

Bacterial Modes of Action for Enhancing of Plant Growth

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Abstract

The greatest issue affecting the sustainability of broad acre cropping both environmentally and economically is the requirement of fertilizers. These are based on mined phosphorous or other mineral ores, ammonia produced through the Harbour-Bosch process and industrially manufactured potash. As global demand for fertilizers increases, the costs associated with the production for each of these major nutrients increases. Biofertilizers such as plant growth promoting bacteria (PGPB) are a possible biotechnology that could alleviate the need for addition of increasing amounts of fertilizers. These bacteria naturally occur in soils and aggressively colonize around plant roots and have been shown to have plant growth promoting effects. PGPB are known to influence plant growth by various direct and indirect mechanisms; while some can affect plant physiology directly by mimicking synthesis of plant hormones, others increase mineral availability and nitrogen content in soil. Here we review the previously characterized modes of action for enhancement of plant growth by PGPB such as nitrogen fixation, nutrient solubilization and production of auxins and enzymes, as well as discussing more recent proposed modes of action such as secondary metabolites.

Keywords: Plant growth promoting bacteria (PGPB); Biofertilizer; Rhizosphere nitrogen fixation; Siderophores; Secondary metabolites

Introduction

Globally, agriculture relies on supplementing cropped soils with macro and micronutrients sourced from mined ores or industrially produced through energy intensive processes. The major outcome of supplementing crops with these fertilizers is a consistent yield, however, rising costs associated with producing these fertilizers is tipped to reach a critical point in the next 20 years, where the costs will exceed the pay off, in terms of yield value [1,2]. This is particularly the case for phosphorous based fertilizers for example, which are derived from mined phosphorous ore, but will also become an increasing issue for other essential micronutrients: including zinc, cobalt, magnesium, iron and manganese, which are also sourced from ore deposits.

The main source of commercially available phosphate is derived from phosphate rock. This is a finite resource, and both its acquisition through mining and local depletion of this resource is predicted to have dire impacts on the natural environment. Aside from environmental impacts associated with the mining of phosphate rock, there are large economic costs, which are set to rise as phosphate rock deposits become scarce. It is estimated that world phosphate rock deposits will be depleted within the next 50-100 years [1].

The global price of phosphate rock has risen by over 700% since 2007 during a 14 month period [1-3], and its price is expected increase in the next significantly 20 years (along with the costs of ammonia and potash) [1]. While demand continues to increase, the cost of mining phosphate rock is increasing due to declining quality and greater expense of extraction, refinement, and environmental management [1,4]. The increases in phosphate and the other macro-nutrients costs will inevitably drive increases in farming input prices, resulting in parallel increases in the cost of food production worldwide.

Beneficial microbes that exist in soils naturally are known as plant growth promoting bacteria (PGPB). The potential of PGPB to reduce dependence on high levels of fertilizer inputs has gained significant increase in interest over recent years [5-7]. *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Azospirillum*, *Bacillus*, *Alcaligenes* and *Arthrobacter* are genera that have been reported to enhance plant growth [8-10] and these PGPB strains are capable of enhancing plant growth either

directly or indirectly (Figure 1) [5]. The direct promotion of plant growth is based on the bacterial production of phytohormones, enzymes, siderophores or amino acids [5]. The indirect enhancement of plant growth promotion by bacteria is largely through the restriction of infection and prevalence of deleterious pathogenic organisms through the bacterial production of antagonistic substances (Figure 1) [5].

Plant-Microbe Interactions in the Rhizosphere

The thin layer of soil surrounding plant roots is known as the rhizosphere, and is an extremely important area for plant-microbe interactions. It is approximately one millimeter wide and characterized by high levels of biological and chemical activities and is comprised of plant, fungi, bacteria and soil constituents [11]. The rhizosphere can contain over 10^{10} microbial cells per gram of root [11] and sustain more than 30,000 species of bacteria [12]. The microbial populations benefit from root exudates including: vitamins, sugars, proteins, carbohydrates, organic acids, amino acids and mucilage [13]. These root exudates are also capable of modifying physical and biochemical properties of the rhizosphere by acting as messengers between plant and microbe [13]. For instance, benzoxazinoids found in the root exudates of maize attract *Pseudomonas putida*, which is a competitive colonizer of the maize rhizosphere with plant beneficial traits [14].

