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- Plant-Growth Promoting Rhizobacteria in Soilless Cannabis Cropping Systems:

Implications for Growth Promotion and Disease Suppression

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Introducing Plant-Growth Promoting Rhizobacteria in Soilless Cannabis Cropping Systems: Implications for Growth Promotion and Disease Suppression

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Introduction

The microbial life associated with plants has been studied systematically for about a century (Ruzzi & Aroca, 2015), and much of the scientific literature agrees upon its pivotal role in supporting plant growth, development and overall health (Backer et al., 2018). Beneficial outcomes for plants are primarily a result of microbe's nitrogen-fixation, inorganic nutrient solubilization, pathogen inhibition and stress amelioration through a wide array of biological, chemical and physical mechanisms (Pandey et al., 2019). These microbes are found within both the bacterial and fungal kingdom (Turner, James & Poole, 2013), and inhabit every part of the plant, albeit in different amounts (Backer et al., 2018; Compant et al., 2019).

The roots and their close vicinity have a rich phytomicrobiome (Vacherone et al., 2013), which in combination with the highly dynamic nature of roots makes the rhizosphere a very biologically and chemically active region (Jones, Nguyen & Finlay, 2009). As such, it influences factors essential to plant survival and health, like nutrient availability and presence of pathogens (Walker et al., 2003 ; Jones, Nguyen & Finlay, 2009). An interest in manipulating the composition of the rhizosphere has emerged, and along with it the notion of Plant-Growth Promoting Rhizobacteria (PGPR). Strains pertaining to this group have been shown to have a substantial impact on the growth, development, health, yield and quality of several commercially important crops, while at the same time decreasing the need for energy-intensive and environmentally detrimental inputs like mineral nutrition and synthetic pesticides (Pandey et al., 2019). From a microbiological point of view, the roots and the rhizosphere are important since they serve as a great energy source, owing to their release of compounds rich in carbon and nitrogen.

The plant responses connected to PGPR are, at the moment (Loon, 2007; Ruzzi & Aroca, 2015, Backer et al., 2018):

Plant growth/development/higher yield promotion
Disease suppression
Improved stress tolerance
Elicitation secondary metabolites

Whereof the first two will be considered in this text, since studies on the last two in the context of cannabis cultivation have not been conducted.

Plant-Growth Promoting Rhizobacteria are bacterial strains acting as biostimulants, biofertilizers and biocontrol agents, and modulate plant responses to the growing environment in a way that can be considered beneficial (Backer et al., 2018). PGPR live either inside (endophytes) , on the surface (epiphytes) or in the close proximity of the root (free-living) (Gray & Smith, 2005), feeding on the nutritious exudates that are secreted and diffused from the roots (Lareen, Burton & Schäfer, 2016). These biomolecules, acting both as communication and defense signals, are rich in photosynthetically fixed carbon, and come in forms of low, as well as, high molecular weight compounds (Walker et al., 2003).

The rhizosphere of crops cultivated in soilless systems is also populated by microorganisms, and these too have the potential to interact either positively or negatively with the plant (Raviv, Lieth & Bar-Tal, 2019). PGPR known to colonize the rhizosphere of field grown plants have been shown to elicit desirable plant responses, like improved yield and quality, when inoculated on plants cultivated in both liquid and solid hydroponic systems (Mia et al., 2005 ; Pagnani et al., 2018). Inoculation refers to the act of introducing microorganisms, or suspensions thereof, into a culture medium. Methods of doing so may vary, but for production purposes, or experimental trials mimicking such settings, a bacterial suspension of known concentration is commonly administered to the growing medium.

Soilless cultures mainly differ from field production in soil by using containerized growing media supplied with nutrient solution (e.g rock wool, coco coir and peat) or

pure nutrient solution to grow plants (Raviv, Lieth & Bar-Tal, 2019). This environment is less complex than soil, and thus allows for more extensive control of outcome by changing parameters like root zone temperature, nutrient uptake and oxygenation. Considering the more controlled root environment of soilless cultures, management and evaluation of PGPR activity might be possible to a higher degree than in soil systems, owing to the absence of a highly diverse native microflora (Romano, Venterino & Pepe, 2020).

Soilless systems can be categorized as either solid or liquid, depending on whether growing media surround the roots or not. Liquid culture signifies that the roots are submerged in nutrient solution, continuously (e.g. Deep Water Culture) or intermittently (e.g. Ebb and Flow). In solid culture, on the other hand, roots are surrounded by growing media, which can be either organic (e.g. coco coir and peat) or inorganic (e.g. rockwool and pumice). The main difference between these categories of growing media lies in the degree to which they interact with the system by processes of decomposition and subsequent release of carbon.

In this report, studies using solid as well as liquid hydroponic systems have been incorporated, and the growing media of the trials have been organic as well as inorganic. However, a caveat is that many of them are so called ‘drain to waste’ and not recirculating in their water/fertigation management approach. Moreover, some of the studies are examining plant-microbe interactions in vitro, and this cannot be taken as sole evidence for processes taking place in the vastly more complex environment of the plant rhizosphere. Nevertheless, they might contribute insight to the more reliable trials in which inoculated plants have been grown for a period of time under conditions more similar to real production.

The naturally occurring microflora of soilless culture is a result of root presence, and dormant microbes inhabiting the seed initiate growth simultaneously with the plant. Hence, all soilless systems have a microflora in the rhizosphere under normal conditions. However, since it is largely dependent on the chemical compounds

released from the roots, there might be variance between systems due to physical differences of the medium surrounding the roots and the water content. For example, liquid systems suspend roots in nutrient solution, and the compounds released from them therefore are subject to comparatively large dilution effects. Solid systems, equipped with drip-emitters, on the other hand, are less disruptive since they do not create the same constant mass flow. The rhizosphere effect reaches further in liquid systems, but the concentration of root exudates decreases much faster than in solid ones (Bar-Tal, Lieth & Raviv, 2019).

Cannabis sativa L. is a dioecious, herbaceous annual plant belonging to the family Cannabaceae (Thomas & ElSohly, 2016). Its geographical origin is yet to be determined for sure, but most likely it is native to central Asia/Southern Eurasia (Clarke & Merlin, 2013). Cannabis is considered a thermophilic heliotroph, which thrives in well-drained soil rich in nutrients. As such, natural locations for cannabis would have been river banks, lakesides, agricultural lands and other locals providing sandy-loamy-alluvial soil (Clarke & Merlin, 2013). There has been a debate concerning the taxonomy for several decades now, and there is still some disagreement about whether to use the popular sativa, indica and ruderalis ssp. distinction or not (Clarke & Merlin, 2013). However, even if not accurate enough for scientific standards, who rather relies on chemotype (Aizpurua-Olaizola, 2016), the historical distinction might be interesting for practical applications in horticulture, since it indicates where a variety comes from geographically, helping in optimizing environmental parameters.

Although having many different uses, the production of cannabis in soilless systems for medical and recreational purposes centers on the plant's production of a chemical group called 'cannabinoids', which is synthesized in resin glands located mostly on and around the inflorescences of female flowers (Happyana et al., 2013). The ratio between the major cannabinoids delta-9-tetrahydrocannbinol (THC) and cannabidiol (CBD) is genetically determined by two genes producing compound specific synthases from the precursor cannabigerolic acid (de Meijer et al., 2003; Andre,

Hausman & Guerriero, 2016). The accumulation pattern of THC and CBD is variety, organ and spatial dependent; For example, the flowers at the top of variety x accumulates THCA quadratically in a certain phase, whereas variety y accumulates THCA linearly in the same phase (Richins et al., 2018).

Growing cannabis for modern western medicine, which is predominantly concerned with single-compound pharmaceuticals administered in exact doses, is a matter of precision and reliability (Bedrocan, 2018; Thomas & ElSohly, 2016; Dansk gartneri, 2019), and thus calls for horticultural methods that ensure high quality consistently. Naturally, the proper genetics are absolutely fundamental to success, but the fulfillment of their potential is highly dependent on the caliber of cultivation technique and management practices (Backer et al., 2019). The focus is on producing the highest amount of cannabinoids in the right proportions for the medical condition to be treated (mostly by changing the THC:CBD ratio).

Hydroponic systems, in comparison to field production in soil, allow for increased control and are thus perfectly suitable for the production of medicinal plants like cannabis. Additionally, the controlled environment is conducive to a lower input of pesticides, which is extremely important in the case of cannabis, since use is discouraged and prohibited in several european medical production facilities (Laegemiddelstyrelsen dk ; Bedrocan, 2018). However, pathogen infection and subsequent disease still occur (Punja et al., 2019), which calls for alternative methods.

Soilless cropping systems are different from field production in soil mainly by virtue of more homogenous growing media, which is developed specifically to suit the needs of crops grown in more controlled environments (Raviv, Lieth & Bar-Tal, 2019). As such, the potential of manipulating the root zone environment to improve plant performance is inherently greater in this type of culture, which established cannabis producers deem a necessity, owing to the wish to meticulously control

content of secondary metabolites, inflorescence yield and pathogens common to soilless cultures.

Objective & Hypothesis

The main purpose of this literature study is to ascertain whether the incorporation of Plant-Growth Promoting Rhizobacteria in soilless cultivation of Cannabis is warranted in terms of positive plant responses and reliability of outcome. However, the nature of these microorganisms, as well as practical aspects of application and measurement relevant for successful use, will also be treated with the hope to shed some light on possible best management practices in production. Hence, the study is cross-disciplinary and focused on attaining answers that may encourage and facilitate experimentation within the cannabis industry. Although the main focus of the text is to elucidate growth promotion and disease suppression in Cannabis soilless systems, other crops and cultivation systems are brought up as comparison. Furthermore, it is hypothesized that the knowledge in the latter domain will be adaptable to cannabis cultivation, and that the principles of growth promotion and disease suppression will be similar.

