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Cyclic Lipopeptides from *Bacillus amyloliquefaciens* PPL: Antifungal Mechanisms and Their Role in Controlling Pepper and Tomato Diseases

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ABSTRACT

Plant diseases caused by fungal pathogens pose a significant threat to global crop production, particularly in economically valuable crops like pepper and tomato. Biological control using microbial agents has emerged as a sustainable alternative to chemical fungicides, with Bacillus amyloliquefaciens PPL receiving attention for its potent antifungal activity. This review focuses on the cyclic lipopeptides (CLPs) produced by the B. amyloliquefaciens PPL strain, particularly the iturin and fengycin families. These CLPs play a crucial role in suppressing fungal pathogens, such as Colletotrichum (pepper anthracnose) and Fusarium oxysporum (tomato wilt), through membrane disruption and inhibition of fungal growth. Furthermore, we discuss the mechanisms underlying the antifungal activity of CLPs, the role of lecithin in enhancing production, and the potential of fengycin isoforms (F1, F2, F3) in disease management. Future perspectives on integrating B. amyloliquefaciens PPL and CLPs into sustainable agricultural practices are also presented.

Keywords: *Cyclic lipopeptides, Bacillus amyloliquefaciens, Antifungal activity, Pepper anthracnose, Tomato Fusarium wilt, Iturin, Fengycin, Sustainable disease management*

1. Introduction

Fungal diseases are among the most significant biotic stresses that affect global agricultural productivity, causing severe losses in food production and economic stability. Two widely cultivated crops, *pepper* (*Capsicum annuum*) and tomato (*Solanum lycopersicum*), are particularly vulnerable to destructive fungal pathogens [1]. These crops hold immense economic and nutritional importance worldwide, with their cultivation supporting the livelihoods of millions of small-scale and commercial farmers. However, the increasing prevalence of diseases such as anthracnose in pepper and *Fusarium wilt* in tomato has emerged as a major concern for growers, threatening both yield and crop quality [2]. Anthracnose, caused by *Colletotrichum* species, is one of the most widespread fungal diseases in pepper, leading to severe fruit rot, significant yield losses, and post-harvest damage. Similarly, *Fusarium wilt* caused by *Fusarium oxysporum f. sp. lycopersici* is a soilborne pathogen that infiltrates tomato plants through root systems, blocks vascular tissues, and causes systemic wilting, ultimately resulting in plant death. The management of these pathogens has relied heavily on the use of chemical fungicides, which have been a primary line of defense for decades. Although effective in the short term, the excessive and indiscriminate use of chemical fungicides has raised several concerns, including environmental contamination, residue accumulation in food, non-target toxicity, and the emergence of resistant fungal strains. These factors underscore the urgent need for sustainable and eco-friendly alternatives for disease management in agriculture. In recent years, biological control using beneficial microorganisms has emerged as a promising and environmentally sustainable approach for controlling plant diseases [3]. Among the microbial agents explored, members of the *Bacillus* genus, particularly *Bacillus amyloliquefaciens*, have received significant attention for their ability to suppress plant pathogens and promote crop growth. *Bacillus amyloliquefaciens* is a Gram-positive, spore-forming bacterium widely distributed in the soil, rhizosphere, and plant surfaces. It has been recognized as a potent biocontrol agent due to its multifaceted mechanisms of action, including competition for nutrients, production of antibiotics, induction of plant systemic resistance, and the secretion of bioactive secondary metabolites.

A major class of secondary metabolites produced by *Bacillus amyloliquefaciens* is cyclic lipopeptides (CLPs), which are amphiphilic compounds with a peptide ring and lipid tail. CLPs exhibit a wide range of biological activities, including antibacterial, antifungal, antiviral, and surfactant properties. These biosurfactants are classified into three main families: iturins, fengycins, and surfactins, each of which plays a distinct role in microbial antagonism.

Among these, iturins and fengycins have been extensively studied for their antifungal properties against plant pathogens [4]. Iturins are known to interact with fungal cell membranes, causing pore formation and subsequent leakage of intracellular contents, which leads to fungal cell death. On the other hand, fengycins exhibit a strong affinity for ergosterol, a critical component of fungal membranes, disrupting membrane integrity and inhibiting fungal growth. Studies have shown that these lipopeptides not only control fungal diseases but also induce plant resistance against multiple pathogens [5]. *Bacillus amyloliquefaciens* strains, such as the PPL strain, are capable of producing multiple isoforms of fengycins (e.g., F1, F2, F3), which exhibit varying levels of antifungal activity.

