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ARTICLE

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MICROBIAL BIOFERTILIZERS: BIORESOURCES AND ECO-FRIENDLY TECHNOLOGIES FOR AGRICULTURAL AND ENVIRONMENTAL SUSTAINABILITY

BAISHALI PANDIT*

Production of healthy crops to meet the demands of the world's expanding population is entirely dependent on the type of fertilisers used to supply the plants with all the essential nutrients, but an increased reliance on chemical fertilisers is destroying the environment's ecology and having a detrimental effect on human health. Therefore, utilising microorganisms as bio inoculants is evidently the most eco-friendly alternative to chemical fertilisers for plant development and soil fertility. Studies on biofertilizers have clearly demonstrated that they could improve agricultural yields significantly without causing environmental disruption.

Introduction

A third of the world's gross domestic output comes from the agriculture sector. However, the world's population is expected to reach 9.5 billion people by 2050 due to the tendency towards population growth, which would increase the need for food. The primary barriers to the production of numerous crops are the scarcity of fertile land, urbanisation, unforeseen weather occurrences linked to climate change, and abiotic and biotic stressors[1]. In order to increase crop production per unit area and achieve the stated aim of food security, additional crucial factors include soil quality, nutrient availability, environmental factors, and the biological health of the soil. Chemical fertilizers are employed excessively in modern high input farming systems and technologies to provide the plant nutrient demand for raising agriculture production globally. Due to low fertilizer usage efficiency, only a small portion (30–40%) of these nutrients are absorbed by the plants; the remainder is lost to the soil and causes environmental degradation. Chemical fertilizers also include radio nuclides and heavy metals, which are difficult to breakdown and are persistent contaminants in nature. The development of sustainable and environmentally friendly technology was necessary to address the pollution issues posing risks to the public health and might limit the use of artificial fertilizers[2]. In order to improve soil fertility and plant growth, it is now possible to use beneficial microbiomes as biofertilizers in sustainable agricultural practices[3-6].

Microorganism communities that are varied, intricate, and interactive are present on all terrestrial plants[7,8]. Microbes may colonize plants by an epiphytic, endophytic, or rhizospheric process[9]. Over the last two decades, there has been a lot of interest in the study of microbial communities that live in various environments and how they collectively contribute to plant growth, development, and protection. According to some reports[10], several of these bacteria inside the plant's microbiome perform various crucial functions in fostering the growth and development of agriculturally significant crop plants. Microorganisms isolated from the rhizosphere are tested for plant growth-promoting (PGP) features as well as effective colonization ability in order to identify effective beneficial microbial strains for usage as biofertilizer[11]. The decomposition of organic matter, improvement of nutrient availability,

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* Assistant Professor, Department of Botany, Surendranath College, Kolkata, e-mail: baishalipandit@gmail.com

production of phytohormones, and contribution to mitigation of abiotic and biotic stresses are just a few of the advantageous qualities of PGP microbes for use as biofertilizer[12,13]. Biofertilizers are live or dormant cells that are applied to soil, seeds, or seedlings in order to increase the availability and absorption of nutrients from the soil. As an alternative to chemical-based fertilizers, using biofertilizers has become recognised as being more cost-effective and ecologically friendly. Significant advancements have recently been made in the creation of efficient biofertilizers for several crops.

Mechanisms of Action of Beneficial Microbes

Both direct and indirect mechanisms that contribute to improved plant health and crop productivity are used by microorganisms to promote plant development[14-16].

Fig.1. Mechanism of Action of Beneficial Microbes

Direct Mechanisms Involved in Plant Growth Promotion

Enhanced Nutrient Availability: The growth of plants fundamentally depends on 16 different micro- and macronutrients, and if any of these nutrients are deficient, the growth of the plant may be dysfunctional or unbalanced. Different soil, climate, and agricultural plant characteristics have an impact on nutrient availability. In order to promote better plant development and agricultural productivity, soil microbes keep soil nutrients at their ideal concentration. Through the solubilization of zinc, potassium, and phosphate, nitrogen fixation, and the production of phytohormones, rhizosphere management, which uses beneficial microorganisms, improves nutrient availability in soil for improved plant development[17,18]. According to recent research[19], mycorrhizal fungi and PGPR are thought to be essential for the availability of nutrients in soil as well as the reduction of stressors. As a result, these microorganisms have grown to be critical parts of the soil ecosystem's efficient operation. Several reports have shown that applying beneficial microorganisms (as biofertilizers) can increase nutrient levels by (i) altering plant metabolism and the composition of root exudates, (ii) influencing the solubility and availability of nutrients, or (iii) increasing interactions with other soil microbes. By secreting substances such as oxalate, gluconate, citrate, catechol, lactate, and pseudobactin, microbes mineralize nutrients[20-22]. In exchange for absorbing the carbon from the plant, arbuscular mycorrhizal fungi boost the availability and absorption of water and minerals in terrestrial plants. Reports show that nutrient-mobilizing microbial inoculants stimulates root and shoot development, improves nutrient absorption, and increases seed output of various crops[23,24].

