

# Review Article

## Ecological Niches of *Bacillus* Species and Their Multifaceted Mechanisms in Combatting Plant Pathogens

### ABSTRACT

The *Bacillus* genus, an integral member of the *Firmicutes* phylum, is characterized by its rod-shaped structure and widespread presence in diverse ecosystems. Known for its resilience, *Bacillus* thrives in various environments, making it a versatile agent in agriculture. Several *Bacillus* species serve as biocontrol agents, effectively managing fungal and bacterial plant diseases. Their efficacy stems from a range of biochemical and metabolic actions, including the production of siderophores that suppress phytopathogens and promote plant growth. *Bacillus* spp. produce hydrolytic enzymes such as chitinases, glucanases, proteases, and cellulases, which degrade pathogen cell components, leading to cellular collapse. Additionally, *Bacillus* secretes antimicrobial compounds like bacillomycin, fengycin, iturin, surfactin, and difficidin, targeting multiple plant pathogens. The production of volatile antimicrobial compounds further enhances their biocontrol potential by inhibiting microbial growth and reproduction. Moreover, *Bacillus* plays a vital role in activating plant defense mechanisms, including Induced Systemic Resistance (ISR). Through the production of elicitor compounds, *Bacillus* triggers the jasmonic acid signaling pathway on host plant, fortifying the plant's biochemical and physical barriers against pathogen invasion. These multifaceted attributes position *Bacillus* as a promising tool for sustainable plant disease management and enhanced crop health.

*Keywords: Bacillus, siderophore, plant pathogens, Induced Systemic Resistance (ISR)*

### Introduction

*Bacillus* group are well known as producers of a wide array of antimicrobials. *Bacillus* species have a unique ability to replicate rapidly, resistant to adverse environmental conditions as well as they have broad spectrum of biocontrol ability. The genus *Bacillus* is one of the predominant bacterial genera found in soil, and several species of this genus have been reported from diverse ecological niches (Fira et al., 2018; Saxena et al., 2020). The aim of the study is to explore the ecological niches of *Bacillus* species and Understand the mechanisms by which *Bacillus* species combat plant pathogens.

## 1. HABITAT

*Bacillus* species are predominantly found in soil environments and are among the most commonly isolated genera from soil samples. They play essential roles in various nutrient cycling and organic matter decomposition (Schallmeyer *et al.*, 2004). Additionally, *Bacillus* species have been identified in various marine habitats such as sediments, seawater, and marine organisms, indicating their adaptability to saline environments (Ivanova *et al.*, 1999). *Bacillus* spores are resilient and often found in air and dust, with research showing their frequent detection in airborne dust samples, facilitating their dispersal across diverse environments (Hussain *et al.*, 2011). *Bacillus* species also appear in various food products, where they may act as contaminants or beneficial probiotics i.e. *Bacillus cereus* is commonly found as a contaminant in rice and dairy products, while *Bacillus subtilis* is used in fermented foods (Schoeni and Wong, 2005). In addition, *Bacillus* species are part of the natural gut flora in humans and animals, contributing to digestion and immune modulation (Hong *et al.*, 2005). *Bacillus* species are also present on plant surfaces and in the rhizosphere, where they colonize plant roots, promote growth by producing phytohormones, and protect against pathogens (Kloepper *et al.*, 2004; Tsetetsi *et al.*, 2022). Furthermore, some *Bacillus* species are extremophiles capable of surviving in harsh conditions, such as high temperatures, salinity, and acidity. They have been isolated from environments like hot springs and salt lakes, highlighting their extreme adaptability (Canganella *et al.*, 2000; Satyanarayana *et al.*, 2005).

## 2. MORPHOLOGY OF *BACILLUS* SPP.

The morphology of *Bacillus* species has been extensively documented in numerous studies. Summarizing findings from various research, *Bacillus* species are generally rod-shaped, a defining characteristic of the genus. Typically, *Bacillus* cells are straight rods, although some species may exhibit slight curvature (Logan and De Vos, 2009; Biology insights, 2024). Cell size varies across species, ranging from 0.5 to 2.5  $\mu\text{m}$  in diameter and 1.0 to 10.0  $\mu\text{m}$  in length. *Bacillus* species are Gram-positive, characterized by a thick peptidoglycan layer in their cell walls that retains the crystal violet stain in Gram staining. However, some may appear Gram-variable due to age-related or compositional changes in the cell wall (Sneath, 1986). A key feature of *Bacillus* species is their ability to form endospores, which are highly resistant to environmental stresses like heat, desiccation, radiation, and chemicals. These spores are generally oval to cylindrical and can be positioned centrally, terminally or sub-terminally within the vegetative cell. In some cases, spore size can cause cell swelling, aiding in identification (Nicholson *et al.*, 2000).

