Research Article

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Isolation, characterization and purification of *Rhizobium* strain to enrich the productivity of groundnut (*Arachis hypogaea* L.)

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Abstract: Groundnut (*Arachis hypogaea* L.) is an important food legume in tropical and subtropical areas because of its ability to adapt to a wide range of agro-climatic regions. Groundnut is usually cultivated in nutrient-poor soil and rain-fed conditions, so average yield tends to be very low relative to potential yield. Even though the nitrogen (N) requirement of groundnut is much higher than cereals due to its high protein content, it has the capacity to meet 60-80% of N-based requirements through symbiotic N fixation via its root nodules. In its symbiotic relationship with legumes, *Rhizobium* fixes N, thereby positively impacting the content of this nutrient. This study aimed to isolate, characterize and purify microbial strains of Rhizobium specific to groundnut in a bid to increase this legume's productivity. The research was conducted in the AICRP-Groundnut laboratory and greenhouse of the Directorate of Research, BCKV, in Kalyani, India during October 2016 to March 2017. Two *Rhizobium* isolates (RhBC and NRA1) were isolated and selected from groundnut pot cultures. After 45 days, NRA1 produced higher plant biomass, longer roots and shoots, more nodules and higher nodule dry weight than RhBC. NRA1 was selected for a future field trial. The two isolated microbial strains will aid in the screening of additional local isolates to test their effectiveness when co-cultured with local groundnut cultivars to increase yield in soil with low fertility.

Keywords: Groundnut, nitrogen, nodulation, *Rhizobium*

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1 Introduction

Groundnut, otherwise more commonly referred to as peanut (*Arachis hypogaea* L.), is an important food legume in tropical and subtropical areas and is grown in 94 countries under different agro-climatic regions (Table 1). Groundnut can grow in poorly fertile and rainfed conditions. This may explain why yield in some countries, such as India and Nigeria, are lower than in Brazil or Argentina, even though it is grown over a larger area in the former countries (FAO 2018). As a result, in countries where groundnut is grown in nutrient-poor conditions, farmers are unable to increase yield (Gunri et al. 2017). Separately, the nitrogen (N) requirement of groundnut is much higher (150-200 kg ha^{-1}) than cereals, due to its high protein content; despite this, groundnut is able to meet 60-80% of its N requirements by symbiotic N fixation via root nodules and only 20-40% from soil N, thereby impacting yield (Singh et al. 2003). The high N-based fertilization of crops in some countries may explain the high yield to area ratio seen in Table 1.

Several studies have shown that *Rhizobium*, a Gram-negative N-fixing soil bacterium, has a positive impact on legumes (Gouda et al. 2018), including groundnut, increasing its productivity while reducing production costs (Lamas et al. 2000). However, the legume-*Rhizobium* symbiosis is very specific (Andrews and Andrews 2017). El-Ghandour et al. (1997) noted that inoculation with *Bradyrhizobium* increased nodulation and N content in groundnut plants, saving >50% of the recommended N fertilizer. Bhuiyan et al. (1998) noted, compared to the control, higher nodulation, dry matter production and nut yield in inoculated (with *Rhizobium* sp.) groundnut. Pulatova et al. (1999) reported that the inoculation of three groundnut varieties with the industrial strain

CIAM0104 *Bradyrhizobium* sp. (*Arachis*) increased the dry mass of plants and 1000 seeds, seed yield, and total number of nodules. Deshwal et al. (2003) found that groundnut seeds coated with one of three *Bradyrhizobium* strains (AHR-2, AHR-5, and AHR-6) showed significantly enhanced germination (as much as a 26% increase), and higher seedling biomass (mostly 80-90% increases), nodule number (in excess of a three-fold increase), nodule fresh weight (as much as a 10-fold increase) and average nodule weight (about a three-fold increase) compared to the uninoculated control. Biswas et al. (2003) noted that *Bradyrhizobium* significantly increased groundnut nodule number, nodule weight, plant height, shoot weight, root weight, pod yield and N content. Rahman (2006) found that while *Bradyrhizobium* inoculation improved groundnut yield significantly, most other yield attributes were not significantly affected. Brahmaprakash et al. (2007) found that nodule number plant^{1} and grain yield improved after inoculation with liquid *Rhizobium* inoculants compared to carrier-based *Rhizobium* inoculants in groundnut. *In vitro* trials conducted by Badawi et al. (2011) showed that *Bradyrhizobium* was unable to produce indole-3-acetic acid (IAA) unlike two other rhizo-symbionts, *Serratia marcescens* and *Trichoderma harzianum*. A local Pakistani *Rhizobium* strain improved vegetative growth and yield parameters of groundnut cv. 'Chakori' in the absence of any fertilizer (Sajid et al. 2011). In contrast, a local Malaysian *Rhizobium* strain improved vegetative growth and yield parameters of groundnut, but only when phosphorous and N fertilizer were supplemented (Hasan and Sahid, 2016). In all these previous studies, groundnut production was enhanced if a *Rhizobium* or *Bradyrhizobium* inoculum was applied to soil.

