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REVIEW ARTICLE

Ecology and biotechnological potential of *Paenibacillus polymyxa*: a minireview

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Abstract Microbial diversity is a major resource for biotechnological products and processes. Bacteria are the most dominant group of this diversity which produce a wide range of products of industrial significance. *Paenibacillus polymyxa* (formerly *Bacillus polymyxa*), a non pathogenic and endospore-forming *Bacillus*, is one of the most industrially significant facultative anaerobic bacterium. It occurs naturally in soil, rhizosphere and roots of crop plants and in marine sediments. During the last two decades, there has been a growing interest for their ecological and biotechnological importance, despite their limited genomic information. *P. polymyxa* has a wide range of properties, including nitrogen fixation, plant growth promotion, soil phosphorus solubilisation and production of exopolysaccharides, hydrolytic enzymes, antibiotics, cytokinin. It also helps in bioflocculation and in the enhancement of soil porosity. In addition, it is known to produce optically active 2,3-butanediol (BDL), a potentially valuable chemical compound from a variety of carbohydrates. The present review article aims to provide an overview of the various roles that these microorganisms play in the environment and their biotechnological potential.

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Introduction

The microbial world is the largest unexplored reservoir of biodiversity which exists in diverse ecological niches, including extreme environments. 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 [1]. *Paenibacillus polymyxa* (formerly known as *Bacillus polymyxa*) has attracted considerable interest because of its great biotechnological potential in different industrial processes and in sustainable agriculture. The genus *Paenibacillus* was created by Ash et al. [2] in 1993 to accommodate the former 'group 3' of the genus *Bacillus*. It comprises over 30 species of facultative anaerobes and endospore-forming, neutrophilic, periflagellated heterotrophic, low G+C gram-positive bacilli. The name reflects this fact, in Latin *paene* means *almost*, and therefore the *Paenibacillus* is almost a *Bacillus*. Comparative 16S rRNA sequence analyses revealed that rRNA group 3 bacilli represents a phylogenetically distinct group and exhibit high intragroup sequence relatedness and is only remotely related to *B. subtilis* the type species of the genus *Bacillus*. The taxon contains various species such as *B. alvei, B. amylolyticus, B. azotofixans, B. gordonae, B. larvae, B. macerans, B. macquariensis, B. pabuli, B. polymyxa, B. pulvifaciens* and *B. validus* [3]. Phenotypically, species of this group react weakly with gram's stain and even young cultures appear gram-negative. They differentiate into ellipsoidal spores which distinctly

swell the mother cell. The combination of morphology and physiology is sufficient to distinguish rRNA group 3 bacilli from all other mesophilic species of *Bacillus* with the exception of *B. circulans, B. lautus, B. lentimorbus* and *B. popilliae.* The latter four species are however, phylogenetically only remotely related to *B. polymyxa* and its relatives and the described rRNA group 3 specific gene probe provides an unequivocal method for distinguishing these taxa [2]. Among the 51713 Firmicutes sequences listed in Ribosomal Database Project (RDP) II, *Paenibacillaceae* comprises 1057 16S rRNA sequences with 74 as *P. polymyxa* (as on January 2008). Complete sequencing of the genome of the plant growth promoting strain *P. polymyxa* E681 is in progress.

P. polymyxa inhabits different niches such as soils, roots, rhizosphere of various crop plants including wheat, maize, sorghum, sugarcane and barley [4, 5], forest trees such as lodgepole pine [6], douglas fir [7] and marine sediments [8] etc. In the rhizosphere, *P. polymyxa* is involved in nitrogen fixation [9,10], soil phosphorus solubilization [11], production of antibiotics [12–17], exopolysaccharides [18], chitinase [19], hydrolytic enzymes [20] and in the enhancement of soil porosity [21] (Table 1). *P. polymyxa* exhibited clear antagonistic activity against soilborne fungal and oomycetic pathogens [9, 18, 22–25] (Table1). The bacterium displays inhibitory activity against human and animal pathogenic microorganisms [8, 26] (Table 1). In another study, the dominant species during hydrogen production from alkaline pretreated sludge was identified as *P. polymyxa* [27]. The present attempt has been made to review available literature on various roles and potentials of *P. polymyxa* in different biotechnological processes.

