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BACILLUS SUBTILIS AS A PLANT-GROWTH-PROMOTING RHIZOBACTERIA: A REVIEW

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ABSTRACT

In addition to rhizospheric soil, Bacillus may be found worldwide. Root-associated Bacillus spp. are known as plant growth-promoting rhizobacteria for various reasons, including the formation of in dole acetic acid (IAA-auxin), phosphate solubilization, and siderophore production (PGPR). One of the most beneficial interactions between plants and microbes is plant growth-promoting rhizobacteria (PGPR). Rhizobacteria of the genus Bacillus may produce spores that persist in the soil for long periods, even in the harshest environments. PGPR enhances plant growth by inducing systemic resistance, antibiosis, and competitive omission. It is possible to use this genus to augment or improve soil fertility with other biocompatible bacteria, such as nitrogen-fixing species *Azospirillum* and *Azotobacter*. Based on this chapter's emphasis on the use of *Bacillus* on various commercially significant crops.
***Keywords*:** Rhizobacteria, *Bacillus subtilis*, Biocontrol potential, Biocontrol mechanism, Biotic stress, Abiotic stress, PGPR

Introduction

Several firms have promoted microbial products that increase the health and development of plants. It has been shown that bacteria from the plant rhizosphere have a favorable effect on plant roots and growth. These bacteria include rhizobacteria that promote plant development (PGPR). Rhizobacteria have direct and indirect impacts on plant growth, which contribute considerably to their favorable effects. The use of compounds that stimulate plant growth and reduce stress is one of the most straightforward methods. (Goswami *et al*., 2016) Root-PGPR interactions demonstrate beneficial effects on plant growth and stress reduction from biotic and abiotic sources. Resistance, antibiosis, and other mechanisms all contribute to the growth of plants (Tripathi *et al*., 2012).

Harmful nematodes, bacteria, weed seeds, and spider webs are just some of the many biotic stresses that plants are subjected to. Their hosts are damaged, their vigor is reduced, and the plants might die due to these agents. Pre- and post-harvest agricultural plant losses are also caused by them (Singla and Krattinger, 2016). Biotic and abiotic stressors adversely impact plant growth, development, yield, and biomass output (Chaudhary *et al*., 2012). Pseudomonas and Bacillus are the most common genera found in PGPR. It is possible to decrease plant stress by using PGPR in the rhizosphere because of its unique properties and close association with plants. Abiotic and biotic stressors may be alleviated by using genetically modified organisms (PGPR) in agricultural production systems (Grover *et al*., 2011, Radhakrishnan *et al*., 2017).

The rhizosphere is the thin film surrounding plants' roots, and it is the principal place for nutrient intake and key physiological, chemical, and biological processes. The rhizosphere is dominated by bacteria, which are the most common microorganisms. It is possible for bacteria to create long-lasting, stress-resistant spores and produce compounds that drive plant development and inhibit pathogen invasion (Glick *et al*., 2007). Abiotic stress tolerance may be improved by applying bacteria to the rhizosphere, particularly in the face of climate change-induced environmental challenges (Ryu *et al*., 2005). *Bacillus subtilis* may improve tolerance to biotic stressors. The expression of particular genes and hormones, such as 1-aminocyclopropane-1-carboxylate deaminase, is involved in inducing disease resistance (ACC). To preserve plant homeostasis, Ethylene restricts root and shoot development. A bacterial ACC alleviates plant stress and sustains normal development under stressful circumstances by degrading the ethylene precursor (ACC). This bacterium's volatile organic compounds (VOCs) assist plants in fighting disease assault (Firmin *et al*., 1986). For example, bacteria release exopolysaccharides and siderophores that prevent the flow of harmful ions and maintain the ionic balance, increase the circulation of water inside plants, and hinder the development of pathogenic germs (Ryu *et al*., 2005). Biocontrol potential and biocontrol mechanisms of *B. subtilis* and the use of *B. subtilis* to maintain and boost field crop yield in the presence of biotic and abiotic stress are all discussed in this paper.

Plant-Growth-Promoting Rhizobacteria (PGPR)

