Biological Forum – An International Journal **15(8a): 28-34(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

A Review on Biocontrol mechanisms of *Pseudomonas* spp.

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(Received: 08 June 2023; Revised: 26 June 2023; Accepted: 25 July 2023; Published: 15 August 2023)
(Published by Research Trend)

ABSTRACT: The improper use of chemicals such as fertilizers and pesticides cause incredible damage to the environment and ecosystems, as well as to the human body. Instead of these dangerous agrochemicals, a biological solution can be offered in the form of microorganisms that promote plant growth without significantly harming the environment. Plant growth promoting rhizobacteria (PGPR), which suppress or prevent phytopathogenic damage is one of the biological approaches to control various phytopathogenic pathogens. *Pseudomonas* PGPR are the best characterized bio controlling PGPRs. Because they are abundant in natural soils, plant roots and are able to utilize many plant exudates as nutrients. In addition to their ability to adhere to soil particles and rhizoplanes, fluorescent pseudomonads are capable of motility, prototrophy, synthesis of antibiotics and production of hydrolytic enzymes, which contribute to bacterial fitness. Moreover, *Pseudomonas* possesses plant growth-promoting traits such as nitrogen fixation, phosphate solubilization, iron chelation and phytohormone production. By analyzing rhizosphere competence and biocontrol traits, novel tools may be developed for managing indigenous and inoculated *P. fluorescens* biocontrol agents. For sustainable agriculture, this will also improve their plant-beneficial properties. This multidimensional utility makes fluorescent *Pseudomonas* an ideal bioagent for agricultural use.

Keywords: *Pseudomonas* PGPR, agrochemicals, antibiotics, hydrolytic enzymes and Phytohormones.

INTRODUCTION

In the late 1970's, *Pseudomonas* sp. was made into a biocontrol agent at the University of California (Weller 1988). Fluorescent species of *Pseudomonas* thrive in a variety of environments. Among its members, this genus has extensive distribution across terrestrial, freshwater and marine habitats, as well as intimate relationships with plants and animals (Noway-Thompson *et al.,* 1997). Due to their multiple functions as plant growth-promoting agents and bio remediation, *Pseudomonas* bacteria have a unique functional and ecological diversity. A pseudomonad is a gram-negative, chemo-heterotrophic motile rod shaped with polar flagella defined by Palleroni (1984). Gardener *et al.,* (2005) described *Pseudomonas* a complex collection of various species. According to comprehensive studies spanning more than forty years, *Pseudomonas* exhibits functional and metabolic heterogeneity. Despite its catabolic adaptation, outstanding root colonizing capabilities and ability to produce antifungal metabolites, the genus *Pseudomonas* embodies as a biocontrol agent. Biocontrol agents among *Pseudomonas* species have been emphasized through fluorescent pseudomonads. Physiologically, *Pseudomonas* exerts its biocontrol activities by directly attacking phytopathogens and by promoting the development of disease resistance in the host plant (Cartieaux *et al.,* 2003). Among common inhabitants of the rhizosphere, fluorescent *Pseudomonas* has been widely studied. *P. aeruginosa, P. aureofaciens, P. chlororaphis, P. fluorescens, P. putida and P. cichorii* are the six species of this genus. They are distinguished from other species because of their yellow-green pigmentation. Apart from the fact that they are well suited to inhabiting the rhizosphere, pseudomonads possess a number of characteristics that make them an (1) effective biocontrol agent as well as a growth promoter (Weller 1988). (2) It grows faster, making it easy to produce in the laboratory (3) consume seed and root exudates (4) colonize and multiply in rhizospheres and spermospheres (5) produce a wide spectrum of bioactive metabolites (antibiotics, siderophores, volatiles and growth-promoting substances) (6) adapt to environmental stresses, (7) colonize plants easily upon reinoculation in soil by seed

bacterization. Some of the soilborne pathogens are suppressed by pseudomonads (Weller *et al.,* 2002).