Many *Bacillus* species are motile, possessing peritrichous flagella, distributed over the cell surface that enable movement, allowing them to colonize new environments and survive under adverse conditions (Henriques and Moran, 2007; Kearns, 2010). On solid media, *Bacillus* species often form large, spreading colonies with a rough, matte surface, with color varying from white to cream depending on the species and medium. Colonies may exhibit a rhizoid or filamentous edge due to cell chaining and motility (Gordon *et al.*, 1973).

*Bacillus* cells often arrange in chains, facilitated by their rod shape and division process. This chaining can occur in both vegetative and spore-forming cells and assists in species identification and classification (Turnbull, 1996). Additionally, some *Bacillus* species produce a polysaccharide capsule, visible as a clear halo around the cell when stained with specific capsule stains. This capsule helps the bacteria evade host immune defenses and can contribute to pathogenicity (Patel *et al.*, 1985).

### **3MECHANISM OF DISEASE CONTROLLING POTENTIAL**

#### **3.1 Siderophore production**

The production of siderophores by *Bacillus* species plays an essential role in disease control, especially in agricultural environments. Siderophores are high-affinity compounds that chelate iron, enabling bacteria to capture this vital nutrient from their surroundings for growth and metabolism. This iron-sequestering capability limits iron availability to plant pathogens, thereby inhibiting their growth (Beneduziet *et al.*, 2012; Saha *et al.*, 2020).

##### **3.1.1 Mechanism of Action of Siderophore**

Siderophores produced by *Bacillus* species are effective in competing with plant pathogens for iron, thereby inhibiting their growth. *Bacillus subtilis* produces siderophores such as bacillibactin, which have a high affinity for ferric iron ( $Fe^{3+}$ ). By sequestering iron, these siderophores limit the availability of this essential nutrient for pathogenic microbes, thus curbing their growth (Zawadzka *et al.*, 2006; Ongena and Jacques, 2008).

##### **3.1.1.1 Suppression of Phytopathogens**

Siderophore production by *Bacillus* species has proven effective in the suppression of various plant pathogens. *Bacillus subtilis* and *Bacillus amyloliquefaciens* successfully controlled pathogen*s*.e. *Fusarium oxysporum* and *Pythium ultimum* by producing siderophores that restricted the pathogens iron access (Milagres *et al.*, 1999; Beneduziet *et al.*, 2012).

##### **3.1.1.2 Enhancement of Plant Growth and Health**

In addition to pathogen suppression, siderophores promote plant growth and health. Siderophores produced by *Bacillus* species aid in plant iron uptake, which is essential for chlorophyll production and overall plant vitality. Healthier plants exhibit greater resistance to infections and are better equipped to handle environmental stresses (Vansuytet *et al.*, 2007; Sánchez *et al.*, 2007).

##### **3.1.1.3 Synergistic Effects with Other Biocontrol Mechanisms**

Siderophore production often operates synergistically with other biocontrol mechanisms, such as antibiotic production and the release of lytic enzymes. *Bacillus* species that produce both siderophores and antifungal antibiotics show enhanced control over root rot caused by *Rhizoctonia solani* compared to strains that produce only one type of biocontrol agent (Duffy and Défago, 1999; Chowdhury and Bae., 2016).

#### **3.1.1.4 Role in Soil Health and Microbial Community Dynamics**

Siderophore production by *Bacillus* species also plays a role in enhancing soil health and shaping microbial community dynamics. Siderophores from *Bacillus* can shift the composition of the microbial community by outcompeting pathogens for iron while supporting beneficial microbes, thereby fostering a healthier soil microbiome and reducing the incidence of plant diseases (Zhao *et al.*, 2014; Liu *et al.*, 2016;).

### **3.2 Production of various enzymes by *Bacillus* spp.**

The *Bacillus* genus is renowned for producing various extracellular enzymes that contribute to the biological suppression of plant pathogens. Key enzymes include chitinases, glucanases, proteases, and cellulases, all of which can degrade the cell walls of pathogenic fungi and other harmful microbes (Santos and Souza, 2017). Some important enzyme by different *Bacillus* spp. and their role.

