Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7737

ISSN: 2582 – 2845

Ind. J. Pure App. Biosci. (2019) *7*(4), 319-328

Research Article

Isolation and Characterization of Endophytic Bacteria from Nodule, Root and Seeds of Greengram (*Vigna radiata* L.)

M. Bhagya[1], K. Nagaraju[2], B. J. Praveen Biradar[3*], G.P. Santhosh[4] and R.C. Gundappagol[5]

[1]Assistant Professor, Department of Agricultural Microbiology, Agricultural College, Kalaburagi

[2]Assistant Professor, Department of Agricultural Microbiology, University of Agriculture sciences, Bengaluru

[3]Assistant Professor, Department of Agricultural Microbiology, Agricultural College, Vijayapur

[4]Assistant Professor, Department of Agricultural Microbiology, University of Agriculture sciences, Dharwad

[5]Professor of Department of Agricultural Microbiology, College of Agriculture, Kalaburagi

*Corresponding Author E-mail: pavitheking21@gmail.com

Received: 26.06.2019 | Revised: 29.07.2019 | Accepted: 10.08.2019

ABSTRACT

Plants are host to one or more endophytic microorganisms. However the endophyte plant interaction is one of the least studied biochemical system in nature. The present experiment was conducted to isolate endophytic bacteria from surface sterilized nodules, roots and seeds of Greengram. Total 10 endophytic bacteria were obtained from nodules, roots and seeds of Greengram. Highest population of endophytic bacteria were observed in nodules (7.5 ×10^5 CFU/g) followed by root (5.6×10^5 CFU/g) and seeds (3.2×10^5 CFU/g). Based on microscopic observation it was found that all the 10 isolates were rod shaped, when studied for gram reaction out of 10 isolates 8 isolates showed negative reaction, out of ten 6 isolates were motile and remaining 4 isolates were non motile and with regards to endospore formation test 4 isolates were observed as endospore forming bacteria and remaining 6 isolates were non endospore formers. With respect to biochemical characterization all isolates showed positive results for catalase test, citrate utilization, phosphate solubilization and casein production tests and all isolates showed negative result for Indole production and Voges-Proskauer test.

Keywords: *Endophytic bacteria, Morphological and Biochemical characterization.*

INTRODUCTION

Plant microbe interactions that promote the plant development and plant health have been the subject of considerable interest. Plants constitute vast and diverse niches for endophytic organisms. Nearly 3, 00,000 plants species exist on the earth, each plant host a numerous number of endophytes (Petrini, 1991).

Endophytic bacteria can be defined as group of beneficial free living soil bacteria that colonizes the internal tissues of plant without showing any external sign of infection on their host.

Cite this article: Bhagya, M., Nagaraju, K., Praveen Biradar, B. J., Santhosh, G.P., & Gundappagol, R.C. (2019). Isolation and Characterization of Endophytic Bacteria from Nodule, Root and Seeds of Greengram (*Vigna radiata* L.), *Ind. J. Pure App. Biosci. 7*(4), 319-328. doi: http://dx.doi.org/10.18782/2320-7051.7737

The role of endophytic microorganisms in plants can be divided into two categories based on types of activity, growth promotion and disease control. Endophytic bacteria are believed to elicit plant promotion in one of two ways:

Directly by producing phytohormones such as auxin or cytokinin or by producing the enzyme 1-aminocyclopropane- 1- carboxylate (ACC) deaminase, which lowers plant ethylene, levels (Anu, 2012).

Indirectly by preventing pathogen infections *via.,* antifungal or antibacterial agents, by outcompeting pathogens for nutrients by siderophore production, or by establishing the plants systemic resistance (Anu, 2012).

Endophytic bacteria exert several beneficial effects on host- plants such as stimulation of plant growth, Nitrogen fixation, phosphate solubilization, iron chelation, induction of resistance in plant against plant pathogens, increased drought resistance, thermal protection, survival under osmotic pressure etc.,

Endophytic bacteria of several genera have been isolated from legume tissues, including *Aerobacter, Aeromonas, Agrobacterium, Bacillus, Curtobacterium, Enterobacter, Erwinia, Mycobacterium, Flavimonas, Pseudomonas* and *Sphingomonas* and others (Struz et al., 1997).

