SUSTAINABLE FARMING USING PLANT GROWTH-PROMOTING BACTERIA

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Abstract. Increased usage of chemical fertilizers poses a severe risk to the environment, causing soil acidification and plant growth inhibition, as well as to human health since heavy metals present in fertilizers can cause kidney dysfunction, blood diseases, and cancer. Therefore, alternative methods are needed to improve plant growth without having concerns regarding soil fertility and public health. In this manner, plant growth-promoting eco-friendly bacteria approach to implement globally sustainable farming. plant growth-promoting bacteria are the group of microbes that can enhance the growth of plants through nitrogen fixation in addition to various other mechanisms, increase plant crop yield, improve tolerance to environmental conditions, and improve its resistance to plant-pathogenic microbes. Pseudomonas and Bacillus species are the most used plant growth-promoting bacteria in promoting plant growth and enhancing properties. In addition, more recent studies demonstrated the high potential of Herbaspirillum and Paenibacillus's promotion of plant growth, making the goal of globally sustainable farming possible; moreover, CRISPR can be employed in other sustainable applications to benefit future agriculture.

Keywords: *CRISPR/Cas9, nitrogen fixation, biofertilizers, antimicrobials, PGPB*

Introduction

A growing human population combined with reduced agricultural land availability represents a significant challenge to the food supply. The global population is expected to cross 9 billion by 2050, with food demand increasing by 70% (Adam, 2021; van Dijk et al., 2021; Daszkiewicz, 2022). To fulfill this demand, chemical fertilizers are used extensively as a source of plant nutrients such as nitrogen (ammonia, nitrate, and urea) and phosphorus (monoammonium phosphate (MAP), diammonium phosphate (DAP), triple super phosphate, orthophosphate, and polyphosphate) to promote plant growth. However, the excessive use of chemical fertilizers represents a serious threat to the environment and human health (de Souza et al., 2015). Chemical fertilizers, in general, harden the soil, lower soil fertility, restrict microbial growth, and pollute ecosystems (Pahalvi et al., 2021). The increased usage of chemical fertilizers results in soil acidification, which lowers phosphate uptake and inhibits plant growth (Kumar et al., 2019). Moreover, chemical fertilizers contain heavy metals like Mercury, Cadmium, and Lead that are absorbed through plant roots and sequentially enter the food chain (Mortvedt, 1995), causing various complications, including kidney dysfunction, nervous system disorders, blood-related diseases, and cancer (Katiyar, 2016; Balali-Mood et al., 2021).

In this sense, plant growth-promoting bacteria (PGPB) are other options that can stimulate and enhance plant growth and preserve soil fertility for sustainable farming and long-term management while minimizing environmental concerns regarding chemical fertilizers. PGPBs are a group of microbes from different genera that can promote and enhance the growth of plants and increase their tolerance and

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resistance to both biotic and abiotic stresses (Dimkpa et al., 2009; Grover et al., 2011; Glick, 2012; Pathania et al., 2020). The rhizosphere, where PGPBs are found surrounding the plant roots, can be defined as the soil region characterized by high microbial diversity and intensive interactions between soil, plant, and microbes (Broeckling et al., 2008).

Microbial formulations of PGPBs, marketed as "Bio-fertilizers," can be directly sprayed on seeds or incorporated into the rhizosphere to colonize and supply the host plant with essential supplemental nutrients (Malusa and Vassilev, 2014; Timmusk et al., 2017). Bio-fertilizers are considered safe alternatives to chemical fertilizers, ensuring a globally sustainable farming (Vejan et al., 2016). Plant growth is improved due to bio-fertilizer treatment, with enhanced seed germination, shoot, and root development, increased biomass, and reduced disease occurrence (Dal Cortivo et al., 2020). Therefore, globally sustainable farming can be implemented using biofertilizers instead of chemical fertilizers. However, biofertilizers have certain drawbacks, such as shorter shelf life and reduced nutrient density compared to chemical fertilizers. Also, biofertilizers are more challenging to store and transport than chemical fertilizers (Malusà et al., 2016; Thomas and Singh, 2019).

PGPBs can promote plant growth directly or indirectly through various mechanisms (*Figure 1*), including nitrogen fixation, phosphate solubilization, iron acquisition, and the production of antimicrobials, ACC deaminase, IAA, and exopolysaccharides (Pathania et al., 2020). *Table 1* summarizes the PGPRs discussed in the review and their associated features to promote the growth promotion of plants.