The *Bacillus amyloliquefaciens* PPL strain, specifically, has demonstrated remarkable efficacy in controlling anthracnose in pepper and Fusarium wilt in tomato through the production of CLPs. *In vitro* and *in vivo* studies have revealed that the iturin A fraction from the PPL strain exhibits higher antifungal activity against *Colletotrichum* species (responsible for pepper anthracnose), while the control of *Fusarium oxysporum* in tomato is largely attributed to the fengycin family, particularly the F1 isoform. Such findings highlight the versatility and specificity of CLPs in targeting different fungal pathogens [6]. Furthermore, studies have shown that the production of lipopeptides can be enhanced through nutritional amendments, such as lecithin supplementation in culture media. Lecithin, a phospholipid, serves as a precursor for the biosynthesis of CLPs, leading to increased yield and antifungal efficacy. This optimization of lipopeptide production enhances the potential of *Bacillus amyloliquefaciens* PPL as a reliable biocontrol agent for field applications. Given the challenges associated with chemical fungicides, the application of *Bacillus amyloliquefaciens* PPL and its CLPs offers a sustainable and eco-friendly solution for disease management in pepper and tomato cultivation. The ability of CLPs to effectively suppress fungal pathogens, coupled with their environmental safety and biodegradability, makes them ideal candidates for integrated pest management (IPM) strategies. However, despite the promising potential of CLPs, challenges such as large-scale production, formulation stability, and field efficacy under varying environmental conditions need to be addressed to facilitate their commercial application [7]. This review aims to provide a comprehensive overview of the antifungal mechanisms and agricultural applications of cyclic lipopeptides produced by *Bacillus amyloliquefaciens* PPL. Specifically, we focus on the role of *iturins* and *fengycins* in controlling pepper anthracnose and tomato Fusarium wilt, the optimization of lipopeptide production, and the identification of key isoforms with enhanced antifungal properties. Additionally, we discuss future perspectives on integrating CLP-based biocontrol strategies into sustainable agricultural systems to reduce the reliance on chemical fungicides and improve crop resilience to fungal diseases.

Table 1: Classification and Key Characteristics of CLPs

CLP Family	Members	Structure	Primary Function	Mechanism of Action
Iturin Family	Iturin A, B, C, Mycosubtilin	Cyclic peptide with lipid tail	Strong antifungal activity	Pore formation in fungal membranes, leading to cell leakage
Fengycin Family	Fengycin A, B (F1, F2, F3)	Cyclic peptide with fatty acid	Antifungal against filamentous fungi	Binds ergosterol, disrupting membrane integrity
Surfactin Family	Surfactin A, B	Cyclic peptide with hydrophobic tail	Biosurfactant, antimicrobial activity	Disrupts surface tension; secondary antifungal role

Table 2: Comparison of CLP Families Based on Antifungal Efficacy

CLP Family	Target Pathogen	Mechanism	Efficiency (% Growth Inhibition)	Specific Roles
Iturin A	*Colletotrichum* spp.	Pore formation in fungal membranes	73–80%	Control of pepper anthracnose
Fengycin F1	*Fusarium oxysporum*	Ergosterol binding and disruption	High efficacy (against Fusarium wilt)	Inhibition of mycelial growth and spores
Fengycin F2, F3	*Fusarium* spp.	Membrane destabilization	Moderate efficacy	Supports synergistic action
Surfactin	General fungi	Surface tension reduction	Low antifungal activity	Assists other CLPs

Table 3: Antifungal Activity of CLPs Under Optimized Culture Conditions

CLP Type	Culture Condition	Yield (mg/L)	In Vitro Activity (%)	Pathogen Targeted
Iturin A	Lecithin supplementation	120–150	80%	*Colletotrichum* spp. (Anthracnose)
Fengycin (F1, F2, F3)	Lecithin supplementation	200–250	85%	*Fusarium oxysporum* (Fusarium wilt)
Surfactin	Basic culture media	50–80	40%	General fungi

Table 4: Antifungal Efficacy of CLPs in Pepper and Tomato

Crop	Pathogen	CLP Family	Efficacy (In Vitro) (%)	Efficacy (In Vivo) (%)
Pepper	*Colletotrichum* spp.	Iturin A	80%	73%
Tomato	*Fusarium oxysporum*	Fengycin (F1, F2, F3)	85%	75–80%

