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Isolation and Identification of Mycorrhiza Helper Bacteria with Biochemical Characterization and Plant Growth Promoting Traits

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ABSTRACT: Mycorrhiza Helper Bacteria (MHB) forms symbiotic associations with ectomycorrhiza and Arbuscular Mycorrhiza (AM). Arbuscular Mycorrhizal Fungi (AMF) colonize the roots of approximately 80% of terrestrial plants. The most common beneficial effect of mycorrhizae is increased uptake of immobile nutrients, especially P, from soil. In the present study, it is mainly focussed on selection of an efficient MHB to enhance AM fungal activity and in turn increase the crop growth. In this study 40 MHBs were isolated from the rhizosphere soils of different crops from different agroclimatic zones of Andhra Pradesh. Among the 40 MHB isolates, most of them were gram positive (+ve), rod shaped and few were motile and endodpore formers. 23 isolates were positive for catalase, 18 isolates were positive for starch hydrolysis, 37 isolates were positive for MR test, 20 isolates were positive for VP test, 18 isolates were positive for casein hydrolysis, 12 isolates were positive for indole production and only one isolate was positive for H_2S production. Fifty five per cent of the isolates were found positive for IAA production, maximum phosphrous solubilization was shown by the NDLCP2 with a Solubilization Index of 4.00 and highest potassium Solubilization Index was shown by the strain NDLCP3 (2.7) and among 40 bacterial isolates 38 bacterial isolates were positive for ammonia production. The MHB isolates which exhibited PGPR traits can be better utilized in agriculture as PGPR to enhance crop productivity.

Keywords: MHB: Mycorrhiza Helper Bacteria, PGPR: Plant growth promotion, IAA, Ammonia production, Solubilization Index.

INTRODUCTION

Mycorrhiza Helper Bacteria (MHB) forms symbiotic associations with ectomycorrhiza and arbuscular mycorrhiza (AM) (Frey-Klett *et al.,* 2007). MHB are not plantspecific, but are clearly selective about the plant species, and the term fungus-specific can be used (Garbaye, 1994). MHB and their culture filtrates were able to stimulate the AM fungal spore germination, MHB effectors that facilitate root colonization could be plant cell wall digesting enzymes, which could enhance the penetration and spreading of the fungus within the root tissues (Mosse, 1962). Direct contact between the spores and bacteria was necessary for the induction of spore germination in *Glomus clarum* (Xavier and Germida 2003). Gram-positive bacterial association with AM fungi is higher, compared to the Gram negative bacteria, but has yet to be verified (Artursson *et al*., 2005). The significance of such interactions is because some of the most important PGPRs including *Bacillus* spp., which are in synergistic interaction with AM fungi, are Gram-positive bacteria (Frey-Klett *et al*., 2007; Francis *et al*., 2010). There are certain rhizospheric microorganisms, especially bacteria, which elucidate the mechanism of selective interaction with surrounding microorganisms and have a positive or neutral effect on mycorrhizal associations, usually this beneficial effect of bacteria on mycorrhiza, the concept of MHB takes into account existence (Frey-Klett *et al.,* 2007) and this need to be further verified. Hence, this study was taken up to select an efficient MHB to enhance mycorrhizal activity and crop productivity.

MATERIALS AND METHODS

Collection of rhizosphere soil of different crops. Soil samples were collected from selected bench mark points of different agro climatic zones of Andhra Pradesh. Rhizosphere soil along with plant roots were collected from each sampling site with the help of scoop. These soil samples were thoroughly mixed and 500-600 g of each sample was brought to the laboratory in a clean ziplock bags and kept in the refrigerator till samples were used for isolation and enumeration of mycorrhiza helper bacteria and physico-chemical and biochemical analysis.

