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REVIEW ARTICLE Agricultural Reviews, Volume Issue : ()

Application of *Pseudomonas* Strains for Biocontrol of Commercial Crops Susceptible to Plant Pathogens: A Review

Ullas Prasanna Sadarahalli, Guruprasad Nadabhaga Manjunatha[1],
Thammaiah Chekkera Kuttappa, Ajith Sheshagiri, Manjunath Kumbar[2]

10.18805/ag.R-2451

ABSTRACT

Indiscriminate use of chemicals as fertilizers and pesticides gives rise to substantial harm to the environment and ecosystem along with animals and humans. More importantly, to replace such hazardous agrochemicals, many biological solutions are available in nature which is present in the form of microorganisms having the capacity to promote plant growth substantially. *Pseudomonas* play a major role in plants like inducing systemic resistance, colonizing plant roots or tissue interior, helping in modulating plant hormone levels, biological control of pathogens and more over regulating the growth-related genes in plants. The *fluorescent pseudomonads* belong to plant growth-promoting rhizobacteria (PGPR) and many strains of *pseudomonas* are well known to enhance plant growth promotion and decrease the severity of many diseases. Biocontrol strains of *pseudomonas* in plant bioassays synthesize one or more several antibiotic compounds. *In vitro*, these antibiotics have been proven as inhibitory compounds and have exhibited active responses for plant health management in field conditions. The current study mainly relies on the effective use of *Pseudomonas spp.* as a potential biocontrol agent for the biological control of many diseases in agriculture and horticultural crops.

Key words: Biocontrol, Natural antagonists, PGPR, *Pseudomonas*, Rhizosphere.

Biocontrol agents

Biocontrol is the suppression of determined endeavors of one organism by one or more other organisms, often represented as natural antagonists. Biocontrol involves living organisms or natural substances to counteract or diminish the damage caused by destructive organisms (pests, weeds and microbial pathogens). They are predominantly used for the diminution of pest residents and generate yields that are free of any pest (Molinari *et al.,* 2019). More broadly, the term biocontrol has been applied to the use of natural products extracted from various sources of microbes. These formulations are the simple mixtures of natural ingredients with activities or complex mixtures with multiple effects on the host as well as the target pathogen (Martins *et al*., 2018). In the present scenario, biological control has a significant role in reducing the effects of pests and diseases along with environmental effects. Biocontrol agents give protection to the crop through the crop period. They multiply easily in the soil and leave no residual problem (De Silva *et al*., 2019). In the present review, we are confidently focusing on the growth promotion effects and antagonistic activities of *pseudomonas* on different commercial crops instead of chemical pesticides.

Pseudomonas

The genus *Pseudomonas* is the most numerous among the cataloged genera of gram-negative bacteria (Gomila *et al.,* 2015). They are aerobic, non-spore-forming, which are straight or slightly curved about 0.5-1.0 µm by 1.5-5.0 µm and are motile employing one or more flagella, ubiquitous in agricultural soils and are well adapted to grow in the rhizosphere. It appears that they retain a very strict aerobic respiratory metabolism with oxygen (Meyer *et al.,* 2011). At the outset, *pseudomonas* is behaviourally versatile with free-living as well as parasitic forms capable of colonizing a wide variety of host organisms and ecological niches within hosts. *Pseudomonas* retain many traits that make them well suited as biocontrol and growth-promoting agents (Rajkumar *et al.,* 2017). Furthermore, the typical *pseudomonas* in nature can exist in biofilms formats, attached to some surface or substrate, or in a planktonic form, as a unicellular organism and are actively swimming using its flagellum (Gamalero *et al.,* 2004). Classification of *Pseudomonas* has been mentioned below in Table 1.

Department of P.G. Studies and Research in Biotechnology, Kuvempu University, Shankaraghatta-577 451, Karnataka, India.
[1]Western Ghats Team, Wildlife Conservation Society, Kodigehalli, Bengaluru-560 097, Karnataka, India.
[2]Department of P.G. Studies and Research in Applied Botany, Kuvempu University, Shankaraghatta-577 451, Karnataka, India.

