Chapter 4

Biological Control of Root Pathogens by Plant- Growth Promoting *Bacillus* spp.

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Additional information is available at the end of the chapter

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1. Introduction

At the present time, among the most important factors limiting production of different crops are soil-borne plant pathogens [1]. Which include the genera *Pythium, Rhizoctonia, Fusarium, Verticillium, Phytophthora spp, Sclerotinia, Sclerotium,* and *Rosellinia* [2]. By this reason, different methods have been used to control these pathogens [3]. Cultural practices and chemical control using synthetic fungicides are the most used control methods [4], however, use of some of these synthetic products has caused various problems due to environmental pollution, with consequences such as toxicity to humans, as well as resistance of certain pathogens to these fungicides [5]. An alternative to reduce the effect of these plant pathogens is the use of antagonistic microorganisms such as: some species of the genus*Bacillus* which is recognized as one of the most effective biological control agent because of their properties on pathogens growth inhibition [6-7]. Biological control has many advantages as an alternative in the integrated management of diseases such as little or no harmful side effects, rare cases of resistance, long-term control, completely or substantially eliminates the use of synthetic pesticides, cost / benefit ratio very favorable; prevents secondary diseases, not symptoms of poisoning and can be used as part of integrated disease management [8]. Generally, the mode of action of *Bacillus* is antibiosis by producing extracellular hydrolytic enzymes which decompose polysaccharides, nucleic acids, other way are: production of antibiotics such as bacitracin, polymyxin, and gramicidin, [9-11], competition to occupy an ecological niche and metabolize root exudates on pathogens affecting their growth [12-13]. Also, activating plant resistance induction when

installed in the roots and leaves which induces plant to produce phytoalexins which give resistance against attack by fungi, bacteria and pathogenic nematodes [14], reducing in these ways disease incidence.

2. Overview of Bacillus

The genus *Bacillus* Cohn was established in 1872, initially with two prominent forming endospores species: *Bacillus anthracis* and *B. subtilis*, actually, this genus has suffered considerable taxonomic changes, until early 1900, taxonomists not only restricted the genus to endospore forming bacteria, having that the number of species assigned to this genus were 146 in the 5th edition of Bergey's Manual of Systematic Bacteriology. Subsequent comparison studies by Smith et al. and Gordon et al. over 1114 strains of aerobic bacteria forming endospores (PGPR) helped to reduce this number to 22 well-defined species same as reported in the 8th edition of Bergey's Manual of Systematic Bacteriology [15]. Bacillales is the order to which Bacillaceae family belongs within the genus *Bacillus*. This genus is characterized by having a rod shape within the group of Gram positive [16-17], and is therefore classified as strict aerobes or facultative anaerobes [18] and integrated by 88 species [15]. A feature associated with this genus is that it forms a type of cell called endospore as a response to adverse growth conditions which distorts the structure of the cell. This spore form is resistant to high temperatures and current chemical disinfectants [19]. This genus is abundant in various ecological niches which include soil, water and air [18, 20], it is also found as food contaminants. Generally, *Bacillus* species used in bio-control are mobile, with peritrichous flagella, but yet some species are of interest in human medicine (*B. anthracis*) which are characterized as being stationary [21].

2.1. Ecology and habits

Distribution and habitat of *Bacillus* are very diverse; some species have been isolated from soil micro-flora adjacent to plants rhizosphere, water, air and food as contaminants [18, 20]. Eco-physiological criteria commonly used to group different species such as vertebrate pathogens, insect pathogens, antibiotics producer, nitrogen-fixing, denitrifying, thermophilic, psychotropic halophilic, alkali and acidifies rows. For example *B. thuringiensis* is considered an insect pathogen and is used as a bio-pesticide, it has been isolated from soil, and abundantly found worldwide [22-24], in soil remains largely in the form of endospores [25], particles of dust in suspension [26], insect bodies sick or dead [27], also is found in stored products [28-29], food [30], marine sediments [32], and even as opportunistic human pathogen [33]. Furthermore, *Bacillus* species are found abundantly in plant leaves [34-38]. In conclusion, the *Bacillus* genus has a cosmopolitan distribution (Table 1).