Isolation of mycorrhiza helper bacteria from rhizospheresoil samples. The method proposed by Vlassak *et al.* (1992) was followed for the isolation of MHB. As per the procedure, 10 g of soil from each soil sample was taken in a conical flask with 90 ml water. The sample was agitated for 15 minutes on a vortex and serial dilution of soil suspensions were prepared. The dilutions prepared were used for the isolation of different bacteria, 100 µl of the respective dilutions of samples were taken and spread on specific sterilized

solid media *i.e.,* yeast extract mannitol agar for Rhizobium, Pikovskaya's medium for Phosphate solubilising bacteria, modified Aleksandrov's medium for potassium releasing bacteria, nutrient agar for total bacteria. The plates were incubated at room temperatures (28±2°C) for 24-72 hours. After incubation colonies formed were counted using digital colony counter and the population was expressed as CFU × dilution factor per gram.

Gram's staining. The suspension of 24 hr old culture was spread on slide using inoculation loop. The smear was air dried and heat fixed. The smear was then flooded with crystal violet solution and 1 min smear was washed gently with tap water. Then the slide was flooded with iodine solution for 1 min, then treated with 95 % ethanol for10-15 seconds and washed with water. Then smear was then flooded with Safranin solution for 1 min. Then slide was washed gently with tap water and air dried. The slide was examined under microscope at 100X power with oil immersion and data was recorded (Barthalomew and Mittewar 1950).

Endospore staining. The smear was prepared by spreading bacterial suspension on slide using inoculation loop. The smear was air dried and heat fixed. The smear was then flooded with malachite green, heat the slides to steaming and steam for 5 minutes adding more stain to smears from time to time, then wash the slides under slowly running water. Counter stain with safranin solution for 30 seconds, wash the smears with distilled water, blot dry the slides with absorbent paper. The slide was examined under microscope at 100X power with oil immersion and data were recorded.

Motility test. Each isolate was spot-inoculated on the centre of semi-solid nutrient agar plates (0.2 % agar) and incubated a 30°C. The diameter of the diffusion of colony was recorded after 24 hours (Elbeltagy *et al*., 2000).

Biochemical and Physiological Characterization. Different biochemical tests performed and the protocols followed are briefly outlined below. Biochemical based characterization was done as per Bergey's Manual of systematic Bacteriology (Holt *et al*., 1994).

Starch hydrolysis (MacFaddin, 2000) : Sterile starch agar plates were spotted with 10 µL overnight broth cultures of the isolates and incubated at 28±2°C for 24-48 h. After incubation, the plates were flooded with iodine solution. The formation of a transparent zone around the colony was taken as positive reaction for the test.

Indole production (Isenberg and Sundheim, 1958): Sterilized SIM agar broth in testube were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28±2°C. After incubation, 10 drops of Kovac's indole reagent was added to each tube. The isolates showing production of red colour were recorded as positive for indole production.

Catalase test (Rangaswami and Bagyaraj 1993): This test was performed to study the presence of catalase enzyme in bacterial colonies. The fresh cultures of pure isolates were taken on glass slides and one drop of H_2O_2 (30 %) was added. Appearance of gas bubble indicated the presence of catalase enzyme.

Methyl Red test (Crown and Gen, 1998): Sterilized glucose-phosphate broth tubes were inoculated with the test culture and incubated at 28±2°C for 48 h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red colour was taken as positive and yellow colour was taken as negative for the test.

Voges Prausker's test (MacFaddin, 2000). The presterilized glucosephosphate broth tubes were inoculated with test cultures and incubated at 37°C for 48 h. After incubation ten drops of Baritt's reagent A was added and gently shaken followed by addition of 10 drops of Baritt's reagent B. Development of pink colour in the broth was taken as positive for the test.

Hydrogen Sulfide Test (Beishir, 1991): Sterilized Hydrogen Sulfide-Indole-Motility agar (SIM agar) stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 hours at 28 ± 2ºC. Visualization of black colour along the line of inoculation indicate positive reaction to the test.

