The potential to use rhizobacteria within agricultural innovations instead of chemical inputs

-taking quality and quantity of the crop into consideration

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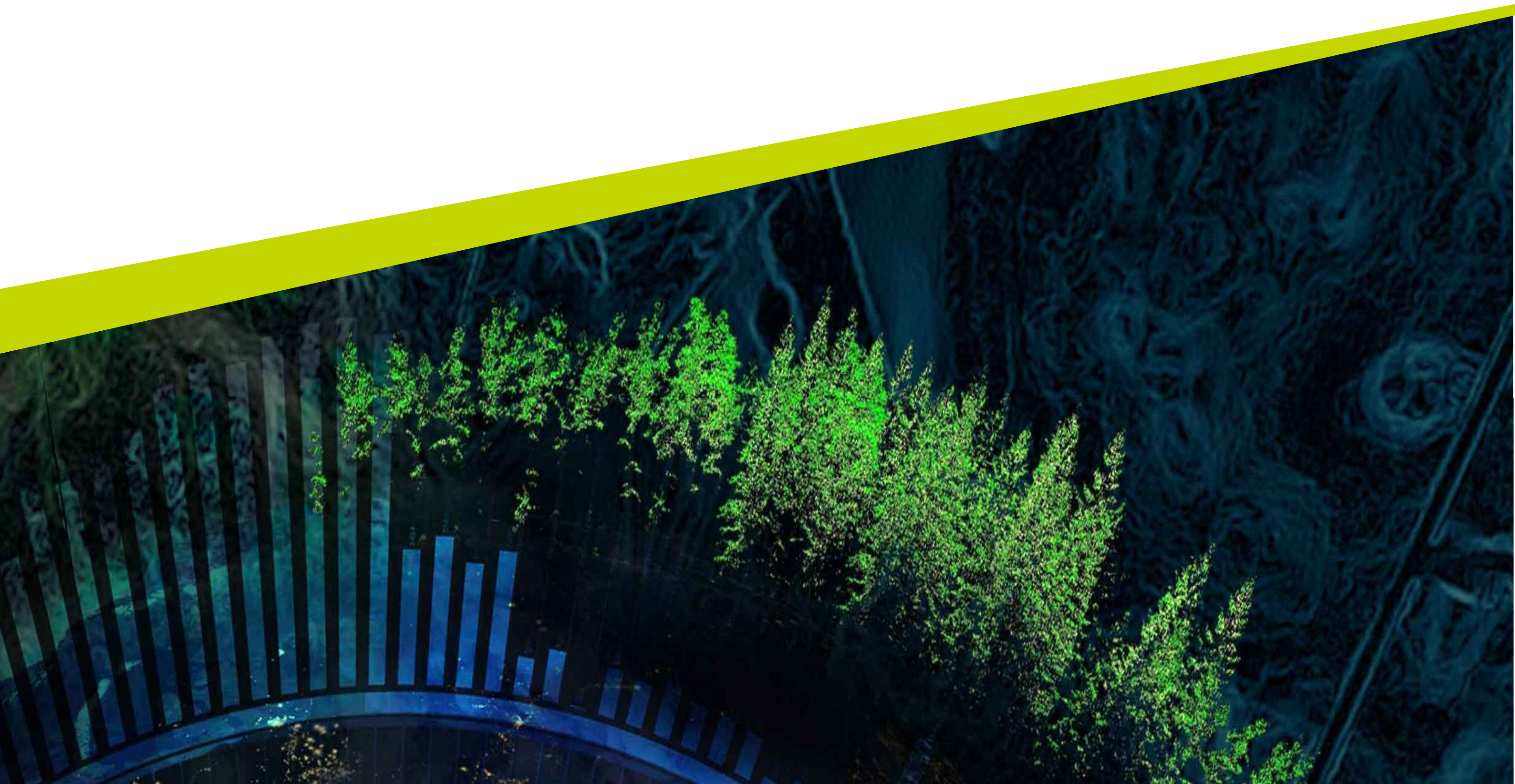

The Potential to use rhizobacteria within agricultural innovations instead of chemical inputs -taking quality and quantity of the crop into consideration

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Abstract

Plant growth promoting rhizobacteria (PGPR) are soil bacteria that occur naturally, colonize the roots of plants and can promote plant growth. PGPR isolated and used as inoculum to seed or plants is mostly marketed as a growth stimulant because it facilitates registration and reduces costs, even though PGPR may also serve as biocontrol products. An example of its effect as a growth stimulant is that it can stimulate an increased growth of lateral roots, which in itself leads to e.g. increased water and mineral uptake and thus a stronger plant. Quantity and quality effects of crops harvested on PGPR treated material will be addressed in this work and will be focussed on food crops. Possible conflicts with organic production will also be addressed. Use in climates like Swedish conditions will be the main focus, however comparisons will be made between Sweden and a global scale. It will be discussed when PGPR will be used to a greater extent, what is required to get there and what knowledge gaps there are. This literature study was conducted by collection of data and search of scientific literature in databases. In conclusion, several of the studies have shown that both the quality and quantity are not only maintained with the use of PGPR, but also improved.