rhizoplane contains plant root surfaces and soil particles that adhere tightly to the rhizoplane, making up the rhizosphere. It has been observed that several soil bacteria may boost plant development by generating plant growth regulators, causing root exudation, and increasing the availability of nutrients to the plant, in addition to preventing soil-borne plant diseases (Kloepper, 1993). Biochemical nitrogen fixation, boosting the rhizosphere's nutrient availability, promoting root surface area, and improving other positive plant-host interactions are all examples of PGPR's potential to improve the nutritional condition of its hosts. Rhizospheric bacteria colonize the roots of leguminous plants, affecting the capacity of seed injected rhizobia to survive and nodulate (Kloepper *et al*., 2004). As a consequence of their synergism, rhizospheric bacteria may harm injected rhizobia through saprophytic competition and benefit them in terms of survival via an increase in their nodulation capacity and N2 fixation efficiency (Kloepper *et al*., 1989). Various processes, including the generation of plant signaling chemicals, bacteriocins, siderophores, plant growth hormones, and the improvement of nutrient availability by rhizospheric microbes, have been documented for this synergism (Bin *et al*., 2005). Root exudates include chemical substances readily absorbed and catabolized by the bacteria that live in the rhizosphere. Due to inoculation, Ramaswami and Oblisami (1986) noticed an increase in nodules (Ramaswami and Oblisami, 1986). The host and the bacteria exchange signals during the nodulation process. Certain rhizobacteria enhance plant development and rhizobial nodulation (Aiyer, 2004). The PGPR may directly impact plant growth through N_2 fixation and the development of biocontrol agents against soil-borne phytopathogens (Choudhary and Shrivastava, 2001). Plant-soil microbe biochemical interactions and signal molecule exchanges have been well documented and discussed (Mago and Shrivastava, 1994).

Bacteria are the most common microbes in the soil's rhizosphere. When competing microorganisms are present in the rhizosphere, rhizobacteria can aggressively colonize plant roots and thrive in all the ecological niches found on the roots at all stages of plant development. A PGPR is a dinitrogen-fixing bacterium that does not alter the host plant's morphology when it associates with the plant. In non-legume plants, rhizobia may act like PGPR, and certain rhizobia are endophytes (Salamone *et al*., 2001). Plant growth promotion may be induced by PGPR in two ways: directly or indirectly.

Nitrogen Fixation by *Bacillus subtilis*

Adding nitrogen to a plant's diet is essential to its development. Nucleic acids, proteins, and enzymes all rely on it. The problem is that nitrogen is in a gaseous state that plants or animals cannot absorb. For plants to absorb nitrogen from the atmosphere, ammonia must be formed. Nitrogenase, an enzyme complex found in nitrogen-fixing bacteria, aids in the process of biological nitrogen fixation (Paulsen *et al*., 2005). Microorganisms that can fix nitrogen are plentiful in the soil's rhizosphere. Symbiotic or non-symbiotic nitrogen fixation is possible. The symbiotic relationship is a win-win situation for microbes and plants (Davison, 1988). There is symbiotic interaction between *Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium*, and nonleguminous trees and shrubs with *Frankia* (Fuhrmann and Wollum, 1989). A non-symbiotic relationship of Cyans (*Nostoc* and *Anabaena*), *Azotobacter, Burkholderia, Enterobacter, Gluconacetobacter*, and *Pseudomonas* is formed (Lewis, 1991). This association may be free-living or endophytic. With the help of nitrogen-fixing microorganisms, seeds, seedlings, or the soil may accelerate plant development, improve soil quality and maintain a stable level of nitrogen (Fuchs *et al*., 2000).

N_2-fixing bacteria have been identified in many different forms, including root nodulating *Rhizobium* spp. (Bohlool *et al*., 1992) and other free-living rhizobacteria, such as *Azospirillum* and *Azotobacter* (Kloepper *et al*., 1992), as well as symbionts such as *Bacillus, Enterobacter* and *Pseudomonas*. Some of these free-living N2-fixing bacteria are known as plant growth-promoting rhizobacteria because they have favorable impacts on plant development when utilized as seed inoculants (PGPR). Because they produce plant growth hormones, fix N_2, and solubilize phosphate, several Bacillus species utilized as biofertilizers may have a direct impact on plant development (Amer *et al*., 2000; Cakmakci *et al*., 2001). Plant development is stimulated by Phosphate-solubilizing Bacillus spp., which increases N, P, potassium (K), and iron intake via improved P feeding (Whitelaw *et al*., 1997). (Fe). By enhancing the effectiveness of biological nitrogen fixation and the availability of Fe and zinc (Zn), phosphate biofertilizers might improve plant development by producing plant growth stimulating chemicals. Many crops have shown a boost in production thanks to the use of Bacillus species.

In agriculture, the capacity to fix atmospheric nitrogen by *Rhizobium* or *Bradyrhizobium* is also commonly employed to benefit crops. Co-infection of PGPR strains with rhizobia improves legume growth, nodulation, and nitrogen fixing. With the addition of PGPR and *Rhizobium/ Bradyrhizobium* spp., root and shoot biomass, nodule dry matter nitrogenase activity and N_2-fixation have been increased in chickpea (Whitelaw *et al*., 1997; Parmar and Dadarwal, 1999), common bean (Grimes and Mount, 1984, Sindhu *et al*., 1999), green gram, and soybean. Combining N_2-fixing and P-solubilizing bacteria inoculations was more successful than doing so alone, probably because it provided plants with a more balanced diet. Dual inoculations with N_2-fixing and P-solubilizing bacteria boosted yields in numerous crops compared to single inoculations. However, the majority of research indicating positive benefits of the above-mentioned PGPR were conducted in warm and subtropical locations with favorable ambient temperatures. In frigid temperatures, these bacteria may be rendered ineffective. It was decided to test the effects of single, dual, and triple inoculations with Rhizobium, N_2-fixing bacteria subtilis (and P-solubilizing bacteria megaterium) on nodulation, plant development, and chickpea seed output in Erzurum plateau's cold highland.

