Chapter 6

Current Understanding and Future Directions of Biocontrol of Plant Diseases by *Bacillus* spp., with Special Reference to Induced Systemic Resistance

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Abstract Plant pathogens represent one of the prime threats to sustainable crop production. Till date, synthetic agrochemicals are considered as effective tools for the management of various biotic stresses such as pathogenic microorganisms and insects in plants. Unfortunately, their injudicious and intensive usages in agriculture pose a serious threat to the environment and all living beings dwelling on the earth. Under such circumstances, the application of beneficial *Bacillus*-mediated management of plant pathogens has emerged as one of the most benevolent and sustainable options. A large number of *Bacillus* species has been identified as promising candidates for managing a number of plant pathogens through induction of systemic resistance in plants. Significant research progress has been attained in the characterisation and understanding of the role of *Bacillus*-induced systemic resistance (ISR) against a wide range of pathogens of crop plants. In this chapter, we aim to provide an overview of the mechanisms of *Bacillus*-induced ISR for instance, elici-

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M. T. Islam et al. (eds.), *Bacilli in Agrobiotechnology*, Bacilli in Climate
Resilient Agriculture and Bioprospecting,
https://doi.org/10.1007/978-3-030-85465-2_6

tors, phytoalexins, lipopeptides, antibiotics, hormones and enzymes to protect plants from various pests. Additionally, glimpses of the research progress in the identification of different *Bacillus* strains and their evaluation as a potential biocontrol agent have also been presented.

Keywords *Bacillus* · Induced resistance · Bio-control · Lipopeptides · Antibiotics

6.1 Introduction

Steady increase in crop yield to feed the ever-increasing population is the greatest challenge faced by the agriculturists worldwide. Apart from factors such as nutrient supply, soil conditions, moisture, degrading cultivable land, and cultivar quality, biotic stresses pose a huge threat to the food security of the ever-increasing global population (Wulff et al. 2011; Islam et al. 2016a, b, 2019a, 2020). Although practices like crop rotation, use of chemical pesticides, and development of better cultivar have been utilised for the management of emerging diseases, these approaches are no longer ecologically and economically sustainable. Thus, application of beneficial microorganisms have emerged as a suitable alternative to the pre-existing traditional approaches (Hardoim et al. 2015). Coordination between microbiota and root endodermis supports plant mineral nutrient homeostasis (Salas-González et al. 2020). Among the beneficial microorganisms studied so far, *Bacillus* spp. have been observed to perform especially well not only in managing diseases but also in increasing nutrient availability, enhancing plant growth, improving beneficial microbial community in the rhizosphere, and inducing sustained defence reactions in the plant (Islam et al. 2016a, b, 2019b).

Bacillus is a genus of Gram-positive and endospore-forming bacteria, which is cosmopolitan in nature and widely spread across a diverse range of environments. They occur naturally in crop fields and aid in crop productivity both directly and indirectly. Inherent traits like durable cell wall, formation of endospores, peptide and antibiotic secretion, and production of antimicrobial enzymes play a significant role in the survival of these ubiquitous organisms even in a highly adverse situation.

A large body of research on the *Bacillus* species has attributed its success in the production of antibiotics and lipopeptides, quorum quenching, lysis of pathogen hyphae, competition for space and nutrients, and induced systemic resistance (Li et al. 2013; Islam et al. 2016a, b, 2019a, b; Surovy et al. 2019; Rayanoothala et al. 2020). Furthermore, *Bacillus* spp. increase nutrient uptake, siderophore production, and promote plant growth. For example, *B. subtilis* increases N uptake, phosphate solubilisation, and siderophore and phytohormone production, and promotes plant growth. *Bacillus* species has also been observed to alter the microbial community in the rhizosphere which facilitated disease suppression (You et al. 2016; Mahapatra et al. 2020).

This chapter aims to review the current understanding of the induction of resistance mechanism in host plant by *Bacillus* species. It deals with the various mechanisms employed by *Bacillus* species to suppress the phytopathogens in the plant rhizosphere and phyllosphere. It also discusses the genomic and molecular bases of disease resistance in plants imparted by the *Bacillus* species. Finally, it summarises the field application of *Bacillus*-based formulation, its prospects and challenges. This report also tries to bridge the knowledge gap and help to develop *Bacillus* species as a reliable and effective strategy for the management of emerging pests and diseases.

6.2 *Bacillus* Diversity and Antagonism

The type of soil plays a crucial role in the management of phytopathogens. Suppressive soils contain a large number of beneficial microbes that are able to inhibit the growth of bacterial and fungal pathogens. Among the biocontrol agents, the *Pseudomonas* genus has been most extensively studied for its anti-fungal metabolites (AFMs) such as pyrrolnitrin, phenazines, pyoluteorin, 2,4-diacetylphloroglucinol (DAPG), and viscosinamide (Nielsen et al. 1999; Nielsen et al. 2000). However, *Bacillus* species has gained popularity in the recent years due to its production of a wide array of antimicrobial substances and the ability of certain strains to promote plant growth (Choudhary and Johri, 2008). A large number of cultivable strains of *B. cereus* and *B. subtilis* have been identified in different studies (Vargas-Ayala et al. 2000), while cultivation-independent studies have demonstrated the existence of a much more number of uncultivable strains of *Bacillus* spp. (McSpadden Gardener 2004).

