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Review

Bacillus spp. as a new direction in biocontrol and deodorization of

organic fertilizers

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Abstract: Bacteria belonging to the genus *Bacillus* are widely distributed in environments fulfilling several important functions. Due to their ability to produce antibiotics and other metabolites, these bacteria limit the development of pathogens and thus promote plant growth. They also play an important role in the deodorization of organic fertilizers, including manure, slurry, and dung. This article describes the characteristics of *Bacillus* spp., their properties, and their application in biocontrol.

Keywords: Bacillus; biocontrol; plant protection; deodorization; odors

1. Introduction

Bacillus spp. are characterized by a great diversity of genetic traits and biotechnological activities. The unique characteristics of these bacteria can be attributed to the high G+C content in the chromosomal DNA, which varies between 32 and 69%. Due to their ability to produce various extracellular enzymes, surfactants, and bacteriocins, *Bacillus* spp. are widely used in food, pharmaceutical, and agricultural industries [1]. These bacteria can promote the growth and development of plants. They also contribute to the formation of tubercular structure of the soil, and thus improving its quality, by forming biofilms. The antagonistic properties of *Bacillus*, which allow these bacteria to play a vital role in biocontrol, have been extensively studied [2]. In recent years, researchers have been showing a great interest in investigating the possibility of utilizing *Bacillus* spp. in the removal of odors arising from organic fertilizers, including manure and slurry [3].

2. Characteristics of the genus *Bacillus*

The genus *Bacillus* includes Gram-positive bacteria of the type *Firmicutes*. They are aerobic or relative anaerobic in nature. A characteristic feature of these bacteria is their spore-producing ability, which gives them the potential to survive in adverse environmental conditions. Owing to this property, they are widely distributed and can be easily isolated from soils, fresh and saline waters, the digestive tract of animals, perishable food materials, and clinical samples [4]. In addition, these bacteria have a high growth rate and an efficient synthesis system, and secrete extracellular proteins [5].

Bacteria of the genus *Bacillus* are long rods and occur as single cells, or in pairs or long chains. The representatives of this genus can be distinguished into three types: mobile, peritrichous or polar (*Bacillus cereus, Bacillus thuringiensis*), and immobile (*Bacillus anthracis* and *Bacillus mycoides*) [6]. Due to their high genetic diversity, these bacteria exhibit a wide range of physiological and metabolic characteristics, and accordingly, they can be differentiated into psychrophilic and thermophilic, acidophilic and alkaliphilic, or saline-resistant and halophilic strains. *Bacillus* spp. can also be classified as chemoorganotrophs, with the exception of two species which are facultative chemolithotrophs. Furthermore, the nutritional requirements of strains within the species vary to a great extent. Some strains are prototrophs and require only one source of organic carbon, while some are auxotrophs and depend on the substrate for important organic compounds. Organic matter, on the other hand, is metabolized via aerobic respiration, fermentation, or a combination of both processes [7].

Bacillus spp. can produce spores (endospores), which refer to the resting forms of bacteria which can survive in adverse conditions, including in the absence of water and nutrients, at high or low temperatures, in the presence of UV radiation, or under unfavorable pH. Spores are formed inside the cell as enclosed in a genophore, with a small amount of cytoplasm and ribosomes with a multilayer sheath made of sugars and proteins saturated with fats, and surrounded by a cell membrane. Dipicolinic acid is an important component that confers the spores with heat resistance. The other factors that facilitate spores to exhibit resistance to undesirable conditions are deep metabolic dormancy, lack of ATP, and presence of SASP proteins [8]. In taxonomic studies, the identification of *Bacillus* spp. is performed based on the morphological features of endospores such as their size, shape, and cytoplasmic appearance. *Bacillus* spores are generally cylindrical, spherical, or ellipsoidal in shape. Within the cells, they may be found in the center, at the end, or on the side [9,10].

Among the species of *Bacillus* genus, some cause diseases in humans and animals. These pathogenic species include *Bacillus licheniformis*, *B. anthracis*, *B. cereus*, and *B. thuringiensis*. The first three species affect humans and animals, while the last one is pathogenic to invertebrates [2].

3. Use of the genus *Bacillus* in biocontrol

Bacteria of the genus *Bacillus* are widely used in biocontrol processes. The biologically active compounds secreted by them can directly inhibit the growth and function of several other bacteria as well as fungi, insects, nematodes, and viruses. They may also limit the growth of pathogens through indirect mechanisms, such as induction of resistance in plants [11].

