves per treatment. A total of 40 damaged leaves were counted across all treatment groups and the number in each group is represented as a percentage of the total number of damaged leaves.

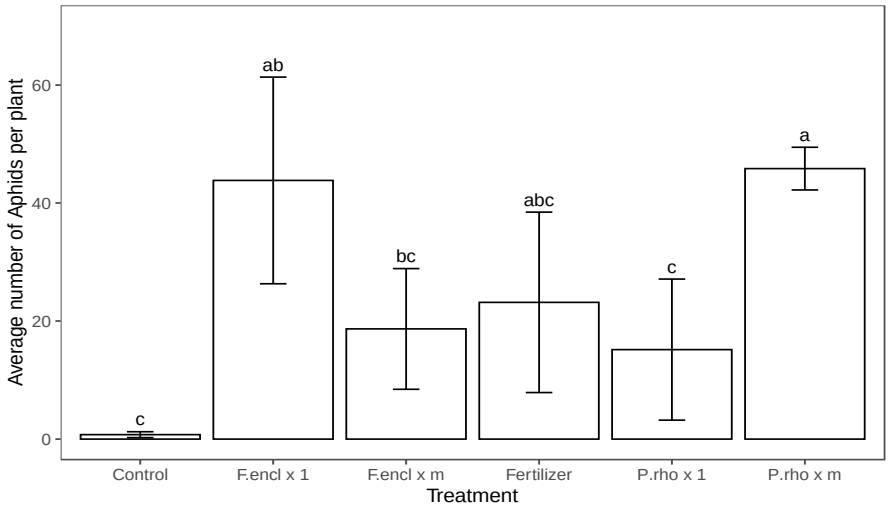

Figure 17. Number of Aphids present on each plant per treatment group measured at Saint Mary's University. Each bar represents the mean ± SD, and each count was replicated three times and averaged. The four highest counts were used for statistical analysis. A non-parametric Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found significant differences (chi-squared = 17.41, df = 5, p-value = 0.0037, n=4) between treatment groups. Letters denote significant differences between groups.

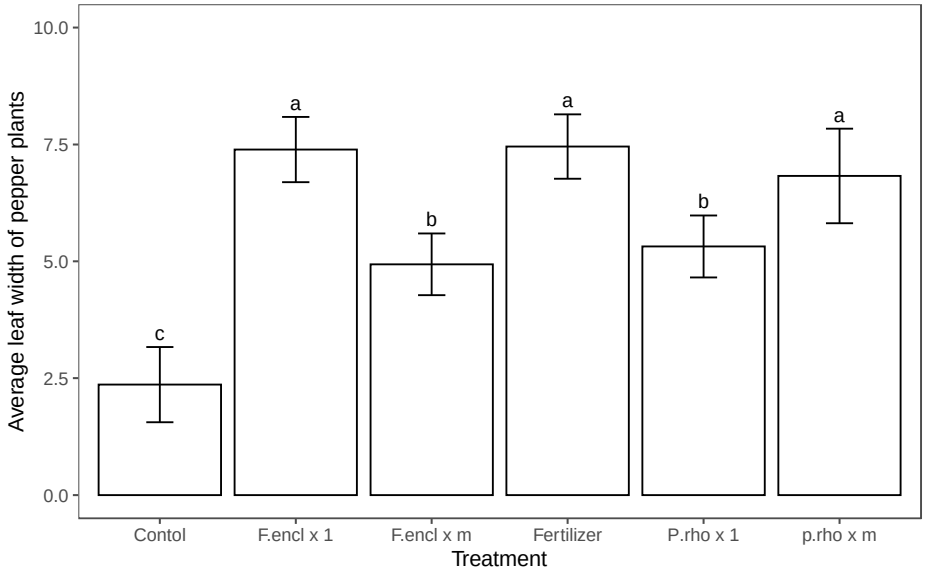

Figure 18. Average leaf width per treatment group measured at Saint Mary's University. Each bar represents the mean ± SD. 15 to 19 leaves were measured and the highest 15 were used for statistical analysis. A non-parametric Kruskal-Wallis was performed followed by a Dunn post hoc test was conducted and found significant differences (chi-squared = 52.569, df = 5, p-value =4.124e-10, n=15) between treatment groups were found denoted by letters.

As seen in *figure 16, F. enclensis* (multiple) and fertilizer treatment had the greatest number of damaged leaves. This did not coincide with *Figure 17,* where *F. enclensis* (single treatment) and *P. rhodesiae* (multiple treatments) had the highest number of aphids present per plant. This difference in results may be due to a type II error and lack of power since the sample size was very small. It has been shown that certain *Pseudomonas* spp. can kill insects in orders Hemiptera (Aphids), Diptera and Coleoptera (Grenier et al. 2006; Péchy-Tarr et al. 2008; Costechareyre et al. 2012). In a study by (Paliwal et al. 2022) on aphid-killing bacteria, *P.fluorescens* was the most toxic amongst all other bacteria studied, to almost all aphid clones. It was found that there was a 69% reduction in aphid populations on *Beta vulgaris* plants inoculated with bacteria immediately before introducing aphids compared to plants with aphids introduced days after bacterial inoculation (Paliwal et al. 2022). Reduction of aphids was seen after 72 hours and

beyond. This study may explain the differences in aphid population per treatment and damaged leaves. It took a minimum of 72 hours after treatment application to observe a reduction in the aphid population. Unfortunately, in this study, the number of aphids per plant was counted immediately after applying treatments and plants subsequently died after two days of accidentally applying a higher concentration acetic acid than intended. 0.1% acetic acid is used to kill/ ward off aphids while keeping the plant alive, due to its high pH. If plants were monitored for a week or after infestation and inoculation, perhaps the results may have favoured the multiple *P. rhodesiae* treatment groups since the concentration of bacteria would be higher after a fresh inoculation. *Figure 18* compares the average leaf width (the widest point across the leaf) in each treatment group. Due to the deterioration of the plants, after acetic acid treatment, it was difficult to obtain the leaf surface area. Instead, the width of the leaf was taken for comparison. Leaves are directly involved in photosynthesis, leading to carbon fixation and production of food (sugars) required for plant growth and in this case pepper production. Plant leaves are also involved in the transpiration and respiration (Liu, Zheng, and Qi 2020). The wider the leaf surface, the more sunlight is trapped by the plant for photosynthesis, which positively correlates with plant growth. The fertilizer treatment, *F. enclensis* (single application) and *P. rhodesiae* (multiple application) had the widest leaf width and there was no significant difference between the three. All other treatment groups had significantly wider leaves than the control group (chi-squared = 52.569, df= 5, p-value =4.124e-10). *P. rhodesiae* confirmed the prediction that additional bacteria applications may enhance plant growth when compared to a single treatment. As for *F. enclensis*, a single bacteria treatment performed better in terms of plant leaf width when compared to multiple treatments. When the soil or plant is saturated with bacteria, there

may be no additional effect of the bacteria on plant growth. Paliwal et al. (2021) showed that certain Pseudomonas spp. can survive over 21 days on plant leaf surfaces when sprayed and (Théraud et al. 2003) demonstrated that yeast such as *Candida albicans* and *Cryptococcus neoformans* can survive up to 26 weeks in a soil and water mixture. This study proved that bacterial treatments faired just as well as the fertilizer treatment and that multiple applications of the treatment may or may not be more beneficial in terms of plant growth and may be specific to the bacterium.

3.2.3 Greenhouse/growth shelf trial- Carrots

Similar to the aforementioned pepper trial, this experiment on carrots assessed the effectiveness of multiple versus single microbial treatments on carrot (*Daucus carota*) growth and the effectiveness of *F. enclensis* as a PGPR.

Carrots are biennial, cool climate crops, that store carbohydrates in enlarged taproots for reproductive growth in the second year (Que et al. 2019). They are usually ready for harvest after 70 to 80 days after planting and require loose, moist soil for optimal growth. In this experiment, carrots were planted in October of 2021 and left to grow for 121 days. Due to time constraints as a result of the temperature change, carrots were planted in the same soil as previously used by the peppers (all treatments were the same). After 21 days of growth, it was noted that the sunlight available in the greenhouse at Saint Mary's University was extremely limited and was suspected to hinder carrot growth. Additionally, since carrots are usually sown in the spring in temperate climates, the low temperature in the greenhouse was also of concern. The carrots were

transferred to a growth shelf in Dr. Zhongmin Dong's laboratory at Saint Mary's University, for the remaining 100 days.

Figure 19. Carrots after harvest after 121 days for P. rhodesiae (single) treatment group.

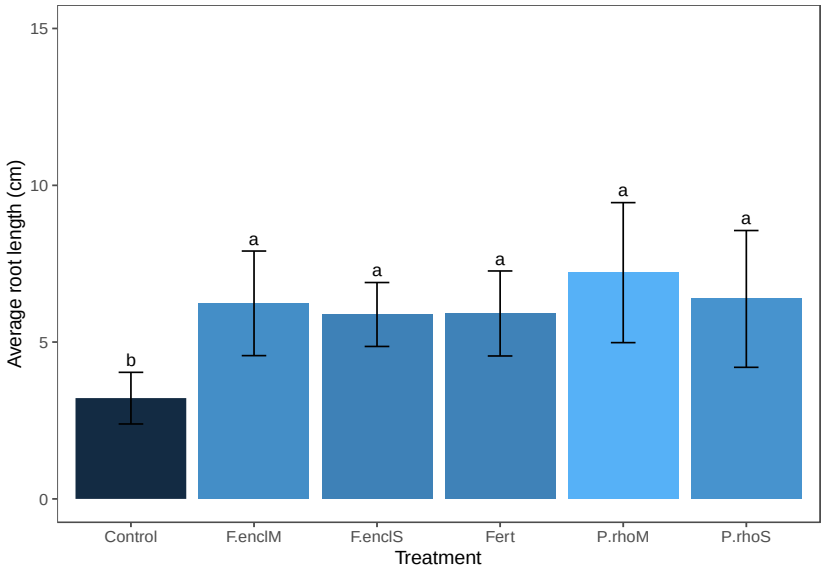

Figure 20. Average carrot root length per treatment group measured at Saint Mary's University. Each bar represents the mean ± SD. A non-parametric Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found significant differences (chi-squared = 18.873, df = 5, p-value = 0.00203, n=10) between treatment groups denoted by letters.

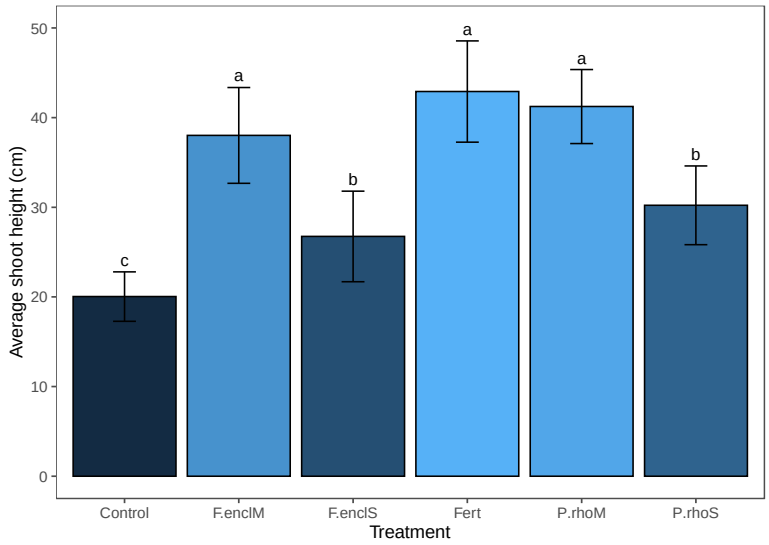

Figure 21. Average carrot shoot height per treatment group measured at Saint Mary's University. Each bar represents the mean ± SD. A non-parametric Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found significant differences (chi-squared = 41.153, df = 5, p-value =8.736e-08, n=10) between treatment groups denoted by letters.

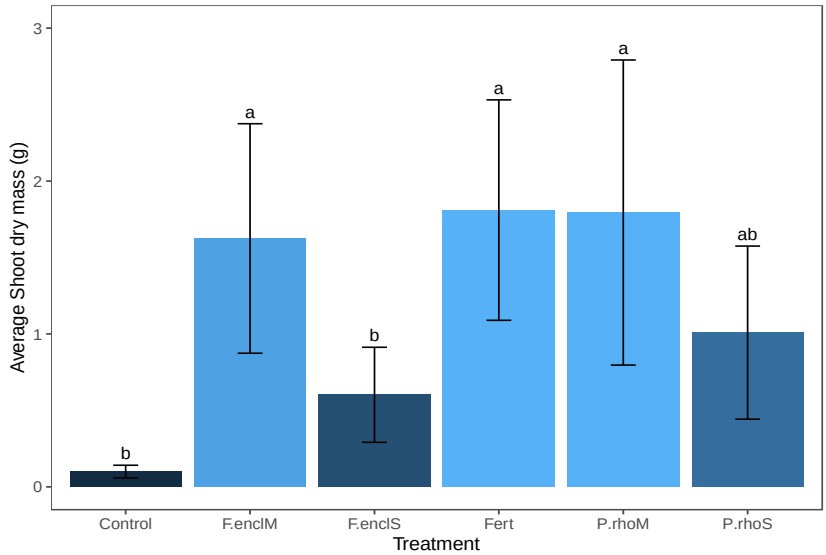

Figure 22. Average carrot shoot dry mass per treatment group measured at Saint Mary's University. Each bar represents the mean ± SD (n=10). A non-parametric Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found significant differences (chi-squared = 32.726, df = 5, p-value =4.265e-06, n=10< 0.05*) between treatment groups denoted by letters.*

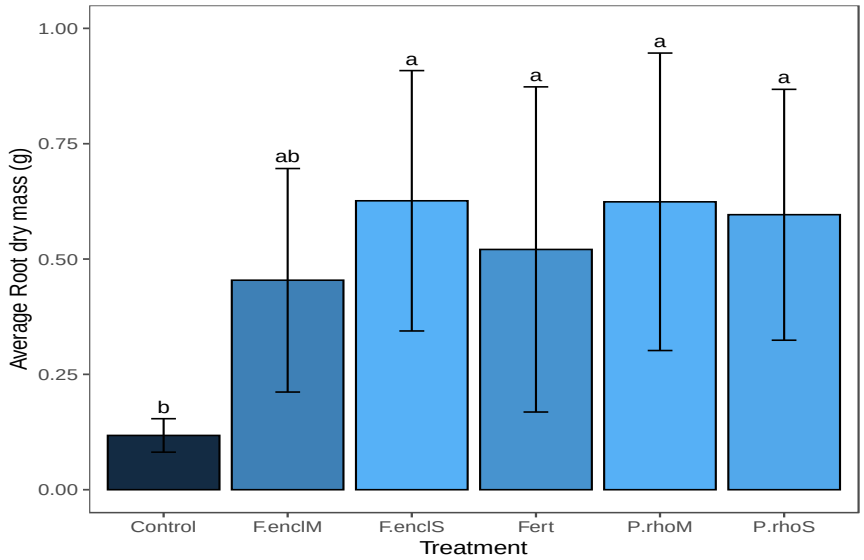

Figure 23. Average carrot root dry mass per treatment group measured at Saint Mary's University. Each bar represents the mean ± SD. A non-parametric Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found a significant differences (chi-squared = 22.335, df = 5, p-value = 0.000452, n=10) between treatment groups denoted by letters.

