

Isolation, Screening and Characterization of PGPR from Rhizosphere of Rice

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ABSTRACT

In the present study 6 strains of pseudomonas, 4 strains of Azotobacter and 2 strain of Bacillus were isolated from Rice rhizospheric soil samples from different villages of Bardoli, Gujarat, India. Primary screenings were carried for their plant growth promoting activities such as IAA production, Phosphate solubilization, siderophore production, N₂ fixation and protease production. The consolidated positive results of PGPR activities were revealed most potential 15 isolates for other PGPR activities such as cellulase production, chitinase production, HCN production and antifungal activity. Overall investigation of the rhizospheric diversity of the rice field of Bardoli region revealed many potential PGPR candidates with significant results which can be utilized as potential biofertilizer for improving soil health and rejuvenization. Looking to overall PGPR activities together, isolate, M2 was found most efficient for all the activities under study.

Key words: Rhizobacteria, PGPR, Biocontrol, Plant growth promoting activities, Biofertilizers

INTRODUCTION

Rice is a dietary staple food for 2.5 billion people, making it the most consumed cereal grain, belonging to the *Graminaceae* family. India globally stands first in rice area and second in rice production, after China. To improve the growth and production of rice Chemical fertilizer and biofertilizer can be used. Chemical fertilizers is cheap but have many limitations such as leaching, pollution of water resources, upset beneficial microbial ecosystems and even contribute to the release

of greenhouse gases. To reduce input of chemical fertilizers yet sustain productivity and maintain the agro ecosystem, bio-fertilizers have become an ideal substitute for fertilization and conditioning of soil. Rhizobacteria aggressively colonize roots of plants, able to multiply and colonize on the roots at all stages of plant growth, survive in the presence of a competing microflora. The rhizobacteria in the rhizosphere can be neutral, detrimental or beneficial for plant growth.

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PGPR promote plant growth by several mechanisms which include induce systematic resistance, produce plant growth regulators like indole acetic acid, gibberellic acid, cytokinins and ethylene, asymbiotic Nitrogen fixation, antagonism against phytopathogenic microorganisms by production of siderophores, antibiotics and cyanide, fungal cell wall lysing enzymes which suppress the growth of fungal pathogens, solubilization of mineral phosphates and other nutrients.

MATERIALS AND METHODS

1. Rhizospheric soil sample collection

Rhizospheric soil samples were collected from rice plants of different village of Bardoli, Gujarat, India. Rhizospheric soil samples of different cultivars of rice like Jaya, Mashuri, Gujarat 3, Gujri, Cholum and Rice 171 were undertaken for study. Soil samples were collected at a depth of 5-10 cm. 17 samples were collected from different sites. Samples were collected in aseptic bags and immediately transported to lab under cold condition (4°C) for further process.

2. Isolation and purification of bacterial culture from the rhizospheric soil

For rhizospheric PGPR, prepare soil suspension, dilutions were made up to 10⁻⁸ grades. On pre-prepared nutrient agar plates, 0.1ml of each dilution was spreaded. After 24h of incubation at room temperature, many colonies were randomly selected on the basis of colony morphology and further purified by streaking on nutrient agar plate¹. The most prominent colonies were isolated and maintained on nutrient agar slant at 4°C for further studies.

3. Growth promoting activities by the isolates

3.1 Determination of Indole Acetic Acid

The production of indole acetic acid (IAA) was assayed by using Salkowski method². Production of pink colour indicate presence of IAA in the medium.

3.2 Phosphate solubilization

The culture of isolates were streaked on the plates of Pikovaskya's medium and incubated in an incubator at 30°C for 7 days. The plates were then examined for formation of halo zone around the colony³.

3.3 Nitrogen fixation

Screening of nitrogen fixing organisms was carried out by using semisolid malate medium. Growth of isolates indicates nitrogen fixation.

3.4 Protease production

The cultures were spot inoculated on skim milk agar plate and incubated for 2 days at 28°C. Proteolytic activities were identified by clear zone formation around the colony.

