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A Review on Biocontrol Efficacy of *Pseudomonas* Against Phytopathogens

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Abstract:
Plant diseases caused by a group of phytopathogens like bacteria, viruses, nematodes, and fungi pose a severe challenge to global agriculture by reducing crop quality and yield. Due to the impact on the environment and resultant health risks, the conventional reliance on chemical pesticides for disease regulation has led to issues. The *Pseudomonas'* ability to produce a diverse range of antagonistic compounds, they have emerged as a potential biocontrol agent as an environmentally friendly and sustainable alternative. Aside from inhibiting phytopathogens, these systems promote plant growth and induce systemic resistance. This review discusses *Pseudomonas'* potential for biocontrol, emphasizes the impact of phytopathogens on crops, and summarizes key studies demonstrating their effectiveness against various plant diseases. Generally, integrated plant disease management and sustainable agriculture hold much promise with *Pseudomonas*-based biocontrol methods.

Keywords: Pseudomonas, Biocontrol, Phytopathogens, Antimicrobial compounds.

1. Introduction:

Plant diseases decrease crop production by weakening plant growth and development and reduces crop quality under pre-harvest and post-harvest conditions there by hindering the agricultural progress and food security (Omran and Baek, 2022). Plant diseases can be grouped into four classes according to the type of pathogen nematode, bacterial, viral, and fungal diseases (Harveson, 2015). Numerous factors influence the incidence and prevalence of plant diseases, such as the host plant varieties, the type pathogens, virulence, environmental factors and management practices (Gai and Wang, 2024). To manage plant disease, various chemical pesticides negatively impact living organisms and the environment (El-Saadony *et al*., 2022).
In an effort to mitigate these effects, alternative disease management stratergies are being employed globally. Among these, the biocontrol approach is safe, eco-friendly and sustainable approach for the disease management. These strategies are employed by microbial based biocontrol agents to shield plants from pathogen infections. Through direct or indirect interactions with the pathogen, they may employ a single or a combination of strategies to prevent or minimize plant disease. The biocontrol

agent secretes antimicrobial chemicals and can engage in indirect interactions with the pathogen, regardless of its pathogenicity while posing competition for nutrients and space (Bonaterra *et al.,* 2022). The number of bacterial species, such as *Bacillus, Pseudomonas, Serratia, Rhizobium, Xanthomonas, Streptomyces, Enterobacter, Agrobacterium, Erwinia, Alcaligenes, Stenotrophomonas*, and *Arthrobacter*, have shown antimicrobial activity (Riseh *et al.,* 2024).

Among biocontrol strategies the use of bacterial-based biocontrol mechanism has proven effective. Among these *Pseudomonas* species produce a variety of metabolites such as siderophores, hydrolytic enzymes, hydrogen cyanide, and phenazines, they have shown antagonistic qualities against a variety of phytopathogens (Arseneault and Filion, 2016; Kabdwal *et al.,* 2023). The majority of *Pseudomonas* biocontrol agents have been isolated from soil and rhizospheres region. They are effective in controlling soilborne diseases by inducing systematic resistance and producing secondary metabolites (Bakli and Zenasni, 2019; Höfte, 2021; Iqbal *et al.,* 2023). The objective of this review is to understand the biocontrol aspects of *pseudomonas* against phytopathogens.

Numerous microbes, including bacteria, fungi, viruses, and nematodes are responsible for plant diseases. These diseases affect crop development, lower the quality of yield, and result in crop losses. (Parrado and Quintanilla, 2024). Although plants have natural cellular immunity, certain phytopathogens can neutralize these defences by enzymatic action (Nazarov *et al.,* 2020). The emergence of new diseases poses a serious threat to global crop output in the modern era of agriculture. The quality and quantity of agricultural products can be severely impacted by these illnesses, which are brought about by nematodes, bacteria, viruses, and fungi. Plant diseases have historically been a persistent problem in the agricultural sector; however, the globalization and climate change have accelerated the occurrence and spread of novel illnesses. Food security and economic stability are seriously hampered by the aforementioned development, particularly in regions heavily dependent on agriculture (Oraon *et al.,* 2024). The following table outlines major plant pathogens, their types, the crops they affect, the diseases they cause, associated symptoms, and relevant literature references

Table 1: Phytopathogens causing different diseases in plants.