2. Cyclic Lipopeptides: Structure and Classification

Cyclic lipopeptides (CLPs) are a diverse group of amphiphilic biosurfactants synthesized non-ribosomally by various species of the *Bacillus* genus, including *Bacillus amyloliquefaciens*. These unique bioactive compounds are composed of a hydrophobic lipid tail and a cyclic peptide moiety, both of which are essential for their ability to interact with biological membranes. The amphiphilic nature of CLPs enables them to exhibit a wide range of biological activities, including antifungal, antibacterial, antiviral, and surfactant properties. By targeting and disrupting cell membranes, CLPs play a critical role in the suppression of plant pathogens, contributing to their potential as biocontrol agents in agriculture. Structurally, CLPs can be broadly classified into three major families: iturins, fengycins, and surfactins. Each family exhibits distinct structural characteristics and functional properties, with iturins and fengycins primarily responsible for antifungal activity, while surfactins contribute more significantly to surface tension reduction and biofilm formation.

2.1 Iturin Family

The iturin family consists of cyclic lipopeptides that include variants such as iturin A, iturin B, iturin C, and mycosubtilin. These CLPs are characterized by a β-amino fatty acid chain linked to a cyclic peptide moiety containing seven amino acid residues. The unique amphiphilic structure of iturins enables them to interact directly with fungal cell membranes, leading to pore formation and the subsequent leakage of intracellular contents. This mode of action results in the disruption of membrane integrity, causing cell lysis and death.

The high antifungal potency of iturins is attributed to their ability to bind to sterol components, such as ergosterol, in fungal membranes. This specific interaction makes iturins highly effective against a wide range of fungal pathogens, including species of *Colletotrichum* and *Fusarium*. In the case of *Bacillus amyloliquefaciens* PPL, the purified iturin A fraction has demonstrated superior antifungal activity (up to 73–80%) against *Colletotrichum* spp., which causes anthracnose in pepper plants. This highlights the crucial role of iturins in the biocontrol of fungal diseases.

2.2 Fengycin Family

The fengycin family comprises CLPs that include two major isoforms: fengycin A and fengycin B, with further subtypes such as F1, F2, and F3 isoforms. Fengycins consist of a β-hydroxy fatty acid tail attached to a decapeptide ring, making them structurally distinct from the iturin family. Unlike iturins, which target membrane sterols, fengycins exhibit strong antifungal activity by directly binding to ergosterol, a key component of fungal membranes. This interaction disrupts membrane integrity, alters permeability, and inhibits fungal growth, ultimately leading to cell death.

Among the fengycin isoforms, studies have shown that fengycin F1 possesses enhanced antifungal activity compared to other isoforms. For instance, *Bacillus amyloliquefaciens* PPL culture filtrates have been found to contain multiple fengycin isoforms (F1, F2, and F3), which collectively suppress the growth of *Fusarium oxysporum f. sp. lycopersici*, the causal agent of Fusarium wilt in tomato. In this context, purified fengycin F1 exhibited the most potent antifungal effect, highlighting the specificity and effectiveness of fengycins in managing fungal diseases.

Fengycins not only inhibit fungal pathogens but also exhibit low cytotoxicity to plants, making them ideal candidates for biocontrol applications. Additionally, their ability to induce systemic resistance in plants further enhances their utility in sustainable disease management programs.

2.3 Surfactin Family

The surfactin family is primarily recognized for its biosurfactant properties and antimicrobial activity. Structurally, surfactins are heptapeptides linked to a β-hydroxy fatty acid chain that forms a cyclic lactone ring. This configuration gives surfactins their remarkable surface activity, enabling them to lower surface tension and form micelles. As a result, surfactins play a critical role in enhancing the motility of *Bacillus* strains, facilitating root colonization, and promoting biofilm formation.

Although surfactins exhibit antimicrobial activity, their role in antifungal action is considered secondary compared to the iturin and fengycin families. Surfactins are believed to act synergistically with other CLPs, enhancing their overall efficacy against fungal pathogens. For example, surfactins contribute to the dispersion and solubilization of fengycins and iturins, improving their availability and delivery at the site of infection.

In addition to their antimicrobial properties, surfactins also have significant environmental benefits due to their biodegradability and low toxicity, making them suitable for use in eco-friendly agricultural practices.

