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Host Specificity and Symbiotic Association Between InDigenous *Rhizobium* Strain and *Arachis hypogaea* Plants

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Abstract

Legume nodules are a major source of accessible nitrogen in the biosphere. A symbiotic interaction between soil bacteria termed rhizobia and other legume plants results in the formation of nitrogen-fixing nodules. The goal of this research was to use bio-inoculants in conjunction with specific legume plant diversity to improve nodulation and plant growth. The method entails the organic selection of 36 rhizobial isolates, of which 6 strains were isolated to determine the efficacy of relative host-specific inoculation on nodulation and development in *Arachis hypogaea* legumes. The six isolates are identified as *Bradyrhizobium yumangenesis* sps. AB5 (NCBI Accession no.: ON724398), *B. japonicum* BD5 (ON724369), *B. elkanii* KT5 (ON729445), *B. arachidis* ET5 (ON729963), *B. liaongense* JN5 (ON734019), and *B. elkanii* NP5 (ON734425). All promising combinations of preferred rhizobial strains were examined in sterile circumstances for improved nodulation and to screen for the best isolate with enhanced features by inoculation in diverse soils. The nitrogen concentration of *Arachis hypogaea* ranged from 1.2 to 2.9 percent. The strains from Amrabad were shown to be highly host specific for *Arachis hypogaea* plants, and when inoculated, they boosted nodulation and plant growth. Because of the useful traits in AB5, more research was done, using this *Bradyrhizobium yumangenesis* sps. AB5 (NCBI: ON724398). This rhizobium species AB5 was studied for its ability to unravel and improve crop production in barren, polluted, and agricultural soils, with improved *Arachis hypogaea* plant features. This method may be employed across the globe of same climatic conditions for the retrieval of plants from soils where agriculture has failed.

Key words: *Arachis hypogaea*,Host specificity, nodulation,*Bradyrhizobium* sp. strain.

Introduction

Nitrogen fixation is regarded as a significant step in the evolution process because vital molecules like proteins and nucleic acids cannot be synthesized by photosynthesis products or by other means. Lightning and combustion are examples of natural processes that can fix nitrogen (N_2) oxidatively, accounting for 10% of total nitrogen fixed. The reductive process is known as the Haber-Bosch process; it is used in the production of industrial ammonia in which H_2 gas reacts with N_2 gas over an iron-based catalyst at high temperature and pressure. This method accounts for 30 percent of total nitrogen fixation. Biological nitrogen fixation (BNF), which is also known to be a reductive process that produces ammonium (NH_4^+), is responsible for the remaining significant N_2-fixation. It does, however, occur at ambient temperature and pressure and

does not require the usage of H_2. BNF is the major support system for life on earth due to its substantial delivery of fixed nitrogen to the biosphere (1).

Biological nitrogen fixation (BNF) was reported by Beijerinck and is carried out by a specialized group of prokaryotes (2). Aquatic organisms like cyanobacteria, free-living soil bacteria like *Azotobacter*, bacteria that create associative relationships with plants like *Azospirillum*, and bacteria that form symbiotic relationships with legumes and other plants like *Rhizobium* and *Bradyrhizobium* are among these prokaryotes (3).

Between rhizobia, the degree of host specificity varies. Some strains have a very narrow host range, whereas others have a very broad range. The establishment of a symbiotic interaction between legume species and rhizobia is a difficult task. Biofertilizer technology is a new age in biological input technology that has resulted in a large increase in annual agricultural production in recent decades (4). *Rhizobium* sp. is well recognized for its symbiotic nitrogen fixing ability through nodulation in legumes, which is critical for soil nitrogen richness and cultivated land management. The importance of microbial nitrogen fixation, particularly symbiotic fixation, to agricultural productivity has fascinated scientists for over a century (5). The inability of an inoculant strain to nodulate under field conditions is caused by a variety of living and nonliving factors. Nitrogen-fixing bacteria (NFB) in the mycorrhizosphere, as well as the exploitation of these bacterial partnerships as a technique to boost plant growth, are two hot topics in biotechnology right now. One of the two major groups of eurosids is the Fabidae, which includes the majority of species capable of endosymbiotic nitrogen fixation (6). For increased nodulation specificity, these types of investigations also require molecular identification of microorganisms, and molecular microbial identification may offer an advantage over culture approaches (7,8). Previous research has shown that gene discovery and characterization can help legumes and non-legume plants with nodulation and productivity. Rhizobia, in addition to nitrogen fixing action with legumes, can boost plant phosphorus nutrition by mobilizing inorganic and organic phosphorus.

