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Research Article

Mung Bean Rhizobacteria Antagonist to *Fusarium oxysporum* and *Rhizoctonia solani*

M Oberai[1]* and V Khanna[2]

[1]*Department of Microbiology, Punjab Agricultural University, Ludhiana, India*

[2]*Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India*

***Corresponding Author:** M Oberai, Department of Microbiology, Punjab Agricultural University, Ludhiana, India.

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Abstract

Rhizobacterial chemicals can prove to be dynamic natural pesticides. In the present study twelve PGPR isolates from mung-bean rhizosphere showed ability to produce various volatile and non-volatile chemicals such as *Hydrogen* cyanide, siderophore, volatile antifungal compounds, diffusible antimetabolites (viz. pyrrolnitrin, cyclic lipopeptides), ammonia, hydrolytic enzymes and phenazine which had a biocidal effect on *Fusarium oxysporum* and *Rhizoctonia solani*. The TLC and HPLC analysis confirmed the production of phenazine by Isolate MO33. The crude filtered extract containing phenazine had anti-mycotic potential against the test pathogens. SEM analysis of the antagonist-pathogens post interaction events showed a biocidal effect on fungal hyphae brought about by these bacterial chemicals, showing scanty, deflated, perforated and shrunken hyphae.

Keywords: Hydrogen Cyanide; Hydrolytic Enzymes; Phenazine; SEM Studies; Siderophore; Volatiles

Introduction

Mung bean (Vigna radiata), known as green gram or golden gram, is one of the most important short duration pulse crops grown in India during the kharif, spring and summer seasons. However, its production and productivity has been stationary as the crop is prone to more than one disease and pests [34]. Fungi such as *Fusarium spp*., *Rhizoctonia solani, Macrophomina phaseolina* and *Pythium spp*., produce severe diseases such as root rot, charcoal rot, damping-off and stem rot as well as mycotoxins in the grain [4,26]. These soil-borne pathogens can be controlled by chemicals but use of the chemical pesticides has several negative effects. The use of microbial antagonists is a safe, effective and eco-friendly method for the control of many of these soil-borne pathogens and this is gaining popularity in recent days. These biological agents promote plant growth by nutrient recycling and other processes [30]. Their biocidal effects can occur via local antagonism to soil-borne pathogens or by induction of systemic resistance against pathogens. Several substances produced by antagonistic rhizobacteria have been tested for pathogen control and indirect promotion of growth in many plants [6].

Induced systemic resistance (ISR) in plants bears a resemblance to pathogen-induced systemic acquired resistance (SAR) under conditions where the inducing bacteria and the challenging pathogen remain spatially separated. Both types of induced resistance allow the uninfected plant more resistance to pathogens. Rhizobacteria belonging to the genera *Pseudomonas* and *Bacillus* are well known for their antagonistic effects and their ability to trigger ISR [6]. Resistance-inducing and antagonistic rhizobacteria might be beneficial in formulating new inoculants with combinations of different mechanisms of action, leading to a more efficient use for biocontrol strategies.

Some successful applications of rhizobacteria have been reported by *in-vitro* assays, green house experiments and field trials [26]. Although the exact mechanism of their action is not known, it is believed that these bacteria secrete antimicrobial substances such as siderophores, HCN, salicylic acid, antibiotics and cyclic lipopeptides (LPs) which act as bioactive metabolites and are effective in controlling diseases [4,27]. Many investigators have tested the antagonistic potential of PGPRs (Plant growth promoting rhizo-

bacteria) and have reported a complex response with a range of antagonistic effects on the test pathogens. The use of microbes or their secretions to control plant pathogens offers an attractive alternative for the control of plant diseases [1]. In the present study we have investigated the antagonist activity of some rhizobacteria isolated from mung bean rhizosphere against the *F. oxysporum* and *R. solani*.

Materials and Methods

The two pathogens, *Fusarium oxysporum* and *Rhizoctonia solani* were procured from the Department of Plant Pathology, Punjab Agricultural University and were maintained on Potato Dextrose Agar (PDA) slants.

