Research Article

Biomanagement of Root Rot of Mungbean (*Vigna Radiata* (L.) Wilczek) Caused by *Rhizoctonia Solani* by Dual Inoculation of Antagonistic Rhizobacteria and Native *Rhizobium*

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Abstract

Fifty three antagonists out of 200 rhizobacterial isolates, isolated from mungbean rhizospheric soils infested with wet root rot, were found to be effective to control the mycelial proliferation of the casual test pathogen *Rhizoctonia solani*, maximum being with Ps132 (42.5%) followed by Ps33 and Ba91 with 41.2% radial inhibition in dual culture agar plate assay. Analysis for diffusible and volatile antifungal metabolites expressed inhibition in the range between 22.8-93.7% and 12.5-93.7% respectively, maximum being with Ps132 in both the cases. Twenty nine potential isolates were selected for further antagonistic traits out of which only 15 were found to produce HCN, however the ammonia production was recorded by all the isolates. Iron chelating affinity *via* siderophore production was recorded in 18 (62.0%) antagonists on Chrome azurol sulphonate agar plate. Six potent antagonists (Ba26, Ba91, Ps97, Ps110 and Ps123, Ps132) selected on the basis of *in vitro* antagonistic traits, were evaluated for their compatibility with native *Rhizobium*, and were tested for their efficacy alone and in combination with *Rhizobium* to control root rot in mungbean varieties ML-2056 and ML-818. Though the isolates alone showed efficient emergence, but Isolate Ba26, Ba91 and Ps97 as dual inoculation with native *Rhizobium* were recorded to induce 100% seedling mergence in case of ML-2056 compared to 93.3% in fungicide treatment. Similarly, in case of ML-818, Ba26, Ba91 and Ps132 recorded 100% germination when inoculated alongside native *Rhizobium* compared to fungicide (89.0%), and negative control 70.0%.

Observations for post emergence wet root rot revealed, seed bacterization with Ba91 maximally reduced the disease incidence up to 82.4%, followed by Ps132 (81.5%) and Ps110 (77.5 %) in combination with *Rhizobium*, compared to fungicide Captan (73.0%). Similar results were recorded in ML-818, where isolate Ps110, Ps132 and Ps97 alongside *Rhizobium* were found most effective to control 82.1, 78.0 and 77.2% of root rot severity in contrast to fungicide (66.0 %) that was comparable to recommended *Rhizobium* alone in ML-818 mungbean plants. Rhizobacterial isolates Ba91, Ps132, and Ps110 inoculations alongside *Rhizobium* were found effective in promoting seed emergence and in controlling the disease severity.

Keywords: Mungbean, Rhizobacteria, *Rhizobium*, root rot, biocontrol, *Rhizoctonia solani*

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Introduction

Mungbean [Vigna radiata (L.) Wilczek], is one of the most important short duration pulse crop, grown in almost all parts of the world with an annual production of around 3.0 million tons [1]. Being third most important pulse crop after chickpea and pigeonpea, mungbean contributes more than half of the global production in India [2]. It is an excellent source of high quality protein and can be consumed in different ways and preparations of high value food contributing a nutritional security for a large fraction of vegetarian people around the world [3]. Due to the development of early maturing varieties, mungbean has now proved to be an ideal crop for spring and summer seasons.

In spite of having large productions, the productivity of mungbean is considerably decreasing in Asian as well as African countries due to many biotic and abiotic factors [4]. However the major loss is due to biotic one because of its susceptible nature to some severe pests and diseases. Among these soil borne fungal pathogens are considered to be the most destructive pathogens, where root rot caused by *Macrophomina phaseolina* (Tassi) Goid [5] and wet root rot by *Rhizoctonia solani* are major threat to mungbean production [6, 7]. The pre-emergence seedling rot or damping off and post emergence water soaked lesions at the lower portion of the stem at later stages inhibit the passage of essential nutrients that halts the plant growth, leading substantial yield loss in mungbean [8]. Adverse climatic and poor soil conditions favour such diseases and present enhanced necrotic lesions [9]

To combat the pathogenic effects of such soil born rotting fungi farmers usually apply fungicides at the time

sowing or at post emergence stage depending upon the severity of the disease [10]. But high pathogenic variability in *Rhizoctonia solani* and development of fungicide resistance, chemical treatment often fails to provide satisfactory results to control this pathogen [11]. Moreover the excessive or non judicious application of chemical fungicides cause non targeted environmental impacts and incorporation of hazardous chemicals in food chain [12, 13] As a result, strict regulations imposed upon the use of chemical pesticides, has created a renewed interest in environmentally sound alternatives.

Use of biological control agents, such as plant growth promoting rhizobacteria (PGPR), associated with many plant species, can be a suitable approach to control such phytopathogens. PGPRs are beneficial to plants as they not only invigorate plant growth through nutrient availability assisted by liberated plant growth regulators, but also by protecting plants from various phytopathogens *via* broad spectrum animetabolites [14]. Due to this property, in addition of being used as bio-fertilizer, several PGPRs with antagonistic traits for the pathogens can also be used as bio-pesticides for the control of several soil/seed borne diseases [15, 16].

Biocidal mechanisms imposed by antagonistic rhizobacteria to control such soil borne pathogens include rapid solubilization and mineralization of essential nutrients, niche exclusion, antibiotics, extracellular lytic enzymes and other diffusible and volatile antifungal metabolites like certain esters, aldehydes, sulfides, terpenoids, Hydrogen cyanide, Ammonia etc. [17]. A large array of rhizobacteria with inimical effect on soil borne fungal pathogens, belonging to *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Clostridium*, *Bradyrhizobium*, *Bacillus*, *Burkhoderia*, *Brevibacterium*, *Serratia*, *Klebsiella* and *Streptomyces* spp. commonly reside the rhizosphere of most of the plants and hence help the plants from escaping the pathogenic effects of soil residing fungal pathogens [18, 19]. Among these antagonistic PGPRs, several species of *Bacillus* and *Pseudomonas* have been well reported for their inhibitory actions against fungal phytopathogens due to their unique ability to liberate a range of antimetabolites [20]. Most of the *Pseudomonas* strains encompass antagonistic activity as can comparatively synthesize a range of antibiotics like aerugine, azomycin, butyrolactones, cepaciamide A, cepafungins, ecomycins, pyoluteorin (Plt), pyrrolnitrin (Prn), 2,4 diacetyl phloroglucinol (DAPG), phenazine-1-carboxylic acid (PCA), phenazine-1-carboxamide (PCN), oomycin A, kanosamine, viscosinamide, zwittermycin-A, rhamnolipids, pseudomonic acid, hydrocyanic acid and ammonia [21, 22]. Bacteria belonging to *Bacillus* spp. also produce a wide variety of antibacterial and antifungal antibiotics including bacilysin, bacillaene, chlorotetain, difficidin, mycobacillin, subtilin, subtilosin A, sublancin and rhizocticins [23, 24]. Among these 2,4-DAPG, PCA and PCN have been reported to efficiently control the plant pathogens [25, 26]. In addition to their direct biocidal inhibitory mechanisms these PGPRs also induce the host plant's defence mechanisms and systemic resistance to pathogens [27, 28]. As a result of these stand-out qualities these boiagents have additionally been utilized as a part of coordinated pest management programmes [29, 30].

Considering the previous reports for the antagonistic behaviour of *Bacillus* and *Pseudomonas*, performance of seed priming of potential antagonistic isolates were tested for their efficacy to control damping off and root rot in mungbean incited by *Rhizoctonia solani* under controlled glass house conditions. Potential antagonistic rhizobacterial isolates were screened from mungbean field soils suppressed with root rot complex diseases.

Materials and Methods

Isolation of rhizobacterial isolates from mungbean rhizoshere

A total of thirty rhizospheric soil samples were collected from mungbean field soils suppressed with root rot complex diseases from different locations of Punjab, Himachal Pradesh, Haryana and Uttar Pradesh. The fresh root adhered soil samples were collected in sterile plastic bags, randomly from different locations by carefully uprooting the plants. From each sample, 10 g of soil was added to 90 ml of distilled sterilized water and vigorously shaken using a shaker for 20-30 minutes. From this, seven fold serial dilutions were made by pipetting 10 ml into additional dilution water. From the final dilution (10^{-7}), aliquots of 0.1 ml each were spread on plates, containing 20 ml of Nutrient agar for *Bacillus* and King's B [31] for *Pseudomonas* and incubated at 25^{o}C for 24 hours. The colonies showing whitish colouration on NA plates and fluorescent yellow to green colouration on Kings B were picked up and were transferred to respective slants for further tests.