PGPR bacteria are found near the rhizosphere (9). Even in the face of a variety of stressors, such as drought, the bacteria promote plant growth (10). Plant growth promoting rhizobacteria (PGPR) are root colonization bacteria (rhizobacteria) that have a beneficial influence on plant growth via direct or indirect methods (11). Plant growth-promoting rhizobacteria is now well-known for its ability to promote growth. Kloepper and Schroth (12) described plant growth boosting rhizobacteria as soil bacteria that colonize the roots of plants following seed inoculation and enhance plant growth. The difficulty of PGPR to colonize plant roots has been attributed for its ineffectiveness (13, 14). The synergistic relationships of these pathogens collectively caused more yield, loss to this key pulse crop than the sum of their individual losses (15, 16). Furthermore, increasing bacterial populations in a plant's rhizosphere during its life cycle can improve microbial behaviour (17-19). In a pair-wise inoculation experiment, the seven most efficient nitrogen-fixing strains can be tested for their competitiveness against less effective strains, and nodule occupancy for the most efficient strain can be identified (20). Greenhouse and field studies with PGPR strains have demonstrated enhanced nodulation and nitrogen fixation in soybean, lentil, pea, chickpea and common bean (21, 22). Plant growth-promoting rhizobacteria (PGPR) are helpful native soil bacteria that colonize plant roots and boost plant development (23), plant growth regulator production (24), and plant water and nutrient intake (25). Antifungal action of PGPR can also inhibit soil-borne plant diseases (26). Because it is nodulated by rhizobia capable of nodulating a wide set of legumes and a vast group of unrelated rhizobia, peanut is regarded

as a promising promiscuous species (27). Peanut-rhizobia symbiosis has been shown to contribute 40.9 kg per hectare of biological nitrogen fixation (BNF) (28). The local or indigenous strains isolated from a geographical location have been proven to be more particular to the crop in that same geography (29) which is the primary motivation for conducting this research.

Materials and Methods

Selection of rhizosphere soils

Undisturbed rhizosphere soils along with relative six legume species [*Arachis hypogaea* (Ground nut), *Glycine max* (Soya bean), *Cicer arietinum* (Chickpea), *Phaseolus vulgaris* (Common bean),*Vigna radiata* (Mung bean), *Cajanus cajan* (pigeon pea)]in triplets were collected from six forest soils of Jannaram (JN)- Adilabad, Eturunagaram (ET)-Warangal, Narsapur (NP)-Medak, Bhadrachalam (BD)-Khammam, Amrabad (AB)-Mahabubnagar and Kataram (KT)-Karimnagar areas. The soil samples are brought to the laboratory in sterile zip-lock covers/bags.

Physico-chemical analysis of soil

There was no past history of chemical fertilizer use in these rhizosphere soils, therefore there was no risk of chemical fertilizer-induced growth inhibition of natural bioinoculants. The alkaline potassium permanganate approach was used to estimate available nitrogen in the soil (30), while the Bray and Kurtz (31) method was used to evaluate available phosphorous. Potassium is determined by flame photometrically (32).

Screening of host-specific rhizobial strains by cultivable method

Soil sample preparation

The soil samples collected from various locations were brought to the laboratory and then followed by the removal of foreign materials such as roots, gravels, stones, and pebbles. Later, using the quartering technique, the bulk amount of soil is reduced to the desired quantity (33, 34). These soil samples were used as a source sample for the isolation of host-specific rhizobium, and for seed germination.

Sowing of arachis hypogaea seed

The seeds of *Arachis hypogaea* were taken from a single lot. For sowing, undamaged and healthy seeds of the proper shape and size were chosen. Ethrel was used to break seed dormancy of the peanut seeds (35). The seeds were first surface sterilized with 0.1 percent $HgCl_2$ solution (36), and then washed 5 times with sterile distilled water to remove any remaining $HgCl_2$ traces. Then the pots are filled with respective soil samples and used to implant seeds in each pot. The seeding was done at an average 2.5 cm depth, using 10 seeds per pot. After germination, the thinning was done, staying three plants per pot. The pots were irrigated on a regular basis. Hoagland's solution course was given from time to time. Three duplicates were maintained.

Root nodule study

The nodules serve as a home for the bacteria, which obtain energy from the host plant and fix free N_2 before converting it to combined nitrogen (37). In exchange, the plant receives fixed nitrogen from nodules and produces food and forage protein (38). The fascinating thing is that nodules that fix higher amounts of N_2 are also awarded more resources, whereas nodules that fix lower amounts are penalized by the host in terms of C allocation (39-41). The most productive symbioses feature nodules with high sink strength that also distribute large amounts of organic N to the host, resulting in a strong, positive feedback on plant development under N-limiting situations (42, 43). This explains why nodule biomass is proportional to the rhizobia's N_2-fixing capacity in many symbioses (43, 44).

