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Isolation and Characterization of *Rhizobium* from Green Gram (*Vigna radiata*)

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Abstract

Nitrogen is a crucial component that acts as building blocks for nucleic acids and proteins. It is abundantly present in atmosphere of the earth but plants are not able to easily utilize it. Diazotrophic microorganisms such as *Rhizobium* convert N_2 and make it available to pulse crops. In the present study,a total of thirty-four *Rhizobium* isolates were recovered, from that twenty-two with gram-negative-rods were biochemically characterized. Most of the isolates were negative for congo red (CR) absorption, glucose-peptone, lactose, and hofer's alkaline assays. These isolates have PGP (plant growth promoting) properties such as solubilisation of mineral, growth hormone, and hydrogen cyanide (HCN) productions along with antagonistic activity. Plant study revealed that SBGR25 treatment has highest germination per cent (98.0 %), shoot (10.3 cm) and total-height (16.6 cm), and dry weight (5.60 g). Hence, the present study suggests that SBGR25 strains could be used as promising bio inoculants for the pulse crop.

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Introduction

The population in the world has increased substantially and expected to increase by 9.9 billion in 2050 mainly in developing countries,[1] hence, the food demand will also increase proportionately to maintain the healthy diet.[2-4] Pulse crops are considered as important food crops throughout the World due to higher protein content, thus, it becomes imperative to increase the production of pulse crops through sustainable agriculture to reduce the dependence on imports.[5-8] Bioinoculants promote the growth and yield of the pulse crops which have

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been used to supplement and/or replace chemical fertilizers.[9-13] Rhizobacteria efficiently colonize plant root and enhancetheir growth by diverse mechanisms such as hormones production, N_2 fixation, mineral solubilization and antagonistic activities against anti-nutritional as well as pathogenic factors.[14,15]

Moreover pulses provide nutrients mainly N to the soil and makes them fertile and healthy. The bacterial colonization forms root nodules and this symbiotic association helps in biological nitrogen fixation (BNF) to make unavailable atmospheric nitrogen to available ammoniacal form for plants.[16-19] In agriculture around 80 % of BNF due to Rhizobiaceae bacteria, generally called as Rhizobia, includes six genera (*Rhizobium*, *Bradyrhizobium*, Meso*rhizobium*, *Azorhizobium*, *Sinorhizobium*, and Allo*rhizobium*).[20-22] The inoculation of *Rhizobium* increases the seed germination efficiency and thereby increase the yield of different pulse crops and shows their potential in plant growth promotion.[23-27] Hence, this study aimed to isolate, identify *Rhiozobium* and investigate PGP activities with an intention of using them for sustainable agriculture.

Materials and Methods

The root nodules along with the rhizosphere soil was collected from the young and healthy seedlings of green gram plant in Sethu Bhaskara Agricultural College and Research Foundation (SBAC&RF), Karaikudi, Sivaganga, Tamil Nadu, India and the isolates were named as SBGR (Sethu Bhaskara Green gram *Rhizobium*).

Isolation of *Rhizobium*

Three healthy nodules were detached carefully from each of five plants chosen randomly. Surface sterilization was carried out using 70 % ethanol for 30 seconds then washed in sterile water for five times, followed by immersing in 0.1% of $HgCl_2$ for 30 seconds for complete removal of surface microorganisms and rinsed for 10 times in sterile water.[28] Then, surface sterilized nodules were transferred to a minimum quantity of sterile water and crushed with blunt end of sterilized glass rod to get nodule suspension. Later,the suspension was diluted and poured to sterile petriplates followed by the addition of yeast extract mannitol agar (YEMA) medium containing congo red[29] and incubated for 24 to 48 h at 30 °C.