Methods

- Analysis of cell numbers and DNA concentrations using standard curves Using exponentially growing cultures of *R. palustris* and *B. subtilis*, cell suspensions with varying cell densities were generated by diluting those with new media or concentrating those with centrifugation at 10,000 X g for 10 minutes. When 2ml of each cell suspension was centrifuged, the precipitated cells were kept at -25°C for DNA extraction, as described in the paper. A Thoma'shemacytometer was used to count the number of cells in the suspensions. There were standard curves for Rps. palustris suspension cell number against the concentration of nifH, which encodes dinitrogenasereductase (Choudhary and Shrivastava, 2001), and cell number in *B. subtilis* suspension cell number vs the concentration of nifC, which encodes the g-polyglutamic acid synthetase (Choudhary and Shrivastava, 2001)

- The activity of nitrogenase may be measured A syringe was used to inject 5 ml of acetylene via a rubber cap into the sealed rectangular glass container after a six-day cultivation period. It was determined that ethylene was produced after two hours of cultivation at 30C with light irradiation, and it was detected by using a gas chromatograph (GC-4000, Tokyo, Japan) equipped with an ionisation detector and a capillary column (Rtaluminabond/KCl/0.32 mm inner diameter, 30 m length, Restek, Bellefonte, PA, USA). All of the oven, injector, and detector temperatures were set at 100C; nitrogen was utilised as a carrier gas at a rate of 3 ml/min. Using a standard curve made from ethylene gas (99.5%), the ethylene concentration was estimated (GL Sciences).
- Measurement of nitrogen and carbon concentrations Culture broths containing cells and medium before and after 7-d culture in the sealed rectangular glass bottlewere frozen at 25C and lyophilized to remove water. The biofilm generated duringthe co-culture was scraped off from the glass surface into the culture broth. Total nitrogen content in the co-culture was determined using the Kjeldahl method using a micro Kjeldahl distillation device (Sibata Scientific Technology, Saitama, Japan) (Sibata Scientific Technology, Saitama, Japan). An NC analyzer was used to determine the lyophilizate's carbon and nitrogen concentrations (Sumigraph NC-220F, Sumika Chemical Analysis Service, Osaka, Japan).

Phosphorous Solubilizing Properties of *Bacillus subtilis*

Phosphate and apatite are the most critical raw ingredients in creating phosphorus fertilizer. There are only finite amounts of phosphorus in mineral raw materials, making them a nonrenewable element supply. According to a list issued by the European Commission in May 2014, raw resources that are critical to the global economy have been updated. Phosphorus ores were among the six new minerals included in the 2011 list (Firmin *et al*., 1986). The European Commission approved a closed-cycle economy package in December 2015, which encompasses the complete product lifetime, waste management, and recyclable materials. So far, organic and waste-derived fertilizers cannot compete with conventional fertilizers made from raw materials. Hence, a proposal to alter the rule on fertilizers is one of the efforts toward adopting such a model. The most significant phosphorus loss is seen in waste streams (Saeid *et al*., 2018). Consequently, one of the most important ways to improve the global and local phosphorus balance is to develop systems for recovering phosphorus from the trash. Waste water by products like sludge and the ashes left over after the treatment process may be used as potential sources of phosphorus (Brears *et al*., 2018).

Chemical processing is necessary to remove phosphorus from these materials since it is inaccessible to plants and cannot be administered directly to the soil (Whitelaw *et al*., 1997). In addition to commercial chemicals, organic acids generated by soil microorganisms may be utilized to treat phosphorus-bearing waste flux (Atlas, 2018). Bacillus rhizospheric bacteria may boost plant growth by enhancing their capacity to provide nutrients to plants. When insoluble inorganic compounds like tricalcium and dicalcium phosphates are dissolved in organic acids, phosphorus is released into the soil for plant use. (Schroder *et al*., 2010; Meena *et al*., 2016), and acid phosphatase activity has increased (the mineralization of organic phosphorous) (Firmin *et al*., 1986).

Not only plants employ this method to convert the soil's inaccessible form of phosphorus, PO43, into the more readily available forms, HPO_{42} and H_2PO_4 (Oteino *et al*., 2015; Cheng *et al*., 2017). Acid phosphatase, an enzyme responsible for the solubilization of phosphorus, is activated in plants, which increases root acid exudation and improves phosphorus acquisition efficiency. As reported and shown in earlier studies (Rodriguez and Fraga, 1999, Awasthi *et al*., 2011), roots have the unique capability of responding to soil nutrient status by increasing the synthesis of organic acids. Stressed plants depend heavily on PSM (phosphate-solubilizing microorganisms) (Haicher *et al*., 2014). In certain circumstances, phosphate deprivation induces phosphate solubilization (Felix and Donald, 2002).