Because white nodules are undeveloped or may have formed as a result of the wrong bacterium, they are not included in the study. N_2

fixation is active and effective in pink or rusty nodules. Effective nodules can turn green and then revert to black, signaling that they are dead, under unfavorable conditions such as nutritional deficit, illness, or water stress (45). Peanut plants have smaller nodules, but since they are densely packed with rhizobium-infected cells, they have stronger nitrogen-fixing activity (46), implying a unique link between peanut nodule size and nitrogen-fixing activity.

Physical parameters such as number, size, fresh and dry weight of the nodules were assessed after selecting relatively large, pink, and efficient root nodules and detaching them from the plants. Leg-hemoglobin estimation is performed.

Isolation of rhizobia from nodules

A healthy plant with intact soil around the roots was uprooted. Following that, the roots were thoroughly cleansed with a jet of water. Rhizobium was isolated from nodules that are pink multi-lobed and located on the top of the root. The nodule is gently detached from the root so that a section of root on the nodule's side remains attached and the nodule is not damaged. The nodules were rinsed well under running tap water before being placed in a tube with a nylon mesh on one end. For roughly five minutes, the other end of the tube was linked to tap.

Thoroughly washed nodules were transferred to a sterile test tube and treated with 0.1% $HgCl_2$ and 70% ethyl alcohol, for 3 min and one min respectively. The test tube is shaken periodically in order to remove the adhering air bubbles and the fresh sterilant gets in touch with the nodules. After three minutes, $HgCl_2$ solution is decanted off and nodule was immersed in alcohol for 1 minute. After the nodule surface gets sterilized it was washed with sterile water for at least ten times so as to remove the sterilants completely. Nodules were crushed with a sterile glass rod with flat end. Care was taken so that test tube may not break during crushing. Suspension obtained after crushing of nodule was used for isolation of *Rhizobium*. Serial dilution of the same was done and 10^{-6} dilution was selected for isolation.

Yeast extract mannitol agar (YEMA) was used as a selective medium for isolation of *Rhizobium* sp. 100µl of 10^{-6} dilution was inoculated on sterile YEMA media plates by spread plate technique and incubated at 28°C 3 to 8 days. Based on the color of the colony and other characteristics, *Rhizobium* was isolated and various other confirmatory tests were performed.

Confirmatory Tests

Confirmatory tests are designed as discussed by Somasegaran and Hoben, 2012 (47).

Congo red test

Congo red can aid the identification of rhizobia from other kinds of bacteria. In broad the Rhizobia absorb the stain weakly while several of the common soil bacteria take it up strongly.

Growth on alkaline medium

A. radiobacter can be isolated by streaking on Hoffer's alkaline medium (pH 11) in which *Rhizobium* growth is inhibited, while *A. radiobacter* grow. YEMA added with 1 ml/lit of thymol blue (1.6% sol.) is adjusted to pH 11. On slants, the growth of *A. radiobacter* and the change in colour of indicator is observed up to 15 days. If no growth (or) change in colour is observed, it may be *Rhizobium*.

Growth in glucose peptone agar

Glucose peptone agar

(Glucose 10 g, Peptone 20.0g, NaCl 5.0g, Agar - 15.0g, Bromo cresol purple 1.0 ml (1.6% alcoholic sol.) pH 7.1) was used to distinguish rhizobia, which generally show little growth on the medium without changing the

pH, whereas *Agrobacteria* grow well shows enormous growth. Observations were taken after 15 days of incubation for growth and change in pH.

Ketolactose test

Most of the strains of *A. tumifaciens* and *A. radiobacter* have been found to produce 3-Ketolactose in lactose containing medium but not rhizobia. The composition of medium used for this test is same as that of the yeast mannitol agar except mannitol is replaced by lactose (10 g/l). The medium is poured in plates and on solidification the inoculum is streaked on it. After incubation when sufficient growth is observed, the media plates were flooded with Benedict's reagent. Development of yellow colored ring of cuprous oxide (after 30 min to one hr) around the growth of organism is indicative of *Agrobacterium* contamination.

Nodulation tests:

Nodulation tests were conducted by the following methods.

Agar tube method

This method is good to study the nodulation and differentiation of symbiotic effectiveness with plants having small seeds. In this method the plants are wholly enclosed within the glass tube.

Preparation of agar tubes

Sufficient JSA medium (1.0g $CaHPO_4$, 0.2g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, 0.2g NaCl, 0.1g $FeCl_3$, 8-15g Agar, 1litre Distilled water, (1ml/litre trace element solution) is added to the medium (15 ml for deep and 20 ml for slope) was put in the tubes (200 mm x 25 mm). The tubes were closed with cotton plugs with uniform depth (20 mm) and modest compactness. The tubes were autoclaved and set as agar deep tubes or slopes as required.