Application of chemical fertilizers and pesticides have side effects, such as leaching out, polluting water basins, destroying beneficial soil microorganisms and eco-friendly insects, and making the crop more susceptible to the attack of pest and diseases. Thus, using endophytes as a biofertilizer is more favorable and encouraging to be used for its ecofriendly nature. Considering the above statement, an attempt was made to isolate the endophytic bacteria from Greengram.

MATERIALS AND METHODS

The present investigation was carried out at Dept. of Agricultural Microbiology, UAS, Bangalore, to isolate and study the synergistic effect of endophytic bacteria and *Bradyrhizobium* in Greengram (*Vigna radiata* L.)

The samples for isolation of endophytic bacteria were collected from the zonal agricultural research station, University of Agricultural Sciences, GKVK, Bengaluru. Fresh root, nodules and seeds were collected from the greengram plant. Five plants grown in different location were collected for isolation. Samples were collected before flowering stage *i.e.,* at 45 days after planting. Selected plants were uprooted, collected in sealed sterile plastic bags and transported aseptically to the laboratory.

Isolation of Endophytic bacteria

The procedure followed for isolation of endophytic bacteria was given by (Manoharan et al., 2016) Initially plants collected from field were washed in running tap water to remove adhering soil particles and nodules were separated from the roots. Eight healthy, pink, unbroken and firm root nodules were selected for isolation, root sections of 1 to 2 cm length and 10-15 seeds used for sowing of crop were selected for isolation of endophytic bacteria.

Selected nodules, roots and seeds were washed thoroughly with distilled water and samples were blotted dry with filter paper and then weighed to have final sample weight of 0.5 g. The surface sterilization of the nodules, root pieces and seeds were done with the following immersion sequence: 70 per cent ethanol for 1 minute, 3 per cent sodium hypochlorite for 5 minutes in young plants and 10 minutes in old plants washed for 1 minute, they were then rinsed four times with sterile water and dried in laminar air flow. Then cut ends of surface sterilized segments were placed on nutrient agar medium using sterilized scalpel with cut surface touching the agar. Plates were incubated for three to four days at 32° C in an incubator and the bacterial isolates were purified by streak plate method and selected for further studies.

Enumeration of endophytic bacterial isolates

Standard plate count method

One gram of sample was crushed under aseptic conditions with sterile pestle and mortar. 1 ml of suspension was transferred to 9 ml of sterile water. From this, 1ml of aliquot is transferred to test tube containing 9ml of sterile water similarly suspension was serially diluted up to 10^{-6} dilution. 1ml from each dilution was transferred to nutrient agar plates with 3 replications each and incubated for three to four days at 32 °C. Numbers of colonies grown on dilution plates are enumerated by following formula.

$$\text{Number of cells per ml} = \frac{\text{number (average of 3 replications) of colonies}}{\text{Amount plated} \times \text{dilution}}$$

Characterization of Endophytic bacteria

Morphological Characterization

All the isolates were examined for the colony morphology viz., colour, shape, ability to form endospores and Gram reaction as per the procedure.

Cell shape

Simple staining technique was followed to study the shape of the endophytic bacterial isolates. A loop full of culture was taken on clean slide, thin smear of bacterial culture was made on the slide, staining was done by using crystal violet and Smear was washed using water and slide was observed under microscope.

Gram staining Technique

Gram staining of the inoculants was carried out as per Huckers modified method. (Rangaswami, 1975) A drop of sterilized distil water was taken on the middle of each clear slide. Then a loopful of bacterial suspension (young culture) was transferred to the sterilized drop of water and a very thin film was prepared on each slide by spreading uniformly. The film was fixed by passing it over the gentle flame for two or three times. The slides were flooded with crystal violet solution and allowed to stand for 30 sec and then washed thoroughly with gentle stream of tap water. The slides were then immersed in iodine solution for one minute and washed thoroughly with 95 per cent alcohol for 10 seconds Alcohol was drained off and washed thoroughly with gentle stream of tap water. The slides were then covered with Safranin for one minute. The slides were washed with distilled water and air dried. The cellular morphology of inoculants was observed under microscope.

Motility test (Aneja, 2006)

Semi solid nutrient agar medium tubes were prepared and endophytic bacterial culture was stabbed into the center of tubes containing semi solid medium and motility was observed by its growth in medium.