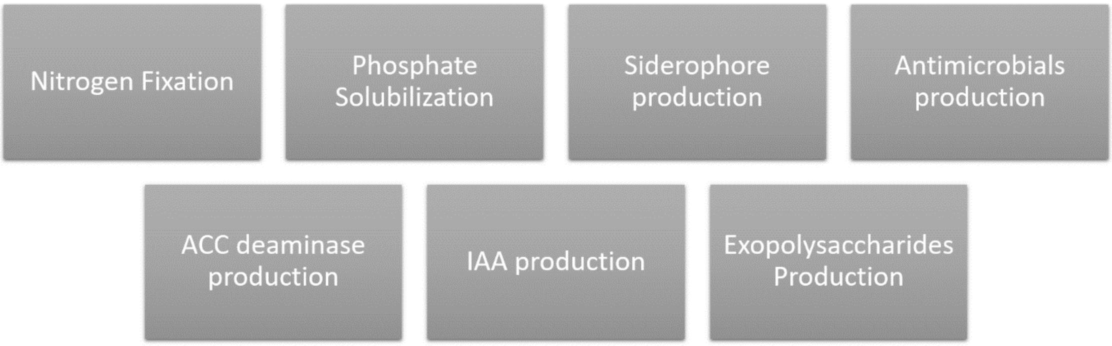

Figure 1. *Bacterial mechanisms used in promoting plant growth*

Nitrogen fixation

Nitrogen fixation refers to the transformation of atmospheric nitrogen, an essential component of proteins and nucleic acids, into biologically reactive forms (nitrates, nitrites, or ammonia) and thus promoting plant growth (*Figure 2*) (Sun et al., 2021). Nitrogen-fixing bacteria, also known as diazotrophs, are capable of growing in the absence of fixed nitrogen sources and fixing atmospheric nitrogen by themselves through reducing nitrogen to ammonia (Dixon and Kahn, 2004). Various microbes perform the nitrogen fixation process either symbiotically, such as *Rhizobium*, *Frankia*, and *Azospirillum*, or symbiotically such as *Pseudomonas*, *Herbaspirillum*, *Paenibacillus*, *Cyanobacteria*, and *Klebsiella* (Franche et al., 2009; Bhat et al., 2015; Shin et al., 2016; Barbu and Boiu-Sicuia, 2021).

Table 1. *Examples of PGPBs assessed for their ability to promote plant growth*

PGPB	Plant	Feature	Ref.
Pseudomonas koreensis & *Pseudomonas entomophila*	Sugarcane	N fixation	(Li et al., 2017)
Herbaspirillum seropedicae SmR1	Maize		(da Cunha et al., 2022)
Paenibacillus polymyxa WLY78	Cucumber		(Li et al., 2022)
Paenibacillus spp.	Green maize and sweet sorghum		(de Aquino et al., 2022)
Curtobacterium citreum CE711	Cassava		(Zhang et al., 2022)
Pseudomonas fluorescens	*Arabidopsis*	P solubilization	(Lee, 2022)
Pseudomonas & Bacillus spp.	Tomato		(Cochard et al., 2022)
Pseudomonas libanensis EU-LWNA-33	***		(Kour et al., 2020)
Paenibacillus xylanexedens	*Chlorella pyrenoidosa*		(Dong et al., 2022)
Pseudomonas strain SP3	Apple	Siderophore production	(Gao et al., 2022)
Bacillus subtilis	Groundnut		(Sarwar et al., 2022)
Achromobacter denitrificans MS3 & *Bacillus aryabhattai* MS3	***		(Sultana et al., 2021)
Leclercia adecarboxylata MO1	Cucumber		(Kang et al., 2021)
Pseudomonas spp. & *Bacillus subtilis*	***	Antimicrobials production	(Ali et al., 2020)
Gluconacetobacter diazotrophicus, *Herbaspirillum seropedicae*, and *Burkholderia ambifaria*	*Cannabis sativa*		(Pellegrini et al., 2021)
Bacillus spp.	Sugar beet		(Farhaoui et al., 2022)
Brevibacterium linens RS16	Rice	ACC deaminase	(Choi et al., 2022)
Enterobacter cloacae & three *Pseudomonas spp.*	*Cyamopsis tetragonoloba*		(Goyal et al., 2022)
Bulkhorderia cepacia	*Capsicum annuum*		(Maxton et al., 2018)
Pseudomonas alcaliphila & *Pseudomonas syringae*	Alfalfa		(Tafaroji et al., 2022)
Paenibacillus xylanexedens PD-R6	Canola plants		(Orozco-Mosqueda et al., 2020)
Paenibacillus polymyxa SK1	*Lilium lancifolium*		(Khan et al., 2020)
Bacillus amyloliquefaciens FZB42	*Arabidopsis thaliana*	Exo-polysaccharides production	(Lu et al., 2018)
Pseudomonas anguilliseptica SAW24	*Vicia faba* L.		(Alaa, 2018)
Thalassobacillus denorans & *Oceanobacillus kapialis*	Rice		(Shah et al., 2017)
Bacillus megaterium & *Paraburkholderia tropica*	Radish	IAA production	(Agustiyani et al., 2022)
Pseudomonas moraviensis B6	Tomato		(Cochard et al., 2022)

