



## ***Pseudomonas*: A bacterial biocontrol agent used for management of soil-borne pathogens**

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<p><b>Research Article</b> Received on February 25, 2023 Revised on March 14, 2023 Accepted on March 28, 2023 Published on April 07, 2023</p> <p><b>Article Authors</b> Rashmi Nigam, Joginder Singh</p> <p><b>Corresponding Author Email</b> <a href="mailto:rashminigampatho16@gmail.com">rashminigampatho16@gmail.com</a></p>	<p>Soil-borne plant pathogens pose significant challenges to agricultural productivity and food security worldwide. Traditional methods of controlling these pathogens, such as chemical pesticides, have raised concerns due to their adverse environmental effects and potential risks to human health. As a sustainable and environmentally friendly alternative, the use of beneficial microorganisms, particularly <i>Pseudomonas</i> species, has gained considerable attention in recent years. This research paper provides an overview of the role of <i>Pseudomonas</i> as a potential biological agent for managing soil-borne plant pathogens. It discusses the mechanisms of action employed by <i>Pseudomonas</i> to suppress pathogen growth and explores the various factors that influence their efficacy. Furthermore, the paper highlights the potential benefits and challenges associated with the use of <i>Pseudomonas</i>-based biocontrol strategies, along with future prospects and research directions in this field.</p>
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Soil-borne diseases pose a significant threat to agricultural productivity worldwide. These diseases are caused by various pathogens that reside in the soil, including bacteria, fungi, and nematodes. Plant growth promoting Rhizobacteria having biocontrol ability and plant growth promoting activities is a viable alternative to minimize the use of chemicals pesticide and their hazardous effects, and to provide protection to the plants against resident pathogen populations. PGPR affect growth of the plant in two ways, direct and indirect. The direct promotion of plant growth by PGPR entails providing the plant with a compound that is synthesized by the bacterium or facilitating the uptake of certain nutrients from the soil.

The indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of one or more phytopathogenic organisms (Negi, 2008). Among PGPRs, most common are *Azotobacter*, *Bacillus* spp., fluorescent *Pseudomonas* spp., *Rhizobium* spp., etc. During last decade much of the research, however, has focused on organisms belonging to *Pseudomonas* species. These organisms have shown great antagonistic activity against several soil borne pathogens of economically important crops (Fernando *et al.*, 2004, Savchuk and Fernando, 2004, Negi *et al.*, 2005).

Among the potential biocontrol agents, *Pseudomonas* species have gained considerable attention due to their ability to suppress soil-borne diseases. This chapter explores the management of soil-borne diseases using *Pseudomonas* as a biocontrol agent, the characteristics of *Pseudomonas*, the mechanisms of disease suppression, and practical strategies for implementing *Pseudomonas* based management techniques.

## Occurrence and Distribution

### Characteristics of *Pseudomonas* as a Biocontrol Agent

*Pseudomonas* is a diverse group of Gram-negative bacteria that inhabit various environmental niches, including soil. Several species of *Pseudomonas*, such as *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas aeruginosa*, have been identified as potential biocontrol agents against soil-borne diseases. Plant growth-promoting Rhizobacteria (PGPR) are the rhizosphere bacteria that can enhance plant growth by different mechanisms like siderophore production, biological nitrogen fixation, phosphate solubilization, inhibition of biofilm formation, rhizosphere engineering, phytohormone production, exhibiting antifungal activity, induction of systemic resistance, production of volatile organic compounds, interference with pathogen toxin production etc. (Sarkar *et al.*, 2022). These bacteria exhibit several key characteristics that make them effective in disease management.

### Antagonistic Activity

*Pseudomonas* produces a wide range of antimicrobial compounds, including antibiotics, enzymes, and siderophores, which can inhibit the growth and activity of soil-borne pathogens. The antibiotics are more effective in suppressing the growth of the pathogen in vitro as well as in situ. There are six classes of antibiotic compounds which are better associated with biocontrol of plant pathogens *viz.* phenazines, pyrrolnitrin, cyclic lipopeptides, pyrrolnitrin, phloroglucinol and hydrogen cyanide (Haas and Defago, 2005).

