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Characterization of Plant Growth Promoting Rhizobial Isolates for Pigeon Pea (*Cajanus cajan* [L.] Mill sp)

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A B S T R A C T

Study was conducted to isolate and characterize Rhizobia from root nodules of pigeon pea from Vijayapur, Kalaburgi and Bagalkot districts of northern regions of Karnataka. Ten Rhizobial isolates and reference strain PPM35B were subjected for nodulation test. All the isolates showed positive result for nodulation. The number of nodules was ranging from 12 to 14.5 per plant. All these isolates were also subjected for functional characterization. The amount of IAA, GA production and ACC deaminase activity by different rhizobial isolates ranged from 16.60 to 22.85 µg IAA/ ml of broth, 11.00 to 14.43 µg/25ml of broth and 44.5 to 73.5 nmoles of α-ketobutyrate/mg/h of broth respectively. The diameter of P, Zn and Si solubilization by the rhizobial isolates was ranged from 4.5 to 10.3 mm, 9.5 to 20.5 mm and 5.5 to 6.5 mm, respectively. These isolates were further subjected for morphological and biochemical characterization. All the isolates were found to be rod shaped and gram negative. All the isolates were positive for citrate utilization, catalase, urease, oxidase, acid and gas production tests whereas negative for Voges proskauer's and indole production test.

Introduction

Pigeon pea (*Cajanus cajan* [L.] Mill sp.), is the second most important kharif pulse crop grown in India after chickpea. In India, it is predominantly grown in rainfed conditions. It is used as a food, fodder and fuel wood. It maintains soil fertility through nitrogen fixation by its microsymbiont *Rhizobium*, as well as from the leaf fall and recycling of the nutrients.

One of the major problems that limit economically successful agricultural production in yield of pigeon pea worldwide is poor soil fertility. One of the way to correct this problem is addition of biofertilizers which improves soil fertility by supplying nutrients needed for optimum crop growth by fixing an atmospheric nitrogen, mineral solubilization *etc.*, (Osman *et al.*, 2011).

Biological nitrogen fixation (BNF) is a

process by which N_2 in the atmosphere is reduced into a biologically useful, combined form of Nitrogen to ammonia by living organisms (Giller, 2001). It is estimated that endosymbiotic biological nitrogen fixation globally represents approximately 90% of all the fixed nitrogen in the terrestrial environment. Mutualism between legumes and rhizobia is regarded as the most important biological mechanism for providing nitrogen to the leguminous plants as an alternate to the expensive nitrogenous fertilizers, also it can make agriculture more productive and sustainable with its eco-friendly nature (Kantar *et al.,* 2003). The activity of *Rhizobium* inhabiting root nodules often results fixing large amounts of nitrogen which ranges from 25 to 201 kg N/ha in grain legumes (Dakora and Kenya, 1997).

In addition to biological nitrogen fixation by rhizobia it also promote plant growth by its plant growth promoting traits through mechanisms that are independent of biological nitrogen fixation (Ahemad and Kibret, 2013, Peix *et al.,* 2001, Alikhani and Yakhchali, 2009), which includes stimulating plant growth directly either by synthesizing plant hormones such as indole-3-acetic acid (IAA), Gibberellic acid (GA) or by promoting nutritional processes such as mineral solublization (Phosphate solubilisation) and production of siderophore, HCN. Through its antagonistic activity it also stimulate plant growth indirectly by protecting the plant against fungal pathogens (Hemissi *et al.,* 2011). When legume plants grow in low nutrient media (Dakora *et al.,* 2002), rhizobia use these excluded compounds to enhance mineral nutrition by production of organic acid to solubilize phosphorous (P) and manganese (Mn) and iron (Fe) is mobilized by production of Siderophores (Carson *et al.,* 2000, Richardson, 2001). IAA released from rhizobia massively proliferate root hair growth and thus enhance the root's absorptive capacity and nutrient uptake in legume system (Yanni *et al.,* 2001). Hence the present study was undertaken to isolate and screen rhizobia for their plant growth promoting activity in addition to their excellent symbiotic effectiveness.