Biochemical Characterization (Aneja, 2006)

Biochemical tests like Hydrogen sulfide production, Catalase test, Citrate utilization, oxidase test, Indole production, MR-VP test, Amylase production, Urease test, Phosphate solubilization, Gelatin utilization, and Casein hydrolysis were carried out to characterize the bacterial isolates.

The biochemical characterization of the isolates was carried out as per the standard procedures as detailed below.

Hydrogen sulfide production Test (Aneja, 2006)

Sulfide indole motility (SIM) media was used to study hydrogen sulfide production. SIM agar stabs were prepared; these stabs were inoculated with endophytic bacterial isolates. After inoculation stabs were incubated at 30 °C for 48 hours. After 48 hours of incubation black coloration along the line of stab inoculation indicated H_2S production.

Catalase test (Aneja, 2006)

A loop full of 24 hour old culture of endophytic bacterial isolate maintained on nutrient agar slants was transferred to a glass tube containing 0.5 ml distilled water and mixed thoroughly with 0.5 ml of 3 per cent hydrogen peroxide solution and observed for the presence of the effervescence.

Citrate utilization test (Aneja, 2006)

Endophytic bacterial isolates were inoculated on Simmons citrate agar media and slants were incubated at 37 °C for 48 hour. Discoloration of medium from green to blue colour due to change in pH indicates positive reaction. Citrate present in the Simmons media was used as its carbon and energy source.

Oxidase test (Aneja, 2006)

Overnight night cultures of bacterial isolates were streaked on the Tripticase soy agar medium and incubated at 30 °C in an inverted position for 48 hour. After incubation period, 3-4 drops of para-amino dimethyl aniline oxalate solution was added on the streaked area and the plates were observed for the color change from pink to maroon and finally to purple within 30 second which indicates a positive reaction.

Indole production test (Aneja, 2006)

Test cultures were inoculated to test tubes containing one per cent tryptone broth and were incubated at 35 °C for 48 hours. After 48 hours of incubation, 1 ml of Kovac's reagent was added to each tube containing tryptone broth and the tubes were kept in Shaking for 10 to 15 minute. And the tubes were allowed to stand and permit the reagent to come to the top. Tubes were observed for the reddening of the alcohol layer within few minutes, it indicated indole production.

MR-VP test (Aneja, 2006)

Methyl Red and Voges Proskauer test (MR-VP) broth prepared in two sets was inoculated with the endophytic bacterial isolates and incubated for 48 hour at 30°C. To the first set of tubes, few drops of alcoholic solution of methyl red was added. The development of distinct red color was indicative of positive reaction for MR test.

α- naphthol solution (5 percent solution in 70 per cent ethyl alcohol) was added to the second set of tubes and shaken gently for 15 minute. The positive reaction of acetyl methyl carbinol production was indicated by development of red color. This indicated positive result for the VP test.

Amylase production test (Aneja, 2006)

The endophytic bacterial isolates were streaked on starch agar medium and incubated at 25 °C in inverted position for 3 to 4 days. Surface of the plates was flooded with iodine solution with a dropper for 30 seconds. Plates were observed for clear zone surrounding the microbial colonies.

Urease test (Aneja, 2006)

The endophytic bacterial isolates were inoculated to the urea agar plates and incubated for 24 to 48 hours. Colour of the medium changes to red color.

Phosphate Solubilization test (Aneja, 2006)

Microorganisms capable of producing a halo or clear zone due to solubilization of phosphate in the surrounding medium which were streaked on plates containing Sperber's medium. The isolates which showed the clear zone were considered as phosphate solubilizer. The clearing zones were measured and recorded.

Gelatin utilization test (Aneja, 2006)

Gelatin is a protein hydrolyzed by enzymes and is tested by gelatin liquefaction. The test cultures were stab inoculated into nutrient gelatin deep tubes, incubated at refrigerated condition for 48 hour and observed for gelatin liquefaction.

Casein hydrolysis (Aneja, 2006)

24 hour old cultures were streaked on Skim milk agar medium plates incubate the plates for 24 to 48 hour at 37 °C in inverted condition. Presence of clearing around the line of growth is observed in the plates.

Compatability test between endophytic bacteria and *Bradyrhizobium* sp.

After isolation and characterization of endophytic bacteria from greengram, endophytic bacteria were tested for their compatibility with the reference *Bradyrhizobium* sp. Beneficial endophytic bacteria grown in nutrient agar broth for a period of 24 hours (10^8 cfu /ml) was used as inoculum. A loop full of endophytic bacterium and reference *Bradyrhizobium* were streaked on opposite side of the medium in the petriplates. The plates were incubated at 32 °C for 48 to 72 hours Compatibility was tested by

overgrowth or by inhibition of *Bradyrhizobium* and observations were recorded.

