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PGPR and Biotechnology Trends in *Bacillus* Spp. *B. subtilis*

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Review Article

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ABSTARCT

Background: Rhizosphere is site of highly production of secondary metabolites by microbes that play key role in pharmacy and agriculture. The Plant Growth Promoting Rhizobacteria (PGPRs) are the most abundant organisms of rhizosphere that comprise a broad range of biological activities. Biotechnology approach researches of the rhizosphere have had helped enormously with management of diseases prevention and enhancing livelihood of both humans and plants.

Scope: The diverse natural bioactivities secondary metabolites of PGPR *Bacillus* genera possess antibacterial, antifungal, anticancer and insecticidal activities. These activities prevent the pathogenicity in soil and indirectly promote plant growth. These natural products are precious commodity in pharmaceutical, drug discovery and drug design.

Conclusion: This review highlights generally PGPR *Bacillus* spp.; particularly *B. subtilis* role in pharmaceutical and agriculture field.

Keywords: Rhizosphere; Bacteria; *Bacillus subtilis*; Plant growth promoting rhizobacteria; Biotechnology

INTRODUCTION

Rhizosphere is the zone around the plant roots where enormous microbes and other important chemical reactions/metabolic pathways process take place for plant growth and development. Since, the Russian Noble laureate, Elie Metchnikoff inception of beneficial microbe used the word Probiotics. Probiotics is defined as 'live microorganisms, administered in adequate amounts confer health benefits'. Over the years probiotic has gained popularity and been used in several clinical and general health scenarios.

In the soil the plant and the associated microorganisms are dependent on each other's whether it's secondary metabolites or mineral. The plant associated microorganisms communities are referred as the plants' other genome or plant microbiome. These microbiomes are part of complex food web that utilizes the plant exudates. Rhizosphere vicinity includes diverse form of life such as bacteria, fungi, oomycetes, nematodes, protozoa, algae, viruses, archaea, and arthropods. These microorganisms colonized within plant boundaries bears greater cell densities and the number of genes outnumbers by far the number of plant cells and genes [1-3].

LITERATURE REVIEW

Plant-associated microorganisms

The plant-associated microorganisms have profound effects on productivity, diseases, nutrition, seeding vigor, seed germination and plant growth. These microbes have shown to play important role in environmental detoxification, nutrient cycling and major resources of industrial, medicinal and agriculture applications.

In mycorrhizosphere, rhizosphere, phyllo-sphere and spermo-sphere plant relying on the microbe's functionality traits photosynthetic compound in return. The plant exudates alter the microbial community, composition and activities. Nitrogen (N) fixation has only been restricted to prokaryotes i.e. bacteria and archaea and as symbiotic association the plant provides niche carbon compounds. *Aspergillus, Bacilus*, *Penicillium* and *Pseudomonas* are well-known Phosphorus (P) solubilizing spp. The extreme ecological (hydrosphere and lithosphere) niches are profound of bacteria as the most dominant organism compared to overall pool of microbes. Accordingly to the genera of bacteria, gram-positive bacteria particularly clostridia, mollicutes, and bacilli in soil mineralize plant-derived compounds, hydrocarbons, pesticides and humus. Gram-negative bacteria for regulation of their virulence traits employ N-Acyl-Homoserine Lactone-based Quorum Sensing System (AHL-QS). However, the AHL molecules can be degraded with a process called Quorum Quenching (QQ) which has been revealed by various leaf associated QQ bacteria such as *Myroides* sp., *Pseudomonas* sp., *Serratia* sp., *Bacillus sphaericus* sp., *Acinetobacter* sp. and *Bacillus* sp. These bacterial quenchers have plant suppressing properties which *in situ* have been found as effective bio-control reagents. disruption of N-acyl homoserine lactone signaling molecules of potato pathogen *Pectobacterium atrosepticum* which cause blackleg and soft rot disease have resulted in prevention of the disease. Also, studies have shown that the combination of strains on soil drench significantly enhance the inhibitory activities and increase seed lipid content. Hence, deciphering the plant microbiome is important for enhancing plant attributes as well as being environmental friendly. Hence, deciphering the plant microbiome is critical in identifying bacteria for further exploitation in improving plant attributes and is environmentally friendly (Tables 1 and 2).