Isolation of rhizobacteria

Soil samples were randomly obtained from mung bean rhizosphere and standard microbiological procedures were used for rhizobacterial isolation [21]. The colonies were selected, purified on nutrient agar, bacillus agar and pseudomonas agar medium and then transferred to respective slants for further use. For further experiments, the isolates were grown at 30° C in respective broths.

Screening of rhizobacteria antagonistic to *F. oxysporum* and *R. solani*

Dual culture test

The antagonistic activity of 40 rhizobacterial isolates against the pathogenic fungi was evaluated using the dual culture test. In this, 20 ml of sterilized PDA (Potato dextrose agar) was poured into a sterile petri dish and allowed to solidify. After it was cooled, 5mm mycelial bit of *Fusarium oxysporum* and *Rhizoctonia solani* were inoculated at the centre of two separate plates. Potential antagonistic bacteria were then streaked 3 cm apart from the fungal inoculum and the plates were incubated at 28°C for 48 h (three replications per culture). Radial growth of the test fungus was measured and percentage growth inhibition was calculated using the equation:

%Inhibition = (R-r)/R x 100

Where, r is the radius of the fungal colony in the presence of antagonist and R is the radius of the fungal colony in control [25]. The test was carried out in triplicates.

Elucidation of antagonistic mechanism

Scanning electron microscopy (SEM) of post-interaction events

Small agar pieces (>1 cm) from the zone of interaction (obtained during dual culture assay) were taken out from each plate and transferred to a dried autoclaved vial. The specimen were fixed overnight at 4 C in 2.5% glutaraldehyde in 0.2M cacodylate buffer (pH 7.3) followed by three repeated washings (15 minutes each) in cacodylate buffer. Then, 1% osmium tetra oxide was added and dehydrated by passage through graded aqueous ethyl alcohol series (30, 50, 70, 90 and 95%) and finally placed in 100% ethanol at room temperature for few minutes. It was then dried, mounted on aluminum stubs and coated with gold-palladium using anion sputtering unit. The samples were then examined under a scanning electron microscope, SEM unit at EMN lab, Punjab Agricultural University, Ludhiana.

Inhibition of fungal growth by diffusibles

The cultures exhibiting the best antagonistic effect in dual culture assay were then co-inoculated with test fungi in 20 ml potato dextrose broth and incubated for a week at 28˚C under stationary condition and the mycelial mass obtained was then filtered, dried and weighed. Also, all the best antagonists obtained were co-inoculated together with the test fungi. Broth inoculated only with fungal bits served as control. The percentage reduction in dry weight of the mycelial mat (filtered and oven dried at 37˚C for 30 minutes) was calculated using the equation:

% Reduction in weight = (w1-w2)/w1x100

Where, w1 is the weight of the test fungus in control flask and w2 with bacterial antagonists [26].

Production of diffusible antimetabolites by rhizobacteria was assayed by method of Kumari and Khanna [23].

Volatiles

Fiddman and Rossal's (1993) sealed plate method was used for testing the production of volatile antimycotic compounds by rhizobacteria. The bacterial lawn of the test bacteria was prepared on nutrient agar medium in a petri-dish and on this was inverted a dish containing PDA inoculated with test fungus (both the test

fungi). Both were sealed with Para film and incubated at 28°C. The fungal radial growth of the pathogen was measured regularly for a period of 5 days.

Culture filtrate antibiosis

For detection of non-volatile antifungal metabolites, selected rhizobacteria were cultured in nutrient broth and incubated at 28° C at 200 rpm for 3 days. The culture was centrifuged at 10000 rpm for 20 min and the supernatant was filter sterilized using 0.2 micrometer filter. The supernatants were supplemented in PDA medium @ 50% i.e. in 2:1 of PDA and supernatant. A piece of actively growing pathogen was placed at the centre of each plate and incubated at 28°C for a week. The same volume of sterile distilled water in PDA medium served as control [22].