Cultural, morphological and biochemical characterization of bacteria

Bacterial cultures isolated on Nutrient agar and King's B medium were further identified by streaking on Bacillus agar and *Pseudomonas* specific, Pigment producing medium (PsP) for *Bacillus* and *Pseudomonas* spp. respectively. Bacterial colonies with desired characteristic pigment/colour on respective media were picked and transferred to Nutrient agar slants for further use. Initial characterization of all the isolates was done on the basis of colony morphology and gram's staining. Further, biochemical characterization of bacterial isolates was done as per the standard methods for bacterial identification [32].

Standard strains

Standard strain of the pathogenic fungi, *Rhizoctonia solani* was procured from department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana and was maintained on Potato Dextrose agar slants. Mungbean nodulating specific native *Rhizobium* was also obtained from department of Microbiology, Punjab Agricultural University, Ludhiana and was maintained on Yeast Mannitol agar slants.

Screening for antagonistic rhizobacteria

In vitro testing of rhizobacteria for inhibition of mycelial growth of test fungi by dual culture agar plate method

The antagonistic rhizobacterial isolates were screened by dual culture plate assay as per the method described by Ahmed *et al* [33]. Ten µl drops from the 10^8 cfu/ml bacterial broth suspension were placed on the margin (2cm away from the fungal disc) of potato dextrose agar (PDA) plates and a 5 mm agar disc from fresh cultures of *Rhizoctonia solani* was placed at the centre of the PDA plate for each bacterial isolate and incubated at 25 ± 3 ºC for five days. The radial growth of the fungal colony towards and opposite direction from the bacterial colony was measured. The percentage growth inhibition was calculated using the following formula:

$$\text{Percentage inhibition} = (D-d) / D \times 100$$

Where, d is the average diameter of the fungal colony opposite the bacterial colony and, D is the maximum diameter of the fungal colony placed in a separate Potato Dextrose agar plate as control. Three replicates were maintained as control.

Assessment of antiphytopathogenic potential of rhizobacterial isolates against the root phytopathogens

Growth inhibition by production of Diffusible antimetabolites

PDA plates covered with a cellophane membrane were overlaid with nutrient agar and inoculated with 100 µl of antagonistic bacterial suspension. After incubation for 48 hrs at 28˚C, the membrane along with the grown bacterial isolate was removed and the plate was inoculated in the middle with 5mm disc of a pure culture of *Rhizoctonia solani* in the inverted position so that fungal hyphae touch the sterile agar plate. After inoculation, plates were incubated at 25˚C for 5 days and the growth of the pathogen was measured at every 24 hr interval [34].

Antagonistic activity via volatile antifungal compounds

One hundred µl of fresh prepared broth culture was spread on nutrient agar medium plate. A second petri dish containing PDA was inoculated with a 5-mm bit of the test fungus and placed over the bacterial culture. The two plates were sealed together with parafilm and further incubated at 25 ˚C. As a control, a petri plate containing nutrient agar medium without bacteria was placed over the PDA medium inoculated with the fungal pathogen. Radial growth of the test fungus was observed over 24 hours intervals for a period of 5 days.

Hydrogen Cyanide (HCN) production

Petri plates containing 10% Trypticase soya agar supplemented with 4.4 g of glycine per litre were spread with 0.1 µl of 24 hrs old bacterial cultures. The plates were inverted with a lid containing filter paper, impregnated with 0.5% picric acid and 2% sodium carbonate. The plates were sealed with parafilm and incubated at 28 ˚C for 3 to 5 days. A change in colour from yellow to orange-brown on the filter paper indicated cyanide production [35].

Production of ammonia

Fresh (24 hrs) grown cultures were inoculated in 10 ml peptone water and incubated for 48-72 hours at 28 ˚C. After the incubation, Nessler's reagent (0.5ml) was added in each test tube. Development of brown to yellow colour was examined as positive test for ammonia production [32].

Siderophore production by Chrome azurol sulphonate (CAS) agar plate method

Siderophore production was remarked by using blue agar succinate medium plates containing the dye chrome azurol sulphonate (CAS). The indicating dye was produced using 60.5 mg CAS that was dissolved in 50 ml deionised water. To this mixture, 10ml of a Fe^{3+} solution (1mmol/l $FeCl_3.6H_2O$ in 10mmol/l HCl) was added. The prepared CAS assay

solution was further mixed with previously prepared hexadecyl tri methyl ammonium bromide (HDTMA) solution (72.9 mg HDTMA in 40 ml distilled water). The dye solution was autoclaved and mixed with 900ml succinate media upon cooling (50-60 ˚C). The medium was allowed to solidify on petri plates. Cultures positive for siderophore production produced a halo of orange around the colony where siderophores had chelated iron that had been bound to the dye.

Compatibility test

Rhizobacterial antagonists selected on the basis of multifunctional traits were evaluated for their compatibility with mungbean specific native *Rhizobium* (recommended culture of Department of Microbiology, PAU, Ludhiana). The bacterial antagonists and *Rhizobium* cultures were cross streaked on Yeast Mannitol agar plates and were observed for overlapping growth indicative of compatible interaction between the paired microorganisms.

Evaluation of antagonistic rhizobacteria induced bioantagonism in mungbean under glasshouse condition

Procurement of seeds

Seeds of two mungbean genotypes "PAU-2056 and ML-818" were selected and procured from Pulses section, Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

Bacterial cultures and seed bacterization

Selected rhizobacterial cultures were inoculated @ 1% in 100 ml of nutrient broth and were incubated at for 24 hours with bacterial count of 10^{7}-^{8} cfu/ml of the broth. The seeds of mungbean selected varieties were washed with 0.1% Mercuric chloride followed by 70% ethanol and then repeatedly with sterile distilled water for surface sterilization. After that, seeds were soaked in selected bacterial broth cultures (10^{7} ml^{-1} broth) individually and in combination with native *Rhizobium* (1:1) for 20-30 minutes before sowing the seeds.

Pathogen culture multiplication and soil preparation

The test fungal pathogen, *Rhizoctonia solani* was mass multiplied in Potato dextrose broth. Mycelial mat was used to inoculate pathogen in soil i.e. 10 g /Kg of the sterilized soil (autoclaved at 121°C for 15 min). Soil was mixed thoroughly to disperse fungal hyphae and spores properly in the soil.

Glasshouse experiment

The pot experiments were conducted in glass house of Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana during *Kharif* 2015 for mungbean. Selected antagonists and their co-inoculation with specific *Rhizobium* were examined for their potential to enhance seedling emergence *via* reducing pre emergence and post emergence wet rot disease severity under glass house conditions, using sterile soil inoculated with pathogen. The experiment was designed with following treatments, with selected culture treatments alone and in combination with *Rhizobium* (1:1). The absolute control with pathogen free soil and untreated seeds, negative control with sick soil and untreated seeds and Fungicide treatment with sick soil and captan treated seeds (2g/Kg seeds) were also maintained as separate treatments. Medium black clayey soil from mungbean fields was autoclaved at 15 lbs (121°C) for 15 minutes for sterilization. Plastic bags (15 x 10 cm) were filled with 250 g sterilized soil inoculated with pathogen mycelial mat i.e. 10g/Kg soil. Ten seeds were sown in each pot. Pots were maintained by regular watering up to maturity.

Treatments for mungbean

1. Absolute control
2. Negative control
3. Fungicide (Captan)
4. *Rhizobium* (R)
5. Ba26
6. Ba91
7. Ps97
8. Ps110
9. Ps123
10. Ps132
11. Ba26+R
12. Ba91+R
13. Ps97+R
14. Ps110+R
15. Ps123+R
16. Ps132+R

Results and Discussion

Isolation of rhizobacteria

The soil under the influence of root exudates supports the nourishment and activity of diverse microbial communities playing a vital role in plant growth promotion and protection from various pathogens. However this diversity is majorly affected by a number of factors such as soil and climatic conditions, type of the crop and other biological influences present in the soil. In an attempt to tap this microbial diversity in the mungbean planted fields with root rot symptoms, two hundred rhizobacterial cultures were isolated from thirty mungbean rhizospheric soil samples collected from different locations of Punjab, Himachal Pradesh, Haryana and Uttar Pradesh. Out of these two hundred isolates were selected each from Kings B (Ps) and Nutrient agar (Ba) medium from the rhizospheric soil samples. Kings B medium has been proposed as a pigment producing medium for the isolation of fluorescent pseudomonads producing fluorescent metabolites (fluorescein and pyorubin) and other strains of *Pseudomonas* spp. that are well known to produce and release non-fluorescent pigmented secondary metabolites such as blue pigmented pyocyanin and blue green pigmented phenazines and their derivatives [31] (Figure 1a). However pigment production is also affected by incubation conditions and varies from species to species in this group. Rhizobacteria isolated from Nutrient agar medium were majorly white to creamish white in colour differing in their colony morphology were found in the isolation trails (Figure 1b).

