Biodiversity of *Bacillus subtilis* group
and beneficial traits of *Bacillus* species useful
in plant protection

Received for publication, June 10, 2015
Accepted, September 10, 2015

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Abstract

Biological control of plant pathogens and plant growth promotion by biological means is the late
tendency in biotechnological approaches for agricultural improvement. Such an approach is
considering the environmental protection issues without neglecting crop needs. In the present study, we
are reviewing *Bacillus* spp. biocontrol and plant growth promoting activity. As *Bacillus* genus is a
large bacterial taxon, with grate physiological biodiversity, we are describing some inter-grouping,
differences and similarities between *Bacillus* species, especially related to *Bacillus subtilis*.

Keywords: *Bacillus subtilis* group, beneficial bacteria

1. Introduction

*Bacillus* genus is a heterogeneous taxon, with ubiquitous spread in nature. *Bacillus* species, such as *Bacillus cereus*, *B. megaterium*, *B. subtilis*, *B. circulans* and *B. brevis* group
are widely exploited for biotechnological and industrial applications [1, 2]. Their beneficial
traits for plant protection and growth promotion comprise the synthesis of broad-spectrum
active metabolites, easily adaptation in various environmental conditions, benefic plant-
bacterial interaction and advantageous formulation process [3].

As plants roots exudates and lysates attracts and stimulate microbial activity in the root-
surrounding soil, the rhizosphere became a highly populated area [4]. Root-microbial interaction
involves many forms of coexistence, such as commensalisms, where microorganisms live on
naturally occurring compounds released from the plant; endophytism, where microorganisms
grow inside plant tissue without adversely affect the host; symbiosis, where plant and microbial
organism are creating favorable conditions for mutual conviviality; parasitism, by which the host
plant is suffering due to microbial attack; and saprophytism, by which the organisms can nourish
with the decaying organic matter [5]. Beneficial *Bacillus* spp. strains can compete other microbes
that could adversely affect crops; they can inhibit phytopathogenic attacks, or induce host-plant
defense system against potential pathogenic attacks, stimulate plant growth, improve nutrient
uptake and reduce some negative environmental traits [3].
2. *Bacillus* genus

The *Bacillus* genus Cohn (1872) includes Gram positive, rod shaped bacterial cells of 0.3-2.2 to 1.2-7.0 µm, most of them motile, with peritrich flagella. These bacteria are chemoheterotrophs that can use various nutritional substrates. They can exhibit either fermentative or both respiratory and fermentative pathway to produce energy. As respiration, *Bacillus* species can be obligate aerobe or facultative anaerobe. The terminal electron acceptor is the molecular oxygen (O₂), replaced at some species by nitrate (NO₃) in special conditions [6]. The morphology and colony size are highly variable characteristics, depending on the environmental conditions. In the presence of specific nutrients, some species can produce pigments. Most species are widespread in nature. Many of the *Bacillus* spp. form resistant endospores, with no more than one endospore in the sporangia cell. The endospores can be distinguished from vegetative cells, as they are refractile and less colored, containing dipicolinic acid, 5 to 15% in dry weight. These endospores are dormant structures, non-reproductive, they can survive without nutrients and resist to extreme physical and chemical agents [7].

Regarding the nomenclature, there is no official classification of prokaryotes [8], however there are such systems used at this time. The widely accepted classification of "Taxonomic Outline of the Prokaryotes" is found in *Bergey's Manual of Systematic Bacteriology*. Lately it was published the "Taxonomic Outline of the Bacteria and Archaea" [9]. According to the List of Prokaryotic names with Standing in Nomenclature (LPSN) in 2014 there were 301 species mentioned to be included in *Bacillus* genus, three of them having subspecies [http://www.bacterio.net/bacillus.html]. Registered bacterial species, not only that they are found in international microbial collections, but they were previously analyzed through molecular and biochemical analysis in order to be validate as novel species. *Bacillus* DNA composition is 32-62% G+C mole [10]. Molecular diversity within populations in order to determine genetic variation between *Bacillus* species, with their similarities and diversity at genetic level are the most accurate when using the molecular techniques. However, gene expression, biochemical and morphological aspects must also be considered.

3. *Bacillus subtilis* and related species

The phylogenetic studies, based on 16S rRNA sequence, suggest five groups of closely related species, *Bacillus cereus*, *B. megaterium*, *B. subtilis*, *B. circulans* and *B. brevis* group. There is also mentioned a group of closely related bacteria, referred to as *Bacillus pumilus* subgroup, as it is included in *Bacillus subtilis* group [11].