Biological Nitrogen Fixation (BNF)

Nitrogen is one of the three macronutrients required for high crop yields. Three quarters of our atmosphere consists of nitrogen gas (N_2) and elemental nitrogen must be transformed to usable forms before

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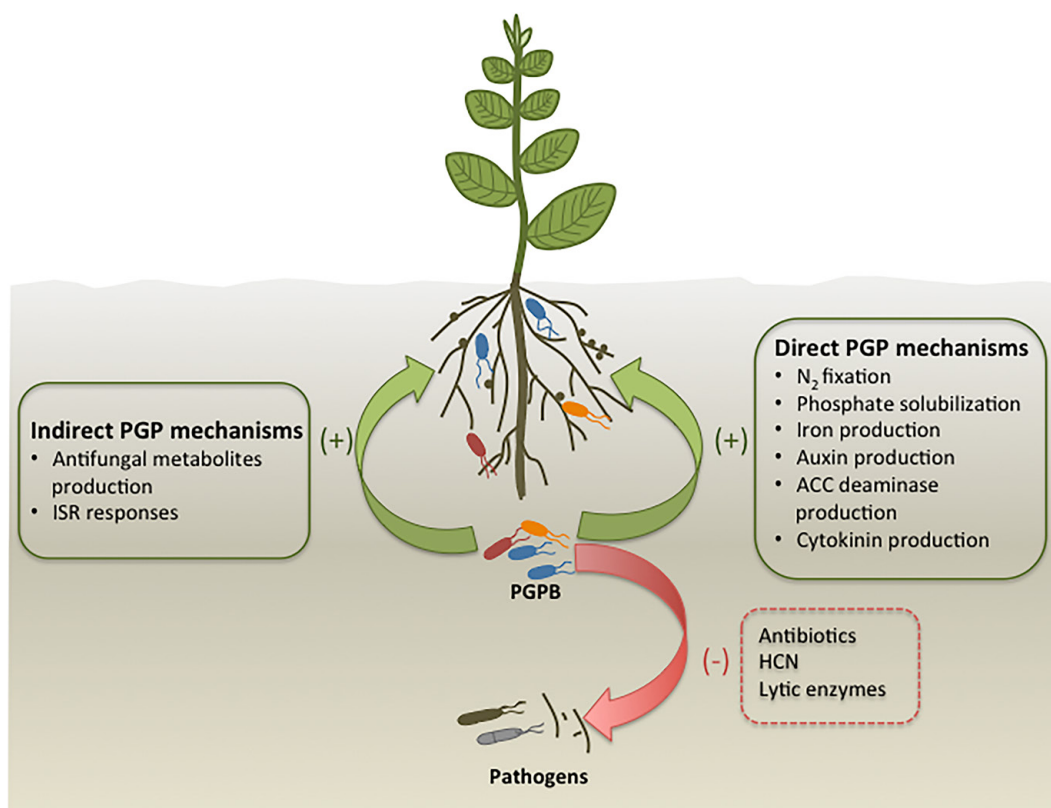


Figure 1: The modes of action for PGPB are based on either direct and/or indirect mechanisms. Direct modes of action for enhancement of plant growth by PGPB include nitrogen fixation [18], nutrient solubilization [25,37] and production of auxins [49], enzymes [69] and secondary metabolites [106]. The indirect modes of action for enhancement of plant growth by PGPB are largely based on warding-off pathogens [90].

it is available for plant uptake. Nitrogen is the major component of chlorophyll, which plays an important role in photosynthesis. It is also a building material of proteins and a major component of DNA and RNA in the form of the nitrogenous bases. In terms of nutrient demand, plants require more nitrogen than any other nutrient, however, there is a very limited amount of nitrogen available in soil due to regular nitrogen loss.