Corresponding Author: Thammaiah Chekkera Kuttappa, Research Associate Wildlife Institute of India, Chandrabani, Autonomous Institution of Ministry of Environment, Forest and Climate Change, Dehradun-248 001, Uttarakhand, India.
Email: ullasprasanna@gmail.com

How to cite this article: Sadarahalli, U.P., Manjunatha, G.N. and Kuttappa, T.C., Sheshagiri, A. and Kumbar, M. (2022). Application of *Pseudomonas* Strains for Biocontrol of Commercial Crops Susceptible to Plant Pathogens: A Review. Agricultural Reviews. DOI: 10.18805/ag.R-2451.

Submitted: 27-12-2021 **Accepted:** 12-05-2022 **Online:** 13-06-2022

Diversity in *Pseudomonas*

The genus *pseudomonas* encompasses the most diverse and economically significant group of bacteria, currently containing more than 200 recognized species. Members of the genus are found in large numbers in all of the crucial natural environments and form intimate associations with plants. Members of the *pseudomonas* genus are highly adaptable as evidenced by the successful colonization of many peculiar environments and their immense deal of metabolic versatility and genetic plasticity (Spiers *et al.,* 2000). Substantially, they also comprise a metabolically versatile group of organisms that are known to engross numerous ecological niches including the rhizosphere and endosphere of many plants (Alfano *et al.,* 2000). On the contrary, the formation of spores occurs in some species, but these strains do not produce spores where there is the occurrence of retractile granules of reserve materials, which often look like spores. Henceforth many isolates are of interest as possible biocontrol agents (Gomils *et al.,* 2015).

Classic strains and novel concepts

Berkeley strains

On contemporary PGPR research can be traced, now nearly 4 decades old, began with the manifestation by the researchers. At the University of California, Berkeley certain isolates of *pseudomonas* that were applied to the seeds or seed pieces could colonize roots and helped to promote the growth of the potato, sugar beet and radish (Joseph *et al.,* 2012).

Dutch strains

A second lineage of contemporary *pseudomonas* biocontrol activity can be traced by bacterization studies with *fluorescent pseudomonads*. These findings were followed by alike reports by Dutch researchers that *pseudomonads* promoted the growth of potatoes (Weller *et al.,* 2012).

Antibiotic producers

The third lineage of contemporary *pseudomonas* biocontrol activity can be traced by bacterization studies with *fluorescent pseudomonads* that produce antibiotics such as phenazine-1-carboxylic acid (PCA) and other derivatives such as 2, 4- diacetylphloroglucinol (DAPG), pyrrolnitrin (Prn) (Mann *et al.,* 1986).

ISR *pseudomonads*

The fourth lineage of contemporary *pseudomonas* biocontrol activity can be traced by some *pseudomonads* colonizing the roots protected plants from various pathogens by including systemic resistance (Vilchez *et al.,* 2000).

Need for biocontrol agents

Across the world, plant diseases are the major cause of yield loss. In certain, the use of chemical pesticides has led to numerous disadvantages which include toxic accretion of toxic residues in the environment. Hereby adaption of pathogens to such chemicals, in turn, lessen its efficiency and thus leads to a disagreeable effect on non-target organisms persuading in the same niche. Moreover, nowadays, consumers are more anxious about pesticide-free foods. This results in the emergence of eco-friendly strategies for plant disease management (McSpadden *et al.,* 2005).

Pseudomonas as biocontrol agents

Global interest in *pseudomonas* as biocontrol agents were evoked by research carried out at the University of California, Berkeley, during the late 1970s (De La-Fuente *et al.,* 2006). However, species of *pseudomonas* are efficient in employing a wide range of organic and inorganic compounds which in turn influences their capacity to live in varied environmental conditions (McSpadden *et al.*, 2005). Among various *pseudomonas*, *fluorescent pseudomonas* has particularly secured attention as a biocontrol agent of choice. *Pseudomonas* formulated as products used as biological control agents has been mentioned in Table 3. Despite this, *pseudomonas* exerts its biocontrol activity through direct antagonism of phytopathogens and induction of disease resistance in the host plant (Cartieaux *et al.,* 2003).