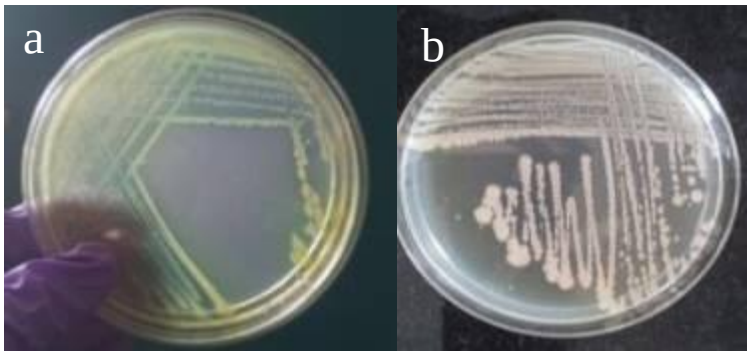

Figure 1 Isolation of rhizobacteria from mungbean rhizosphere, a. Yellow-green pigment producing colonies on Kings B agar medium, b. Creamish white colony on Nutrient agar medium

In support of this research, many workers have also reported the predominance of the fluorescent pseudomonads and spore forming *Bacillus* in rhizosphere and rhizoplane of several pulse crop plants [36, 37]. Likewise, in our previous studies on characterization of antagonistic PGPR from chickpea rhizosphere, the predominace of *Pseudomonas* sp. followed by *Bacillus* and *Serratia marcecens* was reported [20, 34]. Hynes *et al* [38] reported the presence of fluorescent pseudomonads as a major group of rhizospheric bacteria in pea, mungbean, lentil and chickpea. In addition to the *Bacillus* and, *Pseudomonas* and *Serratia*, Yadav *et al* [39] also reported *Azospirillum*, *Alcaligenes*, *Azotobacter*, *Arthrobacter*, *Klebsiella*, *Enterobacter* and *Burkholderia* as dominant bacterial communities of the chickpea and mungbean rhizosphere. Similarly, Sahu and Sindhu [37] reported the predominance of *Pseudomonas*, *Bacillus*, *Enterobacter*, *Proteus* and *Klebsiella* in green gram rhizosphere inferring the presence and their importance in the rhizospheric soils of pulse crops.

In vitro screening for antagonistic rhizobacteria by dual culture plate assay

Sustainable agricultural plant disease management programs require biological agents that can efficiently reduce the pathogenic effects of plant pathogens by multiple mechanisms [40]. Soil borne non-pathogenic rhizobacteria that are able to survive in sick or declined soils are more likely to encompass the ability to compete with such pathogens and also to inhibit their growth for their sustenance, thereby providing the front-line defense for roots when present in the rhizosphere [41, 42]. Antagonistic potential of 200 isolates from mungbean rhizoshere were evaluated against *Rhizoctonia solani* in dual culture plate assay under *in vitro* conditions. Out of the 200 isolates, 53 isolates (26.5%) were found to show inhibitory effect on the radial growth of *Rhizoctonia solani*. On the basis of extent of radial growth inhibition antagonists were characterized into weak (1-25%), modest (25-35%) and strong antagonists (>35%). On that basis most of the antagonists (75.4%) were found to belong the group of moderates.

Ten rhizobacterial antagonists (6 from Nutrient agar and 4 from Kings B) were characterized as strong antagonists of *Rhizoctonia solani*. The range of growth inhibion of *Rhizoctonia solani* ranged from 22.5 to 42.5 % (Table 1). Somewhat similar range was recorded in case of our earlier case study where antagonistic rhizobacterial inhibition of Foc radial growth was observed between 18.2- 41.8% [34]. Isolate Ps132 (42.5%) surpassed isolates

Ps33 and Ba91 with 41.2% of inhibitory effect (**Table 1**, **Figure 2**). Observations revealed that green or blue pigmented isolates picked from Kings B medium showed comparatively more inhibitory effect than the isolates from Nurient agar in dual culture plate assay, this is in support to the observations by Altinok *et al* 2014 where *P. aeruginosa* (P07-1 and 85A-2) and *P. putida* (P11-4) inhibited 70% of the radial growth of wilt fungal pathogen compared to other isolates. Ouhaibi-Ben Abdeljalil *et al* [43] reported growth inhibition of *Rhizoctonia soloni* between 34.44 and 59.26% by *Bacillus* isolates from tomato plants. In corrobation, Abaidoo *et al* [44] also reported the antagonistic potential of *B. subtilis* isolated from soil to inhibit the growth of soil-borne fungal pathogens of cowpea in dual culture experiments. Singh *et al* [45] recorded 75.5% of mycelial growth inhibition of *Rhizoctonia solani* by using rhizobacterial antagonists isolated from mungbean rhizosphere under *in vitro* conditions. Antagonistic activity of rhizobacterial isolates can be attributed to different diffusible and volatile antifungal metabolites and competitions for various nutrients [18].

Table 1 Antagonistic potential of rhizobacterial isolates against *Rhizoctonia solani*

Serial No.	Isolates	Radial growth (cm)	Inhibition over control (%)	Serial No.	Isolates	Radial growth (cm)	Inhibition over control (%)
	Control	**8.0**	**-**	**27**	**Ps24**	**5.6**	**30.0 ± 1.76**
1	Ba2b	6.0	25.0 ± 1.64	28	Ps26	5.9	26.2 ± 0.79
2	Ba3	6.1	23.7 ± 0.44	29	Ps32	5.4	32.5 ± 0.66
3	Ba4b	5.1	36.2 ± 1.79	30	Ps33	4.7	41.2 ± 0.54
4	Ba5	5.6	30.0 ± 1.13	31	Ps48	5.7	28.7 ± 1.20
5	Ba7	5.8	27.5 ± 0.60	23	Ps50b	5.9	26.2 ± 1.17
6	Ba8	5.9	26.2 ± 1.28	33	Ps51	5.6	30.0 ± 0.77
7	Ba11	5.0	37.5 ± 0.98	34	Ps54	5.8	27.5 ± 0.97
8	Ba13	5.1	36.2 ± 1.14	35	Ps61	5.7	28.7 ± 1.76
9	Ba18	5.7	28.7 ± 0.88	36	Ps66b	5.8	27.5 ± 0.38
10	Ba25	5.5	31.2 ± 3.00	37	Ps70	5.7	28.7 ± 0.89
11	Ba26	5.1	36.2 ± 1.31	38	Ps80	5.6	30.0 ± 1.06
12	Ba27	5.8	27.5 ± 0.52	39	Ps82	5.9	26.2 ± 1.10
13	Ba29c	5.4	32.5 ± 0.65	40	Ps87	5.3	33.7 ± 1.08
14	Ba32	5.2	35.0 ± 0.97	41	Ps91	6.0	25.0 ± 0.77
15	Ba40c	5.6	30.0 ± 1.73	42	Ps97	5.0	37.5 ± 0.96
16	Ba41	5.6	30.0 ± 1.73	43	Ps100	5.5	31.2 ± 0.91
17	Ba42	5.4	32.5 ± 0.66	44	Ps109	5.3	33.7 ± 1.09
18	Ba43	5.3	33.7 ± 0.91	45	Ps110	4.8	40.0 ± 0.85
19	Ba46	6.0	25.0 ± 1.14	46	Ps114	5.7	28.7 ± 0.94
20	Ba72	5.4	32.5 ± 0.65	47	Ps115	5.9	26.2 ± 0.82
21	Ba91	4.7	41.2 ± 1.69	48	Ps123	4.8	40.0 ± 1.27
22	Ba84	5.8	27.5 ± 1.58	49	Ps124	5.4	32.5 ± 0.65
23	Ba86	5.2	35.0 ± 0.13	50	Ps125	6.2	22.5 ± 0.85
24	Ps1a	5.9	26.2 ± 3.05	51	Ps132	4.6	42.5 ± 0.65
25	Ps11	6.1	23.7 ± 3.26	52	Ps145	5.8	27.5 ± 0.32
26	Ps21	5.9	26.2 ± 1.64	53	Ps144	5.5	31.2 ± 0.66