Table 1. List of different PGPR application to plant and the plant response.

PGPR	Response	Rhizosphere	Applied plant	Analysis
Paeni *Bacillus polymyxa* - BSP1.1	Higher accumulation of P and K, improved both photosynthesis and water use efficiency	Potato	Capsicum chinense	Vernier scale, Kjeldahl method, portable infrared gas analyser system
Genera *Azospirillum* and *Azotobacter*	Increase growth, yield and nutrients uptake		Maize	flame photometer, Walkley and Black method, Kjeldahl method
Pseudomonas strains- BHUJY13, BHUJY16, BHUJY20 and BHUJY29	Higher plant growth attribute, yield, nutrient contents	Rice	Rice variety	16S rDNA gene sequencing, BLAST, AOAC (1970)
Bacillus cereus, Brevibacillus reuszeri, and *Rhizobium rubi*	Increase growth, yield and nutrients uptake (macro and micro-nutrients)	Tea	Broccoli	chlorophyll meter, fatty acid methyl ester analysis (MIDI system), Kjeldahl method
Azotobacter species, *Nitrobacter* species, and *Nitrosomonas* species	Best performance in terms of growth parameters	Tomato (*Lycopersicon esculentum*)	Tomato (*Lycopersicon esculentum*)	Chrome azural (CAS) agar; tricalcium phosphate agar; salvakowski reagent
Bacillus subtilis (AJ276351)	Better root, shoot and leaves yield; N, P, and K uptakes increased	Forest-growing fields	*Fraxinus americana*	Ultra-PLC electrospray ionization tandem mass spectrometry bioassay for cytokinin; 16S rRNA gene
Bacillus amyloliquefaciens IN937a and *B. pumilus* T4	Increase plant growth and N, P uptake		Tomato	CNS element analyzer; TOC/ TNb analyzer LiquiTOC II
Sinorhizobium meliloti, Bacillus megaterium, Enterobacter sp., *A. chroococum, Pseudomonas* sp.	Shoot Dry Weight (SDW) and shoot N, P and K content	Alfalfa root nodules	Barley and oats	plates containing tricalcium phosphate;
Bacillus megaterium; Pseudomonas fluorescens	Improved the root growth and yield; high nutrient uptake as well as nutrient use efficiency		Maize	standard methods
Azospirillum, Azotobacter, Mesorhizobium and *Pseudomonas*	Improvement in grain yield and plant biomass		Chickpea	standard methods

Table 2. List of PGRs producing PGPR screened from various plant rhizosphere.

PGPR	Plant	PGRs
Azospirillum lipoferum	Wheat	Indole-3-acetic acid
Azospirillum brasilense	Rice	Indole-3-acetic acid
Azotobacter sp.	Maize	Indole-3-acetic acid
Azotobacter sp.	Sesbania	Indole-3-acetic acid
Mesorhizobium loti	Brassica	indole-3-acetic acid, hydrocyanic acid, hrom-azurol, siderophore
Pseudomonas denitrificans	Wheat, maize	Auxin

Plant Growth Promoting Rhizobacteria (PGPRs): *Bacillus*

PGPRs are the most abundant microorganisms among the coexisting microbes in the vicinity. These PGPR facilitates direct or indirectly the plant. PGPR directly are involved in facilitating plant growth as resource acquisition of phosphorus, nitrogen and other essential minerals, or modulating levels of plant hormones. PGPR indulge of synthesizing and degrading organic matter, solubilizing P, fixation of soil N and production of phytohormones, siderophore and ethylene. The genera *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium* spp. comprises the N fixer bacteria [4,5].

