Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(2): 942-948
Received: 16-01-2020
Accepted: 18-02-2020

BH Joshi
Department of Food Quality Assurance, College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, Gujarat, India

RM Dhingani
Department of Food Quality Assurance, College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author:
BH Joshi
Department of Food Quality Assurance, College of Food Processing Technology and Bioenergy, Anand Agricultural University, Anand, Gujarat, India

Assessment and selection of multi-trait plant growth promoting bacteria associated with rice rhizosphere

BH Joshi and RM Dhingani

DOI: https://doi.org/10.22271/phyto.2020.v9.i2p.10978

Abstract
The application of plant growth promoting bacteria is the need of present day for sustainable agriculture. The present study was carried out to assess and select of multi-trait plant growth promoting bacteria associated with rice. Seventeen rhizospheric samples from different rice varieties were collected from different locations of South Gujarat. Total of 98 isolates obtained from different rhizospheric samples were screened out for PGPR activities. Primary screening revealed 15 isolates having potential PGPR activities. These isolates were further evaluated for their ability to produce IAA, phosphate solubilization, siderophore production, protease, cellulase and chitinase enzyme production quantitatively. The result showed that isolate M2 found to produce maximum of 48.4 mg/L IAA, 80 % siderophore and solubilizing 11 mg/L phosphate. Besides these growth promotion traits, M2 also found to produce 14 IU protease, 0.98 IU cellulase and 0.24 IU chitinase. Considering the multi-trait plant growth promotion abilities of M2 isolate it was further characterized and identified tentatively as *Pseudomonas* species.

Keywords: Multi-trait PGPR, rice, *Pseudomonas* species

Introduction
Cultivated rice (*Oryza sativa* L.) is the most important staple crop, and nitrogen is the main input required for rice production. In order to make rice cultivation sustainable, research has been focused on the isolation of mainly diazotrophic bacteria [6, 12, 27] but plant growth-promoting bacteria [27, 29] and antagonistic bacteria [19, 28] have also been reported. Most studies have explored the properties of these isolates in relation to their potential as agronomical inoculants. However, the dynamics of the bacterial community that inhabits cultivated rice has been poorly studied [14]. The competition between native endophytic bacteria and inoculated bacteria may reduce the activity of the inoculated microorganisms and consequently diminish the crop yield. Thus, the study of plant-associated bacteria is important for understanding their ecological role as well as for the application of plant growth-promoting microorganisms.
Cultivated rice (*Oryza sativa* L.) is the most important staple crop, making it the most consumed cereal grain world-wide. Rice is rich in genetic diversity, with thousands of varieties grown throughout the world. Rice is grown in wide spectrum of climates therefore; it is one of the most widely consumed foods of the world. Rice is the only cereal crop that can grow for long periods of time in standing water [1]. Many abiotic and biotic factors affect the yield and quality of rice, where, soil dynamics, plays crucial role and decide the need for various inputs in terms of fertilizers and pesticides. To reduce these chemical inputs with sustainable productivity is the focus area under investigation.
Rice seed priming with plant growth promoting bacteria (PGPB), has been emerged as an alternative supplementation to reduce the chemical inputs with considerable enhancement of seed germination, vigor index and germination speed. Bacteria able to colonize plant root systems and promote plant growth are referred to as Plant Growth Promoting Rhizobacteria (PGPR) [10]. PGPR can affect plant growth either indirectly or directly. Rice growth promotion can be achieved by beneficial bacteria by producing phytohormones, activation of phosphate solubilization and promotion of the mineral uptake [12]. Production of phytohormones is one of the important mechanisms for plant growth promotion. Several bacteria produces phytohormones such as Indole-3 acetic acid (IAA), which induce plant growth by stimulating protein synthesis, flowering and fruiting. Abundant amount of soil phosphorus is in the form of insoluble phosphate which cannot be assimilated by plants directly. PGPB solubilize this phosphate and make available for plant growth. Iron is an essential nutrient of plants, but it is

relatively insoluble in soil [24]. PGPB produces siderophores which stimulate the plant growth directly by increasing the availability of iron in the soil surrounding the roots [10] and also exhibit biocontrol activities against plant pathogens. Moreover, PGPB also shows antagonistic to fungal pathogens by producing different cell wall lysing enzymes like cellulase, chitinase and protease. Present studies were carried out to assess and select multi-trait PGPB for rice growth promotion.