Nitrogen can be lost from the rhizosphere through the following processes:

Denitrification- Oxidative reduction of soil nitrates to atmospheric nitrogen by heterotrophic facultative anaerobic bacteria through a series of gaseous nitrogen oxide intermediates [15].

Volatilization- Loss of the organic form of nitrogen as urea (originated from animal manure, fertilizers and plant materials), which is converted into gaseous ammonia to the atmosphere [16]

Leaching- Once the nitrogen has been converted to nitrate, the excess soil nitrates dissolved in water can move below the root zone under certain conditions. Generally, leaching takes place in sandy soils with low water holding capacity where water penetrates quickly. The amount of leaching is also dependent on subsoil moisture recharge [17]. If subsoil moisture levels are not recharged, water is more likely to be held in soil, therefore reduces the probability of leaching [17].

Certain microbes are able to convert atmospheric nitrogen into utilizable forms of nitrogen for both themselves and plants via biological fixation process [18]. These nitrogen-fixing microorganisms can be divided into two main categories, symbiotic and free-living. It is well

documented that biological nitrogen fixation (BNF) is facilitated by a multimeric enzyme called nitrogenase, which consists of conserved proteins, iron containing dinitrogenase reductase and molybdenum iron dinitrogenase [19]. These enzymes are irreversibly inhibited by molecular oxygen, therefore nitrogen-fixing bacteria have developed evolutionary adaptive mechanisms to limit oxygen exposure via leghemoglobin [20]. The nitrogen fixation capacity of bacteria is highly dependent on moisture content, oxygen concentration and supply of organic substrates in soil [19].

Of all the bacterial species that have the ability to fix atmospheric nitrogen [21], rhizobia are the best described for providing nitrogen to plants, and are well known for their ability to form nodules on roots of legume plants. The success of rhizobium in providing nitrogen to their legume hosts has led to the commercialization of rhizobium inoculants. However, the efficiency of these bacterial inoculants can be greatly affected by rhizosphere nutrient conditions.

Symbiotic nitrogen-fixing bacteria reduce atmospheric nitrogen within the root nodules to ammonia, which is then being used by the host plant [22]. This symbiosis process allows the bacteria to have an exclusive niche and carbon source and in return, the plant will obtain nitrogen. Similar relationships can be observed in aerobic Azotobacter and anaerobic Clostridia with higher plants such as Azolla [22].

Enhancement of Phosphate Availability to Plants by Bacteria

Phosphorus (P) is a crucial element for survival and prosperity of

most organisms including plants and is the second most important plant growth-limiting nutrient after nitrogen. The majority of phosphorus is in its insoluble form, whilst the plants can only absorb phosphorus when it is bonded with oxygen as in monobasic (H_2PO_4^-) and dibasic forms (HPO_4^{2-}) [23]. A low abundance of phosphorus is typical in many agricultural soils, and where phosphates are often complexed to soil constituents, making them unavailable to many organisms [24]. To overcome plant growth limiting phosphate deficiency, phosphate dense fertilizers are applied to crops regularly. However, plants can only absorb limited amount of phosphates and the rest is rapidly converted into insoluble P. There is also an extensive loss of phosphates in agricultural lands via run off and much of the phosphate ends up in water reservoirs.

Phosphate is essential for the optimum growth of most bacteria and has a central role in many metabolic and energy producing pathways. Microorganisms associated with hydrolyzing organic and inorganic phosphates are known as phosphate-solubilizing bacteria (PSB). These organisms are known to solubilize P from substrates and make it available to plants, and hence are a possible alternative to phosphate rich fertilizers. Bacteria of the genera *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are reported as the most effective PSB [25].