Casein hydrolysis (Smibert and Kreig 1981): The isolates were streaked on skim milk agar plates and incubated at room temperature. Hydrolysis of casein as indicated by formation of clear zones around the colonies was considered as positive reaction.

Evaluation of Plant growth promoting characters of MHB isolates

Indole Acetic Acid (IAA) production. Indole acetic acid production was tested according to the procedure proposed by Gordon and Weber (1951). The active culture of each test isolate was raised in 5ml respective broth tubes and incubated. After incubation these cultures were centrifuged. Two drops of O- phosphoric acid was added to 2 mL of supernatant and incubated for 30 min to develop the colour. Development of pink colour was considered as positive for IAA production.

Potassium solubilisation. The isolates were inoculated onto the surface of Aleksandrov's agar medium constituted 0.5 % potassium alumino silicate (Sugumaran and Janarthanam 2007) and incubated at 30ºC for 4-5 days. The diameter of the clearing zones around the colonies was measured (Prajapati and Modi 2012).

KSE (Potassium Solubilization Efficiency) =Z / C x 100

Z- Clearance zone including bacterial growth

C- Colony diameter

Phosphate solubilisation. Sterilized Pikovskaya's medium was poured as a thin layer on to the sterilized petri plates and incubated for 24h. The agar plates were spot inoculated with isolates and incubated at 28±1°C for 4-5 days. Formation of a clear zone around the colonies was considered as positive result for phosphate solubilization.

PSI (Phosphate Solubilization Index) = Z / C × 100

Z- Clearance zone including bacterial growth

C- Colony diameter

Ammonia production (Juanda, 2005). The isolates were tested for ammonia production by inoculating the

isolates into 10 mL of pre-sterilized peptone water in test tubes. The tubes were incubated for 48-72 hours at 36 ± 2^0C and Nessler‘s reagent (0.5 mL) was added in each tube. Change in colour of the medium from brown to yellow colour was taken as a positive test for ammonia production.

RESULTS AND DISCUSSION

Collection of soil samples from Rhizosphere. Soil samples were collected from different regions of Andhra Pradesh which includes Amaravathi, Guntur, Nandyala and Bapatla during *Kharif* and *Rabi*, 2021. The informations like crop growth stage, type of soil and latitude and longitude were recorded (Table 1).

Bacterial population present in different Rhizosphere soil samples. Twenty rhizospheric soil samples obtained from different locations of Andhra Pradesh were used for the isolation of Mycorrhiza helper bacteria. The microbial populations in the rhizosphere soils of different crops were determined and given in the Table 2. Bacterial population ranges between 6.00-7.30 LogCFUg^{-1} soil. Maximum bacterial population was recorded in the sorghum rhizosphere soil, Amaravathi (7.30 Log CFUg^{-1} soil), followed by Bapatla (7.04 Log CFU g^{-1} soil), and least bacterial population was recorded in the blackgram rhizosphere soils, Nandyala (6.00 Log CFUg^{-1} soil).

Different morphotypes isolated from Rhizosphere soils in different crops

Different morphotypes of bacteria obtained from rhizosphere soil samples (Table 2) from blackgram, Amaravathi were 14 (AMTBG1, AMTBG2, AMTBG3, AMTBG4, AMTBG5, AMTBG6, AMTBG7, AMTBG8, AMTBG9, AMTBG10, AMTBG11, AMTBG12, AMTBG13, AMTBG14), from redgram rhizosphere soils, Amaravathi were 6 AMTRG1, AMTRG2, AMTRG3, AMTRG4, AMTRG5, AMTRG6), from sorghum and maize rhizosphere soils, Amaravathi were 9(AMTSG1, AMTSG2, AMTSG3, AMTSG4, AMTSG5, AMTSG6, AMTSG7, AMTMZ1, AMTMZ2), from blackgram and horsegram rhizosphere soils, Bapatlawere 4 (BPTBG1, BPTBG2, BPTHG1, BPTHG2), from chickpea, sorghum and blackgram rhizosphere soils, Nandyala were 7 (NDLCP1, NDLCP2, NDLCP3, NDLSG1, NDLSG2, NDLBG2, NDLBG3).