#### **3.2.1 Chitinases**

Chitinases break down chitin, a primary component of fungal cell walls. *Bacillus* spp. is known for their high chitinase production, which is essential in biocontrol applications (Sharma and Kaur, 2018). *Bacillus subtilis* S1-21 produces chitinase that effectively inhibits fungal pathogens like *Fusarium oxysporum* and *Rhizoctonia solani*, significantly reducing disease severity in tomato plants infected with these fungi (Cao *et al.* 2011).

#### **3.2.2 Glucanases**

Glucanases, such as  $\beta$ -1,3-glucanase, specifically target glucans in fungal cell walls. *Bacillus* spp. produce these enzymes to disrupt fungal hyphae. *Bacillus amyloliquefaciens* strain B10 produces  $\beta$ -1,3-glucanase with potent antifungal activity against *Sclerotinia sclerotiorum*. This enzyme not only inhibited fungal growth but also induced systemic resistance in the host plants (Singhet *al.* 2018).

#### **3.2.3 Proteases**

Proteases degrade proteins in fungal cell walls and other pathogen structures, compromising pathogen integrity. In a study by a protease-producing *Bacillus* strain showed strong antifungal effects against *Botrytis cinerea*. Upon the applied to grapevines, this strain significantly reduced gray mold incidence, demonstrating the agricultural benefits of protease-producing *Bacillus*spp (Li *et al.* 2015; Wang *et al.*, 2016).

### **3.2.3 Cellulases**

Cellulases are enzymes that break down cellulose, a key structural component of the cell walls of many plant pathogens. *Bacillus* spp. that produces cellulases can weaken and lyse these pathogenic cells (Meena and Saha, 2015). *Bacillus megaterium* strain Y16 produces cellulase, which effectively controls *Phytophthora infestans*, the organism responsible for late blight in potatoes. The study found a significant reduction in disease symptoms in plants treated with the cellulase compared to untreated controls (Shafiq *et al.* 2017).

### **3.2.4 Synergistic Effects**

The combined action of multiple enzymes can enhance the biocontrol effectiveness of *Bacillus* spp. *Bacillus* strains produces chitinases, glucanases and proteases which exhibit greater biocontrol activity against a wide range of plant pathogens. This synergistic effect not only improves pathogen suppression but also reduces the likelihood of resistance development (Borriss (2011); Barka and Nowak,2016).

## **3.3 Antibiotics Produced by *Bacillus* spp.**

The *Bacillus* genus is well-known for producing a diverse range of antibiotics effective against various plant pathogens (Tian and Yang, 2011). These antibiotics are vital for biological control and have been extensively studied.

### **3.3.1 Bacillomycin**

Bacillomycin is a lipopeptide antibiotic produced by several *Bacillus* species, including *Bacillus subtilis* and *Bacillus amyloliquefaciens*. It has demonstrated significant effectiveness against fungal pathogens. Bacillomycin produced by *B. amyloliquefaciens* FZB42 exhibited strong antifungal activity against *Fusarium oxysporum*, which causes wilt diseases in various crops. The study revealed that bacillomycin disrupts the integrity of the fungal cell membrane, leading to cell death (Koumoutsiet *al.* 2004; Zhang and Song,2014).

### **3.3.2 Fengycin**

Fengycin is another notable lipopeptide antibiotic produced by *Bacillus* species, known for its potent antifungal properties, particularly against filamentous fungi. A study by Vanittanakomet *al.* (1986) and

Chung and Kim, 2012 reported that fengycin produced by *Bacillus subtilis* effectively inhibited *Botrytis cinerea*, the causative agent of gray mold in grapes and other fruits. The study highlighted that fengycin induces pore formation in the fungal cell membrane, resulting in cell lysis.

### **3.3.3 Iturin**

Iturin is a well-studied antibiotic produced by several *Bacillus* species, including *B. subtilis*, *B. amyloliquefaciens* and *B. thuringiensis*. Iturin exhibits a broad spectrum of activity against fungi and some bacteria. Iturin A, produced by *Bacillus subtilis* RB14-C, significantly inhibited the growth of *Rhizoctonia solani*, a soil-borne pathogen responsible for root rot in various crops. The study revealed that iturin A interacts with the lipid components of the fungal cell membrane, leading to leakage of cellular contents and eventual cell death (Leclère et al. 2005; Zhao and Chen, 2014).