Some studies have found *B. megatarium* to be the most abundantly available species, while some researchers observed *Paenibacillus*, previously known as *Bacillus polymyxa*, to dominate a variety of soils. *Paenibacillus* has the ability to fix atmospheric nitrogen and thus are helpful in meeting the N requirement of a large number of crop plants like Canadian wheat (Priest 1993). *Brevibacillus*, previously known as *Bacillus brevis*, is terrestrial and aquatic in nature (Panda et al. 2014). A member of the *Bacillus* species, *B. sphaericus* is a notable entomopathogen and thus thrives in the habitats of insect larvae like pools, ditches, and lakes (El-Bendary 2006).

Spatiotemporal analysis of the microbial communities in soil, rhizoplane, and rhizosphere revealed that soil type had greater effect than plant type in determining the microbial population diversity (Wieland et al. 2001). Arias et al. (1999) studied the distribution and diversity of *Bacillus* spp. in the soybean phylloplane. Their study revealed that *B. pumilus* is the most widely distributed species in the soybean phylloplane. Although other bacterial strains like *B. brevis*, *B. subtilis*, *B. circulans*, and *B. firmus* were also observed in the phylloplane, their population continued to decline with the growth of the crop and became completely undetectable at 85 days of the cropping season. As mentioned earlier, *Paenibacillus* genus contains many nitrogen fixing bacterial species, like *P. azotofixans*, *P. polymyxa*, and *P. macerans* (Ash et al. 1993). But amongst all the reported species, *P. azotofixans* has been found to be the most efficient, and hence, is abundant in the rhizosphere of wheat, maize, sorghum, banana, sugarcane, and some forage crops (Seldin 1992). *P. azotofixans* is more abundant in bulk soil than the rhizosphere, while its population varied largely with the soil type in the same crop rhizosphere (Rosado et al. 1998, Seldin et al. 1998).

The distribution and diversity of the *Bacillus* species is largely dependent on the interaction between the soil and the plant. The exudates from the plant roots, which play a significant role in determining the microbial distribution, are specific to the plant and are also correlated to a particular soil habitat (Crowley and Rengel 1999; Duineveld et al. 2001). The exudates vary with the stage of the crop as well, which is reflected in the findings of Jaegar and co-workers. The growth stage of the crop could be a crucial factor in determining the rhizobacterial community, as observed by Van Overbreek and van Elsas (2008) in potato. It has been observed that plants encourage the growth of specific microorganisms, from the indefinite microdiversity based on the chemical influences in the rhizosphere. Thus the manipulation of the chemical constituents of the rhizosphere could be done to encourage the growth of promising *Bacillus* strains among the rhizospheric community of microorganisms.

6.3 Mechanism of Induction of Resistance Against Plant Disease by *Bacillus* spp.

When *Bacillus* is introduced in the host-pathogen-environment system, it interacts with all the components in a number of complex mechanisms that affect the growth of the phytopathogen directly or indirectly. These interactions may lead to plant growth promotion, biofilm formation, induced systemic resistance (ISR), competition for nutrients, production of antibiotics, and cell lysis (Fig. 6.1). Strains of *Bacillus* spp. have been recorded to exhibit one or more of these traits that work synergistically with the plant and the environment in suppressing the phytopathogens. Furthermore, these interactions may lead to increased plant growth, vigour, and a shift in the rhizospheric microbial community, which would indirectly reduce the impact of the plant pathogens on the host plant.