The image in *Figure 19* depicts severely underdeveloped carrots after 121 days of growth. These results coincide with the data from *Figures 20 and 23* where there is no significant difference in root length between the bacterial treatment groups and the fertilizers (*Figure 20*) but a difference in root length between all other groups and the control treatment (chi-squared = 18.873, df = 5, p-value = 0.00203). *Figure 23* is also very similar to *Figure 20* except the multiple *F. enclensis* application treatment groups. Unfortunately, this experiment was not designed well enough to objectively assess the effects of each treatment group on carrot roots. Firstly, before planting the carrot seeds, the soil was already compacted since it was previously used for the pepper plants, although manual soil mixing was attempted, it might not have been good enough for the desired results. Carrots thrive in loose moist soil, and this was not the case in this instance, even after planting, the soil became very compact once again and was not loosened until harvest. Another

factor that potentially affected these results is that the soil volume was slightly lower than the 2 L of soil originally placed in the pots since some of the soil on the top surface was removed due to the growth of algae. Upon statistical analysis, there was no significant difference in soil volume overall. Two carrot plants per pot were planted in an attempt to increase sample size, but carrot plants require as much room as possible and this lack of space may result in resource competition leading to small roots. Finally, the last factor that may have had a negative impact on the carrots, is the lack of sunlight in the early stages of growth. Since carrots were grown firstly in the greenhouse in the fall of 2021, the exposure to sunlight was very minimal. This greatly affected the growth of the stem and leaves (what was visible) and this observation led to the removal of the carrots from the greenhouse to the growth shelf. Once plants were placed on the growth shelf, a huge difference in shoot height was observed. These observations can also be seen in *Figures 21 and 22.* In terms of both shoot length and shoot dry mass, the fertilizer treatment, *F. enclensis* (multiple) and *P. rhodesiae* (multiple) significantly higher averages than the singly inoculated treatments and the control treatment. These results are in keeping with the prediction that multiple bacterial treatments are more beneficial to plant growth compared to single treatments. If this experiment were to be repeated addressing all the issues previously stated, the results of the carrot shoot may be reflected in the results of the carrot root. Larger shoots indicated that more photosynthetic carbohydrates should be stored in the root leading to greater root mass. In a similar experiment done by Cassie Burns (Burns & Sit 2021), although carrot root mass was significantly higher than all treatment groups except the fertilizer treatment, carrots root length was significantly lower, increasing laterally versus longitudinally. The observations made in these experiments will be revisited in a later section.

3.2.4 Growth shelf – Barley

This experiment was designed to test the plant growth-promoting ability of 6 treatments including the effectiveness of the *F. enclensis/S. rhizophila* co-culture. *F. enclensis* and *S. rhizophila* were tested as a separate treatment before being combined into a co-culture treatment. This allowed for the contribution of each bacterium to plant growth to be assessed as well as the potential combined effect. When microorganisms are grown together in the same environment, the interactions and products of these interactions can be beneficial or detrimental to the microorganisms themselves or their surrounding environments. When referring to the positive interactions between microorganisms, it has been proven that co-cultures can enhance, levels of peptides, organic acids and volatile organic compounds in fermented foods and lead to the more rapid growth of microbial compounds when compared to monocultures (Canon et al. 2020).

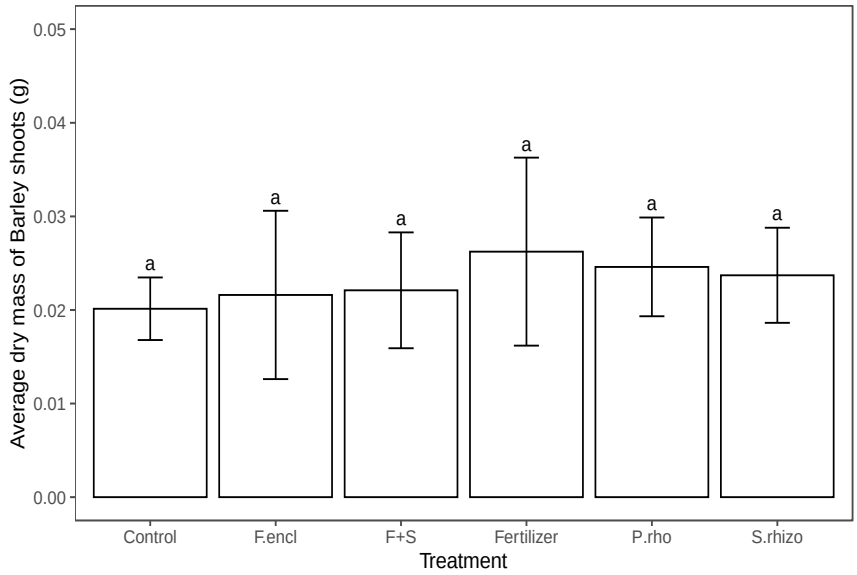

Figure 24. Average barley shoot dry mass per treatment group was measured at Saint Mary's University. Each bar represents the mean ± SD. A one-way ANOVA was performed followed by a Tukey post hoc test. There was no difference (df= 5, F-value= 1.961, Pp = 0.0903, n=20) found between treatment groups denoted by letters.

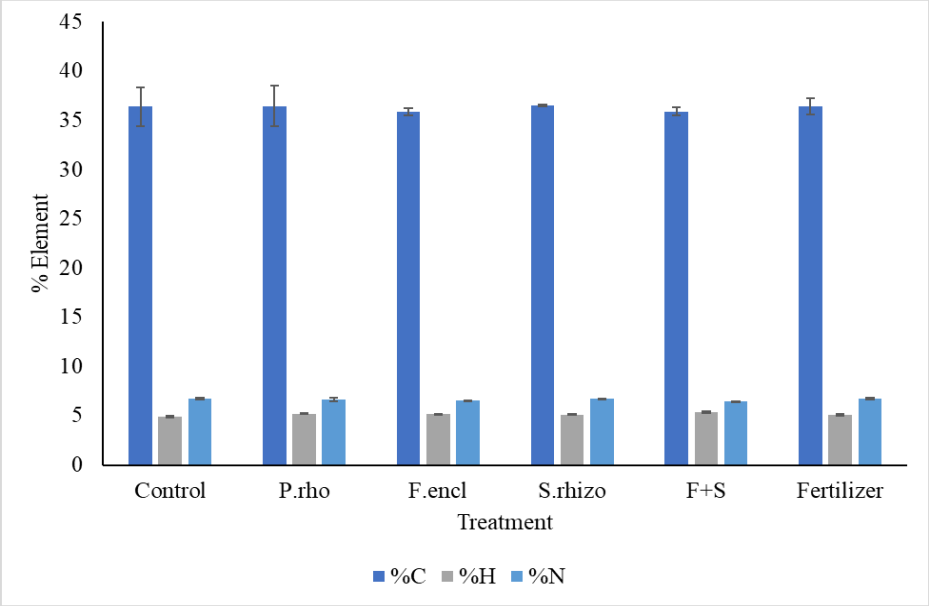

Figure 25. Percent carbon, hydrogen and nitrogen for each treatment, found in barley shoots after 14 days of growth. Elemental analysis for each sample was conducted three times and the average of the three is presented here.

Table 5. carbon-nitrogen ratio of each treatment group after elemental analysis.

Treatment	C:N ratio
Control	5.4
P. rhodesiae	5.5
F. enclensis	5.5
S. rhizophila	5.4
F+S	5.6
Fertilizer	5.4

Based on the results in *Figures 24 and 25* there was no significant difference between all of the percent carbon, nitrogen and hydrogen were all very close in value for all the treatment groups. These results can also be seen in the C:N ratio in *Table 5* where the C:N ratio values are all within the range of ± 0.2. The plant could have been left to grow for longer, and this may have

led to more statistically significant results. A similar study of barley on growth shelves by (K. Blatt-Janmaat 2018) found an increase in barley shoot mass for treatments containing the bacterium *P. rhodesiae*. Since the fertilizer treatment did just as well as the control treatment, this indicates that the fault might be with the study design rather than the treatment groups. Further analysis of barley growth is covered in the section below.

3.2.5 Germination studies-Pepper/ Barley

The effect of fertilizer, *F. enclensis, P. rhodesiae, S. rhizohila* and the *F. enclensis+ S. rhizophila* co-culture on seed germination was observed on seeds of peppers and barley. Habanero pepper germination studies were done in Petri dishes containing treatments and the barley germination studies were done in pots of soil. In the case of peppers, radicle emergence was recognized as germination since peppers take longer to germinate, as compared to the emergence of the shoot for the barley experiment.

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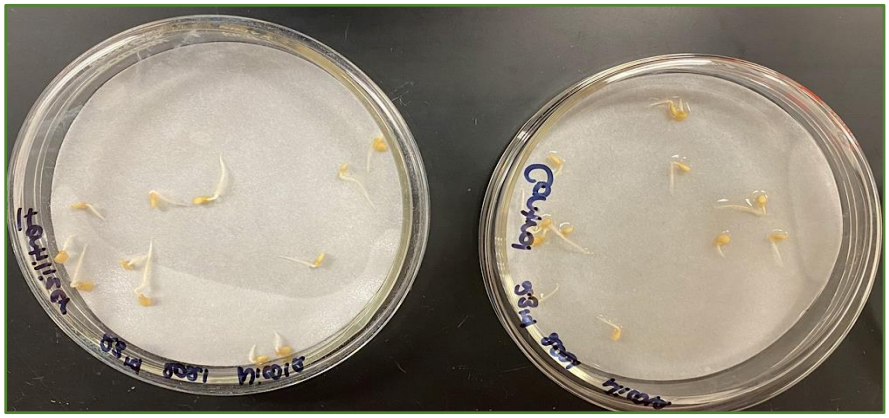

Figure 26. Seedlings of control (right) fertilizer (left) treatments after 7 days of growth. Control treatment had only water.

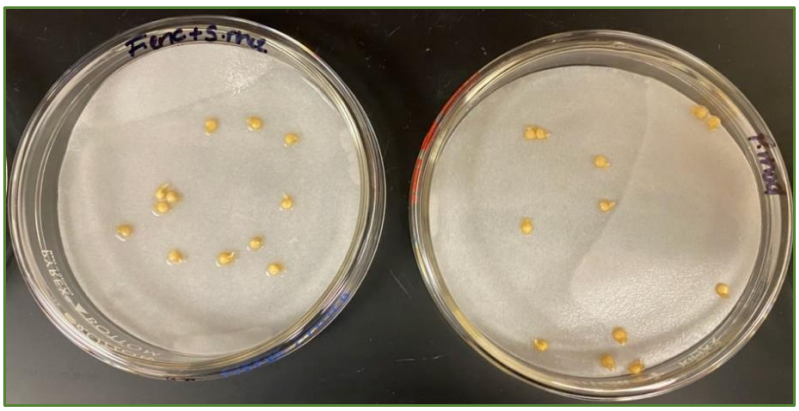

Figure 27. Seeds of the co-culture (left) and P. rhodesiae (right) treatments after 7 days of growth.

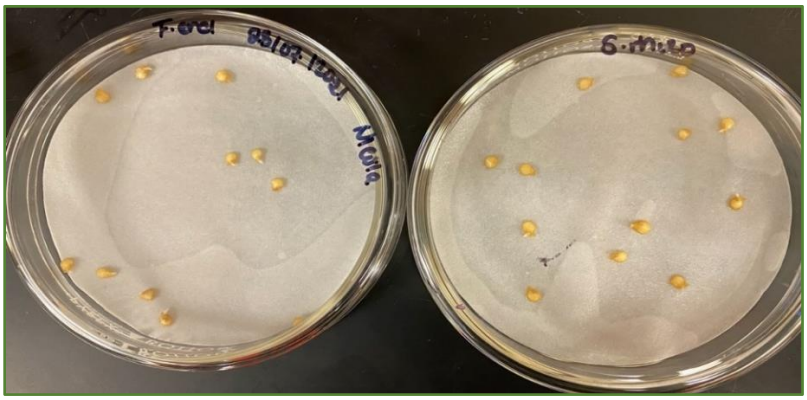

Figure 28. Seeds of F. enclensis (Left) and S. rhizophila (right) treatments after 7 days of growth.

Table 6. Number of seeds germinated and percentage germination for each treatment after 7 days.

Treatment	Total # seeds Planted	Number of seeds germinated	Percentage germination
Control	11	11	100
P. rhodesiae	11	7	63.3
F. enclensis	11	8	72.7
S. rhizophila	11	4	36.4
F+S	11	5	45.5
Fertilizer	11	11	100

As seen in *Figures 26 to 28* after 7 days of growth, the root of the fertilizer treatment and control treatment are visible, compared to the root of the remaining bacterial treatments. This observation led to the theory that the bacteria treatments slowed down the germination rate of plants (in this case carrots) from seed. *Table 6* further confirms this theory with all seeds germinated after 7 days of growth for the control and fertilizer treatments, whereas less than 75% of seeds germinated in the bacterial treatments. It seems that *F. enclensis* and *P. rhodesiae* were more favourable to germination than *S. rhizophila* and the co-culture. Although Habanero seeds have a germination rate of about 65 % (WestCoastSeeds ®) based on the results from the non-bacteria treatments it is unlikely this affected this study. A similar germination study was conducted on barley seeds to assess the effects of each treatment group on barley germination (shoot emergence).

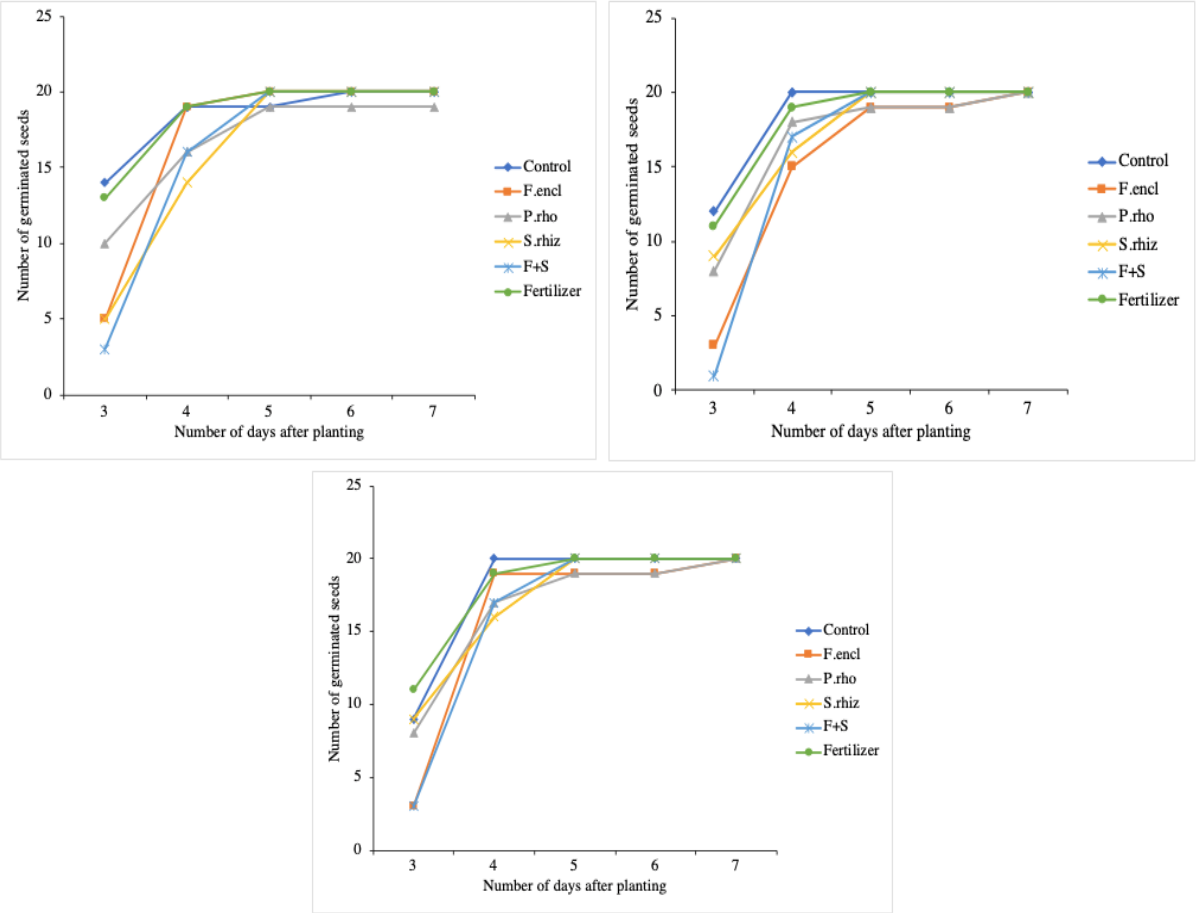

Figure 29.Line graphs depicting the number of germinated barley seeds for three separate experiments recorded over 7 days n=20.

Figure 29 depicts a delay in germination for all bacteria treatments versus the control and fertilizer treatments. All treatments began germinating on day 3 with the control and fertilizer treatments having the most germinated seeds after 3 and four days of growth. Amongst the bacteria treatments, *P. rhodesiae* has the most germinated seeds on day 3 and day 4 in all three experiments. After 5 days of growth, all seeds in all treatment groups germinate. Although *F. enclensis* is only at the lower end of germination on day 3, from day 4 onwards *F. enclensis* germination is on par with the fertilizer and control treatments.

