Unleashing Bacillus Lipopeptides: A Powerful Ally against Plant Fungal Pathogens

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ABSTRACT

Plant fungal pathogens significantly threaten global agriculture, leading to major crop losses. *Bacillus*-produced lipopeptides, including **surfactins, iturins**, and **fengycins,** offer an eco-friendly solution to plant fungal pathogens by disrupting fungal membranes, inhibiting biofilms, and enhancing plant immunity. Used in seed treatments, foliar sprays, and soil amendments, these biodegradable, non-toxic compounds control diseases like *Fusarium* wilt and anthracnose, reducing reliance on chemical fungicides. While challenges like field variability and formulation stability exist, advances in biotechnology are driving their broader adoption, supporting sustainable agriculture and environmental health.

INTRODUCTION

The growing focus on sustainable pest management has underscored the importance of biopesticides, especially those derived from *Bacillus* species. Renowned for their safety and efficacy, *Bacillus*-based products like Serenade® (*Bacillus subtilis*) and Sonata® (*Bacillus pumilus*) constitute about 74% of commercial biopesticides, effectively targeting pathogens like *Fusarium*, *Rhizoctonia*, and *Botrytis* *cinerea*. For example, Serenade ® achieved up to 100% control of *Botrytis cinerea* in lettuce, primarily due to its production of antifungal lipopeptides. Lipopeptides amphiphilic molecules comprising peptide and lipid components—are central to *Bacillus* biocontrol mechanisms. Iturins, fengycins, and surfactins disrupt fungal membranes, inhibit biofilm formation, and enhance plant defenses through induced systemic resistance (ISR). Their stability, coupled with advancements in biosynthesis, makes them vital tools in integrated disease management (IDM). This review highlights their biosynthesis, regulation, and pivotal role in combating fungal pathogens, promoting sustainable agriculture and environmental conservation (Ruiz *et al*., 2020).

1. Biosynthesis of lipopeptides in *Bacillus*

The genus *Bacillus* includes 423 Gram-positive, rod-shaped species known for producing lipopeptides with antimicrobial and surfactant properties. Lipopeptide biosynthesis in *Bacillus* occurs via non-ribosomal peptide synthetases (NRPS), large enzyme complexes that assemble lipopeptides by incorporating amino acids and fatty acids without mRNA templates. This process results in diverse compounds like **Iturins**, **Fengycins**, and **Surfactins**. Synthesized during the stationary phase, their production is regulated by factors such as quorum sensing, nutrient availability, and stress conditions, enabling *Bacillus* to produce bioactive molecules with antimicrobial and antifungal properties. As previously mentioned, lipopeptide biological control of phytopathogens may be supported by the following interactions (**Fig. 1**) (Ruiz *et al*., 2024). *Bacillus* lipopeptides play a key role in biological control through three main interactions: (A) establishment of the bacterium in biofilm or microcolonies on plant roots, (B) direct antibiosis against pathogens in the same environment, and (C) signaling for plant defense activation. These processes contribute to fungal growth inhibition and leaf disease reduction upon **inoculation (Fig 2)** (Ongena *et al*., 2008)**.**

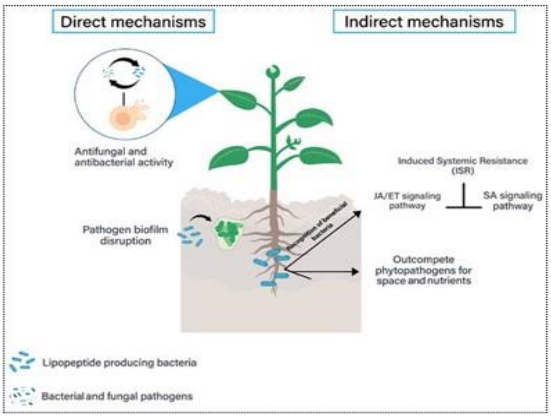

Fig 1. Direct and Indirect Roles of Lipopeptides in Phytopathogen Control

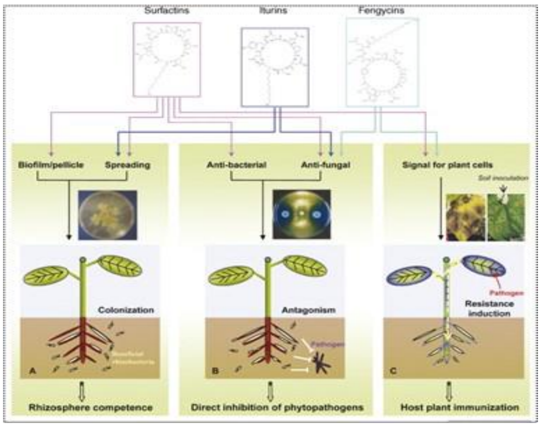

Fig 2. Mechanisms of various *Bacillus* Lipopeptides in Biocontrol

Implication of Lipopeptides in plant tissue colonization

Plants release root exudates that attract Bacillus species through chemotaxis, aiding colonization in the rhizosphere. Lipopeptides (LPs) like surfactins play a key role in biofilm formation and root attachment. Surfactins are essential for stable biofilm formation and effective biocontrol, as seen in *Bacillus subtilis* on Arabidopsis roots. Additionally, LPs like surfactin and mycosubtilin contribute to bacterial motility by reducing surface tension, facilitating the spread of bacteria across root surfaces through swarming. This motility is crucial for establishing bacterial colonies in nutrient-rich root areas (Ongena *et al*., 2008).

