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A Review on Detailed Understanding and Recent Advances of Biocontrol Agent: *Pseudomonas fluorescens*

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ABSTRACT

Indiscriminate use of chemicals as fertilizers and fungicide caused inconceivable detriment to the terrain and ecosystem including creatures and humans. To replace similar type of dangerous agrochemicals, natural result is handed by nature in the form of microorganisms having capacity to promote the plant growth without mainly harming the terrain. One of the natural approaches for the control of different phytopathogenic agents is the use of biocontrol plant growth- promoting rhizobacteria (PGPR), which is able of suppressing or precluding the phytopathogen damage. The biocontrol PGPR belongs to the bacteria genus *Pseudomonas*. Fluorescent pseudomonads are suitable for operation as biological control agents due to their abundant population in natural soils and plant root system and their capability to use numerous plant exudates as nutrient. Fluorescent pseudomonads are known to have important traits in bacterial fitness similar as the capability to cleave to soil patches and to the rhizoplane, motility and prototrophy, conflation of antibiotics, and product of hydrolytic enzymes. In this review important characters, recent advances and importance of *P. fluorescens* in reduction of pesticide pollution has been discussed.

Key words : *Pseudomonas fluorescens, Plant diseases, Biological control, Rhizosphere, Rhizobacteria, Plant defense.*

Introduction

The *Pseudomonas* is motile (one/several polar flagella), and also non sporulating rods type structured with gram negative response and 58 – 69% GC content (Palleroni, 2008). They're catalase positive and chemo-organotrophic, with a rigorously respiratory metabolism. The fluorescent pseudomonads include all *Pseudomonas* species with the capability to produce fluorescent pyoverdine siderophore (s), noticeably *P. aeruginosa, P. syringae, P. putida* and *P. fluorescens* (Bossis *et al.* 2000). *Pseudomonas fluorescens* is acclimated to uplift in soil and colonization of plant roots (Kiely *et al.* 2006), and this implicates also to the certain case of biocontrol agents from this species. Biocontrol strains have markedly been observed at the root face, (i.e. the rhizoplane) often forming microcolonies or abstained biofilms in the grooves between epidermal cells. Particular strains are also able of endophytic colonization. In the inter-cellular spaces of the epidermis and the cortex within the tissues of root, they're substantially found in the intercellular spaces of the epidermis and the cortex within the tissues of root (Duijff *et al.* 1997). They are effective at exercising seed and root exudates for growth and can populate the rhizosphere aggressively. Strains with the capability of bio-control may represent in the order of 10 of all rhizosphere strains, and they've been insulated from a veritably wide range of soils, climatic regions and host plants (Rezzonico *et al.* 2007). Biocontrol agents from *P.*

fluorescens is moreover non-specific in their ability to cover plants from soil phyto-pathogens.

Indeed, each biocontrol strain can generally act in further than one pathosystem, i.e. cover further than one plant species from frequently distinct pathogens, handed the rhizosphere is successfully settled. They've been substantially studied for protection of crop plants from phytopathogenic oomycetes (particularly *Pythium spp*.) and fungi *(Fusarium oxysporum, Gaeumannomyces graminis, Rhizoctonia solani, etc*.), and to a lower extent bacteria (e.g. *Pectobacterium carotovorum*) and nematodes (e.g. *Meloidogyne spp*.). Disease repression by these bacteria frequently results inhibition of phytopathogens in soil or on roots, by competition and D or enmity (Haas and De´fago 2005). Protection of plant may also affect from the direct interactions with the host plants, especially in the case of induced systemic resistance (ISR) (Bakker *et al.* 2007). It resembles a group of common and non-pathogenic saprophytes. In soil, water and plant exterior environments, it can populate. It's a common gram negative, rod- shaped bacterium. Particularly under conditions of low iron availability, secretion a soluble greenish fluorescent pigment (which is called fluorescein) is happened by it. Excepting some strains, it can use NO3 as an electron acceptor in the place of O2 as it is an obligate aerobe. It is motile because of multiple polar flagella. Simple nutritive demands are shown in *P. fluorencens*. In mineral salts media, it grows very well. That mineral salt media is supplemented with any of a large number of carbon sources (Palleroni 1984). Because they're well-conditioned acclimated in soil, *P. fluorescens* strains are being delved considerably for use in operations that need the release and sustain of bacteria in the soil. In agriculture and bioremediation, the supreme among these are biocontrol of pathogens of various organic compounds. It decreases plant diseases by securing the seeds and roots from the fungal infection. They are known to enhance plant growth promotion and reduce harshness of numerous fungal diseases (Hoffland *et al.,* 1996; Wei *et al.,* 1996). The effect happens because of the result of the product of a number of secondary metabolites which are including antibiotics, siderophores and hydrogen cyanide (O'Sullivan and O'Gara, 1992). In 2005, Hass and Defago observed the mechanisms by which *P. fluorescens* controls pathogenic microorganisms in full description. Competitive rejection of pathogens as the result of fast colonization of the rhizosphere by *P. fluorescens* may also be a vital factor in the view of disease control. The present review discusses the circumstance, distribution, growth needs of the *P. fluorescens* and their impact on the plant diseases. It is prevented by the bacteria antagonist in different horticultural and agricultural crops.