2.4 Role of CLPs in Bacillus amyloliquefaciens PPL

In *Bacillus amyloliquefaciens* PPL, iturins and fengycins are the primary cyclic lipopeptides responsible for antifungal activity. These CLPs exhibit complementary mechanisms of action, allowing the PPL strain to target multiple fungal pathogens effectively. For example:

1. Iturins play a dominant role in controlling pepper anthracnose caused by *Colletotrichum* species by disrupting fungal membranes through pore formation.

2. Fengycins are more effective in managing tomato Fusarium wilt caused by *Fusarium oxysporum f. sp. lycopersici*, primarily through ergosterol-binding and membrane destabilization.

The ability of *Bacillus amyloliquefaciens* PPL to produce a diverse array of CLPs, including various fengycin isoforms, further enhances its biocontrol efficacy. Additionally, lecithin supplementation in culture media has been shown to significantly increase the yield and antifungal activity of CLPs, highlighting the potential for optimizing their production for field applications. Cyclic lipopeptides produced by *Bacillus amyloliquefaciens* represent a promising alternative to chemical fungicides for the sustainable management of fungal diseases in crops. By understanding the structure, classification, and antifungal mechanisms of iturins, fengycins, and surfactins, we can harness their full potential in controlling devastating plant pathogens such as *Colletotrichum* and *Fusarium*. The specific role of each CLP family, particularly iturins and fengycins, in *Bacillus amyloliquefaciens* PPL underscores their importance as eco-friendly biocontrol agents for protecting pepper and tomato crops.

3. Mechanisms of Antifungal Action

The antifungal activity of cyclic lipopeptides (CLPs) produced by *Bacillus amyloliquefaciens* PPL arises from their ability to disrupt fungal cell membranes, interfere with key cellular processes, and prevent fungal growth. These mechanisms operate in a multi-faceted manner, enabling the PPL strain to effectively combat a variety of plant pathogenic fungi.

The unique amphiphilic structure of CLPs—comprising a hydrophobic lipid tail and a hydrophilic cyclic peptide moiety—allows them to target fungal membranes and cellular functions selectively.

3.1. Membrane Disruption

Membrane disruption is one of the primary antifungal mechanisms of CLPs. Due to their amphiphilic nature, CLPs interact directly with fungal cell membranes, leading to structural destabilization and eventual cell death.

• **Iturin Family**: Iturins are known to cause pore formation in fungal membranes. The lipid tail of iturins integrates into the phospholipid bilayer, creating transmembrane pores that disrupt membrane integrity. This results in the leakage of ions, nutrients, and other essential intracellular contents, leading to cell lysis.

o For example, the iturin A fraction purified from *Bacillus amyloliquefaciens* PPL exhibited 73–80% antifungal activity against *Colletotrichum* spp., the causal agent of pepper anthracnose.

• **Fengycin Family**: Fengycins exhibit a more targeted mode of action by specifically binding to ergosterol, a vital sterol component of fungal cell membranes. Ergosterol is essential for maintaining the structural integrity and fluidity of fungal membranes.

o Fengycins disrupt the membrane by altering lipid organization, leading to membrane destabilization, loss of cellular function, and inhibition of fungal growth.

o This mode of action is particularly effective against *Fusarium oxysporum* in tomato plants, where the fengycin F1 isoform showed the highest antifungal activity.

The selectivity of CLPs for fungal membranes over plant or animal membranes is due to differences in membrane composition, making CLPs effective yet safe antifungal agents for agricultural applications.

3.2. Inhibition of Fungal Growth

Beyond direct membrane disruption, CLPs, particularly fengycins, exhibit inhibitory effects on key fungal processes, such as spore germination and mycelial growth. This inhibition significantly impairs the ability of fungi to colonize host plants and cause disease.

• **Fengycin Isoforms**: Fengycin isoforms (F1, F2, F3) produced by *Bacillus amyloliquefaciens* PPL disrupt essential cellular processes within fungi.

o They inhibit spore germination by interfering with fungal energy metabolism, particularly by reducing ATP production and mitochondrial function.

o Fengycins also suppress mycelial growth by targeting enzymatic pathways involved in fungal cell wall synthesis and cytoskeletal organization.

o For example, purified fengycin F1 exhibited stronger inhibition of *Fusarium oxysporum* growth compared to other isoforms, demonstrating its specificity and potency.

By impairing fungal growth at multiple stages, fengycins reduce pathogen virulence and enhance the overall biocontrol efficiency of the PPL strain.