Environmental factors such as temperature, concentration of iron, carbon and nitrogen sources can impact on P solubilizing ability of bacteria. PSB are able to solubilize insoluble forms of P such as aluminum phosphate (Al_3PO_4), iron phosphate (Fe_3PO_4) and tricalcium phosphate ($\text{Ca}_3(\text{PO}_4)_2$) [26]. Typically, inorganic phosphate solubilization is initiated as a consequence of the actions of low molecular organic acids such as gluconic and citric acid [26]. Organic P compounds undergo mineralization and the resulting P will be readily available for the plants to absorb [27,28]. This mineralization process is facilitated by enzymes secreted by soil microbes such as phosphatases [29] and phytases [30]. Considering critical impacts of such enzymes in dissolution of complex compounds into usable P, it is highly desirable to develop the bacterial inoculants with high phosphatase and phytase activity to overcome P limiting soils.

Bacterial Siderophore Production

Iron is a vital nutrient for proper plant development. Since it is a cofactor of many metabolic pathways, its deficiency may lead to disruption of many processes including respiration or photosynthesis. In aqueous environments, iron exists as Fe^{3+} and Fe^{2+} , which form insoluble oxides and hydroxides. However, both plants and microorganisms are unable to metabolize insoluble iron oxides [31].

Plants mainly acquire Fe from the rhizosphere and have developed two strategies to acquire iron. The first strategy involves acidification of the rhizosphere, followed by the reduction of Fe^{3+} ions by membrane-bound Fe^{3+} -chelate reductase, and subsequent uptake of Fe^{2+} into root cells [32]. The second strategy involves plants secreting low molecular weight phytosiderophores in order to solubilize and bind iron, which is then transported into root cells via membrane proteins [33,34]. These strategies are often not efficient enough to meet the needs of the plants growing, particularly in calcareous and alkaline soils. Therefore, providing plants with accessible forms of iron is often necessary, particularly for intensively cropped soils [35].

The application of PGPB may be one strategy to increase soil iron availability in the rhizosphere. Bacteria are able to synthesize low

molecular weight siderophores, which have the ability to scavenge Fe^{3+} from the environment [36]. Siderophores have high affinity ligands that are able to pair with ferric ions. They have a strong capacity to chelate ferric ions allowing their solubilization and extraction from most mineral or organic complexes [37].

Siderophores are small peptide metabolic molecules with functional groups, which can provide a set of high affinity ligands to equalize ferric ions. Bacterial siderophores have been classified into three main categories according to their structural features, type of ligands, and their iron coordinating functional groups. They are namely carboxylates, hydroxamates and catecholates [38]. Hydroxamate siderophores have a 1:1 (metal-EDTA complexes) stability constant with Fe^{3+} that nears that of the Fe^{3+} -EDTA complex (10^{30}), whereas catecholates and carboxylates siderophores can form similar complexes with stability constants near that of Fe^{3+} -EDDHA (10^{40}) [39].

The ability to produce siderophores plays a central role in various microorganisms in regards to plant growth promotion. Bacterial siderophores from *Chryseobacterium* spp. C138 isolated from the rhizosphere of *Oryza sativa* are effective in supplying Fe to iron-starved tomato plants when delivered to the roots [40]. Supplementation of fluorescent *Pseudomonas* strains in maize seeds was demonstrated to significantly increase germination percentage and plant growth [41]. Under low iron conditions, co-inoculation of maize with siderophore producing plant growth promoting rhizobacteria, resulted in larger shoot and root lengths and higher dry weights, in comparison with uninoculated plants suggesting application of these bacteria could increase crop productivity in calcareous soils [41].

Studies have shown that plants absorbed radiolabelled iron from siderophores previously exposed to radiolabelled iron [42]. This can be observed in Fe-pyoverdine complex synthesized by *Pseudomonas fluorescens* C7, which was then taken up by *Arabidopsis thaliana* plants in order to fulfill their iron requirements [42].