*Bacillus subtilis* is a well studied bacterial species, ubiquitous in nature with valuable treats useful for biotechnological, industrial and agricultural applications [2]. This specie was first described by Christian Gottfried Ehrenberg in 1835, who entitled it *Vibrio subtilhe* [12]. However, the *Bacillus* genus was established by Ferdinand Cohn in 1872, and *Bacillus subtilis* was defined as the type species by Soule in 1932 [13]. This has three subspecies, *Bacillus subtilis* subsp. *subtilis*, subsp. *spizizenii* and subsp. *inaquosorum* [2]. Along with *B. subtilis* there were described other closely related species with high genetic and/or biochemical similarities. The species that we referred to are *Bacillus amyloliquefaciens*, *B. atrophaeus*, *B. axarquiensis*, *B. licheniformis*, *B. malacitensis*, *B. mojavensis*, *B. pumilus*, *B. sonorenensis*, *B. tequienensis*, *B. vallismortis* and *B. velezensis*. Such species were first called "Bacillus subtilis spectrum" [14], much later they were grouped into "Bacillus subtilis species complex" [2], and now are clustered in "Bacillus subtilis group" [15].

Molecular analyzes are providing the most accurate information regarding phylogeny and evolution. In bacteria, the 16S rRNA molecules are highly conserved, and therefore
suitable for bacterial identification and classification. The comparative analysis of these sequences can establish phylogenetic distance between the microorganisms studied and the degree of relatedness between strains and species. The degree of similarity between Bacillus subtilis and its closely related species is ≥ 99% for 16S rRNA sequence level. With DNA-DNA hybridization, the complementarity’s percentage at genome level is up to 70%, between the species from Bacillus subtilis group [16]. This explains the genetic variability when analyzing the entire genome compared to highly conserved sequence.

The phenotypic and biochemical differentiation between closely related species is difficult to achieve [2]. For example, B. amyloliquefaciens could be distinguished from B. subtilis by the fact that it produces acid from inulin, cells tend to be enchained and the endospores are located in the center of the cell [17].

4. Molecular and microbiological differences between species of Bacillus subtilis group

Bacillus amyloliquefaciens was first described by Fukomoto in 1943, but has been validated and registered in the "Lists of Bacterial Names" only in 1980. Due to the similarities with Bacillus subtilis, B. amyloliquefaciens has received a subspecies status, being classified as "B. subtilis subsp. amyloliquefaciens", as a variety that produces large quantities of extracellular enzymes [14]. Moreover, this specie is the highest producing α-amylase and protease, between bacterial species. Nowadays, B. amyloliquefaciens is self-standing specie with two approved varieties, subsp. amyloliquefaciens and subsp. plantarum [18].

The molecular analysis of Bacillaceae species, using 16S rRNA and the 16S–23S ITS (internally transcribed spacer), to differentiate, in clusters, this bacterial family, based on their phylogeny, included B. atrophaeus in the Bacillus group VI, as related with Bacillus amyloliquefaciens, Bacillus mojavensis and Bacillus subtilis [1]. Regarding the genetic similarity at genome level, Bacillus atrophaeus and B. subtilis type species show only 32% resemblance when analyzing by DNA-DNA hybridization [19]. According to the literature, Bacillus atrophaeus was also referred as “B. subtilis var. subtilis”, “B. globigii”, “B. subtilis var. niger -red strain”, “B. niger” or “B. atrophaeus subsp. globigii [20, 21, 13]. It should be noted that the reference strains DSM 675 and DSM 2277, previously identified as B. subtilis were reclassified as B. atrophaeus [19]. Phenotypic, Bacillus atrophaeus can be distinguished from the type culture of Bacillus subtilis only in certain nutrient substrates, containing tyrosine, on which B. atrophaeus produces a dark brown pigment [20]. The pigment production is also used to differentiate this specie from Bacillus mojavensis and B. vallismortis [21].

Bacillus axarquensis and B. malacitensis were proposed as new species by Ruiz-Garcia et al. (2005). However, Wang et al. (2008) [22] revealed these two species to be heterotypic synonyms of Bacillus mojavensis. The similarity at genome level, between the B. mojavensis and some of the species from B. subtilis group is 12 to 40% by DNA-DNA hybridization with B. amyloliquefaciens, B. atrophaeus, B. licheniformis and B. subtilis. Regarding phenotypic differentiation between of B. mojavensis and B. subtilis was possible only by analyzing the composition of fatty acids [22].