3.3. Synergistic Action

The synergistic interaction between different families of CLPs further enhances their antifungal efficacy. In *Bacillus amyloliquefaciens* PPL, iturins and fengycins work together to provide broad-spectrum antifungal activity against plant pathogens.

• **Iturins** primarily disrupt fungal membranes through pore formation, while fengycins complement this activity by binding to ergosterol and destabilizing the membrane. This combination results in greater membrane damage and accelerated fungal cell death.

• Surfactins, though not as potent in antifungal activity, enhance the availability and dispersion of iturins and fengycins at the site of fungal infection. Surfactins act as biosurfactants, reducing surface tension and promoting the penetration of CLPs into fungal biofilms or tissue.

The combined activity of these CLP families allows *Bacillus amyloliquefaciens* PPL to effectively target fungal pathogens that may exhibit resistance to single mechanisms of action. Such synergistic interactions are particularly beneficial for managing complex diseases, such as anthracnose in pepper and Fusarium wilt in tomato, where multiple fungal species or resistant strains may coexist. The antifungal mechanisms of CLPs, including membrane disruption, inhibition of fungal growth, and synergistic interactions, highlight the versatility and effectiveness of *Bacillus amyloliquefaciens* PPL as a biocontrol agent. By targeting fungal membranes and cellular processes simultaneously, CLPs not only suppress plant pathogens but also minimize the risk of resistance development. The ability of iturins, fengycins, and surfactins to work together ensures broad-spectrum activity, making *B. amyloliquefaciens* PPL an ideal candidate for sustainable disease management in crops like pepper and tomato.

4. Applications in Controlling Pepper and Tomato Diseases

4.1. Control of Pepper Anthracnose

Pepper anthracnose, caused by *Colletotrichum spp.*, is a major fungal disease leading to fruit rot and yield losses. In vitro and in vivo studies have demonstrated that the iturin A fraction from *B. amyloliquefaciens* PPL exhibits significant antifungal activity (73–80%) against *Colletotrichum*. Iturins disrupt the fungal membrane, inhibiting spore germination and reducing lesion development on pepper fruits.

4.2. Control of Tomato Fusarium Wilt

Fusarium wilt, caused by *Fusarium oxysporum*, affects tomato plants by blocking vascular tissues, leading to wilting and death. Fengycin lipopeptides produced by *B. amyloliquefaciens* PPL are primarily responsible for controlling Fusarium wilt. LC-MS analysis revealed that fengycin isoforms (F1, F2, F3) exhibit varying degrees of antifungal activity, with fengycin F1 showing the highest efficacy.

4.3. Enhancing CLP Production with Lecithin Supplementation

Studies have shown that lecithin supplementation enhances the yield and production of fengycin and iturin lipopeptides in the

culture broth of *B. amyloliquefaciens* PPL. This optimization improves the biocontrol efficiency against fungal pathogens in agricultural settings.

5. Future Prospects and Challenges

The potential of *B. amyloliquefaciens* PPL and its CLPs in sustainable agriculture is immense. However, several challenges must be addressed:

- **Field Stability**: Enhancing the stability and persistence of CLPs under field conditions.
- **Formulation Development**: Developing cost-effective formulations for large-scale application.
- **Regulatory Approvals**: Ensuring safety and compliance with regulations for commercial use.
- **Pathogen Resistance**: Monitoring and mitigating the risk of fungal resistance to CLPs.

Future research should focus on genetic and metabolic engineering approaches to optimize CLP production and explore their synergistic effects with other biocontrol agents.

6. Conclusion

Cyclic lipopeptides (CLPs) from *Bacillus amyloliquefaciens* PPL, especially the iturin and fengycin families, provide an eco-friendly and effective approach for managing fungal diseases in economically important crops like pepper and tomato. The potent antifungal mechanisms of CLPs, including membrane disruption, inhibition of fungal growth, and synergistic activity, make them valuable alternatives to chemical fungicides. The enhanced production of CLPs through optimized culture conditions, such as lecithin supplementation, further improves their efficiency and yield. Notably, the specific activity of fengycin isoforms (F1, F2, F3) against *Fusarium* species and the superior antifungal performance of iturin against anthracnose emphasize their versatility. Integrating CLP-based biocontrol strategies into current pest and disease management programs can minimize environmental hazards, reduce the risk of pathogen resistance, and promote sustainable agriculture. *Bacillus amyloliquefaciens* PPL and its CLPs thus represent a promising tool for ensuring agricultural productivity while maintaining environmental balance.

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