Keywords: Biocontrol, Biofertilizer, Biopesticide, Biostimulant, Plant growth promoting rhizobacteria, PGPR, Quality, Rhizosphere Microbiome , Yield

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Abbreviations

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
IAA	Indole acetic acid
ISR	Induced systemic resistance
PGPR	Plant Growth Promoting Rhizobacteria
TA	Total acidity
TSS	Total soluble solids
WHC	Water holding capacity

1. General information about Plant-Growth Promoting Rhizobacteria

One of the most important groups of microorganisms, that may have one of the biggest roles when it comes to stimulation of plant growth and control of plant stress, is plant-growth promoting rhizobacteria (PGPR). PGPR has been proven in several studies to greatly increase root development, water and nutrient uptake as well as utilization of plants, along with improving seed germination (Akhtar et al. 2010). PGPR can be added to seeds or to soil as soil amendments with the intention to support plants as a biostimulant. Multiple studies have shown numerous improvements of the soil including texture, fertility, long-term maintenance of soil health and crop yield. An example of when PGPR can lead to an increase in yield is when auxin is produced by the PGPR. In a study done by Irene Kuiper et al., (Kuiper et al. 2003) *Pseudomonas fluorescens* WCS365, which has the ability to generate auxin, was used on vegetables to study if the yield would increase along with the increased amount of auxin the crop would have available. Among the vegetables which had seeds inoculated with *P. fluorescens* WCS365, cucumber, tomato, sweet pepper as well as radishes were included. This particular strain did not lead to a root or shoot weight increase of any of the other vegetables present, whilst the root weight from the radish showed a quite significant increase compared to that of the control (Kuiper et al. 2003). In another study done by Zhang et al., where *Bacillus subtilis* GB03 was applied on *Arabidopsis thaliana*, it was found that both the chlorofyll content and the efficiency of photosynthesis increased because of abscisic acid sensing as well as the modulation of endogenous signaling of glucose. Thus proving that the bacterium had an important role when it comes to the plants ability to acquire energy (Lugtenberg et al. 2009).

The microorganisms of PGPR often inhibits the possible growth of plant pathogens as well as invasions from pathogenic fungi such as *Penicillium*, *Rhizoctonia* and *Fusarium*. PGPR can also provide protection against a range of bacteria such as *Bacillus spp*. and *Pseudomonas spp*. (Lagerqvist 2014). By producing growth hormones the rhizobacteria can directly stimulate plant growth, as well as improve the uptake of many nutrients (Akhtar et al. 2010).

Many soil amendments can pose a threat to both the agro-ecosystem and human health. Some can bring pollutants such as heavy metals or potential human pathogens. Compared to e.g. animal slurry or fly ash however, PGPR is one of the soil amendments that has a lower risk of these pollutants occurring along with for e.g biochar. This is one of the reasons the application of PGPR has increased in interest lately (Lugtenberg et al. 2009). When applied to the roots of a plant, PGPR can stimulate the growth of crops by improving mineral nutrition as well as increase the tolerance for both abiotic stresses such as salinity and drought, and biotic stresses such as plant pathogens (Lyu et al. 2019).

Continuous use of chemical pesticides disrupt the biological balance of the soil, increase environmental pollution as well as the fact that the chemical products in question leads to an increased production cost for agricultural products. This is one of the reasons to choose a biopesticide such as PGPR instead. When registering PGPR, companies commonly market it as a biostimulant that may have the effect of a biopesticide since it is much cheaper than registering it as a biopesticide. One type that can be used is *Pseudobacteremia-2*, which can provide protection against insect pests and plant diseases. This strain is built up of living cells of rhizobacteria from the genus *Pseudomonas aureofaciens* BS1913 as well as a complex of biologically active substances. Another favorable *Pseudomonas* strain acting as a PGPR is *Pseudomonas chlororaphis* GP72. This particular strain is a non-pathogenic biocontrol agent with a genome that is entirely sequenced (Shen et al. 2013).

