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ASSESSMENT OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM *JATROPHA CURCAS*RHIZOSPHERE

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ABSTRACT

Plant growth promoting rhizo bacteria (PGPR) are able to colonize all the ecological niches found on plant root and promotes plant growth, have profound effects on productivity of plants. In this study total 163 isolates were successfully isolated from *Jatropha curcas* rhizosphere from two distinctive sites of Gujarat state (India).Ten isolates were screened on the basis of their fast growing ability, motility and examined for production of in dole acetic acid (IAA),hydrogen cyanide (HCN), ammonia, exopolysaccharide (EPS)and phosphate solubilisation. DN2, DP3, DR1, SP1 and SAz1 showed good potential ability for plant growth promotion. They were further studied for different salt concentration, pH and temperature.DN2, DP3 and DR1 isolates were grown best at 0.5% NaCl and7 pH. SP1 and SAz1 isolates were shown maximum growth at 1% NaCl and 9 pH. All five isolates have excellent growth under the temperature of 37°C. It is expected that inoculation with these five isolates DN2, DP3 &SP1 (*Bacillus*spp.), DR1 (*Rhizobium*spp.) and SAz1 (*Azotobacter*spp.) have positive impact on plant root, shoot and yield of *Jatrophacurcas* under the field condition.

Keywords: Indole acetic acid, HCN, Ammonia production, EPS, Phosphate solubilisation, *Jatropha curcus* rhizosphere, PGPR

INTRODUCTION

Biodiesel is a renewable source of energy which can be produced locally by growing oil seed producing plants which is eco-friendly also. Due to pressure on edible oils like groundnut, rapeseed, mustard and soya bean etc. non-edible oil of *Jatropha curcas* is evaluated as a diesel fuel extender (Raheman *et al.* 2003). *Jatropha curcus*can grow inwastelands, but its growth is limiteddue to various reasons. Inoculation of beneficial microbeswith *Jatropha* seedsmay improve plant growth by enhancing plant resistance to adverse environmental stresses. Among those beneficial microbes “Plant growth promoting rhizobacteria” (PGPR) actsas a modem of soil fertility and facilitators of plant establishment.

The PGPR are known to participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling and / or seedling growth (Zahir et al., 2004). PGPR can affect plant growth either directly or indirectly. Direct promotion by PGPR entails either providing the plant with plant growth promoting substances that are synthesized by the bacterium or facilitating the uptake of certain plant nutrients from the environment. Indirectly promote plant growth by preventing the deleterious effects of phytopathogenic bacteria, fungi, nematodes and viruses(Glick *et al.* 1999; Antoun and Prevost, 2006).Motile rhizobacteria may colonize the rhizosphere more profusely than the non-motile organisms resulting in better rhizosphere activity and nutrient transformation.

Phosphate in the soil mostly exists in insoluble forms and the concentration of soluble phosphate in soil is very low and plants are able to absorb only the soluble forms, whichare mono and dibasic phosphates. Microorganisms enhance the phosphour (P) availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphates (Chen *et al.* 2006).A number of rhizobacteria were able to produce volatile compounds such as ammonia and hydrogen cycnide,which are reported to play an important role in biocontrol.HCN produced in the rhizosphere seedlings by the selected rhizobacteria is a potential and environmentally compatible mechanism for biological control of weeds and minimizing harmful effects on the growth of desired plants (Kremer *et al.* 2001).Accumulation of ammonia in soil may increase the pH and thus by creating an alkaline condition of soil at 9-9.5,that suppresses the germination andgrowth of fungi as well as nitrobacteria due to its potent inhibiting effect.EPSproduction is an important trait of bacteria because it protects the cells against phagocytosis, phage attack and helps in nitrogen fixation by preventing high oxygen tensions(Tank and Saraf, 2003).IAA producing PGPR strains can able to enhance the growth and development of plants by interfering in the concentration of known phytohormones (Dey *et al.* 2004). The salt concentration, temperature, acidity and alkalinity activities of rhizobia in the rhizosphere are likely to be influenced by the plant genotypes and their symbiosis status. The present study was designed to identify and develop a suitable strain of PGPR which can be used in wastelands for the establishment of seedling of *Jatropha curcas* facilitating high yield.

