RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF NITROGEN FIXING *PAENIBACILLUS* SPP ISOLATED FROM DIFFERENT RHIZOSPHERIC SOIL SAMPLES COLLECTED FROM DIFFERENT PLACES OF ANDHRA PRADESH

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Abstract: The isolation for nitrogen fixing *Paenibacillus* spp remains poorly explored. In this study, the endospore-forming *Paenibacillus* strains were isolated from rhizospheric soil samples of sorghum collected from different places of Andha Pradesh. A total of twenty eight nitrogen fixing *Paenibacillus* strains were isolated based on heat treatment at 70 ^{0}C for 10 minutes and growth on nitrogen free media. Two reference strains and all the twenty eight isolates took 3-4 days to show small to medium, circular, milky white colonies with entire margin. Morphology of two reference *Paenibacillus* strains and all the twenty eight isolates were found to be gram positive, endospore forming, rod shaped and without any pigmentation. The 28 *Paenibacillus* isolates and two reference strains were tested for different biochemical tests. Results revealed that 28 *Paenibacillus* isolates showed similar results to that of the reference stains. Therefore the 28 isolates were confirmed as *Paenibacillus* isolates.

Keywords: Rhizosphere, isolation, Characterization, Endospore, *Paenibacillus* spp.

INTRODUCTION

The genus *Paenibacillus* was proposed by (Ash *et al.*, 1994) and belongs to the phylum Firmicutes, class Bacilli, order Bacillales and family 'Paenibacillaceae'. The name reflects this fact, in Latin paene means almost and therefore the *Paenibacillus* is almost a *Bacillus*. They claimed that strains of *Paenibacillus* have dissimilarity in the consensus region of 16s rRNA as compared to strains of *Bacillus*. This genus houses certain endospore-forming bacteria that differ from species of *Bacillus* (Vos et al., 2009) in some of the following ways: being motile via peritrichous flagella, Gram-positive, Gram negative, Gram-variable and non-pigmented on nutrient agar (Grady et al., 2016).

At that time, the genus *Paenibacillus* encompassed 11 species, including 3 nitrogen-fixing species *Paenibacillus polymyxa* (Grau and Wilson, 1962), *Paenibacillus macerans* (Witz *et al.*, 1967) and *Paenibacillus azotofixans* (Seldin *et al*., 1983). The genus *Paenibacillus* currently comprises more than 150 named species, approximately 20 of which have nitrogen fixation ability, including the following 8 novel species described i.e., *: Peanibacillus sabinae* (Ma *et al*., 2007), *Peanibacillus zanthoxyli* , *Peanibacillus forsythia* (Ma and Chen, 2008), *Peanibacillus sonch*, *Peanibacillus sophorae*, *Peanibacillus jilunlii*, *Peanibacillus taohuashanense* and *Peanibacillus beijingensis*. The first report on nitrogen fixation existed in *Peanibacillus stelifer* and *Peanibacillus jasmali* and first report of novel *Nif*H gene in *Peanibacillus stelifer* (Jin *et al.*, 2011).

To our interest, species of *Paenibacillus* are similar to Bacillus in their action as plant-growth-promoting rhizobacteria (PGPR), but the nitrogen-fixing ability shown by some *Paenibacillus* strains provides them superiority. The genus *Paenibacillus* was the most prominent group in both the rhizosphere (77.8 %) and soil (79 %). The *Paenibacillus* spp inhabits different niches such as soils, roots, and rhizosphere of various crop plants including wheat, maize, sorghum, sugarcane and barley, and forest trees such as lodgepolepine, douglas fir, and marine sediments *etc.*, (Weid *et al.*, 2000). N_2-fixing *Paenibacillus* species have increasingly been used in non legume crop species such as sugar beet, canola, wheat, and conifer species (Bent *et al.*, 2001).

Species of the genus *Paenibacillus*, are promising candidates for crop inoculation not only due to their nitrogen-fixing ability but also by their capacity of solubilization of soil phosphorus, uptake of micronutrients, increase soil porosity, suppress plant pathogens and promotion of plant growth through the production of phytohormones (auxins and cytokinins) and antimicrobial substances (Timmusk *et al*., 2009).

These plant growth promoting abilities of *Paenibacillus* can make them suitable for their application in sustainable agriculture. Keeping this in view the present investigation is on the isolation of N_2 fixing *Paenibacillus* spp from the rhizospheric soil samples by heat treatment and their capability of growing on N-free media. Isolates were identified based on the biochemical characterization of the *Paenibacillus* spp.