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Organism	Reference strain isolated from	Common habitats and comments
B. acidocaldarius	Hot springs	Acid hot springs and soils, enrichment from neutral soils have failed.
B. alcalophilus	Human feces	soil, water, dung
B. alvei	Honeybee larvae suffering from European foulbrood	Soil, this specie is a saprophyte but common in bees with European foulbrood
B. aminovorans	Soil	
B. amyloliquefaciens	Soil	soil, industrial amylase fermentations
B. amylolyticus	Soil	
B. aneurinolyticus	human feces	
B. azotofixans	Soil	soil, rhizosphere of various grasses
B. azotoformans	Soil	Soil
B. apiaries	Dead larvae of honeybee	
B. badius	Human feces	Dust, coastal waters, soil
B. benzoevarans	Soil	Soil
B. brevis	Soil	Foods, soil, seawater, and sediments
B. cereus	Soil	soil, foods, especially dried foods, spices, and milk; seawater and sediments
B. circulans	Soil	Widespread in soil and decomposing vegetables; medicated creams, Relatively scarce in soil.
B. cirroflagellosus	marine mud	
B. coagulans	evaporated milk	Beet sugar, canned foods, especially vegetables; medicated creams, relatively scarce in soil.
B. epiphytus	marine phytoplankton	
B. fastidiosus	Soil	soil, poultry litter
B. firmus	Soil	soil, seawater and marine sediments, salt marshes
B. freudenreichii		Soil, river water, and sewage
B. globisporus	Soil	soil, mud, and water
B. insolitus	Soil	soil, mud, water and frozen foods
B. laevolacticus	Rhizosphere of ditch crowfoot	rhizosphere of plants
B. larvae	honeybee larvae suffering from American foulbrood	Infected brood and honey combs. Presumable in soil around hives of bees
B. laterosporas	Soil	soil, water, dead honeybee larvae, rumen of animals
B. lentimorbus	hemolymph of larvae of Japanese beetle	causes milky disease of scarabaeidae larvae

Organism	Reference strain isolated from	Common habitats and comments
B. lentus	Soil	Seawater, marine sediments, salt marshes and soil. Spices including black and red pepper
B. lecheniformis	Soil	soil, marine and freshwaters; foods, particularly dried foods, spices and cocoa beans, compost, rumen of cattle
B. macerans	unknown	foods and vegetables, compost
B. macquariensis	soil from Macquarie island	Unknown
B. macroides	cow dung	decaying material
B. marinus	seawater	Unknown
B. megaterium	Soil	soil including desert soil, seawater and marine sediments, cocoa bean, dried foods and spices
B. pacificus	sand from seashore	Seawater
B. pantothenticus	Soil	generally considered to be a soil inhabitant but also isolated from pharmaceutical products
B. pasteutii	Soil	soil, water, sewage, urinals
B. polymyxa	Soil	widely distributed in soil, decomposing plant matter and water
B. popilliae	Commercial spore dust	causes milky disease of scarabaeidae larvae
B. psychrophilus	Soil	soil, water, mud, frozen foods vegetables
B. pulvifaciens	dead larvae of honeybee	Unknown
B. pumilus	Soil	Ubiquitous in soil. Also found in seawater and marine sediments. Common in dried foods.
B. racemilactius	rhizosphere of wild lettuce	rhizosphere of plants
B. schlegelii	sediments of eutrophic lake	Unknown
B. sphaericus	Soil	soil, marine and freshwaters sediments and foods
B. stearothermoplilus	unknown	soil, foods including milk, canned foods and sugar beet, dried foods
B. subtilis	Soil	soil, marine and freshwater and sediments, foods including spices, cocoa, pulses, seeds and bread
B. thermoglucosidasius	Soil	Unknown
B. thiaminolyticus	Human feces	Unknown
B. thuringiensis		Pathogenic for lepidopteran larvae, common in soil.
B. xerothermodurans	Soil	Unknown

Table 1. Sources and common habitats of aerobic endospore forming bacteria of *Bacillus* genus, [39].

2.2. Importance how antifungal agents

Many species of *Bacillus* including *B. subtilis*, B. *licheniformis*, *B. pumilus*, *B. amyloliquefaciens*, *B. cereus*, *B. mycoides* and *B. thuringiensis*, are known to suppress growth of several fungal pathogens such as *Rhizoctonia, Fusarium, Sclerotinia, Sclerotium, Gaeummanomyces, Nectria, Pythium, Phytophthora* and *Verticillium* [20, 40-43]. The main property of antagonist bacterial strains is production of antifungal antibiotics [44-45], which seem to play a major role in biological control of plant pathogens [6, 44, 46-49] and post-harvest spoilage fungi [50]. Many of these antifungal substances have been characterized and identified as peptide antibiotics [51]. Antifungal peptides produced by *Bacillus* species: iturins [20, 52-53] are: mycosubtilins [54-55], bacillomycins [56-57], surfactins [58-59], fungistatins [60-61], and subsporins [62-63]. Most of these antibiotics are cyclic peptides composed entirely of amino acids, but some may contain other residues. However, a few antibiotic peptides are linear such as rhizocticins [64]. *Bacillus* spp. produces also a range of other metabolites including chitinases and other cell wall-degrading enzymes [65-68], and volatiles compounds [68-70] which elicit plant resistance mechanisms [14, 71].