As the amount of P in the system increases, so does the organic acid concentration. Because organic acids are considered essential root exudates (Eisenhauer *et al*., 2017), they may displace unavailable phosphate from soil particles and increase phosphate availability for plants (Wu *et al*., 2017).

Sludge incineration ashes from wastewater treatment facilities with the third stage of biological treatment and bones with high phosphorus content are two of the most promising waste sources that might provide phosphorus (Bagyaraj, 2000). The natural capacity of soil bacteria to change phosphorus from unavailable to accessible has previously been characterized in the literature as a biotechnological valorization of these secondary raw materials, often regarded as wastes challenging to employ. Inorganic phosphorus (P) is used as a chemical fertilizer worldwide; however, prolonged use of fertilizers degrades soil quality. Consequently, the current trend is toward more environmentally friendly farming. As an alternative to the more traditional chemical method of freeing phosphate from phosphate-containing materials, soil microorganisms have created organic acid (Wyciszkiewicz *et al*., 2015).

It is critical to produce and identify organic acids, which are well known as a primary mechanism for releasing phosphorus from hydroxyapatite structures during the valuation of new types of raw materials. Three types of Bacillus species were utilized to solubilize phosphorus from three separate sources, two of which were renewable and one of which was not. Aims included determining the concentration of the filtrate's organic acid composition and whether the acids could solubilize low-quality phosphorus resource phosphate, both of which were provided in organic acids. With an eye on future phosphorus fertilizer production, researchers looked at how released P_2O_5 affects the development and pH changes of bacterial cells and the concentration of that P_2O_5.

For optimal development, plants need phosphorus, which is another macronutrient. It is an essential nutrient because of its vital function in metabolic processes, signal transmission, macromolecules production, and photosynthesis (Saeid *et al*., 2014). Plants have difficulty absorbing the vast majority of the phosphorus that is readily accessible because it is insoluble, immobilized, or precipitated. Depending on their needs, plants may use phosphate as monobasic or dibasic ions. There is a high concentration of phosphate solubilizing bacteria in the rhizosphere soil (Wyciszkiewicz *et al*., 2015). As well as making organic acids with low molecular weights like gluconic acid and citric acid and phosphatase enzymes that solubilize inorganic phosphorus into monobasic or dibasic ions, these bacteria that solubilize and mineralize phosphate are capable of solubilizing and mineralizing it (Krishnaraj and Dahale, 2014). Some of the PGPR family members are (Wyciszkiewicz *et al*., 2015) phosphate solubilizers, including the genus Arthrobacter and the genus *Bacillus*, the genus *Beijerinckia* and *Burkholderia,* and the genus *Erwinia* (Hayat *et al*., 2017). *Bacillus, Enterobacter, Erwinia*, and *Pseudomonas* are the best phosphate solubilizers. Additionally, it stimulates plant development by increasing nitrogen-fixing bacteria' ability to fix soluble phosphorus (Wei *et al*., 2018).

Siderophore Production by *Bacillus subtilis*

Another essential ingredient for plants is iron (Fe). Generally, it resides in the form of Fe^{3+} and Fe^{2+}, insoluble hydroxides and oxyhydroxides in an aerobic environment, making it inaccessible to plants (Kloepper, 1992). The tiny molecular weight iron chelators secreted by rhizospheric bacteria, known as siderophores, are particularly effective at binding to iron complexes. Many strains of the Gram-negative pathogen *Pseudomonas, Bacillus, Rhizobium,* and *Azotobacter* have generated siderophores (Kloepper *et al*., 2004). Iron may be solubilized from minerals or organic substances by these PGPR strains under iron-limiting conditions, and stable complexes with heavy metals and radioactive particles can be formed (Kloepper *et al*., 1989). As a result, heavy metals in soil may be alleviated by this capacity. Different methods, such as chelating and releasing iron, ingestion of siderophores-iron complexes directly, or a ligand exchange reaction, are used by plants to assimilate iron from siderophores (Redmon *et al*., 1986, Ryan and Ray, 2004).

Both iron sequestration (Volkmar *et al*., 1998) and heavy metal stress alleviation (Kim *et al*., 1998) are made possible by microbial siderophores, which serve a dual purpose. These Pseudomonad siderophores are well-known because of their strong affinity for ferric ions (Bin *et al*., 2005). Biocontrol pseudomonads have been shown to suppress phyto pathogens, including *Fusarium, Pythium,* and *Aspergillus* species, by producing siderophores (Sticher *et al*., 1997). Phytosiderophores generated by pseudomonads have been shown to suppress *Fusarium oxysporum* potato wilt disease (Van loon *et al*., 1998). In peanuts and maize, it inhibited the growth of phytopathogens *Fusarium moniliforme, Fusarium graminearum,* and *Macrophomina phaseolina* (Rautela *et al*., 2001).