MATERIALS AND METHODS

Soil sample collection

Soil sample from the rhizosphere of *Jatropha curcas*were collected from two different fields D1 and S1 in Gujarat. D1 was fertile land located in Sardarkrushinagar Dantiwada Agricultural University and S1 was sodic soil, both alkaline and saline in nature from the Surendranagar district. The rhizosphere soil was collected digging up to 15 cm in depth with the help of

sterile equipments, samples were placed in aseptic polyethylene bags and stored at 4ºC until the use (Kennedy *et al.* 1995).

Diversity of Rhizobacteria from the rhizosphere of *Jatropha curcas*

The sample quantity of soil (10 g) was suspended in90 ml of sterile normal saline.The soil samples were serially diluted and inoculated on the respective media for isolation of rhizobacteria. Soil suspensions (0.1 ml) were spread on Nutrient agar for non specific bacteria, Yeast Extract Mannitol Agar (YEMA) for *Rhizobium*, Ashby's Mannitol agar for *Azotobacter*, Pikovskaya's agar for phosphate solubilizing bacteria, Martin's Rose Bengal agar for fungi and Actinomycetes agar for *Actinomycetes*. Fast growing prominent colonies were selected for further study. Colonies maintained on the respective slants and motility of the bacterial isolates was examined by hanging drop preparation of 24 hours old broth culture as described by Harrigan and Mccance (1966). Isolates were studied for their morphological characteristics by gram staining.

Phosphate solubilisation

Bacterial isolates were spot inoculated at centre of Pikovaskay's plate and incubated 37°C for 48 to 72 h. Phosphate solubilization was checked in the form of a clear halo formed around the colony representing the production of organic acids.

Ammonia production

Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 10 days at 30°C. The test bacterium was centrifugedand supernatant mixed with 1 ml of Nessler's reagent. The colour development of brown to yellow was a positive test for ammonia production (Dye, 1968).

EPS production

EPS production was studied in basal medium of all different organisms. 5% sucrose was added into the medium as the carbohydrate source (Modi, 1989). 0.1 ml of culture suspension was inoculated into their respective basal medium and incubated on rotary shaker for 5-6 days at room temperature. After incubation, culture suspension was centrifuged and chilled acetone was added into the supernant thrice the volume. The precipitate was collected on a pre-dried filter paper and the precipitate was allowed to dry overnight at 50ºC.

Hydrogen cyanide (HCN) production

Bacterial cultures were streaked on nutrient agar slants and filter paper strip impregnated with 0.5% picric acid and 2.0% sodium carbonate were added to theabove medium. The slants were sealed with parafilm and incubated at 37ºC for 48 h. After the incubation, change in colour from yellow to light brown, brown or reddish brown showed the presence of HCN production (Morrison and Askeland 1983).

Indole acetic acid (IAA) production

Bacterial cultures were grown on Minimal Salt medium (Frankenberger and Poth, 1988) which was amended with 5mM L-tryptophane for 24 hours on shaker at room temperature. Fully grown cultures were centrifuged at 1000 rpm for 20 min. The supernatant (1 ml) was mixed with 1ml of Salkowsky's reagent and incubated in dark incubator for 1h. Then, pink colour development was estimated on spectrophotometer at 536 nm.

Growth under different salinity conditions

The isolates were tested under 0.5%, 1% and 2%concentrationofNaCl. The change in the growth was observed after 3 days of incubation.

Growth at different pH conditions

The isolates were tested at pH values of 3, 5, 7 and 9 and effect of the pH on the growth of isolateswas studied.

Growth at different temperature conditions

The effect of temperature on the growth of isolates was studied at temperatures 25 ºC, 37 ºC and 45 ºC.