The biologically active compounds synthesized and secreted by *Bacillus* spp. include enzymes (glucanases, proteases, cellulases), biosurfactants, bacteriocins, and antibiotics [12,13]. The most important of the secreted enzymes are proteolytic and amylolytic enzymes. Enzymes are macromolecular proteins that mainly act as catalysts to accelerate specific chemical reactions by lowering their activation energy. Proteases are a group of enzymes that catalyze the hydrolysis of peptide bonds, and amylases are enzymes involved in the breakdown of starch and other polysaccharides [14]. The primary function of enzymes produced by the bacteria of *Bacillus* genus is the degradation of fungal cell wall components (chitin and glucans) and lysis of mycelium of phytopathogenic fungi such as *Alternaria alternata* and *Fusarium culmorum*. Some *Bacillus* spp. secrete cellulolytic enzymes, which are considered valuable due to the need to recycle large amounts of cellulose accumulated worldwide. Species that can produce cellulases are *Bacillus subtilis*, *B. cereus*, and *B. licheniformis* [15].

Surfactants are surface-active compounds which accelerate the degradation of hydrophobic nutrient substrates [16]. Biosurfactants are metabolites that affect the hydrophobic properties of cells, thereby facilitating their separation from surfaces or improving their adhesion. These compounds are widely used in environmental bioremediation processes. The term "bioremediation" refers to the process of removing contaminants from soil and groundwater with the use of live microorganisms [17]. Surfactants destabilize the cell membrane by modifying the activity of pores within and ultimately result in the cell death of microorganisms. Biosurfactants produced by the strains of *B. subtilis* can inhibit the growth of pathogens such as *Listeria monocytogenes*, *Legionella pneumophila*, *Candida albicans*, and *Botrytis cinerea* [18].

Bacteriocins are protein metabolites that exhibit antimicrobial activity. However, they are effective in inhibiting the growth of only a small number of species. These compounds differ in their biochemical properties, molecular weight, mechanism of action, and spectrum of activity. Depending on their dose and degree of purification, growth phase of susceptible cells, and environmental conditions, bacteriocins can act as bactericidal or bacteriostatic agents. As these substances have antibacterial and antifungal properties and have also been recognized as safe, they are used as an alternative to chemical food preservatives [19]. Some bacteriocin-producing *Bacillus* strains can also be used as starter cultures for the production of fermented foods, or as probiotics and animal feed additives [20].

Antibiotics are organic compounds produced by bacteria and have the ability to limit the growth and functioning of pathogenic microorganisms. Their action can disrupt cell wall structure and disturb cell membrane functions, affect protein synthesis, and inhibit respiratory chain enzymes [21]. Antibiotics produced by *Bacillus* spp. can inhibit the growth of both Gram-positive and Gramnegative bacteria and phytopathogenic fungi such as *Aspergillus flavus*, *Alternaria solani*, and *Fusarium oxysporum* [22]. Some of the antibiotics produced by these bacteria include bacitracin and gramicidin S.

Among the *Bacillus* spp., *B. thuringiensis* is the only one characterized with the ability to synthesize protein crystalline toxins Cry and Cyt, which are toxic to insects. The degree of toxicity of crystalline toxins depends on their affinity to cell membranes. The mechanism of action of the toxins involves their dissolution in the midgut of the insect and release of prototoxin, which is activated by peptidases. The cut fragments of prototoxin then bind to the receptors on the membrane of cells covering the digestive tract, leading to disturbance in the osmotic balance of cells and thus their lysis. This results in the paralysis of the intestine, thereby restricting food consumption by insects and

finally causing death within a few hours [4]. The toxin crystals of bacteria can be used along with endospores to produce biopesticides. Such a product is environmentally safe and can be used in the place of chemical pesticides for controlling unwanted insects [23].

Bacteria of the genus *Bacillus* are included in the group of Plant Growth-Promoting Rhizobacteria (PGPR) due to their ability to solubilize phosphorus and potassium, as well as produce phytohormones and siderophores [24]. The interaction of these compounds not only improves the performance of plants but also their photosynthetic activity by increasing the content of chlorophyll, sucrose, glucose, fructose, and amino acids in leaves [25].

