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Efficacy of Pseudomonads as biocontrol agents of phytopathogens

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Introduction

Microbial plant pathogens infecting different crops cause great economic losses worldwide. Thus plant diseases need to be controlled to maintain the quality and abundance of these crops. Different approaches have been used to control plant diseases, mainly chemical fertilizers and pesticides. However, environmental pollution and pathogens induced resistance due to excessive use of these agrochemicals; may exclude their successful application. Consequently, researchers have focused on developing alternatives to control plant diseases; among these alternatives is biological control. The term biological control or biocontrol applies to the use of living antagonists to suppress or kill deleterious plant pathogens. The saprophytic microbe which antagonises the pathogen is called the biological control agent (BCA) or bioagent.

Microbe-mediated biological control is considered safe as being close to the natural eco-systems (Coleman-Derr and Tringe, 2014). There are two general ways of applying biocontrol agents against phytopathogens; through addition of large amounts of bioagents to the soil or stimulating the activities of the existing bioagents using various amendments (Conrath, 2011).

Biocontrol mechanisms involve production of antibiotics, volatile organic compounds (VOC), hydrolytic enzymes, competition for space, nutrients and induction of systemic resistance in plants (Faheem *et al*., 2015; Yunus *et al*., 2016). Senthilraja *et al*., (2010) pointed that combination of more than one biocontrol agent which occupy different sites and exert different promoting effects on their host plants is more advantageous than their individual application.

Pseudomonas spp. are soil-borne, Gram-negative bacteria which possess characteristics that make them advantageous for plant disease biocontrol (Palleroni, 2008). Several species of these bacteria produce antibiotics which suppress phytopathogens growth (Yang and Cao, 2012). Moreover, Duke *et al*., (2017) added that surface colonization by *Pseudomonas* spp. is accompanied with induction of host plant defence.

Fluorescent Pseudomonads belong to Plant Growth Promoting Rhizobacteria (PGPR), which play major role in the plant growth promotion, induced systemic resistance and biocontrol of several phytopathogens etc. These effects are attributed to the production of different secondary metabolites including; antibiotics, siderophores and hydrogen cyanide (HCN) (O'Sullivan and O'Gara, 1992). Siderophores are low molecular weight chelating agents of ferric ions produced by bacteria and fungi under conditions of low iron stress (Ngamau *et al*., 2014). According to Decheng *et al*., (2005) most of the plants are able to use bacterial iron siderophore complexes from the soil as a competitive advantage under iron stress, thus restrict proliferation and colonization of root by different phytopathogens. Siderophore-based bioagents have commercial importance as they are safer and do not lead to biomagnification, in addition, they also provide iron nutrition to the plants thereby promoting their growth (Sayyed *et al*., 2007).

Among various biocontrol agents, *P. fluorescens* strains inhabit different environments such as; plant, soil and water surfaces and have been used successfully to control different soil-borne pathogens (Ganeshani and Kumar, 2005). *P. fluorescens* BE8 showed 55% control of *Fusarium oxysporum* f. sp. *cucumerinum* in an *in vivo* pot experiment (Szentes *et al*., 2013). Another strain of *P. fluorescens* inhibited *Xanthomonas oryzae* pv. *oryzae*, the bacterial causal agent of leaf blight in rice (Lingaiah and Umesha, 2013).

The study of Bahrouna *et al*., (2018) demonstrated that bacteria with high biocontrol potency such as; *Rahnella aquatilis* B16C, *P. yamanorum* B12 and *P. fluorescens* B8P produce siderophores, chlorinated phenylpyrrole antibiotics (PRN) and a volatile compound. These are involved in biocontrol potential against many soil-borne fungal pathogens (de Souza and Raaijmakers, 2003). However, Weidner *et al*., (2017) reported that these three bacterial strains did not produce auxins, as they showed no plant growth promoting (PGP) activities in non-infested soil. This is attributable to the fact that biocontrol potency is not always associated with PGP activities, accordingly it's quite difficult to detect a strain of bacteria which encompasses both traits simultaneously.