- ***Nitrogen supplied control***: Nitrogen-controls were provided to a final concentration of approximately 70 ppm N (0.05% KNO_3).
- ***N-deficient control***: Agar tubes without inoculation are planted with seed or pre-germinated seedlings and put as uninoculated N-deficient control.

Microbial Identification by molecular study

Isolation of DNA from the bacterial isolates

The DNA was isolated from the bacterial colony. The obtained DNA sample was analyzed by colony PCR.

DNA was isolated from the Bacterial culture; the DNA was used in PCR to amplify bacterial 16S rDNA PCR Kit (800). Our PCR process uses the rDNA PCR Kit obtained from (TAKARA), Catalog number-RR182A. Using primers from the kit, we amplified a 1500bp amplicon and no amplicon was visible in the negative (no DNA) control and the expected sized amplicon (1500bp) was seen in the positive control. The test amplicon of 1500 bp was purified using magnetic beads and the product sequenced by Sanger's method of DNA sequencing. The sequencing results were assembled and compared with NCBI data base.

PCR analysis

In polymerase chain reaction 16S rRNA Universal primers were used to amplify the small subunit rRNA of each sample's culture DNA. The reaction mixture 50 µl contains 4µl bacterial DNA (nearly 200ng), 1µl Taq-DNA polymerase, 5µl of Taq buffer, 5µl of 2mM dNTP mix, 5 µl of forward primer (10 pM/µl) and 5 µl of reverse primer (10 pM/µl). PCR Amplification was carried out in a Bio-Rad thermo cycler run for 30 cycles. Denaturation was done for 94ºC for 20s, annealing at 48ºC for 20s and extension was done at 72ºC for 40s for each cycle, final extension was carried out for 5min at 72ºC at the end of all 30 cycles. The amplified PCR product of approximately 1542 bp was separated on a 1% agarose gel and purified by Qiagen spin columns (48).

16S rRNA gene Sequencing

The purified 1542bp PCR product was sequenced using universal primers. The complete 16S rRNA gene sequence of the isolate was subjected to BLAST sequence similarity search and Ez Taxon to identify the nearest taxa. The entire related 16S rRNA gene sequence was downloaded from the database (http://www. nbi .nlm.nih-gov) aligned using the celestial – program.

Seed inoculation and plant growth assessment

Sterile soils in triplicates from six forest places were collected and brought to the laboratory. They were taken in pots (Triplicates) and the seeds inoculated with different rhizobial strain of respective species were sown and then the growth parameters of each plant were assessed.

Undamaged and clean seeds of uniform size, selected to a reasonably uniform size were rinsed with 95% ethanol and immersed for 4 minutes in 0.2% $HgCl_2$. Seeds were then washed five times with sterile water. The viable seeds are sown after sterilization and washing, either using two seeds per tube (seedlings later can be thinned to one) or singly, allowing sufficient extra tubes. After 8 weeks of growth, the growth parameters like nodulation efficiency, number of nodules, nodular dry weight, dry weight of the plant and the plant height were assessed.

The presences of nodules, their nature at the time of harvesting that are pink because of leg-hemoglobin were accounted as effective. The rhizobial strains were isolated and tested for host specificity. All probable combination of the selected rhizobial isolates were checked under sterile conditions for improving nodulation to isolate the best host specificity plant.

Estimation of nitrogen in arachis hypogaea

Soil preparation and sowing

Soil is collected, vigorously washed and subjected to sterilization - to remove the nutrients and microorganisms particularly nitrogen fixing microorganisms from the soil to ensure that no external agent acts as source of nitrogen. The seeds of *Arachis hypogaea* from a single batch of the same size were picked and soaked in ethrel solution (5ml in 10 litres of water) for almost 12 hours before being shade dried to break dormancy before being sowed in the pots filled with sterile and nutrient free soil (35). The pots were inoculated with specific bioinoculants (Nitrogen fixing Rhizobia under study) except the controls. Negative control is maintained without any nitrogen source except the nitrogen contained in the seed initially. Positive control is treated with sufficient/unlimited nitrogen source. All the pots are irrigated frequently with Nitrogen-free Hoagsland solution. After 60 days of sowing the plants are extricated for examination of parameters.

Estimation of nitrogen by snell and snell (1949) method

A dried and ground plant sample (0.1 g) is digested completely with H_2SO_4 and heated. Then add H_2O_2 again until it is colorless. The intensity of color developed after treatment with NaOH, sodium silicate, and Nessler's reagent is then measured in the colorimeter at 440nm (blue filter). The obtained OD values are plotted on the standard graph. The nitrogen content is measured using a standard graph (49).