Bacterial Auxin Production

Auxins are plant hormones that are essential for plant development. They have a fundamental role in coordination of many growth and physiological processes in the plant's life. Indole-3-acetic acid (IAA) is the most extensively studied plant hormone and it is a carboxylic acid with a carboxyl group attached through a methylene group to the 3rd carbon of the indole ring [43]. IAA affects plant nutrition and development through altering cell division, extension and differentiation by increasing the rate of xylene and root development [44].

Plant growth promoting bacteria exhibit a variety of characteristics responsible for influencing plant growth, including the production of IAA [45,46]. Indole-3-acetic acid in rhizobacteria helps to loosen plant cell walls, which may facilitate rhizobacteria to absorb various substances secreted by roots [44]. Several studies have suggested that elevated auxin levels, including IAA in host plants, are required for nodule formation [45,47-49]. This was observed when low IAA producing *Bradyrhizobium elkanii* mutants were shown to result in fewer nodules in soybean roots than the wild-type strain [45]. Co-inoculation of low IAA producing mutant of *Rhizobium* sp. NGR234 with soy bean shows that the IAA content in nodules is significantly lower than in nodules induced by the wild-type strain [50]. This supports the concept that some elements of the IAA established in plant nodules is of prokaryotic ancestry and IAA facilitates nodulation.

The amino acid tryptophan is vital for regulating the levels of IAA synthesized by bacteria [51], while anthranilate acts as a precursor for

tryptophan [52]. By this mechanism, IAA biosynthesis is fine-tuned because tryptophan inhibits anthranilate formation by a negative feedback regulation on the anthranilate synthase, resulting in an indirect induction of IAA production [52]. It has been shown that the supplementation of tryptophan could increase IAA production in most rhizobacteria [50].

IAA produced by rhizobacteria may be involved in various plant-bacterial interactions. Most rhizobium strains that have been investigated for plant growth promotion have been found to produce IAA [53]. A recent study investigating the role of IAA synthesized by *Pseudomonas putida* GR12-2 has confirmed that it promoted canola root development by 35-50% over the roots from seeds treated with IAA deficient mutant, and the roots from uninoculated seeds [46].

Reduction of Plant Ethylene Levels by Bacterially Produced 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase

Ethylene is an essential metabolite for normal growth and development of plants [54]. This growth hormone is produced by all plants (particularly in rapidly growing cells) and is capable of inducing various physiological changes [55]. Ethylene has a very low solubility in water, however, it can efficiently diffuse through cell membranes. Plant cells in developing seedlings produce elevated amounts of ethylene to increase cell numbers [56]. However, as shoots expand, phytohormones generate a signal to limit the production of ethylene in order to allow leaf expansion [57]. In pea shoots, it has been shown that when they meet an obstacle during their rapid growth stage, reduced levels of ethylene are produced. This allows lateral expansion of the stem resulting in radial enlargement [58]. It has also been identified that ethylene is responsible for thicker and steadier tree trunks and branches [58]. Moreover, ethylene affects fruit ripening process and elevated ethylene levels in fully ripen fruits resulted in a climacteric event just before the seed dispersal [59].

Apart from being a plant growth regulator, ethylene has a vital role as a growth inhibitor [60]. Levels of ethylene in plants increase under stressful circumstances such as drought, presence of heavy metals, extreme temperatures, radiation, high salt environments and wounding pathogenicity [61]. In such situations, plants will initiate survival strategies, which may result in low crop yields [61]. It has been shown that plants exposed to adverse conditions quickly respond by producing a small peak of ethylene due to the activation of a protective mechanism by plants [62]. If the severe conditions persist for a few days a second peak of ethylene arises. After the second ethylene peak, processes such as senescence, chlorosis and abscission are induced [62].

ACC deaminase is an enzyme produced by PGPB, which can decrease ethylene levels, thus increase stress (salt and drought) tolerance in plants [63]. There is a wide range of bacteria that have been identified as ACC deaminase positive including: *Pseudomonas*, *Ralstonia*, *Serratia*, *Rhizobium*, *Acinetobacter*, *Agrobacterium*, *Achromobacter*, *Alcaligenes* and *Bacillus* [64-67]. These bacteria are capable of taking up the ethylene precursor ACC from plants and converting it into 2-oxobutanoate and ammonia [68]. By decreasing ACC levels in plants, ACC deaminase producing bacteria limit accumulation of high ethylene concentrations in plants.