Materials and Methods

Isolation and purification

Seventeen soil samples of rice rhizosphere were collected from different locations of South Gujarat region. These soils samples were collected from different rhizospheric region of 6 popular varieties of rice under cultivation. Soil samples were collected at a depth of 5-10 cm according to v-shaped method [30]. One gram of this rhizosphere soil was placed in 9 ml of sterilized distilled water under aseptic conditions. The soil suspension was diluted in 10 fold series and 0.1 ml of each dilution was spread on nutrient agar plate. After 24 h of incubation at 37 ºC, each distinct colony was selected and purified by streaking on nutrient agar plate and maintained on nutrient agar slant at 4 ºC for further studies [30].

Screening of PGP bacteria

All isolates obtained from the rhizospheric soil of popular varieties of rice grown under different agro climatic conditions were studied for screening of multiple PGP traits.

IAA production: Bacterial isolates were grown in malate medium supplemented with tryptophan (100 mg/L) as the precursor of IAA and compared to those grown without the addition of tryptophan for its qualitative assessment for IAA production [20]. Qualitative estimation of IAA production was studied using colorimetric methods. The amount of IAA produced was calculated using the standard curve prepared with known concentration of IAA [5].

Phosphate solubilization: Primary screening for phosphate solubilization was carried out on Pikovskaya's agar plate as described by Gaur [4]. Quantitative analysis of solubilization of tricalcium phosphate in liquid medium was carried out as described by King [9]. The potential isolates were inoculated in 25 ml Pikovskaya's broth and incubated for 48 h at 30˚C. The bacterial cultures were centrifuged at 15,000 rpm for 30 min. One milliliter supernatant was mixed with 10 ml of chloromoliblidic acid and volume was made upto 45 ml with distilled water. The absorbance of developing colour was read at 600 nm. The amount of phosphorous was detected from standard curve of KH_2PO_4.

Siderophore production: The screening for siderophore producing bacterial isolate were carried out by inoculated onto chrome azurol S (CAS) blue plates [25] with the modifications described previously [3]. The siderophore test was analyzed for the presence or absence of the orange-yellow halo surrounding the colonies, which indicated the presence or absence of a siderophore, respectively. Further quantitative estimation of siderophore was done by CAS-shuttle assay. In which cultures were inoculated (1% v/v) in sterile succinate medium separately and incubated on rotary shaker at 30 ˚C, 120 rpm. After 36 h of incubation, 0.5 ml of culture supernatant was mixed with 0.5 ml of CAS reagent, and absorbance was measured at 630 nm [17].

$$\% \text{ Siderophore Unit} = \frac{\text{Ar-As}}{\text{Ar}} \text{X } 100$$

Where, Ar = absorbance of reference at 630 nm and As = absorbance of sample at 630 nm

Biological nitrogen fixation: Screening of nitrogen fixing organisms was carried out by using semisolid malate medium (NFB) which include (malic acid, 5 g; KOH, 4 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2$, 0.02 g; NaCl, 0.1 g; $FeSO_4 \cdot 7H_2O$, 0.5 g; : $Na_2MoO_4 \cdot 2H_2O$, 2 mg; $MnSO_4 \cdot 7H_2O$, 10 mg; 0.5% alcoholic solution (or dissolved in 0.2 N KOH) of bromothymol blue, 2 ml; agar, 0.5 %; 1000 ml distilled water, pH 6.8) [2]. Growth of bacterial isolates in NFB medium indicates nitrogen fixation.