S no.	Pathogens	Type	Affected Crops	Disease Caused	Symptoms	Reference
1	*Fusarium oxysporum*	Fungal	Tomato, Banana, Cotton, Pea.	Fusarium Wilt	Wilting, yellowing, vascular browning	Mostert *et al.,* 2017
2	*Phytophthora infestans*	Oomycete	Potato, Tomato	Late blight	Water-soaked lesions, leaf necrosis	Yuen, 2021.
3	*Rhizoctonia solani*	Fungal	Rice, Potato Soyabean	Root Rot	Stem and root lesions, stunted growth	Rahman *et al.,* 2020

4	*Pythium spp*	Oomycete	Various vegetables, cereals	Damping-off	Seedling death, root-rot	Khrieba, 2020.
5	*Alternaria solani*	Fungal	Tomato, Potato	Early Blight	Brown leaf spots.	Schmey *et al.,* 2024
6	*Xanthomonas campestris*	Bacterial	Cabbage, Rice	Bacterial Leaf Spot, Canker	Yellow halos, water soaked lesions	MANASA, 2019.
7	*Collectrotrichum spp*	Fungal	Banana, Mango	Anthracnose	Dark sunken lesions, fruit rot	*Huang et al.,* 2021
8	*Erwinia amylovora*	Bacterial	Apple, Pear	Fire Blight	Wilting, blackening of shoots	Myung *et al.,* 2016
9	*Aspergillus niger*	Fungal	Onion, Groundnuts	Black mould	Black spores on infected plant parts, toxin production (mycotoxins)	Pudake, 2018
10	*Botrytis cinerea*	Fungal	Grapes, Tomato	Gray Mould	Gray spores, fruit rot	Rhouma, 2023

2. *Pseudomonas* and it's antimicrobial compounds:

Pseudomonas acts as an effective biocontrol agent, as it produces a variety of metabolites such as pyoluteorin, pyrrolnitrin, pyocyanin, 2,4-diacetylphloroglucinol (DAPG), hydrogen cyanide, siderophores, hydrolytic enzymes, phenazine-1-carboxylic acid (PCA) and rhamnolipids (Panpatte *et al.,* 2016; Clough *et al.,* 2022; Abo-Zaid *et al.,* 2023). These compounds exert direct toxicity on plant pathogens by disrupting cell membranes, inhibiting respiratory enzymes, and interfering with vital metabolic processes. Their broad-spectrum activity makes them key agents in the suppression of various soilborne and foliar pathogens, contributing significantly to the biocontrol potential of *Pseudomonas* in agricultural systems (Chandurkar *et al.,* 2017; Lia *et al.,* 2022)

The most studied phenomenon of rhizobacteria is their antagonistic or competitive interactions which contribute to their biocontrol activity. Through a variety of mechanisms, including physical displacement of the pathogen, siderophore secretion to prevent the spread of nearby pathogens, synthesis of antibiotics, bacteriocins, and other minor molecules that inhibit pathogen growth, production of enzymes and induction of systemic resistance in the plants they help control the damage caused by pathogens to plants (Sorathiya *et al.,* 2023). An inexpensive, efficient and environmentally acceptable way to manage crop diseases is the biological control of plant pathogens by antagonistic microorganisms. Due to the negative effects of chemical pesticides, the use of biological control agents as a substitute for fungicides is growing quickly in modern agriculture (Ghadamgahi *et al.,* 2022).

2.1. Pyoluteorin:

Pseudomonas species produce pyroluteorin, a chlorinated polyketide antibiotic with strong antifungal ac tion against a range of phytopathogens (Zhang *et al.,* 2020). Recent research has demonstrated its critical role in plant-microbe interactions and biocontrol, where pyoluteorin affects the dynamics of microbial communities in the rhizosphere, in addition to suppressing fungal diseases (Zhao *et al.,* 2024). The regulatory networks controlling the biosynthesis of pyoluteorin have been clarified by advances in genomes and metabolomics. These networks exhibit intricate interactions with other secondary metabolites, including 2,4-diacetylphloroglucinol (DAPG), which collectively improve the ecological fitness of the strains that produce them (Yan *et al.,* 2017) The significance of pyoluteorin in integrated pest control and sustainable agriculture is highlighted by its dual function as a signaling molecule and an antifungal agent (Correa *et al.,* 2022).

A study investigated the capacity of sugarcane rhizobacteria from the rhizosphere to regulate red rot disease in two types of sugarcane (SPF-234 and Co-1148). In field studies, *Pseudomonas putida* strain NH-50 had the greatest efficacy, lowering disease severity by 44–60%. By using PltB gene amplification and HPLC analysis, it was determined that this strain produced the antibiotic pyoluteorin. In vitro, the antibiotics and metabolites also reduced the growth of *Glomerella tucumanensis,* which causes red rot in sugarcane (Hassan *et al.,* 2011).