The application of chemical fertilizers negatively affects the environment and increases production costs, so eco-friendly and cost-effective agro-technologies to increase groundnut production are required, including microbial interventions through biofertilizers such as a *Rhizobium* inoculum. Even though several studies, as documented above, have assessed the effect of *Rhizobium* inoculation on the ability to enhance the growth and productivity of groundnut, there is a constant need to screen local isolates and test their effectiveness against local varieties (de Rosália e Silva Santos et al. 2017). With this in mind, and considering the low productivity of groundnut production in India (Table 1), and previous unsuccessful attempts by Sharma et al. (2011) and Pandeeswari and Kalaiarasu (2017) to increase yield in groundnut after inoculation with *Rhizobium*, our objective was to isolate, characterize and purify a local microbial strain for groundnut to enrich its productivity in soil with low fertility.

2 Materials and methods

2.1 Location of the research

This study was part of an advanced research training program entitled "Isolation, characterization and purification of microbial strains for legume *Arachis hypogaea* L. to enrich productivity". Research was carried out in the laboratory of AICRP-Groundnut and in the greenhouse of the Directorate of Research, BCKV, Kalyani, Nadia, West Bengal, India between October 2016 and March 2017. The experimental procedures related to the isolation, characterization and identification of *Rhizobium* isolates and the efficiency test of selected isolates are described, as follows. .

2.2 Experimental materials

To isolate *Rhizobium*, one high-yielding groundnut variety TG 51 and one advanced line TG 71 were grown in separate pots (Figure 1A) and were not inoculated. TG 51 is a new Trombay groundnut (TG) variety, which was released in 2008 by the Central Sub-Committee on Crop Standards, Release and Notification of Varieties, Ministry of Agriculture, Government of India for cultivation in both *rabi* and summer season (October–May) in Odisha, West Bengal, Bihar and northeastern states of India (zone IV). TG 51 is a Spanish bunch (*Arachis hypogaea* ssp. *fastigiata* var. *vulgaris*) variety bred at the Bhabha Atomic Research Centre (BARC), Trombay by crossing TG 26 with Chico in 1998 (Kale et al. 2009). TG 26 is an early maturing, semi-dwarf, erect variety with high shelling outturn, high pod growth rate, high harvest index and large partitioning efficiency (Kale et al. 1997; Badigannavar et al. 2002) while Chico is an early maturing germplasm line selected from PI 268661 (Bailey and Hammons 1975). TG 71 is an advanced BARC line. It is also an early maturing, semi-dwarf, erect variety with high shelling outturn, high pod growth rate, high harvest index and large partitioning efficiency advanced line for both *rabi* and summer seasons (October–May) in Odisha, West Bengal, Bihar and north-eastern states of India (BCKV 2016).