Estimation of leghemoglobin

The amount of nitrogen (N) fixed in the symbiotic association between the rhizobia and the plant is closely correlated with the amount of leghemoglobin (LHb) content of the root nodules of leguminous plants. A rapid, quantitative method for determining LHb in plant tissue would greatly facilitate research into symbiotic N fixation. Wilson and Reisenauer's cyanmethemoglobin method (1962) was used to determine the concentration of leghaemoglobin (50). 50 to 100mg nodules were collected and crushed in 9 volumes of

Drabkin's solution in a microfuge tube with a glass rod before centrifugation at 12,000rpm for 15 minutes. A 0.2m syringe filter was used to filter the supernatant. The filtrate was collected in a micro cuvette, and its absorbance at 540 nm was measured using a spectrophotometer (51).

Collection of soil samples from different problematical sites

Soil samples from different sites such as polluted soils, barren soils and agriculture soils were taken in triplets and inoculated with rhizobial strains in order to check the improvement in nodulation and development of these respective plants during inoculation in field trials.

Results and Discussion

The steps carried out in the present study have been represented in Figure 1. The steps include selection and collection, soil characteristics, isolation, screening and confirmatory tests for rhizobial strains, isolation of pure cultures, selection of the best isolate, Experiments carried out with the best rhizobial strain particular to legume speciesis studied and later tested in the same relative plants grown in various soils. The results of the same are presented in the following sequence as in Figure 1.

Six forest rhizosphere soils along with respective six legume species (*Arachis hypogaea*, *Glycine max*, *Cicer arietinum*, *Phaseolus vulgaris*,*Vigna radiata*, *Cajanus "cajan*) were collected from Jannaram (JN), Eturunagaram (ET), Narsapur (NP), Bhadrachalam (BD) Amrabad (AB) and Kataram (KT) areas. The physico-chemical characteristics of the soil samples were carried out.All the soils under investigation varied slightly in their soil types, the pH of different soils was slightly acidic and varied from pH 6.2 to 8.3 as shown in Table 1.

For carrying out the study six legume

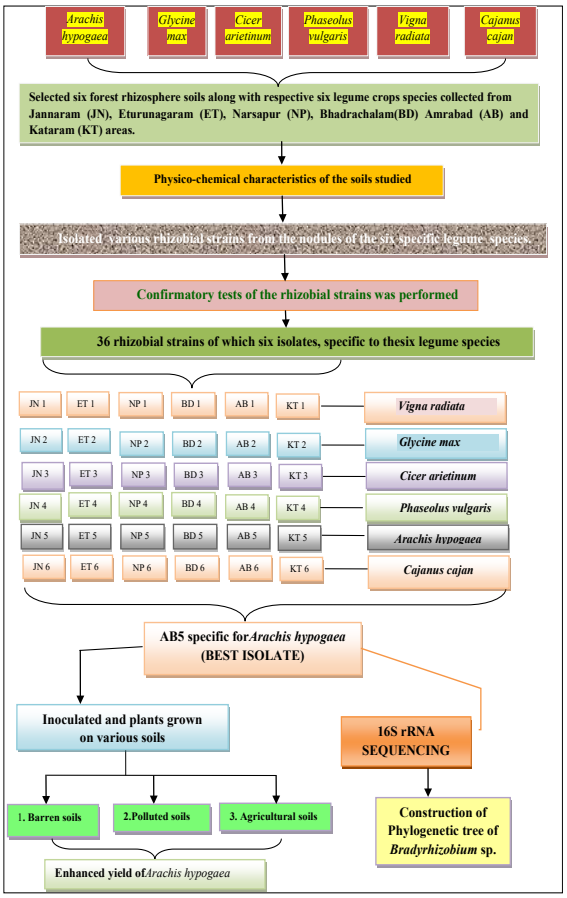


Figure 1: Steps involved in host specificity and effect of nodulation by indigenous *Bradyrhizobium* sp. AB5 in *Arachis hypogaea*

species (*Arachis hypogaea*, *Glycine max*, *Cicer arietinum*, *Phaseolus vulgaris*,*Vigna radiata*, *Cajanus cajan*) were selected and the specific rhizobial strains from the nodules of these six particular legume species were isolated. The confirmatory tests of these isolates were carried out and the pure cultures of these isolates were obtained. Thirty six rhizobial strains, six isolates specific to the six legume species were tested. The strains JN1, ET1, NP1, BD1, AB1, KT1 were specific to *Vigna radiata,*the isolates JN2, ET2, NP2, BD2, AB2, KT2 showed specificity to *Glycine max*strains JN3, ET3, NP3, BD3, AB3, KT3 showed to *Cicer arietinum*, while JN4, ET4, NP4, BD4, AB4, KT4 strain showed

Table 1: Physico-chemical characteristics in 6 forest soils (NPK in kg/hectare)