2.2. Pyrrolnitrin:

Pyrrolnitrin is a halogenated antibiotic that is produced by *Pseudomonas* strains. With its broad-spectrum antifungal activity, pyrrolnitrin suppresses phytopathogenic fungi such as *Botrytis cinerea, Rhizoctonia solani*, and *Fusarium* spp. The potential of *Pseudomonas* species to function as efficient biocontrol agents, enhancing plant health by inhibiting soilborne illnesses, is frequently associated with their synthesis of pyrrolnitrin (Kenawy *et al.,* 2019). It works by interfering with fungal membrane integrity and disrupting mitochondrial respiration. *Pseudomonas* uses complicated control systems and quorum sensing mechanisms, to regulate the biosynthesis of pyrrolnitrin (Pawar *et al.,* 2019). The ecological fitness of *Pseudomonas* and its use in sustainable agriculture are greatly enhanced by pyrrolnitrin because of its strong antifungal qualities and stability in the soil environment (Fischer *et al.,* 2013).

The pathogen *Fusarium graminearum* causes *Fusarium* head blight in cereal crops, the strain of *Pseudomonas chlororaphis* showed that even in the absence of phenazine biosynthesis (via phz operon deletion), the strain still inhibited fungal pathogens, despite earlier studies highlighting the antifungal activity of phenazine-1-carboxamide and pyrrolnitrin against *F. graminearum*. Subsequent genetic and pharmacological investigations verified that the primary molecule exhibiting antifungal activity is pyrrolnitrin rather than phenazines, providing promising bioprotection against *Fusarium* head blight in cereal crops (Huang *et al.*, 2018).

2.3. Pyocyanin:

Pseudomonas aeruginosa is the primary producer of pyocyanin, a blue-green, redox-active phenazine pigment that is an essential virulence factor and quorum sensing (QS) signaling molecule. Two homologous operons, phz1 and phz2, are involved in its production, which starts with chorismic acid and is triggered by QS regulatory proteins. The enzymes PhzM and PhzS mediate the last stages of pyocyanin production, transforming phenazine-1-carboxylic acid into pyocyanin (Abdelaziz *et al.,* 2023). Pyocyanin functions by causing oxidative stress in host cells, interfering with electron transport chains, and reducing cellular respiration, which prevents the growth of bacteria, fungi, and other pathogens. Pyocyanin inhibits pathogens such as, *E. coli*, *S. aureus*, *Klebsiella*, *Listeria monocytogenes*, *Bacillus cereus*, and *Streptococcus pneumoniae*. It also effectively suppresses fungal pathogens such as *Candida spp.*, *Aspergillus fumigatus*, and *A. flavus* (Marrez and Mohamad 2020).

2.4. Diacetylphloroglucinol (DAPG):

2,4-Diacetylphloroglucinol (DAPG) is polyketide antibiotic, which is mostly generated by *Pseudomonas* species, particularly *Pseudomonas fluorescens*, and is essential for the biological control of a number of soilborne plant diseases (Suresh *et al.,* 2022). The biosynthesis of DAPG is governed by the *phl* gene cluster, comprising *phlACBD*, which encodes enzymes responsible for its production, and is regulated by pathway-specific transcriptional regulators such as PhlF and PhlH. PhlF acts as a repressor by binding to the promoter region of the *phlA* gene, while DAPG itself can derepress this binding, leading to autoinduction of its biosynthesis. PhlH modulates DAPG levels by controlling the expression of the DAPG hydrolase PhlG in response to DAPG and its biosynthetic intermediates (Yan *et al.,* 2017).

DAPG has broad-spectrum activity against phytopathogenic bacteria, fungi, plant viruses, nematodes, that cause root infections in both dicots and monocots, including rice, cotton, sugar beets, cucumbers, maize, peas, tobacco, tomatoes, wheat, oats, and bananas. It works by rupturing membrane integrity and blocking the link between ATP synthesis and respiration in the organisms it targets. Moreover, DAPG-producing *pseudomonas* are frequently abundant in crop rhizospheres, improving the soil's inherent ability to control specific diseases (Kankariya *et al.,* 2019). *Pseudomonas fluorescens* DAPG effectively inhibited *Ralstonia solanacearum* and several fungal pathogens causing tomato wilt (Suresh *et al.,* 2021).

2.5. Hydrogen Cyanide:

Hydrogen cyanide (HCN) is a volatile secondary metabolite produced by certain strains of *Pseudomonas.* It plays a significant role in biocontrol by exerting direct toxic effects on a wide range of soilborne and foliar pathogens. HCN inhibits cytochrome c oxidase in the electron transport chain, thereby blocking cellular respiration in target microorganisms. This leads to energy depletion and ultimately cell death (Nandi *et al.,* 2017; Sah *et al.,* 2021). *Pseudomonas chlororaphis* exhibits strong nematicidal activity against root-knot nematodes by secreting HCN. This strain effectively reduced gall formation on tomato plants, comparable to chemical nematicides, highlighting its potential as a sustainable, eco-friendly biocontrol agent (Kang *et al.,* 2018).