Biochemical characterization of rhizobacteria

Biochemical tests of the selected PGPR isolates were carried out to authenticate and identify them according to the Bergey's Manual of Systematic Bacteriology (Kreig and Holt, 1984; Sneath *et al.*, 1986).

RESULTS AND DISCUSSIONS

Total 163 (87 and 76) bacterial isolates were isolated from two sites of *Jatropha curcas* rhizosphere (D1 and S1). Most of the isolates of rhizobacteria isolated from D1 and S1 sites showed maximum bacterial population on nutrient agar plates (51.72% &46.67%), followed bynon symbiotic nitrogen fixers (13.79 % & 12.34%) and phosphate solubilizers (11.49% & 10.0%) (Figure-1 and 2). Out of 87 and 76 isolates, 25 and 20 isolates were selected on the basisof fast growing ability. The isolates shown maximum growth were termed as fast growers. Motility is an important factor in the colonization of plant roots by bacteria (Czaban *et al.*, 2007). Flagellar motility presumably plays an important role in bacteria competition for nutrients through chemotaxis (De Weert *et al.* 2002). Among 25 isolates (D1) and 20isolates (S1), 5 isolates each were selected on the basis of motility test. Microscopic observations were performed to examine characteristics of 10 bacterial isolates such as shape, Gram reaction and motility (Table-1). Phosphate solubilisation, HCN production and IAA production of PGP traits are depicted in Table-2.