Values represent mean ± SE (Standard Error) of three replication

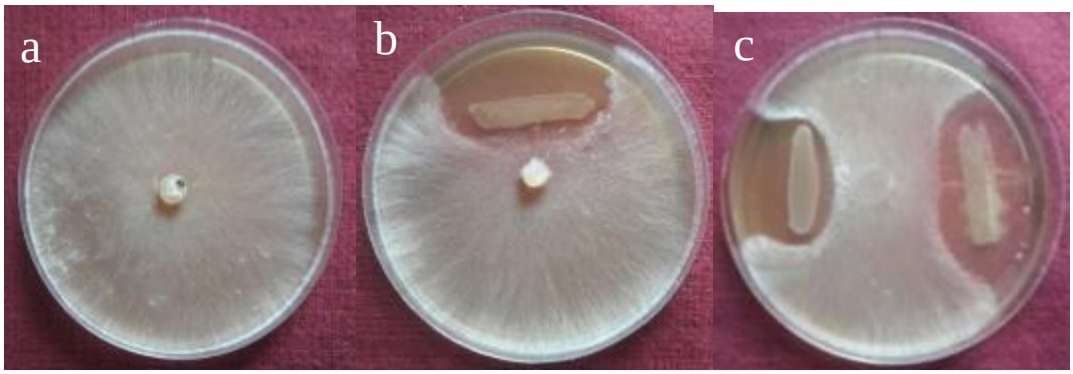

Figure 2 Antagonistic activity of rhizobacterial isolates against for *Rhizoctonia solani*, a: Control, b: Ba91, c: Ps110, Ps132

Characterization of antagonistic isolates

Bacterial antagonists selected on the basis of dual culture assay using *Rhizoctonia solani* as test pathogen, were appraised for morphological and biochemical characteristics as per Bergey's manual of Systemic Bacteriology. Out of 53 *Rhizoctonia* antagonists, 30 were characterized as gram negative by gram staining and the rest 23 were Gram positive. Morphologically, all the isolates were found to be rod shaped. Bacterial cultures isolated on Kings B medium and Nutrient agar medium were further streaked on *Pseudomonas* and *Bacillus* agar medium respectively. All the isolates from Kings B medium produced fluorescent green to blue green coloured pigment on the *Pseudomonas* selective pigment producing PsP medium (Figure 3a). On the other hand cultures isolated from nutrient agar produced dark blue to green colonies on pink coloured *Bacillus* agar medium, indicating their nearness to *Bacillus* genera (Figure 3b). Selected cultures were tested for starch hydrolysis, catalase production, Methyl red, Citrate utilization and Nitrate production test (Table 2). On the basis of their colony characteristics on the selective or differential medium and morphological and biochemical characterization, 23 (43.3%) bacterial antagonists were observed to tentatively belong to *Bacillus* and 30 (56.4%) to *Pseudomonas* spp. The predominance of other genera like *Azotobacter*, *Alcaligenes*, *Burkholderia*, *Klebsiella*, *Enterobacter*, and *Arthrobacter* have also been reported by many researchers in the rhizosphere of leguminous crops revealing their importance as potential plant growth promoting as well as antagonistic agents [46, 63, 39].

Table 2 Cultural, morphological and biochemical characteristics of rhizobacterial isolates

Characteristic of test organism	***Pseudomonas***	***Bacillus***
Gram's reaction	-ve	+ve
Shape	Rods	Rods
Pigment	+/-	-
Pigment colour	Fluorescent green	White
Starch hydrolysis	+	+
Catalase production	+	+
Methyl red test	-	-
VP	-	+
Citrate	+	+
Nitrate reduction	+	+

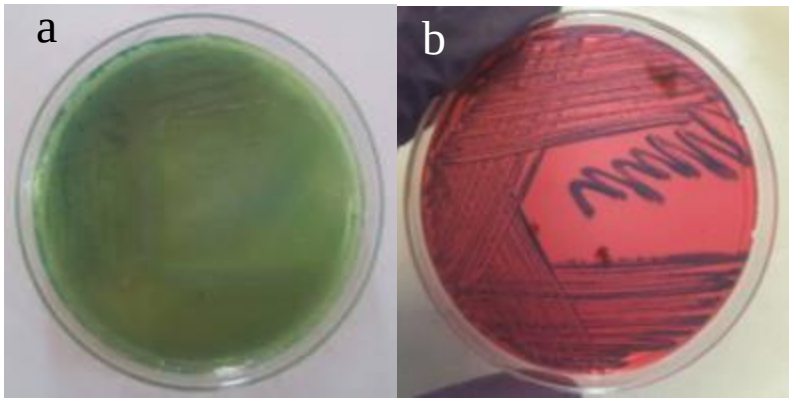

Figure 3 Morphological identification of isolates on selective medium. a. Blue green coloured colonies on Pseudomonas agar P (PsP) medium. b. Dark blue coloured colonies on Bacillus agar medium

Assessment of antiphytopathogenic potential of antagonistic rhizobacteria

The huge microbial diversity sustaining the plant rhizosphere, provides an endless resource of metabolites which could replace agrochemicals to control phytopathogens. Antagonistic bacteria have been used as ideal biological control agents because of their ability of rapid growth and competitive colonization of the rhizosphere [47]. This competitive nature can be addressed to a number of allelochemicals such as siderophores, antibiotics, biocidal volatiles, lytic and detoxification enzymes produced by these soil microbes. The present study focus on the mode of actions of certain allelochemicals released by bioantagonists to impede the fungal pathogen's growth *via* biocidal mechanisms. In context to this, all the *Rhizoctonia* antagonists were assessed for their potential to produce biocidal metabolites and to address their inhibitory effect on the pathogen, under controlled experimental designs.

Inhibitory effect of biocidal volatile

A wide range of low molecular weight volatile antimicrobial compounds produced by a number of rhizobacteria can be implied as a potential mechanism to control various plant pathogens especially that incite the plants in earlier or later stages of growth [43]. The *in vitro* trials using antagonist- pathogen dual cultures in sealed plate method indicates the potential of some microorganisms to produce volatile antifungal metabolites to serve as biocontrol agents. All the 53 antagonists variably inhibited the test fugal growth in the range between 12.5-93.7%. Isolate Ps132 induced maximum inhibition (93.7%) *via* the production of volatile metabolites in the same trend as in the dual culture where it induced 42.5% of inhibition, inferring the contribution of certain biocidal volatiles (**Table 3**, **Figure 4**). However isolates Ps124, Ps115, Ba26, Ba72 and Ba86 were also recorded with same extent of inhibition as that of Ps132 i.e. 93.7 followed by Ps123 (91.2%) and Ps61, Ba5 and Ba84 (87.5%).

Table 3 Effect of volatile antifungal metabolites on suppression of the radial growth of *Rhizoctonia solani*

Serial No.	Isolates	Radial growth (cm)	Inhibition over control (%)	Serial No.	Isolates	Radial growth (cm)	Inhibition over control (%)
	Control	8.0	-	27	Ps24	3.2	60.0 ± 0.76
1	Ba2b	4.8	40.0 ± 0.15	28	Ps26	4.2	47.5 ± 0.65
2	Ba3	5.0	37.5 ± 0.85	29	Ps32	3.2	60.0 ± 1.10
3	Ba4b	1.5	81.2 ± 1.30	30	Ps33	2.8	65.0 ± 1.38
4	Ba5	1.0	87.5 ± 1.10	31	Ps48	2.1	73.7 ± 0.95
5	Ba7	3.0	62.5 ± 1.53	23	Ps50b	2.0	75.0 ± 0.83
6	Ba8	5.1	36.2 ± 0.62	33	Ps51	2.8	65.0 ± 0.39
7	Ba11	5.0	37.5 ± 0.85	34	Ps54	3.0	62.5 ± 1.87
8	Ba13	2.9	63.7 ± 1.73	35	Ps61	1.0	87.5 ± 1.18
9	Ba18	5.0	37.5 ± 0.85	36	Ps66b	7.0	12.5 ± 0.97
10	Ba25	1.0	87.5 ± 0.86	37	Ps70	3.4	57.5 ± 0.84
11	Ba26	0.5	93.7 ± 1.31	38	Ps80	3.6	55.0 ± 0.75
12	Ba27	3.7	53.7 ± 0.97	39	Ps82	2.9	63.7 ± 0.90
13	Ba29c	3.5	56.2 ± 0.49	40	Ps87	0.5	93.7 ± 1.93
14	Ba32	5.0	37.5 ± 0.87	41	Ps91	2.6	67.5 ± 0.47
15	Ba40c	3.4	57.5 ± 0.81	42	Ps97	2.0	75.0 ± 1.49
16	Ba41	2.3	71.2 ± 0.47	43	Ps100	3.5	56.2 ± 0.85
17	Ba42	1.8	77.5 ± 1.12	44	Ps109	3.2	59.8 ± 2.26
18	Ba43	1.9	76.2 ± 0.47	45	Ps110	1.2	85.0 ± 2.54
19	Ba46	5.2	35.0 ± 1.17	46	Ps114	2.5	68.7 ± 0.84
20	Ba72	0.5	93.7 ± 1.14	47	Ps115	0.5	93.7 ± 1.31
21	Ba84	1.0	87.5 ± 0.82	48	Ps123	0.7	91.2 ± 0.38
22	Ba86	0.5	93.7 ± 1.34	49	Ps124	0.5	93.7 ± 0.91
23	Ba91	3.1	55.5 ± 0.13	50	Ps125	2.3	71.2 ± 0.98
24	Ps1a	4.0	50.0 ± 1.96	51	Ps132	0.5	93.7 ± 1.31
25	Ps11	3.7	53.7 ± 2.36	52	Ps145	3.0	62.5 ± 0.92
26	Ps21	4.0	50.0 ± 1.47	53	Ps144	2.8	65.0 ± 1.41

Values represent mean ± SE (Standard Error) of three replication

Results revealed that the pigment producing *Pseudomonas* isolates showed comparatively higher inhibitory effect on the radial growth of *Rhizoctonia solani* than the Nutrient agar isolated *Bacillus* same as in the dual culture assay, representing pseudomonads as potent biocidal volatiles producers compared to bacilli (Table 3). In addition, Fiddman and Rossal [48]. proposed that volatiles produced by *Bacillus* sp. also induce profound adversial effect on the mycelial proliferation of various fungal plant pathogens.