2.6. Siderophores:

Iron is essential for all organisms, supporting key metabolic functions and chlorophyll synthesis in plants. In oxygen-rich soils, it is mostly found as insoluble Fe^{3+}, making it difficult to absorb. To overcome this, plants and microbes release siderophores—iron-chelating compounds that enhance uptake. *Pseudomonas* species produces siderophores under favourable environmental conditions limits the amount of iron available to possible plant diseases, which hinders their capacity to proliferate. Furthermore, this might provide advantages for competition that encourage the growth and development of plants (Abo-Zaid *et al.,* 2023).

Pseudomonas species, particularly *pseudomonas fluorescence,* produce pyoverdine, a high-affinity, greenish-yellow siderophore that gives them a competitive edge in the rhizosphere (Liu *et al.,* 2021; Gutierrez-Urrego and Johnston-Monje, 2024). Pyoverdine's structure includes a dihydroxyquinoline chromophore, a unique peptide, and a carboxylic acid chain, forming stable iron complexes (Cézard *et* *al.,* 2015). Due to their role in limiting iron access to pathogens, siderophores are seen as promising biocontrol tools (Sasirekha and Srividya, 2016). *P. aeruginosa* also produces pyochelin, a siderophore with lower iron affinity than pyoverdine. Both siderophores are regulated by a positive feedback loop, where ferri-siderophore complexes enhance their own production. However, pyochelin's lower affinity limits its effectiveness in competitive environments with stronger iron chelators (Cunrath *et al.,* 2020).

2.7. Hydrolytic Enzymes:

One of the most effective and direct mechanisms involves the secretion of extracellular enzymes by *pseudomonas* spp that degrade the structural components of pathogenic cells. Among these mechanisms, extracellular or hydrolytic enzymes produced by biocontrol bacteria play a direct role in combating phytopathogens by breaking down essential components of their cell walls (Jadhav *et al*., 2017). *Fusarium*, *Sclerotinia*, *Phytophthora*, *Verticillium*, *Rhizoctonia*, and *Pythium* are responsible for yield losses crops. In addition, *Ralstonia solanacearum* and nematodes like *Meloidogyne* causes significant agricultural damage. Lytic enzymes such as chitinase**,** β-1,3-glucanase, catalase, cellulase, and proteases target major biopolymers like chitin, glucan, cellulose, proteins, DNA, and hemicellulose key elements of the pathogen cell wall thereby compromising their structural integrity and inhibiting their ability to infect plants (Riseh *et al.,* 2024).

2.8. Phenazine-1-carboxylic acid (PCA):

Pseudomonas species, such as *Pseudomonas chlororaphis*, *P. fluorescens*, and *P. aeruginosa*, are the main producers of this PCA. Several phenazine derivatives, including pyocyanin, phenazine-1-carboxamide, and 1-hydroxyphenazine, are biosynthesized using PCA as a key precursor. Through a multi-step enzymatic process, chorismic acid is converted into PCA by a highly conserved phz operon (phzABCDEFG), which controls its production. Because PCA can produce reactive oxygen species (ROS), upset cellular redox equilibrium, and damage competing bacteria's capacity to breathe, it has potent antimicrobial effect (Biessy and Filion, 2018). Phenazine-1-carboxylic acid is essential to inhibit soilborne diseases such as *Pythium* spp., *Rhizoctonia solani*, and *Fusarium* spp. (Zeng *et al.,* 2023).

2.9. Rhamnolipids:

Pseudomonas aeruginosa is the primary producer of rhamnolipids, which are biosurfactants distinguished by their amphiphilic structure consisting of one or two rhamnose sugar units connected to chains of β-hydroxy fatty acids. The rhlA, rhlB, and rhlC genes work together to synthesize these glycolipids; rhlA produces the lipid moiety (HAAs), rhlB catalyzes the formation of mono-rhamnolipids, and rhlC is involved in the synthesis of di-rhamnolipids. Quorum sensing systems, especially the RhlRI system, strictly control the production of rhamnolipids and ensure that biosurfactant synthesis happens in response to environmental factors and cell density (Reis *et al.,* 2011). They improve *Pseudomonas'* biocontrol potential in agriculture by encouraging root colonization and aiding in disease suppression. Furthermore, by solubilizing heavy metals and hydrophobic organic contaminants, rhamnolipids are essential for bioremediation and are hence appealing for use in industrial and environmental settings (Maier and Soberon-Chavez, 2000). It has also been proved that rhamnolipids has antifungal properties, evaluated against *Fusarium verticillioides*, a major maize pathogen that causes stalk and ear rot and produces carcinogenic fumonisin mycotoxins (Borah *et al.,* 2016).

3. Conclusion:

Pseudomonas species have great potential as biocontrol agents against a variety of phytopathogens that threaten agricultural productivity. These bacteria are excellent alternatives to chemical pesticides because they can produce a range of antagonistic compounds. *Pseudomonas*-based biocontrol methods continue to experience challenges in the field, such as uneven performance and environmental sensitivity, even though stimulative results observed within lab and greenhouse settings.

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