Materials and Methods

Isolation of Rhizobial isolates

Root nodule samples of pigeon pea were obtained from Vijayapur, Kalaburgi and Bagalkot districts of northern regions of Karnataka. The root nodule samples from 60 days old pigeon pea plants were collected and placed in sterile polythene bags and brought to laboratory for isolation of Rhizobia.

Fresh root nodules of pigeon pea plants were collected and sterilized with 70% ethanol for 4-5 min and 0.1% $HgCl_2$ for 1 min. Nodules were then rinsed, crushed in sterile distilled water, streaked on yeast extract manitol agar (YEMA) plates and incubated at 29±2° C for 3 days (Bhattachary and chandra, 2013). At the end of incubation period, the rhizobial colonies with white, translucent and elevated were selected. These *Rhizobium* colonies were purified and used for the study.

Assessment of nodulation under laboratory condition

The *Rhizobium* like isolates obtained from root nodules of pigeon pea were tested for their ability to nodulate the host plant using small plastic cups filled with sterilized soil. About 4-5 seeds of pigeon pea were sown in each cup. Ten ml of *Rhizobium* culture was inoculated on to each seed. Control treatment was maintained without inoculating culture. After seed germination, only one plant was retained per pot. After 60 days of sowing, the plants were uprooted and nodule count was recorded (Tilak *et al.,* 2006).

Functional characterization of Rhizobial isolates

The Rhizobial isolates were subjected for functional characterization *viz.*, IAA, GA production, ACC deaminase activity, P, Zn and Si solubilization, HCN and siderophore production and Antagonistic activity.

IAA production

Rhizobium isolates were grown in Luria Bertani broth supplemented with 0.01% tryptophan and incubated at 28±2˚ C for 3 days under shaking condition. The broth was then centrifuged at 10,000 rpm for 20 min at 4˚ C to collect the supernatant. The supernatant (2 ml) was mixed with two drops of orthophosphoric acid and 4 ml of the Salkowski's reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink colour indicates IAA production (Gordon and Paleg, 1957).

GA production

Twenty five ml culture supernatant of each isolate was taken in a test tube. To this, two ml zinc acetate was added followed by addition of two ml potassium ferrocyanide after two minutes. This was centrifuged at 10,000 rpm for 15 minutes and the supernatant was collected. To 5 ml of this supernatant, 5 ml of 30 per cent HCl was added and incubated at 20˚ C for 75 mins. The blank sample was treated with 5 ml of 30 per cent HCl. The absorbance of the samples as well as blank was read at 254 nm in an UV-visible spectrophotometer. The amount of GA present in the extract was calculated from the standard curve and expressed in µg per 25 ml of the medium (Paleg, 1965).

ACC deaminase activity

ACC deaminase activity was determined by measuring the production of α- ketobutyrate and ammonia generated by the cleavage of ACC deaminase. The overnight grown bacterial cells in YEMA broth were harvested by centrifugation at 3000 rpm for 5 min, followed by washing twice with 0.1 M Tris-HCl (pH 7.5). Supernatant was discarded and the pellet was resuspended in 200 µl of 0.1 MTris-HCl (pH 8.5). The cells were labilized by adding 5 per cent toluene and then vortexed at highest speed for 30 seconds. Fifty µl of labilized cell suspension was incubated with 5 µl of 0.3M ACC in an eppendorf tube at 28° C for 30 min. Fifty µl of 0.1 M Tris-HCl (pH 8.5) with 5 µl of 0.3M ACC was maintained as negative control. The samples were mixed thoroughly with 500 µl of 0.56 N HCl by vortexing and the cell debris were removed by centrifugation at 10,000 rpm for 5 min. Five hundred µl aliquot of the supernatant was transferred to a glass test tube and mixed with 400 µl of 0.56 N HCl and 150 µl of DNF solution (0.1 g 2,4-dinitrophenylhydrazine in 100 ml of 2 N HCl) and the mixture was incubated at 28° C for 30 min. One ml of 2 N NaOH was added to the sample before measuring the absorbance at 540 nm (Penrose and Glick, 2003).