MATERIALS AMD METHODS

Soil Sample Collection

Rhizospheric soil samples were collected from different places of Andha Pradesh where kharif

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sorghum is cultivated i.e.; from Anantapur, Kadapa, Kurnool and Prakasam districts. A total of twenty seven soil samples were collected and separately bagged, air dried and stored in a refrigerator for further studies.

Isolation of *Paenibacillus* spp. from Soil Samples

Isolation of different *Paenibacillus* isolates was done by method based on selective germination. A soil sample was heat-treated at 70 ^{0}C for 10 min to destroy vegetative cells, inoculated in nutrient broth for a short period (e.g., 4 h) to allow spores to germinate and then heat-treated for second time before plating onto a suitable media to avoid most common *Bacillus* spp. After cooling the dilutions were prepared using soil suspension (from 10^4 to 10^7) and 0.1 ml of suspension was spread on nitrogen-free medium (Glucose: 50 g l^{-1}, K_2HPO_4: 0.2 g l^{-1}, K_2SO_4: 0.1 g l^{-1}, NaCl: 0.2 g l^{-1}, $CaCO_3$: 5.0 g l^{-1}, Agar: 25 g l^{-1}, pH 6.8, prepared in distilled water) and the plates were incubated at 27 ^{0}C for 72 h. Pure cultures were obtained by the streak plate method. These pure cultures of different bacterial isolates were preserved and used for further analysis. (Priest, 2015)

Identification of Bacterial Isolates

Morphological Characterization

All the isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

Colony Morphology

The morphological characteristics of the colony of each isolate were examined on Tryptic Soya agar and selective N-free medium by incubating for specific period. Cultural characterization of isolates such as shape, size, elevation, surface, margin, colour, odour and pigmentation of the colony were recorded.

Microscopy

Gram's Staining

A drop of sterile distilled water was placed in the centre of glass slide. A loopful of inoculum from young culture was taken, mixed with sterile distilled water and placed in the centre of the slide. The suspension was spread on slide using the tip of inoculation loop to make a thin smear. The smear was air dried and fixed though mild heating by passing the slide 3 to 4 times over the flame. The smear was then flooded with crystal violet solution for 1 min and washed gently with flow of tap water. Then the slide was flooded with iodine solution. After incubation at room temperature for 1 min, iodine solution was drained out followed by washing with 95 % ethanol. After that, it was washed with water within 15 to 30 s and blot carefully. The smear was incubated with safranin solution for 1 min. The slide was washed gently in flow of tap water and dried in air. The slide was examined under microscope at 100 X power with oil immersion and data were recorded.

Endospore Staining

Endospore staining was conducted on the isolate for a 4 day old culture. A drop of water was added to a slide, a small amount of the isolate was added with needle or inoculation loop to the water to emulsify it and then it was heat fixed to the slide by passing it over a flame. After heat fixing, the slide was placed over a beaker over boiling water and a piece of bibulous paper was place over the slide. Malachite green stain was then added on top of the bibulous paper, keeping it saturated, for a total of five min. After five min, the slide was rinsed with water and counterstained with safranin for one min, then rinsed again with water (Leboffe and Pierce 2010) and air dried. The slide was examined under microscope at 100 X power with the oil immersion and results were recorded.

Biochemical and Physiological Characterization

Different biochemical tests were performed as per the procedure described by Aneja (2001) and Cappuccino and Sherman (1992). The protocols followed are briefly outlined below.

Starch Hydrolysis

The sterile starch agar plates were streaked with overnight broth cultures of the isolates and incubated at 28 ^{0}C ± 2 ^{0}C for 24-48 h. After incubation, the plates were flooded with iodine solution and drain after a minute. The formation of a transparent zone around the colony was taken as positive reaction for the test.

Indole Production

The sterilized Hydrogen Sulfide- Indole-Motility (SIM) agar slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ^{0}C ± 2 ^{0}C. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing production of red colour were recorded as positive for indole production.

Methyl Red Test

Sterilized glucose-phosphate broth tubes were inoculated with overnight culture and incubated at 28 ± 2 ^{0}C for 48 h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production were taken as negative.

Voges Proskauer's Test

To the presterilized glucose-phosphate broth tubes, over night cultures were inoculated and incubated at 37 ^{0}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.

Citrate Utilization

Culture isolates were streaked on Simmon's citrate agar slants and incubated at 28±2 ^{0}C for 24 h. Streaked slants changes in colour from green to blue indicates the positive reaction for citrate utilization.