Compatible bacterial strains were characterized by their culture conditions, morphological and biochemical characteristics using standard methods.

RESULT AND DISCUSSIONS

Isolation of endophytic bacteria from nodules, roots and seeds of Greengram

A total 10 isolates were obtained from nodules, roots and seeds of greengram and out of which three compatible endophytic bacterial isolates were selected for further studies. Endophytic communities are clearly distinct in different plant species and the diversity of the communities may vary significantly (Justin & Christopher, 2003)

The sterility check was carried out by plating the last washed water confirmed that bacteria obtained on the plates were endophytes (Gyaneshwar et al., 2001). Based on different colony morphologies like colour, shape, surface growth of endophytic bacteria around ten isolates were selected for further morphological, microscopic and biochemical studies to characterize the endophytic bacteria. Similar studies also been reported by (Miche & Balandreau, 2001) on diversity of endophytes and also have focused on characterization of isolates obtained from internal tissues following disinfection of plant surface with sodium hypochlorite or similar agents.

Enumeration of endophytic bacterial population from nodule, root and seeds of Greengram

The observations recorded for enumeration of endophytic bacteria are presented in Table1. The highest number of endophytic bacteria were observed in nodules (7.5×10^5 CFU/g) followed by root (5.6×10^5 CFU/g) and seeds (3.2×10^5 CFU/g).

Results indicated that endophytic bacterial population were more in nodules than in roots and seeds. Similar work was reported by[10] and stated that densities of bacterial endophytes were higher in nodules (6.39 CFU$\times10^5$ g^{-1}) than in root (5.56 CFU $\times10^5$ g^{-1}).

More number of endophytic bacterial populations was observed in nodule compared to roots and seeds may be because nodules have high symbiotic association with the microorganisms compared to root. The normal endophytes concentrations can vary between 10^2 to 10^6 CFU per gram.

Naming of isolates

Endophytic bacterial isolates were named based on the plant part from which they were isolated. Ten different endophytic bacterial isolates were isolated and named. Four isolates from nodule were named as GGNI1, GGNI2, GGNI3 and GGNI4. Three isolates from roots were obtained and named as GGRI1, GGRI2 and GGRI3 and in the same fashion three isolates were taken from seeds and were named as GGSI1, GGSI2, and GGSI3. These were recorded in Table 2.

Morphological and Biochemical characterization of the endophytic bacterial isolates

Morphological Characterization

All 10 isolates were examined for the colony morphology *viz.*, Colour, Cell shape and also isolates were examined for motility of bacteria and ability to form endospores and Gram reaction as per the standard procedures.

Cultural characteristics of the endophytic bacterial isolates on broth and media were observed in the form of cloudiness of broth and colony characters on media respectively. Observations obtained were presented in Table 3.

Four isolates GGNI1, GGSI1 and GGSI3 showed heavy broth cloudiness and slight cloudiness of broth was seen in the isolates GGNI2, GGNI3, GGRI3 and GGSI2 and there was no clouding of broth in the isolates GGNI4, GGRI1 and GGRI2.

Following Observations were recorded for the colony characteristics of isolates. Isolate GGNI1 showed round, whitish green colonies, in GGNI2 dirty white slimy glistening colonies were observed, GGNI3 showed white flat wrinkled colonies and GGRI2 represented white powdery dotted

colonies. While GGRI1 showed yellow raised glistening colonies, GGRI3 showed white dotted raised colonies, and pinkish white slimy colonies were seen in case of GGSI2 remaining 3 isolates *viz.*, GGNI4, GGSI1 and GGSI3 were observed like white dotted colonies.

For Gram reaction test following results were noticed, out of ten isolates eight isolates were Gram negative *viz.*, GGNI1, GGNI3, GGRI1, GGNI4, GGSI1, GGSI2, GGRI2 and GGSI3. And the remaining two isolates were found as Gram positive namely GGNI2, GGRI3.

Shape of the isolates was also studied wherein all the 10 isolates were rod shaped and with respect to endospore formation following observations were drawn, isolates GGNI1, GGNI2, GGNI3 and GGSI3 were endospore formers, remaining six isolates were non endospore formers.