3.5 Cellulase production

Isolates were screened for cellulase activity by inoculating on Carboxymethyl cellulose CMC agar⁴. Plates were prepared and spot inoculated with test organism and incubated at 30°C for 5 days.

4. Biocontrol activities of isolates

4.1 HCN production

Hydrogen cyanide production was assayed by the method suggested by Lorck and Castric^{5,6}. For the production of HCN, isolates were streaked on King's B agar plates⁷.

4.2 Siderophore production

Siderophore production was tested qualitatively using Chrome Azurole S (CAS) agar⁸.

4.3 Antifungal activity

In-vitro antagonistic ability of bacterial isolates against *Fusarium moniliforme* was studied by dual culture technique⁹.

RESULTS AND DISCUSSION

In this study, plant growth promoting rhizobacteria were isolated from rhizospheric soil of rice plants grown in different villages of Bardoli, Gujarat, India. Bacterial isolates were examined for their ability to Plant Growth Promoting Rhizobacteria. In the present study 6 strains of *pseudomonas*, 4 strains of *Azotobacter* and 2 strain of *Bacillus* were isolated from Rice rhizosphere.

1. Isolation of rhizobacteria

On the basis of differences in colony morphology, total 98 isolates from different sampling sites were obtained as shown in Table 1.

2. Plant growth promoting traits of selected isolates

2.1 IAA production

IAA is very crucial phytohormone responsible for the division, expansion and differentiation of plant cells and tissues and also stimulates root elongation. As shown in Fig. 1, isolates H7, M2 and P5 were found to produce more than 45 mg/L IAA than other. While isolates B1, F3, J5 and K3 were found to produce less than 25 mg/L IAA. Remaining isolates were found to produce IAA in the range of 25-45 mg/L.

2.2 Phosphate solubilization

Phosphorus is a primary essential nutrient element for rice production. To enhance phosphorus uptake efficiency, PSB play an important role in supplying phosphate to plants, which is environment friendly and sustainable approach. As shown in Fig. 2, isolates D4, M2 and P5 were found to solubilize more than 10 mg/L phosphate. While isolates H7, I4, J5 and P4 were found to solubilize less than 8 mg/L phosphate and H7, I4 and I5 were also efficient for IAA production.

2.3 Protease production

As shown in Fig. 3, isolates I4, I5, M2 and P4 were found to produce more than 12 IU of Protease and I4 and M2 were also efficient for IAA production, phosphate solubilization and siderophore production. While only 1 isolate J5 was found to produce less than 8 IU of Protease which is cell wall lysing enzyme and is important to inhibit fungal pathogen.

2.4 Cellulase production

Cellulase is very crucial fungal cell wall lysing enzyme and concentration of microbes producing cellulose is very low. As shown in Fig. 4, only 2 isolates M2 and N4 out of 15 selected isolates were found to produce cellulase.

2.5 Chitinase Production

The mycolytic activity of fungal as well as bacterial antagonists is mainly due to the lytic enzymes like chitinase. As shown in Fig.5, only 3 isolates J1, M2 and N4 were found to produce chitinase. Isolate J1 was found to produce 0.32 IU of chitinase and as shown all the other graphs M2 was more efficient for all activities

3. Biocontrol traits of isolates

3.1 Siderophore production

Siderophore is very essential as it act as iron chelator and also plays an essential role in determining the competitive fitness of bacteria to colonize plant roots and to compete for iron with other microorganisms in the rhizosphere. As shown in Fig. 6, isolates D4, F3, H7, I4, I5 and M2 were found to produce more than 50% Siderophore Unit. Isolates H7, I4, I5 and M2 were also efficient for IAA production and phosphate solubilization. Only 1 isolate N4 was found to produce less than 30% Siderophore unit.

3.2 HCN production

HCN is produced by many rhizobacteria and is postulated to play a role in biological control of pathogens. As shown in Table 2 , all the other isolates were positive to produce HCN except isolates J1 and F3.