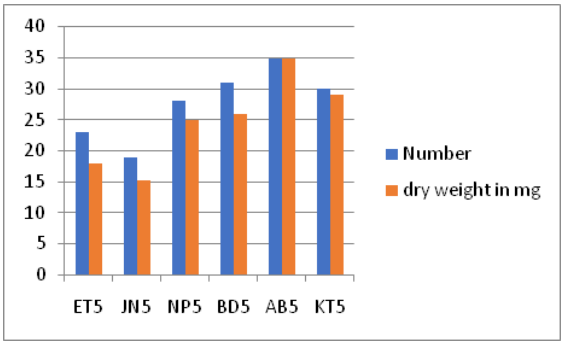
Sample collected from	Coordinates		Soil type	N	P	K	pH
	Latitude	Longitude					
Jannaram	19.1156 N	78.999 E	Red soil	265.06	105.19	160.65	6.9
Eturnagaram	18.338	80.426	Red soil	223.01	110.21	150.05	6.3
Narsapur	17.738	78.284	Deep black soil	272.73	112.32	160.00	6.2
Bhadrachalam	17.668	80.893	Red soil clay	273.03	118.11	157.02	6.7
Kataram	17.544	80.646	Red soil	272.08	104.03	147.00	6.2
Amrabad	16.383	78.833	Sandy loam	244.70	27.20	451.55	8.3

specific features with *Phaseolus vulgaris***,** JN5, ET5, NP5, BD5, AB5, KT5 isolates showed with *Arachis hypogaea* and JN6, ET6, NP6, BD6, AB6, KT6 strains were specific to *Cajanus cajan.* Among all these rhizobial isolates, JN5, ET5, NP5, BD5, AB5, KT5 were specific to*Arachis hypogaea* plantswere studied in detail**.**

The six isolates obtained from the nodules of *Arachis hypogaea*namely AB5, BD5, ET5, JN5, NP5, and KT5 are identified as *Bradyrhizobium yumangenesis* sps. AB5 (NCBI Accession no.: ON724398), *B. japonicum* BD5 (ON724369), *B. arachidis* ET5 (ON729963), *B. liaongense* JN5 (ON734019), *B. elkanii* KT5 (ON729445), and *B. elkanii* NP5 (ON734425).

Field trial studies exposed that the bacterial inoculation by rhizobia AB5 isolate on *Arachis hypogaea*plantseffects the growth and symbiotic characteristics. *Bradyrhizobium sps.* AB5 strains increased the number of nodules, nodular dry weight, dry weight of the plant and plant height compared with the control. A critical perusal of the Table 2 reveals that all the isolates under investigation induced nodulation with *Arachis hypogaea*species. However, the nodulating efficiency varied with the isolate and the plant. A lot of variation is evident with regard to the number, size and dry weight of the nodules induced by different strains.

In perusal, it was observed that AB5 isolate showed highest number of nodules in comparison with the other strains. An enhanced nodulation size was seen in KT5, AB5 and BD5 rhizobial strains. Dry weight of the nodules with the different isolated strains ranged from 15.2 to 35mg. efficient nodules were produced by AB5 isolate in *Arachis hypogaea*plant and also showed a maximum dry weight of 35 mg as seen in Graph1.

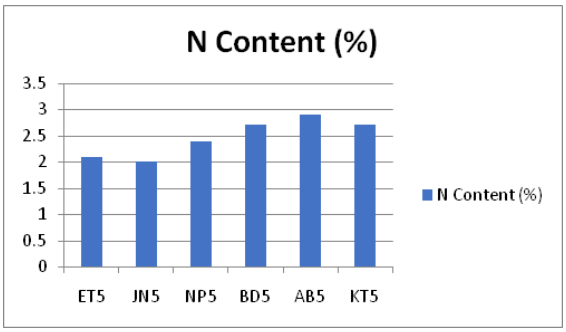


Graph 1: representing the number of nodules and total dry weight of nodules developed by various strains of rhizobium.

Plant height of *Arachis hypogaea*when inoculated with different rhizobium isolates (JN5, ET5, NP5, BD5, AB5, KT5) ranged between 24.6 and 37.8 cm. Maximum height 37.8 cm was induced by the AB5 isolate in*Arachis hypogaea*. The least height of the plant, 24.6cm was recorded with the inoculants ET5 which showed a raise in height with that of control. AB5 strain with comparison to the control showed about 2 times increase in height of *Arachis hypogaea*species.