Characterization

Typical rhizobial colonies were recognized by their appearance on the YEMA plates and a total of 34 distinct isolates were selected and purified by several streaking, and preserved as glycerol stock for further characterization. Morphological characters such as macroscopic andmicroscopic observations were examined by the appearance of colony on CR-YEMA plate and gram staining. The following macroscopic characters such as colony colour, shape, texture, margin, opacity, and growth were observed.[30] For microscopic examination, gram reaction was performed by the preparation of smear followed by staining.[31] Twenty two isolates with gram negative and rod shaped cells were taken for biochemical methods including Methyl Red-VogesPrausker (MR-VP),[32] amylase production,[33] cellulase production[34] and citrateutilization.[35] Then, the isolates were characterized by the authentication test such as growth on CR agar,[36] bromothymol blue (BTB) reaction,[28] glucose peptone agar,[37] lactose assay[38] and hofer's alkaline assay.[39]

Screening of the Isolates

The 22 isolates were estimated for PGP characters (mineral solubilization, indole acetic acid (IAA) production, HCN production and antagonistic activity). For mineral solubilization,the isolates were point inoculated on Pikovskaya's,[40] Aleksandrov`s,[41] and Bunt and Rovira media[42] separately for P, K, and Zn solubilization, and incubated for 5 days at 30°C. The diameters of clear zones around the colonies were measured by solubilization index.[43] Indole production was performed qualitatively by point inoculation of bacterial isolates on trypton soya agar medium[44] and incubated for 5 days at 30 °C. Then, a sterile whatman filter paper saturated with few drops of salkawski's reagent was placed on bacterial growth. Appearance of pink color was marked as +, ++ and +++ for pale, moderate and dark colour for IAA production.

The bacterial isolates were streaked on Nutrient agar plate containing glycine,[45] then a sterile filter paper immersed in the solution of sodium carbonate and picric acid was placedon the top of petri-plate and incubated for 3-5 days. Formation of brown color was marked as +, ++ and +++ for pale, moderate and dark brown for HCN production. The bacterial isolates were tested to study their

effect for antagonism against three important pulse pathogens *viz*., *Colletotrichum* sp., *Macrophomina* sp., and *Aspergillus* sp.Their mycelial disc was placed at one end of the potato dextrose agar plate and bacterium was streaked at opposite side of each plate.[46] The control plate was maintained with fungal mycelium alone and incubated at 30 ºC for 7 days and expressed as per cent inhibition using the formula, C-T/C x 100 where, C and T are the radial mycelia growth in control and treated plates.[47]

Plant Assay

Among the twenty two isolates, eight (SBGR1, SBGR2, SBGR19, SBGR24, SBGR25, SBGR27, SBGR32, and SBGR34) were screened based on mineral solubilization, IAA, HCN production and antagonism taken for germination test. The bacterial isolates were inoculated in yeast extract manitol broth[28] and allowed to grow for 3 daysat 30°C. Exponentially growing cells (1 $OD@_{600nm}$) were inoculated to surface sterilized seeds.The surface sterilized seeds were treated with the isolates for 2 h, air-dried for 30 min. andtwenty five seeds were placed over filter paper in sterilepetri plates. The uninoculated seeds act as a control. Germination percent was calculated by number of emerged seedlings/number of seeds sown x 100.[48] The shoot, root and total length along with fresh and dry weight of seedlings were observed on fifth day of inoculation. For dry matter production, whole seedlings were dried in hot air oven at 70-80 °C till the constant dry weight obtained and expressed as g per 25 seedlings.

Statistical Analysis

All the data were subjected to statistical analysis with softwares, SPSS[49] and Microsoft Excel for Windows 2007 add-ins with XLSTAT Version 2010.5.05.[50] Statistically significant differences between the treatments were analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at 5 % significance level.

Results

Thirty four isolates,obtained from the nodules of green gram, were subjected to morphological characters.