3.3 Antifungal activity

Antifungal activity of isolates was tested against *Fusarium moniliforme*. No zone of inhibition was observed by the selected isolates¹⁰.

4. Characterization of isolates

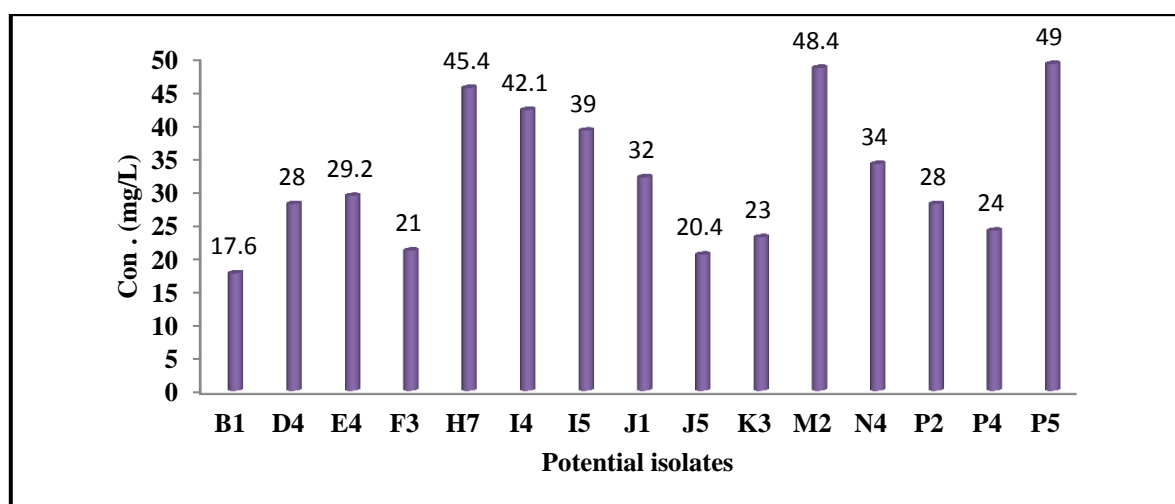
Only 4 isolates from 15 selected isolates were gram positive while remaining 10 were gram negative. Out of 15 selected isolates 10 isolates were found to produce green pigmentation which is a typical character of *Pseudomonas* sp. and 6 isolates were found to grow on cetrinide agar plate which is also a typical character of *Pseudomonas* spp. According to Bergey's manual of systemic bacteriology, isolates K3 and N4 were tentatively identified as *Bacillus* spp., isolates D4, I4, I5, M2, P4 and P5 were tentatively identified as *Pseudomonas* spp., isolates H7, J1, J5 and P2 were tentatively identified as *Azotobacter* spp.

Table 1: Primary screening of rhizospheric diversity to its PGPR potential

Sample	Sampling Site	Cultivar	Isolate No.	PGPR Activities				
				IAA Production	Phosphate Solubilization	Siderophore Production	Nitrogen Fixation	Protease Production
A	Vihan	Jaya	6	4	1	3	2	3
B	Vihan	Gujri	5	2	3	3	5	3
C	Vihan	Gujarat 3	7	2	2	3	5	4
D	Sevni	Mashuri	5	2	2	3	5	3
E	Sevni	Cholum	6	3	3	4	5	6
F	Sevni	171	4	1	1	1	3	2
G	Sampura	Gujri	7	5	3	2	6	3
H	Sampura	Mashuri	7	6	2	4	2	2
I	Asta	Jaya	5	4	3	4	4	4
J	Asta	171	5	4	2	4	3	4
K	Asta	Gajarat 3	6	4	3	3	4	2
L	Dungar	Jaya	8	5	3	5	7	3
M	Dungar	Mashuri	7	7	4	7	6	1
N	Timba	Gujri	5	3	1	5	3	4
O	Timba	Cholum	6	3	2	4	3	1
P	Tarsadi	171	6	5	4	6	3	3
Q	Tarsadi	Cholum	3	3	2	3	3	0