The indirect mechanism involves decomposition of crop residues and production of hydrolytic enzymes, HCN, siderophores, antibiotics etc; suppressing of plant disease to obtain sustainable fertility of the soil and plant growth. The 'induced resistance' have become renowned approach for better plant attributes and eliminating the barriers for sustainable agriculture and agro-ecosystems determine Induced Systemic Resistance (ISR) to be linked with salicylic/jasmonic acid and ethylene pathways. *Phytophthora parasitica* var. *Nicotianae* is soil borne pathogen that causes tobacco black shank disease was prevented with the newly isolate strains PGPR *Paenibacilllus polymyxa* C5. Through confocal laser scanning microscopy the *P. polymyxa* C5 growth in soil was detected on root tip and elongation zone but no growth was detected inside the roots. *Paenibacillus polymyxa* were found to produce cell wall degrading enzymes such as xylanase, protease, chitinases, cellulases and β-1,3-glucanases. The *P. polymyxa* producing β-glucans in cell wall on immune system of experimental animals are used as immune-stimulants or as adjuvant of some animal vaccines.

Genus *Bacillus* represents one of the most diverse genera in the class bacilli. The members of this genus are known for exhibiting a broad spectrum of DNA base and major amino acid cell wall composition. On the basis of 16S rRNA gene sequence analysis, division of *Bacillus* genera revealed phylogenetic heterogeneity with *Gracilibacillus*, *Salibacillus*, *Virgibacillus*, *Aneurinibacillus*, *Brevibacillus*, *Paenibacillus* and *Alicyclobacillus* under phylum Firmicutes.

The classification of *Bacillus* differs on basis of antimicrobial agents productions including enzymes, antibiotics and lippeptides; Systemic resistance inducer; Plant growth promoter and colonization competitors among growth factors including nutrients and space with surrounding microbes. *Bacillus* and related genera are known for the synthesis of wide range of pharmaceutical, agricultural, medicinal and industrial products [6].

Bacillus species have also been recognized as opportunistic pathogen or toxin producer in human or animal hosts. *Bacillus* spp. in gastrointestinal tract under hostile environment showed to enhance the survivability and tolerance in human health. *B. anthracis* that produce PA-LF and PA-EF toxins in both animals and humans are associated with deadly illness. *B. cereus* in association with severe eye infections and food poisoning has been incriminated in a multitude of other clinical conditions such as neonates, intravenous drug abuser, devastating central nervous system infections, fulminant sepsis and anthrax-like progressive pneumonia.

The genus gram-positive, rod shaped, spore-forming, aerobic or facultative anaerobic bacterium designated as a group of soil inhabitants. The soils *Bacilli* endospores (metabolically dormant form of life) ubiquity facilitates easily transfer routes to other feasible and accessible sites including low-biomass and cleanrooms sites in many industries. For many centuries spore-forming bacilli are being explored for preservation and production of food. The bacterial endospore are more resistant and tolerant than vegetative cell to gamma and UV radiation, oxidizing agents and organic chemicals, desiccation, deficient nutrients and heat. Spore-forming *Bacillus* of the aerobic

members and similar genera have been recovered from almost every niche in the environment and sources such as food and vegetables, animal and human gut. During low temperature storage and heat processing, *Bacillus* spp. offers better stability and higher acid tolerance. *Bacillus* spp. exhibits other biological activities such as antimicrobial, antitumor and antiviral activities. Broad spectrums of metabolites that are released by these *Bacillus* strains are beneficial to the environment by increasing availability of nutrient to the plants [7-9].