PGPR of *Pseudomonas*

Most of the bacteria genera are represented as PGPR and many members of the genera *pseudomonas* implement beneficial effects on plants (Vacheron *et al.,* 2015). Whereas, many reports have assessed *pseudomonas* as PGPR and/or biological control agents that can affect plant fitness and are also relevant in biotechnological applications based on integrated plant-bacteria systems. (Montesinos *et al.,* 2002). Effect of *Pseudomonas* on plant growth promotion have been mentioned in Table 4. On the other hand, different PGPR species have the mechanism of instantly enhancing plant development. Mechanism of PGPR have been mentioned in Fig 1. Strains of *pseudomonas* were able to trigger plant growth by different traits like nitrogen fixation, phosphate solubilization, production of organic acids and IAA (Indole Acetic Acid) (Ahemad and Kibret 2014). Several researchers have selected strains of *pseudomonas* that generate gibberellin like substances in culture and reported that they are substantial to plant growth responses. These gibberellin like substances and other growth-promoting compounds were chiefly produced by *pseudomonas* (Kay *et al.,* 2005). *Pseudomonas* mediated induced systemic resistance in plants against pathogens have been mentioned in Table 2**.** Hence *pseudomonas* group possesses several species of rhizobacteria that have been used as strains for rhizobacteria colonization experiments. On the contrary, colonization potential is associated to hold up the nutritional balance

Table 1: Classification of *pseudomonas* according to integrated taxonomic information system (ITIS) (Palleroni 2015).

Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	*Pseudomonadales*
Family	*Pseudomonadaceae*
Genus	*Pseudomonas*

Table 2: *Pseudomonas* mediated induced systemic resistance in plants against pathogens.

