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Bacillus polymyxa: A Potential Probiotic Species

Pratik Katare, KhushalChaudhari, Mahalaxmi Mohan*

**Department of Pharmacology, MGV Pharmacy College, Nashik, Maharashtra, India.*

***Corresponding author:** Email: mm_nasik@yahoo.co.in

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Review

Abstract

Bacillus polymyxa is the potential probiotic species. Bacillus species are highly resistant to heat, spore-forming, and ideal food additives for animals. *B. polymyxa* has the ability to produce a large number of secretory proteins, enzymes and antimicrobial compounds (Bacteriocin). It is found in soil, plant tissues and marine sediments. It may have a role in forest ecosystems and potential future applications as a biofertilizer and biocontrol agent in agriculture. It has a wide range of applications in agriculture, chemotherapy, food technology etc. The present study mainly focuses on all such applications of the B. polymyxa species which will be useful in all the future endeavors for more detail discoveries of the species

Keywords: *Bacillus polymyx*; Bioremediation; Biopesticide; Food technology; Antimicrobial

Introduction

The term probiotic taken as an unchallenged synonym to the beneficial microbe has gained popularity over the years and has found application in several general health and clinical scenarios. Probiotics are live microorganisms, which when administered in adequate amounts confer health benefits to the host [1].

The term "probiotic" originally referred to microorganisms that have effects on other microorganisms[2]. The concept of probiotics involved the notion that substances secreted by one microorganism stimulated the growth of another microorganism. The term was used again [3]to describe tissue extracts that stimulated microbial growth. The term probiotics was taken up by Parker [4],who defined the concept as, "Organisms and substances that have a beneficial effect on the host animal by contributing to its intestinal microbial balance." Later, the definition was greatly improved by Fuller [5], whose explanation was very close to the definition used today. Fuller described probiotics as a "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." He stressed two important claims for probiotics: the viable nature of probiotics and the capacity to help with intestinal balance.

*Bacillus polymyxa*is the potential probiotic species. Spores of various *Bacillus* species including *B.subtilis, B.cereus, B.pumillis, B.clausii, B.amyloliquefaciance, B.coagulance*and *Bacillus licheniformis* are being used as probiotics. Probiotics are live microorganism, which when administered in adequate amounts confer health benefits to the host. It became clear that intestinal microflora had metabolic functions, such as fermenting indigestible dietary residues and endogenous mucus, saving of energy, production of vitamin K, and absorption of ions [6].Probiotics have roles in epithelial cell proliferation and differentiation, and the development and the homeostasis of the immune system [7].Probiotics are not an invention but existed in our traditional foods such as beverages, salty fishes, yogurt, different types of cheeses and so on since olden times [8]. Such food structures contain different types of useful bacteria. It might be that the first real use of food containing Probiotics was fermented milk [9]. Humans learned that fermented milk has good taste. Later they learned how to convert it into cheese, yogurt and so on [8,10]before the discovery of the microscope, humans knew how to prepare different types of milk products with different tastes and structures. This is a result of the action of different microbial reactions induced by different microbes.The public, globally transfer such information for producing such foods from generation to generation till today. We really did not know the starting point for the first use of food containing probiotics, particularly for medicinal applications. However, by one way or the other Probiotics is collectively a part of the fermented food if the active microbes are useful and able to colonize the digestive system. Fermentation using microbes is known from ancient times. One could see the fungi growing in the food by the naked eye. The public knew how to produce bakery and alcoholic products since times immemorial. They knew how to maintain the product quality and testing by maintaining a seed culture from the most successful fermentation processes to use in the next process [8]. However, scientists were interested to give such honour to Van Leeuwenhoek and Hooke in 1665 [11].

It might be that probiotics have been discovered by the first human who used milk products, or might be with substances other than milk such as the other different types of fermented foods. However, climatic conditions for sure favoured traditional sour milk or cultured dairy products. Public distribute stories about the origin of some types of probiotics and that some have religious origin [2]. The author described that some of the probiotics used by the Pharaonic civilization, which the Egyptians still use nowadays. They include milk, seeds, fish and some other products. However, it might be that Ilya Ilyich Metchnikoff, the Nobel Prize winner in Medicine in 1908, at the Pasteur Institute was the first who spotted the effect of what is called now probiotic. He linked the health and longevity to the ingestion of bacteria present in yogurt [10].