Nitrogen, phosphorus, and potassium are some of the most important nutrients supporting the growth and development of plants. One of the main purposes of the application of PGPR microorganisms is to increase the availability of these nutrients [26]. Plants can take up nitrogen only if it exists in the form of ammonia. The process of converting atmospheric nitrogen to the assimilable form (NH3) is called biological nitrogen fixation. Bacillus circulans, Bacillus firmus, Bacillus *pumilus*, *B. cereus*, and *B. licheniformis* are capable of fixing atmospheric nitrogen [27]. Phosphorus is taken up by plants only in the form of phosphate. However, in the soil, most of this element is present in the form of orthophosphoric acid salts that are difficult to solubilize. Phosphatases and phytases produced by different microorganisms can convert these unavailable phosphorus compounds to forms available to plants. Phosphatases are secreted by Bacillus lexus and Bacillus megaterium, while phytases are secreted by Bacillus laevolacticus, B. subtilis, and B. licheniformis [28]. Potassium can be solubilized by bacteria and converted from unavailable to available forms through the production of organic acids or extracellular polysaccharides participating in mineral decomposition, which results in the release of this element into the soil. Some of the potassium-solubilizing species are Bacillus velezensis, Bacillus coagulans, Bacillus edaphicus, B. cereus, B. circulans, B. megaterium, B. subtilis, B. firmus, and B. mycoides [29].

Phytohormones produced by *Bacillus* spp. are gibberellic acid, indolyl-3-acetic acid (IAA), and to a lesser extent, abscisic acid. These compounds support the germination process—the growth of roots and aboveground parts of a plant. They can also make the plant resistant to environmental stress [30]. Among the *Bacillus* spp. *B. subtilis*, *B. velezensis*, and *B. thuringiensis* can produce phytohormones [31,32]. Siderophores are chemical compounds that chelate iron ions. The resulting complexes can be indirectly taken up by plants, which can be beneficial because under natural conditions the uptake of iron ions from the soil by plants is greatly hindered by their low solubility in water. In addition, siderophores can be limit the growth of pathogens because by entering into complexes with these compounds, iron ions become inaccessible to these microorganisms [33].

Bacillus spp., especially *B. subtilis*, can activate the immune response of plants when attacked by a pathogen. Systemic induced resistance (ISR) confers plants with temporary resistance to pathogens. It is usually induced by the signal molecules jasmonic acid and ethylene. The activation of ISR results in the accumulation of phenolics at the infection site, an increase in flavonoid content in tissues adjacent to the infection site, and synthesis and subsequent transport of PR proteins to all plant organs or the secretion of these proteins into the rhizosphere [34]. Induced immunity is extremely important as it determines the status of basal immunity in plant organisms [33].

Bacillus subtilis is also used as a model organism for studies on biofilm formation. A biofilm is a multicellular structure composed of bacteria, and also other microorganisms, surrounded by a layer of organic and nonorganic substances secreted by these organisms. Biofilms can adhere to both biological and abiotic surfaces [35]. Microorganisms form biofilms to protect themselves from adverse external environmental conditions. Moreover, the compact structure of biofilms provides bacteria with high resistance to various bactericidal agents such as antibiotics and disinfectants [36]. Biofilm formed by *B. subtilis* is highly useful in the bioremediation of groundwater and soils, as these bacteria can degrade harmful compounds. The toxin-degrading property of biofilms also enables controlled degradation of toxic compounds in suitable bioreactors. Biofilms also increase the efficiency of microbial processes [37].

Due to these properties, bacteria from the genus *Bacillus* are widely used in the production of biopreparations for plant protection, which can be an ideal substitute for fungicides. Biopreparations not only limit the growth of plant pathogens by exploiting the antagonistic properties of bacteria but also improve plant and soil quality by secreting specific metabolites [26].

Species of microorganism	Trade name of the preparation	Manufacturer/country of production	Targeted organisms
<i>B. subtilis</i> (strain FZB 24)	Rhio-plus	KFZB	Fungi attacking vegetables
		Biotechnick/Germany	(potatoes)
B. subtilis	Serenade	AgroQuess Inc./USA	Fungi attacking vegetables (potatoes) and fruits (mullets, apples)
B. licheniformis SB3086,	EcoGuard	Novozymes/USA	Sclerotinia homoeocarpa
B. amyloliquefaciens GB99, B. subtilis GB122	BioYield	Gustafson/USA	Fungi attacking bedding plants
B. pumillus GB34	Yield Shield	Gustafson/USA	Fungi attacking soybeans
B. subtilis MBI600	Subtilex	Beker Underwood/USA	Fungi attacking soybeans,
			legumes, and cotton

Table 1. Bacillus-based biological plant protection formulations [38,39].