About 83% of the of *Rhizoctonia* antagonists, were found to inhibit ≥ 50% of the mycelial growth in sealed plate assay. In a case study by Prashar and his co-workers [49], six of the ten antagonists were reported to inhibit *Fusarium oxysporum* f. sp *lycopersici* in tomato plants with an average inhibition of 31.21% *via* such metabolites. Some of the species of *Serratia*, *Pseudomonas* and *Bacillus* synthesize and emit complex blends of volatile compounds such as ammonia and hydrogen cyanide that inhibit growth of many phytopathogenic and non phytopathogenic fungi and play an important role in biological control [50, 51].

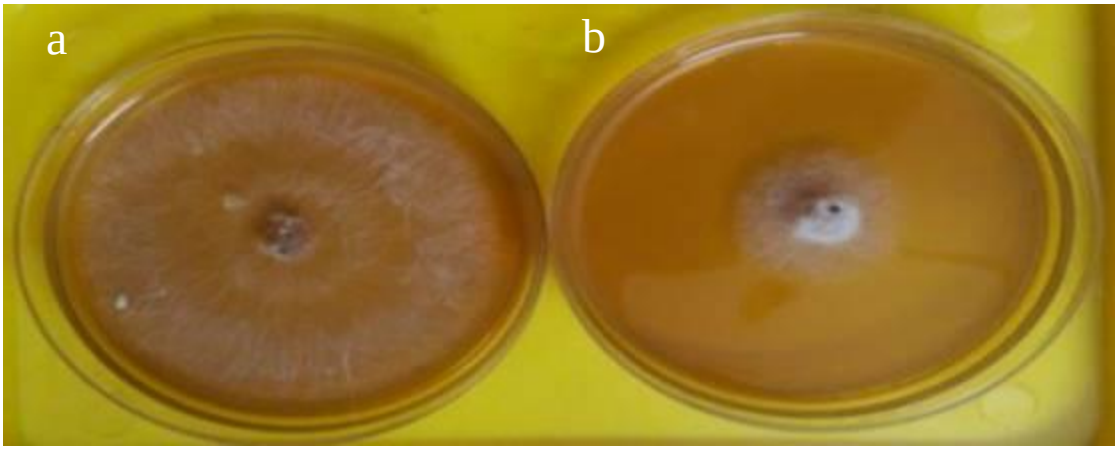

Figure 4 Radial growth inhibition of test pathogen *Rhizoctonia solani* by biocidal volatiles, a: Control, b: Effect of isolate Ps132

Antagonism via Diffusible antifungal metabolite:

A variety of diffusible antimetabolites are considered to be involved in phytopathogen suppression attributing their significant role in biological control mechanisms expressed by PGPR [52]. The non volatile diffusible metabolites include certain antibiotics like 2, 4-diacetylphloroglucinol (DAPG), pyoluteorin, pyrrolnitrin, phenazines and their derivatives such as 2- hydroxyphenazines, phenazine-1-carboxyclic acid (PCA), phenazine-1-carboxamide (PCN) etc. and enzymes involved in cell wall degradation of the pathogens or the detoxification of toxic metabolites released by the pathogens in the soil biosphere [53,54].

Table 4 Effect of rhizobacterial diffusible antifungal metabolites on growth of *Rhizoctonia solani*

Serial No.	Isolates	Radial growth (cm)	Inhibition over control (%)	Serial No.	Isolates	Radial growth (cm)	Inhibition over control (%)
	Control	8.0	-	27	Ps24	3.3	52.8 ± 1.16
1	Ba2b	4.5	35.7 ± 1.66	28	Ps26	3.4	51.4 ± 0.45
2	Ba3	5.1	27.1 ± 1.36	29	Ps32	3.6	48.5 ± 1.17
3	Ba4b	3.1	55.7 ± 0.82	30	Ps33	30	57.1 ± 1.35
4	Ba5	4.1	41.4 ± 0.48	31	Ps48	3.6	48.6 ± 1.19
5	Ba7	4.5	35.7 ± 1.54	23	Ps50b	3.0	57.1 ± 1.96
6	Ba8	5.3	24.2 ± 1.35	33	Ps51	4.8	31.4 ± 0.44
7	Ba11	5.1	27.1 ± 1.32	34	Ps54	1.4	80.0 ± 0.77
8	Ba13	3.7	47.1 ± 1.59	35	Ps61	2.4	65.7 ± 0.93
9	Ba18	5.4	22.8 ± 1.53	36	Ps66b	2.4	65.7 ± 0.94
10	Ba25	3.4	51.4 ± 0.95	37	Ps70	5.4	22.8 ± 1.44
11	Ba26	2.8	60.0 ± 0.45	38	Ps80	5.2	25.7 ± 1.33
12	Ba27	4.6	34.2 ± 1.25	39	Ps82	1.4	80.0 ± 0.59
13	Ba29c	3.1	55.0 ± 1.36	40	Ps87	3.1	55.7 ± 1.56
14	Ba32	3.8	45.7 ± 1.02	41	Ps91	2.8	60.0 ± 0.64
15	Ba40c	3.4	51.4 ± 0.50	42	Ps97	1.1	84.2 ± 1.15
16	Ba41	5.1	27.1 ± 0.55	43	Ps100	4.1	41.4 ± 1.54
17	Ba42	3.5	50.0 ± 1.60	44	Ps109	4.8	31.4 ± 1.23
18	Ba43	3.1	55.7 ± 0.52	45	Ps110	2.3	67.1 ± 0.84
19	Ba46	3.0	57.1 ± 1.14	46	Ps114	3.0	57.1 ± 0.95
20	Ba72	2.8	60.0 ± 1.32	47	Ps115	3.6	48.5 ± 2.35
21	Ba91	4.6	34.2 ± 1.21	48	Ps123	0.7	90.0 ± 1.49
22	Ba84	2.4	65.7 ± 1.53	49	Ps124	5.0	28.5 ± 1.59
23	Ba86	3.2	54.2 ± 0.26	50	Ps125	4.2	40.0 ± 2.01
24	Ps1a	3.2	54.2 ± 1.65	51	Ps132	1.5	93.7 ± 0.99
25	Ps11	2.1	70.0 ± 1.05	52	Ps145	1.8	74.2 ± 2.11
26	Ps21	5.0	28.5 ± 2.47	53	Ps144	3.5	50.0 ± 2.83

Values represent mean ± SE (Standard Error) of three replication

The radial inhibition of *Rhizoctonia solani* was recorded between 22.8-93.7%, where Ps132 exhibited maximum inhibition of 93.7±0.99% followed by Ps123 (90.0 ± 1.49 %) and Ps97 (84.2±1.15%) due to diffusible antifungal metabolites (**Table 4**, **Figure 5**). However Ps11, Ps54, Ps82 and Ps145 isolates were also found efficient to carve more than 70% of the fungal radial growth during membrane plate assay for diffusible metabolites. In support, Giorgio and his co-workers [55] also have reported that an array of rhizobacteria show a negative effect on the growth of various pathogens such as *Botrytis cinerea, Fusarium equiseti, F. oxysporum, F. solani, Phytophthora nicotianae, Rhizoctonia* and *Sclerotinia* spp. *via* the action of diffusible [56].