*** various plants

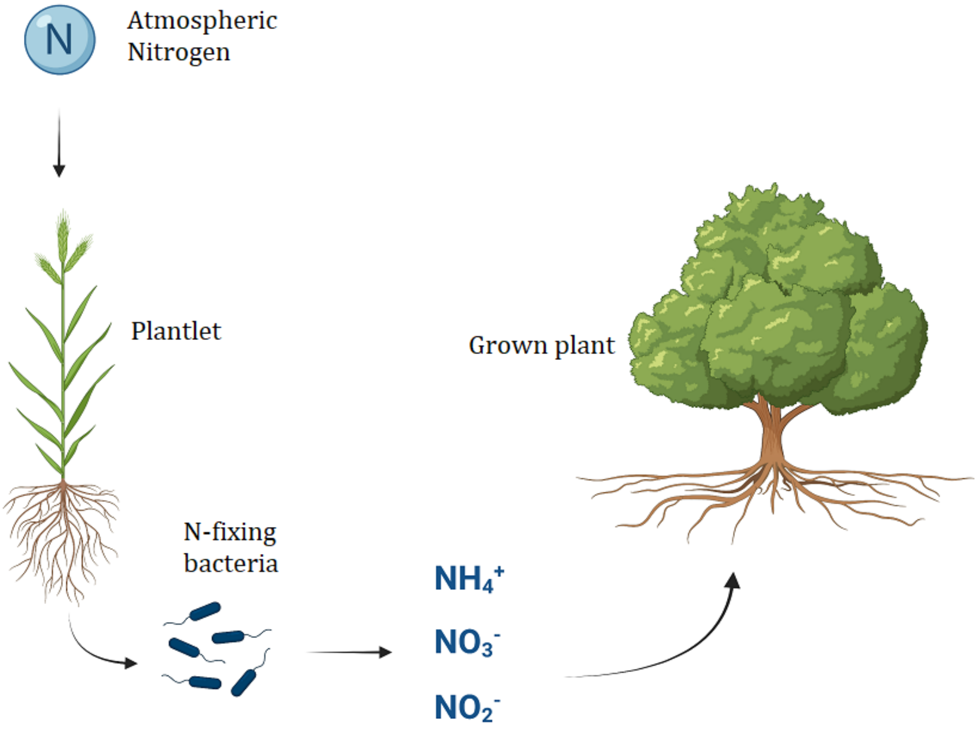

Figure 2. *The process of Nitrogen fixation*

Pseudomonas is a well-studied bacterial genus that has been the focus of scientific research to increase crop yield and minimize the usage of chemical fertilizers (Shaharoona et al., 2006; Kumar et al., 2009; Sivasakthi et al., 2014; Singh et al., 2022). While screening for *Pseudomonas spp.* in the sugarcane rhizosphere, two isolates, *P. koreensis* and *P. entomophila*, were reported to fix nitrogen efficiently and promote the growth of sugarcane (Li et al., 2017). In the same year, another study showed that the genome of *Pseudomonas psychrotolerans* strain PRS08-11306 encodes for biosynthetic genes involved in the nitrogen fixation (Liu et al., 2017). This was verified in a growth assay in which rice seedlings were inoculated with *P. psychrotolerans* in nitrogen-deficient conditions. Multiple growth parameters were estimated after 30 days from the plantation. When compared with the controls, inoculated plants grew significantly in root length, shoot weight (fresh and dried), and lateral root number, confirming *P. psychrotolerans* PRS08-11306's N-fixing potential.

Herbaspirillum seropedicae, a gram-negative bacterium, is a true endophytic diazotroph found in several important crops including maize (Monteiro et al., 2008; Rothballer et al., 2008). The ability of *H. seropedicae* SmR1 in colonization and improving maize growth in the early development phases was assessed in two independent greenhouse experiments, one performed in late spring and the other in early summer (da Cunha et al., 2022). For the control treatments, a potassium nitrate solution was used to obtain two nitrogen concentrations, high (5 mM) and low (Sabet and Mortazaeinezhad, 2018). The results showed that *H. seropedicae* SmR1 increased maize root biomass significantly by 19.43% for the first experiment and 10.51% for the second experiment (compared to the controls), demonstrating its promising applicability as an inoculant for promoting plant growth. In comparison to the controls, the inoculation increased the shoot length in the first experiment only and, in a similarly odd manner,

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significant differences in root length were observed in the second experiment only. In the two experiments, the inoculation led to no significant difference in the shoot dry mass. Therefore, additional field investigations are required due to the seasonal variations that led to variance in findings especially those between the two experiments.

Another important example of diazotrophs is the genus *Paenibacillus* which comprises gram-positive, endospore-forming bacteria (Navarro-Noya et al., 2012). To demonstrate the promising capabilities of *Paenibacillus* strains as PGPBs in enhancing the growth of various plants under low or no nitrogen levels, the effects of *Paenibacillus polymyxa* WLY78 inoculation on the growth of cucumber plants under low nitrogen levels were assessed in a greenhouse cultivation experiment. The plant growth was evaluated on the 30th day after plantation by measuring the dry weight and length of cucumber roots and shoots (Li et al., 2022). After comparing the observations to the non-inoculated control samples, it was found that inoculation with WLY78 markedly increased cucumber growth by increasing root and shoot dry weights by 59.15% and 68.18%, respectively, and root and shoot lengths by 38.52% and 37.61%.

In a two-year field study, six PGPBs were assessed for their ability to improve the growth of the non-legumes crops green maize and sweet sorghum (de Aquino et al., 2022). The subjects were treated with or not with supplemental nitrogen fertilization (50% of N required by plants). The results revealed two strains of *Paenibacillus* (named IPACC38 and IPACC55 in the original article) that caused the highest crop yield in both plants without any nitrogen fertilization. In addition, two strains of *Bacillus subtilis* (IPACC26 and IPACC29) caused a considerable increase in the biomass of sweet sorghum with no supplemental nitrogen. For green maize, the inoculation of *H. seropedicae* (IPACC07) and *Burkholderia* (IPACC10) with no supplemental nitrogen fertilization led to higher cob production. It is also noteworthy to mention that there was no inconsistency in any outcomes during the two crop seasons for both plants.

In conclusion, current research has demonstrated the potential of *H. seropedicae* and *Paenibacillus spp.* to be used in promoting plant growth and improving yield productivity through nitrogen fixation, exhibiting their applicability in the formulation of bio-fertilizers. However, further studies are needed to better understand the bacterial processes and signaling cascades that are responsible for the interaction with plants.

Moreover, in the last three years, research has highlighted the potential of *Curtobacterium* spp. as a nitrogen-fixing alternative to promote plant growth (Mayer et al., 2019; Vimal et al., 2019; Patel et al., 2022; Zhang et al., 2022). *Curtobacterium citreum* CE711, for example, exhibited significantly high nitrogenase activity, the enzyme system responsible for nitrogen fixation, resulting in higher nitrogen retention and accelerated growth of cassava plants, which are known to utilize large amounts of nitrogen fertilizers (Zhang et al., 2022).

