CIBTech Journal of Biotechnology ISSN: 2319–3859 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/cjb.htm
2016 Vol. 5 (2) April-June, pp.15-18/Tanveer et al.
Research Article

BIOCONTROL ACTIVITIES OF FLUORESCENT *PSEUDOMONAS*

Kahekashan Tanveer[1], A.R. Apastambh[2], D.R. More[3] and *M.M.V. Baig[4]
[1]Department of Botany, Yeshwant Mahavidyalaya, Nanded - 431 602, India
[2]Department of Biotechnology, Yeshwant Mahavidyalaya, Nanded - 431 602, India
[3]Department of Botany and Biotechnology, L. B. S. Mahavidyalaya, Dharmabad, District Nanded, India
[4]Department of Botany and Biotechnology, Yeshwant Mahavidyalaya, Nanded - 431 602, India
**Author for Correspondence*

ABSTARCT
Plant growth promoting rhizobacteria are root colonizing bacteria which exert beneficial effect on plant. In this study 10 selected isolates of fluorescent *Pseudomonas* from wheat rhizosphere were studied for their biocontrol activities. All the isolates were positive for HCN production and siderophore production. These were tested for *in-vitro* antagonism towards bacterial plant pathogens like *Xanthomonas axonopodis, Xanthomonas malvacearum,* and fungal plant pathogens like *Alternaria alternata and Fusarium oxysporum.* The isolates Pfw1, Pfw2, and Pfw7 showed antifungal activity against all plant pathogens i.e. *X. axonopodis. X. malvacearum, A. alternata and F. oxysporum*. The maximum zone of inhibition towards fungal pathogen was shown by Pfw8 (7.1cm) against *A. alternata* while maximum zone of inhibition towards bacterial pathogen was shown by Pfw1 (1.6 cm) against *X. axonopodis.*

Keywords: *PGPR, Rhizosphere, HCN Production, Siderophore Production*

INTRODUCTION
Several groups of microorganisms affect plant growth and development. Some of them are plant pathogens while some exert beneficial effect on plants.
The microorganisms which exert beneficial effect on plants are usually present around the roots of plants and are known as Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper and Schroth, 1978). The well known PGPR include *Azotobactor, Azospirillum, Pseudomonas, Bacillus, Enterobactor, Paenibacillus.* The genus *Pseudomonas* is a diverse group bacteria with high levels of physiological and genetic diversity within species (Tanveer *et al.,* 2010) and hence mostly studied among PGPR Several mechanisms have been reported for plant growth promoting activities of PGPR. The mechanism may be direct or indirect.
Direct mechanism includes synthesis of plant hormones like auxins, cytokonin and gibberlin (Nautiyal *et al.,* 2000) resulting in enhanced growth of plants (Baig *et al.,* 2002) while indirect mechanism (biocontrol) includes synthesis of siderophore and antifungal metabolites which suppress plant pathogens (Kloepper, 1993).
Several PGPR inoculants have been currently commercialized that promote plant growth. The use of PGPR inoculants as biofertilizers or antagonists of plant pathogens can be a promising alternative to chemical fertilizers and pesticides.

MATERIALS AND METHODS
Isolation of Fluorescent Pseudomonas
Fluorescent pseudomonas was isolated from wheat rhizosphere by using King's B (KB) agar medium by serial dilution and plating technique. The plates were incubated at 30^0 C for 24 h. Colonies were observed under UV light on a transilluminator. The fluorescent colonies were selected for further studies.
HCN Production
HCN production of the isolates was determined by method described by Wei *et al.,* (1991).
Siderophore Production
Production of siderophore by the isolates was assessed by plate assay. Chrome Azurol S blue agar medium (CAS) was used to detect siderophore production by the isolates of fluorescent pseudomonads (Schwyn and Neilands, 1987).

Antifungal Activity of Fluorescent Pseudomonas

Fungal pathogens were grown on a Potato Dextrose Agar plate. A fungal growth disc from this plate was taken and placed at the center of another PDA plate. Active bacterial culture was then streaked parallelly on both side of the fungal disc 3-4 cm away from the disc. The plates were incubated at 30ºC for 96 hours. Inhibition of fungal growth was recorded after 96 hours of incubation in comparison with control (Ganesan and Gnanamanickam, 1987).