Environmental stressors such as heavy metals in soil, radiation, extreme temperatures, insect predation, high light intensity and high salt concentration are alleviated by the production of ACC deaminase [69]. Thus, co-inoculation of ACC deaminase producing bacteria with

plants subjected to a wide range of biotic and abiotic stressors, can result in enhanced plant tolerance [69].

Enhancement of Plant Growth by Bacterially Produced Cytokinins

Cytokinins are organic plant growth hormones that have the ability to promote cell division (cytokinesis) in plant root and shoots. Naturally occurring cytokinins are adenine derivatives and they carry either an isoprene derived side chain or an aromatic side chain at the N⁶ terminus [70]. They are called isoprenoid, cytokinins or aromatic cytokinins, respectively [71,72]. Isoprenoid cytokinins are commonly found in a range of plant species, however, there is no clear evidence of the common existence of aromatic cytokinins in plants [70]. Cytokinins influence cell division by stimulating the production of proteins required for mitosis and are produced in the meristem where stem cells self-renew and produce daughter cells that separate and give rise to different organ structures [73]. Once synthesized in roots, they travel up the xylem to the other parts of the plant such as leaves, developing fruits and seeds [73].

Seven different genes (IPT: adenosine phosphate-isopentenyltransferase) have been identified as potential cytokinin producers in *Arabidopsis* (*altPT1* and *altPT3* to *altPT8*) [74-76]. *AltPT1*, *AltPT3*, *AltPT5* and *AltPT8*, are expressed in plastids and produce cytokinins with dimethylallyl diphosphate (DMAPP) originated from MEP (Methylerythritol phosphate) pathway [77]. *AltPT4* and *AltPT7* are located in the cytosol and mitochondria respectively, and they produce cytokinins with DMAPP derived from MVA (Mevolonate) pathway [77]. Inoculation of lettuce plants with cytokinin producing bacterium, *Bacillus subtilis* increased the cytokinin content of both shoot and roots [78]. Accumulation of cytokinin, zeatin riboside in inoculated lettuce plants was associated with an overall increase in both root and shoot weights [78].

Cytokinins have recently been found to play a crucial role in resistance to plant pathogens. Plant-derived cytokinins promote resistance against the non-cytokinin producing pathogen, *Pseudomonas syringae* by modulating the defence signalling in *Arabidopsis* [79]. In *Nicotiana tobacum*, cytokinins enhanced overall plant resistance against the virulent hemibiotrophic pathogen, *P. syringae* [80]. This cytokinin-mediated plant resistance is associated with elevated levels of bacteriocidal activities and increased amounts of antimicrobial phytoalexins [80]. In the context of plant biological control via microbes, cytokinins produced by *P. fluorescens* G20-18 have negative influence on the pathogenic activity of *P. syringae* in *A. thaliana* [81].

Enhancement of Plant Growth by Bacterially Produced Secondary Metabolites

In the past, secondary metabolites were considered as elements with a low molecular mass that are not the end products of primary metabolic pathways [82,83]. It was assumed that secondary metabolites were not vital to normal bacterial function, hence had no influence on growth and development of microorganisms. Contrary to this, recent studies have shown that secondary metabolites are fundamental for growing cells as regulators of cellular differentiation processes and also as cellular inhibitors against competing bacteria [84-86].

A vast range of secondary metabolites have been discovered and despite their greater divergence, most secondary metabolites have the same precursors branched into various compounds that result in secondary metabolites [86]. Depending on their structural significance,

secondary metabolites have distinct features that reflect their biological activity. Apart from their involvement in medicine, these bioactive compounds have demonstrated compelling advantages in agricultural and industrial applications [85].

