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Pseudomonas fluorescence: Bio-control Agent for Phytopathogens

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One of the biological approaches for the control of different phytopathogenic agents is the use of biocontrol plant growth promoting rhizobacteria (PGPR), which is capable of suppressing or preventing the phytopathogen damage. The best characterized biocontrol PGPR belong to the bacteria genus *Pseudomonas.* Fluorescent pseudomonads are suitable for application as biological control agents due to their abundant population in natural soils and plant root system and their capability to utilize many plant exudates as nutrient. Fluorescent pseudomonads are known to have important traits in bacterial fitness such as the ability to adhere to soil particles and to the rhizoplane, motility and prototrophy, synthesis of antibiotics and production of hydrolytic enzymes. Moreover, Pseudomonas also possesses plant growth promoting traits such as nitrogen fixation, phosphate solubilisation, iron chelation and phytohormone production. Such multi dimension utility of fluorescent *Pseudomonas* makes them a bioagent of choice to be exploited in the field of agriculture.

Introduction

Soil-borne, non-pathogenic bacteria with the ability to antagonise fungal phytopathogens and thus prevent plant disease represent a realistic alternative to chemical fungicides. These bacteria are known by several names, including biological control agents, plant growth promoting rhizobacteria and biopesticides. Because of their catabolic versatility, their excellent root-colonising abilities and their capacity to produce a wide range of antifungal metabolites, the soil-borne fluorescent pseudomonads have received particular attention. In addition, some *Pseudomonas* have been shown to elicit a disease-resistance response in crop species, a phenomenon known as induced systemic resistance (ISR). This dual activity of *Pseudomonas* further highlights their potential as plant protection products. *Pseudomonas fluorescence* is gram negative, obligate aerobic, rod-shaped, non-pathogenic, motile saprophytes that colonizes soil, water and plant surface. It secretes a soluble greenish fluorescent pigment–fluorescein. Many strains of *P. fluorescence* are known to enhance plant growth and reduce severity of various diseases. The efficacy of bacterial antagonists in controlling fungal diseases was often better as alone, and sometimes in combination with fungicides. Pseudomonads possess many traits that make them well suited as biocontrol and growth-promoting agents (Weller, 1988). These include the ability to grow rapidly *in vitro* and to be mass produced; rapidly utilize seed and root exudates; colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant; produce a wide spectrum of secondary metabolites (i.e. antibiotics,

siderophores, volatiles, and growth-promoting substances); compete aggressively with other microorganisms and adapt to environmental stresses.

Colonization of Plants

Biocontrol strains have noticeably been observed at the root surface forming microcolonies or discontinued biofilms in the grooves between epidermal cells. Certain strains are also capable of endophytic colonization. Within root tissues, they are mostly found in the intercellular spaces of the epidermis and the cortex. Strains with biocontrol ability may represent in the order of 10% of all rhizosphere strains, and they have been isolated from a very wide range of soils, climatic regions and host plants (Rezzonico *et al.,* 2007).

Biocontrol of Phytopathogens by *P. fluorescence*

Biocontrol agents from *P. fluorescence* are rather nonspecific in their ability to protect plants from soil phytopathogens. They have been mostly studied for protection of crop plants from phytopathogenic fungi *viz. Pythium* spp., *Fusarium oxysporum, Gaeumannomyces graminis, Rhizoctonia solani* etc. and to lesser extent bacteria such as *Pectobacterium carotovorum* and nematodes *Meloidogyne* spp.

Mode of Action of *P. fluorescence*

Disease suppression by these bacteria often entails inhibition of phytopathogens in soil or on roots, by following mechanisms.

Mechanism

1. Antibiotic Production
2. Siderophores Production
3. Induced Systemic Resistance
4. Competition
5. Hydrogen Cynide Production
6. Plant Growth Promotion Antibiotic Production

1. Antibiotic Production: The anti-fungal metabolite and surfactants play a major role in the biocontrol capabilities of *P. fluorescence* (Delany *et al.,* 2000). The *P. fluorescence* is very effective antibiotic producer. Many secondary metabolites of *P. fluorescence* acts as antibiotics against plant pathogens. The *P. fluorescence* produces antifungal compounds which are fungistatic, inhibiting spore germination and lysis of fungal mycelia.