Cell morphology and physiological characters of MHB isolates. Out of 40 bacterial isolates, 18 bacterial isolates were Gram negative (G-ve) and remaining 22 isolates were gram positive (+ve). Around 31 bacterial isolates were rod shaped with varied size ranging from small rods to long rods and 9 isolates were cocci shaped, 17 isolates were found motile, while 23 isolates were non motile.

Among 40 isolates five isolates (AMTRG6, BPTHG1, BPTHG2, AMTBG11, AMTBG8, AMTSG6) were endospore formers (Table 3).

In the similar studies Duponnois and Garbaye (1991) isolated 14 Bacterial strains from sporocarps and surface-sterilized mycorrhizas of *L laccata* young plants of Douglas fir in France. Preliminary characterization of bacterial strains showed that approximately equal percentages of gram-positive (42 %) and gram-negative (41 %), cell morphology ranges from long rods, elongated rod chains, large rods and small rods, 3 bacterial isolates shown motility, 2 bacterial strains were positive for sporulation.

BIOCHEMICAL CHARACTERIZATION AND PLANT GROWTH PROMOTING TRAITS OF MHB

Biochemical characterization of MHB

As per Bergey‘s manual of determinative bacteriology, the physiology and biochemical characteristics of the selected isolates were determined. 23 isolates (AMTRG4, AMTRG6, BPTHG1, BPTBG1, BPTHG2, BPTHG1, BPTHG2, BPTBG1, BPTBG2, AMTBG6, AMTBG7, AMTBG8, AMTBG9, AMTBG11, AMTSG4, AMTSG5, AMTSG6, AMTSG7, NDLSG1, NDLCP2, NDLSG2, NDLCP3, NDLCP1, NDLBG2) out of 40 isolates were positive for catalase activity. Eighteen isolates (AMTBG2, AMTBG3, AMTBG5, AMTRG1, AMTRG2, AMTRG5, AMTMZ2, BPTHG2, AMTBG6, AMTBG8, AMTBG11, AMTBG12, AMTBG13, AMTSG4, AMTSG5, AMTSG6, NDLSG1, NDLCP1) out of 40 isolates were positive for starch hydrolysis. Except isolate AMTSG7 remaining 39 isolates are negative for H_2S production. Twelve isolates (AMRG1, AMTRG3, AMTRG6, AMTMZ1, AMTMZ2, AMTBG7, AMTBG11, AMTBG14, AMTSG1, AMTSG4, AMTSG5, NDLSG1) were found to be positive for indole production. Isolates AMTBG1, AMTSG6, AMTSG7 found to be negative for MR test while remaining 37 isolates shown positive result. Twenty isolates (AMTBG4, AMTRG1, AMTRG3, AMTR6, AMTMZ1, BPTHG1, BPTHG2, AMTBG6, AMTBG8, AMTBG9, AMTBG11, AMTBG12, AMTBG13, AMTBG14, AMTSG1, AMTSG2, AMTSG3, NDLCP2, NDLSG2, NDLCP1, NDLBG2)were positive for VP test. Eighteen isolates (AMTBG1, AMTBG3, AMTRG1, AMTRG3, AMTRG6, AMTMZ1, BPTHG1, BPTHG2, AMTBG7, AMTBG10, AMTBG11, AMTBG12, AMTBG13, AMTBG14, AMTSG1, AMTSG4, AMTSG5, NDLSG1) were positive test for Casein hydrolysis (Table 4).

Similar studies were conducted by earlier researchers where Zhao *et al*. (2014) in their study of performed biochemical tests for MHB strain (DZ18). The strain DZ18 was tested positive for oxidase, catalase, indole production, VP test, methyl red test, H_2S production, starch hydrolysis, and gelatine hydrolysis. The phenotypic and biochemical characterization of the DZ18 isolate displayed a broad similarity with the genus *Bacillus*.