When referring to plants and their ability to grow and fight disease, it is important to think about the cost of resistance model. This means that the plant has to divide its energy to focus either on growth or protection. If too much energy is spent on protection, the growth will be stunted and if all the energy is put into growth, the plant will be vulnerable to disease and pest attacks, such as insects or fungi. PGPR disengages that model which makes it possible for the plant to prioritize both growth and protection as well as giving both sides more energy than what was possible before. The presence of PGPR increases the area of the root system providing both more space for the PGPR to live on and more resources both for growth and storage, enabling that the leafy parts of the plant can grow much bigger. This area increase enables an increased ability to perform photosynthesis, where all of the excess energy can be sent down to be stored in the roots. This leads to the roots being able to take up more water, energy and nutrients to stimulate the growth as well as assisting the roots in anchoring deeper down in the soil.

There are some strains of PGPR that have the ability to form what is called a biofilm. Biofilms are by definition “a thin but robust layer of mucilage adhering to a solid surface and containing a community of bacteria and other microorganisms.” (Oxford Languages n.d). The strains of PGPR that can produce biofilm are e.g. *P. fluorescens, Pseudomonas aeruginosa, B. subtilis* and *Paenibacillus polymyxa*. The biofilm is used by the bacteria as a survival strategy, as it can act as a shield in hostile environments. Biofilms can be found on both abiotic and biotic surfaces since it is made of bacteria and also has a very diversified configuration, it can range from tower to flat or simple to complex, as well as having very distinct physiological structures. Biofilms that have been formed in plant-microbe interactions have the ability to modulate not only the microbial behavior but also the growth of the plant. When a plant is actively growing it will use its root tissues to release a higher amount of exudation into the soil surrounding it. The biofilm in turn that is present around the roots will react and be impacted by the nutrients that are in the root exudates (Rafique et al. 2015).

Biofilm is of importance when it comes to agriculture since it can provide protection against the environment as well as assist in nutrient availability. When a bacteria is residing inside the biofilm, it is protected, this is because of the extracellular polymeric substances (EPS) and other proteins in the biofilm. The biofilm has restricted access when it comes to which antimicrobial agents that can be let through, this is because of the matrix of the EPS, which acts as an anion exchanger. This also means that there is a limitation on which other compounds surrounding the biofilm can enter by diffusion (Rafique et al. 2015). Within this matrix, the coordination and production as well as the release of compounds important to the plant, such as nutrition, induced systemic resistance (ISR) and growth promotion. The matrix can therefore be considered to be a sort of mutualistic interference, which the plant host can use to exchange e.g. chemical information as well as exchange solutes (Pieterse et al. 2014). When water is present in relation to biofilm, an aqueous phase is established. Water being present also aids in the exchange of metabolites and toxic metabolite removal, as well as the ability to regulate nutrient availability (Rafique et al. 2015). For *B. subtilis*, the formation of biofilm has proven to be crucial for the bacteria to be able to colonize roots (Pieterse et al. 2014).

1.1 Aim

The purpose of this literature study is to investigate whether the quality and quantity of the harvest can be maintained when using PGPR as biostimulants in crop cultivation instead of chemical inputs (conventional). Quantity and quality effects of crop harvested on PGPR treated soil, seeds and plants will be addressed in this work and will be focussed on food crops. Possible conflicts with organic production will also be addresses. Use in climates like Swedish conditions will be the main focus, however comparisons will be made between Swedish and global conditions. It will be discussed when PGPR will be used to a greater extent, what is required to get there and what knowledge gaps there are.

1.2 Method

This literature study was conducted by collection of data and search of scientific literature in databases. Examples of these are *Scopus, National Center* for *Biotechnology Information* (NCBI), *Google Scholar, PubMed* and *Researchgate.* Information from various actors (authorities, companies, interest organisations, etc.) on the internet were also collected. The following keywords are some of the search terms used: *Biocontrol, Biofertilizer, Biopesticide, Biostimulant, Plant growth promoting rhizobacteria, PGPR, Quality, Rhizosphere Microbiome , Yield.*

1.3 Direct Modes of Action

When in the absence of pathogens, PGPR can act directly, such as enhancing plant growth. Direct mechanisms of PGPR may include biological nitrogen fixation, siderophore production, phosphate solubilization, production of phytohormones such as auxin, gibberellin and cytokinin. When PGPR provides the plant with resources that it needs, it gives the plant the ability to gain a higher yield (Jeyanthi et al. 2018). Siderophores are compounds secreted by PGPR and are advantageous for plants. They have the ability to act as biosensors, chelating agents, biocontrols and bioremediation as well as having a big role in plant growth enhancement and weathering of soil minerals (Ahmed et al. 2014). The increased availability of iron that the siderophores provide the plants with can aid the plant when it is exposed to stress from its surroundings (Midali et al. 2022). This leads to sturdier plants and the amount of crops that can survive harsh weather conditions increases, which is good for farmers who live in places where the weather can vary greatly in short periods of time.