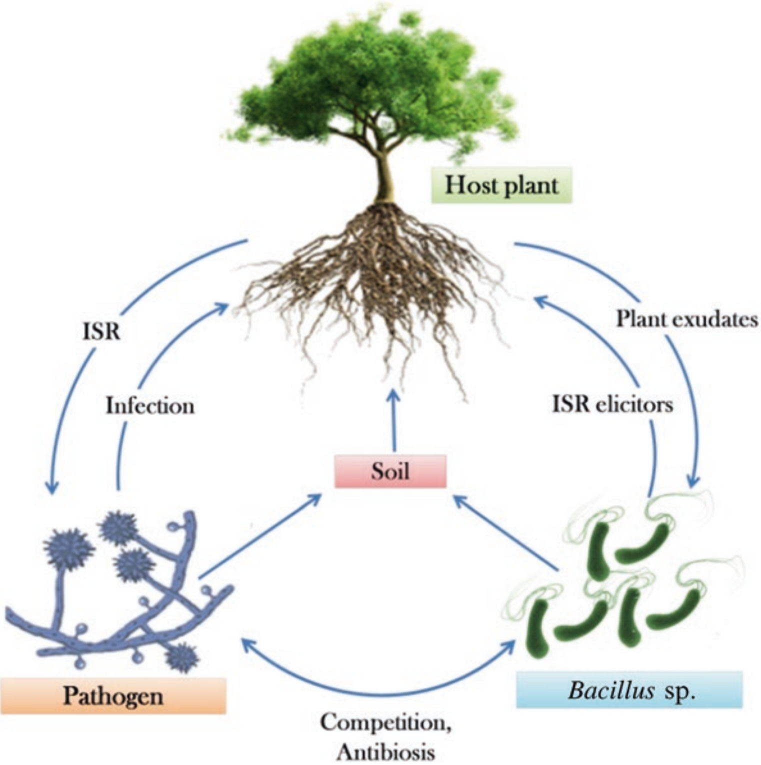

Fig. 6.1 Interactions between *Bacillus* spp., the pathogen and the host plant

6.3.1 Competition for Nutrients

Nutrients are always limiting factors for the growth and development of microorganisms and plants in the any crop fields. Biological control aims at utilizing this competition for nutrient and space to reduce the growth and productiveness of the pathogen by the non-pathogenic organisms. Generally, soil-borne pathogens like *Fusarium* and *Pythium* infect through mycelial contact, which makes them more vulnerable to competition from the non-pathogenic organisms. Micronutrients, such as iron, are extremely essential but its availability is limited and largely dependent on the soil conditions. In such situations, microbes produce siderophores, which are capable of attracting iron from the rhizosphere, thus meeting the iron requirement of the microorganism (Chakraborty et al. 2020a, b). In many instances, *Bacillus* has been found to compete with pathogenic microbes, such as *Erwinia carotovora*, by a similar mechanism, hence, restricting the growth and the development of the pathogen (Kloepper et al. 2004a, b). Studies also revealed that the inoculation of *Bacillus* sp. in the rhizosphere resulted in a shift of the pathogenic microflora from the site of infection. *Bacillus* species was reported to cause rapid colonisation in the tissues

Table 6.1 List of important *Bacillus* strains in respect to their competition, target pathogens, and host plants

Bacteria	Strain	Host	Target pathogen	References
Bacillus subtilis	HU5	Cotton	*Verticillium dahlia*	Li et al. (2013)
B. subtilis	SQR9	Cucumber	*Fusarium oxysporum*	Cao et al. (2011)
B. subtilis	E1R-j	Wheat	*Ustilago tritici*	Baker et al. (1983)
B. subtilis	SB24	Tomato	*Sclerotinia sclerotiorum*	Clayton and Hudelson (1991)
B. Amyloliquefaciens	CM-2 and T-5	Tomato	*Ralastonia solanacearum*	Tong-Jian et al. (2013)
B. Amyloliquefaciens	54	Watermelon	*Acidovorax avenae* subsp. *citrulli*	Jiang et al. (2015)
B. Megaterium	A6	Oilseed rape	*Sclerotinia sclerotiorum*	Hu et al. (2013)
B. Megaterium	B153-2-2	Soybean	*Rhizoctonia solani*	Zheng and Sinclair (2000)
B. pumilus	SE34	Pea	*F. oxysporum f.* sp. *pisi*	Benhamou et al. (1996)
B. cereus	UW 85	Tobacco	*Phytophthora parasitica*	Blagoeva-Nikolaeva et al. (1995)

of tomato plant, which reduced the disease severity and wilting index of *Fusarium* sp. in the host plant (Jangir et al. 2018). Similar results were obtained by Zhang and co-workers (2011) when working with *Fusarium* wilt of banana. They found that *Bacillus* was a rapid coloniser under hydroponic, sand and soil conditions, which inhibited the development of *Fusarium* sp. in the treated banana plant. Many studies have been conducted on the role of competition and colonisation in establishing *Bacillus* species as successful bio-control agents (Table 6.1).

6.3.2 *Synthesis and Excretion of Lytic Enzymes*

Aside from the production of lipoproteins and antibiotics, *Bacillus* has been known to produce a number of defense-related oxidative enzymes like peroxidase (PO) and polyphenol oxidase (PPO), which bring about structural changes in the host cell wall, thus strengthening the defense barriers against the invading pathogen. Several studies conducted so far have revealed that *Bacillus* synthesizes a large number of phytosanitary enzymes, which are enlisted in Table 6.2. Phenol oxidase enzymes produce quinones and release hydrogen peroxide. These enzymes trigger the release of toxic free radicals and polymerise the phenolic compounds into lignin like substances. The resulted metabolites are then deposited in the host cell wall, which

Table 6.2 List of enzymes activated by Bacillus species, along with the target pathogen and host plant

Bacteria	Strain	Host plant	Enzyme activated	Target pathogen	References
Bacillus subtilis	174	Tomato	PO,PPO, PAL	*Fusarium oxysporum*	Akram and Anjum (2011)
B. subtilis	AUBS1	Rice	PAL, PO and PR proteins	*Rhizoctonia solani*	Jayaraj et al. (2004)
B. subtilis	PTA-271	Grapevine	Lipoxygenase, PAL and chitinase	*Botrytis cinerea*	Trotel-Aziz et al. (2008)
B. subtilis	B4	Cucumber	Indole acetic acid	*Colletotrichum orbiculare*	Park et al. (2013a, b)
B. subtilis	SE34 and GBO3	Rice	PO and PAL, PPO	*Xanthomonas oryzae pv. oryzae*	Udayashankar et al. (2011)
B. pumilus	SE34	Pea	Phenolic compounds	*F. oxysporum f.* sp. *pisi*	Benhamou et al. (1996)
B. vallismortis	BS07	Chili pepper	Salicylic acid (SA)	*Phytophthora capsici and Colletotrichum acutatum*	Park et al. (2013a, b)
B. mycoides	Bac J	Sugar beet	Chitinase, b-1,3-glucanase and peroxidase	*Cercospora beticola*	Bargabus et al. (2002)
B. cereus	AR156	Loquat	PAL, PO, chitinase, β-1,3-glucanase, polyphenoloxidase and promoted accumulation of H_2O_2	*Colletotrichum acutatum*	Wang et al. (2014)
B. subtilis	BBG111	Rice	Jasmonic acid (JA) and ethylene (ET) as well as abscisic acid (ABA) and auxin signalling	*Rhizoctonia solani*	Chandler et al. (2015)

affect the growth of the pathogen. In many instances, *Bacillus* spp. have been found to be highly effective in the production of these oxidative enzymes. According to a study by Ramyabharathi et al. (2012), the liquid formulation of *B. subtilis* EPCO16 was capable of eliciting defense related enzymes like catalase and phenylalanine ammonia lyase (PAL) in tomato plants infected with *F. oxysporum* f. sp. *lycopersici.* Spectrophotometric analysis of plants revealed the activity of defense enzymes at their highest on the seventh day after inoculation by the pathogen.