The antibiotic 2, 4- diacetylphloroglucinol (DAPG) produced by *Pseudomonas* particularly has inhibitory action against zoospores of *Pythium* spp. (Desouza, *et al.*, 2003). *Pseudomonas* produces phenazine antibiotic that shows antagonistic activity against *Fusarium oxysporum* and *Gaeumannomyces graminis* (Chin *et al.*, 2003). The anti-fungal metabolite 2,4-diacetyl phloroglucinol play a major role in the biocontrol capabilities of *Pseudomonas fluorescens* (Delany *et al.*, 2000). Karunanithi *et al.* (2000) observed a native isolate of *P. fluorescens* producing an antibiotic compound, pyrrolnitrin, which inhibited growth of *Macrophomina phaseolina* by producing an inhibition zone of 12 mm. Kell *et al.* (1992) indicated that the importance of 2,4-diacetyl phloroglucinol production by strain CHA0 (*P. fluorescens*) is the suppression of soil-borne plant pathogens in the rhizosphere.

### Induced Systemic Resistance (ISR)

*Pseudomonas* can induce systemic resistance in plants, priming their defense mechanisms and making them more resistant to pathogen attack. This phenomenon enhances the plant's ability to combat soil-borne diseases. Indirect growth promotion may also alter the physiology of the plant thereby improving the host defence to pathogens attack *i.e.* induced resistance (Van Peer and Schipper, 1992, Liu, *et al.*, 1995).

### Competition for Nutrients

*Pseudomonas* bacteria can outcompete soil-borne pathogens for essential nutrients, limiting their growth and establishment in the rhizosphere.

### Mechanisms of Disease Suppression by *Pseudomonas*

The successful management of soil-borne diseases by *Pseudomonas* involves a range of mechanisms that collectively contribute to disease suppression. These mechanisms include:

#### Antibiosis

*Pseudomonas* produces antibiotics, such as phenazines, pyrrolnitrin, and pyrrolnitrin, which exhibit broad-spectrum activity against various soil-borne pathogens.

Yuan *et al.* (2012) noted that volatile compound produced by the bacteria reduced the mycelia growth and inhibited spore germination of *F. oxysporum*. The *P. fluorescens* is very effective antibiotic producer. Many secondary metabolites of *P. fluorescens* acts as antibiotics against plant pathogens. The *P. fluorescens* produces antifungal compounds like phenazine-1-carboxylic acid (PCA), 2, 4 – diacetylphloroglucinol (DAPG), pyocinin, pyrrolnitrin, pyoluteorin and oomycin-A which are fungistatic, inhibiting spore germination and lysis of fungal mycelia (Kell *et al.*, 1992, Hass and Defago, 2005).

### **Enzyme Production**

*Pseudomonas* secretes enzymes, such as chitinases, proteases, and glucanases, which degrade the cell walls and structural components of pathogens, leading to their lysis and death.

### **Volatile Organic Compounds (VOCs)**

Some *Pseudomonas* strains release VOCs that have antimicrobial properties. These compounds can inhibit the growth of soil-borne pathogens and disrupt their communication and virulence mechanisms. *Pseudomonas* spp. are known to produce volatile compounds. One such metabolite is HCN (Tripathi and Johri, 2002). Afsharmanesh *et al.* (2010) suggested that fungal growth is mainly inhibited by HCN production and siderophore production. Imran *et al.* (2006) reviewed the role of cyanide in controlling root knot disease of tomato. *Pseudomonas fluorescens* 2-79 produce the antibiotic phenazine-1-carboxylic acid and suppress take-all of wheat caused by *Gaeumannomyces graminis* var. *tritici*. The antibiotic was isolated only from roots of wheat colonized by strain 2-79 in both growth chamber and field studies in USA (Thomashow *et al.*, 1990). Positive correlations between in vitro HCN production and plant protection in the cucumber (*Pythium ultimum*) and tomato (*Fusarium oxysporum* f. sp. *radicis-lycopersici*) pathosystems (Ramatte *et al.*, 2003).

### **Induced Systemic Resistance (ISR)**

*Pseudomonas* activates plant defense responses, such as production of phytohormones and pathogenesis related proteins, thereby enhancing the plant's resistance to soil-borne pathogens.

Many rhizospheric strains of *Pseudomonas* produce indole acetic acid (IAA) which helps in stimulating plant growth (Loper and Schroth, 1986). The phytohormone indole-3-acetic acid (IAA) is known to be involved in root initiation, cell division, and cell enlargement. *P. fluorescens* can produce cytokinins as reported by (Garcia *et al.*, 2001). Cytokinins promote cell divisions, cell enlargement, and tissue expansion and are believed to be the signals for mediation of environmental stress from roots to shoots.

