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Chapter 3

Economically important *Bacillus* and related genera: a mini review

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Abstract

Members of the aerobic spore-forming *Bacillus* and related genera can be recovered from almost every niche in the environment. They are frequently isolated from both the natural habitat (soil and growing plants) and foods. In recent decades, there has been a growing interest in their biotechnological and economic importance. Members of *Bacillus* and related genera are known for the synthesis of wide range of medicinal, agricultural, pharmaceutical and industrial products. The present review is aimed to provide an overview of the various potentials that the organisms belonging to *Bacillus* and related genera have in biotechnological applications.

Keywords

Bacillus, aerobic bacteria, PGP, biocontrol

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Introduction

Microbial diversity is a major resource for biotechnological products and processes. Exploration of microbial diversity holds great promise because of the role of microbes in nutrient cycling, environmental detoxification and novel metabolic abilities in pharmaceuticals and industrial processes and act as a major resource for agricultural, industrial, and medicinal applications¹⁻³. Bacteria are the most dominant group of this diversity which exists in diverse ecological niches, including extreme environments present in both lithosphere and hydrosphere, where their metabolic abilities play a critical role in geochemical nutrient cycling² and producing a wide range of products of industrial significance.

The gram-positive bacteria form an important part of the microbiota in many soils. In particular, the low G+C content gram-positive bacteria, which are divided into three classes — clostridia, mollicutes, and bacilli⁴, play major roles in the mineralization of plant-derived materials, humus, pesticides and hydrocarbons in soil⁵. The traditional genus *Bacillus* represents one of the most diverse genera in the class bacilli. The members of this genus exhibit a wide range of DNA base compositions and major amino acid compositions of the cell walls^{6,7}. It includes aerobic and facultatively anaerobic, rod-shaped, gram-positive spore-forming bacteria⁵. Recently, 16S rRNA gene sequence analysis has revealed a high level of phylogenetic heterogeneity in this genus, on the basis of which a division into different genera was proposed: *Bacillus*, *Alicyclobacillus*, *Paenibacillus*, *Brevibacillus*, *Aneurinibacillus*, *Virgibacillus*, *Salibacillus*, and *Gracilibacillus*⁸. Here the term “*Bacillus* and related genera” is used as an operational term to indicate these organisms.

Common physiological traits important to their survival include production of a multilayered cell wall structure, formation of stress-resistant endospores and secretion of peptide antibiotics, peptide signal molecules, and extracellular enzymes⁹.

Members of *Bacillus* and related genera are used for the synthesis of a very wide range of important medical, agricultural, pharmaceutical and other industrial products. These include a variety of antibiotics, enzymes, amino acids and sugars⁸. They are major elements of the agronomic environment of plants, inhabiting the soil. Several reports have described the biodiversity, antibiotic production and plant growth-promoting effects of *Bacillus* and related genera (Table 1).

***Bacillus* and related genera as Plant growth promoting (PGP) agents**

Microbial production of secondary metabolites that can promote or constrain plant growth has often been found to be finely tuned, controlled by environmental conditions, medium