Plant species	*Pseudomonas* strains	Microbial pathogen	Disease symptoms	References
Radish	*P. fluorescens* WCS374	*Fusarium. oxysporum raphani*	Vascular wilt	Leeman *et al.,* (1995)
	P. fluorescens WCS417	*Alternaria brassicicola*	Necrotic lesions	Ton *et al.,* (2002)
Bean	*Pseudomonas Rhodesiae*	*Botrytis cinerea*	Gray mold	De Meyer and Hofte (1997)
Pepper	*Pseudomonas aureofaciens* 25-33	*Xanthomonas axonopodis*	Bacterial spot	Kang *et al.,* (2007)
Rice	*Pseudomona fluorescens* (pf1)	*Xanthomonas oryzae*	Rice leaf blight	Vidhyasekaran *et al.,* (2001)
Grape	*Pseudomonas aeruginosa* 7NSK2	*Botrytis. cinerea*	Grey rot	Verhagen *et al.,* 2010
Chillies	*Pseudomonas fluorescens* (pf1)	*Colletotrichum. capsici*	Antgracnose, Die-back	Bharathi *et al.,* (2004)
Chickpea	*Pseudomonas fluorescens* (pfl-94)	*Fusarium. oxysporum f.sp cicero*	Chickpea wilt	Saikia *et al.,* (2006)
Lentil	*Pseudomonas putida* (MTCC no: 493)	*Meloidogyne. javanica (nematode)*	Root-knot nematode	Siddiqui *et al.,* (2007)
Peppermint	*Pseudomonas fluorescens* (PFMMP)	*Rhizoctonia. solani*	Root and stolon rot	Kamalakaman *et al.,* (2003)
Mango	*P. fluorescens* (FP7)	*Colletotrichum. gloeosporioides*	Anthracnose	Vivekananthan *et al.,* (2004)
Tea	*Pseudomonas fluorescens* (PFV, PFP, PSV)	*Exobasidium. Vexans*	Blister blight	Saravana kumar *et al.,* (2007)
Tomato and hot pepper	*Pseudomonas fluorescens* (Pfl) *Pseudomonas putida* (PFATR and KKMI)	*Pythium aphanidermatum*	Root rot and wilt	Ramamoorthy *et al.,* (2002)
Banana	*Pseudomonas fluorescens*	*Fusarium oxysporum.f.sp cubense*	*Fusarium* wilt	Thangavelu *et al.,* 2003
Onion	*Pseudomonas fluorescens*	*Alternaria palandui*	Alternaria blight disease	Karthikeyan *et al.,* 2005
Pea	*Pseudomonas fluorescens* *Pseudomonas aeruginosa*	*Erysiphe pisi*	Mildew of Pea	Dahiya J. *et al.,* 2001
Tomato	*Pseudomonas fluorescens* WCS417	*Fusarium oxysporum* f.sp. *lycopersici*	Vascular wilt	Duijff *et al.,* (1998)
Finger millet	*Pseudomonas fluorescens*	*Pyricularia grisea*	Blast disease	Leeman *et al.,* 1995
Wheat	*Pseudomonas fluorescens*	*Microconidium nivale / Fusarium nivale*	Snow mold,Seedling Blight	Amein *et al.,* 2008
Sorghum	*Pseudomonas chlororaphis*	*Macrophomina phaseolina*	Charcoal rot of Sorghum	Das *et al.,* 2008
Turf	*Pseudomonas aureofacians*	*Pythium aphanidermatum*	Dollar spot, Anthracnose	www.ecosoil.com
Mung bean	*Pseudomonas fluorescens*	*Macrophomina phaseolina*	Root rot	Noreen *et al.,* 2015
Cauliflower	Endophytic *Pseudomonas fluorescens* strain PF-1	*R. solanacearum*	Black dot	Singh *et al.,* 2010
Mulberry	*Pseudomonas fluorescens*	*Fusarium proliferatum*	Root rot	Ganeshamoorthi *et al.,* 2008
Pigeon pea	*Pseudomonas fluorescens*	*Heterodera cajani* *Meloidogyne incognita*	Wilt disease	Kumar *et al.,* 2010
Groundnut	*Pseudomonas fluorescens*	*Fusarium udum Aspergillus niger*	Collar rot disease	Senthilraja *et al.,* 2013
Sugarcane	*Pseudomonas fluorescens*	*Colletotrichum falcatum*	Sugarcane red rot	Senthil *et al.,*2011
Barley	*Pseudomonas fluorescens*	*Fusarium graminearum*	Fusarium head blight	Henkes *et al.,* 2011
Lupine	*Pseudomonas fluorescens*	*Fusarium solani*	Root rot	Hewedy *et al.,* 2011
Chilli	*Pseudomonas fluorscens*	*R. solanacearum*	Bacterial wilt	Umesh *et al.,* 2005

Table 3: *Pseudomonas* formulated as products used as biological control agents.