Biodiversity of *P. polymyxa*

Biodiversity studies of indigenous bacterial populations are of great importance for understanding their ecological role in nature as well as to discover new microbial activities. Few studies on the biodiversity within the species of *P. polymyxa* have been carried out, and most of them point out the influence of different factors on the degree of genetic polymorphism. Von der Weid et al. [5] investigated the influence of plant development both at phenotypic and genotypic level by *P. polymyxa* populations naturally occurring in the maize rhizosphere. The investigation(s) suggested that a more homogeneous *P. polymyxa* population was present during the middle stages of maize growth (30 and 60 days after sowing) than in the first stage (10 days) and after 90 days of maize growth. The effect of plant cultivar on the degree of genetic diversity of 67 *P. polymyxa* isolates recovered from the root system of maize planted in a tropical Brazilian soil was evaluated by da Mota et al. [28]. Results revealed a high level of genetic polymorphism among isolates recovered from different cultivars, yielding a total of 54 distinct groups. The influence of long-term cultivation on genetic structure of *P. polymyxa* populations associated with the rhizosphere of durum wheat was investigated in Algerian soils sampled in regions where wheat had been cultivated for 5 and 26 years, 70 years and more than 2000 years. Results indicate that long-term cultivation of wheat in Algerian soils (>70 years) seems to modify rhizospheric populations of *P. polymyxa* by increasing their size, reducing their diversity, selecting a dominant genotype, and increasing the proportion of nitrogen fixers [4].

A more comprehensive study on genetic diversity of *P. polymyxa* strains recovered from different localities was carried out by means of phage IPy1 probing method. A high degree of genetic diversity was observed among the 102 strains, as a total of 53 different hybridization patterns were found [29]. In another study, sequence heterogeneities in 16S rRNA genes from individual strains of *P. polymyxa* were detected by sequence-dependent separation of PCR products by temperature gradient gel electrophoresis (TGGE). Targeting rapidly evolving regions V6, V7 and V8 of 16S rRNA genes resulted in distinct band patterns derived from different *P. polymyxa* strains indicate interstrain (intraspecific) variability [30].

P. polymyxa as a plant growth-promoting rhizobacterium

Soil microorganisms can promote plant growth through the production of different hormones such as cytokinins, auxins and/or ethylene, gibberellins and nitrogen fixing ability or by the suppression of plant diseases caused by deleterious microorganisms [31, 32]. Some spore-forming bacteria, in particular gram-positive bacilli and streptomycetes, have attracted special attention due to their advantages over non-spore formers in product formulation and stable maintenance in soil [33]. Among these plant growth-promoting rhizobacteria (PGPR), *P. polymyxa* is known to have a broad host plant range.

Nitrogen fixing ability by *P. polymyxa* was demonstrated by Guemori-Athmani et al. [4]. These authors measured nitrogenase activity of some representative isolates of *P. polymyxa* recovered from Algerian soil by acetylene reduction assay (ARA). Results showed that only 14 of the 23 strains tested were able to reduce acetylene. Some of them