Bacillus licheniformis was shown to be closely related to B. subtilis and B. amyloliquefaciens after rigorous taxonomic studies using comparative sequencing of 16S rRNA and 16S-23S internal transcribed spacer [1]. It is estimated that 80% of the B.licheniformis genome sequence contains orthologous genes of B. subtilis. However, this two species differ in the amount and position of prophage and insertion sequence elements, operons of the secondary metabolic pathway, antibiotic synthases, extracellular enzymes, and metabolic activities that are
not present in *B. subtilis* [23].

For the differentiation of *Bacillus pumilus* from the other species belonging to "*Bacillus subtilis* group", the ARDRA method, using 16S rRNA gene amplification and digestion with the restriction enzymes *Rsa* I, *Cfo* I and *Hinf* I, could be successfully used. An exception is for *B. pumilus* and *B. amyloliquefaciens* separation. In this case, ITS-RFLP analysis method could be used, and the PCR products from ITS amplification of 16S-23S rRNA enzymatic digested with *Cfo* I manage a good differentiation between these two species [15].

*B. safensis*, *B. stratosphericus*, *B. altitudinis* and *B. aerophilus*, are having a high similarity, of 99.5%, at the 16S rRNA sequence with *Bacillus pumilus* type species [24]. Because of this, these species were designated to constitute the complex of related species called "*B. pumilus* group". However, given that *B. pumilus* is included in "*B. subtilis* group", the highly related species with *B. pumilus* represent a subgroup. The latest studies, however, indicate that in this subgroup should be included seven species *B. pumilus*, *B. safensis*, *B. stratosphericus*, *B. altitudinis*, *B. aerophilus*, *B. xiamenensis* and *B. invictae* [25], all of them with high similarity at molecular level. The diversity and evolution of the species belonging to this group/subgroup was analyzed considering the highly conserved 16S rRNA gene and the multilocus analysis (MLSA) of seven housekeeping genes, *gyr B*, *rpo B*, *aro E*, *mut L*, *pyc A*, *pyr E* and *trp B*, involved in providing the essential life functions of the cells [24].

*Bacillus sonorensis* is mentioned to be phenotypically and genotypically similar to *B. licheniformis* [26]. However, they are ecologically distinct species. This two closely related species can be distinguished based on a few phenotypic traits, such as colony pigmentation on tyrosine agar medium, growth characteristics on glicerol/glutamat-agar, or at pH of 5-6, or in various salt concentrations (5%, 7% and 10% NaCl) and sensitivity to different level of clindamycin [27]. Some differences can be also observed at genetic level by multilocus enzyme electrophoresis (MLEE), genomic DNA reassociation and sequencing of 16S rRNA or *secY* and *rpoB* protein-coding genes [27].

*Bacillus tequilensis* was first mentioned by Gatson et al. in 2006. Initially, it was identified as *B. subtilis* based on conventional biochemical techniques. Further analysis revealed 99% similarity with *B. subtilis* in the 16S rRNA sequence, but significantly different from *B. subtilis* and related species when using pulsed-field gel electrophoresis (PFGE). DNA-DNA hybridization studies showed less than 70% homology between *B. tequilensis* and other species of *B. subtilis* group [28].

Roberts et al. (1996) isolated and identified the first strains of *Bacillus vallismortis*. This specie is referred to be similar with *B. subtilis*, but differences can be seen at *gyrA*, *polC* and *rpoB* genes digestion with restriction enzymes [29].

*Bacillus velezensis*, as it was first mentioned by Ruiz-Garcia et al. (2005) was later found to be a heterotypic synonym of the *Bacillus amyloliquefaciens* [22].