Phosphate solubilization

Overnight grown test culture was inoculated on to Pikovskaya's media and incubated at 28±2˚ C for 5 days. Formation of zone around the colonies on Pikovskaya's medium indicates the phosphate solubilization ability of the organism. The diameter of the zone of solubilization was measured and expressed in mm (Vazquez *et al.*, 2000).

Zinc solubilization

The *Rhizobium* isolates were tested for their ability to solubilize insoluble inorganic zinc on mineral salt agar medium supplemented with ZnO (0.25 %). Ten µl of overnight grown culture was inoculated to mineral salt

agar medium supplemented with ZnO (0.25 %) and incubated for 4 days at 28˚ C. The isolate showing clear zone around the colony on the medium was considered as zinc solubilizer. The diameter of the zone of solubilization was measured and expressed in mm (Di Simine *et al.,* 1998).

Silica solubilization

The overnight grown culture was inoculated on to silicate medium and incubated for 7 days at 28˚ C under dark conditions. Formation of zone around the bacterial colony on silicate medium indicated the silica solubilizing ability of the organism. The diameter of the zone of solubilization was measured and expressed in mm.

Siderophore production

The Yeast extract mannitol agar was prepared using PIPES buffer (30.2 g) and Difco agar (18.0 g) and the pH was adjusted to 6.8 by addition of 0.1 N NaOH before autoclaving (Schwyn and Neilands, 1987). After cooling, the CAS solution (100 ml) was added along the wall of flask with gentle agitation to mix without formation of foam. The CAS agar thus prepared was poured in to the plates. After solidification, the plates were kept in the refrigerator (4˚ C) for 24 h. The overnight culture of isolate (10 µl each) was spotted on these CAS agar plates and incubated at 28 ± 2˚ C for 48 h. Formation of orange coloured zone around the colony was taken as positive for the siderophore production. The diameter of orange coloured zone was recorded.

HCN production

Whatman No. 1 filter paper pads were placed inside the lids of petriplates and sterilized. Yeast extract mannitol agar (YEMA) medium amended with 4.4 g/ l of glycine was prepared and autoclaved. Cool and molten YEMA media was added to the bottom petriplate and allowed for solidification. After solidification, 24 h old cultures were streaked on the solidified media. The filter paper padding inside the lid was soaked in 2 ml sterile picric acid solution (2.5 g/l of picric acid and 12.5 g/ l of Na_2Co_3). Then plates were properly sealed with parafilm in order to retain the gaseous metabolites produced by the test organism and to allow for chemical reaction with picric acid present in the filter paper padding (Wei *et al.,* 1991). Plates were incubated at 30˚ C for 7-8 days and the colour change of filter paper was noted.

In vitro screening of *Rhizobium* isolate against fungal pathogen (*Fusarium oxysporum* f. sp. udum) of pigeon pea

The fungal pathogen was grown on potato dextrose agar plates until they completely cover the agar surface. With the help of a sterile cork borer (10 mm diameter), discs of fungal growth from the plates was taken and placed at the center of the fresh PDA plates. Each test isolate was then streaked parallel on either sides of the fungal disc leaving 1.5 cm distance from the edge of the plate. The PDA plates inoculated with only fungal pathogens were considered respective controls. The plates were incubated at 30˚ C for 96 h. The colony diameter of the fungus in control plate and the plates streaked with *Rhizobium* were recorded **(**Sakthivel and Gnanamanickam, 1987). The zone of inhibition (ZOI) of each fungal pathogen by different isolates were calculated by using the following formula,

ZOI = Colony diameter (control plate) - Colony diameter (in dual inoculated plates)

The per cent inhibition of pathogen was assessed by using the formula given below

$$I = \frac{C-T}{C} \times 100$$

Morphological and biochemical characterization

The isolates were studied for morphological characteristics as per the standard procedure given by Somasegaran and Hoben (1994). The isolates were also studied for their biochemical characteristics *viz*., Urea hydrolysis (Lindstrom and Lehtomaki, 1988), Starch hydrolysis (De Oliverira, 2007), indole production (Kovacs, 1956), Nitrate reductase test (Costilow and Humphreys, 1955), Oxidase test (Cappuccino and Sherman, 1996), Catalase test (Graham and Parker, 1964), Citrate utilization (Simmonds, 1926).