Biocontrol involves the production of antibiotics or inactivation of pathogen virulence traits (Diby *et al.,* 2005). Over 50 fluorescent *Pseudomonas* species have been identified as plant-associated strains (Yamamoto *et al.,* 2000; Mulet *et al.,* 2010). Plant-associated strains are indirectly inhibited by bacterial stimulation of defense responses. By defending plants from pathogens at different developmental stages, *Pseudomonas* improves plant growth and development. Bacteria have evolved antipredatory mechanisms, besides consuming root exudates efficiently, pseudomonads also possess the ability to resist soil predators (De Mesel *et al.,* 2004; Abuzar and Haseeb 2010). The extracellular metabolites produced by *Pseudomonas* sp. are effective as repellents, stressors or toxins against predators. *Pseudomonas* sp. synthesizes a variety of secondary metabolites to protect plants from pathogens to improve bacterial resistance (Gadoury *et al.,*1989). Secondary metabolites are produced by *Pseudomonas* sp, including 2, 4-diacetylphloroglucinol (DAPG, Phl), lipopeptides, phenazines, pyrrolnitrin, pyochelin and hydrogen cyanide (Keel *et al.,* 1992; Haas & Defago 2005). This biocontrol strain also produces several antibiotics. In vitro, antibiotics have been shown to inhibit compounds. In addition, they are effective for managing plant health in the field as well. Root disease suppression is significantly improved when strains producing DAPG are introduced through seeding or soil treatment (Reddy *et al.,* 2009). There is considerable commercial interest in the bacterium *Pseudomonas* sp. as a plant disease suppressant. In the future, plant disease management strategies may be transformed by the introduction of antagonistic bacteria to control plant diseases.

BIOCONTROL MECHANISMS

Over the past few years, research has focused on identifying, characterization and testing of the rhizosphere microorganism population for its ability to function as a biocontrol agent. Antibiotics, siderophores, enzymes and other compounds produced by these microorganisms can limit phytopathogen damage and they can also induce plant resistance to these compounds through systemic resistance. *Pseudomonas* also competes with pathogens for colonization sites and nutrients (Deepak *et al.,* 2016).

2.1. Competition

Keel *et al.,* (1992) have shown that rhizosphere biological buffering systems are challenged by the high diversity, density, metabolic activity and competition of microbial species. Therefore, exogenous foreign microorganisms find it difficult to establish themselves in the rhizosphere. As a result, it is essential to evaluate introduced pseudomonads can colonize roots and protect against major and minor soil pathogens. It has been suggested that rhizobacterial root colonization occurs when rhizobacteria move from an inoculum source to roots, multiply and persist in the soil microbiome (Lemanceau *et al.,*1995; Van Loon *et al.,*1998). Weller *et al.* (2002) describe root colonization as the process by which rhizobacteria introduced into seeds, vegetative propagated plant parts or soil are spread throughout the root system in raw soil by spreading them throughout the root system. Rhizobacteria multiply, and they survive for several weeks in soil microorganisms. A rhizobacterium's rhizosphere competence refers to its ability to invade the root surface, rhizosphere, and/or inside of the root. Establishing an inoculant is crucial. When an inoculant multiplies on the roots and colonizes them, it is more effective in suppressing diseases. The extent to which a biocontrol agent colonizes a plant can influence how efficient it is at controlling fungal infections. Root colonization increases root population density and generates antifungal metabolites throughout the root system.

Rhizosphere microflora is influenced by root exudes, which contain organic acids, sugars and amino acids. Rhizospheres contain a higher concentration of microbes than bulk soil (Hiltner, 1904). The soil's nutrient-dense root exudates and colonization niches are where biocontrol agents compete for nutrients and eventually outnumber harmful microorganisms and pathogens. It inhibits soil pathogens for a short time when inoculated with a biocontrol agent. Consequently, the biocontrol agent must compete with native microflora in the host plant's rhizosphere for nutrients. So, soil microorganisms need nutrients from the rhizosphere to survive. Therefore, native bacteria can prevent pathogens from establishing themselves and causing deleterious effects. As pseudomonads establish themselves in niches and vie for nutrients, antagonistic activity is a general mechanism. The probiotic pseudomonad strains in plants disperse these numbers. Increased competition for nutrients (carbon, nitrogen, and iron) can eliminate fungal pathogens from the soil (Leong 1986; Loper and Buyer 1991). As microorganisms live under nutrient-limited conditions, rhizospheres generate pseudomonads for 3 to 6 hours, which is slower than in nutrient-rich laboratory media (Kamilova 2005; Haas and Defago 2005). Since fluorescent pseudomonads have nutritional access and a high growth rate (Walsh *et al.,* 2001), root populations can act as sinks for nutrients. By sequestering iron Fe^{3+} in the rhizosphere, pseudomonads produce siderophores. Their removal succeeds in controlling the rhizosphere. In this way, they serve to combat pathogens that share the same ecological niche. The fluorescent siderophores form a ferric-siderophore complex that becomes unavailable to other organisms when exposed to ferric iron. However, a very specific receptor on the cell membrane allows the producing strain to utilize this complex (Koster *et al.,* 1993, 1995; Buyer and Leong 1986). Loper and Buyer (1991) found that fluorescent Pseudomonas strains prevented the growth of harmful bacteria.