Bacillus species are ubiquitous in nature but found in higher concentrations in soil, water, and food products that have a plant origin. Strains of *Bacillus* are very good potential candidates to be used as probiotics. Metabolically, *Bacillus* species are very active and previous research has identified a number of useful enzymes and numerous antibiotics they produce. In addition to these secreted products, *Bacillus* remains stable in probiotic products much longer than conventional probiotics due to their ability to form endospores *PaeniBacilluspolymyxa*, also known as *Bacillus polymyxa*, is a Gram-positive bacterium capable of fixing nitrogen. It is found in soil, plant tissues and marine sediments. It may have a role in forest ecosystems [12] and potential future applications as a biofertilizer and biocontrol agent in agriculture [13].

Applications in Agriculture

***P. polymyxa*CR1 with potentials for biopesticides, biofertilization, biomass degradation and biofuel production: -**

The species *B. polymyxa* benefits agriculture through antimicrobial activity, nitrogen fixation, phosphate solubilization, plant hormone production, or lignocellulose degradation. However, no single strain of *Bacillus* has yet been identified in which all of these advantageous traits have been confirmed.

B. polymyxa CR1 was isolated from degrading corn roots from southern Ontario, Canada. It was shown to possess in vitro antagonistic activities against the common plant pathogens Phytophthorasojae P6497 (*oomycete), Rhizoctoniasolani*1809 (basidiomycete fungus), *Cylindrocarpondestructans* 2062 (ascomycete fungus), Pseudomonas syringae DC3000 (bacterium), and *Xanthomonascampestris* 93-1 (bacterium), as well as *Bacillus cereus* (bacterium), an agent of food-borne illness. *B. polymyxa* CR1 enhanced growth of maize, potato, cucumber, Arabidopsis, and tomato plants; utilized atmospheric nitrogen and insoluble phosphorus; produced the phytohormone indole-3-acetic acid (IAA); and degraded and utilized the major components of lignocellulose (lignin, cellulose, and hemicellulose).

Therefore, *B. polymyxa* CR1 has multiple beneficial traits that are relevant to sustainable agriculture and the bio-economy. This strain could be developed for field application in order to control pathogens, promote plant growth, and degrade crop residues after harvest [14].

Phytohormone production

Auxins are hormones that are crucial regulators of gene expression and development throughout a plant's life, participating in cell division, elongation, fruit development and senescence. There are multiple classes of auxins, but the first identified and most abundant in nature is indole-3-acetic acid (IAA). Although plants are able to produce their own phytohormones, they can also utilize foreign sources produced by other organisms [15,16].

Food Technology

The study, that investigated the effect of *B. polymyxa* D05-1 as starter culture during salted fish fermentation, showed that the inoculated samples had lower TVBN and biogenic amine contents than control samples. The reduction percentage of histamine in inoculated samples was 34% at the end of fermentation, compared to control samples. Inoculation of *B. polymyxa* D05-1 could inhibit other bacterial growth to retard the increase of aerobic bacterial counts in salted fish fermentation. The results emphasized that application of *B. polymyxa* D05-1 as a starter culture in salted fish products fermentation is effective to inhibit biogenic amines accumulation and to enhance the safety of salted and fermented fish products [17].

Antibiotic efficacy

Polymyxins are cationic lipopeptide antibiotics active against many species of Gram-negative bacteria. They sequenced the gene cluster for polymyxin biosynthesis from *PaeniBacilluspolymyxa* PKB1. The 40.8 kb gene cluster comprises of three nonribosomal peptide synthetase-encoding genes and two ABC transporter-like genes. Disruption of a peptide synthetase gene abolished all antibiotic production, whereas the deletion of one or both transporter genes only reduced antibiotic production [18].

*P. polymyxa*var. *colistinus* produces the antibiotic colistin. Colistin, also known as polymyxin E, is an antibiotic used as a last resort for multidrug-resistant Gram-negative infections including pneumonia [19].

Biocontrol mechanism

Perhaps the most notable plant-growth-promoting feature of *PaeniBacillus* species comes from their numerous biocontrol capabilities. By inducing the plant's own resistance mechanisms or by producing biocidal substances, *PaeniBacillus* can neutralize a diverse variety of phytopathogens and insect herbivores. *B. polymyxa* alone has been shown to provide protection to cauliflower, pea, ginseng, cucumber, chickpea, peanut, soybean, pepper, and more. Other species of *PaeniBacillus* that have biocontrol properties include *P. alvei, P. brasilensis, P. dendritiformis, P. ehimensis, P. elgii, P. kobensis, P. lentimorbus, P. macerans, P. peoriae, and P. thiaminolyticus*[16].