Method & Material

A systematic search of the scientific literature concerning the use and efficacy of Plant-Growth Promoting Rhizobacteria in horticultural production generally, and Cannabis production specifically, was conducted. As such, the resulting text is of cross-disciplinary nature - touching on plant biology, microbiology, the emerging field of Cannabis production and soilless systems in general. Moreover, the search has been conducted with practical application in mind, and so has focused more on aspects directly related to this - plant responses, colonization techniques, inoculant concentrations , etc., - than more detailed knowledge about the mechanisms behind growth promotion and disease suppression. Hence, there has been a natural filtering,

favoring commercially important horticultural crops and their responses to the applied bacteria in environments as close to those of their real production settings as possible. However, laboratory studies have also been incorporated where there has been a need to give a background more focused on microbiology.

3. Results

3.1. The Rhizosphere and Rhizomicrobiome

3.1.1. Plants and their associated microflora constitute a meta-organism

Together, the plant and its associated microbial life are considered to form a ’meta-organism’ referred to as the ‘holobiont’. The rhizosphere, defined as roots and the soil/growing medium closest to them, represents the below ground part of the holobiont, which is full of both eukaryotes and prokaryotes affecting the plant positively, negatively, or not at all (Dazzo, Garouette & Hartmann, 2019). Basically, plants secrete, and diffuse different carbon sources (amino acids, organic acids, sugars, phenolics, proteins and mucilage), into the soil/growing medium, which then are consumed by microorganisms (Walker et al., 2003). Moreover, cells are sloughed off as a natural part of the roots development, and these also contribute to the carbon pool of the system (Raviv, Lieth & Bar-Tal, 2019). These microorganisms, in contrast to those inhabiting bulk soil, colonize and compete more effectively when the supply of organic inputs to the system is high, thus making the amount of rhizodeposits from plants paramount for their proliferation (Dazzo, Garouette & Hartmann, 2019).

3.1.2. Nutrient flow from the plant to the microflora

Rhizodeposits include all carbon sources released from the plant: both low molecular weight ones with a lower C/N-ratio (amino acids, organic acids , etc.), and high molecular weight ones with a higher C/N-ratio (mucus, proteins , etc.). The former

category is the most common, and its members are termed ’exudates’ ; plant photosynthates that are being transported to, and released from, the root without first being stored in tissue (Walker et al., 2003 ; Dazzo, Garouette & Hartmann, 2019). However, there are multiple factors governing exudation, including the root-soil concentration gradient, permeability of the root plasma membrane and the solute location in root tissues (Jones, Nguyen & Finlay, 2009). The presence of these different nutrient sources makes the rhizosphere a highly dynamic and metabolically active place, which entails a different environment (one where nutrient cycling proceeds at a faster rate) compared to the rest of the soil system, due to the increase in respiration, gas exchange and moisture levels brought about by these plant-microbe interactions (Dazzo, Garouette & Hartmann, 2019).

3.1.3. The spatial dimension of the rhizosphere

Although very active, the rhizosphere does not extend more than approximately 1 centimeter from the roots, and its influence in terms of available nutrients is considered inversely proportional to the distance (Dazzo, Garouette & Hartmann, 2019). This influence on its surroundings is called ‘the rhizosphere effect’, and can be quantified by using the R:S-ratio rhizosphere bulk soil where is the microbial population density. Furthermore, the volume of the rhizosphere radially extended from the root cylinder is considered a function of the following factors: rhizodeposition rate, nutrient diffusion, mechanical properties of the soil matrix as well as the uptake and metabolism of the microbial community (Dazzo, Garouette & Hartmann). The rhizoplane (i.e., root surface) seems to be the location where the effect is most pronounced, but this may vary with plant species/age as well as root architecture, and root systems that are longer and more fibrous have displayed the most far-reaching rhizosphere effect (Dazzo, Garouette & Hartmann, 2019).

3.1.4. Rhizomicrobiome composition and dynamics

The composition of the rhizomicrobiome is dependent on multiple biotic and abiotic factors - soil type, plant species, cultivar and developmental stage - and does not stay the same over time. This is a result of varying rhizodeposit composition and amount, plant ion uptake and release, root respiration, secretion of chelating agents and release of secondary metabolites (Philippot et al., 2013). Furthermore, there is a difference in dominant bacterial species between the plant rhizosphere and bulk soil at the same site, and it has been noted that the rhizosphere community changes over time, which is not the case for bulk soil (Compant et al., 2019).

3.1.5. Common Characteristics of Plant-Growth Promoting Bacteria

Despite being made up of a plethora of different bacteria, those considered PGPR share some important organismal characteristics. Many of them are categorized as broad range r-strategists (Philippot et al., 2013) which simply means that they are metabolically diverse, thus feeding on an array of carbon and nitrogen sources, and that they are mainly limited by their inherent reproductive capacity (Encyclopaedia Brittanica, 2020). Furthermore, the r-strategists go through exponential growth, quick maturation and short life span - which makes them relatively weak competitors (Encyclopaedia Brittanica, 2020). Since colonization is a prerequisite for plant-growth promotion (Romano, Venterino & Pepe, 2020), it comes as no surprise that several of these bacteria are also free-living and motile; they can move and/or stick together. Morphology and behavior aside, these microorganisms are also highly susceptible to their environment: Navigating the bulk soil toward plant roots is achieved by processes of chemo- and aerotaxis; Nitrogen fixation is dependent on nitrogen levels in the soil; Conditions not conducive to proliferation might induce cyst formation (Steenhoudt & Vanderleyden, 2000).

3.1.6. Bacterial Community Structure Is Not Homogenous

Marschner, Crowley & Yang (2004) published results indicating that soil type, root zone environment and plant species all affected the community structure of the

rhizomicrobiome of sorghum, cucumber and wheat. Different fertilizer regimes (nitrogen and phosphorus) gave rise to 2 different rhizosphere community structures, while varying pH (5.9 - 8.1) resulted in three distinct groups. Furthermore, the effects changed when factors were combined, showing the highly dynamic ecology of the root zone. Examining the maize (*Zea mays*) rhizosphere with DNA-sequencing technique, Yang et al. (2017) identified 44 bacterial genera from 28 phyla (where the 9 most abundant made up 90% of the total) in bulk soil and rhizosphere. Some genera were more abundant in bulk soil, others in the rhizosphere, and community structure was a function of time. Notably, different bacterial genera were found to perform the same function in the rhizosphere and bulk soil; i.e., the nitrogen-fixing and phosphorus solubilizing microbes were not the same in these 2 habitats.

So what is the mechanism behind this differentiation in natural systems? While much of the literature has been ascribing a primary role to the root exudates, Dennis, Miller & Hersch (2010) cautions that the evidence is far from conclusive owing to the inherent limitations of the experimental setups. Nevertheless, rhizodeposits - whether of low or high molecular weight - are an important energy source for the rhizomicrobiome, and influence the microbial community structure by differing in chemical composition, thereby favoring the organisms able to metabolize these compounds. Hence, the bacteria in the rhizosphere most responsive to chemotaxis and capable of rapid growth in the presence of nutrient sources, will be the most competitive (Dennis, Miller & Hersch, 2010). The factors influencing these rhizodeposits may be categorized as biotic or abiotic, and are listed in the table below according to Hassan, McInroy & Kloepper (2019):

Biotic Factors Rhizodeposition	Abiotic Factors Rhizodeposition
Plant species	Temperature
Photosynthesis	Humidity
Root architecture	Moisture
Carbon translocation	Rooting depth

Biotic Factors Rhizodeposition	Abiotic Factors Rhizodeposition
Mycorrhiza	Soil texture
Nodulation	Nitrogen deposition

3.2. The Cannabis Rhizomicrobiome

3.2.1. Different Plant Species, Same Principles?

There is insufficient scientific literature on the subject of cannabis rhizomicrobiomes to draw any reliable conclusions, but some studies have been conducted, and these point to similarities with other plant species in terms of microbial makeup and function. For example, cannabis recruits bacteria from surrounding soil, and filters out certain species for endophytic relationships (Taginhasam, M. & Jabaji, S., 2020). Moreover, the plant-bacteria associations in the rhizosphere has been shown to result in plant responses akin to those documented in several other plant species, such as yield increase, growth promotion and increased tolerance to disease (Pagnani et al., 2018 ; Taginhasam, M. & Jabaji, S., 2020).

Furthermore, soil type is the most important factor determining the nature of the bacteria present in the rhizosphere of cannabis, but the makeup of the community changes when moving from bulk soil to endosphere, and plant genotype selection controls the community structure (Winston et al., 2014). This relationship has been established for other plant species as well, and is considered to be under the influence of several factors, for example: rhizodeposit composition, plant ion uptake/ release, root respiration, secretion of chelators and presence of secondary metabolites (Philipott et al., 2013 ; Compant et al., 2019).

3.3. Plant-Growth Promotion

3.3.1. Mechanisms Behind PGPR-Induced Growth and Yield Promotion

PGPRs are known to affect plant growth positively in several ways, either directly (by enhancing plant growth and nutrient acquisition) or indirectly (by contributing to a lower incidence of infection and mortality in the presence of pathogens), and studies using different plant species have demonstrated this phenomenon clearly (Backer et al., 2018). However, the plant responses of Cannabis to PGRP remain relatively under-studied (Lyu et al., 2019), although there is strong evidence for their ability to suppress disease through antagonism (Backer et al., 2019).

Two of the most prevalent hypotheses on how growth and yield promotion results from PGPR activity is increased nutrient cycling and microbial production of plant hormones like auxin (Backer et al., 2018).