Hydrolytic enzymes

For detecting β-1, 4 glucanase, plates containing minimal salt agar medium [23] supplemented with carboxymethyl cellulose (1% w/v) were spot inoculated with rhizobacterial isolates at centre for 48-72h at 30°C. The plates were then flooded with Congo red for 15 min, washed and visualized for CMC hydrolysis.

Similarly, For protease detection, rhizobacterial isolates were spot inoculated on minimum salt agar medium supplemented with casein (1% w/v). The plates were incubated at 28°C for 48h and were then visualized for zone of hydrolysis.

Production of siderophore

Siderophore production by the rhizobacterial isolates was detected by the method of Schwyn and Neilands [32] using chrome azurol S (CAS). Cultures positive for siderophore produced an orange halo around the colony where siderophores had chelated iron bound to the dye.

Production of HCN and ammonia

The antagonistic isolates were screened for the production of hydrogen cyanide as per the method of Bakker and Schippers [5]. Petri plates encompassing 10% Trypticase soya agar (TSA) appended with 4.4 g of glycine per liter were inoculated with bacteria and covered with the lid on which a piece of paper, drenched with 0.5% picric acid and 2% sodium carbonate was placed. The plates were then incubated at 30˚C for 72- 96 h. Color change from yellow to orange-brown of the filter paper indicated the presence of HCN.

Bacterial isolates were also tested for the production of ammonia in peptone water [31]. Overnight grown cultures were inoculated into peptone medium and incubated for 2-3 days at 30˚C. and then 0.5 ml of Nessler's reagent was added. Appearance of a brown to yellow color indicated presence of ammonia.

Phenazine extraction and detection by TLC and HPLC analysis

The rhizobacterial isolates were grown in *Pseudomonas* broth [8] at 37˚C for 48 h for pigment production. Then the pigment rich broth culture was centrifuged at 10000 rpm for 15 min and the supernatant was collected and acidified with 0.1N HCl. The pigment was extracted using benzene (2:1), and the extract was then dried at 37˚C. The residue was then suspended in methanol and the methanol extract was analyzed by TLC using methanol and chloroform solvent (in 1:1) system. The plates were visualized under UV-light and Rf values recorded [29].

The production of phenazine was further detected by reverse phase chromatography was performed using a Varian HPLC system fitted with Lichrosphere RP18 column (Merck KgaA, Darmstadt, Germany) to confirm the presence of phenazines. The resolution of phenazine was achieved at 325 nm under isocratic conditions at a flow rate of 1.0 ml min^{-1} using a mobile phase of methanol-water (60:40 v/v). A 20 µl sample was injected each time for a run of 20 min [7].

This crude methanol extract was filter sterilized through 0.45 µm pore sized filter and its antagonistic potential was tested against the phytopathogens.

Statistical analysis

Data was analyzed using Analysis of variance (ANOVA) appropriate for Completely randomized design (CRD) carrying out the statistical analysis using CPCS1 software developed by Department of Mathematics, Statistics and Physics, PAU, Ludhiana.

Results and Discussion

Dual culture test (plate assay)

Twelve out of the 40 isolates showed antagonistic activity against both *F. oxysporum* and *R. solani* ranging from 4.44 – 33.3% (Table 1) Isolates MO1, MO32 and MO33 displayed strong inhibitory impact on the test fungi (Figure 1). Isolates MO32 and MO33 exhibited maximum inhibition potential against *F. oxysporum* and *R. solani*, respectively (Table 1).

The inhibition of the fungi (Figure 2) could be a consequence of various allelochemicals– volatiles, diffusibles, HCN, ammonia etc. (Table) produced by these antagonists which are toxic and curtail the phyto-pathogen growth by various mechanisms.