The Pseudomonads belong to plant Growth Promoting Rhizobacteria. It is the important group of bacteria that play a major part in the factory growth creation, convinced systemic resistance, natural control of pathogens etc. Numerous strains of *Pseudomonas fluorescens* are known to enhance plant growth creation and reduce inflexibility of colourful conditions. The efficacity of bacterial antagonists in controlling fungal conditions was frequently more as alone, and occasionally in combination with pesticides. The present review refers to circumstance, distribution, medium, growth conditions of *P. fluorescens* and conditions controlled by the bacterial antagonist in different agrarian and horticultural crops were bandied. The literature in this review helps in unborn exploration programmes that aim to raise *P. fluorescens* as an inherent bio-pesticide for the augmentation natural control of numerous conditions of agriculture and horticultural significance.

Taxonomy and Distribution

A large number of strains with disease reduction potentiality are presented as *P. fluorescens*, some of these bio-control strains are actually belonging to this species (Sanguin *et al.* 2008). Numerous of the other strains correspond in fact to nearly- related species from the same '*P. fluorescens*' complex, noticeably *P. kilonensis*, *P. aurantiaca*, *P. thivervalensis* and *P. brassicacearum* (Frapolli *et al.,* 2007), which are frequently tough to distinguish from *P. fluorescens*. In addition, a some strains described as *P. fluorescens* belong to a separate fluorescent Pseudomonas lineage, taxonomically ill- defined and generally appertained to as ARDRA-1 grounded on 16S rRNA gene restriction profiling (Keel *et al.,* 1996). Thus, in this review, we're dealing with *P. fluorescens* biocontrol agents in the vast sense, which is by considering also those present in nearly- related taxa of *P. fluorescens* and mis-represented as *P. fluorescens* in the literature. The current state of the *Pseudomonas* taxonomy makes it tough to assess the phylogenetic distribution of biocontrol agents within *P. fluorescens* and nearly- related fluorescent pseudomonads (Bossis *et al.,* 2000). Still, it seems clear that these taxa

include both biocontrol agents and strains without any egregious biocontrol potentiality, anyhow of whether only true *P. fluorescens* or also related fluorescent pseudomonads are allowed (Sanguin *et al.*, 2008). It's important to note that *P. fluorescens* and close related species are being permitted to include also strains with human pathogenicity potentiality (Wei *et al.*, 2002; Bodilis *et al.*, 2004), but the proof to date isn't completely satisfying in the current taxonomic environment and this issue deserves more explanation. *Pseudomonas fluorescens* and it's nearly-related fluorescent pseudomonads are showing to be generally clonal (Frapolli *et al.*, 2007). Yet, horizontal gene transfer may happen and such a possibility has been taken up for genes involved in the dealings with the plant and D or phytopathogens (Ramette *et al.*, 2003; Blaha *et al.*, 2006). This includes