An Optimal colonization is essential to deliver antifungal compounds to the entire root system of plants through biocontrol through antibiosis. For a plant to respond to disease, only a few bacteria need to colonize it before an ISR response can be initiated. *Pseudomonas* strains generally have short generation

times, which is considered to be an important characteristic. One of the key characteristics of biocontrol is the speed and degree of colonization. On tomato roots one day after seed inoculation, *P. fluorescens* WCS365 microcolonies appeared (Chin-A-Woeng *et al.,* 1997; Bloemberg *et al.,* 2000). Due to their nutritional wealth, which is a small percentage of the root surface area, bacterial antagonists tend to colonize the intracellular junction between root epidermal cells. Dhingani *et al.,* (2013) show that colonization of fluorescent Pseudomonas isolates can promote plant growth. A total of 30 fluorescent *Pseudomonas* isolates were isolated from six locations in Junagadh district, Gujarat, India. The fluorescent *Pseudomonas* possess various PGPR traits that are useful for improving plant growth during colonization of suppressive rhizosphere soils. Other PGPR traits are also present in them. A positive relationship exists between colonization and pathogen suppression in biocontrol systems.

A biocontrol strain will limit the spread of an organism if it cannot compete effectively and use nutrients available in the same ecological niche. Its cell surface contains an ice nucleation protein, so it is a classic example of niche exclusion. At neutral and alkaline pH, iron is less available, causing Fe^{3+} limitation. Iron is an essential cofactor for growth in all organisms. By producing siderophores, fluorescent *Pseudomonas* species chelate iron well. Biocontrol organisms that scavenge iron have an advantage over phytopathogens that don't. (Bakker *et al.,* 1986) Siderophore-deficient mutants were less effective against pathogens than wild-type parental strains.

Antibiotic Production. Biological control agents that produce antibiotics are common and useful for managing plant diseases since they are easily isolated. Antibiotic production depends on a number of factors, including temperature, pH, metal ions, specifically zinc ions and metal ion concentrations (Duffy and Defago 1997). Keel *et al.,* 1992, 1996 have noted that fluorescent pseudomonads inhabiting the rhizosphere are capable of controlling seeds and soil-borne fungi and oomycetes. Plant-beneficial microorganisms facilitate the elimination of pathogens from the rhizosphere by secreting antimicrobial metabolites (Haas and Keel 2003; Handelsman and Stabb 1996; Raaijmakers *et al.,* 2002; Thomashow and Weller 1996). Plants, pathogens and bacteria interact triangularly to regulate *Pseudomonas* antifungal traits (Jain *et al.,* 2011). Antibiotics require efficient colonization (Chin-A-Woeng *et al.,* 2003), so it is not surprising that some strains with antifungal activity in the laboratory do not perform well in the field as biocontrol agents. Only a few cases have been demonstrated that it is possible to identify and quantify antibiotics produced during biocontrol in situ. Secondary metabolites are produced by bacteria in the rhizosphere due to slow growth (Haas and Defago 2005). The most frequently detected antifungal metabolites in *Pseudomonas* biocontrol strains are DAPG, phenazines, pyrrolnitrin, pyoluteorin and volatile hydrogen cyanide. Viscosinamide and Tensin (Nielsen *et al.,* 2001) protect plants from phytopathogens (Nielsen *et al.,* 1999). In many crops, pseudomonads that produce DAPG are useful biocontrol agents for suppressing root and young seedling diseases, such as Tobacco black root rot suppressed by *P. fluorescens* CHA0 (Stutz *et al.,* 1986), Wheat Take-all (Keel *et al.,* 1992), Tomato root rot, crown rot and *Fusarium* wilt (Duffy and Defago 1997). In Sugar beets, F113 inhibits Damping off (Fenton *et al.,* 1992; Shanahan *et al.,* 1992), while *P. fluorescens* Q2-87 (Harrison et al. 1993; Pierson and Weller 1994) and Q8r1-96 (Raaijmakers and Weller 1998) inhibit Take-all. DAPG-producing strains of *P. fluorescens* can prevent the disease, but their mechanism of DAPG is unknown (Raaijmakers *et al.,* 1997). A genetic approach (Thomashow, 1996) and direct isolation of disease suppressive strains producing DAPG from crop rhizospheres has been demonstrated by Duffy and Defago 1997, Raaijmakers and Weller 1998.