Product (Brand name)	Crop	Biocontrol agent	Target pathogens	Company registered trademark
Bio Ject, spotless	Vegetables and Ornamentals ingeen houses	*Pseudomonas aureofaciens* strain TX-1	*Sclerotinia homeocarps* (Dollar spot)	Eco soil system
Bio-save® 10LP, 110	Pome fruit, citrus, cherries and potatoes	*Pseudomonas syringae* ESC-10 and ESC-11	*Botrytis cinerea, Penicillium* spp, *Mucor pyroformis, Geotrichum candidum*	Ecoscience Corp. (Longwood, FL)
EPA registered 1995 Blight Ban A506®	Almond, apple, apricot, blueberry, cherry, peach, pear, potato, strawberry and potato	*Pseudomonas fluorescens* A506	*Erwinia amylovora* and russet including bacteria	www.villagefarms.com NuFarm Inc. (Blurr Ridge, IL) www.nufarm.com
EPA registered Cedemon	Barley and Oats	*Pseudomonas chlororaphis* strain	Leaf stripe, nrt blotch, *Fusarium* spp., spot blotch, leaf spot	BioAgri (Uppsala, Sweden)
EPA registered AtEze	Potential use on cereals Ornamentals and greenhouse-grown vegetables	*Pseudomonas chlororaphis* 63-28	*Pythium* spp., *Rhizoctonia solani, Fusarium oxysporum*	www.bioagri.se Eco soil systems Inc. (San Diego, CA)
EPA registered 2001 FROSTBAN	Fruit crops, almond, potato and tomato	*Pseudomonas fluorescens* A506, 1629RS	Frost-forming bacteria	www.ecosoil.com Frost technology corporation
Frostban A	Wheat, gram, legumes, silage crops, cereals, oil and root crops and ornamental crops	*Pseudomonas fluorescens* strains A506 and 1629RS, *Pseudomonas syringae* 742RS	Frost-forming bacteria	www.ecosoil.com Frost technology corporation
Frostban B	Wheat, gram, legumes, silage crops, cereals, oil and root crops and ornamental crops	*Pseudomonas fluorescens* A506	Frost-forming bacteria	www.ecosoil.com Frost technology corporation
Frostban C	Wheat, gram, legumes, silage crops, cereals, oil and root crops and ornamental crops	*Pseudomonas fluorescens* 742RS	Frost-forming bacteria	www.ecosoil.com Frost technology corporation
Howler®	Cranberries, grapes, strawberries, tobacco, tomato	*Pseudomonas chlororaphis* AFS009	*Botrytis, Colletotrichum Phytophthora, Pythium, Rhizoctonia*	AgBiome Innovations (North Carolina, USA)
Tainio® BioGenesis ITM Non-Polymer	Row crops, trees, potted plants, other plants	20 species representative of 10 genera including *Pseudomonas*	N/A	Tainio Biologicals, Inc (Washington, USA)
N- TEXX® Soil inoculant with humus	Row crops, forage crops, permanent crops, turf, shrubs, indoor plants	*Pseudomonas putida*	N/A	CXI (Texas, USA)

Table 4: Effect of *Pseudomonas* on plant growth promotion.

Bacteria	Plant	Impact on plant	References
Pseudomonas fluorescens	Turmeric	Growth and curcumin content	Kumar *et al.*, 2016
Pseudomonas fluorescens	Wheat	Growth	Shaharoona *et al.*, 2007
Pseudomonas fluorescens	Wheat	Seed yield and shoot dry mass	Behn 2008
Pseudomonas fluorescens	Mustard	Growth and yield attributes	Aeron *et al.*, 2011
Pseudomonas fluorescens	Tomato	Root growth	Belimov *et al.*, 2007
Pseudomonas fluorescens	Marigold	Shoot fresh weight, root dry weight, leaf number, node number	Cappellari *et al.*, 2013
Pseudomonas aeruginosa	Tomato	Fruit yield	Dashti *et al.*, 2012
Pseudomonas fluorescens	Blackberries	Fruit quality	Garcia- Seco *et al.*, 2013
Pseudomonas spp.,	Onion	Onion bud	Harthmann *et al.*, 2009
Pseudomonas fluorescens	Catharanthus roseus	Biomass yield and ajmalicine production	Jaleel *et al.*, 2007
Pseudomonas putida	Cherry trees	Fruit set, plant vegetative growth	Karakurt *et al.*, 2011
Pseudomonas spp.,	Maize	Compatible solutes, antioxidant	Sandhya *et al.*, 2010
Pseudomonas spp.,	Wheat	Growth and yield of wheat	Shaharoona *et al.*, 2007
Pseudomonas putida	Rice	Grain iron	Sharma *et al.*, 2013
Pseusomonas fluorescens	*Zea mays*	Ag-nanoparticles augmented the PGPR-induced increase in root area and root length	Khan *et al.*, 2015
Pseudomonas fluorescens	*Brassica napus*	Increase Pb intake in shoots, increase root length, shoot and dry weight	Sheng *et al.*, 2008
Pseudomonas spp.	Soybean, mung bean, *Triticum*, *aestivum*	Promotes growth of plants	Gupta *et al.*, 2002
Pseudomonas chlororaphis	Sorghum	Extracellular antibiotics, production of volatiles, siderophores, effective root colonization	Das *et al.*, 2008
Pseudomonas fluorescens	Rye	Fe (III) chelating compounds including siderophores	Kurek *et al.*, 2003