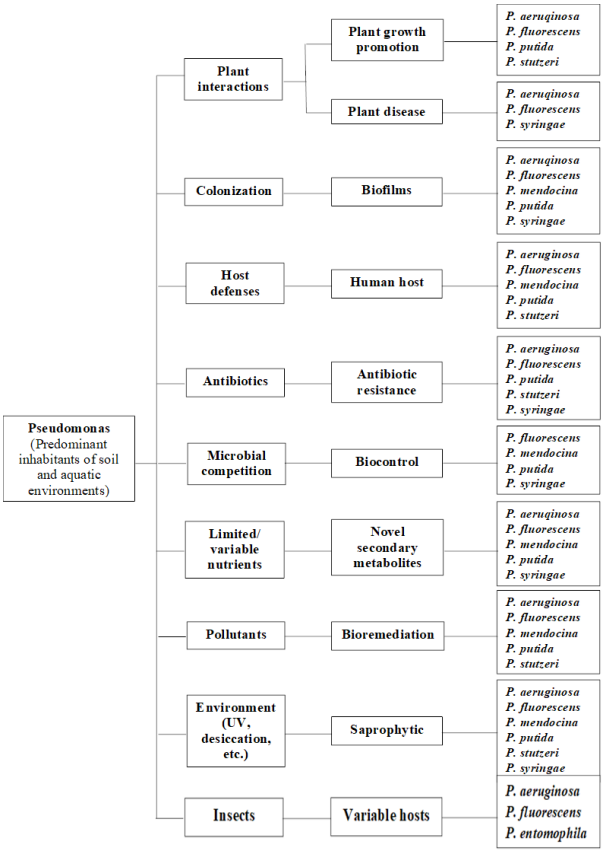

Fig. 1. Environmental niches and functional range of the *Pseudomonas genus*, pointing the broad distribution of the *P. fluorescens* species complex. Members of the *P. fluorescens* species complex are successful pioneers in a wide range of surroundings and territories due to different functional capacities. (Silby *et al.*, 2011)

also the thesis that genes involved in the synthesis of biocontrol compounds might have been acquired from the plant itself (Cook *et al.*, 1995; Ramette *et al.*, 2001). It belongs to Phylum, Proteobacteria (Garrity *et al.*, 2005), class Gamma proteobacteria, family Pseudomonadaceae and biocontrol species is *P. Fluorescens*.

Genome Structure

Presently, two strains of *Pseudomonas fluorescens* have their genomes sequenced fully. *P. fluorescens* Pf-5 genome contains one roundabout chromosome that has 7.1 Mbp and a GC content of 63.3. It contains 87 RNAs and 6137 proteins.5.7 of its genome contributes to secondary metabolism which is the largest of the pseudomonas. One chromosome with 60.5 GC content and 6.43841 Mbp is possessed by the genome of *Pseudomonas fluorescens* PfO-1 (Paulsen *et al.*, 2005). There are 95 RNAs and 5736 proteins. The genome sequencing of *P. fluorescens* SBW25 is still in progress.

Table 1. General genome features of *P. fluorescens*

Genome features	Numbers
Size (base pairs)	7,074,893
G+C content (%)	63.3
Protein-coding genes	
No. similar to known proteins	3,822
No. similar to proteins of unknown function*	890
No. of conserved hypotheticals**	1,113
No. with no database match***	330
Total	6,144
Average ORF size (bp)	1,021
Coding (%)	88.8
rRNA	5
tRNA	71

*It is an important sequence which has similarity to a named protein for which, there is no function, currently attributed.
**A sequence having similarity to a translation of another ORF, still for protein expression no experimental evidence exists.
***The Hypothetical protein, it has no significance in similarity to other sequenced protein.