Plant Hormone Production by *Bacillus subtilis*

Phytohormones are essential growth regulators because they stimulate metabolism and defense mechanisms in plants. Auxins, cytokinins, gibberellins, and Ethylene are among the phytohormones that PGPRs may stimulate. *Rhizobium, Bradyrhizobium, Mesorhizobium, Bacillus, Pantoea, Arthrobacter Pseudomonas, Enterobacter,* and *Burkholderia* all generate phytohormones. Bacteria isolated from the rhizosphere synthesized and released auxins on a large scale. Auxins are vital in plant cell division and differentiation, germination, phototropism and geotropism, the generation of metabolites, and the ability to withstand stress. The amino acid tryptophan, found in the root exudates of plants, acts as a precursor in bacteria to auxin production-bacterial detoxification of tryptophan yields auxins as a by product (Thomashow and Weller, 1996; Ahemad *et al*., 2009).

Some study suggests that tryptophan is not necessary for the production of auxin. If bacteria have several auxin production pathways (Dwivedi *et al*., 1993), they may be used as signaling molecules for bacterial communication and cooperation. All of these processes depend on plant cytokinins, which are crucial for cell division, seed germination, and the slowing of the plant's natural aging process. Bacillus and Pseudomonas were shown to be able to produce cytokinins. Gibberellins have an essential role in fruit ripening, seed germination, and viability (Leeman *et al*., 1995). PGPR bacteria such as Bacillus spp. have been demonstrated to boost gibberellin synthesis in the presence of *Enterococcus faecium*, *Pseudomonas,* and *Promicromonospora* to govern fruit ripening and the plant's capacity to adapt to environmental stresses such as pests, disease, and drought, the ethylene hormone plays a crucial role in plant growth and development. Bacterial synthesis of Ethylene is rare, but the enzyme they produce can counteract Ethylene's adverse effects on plants. *Azospirillum* and *Rhizobium* are among the PGPR strains with a deaminating enzyme that helps plants deal with stress. *Achromobacter* and *Burkholderia* also contain this enzyme. PGR helps promote the expression of genes that encode enzymes for the synthesis of Ethylene. ACC-synthase is both synthetic and oxidative.

***Bacillus subtilis* useful in biotic stress**

When living organisms, such as bacteria, viruses, fungi, parasites, nematodes, insects, and weeds, cause harm to plants, it is known as "biotic stress" (Reitz *et al*., 1993). This stress causes plants to deviate from their optimum physiological state. Because it reduces the expression of genes that are important for excellent yields and alters the plant's metabolism, biotic stress is a severe hindrance to commercial crops. Crop losses in India are attributed to insects, nematodes, and vertebrate pests, which account for up to 25% of those losses. Pesticides such as monocrotophos, aldrin, dichloro-diphenyl-trichloro-ethane (DDT), dieldrin, -benzene hexachloride (BHC), 2,4-dichlorophenoxyacetic acid (2,4-D), chlordane, and endrin are mostly (Vassilev *et al*., 2010; O'Mahony *et al*., 2006): weedicides such as atrazine, simazine, alachlor, metolachlor, and tri, For example, toxicity to beneficial soil microorganisms (Ali *et al*., 1981), influence on nontarget soil community (Schroth and Hancock, 1982), and drop-in helpful nontarget pollinators (Newton *et al*., 2011) are some of the adverse effects of pesticides on the environment. There is a pressing need to find alternatives to biotic stress mitigation that are environmentally friendly.

Nematodes

Instead of synthetic nematicides, biocontrol agents such as bacterial lytic enzymes are advised. Chitosanase and protease may change the egg's structure and prevent nematode egg hatching. The mortality rate of *Meloidogyne incognita* increases when chitinase is used. The cuticle of worms is degraded by lytic enzymes, proteases, and chitinases. Root-knot nematodes, such as M. incognita, may be controlled using PSB such as Bacillus subtilis and *B. megaterium*, which produce secondary metabolites having nematocidal action such as cyclic lipopeptides, surfactin, and iturin. Anti-M.javanica action has been proven by B. cereus' production of chitinase and glucanase. Egg hatching is inhibited by these enzymes, which lowers the survival rate of juveniles. Tomato crops using *B. subtilis* and *Pseudomonas aureus* applications effectively suppressed root-knot nematodes and managed M. incognita, egg counts (Delaplane, 2000 and Ferencz *et al*., 2010).

Fungi

Macrophominia phaseolina, Fusarium exosporium, Sclerotinum rolfissii, and *Trichoderma* spp. were the fungi employed in this investigation. The fungi were cultured at 28°C on Petri plates with Potato dextrose agar medium (PDA) for the first five days. This pathogen was implanted 2.5 cm away from a loopful culture of an older 24-hour-old bacterial strain, each at a distance of 1 cm^2 from the previously active fungal cultures. After 72 hours of incubation at 28°C, the plates were tested for antifungal activity every 24 hours (Anasco *et al*., 2010, Brittain *et al*., 2010).