Phosphate solubilization

After nitrogen, Phosphorus is the second most limiting soil macronutrient for the growth of plants (Miller et al., 2010). It is a fundamental element in the structure of nucleotides (building units of nucleic acids), phospholipids that make up the cell membrane, and adenosine triphosphate which is essential in energy production, nitrogen fixation, and photosynthesis (Kouas et al., 2005; Khan et al., 2009). Phosphate fertilizers are used to provide plants with phosphorus but a larger portion is transformed into insoluble forms of phosphorus and wasted (*Figure 3*) (Rodríguez and Fraga, 1999;

Richardson, 2001). Some phosphate-solubilizing microbes include *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Bacillus*, and *Flavobacterium* (Ambrosini et al., 2012; Anand et al., 2016).

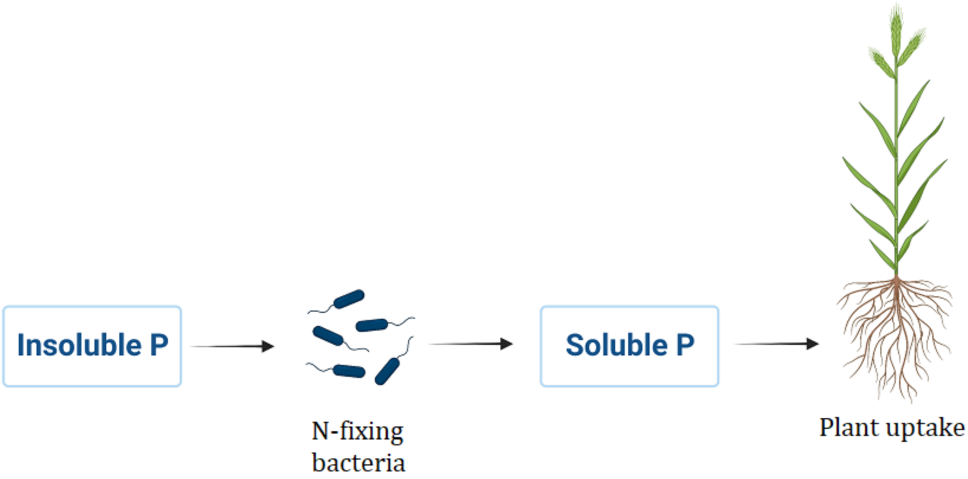

Figure 3. *Phosphate solubilization by PGPB*

Pseudomonas fluorescens is a common phosphate-solubilizing bacterium known to enhance water absorption by plants facilitating plant growth under stress (Otieno et al., 2015). *Bradyrhizobium japonicum* is an endogenous nitrogen-fixing bacterium capable of forming root nodules (Itakura et al., 2009). The ability of the two strains to improve the *Arabidopsis* growth was evaluated in two environments; normal water conditions and drought stress environment (Lee, 2022). Generally, inoculation with the two bacteria positively influenced plant growth. Under normal water conditions, inoculation of *B. japonicum* alone was enough to promote plant growth, possibly since the water was already being added. The coupling of the two strains also promoted plant growth considerably. Under drought stress conditions, *B. japonicum* alone wasn’t capable of inducing plant growth suggesting that the nitrogen-fixing bacterium lacks the function of enhancing water absorption. On the other hand, the inoculation of *P. fluorescens* alone relatively enhanced the plant growth, which is consistent with its known water supply function, but the largest increase was achieved when the plant was inoculated with the two strains together. Despite that there was no direct assessment of the nitrogen or phosphate levels in the soil or the plant, it was assumed that the increase in growth of the inoculated plant was due to the nitrogen fixation and phosphate solubilization capabilities of PGPBs.

In another study that aimed to identify beneficial microbes with a positive effect on the growth of tomato (*Solanum lycopersicum*) seedlings in greenhouse conditions, various bacteria of the genus *Pseudomonas* and *Bacillus* were reported to increase the fresh and dry weights of the root and shoot systems and be good phosphate solubilizers (Cochard et al., 2022). In particular, *B. aryabhattai* B29, *P. moraviensis* B6, and *P. fluorescens* B17 had a significant effect on the root system while the shoot system was significantly affected by the inoculation of *P. palleroniana* B10 and *B. subtilis* B25. In terms of phosphate solubilization, *B. simplex* B19 had the highest solubilizing rate followed by *P. moraviensis* B6, *B. subtilis* B25, and *B. subtilis* B18.

Under drought stress conditions caused by the addition of PEG-8000, phosphorus was efficiently solubilized at various PEG concentrations by the stress-adaptive phosphorus-solubilizing bacteria *Pseudomonas libanensis* EU-LWNA-33 isolated from the Divine Valley of Peace in India (Kour et al., 2020). All these studies reveal the *Pseudomonas* species' great promise and applicability as phosphate solubilizers in the biofertilizers industry.

In addition to its N-fixing potential, *Paenibacillus* were able to solubilize phosphate in a symbiotic system of algae-bacteria in which the effects of the phosphate solubilizing bacteria *Paenibacillus xylanexedens* on the growth of the microalgae *Chlorella pyrenoidosa* were investigated (Dong et al., 2022). Findings indicated that, as compared to the uninoculated algae, the intracellular components of *P. xylanexedens* significantly increased the algal biomass. Also, inoculation with *P. xylanexedens* increased the removal rate of PO_4^{3-} by 9.96% confirming the phosphate-solubilizing potential of *P. xylanexedens*.