Denitrification Test

Sterilized nitrate broth tubes were inoculated with the bacterial cultures and incubated at 28 ^{0}C for 24 h. A

drop each of solution A and solution B were added to the bacterial growth in test tubes. The change in the colour of the medium to red indicated positive test.

RESULTS AND DISCUSSION

Collection of Soil Samples from Different Rhizospheric Soils of Sorghum:

Rhizospheric soil samples were collected from different places of Andhra Pradesh i.e. from Kurnool, Prakasam, Anantapur and Guntur districts where sorghum is grown and geographical indications of the sampling sites were recorded (Table 1).

Isolation of Nitrogen Fixing *Paenibacillus* spp. from Soil Samples:

From the rhizosperic soil samples used to isolate *Paenibacillus* strains on nitrogen-free medium, a total twenty eight isolates were obtained and coding of isolates were done (Table 1). Nitrogen fixing *Paenibacillus* population ranges between 1.1 - 4.1 × 10^6 CFU g^{-1} soil. Maximum nitrogen fixing *Paenibacillus* population was recorded in the rhizospheric soil samples of Tiparajupalle, Kadapa dist (4.1 × 10^6 CFU g^{-1} soil) and minimum nitrogen fixing *paenibacillus* population was recorded in the rhizosphere soils of Yenugumari, Kurnool dist (1.1 × 10^6 CFU g^{-1} soil).

Similar results were observed by Beneduzi *et al.* (2010). They isolated a bacterial strain designated $SBR5^T$ from the rhizosphere of *Triticum aestivum* based on their growth on nitrogen free medium and their resistance to 100 ^{0}C for 10 min. Phylogenetic analysis based on 16S rDNA gene sequence showed that the strain should be considered as nitrogen fixing type strain of genus *Paenibacillus* belong to the species *Paenibcillus riograndensis*.

Cultural and Morphological Characterization of *Paenibacillus* Isolates:

All *Paenibacillus* isolates and reference strains of *Paenibacillus* were studied for colony morphology, cell morphology, gram reaction and endospore formation. (Table 2) The cultural and morphological characteristics of *Paenibacillus* isolates on Tryptic soya agar was presented in Plate 4. Two reference strains took 3-4 days to show small to medium, circular, milky white colonies with entire margin. Reference *Paenibacillus* strains were found to be gram positive, endospore forming, rod shaped and without any pigmentation.

All the twenty eight isolates took 3-4 days to show small to medium and milky white colonies with entire margin. Among 28 isolates 57% of isolates shows glossy, 21% shows smooth and remaining 21% shows translucent surface type. Morphology of all the twenty eight *Paenibacillus* isolates were found to be gram positive, endospore forming, rod shaped and without any pigmentation (table 4.5). Among 28 isolates 21 isolates shows circular shape (ALP-1, KNP-1, KNP-2, ARP-1, AMP-1, KUP-1, PPP-1, AVP-1, PNP-2, PMP-2, CYP-1, ACP-1, KDP-1, ARP-2, PPiP-1, PNP-3, KNP-3, CVpP-1, APP-1, KYP-1 and CVmP-1) and 5 isolates shows irregular shape (PRP-1, CVeP-1, PPiP-2, PKP-3 and CTP-1) and other 2 isolates shows filamentous shape (PMP-1 and KKP-1). The results supports the finding of Vos *et al.* (2009).

Similar results were observed with Carlson *et al.* (2017). They isolated and characterized *Paenibacillus* spp. from GSMNP at Kephart Prong. The colonies on the TSA plates were round with a white, opaque appearance. When isolated colonies were achieved, the size of the colonies were roughly 2-4 mm in diameter. The isolate was Gram-positive, rod-shaped and formed endospores.

Biochemical and Physiological Characterization of *Paenibacillus* Isolates:

The 28 *Paenibacillus* isolates and two reference strains were tested for different biochemical tests. (Table 3) Biochemical and physiological characterization of reference strains showed that two reference *Paenibacillus* strains were negative for indole production, vogues Proskauer's test, gas production, methyl red production and both showed positive for starch hydrolysis and acid production. For citrate utilization *Paenibacillus* spp 1 showed negative and *Paenibacillus* spp 2 showed positive result. For nitrate reduction *Paenibacillus* spp 1 showed positive and *Paenibacillus* spp 2 showed negative result.