Cell Structure and Metabolism

Cells are single, straight or curvy but not spiral. During expontial growth, Cell size is generally (0.7-0.8) X (2.3-2.8) micron. It's motile with polar multitrichous flagellation. It is strict aerobes, chemoorganotrophs, metabolism respiratory, no fer-

mentative. Diffusible fluorescent (yellow-green) pigment is produced by culture on King's B medium. Optimum temperature for growth ranges from 25-30°C. For Starch hydrolysis, Gelatin liquefaction, Catalase test, Oxidase test, Levan formation from sucrose, Citrate application etc., the *P. fluorescens* gives positive response. (Mahaveer Singh Bochalya, *et al.*, 2012) *P. fluorescens* is an aerobe. It is oxidase positive also. It's unfit to grow under anaerobic conditions when placed in the anaerobic GasPak jar. Some strains can use NO_3 rather of O_2 as the electron acceptor. These microbes retain multiple polar flagella for motility. *P. fluorescens* used siderophores too to fulfil the need of iron. (*P. fluorescens* PfO-1, D.O.E. Joint Genome Institute) The siderophore pyoverdine is produced by this bacterium and it is liable for chelating iron only while concentrations are low. This siderophore is liable for the fluorescence of *P. fluoresecens*. This denotes the reason of the fluorescent pigment is only produced when the concentrations of iron are low. When the concentrations of iron are high, pyoverdine isn't required. In fact, colonies won't fluoresce under ultraviolet light. Strain Pf-5 retains numerous extracellular hydrolytic enzymes that degrade polymers found in soil as well as hydrolases used on plant- derived carbohydrates. They're also able of demeaning and using components of plant tissues similar as hydrocarbon molecules, fatty acids and oils. Viscosin is produced by *P. fluorescens*. Viscosin is a peptidolipid that enhances antivirality. (Paulsen *et al.,* 2005) A sulfate transport system is also used by them. Chromate competitively inhibits that, which may be associated to *P. flurorescens's* sensitivity to chromate (Montie *et al.* 1998) *P. flurorescens* can produce certain enzymes similar as heat stable lipases and proteases which are involved in the spoiling of milk. *P. fluorescens* Pf-5 produces four metabolites that are poisonous to fungi or oomycetes and important in biocontrol two well- characterized polyketides pyoluteorin and diacetylphloroglucinol, the chlorinated tryptophan derived HCN (hydrogen cyanide) and pyrrolnitrin, which is formed by oxidation of glycine. The gene clusters for each of this compound are got in the genomic sequence of *P. fluorescens* Pf-5 and are alike to those preliminarily reported. Three preliminarily unknown gene clusters with characteristics of secondary metabolite biosynthetic regions were linked. For the biosynthesis of two siderophores (pyoverdine and pyochelin), combination with gene clusters is done and it's estimated that 5.7 of the *P. fluorescens* Pf-5 genome (<" 400 kb) is devoted to secondary metabolism, the largest percentage(%) among the *Pseudomonas spp.* genomes sequenced to date (Paulsen *et al.*, 2005).

Pathology

In spite of, their commensal nature, *P. fluorescens* are non-pathogenic. It has no virulence factors of other plant pathogens. In *Pseudomonas fluroescens* Pf-5, enzymes that degrade plant cell walls and their factors similar as cellulase, pectinase, or pectin lyase aren't present. Still, it's able of breaking down some plant- deduced carbohydrates, fatty acids, and oils and can hydrolyze proteins causing the decay of milk, meat, and fish. It has also been shown to be an opportunistic pathogen in vulnerable compromised fish like Koi which are generally kept in vicinity garden ponds. There are some species of Pseudomonas that are pathogens. *Pseudomonas syringae* is a plant pathogen that impacts food and biomass product. Diseases include bacterial specks on tomatoes and leaves which affect in suppressed growth and halo scar of beans. These microbes can spread through rain and are seed- borne pathogens (Paulsen *et al.,* 2005). Though *P. fluorescens* generally has low degree of virulency, in 1997 four cases at the National Taiwan University Hospital developed *Pseudomonas fluorescens* bacteremia. These cases had been treated in the chemotherapy room and had begun displaying symptoms similar as sicknesses and chills. Eight societies were separated from catheters and from the blood of the four cases. All isolates were associated as *Pseudomonas fluorescens.* (Hseuh *et al.* 1998)