IAA production. Out of 40 bacterial isolates 55% were found to be positive for IAA production, based on the colour intensity developed after adding the Salkowski reagent and incubation. Of the 40 isolates screened, 22 bacterial isolates (AMTBG2, AMTBG5, AMTRG1, AMTRG2, AMTRG4, AMTRG5, AMTMZ2, AMTBG1, AMTBG6, AMTBG8, AMTBG10, AMTBG11, AMTBG13, AMTBG14, AMTSG3, AMTSG4, AMTSG6, AMTSG7, NDLSG1, NDLCP2,

NDLSG2, NDLCP3) produced IAA while 18 strains did not produced IAA (Table 5).

In the similar studies Yu *et al*. (2016) have isolated many bacteria from maize roots which produced IAA and they belong to the genera *Bacillus, Microbacterium, Psychrobacillus* and *Lysinibacillus.*

Phosphorus solubilisation. Out of 40 bacterial isolates, 30 bacterial isolates have formed solubilisation zone on Pikovskaya's agar plates supplemented with tri calcium phosphate at the rate of 0.5%.

According to Silva Filho and Vidor (2000), solubilization indices below 2 are considered low, between 2 and 3 medium and above 3 it is high. In present study, 10 isolates were found to have no solubilization ability while 17 isolates have shown low solubization indices (SI <2), 11 isolates have shown medium SI (2-3), whereas 27 isolates were found have high phosphorus solubilization ability (>3). Maximum solubilization was shown by the strain NDLCP2 (4.00) followed by NDLCP3 (3.66) (Table 5).

Potassium solubilisation. Among MHB isolates 15 per cent were found to have potassium solubilisation ability. The potassium solubilisation indices were observed in 6 MHB isolates which were in the range of 1.00 to 2.69 and the highest SI was shown by the strains NDLCP3 (2.7) followed by NDLCP1 (2.67) (Table 4.6). Number of MHB isolates as K solubilizers were less as compared to P solubilizers in present investigation, this indicates that the isolates may use different mechanisms or metabolites for solubilizing phosphorus and potassium (Table 5). Similarly, Sarikhani *et al.* (2018) isolated K releasing bacteria and screened them on the Aleksandrov media and the most efficient bacteria was identified as the *Pseudomonas*.

Ammonia prduction. Ammonia production is an important trait that indirectly affect the plant growth. In the present study except two isolates AMTBG2, AMTBG3 remaining 38 isolates were found to be positive for ammonia production by changing colour after addition of Nessler's reagent, indicating that these isolates may accumulate nitrogen in plants and promotes root and shoot elongation which indirectly influencing seed vigor index (Table 5).

In similar studies, Masmoudia *et al*. (2022) isolated rhizobacteria, which were positive for ammonia production where as *Bacillus inaquorsum* and *Bacillus mojavensis* produced highest ammonia concentration about 371 and 370 micro molar respectively.

Table 1: Details of collection of soil samples from rhizosphere soils and their locations across Andhra Pradesh.