Characterization

The macroscopic and microscopic characters were observed and presented in Table 1. Ninety per cent of the isolates were white to light pink in colour except SBGR19, SBGR26, and SBGR33. All the isolates being circular, translucent, gummy with entire margin and grown within 48 h were considered as fast growersexcept two (SBGR1, and SBGR2). Nearly twenty two isolates were gram negative rods which might be the *Rhizobium* and those were taken for biochemical analysisin the respective medium (Fig. 1). The results showed that many isolates were positive for indole production bythe formation of cherry red colourexcept five isolates (SBGR5, SBGR6, SBGR8, SBGR9, and SBGR15). In MRVP test, half of the isolates were positive for MR test and werenegative for VP test except 5 (SBGR3, SBGR5, SBGR6, SBGR8, and SBGR9) while few isolates (SBGR24, SBGR25, SBGR27, and SBGR28) showed positive for both tests. In citrate test, three quarters of isolates remained green in colour except eight (SBGR3, SBGR5, SBGR6, SBGR8, SBGR9, SBGR24, SBGR25, and SBGR34). Only very few isolates (SBGR1, SBGR19, SBGR24, SBGR25, SBGR32, SBGR33, and SBGR34) produced both amylase and cellulase enzymes.

Table 1: Morphological characters of the isolates.

Isolates	Macroscopic observation						Microscopic observation	
	Colony colour	Shape	Texture	Opacity	Margin	Growth	Gram staining	Shape
SBGR1	LP	Circular	Gummy	Opaque	Entire	FG	-	Rod
SBGR2	LP	Circular	Gummy	Opaque	Entire	FG	-	Rod
SBGR3	White	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR4	White	Circular	Gummy	Translucent	Entire	FG	+	Cocci

SBGR5	White	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR6	MW	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR7	White	Circular	Gummy	Translucent	Entire	FG	+	Rod
SBGR8	MW	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR9	Pink	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR10	White	Circular	Gummy	Translucent	Entire	FG	+	Rod
SBGR11	White	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR12	White	Circular	Gummy	Translucent	Entire	FG	+	Rod
SBGR13	White	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR14	White	Circular	Gummy	Translucent	Entire	FG	+	Rod
SBGR15	MW	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR16	MW	Circular	Gummy	Translucent	Entire	FG	+	Cocci
SBGR17	White	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR18	White	Circular	Gummy	Translucent	Entire	FG	+	Rod
SBGR19	Red	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR20	White	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR21	LP	Circular	Gummy	Translucent	Entire	FG	+	Rod
SBGR22	White	Circular	Gummy	Translucent	Entire	FG	+	Cocci
SBGR23	LP	Circular	Gummy	Translucent	Entire	FG	+	Rod
SBGR24	LP	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR25	LP	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR26	Red	Circular	Gummy	Translucent	Entire	FG	+	Rod
SBGR27	LP	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR28	White	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR29	White	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR30	White	Circular	Gummy	Translucent	Entire	FG	+	Cocci
SBGR31	White	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR32	LP	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR33	Red	Circular	Gummy	Translucent	Entire	FG	-	Rod
SBGR34	LP	Circular	Gummy	Translucent	Entire	FG	-	Rod

LP-light pink; MW-milky white; FG-Fast grower, - negative and + Positive.

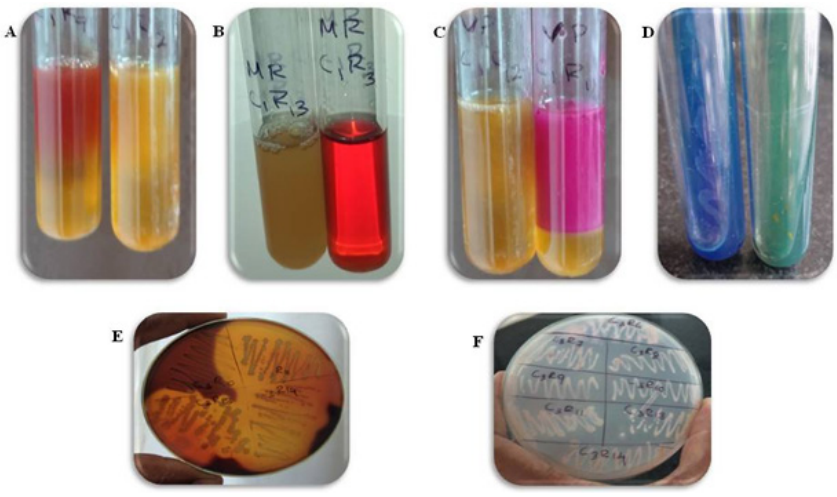

Fig. 1: Biochemical characterization of the isolates. Indole test (A), MR test (B), VP test (C), Citrate utilization (D), Amylase production (E), and Cellulase production (F) of the isolates