(Somova et al. 2001) compared the effects of bacteria concentration on germination rate and root growth of wheat. It was found that the concentration of bacteria can stimulate, inhibit or have no effect on the germination of seeds. The bacteria tested were *Pseudomonas fluorescens* and *Pseudomonas putida*. Concentrations (CFU/mL) higher than 10^8 had an inhibitory effect on both germination rate and root growth. Additionally, a study by (Ndeddy Aka and Babalola 2016), showed that the germination rate was highest in treatment with bacterial cocktails (more than two bacteria). They also found that in the presence of heavy metals (contaminants), seeds containing bacteria inoculants had a higher germination rate and vigour index (a measure of the healthiness of the seed) than uninoculated treatments in a contaminated environment. These studies suggest that bacterial inoculants may not have an overall inhibitory effect on germination but that the formulation and procedure may need to be tweaked for optimal performance.

3.2.6 Root growth analysis- Barley

For the root analysis experiment, the effects of each treatment group on root length and shoot height were assessed. This experiment was conducted twice. In the first attempt, each treatment was added to seed pouches before seeds were placed. This resulted in the germination of seeds in only the control and fertilizer treatment. This observation led to a change in the procedure where seeds were pre-germinated in pouches 1 day before the addition of the treatments. Experiment 1 compared the average root length on day 1 after pre-germination and day 3 after the addition of the treatments.

Figure 30. Barley shoots and roots after 3 days of growth in seed pouches. F. enclensis (left), control (right). For the F. enclensis treatment, the roots are much shorter than the control treatment, whereas the shoots for the F. enclensis treatment are taller than the shoots of the control treatment.

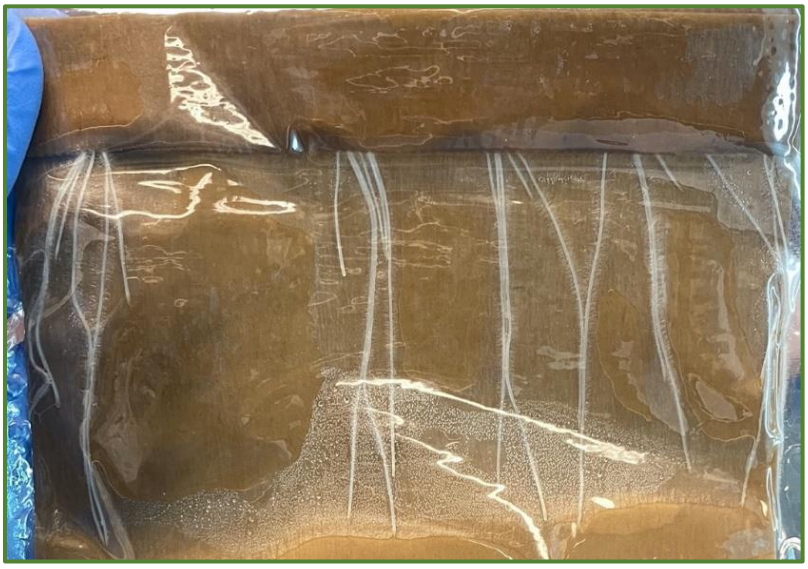

Figure 31.Close-up image of the roots in the control treatment after 2 days of growth. These are very similar to the fertilizer treatment.

Figure 32. Close-up image of the roots of the barley from the F. enclensis treatment after 5 days of growth.

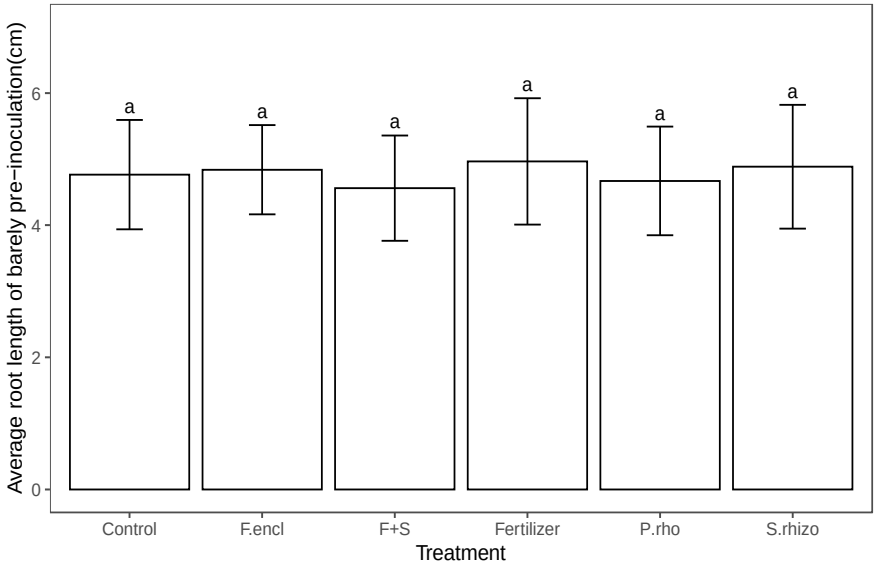

Figure 33. Average barley root length before inoculation per treatment group was measured at Saint Mary's University. This measurement was made after one day of growth with only water. Each bar represents the mean ± SD. A one-way ANOVA was performed followed by a Tukey post hoc test. There was no difference (df= 5, F-value= 0.568, p= 0.725, n=20) found between treatment groups denoted by letters.

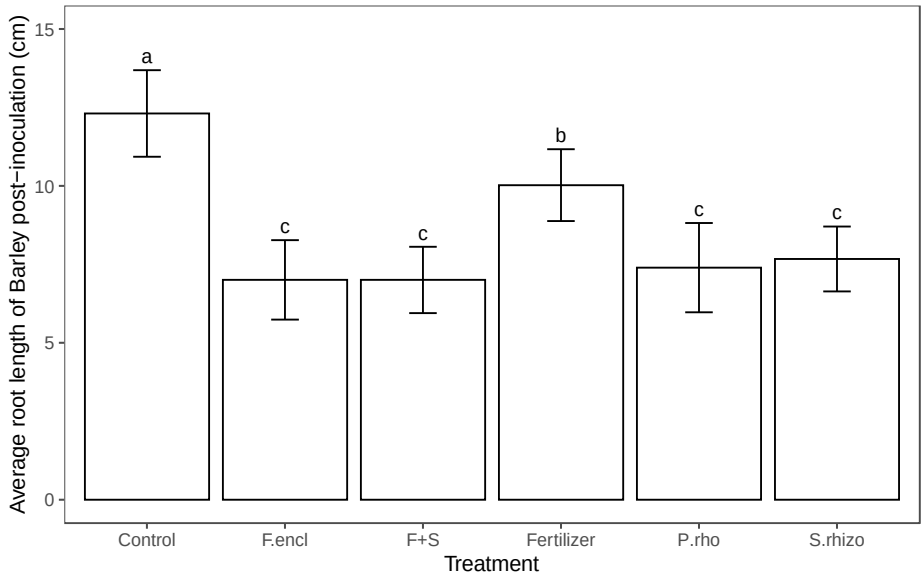

Figure 34. Average barley root length post-inoculation per treatment group measured at Saint Mary's University. This measurement was taken after 3 days of growth. Each bar represents the mean ± SD. A one-way ANOVA was performed followed by a Tukey post hoc test. There was a difference (df=5, F-value=54.98, p-value = <2e-16, n= 20) found between treatment groups, this is denoted by letters.

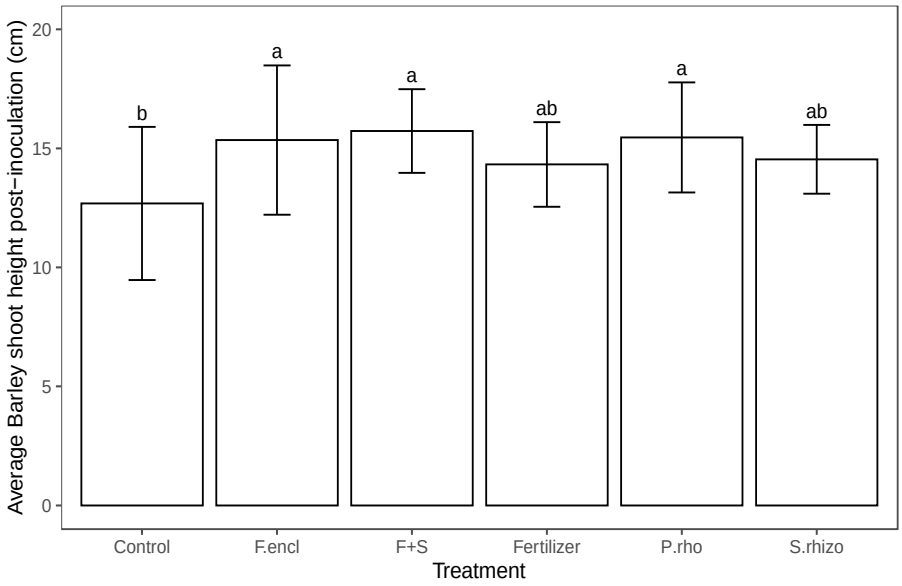

Figure 35. Average barley shoot height (cm) post-inoculation per treatment group measured at Saint Mary's University. This measurement was taken after 5 days of growth. Each bar represents the mean ± SD. A one-way ANOVA was performed followed by a Tukey post hoc test. There was a difference (df=5, F-value= 4.327, p-value = 0.00122, n=20) found between treatment groups, this is denoted by letters.

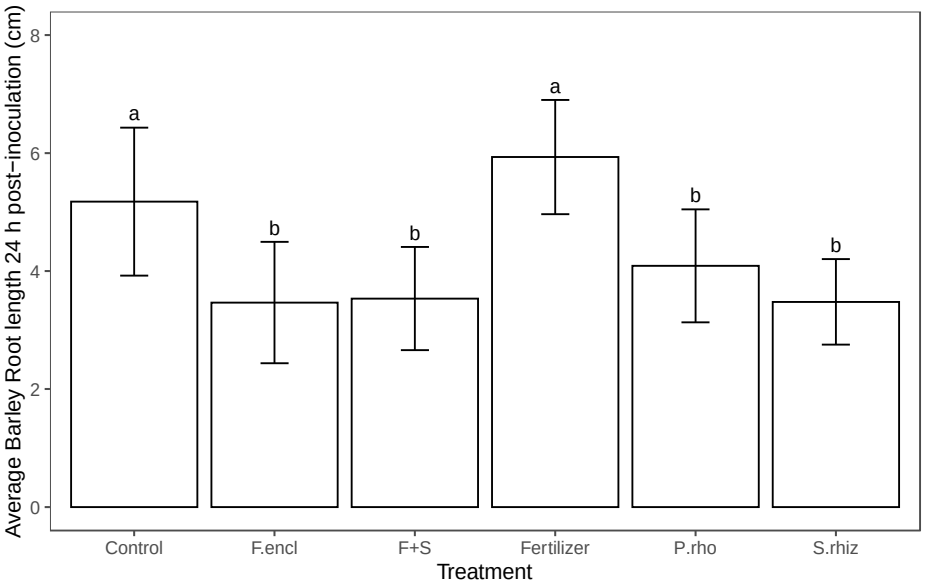

Figure 36. Average barley root length (cm) post-inoculation per treatment group measured at Saint Mary's University. This measurement was taken after 24 hours of growth. Each bar represents the mean ± SD. A one-way ANOVA was performed followed by a Tukey post hoc test. There was a difference (df= 5, F-value= 21.61, p-value= 1.04e-14, n=18) found between treatment groups, this is denoted by

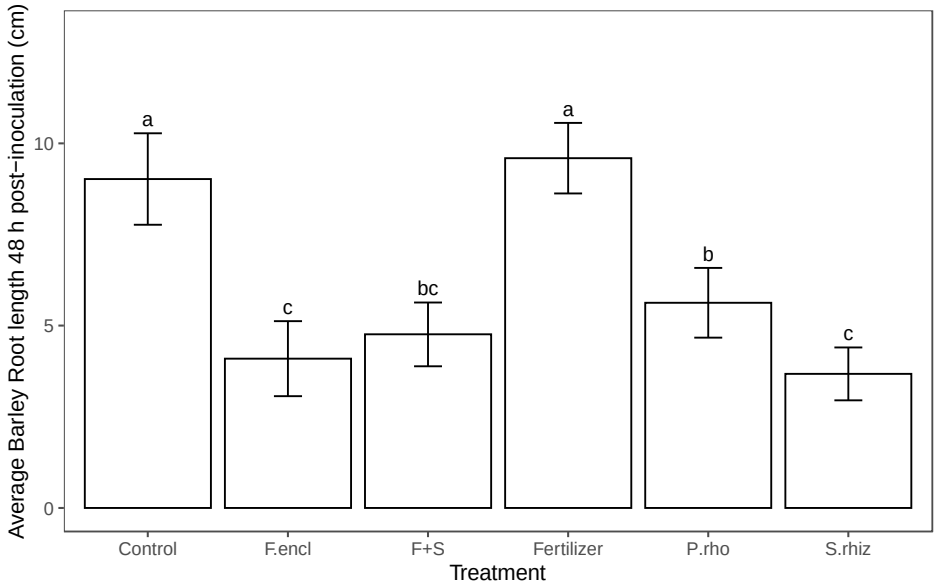

Figure 37. Average barley root length (cm) post-inoculation per treatment group measured at Saint Mary's University. This measurement was taken after 48 hours of growth. Each bar represents the mean ± SD. A one-way ANOVA was performed followed by a Tukey post hoc test. There was a difference (df=5, F-value=80.17, P-value < 2e-16, n=18) found between treatment groups, this is denoted by significant letters.

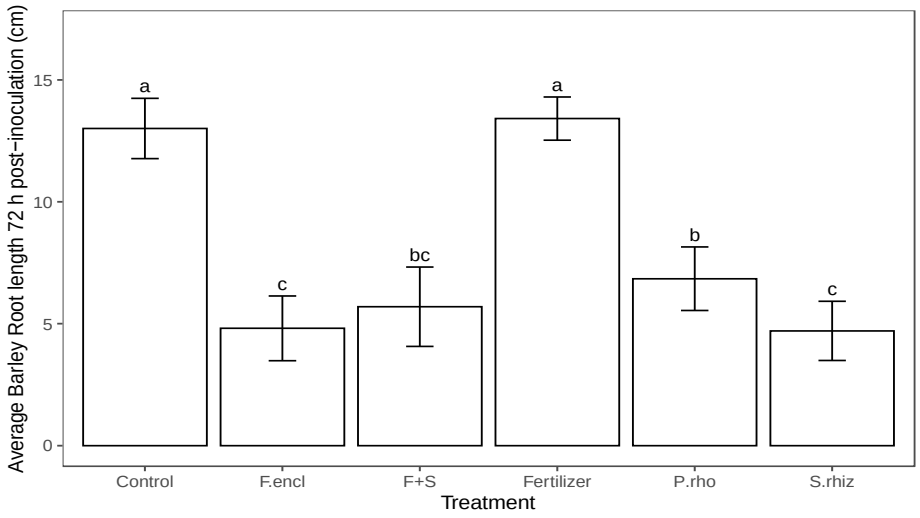

Figure 38. Average barley root length (cm) post-inoculation per treatment group measured at Saint Mary's University. This measurement was taken after 72 hours of growth. Each bar represents the mean ± SD. A one-way ANOVA was performed followed by a Tukey post hoc test. There was a difference (df= 5, F-value= 179.9, p-value< 2e-16, n=18) found between treatment groups, this is denoted by letters.

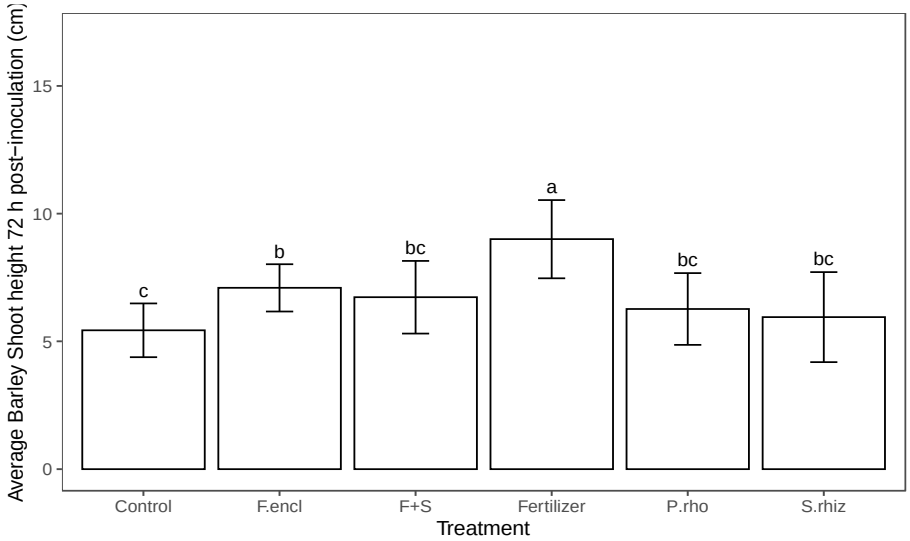

Figure 39. Average barley shoot height (cm) post-inoculation per treatment group was measured at Saint Mary's University. This measurement was taken after 72 hours of growth. Each bar represents the mean ± SD. (n=18). A one-way ANOVA was performed followed by a Tukey post hoc test. There was a difference (df=5, F-value= 11.11, p-value= 1.47e-08, n=18) found between treatment groups, this is denoted by significant letters.