Table 2: Primary screening of selected isolates for other PGPR activities

PGPR Activity	Potential isolates														
	B1	D4	E4	F3	H7	I4	I5	J1	J5	K3	M2	N4	P2	P4	P5
Cellulase Production	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Chitinase Production	-	-	-	-	-	-	-	+	-	-	+	-	-	-	+
HCN Production	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+
Antifungal Production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

**Fig. 1: Comparison of IAA production by potential isolates**

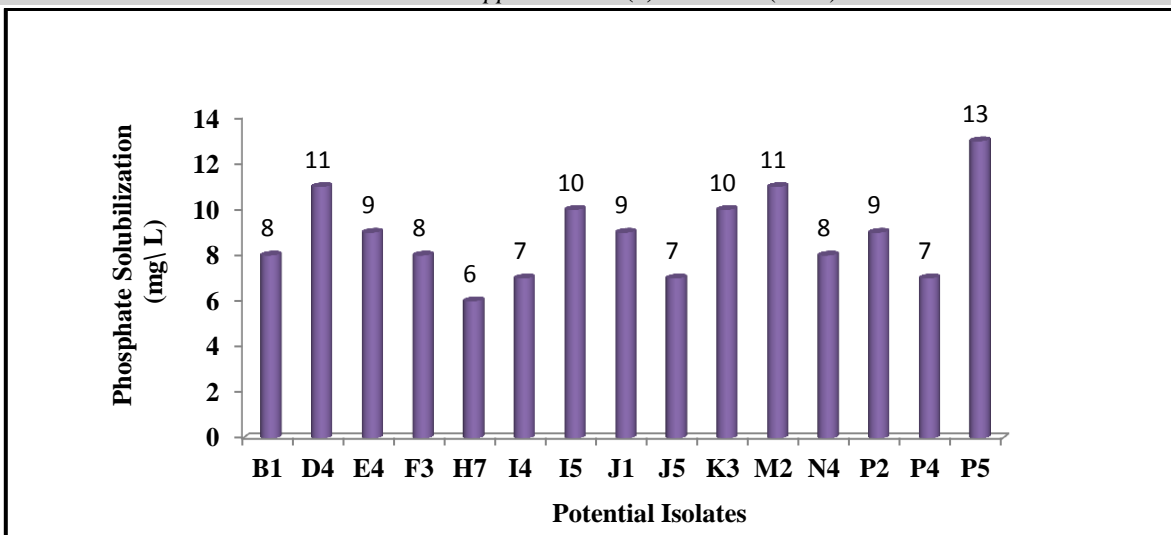