The presumptive tests of *Rhizobium* were conducted for all the twenty two isolates to examine their characters (Fig. 2). Ninety per cent of the isolates were white to pale pink in colour and did not absorb the red colour in CR medium. In BTB test many isolates changed their colour from deep green to yellow. These were considered as fast growers except few (SBGR17, SBGR19, SBGR25, SBGR27, and SBGR28). Thirty one isolates did not change the colour in Glucose peptone agar medium which were considered as positive and might be *Rhizobium* while three isolates (SBGR13, SBGR28, and SBGR33) produced yellow halo around the colony. Twenty nine isolates did not show yellow halo on lactose agar medium and considered as positive for *Rhizobium* except few (SBGR5, SBGR6, SBGR8, SBGR15, and SBGR33). Nearly half of the isolates did not grow on Hofer's alkaline liquid medium which might be *Rhizobium* except 9 isolates (SBGR3, SBGR5, SBGR6, SBGR8, SBGR9, SBGR15, SBGR17, SBGR31, and SBGR33).

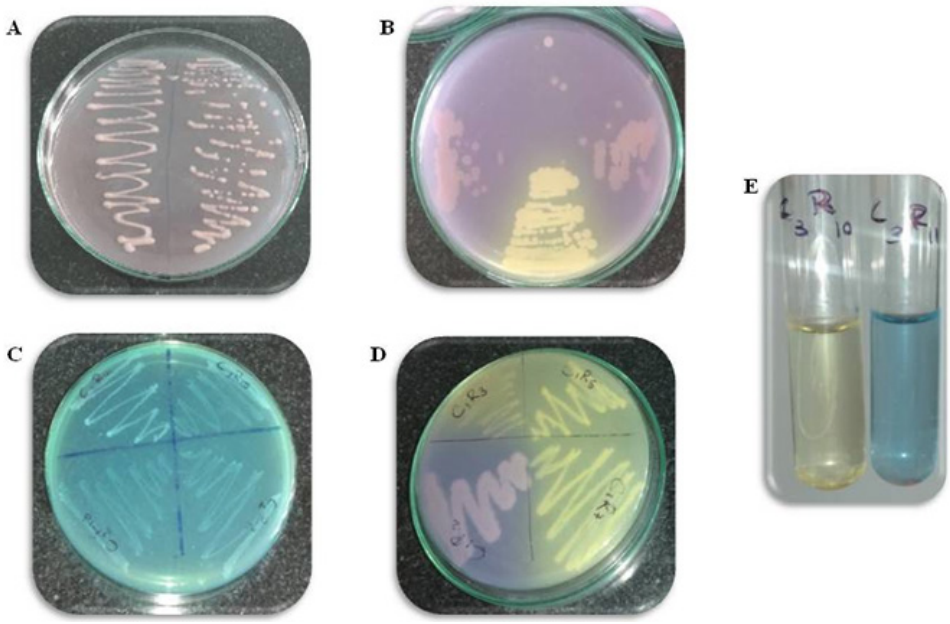

Fig. 2: Cultural and metabolic characterization of the isolates. Growth on CRYEMA medium (A), *Rhizobium* colonies appeared white translucent; Growth on glucose peptone medium (B), *Rhizobium* exhibited poor growth cause little change in pH; Growth on lactose agar medium (C), *Rhizobium* exhibited no yellow colour; Growth on BTB YEMA medium (D), fast growing *Rhizobium* exhibited yellow colour within 3 days of incubation while slow growing bacteria showed colour change in 7 days; Growth on Hofer's alkaline liquid medium (E), *Rhizobium* did not grow

Screening of the Isolates

All the isolates have solubilized phosphorous and highest solubilization index was observed in SBGR28 with 1.71 SI followed by SBGR2 (1.67 SI) while the least was observed in SBGR6 with 1.09 SI. The isolates SBGR8 and SBGR29 showed significantly highest solubilization index for K with 2.00 SI followed by SBGR11 (1.50 SI) while the least was observed in SBGR24 (1.08). Only half of the isolates solubilized zinc, in that SBGR2 had significantly higher solubilization index of 2.60 followed by SBGR28 (2.33 SI) (Table 2).