Figure 30 depicts the difference in root length of barley plants in the control treatment and fertilizer treatment. Although bacterial treatments seem to stunt main/primary root growth, it favours lateral root growth. As you can see in *Figure 32*, *F. enclensis* treatment appears to have more root hairs than the control treatment (*Figure 31*). Several studies conducted on the effect of PGPR on root traits coincide with the observations made in this study, where many PGPR reduce the main root growth but increase the number or length of lateral roots and stimulate root hair elongation (Vacheron et al. 2013). On the contrary studies by (Walker et al. 2012; Zemrany et al. 2007) have reported positive effects of PGPR on root length and biomass. PGPR seems to have varying effects on root growth based on the concentration of the inoculum and the environmental conditions.

In *Figure 33*, which represents the pre-inoculated shoot length, there was no significant difference across all treatment groups (df= 5, F-value= 0.568, p= 0.725). *Figure 34*, 3 days post-inoculation, there is a significant difference in root length between the control group and bacteria treatments and the fertilizer group and bacteria treatments (df=5, F-value=54.98, p-value = <2e-16). *Fictibacullus enclensis*, *P. rhodesiae* and the co-culture outperformed all other treatment groups and had significantly higher shoot height than the control treatment, fertilizer treatment and *S. rhizophila* treatment (*Figure 35*). Similarly, in experiment 2, *figure 36* the control and fertilizer treatment had significantly longer roots after 24 hours of growth compared to the bacterial treatment groups (df= 5, F-value= 21.61, p-value= 1.04e-14*)*. After 48 hours of growth, significant differences in root length of bacteria treatments were observed, df=5, F-value=80.17, P-value < 2e-16, n=18) (*Figure 37*). Among the four bacteria treatments, *P. rhodesiae* and the

co-culture treatment had the longest roots. After 72 hours of growth, the results in *Figure 37* remained consistent with *Figure 38.* The co-culture treatment had a synergistic effect on root length than its monocultured counterparts. Lastly, regarding shoot height after 72 hours (*Figure 39*), the fertilizer treatment had a significantly higher average than other treatments, with the *F. enclensis* being the second-highest average root length and the co-culture, *P. rhodesiae* and *S. rhizophila* all tying for third highest (df=5, F-value= 11.11, p-value= 1.47e-08, n=18*)*. Interestingly, the control treatment had the longest root length, ~ 13 cm but shortest shoot height ~5.4 cm amongst all other treatments (smaller root to shoot ratio). As expected, the fertilizer treatment outperformed all other treatments in both root length and shoot height.

These results are in accordance with studies such as (Vacheron et al. 2013) where the increase in lateral roots such as root hairs leads to the increase in shoot development since lateral roots play a significant role in, the uptake of water, ion, and nutrients (Grover et al. 2021). Due to the large surface area of root hairs, plants are better able to facilitate diffusion and nutrient uptake, contributing significantly to plant development (Grover et al. 2021).

3.2.7 Growth shelf- Bush beans

The bush bean experiment was designed to test the effectiveness of *F. enclensis* and the *F. enclensis/S. rhizophila* co-culture on bush beans. This experiment was conducted twice; although the first session had significant growth, it withered over the 2021 Christmas holidays. The plants were left to grow for 96 days on a growth shelf at Saint Mary's University from January 2022 and watered twice a week. Unfortunately, the growth was limited by the size of the containers and bush beans began to wither a few days before harvest.

The common bean (*Phaseolus vulgaris L.*) is a leguminous crop high in dietary fibre and other vitamins and minerals. Bush beans have a shallow root system that requires fertile soil and lots of sunlight for optimal growth (Keller et al. 2022).

Figure 40. Bush beans plants on a growth shelf, Saint Mary's University.

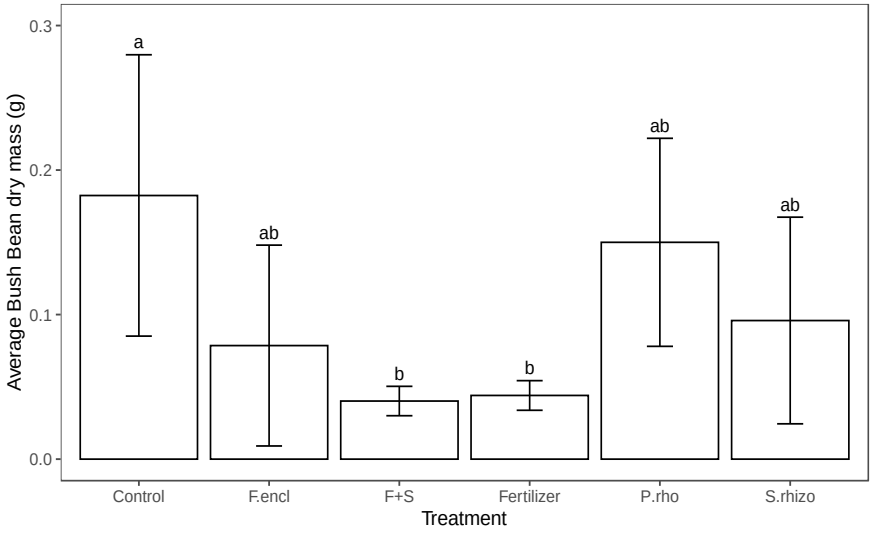

Figure 41. Average bean dry mass per treatment group measured at Saint Mary's University. Each bar represents the mean ± SD. A non-parametric Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found significant differences (chi-squared = 14.386, df = 5, p-value = 0.0133, n=5) between treatment groups denoted by letters.

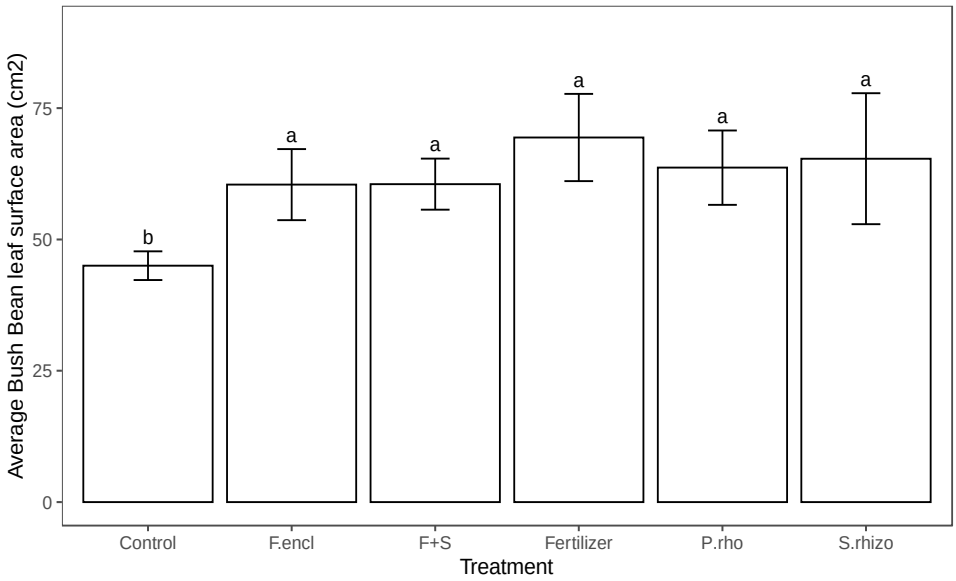

Figure 42. Average bean leaf surface area per treatment group measured at Saint Mary's University. Each bar represents the mean ± SD. (n=10). A non-parametric Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found significant differences (chi-squared = 28.75, df = 5, p-value = 2.596e-<0.05, n=10) between treatment groups denoted by letters.

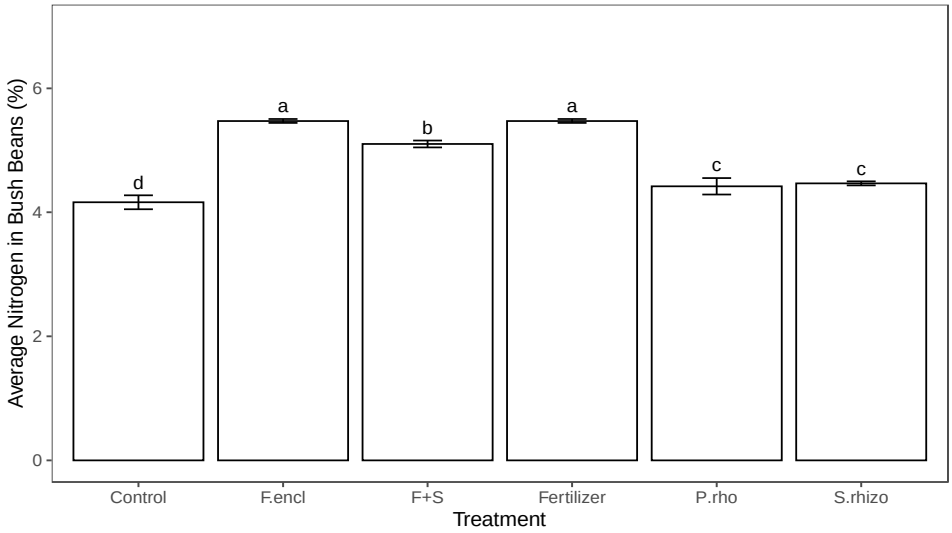

Figure 43. Average % nitrogen per treatment group measured at Saint Mary's University. Each bar represents the mean ± SD. Five beans were sampled per treatment; three measurement replications were made. A non-parametric Kruskal-Wallis test followed by a Dunn post hoc test was conducted and found significant differences (df=5, chi-squared= 17.88, p-value= 0.033, n=3) between treatment groups denoted by letters.

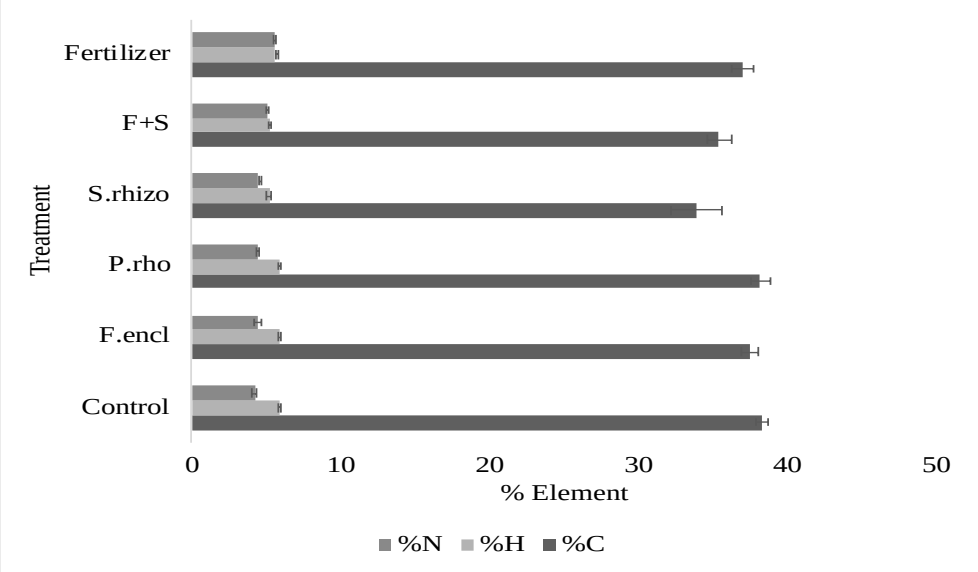

Figure 44. Percent nitrogen, hydrogen and carbon present in the beans of 6 treatment groups.

When comparing the average dry mass of the beans (*Figure 41*), the control treatment and *P. rhodesiae* treatment have the highest been dry mass overall. The fertilizer treatment did very poorly contrary to the expected result chi-squared = 14.386, df = 5, p-value = 0.0133. This signified that the results on beans produced from this experiment may not be the most reliable. One possible explanation for these results is space and insufficient light as a limiting factor. Although the beans seemed extremely healthy in the early stages after planting, after they began flowering, the health of the plants began to deteriorate. Additionally, the light source stood 21 inches above, the low light intensity experienced by the plant may have led to smaller beans. The dry mass of only 5 beans was used for statistical analysis since each treatment group produced varying numbers of beans. The five highest masses were selected for analysis. There was no significant difference in average leaf surface area when comparing the fertilizer and bacteria treatments (*Figure 42*). All other treatments had significantly higher leaf surface

area than the control treatment (chi-squared = 28.75, df = 5, p-value = 2.596e-<0.05,). These results indicate that the bacteria treatments did just as well as the synthetic fertilizer treatment and show great promise for plant growth promotion.

The average % nitrogen, carbon and hydrogen were determined for the bush beans in each treatment. Interestingly, there were significantly different percentages of nitrogen present in each treatment (df=5, chi-squared= 19.88, p-value= 0.033). As seen in *Figure 43*, the fertilizer treatment and *F. enclensis* produced the highest percent nitrogen in the beans. The co-culture treatment had a higher nitrogen content that *S. rhizophila* but a slightly lower content that *F. enclensis*. This demonstrates the effects of both bacteria on nitrogen content. *Figure 44* shows that the co-culture treatment is also in between *S. rhizophila* and *F. enclensis* for percent carbon, again demonstrating the effects of both bacteria on carbon content.

3.3 ACC deaminase quantification and qualification

As previously stated in section *1.2.3* bacteria present in soil produced ACC deaminase in response to the increase in ethylene production by plants under stress (which leads to stunted growth in plants). ACC deaminase breaks down ACC, which is a precursor to ethylene, into α-ketobutyrate and ammonia (*Figure 45*) (S. Gupta and Pandey 2019). Many PGPRs possess the ability to produce ACC deaminase, which by decreasing the levels of stress ethylene, indirectly promotes plant growth. On a quest to investigate the mode of action in which *F. enclensis* potentially promotes plant growth, testing for ACC deaminase production is vital to understanding this novel PGPR. According to Burns and Sit (2021), *F. enclensis* tested positive for IAA production, ammonia production and the potential to fix atmospheric nitrogen.

Confirming its ability to break down ACC would be a great addition to its list of plant growth-promoting properties.

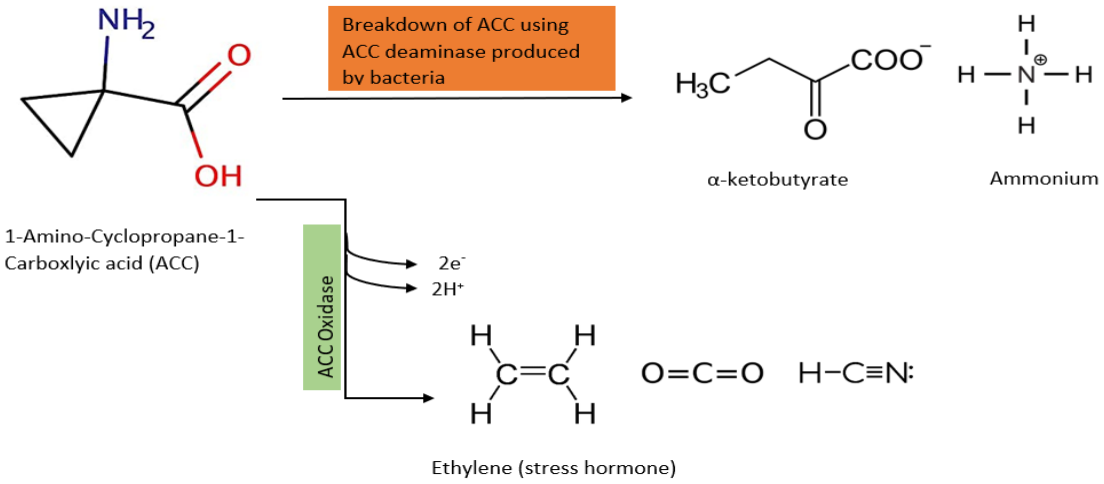

Figure 45. Products of ACC deamination and oxidation.