### **3.3.4 Surfactin**

Surfactin is a cyclic lipopeptide known for its potent surfactant properties and antimicrobial activity. Surfactin produced by *Bacillus* spp. has been shown to suppress a wide range of plant pathogens. Surfactin from *Bacillus subtilis* strain 6051 significantly reduced the incidence of *Pseudomonas syringae* pv. *tomato*, the pathogen responsible for bacterial speck in tomatoes. The study demonstrated that surfactin disrupts biofilm formation by the pathogen, thereby enhancing the plant's defense mechanisms (Bais et al. 2004; Liu and Zhang, 2015).

### **3.3.5 Difficidin and Bacilysin**

Difficidin and bacilysin are polyketide and peptide antibiotics, respectively, produced by *Bacillus* species. These antibiotics have shown effectiveness against both bacterial and fungal pathogens. Difficidin and bacilysin produced by *Bacillus amyloliquefaciens* FZB42 inhibited the growth of *Erwinia amylovora*, the bacterium responsible for fire blight in apples and pears. The study indicated that these antibiotics interfere with protein synthesis in the target pathogens, leading to their suppression (Chenet et al. 2009; Liu and Tian, 2012).

## **3.4 Volatile Organic Compounds Produced by *Bacillus* spp.**

Volatile organic compounds (VOCs) produced by *Bacillus* species play an important role in the biological control of plant pathogens. These low molecular weight compounds can diffuse through air and soil, exerting antimicrobial effects against various plant pathogens (Ryu and Kim, 2003).

### **3.4.1 Acetoin and 2,3-Butanediol**

Acetoin and 2,3-butanediol are well-researched volatile organic compounds (VOCs) produced by *Bacillus* species. These compounds are known to enhance systemic resistance in plants while also directly inhibiting pathogen growth (Chung and Lee, 2012). Ryu *et al.* (2004) demonstrated that *Bacillus subtilis* GB03 produces acetoin and 2,3-butanediol, significantly promoting plant growth and boosting resistance against *Erwinia carotovora* subsp. *carotovora*, which causes soft rot. The study revealed that these VOCs activate the plant's defense mechanisms, resulting in improved disease resistance (Dudareva *et al.*, 2013).

### **3.4.2 Benzaldehyde**

Benzaldehyde is another VOC produced by *Bacillus* spp. that exhibits antimicrobial properties. A study by Kai *et al.* (2009) found that benzaldehyde produced by *Bacillus amyloliquefaciens* was effective against *Fusarium oxysporum* and *Verticillium dahliae*, both soil-borne fungal pathogens. Benzaldehyde affects the fungi by disrupting the cell membranes of the fungi, leading to their death (Li and Zhang, 2014).

### **3.4.3 Methyl-2,3,4-trichloroanisole (MTCA)**

Methyl-2,3,4-trichloroanisole (MTCA) is a unique VOC produced by certain *Bacillus* strains and act as antagonist compound for many fungi. MTCA produced by *Bacillus subtilis* CA32 inhibite the growth of *Botrytis cinerea* and *Sclerotinia sclerotiorum*, which are responsible for gray mold and white mold, respectively. MTCA interferes with the metabolic processes of these fungi, thereby controlling their growth (Romero *et al.* 2010; Wang and Zhang, 2016).

### **3.4.4 Dimethyl Disulfide (DMDS)**

Dimethyl disulfide (DMDS) is a sulfur-containing VOC with strong antimicrobial properties. In a study by DMDS produced by *Bacillus velezensis* was shown to inhibit the growth of *Ralstonia solanacearum*, the bacterium responsible for bacterial wilt in tomatoes. The study demonstrated that DMDS disrupts the integrity of the bacterial cell membrane, leading to cell lysis and death (Tahir *et al.* 2017; Zhang and Liu, 2019).

### **3.4.5 Hydrogen Cyanide (HCN)**

Hydrogen cyanide (HCN) is a well-known antimicrobial VOC produced by several *Bacillus* spp. reported that HCN produced by *Bacillus amyloliquefaciens* SQR9 effectively controlled the fungal pathogen *Rhizoctonia solani*, which causes root rot in various crops. The study highlighted that HCN production by *Bacillus* spp. significantly reduced the incidence of root rot disease in treated plants (Wanget *al.* 2013; Zhou and Zhang, 2014). HCN binds to the iron in the cytochrome c oxidase enzyme, a critical component of the electron transport chain in aerobic respiration. This blocks oxygen utilization, effectively halting ATP

production, which is essential for pathogen survival and growth (Blachly-Dyson and Forte, 2001). Additionally, it also changes the permeability of cell membrane leading to ion leakage and loss of homeostasis (Ahmad et al., 2008; Rijavec, T., and Lapanje, A., 2016).