Protease: Primary screening for protease producing bacterial isolates were carried out using skim milk agar medium. Spot inoculation of each bacterial isolate was carried out. The plates were incubated for 48 h at 30 ˚C. The halo around colonies confirmed the protease production ability of bacterial isolates. Further these protease producers were evaluated for its quantitative production abilities. A loopful culture was inoculated into protease production medium followed by incubation at 48 h at 30 ˚C in shaking condition. Filtrate was used for enzyme activity as described by using McDonald and Chen method [16]. A unit of protease activity may be defined as the amount of enzyme required to release 1 µmol of tyrosine per min under standard condition.

Cellulase: Each of the isolates was spot-seeded on mineral agar medium containing 1% carboxylmethyl cellulose to detect cellulases [23]. Those isolates which produced zone of clearance undergo were reconfirmed by quantitative assay. The quantitative estimation was carried out by inoculating a loopful culture into the cellulase production medium (mineral agar medium+1% CMC). Incubation was carried out at 30 ˚C for 48 h in shaking condition at 120 rpm. Then the filtrate was for determination of enzyme activity. Enzyme activity was measured using DNS method [13]. One unit of cellulase activity was defined as the amount of enzyme required to release 1 µmol of glucose per min under these conditions [13].

Chitinase: Each of the isolates was spot-seeded on a mineral agar medium containing 0.08% colloidal chitin to detect chitinases [23]. Those isolates which produced zone of clearance undergo for quantitative assay. A loopful culture was inoculated into the chitinase production medium, and then it was incubated at 30 ˚C for 48 h in shaking condition at 120 rpm. Filtrate was used for enzyme activity. Chitinase activity was determined by a DNSA method [18]. This method works on the concentration of N-acetyl glucosamine (NAG), which is released as a result of enzymatic action [15].

HCN production: All bacterial isolates were screened for the production of hydrogen cyanide by method described by Lorck [11]. The nutrient broth was amended with 4.4 g glycine/l and the isolates were streaked on modified agar plates. A Whatman filter paper no. 1 soaked in 2 % sodium carbonate in 0.5 % picric acid was placed on the top of the plate. The plates were sealed with parafilm and incubated 30 ºC for 4 days. Development of orange to red colour indicated HCN production.

Antifungal activities: For the screening of antifungal actinomycetes, different test organisms were evaluate against common sugarcane pathogens such as *Aspergillus niger*, *Trichoderma viride* and *Fuserium oxysporium*. These test organisms were grown in sterile potato dextrose broth for 74

h. One milliliter of these cultures of each test organism was seeded in melted potato dextrose agar which was then poured in sterile petridish. After solidification of media spot inoculation of each bacterial isolate was carried out and incubated at 30 ˚C for 72 h. The antifungal bacteria show the zone of inhibition of the test organisms. *In-vitro* antagonistic ability of bacterial isolates was investigated against sugarcane pathogen *Fusarium moniliforme* by dual culture technique [22]. Bacterial isolates were streaked at one side of petri dish (1 cm away from the edge) containing PDA (Potato infusion can be made by boiling 300 g of sliced (washed but unpeeled) potatoes in water for 30 min and then decanting or straining the broth through cheesecloth. Distilled water is added such that the total volume of the suspension is 1 l. 20 g dextrose and 20 g agar powder is then added and the medium is sterilized by autoclaving at 15 p for 15 min). Five mm mycelia plug from seven day old PDA cultures of *Fusarium moniliforme* were placed at the opposite side of petridishes perpendicular to the isolate streak. Petridishes were then incubated at 30 ˚C for 5 days. Petri dishes inoculated with fungal discs alone were served as control. Observations on width of inhibition zone and mycelia growth of test pathogens were recorded.

Characterization of the most efficient multi-trait PGP isolates

The most efficient selected isolate was characterized on the basis of its morphological, cultural and biochemical characteristics as per Bergey's Manual of Systematic Bacteriology [8]. The morphological characteristics of the isolates studied included cell shape, size, arrangement of cells and Gram's nature. The cultural characteristics studied were colony morphology and growth on selected media. The selected isolates were further subjected to the biochemical characterization for identification of organisms up to genus level.