Certain bacteria of the rhizomicrobiome, primarily strains belonging to the *Bacillus*, *Pseudomonas* and *Streptomyces* genera, solubilize different forms of chemically bound phosphorus in the soil, turning it into plant available anions. They do so by producing organic acids (gluconic, lactic, citric etc.) and carbon dioxide, which lower the soil pH, through the respiration and fermentation processes; chelating cations (Ca, Fe, Al) known to bind P over a wide range of pH; releasing enzymes capable of mineralizing organic P; and through siderophores, which have a high affinity for Fe - making the formation of insoluble chemical constellations less probable (Kalayu, 2019).

Other rhizosphere bacteria, *Azospirillum* and *Azotobacter*, for example, are diazotrophs and have the ability to fix and metabolize different forms of nitrogen. This might prove beneficial to the plant, since it mainly takes up and utilizes two types of nitrogen (ammonium and nitrate), whereas diazotrophs can take up and utilize three more: Nitrite, amino acids and molecular nitrogen from the atmosphere.

However, this seems to be the case when the levels of nitrogen in the rhizosphere are comparatively low (Steenhoudt & Vanderleyden, 2000). The nitrogen is transformed by the metabolism of the microbe, and later made available to the plant, which plays a great role in conserving nitrogen in natural ecosystems (Kuzyakov & Xu, 2013). Since soilless systems seldom lack in nitrogen due to continuous fertigation or constant nutrient solution contact with roots, this mechanism might play an insignificant role in growth promotion of soilless crops.

Nevertheless, nitrogen dynamics will potentially play a more important role than phosphorus in soilless systems inoculated with PGPR, owing to its direct effect on aggressive microbial growth in environments not limited in easily available carbon sources. Since interspecies competition is higher in the rhizosphere, and nutrient depletion zones are quickly formed in the close proximity of the roots, there is an ecological need to solve the puzzle of maintaining easily mobilized plant nutrient ions, like nitrate, over time (Kuzayakov & Xu, 2013).

Even though the effects of plant hormones are interconnected, the two most commonly associated with plant growth are auxin and cytokinin. These compounds constitute an important ratio in plant physiology, and their relative proportions decide whether adventitious root or shoot formation will be favored - a high auxin:cytokinin ratio will result in more adventitious roots and a low auxin:ratio will result in more adventitious shoot formation (Hartmann, 2014). Experiments have shown that PGPR indeed promote plant growth by increasing the concentrations of auxin (IAA) as well as cytokinin (Asari et al., 2017).

3.3.2. The Effects of PGPR Vary With Fertilizer Rate

Mia et al. (2005) inoculated hydroponically grown (DWC) banana plants with one of two strains of PGPR bacteria: *Azospirillum brasiliense* and *Bacillus sphaericus*. The presence of these strains in the growing medium increased plant macronutrient accumulation significantly compared to controls, and the response was correlated to

the amount of nitrogen supplied with chemical fertilizer. The ability of the bacteria to increase the banana plant's accumulation of macronutrients, over the board, was greatest when nitrogen was supplied at a rate of 33% compared to controls. *A. brasiliense* generally led to a higher observed accumulation. Phosphorus uptake, for example, increased with 55%, as opposed to 26% for *B. sphaericus*. Fruit yield and quality attributes were also increased; bunch weight increased by 17% for plants inoculated with *A. brasiliense*, and 7% for those inoculated with *B. sphaericus*.

Nosheen, Bano & Ullah (2016) observed similar results, in terms of yield and quality, for ethiopian mustard (*Brassica carinata*) inoculated with *A. brasiliense* and/ or *Azotobacter vinelandii*, both of which stimulated plant growth, presumably through the production and dissemination of phytohormones like IAA. For example, the number of branches were equal to control in plants that had received PGPR, even though the amount of chemical fertilizer had been decreased by 50%. Also, a combination of the two bacteria coupled with half the fertilizer dose yielded a greater amount of seed than those plants that received a 100% fertilizer rate. Other than plant growth promotion, the inocula affected the biosynthesis of glucosinolates, but not in the same way. *A. vinelandii* in combination with a 50% fertilizer rate, stimulated the production of these secondary metabolites, while *A. brasiliense* in combination with the same amount of fertilizer decreased the amount. Moreover, the combination of the two led to a decrease as well.

Growth promotion and increased yield was also observed when inoculating white cabbage (*Brassica oleracea var. Capitata* L.) seeds and seedlings with the bacterium *Paenibacillus polymyxa* at a concentration of 10^8 CFU/mL (Ertan et al., 2016). The plants in this trial were subjected to different nitrogen fertilizer rates, and compared to inoculated and non-inoculated controls grown in a medium devoid of nitrogen. As in Mia et al. (2017) and Nosheen, Bano & Ullah (2016), the improvement of the treated plants was a function of nitrogen fertilizer rates. However, the phenological stage at which inoculation occurred also had a significant impact on results, and

seedling inoculation generally favored plant growth/yield more than seed inoculation.

Ertan et al. (2016) conducted studies on cabbage grown in field conditions, and noted that the nitrogen use efficiency (NUE) decreased after a certain point with increasing fertilizer rates, and was always significantly higher in PGPR-inoculated plants, independent of how much nitrogen that was applied (0-200 kg/ha). However, inoculated plants without additional nitrogen application did not perform as well as inoculated + nitrogen, but they had almost twice the NUE of non-inoculated plants without additional nitrogen applied. Furthermore, the plant's ability to recover nitrogen from the soil was greater in inoculated seeds/seedlings than for controls, and here as well, a considerable difference (> 2x) was noted in comparison between non-fertilized inoculated plants and non-fertilized controls.

3.3.3. Inconsistent Results in Commercial Production Environment

Garcìa, Crowley & Yang (2004) carried out three experiments with two different cultivars of tomato and pepper propagated and grown in commercial greenhouses, using a single *Bacillus* strain isolated from alder. Three different growing media were employed - peat, sand and rockwool - all of which were given a bacterial suspension (1 - 1.5 L/plant [10^8 CFU/ml]) every 20 days after transplanting 2 month old plants. Number of fruits and fruit diameter increased in both peat and rockwool for one tomato variety, but not in the other one. In the case of pepper, inoculation resulted in significantly larger yields in 4/7 harvests. The plants undergoing propagation received one application (10^8 CFU / g peat) at planting and another one 15 days later. In contrast to the other two experiments involving more mature plants, inoculation only seemed to favor height, leaf area and foliage dry weight.

3.3.4. Bacterial Consortia - Application of Multiple Species

In the above studies, strains of PGPR have been inoculated singularly, and exposed to one singular pathogen at a time. However, Liu et al. (2018) performed experiments which indicated a more effective disease suppression and growth promotion on cucumber and bell pepper for a mixture of PGRP - 4 *Bacillus* strains - rather than single strains. By establishing 5 growth parameters - shoot dry weight, root dry weight, root length, root surface area and fine root length - and inoculating with 3 pathogens - *Xanthomonas axonopodis*, *Pseudomonas syringae* and *Pythium ultimum* - a total of 15 growth parameters was achieved. Out of these 15, 13 were increased by the PGPR mixture, in comparison to 8 for the most successful single strain. This points to a broader protective effect when using a mixture of compatible PGPR.

The effect of microbial consortia, as opposed to singular inoculants, has been examined in vitro and in vivo for *Lycopersicum esculentum* by Botta et al. (2013). Here, inoculants consisting of *Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae* and *Burkholderia ambifaria*, alone or in some combination with each other, were applied. Notably, the results differed between in vitro and in vivo, as well as between single strain and consortium. The former category was associated with more significant plant growth promotion - manifested as longer roots, more lateral roots, increased amount of root hairs and size, more leaves and an overall increase in dry matter content.
Concluding analysis showed *A. brasiliense* and *G. diazotrophicus* to be the ones most abundant in the rhizosphere. The latter, however, did not show the same degree of plant growth promotion, and *G. diazotrophicus* did not seem to do as well as in the in vitro environment. Nevertheless, the other three strains stimulated both root- and aerial growth significantly, and a consortium consisting of all four affected dry matter content and overall growth positively.

Inoculating a consortium of PGPR (*A. brasiliense*, *G. diazotrophicus*, *B. ambifaria* and *H. seropedicae*) to the seed of female hemp plants (cv. Finola) cultivated in a greenhouse controlled environment increased leaf area, nitrogen use efficiency,

cannabinoid content and overall quality (Pagnani et al., 2018). The concentrations applied (10^6 and 10^7 cells/mL) varied in efficacy, the lower concentration yielding better results, but they both performed better than controls, which did receive neither inoculants nor nitrogen fertilizer. Inoculated plants (10^6 cells/mL) that had not been fertilized with nitrogen, were on par with those receiving optimal nitrogen fertilizer rates for Cannabis (80 kg/ha), in terms of leaf area expansion and cannabinoid content (THC & CBD). Moreover, analyzing roots grown in vitro with a scanning electron microscope, showed that the consortium successfully colonized the rhizosphere of the inoculated plant (Pagnani et al., 2018).

3.3.5. An Existing Commercial Alternative for Cannabis

Focusing on Cannabis grown in high-intensity indoor cropping systems, Conant et al. (2017) evaluated the effect of a plant derived microbial inoculant (Mammoth P™), which mobilizes phosphorus in the soil and promotes plant root growth, on growth and inflorescence yield. Treated plants produced 16.5% more inflorescence weight, grew 9% taller and had a 18% larger basal stem diameter. Also, there were indications of earlier onset of flowering in treated plants, a phenological response correlated to PGPR inoculation in *Arabidopsis thailana* (Poupin et al., 2013), but data for this observation was not provided by the author. Clonal proliferation was also examined in a parallel trial, but inoculation with Mammoth P™ did not produce significantly different results from control. Application of Mammoth P™ has als been evaluated for other crops (jalapeno, tomato, basil and wheat) by Baas et al. (2016). Here, earlier flowering could be established for jalapeno plants that received Mammoth P™ in addition to fertilizer. This plant species also benefited the most in terms of productivity, whereas tomato and basil treated with the inoculant did not deviate much from those receiving only fertilizer. However, another biostimulant used as comparison, Accomplish™, had a significant effect on tomato fruit yield.