Results and Discussion

Isolation of bacteria from root nodules of pigeon pea

Root nodules of pigeon pea were collected from different regions of northern Karnataka. Sampling details are furnished in Table 1.

Ten *Rhizobium* like isolates were obtained from pigeon pea root nodules. Among ten isolates, AMVPR 6, AMVPR10, AMVPR 32, AMVPR 45, AMVPR 53, AMVPR 79 and AMVPR98 were isolated from Vijayapur district, AMVPR 128, AMVPR 131 from Kalaburgi district and AMVPR178 from Bagalkot district of northern Karnataka.

Assessment of nodulation

All the isolates showed positive result for nodulation test. The *Rhizobium* potency in terms of formation of root nodules in pigeon pea varied from 12.00 to 14.75 number of root nodules per plant (Table 2). The results pertaining to assessment of nodulation in this study are in line with the observations made by Gouri *et al.,* 2011, Saad *et al.,* 2014, Demissie *et al.,* 2018 and Degefu *et al.,* 2018. Naeem *et al.,* (2004) reported that four out of six strains isolated from the *Medicago sativa* re-nodulated the host plant confirming them as the strain of *Rhizobium meliloti.*

Functional characterization of *Rhizobium* isolates

IAA production

All the isolates were positive for IAA production and it was ranged from 16.60 to 22.85 µg IAA/ ml of broth (Table 3). Highest IAA production was observed in the isolate, AMVPR 98 (22.85 µg/ml) followed by the reference strain PPM35B (22.45 µg/ml). The results are in line with the findings of Kucuk and Cevher (2016) who reported that the IAA production in *Rhizobium* isolates ranged between 15.6 to 165.6 µg/ml. IAA production by plant growth promoting rhizobacteria (PGPR) can vary among different species and strains and it is also influenced by culture condition, growth stage and substrate availability (Mirza *et al.,* 2001).

GA production

All the isolates were positive for GA production and it ranged from 11.00 to 14.43 µg/25ml of broth (Table 3). The maximum amount GA production of 14.51 µg/25ml of broth was observed in the reference strain which was on par with the isolates AMVPR98 (14.43 µg/25ml broth). The results of this study are in agreement with the findings of Baba *et al.,* (2015) who reported that *Rhizobium phaseoli* isolated from organic farm and Kupwara district were found to produce 162 µl and 153 µl of GA respectively.

ACC deamination activity

All the isolates showed ACC deaminase activity and it ranged from 44.5 to 73.5 nmoles of α-ketobutyrate/mg/h of broth (Table 3). The reference strain PPM35B

reported 75.5 nmoles of α-ketobutyrate/mg/h ACC deamination activity which was followed by the isolates AMVPR98 and AMVPR79 with ACC deamination activity of 73.5 and 68.5 nmoles of α-ketobutyrate/mg/h respectively. The results pertaining to ACC deaminase activity in this study are also in line with the observation made by Wenbo Ma *et al.,* (2003) and Duan *et al.,* (2009).

P-solubilization

P-solubilization ability of *Rhizobium* isolates ranged from 4.5 to 10.3 mm. Out of ten native isolates, six isolates exhibited P-solubilization ability (Table 4). The isolate AMVPR 98 showed highest diameter of zone of solubilization (10.3 mm) which was followed by reference strain (PPM35B), with zone of solubilization of 9.8 mm. Isolates of *Rhizobium* species differ in the ability to produce organic acids such as acetic, propionic, glycolic, formic, lactic, succinic and fumaric acid and also differ with the synthesis of phosphatase enzyme, the production of organic acids results in a decrease in pH and producing H^+ which replaces the Ca^{2+} and release HPO_4^{2-} to the soil solution (Deubel *et al.*, 2000).