The amount of antibiotics produced by bacilli class was approaching 167 [45], being 66 derived from *B. subtilis*, 23 from *B. brevis* and the remaining antibiotic peptides are produced by other species of *Bacillus*. The main antibiotic producers of this genus are *B. brevis* (gramicidin, tyrothricin) [72], *B. licheniformis* (bacitracin), *B. polymyxa* (polymyxin, colistin), *B. pumilus* (pumulin), *B. subtilis* (polymyxin, difficidin, subtilin, mycobacillin, bacitracin), *B. cereus* (cerexin, zwittermicin), *B. circulans* (circulin), *B. laterosporus* (laterosporin) [14, 68-71].

2.3. Collection and isolation of *Bacillus*

Traditional tools for determining composition of the soil bacterial community and diversity are based largely on *in vitro* culture methods. Typically, solid organic medium is inoculated with dilutions of a suspension of soil, then incubated and the colonies obtained are purified further sub culturing into another medium [73]. Heat treatment or pasteurization is the most used technique for selecting spores. These techniques are very powerful because they are selective to remove all non-spore forming microorganisms, and are very efficient for obtaining populations of bacteria from spores, recommended temperatures oscillate between 65 to 70 ° C for 15 minutes [74-75]. However, heat treatment has to be adapted to certain species because endospores of some strains of bacteria are more resistant to heat than others, while incubation time used can vary from 3 to 30 min [76]. It is recommended to start heating at a relatively low temperature (70 or 75 ° C) and gradually increasing to achieve an optimum temperature [77]. To isolate endospores, some authors have taken advantage of spore tolerance to diverse stress conditions, for example, Koransky et al. [78] concluded that treatment with ethanol (50%) for 1 h is an effective technique to selectively isolate spore- forming bacteria, as effective as heat treatment to 80 ° C for 15 minutes. Patel et al. [79] confirmed this finding by isolating *Bacillus* strains from food residues, both by heating at 65 ° C for 45 minutes and incubation with ethanol. Soil drying may also be used as a selective method to isolation by striking desiccation tolerance of spores, which can therefore survive for long periods of time under these conditions. Drying treatment is probably more gentle that heating

or ethanol incubation. Eman et al. [80] reported that vegetative cells were killed by addition of chloroform (1% v / v) however; this technique has not been validated. An interesting selection process, which is different from classical heat treatment was developed by Travers et al. [81] for isolation of *Bacillus thuringiensis*, which makes use of ethyl (ethyl selection), *B. thuringiensis* is selectively inhibited by sodium acetate (0.25 M), while most unwanted spore-forming species allowed to germinate. Then all non-sporulating bacteria were eliminated by heat treatment at 80 ° C for 3 min. Subsequently, surviving spores are germinated on enriched agar medium. Even if some other species of *Bacillus* are also selected by this method, such as *B. sphaericus* and *B. cereus*, this technique is commonly used for studying the diversity worldwide of *B. thuringiensis* [22, 77]. A modification to the method promotes greater sporulation spore production by stimulating shock before applying stress. For example, some authors suggest suspending one gram of soil in 50 mL of sporulation medium after incubation at 37 ° C under stirring for 48 hours before killing vegetative cells by heat treatment [80], while others proposed in soil suspensions incubate the culture broth at different temperatures for 5 days to allow better maturation of spores [77].

2.4. Biochemical identification

Biochemical test were the traditional method for bacteria identification to specie level, after that, strains are located at the genus taxonomically, based on characteristics of colony growth in artificial medium, form cell unit, presence, number and orientation of locomotive units, Gram stain, spore form and specific environmental conditions of growth and finally the specific use of carbon sources (biochemical tests) gave its metabolic diversity (Table 2 and 3).