Among the molecular methods reliable and commonly used for the differentiation of *Bacillus* spp. and strains are the Amplified Ribosomal DNA Restriction Analysis (ARDRA) method; Internal Transcribed Spacer (ITS) – PCR; the Restriction Fragment Length Polymorphism (RFLP), the Random Amplified Polymorphic DNA; Repetitive Sequence-Based PCR (rep - PCR) including Repetitive Extragenic Palindromic PCR (REP-PCR), Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR), box elements (BOX-PCR) or other repetitive elements (figure 1); the comparison of *gyrB* gene and the Multiplex PCR method using specie specific primers.
5. Beneficial traits with agricultural purposes in *Bacillus subtilis* and related species

The species of *Bacillus subtilis* group, particularly *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens* and *B. pumilus* are intensively studied for their importance in biotechnological industry and agriculture, as phytopathogenic antagonists or plant growth promoters. The terms of "Plant Growth Promoting rhizobacteria" – PGPR, were introduced by Kloepper and Schroth in 1978. They described the PGPR as naturally occurring soil bacteria that have the ability to colonize the roots and stimulate plant growth. Various species of *Bacillus* were than reported as PGPR [30].

The PGPR improve plant physiological state by phytohormones production or by releasing beneficial organic compounds [30]. Polyamines, for example, have important physiological role, being involved in cell division and differentiation, protein synthesis, and membrane stability, moreover they have protective role against various abiotic stresses [31]. The PGPR can also improve the mineral uptake of low-soluble or difficultly to be assimilated minerals such as phosphorus, zinc or silica [32, 33, 34]. Beneficial soil bacteria also have important functions in rhizoremediation and alleviating negative effects of soil stresses on plants [35].

Beside plant growth stimulation, *Bacillus subtilis* and related species are involved in plant protection against phytopathogenic attacks. The mechanisms of action are various. They could act directly against pathogens by producing extracellular lytic enzymes and secondary metabolites with inhibitory growth action [36] or interfere by quorum quenching to disturb cell-to-cell communication of the infectious expression in pathogenic bacteria [37]. They could also compete plant pathogens for the available nutrients and niche [3]. Another important role is the reduction of the infection process by inducing defense responses in the host plant [38].

5.1. Plant growth promotion induced by *Bacillus* spp. strains

Phytohormone production by beneficial *Bacillus* spp. strain involves auxins, cytokinins and gibberellins synthesis. Plant growth regulators with inhibitory action, such as ethylene and abscisic acid in some concentration, could be influenced by *Bacillus* activity [35].

The most studied auxin in *Bacillus* spp. is indole-3-acetic acid (IAA). Its synthesis was detected in large spectrum of *Bacillus* spp. strains. The IAA production is being increased in the presence of its precursors and specific pH conditions [33]. The most important IAA synthesis pathway in *Bacillus* spp. is tryptophan dependent, and involves indole-3-pyruvic acid (IPA). However, IAA could also be derived through Trp-independent pathways, from other aromatic amino acids, such as praline, cysteine, phenylalanine, alanine and methionine [33].

Gibberellins production was confirmed in *Bacillus pumilus* and *B. licheniformis*, The quantitative analysis of gibberelic acid (GA), by gas chromatography coupled with mass spectrometry.
Bacterial cytokinins synthesis has been demonstrated in *Bacillus subtilis* and *B. licheniformis*. In *Bacillus subtilis* culture was quantified 0.8-1.2 μg zeatin riboside/ml [40]. Cytokinin production was also quantified in *Bacillus licheniformis* Am2 culture, were 521 ng zeatin riboside/ml and 1091.9 ng trans zeatin/ml were registered after four to five days of growth in M9 minimal medium supplemented with 0.2% casamino acids, 0.01% thiamine, and 0.2% biotin [41].

Beside plant hormones with stimulatory action, there are also other plant growth regulators with inhibitory activity such as ethylene in high levels. Increased ethylene acts as a stress hormone, and negatively affect plant growth and yield. Improper growth conditions created by soil salinity, drought, water logging, heavy metals or pathogens increase the amount of 1-aminocyclopropane-1-carboxylate (ACC), a precursor for ethylene production. PGPR, including *Bacillus* spp. strains, express ACC-deaminase, decreasing plant ethylene levels expressed in deficient growth conditions improving plant growth and development [35].

Regarding ABA regulation in plants, it was shown that *Bacillus subtilis* GB03 inoculation influence plant sugar sensing and photosynthesis. Volatile organic compounds (VOCs) produced by *B. subtilis* GB03 decrease ABA level in shoots of *Arabidopsis* bacterial treated plants. As hexokinase-dependent sugar signaling is inhibit in reduced ABA levels, and ABA reduction mediated by *B. subtilis* GB03 repress plant sugar sensing. This way, *B. subtilis* GB03 increase seed germination and repress hypocotyl elongation inhibited by glucose signaling. Moreover, it was showed that exogenous applied ABA reduces the chlorophyll content, photosynthetic efficiency and growth of *Arabidopsis* plants, and negatively affects *B. subtilis* GB03 activity on plants. In these respect, reduced ABA levels are necessary for GB03 to be able to express its PGPR potential and enhance photosynthetic activity and chlorophyll content [42].