Antibacterial Activity of Fluorescent Pseudomonas

All the isolates were also tested for in vitro antagonistic activity against bacterial plant pathogen *Xanthomonas oxanopodspv.punicae* causing bacterial blight of pomegranate, *Xanthomonas axonopodspv.malvaserum* causing bacterial blight of cotton. Using dual culture assay (Sakthivel and Gnanmanikum, 1987). The overnight grown culture of bacterial plant pathogen was spread uniformly on King's B agar plates. After drying 10µl of 24 hr. old nutrient broth culture of test organism was spotted on these plates and the plates were incubated at 30°c for 2 to 4hr. the zone of inhibition of bacterial pathogen around the colonies of the *Pseudomonas fluorescence* was measured.

RESULTS AND DISCUSSION

The isolates were identified by morphological characters having rod shaped cells with Gram negative cells and showing fluorescence under UV light (Table 1) and were studied for biocontrol activities such as HCN production and siderophore production. All the isolates were positive for HCN production and siderophore production. The isolates Pfw1, Pfw2, Pfw4, Pfw7, Pfw8, Pfw9 were strong producer of HCN indicated by Colour change of the filter paper from yellow to complete orange. The isolates Pfw3, Pfw5, Pfw6, Pfw10 produced moderate amount of HCN indicated by Colour change of filter paper from yellow to brownish orange (Table 2). Ramatte *et al*., (2003) reported that hydrogen cyanide is a broad-spectrum antimicrobial compound involved in biological control of root disease by many plant-associated fluorescent pseudomonads. The isolate Pfw10 showed maximum zone of clearance (25.6 mm) on CAS agar while Pfw2 showed least zone of clearance (9.87 mm). This showed the ability of isolates to produce siderophore. Siderophore is low molecular weight, extracellular compound and possess high affinity for ferric ions. They sequester ferric iron and this ability provides a competititve advantage to microorganisms. Apastambh *et al.,* (2016) reported that siderophore producing Pseudomonads strains significantly reduced the growth of *A.solani* and *F.oxysporum* in- vitro. Based on the ability of these isolates to synthesize antimicrobial metabolites, they were tested for *in-vitro* antagonism towards bacterial plant pathogens like *X.axonopodis. X. malvacearum,* and fungal plant pathogens like *A. alternata and F.oxysporum.* The isolates Pfw1, Pfw2, and Pfw7 showed antifungal activity against all plant pathogens i.e. *X. axonopodis. X. malvacearum, A. alternata and F. oxysporum*.

Table 1: Morphological Characters of Fluorescent *Pseudomonas*

Sl.	Isolate	Colony Morphology			Cell Shape	Gram Reaction	Fluorescence
		Colour	Shape	Nature			
1	*Pfw*1	Light green	Round	Non-Spreading	Rod	Gram -ve	++
2	*Pfw*2	Green	Irregular	Spreading	Rod	Gram -ve	+++
3	*Pfw*3	Light green	Round	Non-Spreading	Rod	Gram -ve	++
4	*Pfw*4	Green	Irregular	Spreading	Rod	Gram -ve	++
5	*Pfw*5	Green	Irregular	Spreading	Rod	Gram -ve	++
6	*Pfw*6	Light green	Round	Non-Spreading	Rod	Gram -ve	++
7	*Pfw*7	Green	Irregular	Spreading	Rod	Gram -ve	+++
8	*Pfw*8	Light green	Round	Non-Spreading	Rod	Gram -ve	++
9	*Pfw*9	Green	Irregular	Spreading	Rod	Gram -ve	+++
10	*Pfw*10	Light green	Irregular	Spreading	Rod	Gram -ve	++

The maximum zone of inhibition towards fungal pathogen was shown by Pfw8 (7.1cm) against *A. alternata* while maximum zone of inhibition towards bacterial pathogen was shown by Pfw1 (1.6 cm) against *X. axonopodis* (Table 3). The rhizosphere bacteria are the ideal biocontrol agents as they can provide the frontline defense for plant roots against the attack by various root-/soilborne plant pathogens (Manoharachary and Tilak, 2015). Further studies on biocontrol activities of these PGPR can be useful to develop effective biocontrol formulations and can be used as an alternative to synthetic pesticides.