Results and Discussion

Isolation and Purification Bacteria from Rice Rhizosphere

Total of 98 isolates were obtained from different rhizospheric samples collected from different rice varieties grown in South Gujarat region. Result of isolation is shown in Table 1.

Table 1: Isolation of Rhizosphere Bacteria from Different Locations

No.	Sampling site	Sample code	Rice variety grown	No. of isolates obtained
1	Vihan	A	Jaya	6
2	Vihan	B	Gujri	5
3	Vihan	C	Gujarat 3	7
4	Sevni	D	Mashuri	5
5	Sevni	E	Cholum	6
6	Sevni	F	171	4
7	Sampura	G	Gujri	7
8	Sampura	H	Mashuri	7
9	Asta	I	Jaya	5
10	Asta	J	171	5
11	Asta	K	Gajarat 3	6
12	Dungar	L	Jaya	8
13	Dungar	M	Mashuri	7
14	Timba	N	Gujri	5
15	Timba	O	Cholum	6
16	Tarsadi	P	171	6
17	Tarsadi	Q	Cholum	3

Primary Screening of Rhizospheric Bacterial Diversity for its PGPR Potential

All 98 isolates obtained from the extensive isolation from rhizosphere region of 6 different rice varieties grown at different field of South Gujarat region were screened out for IAA production, phosphate solubilization, siderophore production, N_2 fixation and protease production potentials. Majority of bacterial isolates were shown potential for siderophore production, IAA production and nitrogen fixation. While, protease producers and phosphate solubilizers were few in number (Table 2).

When bacterial population was analyzed with respect to rice cultivar and location, it was observed that rhizospheric soil of Mashuri, Jaya and 171 rice varieties were enriched with PGPB diversity. Rhizospheric isolates from rice cultivar Gujarat3 and Gujri were found to show moderate PGPR potential, while isolates from Cholum rice variety was very poor with respect to PGPB diversity (Table 2).

Table 2: Primary Screening of Rhizospheric Bacterial Diversity for its PGPR potential

Sample	Cultivar	No. of Isolates	PGPR Activities				
			IAA Production	Phosphate Solubilization	Siderophore Production	Nitrogen Fixation	Protease Production
A	Jaya	6	4	1	3	2	3
B	Gujri	5	2	3	3	5	3
C	Gujarat 3	7	2	2	3	5	4
D	Mashuri	5	2	2	3	5	3
E	Cholum	6	3	3	4	5	6
F	171	4	1	1	1	3	2
G	Gujri	7	5	3	2	6	3
H	Mashuri	7	6	2	4	2	2
I	Jaya	5	4	3	4	4	4
J	171	5	4	2	4	3	4
K	Gajarat 3	6	4	3	3	4	2
L	Jaya	8	5	3	5	7	3
M	Mashuri	7	7	4	7	6	1
N	Gujri	5	3	1	5	3	4
O	Cholum	6	3	2	4	3	1
P	171	6	5	4	6	3	3
Q	Cholum	3	3	2	3	3	0

Total rhizospheric diversity with respect to its PGPR activities is represented in Fig 1. 70.40% of diversity was found to exhibit siderophore production, 65.30% diversity was found to exhibit N_2 fixation potential and IAA production was found

in 64.28% of total diversity under study. While the protease production and phosphate solubilization activity was found in less than 50% of diversity.