Nutrients uptake improvement of low-soluble minerals enhances plant growth. Phosphorus is one of those minerals; its concentration in soil solution is very low as soluble phosphate. However, its role is particularly important for plant growth and development. Adequate amounts of phosphorus uptake in plants leads to a good development of the root system, an increased productivity, a stronger natural defense mechanism towards the phytopathogenic agents, an improved yield quality and sometime an early crop maturation [43].

In soil, phosphorus can be found as mineral phosphates and phytates. Bio-fertilization with PGPR strains, having extracellular phytase activity, leads to plant growth promotion, even under limited phosphate conditions [44]. Phytase activity and genes encoding for such enzymes have been determined in several *Bacillus* spp. isolated from rhizosphere [36]. Beside their ability to improve phytate hydrolysis and phosphorus assimilation, the uptake of other nutritionally important minerals (Zn\(^{2+}\), Fe\(^{2+}\) and Ca\(^{2+}\)) is enhanced, by limiting chelate-forming phytate [45].

Zinc solubilizing rhizobacteria improve plant growth and yield, and increase Zn concentration of wheat grains. Zn biofortification with PGPR can overcome Zn deficiency in an economical and organic way [32]. Zn mobilizing ability has been reported in different bacterial taxa, including in *Bacillus* genus (*B. thuringiensis, B. megaterium*) [32, 46].

Some beneficial bacteria could produce organic compounds, such as polyamines, with positive influence on plant growth promotion, senescence delay and protective role against abiotic stresses [47, 48, 49]. The polyamines, putrescine, spermine, spermidine and cadaverine, are organic compounds that contain several amino acids. They are largely spread and could be found in bacteria and along with other microorganisms, plants, higher animals...
and mammals. Beside their key role in the genetic material stability, they also have regulatory functions [48]. Regarding polyamines role in bacteria, Wortham et al. (2007) speak about the resistance to acidic environments and protection against reactive oxygen species toxicity conferred by these compounds [49]. In *Bacillus subtilis*, polyamines serve as a signal molecule for bacterial swarming motility and biofilm formation [50]. In plants, the polyamines and their biosynthesis enzymes are involved in many metabolic processes, such as cell division, organogenesis, embryogenesis, floral induction and development, leaves aging, fruits growth and ripening, or in response to certain biotic and abiotic stress factors [48, 50].

### 5.2. Plant protection activity triggered by *Bacillus* spp. strains

*Bacillus* spp. strains could act as biological control agents (BCAs) to provide plant protection against microbial and insect pathogens. BCAs are a promising alternative to chemical pesticides. Therefore, many studies are focused on their interaction with plants, pests, pathogenic and beneficial microorganisms, and understanding their impact to the environment, and implication on animals and humans. Important traits, such as efficacy, formulation, stability and viability were also intensively studied. All these in order to select most efficient and suitable BCAs for plant protection and, in the same time, to promote only the strains neutral to non-target organisms.

The various mechanisms involved in plant protection refer to pathogen inhibitors and extracellular lytic enzymes production, antagonism (figure 2), nutrients and niche competition, quorum quenching and induced defense responses in host plants.

#### 5.2.1. Cellulolytic enzymes

Cellulolytic enzymes synthesized by the BCAs can be involved in two plant defense mechanisms against phytopathogenic fungi. One of these mechanisms triggers elicited biotic defense in plants, preventing them from phytopathogenic attacks. The second course action involves the lytic action of cellulases. Cellulolytic enzymes hydrolyze β-1,4 glycoside bonds, and break down the cell wall cellulose from *Pythium* and *Phytophthora* pathogens. Cellulase activity (figure 3) was found in *B.subtilis* [51], *B.amyloliquefaciens* [52], *B.licheniformis* [53] or *B.pumilus* [54].