Table 1: Economically important *Bacillus* and related genera

<i>Bacillus</i> and related genera	Functions/activities reported
<i>Bacillus</i> spp. ^{12,13}	Plant growth promoting (PGP)
<i>Bacillus</i> spp. ¹⁵	PGP (increasing nutrient availability of the plants)
<i>Bacillus licheniformis</i> ¹⁶	Biofertiliser
<i>Bacillus</i> spp. ¹⁷	Increase plant health and survival rates of micropropagated bananas
<i>Bacillus</i> spp. ¹⁸	Increase the yield, growth and nutrition of raspberry plant
<i>Bacillus megaterium</i> ¹⁹	Improve different root parameters (rooting performance, root length and dry matter content of root) in mint
<i>Bacillus megaterium</i> var. phosphaticum ²⁰	Phosphate solubilising
<i>Bacillus mucilaginosus</i> ²¹	Potassium solubilising
<i>Bacillus pumilus</i> 8N-4 ²²	Bio-inoculant for biofertilizer production
<i>Brevibacillus brevis</i> ²³	Improve <i>in vitro</i> spore germination and growth of <i>Glomus mosseae</i>
<i>Paenibacillus</i> spp. ¹¹	Plant growth promoting (PGP)
<i>Paenibacillus polymyxa</i> ²⁵	Nitrogen fixing ability
<i>Paenibacillus polymyxa</i> ²⁶	Produce plant growth promoting compounds similar in activity to indole-3-acetic acid
<i>Paenibacillus polymyxa</i> ²⁷⁻²⁹	Releases iso-pentenyladenine and unknown cytokinin-like compound which promotes seed germination, <i>de novo</i> bud formation, release of buds from apical dominance, stimulation of leaf expansion and reproductive development and retardation of senescence
<i>Paenibacillus polymyxa</i> ³⁰	Affects growth parameters of wheat and spinach plants
<i>Paenibacillus elgii</i> ³¹	Chitinolytic, antifungal, and mineral phosphate solubilization abilities and enhanced the growth of groundnut and tobacco plant
<i>Lysinibacillus</i> sp. NF-4 ³²	A potential N ₂ fixing bacteria, harbouring <i>nifH</i> gene
<i>Lysinibacillus fusiformis</i> ³³	Plant growth promotion of ginseng
<i>Bacillus subtilis</i> ³⁴	Biocontrol agent, produce different biologically active compounds with a broad spectrum of activity
<i>Bacillus megaterium</i> ³⁵	Produce antifungal metabolite
<i>B. thuringiensis</i> ³⁶	Potent biopesticides
<i>Bacillus sphaericus</i> ³⁶	Potent biopesticides
<i>B. thuringiensis</i>	Infects protozoa, nematodes, flatworms, mites and insects that are either plant pests or human and animal health hazards
<i>Bacillus thuringiensis</i> (kurstaki) ³⁷	Control lepidopteron pests

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Bacillus and related genera	Functions/activities reported
<i>Bacillus sphaericus</i> ³⁸	Control lepidopteron pests
<i>Paenibacillus lentimorbus</i> NRRL B-30488 ²⁴	Biocontrol activity against a phytopathogenic <i>Fusarium oxysporum</i>
<i>Paenibacillus polymyxa</i> strain P13 ³⁹	Produce and secrete a compound, named Polyxin, effective against a wide range of gram-positive and gram negative bacterial species including food-borne pathogens
<i>Brevibacillus laterosporus</i> strain BPM3 ⁴⁰	Strongly inhibited growth of phytopathogenic fungi and gram-positive bacterium
<i>Lysinibacillus fusiformis</i> ⁴¹	Biological activity against <i>Phytophthora cinnamomi</i>
<i>Lysinibacillus sphaericus</i> C3-41 ⁴²	Used as an insecticide
<i>Bacillus subtilis</i> MA9 ⁴⁸	Produce thermostable α -amylase enzyme
<i>Bacillus cereus</i> MK8 ⁴⁸	Produce thermostable α -amylase enzyme
<i>Bacillus subtilis</i> (natto) ⁴⁹	Mediated fermentation of soybean food, <i>natto</i>
<i>B. subtilis</i> (natto) ⁵⁰	Produces amylases, cellulases and important proteases
<i>B. subtilis</i> ⁵¹	Produce significant amounts of D-ribose
<i>B. pumilus</i> ⁵¹	Produce significant amounts of D-ribose
<i>Bacillus</i> spp. ⁵²	Produce antibiotics (low-molecular-weight peptides) which possess different biological activities, including antimicrobial, antiviral, and antitumor activities
<i>Paenibacillus polymyxa</i> strains ⁵³⁻⁵⁵	Produce cell wall degrading enzymes such as β -1,3-glucanases, cellulases, chitinases, proteases, xylanase
<i>Paenibacillus polymyxa</i> ⁵⁶	Cell wall β -glucans that act as immunostimulants or as adjuvant of some animal vaccines
<i>Brevibacillus thermoruber</i> 438 ⁵⁷	Produce exopolysaccharides which plays an important role in medicine, dairy industry, biofilms and corrosion, and their applications in the field of biotechnology

components, and influenced by root activities. Plant-stimulatory effects exerted by plant growth-promoting bacteria (PGPB) might also be due to an enhanced availability of limited plant nutrients such as nitrogen, phosphorus, vitamins and amino acids in the rhizosphere, caused by phosphate-solubilizing and diazotrophic bacteria^{10,11}.