Biopreparations based on *Bacillus* spp. mainly inhibit the growth of phytopathogenic fungi including *A. alternata*, *Fusarium graminearum*, *Rhizoctonia solani*, *Cryphonectria parasitica*, and *Phytophthora capsici*. The activity of biopreparations is determined by the ability of bacteria to synthesize different metabolites with fungistatic activity [38,39].

4. Deodorization of organic fertilizers

The emission of odorous compounds (odors) resulting from livestock production has become a major environmental problem. Bernet and Beline [40] reported that up to 64% of NH₃ emission, 37% of CH₄ emission, and 65% of N₂O emission occur within breeding farms. During poultry farming, a large number of undesirable compounds, including ammonia and its derivatives, pyrazines, thiols, sulfides, hydrogen sulfide, aldehydes, carboxylic acids, and a few hydrocarbons, are released to the atmosphere. Several methods, such as biological, chemical, and physical, have been used for eliminating odors from the environment. Chemical methods include catalytic oxidation, ozonation, and chemical scrubbing; physical methods include adsorption, absorption, condensation, and UV irradiation; and biological methods include the use of biofilters, bioscrubbers, and biosorption in activated sludge devices [41]. High emissions of the abovementioned odorous compounds can have a

significant impact on the health of people working in production facilities and the animals themselves, while also affecting the environment, resulting in acid rain. Therefore, odor reduction is important to protect the health of people, animals, and the environment. However, the commonly used physical and chemical methods are costly and time-consuming, and so the use of biological methods with selected microorganisms is gaining increasing attention [42].

Specific groups of microorganisms can transform most organic compounds. Biological deodorization refers to a combination of gas diffusion and biochemical reactions. In this process, odorous compounds (odors) are combined with water, in which they get dissolved or remain bound to the surface of the molecules. The compounds dissolved in water or bound to water molecules are then absorbed by microorganisms. In contrast, water-insoluble odorous compounds first attach to the cell surface of microorganisms, and with the help of secreted extracellular enzymes, they are converted to soluble forms. The converted forms are utilized by microorganisms as nutrients [43]. The main purpose of using biological treatment techniques is to adjust the environmental conditions (oxygen content, chemical composition, temperature) such that the process of deodorization of odors to acceptable products becomes efficient and cost-effective [44].

The development of biotechnology has enabled the application of biological deodorization using specialized microorganisms on a global scale. The advantages of this odor abatement method are its higher efficiency and lower production costs [43]. Biological treatments are generally safe for the environment because they are mostly carried out at an ambient temperature and do not generate nitrogen oxides or other harmful wastes [45].

Recent studies indicate that *Bacillus* spp. can reduce compounds such as NH₃, H₂S, and SO₂. Kim and co-workers [42] investigated the efficacy of *Bacillus amyloliquefaciens* in the reduction of odor emissions from swine manure under anaerobic conditions. In the experiment, samples were treated with 1%, 5%, 10%, and 100% suspension of *B. amyloliquefaciens* (BA) bacteria. These BA preparations with specific concentrations were made by diluting the liquid bacterial culture containing 1×10^9 cfu/ml with an appropriate amount of distilled water. Unsprayed slurry sample and slurry sample treated with water were used as control. The experiment showed that the addition of BA preparations to the slurry caused a reduction in odor emission in the environment. However, a significant (*P* < 0.05) decrease in emissions of selected compounds (NH₃, H₂S, SO₂), in comparison to the control, was observed only after several days (4–6 days) of treatment of slurry with 10% BA once a day.

Kim et al. [42] demonstrated that the reduction of NH₃ may be related to the bacterial secretion of bacteriocins, which inhibit the growth of different bacteria responsible for odor formation. On the other hand, the reduction of ammonia may be caused by the activity of many extracellular enzymes produced by *B. amyloliquefaciens*, including metalloproteases and proteases. Bacteria of the genus *Bacillus* can also maintain the pH values of slurry through the production of organic acids, which can inhibit the hydrolysis of urea and deamination of other nitrogen compounds, leading to a reduction in NH₃ emission [46,47].