Isolation of *P. fluorescens*

Firstly, we have to collect rhizosphere soil particles loosely adhering to the roots and make fine powder. Then, 10 gm sample of finely pulverized, air dried soil is added into 90 ml sterilized distil water. It will make 1:10 dilution (10-1). On a magnetic shaker, we need to shake the dilution vigorously for 20-30 minutes so that it obtain uniform suspension. 1 ml of suspension (1:10 dilution) is transferred to another 9 ml sterilized distil water to make 1:100 dilution (10-2). Then, we prepare serial dilution 10-3 to 10-7. King's B medium is used for isolation of P. fluorescens. After that, we need to pour 20 ml sterilized, melted and cooled (45° C) King's B medium in each Petri plate. Meanwhile, 1 ml of soil suspension is transferred from aliquot dilution (10-5 to 10-7) to sterilized Petri plates containing King's B me-

dium. Then, we incubate Petri plates at 28 = 2°C for 24 hours. Individual colonies with yellow-green and blue white pigments are detected and marked by viewing under UV light. Then, we pick up individual colony with sterilized loop and again we place it in the fresh king's B medium. At last, single colonies are transferred to King's B medium slants so that it obtains pure culture. Finally, we store in refrigerator at 4°C.

Pseudomonas as Bio Control Agent and Boon to Reduce Pesticide Pollution

The research which is conducted at the University of California, Berkeley, during the late 1970s (Weller 1988) has enlightened the worldwide interest in the *Pseudomonas sp.* as bio-control agents. The *Pseudomonas fluorescent* species are capable of using vast range of organic and inorganic compounds which acquaints them capacity for living in varied environmental conditions. The members of this genus are traced in great numbers in all the major natural surroundings, viz., terrestrial, fresh water, and marine, and they also form close associations with plants and animals. This suggests an importance quantum of physiological and genetic elasticity (Nowak Thompson *et al.* 1997). The bacterium which is belonging to genus *Pseudomonas* are different in the pint of view of functional and ecological noticeable microorganisms owing to their multiple uses as plant growth- promoting agents and bio-remediators. *Pseudomonas* has been identified as complex collection of a great number of species (Gardener *et al.*, 2005). The functional and metabolic heterogeneity of *Pseudomonas* has been well structured from studies which are dating to more than 45 years ago. The species of the *Pseudomonas* assimilates a bio-control agent owing to their catabolic adaptability, and moreover for their outstanding root- colonizing abilities, and their capacity for producing a vast range of anti-fungal metabolites. Within many *Pseudomonas spp.*, fluorescent pseudomonads have gotten particular attention as bio control agent. *Pseudomonas* releases its bio control activity in the way of direct antagonism of phytopathogens and induced of disease resistance in the host plant (Cartieaux *et al.*, 2003).

Fluorescent *Pseudomonas* is a widely studied group within general inhabitants of the rhizosphere. They can be visually prominent from the other *Pseudomonas* species of soil by their ability for producing water-soluble yellow-green pigments. They include of *P. aeruginosa, P. aureofaciens, P. fluorescens, P. chlororaphis, P. putida,* and the *P. cichorii* and *P. syringae* (plant pathogenic species) (Landa *et al.*, 2003; De La-Funte *et al.*, 2006). *Pseudomonas spp.* is well documented to inhabit in the rhizosphere.

Table 2. Summary of information on fully sequenced bacterial strains from the *P. fluorescens*:

Strain	Isolation source	Genome size (Mb)	Yr. isolated/yr. sequenced	Reference (s)
Pf0-1	Loam soil, Sherborn, MA	6.44	1988/2009	Silby *et al.*, 2009; Compeau *et al.* 1988
A506	Pear phyllosphere, California	6.02	1994/2012	Loper *et al.* 2012; Vilo *et al.* 2012
SBW25	Sugar beet phyllosphere, Oxfordshire, UK	6.72	1989/2009	Silby *et al.* 2009
Q2-87	Wheat rhizosphere, Washington State (same field as that for Q8r1-96)	6.37	1987/2012	Loper *et al.* 2012, Pierson *et al.* 1994
Q8r1-96	Wheat rhizosphere, Washington State (same field as that for Q2-87)	6.6	1996/2012	(Loper *et al.* 2012); (Raaijmakers *et al.* 1998)
WH6	Rhizosphere of *Poa* sp. and *Triticum aestivum* at Hyslop Research Farm, Benton County, OR	NA	2008/2010	Banowetz *et al.* 2008), Kimbrel *et al.* 2010
SS101	Wheat rhizosphere, near city of Bergen op Zoom, The Netherlands	6.18	2003/2012	Loper *et al.* 2012), De Souza *et al.* 2003
R124	Tepui orthoquartzite sandstone cave in Guiana Shield, South America	6.3	2007/2013	Barton *et al.* 2013
F113	Sugar beet rhizosphere	6.85	1992/2012	Redondo-Nieto *et al.* 2012, Shanahan *et al.* 1992
NCIM 11764	Culture supplied with potassium cyanide as the sole nitrogen source	6.97	1983/2012	Harris *et al.* 1983), Wilson *et al.* 1994

Pseudomonads are having many traits. Those traits make them well suited as bio-control and growth promoting agents (Weller, 1988). These show their capability to (a) grow up faster which makes them much more easier to produce in mass in the laboratory, (b) immediately, it intakes seed and root hairs, (c) form groups and multiply in the spermosphere and rhizosphere environments and inside of the plant, (d) also generate a large spectrum of bio-active metabolites (siderophores, antibiotics, volatiles, and growth-promoting substances), (e) compete vigorously with the other microorganisms, (f) get habituated to environmental conditions, and (g) easily form group of plants upon subsequent re-inoculation in to the soil by seed bacterization. In the presence of pseudomonads in the soil the soil-borne pathogens are naturally get suppressed (Weller *et al.,* 2002). Some strains are living in close relationship with the plants, which is safeguarding them from infection by pathogens. Otherwise that might cause disease. Control of root diseases by some beneficial bacteria which involves in a mixture of possible mechanisms that may help each other. The primary mechanism of bio-controlling includes mainly the production of antibiotics or suppresses the function of virulent characteristics of pathogens (Diby *et al.,* 2005). Moreover another important mechanism is indirectly inhibiting the pathogens by bacterial stimulation of defencing responses within the plant host. And many of the plant-associated strains belong to fluorescent *Pseudomonas* group, which presently includes more than 50 identified species (Yamamoto *et al.,* 2000; Mulet *et al.,* 2010). *Pseudomonas* is having a great role in nicely growth and development of plant by its ability to safe plants against pathogens during different developmental stages. The benefit of pseudomonads (above mentioned) relies on their capability to proficiently intake root hairs and prevents predation by soil predators like nematodes and protozoa (De Mesel *et al.,* 2004; Abuzar and Haseeb 2010). Bacteria have involved an order of anti-predatory mechanisms, like toxicity. Complex interactions with predators are driven extracellular metabolites of *Pseudomonas sp.* affecting their physiology and behaviour. Secondary metabolite is working particularly on predators, which is acting as repellents, stressors, or toxics. Such secondary metabolites production by bio-control bacteria is having many functions, and metabolites safeguarding plants against the pathogens for improving bacterial resistance (Gadoury *et al.,* 1989). Different variety of organic compounds can be used as energy sources by *pseudomonas sp.* It generates an arrangement of secondary metabolites which is as lipopeptides, pyrrolnitrine, 2, 4-diacetylphloroglucinol, pyochelin, phenazines, and hydrogen cyanide (Keel *et al.,* 1992; Haas and Defago 2005). One or some antibiotic compounds are produced by bio controlling strain of *Pseudomonas sp.* These antibiotics have been tested *in vitro*. These have been known as inhibitory compounds. For the sake of the plant health management, they exhibit active response in the field situation. Some strains are producing DAPG, the anti-fungal compound. During introducing into the rhizosphere through seed or soil treatments, those strains have a vital role for suppressing some root diseases (Reddy *et al.,* 2009). In agriculture, *Pseudomonas sp.* has vital role for suppressing plant disease and management of plant diseases. With the help of antagonistic bacteria, bio-logical control against plant diseases is less convenient than other control methods of plant diseases. However, it possesses the capability of transforming the strategies for management of plant diseases.