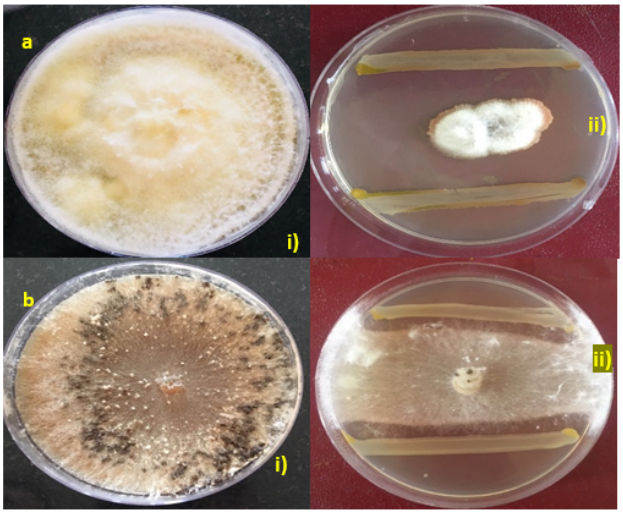

Figure 1: Inhibition of a) *F.oxysporum* i) control ii) inhibition of fungal proliferation and b) *R.solani* i) control ii) inhibition of fungal proliferation by antagonistic rhizobacteria.

Rhizobacterial isolates	Inhibition (%)	
	F. oxysporum	*R. solani*
MO1	22.2 ± 0.14	25.5 ± 0.74
MO4	11.1 ± 0.12	22.2 ± 0.98
MO6	6.67 ± 0.49	16.4 ± 0.87
MO12	11.1 ± 0.51	11.1 ± 0.12
MO17	13.33 ± 0.24	4.44 ± 0.89
MO19	22.2 ± 0.38	14.44 ± 0.26
MO22	11.1 ± 0.44	20 ± 0.54
MO25	16.66 ± 0.52	27.77 ± 0.61
MO39	13.33 ± 0.32	22.2 ± 0.64
MO31	11.1 ± 0.18	21.7 ± 0.55
MO33	28.8 ± 0.12	33.3 ± 0.79
MO32	33.3 ± 0.12	28.8 ± 0.86

Table 1: Inhibition of phytopathogens in dual plate assay.

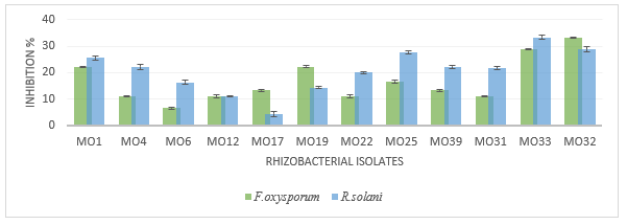

Figure 2: Relative inhibition of *F.oxysporum* and *R.solani* by rhizobacteria.

Our studies are consistent with the work of Hanson and Fernandez [10] who reported 45 bacterial isolates to have biocontrol activity against six fungal pathogens. Ji., *et al*. [12] examined 12 endophytic rhizobacteria for their antagonistic potential against *F oxysporum* and *R solani* and found them to be potent antagonists. Studies by Karimi., *et al*. [17] and Kumari and Khanna [23] also have shown the effectiveness of bacterial strains inhibiting the growth of *F. oxysporum f. sp. Ciceris (Foc)*.

Elucidation of antagonistic mechanism

1. Scanning electron microscopy (SEM) of post-interaction events

The SEM examination of isolate MO32 and *F. oxysporum* and isolate MO33 and *R. solani* by SEM analysis showed deformities in the hyphae of the respective fungi. The hyphae of *F. oxysporum* (treated with MO32) showed (Figure 3) shrinkage with loss of rigidity, deflation and perforations compared with normal hyphae in the control. Also, scanty hyphal growth was pragmatic. Similarly, in case of *R solani* (Figure 4) (treated with MO33) the hyphae were broken or distorted, showing swelling, shrinkage and acerbity.

The SEM studies clearly show the biocidal influence of the culture extracts as depicted by the lysis of mycelial structure.