Fig. 2: Comparison of Phosphate Solubilization by Potential Isolates

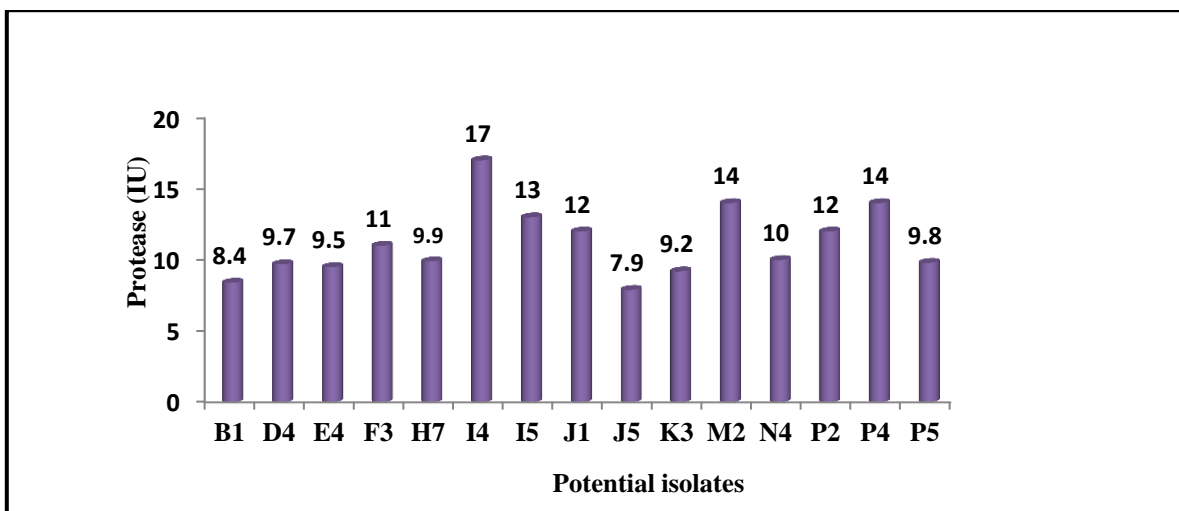


Fig. 3: Comparison of protease production by potential isolates

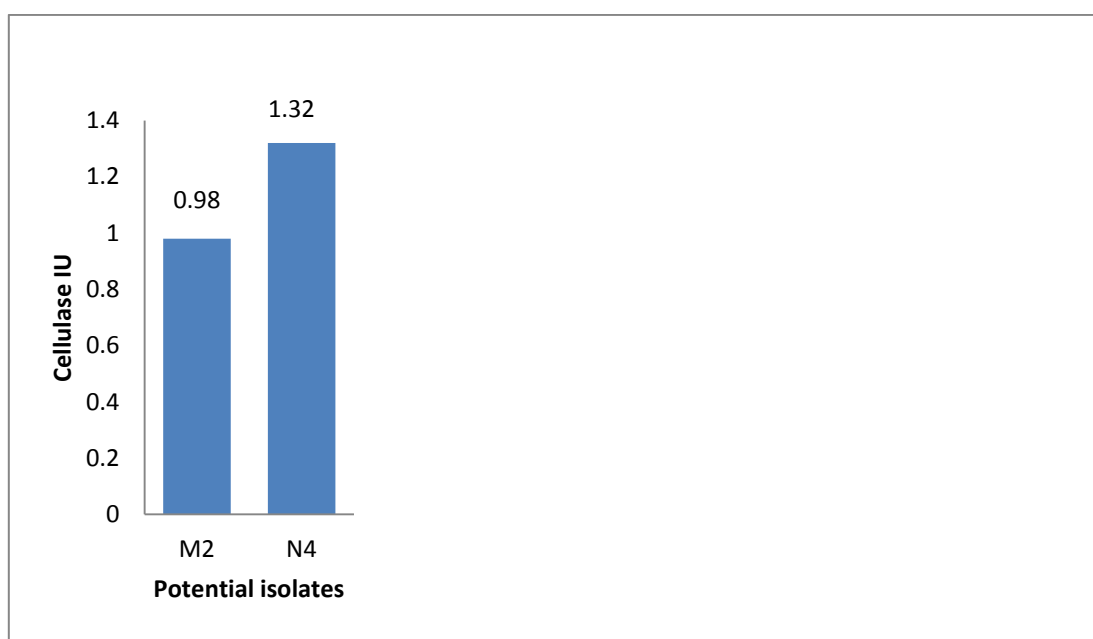


Fig. 4: Comparison of Cellulase Production by Potential Isolates

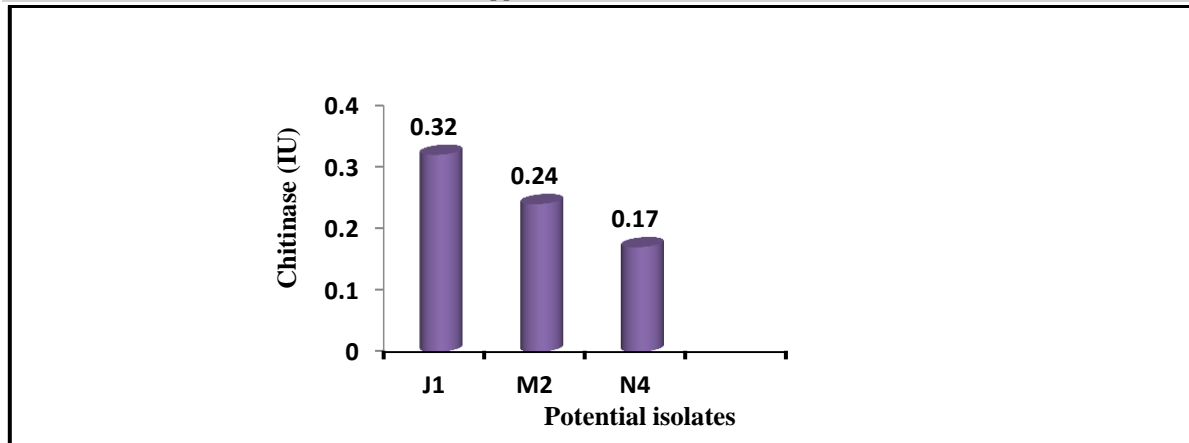


Fig. 5: Comparison of Chitinase Production by Potential Isolates

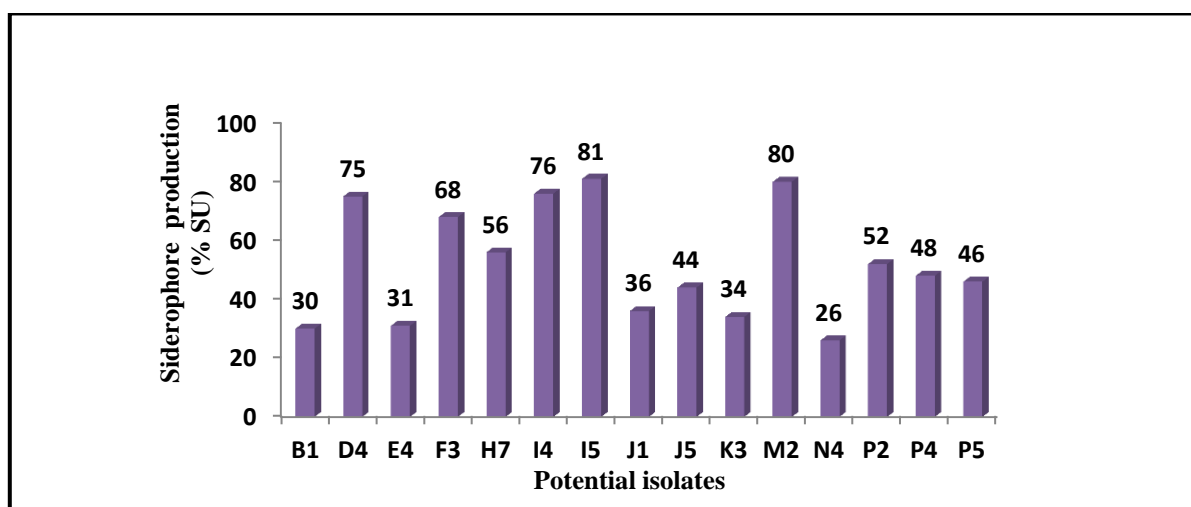


Fig. 6: Comparison of Siderophore Production by Potential Isolates

CONCLUSION

From rhizosperic soil samples of rice, bacteria were collected showed different plant growth promoting activities such as IAA production, phosphate solubilization, siderophore production, N₂ fixation, protease production, cellulase production, chitinase production, HCN production and antifungal activity. The consortia of these 15 isolates can be applied as effective biofertilizer that reduces the cost of chemical fertilizer as well improves the soil health and maintains soil fertility. The consortia further investigated for the compatibility as effective inoculum.

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