Iron acquisition and Siderophore production

Iron is a vital plant micronutrient due to its crucial role in regulating chloroplast function, chlorophyll biosynthesis, and photosynthesis (Schmidt et al., 2020). In addition, Iron is an essential activator of many cellular pathways since it is a co-factor of several vital enzymes (Rout and Sahoo, 2015; Schmidt et al., 2020). In soil, the solubility of ferrous ions (Fe^{+2}) is poor, resulting in the accumulation of the insoluble ferric form (Fe^{+3}) (Rout and Sahoo, 2015). PGPBs can increase iron availability to plants, promoting plant growth through the production of chelator agents with high binding affinity to Fe^{+3} ions known as siderophores. The genera of Siderophore-producing microbes include *Pseudomonas*, *Rhizobium*, *Bacillus*, and *Burkholderia* (Krewulak and Vogel, 2008; Sabet and Mortazaeinezhad, 2018).

In an attempt to evaluate the impact of bacterial siderophores on apple (*Malus baccata*) under iron-deficient conditions, the *Pseudomonas* strain SP3 was found to have a significant effect on iron acquisition by the plant (Gao et al., 2022). Compared with the control seedlings, SP3 increased the iron content in the shoot tissue by 2.74-fold and in the root tissue by 44.6%. In addition, inoculation with SP3 comprehensively promoted plant growth by enhancing the characteristics of the plant root system (i.e., root density, length, surface area, volume, and hair length).

A greenhouse experiment revealed that *Bacillus subtilis* and their consortia positively impacted groundnut growth, yield, and iron content (Sarwar et al., 2022). The analysis indicates that *B. subtilis* inoculation and combination with soil-applied Fe resulted in the highest relative increases of 63% and 86% in shoot fresh and dry weight, respectively. The combined application of *B. subtilis* + Fe-SA (soil-applied iron) increased total Fe-intake by groundnut between 24.14% and 49.75%. In comparison, the *B. subtilis* + Fe-FA (foliar applied iron) treatment increased total Fe-uptake of groundnut between 14.73% and 45.72%, indicating that *B. subtilis* has the potential to promote groundnut growth and development and enhance the iron uptake by the plant.

While investigating the potentiality of new bacterial species to produce siderophores under salt-stress and iron-deficient conditions (Sultana et al., 2021), it was discovered that *Achromobacter denitrificans* MS3 produced the most siderophores (Pathania et al., 2020) under salinity (100 mM NaCl) and iron-limitation stresses. At the same time, *Bacillus aryabhattai* MS3 produced a 43% higher siderophore amount at a higher salt concentration, 200 mM NaCl. This highlights the possibility of involving these two strains in developing biofertilizers to aid plants in developing under various climatic changes.

High accumulation of Zinc in soil has a highly detrimental effect as it causes restricted plant growth, decreased crop yield, and formation of reactive oxygen species leading to the disruption of plant defense mechanisms (Lin and Aarts, 2012; Kang et al., 2017; Bilal et al., 2018; Mossa et al., 2020). Further, it has been suggested that increased Zn levels potentially decrease the chlorophyll content and induce iron insufficiency (Lesková et al., 2017). Accordingly, a study aimed to investigate the effect of the siderophore-producing bacteria *Leclercia adecarboxylata* MO1 on the growth of cucumber plants under zinc stress conditions (Kang et al., 2021). Results revealed, under Zinc stress, the ability of *L. adecarboxylata* MO1 to reduce the accumulation of Zinc, increase the chlorophyll content and promote plant growth in terms of the lengths and fresh weights of the root and shoot systems. Further research is necessary to determine the molecular mechanisms of *L. adecarboxylata* MO1 employed for plant iron acquisition.

Antimicrobials production

Fusarium is one of the most essential plant-pathogenic fungi that causes significant crop loss worldwide through the production of mycotoxins, toxic secondary metabolites which are detrimental to both animals and humans (Nganje et al., 2004; Arie, 2019; Perincherry et al., 2019; Summerell, 2019). Other phytopathogens include different species of *Rhizoctonia*, *Pythium,* and *Aspergillus*. Controlling plant pathogens with chemical fertilizers hurts the environment and human health (de Souza et al., 2015; Katiyar, 2016). Thereby, applying microbes as bio-controls for phytopathogens is safer and more sustainable for achieving healthy and economical crop production. Several PGPBs genera showed promising biocontrol capabilities against a wide range of phytopathogens through the production of various antibiotic compounds and antimicrobial peptides (*Table 2*) (Ali et al., 2020; Pathania et al., 2020).

Table 2. *Phytopathogens are affecting the growth of plants and their fighting microbes*

Phytopathogen	**Biocontrol agent**
Fusarium oxysporum	*Pseudomonas & Bacillus* *Gluconacetobacter diazotrophicus & H. seropedicae & Burkholderia ambifaria*
Sclerotium rolfsii	*Bacillus amyloliquefaciens & Bacillus subtilis*

Bacterial secondary metabolites that play a major role in biocontrolling plant pathogens include phenazines, 2,4-diacetylphloroglucinol (2,4-DAPG), pyrrolnitrin, pyoluteorin, and antimicrobial peptides (*Table 3*) (Mishra and Arora, 2018). Phenazines are a large group of nitrogen-containing natural compounds known for their pharmaceutical potential as therapeutics (Guttenberger et al., 2017). Phenazines are effective antimicrobial agents due to their ability to produce reactive oxygen species inducing DNA damage and cell death (Tupe et al., 2015; Mishra and Arora, 2018). 2,4-DAPG is an antimicrobial metabolite that contributes significantly to the biocontrol of phytopathogens (Almario et al., 2017). Bacteriocins, like subtilomycin, plantozolicin, and subtilosin A, are microbial antibiotic peptides that act on their targets via the cell envelope (Negash and Tsehai, 2020; Khatoon et al., 2022). Several microbial genera, such as *Bacillus*, have been shown to synthesize a wide range of bacteriocins (Li et al., 2020; Khatoon et al., 2022). Lipopeptides, such as bacillomycins, surfactins, iturins, and fengycins, are synthesized also by *Bacillus* spp. with known antibiotic properties against the biological membranes owing to their lipophilic nature (Khatoon et al., 2022).