Our SEM results are in agreement with earlier studies by Agarwal., *et al*. [1] who evaluated the antagonistic effect of *Bacillus MSUA3* against *R. solani* and *F. oxysporum* using SEM and found the hyphae of *F. oxysporum* perforated, and lysed. Antimicrobial acti-

vity of *B. amyloliquefaciens* FNL13 against *F. culmorum* Fc1 using SEM by Baffani., *et al*. [3] and by Kumari and Khanna [22] were also similar.

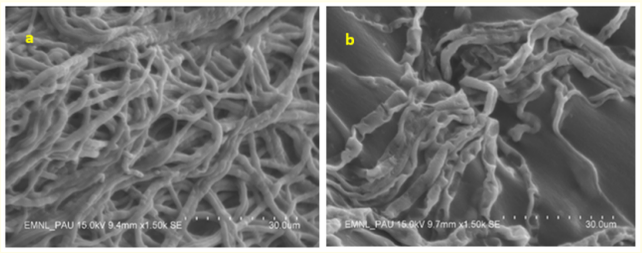

Figure 3: a) Control b) Effects of the antagonistic rhizobacteria MO32 on cell wall integrity of *F. oxysporum*.

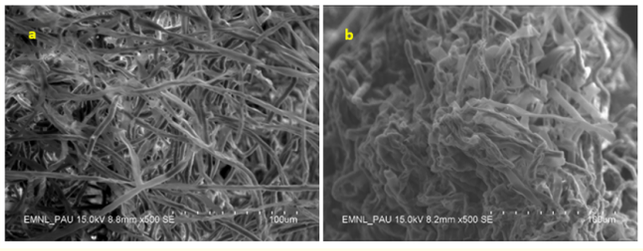

Figure 4: a) Control b) Effects of the antagonistic rhizobacteria MO33 on cell wall integrity of *R. solani*.

Inhibition of fungal growth by bacterial diffusibles

Maximum inhibition of fungal growth was by isolate MO32 both against *F. oxysporum* (92%) and *R. solani* (84%) followed by MO1 and MO33. The impact of the antagonists on *F. oxysporum* was more prominent than on *R. solani*, A cumulative inhibiting effect was seen (96 and 92%) when three rhizo-antagonists were co-inoculated with the phytopathogens respectively, suggesting that the rhizosphere consortia might work as an effective bio-pesticide.

Various diffusibles, volatiles and other anti-metabolites secreted by all the three i.e. MO1, MO32 and MO33, may have acted synergistically to control the pathogenic fungi. Moreover, the twelve antagonistic mung-bean rhizobacteria were adept in the production of diffusible metabolites. The reduction in radial growth of *Fusarium oxysporum* and *Rhizoctonia solani* after 96 h established the effect of these anti-metabolites. Growth inhibition varied from 46.2 – 60.6% among the twelve rhizobacteria, with MO32 and MO33 displaying comparable inhibition in case of *F. oxysporum*, however in case of *R. solani* MO33 and MO1 were at par in inhibiting the pathogen (Table 2). Similar studies by Kumari and Khanna [26] and Kumari., *et al*. [23] showed the potential of rhizobacterial isolates to inhibit *F. oxysporum* and the inhibition percentage elucidated considerable variation (26.4±0.72 to 87.3±0.10%). In another study by Kumar., *et al*. [22] rhizobacterial isolates were also found to exhibit antagonistic activity ranging from 79.8- 84.4% against *F. oxysporum* [2].

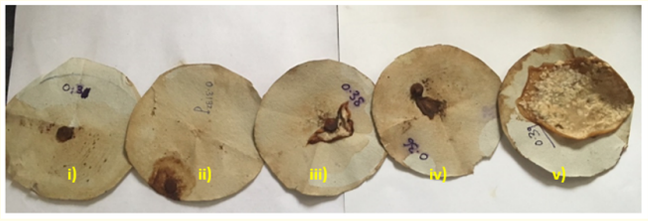

Figure 5: Biomass reduction of R. solani by broth assay clearly depicted. Mycelial mat in presence of i) MO1+MO32+MO33 ii) MO33 iii) MO1 iv) MO 32 v) control.