### **Strategies for Implementing *Pseudomonas* based Disease Management**

The utilization of *Pseudomonas* as a biocontrol agent for managing soil-borne diseases holds great promise in sustainable agriculture. There are various strategies and practical approaches for implementing *Pseudomonas* based disease management. These strategies encompass selection of effective *Pseudomonas* strains, application methods, integration with cultural practices, formulation, and delivery systems. By understanding and implementing these strategies, farmers and agricultural professionals can harness the full potential of *Pseudomonas* for effective disease suppression.

### **Selection of Effective *Pseudomonas* Strains**

Different *Pseudomonas* strains vary in their antagonistic potential against specific soil-borne pathogens. It is crucial to identify and select strains that exhibit high biocontrol efficacy against the target pathogens. The success of *Pseudomonas*-based disease management relies on the careful selection of appropriate *Pseudomonas* strains that exhibit high biocontrol efficacy against target pathogens. Consider the following key considerations for strain selection.

### **Pathogen-Specific Activity**

Different *Pseudomonas* strains possess varying antagonistic potential against specific soil-borne pathogens. Conduct thorough research to identify strains that have demonstrated efficacy against the target pathogen responsible for the soil-borne disease.

## Compatibility with Target Crop

Ensure that the selected *Pseudomonas* strains are compatible with the target crop, considering factors such as pH requirements, environmental conditions, and host specificity.

## Persistence and Colonization Ability

Evaluate the ability of the *Pseudomonas* strains to persist and colonize the rhizosphere, as this influences their long-term efficacy in disease suppression.

## Biocontrol Traits

Consider the production of antimicrobial compounds, enzymes, and induced systemic resistance (ISR) elicitation as desirable traits in the selected *Pseudomonas* strains.

## Application Methods

The successful implementation of *Pseudomonas* based disease management depends on choosing appropriate application methods. *Pseudomonas* can be applied as seed treatments, soil drenches, or foliar sprays, depending on the target pathogen and crop. Optimal application techniques should ensure efficient delivery of the biocontrol agent to the target site. Consider the following methods based on the target pathogen, crop and disease severity:

### Seed Treatments

Coating or inoculating seeds with *Pseudomonas* formulations prior to sowing can provide early protection against soil-borne pathogens. *Pseudomonas fluorescens* was applied as seed treatment (2 g/kg seed) and the results showed less root rot incidence and nematode population along with increased pod yield (Latha *et al.*, 2000).

### Soil Drenches

Application of *Pseudomonas* suspensions or formulations directly to the soil around the plant's root zone can enhance colonization and antagonistic activity against soil-borne pathogens.

## Foliar Sprays

In some cases, foliar application of *Pseudomonas* formulations can provide additional protection against foliar diseases caused by soil-borne pathogens or systemic infections.

## Stomatal Infiltration

*Pseudomonas* suspensions are applied under low pressure, allowing the bacteria to infiltrate the stomata on the leaf surface. This method facilitates the colonization of *Pseudomonas* in the leaf tissues and triggers induced systemic resistance (ISR) in the plant.

## Transplant Root Dips

For seedlings or transplants, a root dip method can be employed to enhance the establishment of *Pseudomonas* around the root system. The root dip method involves immersing the roots of seedlings or transplants in a *Pseudomonas* suspension or formulation before planting. This ensures immediate contact between the bacteria and the root system, promoting rapid colonization and disease suppression.

## Incorporation in Growing Media

When starting plants in containers or greenhouses, *Pseudomonas* formulations can be incorporated into the growing media. This allows the bacteria to colonize the rhizosphere and provide continuous disease protection. *Pseudomonas* can be mixed into the potting mix or added as a granular formulation during the preparation of growing media.

## Irrigation and Root Zone Application

Incorporating *Pseudomonas* formulations into irrigation water or applying them directly to the root zone via drip irrigation systems can promote colonization and disease suppression.

## Integration with Cultural Practices

*Pseudomonas* based disease management should be integrated with other cultural practices, such as crop rotation, soil amendment and sanitation to achieve comprehensive disease suppression.

To maximize the efficacy of *Pseudomonas* based disease management, it is essential to integrate it with other cultural practices. Consider the following approaches:

### **Crop Rotation**

Rotate susceptible crops with non-host or resistant crops to disrupt the life cycles of soil-borne pathogens and reduce disease pressure.