Modern Approaches for Improvements of *Pseudomonas Fluorescen*s Efficacy as Biocontrol Agents

The strains of indigenous *pseudomonad's* (siderophore producing) efficacy of bio-control against *Fusarium* wilt of Tomato

For *in vitro* antagonism, two strains of bacteria are taken in this study. The antagonism is against the pathogen of tomato wilt, *Fusarium oxysporum* (sp. *lycopersici*). Those two bacterial strains are taken for siderophore production. The strain, SP_{S9} generates 59.30 microgram per ml of siderophore and 52.74 microgram ml of siderophore is generated by SP_{S20}. In deferrated SSM, they show the zone of fungal inhibition of 3.8 centimetres and 3.4 centimetres for SP_{S20} and SP_{S9} respectively. In SSM, with the increasing of iron, the decreasing of the size of zone is found. In SSM, with the iron of 2.5 micrometre, 5 micrometre, 7.5 micrometre and 10 micrometre and without iron, the zone of fungal inhibition is 2.1 cm, 1.5 cm, 0.9 cm, 0.6 cm and 3.4 cm respectively for SP_{S9}. For SP_{S20}, the zone of fungal inhibition is 2.2 cm, 1.7 cm, 1.0 cm, 0.7 cm and 3.8 cm respectively. Through the assay of greenhouse, the strains potential of bio-control is assessed. The seedlings of to-

mato which is inoculated with SP_{S20}, shows 75% of disease control. The seedlings, inoculated with SP_{S9} shows 100% disease control. Both of the cases, the dry weight as well as fresh weight get increased. Finally, the strains, SP_{S9} and SP_{S20} get identified in genetic methodology as *P. fluorescens*. (Arya *et al.*, 2018).

Effect of fermentation temperature and duration on bio-control efficacy of freeze-dried cells of *P. fluorescens* Pf153

The study was to know whether there is any effect on the bio-control efficacy of *P. fluorescens* Pf153 by changing in the fermentation conditions. The determination of effect of fermentation temperature on the bio-control efficacy of *P. fluorescens* Pf153, freeze-dried cells was the main goal of this study.

In fermenter and flasks, *P. fluorescens* cells are grown at 28 degree Celsius and 20 degree Celsius respectively. At the beginning of the stationary phase and the mid log, it is harvested respectively. By testing, it gets to know that the freeze-dried cells get survived at 25 degree Celsius at the time of storage. In storage, cells which are fermented at 20 degree Celsius get survived better than those cells which are grown at 28 degree Celsius, regardless of the time of harvesting. In all temperature or duration combinations of production, Pf153 has great efficacy in in vitro tests. Against all strains of *Botrytis cinerea*, it was great effective in comparison of the non-treated control. However, among temperature or duration combinations, there are no noticeable differences. On the separated leaves of *Vicia faba*, it is observed in bioassay that young cells have a great positive effect on the bio-control efficacy when it gets fermented at 28 degree Celsius.

This study explains that there has great effect on the bio-control efficacy of *P. fluorescens* Pf153 because of fermentation parameters (Bisutti *et al.,* 2020)

***P. fluorescens* ZX produced VOCs' anti-fungal activity and on the post-harvest citrus, the bio-control of blue mold decay**