3.4. Disease Suppression

Disease suppression - meaning that pathogens do not persist in the system, alternatively do no harm - is a natural process in soilless systems, emerging from the activity of certain members of the rhizosphere microflora, as well as the overall ecological diversity of the biome. For example, the practice of reusing rockwool-slabs has been observed to increase the suppressive capacity against certain fungal pathogens common to soilless systems. Moreover, in vitro trials have established an suppressive effect of recirculated nutrient solution in liquid soilless systems, and this positive effect did not appear after sterilization, indicating that it was a result of microbial activity (Bar-Tal, Lieth & Raviv, 2019). As a matter of fact, a great proportion of the soilless root microflora is made up of Pseudomonas sp.; a bacterial general strongly associated with disease suppression (Vallance et al., 2010). Hence, disease suppressiveness of a system is not an attribute originating with the inoculation of PGPR, but might still be affected by it.

3.4.1. Mechanisms of Disease Suppression by PGPR

Since a very limited number of pesticides are allowed in the cultivation of cannabis, there is a pressing need to come up with alternatives for the most prevalent diseases, like grey mold caused by *Botrytis cinerea* and root rot caused by *Fusarium* and *Pythium* species. PGPR are known to make plants more disease suppressive, which is achieved either by acting as antagonists, competing for resources and space or inducing systemic resistance (ISR) in the plant. These mechanisms are generally associated with antibiosis, siderophore release, timing, and priming of the plant's immune system, respectively (Choudhary et al., 2007).

3.4.2. Induced Systemic Resistance - Priming the Plant's Immune System

ISR is a pathogen resistance mechanism, stemming from prior infection, in which the plant's ability to withstand and counteract an attack has been increased. This type of

resistance is distinguished by the signaling pathways used (jasmonic acid and ethylene), and this has implications for the type of pathogens which can be effectively resisted - ISR has shown to be most active against necrotrophic pathogens (Choudhary et al., 2007). PGPR (e.g., *Bacillus* & *Pseudomonas spp*.) have the ability to act as elicitors of ISR in plants, which might counteract foliar as well as root infecting pathogens. PGPR-associated resistance in plants is induced by the microbial production of siderophores, rhamnolipids and volatile compounds (Choudhary et al., 2007).

Necrotrophic *Pythium* and *Fusarium* fungi have been observed on cannabis plants grown in soil and hydroponics (recirculating DWC) both, causing root- and crown rot, as well as damping-off disease (Punja, 2017 ; Punja, 2018). Affected tissues included bark, cortex and pith, but wounding was a prerequisite for infection to occur (Punja, 2018). In the hydroponic cropping system, approximately 1% of the plants got infected, and the pathogen species were determined to be the same as those infecting other horticultural crops, like tomato and cucumber (Punja, 2017).

Liu, Kloepper & Tuzun (1995) inoculated cucumber seedlings grown in a soilless peat mix with *Pseudomonas putida* and *Serratia marcescens* by dipping the roots in bacterial suspension to examine the effect of ISR on Fusarium wilt caused by *Fusarium oxysporum*. To ensure that potential biocontrol was not achieved by microbial competition/antagonism, the root system of the cucumber plants was split in two; one part inoculated with the pathogen and the other with the biocontrol agent. Both PGPR treatments resulted in fewer dead plants than controls: 3.2 (*P. putida*), 3.8 (*S. marcescens*) and 6.8 (pathogen inoculated controls) per 10 plants. Moreover, the spread of the pathogen in the plant was significantly slowed down by *Pseudomonas*, even though this bacteria itself did not spread through the plant.

3.4.3. PGPR-Pathogen Competition for Resources

It has been suggested that the biocontrol of *F. oxysporum* using *P. putida* is not only a matter of triggering ISR in the plant, but also involves microbial release of Fe-chelating siderophores, which was shown in experiments on carnation (Duijff et al., 1993). However, when the iron concentration increased in the growing medium, this effect decreased, and at 200 micromolar [FeCl3] inhibition of fungal growth ceased altogether. Although this part of the experiment was done in a petri dish to elucidate the mechanism, significant decrease of disease by *P. putida* was observed on plants growing in soil inoculated with *F. oxysporum* and connected to the availability of iron in this medium as well.

3.4.4. Antibiosis

Damping-off disease caused by *Rhizoctonia solani* has been successfully mitigated by inoculation with *Bacillus subtilis* on container grown tomato seedlings (Asaka & Shoda, 1996). 16.7% of the plants treated with both the biocontrol agent and pathogen were infected, whereas the number was 85.2% for those exposed only to the pathogen. Moreover, shoot length and dry weight were significantly lower for the latter group, but not for the former. This suppressive effect was attributed to the production of the two antibiotics Iturin A and Surfactin, which were recovered from soil at the end of the experiment.

3.4.5. Some Species and Strains More Effective Than Others

Screening over 600 bacterial isolates obtained from natural soil, Paulitz, Zhou & Rankin (1992) found 5 isolates of the *Pseudomonas* genera to be very effective microbial antagonists against *Pythium aphanidermatum* in cucumber cultivar ‘corona’. These beneficial rhizobacteria successfully mitigated pathogen root colonization, germination and motility. The pathogen concentration was 500 zoospores/ml after application in the nutrient solution, and had decreased to 35 CFU/ml after 4 hours. In terms of plant mortality for infected samples, one specific strain of *Pseudomonas* reduced it from 7/10 to 1/10 plants.

Hydroponically cultivated chrysanthemums have also been shown to have decreased disease instance and severity against *Pythium aphanidermatum* and *Pythium dissotocum* when inoculated with beneficial rhizobacteria from the *Pseudomonas* and *Bacillus* genera (Liu et al., 2007). Strains of *P. fluorescens, P. corrugata, P. chlororaphis* and *B. cereus* were all highly suppressive toward the pathogens, albeit some were more effective against *P. aphanidendrum* than *P. dissotocum* and vice versa. However, there was a clear relationship between nutrient solution temperature and disease severity, on the one hand, and PGPR efficiency in suppressing disease, on the other.

The severity of the disease was proportional to the increase in temperature, and *P. aphanidendrum* infection caused more damage than *P. dissotocum* at the maximum temperature (34 °C). Although some PGRP strains also were more active at higher temperatures, and thus had more positive effect, like increasing root volume, there was generally a better response at lower temperatures (20-24 °C). Antagonism by competition for space to colonize were proposed as the mechanisms behind the suppressive capacity, which was reflected by the need to inoculate with PGRP at least 7 days before pathogen introduction in the system for successful results. Antibiosis was also suggested to play a role, which was a hypothesis extrapolated from in vitro trials.

3.4.6. Disease Suppression via Roots is Not Necessarily Local

The fact that PGPR may counteract the colonization and damage caused by root-associated pathogens is rather intuitive, but Nie et al. (2017) have supplied evidence for their suppression of the foliar pathogen *Botrytis cinerea* as well. Submerging roots of young tomato plants in a solution containing a strain of the rhizobacteria *Bacillus cereus* (5 x 10^8 CFU/ml) resulted in significantly smaller necrotic leaf spots and fungal growth following foliar application of the pathogen (1 x 10^6 spores/ml). This response was attributed to induction of ISR, as the microbes did not come in contact with each other. Also, there was a higher accumulation of hydrogen

peroxide systemically in treated plants, which is known to be a part of the plant's immune response. Suppression of *Botrytis cinerea* from *Bacillus* in tomato has been documented elsewhere as well (Garcìa, Crowley & Yang, 2004).

Rhizosphere colonization by inoculated bacteria

3.5.1. How Inoculated Bacteria Find Their Way To the Roots

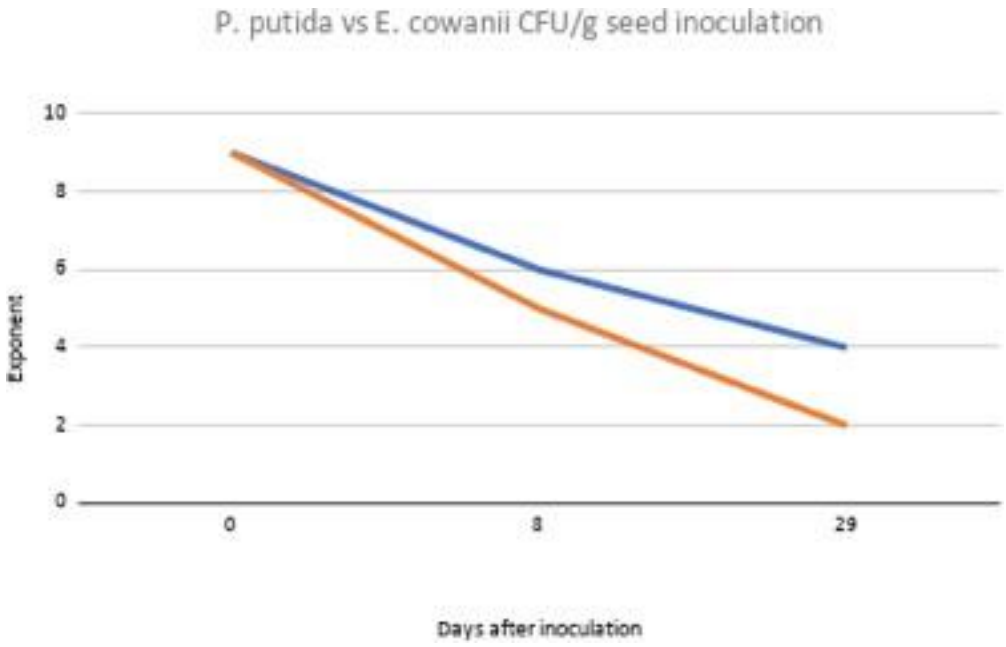
Successful colonization is a precondition for the benefits bestowed on plant growth and health by PGPR (Sultana, Desai & Reddy, 2016 ; Liu et al., 2007), and factors affecting this process positively should therefore be examined. In their extensive review on the topic, Benizri, Baudoin & Guckert (2001) pointed out several important abiotic and biotic factors in root colonization. First and foremost, bacteria need to reach the zone of colonization,which is dependent on movement and navigation. Bacteria, depending on biological structure, may move actively with the propelling force generated by flagella, or passively with the help of external factors like water mass flow and adhesion to soil particles and roots. In the case of active movement - motility - chemotaxis is paramount to the bacteria finding the roots, and the flagella have the ability to respond to a gradient of chemoattractants (rhizodeposits) released by the plant.