Phytohormone Production: Phytohormones or plant growth regulators, are produced by plants and bacteria in very small quantities. These hormones affect a variety of physiological processes, such as cell division, development, gene expression, and stress responses, as well as root and shoot growth, shape, flowering, senescence, and seed growth[25]. The ability of plant roots to absorb nutrients and water is enhanced by phytohormones because they lengthen root hair and increase root surface area. Increased metabolic activity as a result of phytohormone synthesis aids in defense, proper cellular function, and abiotic stress management[25]. During biotic and abiotic stress, hormone-secreting microorganisms that promote plant development either secrete hormones or change the concentration of hormones inside the plant. Phytohormones may be divided into five classes: auxin, cytokinins, gibberellins, ethylene, and abscisic acid. Other classes have also been found, such as strigolactones, jasmonates, and brassinosteroids, which serve as targets for metabolic engineering to create agricultural plants that can survive abiotic stress. Only a small number of bacteria are known to release gibberellins, whereas the majority of PGPRs are known to generate cytokinins, auxins, and ethylene. Although *Pseudomonas* has been noted as a superior IAA generator, *P. putida* outperforms *P. fluorescens* in this regard. The genome of the *Bacillus thuringiensis* strain RZ2MS9 has been shown by scientists[26] to possess the whole set of genes necessary for the production of indole acetic acid.

Numerous microbial taxa have been shown to produce cytokinins and gibberellins, which are bioactive hormones that influence a variety of developmental processes including blooming, fruit setting, stem elongation, root hair growth, and seed germination.

Indirect Mechanisms Contributing Towards Plant Growth Stimulation

Siderophore Production: A lack of iron can result in improper respiration and photosynthesis since it is one of the essential components involved in plant metabolism. Aerobic settings contain iron as $Fe^{3+,}$ which is a significant resource in soil. When consumed by microorganisms and plants as Fe^{+2} form, Fe^{3+} rapidly produces hydroxides and oxyhydroxides, rendering it inaccessible to them[27-29]. Through the secretion of siderophores, which are chelating substances with low molecular weight, ferrous is acquired. After the siderophore and Fe^{3+} have formed a complex, the Fe^{3+} form is converted to Fe^{2+} and released into the cell. This readily absorbed Fe^{2+} is either directly taken up as an iron-siderophore complex or the iron is transferred via a ligand. Oxygen and nitrogen, two elements rich in electrons, make up siderophores and attach to cations. *Pseudomonas*, *Aeromonas*, *Azotobacter*, *Bacillus*, *Rhizobium*, *Streptomyces*, *Burkholderia*, and *Serratia* are among the microorganisms that produce siderophores[30, 31]. Fluorescent *Pseudomonas* species that produce siderophores improved the iron nutrition of graminaceous and dicot plants. According to several reports, inoculating pepper with siderophore-producing *Bacillus subtilis* significantly suppressed the Fusarium wilt disease brought on by *Fusarium oxysporum*. Green gram (*Vigna radiata*) plant disease was discovered to be controlled by siderophore-producing *Pseudomonas* species, which also promoted plant development. Similarly, inoculation by *Pseudomonas aeruginosa* that produces siderophores suppressed the illness in paddy and chilly.

Enzyme Production: Any organism's metabolic activity is controlled by the activity of numerous enzymes. Structure-rich biomolecules in soil are depolymerized and mineralized by extracellular enzymes released by bacteria, archaea, and fungus. Activity of these enzymes may be changed to aid in carbon sequestration, bioremediation, and the stimulation of plant growth.*Pseudomonas*, *Bacillus*,*Xanthomonas*, and *Agrobacterium sp.* produce proteases and lipases, as do other PGPRs. Ascorbate peroxidase (APX), catalase (CAT), glutathione/thioredoxin peroxidase (GPX), and glutathione S-transferase all contribute to the reduction of stress under abiotic stress circumstances[32]. The hydrogen peroxidase enzyme also serves as a signalling molecule during biotic and abiotic stress, photosynthesis, and the cell cycle.