Chitin is an important polysaccharide, which imparts structural rigidity and integrity to the fungal cell wall. But, the presence of hydrolysing enzymes like chitinase and glucanase breaks down the glycosidic bonds holding the constituting polysaccharides together, that leads to cell leakage and cell lysis. Many species of the *Bacillus* spp. are known to produce chitinolytic enzymes and suppress the phytopathogens (Podile and Prakash 1996). Wang et al. (2004) observed that two types of chitinase were produced by *B. amyloliquefaciens* V656, which were highly effective in inhibiting the growth of *F. oxysporum* growth. *B. thuringiensis* is known to inhibit the growth of *Sclerotium rolfsii* in soybean plant by a similar mechanism. Studies by Liu et al. (2010) revealed the production of chitinase from *B. thuringiensis* sub sp. *colmeri,* which was instrumental in preventing spore germination of various phytopathogenic fungi. Generally, bacteria possess a variety of chitinases for breaking down the wide array of chitin molecules that naturally exist in nature. Ruiz-Sanchez (2007) observed that five different types of chitinase (with 42, 49, 53, 62, and 66 kDa) were synthesised by *B. licheniformis*.

6.3.3 Production of Lipopeptides and Antibiotics

Bacillus spp. are considered to be the producers of a vast array of antimicrobial compounds such as bacillomycin and zwittermycin A (Mondol et al. 2013). According to studies, 4-5% of the *Bacillus* genome is devoted for the production of antimicrobial compounds (Stein 2005). Among these compounds, lipopepetides of the surfactins, iturins, and fengycin group are most widely studied. Surfactins are antiviral, antibacterial agents, while iturins and fengycins, also known as plipastatins, exhibit highly effective antifungal activities. The lipopeptides (LPs) interact with the each other in a synergistic way, and bring about an alteration of the cell membrane permeability of the microorganisms. The LPs produced by *Bacillus* are non-ribosomal in nature and are produced by non-ribosomal peptide synthetases (NRPSs) or hybrid polyketide synthases and non-ribosomal peptide synthetases (PKSs/NRPSs). The efficacy of biocontrol of plant diseases by lipopeptides produced by *Bacillus* spp. is well established (Chakraborty et al. 2020a, b).

The surfactins are excellent biosurfactants, with notable foaming ability. They attach themselves to the lipid layers, and thus hamper the integrity of the microbial membranes. The surfactins induce pore formation, which is followed by complete solubilisation and disruption of the membranes. Studies also reveal that the surfactins are unable to function in the presence of cholesterol in the phospholipid bilayer, which explains why they are inactive as antifungal compounds (Meena and Kanwar 2015).

Iturins are a group of heptapepetides linked to a β-amino fatty acid chain. They are named so after their place of discovery, Ituri, in the Democratic Republic of the Congo. The family of iturins mainly include iturins A, C, D, and E; bacillopeptin; bacillomycins D, F, L and LC; and mycosubtilin (Mnif and Ghribi 2015). They are highly fungitoxic in nature but with limited antiviral and antibacterial activity. The iturins are known to form ion-conducting pores, which lead to the permeablilisation of the membranes, leading to fungal toxicity.

The fengycins, also known as plipastatins, are a group of lipodecapeptides attached to a β hydroxy fatty acid chain (Wang et al. 2015). Fengycins are highly fungitoxic in nature, especially against filamentous fungi; however, they are less effective as compared to surfactins and iturins. The mechanism of fungitoxicity of the fengycins is elusive so far. However, these natural compounds have been shown to induce structural imbalance in the microbial membrane (Ongena and Jacques, 2008). Other antibiotics produced by *Bacillus* species include bacteriocins, bacillaene, difficidin, oxidifficidin, sporulenes A–C, bacisubin, and bacilysocin. All these antibiotics exhibit antifungal and antibacterial activity at varying levels of concentrations.

The antibiotics produced by *Bacillus* have a crucial role in its biocontrol activities. The LPs are attributed to biofilm formation by the bacteria in the soil and water surfaces. They bring about the flagella-driven motility of the bacteria, leading to biofilm spread over the plant and soil surfaces. They are also responsible for reducing the surface tension, which helps in further spread of the bacteria. Bais and collaborators (2004) reported that *B subtilis* strain 6051 produced surfactin molecules that resulted in biofilm formation in the roots of *Arabidopsis* plant.

It has been reported by Asaka and his co-workers (1996) that *B subtilis* strain RB14 produces iturin A, which is instrumental in inhibiting the growth of *Rhizoctonia solani* in tomato. Leclere and co-workers (2005) found that mycosubtillin, produced by *B. subtilis* ATCC, helps in the reduction of *Pythium aphanidermatum* infection. The combined role of fengycin and iturin in inhibiting the growth of *Podosphaera fusca* in melon leaves was demonstrated by Romero and co-workers (2007). Ongena and Jacques (2008) observed that *B. subtilis* S499 produces all three families of LPs that help in the management of plant diseases by this strain. This strain also inhibits the growth of *Botrytis cinerea* in wounded apple fruits by the production of lipopeptide-enriched extracts, which contained high doses of fengycins in it.