2.3 Isolation of efficient *Rhizobium* isolates

Rhizobium was isolated from root nodules of two healthy and actively growing plants of each cultivar by carefully uprooting whole plants at 45 days after emergence, which is characterized by maximum nodulation, to obtain intact roots with adjoining root nodules. Detached nodules were washed under running tap water until adhering soil particles were fully removed followed by a wash in distilled water. Nodules were dipped in 0.1% mercuric chloride solution for 30 s, followed by 70% ethanol for another 30 s and rinsed thoroughly (10 times) with sterilized distilled water (Russell et al. 1982). Surface-disinfected nodules were transferred to test tubes containing 5 mL of sterilized distilled water where they were crushed with a sterilized glass rod to obtain a milky suspension. A 0.1 mL aliquot of this suspension was plated on yeast extract mannitol agar (YEMA) medium plates (HiMedia, Mumbai, India) containing Congo red following the pour plate technique (Kenasa et al. 2014). YEMA medium contains 10.0 g L^{-1} mannitol, 1 g L^{-1} yeast extract, 0.5 g L^{-1} K_2HPO_4, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.1 g L^{-1} NaCl, 0.025 g L^{-1} Congo red, and 20.0 g L^{-1} agar powder (Kenasa et al. 2014). Culture medium pH was adjusted to 6.8±0.2 at 25°C (Pelczar et al. 1977), then autoclaved. Sterilized YEMA medium was poured into Petri dishes with plated *Rhizobium* suspension. After medium

Figure 1: (A) Groundnut (*Arachis hypogaea* L.) varieties TG 51 (left) and TG 71 (right) were grown in separate pots to isolate *Rhizobium* isolates. (B) Pure *Rhizobium* isolate grown in plate with YEMA medium. (C) Catalase test: appearance of gas bubbles indicated the presence of catalase in the bacterial isolates. (D) Citrate test: after incubation for 24 h, green turned to blue, indicating a positive result (i.e., citrate utilization). (E) Indole test: formation of a red ring indicated a positive result (i.e., indole production) but a yellow ring indicated a negative result. (F) Congo red test: *Rhizobium* colonies formed on Congo red YEMA medium are white and lack the ability to absorb Congo red from YEMA medium. Common contaminants are *Agrobacterium* colonies on YEMA media. *Agrobacterium* readily absorbs Congo red and becomes pink initially and then turns deep black (top right of Petri dish). (G) Bromothymol blue test: Positive samples are yellow, indicating acid production, after incubation for 48 h at 28 °C. (H) *Rhizobium* isolates (RhBC, NRA1) were transferred to 250 mL conical flasks containing 40 mL yeast-extract mannitol and incubated for three days. (I) Prepared surface-sterilized seeds for sowing in pots. (J1, J2) Air-dried seeds with *Rhizobium* isolates were sown in pots containing sterilized soil and placed in a greenhouse. (K) Sampling procedure: Plants in each pot were uprooted and cleaned at 45 days after inoculation with *Rhizobium* isolates

Table 1: Countries around the world that produce groundnut (with shells), listed alphabetically in terms of harvested area and yield, including official, calculated and unofficial figures (FAO, 2018). Data presented is the most recent data.

Country	Harvested area (ha)	Yield (hg ha^{-1})	Country	Harvested area (ha)	Yield (hg ha^{-1})
Algeria	3.391	20.914	Iran (IR)	3.364	43.881
Angola	343.127	7.123	Iraq	1.125	33.705
Argentina	341.838	29.286	Israel	3.100	51.613
Australia	6.932	28.250	Jamaica	2.332	12.453
Bangladesh	35.726	17.428	Japan	6.550	23.664
Barbados	9.00	18.440	Kazakhstan	30	14.341
Belize	133	10.752	Kenya	13.442	24.516
Benin	159.414	8.607	Kyrgyzstan	190	12.089
Bolivia	19.207	10.823	Lao (PDR)	26.680	23.688
Botswana	1.145	3.924	Lebanon	1.689	33.065
Brazil	154.556	36.542	Liberia	8.009	7.764
Bulgaria	181	21.313	Libya	9.123	18.018
Burkina Faso	420.000	7.993	Madagascar	82.694	7.050
Burundi	13.657	5.378	Malawi	369.987	7.435
Cabo Verde	368	5.236	Malaysia	79	29.401
Cambodia	18.000	15.873	Mali	432.354	8.658
Cameroon	453.826	16.475	Mexico	56.273	17.057
Central African Republic	112.426	12.020	Morocco	15.345	23.729
Chad	971.303	10.708	Mozambique	396.644	2.853
China (incl. Taiwan)*	4.541.541	36.741	Myanmar	989.174	15.896
Colombia	1.766	11.264	Namibia	657	3.212
Comoros	1.135	8.426	Nicaragua	42.280	44.081
Congo	52.612	4.995	Niger	771.075	5.882
Congo (DR)	495.000	8.514	Nigeria	2.680.000	11.301
Costa Rica	197	11.366	Pakistan	89.469	9.755
Côte d'Ivoire	83.019	13.459	Peru	4.089	17.065
Cuba	6.492	10.501	Philippines	23.522	11.870
Cyprus	76	34.474	Rwanda	26.065	4.320
Dominican Republic	4.370	13.398	Saudi Arabia	422	40.476
Ecuador	17.966	12.187	Senegal	880.000	8.170
Egypt	58.000	32.908	Somalia	8.199	10.507
Eritrea	3.156	7.253	South Africa	22.600	7.823
Eswatini	5.660	3.951	Spain	456	29.804
Ethiopia	74.861	17.317	Republic of Korea	4.334	26.293
Gabon	18.012	10.346	Sri Lanka	13.679	20.655
Gambia	118.452	9.274	Sudan	2.315.040	7.888
Georgia	211	9.912	Syria	5.261	26.865
Ghana	336.450	12.400	Tajikistan	2.891	30.001
Greece	512	35.597	Tanzania (UR)	780.000	7.051
Guatemala	2.027	49.168	Thailand	19.335	16.574
Guinea	271.242	13.853	Uganda	420.000	5.000
Guinea-Bissau	38.767	11.540	USA	626.060	41.186
Guyana	300	6.433	Uzbekistan	1.634	42.984
Haiti	42.232	8.810	Venezuela	483	30.455
Hungary	1	9.998	Vietnam	184.792	23.117
India	5.800.000	11.822	Zambia	216.569	7.333
Indonesia	366.256	13.786	Zimbabwe	200.000	2.900