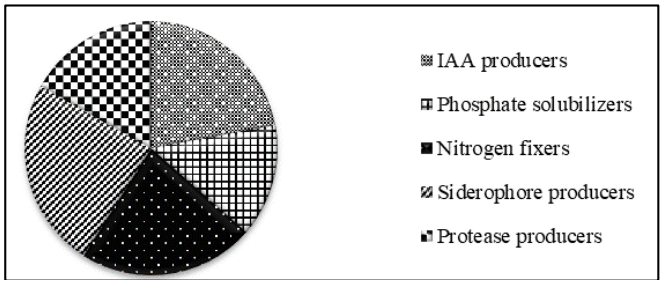

Fig 1: Comparision of PGPR traits of different isolates

Fifteen isolates shown multi-trait PGPR potential were further investigated for cellulase production, chitinase production, HCN production as well as antifungal activity. As shown in Table 3 isolates M2 and N4 were found to produce cellulase. Isolate M2 was also found to produce chitinase as well as HCN. As cellulase and chitinase are fungal cell wall degrading enzymes and essential for fungal inhibition. Microbial production of HCN has been reported as an important antifungal trait to control root infecting fungi. All the 15 isolates were negative for Antifungal production (Table 3).

Table 3: Primary Screening of Selected Isolates for Biocontrol Potentials

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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+: Positive; - : Negative

Secondary Screening of Selected PGPR Isolates

All 15 isolates were further thoroughly investigated for their quantitative ability for the various PGPR activities.

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 2, isolates H7, M2 and P5 were found to produce more than 45 mg/L IAA.

Phosphate solubilization: Phosphorus is a primary essential nutrient element for rice production. The bioavailability of soil inorganic phosphorus in the rhizosphere varies considerably with plant species, nutritional status of soil, presence of effective microorganisms and soil conditions. 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 3, isolates D4, M2 and P5 were found to solubilize more than 10 mg/L phosphate.

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 4, isolates D4, F3, H7, I4, I5 and M2 were found to produce more than 50% Siderophore Unit.

Protease production: Protease is cellwall lysing enzyme and is important to inhibit fungal pathogen. As shown in Fig 5, isolates I4, I5, M2 and P4 were found to produce more than 12 IU of Protease.