By quickly responding to chemotaxis, the bacteria may reach the spermosphere of the plant before competing microbes. However, it must also be able to adhere to the root surface. For example, *A. brasiliense* goes through 2 phases of adhesion: adsorption (weak) and anchoring (strong). These are created with proteins and exopolysaccharides formed by the bacteria, but there are also cases where the plant secrete glycoproteins - molecules causing the bacteria to agglutinate and colonize the roots more efficiently. This phenomenon of bacterial populations adhering to each other, as well as to an external surface, allows the formation of biofilms, which has been linked to significantly (as much as 2.5 log CFU/g difference) better colonization

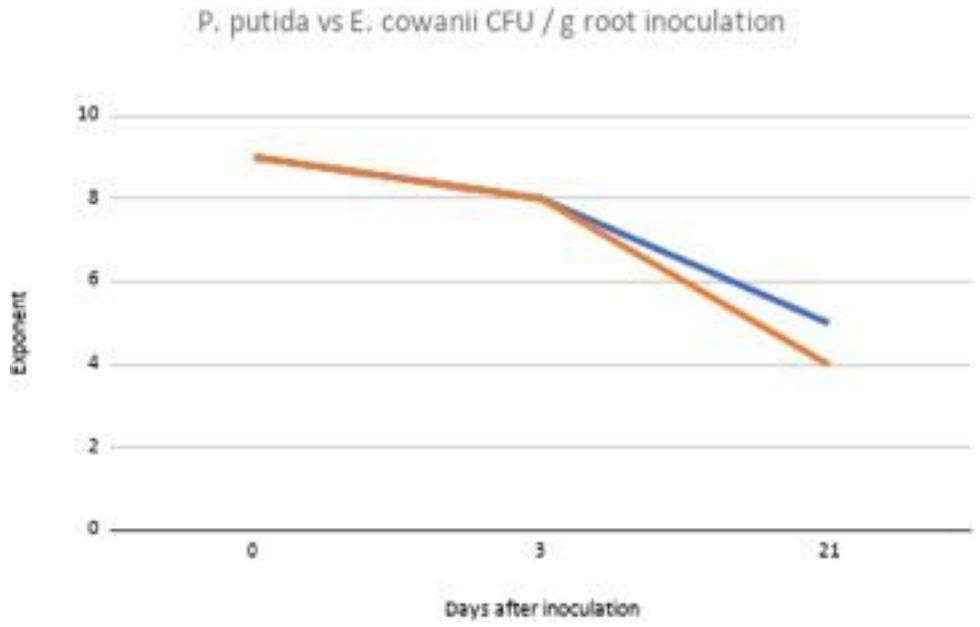
and survival on plant roots in vitro and in vivo when inoculating with *A. vinelandii* (Altaf & Ahmad, 2017).

3.5.2. Methods of Application and Their Effect on Outcome

In a study analysing colonization of inoculated *Pseudomonas putida* and *Enterobacter cowanii* in relation to two different application techniques -submerging tomato seeds or roots in a bacterial suspension - Götz et al. (2006) combined plate culture evaluation and microscopy to estimate the colonization efficiency and patterns over time and space. The results from both methods were in accordance with each other, and confirmed that PGPR populations are heterogeneously distributed in the rhizosphere; seed inoculation colonized the upper root system more densely, while root inoculation yielded a more uniform distribution. Moreover, the bacterial strains differed in number (CFU/g roots) throughout the experiment - *P. putida* outnumbered, as well as, decreased at a slower rate than *E. cowandii* - and root inoculation resulted in greater PGPR colonization by an order of 1-3 magnitudes depending on when samples were taken (see image *1.1*).



1.1 Trends in bacterial population density over time when seed (above) vs root (below) inoculation was applied.



There are several available methods for measuring colonization efficiency and persistence of the bacterial inocula, whereof viable counts and different forms of microscopy might be preferable in commercial cultivation systems, given their relatively low complexity in comparison to molecular and genetic sequencing techniques. These also fit the controlled environment soilless setup, since it is very difficult to distinguish between inoculated and indigenous bacteria in highly diverse systems, like soil (Romano, Venterino & Pepe, 2020). Light microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are all used in the study of rhizosphere colonization, and are often combined with staining or fluorescens to discern inoculated bacteria from indigenous. Nevertheless, these are still not necessarily exact measurements of bacteria colonization and persistence, since it is difficult to make out dead cells from live ones, and bacterial populations are not homogenous over space and time. For example, a certain PGPR might colonize certain parts of the rhizosphere effectively, but not be able to persist for the rest of the plant's life cycle (Romano, Venterino & Pepe, 2020).

Khalil & Alsanius (2001) applied the methods of Sole Carbon Source Utilization and Phospholipid Fatty Acid Profile to determine root zone microbial community structure and functionality in a recirculating hydroponic system where tomato was grown in peat and rockwool. This approach allowed non-destructive analysis in a commercial greenhouse setting, which was not limited in scope by the difficulties of morphology and culturability. Moreover, the nature of the methodology made possible multiple tests during the course of the culture, delivering a broad-scale perspective on the microbial dynamics over time - an important aspect since microbial populations in the rhizosphere change over time due to biotic and abiotic factors alike (Dazzo et al., 2019 ; Turner, James & Pool, 2013).

Cannabis in Soilless Systems

3.6.1. A Highly Controlled Business

Although cannabis is cultivated in many different systems - field, greenhouse and indoor grow chambers - several of the larger producers globally have focused primarily on the more controlled environments, and new facilities are often hybrids between greenhouses and indoor growth chambers. This kind of system is common in the european production of medical cannabis, and is used by large producers like Bedrocan in Holland and Aurora in Denmark (Bedrocan ; Aurora nordic). There are several reasons for a controlled environment in the production of cannabis from a quality perspective, especially when the biomass is intended for medicinal purposes. For example, cannabis may be prescribed for treating pain and nausea in cancer patients, who are immunocompromised, and contaminants (primarily fungi like *Aspergillum spp*. and bacteria like *E. coli*). However, the global trend is to establish best practices that ensure minimal contamination from disease-causing microorganisms as well as pesticides (https://mjbizmagazine.com/going-global-join-the-gmp-parade/).

3.6.2. Common Growing Conditions

Typically, each cropping cycle takes 2-3 months from seed to harvest, depending on the variety, and a longer photoperiod (18-24 h) in the vegetative phase is decreased to a shorter (12 h) for the induction of the flowering phase. Usually, cuttings are taken from mother plants and transplanted into some kind of rooting medium (rock wool, coco coir, peat , etc.), and transplanted once again after the plant has established itself. The environmental parameters suitable for cannabis cultivation vary depending on variety and are interconnected, therefore it is difficult to say something general about these. However, relatively high temperatures (25-30 °C) and light intensities have been observed to result in increased photosynthesis and THC accumulation (Chandra et al., 2008 ; Potter & Duncombe, 2012).

Then there is a meticulously monitored and controlled post harvest process aiming to dry the inflorescences properly in order to prevent mold and to ensure a maintenance of the cannabinoid and terpene content. Temperature, ventilation and light are all important factors in this process (Jin, Jin & Chen, 2019). The dutch regulation on medicinal cannabis inflorescences states that the water content of packageable flowers must be between 5-10% (Hazekamp, Sijrier & Verpoorte, 2006). Bedrocan, supplying european pharmacies with dried cannabis inflorescences, also treat the harvested and dried material with gamma radiation to ensure minimal counts of microorganisms before packaging to ensure that microbial viable counts do not exceed 100 CFU/g inflorescence (Hazekamp, 2016). However, this is not common practice, which makes the environmental conditions during cultivation, post harvest and storage paramount for supplying a safe product.

In terms of rhizosphere environment, rockwool is often used as a substrate in combination with drippers to maximize control over nutrient and water dynamics. There have been some studies done recently of cannabis cultivation in controlled environments, using coco coir and organic fertilizer, which indicate that optimal fertilizer rates exist, and that they differ between the vegetative and generative phases (Caplan, Dixon & Zheng, 2016;2017). Moreover, another study by the same authors (Caplan, Dixon & Zheng, 2019), established a connection between induced

drought stress (-1,5 MPa) in the end of the generative phase and increased levels of major cannabinoids like THC and CBD, while inflorescence biomass was not different from controls. Notably, the increased cannabinoid yield was greater in the coir with the lower water holding capacity, pointing to the importance of the root zone environment for maximizing productivity.

Studies on the ionic factors (pH and EC) of the root zone are not well studied but some literature has registered plant responses to these parameters and have observed that cannabis in solid and liquid hydroponic systems tolerate, and do not show any decrease in well-being when root zone pH and EC are within the ranges of 5.5 - 7.4 and 1.6 - 3.0 mS/cm, respectively (Caplan, Dixon & Zheng, 2017 ; Cockson et al., 2019).