	B. amylolique-faciens	*B. pumilus*	*B. subtilis*	*B. licheniformis*	*B. thuringiensis*	*B. cereus*	*B. mycoides*	*B. fastidiosus*	*B. firmus*	*B. lentus*	*B. megaterium*	*B. bodius*	*B. antracis*
Cell diameter"/>1.0 um	-	-	-	-	+	+	+	+	-	-	+	-	+
Parasporal crystals	-	-	-	-	d	-	-	-	-	-	-	-	-
Anaerobic growth	-	-	-	+	+	+	+	-	-	-	-	-	+
Voges Proskauer test	+	-	+	+	d	+	+	NG	-	-	-	-	+
egg yolk lecithin's	-	-	-	-	+	+	+	-	-	-	-	-	+
growth in lysozyme	-	d	d	d	+	+	+	ND	-	-	-	-	+
Acid from													
d-glucose	+	+	+	+	+	+	+	NG	+	+	+	-	+

	B. amylolique-faciens	*B. pumilus*	*B. subtilis*	*B. licheniformis*	*B. thuringiensis*	*B. cereus*	*B. mycoides*	*B. fastidiosus*	*B. firmus*	*B. lentus*	*B. megaterium*	*B. bodius*	*B. antracis*
l- arabinose	d	+	+	+	-	-	-	NG	-	+	d	-	-
d-xylose	D	+	+	+	-	-	-	NG	-	+	d	-	-
d-mannitol	+	+	+	+	-	-	-	NG	+	+	d	-	-
hydrolysis of													
Starch	+	-	+	+	+	+	+	-	+	+	+	-	+
Casein	+	+	+	+	+	+	+	-	+	d	+	+	+
nitrate reduction	+	-	+	+	+	+	+	-	d	d	d	-	+
degradation of tyrosine	-	-	-	-	ND	+	ND	-	d	-	d	+	d
Growth in 7% NaCl	+	+	+	+	+	d	d	-	+	d	d	ND	+
Growth at													
10°C	ND	+	d	-	d	d	d	+	d	ND	+	-	-
50°C	d	d	d	+	-	-	-	-	-	-	-	+	-
55°C	ND	-	-	+	-	-	-	-	-	-	-	-	-
Utilization of													
Citrate	d	+	+	+	+	+	d	-	-	-	+	-	D
Propionate	ND	-	-	+	ND	ND	ND	-	-	-	ND	-	ND

Table 2. Differential characteristics of *Bacillus* species with ellipsoidal spores (Group I), [39]. += 90 or more of strains positive catalase; - = 10 or more of strains negative catalese; d= substancial proportion of specie differ; ND= Not done; NG= no growth.

2.5. Molecular identification

Bacillus species with diverse physiological traits require development of biochemical tests for identification [82]. But advances in chromatographic analysis using whole cell fatty acid methyl esters (FAME) profiles allows doing this technique sufficiently sensitive and reliable for grouping *Bacillus* to specie level [83-84]. Identification has become even more sensitive, by analysis of ribosomal DNA regions (16S rDNA) sequencing [85-87], and sequence analysis of gyrase B (gyrB) which has proved immensely valuable information for phylogenetic analysis of bacteria [88-90]. Using 16S rDNA sequence, have been identified 5 groups within

the genus *Bacillus*, where group 1 (group *B subitilis*) comprises species *B. amyloliquefaciens, B. subtilis, B. pumilus* and *B. licheniformis* [9, 39, 91].

Test	*Bs*[y]	*Ba*	*Bl*	B1	B3	B9	B13
Gram staining	+z	+	+	+	+	+	+
Flagella staining	+	+	+	+	+	+	+
RYU Test	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Oxidation	+	+	-	+	-	+	+
Fermentation	+	+	+	+	+	+	+
Motility	+	+	+	+	+	+	+
Spore Posicion							
Terminal	-	-	-	-	-	-	-
Central	+	+	+	+	+	+	+
Subterminal	-	-	-	-	-	-	-
Colony Growth:							
45ºC	+	+	+	+	+	+	+
65ºC	-	+	-	+	-	-	-
pH Growth at 5.7	+	+	+	+	+	+	+
NaCl Growth:							
7%	+	+	+	+	+	+	+
5%	+	-	+	-	+	+	+
3%	+	-	+	-	+	+	+
citrate utilization	+	+	+	+	+	+	+
Anaerobic growth in glucoseglucose	-	+	+	+	+	+	+
Glucose							
Acidic Forms:							
Arabinose	+	+	+	+	+	+	+
Manitol	+	-	+	-	+	+	+
Xylose	+	+	+	nd	nd	nd	nd
Voges-Proskauer	+	+	+	+	+	+	+
Hydrolysis starch	+	+	+	+	+	+	+

Table 3. Results on identification of *Bacillus* isolates B1, B3, B9 and B13 by biochemical tests, [9], and Bs = *Bacillus subtilis*, Ba = *B. amyloliquefaciens*; Bl = *B. licheniformis*. Positive test Z = +; negative = -, nd = not determined. [9].