The production of antibiotics was also linked to the induction of defense reactions in a number of treated plants. In tomato and bean, fengycins and surfactins were directly attributed to induce ISR. *B subtilis* S499 inhibited the growth of *Colletotrichum lagenarium* in cucumber plants by the stimulation of systemic resistance. Potato tubers exhibited an accumulation of phenolics when being treated with purified fengycins, which activate the phenylpropanoid pathway. The activation of phenylpropanoid pathway leads to the accumulation of mRNAs, encoding phenylalanine ammonia lyase (PAL).

Although the antibiotics are highly potent in inhibiting the growth of harmful microorganisms, they have not been reported to produce any phytotoxicity in the host plants. These compounds are able to induce a cascade of biochemical reactions that stimulated the defense system of the plant against the pathogen, without causing any adverse effects on the cellular integrity of the plant. Researchers believed that the differing compositions of phytosterol from that of bacterial and fungal compositions are mainly responsible for the attenuation of the disruption of plant membranes.

6.4 Induction of Resistance in Plants

Several lines of evidence suggest that strains of *Bacillus* spp. elicit systemic resistance in the plants. Induced systemic resistance (ISR) was elicited in plants by *Bacillus* spp. in response to a number of biotic stresses like fungi, bacteria, viruses, nematodes, and insects. *Bacillus* spp. produce LPs which are considered to be the key components in ISR elicitation in plants (Rahman et al. 2015). The ISR modulates a number of cytological and biochemical processes in the host plant such as lignin deposition in the plant cell wall, production of phytoalexins, and synthesis of other antimicrobial substances like peroxidases and β-1, 3-glucanases (GarcíaGutiérrez et al. 2013). Among the LPs produced, surfactin is essential for ISR elicitation as observed by Chowdhury and his collaborators in 2015. As discussed earlier, the iturin and surfactin family of lipopepetides are highly effective in suppressing phytopathogens. After analysis of gene expression, it was observed that surfactin activated salicylic acid (SA) regulated pathway, while mycosubtillin of the iturin family, had the ability to activate both jasmonic acid (JA) and salicylic acid (SA) pathways in grapevine (Farace et al. 2015). Similar results were observed in strawberry, which helped in the successful inhibition of *Colletotrichum gleosporioides* (Yamamoto et al. 2015). The role of LPs was also demonstrated in maize (Gond et al. 2015), rice (Chandler et al. 2015), and tomato (Abdallah et al. 2017). The ISR generally leads to plant growth promotion as seen in strain *B. subtilis* 21-1. Lee and his co-workers (2014) observed that *B. subtilis* 21-1 helps in disease suppression of vegetable crops, by activating the plant defense system, as well as helps in plant growth promotion. It can be concluded that *B. subtilis* elicited plant defense system and growth promotion of the host, which collaborates to inhibit the phytopathogens.

The phytopathogens differ considerably in their morphology and cellular compositions. Hence, *Bacillus* employs different mechanisms against these phytopathogens (Fig. 6.2). It synthesises lipopeptides against some, while it produces enzymes for managing other pathogens. A brief description of the mechanisms used by *Bacillus* against phytopathogens is provided in the following section.

6.4.1 Bacillus *Against Fungi*

Extensive research has been conducted on the mechanism of *Bacillus* for the inhibition of fungal growth. Chitin forms a major constituent of the cell wall of all pathogenic fungi; hence, biocontrol agents such as *Trichoderma, Alteromonas,* and *Serratia* produce different type of chitinases (Elad et al. 1982). *Bacillus* has also been reported to produce chitinolytic enzymes for lysing the fungal cell wall (Mitchell and Alexander, 1962). *Bacillus* species was observed to reduce the radial growth *of Penicillium* sp. (72%), *Aspergillus niger* (64.58%), *Aspergillus*

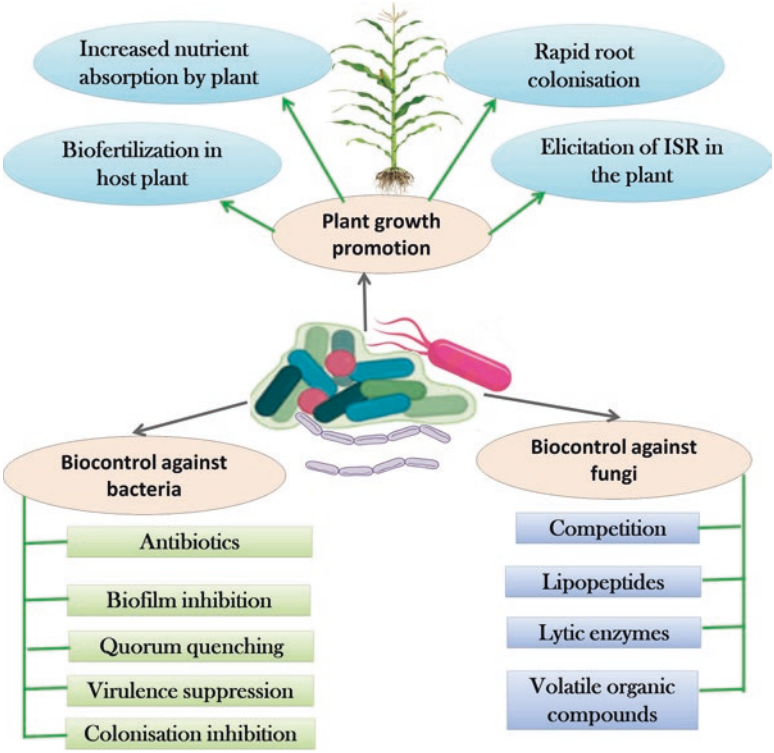

Fig. 6.2 Mechanisms used by *Bacillus* sp. as a growth promoter and biocontrol agent

fumigatus (51.61%), *Fusarium* sp. (39.13%), *Curvularia* sp. (37.50%), *Aspergillus flavus* (35.43%), and *Alternaria* sp. (31.82%), after 6 days of incubation. The inhibitory action was attributed to chitinolytic activity and hyperparasitism as concluded by Basha and Ulaganathan (2002). It was observed by Bargabus et al. (2002, 2004) that *B. mycoides* isolate BacJ and *B. pumilus* isolate 203–7 were highly effective in controlling the *Cercospora* leaf spot of sugar beet to a considerable extent.