Certain PGPR can be used to lower the amount of chemical nitrogen fertilizer needed for growing crops such as maize. In a study where maize was inoculated with PGPR, the amount of nitrogen that the crop could take up increased significantly in all parts of the plant (Kuan et al. 2016). PGPR with the ability to fixate nitrogen is one of the keys to maintaining a fertile soil. Microorganisms can use two different paths when it comes to biological nitrogen fixation, either by non-symbiotic nitrogen fixing or by symbiotic nitrogen fixing. Strains from the non-symbiotic part include *Acetobacter*, *Azoarcus* and *Azotobacter* while strains belonging to the symbiotic side include *Rhizobium*, *Sinorhizobium* and *Bradyrhizobium* that have a more limited host range. Apart from nitrogen, phosphorus is one of the most essential nutrients for plants. Depending on several factors, such as pH, temperature, what microbes are available in the soil or what the moisture level is, the ability of plants to take up phosphorus is affected. The PGPR can lower the rhizospheric pH by the secretion of organic acids, which in turn releases the phosphorus, and makes it available to the plants. PGPR can assist in the availability of both organic and inorganic phosphorus, by using for example microbial turnover, and can facilitate the organic phosphorus mobility or by directly mineralising and solubilising inorganic phosphorus (Jeyanthi et al. 2018).

Depending on the type of PGPR used, different phytohormones can be produced. When the PGPR produces gibberellins and indole acetic acid (IAA) in rhizospheric soil, they are responsible for the increase in the root's surface area and boosted the number of lateral root tips. This helps strengthen the structure of the roots, and leads to the possibilities for PGPR colonization to increase and the bacteria found in the rhizospheric soil decreases the accumulation of ethylene (Riaz et al. 2020). Gibberellins are also known for their modification of the morphology of plants, such as extensions of stem tissue, though they can also promote root growth by their ability to regulate root hair abundance. Gibberellins are involved in other processes that are essential for plant development as well, such as seed germination, flowering and the setting of fruit. They are usually found in bacteria inhabiting the root system of plants, such as *Agrobacterium, Bacillus, Pseudomonas and Rhizobium* (Jeyanthi et al. 2018).

IAA assists plant growth and development in many ways, one being its big role when it comes to the plant-growth promoting abilities of rhizobacteria. The biosynthesis of IAA has been proven to be related to the enhanced proliferation of roots for various strains of PGPR. When PGPR is used to inoculate plants, lateral roots along with root hairs increase while the primary root in question often becomes shortened in length. IAA is also one of the reasons the root exudate increases when PGPR is used, this is a result of IAA loosening the cell walls of the plant (Jeyanthi et al. 2018).

Another class of phytohormones are cytokinins, which is known for being in charge of cell enlargement and division. Several strains of PGPR have the ability to produce cytokinins, such as *P. fluorescens, Rhizobium sp., B. subtilis* and *Azotobacter sp.* to name a few. When plants have been provided with cytokinin exogenously, the resulting effects vary from either an enhancement in root development or root hair formation, as well as the possibility of an enhanced cell division, shoot initiation or the inhibition of root elongation (Jeyanthi et al. 2018).

PGPR strains that produce abscisic acid (ABA) have a big role in the ability to handle water-stressed environments. By closing of the stomata ABA can combat stress, this is crucial for plant survival in weather conditions with high temperatures and little to medium precipitation. PGPR strains known to produce ABA are *Azospirillum sp.*, *Bradyrhizobium japonicum* and *Rhizobium sp*. (Jeyanthi et al. 2018).

Ethylene is not only known as a regulator of plant growth but also as a stress hormone. Essential to plant development and growth, the concentration of ethylene in a plant's root tissues can lead to it having varying effects. If the concentration of ethylene is too high, it can have a negative effect and cause harm to the plant. Negative effects that are the result of too high ethylene levels are inhibited stem and root growth, defoliation as well as reduced crop yield and performance as a result of premature senescence. When in stressful conditions, such as drought, heavy metals, water logging or salinity, the amount of ethylene in the plant increases significantly, which impacts its growth. Many PGPR have the ability to reduce the amount of ethylene that is formed by the use of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Jeyanthi et al. 2018). With this enzyme the PGPR has the capability to prevent some of the ethylene effects or at least lessen them.