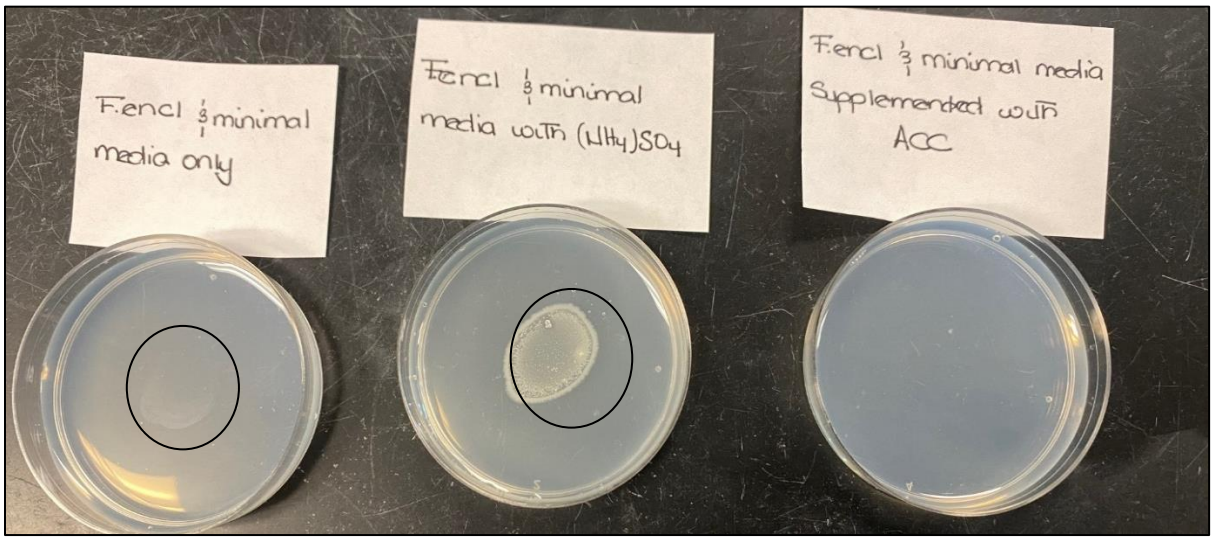

Figure 46 Qualitative analysis of ACC deaminase production in F. enclensis. From left to right, F. enclensis on only minimal media, minimal media with $(NH_4)SO_4$ as a sole source of nitrogen and minimal media supplemented with ACC after 3 days of growth. Black circles indicate growth.

Figure 47. Qualitative analysis of ACC deaminase production in P. rhodesiae. From left to right, P. rhodesiae on only minimal media, minimal media with $(NH_4)_2SO_4SO_4$ as a sole source of nitrogen and minimal media supplemented with ACC after 3 days of growth. Black circles indicate growth.

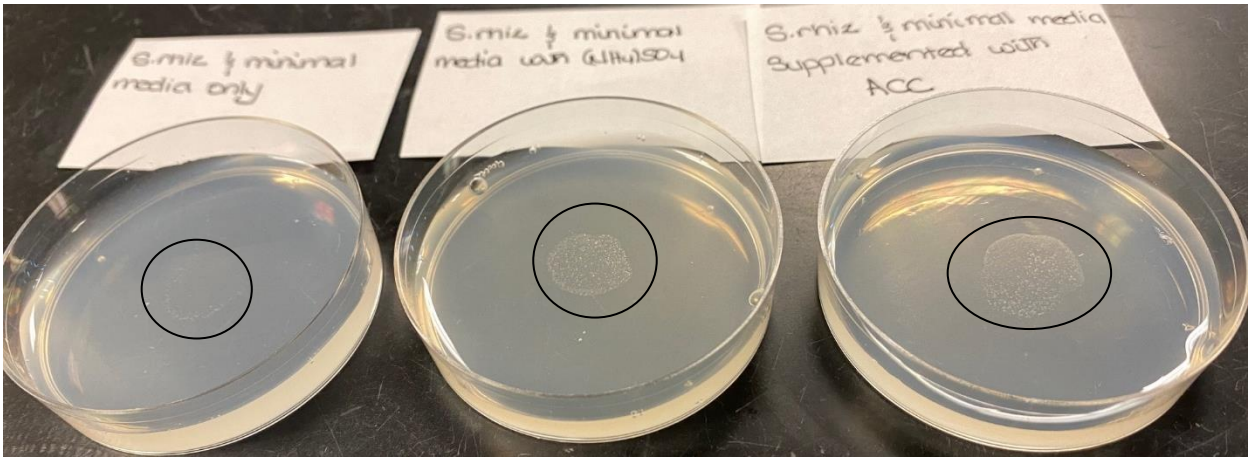

Figure 48. Qualitative analysis of ACC deaminase production in S. rhizophila. From left to right, S. rhizophila on only minimal media, minimal media with $(NH_4)SO_4$ as a sole source of nitrogen and minimal media supplemented with ACC after 3 days of growth. Black circles indicate growth.

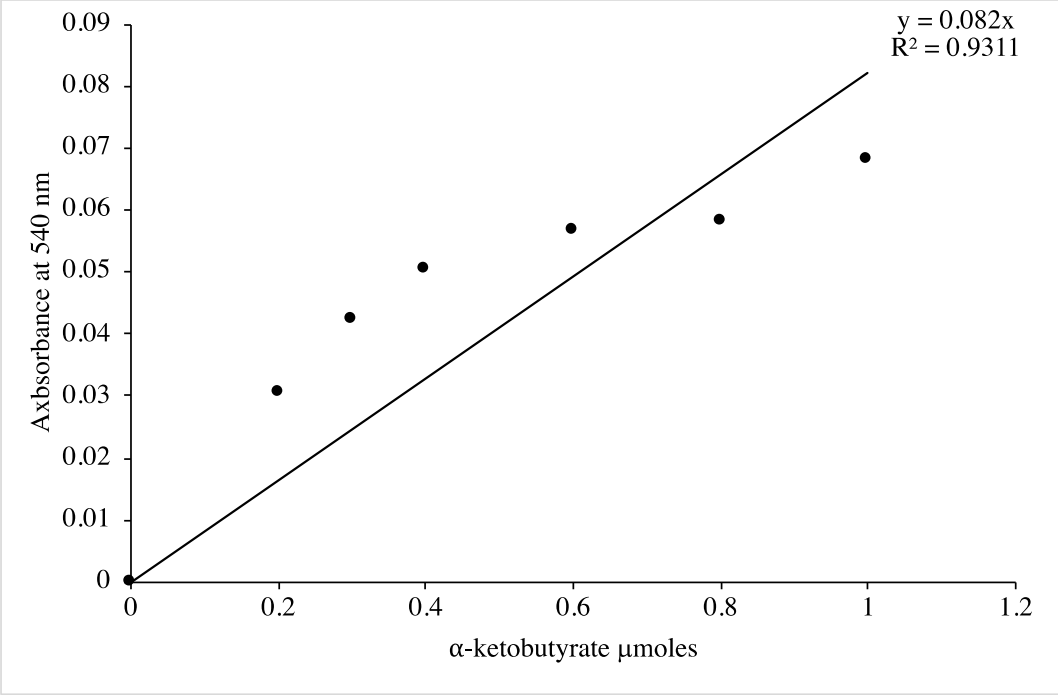

Figure 49. Standard curve of α-ketobutyrate concentration (0.1 -1.0 µmoles) versus absorbance at 540 nm.

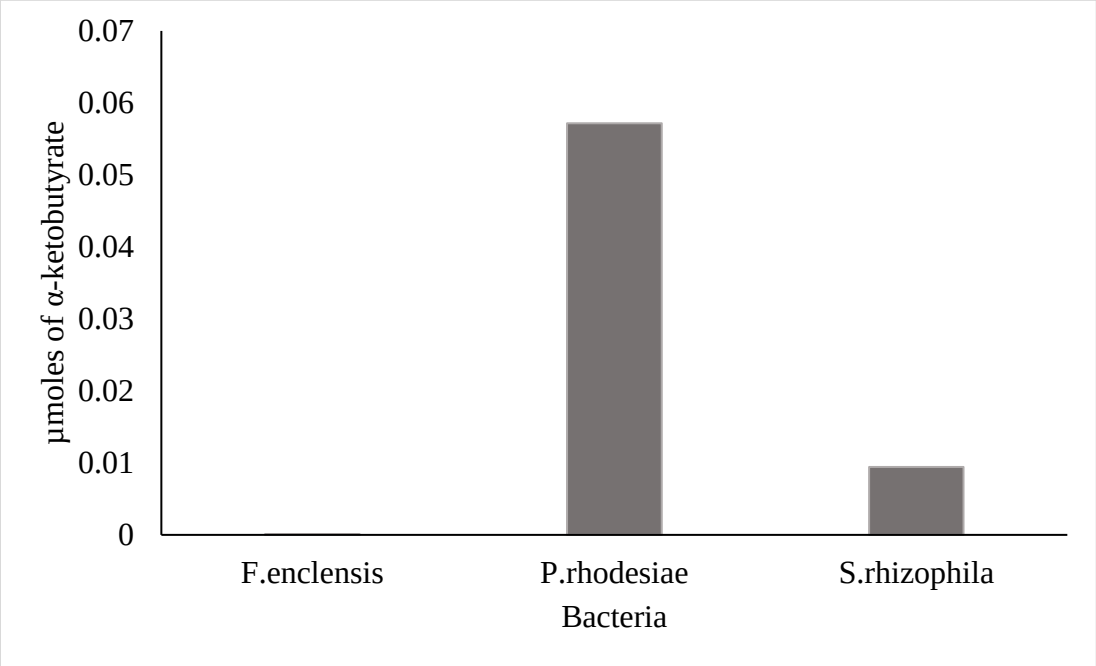

Figure 50. Average α-ketobutyrate (µmoles) produced from each bacterium.

Figure 46, 47, and 48, depicts the qualitative assessment of each bacteria's ability to synthesize ACC deaminase. The two control treatments – minimal medial with no source of nitrogen (negative control), and minimal media with $(NH_4)_2SO_4$ (positive control) – are used as references to qualitatively assess the ability of each bacterium to produce ACC deaminase. *Fictibacillus enclensis, Figure 46* has substantial growth on nitrogen-free media but has no growth on media supplemented with ACC. Several studies have cited other *Fictibacillus* species and their inability to produce ACC deaminase (Apimeteethamrong and Kittiwongwattana 2019; Lee et al. 2021). A study by Lee et al. (2021) found that the bacterium *Fictibacillus solisalsi* tested negative for ACC deaminase production, positive for IAA production, nitrogen fixation, and negative for siderophore production (Dunne et al. 1998; Ryan et al. 2009; Schmidt et al. 2012). *Pseudomonas rhodesiae* had growth only on the positive control and the plate supplemented with ACC although very minimal. *Pseudomonas* species are known for their ACC deaminase-producing ability. There are a lot of species containing the *AcdS* gene, which codes for ACC deaminase, including *P. rhodesiae* (Nascimento et al. 2014; Rolón-Cárdenas et al. 2020). *P. rhodesiae* is known to solubilize, produce siderophores, synthesize IAA and produce ACC deaminase (Rolón-Cárdenas et al. 2020).

Stenotrophomonas rhizophila had minimal growth of the positive control, negative control and the minimal media supplemented with ACC. The qualitative analysis shows a slightly positive result for ACC deaminase production in *S. rhizophila.* The direct growth-promoting properties and indirect plant growth-promoting properties against soil-borne plant pathogens of *S. rhizophila* have been well documented over the years (Dunne et al. 1998; Ryan et al. 2009;

Schmidt et al. 2012). It tests positive for IAA production (Schmidt et al. 2012)(Schmidt et. Al 2012). Since *S. rhizophila* grew on all three media with similar intensity, it is difficult to conclude from this qualitative test whether it definitively is an ACC deaminase producer.

The conversion of ACC to α-ketobutyrate and ammonia produces 1 mole of each compound (Singh et al. 2015). *Figure 50* shows the number of moles of α-ketobutyrate produced by each bacterium. This figure is directly related to the amount of ACC synthesized. *P. rhodesiae* as expected produced the greatest amount of α-ketobutyrate, followed by *S. rhizophila*. *F. enclensis* produced little to no α-ketobutyrate. These results are in line with the literature and the qualitative analysis.

4 Conclusion

To summarize, the bacterium *F. enclensis* was quantified and its growth curve was observed and compared to the positive control (*P. rhodesiae*) and its co-culture counterpart *S. rhizophila*. There were no contact-dependent or independent inhibitory effects between *F. enclensis* and *S. rhizophila* which serve as a positive indication for proceeding with bioassays containing this co-culture.

When comparing treatments with and without the biochar formulation, in the barley field trial, no results were indicating enhanced growth for either treatment with no preference for a particular PGPR. The barley field trial produced results that were inconclusive due to the limitation in the water supply and infestation by barley flea beetles. This experiment has the potential to produce clear results but all other factors affecting plant growth must remain consistent during the study. Experiments comparing the effects of multiple bacterial applications versus single applications showed some promise. The greenhouse pepper trial produced larger leaves in the fertilizer treatment, *P. rhodesiae* (multiple applications) and *F. enclensis* (single application). *Fictibacillus enclensis* surprisingly did just as well as the fertilizers treatment and positive control, providing further confirmation of its plant growth-promoting abilities. In terms of the pest control properties of the treatments, there was a greater number of aphids present on treatments with a larger leaf size. This may have been due to the larger leaf surface area of plants and not necessarily the treatment applied. Conclusive results on pest control properties could not have been made due to the untimely death of pepper plants. The experiment on carrots had inconclusive results in terms of the carrot root but a very clear preference for the fertilizer

treatment and multiple bacteria applications when comparing shoot height. The *F. enclensis* (multiple) did just as well as the positive control (*P. rhodesiae*) and the fertilizer treatment. The conclusion is that multiple applications of biofertilizers may prove to be advantageous depending on the type of plant grown.

Investigating the properties of *F. enclensis* and its co-culture on seed germination led to the conclusion that the bacteria treatments delayed germination. This was confirmed by the study on both pepper seeds and barley seeds. It is suspected that the concentration of the bacteria is responsible for the delayed germination and not necessarily the bacterial species. Further investigation of the effects of the concentration of *F. enclensis* and *P. rhodesiae* on seed germination is advised.

Although the bacterial inoculants decreased root growth in barley plants longitudinally, it was observed that the lateral growth of root hair was extremely enhanced. This lateral root growth proved to be advantageous because, although the control treatment had a longer root system all bacterial treatments had longer shoots compared to the control. The fertilizer treatment as expected performed better across the board but the difference in average shoot height although significantly different had a very similar average shoot height as *F. enclensis*, the co-culture and *P. rhodesiae*.

The co-culture experiments proved that there was no synergistic effect on plant growth. In terms of both the barley root growth experiment and the % nitrogen in the bush bean experiment, the

co-culture treatment produced results between *F. enclensis* and *S. rhizophila* but never surpassed the two.

F. enclensis tested negative for ACC deaminase production while *P. rhodesiae* tested positive and produced the most α-ketobutyrate among the three. *S. rhizophila* showed minimal α-ketobutyrate production and grew on ACC supplemented plates.

In summary, *F. enclensis* showed great promise as a PGPR with significant results in peppers, barley, carrots, and bush beans. *Fictibacillus enclensis* produced significantly higher % nitrogen in bush beans than all other treatments except the fertilizer treatment *F. enclensis* does not produce ACC deaminase and the *F. enclensis* + *S. rhizophila* co-culture was not synergistic to plant growth promotion.

5 Future Work

Expanding the types of crops the *F. enclensis* biochar formulation is advantageous in terms of plant growth and crop production is a direction that this project needs to continue to take over the next few years.

Testing the effect of varying bacterial concentration on germination and seed growth will be very important to create the best formulation for commercialization because it has been proven that there is a delicate balance between bacteria concentration and results produced. This project will continue to test the effects of the number of applications on plant growth as well as to retest a field trial. Understanding how *F. enclensis* performs in the natural environment will be extremely valuable to its commercialization and formulation. There will be an investigation on the survivability of *F. enclensis* after application in the greenhouse and field to determine the duration in which the plant experiences the effects of this PGPR, and a test for the acds gene to verify that *F. enclensis* is not an ACC deaminase producer. Additional co-culture studies to investigate potential synergistic effects on plant growth will also be conducted.