2.6. Antifungal effect *in vitro*, greenhouse and field

Bacillus species have been reported also as growth promoters of certain crops [92], and with antifungal properties, for example *B. amyloliquefaciens* has been reported as a specie with antifungal activity against *Colletotrichum dematium*, *Colletotrichum lagenarium*, *Rosellinia necatrix*, *Pyricularia oryzae*, *Agrobacterium tumefaciens*, *Xanthomonas campestris* pv. *campestris* and *Xanthomonas campestris* pv. *vesicatoria in vitro* and *in vivo* [93-95]; antagonistic to *Botrytis elliptica*, under greenhouse conditions [96]; antagonistic to *Botrytis cinerea* in postharvest [97], in the biological control of *Rhizoctonia solani*, *Fusarium* spp. and *Pythium* spp. [98], as well as inductor of resistance mechanisms in plants [99]. *Bacillus licheniformis* is reported as a fungicide against a variety of pathogens, both as a preventive and curative particularly leaf spots and blights, and a growth-promoting bacteria with production likely gibberellins [100]. *Bacillus subtilis* is the most studied and has been reported as growth promoter and antagonistic to a variety of pathogens such as *Phytophthora cactorum*, *Sclerotium cepivorum*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria carthami*, *Phytophthora capsici*, and *Fusarium solani* among others, in different cultures and evaluated *in vitro*, greenhouse and field level [101-103], so that *Bacillus* strains can be using as an alternative in biological control for management plant disease.

2.6.1. In vitro studies

Results of *in vitro* research using *Bacillus* spp. as biocontrol agent against various soil pathogens, have reported positive responses through observing a negative effect on pathogen growth (Figure 1), per example against *Alternaria dauci* and *Rhizoctonia solani*, foliage and soil pathogens, respectively. In the Table 4 and 5, are showed some effect on pathogen mycelia inhibition by action of *Bacillus*, up to 50% compared to treatment control. Furthermore, in the case of *A. dauci*, greater control was observed with biocontrol agents compared to chemical treatment.

Treatments	Mycelia Inhibition (%)
Strain *Bacillus* B3*	35.55 a
Strain *Bacillus* B9	40.44 ab
Strain *Bacillus* B15	29.44 ab
L. tridentata extract (4000 ppm)	22.22 b
L. tridentata extract (2000 ppm)	11.11 c
Witness [1]	0 d

Table 4. *In vitro* mycelia inhibition of *Rhizoctonia solani* with *Bacillus* spp. strains and *Larrea tridentata* extract. * Summated dozes of *Bacillus* strains were $1x10^6$ cfu / ml, [1] without agrochemicals, [46]. Values in the same column followed by different letters are significantly at $p < 0.05$.

Treatments	Mycelia inhibitions (%)
Strain *Bacillus* B1*	53.44[a]
Strain *Bacillus* B3	48.44b
Strain *Bacillus* B9	40.31c
Strain *Bacillus* B13	46.25b
Strain *Bacillus* B15	0f
Strains *Bacillus* Mix	0f
Q-L 2000-2000 ppm	14.06d
Q-L 2000-1000	4.06e
Q-L 1000-2000	1.88ef
Q-L 1000-1000	0f
Witness[1]	0f

Table 5. *In vitro* mycelia growth inhibition of *Alternaria dauci* by *Bacillus* spp. and chitosan-Larrea (Q-L) suspensions. * Strains of *Bacillus subtilis*. Values in the same column followed by different letters are significantly at p <0.05, [44].