A major risk associated with antibiotic-producing biocontrol agents is antibiotic resistance among human and animal pathogens. The transfer of antibiotic genes to related strains appears feasible because conjugative transfers require quorum sensing and high concentrations of microbes (Zhang *et al.,* 2003). In roots with mucoid layers, pseudomonads are capable of forming microcolonies (Chin-A-Woeng *et al.,* 1997). It takes so long to register biocontrol products based on antibiotic-producing microorganisms during the rhizosphere since genetic material exchanges frequently.

Induced Systemic Resistance (ISR). The ISR, which can be described as a broad-spectrum plant immune response is triggered by bacteria living in association with plant roots. *P. fluorescens* triggers the ISR response to fight plant pathogens (Van Loon and Bakker 2006; Van Wees *et al.,* 1997; Kamilova *et al.,* 2005). Plants immunized with these microbes are more likely to show stronger defense responses after pathogen attack (Van Peer *et al.,* 1991). Beneficial microbes cause immunity in distant plant parts, such as leaves, resulting in the ISR response. Beneficial microbes induce the ISR response, which kills bacteria, fungi and viruses (Van Loon *et al.,* 1998; Van Loon 2007), but the response appears to be random (Verhagen *et al.,* 2003). In ISR-producing microorganisms, Van Loon and Bakker (2006) ; Van Wees *et al.* (1997) found that they are host specific. In many plant-pathogen systems where the bacterium and challenging pathogen remained spatially separated, the ISR response was observed. In general, it is known that jasmonate and ethylene regulate ISR. ISR is induced by biocontrol pseudomonads (Ongena *et al.,* 2004; Ton *et al.,* 2002; Zehnder *et al.,* 2001). The main inducers of the ISR response in plants are live microbes, including *Bacillus*, *Pseudomonas* and *Trichoderma* as well as dead microbial cells and some of the products of bacterial metabolism, such as siderophores, lipopolysaccharides, salicylic acid, pyocyanin and pyochelin, as well as organelles. There is also evidence that AHL signal molecules (Schuhegger *et al.,* 2006), volatile 2,3-butanediol (Ryu *et al.,* 2003),

phloroglucinol (Iavicoli *et al.,* 2003) and some c-LPs trigger the ISR response.

PLANT GROWTH-PROMOTING RHIZOBACTERIA

In addition to its many biocontrol properties, *pseudomonas* has been shown to facilitate plant host proliferation in multiple ways (Weller, 2007), making it an excellent biocontrol agent. In addition to facilitating nutrient uptake from the environment, the bacterium synthesizes a compound that helps the plant absorb nutrients from its environment. Plant Growth is influenced by four direct mechanisms: phytohormones, nitrogen fixation, siderophores and phosphorus solubilization.

Phytohormones and their effects

Indole -3- Acetic Acid. The rhizosphere strain of Pseudomonas, IAA (indole acetic acid), produces IAA (indole acetic acid), a phytohormone that stimulates plant growth (Loper and Schroth 1986). By producing IAA, microorganisms increase root length and surface area, facilitating maximum water and nutrient absorption (Salisbury 1994). Microbes produce IAA, which increases seedling survival by improving seedling anchoring ability and water and nutrient absorption (Patten and Glick 2002) by increasing root length and secondary roots. IAA-producing auxins, which increase grain yields and branches (Asghar *et al.,* 2002, 2004). *P. putida* plays an important role in host root development was reported by Patten and Glick (2002).

Cytokinins. A study conducted by Garcia *et al.* (2001) reported that *P. fluorescens* produces cytokinin, which stimulates cell division, cell enlargement and tissue expansion.

1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase. Root growth is inhibited by ethylene a gaseous phytohormone, when stimulated physically or chemically. In a study conducted by Glick *et al.,* (1998) found that some strains of PGRP produced an enzyme called ACC deaminase, which breaks down ACC, a precursor of ethylene biosynthesis. Plant roots elongate when the microorganisms produce ACC deaminase enzyme, thereby reducing ethylene concentration (Glick *et al.,* 1994). Shah *et al.* (1998) reported that introducing ACC deaminase genes into *Pseudomonas* spp. by producing ACC deaminase enzyme, bacteria release stress, resulting in the extension of seedling roots. According to Burd *et al.* (1998), *Pseudomonas* strains producing ACC deaminase enzyme stimulate plant growth under stressful conditions, such as floods (Grichko and Glick 2001).