As well as enzymes and other metabolites that degrade cell walls, bacteria create other microorganisms' development and activity (Romero *et al*., 2007). Furthermore, fengycin, surfactin, and iturin are all antimicrobial lipopeptides produced by *B. subtilis*. Lipopeptides are amphiphilic molecules with low molecular weight. B. subtilis-derived surfactants and antibacterial chemicals are gaining interest. A variety of biocontrolagents include lipopeptide genes, and several have been marketed for their ability to manufacture antibiotics and reduce fungal root pathogen populations. Lipopeptides have been shown to protect plants both before and after harvest by inhibiting pathogenic fungus directly or by developing systemic resistance in host plants by (Romero *et al*., 2017). Antibiotics produced by *B. subtilis* strains PCL1608 and PCL1612, mainly iturin A, are the primary mechanism for controlling Fusarium oxysporum and Rosellinia necatrix, respectively. Previous investigations show that iturin A has antifungal action against a number of target fungi, which is consistent with our findings. To combat pear-ring-rot disease, a research found that *B. amyloliquefaciens* L-1 was an effective biocontrol agent Bacillus strain 6051 generates surfactin and forms a robust, durable biofilm, making it an excellent biocontrol agent for harmful bacteria. The lipopeptides generated by *B. subtilis* include fengycins, iturins, and surfactins, all of which are antifungal and antibacterial antibiotics. Surfactants have high antibacterial action, but little effect on fungus, according to (Tinker, 1980). Mycosubtilin, D, F, and L bacillomycins, as well as bacillopeptin are subcategories of iturins. Iturins are a good biopesticide because they are antibacterial against fungus and yeast.

There are plipastatins, such as fengycins, which are less hemolytic than surfactins and iturins but have substantial antifungal action and inhibit bacterial and fungal development. Bacteriocins, a class of (Wang *et al*., 2015) peptide antibiotics produced by *B. subtilis*, are critical to the host's innate immunity. A bacteriocin's genetic and biochemical features place it into one of four categories. As an antibiotic, lantibiotics, which belong to class 1, are extensively used. The antibacterial activity and chemical structure of antibiotics generated by B. subtilis are used to classify them into A and B categories. Summary: Bacillus subtilis strains may operate as biocontrol agents against pathogenic fungi, which makes them useful in the fight against illness. Pathogenic fungi may be controlled by a variety of ways, both direct and indirect. Lipopeptides, the capacity to generate endospores, the ability to build biofilms on root surfaces, the ability to develop host systemic host resistance, and the ability to drive plant growth are just a few of the characteristics of this bacterium Wild *B. subtilis* strains are more likely to produce biofilms than laboratory or commercial strains.

Bacteria

Enzymes that break down cell walls and other compounds that inhibit the development or activity of other microorganisms are produced by bacteria. Antibiotic lipopeptides such as fencing, surfactin, and iturin are known to be synthesized by *B. subtilis* strains. Lip peptides are amphiphilic low molecular weight molecules. B. subtilis-derived surfactants and antibacterial chemicals are gaining interest. Many species and strains of biocontrol agents have lip peptide genes, and some of them have been marketed for their improved ability to manufacture antibiotics and reduce fungal root infections. As (Romero *et al*., 2007) observed, lip peptides protect plants against pathogenic fungus and host plant resistance in both pre-and post-harvest circumstances. *B. subtilis* strains PCL 1608, and PCL1612 generate a high amount of antibiotics, mainly iturin A, which acts as the primary mechanism underpinning the control of *Fusarium oxysporum* and *Rosellinia necatrix* in the study. In earlier studies, return A has been shown to have antifungal action against a wide range of fungi. In recent research, *B. amyloliquefaciens* L-1 was an effective biocontrol agent for pear ring-rot. Bacillus strain 6051 generates surfactin, which indicates that it might be used as a biocontrol agent against pathogenic microorganisms.

***Bacillus subtilis* useful in abiotic stress**

Abiotic stressors across the globe negatively impact crop growth and agricultural output. Abiotic factors like high temperature, salt, and drought may harm rain-fed agro-systems like those in India. Drought and salinity would impact more than half of arable land by 2050, according to an estimate of 20 percent of total cultivated and 33 percent of irrigated agricultural areas affected by salt stress. Agricultural sustainability is threatened by pathogens such as fungus, bacteria, nematodes, and viruses. Intriguingly, the spread of diseases and insects is also influenced by abiotic conditions such as high temperatures and dryness. In order to increase agricultural output, it is often necessary to mitigate the impacts of many abiotic and biotic stressors on plants at the same time.

Drought

Polyethylene glycol (PEG 6000) was used to make nutrient broth with a range of water potentials (-0.05, -0.15, -0.30, -0.49, -0.73 MPa) injected with 1% of overnight grown bacterial cultures in NB. A spectrophotometer measured the optical density at 600 nm after a 24-hour incubation at 28°C under shaking conditions (120 rpm).