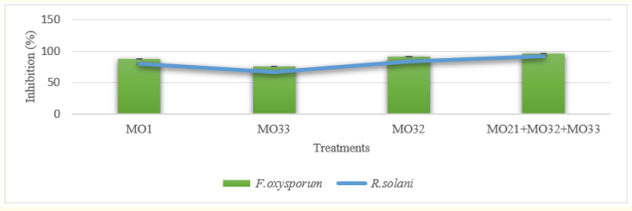

Figure 6: Inhibition of mycelial proliferation (in broth assay).

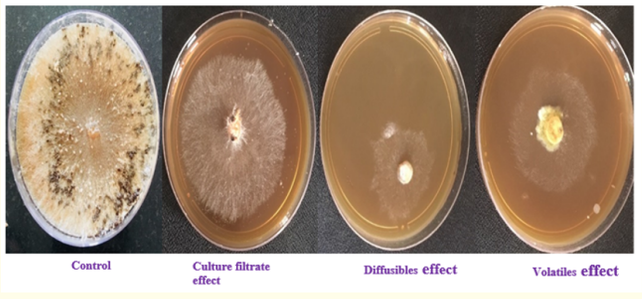

Figure 7: Inhibition of *R. solani* by diffusibles, volatiles etc. secreted by rhizobacteria isolate MO33.

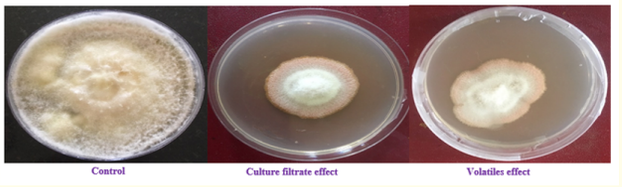

Figure 8: Inhibition of *F. oxysporum* by diffusibles, volatiles etc. secreted by rhizobacterial isolate MO32.

Rhizobacterial isolates	*R solani Inhibition (%)*			*F.oxysporum Inhibition (%)*		
	Diffusibles	VACs	Filtrates	Diffusibles	VACs	Filtrates
MO1	60.0 ± 0.83	56.8 ± 0.25	59.3 ± 0.14	56.25 ± 0.147	55.625 ± 0.627	59.37 ± 0.823
MO4	47.5 ± 0.71	40.0 ± 0.35	57.5 ± 0.24	50.00 ± 0.248	42.50 ± 0.847	62.50 ± 0.146
MO6	53.7 ± 0.94	38.7 ± 0.30	55 .0 ± 0.54	56.25 ± 0.147	37.25 ± 0.604	56.25 ± 0.894
MO12	58.7 ± 0.14	26.2 ± 0.28	57.5 ± 0.32	58.75 ± 0.341	30.00 ± 0.494	58.75 ± 0.646
MO17	46.2 ± 0.65	38.7 ± 0.15	61.2 ± 0.85	46.25 ± 0.644	40.00 ± 0.348	61.25 ± 0.848
MO19	51.2 ± 0.4 3	33.7 ± 0.4 4	50.0 ± 0.64	50.00 ± 0.794	35.00 ± 0.148	51.25 ± 0.146
MO22	52.5 ± 0.59	27.5 ± 0.58	57.5 ± 0.70	55.00 ± 0.249	27.50 ± 0.594	60.00 ± 0.147
MO25	50.0 ± 0.94	27.5 ± 0.85	63.7 ± 0.85	51.25 ± 0.145	31.37 ± 0.892	65.00 ± 0.697
MO31	51.2 ± 0.76	43.7 ± 0.74	58.7 ± 0.54	55.00 ± 0.540	35.00 ± 0.994	62.50 ± 0.890
MO32	55.0 ± 0.64	55.0 ± 0.91	82.5 ± 0.59	60.625 ± 0.746	47.50 ± 0.476	81.25 ± 0.447
MO33	61.8 ± 0.89	43.7 ± 0.56	67.5 ± 0.60	60.00 ± 0.194	55.00 ± 0.894	66.25 ± 0.194
MO39	43.7 ± 0.41	33.7 ± 0.79	56.2 ± 079	48.75 ± 0.641	35.00 ± 0.944	57.12 ± 0.184

Table 2: % Inhibition of phytopathogens by various antifungal metabolites.