Sr. No.	District	Mandal	Code	Crop	Code	Crop growth stage	Soil type	Latitude and longitude
1.	Guntur	Amaravathi	AMT	Black gram	BG	Grain filling	Red soil	16.569578°N, 80.372508°E
2.	Guntur	Amaravathi	AMT	Black gram	BG	Grain filling	Red soil	16.569931°N, 80.372618°E
3.	Guntur	Amaravathi	AMT	Red gram	RG	Flowering	Black soil	16.569960°N, 80.372707°E
4.	Guntur	Bapatla	BPT	Black gram	BG	Grain filling	Black soil	15.274658°N, 80.530525°E
5.	Guntur	Amaravathi	AMT	Red gram	RG	Grain filling	Red soil	16.569040°N, 80.372498°E
6.	Guntur	Amaravathi	AMT	Maize	MZ	Flowering	Black soil	16.569040°N, 80.372438°E
7.	Guntur	Bapatla	BPT	Horse gram	HG	Flowering	Sandy loam	15.269345°N, 80.587641°E
8.	Guntur	Amaravathi	AMT	Maize	MZ	Flowering	Black soil	16.274658°N, 80.530525°E
9.	Guntur	Amaravathi	AMT	Sorghum	SG	Grain filling	Black soil	16.5730°N, 80.3575°E
10.	Guntur	Bapatla	BPT	Sorghum	SG	Grain filling	Black soil	15.9039°N, 80.4671°E
11.	Kurnool	Nandyala	NDL	Black gram	BG	Grain filling	Black soil	15.213773°N, 78.322746°E
12.	Kurnool	Nandyala	NDL	Black gram	BG	Grain filling	Black soil	15.214252°N, 78.323038°E
13.	Kurnool	Nandyala	NDL	Black gram	BG	Grain filling	Black soil	15.213700°N, 78.322860°E
14.	Kurnool	Nandyala	NDL	Sorghum	SG	Grain filling	Black soil	15.213544°N, 78.322839°E
15.	Kurnool	Nandyala	NDL	Sorghum	SG	Grain filling	Black soil	15.210564°N, 78.324324°E
16.	Kurnool	Nandyala	NDL	Sorghum	SG	Grain filling	Black soil	15.4777°N, 78.4873°E
17.	Kurnool	Nandyala	NDL	Chick pea	CP	Flowering	Black soil	15.2304°N, 78.3174°E
18.	Kurnool	Nandyala	NDL	Chick pea	CP	Flowering	Black soil	15.1880°N,78.2640°E
19.	Kurnool	Nandyala	NDL	Chick pea	CP	Flowering	Black soil	15.210264°N, 78.324513°E
20.	Kurnool	Nandyala	NDL	Chick pea	CP	Flowering	Black soil	15.2026°N, 78.3735°E

Table 2: Bacterial population and their morphotypes present in different rhizosphere soil samples.

Sr. No.	Crop	Total Bacterial population (LogCFUg^{-1} of soil)	Number of morphotypes
1.	Blackgram	6.11	2
2.	Blackgram	6.00	2
3.	Red gram	6.32	3
4.	Horse gram	6.54	2
5.	Red gram	6.78	3
6.	Maize	6.95	2
7.	Sorghum	7.30	2
8.	Sorghum	6.78	1
9.	Sorghum	7.30	2
10.	Sorghum	6.59	2
11.	Blackgram	7.04	3
12.	Blackgram	6.40	3
13.	Blackgram	6.41	2
14.	Blackgram	6.94	2
15.	Chick pea	6.96	1
16.	Chick pea	6.75	1
17.	Sorghum	6.41	2
18.	Blackgram	6.00	2
19.	Blackgram	6.75	2
20.	Chick pea	6.85	1

Table 3: Cell morphology and physiological characters of MHB isolates.