Cheng *et al*. (2002) Increased germination and plant biomass, as well as increased photosynthetic capacity and decreased stress-induced ethylene production, were observed at varied NaCl concentrations (50–125 mg/L) in sugar beet.

According to (Vardharajula *et al*., 2011) Increased plant biomass, water content, and the ratio of root-to-soil adsorption, as well as a reduction in leaf water loss, were all achieved by using these PGPRs. Proline, sugars, free amino acids and electrolyte leakage were enhanced as a result of the osmoregulation effects of these microorganisms. Antioxidant enzymes ascorbate peroxidase, catalase, and glutathione peroxidase activity was decreased after inoculation.

Wang *et al*. (2012) Reduced leaf monodehydroascorbate (MDA) and relative electrical conductivity, respectively, while increasing leaf proline content by 3.45-fold. Also maintained leaf chlorophyll content under drought stress when cucumber plants were treated with these PGPRs; Aside from that, compared to controls, the bacteria significantly increased the activity of the superoxide dismutase enzyme (SOD) and mitigated the down-regulation in cucumber leaves of the genes encoding the large and small subunits of cytosolic ascorbate peroxidase (RAP), rbcL, and rbcS, respectively, of ribulose-1,5-bisphosphate carboxy/ oxygenase (Rubisco).

Gagné-Bourque *et al*. (2016) found that this bacterium had a favourable effect on plant development in both drought stress and nonstress situations. There was a considerable increase in shoot and root biomass of 26.6 percent and 63.8%, respectively, when the plant was exposed to an 8-week drought stress compared to non-inoculated plants cultivated under the same circumstances. Several sugars, most notably sucrose and fructans, as well as important amino acids such asparagine, glutamic acid, and glutamine were found in greater concentrations in colonised plants than in non-colonized plants after exposure to this bacteria. As a result of the buildup of GABA in the leaves of plants.

Salinity

One percent of overnight-raised cultures in NB were injected into the nutrient broth with NaCl concentrations ranging from 1 percent to 20 percent. The optical density at 600nm was used to measure growth after 24 hours of incubation at 28°C under shaking conditions (120 rpm).

According to (Shukla *et al*., 2012), "When comparing inoculated and uninoculated plants, the length, weight, dry weight, and biomass of the infected plants were all greater. Plants that had been infected had considerably greater percentage water content (PWC) in the shoots and roots than plants that had not been inoculated. Higher K^+/Na^+ and Ca^{2+}, Phosphorus and Nitrogen levels were also found in the infected plants. Inoculated plants had a greater content of IAA in both shoot and root explants.

Bano and Ullah have a lot in common. (Bano and Ullah, 2015) PGPR inoculation and co-inoculation on maize under induced salt stress increased shoot and root length and fresh and dry mass of shoot and root. These PGPRs improved the accumulation of osmolytes and the activity of antioxidant enzymes, including superoxide dismutase and peroxidase, in the maize variety when inoculated and co inoculated.

According to Heydarian *et al*. (2016) Under the absence of salt, bacteria inoculated into plants enhanced shoot length, whereas in moderately salty circumstances, they boosted seed output by 30–50 percent. The synthesis of ACC deaminase was a factor in this increase in growth under salt stress.

According to Sharma *et al*. (2016) the total nitrogen (N) concentration increased significantly (up to 76%) compared to the non-inoculated control. To cope with salt stress, peanut seedlings that had been injected with bacteria maintained their ion homeostasis as well as accumulated fewer reactive oxygen species (ROS).

Synergistic interactions between *B. subtilis* and root nodule bacteria

When a new strain of rhizobia is employed to inoculate plant roots, the number of nodules that form may be reduced. Introducing new varieties may not be able to keep up with the already well-established domestic varieties. Nodulation is a process in which the host and bacteria exchange signals that lead to rhizobia being established in host tissues, nodulation, and increased plant development via higher nutrient absorption from the surrounding soil (Tilak *et al*., 2006). Both root nodule bacteria and B. subtilis seem to have a good impact on plant disease control and growth when applied to the roots of plants. There are several types of microbes that are connected with roots, including those that are free-living, rhizosphere-dwelling or endophytic (Sharma *et al*., 2016; Tilak *et al*., 2006). In certain situations, these microbes manufacture their own phytohormones. *B. subtilis* and arbuscular mycorrhizal fungi synergistically help in P solubilization, according to Zaidi *et al.* (2009).