PGPR belonging to *Bacillus* genera are found of novel compounds that demonstrate vital bioassay activities. The application of *Bacillus* sp. have shown to enhance plant various attributes such as peroxidase, lyase, siderophores and indole acetic acid. *Bacillus* spp. as rhizobacteria consortium with two micropropagated bananas showed that the microbes had a prospective way in increasing the survival and health rate of plants in commercial nurseries. *Bacillus* spp. have shown to possess pathogen exclusion, immuno-modulatory, antimicrobial, anti-oxidant and food fermentation abilities. *Bacillus* is the most abundant genus in the rhizosphere and the PGP activity/mechanism for many years of these strains has been known. Gram-positive *Bacillus amyloliquefaciens* (subsp. plantarum FZB42) as a model bacterium that unravels the interaction between plants and microbe of the *Bacilli* genus. The FZB42 is well-known commercial biofertilizer and biocontrol agent, whom 10% of the genome encodes anti-microbial metabolites and their corresponding immunity genes. Bacilli have been found to increase growth, yield and nutrition of the plant raspberry under organic growing conditions. In pepper and cucumber plants root, the *B. mucilaginosus* and *B. megaterium* as potassium and phosphate solubilizing agents have improved in nutrient limited soil by consistent potential in increase in plant growth, availability and uptake of nutrients by the plant. The expression of marker genes of jasmonic acid, ethylene and salicylic acid had also increased in seedling treated with cellular suspension of *Bacillus* T-5 strain. *Bacillus megaterium* showed consistent of improving various root attributes such as root dry matter, root length and rooting performance. *B. pumilus* as a bio-inoculant in Mongolia has been proposed to increase the Orkhon wheat variety crop yield [10].

B. thuringiensis, a versatile pathogen, capable of infecting a wide range of organisms hazardous to animal/human or plant including flatworms, nematodes, protozoa, insects and mites. *Brevibacillus laterosporus* isolated from Assam Nambar Wild Life Sanctuary, India hot water spring inhibited the growth of phytopathogenic fungi including *Rhizoctonia oryzae*, *Magnaporthe grisea*, *Fusarium semitectum* and *F. oxysporum* along with *Staphylococcus aureus* bacterium. *Bacillus* strains produce variety of small extracellular effectors molecules and antimicrobial peptides to interact with the internal milieu of the host, thus possessing bio-therapeutic potential. *B. megaterium* isolated from tea rhizosphere comprised biological active compounds that altered the plant growth; reduce disease intensity and producing antifungal metabolites. *Paenibacillus alvei* K165 or *Fusarium oxysporum* F2 (nonpathogenic) in the stem of aubergines had been observed with expression of Pathogenesis-Related Proteins (PRP) i.e. PRP1 and PRP2, inhibiting *V. dabliae* growth. *Pseudomonas* and *Bacillus* genera species have been exploited and are considered as key player for future integrated research strategies for PGPR and bio-control agents. The application of *Bacillus amyloliquefaciens* cellular suspension treated with seedlings had been recorded with higher activities of peroxidase, phenylalanine ammonia lyase and polyphenol oxidase. *Ralstonia solanacearum*, a soil borne pathogen that causes Tobacco Bacterial Wilt (TBW) worldwide has shown to be control by *B. amyloliquefaciens*. Cofocal laser scanning microscopy, the B. *amyloliquefaciens* FZB42 produced surfactant, polyketides and non-ribosomal metabolites which mediate plant defense system by significantly expression of higher *PDF 1.2* gene, suppressing pathogenic microbes.

Bacillus subtilis

Bacillus subtilis naturally being present in the immediate vicinity of plant roots have been found to improve plant growth and to have a stable contact with higher plants. The *B. subtilis* genome has been sequenced, making it easier for understanding the bacterium lifestyle. Interestingly, the components of this genome have encoded many pathways for the use of plant molecules. At stationary phase when B. subtilis stops growing then for its survival the organism produce high amount of secondary metabolites that exhibits antibiotics and enzymatic activities such as carbohydrases and proteases. *B. subtilis* producing volatile compounds are known for their important role in promoting the plant growth and triggering ISR (Induced Systemic Resistance) in plants as a result of defense mechanism. Furthermore the *B. subtilis* endosporic and enzymatic products were found highly active against many fungal pathogens. The US FDA (US Food and Drug Administration) have declared *B. subtilis* as Generally Recognized As Safe (GRAS) organisms that can thus be processed in food industries. *Bacillus subtilis* without producing endotoxins and as recombinant proteins, without using filtration, directly secretes proteins into the medium. Also the growth of biofilms is perceived easily when *B. subtilis* is inseminated on the roots of *Arabidopsis thaliana*.