Sr. No.	Sample code	Grams reaction	Cell shape	Motility	Sporulation
1.	AMTBG1	-Ve	Short Rods	Non motile	Negative
2.	AMTBG2	-Ve	Short Rods	Motile	Negative
3.	AMTBG3	-Ve	Short Rods	Motile	Negative
4.	AMTBG4	-Ve	Cocci	Non motile	Negative
5.	AMTBG5	-Ve	Short Rods	Motile	Negative
6.	AMTRG1	+Ve	Cocci	Non motile	Negative
7.	AMTRG2	+Ve	Rods	Motile	Positive
8.	AMTRG3	-Ve	Short Rods	Motile	Negative
9.	AMTRG4	-Ve	Rods	Non motile	Negative
10.	AMTRG5	+Ve	Rods	Non motile	Positive
11.	AMTRG6	+Ve	Cocci	Non motile	Negative
12.	AMTMZ1	+Ve	Cocci	Non motile	Negative
13.	AMTMZ2	+Ve	Rods	Non motile	Positive
14.	BPTHG1	-Ve	Rods	Non motile	Negative
15.	BPTHG2	+Ve	Rods	Motile	Positive
16.	BPTBG1	-Ve	Rods	Motile	Negative
17.	BPTBG2	+Ve	Cocci	Non motile	Negative
18.	AMTBG6	+Ve	Rods	Non motile	Negative
19.	AMTBG7	+Ve	Rods	Non motile	Negative
20.	AMTBG8	+Ve	Rods	Non Motile	Positive
21.	AMTBG9	-Ve	Short Rods	Non motile	Negative
22.	AMTBG10	-Ve	Rods	Non motile	Negative
23.	AMTBG11	+Ve	Rods	Motile	Negative
24.	AMTBG12	+Ve	Rods	Non motile	Negative
25.	AMTBG13	+Ve	Rods	Motile	Negative
26.	AMTBG14	-Ve	Rods	Non motile	Negative
27.	AMTSG1	+Ve	Rods	Non motile	Negative
28.	AMTSG2	-Ve	Short Rods	Motile	Negative
29.	AMTSG3	-Ve	Rods	Non motile	Negative
30.	AMTSG4	-Ve	Rods	Motile	Negative
31.	AMTSG5	+Ve	Cocci	Motile	Positive
32.	AMTSG6	+Ve	Rods	Non motile	Negative
33.	AMTSG7	+Ve	Rods	Non motile	Negative
34.	NDLSG1	+Ve	Cocci	Non motile	Negative
35.	NDLBG3	+Ve	Cocci	Non motile	Negative
36.	NDLCP2	-Ve	Short Rods	Motile	Negative
37.	NDLSG2	+Ve	Rods	Motile	Negative
38.	NDLCP3	+Ve	Rods	Motile	Negative
39.	NDLCP1	-Ve	Rods	Motile	Negative
40.	NDLBG2	+Ve	Rods	Motile	Negative

Table 4: Biochemical characterization of Mycorrhiza helper bacteria.

Sr. No.	Isolate code	Catalase	Starch Hydrolysis	H_2S Test	Indole Production	MR	VP	Casein hydrolysis
1.	AMTBG1	–	–	–	–	–	–	+
2.	AMTBG2	–	+	–	–	+	–	–
3.	AMTBG3	–	+	–	–	+	–	+
4.	AMTBG4	–	–	–	–	+	+	–
5.	AMTBG5	–	+	–	–	+	–	–
6.	AMTRG1	–	+	–	+	+	+	+
7.	AMTRG2	–	+	–	–	+	–	–
8.	AMTRG3	–	–	–	+	+	–	+
9.	AMTRG4	+	–	–	–	+	+	–
10.	AMTRG5	–	+	–	–	+	–	–
11.	AMTRG6	+	–	–	+	+	–	+
12.	AMTMZ1	–	–	–	+	+	+	+
13.	AMTMZ2	–	+	–	+	+	+	–
14.	BPTHG1	+	–	–	–	+	+	+
15.	BPTHG2	+	+	–	–	+	–	+
16.	BPTBG1	+	–	–	–	+	–	–
17.	BPTBG2	+	–	–	–	+	–	–
18.	AMTBG6	+	+	–	–	+	+	–
19.	AMTBG7	+	–	–	+	+	–	+
20.	AMTBG8	+	+	–	–	+	+	–
21.	AMTBG9	+	–	–	–	+	+	–
22.	AMTBG10	–	–	–	–	+	–	+
23.	AMTBG11	+	+	–	+	+	+	+
24.	AMTBG12	–	+	–	–	+	+	+
25.	AMTBG13	–	+	–	–	+	+	+
26.	AMTBG14	–	–	–	+	+	+	+
27.	AMTSG1	–	–	–	+	+	+	+
28.	AMTSG2	–	–	–	–	+	+	–
29.	AMTSG3	–	–	–	–	+	+	–
30.	AMTSG4	+	+	–	+	+	–	+
31.	AMTSG5	+	+	–	+	+	–	+
32.	AMTSG6	+	+	–	–	–	–	–
33.	AMTSG7	+	–	+	–	–	–	–
34.	NDLSG1	+	+	–	+	+	–	+
35.	NDLBG3	–	–	–	–	+	–	–
36.	NDLCP2	+	–	–	–	+	+	–
37.	NDLSG2	+	–	–	–	+	+	–
38.	NDLCP3	+	–	–	–	+	–	–
39.	NDLCP1	+	+	–	–	+	+	–
40.	NDLBG2	+	–	–	–	+	+	–