Further in the future, when formulations are perfected, the commercialization of this biofertilizer would be a great addition to the environmentally friendly alternatives to chemical fertilizers and would have a tremendous impact on the Nova Scotia Agricultural industry and economy.

6 References

Agegnehu, Getachew, Adrian M Bass, Paul N Nelson, and Michael I Bird. 2016. “Benefits of Biochar, Compost and Biochar–Compost for Soil Quality, Maize Yield and Greenhouse Gas Emissions in a Tropical Agricultural Soil.” *The Science of the Total Environment* 543 (Pt A): 295–306. https://doi.org/10.1016/j.scitotenv.2015.11.054.

Ahmed, E, and S J M Holmström. 2014. “Siderophores in Environmental Research: Roles and Applications.” *Microbial Biotechnology* 7 (3): 196–208. https://doi.org/https://doi.org/10.1111/1751-7915.12117.

Ait Barka, Essaïd, Jerzy Nowak, and Christophe Clément. 2006. “Enhancement of Chilling Resistance of Inoculated Grapevine Plantlets with a Plant Growth-Promoting Rhizobacterium, Burkholderia Phytofirmans Strain PsJN.” *Applied and Environmental Microbiology* 72 (December): 7246–52. https://doi.org/10.1128/AEM.01047-06.

Alizadeh, Omid. 2011. “Effect of Plant Growth Promoting Bacteria on Crop Growth.” *American-Eurasian Journal of Sustainable Agriculture* 5 (July): 344–49.

Ansari, Abid A, and Sarvajeet Singh Gill. 2013. *Eutrophication: Volume 2*. Dordrecht: Dordrecht: Springer Netherlands.

Apimeteethamrong, Suchitra, and Chokchai Kittiwongwattana. 2019. “Diversity and Plant Growth Promoting Activities of Rice Epiphytic Bacteria.” *Current Applied Science and Technology* 19 (2): 66–79.

Arshad, M, B Saharoona, and T Mahmood. 2008. “Inoculation with Pseudomonas Spp. Containing ACC-Deaminase Partially Eliminates the Effects of Drought Stress on Growth, Yield, and Ripening of Pea (Pisum Sativum L.)*1 *1Project Supported by the Higher Education Commission,A Islamabad, Pakistan (No. PIN .” *Pedosphere* 18 (5): 611–20. https://doi.org/https://doi.org/10.1016/S1002-0160(08)60055-7.

“Article: EU Commits to 50% Cut in Agchem Use by 2030 | IHS Markit.” n.d. https://ihsmarkit.com/research-analysis/eu-commits-to-50-cut-in-agchem-use-by-2030.html.

Backer, Rachel, J. Stefan Rokem, Gayathri Ilangumaran, John Lamont, Dana Praslickova, Emily Ricci, Sowmyalakshmi Subramanian, and Donald L. Smith. 2018a. “Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to

Commercialization of Biostimulants for Sustainable Agriculture." *Frontiers in Plant Science*. Frontiers Media S.A. https://doi.org/10.3389/fpls.2018.01473.

Backer, Rachel, J Stefan Rokem, Gayathri Ilangumaran, John Lamont, Dana Praslickova, Emily Ricci, Sowmyalakshmi Subramanian, and Donald L Smith. 2018b. "Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture ." *Frontiers in Plant Science* . https://www.frontiersin.org/article/10.3389/fpls.2018.01473.

Bagheri, Negar, Masoud Ahmadzadeh, Pierre Mariotte, and Gholamreza Salehi Jouzani. 2022. "Behavior and Interactions of the Plant Growth-Promoting Bacteria Azospirillum Oryzae NBT506 and Bacillus Velezensis UTB96 in a Co-Culture System." *World Journal of Microbiology and Biotechnology* 38 (6): 101. https://doi.org/10.1007/s11274-022-03283-8.

Bakker, Peter A H M, Corné M J Pieterse, Ronnie de Jonge, and Roeland L Berendsen. 2018. "The Soil-Borne Legacy." *Cell* 172 (6): 1178–80. https://doi.org/https://doi.org/10.1016/j.cell.2018.02.024.

BENGTSSON, JANNE, JOHAN AHNSTRÖM, and ANN-CHRISTIN WEIBULL. 2005. "The Effects of Organic Agriculture on Biodiversity and Abundance: A Meta-Analysis: Organic Agriculture, Biodiversity and Abundance." *The Journal of Applied Ecology* 42 (2): 261–69. https://doi.org/10.1111/j.1365-2664.2005.01005.x.

Bennetzen, Eskild H, Pete Smith, and John R Porter. 2016. "Agricultural Production and Greenhouse Gas Emissions from World Regions—The Major Trends over 40 Years." *Global Environmental Change* 37: 43–55. https://doi.org/10.1016/j.gloenvcha.2015.12.004.

Berendsen, Roeland L, Gilles Vismans, Ke Yu, Yang Song, Ronnie de Jonge, Wilco P Burgman, Mette Burmølle, Jakob Herschend, Peter A H M Bakker, and Corné M J Pieterse. 2018. "Disease-Induced Assemblage of a Plant-Beneficial Bacterial Consortium." *The ISME Journal* 12 (6): 1496–1507. https://doi.org/10.1038/s41396-018-0093-1.

Bhattacharyya, P N, P N Bhattacharyya, D K Jha, and D K Jha. 2012. "Plant Growth-Promoting Rhizobacteria (PGPR): Emergence in Agriculture." *World J Microbiol Biotechnol* 28 (4): 1327–50. https://doi.org/10.1007/s11274-011-0979-9.

Bindraban, Prem S, Marijn van der Velde, Liming Ye, Maurits van den Berg, Simeon Materechera, Delwendé Innocent Kiba, Lulseged Tamene, et al. 2012. "Assessing the

Impact of Soil Degradation on Food Production." *Current Opinion in Environmental Sustainability* 4 (5): 478–88. https://doi.org/https://doi.org/10.1016/j.cosust.2012.09.015.

Blatt-Janmaat, Kaitlyn. 2018. "Codependence : The Influence of Targeted Soil Inoculation on Vegetative Growth, Chemical Composition, and Fruit Fermentation." Halifax, N.S. : Saint Mary's University.

Blatt-Janmaat, Kaitlyn L., Stephanie L. MacQuarrie, and Clarissa S. Sit. 2020. "Does Size Matter? An Investigation into the Impact of Coarse and Fine Ground Inoculated Biochar on Hordeum Vulgare (Barley) Growth and Yield." *Rhizosphere*. https://doi.org/10.1016/j.rhisph.2020.100184.

Borah, Madhusmita, Saurav Das, Sudipta Sankar Bora, Robin Chandra Boro, and Madhumita Barooah. 2021. "Comparative Assessment of Multi-Trait Plant Growth-Promoting Endophytes Associated with Cultivated and Wild Oryza Germplasm of Assam, India." *Archives of Microbiology* 203 (5): 2007–28. https://doi.org/10.1007/s00203-020-02153-x.

Brito, Luciana F, Marina Gil López, Lucas Straube, Luciane M P Passaglia, and Volker F Wendisch. 2020. "Inorganic Phosphate Solubilization by Rhizosphere Bacterium Paenibacillus Sonchi: Gene Expression and Physiological Functions." *Frontiers in Microbiology* 11 (December): 588605. https://doi.org/10.3389/fmicb.2020.588605.

Brown, J. Kirk. 2011. "Biotechnology Laboratory Textbook." https://www.bio-rad.com/fr-ca/category/biotechnology-laboratory-textbook?ID=LKN6R715.

Canon, Fanny, Thibault Nidelet, Eric Guédon, Anne Thierry, and Valérie Gagnaire. 2020. "Understanding the Mechanisms of Positive Microbial Interactions That Benefit Lactic Acid Bacteria Co-Cultures ." *Frontiers in Microbiology* . https://www.frontiersin.org/article/10.3389/fmicb.2020.02088.

Cao, Zhiping, Xuemei Han, Cheng Hu, Jun Chen, Dianpeng Zhang, and Yosef Steinberger. 2011. "Changes in the Abundance and Structure of a Soil Mite (Acari) Community under Long-Term Organic and Chemical Fertilizer Treatments." *Applied Soil Ecology : A Section of Agriculture, Ecosystems & Environment* 49: 131–38. https://doi.org/10.1016/j.apsoil.2011.06.003.

Chen, Jiao, Shaoyu Lü, Zhe Zhang, Xuxia Zhao, Xinming Li, Piao Ning, and Mingzhu Liu. 2018. "Environmentally Friendly Fertilizers: A Review of Materials Used and Their Effects

on the Environment." *Science of The Total Environment* 613–614: 829–39. https://doi.org/https://doi.org/10.1016/j.scitotenv.2017.09.186.

Chen, Y P, P D Rekha, A B Arun, F T Shen, W.-A. Lai, and C C Young. 2006. "Phosphate Solubilizing Bacteria from Subtropical Soil and Their Tricalcium Phosphate Solubilizing Abilities." *Applied Soil Ecology* 34 (1): 33–41. https://doi.org/https://doi.org/10.1016/j.apsoil.2005.12.002.

Chouhan, Gowardhan Kumar, Jay Prakash Verma, Durgesh Kumar Jaiswal, Arpan Mukherjee, Saurabh Singh, Arthur Prudêncio de Araujo Pereira, Hongwei Liu, Elsayed Fathi Abd_Allah, and Brajesh Kumar Singh. 2021. "Phytomicrobiome for Promoting Sustainable Agriculture and Food Security: Opportunities, Challenges, and Solutions." *Microbiological Research* 248: 126763. https://doi.org/https://doi.org/10.1016/j.micres.2021.126763.

COMPANT, Stéphane, Brion DUFFY, Jerzy NOWAK, Christophe CLEMENT, and Essaid AIT BARKA. 2005. "Use of Plant Growth-Promoting Bacteria for Biocontrol of Plant Diseases : Principles, Mechanisms of Action, and Future Prospects." *Appl Environ Microbiol* 71 (9): 4951–59. https://doi.org/10.1128/AEM.71.9.4951-4959.2005.

Costacurta, Antonia, and Jos Vanderleyden. 1995. "Synthesis of Phytohormones by Plant-Associated Bacteria." *Critical Reviews in Microbiology* 21 (1): 1–18. https://doi.org/10.3109/10408419509113531.

Costechareyre, Denis, Séverine Balmand, Guy Condemine, and Yvan Rahbé. 2012. "Dickeya Dadantii, a Plant Pathogenic Bacterium Producing Cyt-like Entomotoxins, Causes Septicemia in the Pea Aphid Acyrthosiphon Pisum." *PloS One* 7 (1): e30702. https://doi.org/10.1371/journal.pone.0030702.

Dastager, Syed G, Rahul Mawlankar, Krishnamurthi Srinivasan, Shan-Kun Tang, Jae-Chan Lee, V Venkata Ramana, and Yogesh S Shouche. 2013. "Fictibacillus Enclensis Sp. Nov., Isolated from Marine Sediment." *Antonie van Leeuwenhoek* 105 (3): 461–69. https://doi.org/10.1007/s10482-013-0097-9.

Dey, R, K K Pal, D M Bhatt, and S M Chauhan. 2004. "Growth Promotion and Yield Enhancement of Peanut (Arachis Hypogaea L.) by Application of Plant Growth-Promoting Rhizobacteria." *Microbiol Res* 159 (4): 371–94. https://doi.org/10.1016/j.micres.2004.08.004.

Dileep Kumar, B. S., I. Berggren, and A. M. Mårtensson. 2001. “Potential for Improving Pea Production by Co-Inoculation with Fluorescent Pseudomonas and Rhizobium.” *Plant and Soil*. https://doi.org/10.1023/A:1004896118286.

Dunne, C, Y Moënne-Loccoz, J McCarthy, P Higgins, J Powell, D N Dowling, and F O’Gara. 1998. “Combining Proteolytic and Phloroglucinol-producing Bacteria for Improved Biocontrol of Pythium-mediated Damping-off of Sugar Beet.” *Plant Pathology* 47 (3): 299–307. https://doi.org/10.1046/j.1365-3059.1998.00233.x.

Egamberdieva, Dilfuza, Stephan Wirth, Undine Behrendt, Elsayed F Abd_Allah, and Gabriele Berg. 2016. “Biochar Treatment Resulted in a Combined Effect on Soybean Growth Promotion and a Shift in Plant Growth Promoting Rhizobacteria .” *Frontiers in Microbiology* . https://www.frontiersin.org/article/10.3389/fmicb.2016.00209.

Etesami, Hassan, and Dinesh K Maheshwari. 2018. “Use of Plant Growth Promoting Rhizobacteria (PGPRs) with Multiple Plant Growth Promoting Traits in Stress Agriculture: Action Mechanisms and Future Prospects.” *Ecotoxicol Environ Saf* 156: 225–46. https://doi.org/10.1016/j.ecoenv.2018.03.013.

Fahad, Shah, Saddam Hussain, Asghari Bano, Shah Saud, Shah Hassan, Darakh Shan, Faheem Ahmed Khan, et al. 2015. “Potential Role of Phytohormones and Plant Growth-Promoting Rhizobacteria in Abiotic Stresses: Consequences for Changing Environment.” *Environmental Science and Pollution Research* 22 (7): 4907–21. https://doi.org/10.1007/s11356-014-3754-2.

Felici, Cristiana, Lorenzo Vettori, Enrico Giraldi, Laura Maria Costantina Forino, Annita Toffanin, Anna Maria Tagliasacchi, and Marco Nuti. 2008. “Single and Co-Inoculation of Bacillus Subtilis and Azospirillum Brasilense on Lycopersicon Esculentum: Effects on Plant Growth and Rhizosphere Microbial Community.” *Applied Soil Ecology* 40 (2): 260–70. https://doi.org/https://doi.org/10.1016/j.apsoil.2008.05.002.

Fincheira, Paola, and Andrés Quiroz. 2018. “Microbial Volatiles as Plant Growth Inducers.” *Microbiological Research* 208 (April 2017): 63–75. https://doi.org/10.1016/j.micres.2018.01.002.

“Food and Agriculture Organization of the United Nations.” 2013. International Organization. 2013. https://doi.org/10.1017/S0020818300006160.

Fukushima, Tatsuya, Benjamin E Allred, and Kenneth N Raymond. 2014. “Direct Evidence of Iron Uptake by the Gram-Positive Siderophore-Shuttle Mechanism without Iron Reduction.” *ACS Chemical Biology* 9 (9): 2092–2100. https://doi.org/10.1021/cb500319n.

García-Gómez, Pablo, Goizeder Almagro, Ángela María Sánchez-López, Abdellatif Bahaji, Kinia Ameztoy, Adriana Ricarte-Bermejo, Marouane Baslam, et al. 2019. “Volatile Compounds Other than CO 2 Emitted by Different Microorganisms Promote Distinct Posttranscriptionally Regulated Responses in Plants.” *Plant Cell and Environment* 42 (5). https://doi.org/10.1111/pce.13490.

Glick, Bernard R. 1999. *Biochemical and Genetic Mechanisms Used by Plant Growth Promoting Bacteria*. London ; Imperial College Press.

———. 2012. “Plant Growth-Promoting Bacteria: Mechanisms and Applications.” Edited by M Shoda, T Ano, and G Comi. *Scientifica* 2012: 963401. https://doi.org/10.6064/2012/963401.

Gong, Wei, Wei Gong, Xiaoyuan Yan, Xiaoyuan Yan, Jingyan Wang, and Jingyan Wang. 2012. “The Effect of Chemical Fertilizer on Soil Organic Carbon Renewal and CO2 Emission—a Pot Experiment with Maize.” *Plant and Soil* 353 (1): 85–94. https://doi.org/10.1007/s11104-011-1011-8.

Gowtham, H. G., M. Murali, S. Brijesh Singh, T. R. Lakshmeesha, K. Narasimha Murthy, K. N. Amruthesh, and S. R. Niranjana. 2018. “Plant Growth Promoting Rhizobacteria- Bacillus Amyloliquefaciens Improves Plant Growth and Induces Resistance in Chilli against Anthracnose Disease.” *Biological Control*. https://doi.org/10.1016/j.biocontrol.2018.05.022.