![Figure 2. Antifungal activity expressed by different *Bacillus* spp. strains. A – *Bacillus* spp. antagonistic activity against *Fusarium solani*. B – Fungal cell wall degradation, cell lysis and cytoplasm bleeding due to *Bacillus* spp. extracellular enzymes.](image)

![Figure 3. Cellulase activity exposed on Luria Bertani medium supplemented with carboxy-methyl cellulose (CMC), reveal a clear halo of CMC degradation, after two days of *Bacillus* spp. strains incubation and Congo red stain.](image)
5.2.2. Chitinase activity

Chitin is a difficult biodegradable matter. For its hydrolysis, β-1,4 N-glycosidic linkages are cutted by the chitinolytic enzymes [55]. Chitinases are synthesized by numerous species of the Bacillus genus, including B.subtilis, B.amyloliquefaciens, B.licheniformis [55], or B.thuringiensis [56]. Among the potential applications of chitinases are bioremediation and bioconversion of chitin food waste, namely N-acetylglucosamines (NAG) and chito-oligosaccharides [55]. However, they can also be used for their anti-fungal properties and insect biological control [55, 56].

5.2.3. Antibiotic compounds

The most effective mechanism in biological control of plant pathogens is the antibiotic synthesis from beneficial microorganisms. In Bacillus spp., such compounds are released during sporulation, and in the stationary growth stage. Among the antibiotic compounds synthesized by the Bacillus bacteria, kanosamine, zwitermicin A, iturins, bacitracin, gramicidin, fengycin or plipastatin, kurstakin and surfactins are mentioned [3].

Kanosamine (3-amino-3-deoxy-D-glucose) has a strong inhibitory activity against Oomycetes fungal pathogens and moderate activity against various other fungi from Ascomycetes (Aspergillus flavus, Botrytis cinerea, Sclerotinia spp., Venturia spp.), Basidiomycetes (Rhizoctonia solani, Ustilago maydis) and Deuteromycetes fam. (Alternaria spp., Colletotrichum spp., Helminthosporium spp., Fusarium spp., Phomopsis obscurans, Verticillium spp) and some bacteria [57]. Kanosamine synthesis was evidenced in several species of Bacillus spp., such as B.pumilus and B.subtilis [58], or B.cereus [57].

Zwitermicina A is a linear aminopolyol with a broad spectrum of inhibitory activity against certain gram-positive and gram-negative bacteria, or eukaryotic organisms, including Oomycetes [58]. It also enhances the insecticidal activity of the toxin protein from B.thuringiensis. However, zwitermicina A synthesis has not been evidenced in Bacillus subtilis and related species.

Iturins is a large family of antibiotic compounds, which includes bacillomycin D and F, bacillopeptin (or bacillomycin L), ituin A and C, and mycosubtilin [59]. Iturins have strong antibiotic effect and moderate activity as surfactants, and they are mentioned to enhance swarming motility [59, 60]. Iturin synthesis have been shown in various B.amyloliquefaciens, B. licheniformis, B. pumilus and B. subtilis strains [60].

Bacitracin is an antibiotic compound with bactericidal activity, synthesized by some strains of B.licheniformis and B. subtilis [61, 62].

Fengicin A and B, also called plipastatinare lipopeptide antibiotics. These compounds were found to be useful in the biological control of mosquito larvae [63] and plant pathogens degrading their cell structure and permeability [64, 65]. They are also mentioned to induce systemic resistance (ISR) in plant [66]. Used as biosurfactants, they can inhibit biofilm formation on some bacteria [67], and degrade polycyclic aromatic hydrocarbons (PAHs) [68]. These metabolites are produced by various species of the Bacillus genus, such as B.subtilis [60], B.amyloliquefaciens [69], or B.licheniformis [70].

Kurstakins are non-ribosomal lipopeptides produced by Bacillus thuringiensis. Further investigation revealed kurstakin production in other more species, but only from "Bacillus cereus group" except for B.anthracs and B.cytotoxicus. For these reason kurstakin is considered a biomarker for this group of species [71].

Surfactins include bamylocin A, esperin, lichenysin, pumilacidin and surfactin [59]. Surfactins have antimicrobial [72, 73], antiviral [74], anti-mycoplasmatic [75]. These lipopeptides have an amphiphilic character. The most popular uses are as biosurfactant and...
antibiotic. Because of these properties, surfactins are good candidates in solving some of the global issues in medicine, industry and environmental protection [76]. They are also among the best biosurfactants, for biotechnological applications and environmental protection, mainly used for emulsifying, foaming, and oil recovery [77], remediation of heavy metals contaminated soils [78, 79] and biological control of plant diseases [80, 81] and pests [82].