Bacillus is the most abundant genus in the rhizosphere, and the PGP activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved^{12,13}. There are a number of metabolites that are released by these strains¹⁴, which strongly affect the environment by increasing nutrient availability of the plants¹⁵. Being present naturally in the immediate vicinity of plant roots, *Bacillus subtilis* is able to maintain stable contact with higher plants and promote their growth. *Bacillus licheniformis* when inoculated on tomato and pepper shows considerable colonisation and can be used as a biofertiliser without altering normal management in greenhouses¹⁶. Jaizme-

Vega *et al.*¹⁷ evaluated the effect of a rhizobacteria consortium of *Bacillus* spp. on the first developmental stages of two micropropagated bananas and concluded that this bacterial consortium can be described as a prospective way to increase plant health and survival rates in commercial nurseries. *Bacillus* is also found to have potential to increase the yield, growth and nutrition of raspberry plant under organic growing conditions¹⁸. *Bacillus megaterium* is very consistent in improving different root parameters (rooting performance, root length and dry matter content of root) in mint¹⁹. The phosphate solubilizing *Bacillus megaterium* var. *phosphaticum* and potassium solubilising *Bacillus mucilaginosus* when inoculated in nutrient limited soil consistently increased mineral availability, uptake and plant growth of pepper and cucumber, suggesting its potential use as fertilizer^{20,21}. The *Bacillus pumilus* 8N-4 has been proposed to be used as a bio-inoculant for biofertilizer production to increase the crop yield of wheat variety Orkhon in Mongolia²².

Brevibacillus brevis was found to improve *in vitro* spore germination and growth of *Glomus mosseae* by increasing the presymbiotic growth (germination rate growth and mycelial development)²³. *Paenibacillus* spp. isolated from milk was found to possess PGP traits²⁴. Nitrogen fixing ability by *Paenibacillus polymyxa* was demonstrated by Guemori-Athmani *et al.*²⁵. The production of plant growth promoting compounds by *P. polymyxa* similar in activity to indole-3-acetic acid has been suggested to stimulate growth in crested wheatgrass²⁶. It also releases iso-pentenyladenine and one unknown cytokinin-like compound during its stationary phase of growth which promotes seed germination, *de novo* bud formation, release of buds from apical dominance, stimulation of leaf expansion and reproductive development and retardation of senescence²⁷ in wheat^{28,29}. The effect of inoculation with *P. polymyxa* on growth parameters of wheat and spinach plants was observed³⁰. *Paenibacillus elgii* which was positive for chitinolytic, antifungal, and mineral phosphate solubilization abilities enhanced the growth of groundnut in terms of shoot height, root length, total chlorophyll, and fresh and dry weight when applied alone or in combination with chitosan. The plant growth-promoting activity of *P. elgii* was seen in tobacco in a specially designed gnotobiotic setup indicating its capability to promote growth of at least groundnut and tobacco³¹.

A potential N₂ fixing bacteria, *Lysinibacillus* sp. NF-4 harbouring *nifH* gene, was isolated from raw coir pith³². *Lysinibacillus fusiformis* was reported to be positive for most of the plant growth promoting traits, indicating their role in growth promotion of ginseng³³.

***Bacillus* and related genera as biocontrol agents**

Production of extracellular enzymes by biologic control bacteria is a well-documented phenomenon that has long been thought to be involved in the lysis of the phytopathogenic

fungal cell wall. *Bacillus subtilis* is widely recognized as a powerful biocontrol agent. In addition, due to its broad host range, its ability to form endospores and produce different biologically active compounds with a broad spectrum of activity, *B. subtilis* as well as other *Bacilli* are potentially useful as biocontrol agents³⁴. *Bacillus megaterium* from tea rhizosphere is able to produce antifungal metabolite and thus it helps in the plant growth promotion and reduction of disease intensity³⁵. Research of almost 85 years reveals that *Bacillus* spp., especially *B. thuringiensis* and *Bacillus sphaericus* are the most potent biopesticides³⁶. Available information depict that *B. thuringiensis* is a versatile pathogen capable of infecting protozoa, nematodes, flatworms, mites and insects that are either plant pests or human and animal health hazards³⁷. Two strains [*Bacillus thuringiensis* (kurstaki) and *Bacillus sphaericus*] have the ability to help in the control of the lepidopteron pests³⁸.

Paenibacillus lentimorbis NRRL B-30488 isolated from milk showed biocontrol activity against a phytopathogenic *Fusarium oxysporum* by alteration and distortion of the hyphal cell wall through the action of its chitinase and β -1,3-glucanase enzymes²⁴. *P. polymyxa* strain P13, isolated from Argentinean regional fermented sausages, was found to produce and secrete a compound, named polyxin, that inhibited the growth of *Lactobacillus* strains. This antimicrobial compound is effective against a wide range of gram-positive and gram negative bacterial species including food-borne pathogens³⁹.