* Values for Taiwan are 19.562 and 31.608, respectively.

solidified, Petri dishes were sealed by Parafilm (Praxor Instruments and Scientific Co. (Proxor Group), Porur, Chennai, India) to avoid contamination and incubated at 28±1°C for 24-48 h. White, translucent, elevated and mucilaginous *Rhizobium* colonies formed after 24-48 h of incubation, and typical single colonies were restreaked 3-4 times on fresh YEMA plates to obtain pure cultures (Figure 1B). The Gram reaction of the isolates was tested by Gram staining (Arora 2003) and biochemical tests were performed on the isolates, as indicated next.

2.4 Morphological characterization of isolated *Rhizobium* isolates

Circular *Rhizobium* colonies with a raised smooth edge and musky odor were observed under an Olympus CH20i Biological Microscope (Vision Micro Systems, Kolkata, India) following Gram staining. Pink rods were characterized as Gram-negative.

2.5 Qualitative biochemical characterization of isolated *Rhizobium* isolates

2.5.1 Oxidase test

Oxidase activity of *Rhizobium* was determined by the oxidase test (Steel 1961). Oxidase reagent was prepared by dissolving 1% *N*, *N*, *N*, *N*-tetramethyl-*p*-phenylene diamine (HiMedia) in warm water and stored in a dark bottle at 25-30°C. A strip of filter paper (IndiaMART, Kolkata, India) was dipped in this reagent then 24 hold *Rhizobium* colonies (one colony/strip) were transferred to the filter paper strip with a sterile needle (i.e., the wet filter-paper method). Oxidase-positive colonies turned lavender, then dark purple to black within 5 min.

2.5.2 Catalase test

Catalase (CAT; EC 1.11.1.6) activity of *Rhizobium* was determined by the CAT test by placing 24 h colonies on a glass slide and adding one drop of 30% H_2O_2 (Merck Life Science Private Limited, Mumbai, India) (Figure 1G). The appearance of gas bubbles indicated the presence of CAT (MacFaddin 1980).

2.5.3 Citrate test

The ability to use citrate was determined by replacing mannitol in YEM agar with an equal amount of sodium citrate (Ranbaxy Lab. Ltd., S.A.S. Nagar, India) and bromothymol blue (Fisher Scientific, Thermo Electron LLS India Pvt. Ltd., Kolkata, India) (25 mg L^{-1}). Fresh cultures on modified media were incubated for 48 h (Koser 1923). After incubation, a positive result was indicated when green turned to blue (Figure 1D).

2.5.4 Gelatinase test

Log phase cultures from YEM broth were swabbed onto the surface of YEM agar plates containing 0.4% (*w*/*v*) gelatine (India Gelatine & Chemicals Ltd., Vapi, India) to examine gelatinase activity. Plates were incubated at 28±1°C for 7 days (Sadowsky et al. 1983). The formation of a clear zone around the culture indicates a positive result.