Mechanism of action by the Gnotobiotic System to Study *B. polymyxa* effects in plants

The mechanisms involved in these interactions are particularly complicated because the interactions occur in a dynamic environment. Therefore, mechanistic studies can greatly benefit from reductionist approaches, provided that one is cautious with interpretations derived from such simplified experimental protocols.

The researcher has used the gnotobiotic system to study interactions between *B. polymyxa* and the model plant *A. thaliana,* in order to exclude the un-controlled variations in experimental conditions associated with studies carried out on plants grown in soil. *A. thaliana* plant was chosen as the model plant because it provides a good experimental system for genetic studies, many transposon-tagged mutants have been generated, and its entire genomic sequence is available in the database, thus facilitating the identification of genes scored in the approach employed.

*E. carotovora*species. was chosen as a pathogen to study induced resistance since it is a pathogen of very wide host range and therefore provides a system for the study of nonspecific plant-pathogen interaction [20].

Plant growth-promoting rhizobacteria

B. polymyxa is known to be a PGPR (Plant growth-promoting rhizobacteria). The induction of systemic resistance against *E. carotovora* via the root system would indicate the mechanism to be ISR (Induced Systemic Resistance). ISR is a resistance mechanism in plants that is activated by an infection. Its mode of action does not depend on direct killing or inhibition of the invading pathogen, but rather on increasing the physical or chemical barrier of the host plant [20].

However plants seemed to suffer from inoculation-stunted root system were generally observed and overall plant growth was reduced by about 30%. When the author and his colleague observed the stress response, they decided to study the expression of genes connected to biotic stress responses. It has been earlier discovered that ET, JA, & SA pathways were activated in biotic stress situations [21]. Hence we analyzed the expression of marker genes for each of these pathways: HEL (heveine-ET Pathway; [22]ATVSP (vegetative storage protein acid phosphatase-JA pathway; [23] and PR-1 SA Pathway; [24], were chosen. RT-PCR experiments showed that all three marker genes were over-expressed in plants previously treated with *B. polymyxa*, at induction levels varying from 2-6 folds. The authors inferred that this was relatively small but significant increase and is indicative of a "mild" biotic stress [25].

B. polymyxa colonization of *A. thaliana* root and its endophytic mode of action

The author started the bacterial colonization at the tip of the root, invasion of root occurred after 2 hrs of infection and population of the zone in the differentiation region was observed within 5 hrs at which a significant invasion of root tissue was evident. By 24 hrs, severe damage to the root was seen. Thus, *B. polymyxa* has two preferred zone of infection. The first one is located at the root tip in the zones of the elongation, which sometimes results in the loss of the root cap. The other colonization region was observed in the differentiation zone [25].

B. polymyxa inoculation enhances plant drought tolerance

It has been reported that *B. polymyxa* inoculation is most effective in relatively harsh and poor quality conditions.

A. thaliana plants were exposed to drought stress. Experiments were performed in the gnotobiotic system. It was observed that *B. polymyxa* treated plants were more resistant and tolerated drought stress significantly better than control plants [25].

Antimicrobial activity

Several small peptide antibiotics, for example *Polymyxin B*, are produced by *Bacillus polymyxa*. The author has experimented and concluded that by the isolation from Argentinean regional fermented sausages of a strain that produces a new antimicrobial activity similar to the *B. polymyxa* species. The inhibitory spectrum was determined against Gram-positive and Gram-negative organisms, including the food-borne pathogens *Bacillus cereus* and *Escherichia coli*[15].

Other Medical Applications

In addition to a diverse array of antimicrobials, *PaeniBacillus* produce other compounds that may be useful in medicine and dentistry. Their exo-polysaccharides (EPS) have antioxidant and anti-tumour properties, while mutanase enzymes may help to reduce tooth decay [16].

Bioremediation

A variety of industries including petroleum, textiles, pulp and paper, and other chemical industries can unintentionally or intentionally release large amounts of organic pollutant compounds and heavy metals. *PaeniBacillus* species may be utilized in the removal or degradation of these environmental pollutants, through bioflocculation or enzymatic activities.

Often used for wastewater treatment, flocculation is a process that removes suspended particles from liquids, frequently through the addition of chemicals [flocculants] that promote aggregation [16].

Microencapsulation of *B. polymyxa*

One of the challenges for food technology regarding the addition of probiotics in food is to ensure that these microorganisms arrive alive and in good condition to their site of action within the body of the host, and ensure implementation in a wider range food. The survival of these bacteria depends on the vehicle in which they were built, therefore, it is necessary to generate strategies to ensure their survival in the matrix where they are incorporated, during storage and their passage through the gastrointestinal tract [26].