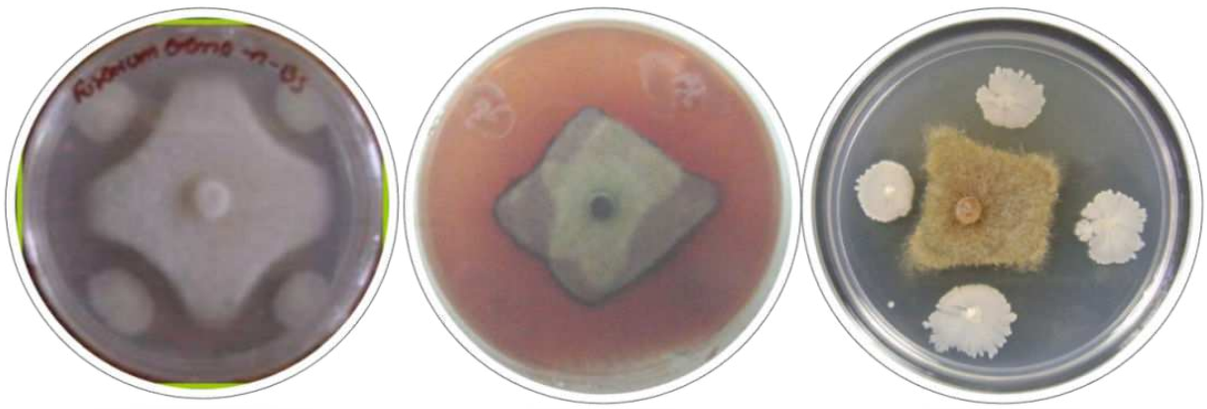

Figure 1. Effect *B. subtilis* in inhibition of mycelia growth of *Fusarium sp., Alternaria dauci* and *Rhizoctonia solani*.

2.6.2. Greenhouse studies

Results under greenhouse conditions, present good evidence of *Bacillus* as biocontrol source for pathogens involved in diseases of root and plant foliage, to cause a decrease in disease development in both incidence and severity. In table 6 is showed that application of *Bacillus* on carrot foliage allowed a control of *A. dauci* incidence up to 25%, which represents a control to 2 times more than the chemical treatment used for its control.

	Incidence	Severity
Treatments	**%**	
Strain *Bacillus* B1	25d	0.5 de
Strain *Bacillus* B3	0e	0 e
Strain *Bacillus* B9	25d	0.5 de
Strain *Bacillus* B13	25d	0.5 de
Strain *Bacillus* B15	50c	1 d
Strains *Bacillus* Mix	50c	1 d
Q-L 2000-2000 ppm	50c	3 c
Fungicides synthetics Mix*	75b	4.24 b
Witness	100[a]	6.75 a

Table 6. Product effect of *Bacillus* based biological products and chemicals on incidence and severity of *Alternaria dauci* on carrot plants under greenhouse conditions. * Chlorothalonil, iprodione, propiconazole, thiabendazole and fluazinam, [44].

Likewise *Bacillus* use has favored not only reduction of symptoms and therefore incidence, but also helps to promote plant growth, which is expressed in greater plant height, as shown in Figure 2, there is an increase in tomato plants height by effect of a microcapsule formulation of *Bacillus* applied in the management of disease caused by *F. oxysporum* and *R. solani*, in contrast to the use of synthetic chemicals [104].

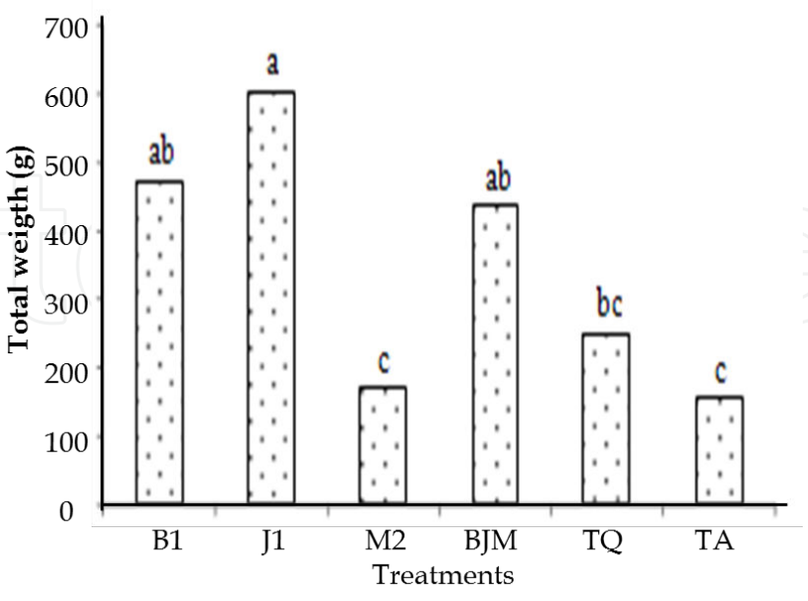

Figure 2. Average fruit yield of tomato plants cv. Floradade under greenhouse conditions subjected to different treatments with microcapsules containing strains of *Bacillus subtilis*, a chemical control (TQ) and a blank (TA).