Results presented in table 4.6 reveal that for indole production only 3 isolates (ARP-1, AVP-1 and KNP-3) showed positive results and remaining 25 isolates showed negative result. For citrate utilization 6 isolates (ALP-1, PRP-1, CYP-1, PPiP-2, PMP-1 and KYP-1) showed positive result remaining 22 isolates showed negative result. For methyl red test 6 isolates (KUP-1, PRP-1, CYP-1, PPiP-1, PKP-3 and APP-1) showed positive result and remaining 22 isolates showed negative result. For vogues Proskauer's test 5 isolates (ARP-1, AVP-1, ARP-2, PPiP-2 and CVpP-1) showed positive result and remaining 23 isolates showed negative result. For gas production only 1 isolate KUP-1 showed positive result and remaining 27 isolates showed negative result. For acid production all the 28 isolates showed positive result. For nitrate reduction 19 isolates (KNP-1, KNP-2, KUP-1, PRP-1, PPP-1, CVeP-1, AVP-1, KKP-1, PNP-2, CYP-1, ACP-1, KDP-1, PPiP-1, PPiP-2, PNP-3, KNP-3, CVpP-1, KYP-1 and CVmP-1) showed positive result and remaining 9 isolates showed negative result. For starch hydrolysis 21 isolates (ALP-1, KNP-2, KUP-1, PRP-1, PPP-1, CVeP-1, AVP-1, PNP-2, CYP-1, ACP-1, PMP-2, KDP-1, ARP-2, PPiP-2, PNP-3, KNP-3, PKP-3, CVpP-1, CTP-1, KYP-1 and CVmP-1) showed positive result and remaining 7 isolates showed negative result.

Similar results were observed by Khianngam *et al.* (2009). They isolated three xylanase-producing bacteria, $S5\text{-}3^T$, $X13\text{-}1^T$ and $MXC2\text{-}2^T$ from Thailand

soils. The strains were characterized based on their phenotypic, morphological, cultural, physiological and biochemical and chemotaxonomic characters. The study of 16S rRNA gene sequence revealed that they belongs to the genus *Paenibacillus*. They showed negative results for methyl red test, indole production, growth on Simmons' citrate, oxidase and nitrate reduction.

Table 1. Details of the Rhizospheric Soil Samples Collected from Sorghum Crop from Different Places of Andhra Pradesh

S.No	Latitude No	Longitude No	District	Mandal	Village	Soil Type	Rhizosperic/ Non Rhizospheric Soil	Coding of *Paenibacillus* isolates
1	15^0 46' 41.4' N	78^0 47' 71.6' E	Kurnool	Nandyal	Nandyal	Black	R	KNP-1, KNP-2, KNP-3
2	15^0 35' 37.3' N	77^0 73' 43.4' E	Kurnool	Dhone	Obulapuram	Red	R	
3	15^0 31' 37.8' N	77^0 79' 97.4' E	Kurnool	Dhone	Devarabanda	Red	R	KDP-1
4	15^0 33' 14.1' N	77^0 80' 82.0' E	Kurnool	Dhone	Rekulakunta	Red	R	-
5	15^0 36' 52.2' N	77^0 82' 88.2' E	Kurnool	Dhone	Ungaranigundla	Red	R	KUP-1
6	15^0 30' 26.6' N	77^0 79' 35.3' E	Kurnool	Peapully	Yenugumari	Black	R	KYP-1
7	15^0 28' 37.6' N	77^0 77' 69.7' E	Kurnool	Peapully	Kothakota	Sandy	R	KKP-1
8	15^0 14' 60.7' N	77^0 66' 53.3' E	Anantapur	Gooty	Vennedoddi	Red	R	AVP-1
9	14^0 53' 64.8' N	77^0 74' 25.2' E	Anantapur	Bathilapalli	Ragavampalli	Red	R	ARP-1, ARP-2
10	14^0 50' 17.0' N	77^0 74' 01.9' E	Anantapur	Bathilapalli	Putlamari	Black	R	APP-1
11	14^0 54' 86.0' N	77^0 74' 90.8' E	Anantapur	Bathilapalli	Lingareddypalli	Black	R	ALP-1
12	14^0 50' 74.6' N	77^0 81' 58.3' E	Anantapur	Tadimari	Madimokulpalli	Black	R	AMP-1
13	14^0 58' 19.0' N	77^0 89' 69.8' E	Anantapur	Tadimari	Chilakondaiahpalli	Black	R	ACP-1
14	14^0 47' 56.1' N	78^0 14' 74.8' E	Kadapa	Lingala	Lingala	Black	R	-
15	14^0 36' 77.7' N	78^0 27' 58.3' E	Kadapa	Vempula	Velpula	Red	R	CVpP-1
16	14^0 37' 02.8' N	78^0 33' 49.5' E	Kadapa	Vempula	Vemula	Black	R	CVmP-1
17	14^0 37' 17.2' N	78^0 44' 58.3' E	Kadapa	Vempalle	Vempalle	Black	R	CVeP-1
18	14^0 40' 51.1' N	78^0 55' 67.2' E	Kadapa	Pedlimari	Tiparajupalle	Black	R	CTP-1
19	14^0 44' 59.2' N	78^0 64' 48.6' E	Kadapa	Pedlimari	Ysr Nagar	Red	R	CYP-1
20	14^0 42' 14.2' N	78^0 57' 41.0' E	Kadapa	Pedlimari	Minnaiahgaripalli	Red	R	-
21	15^0 30' 47.3' N	79^0 88' 41.5' E	Prakasam	Kundukur	Pandalapadu	Red Sandy	R	PPP-1
22	15^0 30' 76.7' N	79^0 86' 17.6' E	Prakasam	Jarugumalli	Ramachandrapuram	Black	R	PRP-1
23	15^0 31' 54.3' N	79^0 85' 47.1' E	Prakasam	Jarugumalli	Paidupadu	Black	R	PPiP-1, PPiP-2
24	15^0 31' 70.9' N	79^0 88' 58.8' E	Prakasam	Jarugumalli	Narsingolu	Black	R	PNP-2, PNP-3
25	15^0 40' 14.8' N	79^0 85' 58.0' E	Prakasam	Kondepi	Kattavaripalem	Black	R	PKP-3
26	15^0 45' 93.5' N	79^0 86' 23.8' E	Prakasam	Kondepi	Anakarlapudi	Black	R	-
27	15^0 45' 93.5' N	79^0 87' 3.1' E	Prakasam	Nutalapadu	Madduluru	Black	R	PMP-1,PMP-2