Zn solubilization

Out of ten isolates, six isolates exhibited Zn solubilization ability (Table 4). The diameter of zone of zinc oxide solubilization was ranged from 9.5 to 20.5 mm. It was highest in reference strain PPM35B (20.5mm) which is followed by AMVPR98 (10.3mm).

The ability of zinc solubilization by zinc solubilizing bacteria may be due to production of organic acids in the culture medium which might have helped in the solubilization of the zinc salts (Pannerselvam *et al.,* 2013). The solubilization of zinc by bacteria might be also due to other mechanisms which includes proton extrusion and production of chelating agents.

Si solubilization

With respect to silica solubilization, out of ten native isolates only one isolate AMVPR98 (6.5 mm) showed magnesium trisilicate solubilization, which is followed by reference strain PPM35B (5.5 mm) (Table 4).

Table.1 Sample details of native isolates of Rhizobia

Sl. No	Isolate name	Village name	District	Type of soil	Latitude (N)	Langitude (E)	Elevation
1	AMVPR06	Kannur	Vijayapur	Black soil	17^0 03' 070"	75^0 41' 961"	578
2	AMVPR10	Mahaveer nagar	Vijayapur	Red soil	17^0 01'087"	75^0 14' 048"	578
3	AMVPR32	Inchageri	Vijayapur	Red soil	17^0 26' 921"	75^0 28' 462"	535
4	AMVPR45	Lamana tanda	Vijayapur	Black soil	17^0 32' 399"	75^0 38' 183"	592
5	AMVPR53	Lamana tanda	Vijayapur	Black soil	17^0 27' 398"	75^0 38' 105"	592
6	AMVPR79	Hadalsang	Vijayapur	Black soil	17^0 53' 108"	75^0 50' 467"	504
7	AMVPR98	Hadalsang	Vijayapur	Black soil	17^0 54' 040"	75^0 51' 901"	511
8	AMVPR128	Alagudda	Kalaburgi	Black soil	17^0 '43 "753	76^0 '91 "643	491
9	AMVPR131	Dharmapura	Kalaburgi	Black soil	17^0 '23 "088	76^0 90' "261	432
10	AMVPR179	Sokanadagi	Bagalkot	Black soil	$16^0$23 '409 "	75^0 56' 645"	528

Table.2 Assessment of nodulation for native Rhizobial isolates obtained from Northern Karnataka

Sl. No	Isolate name	Number of nodules/plant
1	AMVPR06	12.50
2	AMVPR10	13.25
3	AMVPR32	13.75
4	AMVPR45	13.00
5	AMVPR53	14.00
6	AMVPR79	14.50
7	AMVPR98	14.75
8	AMVPR128	13.50
9	AMVPR131	13.10
10	AMVPR178	12.00
11	Reference strain(PPM35B)	13.25

Table.3 Quantitative estimation of growth hormones produced by native Rhizobial isolates obtained from Northern Karnataka

Isolates	IAA production (µg/ ml)	GA production (µg/ 25 ml)	ACC deaminase production (nmoles of –α-ketobutyrate/mg/h)
AMVPR-06	16.88	11.34	50.5
AMVPR-10	16.60	11.96	48.5
AMVPR-32	20.50	13.40	60.5
AMVPR-45	17.30	11.00	44.5
AMVPR-53	22.25	13.97	63.5
AMVPR-79	21.45	14.23	68.5
AMVPR-98	22.85	14.43	73.5
AMVPR-128	21.65	13.40	57.5
AMVPR-131	18.60	11.67	45.5
AMVPR-178	17.75	12.30	46.5
Reference strain (PPM35B)	22.45	14.51	75.5
S. Em. ±	**0.16**	**0.21**	**0.5**
C.D @ 1%	**0.503**	**0.64**	**1.57**

Table.4 Functional characteristics of native Rhizobial isolates obtained from Northern Karnataka

