

***In vitro* screening for biocontrol activity of pink pigmented facultative methylotrophs against phytopathogens**

Pink pigmented facultative methylotrophs (PPFMs) are physiologically an interesting group of bacteria that are capable of growing on single carbon compounds such as methanol and methylamine, as well as on C₂, C₃, and C₄ molecules (Chistoserdova *et al.*, 2003). These are associated with the roots, leaves and seeds of most terrestrial plants and many are thought to be phytosymbionts (Trotsenko *et al.*, 2001). Several beneficial aspects such as stimulation of seed germination, plant growth promotion, production of phytohormones and induction of defense responses in rice and peanut against *Rhizoctonia solani* Kuhn., *Aspergillus niger* Tiegh. and *Sclerotium rolfsii* Sacc. have been reported for *Methylobacterium* (Omer *et al.*, 2004; Madhaiyan *et al.*, 2004; 2006). Considering use of PPFM as biocontrol agents against different phytopathogens, an investigation was undertaken to evaluate biocontrol potential of PPFM isolates against different pathogens of chilli under *in vitro* conditions.

In this experiment, we employed seven PPFM isolates made from the rhizosphere soil, phyllosphere and roots of chilli (*Capsicum annum* L.) collected from major chilli growing areas of North Karnataka. These PPFM isolates were screened for their ability to inhibit growth of phytopathogens (*Colletotrichum capsici* (Syd. & P. Syd.), *S. rolfsii*, *Fusarium oxysporum* Schlecht., *Cercospora capsici* Heald & F.A. Wolf., *Xanthomonas campestris* (Pammel) Dowson) by well established dual culture technique and zone of inhibition was calculated.

Mechanisms of antagonistic activity like production of siderophores and HCN by PPFM isolates was tested. To estimate different types of siderophores produced, PPFM isolates were grown on Iron deficient Ammonium Mineral Salts medium, followed by separation of cells by centrifugation and concentrating the supernatant by evaporating it to dryness. Catechol type of siderophore was extracted on ethyl acetate extracts of the culture supernatant twice with an equal volume

of solvent at pH 2.0. The ethyl acetate layer was removed and evaporated to dryness and the residues were dissolved in a minimum quantity of distilled water while hydroxamate types were measured from the untreated culture supernatant of PPFM isolates (Modi *et al.*, 1985). One volume of the Hathway's reagent (Reeves *et al.*, 1983) was added to one volume of the sample and the development of wine colour showed the presence of catechol type of siderophores while the development of orange colour showed the presence of hydroxamate type of siderophore and HCN production was assessed as per the method of Wei *et al.* (1991).

All the PPFM isolates tested inhibited *C. capsici* and *S. rolfsii* whereas six isolates of PPFM inhibited growth of *F. oxysporum* on potato dextrose agar medium. None of the isolates tested inhibited *C. capsici* and *X. campestris* in dual culture assay. The PPFM6 produced significantly highest zone of inhibition against *C. capsici*, *S. rolfsii* and *F. oxysporum* (Table 1) which was significantly different from other isolates. A positive role is played by phyllosphere antagonistic microorganisms, which protect the plants from pathogenic microorganisms and thus improve their healthiness (Patkowska, 2003). The inhibition of phytopathogens by PPFM isolates has already been reported by Poorniammal *et al.* (2010) who documented that *Methylobacterium* sp. isolate CO 47 significantly reduced the linear mycelial growth of *R. solani* to an extent of 52.2 per cent over control with an inhibition zone of 1.4 cm.

In the present study, none of the isolates could produce HCN (Table 1), which was indicated by the no change in colour of the filter paper. Similar results were obtained by Poorniammal *et al.* (2009). All PPFM isolates tested produced catechol type of siderophores (Table 1) which was indicated by the development of wine colour in the culture supernatant upon addition of the Hathway's reagent. Similarly, Vaidehi and Sekar (2012) also observed catechol type siderophore production by PPFMs.

Table 1. Screening of pink pigmented facultative methylotrophs (PPFM) isolates for biocontrol efficiency

Isolates code No.	Zone of Inhibition (mm) by different pathogens					Mechanism of activity	
	<i>Colletotrichum capsici</i>	<i>Sclerotium rolfsii</i>	<i>Fusarium oxysporum</i>	<i>Cercospora capsici</i>	<i>Xanthomonas campestris</i>	HCN production	Siderophore Production (Catechol type)
PPFM 6	08.00	10.00	18.00	-	-	(-)	(+)
PPFM 35	05.00	08.50	11.00	-	-	(-)	(+)
PPFM 38	03.50	03.00	00.00	-	-	(-)	(+)
PPFM 65	03.80	03.00	05.00	-	-	(-)	(+)
PPFM 99	04.50	07.00	05.00	-	-	(-)	(+)
PPFM 140	04.00	04.00	08.00	-	-	(-)	(+)
PPFM 170	06.00	08.00	09.00	-	-	(-)	(+)
S.Em.±	0.12	0.10	0.11	-	-	-	-
C. D.	0.47	0.45	0.46	-	-	-	-

(+) Produced, (-) Not produced

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