Studies revealed that many *Bacillus* strains such as *B. lentimorbus* and *B. cereus* are able to inhibit the development of coffee rust. It has been observed that the bacterium prevented the pathogen from developing uredospores, which resulted in suppression of the pathogen up to 50% (Shiomi et al. 2006). The wide array of volatile compounds produced by *B. velezensis* ZSY-1 was studied by Gao et al. in 2017. They observed that these compounds possessed significant antifungal activities. *Bacillus* was able to inhibit the growth of *B. cinerea, Monilinia fructicola, Colletotrichum lindemuthianum, Alternaria solani,* and *F. oxysporum* f. sp. *capsicum*. It was also observed that benzothiazole, pyrazine, and phenolic compounds played a crucial role in inhibiting fungal diseases in tomato such as grey mould and early blight.

6.4.2 **Bacillus** ***Against Nematodes***

Limited studies have been conducted on the interaction between *Bacillus* and plant pathogenic nematodes. In some instances, the involvement of LPs has been correlated with the reduction in nematode infestation (Hallmann 2001). Hallmann (2001) observed that bacterial endophytes successfully inhibit the infestation of *Meloidogyne incognita* through extensive root colonisation and elicitation of ISR in the host plant. Reitz et al. (2000) observed that the bacterial endophytes, including *Bacillus*, produced a number of lipopeptides, which successfully controlled potato cyst nematode, *Globodera pallida*. Hallmann and his co-workers (1997) studied the role of endophytic bacteria in inhibiting root-knot nematode, *Meloidogyne incognita*, in cotton roots. They also observed that the nematodes helped the endophytic bacteria in entering the host plant which aided in successful colonisation of the plant roots. Mendoza and Sikora (2009) observed a significant reduction in *Radopholus similis* infestation in banana plants, when a combined treatment of *B. firmus* and *F. oxysporum* was applied on the banana plant.

6.4.3 **Bacillus** ***Against Bacterial Pathogens***

Phytopathogenic bacteria communicate among each other by the production of density-dependent autoinducers (AI), which is known as quorum sensing. Quorum sensing plays a crucial role in establishing the virulence factors in bacteria, which determines the disease severity. In the case of Gram-negative bacteria, *N*-acyl homoserine lactones (AHL) are mostly used as signalling molecules during quorum sensing. *Bacillus* species, however, have the ability to produce quorum quenching molecules that modify the AIs and hinder the bacterial signalling, thus affecting the virulence of the pathogen. *Bacillus* possesses *aiiA* gene encoding N-acyl homoserine lactonase (AiiA) enzyme, which brings about the hydrolysis of AHLs. *Bacillus* species, such as *B. cereus, B. subtilis, B. firmus,* and *B. toyonensis*, have been known to suppress a number of plant pathogenic bacteria by inhibiting quorum sensing, such as *Pseudomonas aeruginosa*, *Agrobacterium tumefaciens*, and *Pectobacterium carotovorum (Erwinia carotovora).* In addition to directly using the AiiA-producing *Bacillus* species to suppress the virulence of phytopathogenic bacteria (Dong et al. 2004), *aiiA* can be heterologously expressed in other bacterial strains (Molina et al. 2003) or in genetically modified plants (Ouyang and Li 2016).

6.4.4 **Bacillus** ***Against Viral Phytopathogens***

Bacillus species has been found to provide resistance in plants against many viruses, such as Tomato Mottle Virus (ToMov) and cucumber mosaic virus. The application of *Bacillus* leads to the reduction in visible symptoms as well as reduced viral accumulation as is evident from ELISA analysis (Murphy et al. 2000). Some studies indicate that the *Bacillus* species results in an accumulation of IAA, leading to growth enhancement in the plants, that results in systemic protection against the virus as observed by Murphy and his co-workers (2000), in tomato against CMV. However, according to some researchers, *Bacillus* elicits ISR in the virus affected plants, that triggers the expression of PR genes, leading to an accumulation of PR proteins like chitinase and β 1,3-glucanase, as observed by Wang et al. (2009) in tobacco plant against TMV. It is known that NPR1 regulates SAR and ISR resistance pathways while Coi1 regulated ISR pathway. *Bacillus* species has been observed to induce the expression of both *NPR1* and *Coi1* genes in the TMV-resistant tobacco plants, indicating a correlation between the gene expression and disease resistance.