In qualitative assay of IAA and HCN, most of the isolates showed pink colour appearance by the addition of salkawski reagent and brown colour in picric acid filter paper. *Rhizobium* isolates tested for antagonistic activity towards three fungal pathogens (*Colletotrichum* sp.,*Macrophomina* sp.,and *Aspergillus* sp.) have been furnished in

table 2 and fig. 3. The result showed only a very few strains such as SBGR1, SBGR19, SBGR24, SBGR25, SBGR27, SBGR32, and SBGR34 which have inhibited all the three tested pathogens. Among them, the isolate SBGR34 showed significantly highest inhibition of 55.6 %, 61.1 %, and66.7 % for *Colletotrichum* sp., *Macrophomina* sp., and *Aspergillus* sp., respectively.

Table 2: Screening of the isolates for PGP activities.

Isolates	Mineral solubilization			IAA	HCN	Antagonistic activity		
	P	K	Zn			*Colletotrichum* sp.	*Macrophomina* sp.	*Aspergillus* sp.
SBGR1	1.20^{h}	-	1.80^{e}	+++	+++	50.0^{b}	44.4^{d}	55.6^{c}
SBGR2	1.67^{b}	1.14^{h}	2.60^{a}	++	+++	44.4^{c}	-	66.7^{a}
SBGR3	1.20^{h}	-	-	+	-	33.3^{e}	-	-
SBGR5	1.22^{f}	1.17^{g}	-	-	-	27.8^{f}	-	-
SBGR6	1.09^{m}	1.40^{C}	-	-	-	-	-	-
SBGR8	1.22^{f}	2.00^{a}	-	-	-	-	-	-
SBGR9	1.14^{k}	1.20^{f}	-	-	-	-	-	-
SBGR11	1.20^{h}	1.50^{b}	-	++	-	27.8^{f}	-	-
SBGR13	1.14^{k}	-	-	++	-	27.8^{f}	-	-
SBGR15	1.14^{k}	1.17^{g}	1.70^{g}	-	-	-	27.8^{e}	-
SBGR17	1.25^{e}	1.20^{f}	1.33^{k}	++	-	44.4^{c}	-	-
SBGR19	1.20^{h}	-	1.50^{i}	+++	+++	38.9^{d}	50.0^{c}	61.1^{b}
SBGR20	1.33^{c}	-	-	+++	-	33.3^{e}	-	-
SBGR24	1.27^{d}	1.08^{j}	1.75^{f}	+++	+++	44.4^{c}	50.0^{c}	61.1^{b}
SBGR25	1.12^{l}	1.11^{i}	1.86^{d}	++	+++	55.6^{a}	44.4^{d}	66.7^{a}
SBGR27	1.20^{h}	1.20^{f}	1.50^{i}	+++	+++	50.0^{b}	55.6^{b}	61.1^{b}
SBGR28	1.71^{a}	-	2.33^{b}	-	++	33.3^{e}	-	-
SBGR29	1.20^{h}	2.00^{a}	-	+	+	50.0^{b}	-	-
SBGR31	1.19^{i}	-	-	+++	-	27.8^{f}	-	-
SBGR32	1.21^{g}	1.25^{e}	1.60^{h}	++	++	38.9^{d}	55.6^{b}	61.1^{b}
SBGR33	1.15^{j}	1.17^{g}	2.09^{c}	++	++	27.8^{f}	-	46.7^{d}
SBGR34	1.14^{k}	1.33^{d}	1.43^{j}	+++	+++	55.6^{a}	61.1^{a}	66.7^{a}

+, ++ and +++ for pale, moderate and dark colour.