With respect to the depth of the root AB5 measured 12 cm, which was the highest among other rhizobial strains. There was an increase in

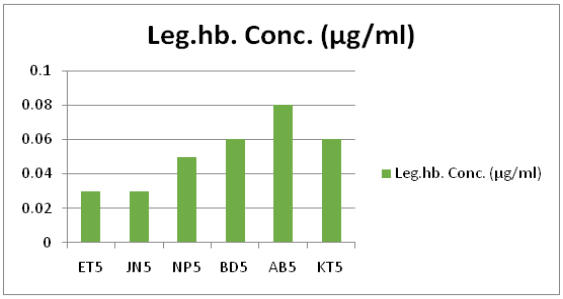
the size of the root by 1.6 times compared to the control as tabulated in Table 2.



Graph 2: Representing the Nitrogen content in Arachis hypogaea by various rhizobium strains.

Increase in nitrogen content of the plant *Arachis hypogaea*was significant with inoculations of AB5, BD5 and KT5 isolates. Maximum nitrogen content recorded was about 2.9 percent and the least recorded was 2 percent in the plants inoculated with host specific strains as shown in Graph 2. The uninoculated plants showed significantly low efficiency among all the plants (Table 2) which indicates that the isolates were increasing the nitrogen content not only in the plant but also increasing the fertility of the rhizosphere soils. With respect to the effect of dry weight on shoot and root with rhizobial strains on *Arachis hypogaea*, it was observed that the AB5 strain were found to be highly host specific and the dry weight of shoot and root was maximum 1.61g and 0.72g respectively, as recorded in Table 2.

As previously indicated, the amount of nitrogen (N) fixed in the symbiotic relationship between the rhizobia and the plant is closely connected with the amount of leghemoglobin (LHb) content of the root nodules, and there is a difference in LHb concentrations between strains. LHb concentrations ranged from 0.03 to 0.08 µg/ml. The outcomes are depicted in the Graph 3.



Graph 3: Representing the concentration of leghemoglobin in Arachis hypogaea and various rhizobium strains.

Table 2: Screening and isolation of different indigenous rhizobial strains from host-specific *Arachis hypogaea* species from forest areas of Telangana.

Name of the plant	Rhizobial strain	Nodulation				Plant Height(cm)		Plant dry weight (gm)		N Content (%)
		No.	Size (mm)	Leg.hb. Conc. (µg/ml)	Dry weight (mg)	Shoot	Root	Shoot	Root	
Uninoculated control	-	-	-		-	18.4 ±0.3	7.4 ±0.02	0.28 ±0.02	0.42 ±0.03	0.7 ±0.03
Arachis hypogaea	ET5	23	1.5	0.03	18±0.03	24.6±0.5	8	0.95	0.64	1.2
	JN5	19	2	0.03	15.2±0.03	25 ±0.5	8	0.88	0.57	2
	NP5	28	2	0.05	25±0.03	29 ±0.5	10	0.98	0.58	2.4
	BD5	31	1.5	0.06	26±0.03	35 ±0.5	11	1.02	0.68	2.7
	AB5	35	3	0.08	35±0.05	37.8 ±0.5	12	1.61	0.72	2.9
	KT5	30	2.5	0.06	29±0.03	35 ±0.5	11	1.01	0.67	2.7

Among all these rhizobial isolates,JN5, ET5, NP5, BD5, AB5, KT5, specific to *Arachis hypogaea* plantsstudied in detail, it can be concluded that AB5 isolate was the best isolate. Because of desirable characters of AB5, nucleic acid sequencing was performed and identified as *Bradyhizobium sp*. AB5 (ON724398).

Further studies were carried on with this strain on various soils like agricultural soils, polluted soils and barren soils with *Arachis hypogaea* plants. All the possible combinations of the potential B*radyrhizobium* sp. AB5 was also tested under field conditions for evaluating the improvement of nodulation and growth in these plants through inoculation in field trials for the categorization and amelioration of crop production in barren, polluted and agricultural soils. The nodulation ability of this isolate was confirmed by inoculation tests. The results showed a considerable increase in nodulation, size and nodular dry weight. There was an enhanced growth rate seen in the plant and also an increase in its dry weight. The nitrogen content was high in the plants. It can be concluded that the *Bradyhizobium sp*. AB5 played an essential role in the development of plants by showing significant increase in nodulation properties even in barren and polluted soils as shown in Table 3.

The results indicate that the *Bradyhizobium sp*. AB5 is host-specific to the plant*Arachis hypogaea*collected from different rhizosphere soils. This strain also increased the fertility of the barren soils that in turn increases the yield of the plant which is not possible with the wild type plants in such soils.