Role of Lipopeptides in Direct Antagonism Against Phytopathogens

Once established in the phytosphere, *Bacillus* isolates deploy their antibiotic arsenal, including lipopeptides (LPs), which aid in biocontrol. Surfactins help with root colonization and control *Pseudomonas syringae* in Arabidopsis, though their exact mechanism remains unclear. Iturins and fengycins also contribute to biocontrol, with iturin A controlling *Rhizoctonia solani* in tomatoes and mycosubtilin reducing seedling infection by *Pythium aphanidermatum*. In the phyllosphere, LPs inhibit pathogen growth, such as *Podosphaera fusca* on melon leaves and *Botrytis cinerea* on apple fruits. Bacillus strains producing all three LP families influence both pathogens and other rhizosphere microbes. Over the past 15 years, the list of bacteria identified as Induced Systemic Resistance (ISR) inducers has expanded significantly, including Gram-negative bacteria from genera like *Pseudomonas* and *Serratia*, as well as Gram-positive bacteria, particularly *Bacillus* species (Table 1) (Ongena *et al*., 2008; Sánchez *et al*., 2016).

Table 1. List of *Bacillus* Strains Reported as Inducers of Plant Systemic Resistance and Their Associated Pathosystems (Ongena *et al*., 2008).

Species and Strain	Pathosystem
Bacillus subtilis	
GBO3	Cotton / *Meloidogyne*; Cucumber / *Erwinia*, beetle; Arabidopsis / Erwinia
IN937b	Tomato / *Cucumber mosaic cucumovirus* (CMV); *Tomato mottle virus* (TMV)
S499	Cucumber / *Colletotrichum*; Tomato / Pythium; Bean / *Botrytis*
FZB-G	Tomato / *Fusarium*
BacB	Sugar beet / *Cercospora*
Bacillus pumilus	
SE34	Tobacco / *Peronospora; Arabidopsis / Pseudomonas; Cucumber* / beetle; Tomato / *Fusarium, Phytophthora*, CMV, TMV
T4	Tobacco / *Pseudomonas*; *Arabidopsis / Pseudomonas*
INR-7	Loblolly pine / **Cronartium**; Cucumber / beetle
203–6	Sugar beet / *Cercospora*
Bacillus amyloliquefaciens	
IN937	Tomato / CMV, TMV; Cucumber / beetle; Arabidopsis / *Erwinia*
EXTN-1	Tobacco / *Pepper mild mottle virus (*PMV); Cucumber / *Colletotrichum*; Arabidopsis / PMV
Bacillus thuringiensis	Berliner: Coffee / *Hemileia*
Bacillus mycoides	BacJ: Sugar beet / *Cercospora*
Bacillus pasteurii	C-9: Tobacco / *Peronospora*
Bacillus sphaericus	B43: Potato / nematode
Bacillus cereus	B1: White clover / nematode

Ecological Fitness of *Bacillus* in Rhizosphere: Impact of Nutrient and Environmental Factors on Biocontrol Metabolite Production

The ecological fitness of *Bacillus* in rhizosphere-based biocontrol strategies depends on various factors like nutrient availability, microbial community complexity, and soil conditions (e.g., pH, temperature, oxygen). In vitro studies have shown that carbon, nitrogen sources, and iron affect the production of biocontrol metabolites, such as surfactins, fengycins, and iturins. Nutrient-starved states in the rhizosphere can limit bacterial growth and modulate antibiotic gene expression. Quorum sensing mechanisms, biofilm formation, and regulatory pathways, such as those involving CodY, DegU, and AbrB, influence the production of these secondary metabolites, which are essential for *Bacillus*' biocontrol activity (Sani *et al*., 2024).

Concluding remarks and future perspectives

Bacillus-derived lipopeptides, such as surfactins, iturins, and fengycins, present a promising eco-friendly alternative for controlling plant fungal pathogens. These compounds effectively inhibit fungal growth, disrupt biofilms, and enhance plant resistance, making them valuable in sustainable agricultural practices. Their use in managing diseases like *Fusarium* wilt and anthracnose offers a significant reduction in reliance on chemical fungicides. Future efforts should focus on optimizing the large-scale production and stability of *Bacillus* lipopeptides. Enhancing biotechnological methods, improving formulations, and conducting comprehensive field trials are crucial for overcoming current limitations. Exploring the combined use of lipopeptides with other biocontrol agents and evaluating their long-term environmental impact will be key to integrating these compounds into sustainable agricultural practices, ensuring both plant protection and environmental health.

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