2.6.3. Field experience

Most research has been conducted in laboratory or greenhouse, and virtually no field-level assessments have been reported. A study carried out using *Bacillus* spp. for control of diseases caused by soil fungi including: *F. oxysporum, R. solani* and *P. capsici* in pepper and tomato crops allowed control of diseases incidence as seen in Table 7, use of *Bacillus* at transplanting can reduce disease incidence in contrast to traditional treatments (fungicide application) as Folpat, Captan, Mancozeb much as 64% and 72% compared to untreated control with the most efficient strain, only 36 and 40 respectively with less efficient biological treatment.

	Harvest 1	B/TT	B/T	Harvest 2	B/TT	B/T	Harvest 3	B/TT	B/T
Treatments	**Incidence %**			**Incidence %**			**Incidence %**		
Bacillus B1	2.71 a	36	33	10.87 c	33	19	19.59 c	34	20
Bacillus B2	2.71 a	36	33	13.04 c	39	23	28.80 c	50	30
Bacillus B9	3.80 a	50	47	11.41 c	34	20	20.65 c	36	21
Bacillus B13	4.89 a	64	60	11.41 c	34	20	25.54 c	44	26
Bacillus Mix	3.25 a	43	40	15.76 c	48	28	20.11 c	35	21
Traditional treatment	7.61 a	100	93	33.13 b	100	59	57.61 b	100	60
Control	8.15 a	107	100	55.97 a	169	100	96.74 a	168	100

Table 7. Effect of *Bacillus* and commercial products on root rot incidence in *Capsicum annum* at different harvest times, [9]. Variance analysis used transformed data by arcsine. B/TT= *Bacillus* vs traditional treatments; B/T = *Bacillus* vs Control [9].

Furthermore, the suppressive effect was maintained over time or among harvest times, this indicates that *Bacillus* strains suppressed disease caused by soil fungi and maintained their remedial effect through harvest times as seen in Table 8 where disease incidence and severity was lower than that offered by the traditional treatment performed by the farmer.

Treatments	Incidence	Severity
	%	
Strain *Bacillus* B1	2.1 c	2.35 b
Strain *Bacillus* B3	3.05 ac	3.10 ab
Strain *Bacillus* B9	3.00 ac	3.05 ab
Strain *Bacillus* B13	2.75 bc	2.85 b
Strains *Bacillus* mix	2.90 ac	3.00 ab
Treatments Fungicides	3.5 ab	3.25 ab
Witness	3.85 a	3.85 a

Table 8. Effect of four strains of *Bacillus* and commercial products on severity of wilt and root rot by *Fusarium* spp., *Rhizoctonia solani* and *Phytophthora capsici*, on pepper (*Capsicum annum*) using scales to wilt and root rot, [9].

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In the case of tomato same behavior was observed for disease development with respect to the presence of *Bacillus*, Table 9.

Treatments	Incidence (%)	Severity
Bacillus B1	0.0 d	0.0 c
Bacillus J1	0.0 d	0.0 c
Bacillus M2	12.0.c	1.5 c
B1J1M2 Mix	0.0 b	0.0 c
QT*	27.0 b	3.5 b
AT**	75.0 a	5.0 a
CV (%)	10.4	1.2

Table 9. Disease incidence and severity at harvest time of tomato plants cv. Florade subjected to different treatments with microcapsules containing strains of *Bacillus subtilis.* *Chemical control treatments; ** Absolute control treatment, Values with same letters area not statistically different (Tukey, p<0.01). [104].

The application in field of *Bacillus* sp on melon crops (Figure 3) for the management of disease caused by *F. oxysporum*, showed an effect in reducing disease incidence in 41% compared to the conventional chemical treatment (TA), and increases in yields of 26.5% higher than TA, however there were no significant differences in the brix degrees, consistency of fruit, but an increase in 12% in the number of fruits and 20% in the length guide, leaves and stem diameter was observed [105].

Figure 3. Treatments effect with *Bacillus subtilis* in fields on melon crops with high incidence of *Fusarium oxysporum.*

2.7. Effect on plant development and growth

The effects obtained by applying *Bacillus* as fungicide were positive for crop development, because *Bacillus* stimulated biomass production, increased number of flowers and fruiting mooring, as seen in Table 10, where *Bacillus* was applied, plants had an increase in height, flowering and fruiting compared to traditional crop management through use of synthetic agrochemicals. It is noteworthy that use only at the time of transplantation *Bacillus* and 20 days after the second application, which is manifested by loss of effect at 84 days, but yet still persists pathogen control, before such situation should be applied in successive moments to keep a *Bacillus* greater crop protection coverage.