Table 3. *Antimicrobials and their associated biological activity*

Antimicrobial agent	Biological Activity	Producing microbe
Phenazines	Generating reactive oxygen species & Promoting DNA damage and cell death	*Pseudomonas*
2,4-DAPG	Biocontroling of phytopathogens	*Pseudomonas*
Bacteriocins	Attacking the cell envelope of their targets	*Pseudomonas & Bacillus*
Lipopeptides	Disrubtion of the biological membranes	*Bacillus*

While screening for biocontrol rhizobacteria in multiple agro-ecological areas in Pakistan, various strains of *Pseudomonas* spp. and *Bacillus subtilis* exhibited more than 65% antifungal activity against a range of phytopathogens through the production of various compounds with known antimicrobial activity (Ali et al., 2020). *Pseudomonas* species produced various antifungal compounds including 2,4-DAPG, pyochelin, pyoverdines, phenazines, rhamnolipids, and violacein. *Bacillus subtilis* strains produced the lipopeptides surfactin and fengycin. Furthermore, both species secreted cell wall degrading enzymes, namely proteases and cellulases, to restrict the growth and proliferation of phytopathogens upon contact.

A microbial consortium comprising *Gluconacetobacter diazotrophicus*, *Herbaspirillum seropedicae*, and *Burkholderia ambifaria* induced a 71% inhibition rate, in vitro, against *Fusarium oxysporum* f. sp. *cannabis* (FOC) that cause wilting, shrinking and damage of *Cannabis sativa* plants (Pellegrini et al., 2021). Using each species separately also induced similar inhibition values. In a pre-emergence experiment (before germination), FOC infection greatly affected germination, with a 45% drop compared to the control. Lower disease intensity and improved germination were observed for the FOC-infected plants inoculated with the consortium (11% lower than the control). In other words, the inoculation enhanced the plant response to infection, suggesting that using the proposed consortium should be considered an effective biocontrol strategy against FOC infections.

Sclerotium rolfsii is another important plant-pathogenic fungus that results in considerable losses in crop production of various plants, including sugar beet (*Beta vulgaris*), which accounts for around 30% of worldwide sucrose supply (Rasu et al., 2013; Dwivedi and Prasad, 2016; Zhang et al., 2017; Paul et al., 2021). Multiple bacterial strains of the genus *Bacillus* significantly inhibited the *S. rolfsii* growth (more than 50%), in both in vitro and in vivo conditions through the production of various lipopeptides such as bacillomycin, surfactin, iturin, and fengycin (Farhaoui et al., 2022). In vitro, after 6 days of incubation, *B. amyloliquefaciens* MW644651 caused the largest inhibition rate, 70.35%, followed by *B. subtilis* MW644678 (67.68%). For the in vivo assessment, isolates were applied in a pre-emergence experiment on the sugar beet seedlings. *B. amyloliquefaciens* MW644651 recorded the lowest incidence rate, 20%, followed by two *B. subtilis* strains, MW644686 (30%) and MW644678 (41%). In greenhouse experiments, three *B. subtilis* isolates promote plant growth significantly. These results highlight the great antagonistic effect of *B. subtilis* strains which can be applied as a potential biocontrol agent in sugar beet plants.

ACC deaminase production

Plants respond to various stresses (infections, drought, high temperatures, and salinity) through different mechanisms (*Figure 4*), one of which involves the production of Ethylene, a stress phytohormone responsible for healthy plant development and resistance to both biotic and abiotic stresses. However, increased levels of Ethylene have a negative impact on plant growth and crop yield (Montero-Palmero et al., 2014; Thao et al., 2015; Bleecker and Kende, 2000). One mechanism that reduces the production of endogenous Ethylene involves the breakdown of 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of Ethylene, into α-ketobutyric acid and ammonia through the ACC deaminase enzyme activity (Glick et al., 1998). PGPBs exhibiting ACC deaminase activity include *Pseudomonas*, *Citrobacter*, *Serratia*, *Burkholderia*, *Bacillus*, and *Enterobacter* (Maxton et al., 2018; Pathania et al., 2020; Riaz et al., 2021). Since Ethylene is generally produced in response to stress conditions, ACC deaminase-positive strains must be inherently tolerant to these stresses.