Table 2: Antimicrobial Compounds Produced by Fluorescent *Pseudomonas*

Sr. No.	Isolates	HCN Production	Isolates	Siderophore Production (Zone of Clearance in mm)
1	*Pfw1*	+++	*Pfw1*	10.2
2	*Pfw2*	+++	*Pfw2*	9.87
3	*Pfw3*	++	*Pfw3*	20.45
4	*Pfw4*	+++	*Pfw4*	13.5
5	*Pfw5*	++	*Pfw5*	21.54
6	*Pfw6*	++	*Pfw6*	15.64
7	*Pfw7*	+++	*Pfw7*	10
8	*Pfw8*	+++	*Pfw8*	10.5
9	*Pfw9*	+++	*Pfw9*	11.5
10	*Pfw10*	++	*Pfw10*	25.6

Table 3: Antifungal and Antifungal Activity of Fluorescent *Pseudomonas*

Sr. No.	Isolates	Mean Zone of Inhibition (cm)			
		Alternaria Alternata	***Fusarium Oxysporum***	***Xanthomonas Axonopodis***	***Xanthomonas Malvacearum***
1	*Pfw1*	6.45	5.95	1.6	0.75
2	*Pfw2*	6.95	5.25	1.2	0.75
3	*Pfw3*	-	-	-	-
4	*Pfw4*	-	-	-	-
5	*Pfw5*	-	5.05	1.3	0.7
6	*Pfw6*	6.85	-	1.3	0.65
7	*Pfw7*	6.9	5.45	1.1	0.8
8	*Pfw8*	7.1	-	-	-
9	*Pfw9*	6.75	4.85	-	-
10	*Pfw10*	-	-	-	-
11	*Control*	-	-	-	-

REFERENCES

Apastambh AR, Tanveer K and Baig MMV (2016). Isolation and Characterization of Plant Growth Promoting Rhizobacteria from Banana Rhizosphere. *International Journal of Current Microbiology and Applied Sciences* **5**(2) 59-65

Baig MMV, Baig MIA and Muley SM (2002). Enhanced Growth of Groundnut by Plant Growth Promoting Rhizobacteria. *International Arachis Newsletter* **22** 60-63.

Ganesan P and Gnanamanickam SS (1987). Biological control of *Sclerotium rolfsii sacc* in pea nut by inoculation with *P. fluoresencs. Soil Biology and Biochemistry* **19** 35–38.

Tanveer K, Apastambh AR, More DR and Baig MMV (2010). Diversity of Plant Growth Promoting *Pseudomonads* from Banana Rhizosphere. *Journal of Pure and Applied Microbiology* **4**(2) 551-556.

Kloepper JW (1993). Plant-growth-promoting rhizobacteria as biological control agents, In: *Soil Microbial Ecology* F.B. Jr., Metting, edition, (Marcel Dekker Inc., New York, USA) 255-273.

Kloepper JW and Schroth MN (1978). Plant growth promoting rhizobacteria on radishes. In: *Proceedings of IVth International Conference on Plant Pathogenic Bacteria* 879–882

Manoharachary C and Tilak KVBR (2015). Biotechnology and bioprospecting of fungi and microorganisms. In: Khurana P, Singh M (edition) *Biotechnology Progress and Prospects*, (Studium Press LLC, Houston, Texas) 124–141.

Nautiyal CS, Bhadauria S, Kumar P, Lal H, Mondal R and Verma D (2000). Stress induced phosphate solubilization in bacteria isolated from alkaline soils. *FEMS Microbiolgy Letters* **182** 291–296.

Ramatte A, Frapolli M, Defago G and Moenne Loccoz Y (2003). Phylogeny of HCN synthase-encoding hcnBC genes in biocontrol fluorescent pseudomonads and its relationship with host plant species and HCN synthesis ability. *Mol ecular Biology Plant Microbial Interaction* **16** 525–535.

Sakthivel N and Gnanamanickam SS (1987). Evaluation of Pseudomonas fluorescens for Suppression of Sheath Rot Disease and for Enhancement of Grain Yields in Rice (Oryza sativa L.). *Applied and Environmental Microbiology* **53**(9) 2056-9.

Schwyn B and Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* **160** 47-56.

Wei G, Kloepper JW and Sadik T (1991). Induction of systemic resistance of cucumber to *Colletotrichum orbiculate* by select strains of plant growth promoting rhizobacteria. *Phytopathology* **81** 1508-1512.