Table 2. Morphological Characteristics of *Paenibacillus* Isolates on TSA Agar from Different Rhizospheric Soils of Sorghum

S. No.	Isolate name	Colony Morphology on TSA agar							Cell Morphology		
		Size	Shape	Colour	Elevation	Surface	Margin	Pigmentation	Gram reaction	Shape	Endospore
1	ALP-1	Medium	Circular	Milky white	Convex	Smooth	Entire	Negative	Gram +ve	Rod	Positive
2	KNP-1	Small	Circular	Milky white	Wrinkled hallow	Smooth	Entire	Negative	Gram +ve	Rod	Positive
3	KNP-2	Small	Circular	Milky white	Convex	Glossy	Entire	Negative	Gram +ve	Rod	Positive
4	ARP-1	Medium	Circular	Milky white	Flat	Glossy	Entire	Negative	Gram +ve	Rod	Positive
5	AMP-1	Medium	Circular	Milky white	Wrinkled hallow	Glossy	Entire	Negative	Gram +ve	Rod	Positive
6	KUP-1	Small	Circular	Milky white	Convex	Glossy	Entire	Negative	Gram +ve	Rod	Positive
7	PRP-1	Medium	irregular	Milky white	Raised	Translucent	Entire	Negative	Gram +ve	Rod	Positive
8	PPP-1	Medium	Circular	Milky white	Flat	Glossy	Entire	Negative	Gram +ve	Rod	Positive
9	CVeP-1	Small	Irregular	Milky white	Wrinkled hallow	Glossy	Entire	Negative	Gram +ve	Rod	Positive
10	AVP-1	Small	Circular	Milky white	Raised	Glossy	Entire	Negative	Gram +ve	Rod	Positive
11	KKP-1	Medium	Filamentous	Milky white	Convex	Translucent	Entire	Negative	Gram +ve	Rod	Positive
12	PNP-2	Small	Circular	Milky white	Raised	Glossy	Entire	Negative	Gram +ve	Rod	Positive
13	PMP-2	Medium	Circular	Milky white	Convex	Glossy	Entire	Negative	Gram +ve	Rod	Positive
14	CYP-1	Small	Circular	Milky white	Wrinkled hallow	Glossy	Entire	Negative	Gram +ve	Rod	Positive
15	ACP-1	Medium	Circular	Milky white	Convex	Smooth	Entire	Negative	Gram +ve	Rod	Positive
16	KDP-1	Small	Circular	Milky white	Flat	Translucent	Entire	Negative	Gram +ve	Rod	Positive
17	ARP-2	Small	Circular	Milky white	Flat	Glossy	Entire	Negative	Gram +ve	Rod	Positive
18	PPiP-1	Medium	Circular	Milky white	Raised	Translucent	Entire	Negative	Gram +ve	Rod	Positive
19	PPiP-2	Medium	irregular	Milky white	Convex	Glossy	Entire	Negative	Gram +ve	Rod	Positive
20	PNP-3	Medium	Circular	Milky white	Convex	Glossy	Entire	Negative	Gram +ve	Rod	Positive
21	KNP-3	Small	Circular	Milky white	Flat	Translucent	Entire	Negative	Gram +ve	Rod	Positive
22	PKP-3	Small	Irregular	Milky white	Convex	Smooth	Entire	Negative	Gram +ve	Rod	Positive
23	CVpP-1	Medium	Circular	Milky white	Raised	Glossy	Entire	Negative	Gram -ve	Rod	Positive
24	PMP-1	Medium	Filamentous	Milky white	Convex	Smooth	Entire	Negative	Gram +ve	Rod	Positive
25	APP-1	Small	Circular	Milky white	Flat	Translucent	Entire	Negative	Gram +ve	Rod	Positive
26	CTP-1	Small	Irregular	Milky white	Raised	Smooth	Entire	Negative	Gram +ve	Rod	Positive
27	KYP-1	Small	Circular	Milky white	Convex	Glossy	Entire	Negative	Gram +ve	Rod	Positive
28	CVmP-1	Medium	Circular	Milky white	Convex	Glossy	Entire	Negative	Gram +ve	Rod	Positive
29	Paenibacillus spp	Medium	Circular	Milky white	Convex	Smooth	Entire	Negative	Gram +ve	Rod	Positive
30	Paenibacillus spp	Medium	Circular	Milky white	Raised	Translucent	Entire	Negative	Gram +ve	Rod	Positive