4. Discussion

The scientific literature implies a significant correlation for at least some of the claimed benefits of PGPR on cannabis plant health, while others remain to be examined more rigorously. However, extrapolating from literature exploring the plant-bacteria interaction in other crop species grown in similar settings might be a valuable tool for initiating increased industrial experimentation with beneficial bacteria inoculation - especially since the studies on cannabis specifically report plant responses similar to other, more researched, crops. Some researchers have pointed out that greenhouse in planta experiments, although time consuming, are effective screening methods, since bacterial traits (like IAA production and nitrogen fixation) do not necessarily predict growth promotion (Akinrinlola et al., 2018). For example, they noticed in their randomized trials - examining beneficial effects of 12 different *Bacillus* strains on corn, soybean and wheat - that some highly effective growth promoters were associated with few growth promotion traits beforehand, and vice versa.

This approach might fit the emerging cannabis industry perfectly, since there is a great interest in exploring new territory, and the cultivation companies invest large sums in state-of-the-art facilities as well as research and development. Additionally, the push towards highly controlled production environments could be a suitable system for PGPR given their homogenous root zone conditions, which might decrease the variability in outcome associated with inoculated members of the rhizomicrobiome. For example, controlling the moisture levels of the growing medium, along with the availability of nutrients could potentially go a long way in making sure that the bacteria do what we want them to do, like fixing molecular nitrogen from the air or produce auxin (Steenhoudt & Vanderleyen, 2000). In this way, the grower would develop the knowledge about a certain bacteria, or consortium, in the specific cropping system used, which is the name of the game if the end goal is optimizing production.

The nature of the soilless system affects the microbial life in the root zone by changing the ion and exudate concentration gradient; Systems with solid growing media result in steeper gradients, while those suspending roots in nutrient solution display a more far-reaching dispersal of molecules from the roots (Raviv, Lieth & Bar-Tal, 2019). This is due to dilution, and the same principle is applicable to solid growing media receiving pulse irrigation. In other words, water flow increases the movement of compounds and particles, which means that irrigation frequency and volume are important factors for the gradient, and thus chemotaxis, of solid systems. As such, they should be considered when inoculating PGPR into soilless cultures, since many of these bacteria were shown to navigate their way to the rhizosphere by sensing the chemical composition of their environment.

The release of root exudates is dependent on many factors, but one with massive implications in soilless culture is the ammonium:ammonium + nitrate ratio (RN). Root exudation has been shown to drop exponentially in response to increasing (RN), which could possibly affect microbial life in a similar manner. However, rhizosphere microorganisms take up both ammonium and nitrate more readily than

plant roots, because of their rapid growth patterns and high surface area-to-volume ratio (Kuzyakov & Xu, 2013), and so the increase of (RN) and decrease in exudate release might not limit their proliferation in reality - they may only shift nitrogen source and make mineral nitrogen less available to the plant in the short term.

Looking at the physical attributes of rock wool, and the drip irrigation systems utilized by several large-scale european producers of medicinal cannabis, could lead to some insight about whether it is feasible to incorporate PGPR into these systems. However, it is not only the ion and exudate concentration gradient - or rhizosphere effect - that is of interest here, but also the ability of the bacteria to successfully colonize and persist in the rhizosphere. We have seen that many of the bacteria hitherto used as PGPR are motile, which means that they are able to move toward the roots, provided that the water content of the growing media is high enough.

Moreover, even though practically sterile in the absence of carbon releasing plant roots, the inert rock wool will quickly become populated once a crop is introduced (Raviv, Lieth & Bar-Tal, 2019). Initially, however, there is an unbalanced microflora which favors the establishment of inocula. Colonization is essential to achieve growth promotion and biocontrol activity, and dependent on both biotic (motility, adhesion, growth rate, genotype selection) and abiotic (medium physical characteristics, nitrogen availability, application technique and temperature) factors. Hence, successful establishment of PGRP in soilless systems should take these into account. But the highest number of viable cells on roots (CFU/g) is not necessarily associated with the greatest amount of growth promotion. Pillay & Nowak (1997) showed that the shoot biomass of in vitro grown tomato seedlings inoculated with a PGPR strain of *Pseudomonas* was increased by 61.6% at a rhizoplane root population density of 1.3 x 10^9 CFU/g, but not at all at 7.9 x 10^10 CFU/g. For now, it is reasonable to suppose the same principle for cannabis, considering that the little literature available on the topic indicates better results with lower inocula concentrations.

The fact that even inert growing media becomes colonized by microbial life naturally raises the question if these bacteria could not benefit the plant in similar ways under suitable environmental conditions. There seems to be some merit to this notion; disease suppression of *Pythium aphanidermatum* and *Fusarium oxysporum* is developed by the naturally occuring microflora in some soilless systems when mineral wool is reused between cropping cycles. However, it is not there from the beginning, and possibly not effective enough (Raviv, Lieth & Bar-Tal, 2019). This raises the important question of microbial temporal dynamics: How does the microflora change over time and what are the effects on the plant responses desired when applying PGPR? If the goal is to have maximal growth promotion, there is likely a certain period in the plant's life cycle where this promotion yields optimal results. For example, Caplan, Dixon & Zheng (2017) showed that positive effects from optimal fertilizer rates in the vegetative phase of cannabis carried over into the generative phase.

It is abundantly clear that the carbon bound in rhizodeposits serve as a primary source of energy for the bacterial microflora in the rhizosphere, and hence also for inoculated Plant-Growth Promoting Rhizobacteria able to successfully colonize this area - Even more so since many of the species may be categorized as opportunistic r-strategists with a capacity to metabolize a wide array of chemically bound carbon. Although some of the factors influencing exudation are complex and hard to manipulate, others - like the chemical gradient from root to growing medium - could potentially be a target for improvement in soilless systems, since it is possible to choose growing media with physical properties fitting for dispersing compounds more readily due to, for example, higher hydraulic conductivity.

Rockwool, in which high-quality european medicinal cannabis (Bedrocan & Aurora Nordic) is grown, has an exceptionally high hydraulic conductivity (5-20 cm/min) and would therefore be likely to create rapid dispersion (Raviv, Lieth & Bar-Tal, 2019). However, this might not be conducive to a rhizosphere richer in rhizodeposits, if the dilution rate is greater than the diffusion/secretion rate. Also, if experimenting

in order to approximate such a value, one would need to consider the fact that the water retention capacity of stone wool drops quickly when the matric potential goes lower than (-1) kPa (Raviv, Lieth & Bar-Tal, 2019).

Factors stemming from the system and its management practices, like nutrient regime and pH, was shown to alter bacterial community structure. This could be seen as an advantage in soilless systems, as opposed to soil, since these are more controllable. For example, equilibrium dynamics of mineralization and immobilization in field conditions are highly variable due to climatic fluctuations, and this affects the RN, which in turn affects rhizosphere pH and exudated carboxylic acids (Raviv, Lieth & Bar-Tal, 2019). In soilless culture, especially climate controlled indoor cannabis cultivation, parameters like added nitrogen sources, growing media chemical attributes (CEC, functional carboxyl groups , etc.) and temperature of the root zone are likely not subject to the same fluctuations.

Moreover, experimentation on different growing media is warranted, since soil type was shown to be the most important factor in determining the microflora of the cannabis rhizosphere. Examining how the use of rockwool and coir affects the cannabis bacterial microflora - focusing on water, oxygen, carbon and nitrogen dynamics - should yield some insight into this since these parameters have proven to be essential in the microflora of the rhizosphere as means of transport, navigation and growth. Especially considering that many of the above studies indicated a superior effect of 'root-drench' inoculation, as opposed to seed inoculation, implying a need for the introduced microorganisms to find their way to the roots.

Since PGPR in the studies including a dimension of fertilization pointed towards a maximum efficiency of the bacteria to act as growth promoters and yield enhancers when the amount of fertilizer supplied was less than normal, the question of what this would confer in the case of soilless cannabis crops. Optimal fertilizer rates have been explored, and it is not known if these are too high to make the PGPR effective. Also, even if the effect of PGPR under such optimal fertilizer rates would turn out

positive, i.e., they successfully promote growth and increase yields, it might not compensate for the biomass lost by deviating from the established fertilizer rates.

Moreover, the management practice of induced water stress in the late generative phase has been shown to significantly increase cannabinoid yield, and as opposed to inoculation with bacteria, it is free and relatively easy to control (Caplan, Dixon & Zheng, 2019). However, this might not necessarily make superfluous the use of PGPR in the culture, as they may still play an important role as biological control agents and biomass promoters. When considering increasing cannabinoid content and biomass both, it is important to understand that there is a compromise to be made here; at a certain point, an increase in plant inflorescence biomass will entail a dilution of the cannabinoid content in the resin glands (Caplan, Dixon & Zheng, 2017).

Disease suppression is well-documented in the literature regarding PGPR, and it seems feasible to suggest its use in the soilless cultivation of soilless cannabis as well, since the pathogens involved are the same as in other, more studied, horticultural crops, where the mechanisms of induced systemic resistance and antagonism are considered effective methods of combating plant mortality and yield/ quality reduction stemming from disease. The inocula concentrations seem to be similar to those showing promise for growth promotion, which suggests that it might be possible to get desired disease suppression without compromising the seemingly concentration-dependent effect on growth promotion.

However, the literature indicates that some bacterial species and strains are more effective in suppressing disease than promoting growth, and vice versa. This speaks for the use of bacterial consortia, since it could include species with different lifestyles and functionality. Moreover, because most systems are subjected to some degree of fluctuation, it might be advisable to also consider the ability of the different organisms of the consortium to survive and thrive in slightly different environmental conditions. For example, if there is only one bacterium added to act as PGPR, and its

biocontrol/growth promotion effect is considerably hampered by some abiotic factor (temperature, nitrogen availability, water content , etc.) - while the system might occasionally be exposed to such conditions - then there should perhaps be another bacteria in the mix that can ‘take over’ under these circumstances. Indeed, the beneficial effect of consortia in comparison to single strain inocula was demonstrated for some commercially important crops by Liu et al. (2018).