Effect of *Rhizobium* Inoculation on Germination of Lentil Seed

Based on these PGP attributes, eight strains (SBGR1, SBGR2, SBGR19, SBGR24, SBGR25, SBGR27, SBGR32, and SBGR34) were chosen for plant study. These isolates were inoculated to the surface sterilized green gram seeds. Table 3 and fig. 4 showed the effect of rhizobial isolates ongermination per cent. The less significant variation were observedamong the treatments. The isolate, SBGR25 hashigh per cent germination of 98.0 % while uninoculated control showed least (84.0 %).The shoot, root and total height of germinated seedlings were measured and the result showed that the green gram seed treated with SBGR25 showed significantly highest shoot height of 10.3 cm followed by SBGR1 (7.95 cm). The root height of SBGR24 treatment showed highest with 6.35 cm and it was on par with SBGR25 treatment (6.00 cm). Similarly, the total plant height was significantly highest in SBGR25 with 16.6 cm followed by SBGR1 (13.2 cm). In all the cases, the untreated control showed least values of 4.35, 4.10 and 8.50 cm, respectively for shoot, root, and total plant heights. The fresh weight of SBGR25 showed highest value of 5.60 g and was on par with SBGR1 (5.54 g) and the least being observed in SBGR19 (4.25 g) while the dry weight was highest in SBGR2 (0.59 g)at par with SBGR25 (0.58 g).

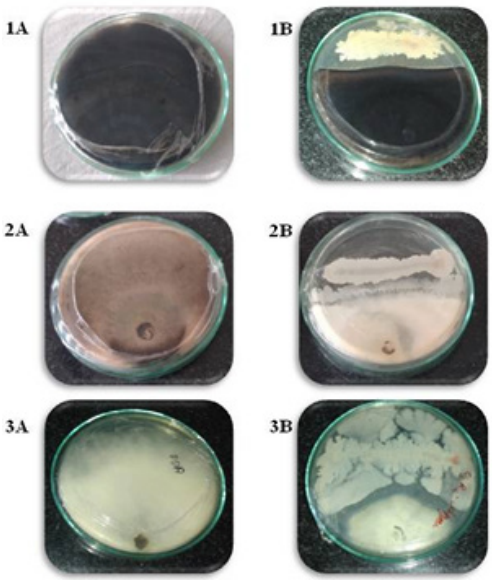

Fig. 3: Antagonistic activity of Rhizobium isolates. The antagonistic activity of the isolates was performed in PDA medium. It showed the growth of *Colletotrichum* sp. (1), *Macrophomina* sp. (2), and *Aspergillus* sp. (3) in control plate (A) and these pathogens were inhibited by *Rhizobium* (B)

Table 3: Effect of Rhizobium on germination and seedling growth of green gram.

Treatments	Germination percentage	Shoot height (cm)	Root height (cm)	Plant height (cm)	Fresh weight (g)	Dry weight (g)
Control	84.0 (±4.00)b	4.35 (±0.15)^{f}	4.10 (±0.10)^{d}	8.50 (±0.25)^{d}	4.92 (±0.20)^{ab}	0.54 (±0.02)^{ab}
SBGR1	94.0 (±6.00)ab	7.95 (±0.05)^{b}	5.20 (±0.20)^{b}	13.2 (±0.25)^{b}	5.54 (±0.15)^{a}	0.44 (±0.00)^{c}
SBGR2	90.0 (±6.00)ab	7.80 (±0.10)^{bc}	4.55 (±0.15)^{cd}	12.4 (±0.25)^{bc}	4.95 (±0.05)^{ab}	0.59 (±0.05)^{a}
SBGR19	92.0 (±4.00)ab	7.20 (±0.10)^{de}	4.60 (±0.10)c^{d}	11.8 (±0.20)^{c}	4.25 (±0.20)^{d}	0.50 (±0.03)^{b}
SBGR24	96.0 (±4.00)ab	6.70 (±0.20)^{e}	6.35 (±0.10)^{a}	12.7 (±0.30)^{bc}	4.46 (±0.05)^{c}	0.45 (±0.01)^{c}
SBGR25	98.0 (±2.00)a	10.3 (±0.15)^{a}	6.00 (±0.15)^{a}	16.6 (±0.30)^{a}	5.60 (±0.08)^{a}	0.58 (±0.04)^{a}
SBGR27	94.0 (±2.00)ab	7.40 (±0.10)^{cd}	4.90 (±0.20)^{bc}	12.3 (±0.30)^{bc}	4.90 (±0.10)^{ab}	0.56 (±0.01)^{ab}
SBGR32	86.0 (±2.00)ab	7.55 (±0.25)^{bcd}	4.55 (±0.15)^{cd}	12.1 (±0.40)^{bc}	4.80 (±0.05)^{b}	0.45 (±0.01)^{c}
SBGR34	94.0 (±2.00)ab	7.30 (±0.20)^{cd}	4.55 (±0.25)^{cd}	11.9 (±0.45)^{c}	4.59 (±0.08)^{c}	0.50 (±0.02)^{b}