### **Soil Amendment**

Incorporate organic matter, compost, or bio fertilizers into the soil to improve soil health, nutrient availability, and microbial diversity, creating a more favourable environment for beneficial *Pseudomonas* populations.

### **Sanitation Practices**

Remove and destroy crop residues, weeds, and infected plant material to minimize the survival and spread of soil-borne pathogens.

### **Balanced Nutrition**

Ensure balanced nutrient management practices to promote plant health and vigour, which can enhance the effectiveness of *Pseudomonas* mediated disease suppression.

### **Formulation and Delivery Systems of *Pseudomonas* for Disease Management**

The successful implementation of *Pseudomonas* based disease management relies not only on selecting effective strains and application methods but also on developing appropriate formulations and delivery systems. This chapter explores the crucial aspects of formulating *Pseudomonas* for optimal viability, stability, and delivery to effectively combat soil-borne diseases. The different formulation techniques, considerations for formulation development, and innovative delivery systems to enhance the efficacy of *Pseudomonas* in disease management.

### **Importance of Formulation and Delivery Systems**

Formulation refers to the process of incorporating *Pseudomonas* cells or their metabolites into a carrier matrix or medium to create a stable, user-friendly product.

Delivery systems encompass the methods and technologies used to transport the formulated *Pseudomonas* to the target site, ensuring efficient colonization and disease suppression. Effective formulation and delivery systems provide several advantages, including:

### **Enhanced Shelf Life**

Formulations can extend the viability and shelf life of *Pseudomonas*, allowing for storage and transportation without compromising their biocontrol efficacy.

### **Improved Viability and Stability**

Proper formulation techniques protect *Pseudomonas* cells from environmental stresses, such as temperature fluctuations, desiccation and UV radiation, thereby maintaining their viability and activity.

### **Controlled Release**

Formulations can be designed to release *Pseudomonas* cells or their metabolites slowly, ensuring a sustained presence and activity in the rhizosphere.

### **Ease of Application**

Well-formulated products and innovative delivery systems simplify application processes, making them more user-friendly and compatible with different agricultural practices.

### **Formulation Techniques**

Various formulation techniques can be employed to develop *Pseudomonas* based products. The choice of technique depends on the desired product characteristics, target pathogen, and application method. Some commonly used formulation techniques include:

### **Liquid Formulations**

Liquid formulations involve suspending or dissolving *Pseudomonas* cells or their metabolites in water or suitable aqueous solutions. These formulations are generally easy to produce, handle, and apply through irrigation systems, sprayers or seed treatments.

## Solid Formulations

Solid formulations provide stability and protection to *Pseudomonas* cells. Examples include granules, powders, or pellets that can be applied directly to the soil or incorporated into seed coatings. Solid formulations are advantageous for their longer shelf life and ease of storage.

## Encapsulation

Encapsulation involves encapsulating *Pseudomonas* cells or their metabolites within protective matrices, such as alginate beads, hydrogels, or microcapsules. This technique provides enhanced protection, controlled release, and improved viability during storage and application.

## Dry Formulations

Dry formulations typically involve freeze drying or spray drying *Pseudomonas* cells to remove water and preserve their viability. These formulations are lightweight, easy to transport, and can be rehydrated before application.

## Conclusion

*Pseudomonas*, a diverse group of beneficial bacteria, holds great promise as a biological agent for the management of soil-borne plant pathogens. This research paper has provided a comprehensive overview of the role of *Pseudomonas* in suppressing the growth and activity of these pathogens, highlighting its potential as a sustainable and environmentally friendly alternative to chemical pesticides. The application strategies for *Pseudomonas* based biocontrol include seed and soil treatments, as well as the development of innovative formulations and delivery systems. Integration of *Pseudomonas* based biocontrol with other management practices, such as cultural methods and chemical controls, can enhance its effectiveness and provide a holistic approach to disease management. The use of *Pseudomonas* as a biological agent offers numerous benefits. It promotes environmental sustainability by reducing the reliance on chemical pesticides and minimizing their detrimental effects on ecosystems.

Moreover, the integration of *Pseudomonas* based biocontrol into integrated pest management (IPM) programs contributes to the development of sustainable agricultural systems. However, further research is needed to address challenges, optimize application strategies, and integrate *Pseudomonas* based biocontrol into sustainable agricultural practices. Continued exploration of this field will contribute to the development of effective and environmentally friendly solutions for soil-borne disease management, ensuring the long-term health and productivity of agricultural systems.

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