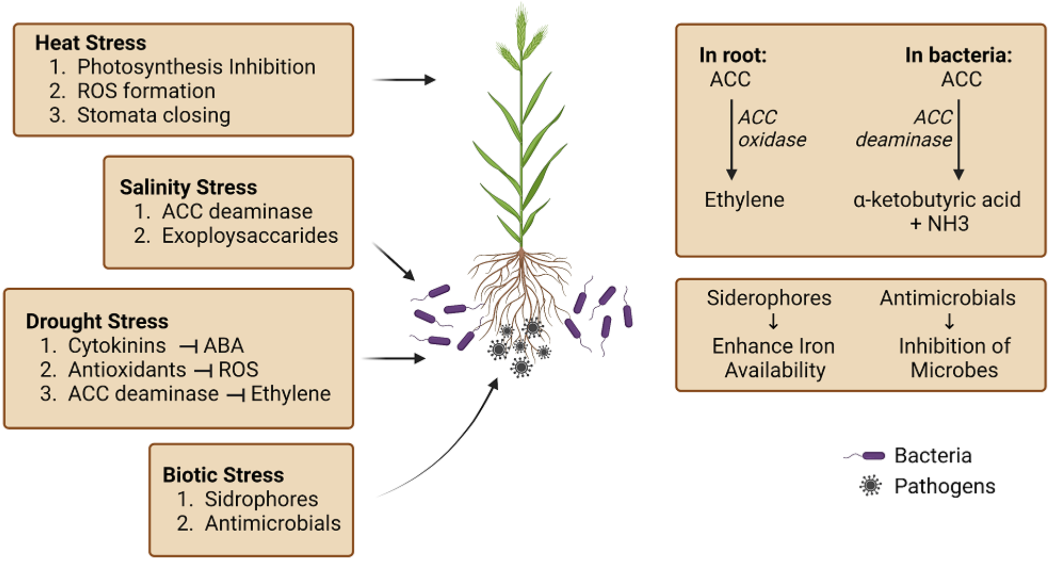

***Figure 4.** Plant response to various stresses*

Rice (*Oryza sativa* L.), one of the most widely consumed grain crops worldwide, is negatively affected by increased temperatures in terms of growth and grain productivity (Kilasi et al., 2018). Accordingly, a study aimed to evaluate the colonization efficiency of the ACC deaminase-producing bacteria *Brevibacterium linens* RS16 in order to improve heat-stress tolerance in the plant (Choi et al., 2022). Indeed, *B. linens* RS16 was able to colonize the rice endosphere and reduce the Ethylene levels with its ACC deaminase activity. Compared with the non-inoculated controls, Ethylene levels were observed to drop by 24.9% and 24.4% at 40°C and 45°C, respectively, after inoculating

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the plants with *B. linens* RS16. This highlights the promise of *B. linens* RS16 as PGPB in promoting plant heat-stress tolerance.

In like manner, heat-resistant strains with ACC deaminase activity were screened from the rhizosphere of a commercial crop in India, *Cyamopsis tetragonoloba* (Goyal et al., 2022). Under stress conditions, four isolates positively impacted the plant growth, one was identified as *Enterobacter cloacae* and the other three belonged to the genus *Pseudomonas*, *P. hibiscicola*, *P. koreensis*, and *P. extremorientalis*. In response to moderate stress, all *Pseudomonas* and *Enterobacter* strains sustained their growth at temperatures 30-50°C and 12% NaCl as salinity stress. Under increasing stress, the only species that could continue to develop at 60°C was *Pseudomonas hibiscicola*, whereas *Pseudomonas extremorientalis* was the only one that could do so at 18% NaCl. Therefore, the latter strains will be particularly valuable in developing biofertilizers for utilization in areas with a harsh climate, especially with high soil salinity and extreme temperatures.

In addition to high temperatures, drought and salt stress substantially affect plant physiological growth factors. On this subject, the ACC deaminase-producing bacteria *Bulkhorderia cepacia* was reported to induce drought and salt resistance in cayenne pepper (Maxton et al., 2018) plants (Maxton et al., 2018). Among three other isolates, *B. cepacia* had the highest ACC deaminase activity in normal conditions. When treated with different NaCl concentrations, plants inoculated with *B. cepacia* showed the best tolerance. Similarly, plants inoculated with *B. cepacia* survived better in different times of watering periods owing to the production of exopolysaccharides which induced drought tolerance.

Under salinity stress conditions, numerous microbes from the rhizosphere of different plants in Iran were assessed for PGPB traits in greenhouse experiments (Tafaroji et al., 2022). Among 181 isolates, *Pseudomonas alcaliphila* significantly promoted the growth of the alfalfa (*Medicago sativa*) plant in terms of the shoot length, shoot dry weight, and chlorophyll content. Another isolate, *Pseudomonas syringae*, showed better effects on the root dry weight and root length.

In *Paenibacillus* genera, high ACC deaminase activity was reported in *Paenibacillus xylanexedens* PD-R6 that was isolated from *Phoenix dactylifera* seedling roots (Orozco-Mosqueda et al., 2020). Additionally, it lengthened the roots of canola plants in both salty and normal conditions. Other species, *Paenibacillus polymyxa* SK1 isolated from *Lilium lancifolium* bulbs, produced ACC deaminase in addition to other important compounds such as IAA, siderophores, and organic acids making this strain a useful source of disease prevention and plant growth stimulation in sustainable agriculture (Khan et al., 2020).

Exopolysaccharides production

The ability to secrete polysaccharides is an essential PGPB trait since it enables the microbe to establish a biofilm matrix on soil particles and plant roots enhancing the texture and water content of the soil, nutrient supply to plants, and defense against phytopathogens (Rinaudi and Giordano, 2010; Bogino et al., 2013; Pandit et al., 2020). Even on sandy soils, bacterial exopolysaccharides were able to maintain the soil moisture and sustain the growth of plants (Khan et al., 2017).

To reflect the role of exopolysaccharides in mitigating drought stress in plants, an exopolysaccharide-deficient mutant of *Bacillus amyloliquefaciens* FZB42 was engineered and inoculated with *Arabidopsis thaliana* (Lu et al., 2018). The outcomes showed that plants' ability to induce drought resistance was substantially reduced. *B. amyloliquefaciens* FZB42 improved *Arabidopsis* root and shoot system growth, drought resistance, and plant survival rate. On the other hand, plants inoculated with the mutant strain were less able to endure the drought. Besides, the mutant strain could not establish a biofilm or colonize the plant root.

Under different NaCl concentrations, the role of exopolysaccharides biofilms as salinity tolerance promoters in faba bean (Alaa, 2018) was investigated (Alaa, 2018). Among 20 *Pseudomonas* strains tested, *P. anguilliseptica* SAW24 exhibited the highest activity for the formation of biofilm and exopolysaccharides production at 150 mM NaCl, which was determined based on the optical density (at 570 nm) as an indicator for the biofilm formation as well as the glucose levels in the bacterial cells. In addition, SAW24-inoculated plants had the highest values of plant height as well as fresh and dry weights.