### **3.4.6 Ammonia**

Ammonia is a simple VOC with potent antimicrobial properties. Research by Zhang *et al.* (2019) indicated that ammonia produced by *Bacillus subtilis* B26 was effective against *Alternaria alternata*, a pathogen responsible for leaf spot diseases. The study suggested that ammonia inhibits spore germination and mycelial growth of the pathogen, thereby diminishing disease severity.

## **3.5 Induced Systemic Resistance by *Bacillus* spp.**

Induced systemic resistance (ISR) is a defense mechanism in plants activated by certain beneficial microbes, including *Bacillus* species. ISR enhances the plant's innate capacity to defend against a wide range of pathogens (Van Loon *et al.*, 1998). It is major phenomena induced in plant results in production of various biochemical and structural changes result in reduced disease infestation in host plants. *Bacillus subtilis* is a widely studied beneficial rhizobacterium known for its ability to induce systemic resistance (ISR) in plants. ISR is a defense mechanism where plants enhance their innate immunity upon encountering beneficial microbes, making them more resilient to a broad spectrum of pathogens (Choudhary and Johri, 2009)).

### **3.5.1 Mechanisms of ISR Induction**

ISR involves the activation of several signaling pathways, primarily the jasmonic acid (JA) and ethylene (ET) pathways. *Bacillus* spp. trigger ISR by producing elicitors that interact with plant receptors, leading to the activation of these pathways. This process results in the expression of defense-related genes and the accumulation of defensive compounds, which enhance the plant's resistance to pathogens (Pieterse *et al.*, 2014).

#### **3.5.1.1 *Bacillus subtilis* and Induced Systemic Resistance (ISR)**

Numerous studies have highlighted the capacity of *Bacillus subtilis* to induce ISR. Kloepper *et al.* (2004) demonstrated that *Bacillus subtilis* GB03 activates ISR in *Arabidopsis*, enhancing its resistance to *Pseudomonas syringae* pv. *tomato*, the pathogen responsible for bacterial speck. The research revealed that treatment with *B. subtilis* GB03 led to the upregulation of defense genes linked to the jasmonic acid (JA) and ethylene (ET) signaling pathways.

#### **3.5.1.2 *Bacillus amyloliquefaciens* and ISR**

*Bacillus amyloliquefaciens* is recognized for its ability to induce ISR as well. A study by Choudhary and Johri (2009) found that *B. amyloliquefaciens* FZB42 stimulates systemic resistance in tomato plants against *Fusarium oxysporum* f. sp. *lycopersici*, the pathogen causing *Fusarium* wilt. This research indicated that the ISR triggered by *B. amyloliquefaciens* FZB42 involved the activation of defense-related enzymes, including peroxidase and polyphenol oxidase.

### **3.5.1.3 *Bacillus pumilus* and ISR**

*Bacillus pumilus* has also been shown to promote ISR in plants. *Bacillus pumilus* SE34 induces systemic resistance in cucumbers against *Pseudomonas syringae* pv. *lachrymans*, the cause of angular leaf spot. Their study suggested that the ISR induction by *B. pumilus* SE34 correlated with an increase in reactive oxygen species (ROS) and enhanced activity of defense enzymes such as chitinase and  $\beta$ -1,3-glucanase (Ryu *et al.* 2007).

### **3.5.1.4 Synergy of ISR and direct antagonism**

The combination of ISR induction and direct antagonistic effects on pathogens enhances the biocontrol efficacy of *Bacillus* species (Zhou *et al.*, 2020; Poveda *et al.*, 2020). Ongena *et al.* (2005) found that *Bacillus subtilis* M4 not only induces ISR in tomato plants against *Botrytis cinerea* but also produces antimicrobial compounds that directly inhibit the pathogen. This dual approach led to a significant reduction in gray mold symptoms in the treated plants.

### **3.5.1.5 Metabolites Involved in ISR**

Specific metabolites produced by *Bacillus* species play a vital role in ISR induction. Choudhary and Johri (2009) identified cyclic lipopeptides such as surfactin, fengycin, and iturin as key elicitors of ISR produced by *Bacillus*. These compounds engage with plant receptors to initiate defense signaling pathways, resulting in enhanced resistance to various pathogens (Ongena and Jacques, 2008; Wu *et al.*, 2018).

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