The *B. subtilis* as a powerful biocontrol agent is widely recognized. In addition, its ability to produce broad spectrum of different activity biologically compounds and endospores forms, and due to its broad host range. *Bacillus subtilis*, generates the most powerful lipopeptide type biosurfactant (comprising seven amino acids bonded to hydroxy and carboxyl groups on long chain fatty acids i.e. C13-C15) but its commercial application is limited due to its high cost production and low yields.

Bacillus subtilis strain has been shown to significantly inhibit black shank disease caused by *Phytophthora nicotianae* on tobacco. *B. subtilis* strain SR/B-16 originally isolated from ornamental farm rhizosphere has demonstrated antifungal activity as well as stabilizing P and N of the soil. *Bacillus subtilis* Tpb55 strain have shown to significantly inhibit growth of the fungal *P. nicotianae*, oomycete causing black shank disease in tobacco. The Scanning Electron Microscopy (SEM) has shown hyphae deformity, protoplasm leakage and hyphae rupture of the fungal with treatment of *Bacillus subtilis* Tpb55. The distribution of bacteria had shown a diffusion distribution around root meristem as well as filling small part of the intercellular space and vascular bundles. Black rot causative agent *Xanthomonas compestris* pv. *campestris* is one of the most destructive and limiting yield pathogen of cruciferous crops. *Brassica campestris* rhizosphere soil isolates (siderophores, autolysins and lactonases) i.e. SE and KA19 which showed inhibition of 11 mm whereas in combination of both isolates a resultant of 18.11 mm inhibition radius was observed. These isolates (KA19 and SE) phylogenetic analysis illustrated homology relation with *Bacillus thuringiensis* and *Pseudomonas aeruginosa*.

Trends in biotechnology: *Bacillus*

The most efficient competency between the plant and microbe's interaction can bring about beneficial effect to both plant and rhizosphere microbes. Since the banning of soil fumigant (methyl bromide), the European Union in 2010 had created demand of alternate effective novel crop protectants. Therefore, the use of biological control agents for the management of plant pathogens is considered safer and sustainable than chemical pesticides.

Plants pathogen responsible for economic losses of crop can be dealt with bio-control agents that are not offensive to environment and human health. For developing a well effective biocontrol agent, a better understanding of the antagonist mode of action can be achieved with high efficacy, economically feasible and ecological fit. The new bio-control approach is based on microbial growth (cellular density) and developing microbes with microsensor properties or called as 'environment agent'. These microbes have been used for developing analogs that can

determine/sense virulence molecule. Numerous microorganisms have been depicted as possible biocontrol agents such as *Pseudomonas flurescens*, *Hypericum gramineum* and some species of *Streptomyces*.

Commonly heat is used for controlling microbial contamination. The moderate heat i.e. <75°C is used for pasteurization, process used for killing pathogenic vegetative cells. Higher heat treatment i.e.121°C >5 min also called sterilization is used for retorting or canning to inactivate *Bacillus* and *Clostridium* species spores. D-value is used in thermal processing which is defined as "the time required at any specific temperature to inactivate 90% of the bacterial population of concern". D-values are applied with heat association factors such as environmental conditions, storage, sugar content, pH, water activity and organism growth status including lag, log and stationary phase (Table 3).

Table 3. Reported D-values (100) min for spores of *Bacillus* strains.