2.5.5 Indole test

The indole test followed the procedure of MacFaddin (2000) and Hemraj et al. (2013). Tryptone (Jeevan Chemicals & Pharmaceuticals, Mumbai, India) broth medium was prepared and poured into 10 mL test tubes (4 mL/test tube; Garg Process Glass India Pvt. Ltd., Mumbai, India) then autoclaved (121°C; 15 psi; 15 min). *Rhizobium* was inoculated in broth and incubated at 30±2°C for two days. Uninoculated broth served as the control. After incubation, 1 mL of Kovac's reagent (isoamyl alcohol, *para*-dimethylaminobenzaldehyde and concentrated hydrochloric acid) was added to each test tube, including the control. Tubes were shaken gently every 10-15 min and allowed to stand until the reagent surfaced. The formation of a red ring indicated a positive result, but a yellow ring indicated a negative result (Figure 1E).

2.5.6 Congo red test

Rhizobium colonies that form on Congo red YEMA medium are white, translucent, glistering, elevated and small. Common contaminants are *Agrobacterium* colonies on YEMA media. Most *Rhizobia*, including *Bradyrhizobium*, lack the ability to absorb Congo red, added at a final concentration of 0.0025% (*w*/*v*), from YEMA medium (Kneen and LaRue 1983; Ondieki et al. 2017) while *Agrobacterium*

absorbs Congo red readily and becomes pink initially and then turns deep black (Fentahun et al. 2013; Figure 1C).

2.5.7 Methyl red test

Rhizobium was inoculated into sterile test tubes containing methyl red-Voges Proskauer broth (Manasa et al. 2017) at 5 mL/test tube, incubated at 30±2°C for two days, after which 5 mL of methyl red was added to each tube. Red broth indicates a positive result but yellow indicates a negative result (Dinesh et al. 2015).

2.5.8 Starch hydrolysis test

Starch agar medium was prepared (Hemraj et al. 2013). Medium was poured into sterile Petri dishes and allowed to solidify. *Rhizobium* was inoculated into Petri dishes and incubated at 30±2°C for 4 days. After incubation, 5 mL of iodine solution (0.340 g iodine and 0.660 g potassium iodide in 100 mL distilled water) (Küpper et al. 2011) was added. Formation of a clear zone around colonies indicated a positive result, i.e., the ability to hydrolyze starch (Hemraj et al. 2013).

2.5.9 Assay to assess the utilization of carbon sources

Rhizobium was also examined for its ability to utilize various carbon sources: glucose, mannitol, sucrose, lactose, galactose, raffinose, mannose, xylose and cellobiose (HiMedia). To determine carbon utilization pattern, 80 µL of 10% (w/v) filter-sterilized solutions of these carbohydrates was added to 5 mL of mannitol-free YEMA. Medium was inoculated with exponentially growing cultures (108 cells mL^{-1}) (Kumar et al. 2014). Inoculated broth was incubated at 28±2°C under constant shaking (150 rpm). Optical density was assessed at 610 nm on a Hach DR 3000 spectrophotometer (Hach, Loveland, CO, USA) after seven days of incubation as a measure of growth.

2.5.10 Bromothymol blue (BTB) test

YEM was enriched with 1% (*w*/*v*) bromothymol blue (HiMedia) to selectively identify fast and slow growing *Rhizobium* isolates. All isolates were grown on BTB-added YEM media. Positive fast-growing strains were yellow, indicating acid production, after incubation for 48 h at 28°C (Shahzad et al. 2012; Figure 1F).

2.5.11 Voges-Proskauer test

The *Rhizobium* isolates were inoculated separately into test tubes containing methyl red-Voges Proskauer broth at 5 mL/sterile test tube and incubated at 30±2°C for two days. After incubation, Barrit's reagents A and B (5 mL each) were added. The development of a red colour indicated a negative result (McDevitt 2009).