1.4 Indirect Modes of Action

Indirect plant growth promotion includes some of PGPRs actions as a biocontrol and acts by decreasing level of disease, priming of induced systemic resistance (ISR), antibiosis and competing for nutrients and niches (Lugtenburg 2009).

Studies have been done for e.g. tomatoes, which is a plant that can be affected by a number of bacterial diseases. For example, the bacterial disease caused by C*lavibacter michiganensis ssp. michiganensis* can lead to up to a 100% loss of the crop yield. It has however been reported that when treated with *B. subtilis (Quadra 136 and 137) and Trichoderma harzianum (R), Rhodosporidium diobovatum (S33)* the disease occurred with less frequency (in greenhouse conditions). Studies investigating PGPRs ability to suppress fungal disease have also been made in an article of Girish & Umesha (2005), implying less frequency of fungal disease when applying PGPR. Brown rot caused by *Moniliana laxa* (Ehr.) can destroy the whole annual crop of apricots and *Burkholdria gladii OSU 7* have been shown to have potential to be able to suppress brown rot of apricots (Altindag et al. 2006).

Parasitic nematodes can also cause big losses of crop yield, but for example *B. spp. and Pseudomonas spp.* have shown capability to counteract nematodes. PGPR has especially risen in interest as a more environmentally friendly alternative instead of nematicides, since the usually used chemical products have shown several negative effects on public health and environmental safety (Kaymak 2011).

1.4.1 The Biocontrol Mechanisms

The biocontrol ability of PGPR includes multiple mechanisms, such as signal interference, predation and parasitism along with improving ISR. Signal interference involves the degradation of homoserine lactones (AHLs), which have shown to be a part of forming biofilm. Having an impact on formation of biofilm could ease biocontrol by letting PGPR act on the roots while also inhibiting other microorganisms. The correlation between use of PGPR and the mechanism behind predation and parasitism is not fully established, but studies have shown enzymatic destruction of the fungal cell wall as a mechanism for predation and parasitism by fungi.

The biocontrol action of priming ISR of the plant means that the PGPR sensitizes the plant and raises a sensitivity threshold which helps the plant to faster recognize threats during stress. The exact mechanisms behind the priming of ISR is not concluded and need further research (Pieterse et al. 2014). However, the transcription factor MYB72 in root cells is known to regulate the initiation in the roots of the plant and the transcription factor MYC2 regulates systemic effects in the other tissues of the plant (Roeland 2012). Examples of PGPR that have been shown to induce systemic resistance in e.g. radish and cucumber are specific strains of *Pseudomonas* (Van Loon & Bakker 2005).

1.4.2 Bacteria Indigenous to soil

Although the PGPR has to compete with other bacteria for root colonization and for nutrients in the rhizosphere, those already present bacterias can be beneficial for the PGPR too. The interactions between other microbes facilitates as nutrient sources since some microbes can convert plant exudates in a favorable form to be facilitated by another microbe (Bhattacharyya & Jha 2011).

1.5 Co-Applying PGPR with Biochar

PGPR can work as a biostimulant and biocontrol by itself, but recent studies shows that co-applying PGPR with other soil amendments such as biochar displayed a good potential for improved soil quality and agronomic productivity. The biochar increases the nutrient status in the soil and creates a pleasant environment for the PGPR to grow in. This leads to better efficiency of the PGPR to act on the plant by stimulating growth and resistance. Studies have shown that the mineral nutrient content of the soil has increased when co-applying biochar and PGPR, compared to application of them separately (Malik et al. 2022).

1.5.1 Enhanced Water Holding Capacity of Soil

Biochar has a large surface area-to-volume ratio, which gives it the potential of improving water holding capacity (WHC). PGPR on the other hand has no proven capability of improving WHC, but rather improves a crop plant's drought tolerance. When co-applied, studies have shown an increased soil WHC of around 18-24% compared to application of them separately. Even though there are results showing this, the underlying explanation is yet to be explored. The observed increased soil WHC is however something that, along with improved pH and nutrient availability, is believed to lead to a bigger and diverse microbial biomass. Some studies have also implied that the effects of co-applying biochar and PGPR, ease the nodulation process and enhance a rhizobium symbiotic performance. More than the potential of co-applying these two is yet to be further researched and long-term field experiments should be done to be able to explore the co-application further (Malik et. al. 2022).

