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# *Brevibacillus Spp.* in Agroecology: The Beneficial Impacts in Biocontrol of Plant Pathogens and Soil Bioremediation

**Ahmed IS Ahmed[1*], Amal M Omer[2], Amr IM Ibrahim[1] and Mohamed K Agha[1]**

[1]*Plant Protection Department, Desert Research Center, Cairo, Egypt*
[2]*Soil Fertilization and Microbiology Department, Desert Research Center, Cairo, Egypt*

[*]**Corresponding author:** Ahmed IS Ahmed, Plant Protection Department, Desert Research Center, Cairo, Egypt, Tel: +201003871788; Email: ahmed_drc@yahoo.com

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**Abstract**

The genus *Bacillus* and related genera are distributed vastly in nature and contain thermophilic, psychrophilic, alkalophilic, acidophilic, and halophilic bacteria that utilize a wide range of carbon sources for autotrophs or heterotrophic growth. A lot of bioactive metabolites have been detected, as bioactive compounds produced by biocontrol agents such as the bacterial genus of *Brevibacillus*. In this review, we summarize the general characteristics and properties of the *Brevibacillus* genus as biocontrol agents, taxonomy, phylogeny, identification and the impact of *Brevibacillus* as a biological control agent of plant disease combat and soil bioremediation.

## Keyword:

*Brevibacillus*; Bioremediation; Chemical pesticides; Morphology; Biocontrol agents; Phylogeny

## Introduction

The agricultural is one of the most significant sectors in related to productivity and economies around the globe in achieving food security and to benefit directly from the natural resources, forestry, and other benefits. In the same time, the agricultural sector suffers a lot of challenges and problems for many reasons, including climate change, water scarcity and excessive use of chemical pesticides, chemical fertilizers. Population growth also is the severe challenge where in the year 2050 it is estimated that the world's population will have reached 9.7 billion, and worldwide crop production will need to increase two-fold in order to cover the demand by this time [1,2].

A large number of phytopathogens are causal disease agents in all plant types. Their effects range from low to higher impacts in large areas planted to economical food crops. Phyto pathogens are challenges to control because the pathogenic populations are variable in time, space, and genotypic variations. At the scientific and political stage, there is a need to know that there is a link between plant diseases and food security [3,4].

The environmental stresses like soil salinity, drought, and lack or increase nutrients, had also been affected plants with herbicides are important challenges in plant production. An alternative to conventional methods for improving plant disease resistance is biological control methods [5]. Biological control of plant diseases can be defined as the use of organisms to influence the activities of a plant pathogen. Biocontrol organisms can be fungi, bacteria, and others.

*Bacillus* as a bacterial genus has a wide range of phenotypic strains of Gram-positive or Gram-variable, aerobic, endospore-forming, facultative anaerobic and rod-shaped [6,7]. *Bacillus brevis* was first characterized in 1900 [8] and was taxonomies as the type species *Brevibacillus brevis* of a new genus, *Brevibacillus*, with nine other species [9]. Recently there are more than twenty two species with validly published names under the genus *Brevibacillus* [9,10]. Determination the species is difficult because the poor reactivity of strains in conventional identification tests strains have useful differential phenotypic characteristics [10]. In *Brevibacillus*, the HV region sequence is highly conserved within a species but has diverged sufficiently between species to enable identification and grouping of *Brevibacillus* species by sequence comparisons of the HV region [10,11]. *Brevibacillus* is one of the most widespread genera of Gram-positive bacteria, recorded from the diverse environmental habitats [5,10,11]. The high growth rate, better transformation efficiency by electroporation, production of the negligible amount of extracellular protease, and the constitutive expression of heterologous proteins so some strains of *Brevibacillus* genus are excellent as laboratory models. For various applications, this genus continues to be a source of various enzymes of effective bio control interest due to their ability to suppress some different species of the phytopathogens, ability to act as bio control agent [12,13,14]. This review article summarized the properties of *Brevibacillus* spp. as biological agents in plant disease control and bioremediation.

## Morphology and Taxonomy of *Brevibacillus*

The genus *Brevibacillus* was originally proposed by Shida et al. [9] based on 16S rRNA gene sequence analyses of eleven species, it has belonged to the genus *Bacillus* and *B. brevis* was designated the type species [9]. This genus is Gram-positive or Gram-variable [12]. Ellipsoidal endospores are in swollen sporangia. Most species of this genus are aerobically on nutrient agar, yellowish grey, and smooth colonies. The DNA G+C content ranges from 40.2 to 57.4 mol% this description is according to Logan et al. [12]. Determine the phenotypic like acid production from carbohydrates) of members of the genus *Brevibacillus* is difficult [12]. Most species of *Brevibacillus* are difficult to distinguish from each other based solely on routine phenotypic tests.