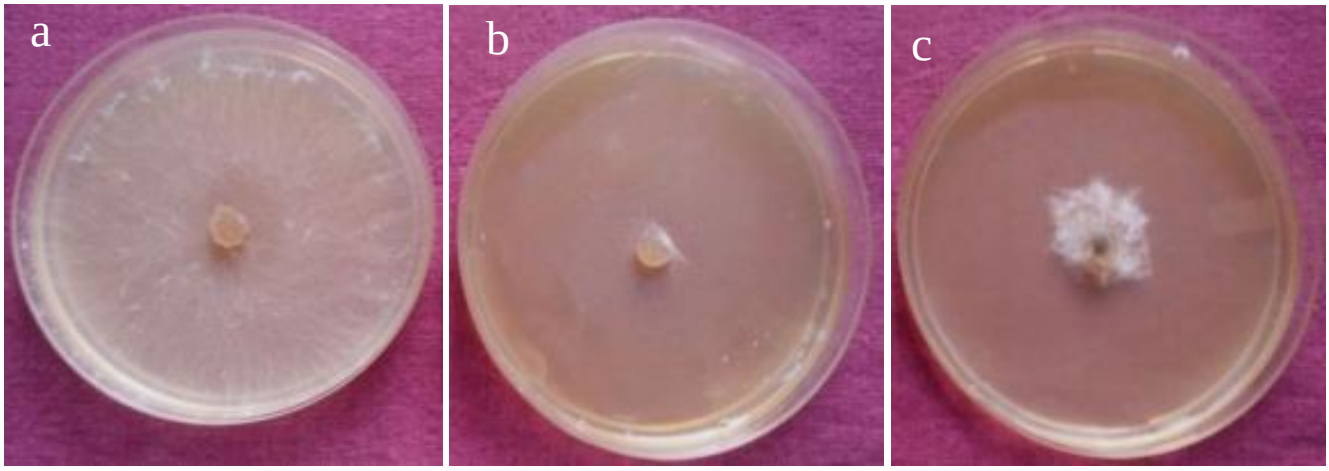

Figure 5 Radial growth inhibition of *Rhizoctonia solani* by biocidal diffusibles, a: Control, b: Effect of isolate Ps132, c: Effect of isolate Ps97

Most of the diffusible antibiotics are involved in the inhibition of cell wall and protein synthesis at several stages along with other vital mechanisms, thereby playing a very important role in reduction of vegetative as well as reproductive growth of plant pathogens. Moreover various enzymes and certain antibiotics like lipopeptides, pyoluteorin, pyrrolnitrin, phenazines and their derivatives also play a crucial role to mortify the effect of toxic metabolites such as fusaric acid produced by *Fusarium* sp. [56]. Furthermore flourescent *Pseudomonas* species produce extracellular metabolites like phenazine and di-acetyl phloroglucinol that are mainly implicated in inhibitory effect on various pathogens associated with plant diseases. Thus inhibition of the pathogens through antibiosis due to wide action spectrum is considered more effective than the other mechanisms [57].

Determinants of biocidal volatiles (Hydrogen Cyanide and Ammonia production)

Hydrogen cyanide and ammonia belong to volatile antifungal metabolites and play a very important role in inhibiting the spore germination and mycelial growth of various fungal phytopathogens [25]. On the basis of antagonistic potential of isolates recorded by dual culture, volatile and diffusible antimetabolite assay, 29 isolates were selected for further volatile determinants i.e. ammonia and hydrogen cyanide production. Only 15 isolates (51.72%) were found to produce HCN, out of which five isolates were found to be strong followed by seven moderate and three weak hydrogen cyanide producers on the basis of intensity of colour (yellow/yellowish-orange/orange-red) produced (**Table 5**, **Figure 6**). In contrast, all the 29 isolates were positive for ammonia liberation where, 8 isolates (27.5%) were recorded as strong, 11 (37.93%) as moderate and 10 isolates (34.48%) as weak ammonia producer on the basis of colour intensity recorded (Table 5, **Figure 7**). Further, quantitative evaluation revealed the potential of Ps91 (10.7±0.24 µm/ml) as highest ammonia producer among antagonists followed by Ps97 (8.4±0.28 µm/ml). In one of our ealier reports, similar results were assessed where 77.5% of the Foc antagonists were found to produce ammonia, whereas only 30% of the antagonists were recorded to produce hydrogen cyanide [34]. In addition to *Bacillus* and *Pseudomonas* sp. reports are there that *Mesorhizobium* spp. also produce HCN, and ammonia along with some enzymes like catalase, chitinase etc [58]. Ammonia inhibits cell cycle progression and thus inhibits the bacterial growth whereas HCN mainly affects the respiratory chain i.e. electron transport chain of the pathogens and thus makes them ATP deficient for further growth and development. Reports for the toxic effect of HCN released by rhizospheric bacteria to subterranean animals and phytopathogenic organisms have been well documented as an important mechanism in biological control of soil borne pathogens [59, 60].

Table 5 Evaluation of *Rhizoctonia solani* antagonists for the production of HCN and ammonia

Serial No.	Isolates	HCN production	Ammonia production		Siderophore production
		Class	Class	μM/ml	Presence (+)/ Absence (-)
1	Ba4b	++	+	4.7±0.05	+
2	Ba13	-	+++	6.2±0.22	+
3	Ba25	-	++	4.3±0.15	+
4	Ba26	-	++	5.8±1.00	-
5	Ba40	-	+	1.7±0.08	-
6	Ba41	-	+	3.4±0.61	-
7	Ba42	++	++	3.5±0.24	+
8	Ba43	++	+++	3.1±0.06	-
9	Ba46	+++	+++	4.6±0.08	-
10	Ba72	-	+	3.3±0.21	+
11	Ba84	-	++	3.7±0.05	-
12	Ba86	+++	++	6.5±0.03	-
13	Ba91	+++	+	1.8±0.04	-
14	Ps1a	+	++	6.9±0.06	+
15	Ps24	-	++	3.8±0.08	-
16	Ps32	-	++	5.4±0.21	+
17	Ps70	-	++	7.7±0.06	-
18	Ps80	-	+	1.2±0.05	+
19	Ps82	++	+++	4.1±1.01	+
20	Ps87	-	+	3.1±0.05	+
21	Ps91	++	+++	10.7±0.24	-
22	Ps97	+	+++	8.4±0.28	-
23	Ps109	-	+	1.6±0.36	+
24	Ps110	++	+++	6.3±0.84	+
25	Ps114	-	+	2.8±0.05	+
26	Ps115	+++	+++	5.3±0.63	-
27	Ps123	++	+	3.9±0.09	+
28	Ps132	+++	++	4.8±0.05	+
29	Ps144	+	++	3.5±0.02	-

For HCN and ammonia production: + represents weak, ++ represents moderate, +++ represents strong producers, - represents non producers;
For siderophore producers: + represents positive for siderophore production, - represents negative for siderophore production.

Detection of siderophore production

One of the allelochemicals involved in the direct as well as indirect antagonism and induction of resistance in hosts plants by PGPR include the production of siderophores, the high-affinity ferric iron chelators, synthesized and liberated by many microorganisms under iron deprivation conditions [61]. Conversion from blue to golden yellow or yellow-orange colour after 24-48 hours of incubation with rhizobacteria spotted on Chrome azurol sulphonate (CAS) medium confirmed the production of siderophores, reaching a maximum after 5 days. Clear halo zones for siderophore production were found to start after 24 hours of incubation, reaching at maximum circumference after four days, when organism had entered into stationary phase (**Figure 8**). Out of 29 *R. solani* antagonists, 18 (62.0%) isolates produced distinct halo zones on CAS plates indicating siderophore production and out of these mungbean isolates, 10 belonged to *Pseudomonas* and 8 to *Bacillus* spp. respectively. Gupta *et al* [62] reported the halo orange zones after 24-72 hrs of incubation of rhizobacterial isolates spotted on CAS agar plates. In a similar study, Joseph *et al* [63] reported the siderophore production by 74.2% of the *Pseudomonas* isolates from chickpea rhizosphere, where only 12.5% of *Bacillus* were able to produce siderophores. Cabaj and Kosakowska [64] evaluated the iron chelating potential of two heterotrophic bacteria i.e. *Micrococcus luteus* and *Bacillus silvestris* isolated from southern Baltic sea, on CAS agar medium revealing their mechanism of survival in such dilute conditions. Gupta and co-workers [65] also reported siderophore production by certain strains of *Pseudomonas, Enterobacter, Brevibacillus, Bacillus* and *Azospirillum* with largest halo of 2.6 cm by *P. fluorescence* PFII on CAS agar medim.