- Phenazine-1-Carboxylic Acid (PCA)
- 2, 4 – Diacetylphloroglucinol (DAPG)
- Pyocinine
- Pyrrolnitrin
- Pyoluteorin
- Oomycin-A

2. Siderophores Production: In a context of biological control, competition for iron involves the synthesis of siderophores of higher affinity compared with siderophores used by phytopathogens. Iron acquisition entails the production of iron transporters (siderophores), noticeably fluorescent pyoverdines. Once complexed to ferric iron in soil or the root zone, the siderophores are then taken up using outer membrane receptors. (Lemanceau *et al.,* 1992). Siderophores are extra cellular, low-molecular weight compounds with very high affinity for ferric iron. As siderophore sequester the limited supply of iron in the rhizosphere, they limits it's availability to pathogens and ultimately suppress their growth.

- Ferribactin

- Ferrichrome
- Ferroxamine B
- Pseudobactin
- Pyochelin
- Pyoverdine

3. Induced Systemic Resistance: Plant protection may also result from direct interactions with the host plants, especially in the case of induced systemic resistance (ISR). Several strains can induce an ISR response in the plant, which makes the plant more efficient in fighting back against pathogens (Bakker *et al.,* 2007). The *P. fluorescence* induces systemic resistance in plants that is phenotypically similar to Systemic Acquired Resistance (SAR). Induction of resistance by *P. fluorescence* is mainly through the:

- Production of phytoalexins,
- Increased lignifications
- Production of PR-protein in the induced plants

4. Competition: As strains from *P. fluorescence* colonize the rhizosphere aggressively, competition with root pathogens for nutrients such as organic substrates released by seeds and roots as well as micronutrients (iron) and root surface colonization has been proposed as an important trait for biological control (Kamilova *et al.,* 2005). The *P. fluorescence* pre-empt the establishment of other rhizosphere microorganisms through competition for favoured sites on the roots and in the rhizosphere.

5. Hydrogen cyanide production: Hydrogen cyanide (HCN) is representative of class of volatile inhibitors. The *P. fluorescence* produces HCN which can check growth of phytopathogen although the producer bacterium itself resistance

6. Plant Growth Promotion Antibiotic Production: The *P. fluorescence* promotes plant growth by production of phytohormones such as Auxins and Gibberellins and also by Phosphate solubilisation.

P. fluorescence for Soil Health Enhancement

Suppressive soils are soils in which phytopathogenic fungi are unable to persist or are present but fail to induce severe disease symptoms on susceptible crops. This phenomenon, although rare, has been well characterised and there is strong evidence that disease suppression is the result of the presence of certain rhizobacteria with antifungal activity. Several studies have demonstrated that *Pseudomonas* strains with the ability to produce the antifungal metabolite 2, 4-diacetylphloroglucinol (Phl) can be isolated at high frequencies from soils.

Inoculant Delivery Systems for *P. fluorescence*

Although the vast body of research on *Pseudomonas* deals with their capacity to control soil-borne fungal pathogens, there has been limited success developing commercially viable products. Inconsistency under field conditions has often been cited as the principal reason preventing the commercial use of many. An equally important, if not over-riding bottleneck, however, is the lack of suitable inoculants formulations that allow *Pseudomonas* cells to survive for long periods under storage at concentrations high enough to afford biocontrol (McQuilken *et al.,* 1998). Current seed coating and pelleting procedures require a drying step, which often results inconsiderable reductions in inoculant viability (Shah-Smith and Burns *et al.,* 1997). Evidence suggests that the addition of nutrients to seed pellets may be a useful strategy for improving inoculants survival (Moenne-Loccoz *et al.,* 1999). Furthermore, carbon sources and minerals have been shown to have an important role in antifungal metabolite production by *Pseudomonas*,

suggesting that nutrient amendments to formulations may also be a useful strategy for improving biocontrol efficacy (Duffy and Defago, 1999). Without doubt, however, further research is required on the development and optimisation of microbial inoculants formulations, which will be compatible with current seed coating technologies. Furthermore, because survival during seed coating/pelleting and during storage at ambient temperatures is critical for the development of microbial inoculants products, it seems logical that these traits should form an integral part of any screening process for the selection of new *Pseudomonas*.

Conclusion

The prospect of manipulating crop rhizosphere microbial populations by inoculation of beneficial bacteria i.e. *P. fluorescens* to increase plant growth has shown considerable promise in laboratory and greenhouse studies. The success of *P. fluorescens* based products will depend on our ability to manage the rhizosphere to enhance survival and competitiveness of these beneficial microorganisms. Sequencing the genome provided further information of its environmental interactions and its metabolic capabilities, which can be used to control plant diseases. The major limitations of this biocontrol agent are its shelf life and inconsistent field performance. Unlike chemical pesticides, biocontrol agents need support even after their application to get established in targeted niche. Therefore, for the success of biological control, one has to ensure not only the quality of biocontrol agent applied but also its establishment in natural ecosystem to thrive and compete well with the pathogens.

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