Due to the reduced concentration and activity of numerous antioxidant enzymes, including catalase, glutathione reductase (GR), ascorbate peroxidase, manganese-dependent superoxide dismutase (MnSOD), peroxidase (POD), and POD, salinity stress tolerance in wheat was improved by inoculation of PGPRs. When *Pseudomonas sp.* Rh323 and *Pseudomonas sp.* were inoculated into rice plants, strong polyphenol oxidase activity in the leaves was observed along with maximum phenylalanine ammonia-lyase and peroxidase activity in the treated plants. The growth of pathogenic fungi such as *Botrytis*, *Rhizoctonia*, *Sclerotium*, *Phytophthora*, *Pythium*, and *Fusarium* was also discovered to be inhibited by the synthesis of lytic enzymes by beneficial bacteria, such as chitinases, lipases, proteases, cellulases, and -1,3 glucanases. On medium plates, *Pythium aphanidermatum* and *Rhizoctonia solani* growth were inhibited by five *Pseudomonas* strains that were shown to generate chitinase and cellulases. Under sterilised conditions, combining these antagonistic *Pseudomonas* strains with the *Mesorhizobium sp. Cicer* strain significantly increased nodule biomass. PGPRs inoculation can prevent diseases such as rhizome rot and leaf blight in turmeric, collar rot in peanuts, and early blight in tomatoes.

Antibiotic Production: Antibiotic synthesis is the most common tactic used to survive during microbial competition[33]. Antibiotics are low-molecular-weight heterogenous chemicals that are harmful to competing microbial strains. Aldehydes, ketones, alcohols, and sulphides are examples of volatile antibiotics. Phenylpyrrole, cyclic lipopeptide amino polyols, and heterocyclic nitrogenous compounds are examples of non-volatile antibiotics. Antibiotics may have antibacterial, antiviral, antioxidant, anticancer, anti-helminthic, phytotoxic, and/or cytotoxic properties. At low concentrations, they may also operate as chemicals that encourage plant development. The antibiotic-producing strain and the competing strain with IAR both give survival strategies as a result of the bacteria developing IAR (intrinsic antibiotic resistance) against the antibiotics. Antibiotics generated by PGPR are also antagonistic compounds created against phytopathogens. Antibiotics prevent the growth of harmful organisms by altering the cell membrane, preventing translation, stopping at the stage of ribosomal RNA production, and inhibiting the creation of cell walls.

PGPR strains have been found to produce various types of antibiotics such as 2,4-diacetyl phloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA), phenazine-1-

carboxamide, pyroluteorin, pyrrolnitrin, oomycin A, viscosinamide, butyroaminectone, kyanoaminectone, zymicrolactone, zymicrolactone A, rhamnolipids, cepacyamide A, ecomycins, pseudomonic acid, azomycin and cepafungins[34]. Besides this, various *Bacillus* strains have been reported to produce antibiotics like mycosubtilin, bacillomycin D, iturins, fengycin, surfactin and zwittermicin A, while on the other hand fluorescent *Pseudomonas* produces pyoluteorin, phenazines, oomycin A, 2,4-diacetyl phloroglucinol, viscosin and massetolide A.

Induced Systemic Resistance: In response to any pathogenic assault, plants have defensive mechanisms that include systemic acquired resistance (SAR) and induced systemic resistance (ISR). Two powerful responses, the ethylene and jasmonate pathways, named for the signalling molecules involved, are used to defend against pathogen attacks on plants. In the case of ISR, signalling molecules include flagellar proteins, the O-antigen side chain, chitin, pyoverdine, lipopeptide surfactants, and salicylic acid. The secretion of phytohormones, PAMPs (pathogen associated molecular patterns), MAMPs (microbes associated molecular patterns), and the production of elicitor molecules, such as volatile organic compounds, siderophores, phytases, and miRNAs, are some of the different strategies used by biocontrol agents to ensure ISR in plants. After inoculation with *Bacillus amyloliquefaciens*, there was a reduction in disease incidence of around 80% in tomato spotted wilt virus, and the disease control was connected with salicylic acid signalling system.

Induced systemic resistance against pathogens resulted in deposition of callose, lignin and phenolics in epidermal and cortical cell walls, boosted expression of stress genes and overproduction of enzymes including peroxidases, phenylalanine ammonia lyase, chitinase and polyphenol oxidase along with increased production of phytoalexin. Salicylic acid stimulated the production of catechin and pro-anthocyanidins in the poplar plant and inhibited the growth of the foliar root fungus *Melamspora larcipopulina*[35]. According to recent reports[36], strain RS11 controlled defence against the necrotrophic caused by *Botrytis cinerea* and *Alternaria alternata* as well as genes involved in the manufacture of ethylene and jasmonic acid.