Other strains have been observed to alleviate salt stress in plants growing in saline conditions. In Pakistan, two salt-resistant bacteria, *Thalassobacillus denorans* (Shah et al., 2017) and *Oceanobacillus kapialis* (Shah et al., 2017), were isolated and tested for their potential to improve rice plant growth in salty soils with various NaCl concentrations (Shah et al., 2017). When compared to un-inoculated seeds, it has been found that seeds inoculated with bacterial strains had a significantly increased germination percentage and rate. Plants grown from inoculated seeds had longer roots and shoots than untreated ones. Furthermore, Chlorophyll a, Chlorophyll b, and carotenoid levels significantly increased in 28-day-old plants treated with bacterial strains under various salinity conditions.

These studies, among others, prove the essential role of bacterial exopolysaccharides in the development of abiotic stress resistance in plants and the great potential of different bacterial strains to be employed for phytoremediation under different stress conditions.

IAA production

Besides Ethylene, IAA belongs to a very important group of phytohormones, Auxins, that stimulates plant growth, seed germination, root development, synthesis of various metabolites, photosynthesis, and tolerance to stress conditions (Spaepen and Vanderleyden, 2011; Kunkel and Johnson, 2021). The screening for PGPB in rhizospheric soils of various plants to evaluate their effect on the growth of radish (Agustiyani et al., 2022) resulted in discovering seven isolates that produced IAA in considerable amounts (Agustiyani et al., 2022). Further analyses were conducted to assess other PGP traits such as phosphate solubilization and nitrogen fixation and revealed that *Bacillus megaterium* and *Paraburkholderia tropica* were the most potent isolates. In a previously discussed study that isolated endophytic PGPR of the genus *Pseudomonas* and *Bacillus* from tomato roots, IAA production was also considered (Cochard et al., 2022). It was discovered that *Pseudomonas moraviensis* B6 produced the most IAA. In addition, the two *Bacillus subtilis* strains, B18 and B25, and *Pseudomonas fluorescens* B3 were also shown to produce IAA in considerable amounts.

Applications of CRISPR technology for sustainable farming

The CRISPR/Cas9 system is a powerful gene editing tool that allows for precise, selective DNA sequence alterations at any particular spot in the genome with much higher efficiency than traditional methods (Singh and Ramakrishna, 2021). More recently, several CRISPR-Cas systems have been introduced, enhancing the applicability of this cutting-edge technology in many other aspects (Pickar-Oliver and Gersbach, 2019).

Determining a protein's subcellular distribution is a key step in identifying the protein’s function. In 2019, researchers developed a CRISPR/Cas9-mediated endogenous gene tagging system that includes the insertion of fluorescent tags to proteins of the pathogenic fungus *Fusarium oxysporum* and observing the accumulation and regulation of these proteins during the fungal development through the detection of a fluorescent signal (Wang and Coleman, 2019). This demonstrated the method's effectiveness in characterizing proteins and determining their function.

CRISPR-Cas technology was employed in understanding the interactions between plants and microbes, which provided useful knowledge about the genetic factors and mechanisms in microbes leading to both beneficial and detrimental plant-microbe interactions by subjecting the plants and/or microbes to gene elimination, incorporation, substitution, and transcription restriction using CRISPR technology (Bisht et al., 2019; Singh and Ramakrishna, 2021).

Effective plant modifications against bacteria, fungus, oomycetes, and viruses

CRISPR technology can be used to characterize the plant-microbe interactions and manipulate these interactions for plant protection, agriculture, and other applications. Tomato bacterial speck disease results in bacterial leaf colonization due to stomata opening as a response to the binding of bacterial coronatine, produced by *Pseudomonas syringae*, to the SlJAZ2 co-receptor in tomatoes (Melotto et al., 2017). Using an innovative CRISPR/Cas9-mediated approach for crop management, researchers were able to create a resistant variant of the SlJAZ2 gene in tomatoes that don't respond to coronatine and provided resistance against the bacterial speck disease without affecting other stomatal characteristics, such as the rate of transpiration and necrotrophic resistance (Ortigosa et al., 2019), moreover, in Apple, there is Fire blight caused by *Erwinia amylovora* and using CRISPR they targeted DIPM-1, 2 and 4 genes. As a result, agronomists have had to constantly rise to the challenge of creating new varieties of plants that are resistant to phytopathogens. CRISPR/Cas has developed as an adjunct to conventional plant crossing and genetic modification methods. Plants resistant to bacterial, viral, and fungal diseases have been created using CRISPR gene editing technology.

CRISPR can be employed in other sustainable agriculture applications to benefit future agriculture. With the newly developing gene editing technology, it is possible to better understand the different aspects of plant-microbe interactions, including identifying plant genes influencing the rhizosphere microbiota and the effect of both beneficial and pathogenic microbes on the host plant.

Concluding remarks

Pseudomonas and *Bacillus* species are considered the most common PGPB genera used due to their extraordinary capabilities in promoting plant growth, increasing crop yield, and improving plant tolerance to stress conditions through nitrogen fixation, solubilization of micronutrients, production of siderophores and antimicrobials, and induced systemic resistance among other mechanisms. As a result, the usage of these bacteria might give an ecologically friendly substitute to toxic chemical fertilizers, therefore making the goal of globally sustainable farming possible. Over and above that, novel bacterial genera, including *Herbaspirillum* and *Paenibacillus*, were discovered to be promising bioinoculums for the alleviation of different stress conditions through the employment of similar mechanisms.

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