The rod-shaped cells of *Brevibacillus* species are usually round-ended, and occur singly, in pairs, and in chains. Cell diameters range

from 0.7–1.0 μm and lengths from 3.0–6.0 μm, but the cells of a particular strain are quite regular in size, and individual species normally have dimensions within fairly narrow limits. Most of the species of *Brevibacillus* do not have distinctive sporangial morphologies; the spores are ellipsoidal, lie sub-terminally or perhaps terminally, and swell the sporangia slightly or moderately The notable exception is the unique sporangial morphology of *Brevibacillus laterosporus* which produces Parasporal Bodies (PBs) which laterally displace the spore in the sporangium, and which remain attached to the free spore. The ellipsoidal spores of this species may lie centrally, paracentrally, or subterminally, and they characteristically swell the sporangia into spindle shapes this detailed characterization is according to Logan et al. [12]. Characterization and features of *Brevibacillus* spp. were mentioned in some previous studies (*Brevibacillus agri* [15-17], *Brevibacillus aydinogluensis* [18], *Brevibacillus borstelensis* [9,17], *Brevibacillus brevis* [8,9], *Brevibacillus centrosporus* [16,17], *Brevibacillus choshinensis* [9,19], *Brevibacillus fluminis* [20], *Brevibacillus formosus* [9,17], *Brevibacillus fulvus* [19], *Brevibacillus gelatini* [18], *Brevibacillus ginsengisoli* [19], *Brevibacillus halotolerans* [20], *Brevibacillus invocatus* [15], *Brevibacillus laterosporus* [9,21], *Brevibacillus levickii* [11], *Brevibacillus limnophilus* [22], *Brevibacillus nitrificans* [23], *Brevibacillus panacihumi* [24], *Brevibacillus parabrevis* [9,25], *Brevibacillus reuszeri* [9,17], *Brevibacillus sediminis* [26]).

## Isolation and Identification of *Brevibacillus* Strains

*Brevibacillus* strains were isolated from the natural environment such as soils, where they appear as saprophytes, while some isolates also were isolated from human illness. *Brevibacillus* species also isolated from heat treatment of specimens. The *Brevibacillus* spores in different environments but does not necessarily indicate that the organisms were able to metabolites production, but some isolates from different habitats produce the metabolites to make the *Brevibacillus* cells active.

In order to identify the unknown bacteria, examination of seven characteristics are necessary, e.g. (1. cell morphology, 2. colony morphology, 3. oxygen requirements for growth, 4. Gram stain reaction, 5. presence of endospores in a culture, 6. carbon source utilization, and 7. Motility), finally comparison according to Bergy's manual. According to [27] gramicidin A and gramicidin S, the first linear and cyclic peptide antibiotics used clinically. After reclassifying *B. brevis* classified into *Brevibacillus*, the genus remained a reliable source for novel Antimicrobial Peptides (AMPs). AMPs produced by *Brevibacillus* spp. and the *B. brevis* were classified based on biosynthesis pathway and structural traits, where two groups of AMPs are recognized: 1 AMPs that target cell-surface components, i.e., cell wall, membrane and membrane-bound protein, and 2 AMPs that target intracellular components such as ribosomes, and the synthesis machinery for DNA and RNA [27]. The previous research reports divided all AMPs into four groups: sidechain-linked, linear polypeptide, sidechain-backbone linked, and backbone-backbone linked circular group. It has been served as a biological controlling agent with various biological activities [27].

## Characterization and General Features of some *Brevibacillus* Species

*Brevibacillus thermoruber*: Optimal growth at 45–48°C, high G/C content (57 ± 0.8 mol %) and endocellular, nondiffusible pigment [9].

*Brevibacillus agri*: Non-pigmented Colonies, Catalase positive. Strictly aerobic. Growth at pH 5.6–5.7. The growth temperature 28°C. The DNA buoyant density 1.7055–1.7084 g/cm$^3$; the G/C content 52–55 mol% [16,17].

*Brevibacillus borstelensis*: Produces soluble brown-red pigment on nutrient agar. Strictly aerobic. Catalase positive and oxidase negative. Growth occurs at pH 5.5–5.6. Specific S-layer proteins present. High G/C content [9,17].

*Brevibacillus centrosporus*: Non-pigmented colonies. Catalase positive. Strictly aerobic.The optimum growth temperature 28°C. The DNA buoyant density 1.7025–1.7045 g/cm3; G/C content 49–51 mol% [16,17].

*Brevibacillus choshinensis*: Utilization the citrate, ammonium and acid production. High G/C content [9,19].

*Brevibacillus formosus*: Smooth, flat, circular, and entire colonies. Strictly aerobic. Catalase positive and oxidase negative. Growth occurs at pH 5.5–5.6. Specific S-layer proteins present. High G/C content [9,17].