Production of Hydrogen Cyanide and Ammonia

The generation of hydrogen cyanide (HCN) and ammonia by the PGPRs is a crucial characteristic for biocontrol action. According to recent reports, HCN has been shown to chelate metal ions, release phosphorus into soil, and act as a highly toxic metabolite that inhibits the growth of phytopathogens. The use of PGPR strains as a biocontrol agent in agriculture is ensured by the production of hydrogen cyanide[37]. As they affect the development and productivity of different crops, PGPB strains that produce HCN are utilised as biofertilizers[38, 39]. Along with HCN, rhizobacterial strains also create ammonia, which gives plants nitrogen and aids in biomass production and root and shoot elongation. Sendi et al. demonstrated that three pathogenic strains belonging to *Fusarium sp.*, *Macrophomina sp.*, and *Alternaria sp.* were inhibited by up to 71% of twelve bacterial strains (endophytic and rhizospheric isolates) collected from field-grown common bean plants. The synthesis of xylanases, chitinases, siderophore, HCN, and indole-3-acetic acid as well as phosphate-solubilizing activity were discovered by biochemical study of the antagonistic and plant growth-promoting activities. In vitro testing of 39 *Bacillus* isolates by Pathak et al.[48] revealed that 48.7% of the isolates were IAA producers, 38.4% had the capacity to solubilize phosphate, and 71.8% had the capacity to produce ammonia. The capacity to produce protease and hydrogen cyanide was shared by all the isolates[40].

Types of Biofertilizers

Biofertilizers are made from active or dormant microorganisms that improve nutrient absorption and plant performance in the rhizosphere. The process for making biofertilizers is simple, and installation costs are low. They can be made up of one, or a combination of two or more different microbial strains, or plant growth-promoting bacteria, and AM or arbuscular mycorrhiza. According to distinct categories, biofertilizers can be categorised as follows:

Nitrogen-fixing Microbes: Biological nitrogen fixation (BNF) is the process by which diazotrophic microorganisms convert atmospheric nitrogen into ammonia. BNF enables the total nitrogen content to be replenished, and fixed nitrogen controls crop development and production. Numerous bacterial strains with various physiologies, such as anoxygenic (*Rhodobacter*) or oxygenic (*Anabaena*) phototrophs, anoxygenic (*Clostridium)*, facultatively anaerobic (*Klebsiella*), heterotrophs, and chemolithotrophs *(Leptospirillum ferrooxidans*), are capable of nitrogen fixation. Diazotrophic bacteria can form symbioses with legumes causing the development of root nodules. The crucial enzyme known as nitrogenase is responsible for turning dinitrogen into ammonia during the nitrogen fixation process. At least 20 nitrogen fixation (nif) genes in aerobic diazotrophic bacteria encode nitrogenase. Nif genes are arranged in seven operons in the model organism *Klebsiella pneumoniae*, which span over 24 kb of DNA and are found on either plasmids or chromosomes. These operons contain a variety of nif genes, including structural, regulatory, and supplementary genes.