Volatile antifungal compounds (VAC)

Volatile compounds such as alkanes, aldehydes, ammonia, esters, ketones, sulfides and terpenoids produced by some rhizobacteria are known to exhibit antifungal activity against phytopathogens. Certain volatile antifungal compounds are known to be effective against *F. oxysporum* and *R. solani*. In the current study substantial reduction in the growth of the pathogen in the presence of VACs was observed (Table 2). The growth inhibition by VAC varied between 26.2 – 56.8% against both the pathogens.

Similar antifungal activity by volatile organic compounds of rhizobacteria has been reported as an antagonistic armor against phytopathogens by Kumari., *et al*. [24]. Eleven isolates from chickpea rhizosphere were capable of inhibiting *F oxysporum f. sp. ciceris*, with maximum inhibition by Ps-14c (39.4%). About 65.7% of the rhizobacterial antagonists were reported to produce antifungal volatile compounds as apparent from the decrease in growth of *Rhizoctonia sp*.in sealed plate technique with inhibition varying between 27.6 and 66.6% [18].

Culture filtrate antibiosis

Three days old culture filtrates of the mung bean rhizobacterial isolates displayed inhibitory to growth of both the test pathogens i.e. *F oxysporum* and *R. solani*. The percentage inhibition ranged from 51.25 t0 81.25% with former and 50 -80.25% with the later. This test basically confirms the production of certain non-volatile antimycotic elements by the antagonistic rhizobacteria. Similar to this study, Kumari., *et al*. [23] also reported that three days old culture filtrates of the bacterial antagonists inhibited the growth of Rhizoctonia sp.in the range of 13.3-71.75%. Mishra., *et al*. [27] also found four days old culture filtrate of *B. subtilis* MA-2 completely inhibiting the growth of *Alternaria alternata* and *Curvularia andropogonis*.

Production of glucanase and protease

Biocontrol agents are known to produce number of hydrolytic enzymes including glucanases, proteases [14] etc.

Production of hydrolytic enzymes by PGPR is an important mechanism used against phytopathogens helping in sustainable plant disease management. These enzymes break down the cell wall of fungal pathogens leading to cell death [14]. Karimi., *et al*. [15] demonstrated production of proteases and β-1, 4 glucanase by the antagonistic rhizobacterial isolates from the chickpea rhizosphere, which is similar to results reported herein.

Production of siderophore

All of the twelve potent rhizobacterial antagonist showed a distinct orange halo on CAS plates indicating siderophore production. The orange halos diameter ranged from 12-40 mm, where the ha-

lo-zone was observable after 72 hrs of incubation, with maximum diameter observed on 15th day of incubation. The maximum diameter observed was 40mm (for MO32). Also, the biocontrol agents possessed the ability to produce both type of siderophores, however, catechol type siderophore was produced in higher amounts compared to the Hydroxamate type.

Kumari., *et al*. [23] reported siderophore production index ranging from 6 mm to 28.6 mm by rhizobacteria isolated from chick pea rhizosphere. Gupta and Gopal [11] also examined ten rhizobacteria isolated for siderophore production and found six of them positive with diameter of the orange halos ranging from 6.7 to 46 mm.

Rhizobacterial isolates	Siderophore production (mm)	HCN Production	NH_3 Production	Glucanase production	Protease production
MO1	36	+	+++	+	+
MO4	28	+	+++	-	+
MO6	32	-	+	-	+
MO12	29	-	+	+	+
MO17	24	-	+++	+	-
MO19	14	+	+++	+	-
MO22	25	+	+	-	+
MO25	12	-	+++	-	+
MO31	26	+	+	-	+
MO32	40	+	+++	+	+
MO33	38	+	+++	+	+
MO39	30	-	-	-	+

Table 3: Antimetabolite profile of biocidal rhizobacterial isolates.