1.6 Yield

There are some PGPR strains that can produce quite large amounts of gibberellins, which in turn can result in a higher yield in plants such as pepper, grapevine and tomatoes. In the case of pepper, a strain of PGPR called *Serratia nematodiphila* can increase the chances of survival when the pepper is exposed to stress caused by low temperatures. This means that the yield will increase since more plants have gained the ability to survive and produce fruit when this particular PGPR is used. The strain *Burkholderia phytofirmans* is a PGPR used to reduce and prevent chilling damage caused by low temperature stress for young grapevines. When it comes to tomatoes, plants inoculated with a combination of *Pseudomonas frederikbergensis* OS261 and *Pseudomonas vancouverensis* OB155 were found to have an increase in the leaf tissues' antioxidant activity as well as being able to handle lower temperatures and low temperature stress (Backer et al. 2018).

The *Azospirillum*, *Azotobacter* and *Pseudomonas* strains have through studies been shown to have great effects on both seed germination and seedling growth. When cereals were inoculated with *Azospirillum,* enhancements could be seen in various plant parameters, such as plant height and leaf size as well as significant increases in the plants biomass (Gholami et al. 2009). Another study done on wheat and straw tested the effect of *Enterobacter* on the yield and growth.When inoculated with *Enterobacter,* the wheat had a yield increase of 7.6 - 14.2% and the straw had a yield increase of 6.8 - 13.6% (Kumar et al. 2014).

BioAgri have a strain of PGPR called *Pseudomonas chlororaphis* MA342 that they use in some of their organic seed dressing. The three seed dressings that have this strain as their active substance are called Cerall, Cedomon and Cedress, and were created with the help of the Swedish University of Agricultural Sciences in Uppsala. Since they all have the same active substance, the resulting advantages are mostly the same, the only difference is to which crop what seed dressing is applied. According to BioAgri, some of the advantages with using Cerall, Cedomon or Cedress are that it can be used in organic production, the active substance is a bacterium that can already be found in the soil, its biodegradable as well as being able to increase the yield of crops. Cerall is currently used for both spring and winter wheat where it acts as a protection against common wheat bunt. Cedomon is used for oats and barley and this seed dressing can help combat leaf spot disease. Lastly Cedress is used to fight diseases in carrots and peas (BioAgri 2021).

1.7 Quality

Regarding the quality of the crop that has been harvested from PGPR treated soil, not a lot of research seems to have been done. But some of the studies published is bringing more attention to the crop quality aspect in application of PGPR as a biostimulant. One study by Nam et al. (2023) evaluates a group of three types of PGPR as potential biofertilizers for strawberries and includes a quality analysis. The analysis of the strawberries grown in PGPR treated soil includes a sensory evaluation, total acidity (TA), total soluble solids (TSS), color (lightness and chroma), and volatile compounds.

The sensory evaluation was based on a blind taste-test with a group of people testing three different groups of strawberries; two groups from PGPR treated soil (one group with 0.24% PGPR and one group with 0.48% PGPR) and one control. No significant differences were seen regarding firmness, sweetness, and tartness nor regarding the overall appearance, texture and flavor.

Regarding TA, there was no significant difference between the three differently treated groups of strawberries. TSS however did differ with one of the groups treated with PGPR. The strawberries treated with a higher concentration of PGPR, had a higher number of TSS compared to the other two groups. Differences in color was also observed in the two groups treated with PGPR, which had a darker colour than the control group of strawberries with an observed higher chroma value. Based on these results, it was concluded the strawberries with darker colour and higher number of TA and TSS were more mature strawberries. The identical period of cultivation therefore implies that the PGPR had a ripening enhancing function for the strawberries.

Volatile compounds giving fruity and sweet flavor were observed being the highest in the group of the strawberries treated with the higher concentration of PGPR (0.48%), being the second highest in the group treated with lower a concentration (0.24%) and being the lowest in the control group.

This study showed ripening enhancing function along with improvements of multiple indicators of quality, such as higher levels of fruitiness and sweetness. Based on this, PGPR is suggested to be able to support plants to improve quality (Nam et al. 2023).

Another study by Song et. al. (2015) investigated the effects in tomatoes and spinach when using PGPR. A field experiment was done and based on using vermicompost of three levels (none, low and high), with and without PGPR.

The result showed increased concentrations of nitrate, vitamin C, soluble solids, pH, soluble protein and carbohydrate whether or not PGPR was applied. However, when combined with PGPR, a decrease in nitrate concentration was observed. The result also showed that when applying PGPR, the vitamin C level of tomato increased by 12.4% in the crop from soil with low doses of vermicompost, and increased by 27.5% in the crop from soil with high doses. Soluble protein of spinach also had an increase by 24.5% in the crop from soil with low doses and an increase by 27.5% in the crop from soil with high doses.