Isolates	Zone of P- solubilization (Dia in mm) & amount of Pi released(µg/ ml)	Zone of Zn-solubilization (Dia in mm)	Zone of Si-solubilization (Dia in mm)	HCN production	Siderophore production (Dia in mm)
AMVPR-06	4.5(1.14)	-	0.0	-	-
AMVPR-10	-	-	0.0	-	-
AMVPR-32	7.5(1.76)	10.5	0.0	++	8.5
AMVPR-45	-	-	0.0	-	-
AMVPR-53	8.5(2.31)	11.5	0.0	++	9.0
AMVPR-79	8.8(2.45)	12.5	0.0	+++	9.5
AMVPR-98	10.3(3.14)	16.5	6.5	+++	10.5
AMVPR-128	5.3(1.28)	9.5	0.0	++	6.5
AMVPR-131	-	-	0.0	-	-
AMVPR-178	-	-	0.0	+	-
Reference strain (PPM35B)	9.8(2.89)	20.5	5.5	+++	10.3

Note:
(-) indicates no zone of solubilisation (For P, Zn, Si solubilization)
(–) indicates - No HCN production
(+) indicates - Weak HCN production
(++) indicates - Moderate HCN production
(+++) indicates - Strong HCN production

Table.5 *In vitro* screening of Rhizobial isolates against *Fusarium oxysporum* f. sp. udum fungal pathogen of pigeon pea

Sl. No	Isolates	Per cent inhibition
1	AMVPR-06	0.00
2	AMVPR-10	0.00
3	AMVPR-32	48.75 (43.84)
4	AMVPR-45	0.00
5	AMVPR-53	53.75 (46.68)
6	AMVPR-79	68.75 (55.45)
7	AMVPR-98	73.75 (58.59)
8	AMVPR-128	0.00
9	AMVPR131	0.00
10	AMVPR178	0.00
11	Reference strain (PPM35B)	72.5 (57.78)
	S.Em. ±	**0.893**
	C.D @ 1%	**2.850**

Note: Figures in parentheses indicate Arcsine transformed values
Inhibition description: (0) indicates no inhibition

Table.6 Morphological characteristics of native Rhizobial isolates of Northern Karnataka

Isolates	Colony morphology					Cell morphology	
	Colour	Elevation	Surface	Shape	Margin	Gram reaction	Cell shape
AMVPR-06	Milky white	Convex	Moist	Circular	Entire	G-ve	Rod
AMVPR-10	Milky white	Raised	Moist	Circular	Entire	G-ve	Rod
AMVPR-32	Milky white	Raised	Moist	Circular	Entire	G-ve	Rod
AMVPR-45	Milky white	Raised	Moist	Circular	Entire	G-ve	Rod
AMVPR-53	Milky white	Convex	Moist	Circular	Entire	G-ve	Rod
AMVPR-79	Milky white	Convex	Moist	Circular	Entire	G-ve	Rod
AMVPR-98	Milky white	Convex	Moist	Circular	Entire	G-ve	Rod
AMVPR-128	Milky white	Raised	Moist	Circular	Entire	G-ve	Rod
AMVPR-131	Milky white	Convex	Moist	Circular	Entire	G-ve	Rod
AMVPR-178	Milky white	Raised	Moist	Circular	Entire	G-ve	Rod
Reference strain (PPM35B)	Milky white	Convex	Moist	Circular	Undulated	G-ve	Rod

Table.7 Biochemical characteristics of native Rhizobial isolates obtained from Northern Karnataka

Sl. No.	Isolate No.	Biochemical tests									
		Catalase Test	Starch hydrolysis	Citrate utilization	Indole production test	Methyl red test	Voges proskauer test	Urease test	Nitrate reduction test	Acid and gas Production	Oxidase test
	AMVPR-06	+	-	+	-	-	-	+	-	+	+
	AMVPR-10	+	-	+	-	-	-	+	-	+	+
	AMVPR-32	+	-	+	-	-	-	+	-	+	+
	AMVPR-45	+	-	+	-	-	-	+	-	+	+
	AMVPR-53	+	-	+	-	-	-	+	-	+	+
	AMVPR-79	+	+	+	-	+	-	+	-	+	+
	AMVPR-98	+	+	+	-	+	-	+	-	+	+
	AMVPR-128	+	-	+	-	-	-	+	-	+	+
	AMVPR-131	+	-	+	-	-	-	+	-	+	+
	AMVPR-178	+	-	+	-	-	-	+	-	+	+
	Reference strain(PPM35B)	+	+	+	-	+	-	+	-	+	+