Bacillus, Acinetobacter, Enterobacter, Pseudomonas, and *Sinorhizobium* have all been found in the soil and rhizosphere. The roots of soy beans were shown to provide a breeding ground for several bacteria, including *Bacillus, Enterobacteria, Arthrobacter, Mycobacteria, Cellulobacterium* and *Pseudomonas*. However, inoculation with *P. putida*, *Pseudomonas fluorescens*, or a *Bacillus* strain improved root nodulation, enzyme production, and plant development as compared to non-inoculated plants even if a PGPR was used. In the roots of the halophyte shrub *Prosopis strombulifera*, endophytic diazotrophic bacteria have been shown to manufacture plant growth hormones (JA, GA_3, IAA, and ABA). It has been shown that bacteria, such as *Pseudomonas* and *Ochrobactrum,* are capable of producing IAA when exposed to harsh circumstances. Cytokinins were shown to be generated by the three *Bacillus* species: *B. megaterium*, *B. cereus*, and *B. subtilis*. On geranium, researchers used a combination of arbuscula rmycorrhizal (AM) fungus and *Bacillus subtilis* to boost production by 49.4 percent, while yield rose by 59.5 percent when the two fungi were used together. There was an increase in biomass output, but the dry weight oil content didn't change considerably. A synergistic impact on plant development is shown by *B. subtilis* when combined with AM fungi. Increased plant growth, enzyme synthesis, antioxidants, P solubilization, biocontrol action, root nodulation, and nitrogen fixation may be achieved with the use of the combination application. More effort should be undertaken to produce a commercial formulation of Bacillus PGPR strains, particularly those that quickly generate endospores; this is based on the data.

Conclusion

When it comes to biologicals, which include a variety of natural elements, including microscopic organisms, you're an expert since you're an organic producer. Microorganisms, which include fungus and bacteria, defend plants against disease, insects, and weeds with their tiny structures. As illness and insects develop resistance to chemicals, they become less effective. They are a feasible and sustainable alternative to pesticides.

One thing you may not have known is that not all microorganisms are the same. There are several strains and strengths of the bacterium *Bacillus subtilis*, a commonly used and efficient component in antifungal products.

In the same way as saying, "Let's go acquire a vehicle," Keith Giertych, of Growth Items, who distributes a wide range of horticultural and agricultural products across the world, explains.

Bacillus subtilis, on the other hand, is a nice man in a world full of bad ones. *Bacillus subtilis* "is deemed a benign bacterium since it does not exhibit features that cause illness," according to an EPA Toxic Substances Control Act study. Humans, animals, or plants cannot get ill from it since it is neither toxic or harmful to them. "The danger of this bacteria being used in a fermentation facility is minimal."

Like a plant breeding programme that creates a cucumber with all the desired features a farmer is looking for, the strength and effectiveness of bacillus is mainly dependent on how it is chosen and colonized in the laboratory.

Sr no.	Author	Findings
1.	(Shambhavi *et al.*, 2017)	"The utilization of inorganic fertilizers increases the crop production; however it causes long term degradation of soil fertility over the years"
2.	(Kumar *et al.*, 2019)	"In order to fulfill the world food security, excessive usage of inorganic fertilizers were practiced that in turn became one of the vital reasons for declining in soil fertility"
3.	(Bargaz *et al.*, 2018).	"Utilization of fertilizers greatly concern with biological characteristic changes which in turn indicate the soil fertility rate"
4.	(Afzal *et al.*, 2019.	"PGPR, the main ingredients of bacterial biofertlizers has hefty relationship with different species relevant to host plants. Rhizospheric and Endophytic, the two prime classes of relationship which are found in the intercellular"

It is possible to use commercially available microbes to stimulate plant growth, which has been done successfully. Researchers have discovered microorganisms in the plant's rhizosphere that benefit roots. Both directly and indirectly, research shows that plant roots with high concentrations of plant growth-promoting rhizobacteria (PGPR) are beneficial to plant growth. Inducing systemic resistance (ISR), antibiosis, and competitive omission of helpful microorganisms may help plants grow. As the needs for organic and sustainable production systems and climate change increase, these rhizospheric bacteria need to be used more extensively. Some Bacillus species can produce endospores that tolerate harsh environmental conditions and release chemicals that help plants grow and thrive. Species of Bacillus. It is, therefore, possible to increase stress tolerance and adapt to climate change via the appropriate use of beneficial microorganisms. To assist plants in recovering from stress, the Bacillus subtilis strain (GB03) produces volatile organic compounds (VOCs). Another way Bacillus species help maintain a healthy ecosystem is by producing exopolysaccharides and siderophores that block or stop detrimental ions from passing through the plant's roots. These compounds also limit the growth of pathogenic microorganisms. If new biocontrol agents are discovered, a thorough study of Bacillus species and strains is needed. Researchers may better identify and assess new bacteria that alter microbial life to increase the favorable interactions between plants and chosen microorganisms. Using technology, it is feasible to discover PGPR that improves stress tolerance, soil fertility, nutrient absorption, and crop output. In future research, there will be a lot more effort to identify beneficial Bacillus isolates that create plant-associated microbial communities and enhance plant health. All three of these disciplines may work together to create new strategies to cope with both biotic and abiotic stresses.

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