It was concluded in the study that there was an improvement of crop quality when applying vermicompost especially with PGPR, compared to chemical fertilizer treatment. Use with PGPR showed generally decreased levels of nitrate, increased levels of vitamin C in tomatoes and an increase of protein, vitamin C and carbohydrate in spinach (Song et al. 2015).

1.8 Environmental Conditions

The effectiveness of biofertilizers is something that has been studied a lot since it has shown to vary quite a lot due to several different plausible causes. In trials PGPR have shown clear improvements of growth but when used by farmers in practice the same growth improvements did not always occur. Multiple studies conclude that the main factors deciding the effectiveness are environmental conditions including local soil condition and climate, along with type of crop and biofertilizer used (Symanczik et al. 2020).

1.8.1 Soil Condition

PGPR that support phosphate solubilizing and nitrogen fixation have a higher effectivity in certain soils than in others. Nitrogen fixers show best effectivity in soil with increasing levels of organic carbon and plant-available phosphorus levels higher than 45 kg P/ha. The phosphate solubilizers show a higher effectivity in soil with a relatively high plant-available phosphorus levels of around 25-35 kg P/ha but with a low organic carbon content. If the soil conditions are too deviant from the optimal conditions for PGPR used as a biostimulant the growth stimulus will be lacking (Symanczik et al. 2020).

1.8.2 Climate

PGPRs effectiveness is also affected by climate. In dry regions, tropical and continental climates, PGPR has been shown to be more effective than in other climates. In these climates the soil has a low fertility and low soil organic matter, but this also means a lower amount of indigenous soil microbes which leaves plenty of space for PGPR to thrive. In the drier climates PGPR reduces stress, such as drought in the plant, and with its biostimulant actions the PGPR can secure a high yield (Symanczik et al. 2020).

In Europe, a lot of studies have been performed to further investigate biofertilizers that have the potential to replace parts of the current mineral fertilizer input in the temperate zone. The research-institute FIBL have done studies with the objective to identify which biofertilizers have the ability to best improve the cultivation of certain food crops; tomatoes, maize and wheat. The goal of the studies were also to conclude what are the main factors that decides the effectiveness of the biofertilizers. The conclusion was that the main determining factors are culture system and fertilizer type. In general the improvement of growth by biofertilizers was most noticeable with low organic matter and low soil phosphorus levels. Phosphate solubilizers have been suggested to have an impact on efficiency, for example by PGPR (Symanczik et al. 2020).

1.8.3 Formulation of biofertilizers

The effectiveness of biofertilizers varies depending on in what form they are applied, different types of cultivation and the local environmental conditions. Biofertilizers can come in different formulations with slightly different advantages and disadvantages. There are liquid biofertilizers and two types of solid biofertilizers: carrier-based or freeze-dried. Carrier-based material is easy to produce and therefore cheaper, which makes it less of an investment. On the other hand they have a low shelf-life, are temperature sensitive and have low cell counts which makes them more prone to contamination. Freeze-dried powders, however, have high cell counts, are contamination free and also have a longer shelf-life but are very expensive. Liquid biofertilizers are also quite expensive but do have a longer shelf-life, are temperature tolerant, have high cell counts, are contamination-free and are for all these reasons considered to be the most effective formulation. PGPR is available in all types of formulations (Symanczik et al. 2020).

1.9 Organic Production

In recent years, PGPR has started to become used commercially. Strains such as *Pseudomonas, Bacillus* and *Azotobacter* have proven to have plant production enhancing abilities, making them sought after as biofertilizers (Symanczik et al. 2020). PGPR is considered a healthier option for the soil and environment than chemical fertilizers. Being an organic fertilizer means that instead of affecting the soil's health in a negative way, the PGPR can improve the soil's health and its ability to retain nutrients and water. One reason as to why organic farming is not done on a larger scale is because of its association with lower yields as well as the fact that it is often more expensive. Therefore, the use of chemical fertilizers will continue as long as it is more affordable and predictable for farmers in the long run. In comparison to other organic fertilizers, PGPR can increase the yield of crops, they also possess several beneficial factors for the plants, such as the suppression of pathogens and insect pests as well as the ability to increase root growth. PGPR are often not very host specific, so they can be used on a wide range of hosts to promote growth and protect from disease, though there are some requirements that need to be met. The host plant and the PGPR must have a compatible communication, otherwise the PGPR will find it hard to establish itself and produce biofilm. If that were to happen other bacteria with a stronger compatibility could take over the host. But PGPR is still a very suitable option as a fertilizer for many different species (Kumar et al. 2021).