*Brevibacillus brevis*: Strictly aerobic, produces gramicidin, 45–56 mol% as range of G/C values [8,9].

*Brevibacillus parabrevis*: Positive for reduction of nitrate to nitrite, utilization of ammonium, hydrolysis of casein, gelatin, DNA, and Tween 60. From 51.3 to 53.3 mol as % G/C content ranges [9,25].

*Brevibacillus reuszeri*: Strictly aerobic. Catalase positive and oxidase negative. Growth occurs at pH 5.5–5.6. Specific S-layer proteins present. 46.4 to 46.7 mol% as G/C content range [9,17].

*Brevibacillus invocatus*: Brownish-yellow colonies, positive in catalase. from 15 to 35C as growth temperature, pH 6–8. Range of G/C content is 49.1–49.8 mol% [15].

*Brevibacillus limnophilus*: Temperature for growth ranged from 20 to 45°C; optimum growth temperature 30–35°C. Optimum pH 7.0–7.5. Main quinone MK-7.G/C content 51.9 mol% [22].

*Brevibacillus levickii*: Utilizes K antiport system and similar energy systems for the uptake of L-glutamic acid [11].

*Brevibacillus ginsengisoli*: At 20–42 ℃ Growth temprature, pH 5.0–8.5, iso-C15:0, iso-C14:0, and anteiso-C15:0 are the major cellular fatty acids.G/C content 52.1 mol% [19].

*Brevibacillus panacihumi*: Aerobic, grow at 15–42°C (optimum 30°C) and at pH 5–9 while (optimum, pH 7). The major cellular fatty acids iso-C15:0, anteiso-C15:0, iso-C14:0, and iso-C16:0. G/C content 50.1–50.5 mol% [24].

*Brevibacillus fluminis*: White pigment, strictly aerobic and motile. The G/C content 52.4 mol% [20].

*Brevibacillus aydinogluensis*: Moderately thermophilic. The major fatty acids iso-C15: 0 (39.30%), anteiso-C15: 0 (26.10%), and iso-C16: 0 (14.75%).The major isoprenoid quinone MK-7. G/C content 56.09 mol% [18].

*Brevibacillus nitrificans*: Grows at pH 5–8 growth temprature, with optimum growth at pH 7. The major isoprenoid quinone MK-7. G/C content 54.1 mol% [23].

*Brevibacillus laterosporus*: Rapidly lose their characteristic appearance and assume a fusiform or spindle shape with a swollen

middle and pointed ends. Flat, transparent, and irregular colonies [9,21].

## Biocontrol and Bioremediation

*Brevibacillus brevis* produced useful peptide antibiotic gramicidin S, which attacks the lipid bilayer of the membranes of organisms [28]. *Brevibacillus* strains with antifungal properties are potentially valuable biocontrol agents. These include a *Bacillus brevis* strain active against fusarium wilt of pigeon pea [29] and a *Brevibacillus laterosporus* effective against wheat foliar necrotrophic [30]. Edwards and Seddon [13] found that gramicidin S produced by *B. brevis*, is sporicidal to conidia of *B. cinerea*, and is less inhibitory towards growth of mycelium. Omar and Ahmed [27] studied inhibitory and antagonistic impact of some rhizobacteria against various isolates of *Fusarium* on Sage plants, they concluded that, Rhizo-bacterial strains namely *Brevibacillus brevis Brevibacillus agri*, and *Brevibacillus formosus* which have high effects in suppression of Salvia officinalis wilt and root rot diseases effectiveness probably due to the production of several inhibitory metabolites like HCN, chitinase, and siderophore. Ahmed [28] studied the effect of *Brevibacillus formosus* strain DSM 9885 and *Brevibacillus brevis* strain NBRC 15304 as a biological control agent on potato brown leaf spot disease caused by *Alternaria alternata*, the study demonstrated that the use of the tested *Brevibacillus* strains could enhance resistance to brown leaf spot in potato. The linear mycelial growth and spore germination of *A. alternata* was inhibited by both of tested bacterial isolates with reduction of disease symptoms, where the effects were determined *in vitro* through detached leaves and under greenhouse conditions. Protein profiling by SDS-PAGE revealed that some bands of protein are produced due to the *Brevibacillus* as biocontrol agents. So Ahmed [28] concluded that *B. formosus* strain DSM 9885, and *B. brevisstrain* NBRC 15304 could be considered as part of management tools for reducing the impact of *A. alternata* causing brown leaf spot disease on potato [31,32].