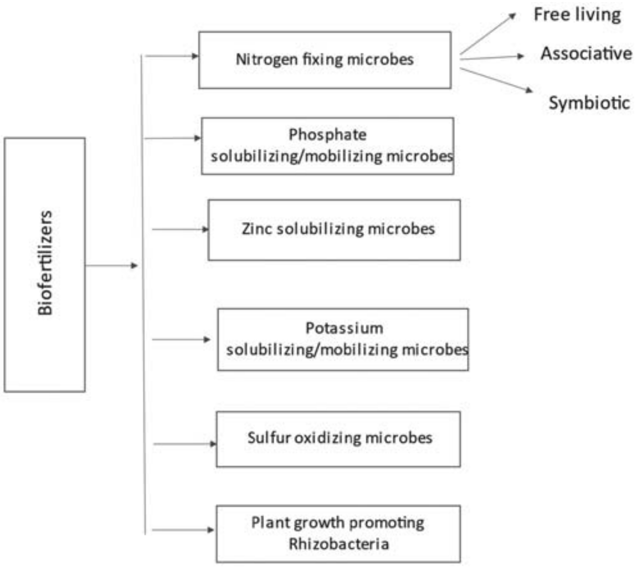

Fig. 2 Types of Biofertilizers

Symbiotic Nitrogen-fixing Microbes: *Mesorhizobium*, *Azorhizobium*, *Allorhizobium*, *Rhizobium*, and *Sinorhizobium* species (together referred to as *Rhizobium*) are responsible for the symbiotic association with the roots of legumes. On leguminous plants, different *Rhizobium* strains produce nodules that aid in the enhancement of soil fertility, plant nutrition, and growth[40]. Leghemoglobin is produced in nodules, which aids in maintaining the low oxygen concentration required for the action of oxygen-sensitive nitrogenase. In comparison to non-leguminous plants, Rhizobium's nitrogen-fixation mechanism allows legumes to rely less on artificial fertilisers[41]. According to certain reports, inoculating soybean with several *Rhizobium* strains improved growth and yield components such as the number of branches yielding pods per plant, the total number of pods per plant, and the number of seeds per plant and per pod. Similarly, increased nodulation, shoot/root weight, and pea seed output were achieved by inoculating *Rhizobium leguminosarum* strains onto pea and lentil seeds.

Free-living Nitrogen Fixing Bacteria: One of the most important species of diazotrophic bacteria that are free-living is *Azotobacter*. The rhizosphere of a variety of non-legume crops, such as cotton, wheat, rice, and vegetables, often contains several *Azotobacter* strains like *Azotobacter chroococcum*, *Azotobacter insignis*, *Azotobacter beijerinckii*, *Azotobacter macrocytogens*, and *Azotobacter vinelandii*. These bacteria have the capacity to fix up to 2-18 mg N/g of carbon employed in culture medium. Some *Azotobacter* strains have been shown to release bioactive substances like phytohormones, which improve mineral intake through promoting root development and operate as potential biocontrol agents. Under conditions of iron shortage, *Azotobacter vinelendii* secretes azotobactin siderophore. Inoculating wheat (WH291) with *Azotobacter chroococcum* strain A103 resulted in a 16.3% increase in grain yield. Seed treatment with *Bacillus strain SYB101* increased the yield of seeds by 32.6% for the wheat variety WH711 and by 23.1% for the wheat variety Raj3765 when compared to the uninoculated control treatment. Cyanobacteria like *Anabaena, Nostoc, Aulosira*, *Calothrix*, also known as blue green algae (BGA) are also a significant free-living community that fixes nitrogen.

Associative Nitrogen-fixing Microbes: *Azospirillum* species associate with plant roots and produce substances which promote plant development, such as IAA, gibberellins, and cytokinin. Currently, 17 different Azospirillum species have been identified, although *Azospirillum brasilense* and *Azospirillum lipoferum* have received the most attention[41]. In comparison to uninoculated control plants inoculation of *A. lipoferum* strain JA4 in plants, increased plant development (higher height and dry weight of root as well as shoots). Also, in comparison to single inoculation with *Rhizobium* alone or uninoculated plants, double inoculation of *Rhizobium* species with *Azospirillum* and/or other PGPR strains significantly increased nodule number, nitrogen fixation, plant biomass, and total nitrogen contents of several legumes.

Phosphate-solubilizing/Mobilizing Microbes: An essential macronutrient for plant growth and development is phosphorus[42]. The most common forms of phosphorous are tricalcium, dicalcium phosphate, and minerals. Phosphate-solubilizing bacteria perform the solubilization and mineralization process in soil, which involves converting phosphate from its organic form to its inorganic form. Citric acid and gluconic acids, two organic acids secreted by PSB, solubilize the organic phosphate reservoirs. In order to mineralize the organic phosphate reservoirs, PSB also secretes phytases and nucleases enzymes. PSBs are also well recognized for producing secondary metabolites that promote plant development. It is interesting to note that ability to produce indole acetic acid was associated with improved phosphate solubilizing activity of rhizobacteria. *Escherichia phosphaticum*, *Bacillus*, *Pseudomonas, Achromobacter, Brevibacterium, Erwinia sp., Flavobacterium sp., Micrococcus sp., Rhodococcus sp., Serratia phosphaticum, Acytonema, Calothrix brauna,* and *Tolypothrix ceylonica, Burkholderia*, and many fungi including *Fusarium sp., Saccharomyces cerevisiae, Torula thermophila, Penicillium, Cephalosporium sp., Aspergillus sp.*and *Alternaria sp.,* were reported to show phosphate solubilization. *Bacillus, Escherichia, Arthrobacter*, and *Pseudomonas* are phosphorus-solubilizing microbial strains that have been reported to solubilize up to 30-35 kg P_2O_5/ ha. Inoculating peanut with PSB *Rhodopseudomonas palutris*, PSB *Burkholderia cepacia* ISOP5 may increase the yield of crop by 12.5% and 19.5%. The protein composition and nitrogen absorption of peanut seeds were enhanced by these treatments. An increase in the content of genes involved in the mineralization of organic phosphorus and the solubilization of inorganic phosphorus was also brought about by the inoculation of these bacteria. Scientists investigated the improvements in P availability brought about by silicon addition, phosphate-solubilizing bacteria inoculation, and arbuscular mycorrhizal fungus (AMF) inoculation. Comparatively to employing each of them alone, the combination strategy of applying Si together with AMF and PSB was shown to be quite effective in increasing the P availability and its absorption by plants. Another related research[43] assessed the effects of coinoculating PSB with an arbuscular mycorrhizal fungus to encourage sunchoke growth and production in outdoor settings. The findings indicated that PSB had an advantageous impact on AMF spore density and colonisation rate. The growth and output of sunchoke were favourably associated with both AMF and PSB inoculation.