Table 1 Characteristics of *Paenibacillus polymyxa*

Strain	Origin	Activity	References
P. polymyxa strain B1 and B2	Wheat rhizosphere	Nitrogen fixation	[10]
P. polymyxa CF43	Wheat rhizosphere	Enhancement of soil porosity	[21]
P. polymyxa PMD216 and PMD230 *P. polymyxa* PMD112 and PMD128 *P. polymyxa* PMD66	Wheat rizoplane, Wheat rhizosphere, Soil	Production of auxin and other indolic and phenolic compounds	[92]
P. polymyxa strain B2	Wheat rhizosphere	Cytokinin production	[23]
P. polymyxa strain B5 and B6	Soil around peanut roots	Production of exopolysaccharides, biocontrol against *Aspergillus niger* in roots and seeds of peanut plants	[18]
P. polymyxa SCE2	Soil (Brazil)	Proteases production, production of antimicrobial compounds active against human pathogenic microorganisms	[12, 26, 54]
P. polymyxa strains CM5-5 and CM5-6	Barley rhizosphere	Production of hydrolytic enzymes, multi-target and medium-independent type of fungal antagonism	[20]
P. polymyxa	Soil, wheat rhizosphere and rizoplane	Production of chitinase	[19]
B. polymyxa ATCC842^T	-	Production of xylanase	[52]
P. polymyxa EJS-3	Root tissue of *Stemona japonica*	Production of fibrinolytic enzyme	[57]
P. polymyxa ATCC 12321	Spoiled starch	2, 3-butanediol (BDL) production	[79]
P. polymyxa T129	Soil	Biocontrol against *Fusarium oxysporum*	[22]
P. polymyxa strains B5 and B6	Wheat rhizosphere	Biocontrol of the oomycete plant pathogens *Phytophora palmivora* and *Phytim aphanidermatum*	[24]
P. polymyxa strains B2, B3 and B4	Wheat rhizosphere	Increased resistance to plant pathogens (biotic stress) and drought resistance (abiotic stress)	[40]
P. polymyxa JB115	Soil	Production of β-glucan	[78]
P. polymyxa 1460	Soil	Production of lectin	[56]
P. polymyxa E681	Winter barley roots	Fusaricidin biosynthesis, biocontrol of fungal pathogens on sesame plants	[13, 41]
P. polymyxa OSY-DF	Fermented foods	Co-production of polymyxin E1 and lantibiotic	[17]
P. polymyxa strain M	Marine sediment	Antagonistic activity against *Vibrio* species	[8]
P. polymyxa P13	Fermented sausages	Polyxin production and biosorption of heavy metals	[16, 66]
P. polymyxa BY-28	Soil	Flocculants production	[75]
P. polymyxa strain B1 and B2	Wheat rhizosphere	Formation of biofilm	[25]

were very active: strain SGH1 reduced C_2H_2 at a similar rate to *P. azotofixans* ATCC 35681T, which is a very efficient nitrogen-fixing bacterium [34]. However, it hasn't been demonstrated that plant growth promotion by *P. polymyxa* is primarily correlated with its nitrogen-fixing ability [10, 35].

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 wheatgrass [36]. 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 senescence [37] in wheat [10, 38]. The effect of inoculation with *P. polymyxa* on growth parameters of wheat and spinach plants and the activities of enzymes present in the leaves of these plants such as glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione reductase and glutathione S-transferase were observed [39].

The *in vitro* antagonistic activity of *P. polymyxa* against the fungus *Gaeumannomyces graminis* var. *tritici* that causes take-all off wheat and the plant pathogenic fungus *Fusarium oxysporum* that causes *Fusarium* wilt disease has been reported by Heulin et al. [9]. In a previous study, Timmusk and Wagner [40] reported that natural isolates of *P. polymyxa* induces drought tolerance and antagonizes pathogens in *Arabidopsis thaliana* (Table 1). These effects were observed both in a gnotobiotic system and soil [24]. These studies indicated that, aside from the beneficial effects observed, inoculation of *A. thaliana* by *P. polymyxa* (in the absence of biotic or abiotic stress) resulted in a 30% reduction in plant growth, as well as a stunted root system, compared to non-inoculated plants. This indicated that there was a mild pathogenic effect [24, 40] and under these conditions, *P. polymyxa* could be considered as a deleterious rhizobacterium. Characterization of colonization process was done to understand the relationship between the beneficial and harmful effects of *P. polymyxa* on *A. thaliana* by Timmusk et al. [25]. They studied colonization of plant roots by a natural isolate of *P. polymyxa* which had been tagged with a plasmid-borne *gfp* gene and observed that the bacteria colonized predominantly the root tip, where they formed biofilm. Ryu et al. [41] demonstrated that *P. polymyxa* strain E681 effectively controlled pre-emergence and post-emergence damping-off diseases on sesame plants (Table 1). A positive effect of the association of *P. polymyxa* and arbusolar michorrizae fungi in biocontrol of *Pythium* damping-off in cucumber has been demonstrated by Li et al. [42].