Among the technologies proposed to ensure cell viability of these microorganisms in food, the microencapsulation technique offers protection to probiotic microorganisms against adverse conditions. Several studies are related to this subject such as research carried out by KhosraviZanjani, Tarzi, Sharifan, &Mohammadi [27], who aimed to study the protective role of this technique in the bacterias*LactoBacilluscasei* and *Bifidobacteriumbifidum*, via emulsion technique. Mokarram, Mortazavi, Najafi, &Shahidi, [28], demonstratedthatmicroencapsulationof*LactoBacillus acidophilus* and *LactoBacillusrhamnosus*, ensures its survival against temperature and pH changes. Also in the study of Ding & Shah [29], the spray-drying technology was the most often used in the food industry for probiotics microencapsulation [27-30].

Microencapsulation under the spray drying technique is an alternative to maintain the integrity of the probiotic strains facilitating their incorporation in various food and pharmaceutical matrices. Also protects the bacteria from temperatures of 70°C and retain their stability after being subjected to conditions simulating the digestive system such as pH 2.5 and bile salt concentration of 0.3% w/v, ensuring the minimum concentration of 1x106 CFU/gr, recommended by the Food and Drug Administration (FDA), Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization [30].

Production of Exopolysaccharides from *PaeniBacillus species*

PaeniBacillus Species is successfully used by the author for the production of exo-ploysacharides. In addition, this technology for EPS production is prevailing because it is more environmentally friendly. EPSs reported from various references constitute a structurally diverse class of biological macromolecules with different applications in the broad fields of pharmacy, cosmetics and bioremediation. The different structural characteristics of EPS from *PaeniBacillus Species*. showing various bioactivities were been investigated by the author. The sources and chemical compositions of the EPSs are summarized in Table 1 [31].

Production of carboxymethyl cellulose

Mango peel, a solid mango processing waste, comprises 15-20% of total fruit weight. This, being a rich source of lignocelluloses, was used as substrate for carboxymethylcellulase (CMCase) production using *PaeniBacilluspolymyxa*. Carboxymethylcellulase (CMCase), a major constituent of cellulase complex is widely used in chemicals, fuel, food, animal feed, brewery and wine, textile and laundry, pulp and paper and agro-based industries [32].

Tooth decay preventive formulation

Mutanases also called (1→3)-α-glucanases, from *PaeniBacillus* may be useful to help prevent tooth decay. These enzymes break down branched (1→3),(1→6)-α-D-glucans (mutans) which are produced by commensal streptococci and which form a major component of dental biofilm [plaque] that can defeat cariogenic bacteria [19].

Future Perspective

Improvement of biological control capacity of *PaeniBacilluspolymyxa* E681 by seed pelleting on sesame

The main obstacle of sesame cultivation is the occurrence of damping-off diseases and wilt caused by a complex of soil-borne pathogens in fields cultivated for two or more successive years. To protect sesame seedlings against these diseases, *PaeniBacilluspolymyxa* E681, a plant growth-promoting rhizobacterium (PGPR) previously shown to suppress disease incidence and promote growth on cucumber and pepper in the greenhouse and field experiments, was evaluated for its capacity for biological control and growth promotion *in vitro* and *in situ*. Seed treatment with strain E681 alone did not show consistent protection. Therefore, seed pelleting with strain E681 was attempted to increase the seed size and improve the stability and effectiveness of biocontrol capacity by strain E681 [33] .*P. polymyxa*might have possible future applications asa soil inoculants in agriculture and horticulture [34].

Biofilms of *P. polymyxa*growing on plant roots have been shown to produce exopolysaccharides which protect the plants from pathogens. The interactions between this bacterial species and plant roots also cause the root hairs to undergo physical changes [16].

Conclusion

Bacillus polymyxa is a live microorganism, which has many beneficial uses and applications. *B. polymyxa* shows its application in agricultural field as biopesticides, biofertilization, biomass degradation and biofuel production, and phytohormone production at large. They are also useful in food technology as they have an ability to preserve the salty fishes from bacterial attack by inhibiting biogenic amines accumulation and to enhance the safety of salted and fermented fish products. It also provides an antibiotic activity by producing colistin. Being a bio-controlling species *Bacillus polymyxa*also provides protection to various plants. Along with plant, it also protects the environment as is a bioremediation type of species.

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Conflict Of Interest

The authors do not have any conflict of interest.

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