*Brevibacillus* is playing an important role as bio-remediation factor to combat the contamination resulted by toxic metals and reduce the environmental pollution in agricultural soils, water, and the atmosphere. Toxic metals can also have massive detrimental effects on soil ecosystems and the environment [33]. Soil contamination by metal is seriously problematic because of the strong adsorption of many metals to the surfaces of soil particles. From a physiological point of view, metals fall into three main categories: 1) basically non-toxic e.g. Ca and Mg, 2) essential but harmful at high concentrations, typically Fe, Mn, Zn, Cu, Co, Ni and Mo, and 3) toxic e.g. Hg, Pb or Cd [34]. Soil remediation contaminated with heavy metals also problem because metals are difficult to degrade. Bioremediation, i.e. the use of living organisms to manage or remediate heavy metal-polluted soils, is an emerging technology. It is defined as the elimination, reduction or transformation of polluting or contaminating substances through the use of biological tools, and it is a genuine option for removing industrial pollution from the environment [35]. Soil microorganisms play important roles in bioremediation or biotransformation processes [36]. Microorganisms can interact with metal contaminants and transform these metals from one chemical form to another by changing their chemical and physical states through addition or removal of oxidation electrons [37-39], or by stimulating changes in the microbial population balance, for instance by using it as a biological control tools against plant pathogens [40]. Some *Brevibacillus spp.* were able s synthesis 3.8 mg L-1 indole-3-acetic acid (IAA) *in vitro* and this might have contributed to the beneficial effects noticed, since the production of IAA or ethylene has been suggested as a mechanism for plant growth promotion under heavy metal stress. Mullen et al. [38] mentioned that soil bacteria associated with the clay and organic fractions of the soil microenvironment and would be expected to engage in the metal dynamics typically attributed to these soil fractions as well as a strictly physical cellular interface should have a great ability for sorbing metals from solutions. Another mechanism by which *Brevibacillus* sp. could have contributed to the protection of plants against Pb toxicity is by induction of root exudates which have a variety of roles. Some previous research reports mentioned to the valuable role of bacteria or mycorrhizal-colonization in plants growing in metal-contaminated sites [41-44]. *B. brevis* behaved as a PGPR, but its notable effect on root biomass was greater than that shown on the shoot. *B. brevis* also played an effective role as a Mycorrhizae-Helper (MH) bacterium [45-47].

Mycorrhizal root development, when related to *B. brevis*, was less negatively affected by Ni stress conditions. These findings could be relevant for improving growth of plant and nutrient. Different strategies would be involved in prohibiting plant toxicity disadvantage. Changes in uptake of metal and internal transportation storage could give tolerance of metal to the host plant [48]. Applying the microbial mixture (AM fungus and/or *B. brevis*) probably induce tolerance to Ni by affecting metal availability and uptake. Changes in root exudates, pH and physicochemical characteristics of the soil [49] may be involved and such changes could decrease metal root uptake or translocation from root to shoot tissue. The benefits that legume plants obtain from the AM symbiosis under Ni stress are more relevant in co-inoculations with *B. brevis*. The *B. brevis* strain studied has a cellular mechanism as bio-sorption and bioaccumulation that involved in the detoxification of Zn in the medium of growth. Metabolic abilities may be related to the Zn tolerance and also to the Zn reduction in the medium Burd et al. [47] suggested that this bacteria used to plant protection against the inhibitory effects of high concentrations of Zn is related to their ability to encourage plant growth by synthesis of Indol, as has been shown for many PGPRs [50-52], so, PGP bacteria can facilitate plant growth by modifying the plant hormonal balance. Another study aimed to investigate the effect of *B. brevis* on the axenic development of *G. mosseae* in related to the rate of spore germination and hyphal development under different levels of Zn added to a water agar medium. Vivas et al. [50] reported that *G. mosseae* spores provided a 56% increase in growth of mycelium without Zn, and more than 130% increase with 200 mg Zn/mL, when treated with the bacterium inoculum, in comparison with uninoculated spores. Moreover, in a subsequent study, the same bacterium not only stimulated presymbiotic mycorrhizal fungal development but also the quality and quantity as metabolic characteristics of mycorrhizal colonization, with the improvement being for vitality of arbuscular and its activity.

Ruíz-Lozano et al. [51] reported that the isolation of efficient metal-adapted microorganisms could be a usefully bio-technological tool for inoculation purposes in contaminated soils, they concluded that this sustainable system can be an important strategy in order to promote bio-remediation of heavy metal-polluted soils. A strain identified as *Bacillusbrevis* was isolated from soil contaminated with hexachlorocyclohexane where it degraded this polluting pesticide, another strain of this species was found to degrade the insecticide teflubenzuron reported that *B. borstelensis* gain a great ability to degrade the fungicide carbendazim from agricultural fields at high rates, especially when mixed with *Streptomyces albogriseolus* found that *Brevibacillus agri* strain CAT37 was able to degrade the thiols,

decanethiol and dodecanethiol, and their corresponding autoxidation products, effectively after 30 days of incubation at 37 ℃ [52,53].

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