So far, most studies on the biocontrol activity of *P. polymyxa* have been concentrated on the production of different antibiotic substances. Fusaricidin, a peptide antibiotic consisting of six amino acids, has been identified as a potential antifungal agent from *P. polymyxa* E681 [13] (Table 1). Various analogs of fusaricidins were isolated and characterized from *P. polymyxa*; these included LI-F03, LI-F04, LI-F05, LI-F06, LI-F07, and LI-F08 [43,44] as well as fusaricidins A–D [14,15] (Table 1). Fusaricidins have an excellent antifungal activity against plant pathogenic fungi such as *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium thomii* and fusaricidin B has particularly antagonistic activity against *Candida albicans* and *Saccharomyces cerevisiae*. Fusaricidins also have an excellent germicidal activity to gram-positive bacteria such as *Staphylococcus aureus* [14, 15]. In addition, they have antifungal activity against *Leptosphaeria maculans*, which causes black root rot of canola [45]. Antagonistic activity of *P. polymyxa* was also demonstrated against the nematode *Meloidogyne javanica*. The inoculation of *P. polymyxa* alone or together with *Rhizobium* increased lentil plant growth both in *M. javanica*-inoculated and -uninoculated plants [46] (Table 1).

P. polymyxa as antimicrobial agent

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 pathogens. It has bacteriocin-like properties such as proteinaceous nature (sensitive to proteases), insensitivity to organic solvents and chelators, stability to heat (up to 10 min at 90°C), and acidic pH but instability in alkaline conditions [16]. Two antimicrobials were isolated from *P. polymyxa* strain OSY-DF: polymyxin E1, which is active against gram-negative bacteria and an unknown 2,983-Da compound showing activity against gram-positive bacteria. The antimicrobial peptide, designated paenibacillin, is active against a broad range of food-borne pathogenic and spoilage bacteria, including *Bacillus* spp., *Clostridium* sporogenes, *Lactobacillus* spp., *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Listeria* spp., *Pediococcus cerevisiae*, *Staphylococcus aureus* and *Streptococcus agalactiae*. Furthermore, it possesses the physico-chemical properties of an ideal antimicrobial agent in terms of water solubility, thermal resistance, and stability against acid/alkali (pH 2.0 to 9.0) treatment. The peptide was unequivocally characterized as a novel lantibiotic. Lantibiotics are a group of antimicrobial compounds and have been used as biopreservatives in a number of food products [47]. Co-production of polymyxin E1 and a lantibiotic from *P. polymyxa* strain OSY-DF are potentially useful in food and medical applications [17]. *P. polymyxa* also produces pyrazine metabolites which was stimulated by valine supplementation [48]. In 2005, Stern et al. [49] evaluated anti-*Campylobacter* activity of three *P. polymyxa* strains from poultry production environments. In this study, they performed bacteriocin-based treatment to reduce *Campylobacter jejuni* colonization in poultry. Bacteriocin treatment dramatically reduced both intestinal levels and frequency of chicken colonization by *C. jejuni*. Feeding bacteriocins before poultry slaughter appears to provide control of *C. jejuni* to effectively reduce human exposure. This advance is directed toward on-farm control of pathogens, as opposed to the currently used chemical disinfection of contaminated carcasses. Recently, the potential of *P. polymyxa* as probionts in both *in vitro* and *in vivo* conditions to reduce mortality of shrimp larvae exposed to Vibrios was evaluated [8].