Finally, some thoughts on the naturally occurring microflora of these systems, and its possible interactions with the inoculated one. The concentration of bacterial cells in the nutrient solution will be lower than on the roots, and the organic coco coir will favor fungal growth over bacteria whereas the opposite is true in the case of inorganic rockwool. Moreover, the latter is more conducive to pathogen attack by Pythium and the former to Fusarium. Aerobic bacteria, with a large portion of the culturable ones pertaining to Pseudomonas sp., are the most common in both solid (organic and inorganic) and liquid systems (NFT and DWC). Unlike the fungal growth, the number of bacterial cells in the rhizosphere plateaus, and stabilizes at 10^10 CFU/g root (Vallance et al., 2010). Hence, it is advisable to consider these factors when experimenting with PGPR-inoculation, since they may affect the outcome. For example, inoculation after native bacterial cells have plateaued would perhaps entail an inability of the PGPR to colonize the rhizosphere. Similarly, inoculation with beneficial fungi, like Trichoderma, could be more effective in coco coir to combat root pathogens.

Conclusion:

Plant-Growth Promoting Rhizobacteria have the ability to positively influence growth, yields and disease suppression. Even though the mechanisms behind these changes has not been fully elucidated, the scientific consensus is that beneficial outcomes are resulting from the influence of the bacteria on processes like nutrient cycling, hormone concentrations in planta, induced systemic resistance and pathogen antagonism. The rhizosphere is a highly dynamic space, owing to the plant’s release

of easily available carbon in the form of rhizodeposits, and even relatively sterile environments (e.g., rockwool) quickly become colonized when plants are introduced. Motile bacteria in the bulk soil respond to the chemical concentration gradients created by the roots through the processes of chemo- and aerotaxis.

In natural ecosystems, the competition is significant, and it may be difficult to successfully incorporate PGPR since there exist indigenous bacteria more adapted to the conditions, and competition for nutrients in the rhizosphere is hard. Nevertheless, inoculation in soilless systems shows promise, but there are some important considerations to be made for achieving colonization and proliferation. Bacterial species, plant species, inoculation makeup, inoculation concentration, inoculation timing, growing medium and management practices are all factors that influence the outcome. Measurements of the microflora in cropping systems can be achieved by applying different methods, but they all come with their own caveats - the low proportion of cultivable rhizosphere bacteria being one. However, this might not be a problem for industrial applications, since it has been noted that analyzing plant growth responses by growing out sufficing replicates may be a more reliable approach.

The different physical and chemical characteristics of soilless systems significantly affect the microbial community structure and functionality; mechanical matrix properties, porosity, fertilizer regime, pH and cation exchange capacity all have an impact on the microflora. Additionally, the physical nature (organic/inorganic) might also have an effect, owing to the release of carbon and the initial microbial load of the growing medium. Indeed, less microbial competition seems to favor colonization of PGPR, which in turn is a prerequisite for growth promotion, and to some degree, disease suppression.

Cannabis rhizomicrobiome makeup is determined by soil type and plant genotype selection - which implies that the mechanism is the same as for other horticultural crops - and consists of both epi- and endophytic bacterial microflora. Yield and

quality increases have been achieved by inoculating potted cannabis plants with different consortia of PGPR in controlled environments. However, more research is needed, and it should be bacteria strain, plant variety and cultivation system specific - since all of these parameters influence the outcome. Disease suppression through ISR and antagonism has not been studied specifically for cannabis, but since the most common pathogens - *Pythium aphanidermatum*, *Fusarium oxysporum*, *Botrytis cinerea* and *Rhizoctonia solanum* - have been thoroughly studied in other soilless crops with positive results, they should be evaluated experimentally for cannabis production as well.

References

Jin, D., Jin, S.X. and Chen, J. (2019) Cannabis Indoor Growing Conditions, Management Practices, and Post-Harvest Treatment: A Review. American Journal of Plant Sciences , 10, 925-946. https://doi.org/10.4236/ajps.2019.106067

Caplan, D., Dixon, M. and Zheng, Y. (2017) Optimal Rate of Organic Fertilizer during the Vegetative-Stage for Cannabis Grown in Two Coir-Based Substrates. HortScience , 52, 1307-1312. https://doi.org/10.21273/HORTSCI11903-17

Caplan, D., Dixon, M. and Zheng, Y. (2017) Optimal Rate of Organic Fertilizer during the Flowering Stage for Cannabis Grown in Two Coir-based Substrates. HortScience, 52, 1796-1803. https://doi.org/10.21273/HORTSCI12401-17

Caplan, D., Dixon, M. and Zheng, Y. (2019) Increasing Inflorescence Dry Weight and Cannabinoid Content in Medical Cannabis Using Controlled Drought Stress. HortScience, 54, 964-969. https://doi.org/10.21273/HORTSCI13510-18

Cockson, P., Landis, H., Smith, T., Hicks, K. and Whipker, B.E. (2019) Characterization of Nutrient Disorders of Cannabis sativa. MDPI Applied Sciences, 9, 4432. https://doi.org/10.3390/app9204432

Backer, R., Schwinghamer T., Rosenbaum P., McCarty V., Eichhorn., Bilodeau S., Lyu D., Ahmed MB., Robinson G., Lefsrud M., Wilkins O. and Smith DL, (2019) Closing the Yield Gap for Cannabis: A Meta-Analysis of Factors Determining Cannabis Yield. Frontiers in Plant Science, 10, 495. https://doi.org/10.3389/fpls.2019.00495

Happyana, N., Agnolet, S., Muntendam, R., Van Dam, A., Schneider, B., Kayser, O. (2013) Analysis of cannabinoids in laser-microdissected trichomes of medicinal Cannabis sativa using LCMS and cryogenic NMR. Phytochemistry, 87, 51-9.

Aizpurua-Olaizola, O.S. (2016) Evolution of the Cannabinoid and Terpene Content during the Growth of Cannabis sativa Plants from Different Chemotypes. Journal of Natural Products , 79, 324-331. https://doi.org/10.1021/acs.jnatprod.5b00949

Clarke, R. and Merlin, M. (2016) Cannabis: Evolution and Ethnobotany. University of California Press, Oakland, CA.

Thomas, B.F. and ElSohly, A.M. (2016) The Analytical Chemistry of Cannabis: Quality Assessment, Assurance, and Regulation of Medicinal Marijuana and Cannabinoid Preparations. Elsevier Inc & RTI International, Amsterdam, Netherlands.

Raviv, M.J., Lieth, H. and Bar-Tal, A. (2019) Soilless Culture: Theory and Practice. Academic Press, United Kingdom.

Bedrocan Primer for Medicinal Cannabis (2018): https://bedrocan.com/wp-content/uploads/a-primer-to-medicinal-cannabis_lr.pdf/ Downloaded 2019-12-05

Ruzzi, M. & Aroca, R. (2015) Plant-growth promoting bacteria act as biostimulants in horticulture. Scientia Horticulturae, 196, 124-134. http://dx.doi.org/10.1016/j.scienta.2015.08.042/

Backer, R., Rokem, JS., Ilangumaran., G., Lamont J., Praslickova D., Ricci E., Subramanian, S. and Smith, DL. (2018) Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. Frontiers in Plant Science, 9 , 1473. http://10.3389/fpls.2018.01473/

Pandey, A., Tripathi, A., Srivastava, P., Choudhary, K.K., Dikshit, A. (2019) Plant-growth promoting microorganisms in sustainable agriculture. Role Of Plant-Growth Promoting Microorganisms In Sustainable Agriculture And Nanotechnology. Woodhead Publishing, Duxford.

Turner, R.T., Euan, J.K. & Poole, S.P.. The Plant Microbiome. (2013) Genome Biology, 14, 209. http://genomebiology.com/2013/14/6/209/

Compant, S., Samad, A., Faist, H. & Sessitsch, A. A Review On The Plant Microbiome: Ecology, Functions And Emerging Trends In Microbial Applications. (2019). Journal of Advanced Research, 19, 29-37. https://doi.org/10.1016/j.jare.2019.03.004

Mia, A.B., Shamsuddin, Z.H., Wahab, Z., Marzhia, M. High-yielding and quality banana production through plant-growth promoting rhizobacterial inoculation. (2005) Fruits, 60, 179-185. https://10.1051/fruits:2005024/

Liu, W., Sutton, J.C., Grodzinsky, B., Kloepper, J.W. & Reddy, M.S. (2007) Biological Control of Pythium Root Rot of Chrysanthemum in Small-scale Hydroponic Units. Phytoparasiticum, 35, 159-178.

Conant, T.R., Walsh, P.R., Walsh, M., Bell, W.C., Wallenstein, D.M. (2017) The Effect of a Microbial Biostimulant, Mammoth P, on *Cannabis sativa* Bud Yield. Journal of Horticulture, 4, 1. http://0.4172/2376-0354.1000191/

Winston, M.E., Hampton-Marcell, J., Zarraonaindia, I., Owens, S.M. & Moreau, C.S., (2014) Understanding Cultivar-Specificity and Soil Determinants of the Cannabis Microbiome. PLoS ONE, 9, 6. http://10.1371/journal.pone.0099641/

Punja, Z.K., Collyer, D., Scott, C., Lung, S., Holmes, J., & Sutton, D. (2019) Pathogens and Molds Affecting Production and Quality of Cannabis sativa L. Frontiers in Plant Science. 10,1120. http://10.3389/fpls.2019.01120/

Steenhoudt, O. & Vanderleyden, J. (2000) Azospirillum, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiology Reviews, 24, 487-506.