Antibiotics produced by bacteria are some of the best known secondary metabolites and approximately 12,000 antibiotics have been identified [87]. The majority of bacterial antibiotics are produced by filamentous bacteria of the genus, Actinomyces [86]. Parallel to the screening for new antibiotics, research efforts have been targeted towards identifying bioactive compounds with other biological activities such as herbicides, immunosuppressant agents, insecticides, plant growth promoter/inhibitors, enzyme inhibitors and antihelmintics.

Functional relevance of secondary metabolites produced by bacteria has yet to be determined in terms of their effects on the soil bacterial ecology. It has been found that microbial bioactive agents such as antibiotics are not only beneficial to hosts against various microbial competitors, but also against other organisms including insects, parasites and plants [86,88]. Most potential secondary metabolites are present in very low amounts in the rhizosphere.

Bacterial Antibiotic Production Enhances Plant Growth

The production of antifungal antibiotics depends on biocontrol ability and the degree of bacterial colonization in plant roots [89]. Bacterially produced antibiotics are mainly low molecular weight organic compounds with heterogeneous groups, which can have deleterious effect on pathogenic of microorganisms. PGPB can act as antagonistic agents against plant pathogens by producing one or more antibiotics. There are six classes of biocontrol antibiotic agents depending on their mode of action: phenazines, phloroglucinols, pyrrolnitrin, pyoluteorin, hydrogen cyanide (HCN) and cyclic lipopeptides [90].

Phenazines

Phenazines are the pigments produced by eubacteria [91]. Production of phenazines varies in different organisms and these phenazines are released at high levels (milligrams to gram per liter) during bacterial growth *in vivo*. Phenazines absorb in both the UV and visible range that alter due to arrangement of the heterocyclic ring [92]. Functional groups present in heterocyclic ring are subjected to the variations in their biological activity [92].

Phenazine production has been demonstrated in various experiments and is common among bacteria with high guanine and cytosine (G+C) content [93]. Fluorescent pseudomonads including *P. fluorescence* and *P. chlororaphis* are regarded as being the highest producers of phenazines [94]. Phenazine producing fluorescent pseudomonads typically produces two or more phenazines except *P. fluorescence*, which produces only phenazine-1-carboxylic acid (PCA). In addition to PCA, *P. chlororaphis* produces phenazine-1-carboxamide (PCN), whereas *P. aeruginosa* produces 5-N-methyl-1-hydroxyphenazine (PYO) [94]. Not only PYO, *P. aeruginosa* is also capable of synthesizing PCN, aeruginosin A (5-methyl-7-amino-1-carboxymethylphenazinium betaine) and aeruginosin B (5-methyl-7-amino-1-carboxy-3-sulfophenazinium betaine) [94]. A microorganism that belongs to Enterobacteriaceae is *Pantoea agglomerans* Eh 1087, which produces alanylgriseoluteic acid (AGA). Similarly, phenazines have been identified in marine bacteria known as *Pelagiobacter variabilis* and *Vibrio* sp. SANK73794 [95].

Phenazine is a bioactive metabolite that inhibits the growth of microorganisms. This has been confirmed in field grown wheat, where

a concentration of 100 nM of PCA inhibited growth of certain Gram-positive bacteria and fungi [96]. Further investigation revealed that PCA produced by *P. fluorescence* in the wheat rhizosphere was linked to the inhibition of the wheat pathogen, *Gaeumannomyces graminis* var. *tritici* [97,98]. Similar studies of the plant pathogenic fungus, *Rhizoctonia* spp. in wheat revealed that the application of phenazine producing *Pseudomonas* spp. restricted *Rhizoctonia* caused root rot [99].

Phenazine also enhances the survival of bacteria in anaerobic conditions. In such conditions, endogenous phenazines enhance the survival of *P. aeruginosa* by facilitating extracellular electron transfer [100]. Moreover, PCA has the ability to increase iron bioavailability via reducing Fe³⁺ to Fe²⁺ [101]. This is evident when a mutant form of *P. aeruginosa* that is unable to form siderophores can enhance formation of biofilms in the presence of PCA, making iron more bioavailable in the environment [101].