P. polymyxa as biotechnological agent in industrial processes

Different strains of *P. polymyxa* were reported to produce cell wall degrading enzymes such as β-1,3-glucanases, cellulases, chitinases, proteases [50, 51] and xylanase [52] along with hydrolytic pathway. *P. polymyxa* encodes two homologous β-glucosidases, BglA and BglB, presenting different quaternary structures and substrate specificities. BglA is highly specific against cellobiose and BglB acts as an exo-β-glucosidase hydrolyzing cellobiose and cellodextrins of higher degree of polymerization [53]. *P. polymyxa* produced a great amount of extracellular protease activities with molecular masses of 20, 35, 50 and 210 kDa in thiamine/biotin/nitrogen broth (TBN broth) at neutral pH when compared with the other four media (Luria-Bertani broth, glucose broth, trypticase soy broth and a defined medium). Quantitative measurement revealed that the best proteolytic activity (~300 arbitrary units (AU) x mg of protein) was reached after 72 h of growth in TBN broth. Neutral-alkaline proteases constitute a very large and complex group of enzymes, with both nutritional and regulatory roles in nature. The major applications of these enzymes are in detergent formulation, food industry, leather processing, chemical synthesis and waste management [54] (Table 1). Ishii et al. [55] have reported the production of flavin reductase from *P. polymyxa* A-l that couples efficiently with desulfurizing enzymes (DszA and DszC).

Enzyme-lectins LI and LII from *P. polymyxa* 1460 showed an increase in their proteolytic activity when incubated with the carbohydrate moiety of the wheat-root exocomponent fraction. This increase may be associated with the presence of lectin-specific carbohydrates in the root fraction. The lectins of the nitrogen-fixing paenibacilli also enhance cellulose degradation in the plant cell, thus increasing the activity of β-glucosidase in the wheat-root cell wall [56]. Two novel extracellular fibrinolytic enzymes (118 and 49 kDa) produced by *P. polymyxa* were isolated from the endophytic strain EJS-3 recovered from the root tissue of *Stemona japonica* (Blume) Miq, a chinese traditional medicine (Table 1). The amount of fibrinolityc activity measured in the culture supernatant was ~100 U/mL. Fibrinolytic enzymes prevent or cure thrombotic diseases by degrading the fibrin in the blood clot [57].

Microbial exopolysaccharides (EPSs) are the primary or secondary metabolites produced by a variety of microorganisms. These EPSs have been widely used within bioindustries as foods [58], medicines [59] and cosmetics [60] as well as for the removal of metal ions from waste water [61, 62] and mineral processing [63], because the production cost of microbial EPS is lower than that of algal or plant polysaccharides [64]. Additionally, bacterial EPS is non-toxic, biodegradable and environmentally friendly [65]. *P. polymyxa* strain P13, was described as EPS producer by Acosta et al. [66]. These authors found that 100 ml of a stationary phase P13 culture formed 27 (±4) mg (±SD) and 15(±4) mg (±SD) EPS in BHI medium containing 1 M NaCl and in control BHI medium, respectively. This strain exhibited significant biosorption capacity of Cu(II) which is originated from several industries. EPS production was associated with hyperosmotic stress by high salt (1 M NaCl), which led to a significant increase in the biosorption capacity of whole cells [66] (Table 1). The absorption of *P. polymyxa* cells or EPS production by these microorganisms on the surface of several minerals have been reported as a method to selectively separate metal ions from binary mixture such as sphalerite and galena, galena and pyrite, suggesting their use in biomineral processing by means of microbial flotation and flocculation [67-69]. Bioflocculation of high-ash Indian coals using *P. polymyxa* showed a decrease in ash by 60%, suggesting that selective flocculation of coal is possible [70]. Some bacteria such as *Rhodococcus erythropolis* S-1 [71], *Alcaligenes cupidus* KT201 [72], *Aspergillus* sp. JS-42 [73], *Phormidium* J-1 [74] and *P. polymyxa* BY-28 [75] (Table 1) are commonly known for flocculants production.