Note:
(+) indicates positive for the test
(-) indicates negative for the test

Siderophore production

Out of ten, five isolates were found to be positive for siderophore production. The diameter of zone of clearance on CAS agar in different isolates ranged from 6.5 to 10.5 mm (Table 4). Maximum diameter of zone of clearance was observed in the isolate AMVPR98 (10.5 mm). Siderophores are known to bind to the available form of iron (Fe^{3+}) in the chickpea rhizosphere thus making it unavailable to the phytopathogens and consequently protects the plant health (Wani and Khan, 2013).

HCN production

Out of ten isolates, six isolates, as well as reference strain were able to produce HCN. Among six isolates, 2 isolates *viz.*, AMVPR98 and AMVPR79 were strong (+++) HCN producers. The reference strain has also exhibited strong (+++) HCN production ability. The isolates *viz.*, AMVPR32, AMVPR53, AMVPR128 exhibited moderate (++) HCN production. The isolate AMVPR178 was weak (+) HCN producer (Table 4).

In vitro screening of *Rhizobial* isolates against fungal pathogen (*Fusarium oxysporum* f.sp. udum) of pigeon pea

With respect to *In vitro* screening of *Rhizobium* isolates against *Fusarium oxysporum* f.sp. udum, Out of ten isolates, four isolates exhibited potential to inhibit mycelial growth of *Fusarium oxysporum* f. sp. udum (Table 5). Per cent inhibition was ranged from 48.75 to 73.75.

The maximum per cent inhibition of 73.75 was observed in AMVPR98. These results are in close agreement with the findings of Sindhu *et al.*, (2010) and Subhani *et al.*, (2013) who also reported reduction of Fusarium wilt of chickpea by *Rhizobium* sp. Similarly, Kucuk *et al.*, (2013) also observed the inhibition of some *Fusarium* sp. due to *Rhizobium* strains.

Characterization of the selected isolates for morphological traits

With respect to the morphological characterization, all the ten native isolates were found to be rod shaped and showed gram negative for gram staining reaction (Table 6). All the isolates were milky white in colour, circular in shape and has moist surface. The elevations of colonies were convex in case of AMVPR06, AMVPR53, AMVPR79, AMVPR98, AMVPR131 isolates and reference strain (PPM35B). Whereas raised elevation in case of AMVPR10, AMVPR32, AMVPR45, AMVPR128 and AMVPR 178. The margins of the colonies of all the isolates were entire where as it was undulated in reference strain. Results are in concurrence with the findings of Deka and Azad (2006) who reported that cells of the isolates of *Rhizobium* were rod shaped, motile and Gram negative.

Characterization of the selected isolates for biochemical traits

With respect to biochemical characterization, all the isolates showed positive for citrate utilization, urease, citrate, acid and gas production and oxidase tests and negative for indole production, nitrate reduction and Voges proskauer's tests. Only two isolates, AMVPR 79 and AMVPR98 were positive for methyl red test and starch hydrolysis. Similarly, Prajapati *et al.*, (2018) reported that *Rhizobium* isolates were positive for biochemical test *viz.*, catalase test, starch hydrolysis test.

In conclusion the pigeon pea is an important legume plant widely cultivated and consumed

in different parts of Karnataka. It is also used in co-cropping and intercropping systems for enhancing soil fertility through its symbiotic association with rhizobia. The present study revealed the presence of plant growth promoting rhizobial strains in the root nodules of pigeon pea plants growing in northern regions of Karnataka. The isolates have the ability to produce IAA, GA, HCN and siderophores, Solubilization of inorganic phosphate, Zinc oxide and magnesium trisilicate and have an antagonistic activity against pigeon pea Fusarium wilt caused by *Fusarium oxyporum* f. sp. udum. Hence, the isolated efficient plant growth promoting Rhizobial isolates could be used as inoculants to improve the yield of pigeon pea.

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