Potassium-solubilizing Microbes: After nitrogen and phosphorus, potassium is the third most important nutrient for plants[44]. As only a tiny portion of potassium of soil (between 2% and 3%) is accessible to plants, a system of continuous potassium replenishment in soil solution is required for it to be available to crop plants. Significant physiological processes in plants including the generation of starch, root development, and stomatal movement are impacted by potassium. Potassium deficit results in sluggish, incomplete root development, tiny seeds, increased susceptibility to disease, and decreased crop output. The potassium that is present in the soil and rhizosphere is converted into soluble form by PGPRs. Some of the potassium solubilizing microbes (KSMs) are *Acidothiobacillus, Enterobacter hormaechei, Pseudomonas, Sphingomonas, Bacillus circulans, Burkholderia*, and *Acidothiobacillus ferrooxidans*. Application of KSMs may be a potential tactic for increasing agricultural output because organic acid released by microorganisms causes potassium to dissolve.In sandy loam soils, some workers treated faba bean (*Vicia faba* L., cv. Giza 843) plants with 50 and 100% of potassium dose with or without potassium solubilizing bacteria (PSB) and 40 kg of humic acid (HA). The soil treated with HA and PSB had the highest potassium usage efficiency (KUE) (40%) of all the soil samples. Humic acid and PSB applied to plants fertilised at 50% of the recommended rate resulted in the greatest growth and production of faba bean plants. As a result of HA and PSB treatment, the amount of chlorophyll and carbohydrates in the leaves rose by 36 and 50%, respectively, above the control. In comparison to complete mineral fertilisation without bacterial inoculation, the seed and straw yields increased by 14% and 19% when half the K needs for faba beans were added in a mineral form with 40 kg of HA and PSB. Raji and Thangavelu[33] extracted fifteen culturable saxicolous (rock-dwelling) bacterial isolates from two locations, each with a different degree of K solubilizing capacity. By 16S rRNA gene sequencing, four isolates that were potential K solubilizers were found to be *Bacillus subtilis*, *Bacillus cereus*, *Bacillus licheniformis*, and *Burkholderia cepacia.* Different bacterial strains solubilized potassium differently, and K sources had a big impact. Under in vitro circumstances, isolated KSB generated several organic acids, indole acetic acid, and siderophore. In both sterilised and non-sterile soils grown in greenhouse environments, KSB inoculation enhanced tomato plant development characteristics such as plant height, leaf area, total root length, root/shoot ratio, and tissue K content.