Nitrogen Fixation. The nitrogen fixation ability of *Pseudomonas* microorganisms was reported by Anderson in 1955. In 1992, Young reported nitrification and nitrogenase protection against oxygen deactivation were not observed. However, according to several researchers (Desnoues *et al.,* 2003; Krotzky and Werner 1987), pseudomonad strains are capable of these behaviors. The genetic structure of genes encoding nitrogenase enzyme was examined in detail by using *P. stutzeri* A15 (A1501). It was isolated from rice (Desnoues *et al.,* 2003) as a model of nitrogen fixation. Accordingly, one can categorize *Pseudomonas* spp. as nitrogen fixers based on their physiological properties, nitrogenase assays, phylogenetic studies and the detection of NIFH DNA by hybridization or PCR analysis (Chan *et al.,* 1994; Vermeiren *et al.,* 1999). Prokaryotes have been assigned new genera to reflect the fact that *Pseudomonas* species are nitrogen-fixing strains. Chan *et al.,* 1994 renamed many genera as a result of the discovery of nitrogen-fixing strains of *Pseudomonas* species.

Phosphorus Solubilization. According to Stevenson and Cole (1999), insoluble phosphates are converted into soluble forms using phosphate-soluble bacteria (PSB). Phosphorous-rich soils provide 0.1 percent of phosphorus to plants. *Bacillus*, *Enterobacter, Erwinia* and *Pseudomonas* are most commonly found solubilizing phosphate to increase phosphate availability. Several phosphate-solubilizing bacteria colonize plant rhizospheres by solubilizing insoluble inorganic phosphatic compounds. A PGPR strain is generally dependent on carbon, nitrogen and metal ions to solubilize phosphate (Kim *et al.,* 1998). As a secondary oxidation product of glucose metabolism, phosphate-solubilizing bacteria produce ketogluconic acid, a secondary oxidation product of phosphate-solubilizing bacteria. Gluconic acid and ketogluconic acid are produced by the enzyme glucose dehydrogenase (GDH) when bacteria oxidize glucose. These acids dissolve phosphate by dissolving it.

Siderophores. In all living organisms, iron plays an important role in respiration, photosynthesis and nitrogen fixation. The earth's surface is abundant with iron, but soil organisms like plants and microbes struggle to get enough iron. Iron in soil is mostly present in the form of insoluble, ferric hydroxides. Some plants and microorganisms can scavenge iron from soil by using organic molecules called siderophores, which have a low molecular weight. Bacteria typically possess catecholate-type siderophores, which bind iron with two oxygen atoms or nitrogen atoms. Many bacteria, including *Pseudomonas* spp., have high affinity iron uptake systems when Fe^{3+} concentrations fall (Braun 1985; Neilands 1982). The siderophore complex consists of Fe^{3+} chelating molecules and outer membrane receptor proteins that have a high affinity for Fe^{3+} siderophores (De Weger *et al.,* 1986). As a result of iron starvation, phytopathogens are inhibited. This antagonism, however, won't occur once iron concentrations are sufficient (Geels and Schippers 1983). In order to explain the increase in plant growth caused by *Pseudomonas* spp. (Kloepper *et al.,* 1980). As siderophores bind Fe^{3+}, they require a specific mechanism to uptake it, making this vital element unavailable to other rhizomicroorganisms. *Pseudomonas* invade plant roots shortly after inoculation by limiting Fe^{3+} concentration. As Raaijmakers et al. 1995 demonstrated, deleterious species fail to obtain sufficient iron for optimal growth because they produce either no siderophores at all or inefficient siderophores. Several fluorescent

pseudomonads produce siderophores and some plants absorb bacterial siderophores (Bitter *et al.,* 1991). By reducing the number of deleterious microorganisms, plants develop in a favourable environment. Pyoverdine or pseudobactin is a fluorescent yellow-green siderophore. Pyochelin is a siderophore with a lower iron affinity.

CONCLUSIONS

Despite being applied to their target niche, biocontrol agents still need support in order to succeed. For biological control to be effective, the biocontrol agent must not only be of high quality, but also established in the natural environment so it can thrive and compete effectively with pathogens. In addition to ensuring persistence in the field and compatibility with chemical and biological seed treatments, better formulations are a key focus. Future bioagents using *P. fluorescens* will have a very high cost-benefit ratio. Based on this, the first assumption is to isolate *P. fluorescens* from the rhizosphere of a wide variety of field crops which exhibit enhanced antagonistic activity against soil-borne fungal pathogens in native environments, and then determine whether selected bacteria can suppress soil-borne fungal pathogens in vitro.

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How to cite this article: Prashanth Kumar A., Nikhil B.S.K., Ashwin Kumar B. and Gajanand P. (2023). A Review on Biocontrol mechanisms of *Pseudomonas* spp.. *Biological Forum – An International Journal*, *15*(8a): 28-34.