When it comes to horticulture, biofertilizers are quite commonly applied for a wide variety of reasons, such as their ability to replenish the soil microbial populations. When it comes to bananas and apples, *Azotobacter* strains and the arbuscular mycorrhizal fungus *Glomus*, used either separately or together, have been proven to enhance the fruit quality as well as increase plant growth. These bacteria can also be used for restoration of ecosystems as well as arable farming. Studies have shown that *Azospirillum* in combination with *Pseudomonas* and when applied to cotton plants and maize, increased the yield of grain, while in combination with *Azotobacter* increased the yield of rice, sorghum wheat and pearl millet (Symanczik et al. 2020).

An example of PGPR improving the soil is *Bacillus amyloliquefaciens* and *Pseudomonas putida.* When these two species of PGPR were used, the negative impact of the pesticide glyphosate, imidacloprid and carbendazim decreased, while the health and fertility of the soil was maintained. Though the introduction of a bacterium not native to an ecosystem may have a negative impact on the future of said ecosystem and its further generations. These consequences have not yet been explored to a sufficient degree, but possible effects could include a change in the microbiome of the plant, the way pollinators behave around it (meaning that the plant could become under pollinated), render the plant unable to fight particular diseases as well as how the nutrients of the plants are circulated around (Moore et al. 2022).

In turn, changes in a particular ecosystem may have an impact on neighboring ecosystems, though the effects might be positive or negative. When used on an ecosystem, the PGPR might leave behind a legacy that decides whether it will continue to persevere in said system or not. Further effects from the legacy may include a shift in the temporal dynamics and the interaction network. Change in the horizontal gene transfer from PGPR to the resident taxa can also occur, but for that to happen there would have to be plasmids in the PGPR, which there usually is not, so this is not as likely to happen. PGPR could also leave a legacy behind when it comes to altering the niche, such as changes in the pH of soil, that will then lead to a change in the biogeochemical cycles (Moore et al. 2022). More studies are needed on this subject.

1.10 Discussion

At this time, great variations can be observed when it comes to the results of PGPR used on a large scale. In some cases improvements can be seen on the crops, but occasionally no effect is observed. Why these differences occur has at the moment no simple explanation. Therefore it is important that more research is done on the subject, so that PGPR has a future in being used on a large commercial scale.

Efficiency of using PGPR is an aspect that is important to take into account when investigating the potential of PGPR to replace chemical inputs. Since there are many factors that can affect PGPRs ability as a biofertilizer, such as soil condition, climate and formulation of the PGPR, the effectiveness of different PGPRs can be quite unique for each type. It is not given that one specific strain that shows very positive results as a biofertilizer in one climate with specific soil conditions, will show the same results in another cultivation with different conditions. A very common problem in the investigation of PGPRs effect in cultivations is that clear positive effects can be observed in the laboratory and in greenhouse experiments but when applied by farmers at a bigger scale, the positive effects do not show (Backer 2018).

To ensure efficiency it is important to choose a relevant PGPR for the specific cultivation. Sometimes this means for example using a PGPR product in liquid form over solid form would be necessary to ensure crop with good yield and quality. However, since liquid form often is more expensive, PGPR could also in this example end up not being economically efficient (Symanczik et al. 2020). Solutions for improving PGPR products efficiency includes combination of multiple strains that each have different effects to collectively have strong efficiency e.g. combining one nitrogen fixer, one growth stimulant and one biopesticide. Combining PGPR with other microorganisms such as fungi or algae can also create consortiums with strong efficiency. However, when creating consortiums it is important to combine strains that are compatible and do not compete with each other (Niu et al. 2020). A solution that is being more and more explored is combining PGPR with different organic soil amendments such as biochar, vermicompost, animal slurry or flyash. Soil amendments have shown abilities to create beneficial environments for PGPR, while also maintaining good soil quality for the plants. Being able to recycle these byproducts from the food industry in agriculture would also be sustainable (Malik 2022).

In order for PGPR to be used to a greater extent, more knowledge and research is needed on the subject. There are more factors to study and to understand when it comes to farmers using PGPR´s, and therefore they are a bit more complicated to use than chemical fertilizers. A strain must have compatibility with the crop it is paired with for any effects to take place and the biofilm to form, a strain that is not compatible may even have negative effects on the plant.

Since PGPR can strengthen the plants ability to withstand and survive for example temperature and water stress, it could aid farmers in Sweden when temperatures become too high or low, or when the crops are exposed to drought.

Several studies have shown that both the quality and quantity are not only maintained with the use of PGPR, but also improved.

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