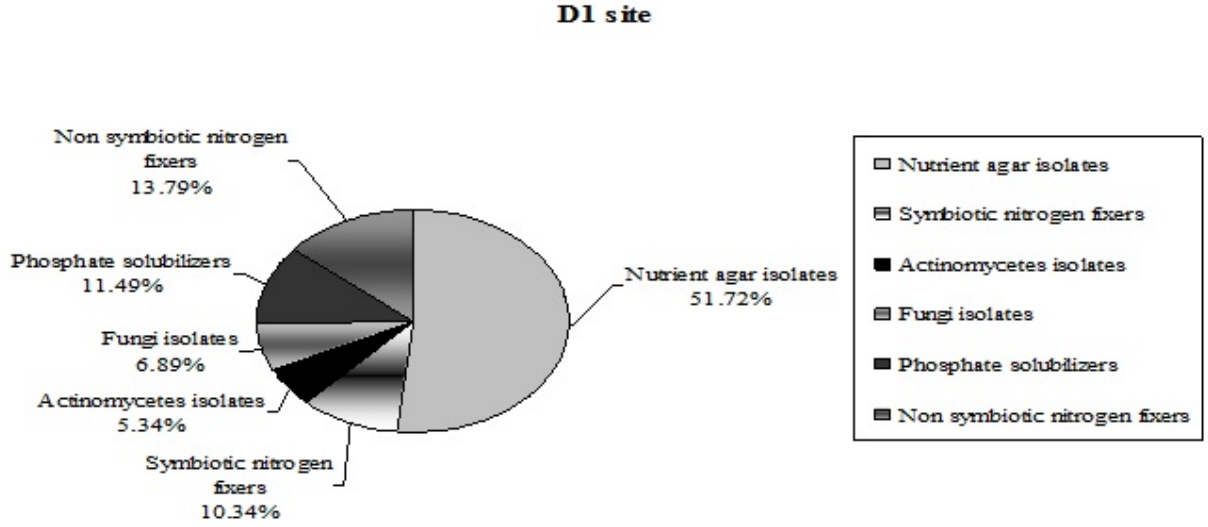

Figure-1. The % of isolates in D1

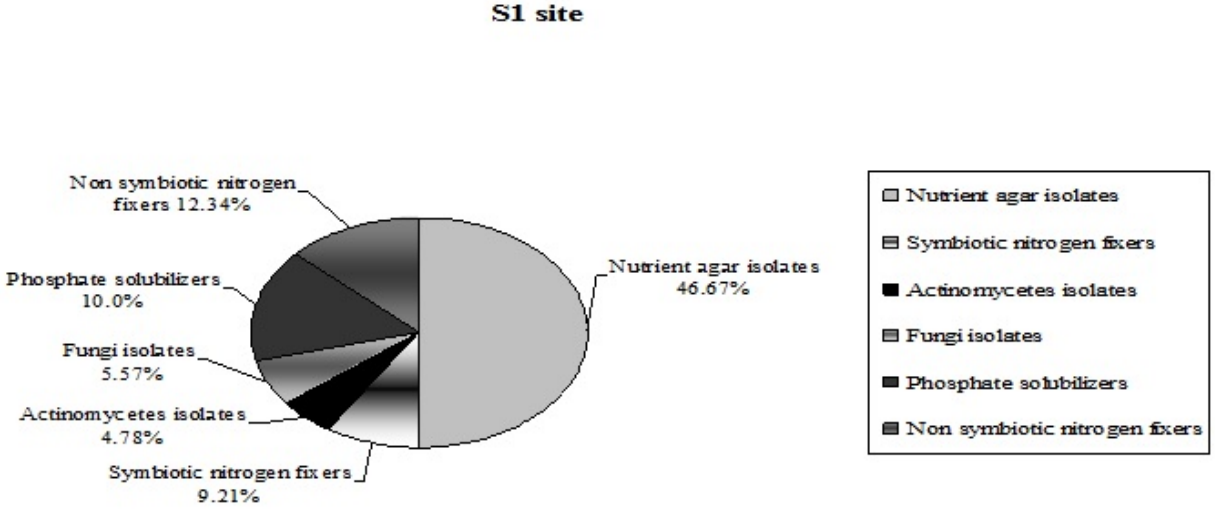

Figure-2. The % of isolates in S1

Table-1. Morphological characteristics of isolates.

Isolates	Cell shape	Motility	Gram reaction
DN2	Rod	Motile	Gram positive
DN4	Cocci	Motile	Gram positive
DN8	Rod	Motile	Gram negative
DP3	Rod	Motile	Gram positive
DR1	Rod	Motile	Gram negative
SN2	Rod	Motile	Gram negative
SN7	Rod	Motile	Gram positive
SN8	Cocci	Motile	Gram positive
SP1	Rod	Motile	Gram positive
SAz1	Rod	Motile	Gram negative

Table-2. Phosphate solubilisation, HCN and IAA production by isolates.

Isolates	Phosphate solubilization	HCN production[a]	IAA Production[b]
Control	-	-	-
DN2	Phosphate solubilize	++	+++
DN4	Phosphate solubilize	+	-
DN8	Phosphate solubilize	-	-
DP3	Phosphate solubilize	+++	++
DR1	Phosphate solubilize	++	-
SN2	Phosphate solubilize	+	++
SN7	Phosphate solubilize	-	-
SN8	Phosphate solubilize	-	-
SP1	Phosphate solubilize	++	++
SAz1	Phosphate solubilize	++	+

[a]HCN production is shown on the basis of colour development on filter paper, i.e.⁻No colour development; ⁺light brown (low); ⁺⁺brown (moderate); ⁺⁺⁺Reddish brown (high). [b]IAA production is shown as ⁻No production; ⁺weak producer; ⁺⁺Medium producer; ⁺⁺⁺ Good producer.