2.6 Testing efficacy of selected *Rhizobium* on growth and productivity of groundnut

2.6.1 Preparation of inocula

Rhizobium isolates for groundnut plants were tested in greenhouse-based pot experiments. Before inoculation of groundnut seeds, isolated *Rhizobium* isolates were grown in 250 mL conical flasks containing 40 mL YEMA for *Rhizobia* (Somasegaran and Hoben 1985) and incubated in a shaking incubator at 150 rpm for 3 days (Figure 1H), shaken only for 8 h each day at 28-30°C. One ml (containing 108 cells) of the bacterial culture at their logarithmic stage of growth was inoculated to surface-sterilized seeds, as indicated above, and dried under shade for 30 min prior to sowing in pots at 5 seeds pot^{1}. Surface-sterilized seeds were soaked in 48 h cultures of *Rhizobium* isolates for 30 min, using pure carboxymethylcellulose sodium salt (Merck Specialities Pvt. Ltd., Mumbai, India) as the adhesive (Figure 1I).

2.6.2 Pot culture experiment to assess the efficiency of selected *Rhizobium* isolates

Growth-promoting and nodulation efficiency of the two *Rhizobium* isolates were studied on groundnut. Surface sterilized seeds were soaked in 48 h old cultures of *Rhizobium* isolates for 30 min (Figure 1I), air dried under shade, then sown in pots (18 cm × 19 cm) containing sterilized soil. Each treatment was repeated twice (Figure 1J). Pots were placed in a greenhouse at 27.3°C (max.)/13.7°C (min.) and 85% (8 A.M.) to 53% (2 P.M.) relative humidity. Plants were uprooted and cleaned with tap water 45 days after inoculation (Figure 1K).

2.7 Data recording and analysis

Data on root and shoot length, plant height, dry matter plant^{-1}, nodule number plant^{-1}, and nodule dry weight plant^{-1} were measured from the pot culture greenhouse experiment (four plants/pot). Data was statistically analyzed, including mean separation, ANOVA, and the *F*-test at $P \leq 0.05$, by STAR (Statistical Tool for Agricultural Research; IRRI 2014).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Isolation of efficient *Rhizobium* isolates

Two *Rhizobium* strains ('RhBC' and 'NRA1') were isolated from the root nodules of groundnut grown in pots (Figure 1A).

3.2 Morphological characteristics of *Rhizobium* isolates

Both isolates grew well on YEMA medium. Phenotypically, both isolates grew quickly within 3-5 days of incubation and failed to absorb Congo red in this medium. Colonies of both isolates in YEMA medium were circular, mucoid, white and translucent. Both isolates were Gram-negative and rod-shaped. In Congo red medium, *Rhizobium* colonies were white, translucent, glistening, elevated and comparatively smaller than stained colonies of other non-*Rhizobium* isolates. The morphological characteristics of *Rhizobium* isolates were similar to those reported by Arora (2003).

3.3 Biochemical characteristics of *Rhizobium* isolates

Several qualitative biochemical tests were conducted as described by Lowe (1962): oxidase and catalase production, citrate utilization, gelatinase activity, indole, methyl red, and Congo red tests, Voges Proskauer test, and starch hydrolysis.

The results of morphological and biochemical characteristics of selected isolates are summarized in Table 2. It was found that both isolates ('RhBC' and 'NRA1') gave positive results for the oxidase, catalase, and Voges Proskauer tests but negative results for citrate utilization, gelatinase, indole, Congo red, starch hydrolysis, BTB and methyl red tests. Shahzad et al. (2012) also used the same tests to confirm that similar isolated bacterial strains were *Rhizobium* spp. Erum and Asghari (2008), Singh (2008) and Shahzad et al. (2012) noted positive sugar tests during the isolation and characterization of *Rhizobium meliloti*.

3.4 Carbohydrate utilization by *Rhizobium* isolates

The results of carbohydrate utilization by *Rhizobium* are summarized in Table 3. Both *Rhizobium* isolates ('RhBC' and 'NRA1') utilized several of the nine carbohydrates as the sole carbon source. Both isolates preferred monosaccharides over disaccharides. Xylose is preferred over other monosaccharides (Ishihara et al. 2002), supporting better growth than glucose and galactose while in disaccharide utilization, all the *Rhizobium* isolates showed better growth when fed lactose and sucrose (Table 3).