S.no	D-value (100) min	*Bacillus* strain
1	3.5	*B. cereus* in infant formula with 50% total solids
2	2.78	*B. cereus* in water
3	1.9	*B. cereus* in phosphate buffer
4	2.3	*B. licheniformis* in UHT milk
5	2	*B. cereus* in pork luncheon meal
6	1.8	*B. cereus* in infant formula with 10% total solids
7	1.6	*B. megaterium* in tomato juice
8	0.5	*B. subtilis* in phosphate buffer
9	0.4	B. cereus in beef slurry

DISCUSSION

The genus endospore-forming bacteria, is used for several manufactured industrial products including bioinsecticides, surfactants and extracellular enzymes such as proteases and amylases. *B. cereus* MK8 and *B. subtilis* MA9 strains isolated from eastern Himalayan region were found of producing thermostable α-amylase enzyme. The *Bacillus*-based biopesticides/biocontrolling agents fundamental role have been proven to be highly effective against wide spectrum of plant pathogens. *B. cereus* spores inactivation in rice has been validated with method of sterilization with temperature 120°C for 1 hr. A total of 85 years research on *Bacillus* spp., especially on *B. sphaericus* and *B. thuringiensis* raised the numbers of potent biopesticides. Two species including *B. sphaericus* and *B. thuringiensis* as biocontrol agent have ability to help in controlling the lepidopteron pests. *Bacillus* species are much of interest to researchers as biological control agents for comprising harder and resistant endospres along with inhibiting the growth of broad spectrum of other bacteria pathogens. The species reported to require simple ingredients for its growth and which release indigenous proteins includes *B. licheniformis*, *B. lentus*, *B. subtilis*, *B. amyloliquefaciens* and *B. thuringiensis* in Third edition of the Food Chemicals Codex (https://www.nap.edu/catalog/19642/food-chemicals-codex-third-edition).

These microbes excrete hydrolytic enzymes such as protease and chitinases and other lipopetide compounds that harnessed phytopathogens control and number of antibiotics in the extracellular vicinity. Important physiological traits that *Bacillus* used for its own survival commonly includes signal molecules, extracellular enzymes, antibiotic

secretion, formation of stress-resistant endospores and production of multilayered cell wall structure. The inoculums of *B. pumilus* INR7 showed a significant decrease in *Xanthomonas axonopodis* pv. *vesicatoria* causing disease in pepper. A single dipping of plant before transplantation in field elicited has been recorded with induces resistance. Addition of benzothiadiazole, a chemical inducer with *Bacillus* strain INR7 showed to stimulated expression defense marker genes *CaPR1, CaPR4* and *CaTin1* in pepper plant. The Benzothiadiazole (BTH) onto tobacco and pepper supported higher density population of plant roots and had no adversely effect in field and neither on rhizosphere colonization. Thus, PGPR and BTH combination under field conditions had shown to enhanced the induced resistance capacity of plant.

CONCLUSION

Biotechnological products and processing is highly dependent on diversity of these microbes. In the world, there is significant potential gained in reducing dependency on nitrogenous fertilizers and increase interest in research of using biological approach for fixing N. It involves those living organisms (microbes) that convert atmospheric N_2 (78 %) to NH_3, which then the plant can uptake N content and use it for growth. Biotechnology as in industrial, medicinal and agriculture products, similar have played key role in management of plant pathogens using biological control agents. *Bacillus* spp. with biotechnological tools has also been found with bio-mineralization, bio-weathering, bio-fertilizer and bio-pesticides abilities. A thermophilic bacterial strain (*Brevibacillus thermoruber* 438) that exhibited exopolysaccharides compounds which plays an important role corrosion, biofilms, dairy industry and in medicine applications in the field of biotechnology. *B. licheniformis* is an attractive host for the biotechnological production including cloning on industrial scale.

Rhizosphere is a unique niche that provides habitation and nutrition to PGP microorganisms. PGPRs may be highly effective however the choice and delivery of these PGPR needs to be of rational approach for ensuring their true potential and prosperousness. The reports reviewed in this work demonstrate effectiveness generally of *Bacillus* genera and particularly the *B. subtilis* spp. in biotechnological applications. Thus, additional comprehensive research to exploit the potential of PGPR definitely will improve the agricultural sector.

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