Values are mean ± SE (n=3) and within each row, values followed by same letters are not significantly different from each other according to DMRT (p≤0.05).

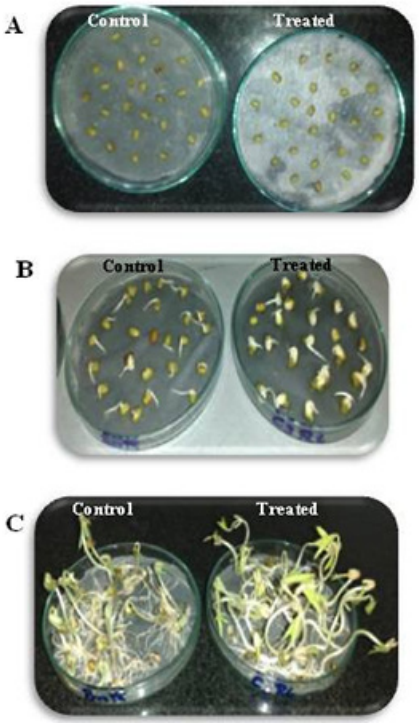

Fig. 4: Effect of *Rhizobium* isolates on seed germination and growth. The plate showing the germination of green gram seeds on 0th (A), 1st (B) and 5th (C) day of experiment

Discussion

The atmospheric nitrogen is not readily available to plants because of its inert nature. The triple bond between two N molecules of atmospheric N_2 must be broken for converting it to available form and bind with C, H, and O to form the building blocks of living organisms, for example, nucleotides, amino acids and proteins.[51] Therefore, nitrogen fixation is essential for agriculture.[52] The ability to reduce atmospheric N is restricted only to diazotrophs and a few diazotrophic microorganisms live in a symbiotic relationship with legume plants (*Rhizobium*), non-legumes (*Frankia*), waterfern, cycads and angiosperms (*Nostoc*), and also free living bacteria in the soil (*Azotobacter*).[53] The inoculation of *Rhizobium* increases the plant growth promotion by increasing seed germination, shoot-root length, plant growth parameters thus ultimately increase the yield of the crops.[24,26,27] In this study, the *Rhiozobium* was isolated from the nodules of green gram and investigatedfor their PGP activities.

Isolation of Root Nodule Bacteria

Rhizobium have been isolated from many pulse crops such as green gram, black gram, cow pea and faba bean[54-57] and here, we isolated 34 strains from green gram.

Characterization of *Rhizobium*

Phenotypically, many strains develop colony within 2-3 days and would not absorb the indicator,congo red and remain white, translucent, glistening, elevated and comparatively smaller. These characters were similar to *Rhizobium*as reported earlier.[58] Several biochemical tests such as methyl red, and Voges Proskauer test, citrate utilization, indole, gelatinase activity, and starch hydrolysis were conducted as described by researcher.[59] Similarly, the present study carried IMViC tests (Fig. 1), which showed that the medium remains red in colour after the inoculation followed by incubation for MR test and changed to yellow for VP test for many isolates. Many strains showed positive result for *Rhizobium* in Simmon's citrate agar medium.