Solubilization of phosphatewas exposedby all 10 isolates from theboth locations. Inoculation of many phosphate solubilizing bacteria (PSB) including *Pseudomonas*, *Bacillus*, *Rhizobium*, *Flavobacterium*, *Micrococcus*, *Burkholderia*, *Achromobacter*, *Erwinia* and *Agrobacterium* increased solubilization of fixed soil phosphates and applied phosphates ensuring higher crop yields (Trivedi, 2008). Maximum HCN production was observed in DP3 isolate, compared to other isolates. 4 isolates, namely DN2, DR1, SP1 and SAz1 developed medium brown color and, therefore, had moderate production of HCN. The remaining 2 isolates, DN4 and SN2 showed low production of HCN by as they developed light brown color. Various secondary metabolites secreted by *Pseudomonas* spp., including HCN have been found to be inhibitory against different phytopathogens (Siddiqui, 2006). DN2, DP3, SN2, SP1 and SAz1 isolates induced IAA production. Isolate DN2 was found to be good producer of IAA. DP3, SN2, SP1 were found to be medium production of IAA in comparison to the weak producer isolate SAz1.Many plant growth bacteria, such as *Azotobacter Paspali* (Surette *et al.* 2003), *Azospirilium brazilance* (Dobbelaere *et al.* 2001), *Pseudomonas putida* (Leave and Lindow, 2005) which stimulate the growth ofroots can produce at least small amounts of the auxin indole 3-acetic acid (IAA).

Ammonia production and EPS production was detected in all 10 isolates in the range of 17.1 to 79µg/ml and 15.3 to 32.5 mg/ml respectively (Table-3). Maximum amount of ammonia production was observed in DR1 (79µg/ml) followed by DP3 (48 µg/ml), DN2 (45 µg/ml), SAz1 (34 µg/ml) and SP1 (32 µg/ml) isolates after 10 days of incubation. Joseph *et al.* (2007) was reported production of ammonia was commonly detected in the isolates of *Bacillus* (95%) followed by *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45.0%).Highest EPS production was observed in DN2 (32.5 mg/ml) and DP3 (32.2 mg/ml) isolates followed by SAz1 (30.3 mg/ml), DR1 (30.0 mg/ml) and SP1 (29.0 mg/ml). The *Rhizobium* to produce more polysaccharide helping to promote the infection and enhance nodulation of legumes (Ghosh et al. 2005). DN2, DP3& DR1 isolates from D1 sample and SP1 & SAz1 from S1 sample have good characteristics of PGPR.

The growth of organisms was observed under the different salinity, pH and temperature conditions. Maximun growth was observed in DN2 (85.57 %), DP3 (89.23 %) and DR1 (84.01 %) isolates at 0.5% salinity and SP1 (98.96 %) and SAz1 (99.23 %) isolates at 1% salinity. The growth of all five organisms were decreased at 2% salinity. At pH value3, the growth of all five isolates was decreased and only DN2 and DR1 isolates were able to grow at 5 pH. DN2 (94.57 %), DP3 (99.39 %) and DR1 (97.13 %) isolates grew best at 7 pH. SP1 (81.22 %) and SAz1 (77.25 %) isolates were grew best at 9 pH. The growth of all five isolates DN2 (91.21 %), DP3 (99.69 %), DR1 (95.52 %), SP1 (67.11 %) and SAz1 (95.34 %) was best in the temperature of 37°C. The lowest growth for all five bacteria was observed at 45ºC (Table-4).It was observed that increase in temperature has inhibitory effect on the growth of organisms. On the basis of cultural, morphological and biochemical characteristics, isolates DN2, DP3 & SP1 were identified to belonging to *Bacillus* spp., DR1 isolate was identified as *Rhizobium* spp. and SAz1 isolate was identified as *Azotobacter* spp. (Table-5, Figure-3).

Table-3. Ammonia production and EPS production by selected isolate.

Isolates	Ammonia production (µg/ml)	EPS production (mg/ml)
Control	-	-
DN2	45.0	32.5
DN4	31.3	20.1
DN8	39.1	24.2
DP3	48.0	32.2
DR1	79.0	30.0
SN2	23.2	25.1
SN7	25.4	21.2
SN8	17.1	15.3
SP1	32.0	29.0
SAz1	34.0	30.3

Table-4. Growth% of PGPR isolate sat different salinity, pH and temperature.