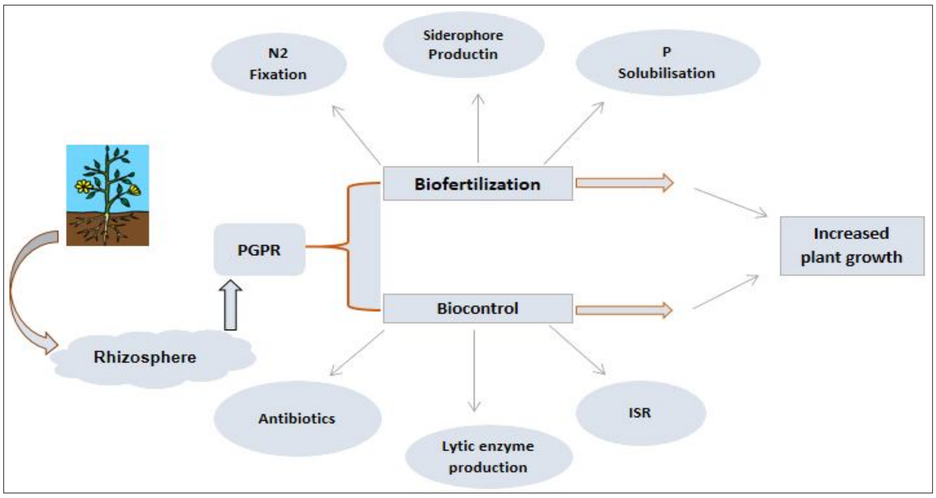

Fig 1: Mechanism of PGPR.

from roots to microbes, as well as, the genes associated with rhizosphere colonization (Compant *et al.,* 2005) and certain genes are involved in rhizosphere colonization. Initially, a method called promoter trapping technology (IVET) accelerates the isolation of *pseudomonas fluorescens* genes which exhibited high levels of expression in the rhizosphere (Siddiqui *et al.,* 2006). Furthermore, this method identified 20 genes that were brought about during rhizosphere colonization and the models of expression were analyzed. As a result, fourteen genes showed significant homology to sequences in Genbank that are intricated in nutrient acquisition, stress response, or secretion (Gamalero *et al.,* 2004).

The current study mainly emphasizes the use of *pseudomonas* as biocontrol control agents in various crops and primarily focuses on *pseudomonas-mediated* induced systemic resistance in plant species against microbial pathogens. Microbial biological control agents are a mode of action that is used whenever microorganisms interact, communicate and regulate their co-existence between microbial cells and between microorganisms and plants. Biocontrol agents from *pseudomonas fluorescens* and also closely associated species have become eminent models for the analysis of plant protection mechanisms and secondary mechanisms. This has further resulted in a good understanding of their direct reaction to the pathogens. Likely, these biocontrol agents are also able to intrude with the functioning of the rest of the rhizosphere microbial community. Despite this, the *pseudomonas* inoculants may perform uncertainly from one field to another and also from one year to the next, as a consequence of variability in root colonization or expression of biocontrol traits. Henceforth, while the selection of particular strains superior root colonization and effectual functioning in the rhizosphere are key measures.

CONCLUSION

In the current scenario, unlike chemical pesticides, biocontrol agents require very adequate support for their application to get commenced in the targeted niche. Environmental, as well as consumer concerns, have concentrated on the expansion of biological control agents as an alternative. *Pseudomonas* is one such proven biological control agent. Most of the success reports by certain scientists around the world have characterized diverse *pseudomonas* strains that are fundamentally able to control several fungal, bacterial and nematode diseases in cereals, horticultural crops, oilseeds and others. In this view, PGPR is a group of bacteria that actively colonize plant roots and thus increase plant growth and yield. This can only be achieved through a better understanding of the biocontrol mechanisms, plant-microbe interactions and processes. Thus the future relies on the development of microbial consortia which can provide a more stable ecological community by improving plant growth and working effectively against a broad spectrum of pathogens.

Conflict of interest: None.

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