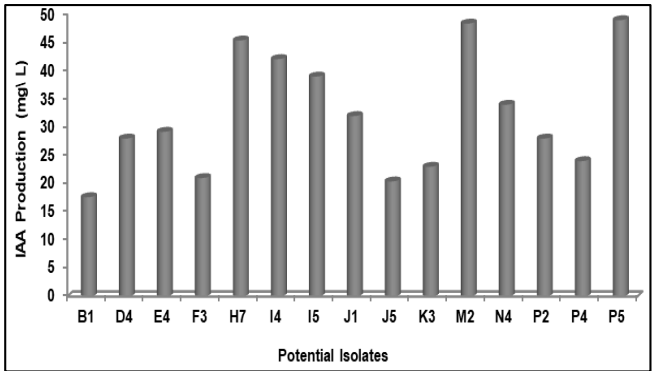

Fig 2: IAA production by selected multi-trait PGP bacterial isolates

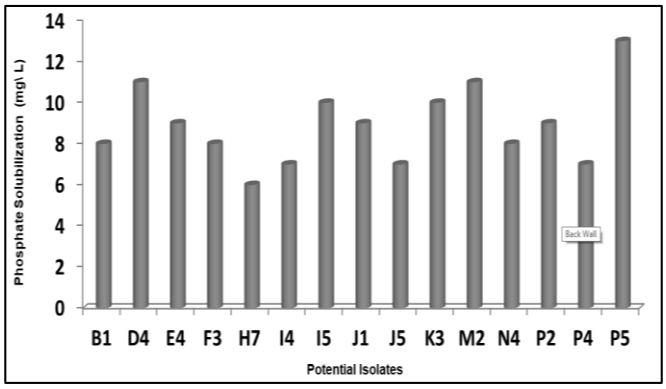

Fig 3: Phosphate solubilization by selected multi-trait PGP bacterial isolates

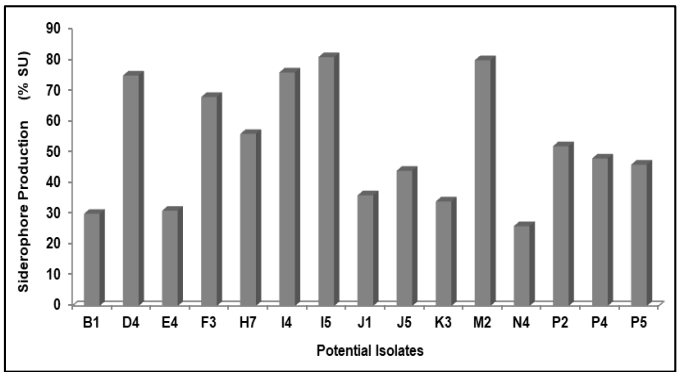

Fig 4: Siderophore production by selected multi-trait PGP bacterial isolates

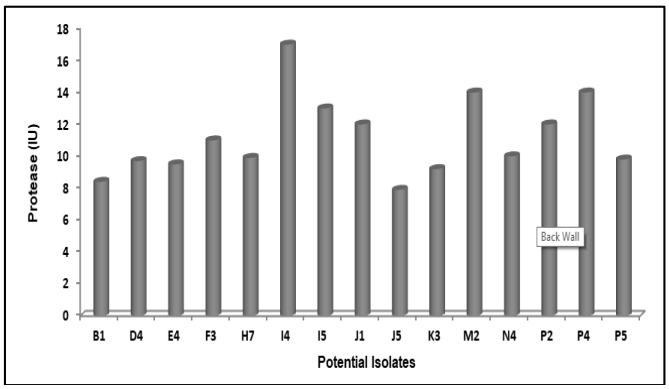

Fig 5: Protease production by selected multi-trait PGP bacterial isolates

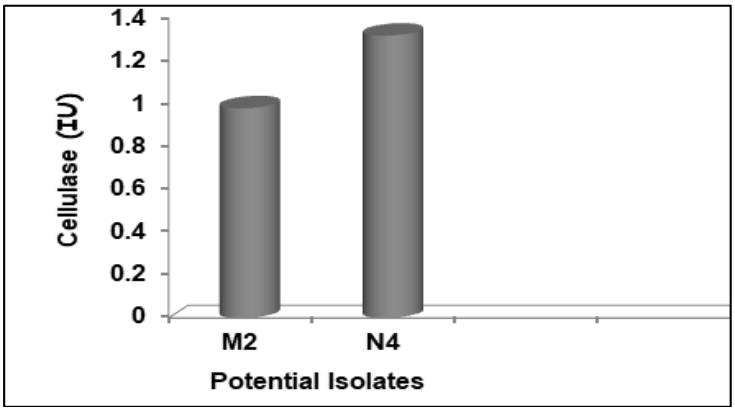

Fig 6: Cellulase production by selected multi-trait PGP bacterial isolates

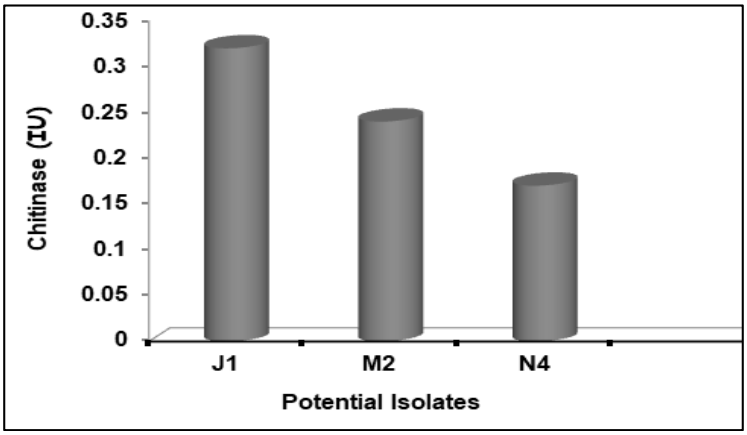

Fig 7: Chitinase production by selected multi-trait PGP bacterial isolates

Cellulase Production: Cellulase is very crucial fungal cell wall lysing enzyme and concentration of microbes producing cellulose is very low. As shown in Fig 6, only 2 isolates M2 and N4 out of 15 selected isolates were found to produce cellulase.

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 7, only 3 isolates J1, M2 and N4 were found to produce chitinase.