Production of HCN and ammonia

HCN and ammonia are chemicals produced by rhizobacteria and are known to have inhibitory effect on the growth of phytopathogens. The bacterial isolates showed moderate to considerable cyanogenesis and ammonia production. Among these, MO1, MO32, MO25, MO33 and MO31 were high HCN and ammonia producers. These results are similar to the study by Kremer and Souissi [17] who found that 32% of total 2000 rhizobacterial isolates were cyanogenic. In another work [11] four among the ten *P fluoresens* strains were strong HCN producers while the remaining six were moderate producers [11]. Dastager., *et al*. [6] found *Micrococcus sp* N11-0909 isolated from cowpea rhizosphere to be a strong ammonia producer. Kumar., *et al*. [20] tested ten strains of *Pseudomonas* from chickpea rhizosphere and reported ammonia production by them.

Phenazine extraction and detection by TLC and HPLC analysis

Phenazines are heterocyclic secondary metabolites possessing antibiotic properties. Phenazine - 1- carboxylic acid (PCA) is produced by fluorescent pseudomonads. MO32 and MO33 two rhizobacteria manifesting, the characteristic fluorescent pigmentation were analyzed for Phenazine production. Our results (Figure 9) in compliance with studies by Devnath., *et al*. [9] and Alagwadi., *et al*. [3] who reported Rf value for phenazine ranging from 0.70 - 0.85.

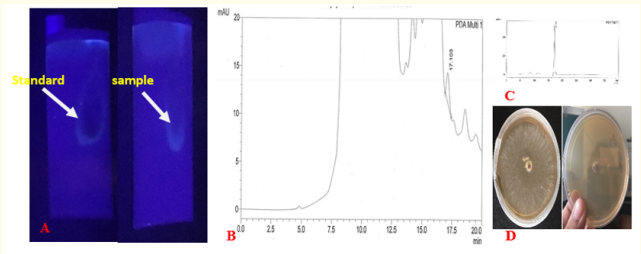

Figure 9: A) TLC analysis showing spots corresponding to standard (left) and the sample (right) B) HPLC analysis indicating 17.03 min retention time C) PCA standard HPLC chromatogram D) Anti-fungal effect of crude extract after 96 hours of incubation (left) control (right) fungal inhibition in presence of crude extract.

The phenazine (i.e PCA) present in rhizobacteria is known to impart strong antifungal ability. They uually act by stimulating the induced systematic pathway in plants which leads to disease supression. Not only phenazine production imparts them biocidal activity but also helpd them toperpetuate in rhizosphere.

HPLC analysis carried out supported TLC results for MO33 but no peak was detected in case of MO32. The Phenazine level in MO32, therefore, was below detection level. The retention time recorded was 17.03 min for sample compared to 17.1 for the standard, clearly depicting presence of the metabolite phenazine. The amount of PCA produced was 5.6 µg/ml. The methanolic extract depicted a strong antifungal activity (Figure 9). Similar results were obtained by Kavitha., *et al*. [19] who reported a broad spectrum of phenazines produced by *Pseudomonas genera*.

Conclusions

The rhizobacterial isolates clearly depict their ability to produce various metabolites as hydrogen cyanide production, siderophores, ammonia and various diffusibles. The HPLC analysis and other biochemical tests confirms their presence and the inhibitory effect of these metabolites is clearly elucidated in the present studies. Also, the inhibition of both the test pathogens by the crude methanol extract strengthens our results. Thus, it can be said that the secondary metabolites detected in rhizobacteria contribute towards their biocidal potential against both the phytopathogens, thereby, advocating the scope of these rhizobacteria as potent bio-pesticides which will be prolific substitute to the chemicals used these days.

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