P. polymyxa JB115 was isolated from Korean soil as a glucan producer (Table 1) for the development of animal feed additives. It has a β-(1→3)- and β-(1→6)-linked glucan parastructure which are known as biological response modifiers (BRMs) and natural immunomodulators [76] and the β-(1→3) backbone is essential for antitumor activity [77]. High molecular weight glucan (above 100 kDa) can be used as an animal feed additive for immune-enhancement and as a potential antitumor agent for livestock [78].

P. polymyxa produces optically active 2,3-butanediol (BDL), at a high optical purity of more than 98% from a variety of carbohydrates [79]. One mol glucose is converted to 2 mol pyruvate, which is consequently converted to 1 mol BDL and 2mol NADH. Since only 1 mol NADH is reoxidized in the formation of 1 mol BDL, other metabolites must be generated to recycle the NADH. Theoretically, maximum yield of BDL from glucose is 0.67 $mol.mol^{-1}$ and the ratio of BDL to ethanol produced is 1 $mol.mol^{-1}$ in the case of no production of acetate and lactate under anaerobic conditions. Generally, anaerobic cultivation has been considered as the most suitable technique for enhancing BDL production as compared to microaerobic cultivation because aeration decreased the optical purity of BDL produced by *P. polymyxa* [80, 81]. Effect of different parameters such as pH, O_2 supply and substrate concentration on BDL production and their purity

have been investigated under anaerobic and microaerobic environments by Nakashimada et al. [80, 81]. BDL is also known as 2,3-butylene glycol, or 2,3-dihydroxybutane, or dimethylethylene glycol. It can be converted to 1,3-butanediene, which is a substance used in the production of synthetic rubber. In addition, many other derivatives for potential uses as anti-freeze agents (levo-form of 2,3-BDL), solvents, and plastics can also be prepared from 2,3-BDL. It can also be used as a flavoring agent in food products when converted to a diacetyl by dehydrogenation. Esterification of butanediol forms precursors of polyurethane for use in drugs, cosmetic products, and lotions etc [82]. It can be considered as effective liquid fuel additive as its heating value is 27,198 Jg^{-1} which is similar to other liquid fuels, such as ethanol (29,055 Jg^{-1}) and methanol (22,081 Jg^{-1}) [83].

Some other bacteria such as *Aerobacter indoiogenes* [84], *Aerobacillus polymyxa* [85], *Klebsielia pneumoniae* [86, 87], *Enterobacter cloacae* NRRL B-23289 [88], *Enterobacter aerogenes* [89], *Vibrio cholerae* El Tor biotype strain N16961 [90], *Klebsiella oxytoca* [91], etc. are also known to secrete 2,3-BDL as end product.

Conclusion

P. polymyxa produces a wide variety of secondary metabolites, including plant growth-regulating substances, hydrolytic enzymes, antibiotic compounds and has nitrogen fixing ability. It can also produce optically active 2,3 butanediol, a valuable chemical compound whose derivatives have a large employment in the production of several compounds. These properties together with its endospore forming potential enables it to resist a wide range of environmental stresses, making it a promising biotechnological agent in sustainable agriculture, on-farm control of pathogens and several industrial processes. Flocculants production by *P. polymyxa* has drawn attention for their bio-degradability, efficiency and harmlessness. It has been used for flocculation and flotation of various minerals including hematite, pyrite and chalcopyrite, wastewater treatment, tap water production and the fermentation industry. However, there is a need to understand the roles and diversity of *P. polymyxa,* as complete genome sequence data is not available.

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