Brevibacillus laterosporus strain BPM3 isolated from mud of a natural hot water spring of Nambar Wild Life Sanctuary, Assam, India, strongly inhibited growth of phytopathogenic fungi (*Fusarium oxysporum* f. sp. *ciceri*, *F. semitectum*, *Magnaporthe grisea* and *Rhizoctonia oryzae*) and gram-positive bacterium (*Staphylococcus aureus*)⁴⁰.

A strain of *Lysinibacillus fusiformis* isolated from the roots of *Persea americana* (avocado) trees was identified as a potential biocontrol agent as it showed biological activity against *Phytophthora cinnamomi*⁴¹. *Lysinibacillus sphaericus* C3-41 (previously, *Bacillus sphaericus* C3-41) is used as an insecticide because its spores release endotoxins that kill mosquito larvae. Because the bacteria is short-lived and are not harmful to humans and other animals, *Lysinibacillus sphaericus* C3-41 is an ideal insecticide⁴².

Bacillus and related genera as biotechnological agents in industrial processes and production

Each single strain of organism produces a large number of enzymes which include the function such as hydrolyzing, oxidizing or reducing, and metabolic in nature. But the absolute and relative amounts of the various individual enzymes produced vary markedly between species and even between strains of the same species. Amylase, lipase, protease

and cellulase constitute a very important part of microbial enzymes that are used in food, pharmaceutical, textile, paper, leather, and other industries. The world market for industrial enzymes is estimated to be US\$1.6 billion, split between food enzymes (29%), feed enzymes (15%), and general technical enzymes (56%)⁴³.

It is estimated that *Bacillus* spp. enzymes make up about 50% of the total enzyme market⁴⁴. Bacterial amylase preparations at elevated temperatures give rapid liquefaction of starch. A significant application of the bacterial enzyme is in the continuous process for desizing of textile fabrics^{45,46}. Another is in preparing modified starch sizing for textiles⁴⁵ and starch coatings for paper⁴⁷. Two *Bacillus* strains [*Bacillus subtilis* MA9 and *Bacillus cereus* MK8] isolated from eastern Himalayan region has been reported to produce thermostable α -amylase enzyme⁴⁸.

Food fermentations mediated by *Bacillus*, can provide insight into some of the potential industrial properties of the genera or species involved. *Bacillus subtilis* (natto) is used in Japan for producing the fermented soybean food, natto⁴⁹. While *B. subtilis* (natto) produces many enzymes, including amylases and cellulases, the most important enzymes in the production of natto are proteases; two proteases having a pH optima of 8.5 and 10.3–10.8 have been characterized⁵⁰. The proteases are responsible for the main flavor, through hydrolysis of soybean protein.

D-Ribose is frequently used as a flavor enhancer in food, health food and animal feed. Several strains of *B. subtilis* and *B. pumilus* and their mutants are reported to produce significant amounts of D-ribose⁵¹. Recent developments in genetic engineering and fermentation technology have contributed to improvements in D-ribose productivity by *Bacillus* fermentations.

Most members of the genus *Bacillus* are able to produce antibiotics. Interestingly, the majority of these antibiotics are low-molecular-weight peptides, which possess different biological activities, including antimicrobial, antiviral, and antitumor activities⁵².

Different strains of *Paenibacillus polymyxa* were reported to produce cell wall degrading enzymes such as β -1,3-glucanases, cellulases, chitinases, proteases^{53,54} and xylanase⁵⁵. β -glucans, or glucose polymers found in the cell walls of *Paenibacillus polymyxa*, have been found to have beneficial effects on the immune system of experimental animals. This investigation supported the idea that β -glucans isolated from *Paenibacillus polymyxa* can be used as immunostimulants or as adjuvant of some animal vaccines⁵⁶.

In their study, Radchenkova *et al.*⁵⁷ isolated a thermophilic bacterial strain, *Brevibacillus thermoruber* 438, which was a high producer of exopolysaccharides which plays an

important role in medicine, dairy industry, biofilms and corrosion, and their applications in the field of biotechnology.

Conclusion

The reports reviewed in this work demonstrate just how effective *Bacillus* and its related genera are in playing important roles as dominant bacteria in biotechnological applications as well as industrial processes and products. And, with recent advances in biotechnology, the economic contributions that these organisms can make in biotechnological applications and industrial processes can be exploited further for large scale benefit of mankind.

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