Table 3. Biochemical Characterization of *Paenibacillus* Isolates Collected from Different Rhizospheric Soils of Sorghum

S.no	Isolate name	Indole production	Citrate utilization	Methyl red test	Voges - Proskauer's test	gas production	Acid production	Nitrate reduction	Starch hydrolysis
1	ALP-1	-	+	-	-	-	+	-	+
2	KNP-1	-	-	-	-	-	+	+	-
3	KNP-2	-	-	-	-	-	+	+	+
4	ARP-1	+	-	-	+	-	+	-	-
5	AMP-1	-	-	-	-	-	+	-	-
6	KUP-1	-	-	+	-	+	+	+	+
7	PRP-1	-	W(+)	+	-	-	+	+	+

8	PPP-1	-	-	-	-	-	+	+	+
9	CVeP-1	-	-	-	ND	-	+	+	+
10	AVP-1	+	-	-	+	-	+	+	+
11	KKP-1	-	-	ND	-	-	+	+	-
12	PNP-2	-	-	-		-	+	+	+
13	PMP-2	-	-	-	-	-	+	-	+
14	CYP-1	-	+	+	-	-	+	+	+
15	ACP-1	-	-	-	-	-	+	+	+
16	KDP-1	-	-	-	-	-	+	+	+
17	ARP-2	-	-	-	+	-	+	-	+
18	PPiP-1	-	-	+	ND	-	+	+	-
19	PPiP-2	-	W(+)	-	+	-	+	+	+
20	PNP-3	-	-	-	-	-	+	+	+
21	KNP-3	+	-	-	-	-	+	+	+
22	PKP-3	-	-	+	-	-	+	-	+
23	CVpP-1	-	-	-	+	-	+	+	+
24	PMP-1	-	+	-	-	-	+	-	-
25	APP-1	-	-	+	-	-	+	-	-
26	CTP-1	-	-	-	-	-	+	-	+
27	KYP-1	+	+	-	-	-	+	+	+
28	CVmP-1	-	-	-	-	-	+	+	+
29	Reference strain 1	-	-	-	-	-	+	+	+
30	Reference strain 2	-	+	-	-	-	+	-	+

CONCLUSION

A total of twenty eight *Paenibacillus* strains were isolated from different rhizospheric soil samples based on heat treatment at 70 ^{0}C for 10 minutes and growth on nitrogen free media. All the twenty eight isolates took 3-4 days to show small to medium, circular, milky white colonies with entire margin. Morphology of isolates were found to be gram positive, endospore forming, rod shaped and without any pigmentation. Biochemical tests of *Paenibacillus* isolates showed similar results to that of the reference stains. Therefore the 28 isolates were confirmed as *Paenibacillus* isolates.

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