In the present study, with *Bradyrhizobium sps.* AB5 strain, there was increase in number of nodules in *Arachis hypogaea* which is similar to the work of Ruben Dario et al (2011) on other leguminous plants. Ruben Dario et al. (2011) observed that the commercial strain *B. japonicum* improved nodulation capacity of Argentinean commercial soybean and contributed to a higher yield (52). A simple increase in the number of nodules is not a sufficient indicator for considering peanut as an efficient nitrogen fixer, but an increase in nodule weight is a good indicator of efficient nitrogen fixation in peanut (53). With respect to the nodule weight, dry weight of the shoot, root and plant height with rhizobial isolate on *Arachis hypogaea* therewas a considerable rise observed which is in accordance with Faridul Alam et al. (2015) which states, soybean plants of all genotypes inoculated with Rhizobium sp. BARIRGm901 produced greater nodule numbers, nodule weight, shoot and root biomass, and plant height than non-inoculated plants (54). This study also revealed an increase in nitrogen content in *Vigna radiata*sp. treated with the rhizobial strain that usually increased

Table 3: Effects of Nodulation in Arachis hypogaea by Bradyrhizobium sps. AB5 isolate in agricultural, barren and polluted soils

Name of the plant	Soil types	No. Nodulation Size (mm)		Leg.hb. Conc. (µg/ml)	Dry weight (mg)	Plant Height(cm) Shoot	Root	Plant dry weight (gm) Shoot	Root	N content (%)
Control		-	-		-	18.30 ±0.5	13.20 ± 0.3	4.97 ±0.3	0.463 ±0.04	0.90 ±0.03
Arachis hypogaea	Agricultural soils	45	3	0.08	44.1	47	15	7.5	2.5	3.1
	Polluted soils	40	3	0.08	34.3	41.5	12	7.1	2.23	2.8
	Barren soils	41	3	0.08	39.77	37.5	12	7.2	2.31	2.5

the fertility of the soil (55). Jones (1998) showed that higher nodulation and biomass yields of inoculated plants could be attributed to high nitrogen fixation incorporated into nitrogen biosynthesis (56). The highest nitrogen contents and uptake were recorded in inoculated soybeans might be attributed to the greater ability of these plants to fix and assimilate nitrogen (57-60). The field trials was carried out with this isolate *Bradyhizobium sp*. AB5 (ON724398) on various soils like barren, polluted and agricultural soils on *Arachis hypogaea*. When grown in various types of soils *Arachis hypogaea* showed enhanced characters of the plant, i.e. number of nodules, size of the nodule, dry weight of root and shoot, plant height. There was an increase in nitrogen content indicating that this strain not only increases the fertility in all soil types but also increases the crop production without the use of chemical fertilizers. Rhizobia often spread from their initial habitats (61); however, the success of their introduction into new environments relies upon their ability to adapt to numerous biotic and abiotic factors (62).With respect to estimation of nitrogen accumulation with rhizobial strain AB5 inoculations, a similar study was carried using rhizobia (63). Plank (1989), reveals that there was 3.5% to 4.5% nitrogen accumulation in groundnut (64); while Oscar Yédéou Didagbé et al. (2014) shows that the nitrogen accumulation was between 1.3% and 3.9% in groundnut (65). Variation in nitrogen accumulation ranged between 2.3% to 3.75% with AB5 strain inoculated plants which are in accord to the above studies. Sulfab et al. 2011 (66) states that mineral nitrogen acts as a starter dose on the growth and production of *Vigna radiata*, which reveals that AB5 rhizobial strain can be used as a bioinoculant, based on the results.

These studies were aimed for improvement in nodulation and growth of the *Arachis hypogaea* plants through inoculation in field trials and thereby increase crop production by *Bradyrhizobium* sp. AB5 (ON724398) strain in barren, polluted and agricultural soils. The results indicate that the rhizobial strains AB5 showed host-specific characteristics to the plant *Arachis hypogaea* collected from different rhizosphere soils. The plant growth promoting characteristics of *Bradyrhizobium* sp. AB5 strain increased the quantity of seed per pod and efficiency in plants.

Conclusion

Our present perceptive of the premature events in nodulation is based on recognition and description of symbiotic association of rhizobium species with the host legume plant. Further characterization showed that this strain helped in providing interesting results. Approaches targeting downstream of the early nodulation events helped to provide a more widespread view of the association between nodule growth and a complete parameter of nodulation in legumes. The above method is an easy and cost effective method for the selection of efficient rhizobium bioinoculants like *Bradyrhizobium* sp. AB5 for its application to the legume plants in soils of same geographical region and it can be applied globally. This technique reduces the use of toxic fertilizers which the farmers do, to increase the fertility of the soil. The use of chemical fertilizers can also have a negative impact on human health and on our environment. All these can be avoided by using natural rhizobial isolates like *Bradyrhizobium* sp. AB5 which are non pathogenic but eco-friendly that can increase the fertility of various soil types, provide nutrients to the plants and in turn augment the yield of the plant because of its symbiotic association.

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