Hydrogen sulfide (H₂S) and sulfur dioxide (SO₂) are identified as the most dangerous volatile sulfur compounds (VS; volatile sulfur gases) among the odorous substances emitted during manure decomposition under anaerobic digestion conditions [48]. Of these, hydrogen sulfide can be metabolized into elemental sulfur or sulfate by aerobic bacteria or anaerobic photosynthetic bacteria. Ushida et al. [45] and Nakada and Ohta [49] showed that *Bacillus* can efficiently degrade H₂S in vitro. Presumably, the H₂S-degrading activity of these bacteria may be related to the reduction of the

pH value of fecal matter. This may prevent sulfate reduction reactions by sulfur-reducing microorganisms and the metabolism of sulfur-containing amino acids by anaerobic bacteria [50–52]. The effect of microorganisms in reducing SO₂ production during manure decomposition has not been clearly understood so far. In a 2014 study by Kim et al., a slight reduction in sulfur dioxide emissions was observed only around day 6 of applying 10% BA formulation once daily. This finding may be related to the degradation of VS by *B. amyloliquefaciens* bacteria or a reduction in the growth and activity of sulfur-reducing bacteria in the sample.

Mpofu and co-workers [53] studied the ability of *B. licheniformis* strain TAB7 to biotransform five monocyclic phenolic compounds—ferulate, vanillate, *p*-coumarate, caffeate, and vanillin and one nonphenolic compound—cinnamate, which are released in large quantities during compost decomposition. The results showed that the tested strain was able to transform five of the listed compounds. Ferulate, *p*-coumarate, vanillate, and vanillin were transformed within 6 hours, while the biotransformation of caffeate took up to 24 hours. It was observed that *B. licheniformis* TAB7 synthesized some enzymes that catalyzed the degradation reaction of the studied phenolic compounds, leading to their transformation. Additionally, the tested strain can produce IAA, and therefore act as a compost-deodorizing agent and plant growth promoter [54].

Although biological deodorization of organic fertilizers using *Bacillus* spp. has been identified as an interesting alternative to physical and chemical methods, further research is needed, especially focusing on the effectiveness of this method in the natural environment and the most effective form in which microorganisms should be delivered into the environment [53].

Borowski and co-workers [55] investigated and described the effectiveness of three microorganisms-based products to reduce ammonia from poultry manure. Each preparation contained the same mixture of microorganisms *Pseudomonas fluorescens*, *Enterococcus faecium*, *B. subtilis*, *B. megaterium*, *Leuconostoc mesenteroides*, and *Lactobacillus plantarum*. The first preparation was a suspension of microorganisms deposited on perlite-bentonite medium (PBM). Their number in the final preparation varied between $9.6 \times 10^6-1.9 \times 10^8$ CFU g⁻¹, depending on the strain. The second preparation was made in the form of spray-dried microcapsules (SDM). In this case, a suitably prepared mixture consisting of maltodextrin N, trehalose and microorganisms was subjected to high temperature in spray dryer. The number of individual strains after application varied between $8.8 \times 10^8-5.0 \times 10^9$ CFU g⁻¹. The last type of preparation was a freeze-dried powder (FDP) containing the previously mentioned bacterial strains. The number of bacteria in the finished preparation, depending on the strain, ranged from $5.5 \times 10^6-8.1 \times 10^7$ CFU g⁻¹. The experiment was conducted in specially prepared laboratory installation in which fresh poultry manure was placed and treated with a selected preparation.

The odor concentration in exhaust air was measured by gas chromatography coupled with mass spectrometry and solid-phase microextraction. The main volatile compounds released from manure were nitrogen-and sulfur-containing compounds. The spray-dried microcapsule formulation proved to be the most effective formulation in deodorization of odors. The results indicated a reduction in the concentration of released ammonia by 93%, indole by 88%, and pyridine by 69%. Among sulfur-containing compounds, the concentration of hydrogen sulfide was decreased by 52%, and that of dimethyl trisulfide and dimethyl tetrasulfide by almost 99%, compared to the control. The freeze-dried powder reduced ammonia concentration by 52%, indole concentration by 60%, while pyridine concentration was reduced by less than 40%. The use of FDP reduced the number of sulfuric odorants by only 41%. For the PBM formulation, the value of reduced concentration of individual

nitrogen-containing odorous compounds did not exceed 30%. However, it showed the highest percentage of hydrogen sulfide reduction (74%) compared to the control [55].

5. Summary

Bacteria of the genus *Bacillus* are widely used in various industries due to their beneficial properties. Their colonizing ability, rapid multiplication rate, and endospore-forming potential have favored the production of commercial preparations containing these bacteria for biological plant protection. There is a great interest in investigating the possibility of improving crop and soil quality by making use of their activity. The use of *Bacillus* spp. in the process of deodorization of organic fertilizers, such as manure and slurry, has become a topic of interest, and research results suggest that these bacteria can efficiently remove odor and their application is environmentally friendly.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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