Grant, Kathryn R, Maree Brennan, and Stephen P Hoad. 2020. “The Structure of the Barley Husk Influences Its Resistance to Mechanical Stress.” *Frontiers in Plant Science* 11: 614334. https://doi.org/10.3389/fpls.2020.614334.

GRENIER, Anne-Marie, Gabrielle DUPORT, Sylvie PAGES, Guy CONDEMINE, and Yvan RAHBE. 2006. “The Phytopathogen Dickeya Dadantii (Erwinia Chrysanthemi 3937) Is a Pathogen of the Pea Aphid.” *Applied and Environmental Microbiology* 72 (3): 1956–65. https://doi.org/10.1128/AEM.72.3.1956-1965.2006.

Grover, Minakshi, Shrey Bodhankar, Abha Sharma, Pushpendra Sharma, Jyoti Singh, and Lata Nain. 2021. “PGPR Mediated Alterations in Root Traits: Way Toward Sustainable Crop Production .” *Frontiers in Sustainable Food Systems* .

https://www.frontiersin.org/article/10.3389/fsufs.2020.618230.

Gupta, Shikha, and Sangeeta Pandey. 2019. “ACC Deaminase Producing Bacteria With Multifarious Plant Growth Promoting Traits Alleviates Salinity Stress in French Bean (Phaseolus Vulgaris) Plants.” *Frontiers in Microbiology* 10: 1506. https://doi.org/10.3389/fmicb.2019.01506.

Gupta, Vadakattu V S R, and Anil K Sharma. 2021. *Rhizosphere Biology: Interactions Between Microbes and Plants*. 1st ed. 20. Singapore: Singapore : Springer Singapore : Imprint: Springer.

Heath, Katy D, Justin C Podowski, Stephanie Heniff, Christie R Klinger, Patricia V Burke, Dylan J Weese, Wendy H Yang, and Jennifer A Lau. 2020. “Light Availability and Rhizobium Variation Interactively Mediate the Outcomes of Legume–Rhizobium Symbiosis.” *Am J Bot* 107 (2): 229–38. https://doi.org/10.1002/ajb2.1435.

Herrmann, Laetitia, and Didier Lesueur. 2013. “Challenges of Formulation and Quality of Biofertilizers for Successful Inoculation.” *Appl Microbiol Biotechnol* 97 (20): 8859–73. https://doi.org/10.1007/s00253-013-5228-8.

Honma, Mamoru, and Tokuji Shimomura. 1978. “Metabolism of 1-Aminocyclopropane-1-Carboxylic Acid.” *Agricultural and Biological Chemistry* 42 (10): 1825–31. https://doi.org/10.1080/00021369.1978.10863261.

Igiehon, Nicholas O, and Olubukola O Babalola. 2017. “Biofertilizers and Sustainable Agriculture: Exploring Arbuscular Mycorrhizal Fungi.” *Applied Microbiology and Biotechnology* 101 (12): 4871–81. https://doi.org/10.1007/s00253-017-8344-z.

Izydorczyk, Grzegorz, Urszula Sienkiewicz-Cholewa, Sylwia Baśladyńska, Daria Kocek, Małgorzata Mironiuk, and Katarzyna Chojnacka. 2020. “New Environmentally Friendly Bio-Based Micronutrient Fertilizer by Biosorption: From Laboratory Studies to the Field.” *Sci Total Environ* 710: 136061. https://doi.org/10.1016/j.scitotenv.2019.136061.

Khan, Mohammad Saghir, Mohammad Saghir Khan, Almas Zaidi, Almas Zaidi, Parvaze A Wani, and Parvaze A Wani. 2007. “Role of Phosphate-Solubilizing Microorganisms in Sustainable Agriculture — A Review.” *Agronomy for Sustainable Development* 27 (1): 29–43. https://doi.org/10.1051/agro:2006011.

Khan, Muhammad Aaqil, Sajjad Asaf, Abdul Latif Khan, Arjun Adhikari, Rahmatullah Jan,

Sajid Ali, Muhammad Imran, Kyung-Min Kim, and In-Jung Lee. 2019. “Halotolerant Rhizobacterial Strains Mitigate the Adverse Effects of NaCl Stress in Soybean Seedlings.” Edited by Yoshinari Uehara. *BioMed Research International* 2019: 9530963. https://doi.org/10.1155/2019/9530963.

Kolton, Max, Yael Meller Harel, Zohar Pasternak, Ellen R. Graber, Yigal Elad, and Eddie Cytryn. 2011. “Impact of Biochar Application to Soil on the Root-Associated Bacterial Community Structure of Fully Developed Greenhouse Pepper Plants.” *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.00148-11.

Korir, Hezekiah, Nancy W. Mungai, Moses Thuita, Yosef Hamba, and Cargele Masso. 2017. “Co-Inoculation Effect of Rhizobia and Plant Growth Promoting Rhizobacteria on Common Bean Growth in a Low Phosphorus Soil.” *Frontiers in Plant Science*. https://doi.org/10.3389/fpls.2017.00141.

Kramer, Jos, Özhan Özkaya, and Rolf Kümmerli. 2020. “Bacterial Siderophores in Community and Host Interactions.” *Nature Reviews. Microbiology* 18 (3): 152–63. https://doi.org/10.1038/s41579-019-0284-4.

Lazcano, Cristina, Cristina Lazcano, María Gómez-Brandón, María Gómez-Brandón, Pedro Revilla, Pedro Revilla, Jorge Domínguez, and Jorge Domínguez. 2013. “Short-Term Effects of Organic and Inorganic Fertilizers on Soil Microbial Community Structure and Function: A Field Study with Sweet Corn.” *Biology and Fertility of Soils* 49 (6): 723–33. https://doi.org/10.1007/s00374-012-0761-7.

Lee, Song Min, Ji-Youn Kim, Hee Sook Kim, Ka-Yoon Oh, Kwang Hui Lee, Sang-Hyeon Lee, and Jeong Su Jang. 2021. “Characterization of Potential Plant Growth-Promoting Rhizobacteria as Biological Agents with Antifungal Activity, Plant Growth-Promoting Activity, and Mineral Solubilizing Activity.” *Journal of Life Science* 31 (7): 641–53.

Lentz, R D, and G A Lehrsch. 2010. “Nutrients in Runoff from a Furrow-Irrigated Field after Incorporating Inorganic Fertilizer or Manure.” *J Environ Qual* 39 (4): 1402–15. https://doi.org/10.2134/jeq2009.0374.

Lin, Yao Nan, Kai Ming Chang, Shiang Cheng Jeng, Ping Yu Lin, and Ray Quen Hsu. 2011. “Study of Release Speeds and Bacteria Inhibiting Capabilities of Drug Delivery Membranes Fabricated via Electrospinning by Observing Bacteria Growth Curves.” *Journal of*

Materials Science: Materials in Medicine 22 (3): 571–77. https://doi.org/10.1007/s10856-011-4249-z.

Liu, Ting, Xiaoyun Chen, Feng Hu, Wei Ran, Qirong Shen, Huixin Li, and Joann K Whalen. 2016. "Carbon-Rich Organic Fertilizers to Increase Soil Biodiversity: Evidence from a Meta-Analysis of Nematode Communities." *Agriculture, Ecosystems & Environment* 232: 199–207. https://doi.org/https://doi.org/10.1016/j.agee.2016.07.015.

Liu, Wensheng, Li Zheng, and Danhui Qi. 2020. "Variation in Leaf Traits at Different Altitudes Reflects the Adaptive Strategy of Plants to Environmental Changes." *Ecology and Evolution* 10 (15): 8166–75. https://doi.org/10.1002/ece3.6519.

Ma, Hua, Vyacheslav Shurigin, Dilfuza Jabborova, Jeane Aril Dela Cruz, Thomas Edison Dela Cruz, Stephan Wirth, Sonoko Dorothea Bellingrath-Kimura, and Dilfuza Egamberdieva. 2022. "The Integrated Effect of Microbial Inoculants and Biochar Types on Soil Biological Properties, and Plant Growth of Lettuce (Lactuca Sativa L.)." *Plants (Basel)* 11 (3): 423. https://doi.org/10.3390/plants11030423.

Madsen, Esben Bjørn, Lene Heegaard Madsen, Simona Radutoiu, Magdalena Olbryt, Magdalena Rakwalska, Krzysztof Szczyglowski, Shusei Sato, et al. 2003. "A Receptor Kinase Gene of the LysM Type Is Involved in Legumeperception of Rhizobial Signals." *Nature* 425 (6958): 637–40. https://doi.org/10.1038/nature02045.

Mahanty, Trishna, Trishna Mahanty, Surajit Bhattacharjee, Surajit Bhattacharjee, Madhurankhi Goswami, Madhurankhi Goswami, Purnita Bhattacharyya, et al. 2017. "Biofertilizers: A Potential Approach for Sustainable Agriculture Development." *Environ Sci Pollut Res Int* 24 (4): 3315–35. https://doi.org/10.1007/s11356-016-8104-0.

MARIMUTHU, S, P SUBBIAN, V RAMAMOORTHY, and R SAMIYAPPAN. 2002. "Synergistic Effect of Combined Application of Azospirillum and Pseudomonas Fluorescens with Inorganic Fertilizers on Root Rot Incidence and Yield of Cotton." *Zeitschrift Für Pflanzenkrankheiten Und Pflanzenschutz (1970)* 109 (6): 569–77.

Mayak, S, T Tirosh, and B R Glick. 1999. "Effect of Wild-Type and Mutant Plant Growth-Promoting Rhizobacteria on the Rooting of Mung Bean Cuttings." *Journal of Plant Growth Regulation* 18 (2): 49–53. https://doi.org/10.1007/PL00007047.

McBratney, Alex, Damien J Field, and Andrea Koch. 2014. "The Dimensions of Soil Security."

Geoderma 213: 203–13. https://doi.org/https://doi.org/10.1016/j.geoderma.2013.08.013.

Mir, Mohammad Yaseen, Nowsheen Shameem, and Javid Ahmad Parray. 2019. *Sustainable Agriculture: Biotechniques in Plant Biology*. Springer.

Miransari, M. 2016. "1 - Use of Biotechnology in Soybean Production Under Environmental Stresses." In , edited by Mohammad B T - Environmental Stresses in Soybean Production Miransari, 1–22. San Diego: Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-801535-3.00001-2.

Munusamy, Umaiyal. 2021. *Plant Growth Promoting Bacteria* . Burlington, Ontario: Delve Publishing.

Nascimento, Francisco X, Márcio J Rossi, Cláudio R F S Soares, Brendan J McConkey, and Bernard R Glick. 2014. "New Insights into 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase Phylogeny, Evolution and Ecological Significance." *PLOS ONE* 9 (6): e99168. https://doi.org/10.1371/journal.pone.0099168.

Naseem, Muhammad, Martin Kaltdorf, and Thomas Dandekar. 2015. "The Nexus between Growth and Defence Signalling: Auxin and Cytokinin Modulate Plant Immune Response Pathways." *Journal of Experimental Botany* 66 (16): 4885–96. https://doi.org/10.1093/jxb/erv297.

Ndeddy Aka, Robinson Junior, and Olubukola Oluranti Babalola. 2016. "Effect of Bacterial Inoculation of Strains of Pseudomonas Aeruginosa, Alcaligenes Feacalis and Bacillus Subtilis on Germination, Growth and Heavy Metal (Cd, Cr, and Ni) Uptake of Brassica Juncea." *International Journal of Phytoremediation* 18 (2): 200–209. https://doi.org/10.1080/15226514.2015.1073671.

Neubauer, Uta, Gerhard Furrer, Achim Kayser, and Rainer Schulin. 2000. "Siderophores, NTA, and Citrate: Potential Soil Amendments to Enhance Heavy Metal Mobility in Phytoremediation." *International Journal of Phytoremediation* 2 (4): 353–68. https://doi.org/10.1080/15226510008500044.

Newton, Adrian Clive, Andrew J Flavell, Timothy S George, Philip Leat, Barry Mullholland, Luke Ramsay, Cesar Revoredo-Giha, et al. 2011. "Crops That Feed the World 4. Barley: A Resilient Crop? Strengths and Weaknesses in the Context of Food Security." *Food Security* 3 (2): 141–78. https://doi.org/10.1007/s12571-011-0126-3.

Nyenje, P M, J W Foppen, S Uhlenbrook, R Kulabako, and A Muwanga. 2010. “Eutrophication and Nutrient Release in Urban Areas of Sub-Saharan Africa — A Review.” *Sci Total Environ* 408 (3): 447–55. https://doi.org/10.1016/j.scitotenv.2009.10.020.

Orozco-Mosqueda, Ma del Carmen, Ma del Carmen Rocha-Granados, Bernard R Glick, and Gustavo Santoyo. 2018. “Microbiome Engineering to Improve Biocontrol and Plant Growth-Promoting Mechanisms.” *Microbiological Research* 208: 25–31. https://doi.org/https://doi.org/10.1016/j.micres.2018.01.005.

“Overview of Canada’s Agriculture and Agri-Food Sector - Agriculture.Canada.Ca.” n.d. https://agriculture.canada.ca/en/canadas-agriculture-sectors/overview-canadas-agriculture-and-agri-food-sector.

Paliwal, Deepa, Amanda J Hamilton, Glyn A Barrett, Fabrizio Alberti, Helmut van Emden, Caroline L Monteil, Tim H Mauchline, et al. 2022. “Identification of Novel Aphid-Killing Bacteria to Protect Plants.” *Microbial Biotechnology* 15 (4): 1203–20. https://doi.org/https://doi.org/10.1111/1751-7915.13902.

Pan, Hongmiao, Yongbin Zhang, Gui-Xin He, Namrata Katagori, and Huizhong Chen. 2014. “A Comparison of Conventional Methods for the Quantification of Bacterial Cells after Exposure to Metal Oxide Nanoparticles.” *BMC Microbiology* 14 (1): 222. https://doi.org/10.1186/s12866-014-0222-6.

Patrick, Achard, Cheng Hui, De Grauwe Liesbeth, Decat Jan, Schoutteten Hermien, Moritz Thomas, Van Der Straeten Dominique, Peng Jinrong, and Harberd Nicholas P. 2006. “Integration of Plant Responses to Environmentally Activated Phytohormonal Signals.” *Science* 311 (5757): 91–94. https://doi.org/10.1126/science.1118642.

Péchy-Tarr, Maria, Denny J Bruck, Monika Maurhofer, Esther Fischer, Christelle Vogne, Marcella D Henkels, Kelly M Donahue, Jürg Grunder, Joyce E Loper, and Christoph Keel. 2008. “Molecular Analysis of a Novel Gene Cluster Encoding an Insect Toxin in Plant-Associated Strains of Pseudomonas Fluorescens.” *Environmental Microbiology* 10 (9): 2368–86. https://doi.org/10.1111/j.1462-2920.2008.01662.x.

Penrose, Donna, and Bernard Glick. 2008. “Quantifying the Impact of ACC Deaminase-Containing Bacteria on Plants.” In *Plant Surface Microbiology*, 489–502. https://doi.org/10.1007/978-3-540-74051-3_26.

Puga, A. P., C. A. Abreu, L. C.A. Melo, and L. Beesley. 2015. “Biochar Application to a Contaminated Soil Reduces the Availability and Plant Uptake of Zinc, Lead and Cadmium.” *Journal of Environmental Management*. https://doi.org/10.1016/j.jenvman.2015.05.036.

Que, Feng, Xi-Lin Hou, Guang-Long Wang, Zhi-Sheng Xu, Guo-Fei Tan, Tong Li, Ya-Hui Wang, Ahmed Khadr, and Ai-Sheng Xiong. 2019. “Advances in Research on the Carrot, an Important Root Vegetable in the Apiaceae Family.” *Horticulture Research* 6 (1): 69. https://doi.org/10.1038/s41438-019-0150-6.

Ramírez-Rivera, Emmanuel de Jesús, Ingrid Mayanin Rodriguez-Buenfil, Mirielen Pérez-Robles, Julio Enrique Oney-Montalvo, Witoon Prinyawiwatkul, Adán Cabal-Prieto, Manuel Octavio Ramírez-Sucre, and José Andrés Herrera-Corredor. 2021. “Development and Validation of a Methodology for the Sensometric Characterisation of High-pungency Peppers: A Case Study of Habanero Pepper (Capsicum Chinense Jacq.).” *International Journal of Food Science & Technology* 56 (2): 573–86. https://doi.org/10.1111/ijfs.14735.

Ramsden, Jeremy J. 2010. “What Is Sustainability?” *Nanotechnology Perceptions* 6 (3): 179–95. https://doi.org/10.4024/N14RA10A.ntp.06.03.