Punja, Z.K., (2018) Flower and foliage-infecting pathogens of marijuana (Cannabis.sativa L.) plants, Canadian Journal of Plant Pathology, 40, 4, 514-527. http://10.1080/07060661.2018.1535467/

Punja, Z.K. & Rodriguez, G. (2018) Fusarium and Pythium species infecting roots of hydroponically grown marijuana (Cannabis.sativa L.) plants. Canadian Journal of Plant Pathology, 40, 4, 498-513. http://10.1080/07060661.2018.1535466/

Marschner, P., Crowley, D. & Yang, H.C. (2004) Development of Specific Rhizosphere Bacterial Communities In Relation To Plant Species, Nutrition and Soil Type. Plant and Soil, 261, 199-208.

Taginhasam, M. & Jabaji, S. (2020) Cannabis Microbiome and The Role of Endophytes in Modulating the Production of Secondary Metabolites. MDPI Microorganisms, 8, 355. http://10.3390/microorganisms8030355/

Philippot, L., Raiijmakers, M.J., Lemanceau, P. & van der Putten, H.W. (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nature Reviews Microbiology, 11.

Akinrinlola, R., Yuen, G.Y., Drijber, A.R. & Adesemoye, O.E. (2018) Evaluation of Bacillus Strains for Plant Growth Promotion and Predictability of Efficacy by In Vitro Physiological Traits. International Journal of Microbiology, http://10.1155/2018/5686874/

Botta, A.L., Santacecilia, A., Ercole, C., P., Cacchio & Del Gallo, M. (2013). In vitro and in vivo inoculation of four endophytic bacteria on Lycopersicon esculentum. New Biotechnology, 30, 6.

Pillay, V.K. & Nowak, J. (1997). lnoculum density, temperature, and genotype effects on in vitro growth promotion and epiphytic and endophytic colonization of tomato (Lycopersicon esculentum L.) seedlings inoculated with a pseudomonad bacterium. Canadian Journal of Microbiology. 43, 354-361.

Lareen, A., Burton, F., & Schäfer, P. (2016) Plant root-microbe communication in shaping root microbiomes. Plant Molecular Biology, 90, 575–587.

Lyu, D., Backer, R., Robinson, W.G. & Smith, D.L. (2019) Plant Growth-Promoting Rhizobacteria for Cannabis Production: Yield, Cannabinoid Profile and Disease Resistance. Frontiers in Microbiology, 10, 1761. http://10.3389/fmicb.2019.01761/

Ilangumaran, G. & Smith, D.L. (2017) Plant Growth Promoting Rhizobacteria in Amelioration of Salinity Stress: A Systems Biology Perspective. Frontiers in Plant Science, 8, 1768. http://10.3389/fpls.2017.01768/

Romano, I., Ventorino, V. & Pepe, O. (2020) Effectiveness of Plant Beneficial Microbes: Overview of the Methodological Approaches for the Assessment of Root Colonization and Persistence. Frontiers in Plant Science, 11, 6. http://10.3389/fpls.2020.00006/

Walker, T.S., Bais, P.H., Grotevold, E. & Vivanco, M.J. (2003). Root Exudation and Rhizosphere Biology. Plant Physiology, 132.

Liu, L., Kloepper, J.W. & Tuzun, S. (1995). Induction of Systemic Resistance in Cucumber Against Fusarium Wilt by Plant-Growth Promoting Rhizobacteria. The American Phytopathological Society, 85,6, 695-698.

Choudhary, K.D., Prakash, A., & Johri, B.N. (2007). Induced systemic resistance (ISR) in plants: mechanism of action. Indian J. Microbiol, 47, 289–297.

Jones, D.L., Nguyen, C., & Finlay, R.D. (2009). Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant and Soil, 321, 1, 5-33.

Danhorn, T. & Fuqua, C. (2007). Biofilm formation by plant-associated bacteria. Annual Reviews in Microbiology, 61, 401–22.

van Loon, L.C. (2007). Plant responses to plant growth-promoting rhizobacteria. European Journal of Plant Pathology, 119, 243–254. http://10.1007/s10658-007-9165-1/

Berg, G. & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiological Ecology, 68, 1–13.

Nosheen, A., Bano, A. & Ullah, F. (2016). Bioinoculants: A sustainable approach to maximize the yield of Ethiopian mustard (Brassica carinata L.) under low input of chemical fertilizers. Toxicology and Industrial Health, 32, 2, 270–277.

Vacherone, J., Desbrosses, G., Bouffaud, M., Touraine, B., Loccoz-Moenne, Y., Muller, D., Legendre, L., Wisniewski-Dyé, F. & Pringent-Combaret, C. (2013). Plant-Growth Promoting Rhizobacteria and Root System Functioning. Frontiers in Plant Science, 4, 356. http://10.3389/fpls.2013.00356/

Pagnani, G., Pellegrini, M., Galieni, A., D'Egidio, S., Matteucci, F., Ricci, A., Stagnari, F., Sergi, M., Lo Sterzo, C., Pisante, M. & Del Gallo, M. (2018). Plant growth-promoting rhizobacteria (PGPR) in Cannabis sativa 'Finola' cultivation: An alternative fertilization strategy to improve plant growth and quality characteristics. Industrial Crops & Products, 123, 75-83. https://doi.org/10.1016/j.indcrop.2018.06.033/

Baas, P., Bell, C., Mancini, M.L., Lee, M.N., Conant, R.T. & Wallenstein, M.D. (2016). Phosphorus mobilizing consortium Mammoth PTM enhances plant growth. PeerJ, 4, 2121. http://10.7717/peerj.2121/

Yildirim, E., Turan, M., Dursun, A., Ekinci, M., Kul, R., Parlakova-Kaargoz, F., Donmez, F.N. & Kitir, N. (2016) Integrated Use of Nitrogen Fertilization and Microbial Inoculation: Change in the Growth and Chemical Composition of White Cabbage. COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS, 47, 19, 2245–2260. http://dx.doi.org/10.1080/00103624.2016.1228955/

Sultana, U., Desai, S. & Reddy, G. (2016). Successful colonization of roots and Plant growth promotion of sorghum (Sorghum bicolor L.) by seed treatment with Pseudomonas putida and Azotobacter chroococcum. World Journal of Microbiology, 3, 1, 43-49.

Jetiyanon, K. & Kloepper, J.W. (2002). Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. Biological Control, 24, 285–291.

García, J.A.L., Probanza, A., Ramos, B., Palomino, M. & Gutiérrez Mañero, F.J. (2004). Effect of inoculation of Bacillus licheniformis on tomato and pepper. Agronomie EDP Sciences, 24, 4, 169-176.

Liu, K., Hu, C-H. & Kloepper, J.W. (2018). Mixtures of Plant-Growth Promoting Rhizobacteria Enhance Biological Control of Multiple Plant Diseases and Plant-Growth Promotion in the Presence of Pathogens. Plant Disease, 102, 67-72.

Benizri, E., Baudoin, A. & Guckert, A. (2001). Root Colonization by Inoculated Plant Growth-Promoting Rhizobacteria. Biocontrol Science and Technology, 11, 5, 557-574.

Hassan, M.K., McInroy, J.A. & Kloepper, J.W. (2019). The Interactions of Rhizodeposits with Plant Growth-Promoting Rhizobacteria in the Rhizosphere: A Review. Agriculture, 9:142. http://10.3390/agriculture9070142/

Dazzo, F.B., Garoutte, A. & Hartmann, A. (2019). Rhizosphere. Encyclopaedia of Microbiology, 4th edition.

Poupin, M.J., Timmermann, T., Vega, A., Zuñiga, A. & González, B. (2013). Effects of the Plant Growth-Promoting Bacterium Burkholderia phytofirmans PsJN throughout the Life Cycle of Arabidopsis thaliana. PLoS ONE, 8, 7. http://10.1371/journal.pone.0069435/

Khalil, S. & Alsanius, B. (2001). Dynamics of the indigenous microflora inhabiting the root zone and the nutrient solution of tomato in a commercial closed greenhouse system. Gartenbauwissenschaft, 66, 4, 188–198.

Yang, Y., Wang, N., Guo, X., Zhang, Y. & Ye, B. (2017). Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. PLoS ONE, 12, 5. https://doi.org/10.1371/journal.pone.0178425.

Paulitz, T.C., Zhou, T. & Rankin, L. (1992). Selection of rhizosphere bacteria for biological control of Pythium aphanidermatum on hydroponically grown cucumber. Biological Control, 2, 3, 226-237.

Duijff, B.J., Meijer, J.W., Bakker, P, A.H.M. & Schippers, B. (1993) Siderophore-mediated competition for iron and induced resistance in the suppression of fusarium wilt of carnation by fluorescent Pseudomonas spp. Netherlands Journal of Plant Pathology 99, 277–289. https://doi.org/10.1007/BF01974309

Nie, P., Li, X., Wang, S., Guo, J., Zhao, H. & Niu, D. (2017) Induced Systemic Resistance against Botrytis cinerea by Bacillus cereus AR156 through a JA/ET- and NPR1-Dependent Signaling Pathway and Activates PAMP-Triggered Immunity in Arabidopsis. Frontiers in Plant Science, 8, 238. http://10.3389/fpls.2017.00238/

Altaf-Musheer, M. & Ahmad, I. (2017). In vitro and In vivo biofilm formation by Azotobacter isolates and its relevance to rhizosphere colonization. Rhizosphere, 3, 1, 138-142.

Vallance, J., Déniel, F., Floch, G., Guérin-Dubrana, L., Blancard, D. & Rey, P. (2011) Pathogenic and beneficial microorganisms in soilless cultures. Agronomy for Sustainable Development, 31, 1, 191-203. http://10.1051/agro/2010018/

Hazekamp A. (2016). Evaluating the Effects of Gamma-Irradiation for Decontamination of Medicinal Cannabis. Frontiers in Pharmacology, 7, 108. https://doi.org/10.3389/fphar.2016.00108

https://www.britannica.com/science/r-selected-species

https://laegemiddelstyrelsen.dk/en/special/medicinal-cannabis/companies/pilot-programme/cultivation-agents