Zinc Solubilizing Microbes: Zinc contributes to the production of enzymes, proteins, as well as chlorophyll in plants.Chlorosis, low membrane integrity and leaf size, delayed shoot growth, decreased grain yield, pollen formation, root development, water uptake and transport, and increased susceptibility to heat, light, and fungal infections are all symptoms of zinc deficiency in plants[43].Inoculating the crop with zinc-solubilizing microorganisms is an efficient method of supplying zinc to the plant. The organic acids produced by microbes sequester zinc cation from zinc complexes in soil by formation of chelated ligands, siderophores, and the redox system found on cell membranes. *Pseudomonas sp., Rhizobium spp., Bacillus aryabhattai, Thiobacillus thioxidans*, and *Azospirillum sp.* are notable zinc-solubilizing microbes. From *Capsicum annuum* L., Bhatt[34] identified bacteria that solubilized zinc; isolate CDK25 was discovered to be the most powerful. Additionally, isolate CDK25 (identified as *Bacillus megaterium*) has several PGP characteristics, including the ability to solubilize phosphate, produce phytase, indole acetic acid, and produce siderophores.By cultivating the 50 bacterial strains on tris-minimal agar medium with insoluble Zn compounds (ZnO and $ZnCO_3$), Batool et al.[35] tested the bacteria's ability to bind zinc in vitro. Six possible zinc solubilizing bacteria (ZnSB) (ZnSB7, *Paenibacillus polymyxa*; ZnSB11, *Ochrobactrum intermedium*; ZnSB13, *Bacillus cereus*; ZnSB21, *Streptomyces*; ZnSB24, *Stenotrophomonas maltophilia*; and ZnSB25, *Arthrobacter globiformi*) were selected based on Zn solubilization efficiency. In a pot experiment, chickpea seeds inoculated with ZnSB13 showed the highest levels of phosphatase, dehydrogenase, and microbial activity as well as the highest soil Zn availability. Chickpea dry biomass, nodulation, and yield significantly increased after ZnSB13 strain inoculation due to enhanced net photosynthetic rate, transpiration rate, stomatal conductance, and water usage efficiency. Additionally, the inoculation of ZnSB13 resulted in the greatest increases in grain N, grain P, and grain Zn concentrations in the chickpea root, shoot, and grains, indicating better Zn biofortification in chickpea.

Sulphur Oxidizing Microbes: Plants require large amounts of the macronutrient sulphur because it is a component of macromolecules like amino acids (cysteine, cystine, and methionine) and plays a role in the regulation of several enzymes, including glutathione reductase, superoxide dismutase, ascorbate peroxidase, monodehydro-ascorbate reductase, and ascorbate reductase. Low lipid content, chlorosis, and decreased plant development and yield are all symptoms of sulphur deficiency. Both organic and inorganic sulphur are present in soil, and sulfur-oxidizing bacteria (SOB) like *Xanthobacter, Alcaligenes, Bacillus, Pseudomonas, Thiobacillus sp.* convert organic sulphur into plant-useable inorganic sulphur (i.e., SO_4^2 form). Other plant growth-promoting properties were also displayed by bacteria that oxidised sulphur. From salty and sulfidic ecosystems in Iran, some scientists identified salt-tolerant sulfur-oxidizing bacteria of the species Halothiobacillus. Eight strains from each of the three Halothiobacillus species—*H. neapolitanus, H. hydrothermalis*, and *H. halophilus*—were identified. During the oxidation of thiosulfate and elemental sulphur, salinity (0, 0.5, 1, 2 and 4 M NaCl) had a substantial effect on bacterial biomass and sulphate production. *H. neapolitanus* strain 19 produced the most biomass and sulphate at 0.5 and 1 M NaCl concentrations, indicating that these bacteria may be used to boost the sulphate storage in salty soils and for crop development. Gilani et al.[38] did a similar study to examine the effects of *Thiobacillus* and various sulphur fertiliser levels on growth and physiological parameters in the replacement intercropping of sesame and mung bean. Three amounts of sulphur fertiliser were used: control level (S0), 50% of the recommended amount (S1), and 100% of the recommended amount (S2). Two levels of Thiobacillus bacteria were utilised: T0 and T1. The findings demonstrated that the investigated treatments had a substantial impact on the growth indices, chlorophyll a, chlorophyll b, total chlorophyll, biological yield, and grain yield of sesame and mung bean.