In cultural and metabolic characterization, among twenty two isolates, nineteen were considered as positive for *Rhizobium.* Hence, they did not change colour in Glucose peptone medium. Similarly, no growth was found in Hofer's alkaline liquid medium for the following thirteen isolates viz., SBGR1, SBGR2, SBGR11, SBGR13, SBGR19, SBGR20, SBGR24, SBGR25, SBGR27, SBGR28, SBGR29, SBGR32, and SBGR34. Therefore these were considered as positive and a few evidences also showed that these characters confirm the *Rhizobium* spp.[38]

Screening of the Isolates

The mineral solubilization of SBGR2, SBGR15, SBGR17, SBGR24, SBGR25, SBGR27, SBGR32, SBGR33, and SBGR34 showed that these have solubilized all the ions tested (P, K and Zn). The phosphorous solubilization of SBGR[28] isolate had the highest solubilization index of 1.71 followed by SBGR2 (1.67). SBGR8 and SBGR29 isolates showedpotassium releasing ability with 2.00 SI while SBGR2 showed highest zinc solubilization index of 2.60.The P and Zn solubilization was observed in *R.radiobacter* LB2 and this strain showed highest zinc solubilization of 2.6 zsi

(zinc solubilization index) which enhanced the yield of lettuce crop even in saline conditions.[60] Few scientists also observed the dissolution of potassium from gluconite ore by *R.leguminosarum*(25.88 %), and R.rhizogenes(23.60 %)[61]

IAA production was qualitatively estimated for these strains and the results showed the formation of pink colour colonies on trypton soya agar medium when added with salkawski reagent. Few reports evaluated the effect of *R. leguminosarum* bv*. trifolli* strain E-11 on rice by growth due to production of IAA[62-64] and cytokinin where *R. radiobacter* LB2 produced high amount of IAA (110.7 µg ml^{-1}).[60]

Rhizobia has the ability to inhibit soil-borne pathogenssuch as *Fusarium*, *Sclerotium*, *Macrophomina*, and *Rhizoctonia*[65-67] by the production of compounds such as antibiotics, mycolytic enzymes, HCN, and siderophore. Here also, HCN production was observed qualitatively on glycine nutrient agar medium. Nearly half of the isolates showed brown colour in picric acid filter paper. Dual culture technique for pathogenecity test revealed that the isolate SBGR34 has significantly highest inhibition of 55.6, 61.1, and 66.7 % for *Colletotrichum* sp., *Macrophomina* sp., and *Aspergillus* sp., respectively followed by SBGR25 (55.6, and 66.7 % for *Colletotrichum* sp., and *Aspergillus* sp., respectively).

Plant Assay

In the germination assay, SBGR25 treated seeds have significantly high per cent germination of 98.0 %. Few reports found that Brady *rhizobium* strains (AHR-2, AHR-5, and AHR-6) significantly increased the germination by 26% along with higher seedling biomass (80-90% increases) in ground nut crop.[68] The treatment, SBGR25 showed significantly highest shoot, total height and fresh weightwith 10.3 cm, 16.6 cm and 5.60 g, respectively. Many reports have concluded that the *Rhizobium* inoculation enhance the shoot and root length, dry matter production and the yield of crop plants.[69-75]

The present study reports that based on morphological, biochemical and cultural characteristics, the root nodule bacteria isolated from green gram nodules might be *Rhizobium.* Most of these strains have PGP traits such as mineral solubilisation, production of IAA and HCN along with antagonistic property against pathogens can enhance the plant growth and promotion. It was evident in germination test that the strain SBGR25 showed highest plant attributes and suggest that this strainmay be used as promising bio-inoculant to increase the yield of pulse crops. Further, molecular identification of isolates is needed for their confirmation as *Rhizobium*and also field studies are required for their evaluation.

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Conflict of Interest

The authors declare no conflicts of interest.

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