Characterization of isolates

Multi-trait PGP activity is shown by the M2 isolate and therefore it was selected for the further characterization for identification. Microscopic observation of M2 isolate revealed it as long rods, present in single or short chain and Gram negative in nature. The colony showed greenish pigmentation and also able to grow in presence of cetrimide. They were catalase and oxidase positive, and displayed oxidative utilization of glucose. It gives indole test positive, methyl red test negative, Voges–Proskauer test negative, and citrate utilization test positive (Table 4). According to Bergey's manual of systemic bacteriology, it is identified as a *Pseudomonas* species [8].

Table 4: Characterization of selected multi-trait PGP isolate for rice growth promotion

Isolate	**M2**
Morphological Characteristics	
Size	Long
Shape	Rod
Arrangement	Single and in chain
Gram's nature	Negative
Cultural Characteristics	
Size	Large
Shape	Round
Margin	Entire
Opacity	Translucent
Elevation	Flat
Consistency	Moist
Pigmentation	Green
Texture	Smooth
Optimum pH for Growth	7
Optimum Temperature for Growth	30
Growth on Cetrimide agar plate	**Positive**
Biochemical Characteristics	

Utilization of: Glucose Maltose Lactose Mannitol Xylose Sucrose	 + - - - + +
Methyl red test	-
Voges-Proskauer test	-
Citrate utilization	+
Indole production	+
Urea hydrolysis	-
Phenyl alanine deamination	+
Nitrate reduction	-
Ammonia production	+
Gelatin hydrolysis	+
Catalase	+
Oxidase	+
Growth on TSI slant: Gas H_2S Lactose fermentation	 - - -

+ : Positive; - : Negative

Conclusion:

The current study revealed that rhizospheric samples collected from different paddy fields of South Gujarat region harbor bacterial diversity with multiple plant growth promoting potentials. Extensive screening showed M2 as most efficient multi-trait PGP candidate. This was identified as *Pseudomonas* species which is versatile and ideal for growth promotion of paddy cultivars to reduce dependence on chemical inputs. Further studies should carried out to evaluate potential of this *Pseudomonas* species in field condition to develop as an effectual bioinoculant formulation.