In the study, blue mold (causal organism: *Penicillium italicum*) affected post-harvest fruits of citrus are taken for treating with VOCs (volatile organic compounds) which are generated by *P. fluorescens* ZX which is incubated on NA & in NB. The citrus fruits are also treated with the pure individual components of volatile organic compounds. The volatile organic compounds from *P. fluorescens* suppress the growth of mycelia of *P. italicum* by 42.14%. Also by VOCs, *P. italicum's* germination of conidia gets suppressed by 77.86%. It is expressed in *in vivo* tests that VOCs suppress the incidence of blue mold diseases and the size of lesion on the fruits greatly. The volatile organic compounds which are generated by bacterial fluid shows higher bio-control efficacy in comparison with VOCs from NA plates. The hyphae and conidia of *P. italicum* get abnormalities morphologically by the result of the exposition to VOCs from bacterial fluid. The pure individual compounds which are consisting of the volatile organic compounds are taken for *in vitro* experiments. The experiments explain about the active components of VOCs i.e. sulphur compounds and organic acids with DMTS (dimethyl trisulphide) and DMDS (dimethyl disulphide). The active components express the highest anti-fungal activity. *In vivo* experiments, the perfect blue mold inhibition in citrus fruits are carried out through the help of 100 microL per L concentration of DMDS and 10 microL per L concentration of DMTS. The exhibition of organic acids for preventing blue mold is possible but it should be adequately low concentration because of avoiding the promotion for physiological damages and diseases on the citrus fruits. (Wang *et al.,* 2021)

The ability of survival & the release with control of *P. fluorescens* VUPF506, alginate-micro encapsulated and the influence of them on bio-control of *Rhizoctonia solani* on potato

There is a great challenge of conserving plant probiotic bacteria efficacy in soil so that plant diseases can be prevented through biological control. In unfavourable surroundings situation, a vital step for conserving pro-biotic ability and viability is micro-encapsulation technique. Selection of a proper coating for the encapsulation of probiotic is the actual purpose of the study. The ability of survival and the release with control of alginate (the combination with carboxymethyl cellulose, peanut butter and whey protein concentrate) en-capsulated *P. fluorescens* VUPF506 are assessed. In addition, the en-capsulated cells are assessed in potato plants through *in vivo* experiments for the prevention of *Rhizoctonia solani*. The assessment explains retaining more than 80 percentages of the cells of bacteria by all examined wall material. For more than two months, the alginate-whey protein concentrate microcapsules (Alg-WPC) give a good controlled release. Amazingly, the experiment in greenhouse

shows the most effective treatment and it was potato plant treatment with alginate-whey protein concentrate microcapsules. 90% of pathogen can be suppressed with the help of that treatment. The assessment proves that the microcapsule (Alg-WPC) is most promised combination for the improvement of the survival ability of *P. fluorescens*. As it contains worthy amino acids, we can utilise it as a fertilizer. (Fathi *et al.* 2021)

Conclusion

Bio-control agent, dissimilar to chemical pesticides has the requirements of support even though many people have paid interesting attention for its application for getting set on particular targeted areas. There should be some features for a successful bio-control agent. People should assure the quality of bio-control agents. For vigorous development and competition with pathogens, bio-control agent should get established in natural environments. There is another aim that one has to consider. For assuring the survivability and getting compatible with seed treatment, better formulations of bio-control agents should be developed. *P. fluorescens* plays an important role as a bio-control agent. One should have good future expectation from it because of providing better benefit-cost ratio. It is a good promising bio agent. The bacterial antagonism of *P. fluorescens* is very effective to control soil-borne fungal pathogens. In future the areas of focus should be in the promotion of *P. fluorescens* as a potential biopesticide preventing many agricultural and horticultural crops diseases. Also, *Pseudomonas* possesses factory growth- promoting traits similar as nitrogen obsession, phosphate solubilization, iron chelation, and phytohormone product. Similar multidimensional mileage of fluorescent Pseudomonads makes them a bioagent of choice to be exploited in the field of agriculture. Numerous strains of *Pseudomonas fluorescens* show implicit for natural control of phytopathogens especially root pathogens. In taxonomic terms, several of them are actually *P. fluorescens sensu stricto*, when others belong to neighbouring species of the '*P. fluorescens*' complex or to ill- defined affiliated species within the fluorescent *Pseudomonas spp*. These bacteria have come prominent models for rhizosphere ecological studies and analysis of bacterial secondary metabolism, and in recent times knowledge on their plant-salutary traits has been vastly enhanced by widening the focus beyond the case of phytopathogen- directed enmity. Genomic analyses of rhizosphere capability and biocontrol traits will probably lead to the development of new tools for effective operation of indigenous and invested *P. fluorescens* biocontrol agents and a better exploitation of their plant-salutary parcels for sustainable farming.

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