Isolates	Salinity (% NaCl)			pH				Temperature		
	0.5	1	2	3	5	7	9	25ºC	37ºC	45ºC
DN2	85.57	82.34	66.93	3.48	32.52	94.57	60.16	55.84	91.21	43.56
DP3	89.23	78.21	7.73	2.47	2.83	99.39	70.46	83.26	99.69	62.18
DR1	84.01	74.36	67.56	6.17	40.29	97.13	57.35	94.27	95.52	30.49
SP1	83.99	98.96	76.37	1.78	1.99	38.73	81.22	34.82	67.11	32.11
SAz1	90.14	99.23	88.37	0.81	4.65	40.02	77.25	75.52	95.34	41.12

Table-5. Biochemical characterization of PGPR.

Biochemical characterization	DN2	DP3	DR1	SP1	SAz1
Catalase test	+	+	+	+	-
Oxidase test	+	+	-	+	-
Citrate utilization test	-	-	+	-	-
M-R test	-	-	+	-	-
V-P test	-	-	-	-	-
Oxidation-fermentation test	-	-	-	-	-
Sugar utilization test	Acid/Gas	Acid/Gas	Acid/Gas	Acid/Gas	Acid/Gas
Glucose	+/-	+/-	+/-	+/-	-/-
Mannitol	+/-	-/-	+/-	+/-	-/-
Sucrose	+/-	-/-	+/+	+/-	-/-
Lactose	-/-	-/-	+/-	+/-	-/-
Xylose	+/-	-/-	+/+	-/-	-/-
Galactose	-/-	-/-	+/+	+/-	-/-
Indole production test	+	+	+	+	+
H_2S production test	-	-	-	-	-
Urea hydrolysis test	-	-	-	-	-
Ammonia production test	+	+	+	+	+
Nitrate reduction test	-	-	-	+	-
Starch hydrolysis test	+	-	-	+	-
Gelatin hydrolysis test	-	-	-	-	-
Casein hydrolysis test	+	+	-	-	-
Motility agar stab test	+	+	+	+	+
Phenylalanine deamination test	-	-	-	-	-
Dehydrogenase test	-	-	-	-	-
TSI agar test					
Slant	Acidic	-	Alkaline	-	-
Butt	Acidic	-	Acidic	-	-
H_2S	-	-	-	-	-
Gas	-	-	-	-	-

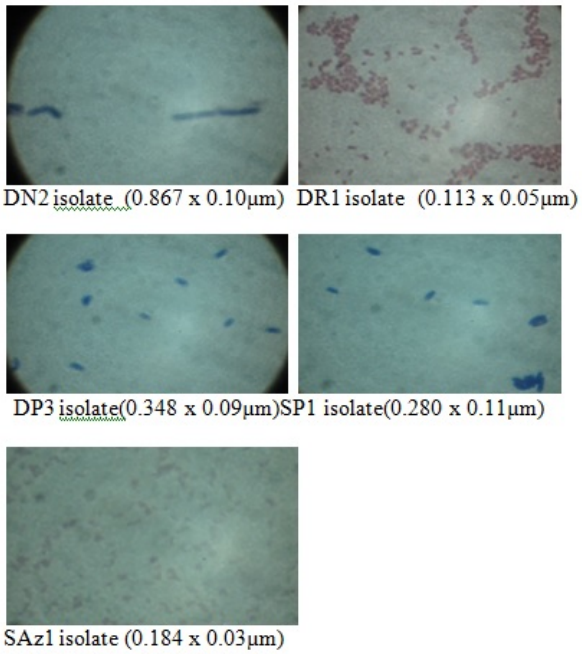

Figure-3. Morphological characteristics of PGPR.

CONCLUSIONS

It has been assumed that after this study, five bacterial strains have significant plant growth promoting attributes. Therefore we suggest that *Bacillus* spp. DN2, DP3 & SP1, *Rhizobium* spp. DR1 and *Azotobacter* spp. SAz1 were suitable PGPRfor enhancement of plant growth productivity of *Jatropha curcas*. Inoculation with these identified bacteria may also improve the environment and quality of rural life. Moreover, these isolates have positive impacts on soil characteristics and health necessary for better growth of planted biomass.

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