Phloroglucinol

Phloroglucinol is a benzenetriol, mainly used in pharmaceutical production of Flopropione (antispasmodic agent) [102]. Phloroglucinol is synthesized commercially via a number of processes, however selective trinitration of benzene is the widely used technique in the world. Phloroglucinols are naturally found in certain plant species and are also produced by microorganisms [94]. *Pseudomonas fluorescence* produces phloroglucinol with a type III polyketide synthase [103]. Synthesis begins via the condensation of three malonyl-CoAs which are coenzyme A derivatives of malonic acid [103]. While de-carboxylation of 3,5-diketoheptanedioate, cyclization of the activated product leads to the formation of phloroglucinol [103].

The 2,4-diacetylphloroglucinol (DAPG) is the widely studied phloroglucinol produced by Pseudomonads. It can cause membrane and zoospore damage in *Pythium* spp. This antibiotic acts as an inhibitor to aldose reductase, an enzyme involved in metabolism of glucose to fructose [104]. DAPG is not only a direct antagonist of plant pathogen but also it is resistive in *Arabidopsis thaliana* against *Peronospora parasitica*, a water mold [105]. Although, DAPG exhibits antifungal activity, it also acts as a plant growth stimulator. DAPG produced by *P. fluorescence* isolates containing the *phID* gene that can stimulate lateral root formation in tomato seedlings by inhibiting primary root development [106]. This indicates that DAPG can change the root architecture by merging with an auxin dependent signaling pathway [106].

Enhancement of Plant Growth by Bacterially Produced Antifungal and Antibiotic Products

Pyrrolnitrin (3-chloro-4-(3-chloro-2-nitrophenyl) pyrrole) is an antifungal antibiotic produced by many fluorescent and non-fluorescent bacterial strains, and it is initially isolated from *Burkholderia pyrrocinia* [107]. Pyrrolnitrin inhibits the growth of fungi, yeast and bacteria by reacting with phospholipid components, which leads to burst cellular membrane [108]. Predominantly, this antibiotic is used to restrain the growth of dermatophytic fungi, such as Trichophyton species [109]. Studies performed on *P. aureofaciens* indicated pyrrolnitrin has a relatively closer arrangement to tryptophan (IAA synthesis precursor) [110]. Further analysis revealed that the addition of D-tryptophan in growth medium can increase the pyrrolnitrin production, whereas L-tryptophan had no significant effect on the pyrrolnitrin production [110].

A type of pyrrolnitrin produced by *P. fluorescence* BL15 strain has

been demonstrated to inhibit the damage caused by *Rhizoctonia solani* during damping-off of cotton plants [111]. *Pseudomonas fluorescens* BL15 has been shown to produce other antifungal metabolites including HCN, chitinase and 2-hexyl-5-propyl-resorcinol [112]. Pyrrolnitrin (approximately 10 µg/mL) is capable of inhibiting the growth of *Saccharomyces cerevisiae*, *Penicillium atrovirens* and *P. oxalicum* [113]. It affects the terminal electron transport system between succinate or NADH (reduced nicotinamide adenine dinucleotide) and coenzyme Q [113]. At such level, pyrrolnitrin inhibits endogenous and exogenous respiration immediately after its addition to the system.

Conclusion

It is clear that microbes have the potential to enhance plant growth through various mechanisms and may help to reduce chemical fertilizer inputs. Whilst some modes of actions such as auxin, enzyme production and nutrient (phosphate and iron) solubilization have been well studied, there are still many areas that require further investigation. One promising target is the area of secondary metabolite production by PGPB as many of these metabolites have not been well characterized and thus further understanding is required for identification of modes of action for plant growth promoting strains. The nature of these molecules and the fact that they are often produced in very small amounts, requires the use of modern separation and analytical techniques such as high performance liquid chromatography and gas/liquid chromatography–mass spectrometry.

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