Rangel de Souza, A. L.S., S. A. De Souza, M. V.V. De Oliveira, T. M. Ferraz, F. A.M.M.A. Figueiredo, N. D. Da Silva, P. L. Rangel, et al. 2016. “Endophytic Colonization of Arabidopsis Thaliana by Gluconacetobacter Diazotrophicus and Its Effect on Plant Growth Promotion, Plant Physiology, and Activation of Plant Defense.” *Plant and Soil*. https://doi.org/10.1007/s11104-015-2672-5.

RAUPACH, G S, and J W KLOEPPER. 1998. “Mixtures of Plant Growth-Promoting Rhizobacteria Enhance Biological Control of Multiple Cucumber Pathogens.” *Phytopathology* 88 (11): 1158–64. https://doi.org/10.1094/PHYTO.1998.88.11.1158.

Raza, Waseem, Jichen Wang, Yuncheng Wu, Ning Ling, Zhong Wei, Qiwei Huang, and Qirong Shen. 2016. “Effects of Volatile Organic Compounds Produced by Bacillus Amyloliquefaciens on the Growth and Virulence Traits of Tomato Bacterial Wilt Pathogen Ralstonia Solanacearum.” *Applied Microbiology and Biotechnology* 100 (17): 7639–50. https://doi.org/10.1007/s00253-016-7584-7.

Riefler, Michael, Ondrej Novak, Miroslav Strnad, and Thomas Schmülling. 2006. “Arabidopsis Cytokinin Receptor Mutants Reveal Functions in Shoot Growth, Leaf Senescence, Seed

Size, Germination, Root Development, and Cytokinin Metabolism." *The Plant Cell* 18 (1): 40–54. https://doi.org/10.1105/tpc.105.037796.

Roba, Tesfaye Burju. 2018. "Review on: The Effect of Mixing Organic and Inorganic Fertilizer on Productivity and Soil Fertility." *OALib*. https://doi.org/10.4236/oalib.1104618.

Rolón-Cárdenas, Gisela Adelina, Jackeline Lizzeta Arvizu-Gómez, Juan Ramiro Pacheco-Aguilar, Juan Vázquez-Martínez, and Alejandro Hernández-Morales. 2020. "Cadmium-Tolerant Endophytic Pseudomonas Rhodesiae Strains Isolated from Typha Latifolia Modify the Root Architecture of Arabidopsis Thaliana Col-0 in Presence and Absence of Cd." *Brazilian Journal of Microbiology* 52 (1): 349–61. https://doi.org/10.1007/s42770-020-00408-9.

Roosa, Stephen A. 2008. *Sustainable Development Handbook*. Lilburn, GA : Boca Raton, FL: Lilburn, GA : Fairmont Press .

Rostaei, Maryam, Sina Fallah, Zahra Lorigooini, and Ali Abbasi Surki. 2018. "The Effect of Organic Manure and Chemical Fertilizer on Essential Oil, Chemical Compositions and Antioxidant Activity of Dill (Anethum Graveolens) in Sole and Intercropped with Soybean (Glycine Max)." *Journal of Cleaner Production* 199: 18–26. https://doi.org/10.1016/j.jclepro.2018.07.141.

Ryan, Robert P, Sebastien Monchy, Massimiliano Cardinale, Safiyh Taghavi, Lisa Crossman, Matthew B Avison, Gabriele Berg, Daniel van der Lelie, and J Maxwell Dow. 2009. "The Versatility and Adaptation of Bacteria from the Genus Stenotrophomonas." *Nature Reviews. Microbiology* 7 (7): 514–25. https://doi.org/10.1038/nrmicro2163.

Ryu, Choong-Min, Mohamed A Farag, Chia-Hui Hu, Munagala S Reddy, Han-Xun Wei, Paul W Paré, and Joseph W Kloepper. 2003. "Bacterial Volatiles Promote Growth in <Em>Arabidopsis</Em>" *Proceedings of the National Academy of Sciences* 100 (8): 4927 LP – 4932. https://doi.org/10.1073/pnas.0730845100.

Saini, Preeti, and Veena Khanna. 2013. "Preliminary Screening for ACC-Deaminase Production by Plant Growth Promoting Rhizobacteria." *Journal of Pure and Applied Microbiology* 7 (March): 573–76.

Saleem, Muhammad, Muhammad Arshad, Sarfraz Hussain, and Ahmad Saeed Bhatti. 2007. "Perspective of Plant Growth Promoting Rhizobacteria (PGPR) Containing ACC

Deaminase in Stress Agriculture." *Journal of Industrial Microbiology and Biotechnology* 34 (10): 635–48. https://doi.org/10.1007/s10295-007-0240-6.

Savci, Serpil. 2012. "Investigation of Effect of Chemical Fertilizers on Environment." *APCBEE Procedia* 1: 287–92. https://doi.org/https://doi.org/10.1016/j.apcbee.2012.03.047.

Schacht, Veronika, Larissa Neumann, Santosh Sandhi, Limei Chen, Torsten Henning, P Klar, Karsten Theophel, Sylvia Schnell, and Michael Bunge. 2012. "Effects of Silver Nanoparticles on Microbial Growth Dynamics." *Journal of Applied Microbiology* 114 (September). https://doi.org/10.1111/jam.12000.

Schmidt, Christoph Stephan, Mohamadali Alavi, Massimiliano Cardinale, Henry Müller, and Gabriele Berg. 2012. "Stenotrophomonas Rhizophila DSM14405T Promotes Plant Growth Probably by Altering Fungal Communities in the Rhizosphere." *Biology and Fertility of Soils* 48 (8): 947–60. https://doi.org/10.1007/s00374-012-0688-z.

Schulz-Bohm, Kristin, Saskia Gerards, Maria Hundscheid, Jasper Melenhorst, Wietse de Boer, and Paolina Garbeva. 2018. "Calling from Distance: Attraction of Soil Bacteria by Plant Root Volatiles." *The ISME Journal* 12 (5): 1252–62. https://doi.org/10.1038/s41396-017-0035-3.

Shaaban, M., S. Bashir, K.A. Kubar, U. Ali, M.S. Rana, R. Hu, L. Van Zwieten, et al. 2018. "A Concise Review of Biochar Application to Agricultural Soils to Improve Soil Conditions and Fight Pollution." *Journal of Environmental Management*.

Sharma, Tamanna, Jyoti Sharma, and Aqib Javed. 2022. "Chapter-2 Soil Fertility and Plant Nutrition." *Chief Editor Dr. RK Naresh*, 13.

Shen, Jianbo, Lixing Yuan, Junling Zhang, Haigang Li, Zhaohai Bai, Xinping Chen, Weifeng Zhang, and Fusuo Zhang. 2011. "Phosphorus Dynamics: From Soil to Plant." *Plant Physiology* 156 (3): 997–1005. https://doi.org/10.1104/pp.111.175232.

Shin, Heungsop, Hwa-Soo Shin, Gary R Dewbre, and Maria J Harrison. 2004. "Phosphate Transport in Arabidopsis: Pht1;1 and Pht1;4 Play a Major Role in Phosphate Acquisition from Both Low- and High-Phosphate Environments." *The Plant Journal* 39 (4): 629–42. https://doi.org/https://doi.org/10.1111/j.1365-313X.2004.02161.x.

Singh, Rajnish P, Ganesh M Shelke, Anil Kumar, and Prabhat N Jha. 2015. "Biochemistry and Genetics of ACC Deaminase: A Weapon to 'Stress Ethylene' Produced in Plants."

Frontiers in Microbiology 6: 937. https://doi.org/10.3389/fmicb.2015.00937.

Skrzypczak, D, A Witek-Krowiak, A Dawiec-Liśniewska, D Podstawczyk, K Mikula, and K Chojnacka. 2019. "Immobilization of Biosorbent in Hydrogel as a New Environmentally Friendly Fertilizer for Micronutrients Delivery." *Journal of Cleaner Production* 241: 118387. https://doi.org/10.1016/j.jclepro.2019.118387.

Somers, E, J Vanderleyden, and M Srinivasan. 2004. "Rhizosphere Bacterial Signalling: A Love Parade Beneath Our Feet." *Critical Reviews in Microbiology* 30 (4): 205–40. https://doi.org/10.1080/10408410490468786.

Somova, L A, N S Pechurkin, A B Sarangova, and T I Pisman. 2001. "Effect of Bacterial Population Density on Germination Wheat Seeds and Dynamics of Simple Artificial Ecosystems." *Advances in Space Research* 27 (9): 1611–15. https://doi.org/10.1016/S0273-1177(01)00257-5.

Spaepen, Stijn, and Jos Vanderleyden. 2011. "Auxin and Plant-Microbe Interactions." *Cold Spring Harb Perspect Biol* 3 (4): a001438–a001438. https://doi.org/10.1101/cshperspect.a001438.

Spångberg, J, P.-A Hansson, P Tidåker, and H Jönsson. 2011. "Environmental Impact of Meat Meal Fertilizer vs. Chemical Fertilizer." *Resources, Conservation and Recycling* 55 (11): 1078–86. https://doi.org/10.1016/j.resconrec.2011.06.002.

Sun, Feifei, Yun DAI, and Xiaohua Yu. 2017. "Air Pollution, Food Production and Food Security： A Review from the Perspective of Food System." *Agricultural Sciences in China* 16 (12): 2945–62. https://doi.org/10.1016/S2095-3119(17)61814-8.

Théraud, M, Jean-Pierre Gangneux, L Preney, and C Guiguen. 2003. "Comparative Survival of Clinical and Environmental Isolates of Candida Albicans and Cryptococcus Neoformans in Natural and Experimental Conditions." *Journal de Mycologie Medicale* 13 (June): 93–97.

Thirkell, Thomas J, Michael D Charters, Ashleigh J Elliott, Steven M Sait, Katie J Field, and Richard Bardgett. 2017. "Are Mycorrhizal Fungi Our Sustainable Saviours? Considerations for Achieving Food Security." *The Journal of Ecology* 105 (4): 921–29. https://doi.org/10.1111/1365-2745.12788.

Ueguchi-Tanaka, Miyako, Masatoshi Nakajima, Etsuko Katoh, Hiroko Ohmiya, Kenji Asano,

Shoko Saji, Xiang Hongyu, et al. 2007. “Molecular Interactions of a Soluble Gibberellin Receptor, GID1, with a Rice DELLA Protein, SLR1, and Gibberellin.” *The Plant Cell* 19 (7): 2140–55. https://doi.org/10.1105/tpc.106.043729.

Ugulu, Ilker, Kafeel Ahmad, Zafar Iqbal Khan, Mudasra Munir, Kinza Wajid, and Humayun Bashir. 2020. “Effects of Organic and Chemical Fertilizers on the Growth, Heavy Metal/Metalloid Accumulation, and Human Health Risk of Wheat (Triticum Aestivum L.).” *Environmental Science and Pollution Research*. https://doi.org/10.1007/s11356-020-11271-4.

Vacheron, Jordan, Guilhem Desbrosses, Marie-Lara Bouffaud, Bruno Touraine, Yvan Moënne-Loccoz, Daniel Muller, Laurent Legendre, Florence Wisniewski-Dyé, and Claire Prigent-Combaret. 2013. “Plant Growth-Promoting Rhizobacteria and Root System Functioning.” *Frontiers in Plant Science* 4: 356. https://doi.org/10.3389/fpls.2013.00356.

Vessey, J Kevin. 2003. “Plant Growth Promoting Rhizobacteria as Biofertilizers.” *Plant and Soil* 255 (2): 571–86. https://doi.org/10.1023/A:1026037216893.

Walker, Vincent, Olivier Couillerot, Andreas Von Felten, Floriant Bellvert, Jan Jansa, Monika Maurhofer, René Bally, Yvan Moënne-Loccoz, and Gilles Comte. 2012. “Variation of Secondary Metabolite Levels in Maize Seedling Roots Induced by Inoculation with Azospirillum, Pseudomonas and Glomus Consortium under Field Conditions.” *Plant and Soil* 356 (1–2): 151–63. https://doi.org/10.1007/s11104-011-0960-2.

Wang, Liyun, Daming Fan, Wanwan Chen, and Eugene Terentjev. 2015. “Bacterial Growth, Detachment and Cell Size Control on Polyethylene Terephthalate Surfaces.” *Scientific Reports* 5 (October). https://doi.org/10.1038/srep15159.

Wang, Zhan-biao, Jing Chen, Shu-chun Mao, Ying-chun Han, Fu Chen, Li-feng Zhang, Ya-bing Li, and Cun-dong Li. 2017. “Comparison of Greenhouse Gas Emissions of Chemical Fertilizer Types in China’s Crop Production.” *Journal of Cleaner Production* 141: 1267–74. https://doi.org/10.1016/j.jclepro.2016.09.120.

Weber, Kathrin, and Peter Quicker. 2018. “Properties of Biochar.” *Fuel (Guildford)* 217: 240–61. https://doi.org/10.1016/j.fuel.2017.12.054.

Weinberg, E D. 2004. “Suppression of Bacterial Biofilm Formation by Iron Limitation.” *Medical Hypotheses* 63 (5): 863–65. https://doi.org/https://doi.org/10.1016/j.mehy.2004.04.010.

Weithmann, Nicolas, Julia N. Möller, Martin G.J. Löder, Sarah Piehl, Christian Laforsch, and Ruth Freitag. 2018. “Organic Fertilizer as a Vehicle for the Entry of Microplastic into the Environment.” *Science Advances*. https://doi.org/10.1126/sciadv.aap8060.

World Development Report 2008: Agriculture for Development. 2007. *World Development Report*. The World Bank. https://doi.org/doi:10.1596/978-0-8213-6807-7.

Xiong, Bijing, Youchi Zhang, Yanwei Hou, Hans Peter H Arp, Brian J Reid, and Chao Cai. 2017. “Enhanced Biodegradation of PAHs in Historically Contaminated Soil by M. Gilvum Inoculated Biochar.” *Chemosphere* 182: 316–24. https://doi.org/https://doi.org/10.1016/j.chemosphere.2017.05.020.

Yang, Bo, Zhengqin Xiong, Jinyang Wang, Xin Xu, Qiwei Huang, and Qirong Shen. 2015. “Mitigating Net Global Warming Potential and Greenhouse Gas Intensities by Substituting Chemical Nitrogen Fertilizers with Organic Fertilization Strategies in Rice–Wheat Annual Rotation Systems in China: A 3-Year Field Experiment.” *Ecological Engineering* 81: 289–97. https://doi.org/10.1016/j.ecoleng.2015.04.071.

ZEMRANY, HAMDY E L, Sonia CZARNES, Paul D HALLETT, Serge ALAMERCERY, René BALLY, and Lucile JOCTEUR MONROZIER. 2007. “Early Changes in Root Characteristics of Maize (Zea Mays) Following Seed Inoculation with the PGPR Azospirillum Lipoferum CRT1.” *Plant and Soil* 291 (1/2): 109–18. https://doi.org/10.1007/s11104-006-9178-0.

Zhao, Xiulan, and Dingyong Wang. 2010. “Mercury in Some Chemical Fertilizers and the Effect of Calcium Superphosphate on Mercury Uptake by Corn Seedlings (Zea Mays L.).” *Journal of Environmental Sciences* 22 (8): 1184–88. https://doi.org/https://doi.org/10.1016/S1001-0742(09)60236-9.

Zhao, Yunchen, Ping Wang, Jianlong Li, Yuru Chen, Xianzhi Ying, and Shuying Liu. 2009. “The Effects of Two Organic Manures on Soil Properties and Crop Yields on a Temperate Calcareous Soil under a Wheat–Maize Cropping System.” *European Journal of Agronomy* 31 (1): 36–42. https://doi.org/10.1016/j.eja.2009.03.001.

Zhou, Cheng, Zhongyou Ma, Lin Zhu, Xin Xiao, Yue Xie, Jian Zhu, and Jianfei Wang. 2016. “Rhizobacterial Strain Bacillus Megaterium BOFC15 Induces Cellular Polyamine Changes That Improve Plant Growth and Drought Resistance.” *International Journal of Molecular*

Sciences . https://doi.org/10.3390/ijms17060976.

Zulfiqar, Faisal, Míriam Navarro, Muhammad Ashraf, Nudrat Aisha Akram, and Sergi Munné-Bosch. 2019. “Nanofertilizer Use for Sustainable Agriculture: Advantages and Limitations.” *Plant Science*. https://doi.org/10.1016/j.plantsci.2019.110270.