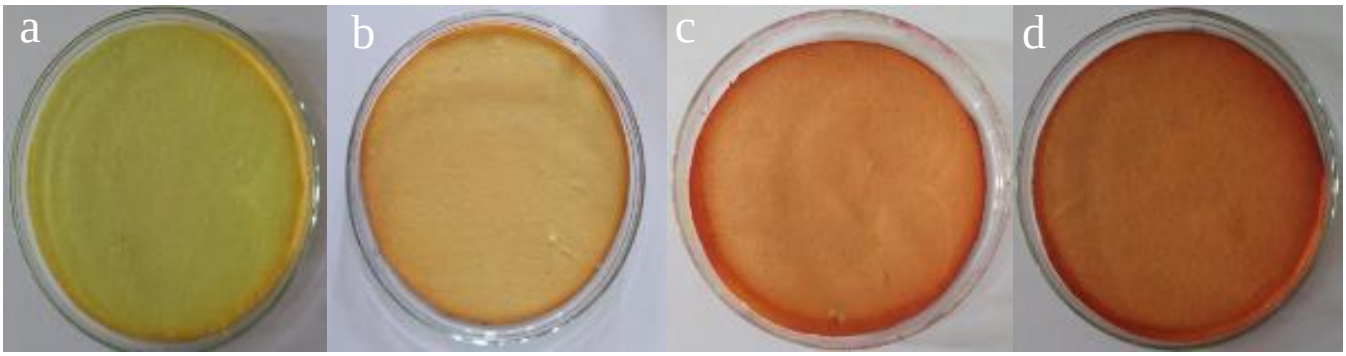

Figure 6 Hydrogen cyanide production by antagonistic isolates, a: Control, b: Weak HCN producer, c: Moderate HCN producer, d: Moderate HCN producer, e: Starong HCN producer.

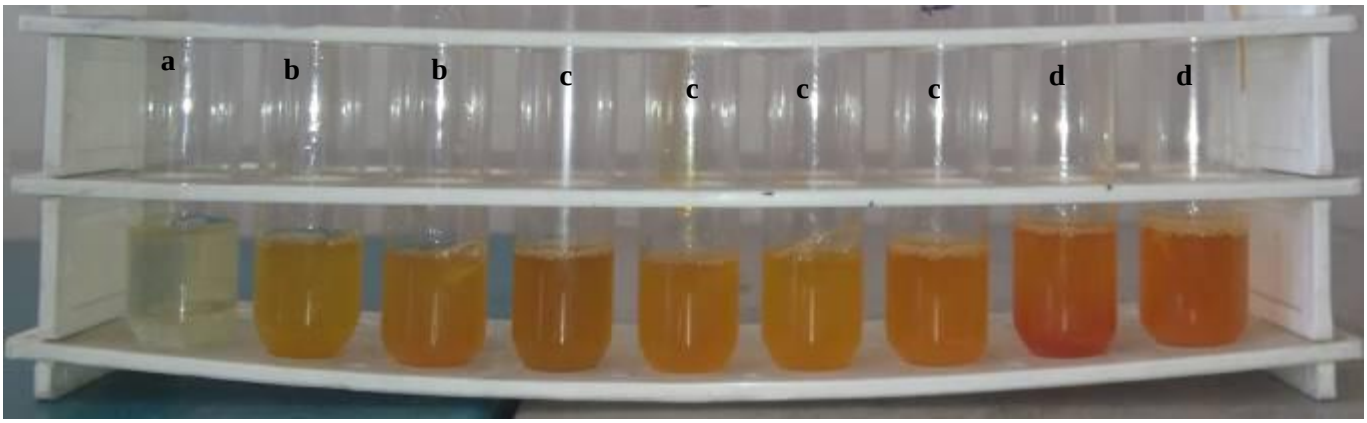

Figure 7 Ammonia production by antagonistic isolates, a: Control, b: Weak ammonia producers, c: Moderate ammonia producers, d: Strong ammonia producers

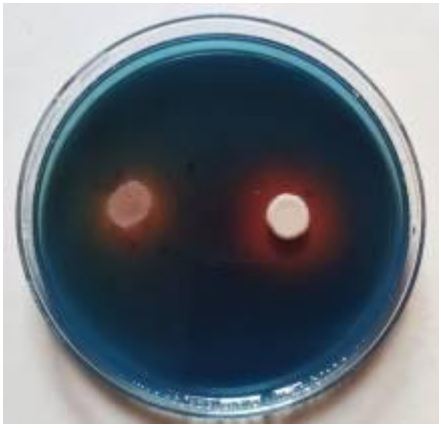

Figure 8 Yellow orange halo zones on CAS agar media for siderophore production

Several reports for the production of siderophores by the biocontrol agents in quantities sufficient to induce the host resistance against plant pathogens have been well documented [66, 67]. Moreover their role in depriving the pathogen of the available Fe^{3+} ions, essentially required for their metabolic pathways also plays a significant role in controlling the pathogens in the soil that has been emerged as a supporting medium for siderophore production.

Compatibility test

Primarily on the basis of *in vitro* analysis for antagonistic traits six (Ba26, Ba91, Ps97, Ps110, Ps123, Ps132) *Rhizoctonia solani* antagonists, were further evaluated for their compatibility with mungbean native *Rhizobium* respectively. The overlapping growth of bacterial antagonists with respective rhizobial cultures on Yeast Mannitol agar plates was determined as compatible interaction between the paired microorganisms (**Figure 9**). All the mungbean isolated antagonists showed positive interaction with respective rhizobial cultures, indicating their synergistic action for plant growth promoting actions.

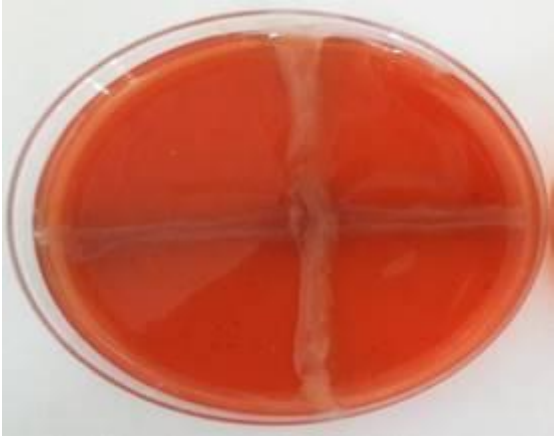

Figure 9 Compatibility test between potential antagonists and native mungbean specific *Rhizobium*

Evaluation of antiphytopathogenic potential of bioantagonists to reduce the root rot severity under glasshouse conditions

The selected (Ba26, Ba91, Ps97, Ps110, Ps123, Ps132) antagonists, were evaluated as single as well as co-inoculants with respective mungbean nodulating *Rhizobium*, and Captan (2g/Kg seeds) as a separate treatment, to evaluate their potential to reduce pre emergence seedling rot and post emergence wet root rot severity in two mungbean varieties (ML-818 and ML-2056 varieties) challenged with *Rhizoctonia solani*, under glass house conditions.

Impact of antagonistic rhizobacteria on seedling development under glasshouse conditions

Observations for the impact of selected PGPR on the seed germination of two mungbean varieties (ML-2056 and ML-818), under glass house conditions, revealed that though the antagonists alone were efficient to induce the seed emergence but the highest seedling emergence was recorded in case of their co-inoculation with *Rhizobium*, indicating their synergistic effect to reduce the pre emergence damping off and to enhance the seed development (**Table 6**).

Table 6 Effect of antagonistic rhizobacteria on seedling emergence of mungbean challenged with *Rhizoctonia solani*

Serial No.	Treatments	Seedling emergence (%)	
		ML-2056	ML-818
1	Absolute control	96.9±1.20	94.6±0.56
2	Negative Control	73.3±1.25	70.0±0.25
3	Fungicide (Captan)	93.3±0.28	89.0±0.63
4	*Rhizobium* (R)	90.0±2.51	86.6±1.25
5	Ba26	93.3±0.96	96.6±1.04
6	Ba91	90.0±2.74	93.3±0.96
7	Ps97	80.0±1.86	90.0±0.82
8	Ps110	96.6±1.96	96.6±1.05
9	Ps123	96.6±1.56	86.6±1.23
10	Ps132	87.0±1.45	90.0±1.89
11	Ba26 + R	100±1.47	100±1.02
12	Ba91 + R	100±1.25	100±0.86
13	Ps97 + R	100±1.65	96.6±0.79
14	Ps110 + R	94.0±0.63	83.3±1.63
15	Ps123 + R	97.0±0.68	100±1.05
16	Ps132 + R	96.0±1.00	100±0.65
17	CD at 5%	3.4	2.9

Values represent mean ± SE (Standard Error) of three replication, Critical difference (CD) values were calculated at the $p \geq 0.05$ level to signify the differences between the treatments.

Seed bacterization with Ba26, Ba91 and Ps97 alongside *Rhizobium* were recorded to induce the emergence in all the seeds sown in the respective pots indicating the 100% germination in these treatments, followed by Ps123+R (R=*Rhizobium*) with 93.3% germination (**Figure 10a**). In contrast to these, the fungicide treatment also recorded

93.3% of the seed development compared to 100% germination in some rhizobacterial treatments in ML-2056. Similarly in mungbean variety ML-818, Ba26, Ba91 and Ps132 were also recorded with 100% germination compared to fungicide 89.0%, and negative control 70.0% when inoculated alongside native *Rhizobium* (Table 6, Figure 10b). In support to this study, rhizobacterial seed treatment was effectively recorded with percentage germination of tomato seeds in the range between 83.3 to 100% in contrast to 75% noted in the untreated control ones in *Sclerotinia sclerotiorum* affected soil under pot conditions [43]. Landa and his co-workers [68] also reported *Pseudomonas fluorescens* RG and *Bacillus megaterium* RGAF to enhance the 100% germination of chickpea compared to negative control.