6.5 Genomics and Molecular Basis of Induction Resistance by *Bacillus* on Plant

Bacillus has been known to produce a number of antimicrobial compounds that aid in plant disease management. *B. subtilis* has been considered to be a model microorganism for the analysis of gene functions, as it encodes for a number of antibiotics and lytic enzymes in order to manage phytopathogens. Research on the genomics and molecular basis of resistance induction may help in the development of biologically feasible weapons against plant diseases. Fungal cell walls are made of chitin that provides mechanical strength to the fungus. According to a study, *B. subtilis* CHU26, isolated from a potato field in Taiwan, exhibited strong chitinase activity in vitro, that was successful in inhibiting the growth of *Rhizoctonia solani.* Further investigations revealed the presence of chitinase encoding gene *chi18* that was responsible for this antimicrobial activity (Yang et al. 2009).

Hydrogen peroxide is known to exhibit cytotoxic activity in organisms by the production of hydroxyl radicals that react to lipids, proteins, and nucleic acids. However, *B. subtilis* has been able to mitigate the H_2O_2 stress by the production of enzymes catalase and an alkyl hydroperoxide reductase, encoded by genes *katA* and *ahpC*, respectively (Broden et al. 2016).

For evaluating the role of *Bacillus* in stimulation of ISR, Lee and co-workers (2015) studied strain HK34 of *B. amyloliquefaciens* against *Phytophthora cactorum* by applying *Bacillus* on the leaves and roots of *Panax ginseng*. It was observed that the bacterium was able to reduce the pathogen growth up to 99.1%. The inhibition of the pathogen was found to correlate with the expression of *PgCAT*, *PgPR5*, and *PgPR10* genes. These outcomes showed the ISR-eliciting potential of strain HK34.

Table 6.3 List of genes of *Bacillus* species encoded for the production of various antimicrobial metabolites that help them in biocontrol of phytopathogens

Strain	Gene cluster	Products/function	Reference
Bacillus sp.	*srf*A	Surfactin	Hsieh et al. (2004)
Bacillus sp.	*fen*B	Fengycin	Ramarathnam et al. (2007)
Bacillus sp.	*bac*A	Bacilysin	Mora et al. (2011)
B. subtilis	*Eps* A-O operon	Synthesis of biofilm matrix	Vlamakis et al. (2013)
*B. subtilis*168	*deg*Q	Secretion of degradative enzymes	Parashar et al. (2013)
B. subtilis	*Pks* gene cluster	Bacillaene	Muller et al. (2014)
B. subtilis 916	locA, locB, locC and locD, gene cluster	Locillomycins	Luo et al. (2015)
B. subtilis	*tyr*Z	Growth and biofilm formation	Williams-Wagner et al. (2015)
B. subtilis	*tap*A	Surfactin synthesis	van Gestel et al. (2015)
B. subtilis	*Apr*E and *Npr*E	Extracellular proteases	Barbieri et al. (2016)
B. subtilis	*lta*S	Lipoteichoic acid (LTA) synthase	Kasahara et al. (2016)
B. subtilis	SWR01 *min*J	Swarming	Gao et al. (2016)

Ryu et al. (2004) worked on the production of volatile compounds, produced by *Bacillus* species. They observed that *B. subtilis* GB03 synthesised 2,3-butanediol and 3-hydroxy-2-butanone (acetoin) in abundance, which helped in the induction of ISR. Furthermore, the volatiles produced by the bacteria were controlled by the expression of genes *CHIB*, *GST2,* and *ERF1*, which were known for the biosynthesis of ethylene. Recent studies have been able to discover a number of gene clusters in *Bacillus* species, which are shown in Table 6.3.

6.6 Commercial Applications of *Bacillus* Species

Bacillus strains have been utilised commercially for the generation of a variety of products. It is used not only as a potent host for genetic modifications but also as a source of naturally obtained biocontrol compounds (Bunk et al. 2010). Furthermore, *Bacillus* species produces stress-resistant endospores that contribute towards better environmental stability and longer shelf-life of the products. Among the wide array of *Bacillus* species discovered so far, *B. thuringiensis* is the most widely exploited with more than 70% of the market share (Ongena and Jacques 2008). Different companies utilise different mechanisms of the biocontrol agent in developing its products. For example, Bio-Yield, produced by 3Bar Biologics Inc., USA, contains a combination of two *Bacillus* species, *B. amyloliquefaciens* GB99 which is responsible for the elicitation of ISR. The *B. subtilis* GB122 inhibits soil-borne pathogens by the production of lipopeptide iturin. Also the product contains chitosan, which is effective against insects and nematodes (Kloepper et al. 2004a, b). Another product, Yield Shield produced by Bayer CropScience Inc., USA, is composed of *B. pumilus* GB34, which activates the plant defense system and promotes plant growth as well (Jeong et al. 2014). Similarly, many other *Bacillus*-based products have been developed commercially that can be utilised for the successful management of phytopathogens (Table 6.4).