Table 5: Characterization of MHB for Plant growth promoting traits.

Sr. No.	Isolate code	IAA	P Solubilization Index	K Solubilization Index	Ammonia production
1.	AMTBG1	–	1.39	–	+
2.	AMTBG2	+	1.09	–	–
3.	AMTBG3	–	–	–	–
4.	AMTBG4	–	1.05	–	+
5.	AMTBG5	+	1.35	–	+
6.	AMTRG1	+	–	–	+
7.	AMTRG2	+	1.36	–	+
8.	AMTRG3	–	–	–	+
9.	AMTRG4	+	2.06	1.39	+
10.	AMTRG5	+	2.13	–	+
11.	AMTRG6	–	–	–	+
12.	AMTMZ1	–	2.16	1.02	+
13.	AMTMZ2	+	1.28	–	+
14.	BPTHG1	–	1.29	–	+
15.	BPTHG2	–	2.23	1.25	+
16.	BPTBG1	+	2.4	–	+
17.	BPTBG2	–	1.09	–	+
18.	AMTBG6	+	2.34	–	+
19.	AMTBG7	–	1.34	–	+
20.	AMTBG8	+	2.39	–	+
21.	AMTBG9	–	1.32	–	+
22.	AMTBG10	+	2.65	–	+
23.	AMTBG11	+	1.06	–	+
24.	AMTBG12	–	2.92	–	+
25.	AMTBG13	+	–	–	+

26.	AMTBG14	+	–	–	+
27.	AMTSG1	–	1.23	–	+
28.	AMTSG2	–	–	–	+
29.	AMTSG3	+	1.26	–	+
30.	AMTSG4	+	1.49	–	+
31.	AMTSG5	–	2.39	1.00	+
32.	AMTSG6	+	2.66	–	+
33.	AMTSG7	+	1.98	–	+
34.	NDLSG1	+	–	–	+
35.	NDLBG3	–	1.02	–	+
36.	NDLCP2	+	4.00	1.45	+
37.	NDLSG2	+	1.34	–	+
38.	NDLCP3	+	3.66	2.7	+
39.	NDLCP1	–	–	2.69	+
40.	NDLBG2	–	–	–	+

CONCLUSION

The results of the present investigation revealed various morphological, biochemical and PGPR characters of MHB isolates. Based on these characters it can be concluded that certain MHB isolates which possess positive characters can be better utilized for enhancing the AM fungal activity and plant growth promotion. And further the two MHB isolates NDLCP2 and NDLCP3 were very efficient and which can be utilized for enhancing the AM fungal activity and crop growth by conducting further pot and field experiments.

FUTURE SCOPE

Although there have been a substantial number of studies of interactions between AM fungi and Bacteria, the underlying mechanisms of these associations are not very well understood, and the proposed mechanisms still need further experimental confirmation. More insight into these mechanisms will enable optimization of the effective use of AM fungi in combination with their bacterial partners as a tool for increasing crop yields. In order to better study bacteria–AM fungal interactions in soil it is beneficial to have means to specifically identify the active bacterial populations in the complex soil community, because these have the potential to exert the greatest effect on their immediate environment.

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