5.2.4. Antifungal volatile compounds
Microbial volatile compounds, like aldehydes, ketones, alcohols, aliphatic alkenes, organic acids, sulphides [83, 84], can interfere as biological control mechanisms against fungal phytopathogens. Among the organic volatile compounds synthesized by some strains of Bacillus spp. bacteria include iturin, ammonia, acetoin, indole, hydrogen sulfide may occur in the biocontrol mechanism, and can trigger the defense response in plants, by inducting phytoalexin formation [85].

5.2.5. Quorum quenching
Bacterial communication inside their population is possible by quorum sensing molecules or N-acyl homoserine lactone (AHL) and oligopeptide as signaling factors. In pathogenic bacteria, the infectious activity is triggered by such signaling molecules. The pathogenicity is expressed only in certain population density, and quorum sensing is the communication mechanism involved in informing about cell density. AHL lactonase, can hydrolyze quorum sensing signaling molecule, and inhibit bacterial communication systems. This way, AHL lactonase producing microorganisms can act as potential biocontrol agents. Such mechanism of biological control is even more useful if the pathogenic bacteria are multi-drug tolerant [86].

AHL lactonase activity expressed by microorganisms is named quorum quenching effect. In biocontrol Bacillus spp. strains, such mechanism was analyzed and it has been shown in Bacillus licheniformis [87], B.cereus [88], B.thuringiensis [89] strains.

5.3. Nutritional and space competition
One of the competitive factors in nutrient uptake is siderophore formation, capable to specifically bind the ferric iron (Fe$^{3+}$) into high-affinity iron chelating compounds. This essential element, although is abundant in nature has a very low solubility. For microorganisms, ferric iron bioavailability is possible by siderophore formation that makes it soluble from the mineral phases. The Fe-siderophore complex has high specificity and can be used only by the issuing microorganisms. The high-affinity Fe-siderophore compounds create a competition between soil microbials to iron uptake. Siderophore formation in Bacillus sp. is not that well documented as in other bacterial species, such as Pseudomonads. However, specific iron uptake was mentioned in plant growth-promoting B.subtilis and B.amyloliquefaciens strains [30].

Swimming and swarming motility enhance the colonization ability of beneficial bacteria, reflecting in better space competitiveness. Bacillus species are among the most prominent bacteria found to colonize soil and plant roots. Bacillus root-colonization and biofilm formation is a prerequisite of phyto-stimulation [30]. The ability to stimulate plant growth, compete and suppress plant pathogens is enhanced when roots are better colonized by beneficial bacteria [44].

5.4. Induced systemic resistance in plants
Induced systemic resistance (ISR) is an enhanced state of defensive capacity, elicited by specific environmental stimuli [38]. Rhizobacteria-mediated ISR enhance the innate defensive
capacity against a broad spectrum of pests and pathogens attack [90]. In plants, rhizobacteria induced resistance has been demonstrated to be systemically expressed, by beneficial bacteria seed inoculation that triggered defensive mechanism against foliar disease [91].

Several *Bacillus amyloliquefaciens*, *B. cereus*, *B. mycoides*, *B. pasteurii*, *B. pumilus*, *B. sphaericus*, and *B. subtilis* strains were mentioned to elicit ISR in a wide range of host plants, where they reduced diseases incidence or severity. Results on *Bacillus* eliciting ISR have been reported against systemic viruses, pathogenic bacteria, damping-off, crown rotting, stem-blight, and leaf-spotting fungal pathogen, blue mold, and late blight diseases, or root-knot nematodes [38]. Therefore, biological controlled strategy using ISR mediating bacteria are promising to be valuable for agriculture application.

6. Conclusions

*Bacillus* species demonstrated to have a wide spectrum of plant protection and growth promoting abilities. They are considered of great importance for biotechnological industry and agriculture. The greatest advantage of using *Bacillus* species as biological inoculant in agriculture and related fields is the ability to produce endospores with prolong viability and high resistance [92, 13], which makes them easy to be formulated in various types and stored in simple conditions, marketing them similar to chemical fungicides in a certain manner. Mixtures of plant-beneficial bacterial-strains with different mechanisms of actions has been suggested as more reliably than individual strains [93].

7. Acknowledgements

This work was financed by Operational Program Human Resources Development 2007-2013, project no. POSDRU/159/1.5/S/132765 using European Social Fund.

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