Table 6.4 Commercial phytosanitary products obtained from *Bacillus*

Name of the product	*Bacillus* strains	Target pathogen	Crop	Company
Bio-yield	*B. subtilis* GB122, *B. Amyloliquefaciens* GB99	*Fusarium, Rhizoctonia, Pythium,*	Bedding plants	3Bar biologics, USA
Nacillus	*B. subtilis Antumávida, B. subtilis Vilcún, B. licheniformis Mallerauco Brevi, Bacillus brevis Maguellines Brevi, Bacillus brevis Maguellines I*	*Clavibacter pseudomonas, Xanthomonas, Acetobacter*	Vegetables, blueberry Cherry, hazelnut tree, tomato, pear, kiwi tree,	Bio Insumos Nativa, Chile
Yield shield	*B. pumilus* GB34	*Fusarium sp., Rhizoctonia solani,*	Soybean	Bayer CropScience, USA
Votivo	*B. firmus* I-1582	*Pratylenchus, Meloidogyne*	Corn, cotton, soybean,	Bayer CropScience, USA
Dipel	*B. thuringiensis* subsp. *kurstaki* HD-1	*Helicoverpa, Diatraea saccharalis, Grapholita molesta, Diaphania nitidalis, Plutella xylostella, Argyrotaenia sphaleropa*	Citrus, pineapple, tomato, cotton, apple tree, sugarcane, melon, cabbage,	Valent BioSciences, USA
AvoGreen	*B. subtilis*	*Colletotrichum gloeosporioides, Cercospora sp.*	Avocado	Ocean agriculture, South Africa
Ecoshot	*B. subtilis*	*Botrytis cinerea*	Citrus, legumes, vegetables, grape	Kumiai chemical industry, Japan
HiStick N/T, Subtilex	*B. subtilis* MB 1600	*Fusarium sp., aspergillus sp., Rhizoctonia sp., Pythium sp.*	Vegetables, ornamentals	Becker underwood, Ames, IA, USA
Kodiak	*B. subtilis* GB03	*Fusarium sp., aspergillus sp., Rhizoctonia sp., Alternaria sp.*	Legumes	Gustafson Inc., Dallas, Texas, USA
Biosafe	*B. subtilis*	*Xanthomonas axonopodis pv. Phaseoli*	Bean	Laboratorio de Biocontrole Farroupilha, Brazil

6.7 Mode of Application of *Bacillus* Species

The method of application of the biocontrol agent depends on its mode of action. Among the different methods of application, seed coating is the most extensively used, as it is easy to use and can be applied efficiently with a little quantity of inoculum. When the microbes are introduced as granular applications, the biocontrol agent is applied with a mixture of marble, peat, perlite, charcoal, and soil, with an aim of enhancing the contact between the plant roots and the biocontrol agent.

In case of *Bacillus*, both liquid and powdered formulations are available commercially, which are applied in the form of pellets, as well as soil drench, foliar spray, or as seed dressing. When *Bacillus* was applied as liquid formulation on tomato plant, it was able to successfully reduce the symptoms of wilt, as well as increase the shelf-life of *Bacillus* (Ramyabharti et al. 2016). Gao and his co-workers (2015) researched upon the most successful method of *B. subtilis* application against *Blumeria graminis* in wheat. They observed that all the preparations, i.e., cell-free culture, non-protein fermentation liquid, and crude proteins, were able to reduce the infection to some extent. However, fermentation liquid formulation was the most effective among all the preparations studied. Hsieh and his collaborators (2009) observed that talc-based formulation of *B. subtilis* and *P. fluorescens* was able to reduce the Banana Bunchy Top Virus incidence up to about 52% and helped in growth promotion of the plant under field conditions.

According to the studies conducted by Selim and his co-workers (2017), the application of endophytic bacteria as both soil drench and talc-based formulations was able to manage *Rhizoctonia solani* in cotton. But among the two formulations studied, soil drench was found to be more effective. However, Yamamoto and co-workers (2015) found that *Bacillus amyloliquefaciens* S13–3 was highly efficient in managing anthracnose in strawberry when applied as foliar spray. Spraying of fermentation liquid formulation of *Bacillus* sp. significantly improves growth, yield, and content of antioxidants in strawberry fruit (Rahman et al. 2015).

After the analysis of each method, Hallman and his co-workers (1997) observed that every method had its pros and cons. However, seed treatment was found to be the most efficient when ease of application, economic feasibility, and environmental stability was taken into consideration. They further stated that the combined application as seed treatment, soil drench, and foliar spray would be able to enhance the colonisation of the bacteria and also increase the benefits manifold.

6.8 Conclusion

Bacillus sp. is considered as the new tools for the promotion of sustainable agricultural system. A lot of information on biocontrol of plant diseases by *Bacillus* spp. has been generated so far by different researchers throughout the world. In this chapter, we summarised the details of the recent advances on mode of action, molecular basis of pathogenic antagonism, application in field level and formulations with their stability potential of *Bacillus* spp. In these aspects, this chapter is unique, informative and a good source of molecular insights for the future researchers to deal with the *Bacillus* biocontrol agent in the crop field without harming the nature.

In this chapter, we summarised the mode of actions involved in successful antagonism and the PGPR activities that include – secretion of lytic enzymes, production of lipoproteins, and antibiotics that are explained with examples. The mechanism of induced systemic resistance against plant pathogens depends on interactions of host-pathogen and environment. One or more *Bacillus* strains can work synergistically with plant and environment in suppressing the phytopathogens. The mechanism is varied according to the application of *Bacillus* sp. against fungus, bacteria, virus and nematodes. The most important part of this chapter is the genomics and molecular basis of induction of systemic resistance by the *Bacillus* spp. *Bacillus* is known to produce a number of antimicrobial compounds that deal with plant diseases resistance mechanism. In this case, the spore producing ability and genetically modified host with *Bacillus* genes may provide an effective solution in their colonisation simultaneously along with other beneficial microbes. But for successful colonisation, understanding ecological requirements are mandatory.

Among the wide functional assay of *Bacillus* sp. so far, *B. thuringiensis* is the most widely exploited even more than 70% of the market shares. But there are lots of conflicts on its commercialisation and acceptation country to country. So, successful application and commercialisation depends on the practical understanding on mode of action, methods of application, ecological distribution and interacting environment. But for more popularity among the farmers, a perfect demonstration of benefit:cost ratio would be mostly required.

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