The co-inoculation with native *Rhizobium* recorded such a positive influence on germination that was found better than the fungicide, might be due to the adverse effect of chemical fungicide on germination (in sterile soil containing no beneficial microbes). Similar results were recorded in our previous study where combination of rhizobia with bioantagonists was observed to enhance the seedling emergence in GPF-2 and JG-41 chickpea varieties under glasshouse conditions [20]. Zongzheng *et al* [69] also demonstrated the efficiency of a *B. subtilis* strain SYI1, to increase the chickpea germination up to 64% over the uninoculated control.

Negative control **Fungicide** **Isolate – Ps97** **Ps97+ *Rhizobium***

Figure 10a Relative seedling emergence between different treatments in munbean variety ML-2056

Negative control **Fungicide** **Isolate – Ps132** **Ps132+ *Rhizobium***

Figure 10b Relative seedling emergence between different treatments in munbean variety ML-818

Enhancement in the sprout development tendency in such sick soils can be attributed to the production of various antimetabolites that reduce that pathogenic effects of such fungal pathogens and help the seeds from damping off. In addition, PGPR have been well reported to produce phytoregulators, specifically IAA and gibbrellins that help the seeds to develop sprouts [28]. Islam *et al* [70] reported certain IAA producing antagonistic strains of *P. stutzeri*, *B.*

subtilis, *Stenotrophomonas maltophilia* and *B. amyloliquefaciens* to improve the seedling emergence and growth of cucumber plants under glass house conditions. One of the mechanisms in the improvement of seed development under abiotic or biotic challenged conditions could be due to the activity of ACC-deaminase produced by PGPR, by reducing the stress ethylene levels, influencing seed development at initial stages [71]. In addition to plant growth regulators and stress reducing mechanisms, a number of researchers have reported PGPR to indirectly enhance the seed germination and vigour index by reducing the incidence of pathogenic seed mycoflora, detrimental to plant growth [72, 73], as in corroboration to the present study.

Impact of antagonistic rhizobacteria on disease prevalence under glasshouse conditions

The first sign of post emergence wet root rot appeared 20 days after sowing, with drooped seedlings, rotten stem at the lowest portion leaving almost dead plants in negative control after 35 days. However symptoms were comparatively less and appeared late in bacterized and fungicide treated plants compared to negative control plants. The disease severity was noticeably reduced by rhizobacterial isolates co-inoculated with native *Rhizobium* indicating the efficacy of both the bacteria to synergistically reduce the pathogenic effect of *Rhizoctonia solani*. Percentage disease reduction was recorded by taking total disease severity in negative control as standard. Observations revealed that even absolute control containing soil of the field having the history of mungbean cultivation, also showed root rot symptoms even after sterlization. Around ≥ 90.0% root rot incidence was recorded in negative control in both the varieties. Observations revealed that although the root rot severity was high in ML-2056 in negative control i.e. 95.2% contrast to ML-818 (90%), but the disease was controlled more efficiently in ML-2056 by the fungicide and rhizobaterial treatment, however co-inoculation with *Rhizobium* was almost equally efficient in both the varieties. In ML-2056, seed bacterization with Ba91 maximally reduced the disease incidence up to 82.4±0.74%, followed by Ps132 (81.5±0.96%) and Ps110 (77.5 ± 0.32%), when used in combination with *Rhizobium*, that was found effective than the fungicide with 73.0±0.64% reduction in disease severity compared to negative control (taken as standard) (**Table 7**, **Figure 11a**). Somewhat similar results were recorded in ML-818, where isolate Ps110, Ps132 and Ps97 were found most effective to control 82.1 ±1.36, 78.0 ± 0.38 and 77.2 ± 1.40 % of root rot severity in contrast to fungicide, recorded to reduce 66.0 ± 0.35% of the disease incidence, comparable to recommended *Rhizobium* in ML-818 mungbean plants (Table 7, Figure 11b).

Table 7 Reduction of root rot severity by antagonistic rhizobacteria in mungbean

Serial No.	Treatments	Reduction in disease severity (%)	
		ML-2056	ML-818
1	Absolute control	92.6 ± 0.96	93.0 ± 0.41
4	Fungicide (Captan)	73.0 ± 0.64	66.0 ± 0.63
5	*Rhizobium* (R)	68.8 ± 0.69	66.1 ± 0.35
6	Ba26	70.6 ± 1.20	69.6 ± 0.81
7	Ba91	76.5 ± 0.34	48.9 ± 0.41
8	Ps97	71.6 ± 0.94	63.2 ± 0.63
9	Ps110	60.0 ± 0.23	77.2 ± 0.85
10	Ps123	71.0 ± 0.74	70.4 ± 0.34
11	Ps132	74.7 ± 0.56	52.3 ± 0.45
12	Ba26 + R	71.9 ± 0.23	67.0 ± 0.42
13	Ba91 + R	82.4 ± 0.74	70.0 ± 0.23
14	Ps97 + R	71.9 ± 0.82	77.2 ± 1.40
15	Ps110 + R	77.5 ± 0.32	82.1 ± 1.36
16	Ps123 + R	70.5 ± 0.55	70.6 ± 0.64
17	Ps132 + R	81.5 ± 0.96	78.0 ± 0.38
18	CD at 5%	3.6	5.3

Values represent mean ± SE (Standard Error) of three replication, Critical difference (CD) values were calculated at the $p \geq 0.05$ level to signify the differences between the treatments.

In a similar report, Pf1-Bs16 and Pf1-Py15 recorded disease severity of 16.66 and 24.99% disease incidence and reduced the disease (81.8%) and (72.7%), respectively against 91.63% disease incidence in control in mulburry [74]. It has been well reported by many workers, that even if a consortium of antagonistic rhizobacteria do not always result in synergistic actions to reduce the phytopathogenic effects, but diverse strains may have diverse mechanisms and their combinations can provide a spectrum of cumulative activity for maintaining the plant health and productivity [75, 34]. Efficacy to descend the disease by rhizobacterial treatment is not only limited to root rot, but

these are also effective against other diseases such as, stem rot caused by *Sclerotinia sclerotiorum*, damping off by *Phytophthora*, leaf rot by *Alternaria* spp. etc. by various antagonistic mechanisms [76, 43]. Exploring such antagonistic mechanisms in plant beneficial rhizobacteria along with plat growth promoting traits can help reducing such soil borne fungal pathogens and to enhance the plant's growth and hence productivity. Moreover co-inoculation of two or more of these cultures or along with native rhizobium/rhizobacteria can induce better results compared to single inoculants on the condition of their synergistic effects when applied in combination.

Negative control Fungicide Isolate – Ba91 Ba91+ *Rhizobium*

Figure 11a Relative root rot incidence between different treatments in mungbean variety ML-2056

Negative control Fungicide Isolate – Ps110 Ps110+ *Rhizobium*

Figure 11b Relative root rot incidence between different treatments in mungbean variety ML-818

Conclusion

With increasing yield losses due to soil borne fungal pathogens and adverse effects of chemical fertilizers and pesticides, the need for environment friendly and cost effective alternative has directed the focus towards several species of plant growth promoting rhizobacteria with various antagonistic mechanisms by which they can control the phytopathogenic effects in the crop plants. In context to this, in the present study, potential rhizobaterial antagonists were screened for impeding effect on root rot pathogen *Rhizoctonia solani via* diffusible and volatile antifungal

metabolites under *in vitro* condition to control root rot severity in two mungbean cultivars under glass house using sick soil. Results revealed that though the selected antagonistic isolates alone were efficient in contrast to control but co-inoculation with mungbean specific native *Rhizobium* showed better results in enhancing the seed germination *via* reducing pre emergence severity of seedling rot or damping off and post emergence root rot incidence caused by *Rhizoctonia solani* in both the mungbean varieties (ML-2056 and ML-818) Depending upon the performance in laboratory as well as glass house conditions, isolate Ba26, Ps132 and Ps110 were spotted as potential *Rhizoctonia solani* antagonists with strong antagonistic mechanisms when applied as dual inoculants with native *Rhizobium*. These dual inoculation were found even better than the fungicide (Captan) treatment to control seedling as well as root rot. These rhizobacterial inoculations can be used as biocontrol agents against *Rhizoctonia solani* in wet root rot affected soils, on the condition of their similar effectiveness under field conditions. Further investigations need for their performance in different combination to select consortia of two or more cultures to evaluate the synergistic potential of antagonists to formulate various combinations of these for assessment of better management effects for such soil borne phytopathogens.

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