Volatile compounds (VOCs) produced by soil microbes promoted plant growth, showed high antimicrobial and nematicidal activities, and induced systemic resistance in several crops (Audrain *et al*., 2015). The genus *Pseudomonas* is well known of producing large arrays of antifungal and plant growth-promoting metabolites, such as VOCs (Yan *et al*., 2017). In the study of Raza *et al*., (2016), VOCs produced by a biocontrol strain *P. fluorescens* WR-1 inhibited the growth and virulence characters of *Ralstonia solanacearum*; the bacterial causal agent of tomato wilt. VOCs produced by this strain showed more growth inhibition of *R. solanacearum* in natural soil than in sterilized soil. This is attributed to the contribution of soil indigenous microbes which also produced antimicrobial VOCs (Hernández-León *et al*., 2015). The VOCs produced by *P. fluorescens* B-4117 inhibited the growth of several *Agrobacteria* such as; *A. tumefaciens* and *A. vitis* (Dandurishvili *et al*., 2011). Moreover, *P. fluorescens* strains UM16, UM240, UM256 and UM270 produced VOCs which inhibited the growth of phytopathogenic *Botrytis cinerea* (Hernández-León *et al*., 2015).

Recently, Singha *et al*., (2016) revealed the impact of bio-protective microbes isolated from rhizosphere soil on plant growth promotion and triggering defence responses in rice plant when inoculated with *Rhizoctonia solani*. Rice plants co-inoculated with *P. fluorescens* PF-08 and *Trichoderma harzianum* UBSTH-501 strains demonstrated significant increase in the shoot and root biomass, and defence related biomolecules. Direct promotion mechanisms of these bioagents were associated with the reduction in pathogen population in the rhizosphere through production of antimicrobial metabolites such as antibiotics (Harman *et al*., 2004), mycoparasitism, competition for space and nutrients (Wang *et al*., 2013). However, indirect promotion effects were attributable to the enhancement in population of different beneficial soil microbes, and triggering defence genes inside the plant leading to suppression of growth and reproduction of phytopathogens.

Bacteriocins are ribosomally encoded proteins which expressed high specificity by killing bacteria closely related to the producer strain (Ahmad *et al*., 2016). *P. fluorescens* strain SF4c isolated from wheat rhizosphere soil produced a bacteriocin similar to phage tail-like pyocins of *P. aeruginosa*. This tailocin has antimicrobial activity against many phytopathogenic strains of Xanthomonads and Pseudomonads, without affecting the microbial community of the plant growth promoting rhizobacteria (PGPRs) such as *P. protegens* CHA0. Screening against number of phytopathogenic strains revealed that SF4c tailocin has inhibitory activity against *X. axonopodis* pv. *vesicatoria* (X. cv Bv5–4a). Therefore, this SF4c tailocin could be used as a biocontrol agent of bacterial leaf spot disease. The Atomic Force Microscopy (AFM) demonstrated that this tailocin adheres and causes damage to the cell envelope of X. cv Bv5-4a. This caused rapid leakage of intracellular materials with subsequent decrease of bacterial cell volume, leading finally to lysis of these cells (Fernandeza *et al*., 2017).

In conclusion, saprophytic Pseudomonads especially fluorescent ones are highly effective bioagents for control of most plant pathogenic microbes. They have multiple modes of action, are normal inhabitants of roots surfaces (rhizospheres) and leaves surfaces (phylloplanes), highly adapted to live as saprophytes and endophytes within plants. However, their potentials as bioagents could be enhanced by inoculating them into plants in association with suitable amendments, thus providing nutrients necessary for their growth, proliferation and colonization of plants surfaces, hence become more advantageous over deleterious phytopathogens. Moreover, it is better to apply more than one species of these Pseudomonads simultaneously to have several modes of action thus obtaining better biocontrol potency.

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