Plant Growth Promoting Rhizobacteria: The PGPR bacteria are naturally occurring, free-living, and found in the rhizosphere. These bacteria generate and release metabolites that, after colonising plant roots, stimulate plant development. After inoculation, PGPR aids the plant's resistance to biotic stress, salinity, and drought stress[43]. Inoculating rapeseed with six phosphorus-solubilizing bacteria (three *Bacillus* strains, one *Serratia*, one *Arthrobacter*, and one *Pantoea* strain) increased crop production by 21–44% even in the absence of P treatment in the field. Some of the PGPR strains have several advantageous properties, and their inoculation may have a synergistic impact that increases crop production. In a different experiment, blackberry roots were inoculated with *P. fluorescens* strain N21.4, which can produce siderophores and chitinases. This led to improved plant growth and fruit production as well as an increase in the promotion of total phenolics, flavonols, and epicatechins/catechins metabolites. In comparison to single inoculations of PGPR (*Rhizobium* or *Pseudomonas fluorescens*), PSB (*Bacillus polymyxa*), or control uninoculated plants in wheat, coinoculation of PSB and PGPR strains, i.e., *Bacillus polymyxa, Rhizobium*, and *Pseudomonas fluorescens*, significantly improved nutrient uptake. The effects of a consortium of four bacterial strains—*Azospirillum brasilense, Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae*, and *Burkholderia ambifaria*—on the development of *Allium cepa* L. and the health of the soil were assessed by certain scientists. In comparison to the control under field conditions, the results revealed that bacterial administration had a beneficial effect on plant development, increasing plant height (+18%), total chlorophylls (+42%), crop yields (+13%), and bulb dry matter (+3%). Out of 36 plant growth-promoting bacteria (ST-PGPB) isolated from sodic soil in eastern Uttar Pradesh, India, researchers characterised *Bacillus pumilus*strain JPVS11 as being the most efficient. At various NaCl concentrations (0–1200 mM), this strain produced IAA, ACC deaminase activity, P-solubilization, proline buildup, and exopolysaccharides (EPS). Rice (*Oryza sativa* L.) variety CSR46 was the subject of a pot experiment with varying NaCl concentrations (0, 50, 100, 200, and 300 mM) both with and without the addition of *Bacillus pumilus* strain JPVS11. A significant improvement in plant height (12.90-26.48%), root length (9.55-23.09%), chlorophyll content (10.13-27.24%), carotenoids (8.38-25.44%), plant fresh weight (12.33-25.59%), and plant dry weight (8.66-30.89%) was observed from 50 to 300 mM NaCl concentration in inoculated plants as compared to non-inoculated plants after *Bacillus pumilus* strain JPVS11 inoculation.

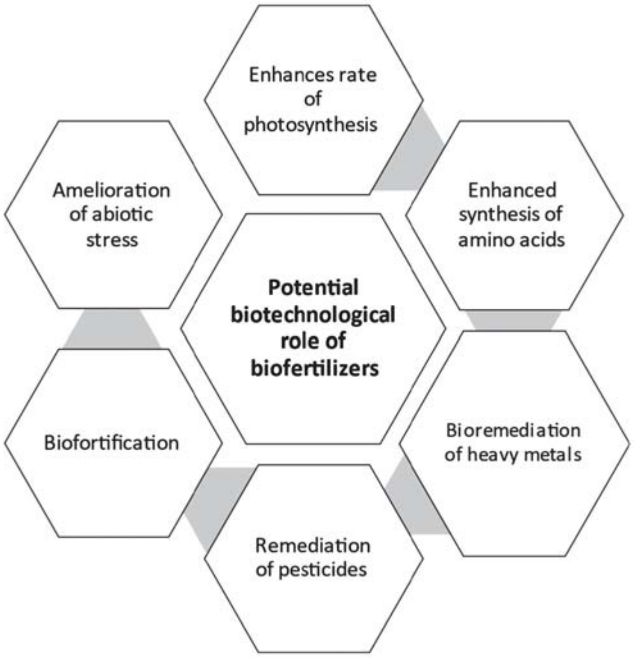

Fig.3 Potential Biotechnological Role of Biofertilizers

Conclusion

A potential method for increasing crop yields and decreasing the usage of chemical fertilisers is biofertilizer inoculation[44.45]. This method aims to create sustainable agriculture while protecting the environment. In order to facilitate the accessibility of nutrients like N, P, K, Zn, and S, as well as the modulation of phytohormones, the suppression of plant diseases, and the reduction of abiotic stresses, a variety of plant growth-promoting microbes have been characterised for advantageous traits. Under greenhouse and field conditions, inoculations of a single beneficial microbe or a group of them have been shown to increase plant biomass and crop output[45]. However, there are several factors that can sometimes prevent crops from growing under field settings in a variety of agricultural habitats, and the inoculation of multifunctional PGPR strains does not increase plant growth or crop output and agri-product quality. Since microbe growth circumstances vary frequently between laboratory and greenhouse environments, microorganisms may not survive or operate well in the field. To improve their lifespan and efficiency and boost crop output, these helpful biofertilizer strains are now amended with organic ingredients, cell protectants, and nanoparticles. Additionally, the discovery of genes and the sequencing of many microbial genomes offered a potent tool to facilitate the production and release of PGP compounds by the helpful bacteria. Additionally, technological advances like the emergence of next-generation sequencing, gene editing, bioengineering of microbial communities, in silico protein modelling, and synthetic biology[45]may enable the manipulation of plants and microbes to deliver short- and long-term solutions for improving crop productivity to feed the world in a more sustainable way. ❐

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