References

1. Borgstrom G. Too many: an ecological overview of earth's limitation. Collier Books, New York. 1969, 400.
2. Döbereiner J, Day JM. Associative Symbioses in Tropical Grasses: Characterization of Microorganisms and Dinitrogen-fixing Sites, In: "Proc. 1st Int. Symp. On Nitrogen Fixation", (Eds.): Newton WE, Nyman CJ, Washington State University Press, Pullman, U.S.A., 1976, 518,
3. Fiss E. Modification of the Chrome Azurol S Medium for Detection of Siderophore Production by Mycobacteria, In: Abstr., 90th Annu. Meet. Am. Soc. Microbiol., Washington, D.C. U-73, 1990, 153,
4. Gaur AC. Phosphate solubilizing microorganisms as biofertilizers. Omega Scientific Publishers, New Delhi, India. 1990; 5761:114.
5. Gordon SA, Weber RP. Colorimetric estimation of indole acetic acid. Plant Physiol. 1951; 26:192-195.
6. Gyaneshwar P, James EK, Mathan N, Reddy PM, Reinhold-Hurek B, Ladha JK. Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. J. Bacteriol. 2001; 183:2634-45.
7. Hiifte M, Woestyne MV, Verstraete. Role of siderophores in plant growth promotion and plant protection by fluorescent *Pseudomonads*. *In*: Biochemistry of metal micronutrients in the rhizosphere. Manthey JA, Crowley DE Crowley DG, Luster DG. (Eds.). Lewis Publishers. Boca Raton, FL., USA., 1994, 81-92
8. Holt JG, Krieg NR, Sneath P, Staley J, Williams S. Bergey's manual of determinative bacteriology, 9th ed. Baltimore MD, Williams, Wilkins. 1994, 787.
9. King JE. The colorimetric determination of phosphorus. Biochem. J. 1932; 26(2):292-297.
10. Kloepper JW, Schroth MN. Plant growth-promoting rhizobacteria on radishes. 4th Int. Conf. Plant Pathogen Bacteria, Angers France, 1978; 2:879-882.
11. Lorck H. Production of hydrocyanic acid by bacteria. Physiol. Plant. 1948; 1:142-146.
12. Malik K, Bilal R, Mehnaz S, Rasul G, Mirza M, Ali S. Association of nitrogen-fixing, plant-growth-promoting rhizobacteria (PGPR) with Kallar grass and rice. Plant Soil. 1997; 194(1):37-44.
13. Mandels M, Andreotti R. Roche C. Measurement of saccharifying cellulase, Biotechnology and Bioengineering Symposium, 1976; 6:21-33.
14. Mano H, Tanaca F, Nakamura C, Kaga H, Morisaki H. Culturable endophytic bacterial flora on the maturing leaves and roots of rice plants (*Oryza sativa* L.) cultivated in a paddy field. Microb. Environm. 2007; 22(2):175-185.
15. Massimiliano F, Jean-Louis L, Federici F. Chitinolytic enzymes activity of *Penicillium janthinellum* P9 in bench top bioreactor. J. Ferment. Bioeng. 2007; 86:620-622
16. McDonald CE, Chen LL. Lowry modification of the Folin reagent for determination of proteinase activity. Ann. Biochem. 1965; 10:175-186.
17. Meyer JM, Abdallah MA. The Fluorescent pigments of *Fluorescent pseudomonas*: Biosynthesis, purification and physicochemical properties. Journal of General Microbiology. 1978; 107:319-328.
18. Miller GL. Use of dinitrosalicyclic acid reagent for determination of reducing sugars, Biotechnology and Bioengineering Symposium, 1959; 5:193-219,
19. Naik S, Shashikala J, Krishnamurthy Y. Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities in vitro. Microbiol. Res. 2009; 164(3):290-296.
20. Okon Y, Albercht SL, Burris RH. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. Appl. Environ. Microbiol. 1977; 22:85-88.

21. Prakamhang J, Minamisawa K, Teamtaisong K, Boonkerd N, Teaumroong N. The communities of endophytic diazotrophic bacteria in cultivated rice (*Oryza sativa* L.). Appl. Soil. Ecol. 2009; 142(2):141-149.
22. Rabindran R, Vidhyasekaran P. Development of a formulation of *Pseudomonas fluorescens* Pfalr2 for management of rice sheath blight. Crop Protection. 1996; 15:715-721.
23. Renwick A, Campbell R, Coe S. Assessment of in vivo screening systems for potential biocontrol agents *Gaeumannomyces graminis*. Plant Pathol. 1991; 40:524-532.
24. Salisbury FB, Ross CW. Plant Physiology. Wadsworth, California, 1992, 451-497,
25. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Anal. Biochem. 1987; 160:47-56.
26. Ulhoa CJ, Peberdy JF. Regulation of chitinase synthesis in *Trichoderma harzianum*. J. Gen. Microbiol. 1991; 14:2163-2169.
27. Verma S, Ladha J, Tripathi. A Evaluation of plant growth promoting and ability of endophytic diazotrophs from deep water rice. J. Biotechnol. 2001; 91:127-141.
28. Yang JH, Liu HX, Zhu GM, Pan YL, Guo JH. Diversity analysis of antagonists from rice associated bacteria and their application in biocontrol of rice diseases. J. Appl. Microbiol. 2008; 104:91-104.
29. Yasmin S, Bakar M, Malik K, Hafeez F. Isolation, characterization and beneficial effects of rice associated plant growth promoting bacteria from Zanzibar soils. J. Basic Microbiol. 2004; 44(3):241-252.
30. Zarrin F, Saleemi M, Zia M, Sultan T, Aslam, Riaz -Ur-Rehman M *et al*. Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. African J. Biotechnol. 2009; 8(2):219-225.