			56 days			84 days
	Height	Flowers	Fruits	Height	Flowers	Fruits Frutos
Treatments	(cm)	(No.)	(No.)	(cm)	(No.)	(No.)
Bacillus B1	39.95 ab	3.60 a	1.25 a	61.20 ab	13.35 a	6.00 a
Bacillus B3	42.75 a	3.60 a	1.05 a	60.05 ab	10.70 a	5.05 a
Bacillus B9	41.20 ab	3.05 a	1.05 a	59.40 ab	9.59 a	4.50 a
Bacillus B13	43.75 a	3.50 a	1.35 a	66.40 a	14.35 a	4.90 a
Bacillus mix	40.90 ab	3.15 a	1.40 a	63.55 ab	12.10 a	6.00 a
Traditional treatments	35.30 ab	2.75 a	0.60 a	58.10 ab	10.45 a	5.95 a
Control	32.80 b	2.65 a	0.55 a	55.55 b	12.00 a	5.00 a

Table 10. Effect of *Bacillus* strains on pepper (*Capsicum annuum*) cv. Caballero development, at 56 and 84 days after inoculation under field conditions. [9].

In a similar way, positive effects of *Bacillus* application were observed on crop yield, by prevent soil pathogen attack. In Table 11, is showed that application of *Bacillus* increased pepper yields in contrast to the traditional crop management by up to 74% cumulative assessment in three harvest times.

Treatment	Cut 1 (kg)	B/TT (%)	Cut 2 (kg)	B/TT (%)	Cut 3 (kg)	B/TT (%)	Yield (kg)	B/TT (%)
BacillusB1	4.38 a	100	4.01 a	150	6.69 a	421	15.10 a	174
BacillusB3	3.32 ab	76	3.05 ab	114	4.01 b	252	10.39 b	120
Bacillus B9	2.73 ab	62	2.40 ab	90	4.04 b	254	9.17 b	106
Bacillus B13	3.79 ab	86	3.41 ab	127	4.01 b	252	11.16 ab	129
Bacillus mix	3.14 ab	72	2.80 ab	104	4.30 b	270	10.25 b	118
Traditional Treatments	4.39 a	100	2.68 ab	100	1.59 c	100	8.67 b	100
Control	1.79 b	41	1.71 b	64	0.57 c	36	4.08 c	47

Table 11. Effect of *Bacillus* strains on pepper (*Capsicum annuum*) cv. Caballero development at 99, 113 and 146 days after inoculation under field conditions. B / TT = *Bacillus* vs. traditional treatment, [9].

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Bacillus favors growth of different plant parts such as stems and leaf area (Tables 12 and 13, Figure 4).

Figure 4. Effect of *Bacillussubtilis* in the biomass production of pepper plants (root). Treatments *Bacillus* and treatments without *Bacillus.*

Treatments	Height (cm)	Leaf area (cm2)
Bacillus B1	119.47 a	6857.01 b
Bacillus J1	118.65 a	7762.92 a
Bacillus M2	102.95 b	5393.32 b
Mixture B1J1M2	121.05 a	7022.90 a
*TQ	99.05 b	4007.51 c
**TA	98.9 b	4302.63 bc
CV (%)	3.11	9.29

Table 12. Height and leaf area of tomato plants cv. Floradade subjected to different treatments with microencapsulated strains of *Bacillus subtilis.* *: Chemical control treatment; **Absolute control treatment, Values with same letters are not statistically different (Tukey, p≤0.01), [104].

Treatments	Leaves	Stems	Roots
Bacillus B1	116.41 a	30.31 ab	31.28 c
Bacillus J1	107.57 a	31.92 ab	38.76 b
Bacillus M2	87.67 b	27.90 b	32.04 c
Mixture B1J1M2	94.51 b	34.69 a	96.63 a
*TQ	1 c	28.06 b	33.38 bc
**TA	26.21 d	14.57 c	13.92 d
CV (%)	6.54	8.32	6.97

Table 13. Biomass (g) production of tomato plants cv. Floradade subjected to different treatments with microcapsules containing strains of *Bacillus subtilis.* *Chemical control treatment; **: Absolute control treatment. Values with same letters are not statistically different (Tukey, p≤0.01), [104].

3. Conclusions

Use and application of biological control agents, such as *Bacillus* spp. prevents negative effects of pathogen attack on crops, providing an attractive option for sustainable agriculture due to their stimulating effects on plant growth, biomass production and its potential to increase plant production. In this chapter are mentioned clear and efficient biocontrol of plant pathogenic fungi by Bacillus strains, as evidence of lower disease incidence and severity. For that reason, it is suggested that B. subtilis can be incorporated to integrated management disease, where these strain may be used as biocontrol agent as well as biofertilizer.

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