Table 2: Morphological and biochemical characterization of *Rhizobia* isolates

Biochemical tests	*Rhizobium* isolates	
	RhBC	NRA1
Gram test	-	-
Morphology	Rods	Rods
Biochemical tests:		
Oxidase test	+	+
Citrase test	-	-
Catalase test	+	+
Gelatinase test	-	-
Indole test	-	-
Congo red test	-	-
Vogas Proskauer test	+	+
Starch hydrolysis test	-	-
Methyl Red test	-	-

(+) positive result, (-) negative result

Table 3: Utilization of carbon sources by *Rhizobium* isolates

Different carbon utilization tests	*Rhizobium* isolates	
	RhBC	NRA1
Glucose	+	+
Mannitol	+	+
Sucrose	+	+
Lactose	+	+
Galactose	+	+
Mannose	+	+
Xylose	+	+
Cellobiose	-	-

(+) positive result, (-) negative result

3.5 Testing the efficiency of selected *Rhizobium* isolates

The *Rhizobium* isolates showed significant positive effects on the growth and nodulation of groundnut plants compared to the uninoculated control (Table 4) 45 days after plant emergence. Compared with the uninoculated control, the maximum amount of biomass per plant was found in TG 51 when inoculated with NRA1, which was statistically similar with TG 71 also inoculated by NRA1, followed by TG 51 and TG 71 which were inoculated with RhBC. Similar to plant biomass plant^{-1}, TG 51 inoculated by NRA1 and TG 71 inoculated with NRA1 showed statistically higher values than TG 51 and TG 71 inoculated with RhBC for other growth parameters such as shoot and root length, nodule number, and nodule biomass plant^{-1}.

NRA1 performed better than RhBC in pot culture in terms of root and shoot length, plant height, dry matter plant^{-1}, nodule number plant^{-1}, and nodule dry weight plant^{-1} and was thus selected for a future field trial.

The results of the present study are similar to those of earlier studies. Rahman (2006) reported that *Bradyrhizobium* inoculation affected yield significantly, but most yield attributes were not significantly affected. Brahmaprakash et al. (2007) reported that nodule number plant^{-1} and grain yield were improved due to inoculation of liquid *Rhizobium* inoculants compared to carrier-based *Rhizobium* inoculants in all tested crops. Deshwal et al. (2003) found that groundnut seeds coated with *Bradyrhizobium* strains had significantly enhanced germination, as well as higher seedling biomass, nodule number, nodule fresh weight and average nodule weight compared to uninoculated and uninfected controls. Biswas et al. (2003) reported that increased levels of *Rhizobium* inoculum significantly increased nodule number, nodule weight, plant height, shoot weight, root weight, pod yield and N content in groundnut seed. In their study, there was a positive correlation between nodule weight, plant height, shoot weight, root weight, pod yield and N content in seeds and total number of nodules as a result of the application of inoculum. Thus, groundnut production can be considerably enhanced by supplying effective inoculum to farmers.

4 Conclusion

Rhizobium isolate NRA1 was isolated from soil in a groundnut plant-based pot experiment. After the isolate was purified, it was characterized by morphological and biochemical parameters. Isolate NRA1 was superior to RhBC strain in terms of nodulation efficiency, plant dry matter and plant nitrogen uptake and has been selected for a future

Table 4: Effect of *Rhizobium* isolates on dry biomass, root length, shoot length, nodulation, nitrogen content and leaf colour score of groundnut under pot culture

Rhizobium isolates	Groundnut variety	Plant biomass (g plant^{-1})	Shoot length (cm)	Root length (cm)	Nodule no. plant^{-1}	Nodule biomass (g plant^{-1})
RhBC	TG 51	6.80	16.85	19.50	24.50	0.05
	TG 71	5.50	16.25	18.98	23.30	0.04
NRA1	TG 51	7.50	20.05	29.00	28.00	0.09
	TG 71	7.44	19.65	28.50	27.50	0.07
Control	TG 51	3.84	15.00	16.05	00.00	0.00
	TG 71	3.82	14.75	15.55	00.00	0.00
F-test		*	*	*	*	*
SEm±		2.12	1.54	2.97	4.85	0.01

* Significant at 5% level

field trial. To increase yield in groundnut requires the isolation of suitable and effective microbial strains. The methodology used in this study will be helpful to screen local *Rhizobium* isolates and test their effectiveness with local groundnut varieties. Ultimately, the objective is to enrich productivity of groundnut in infertile soil.

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