GGNI1, GGNI2, GGNI4, GGRI1, GGRI2 and GGSI1 were motile remaining GGNI3, GGRI3, GGSI2 and GGSI3 were non motile.

Ten endophytic bacteria isolated from different tissue of the Greengram plant was assessed using phenotypic characterization methods. Colony morphology gave an indication of the variation among the endophytic bacterial isolates. The isolates studied were chosen for their dominance as well as uniqueness or differences with others in colony morphology. The results are in agreement with the findings of (Sanjay et al., 2014) The isolates selected from three plant part were named according to the plant part from which they were isolated.

Out of ten endophytic bacterial isolates eight endophytic bacterial isolates were Gram-negative only two isolates were identified as Gram-positive. Earlier researchers have reported a predominance of Gram-negative bacteria in the tissues of various plants (Stoltzfus et al., 1997).

Motility is an important characteristic for endophytic bacteria due to motility of these endophytes there is an advantage of spreading endophytes into host. Out of ten isolates, six isolates were motile and four isolates were non motile. Studies on *Glycine max* and *Glycine soja* revealed that when grown on 2 per cent agar, 78 per cent of the endophytic bacterial isolates were found to be motile (Hung, & Annapurna, 2004).

Biochemical Characterization

Biochemical tests were carried out for further characterization of the endophytic bacterial isolates and the results were presented in the Table 5 and Table 6.

All the isolates were shown positive result for Phosphate solubilization, Casein production, Catalase test and Citrate utilization test. In MR test all the isolates showed positive result except GGNI1. All isolates showed negative result for indole production test and VP test. Four isolates showed positive result for oxidase test namely GGNI1, GGNI2, GGNI3, and GGSI2. The remaining isolates were negative for oxidase test. GGNI1 and GGRI1 showed negative result for Hydrogen sulfide production test. GGNI2, GGRI1, GGRI3, GGSI2, GGSI3 showed negative result for amylase production test.GGNI2, GGNI3,GGNI4,GGRI1,GGRI3,GGSI1,GGSI2 showed positive result for urease test, GGNI1,GGRI1, GGRI2,GGSI1 were showed positive result for gelatin utilization test.

All the isolates gave positive result for Catalase tests this result indicated that they can produce Catalase enzyme. Similar result was observed by (Son et al., 2006), who had conducted experiment on isolation, identification and screening of endophytic bacteria. Catalase is the enzyme which helps bacteria to avoid cellular toxicity.

All the isolates showed positive result for phosphate solubilization test because these endophytes possess the capacity to solubilize phosphates by releasing organic acids as shown with endophytic bacteria of soybean in phosphate assimilation (Hung & Annapurna, 2004). Similar Phosphate solubilization by *Bacillus* sp. isolated from salt stressed environment had been observed by earlier researchers.

Study of urease activity is an important biochemical characteristic for endophytic bacteria because urease is an enzyme which splits urea to simple forms of nitrogen which can be readily absorbed by the

plants thus helps growth promotion. In urease test out of ten isolates seven isolates showed positive result. The result was similar with the results obtained by (Kumaresan & Suryanarayanan, 2001) who investigated on occurrence and distribution of endophytic bacteria in a legume community

Compatability test for endophytic bacterial isolates with reference *Bradyrhizobium* sp.

The isolates GGNI1, GGRI2, GGSI1 were compatible with each other and with *Bradyrhizobium* sp. Compatible isolates were selected for further identification and studies in green house condition. Picture showing compatability between endophytic bacteria with *Bradyrhizobium* sp. were showed in plate 1.

Compatible endophytic bacterial isolates such as GGNI 1, GGRI2 and GGSI1 were identified as *Pseudomonas* sp. *Enterobacter hormaechei*, and *Enterobacter* sp. based on morphological, microscopic and biochemical characteristics. Results were represented in Table 7. Result proves that both *Bradyrhizobium* sp. and endophytic bacteria can coexist in same plant and there is no antagonistic effect among each other hence they showed compatibility.

Table 1: Endophytic bacterial population in Greengram

Plant part	Population count (No. ×10 ^{5}CFU/g)
Nodules	7.5
Roots	5.6
Seeds	3.2

Table 2: Naming of endophytic isolates

Plant part	Isolats
Nodule	GGNI1
	GGNI2
	GGNI3
	GGNI4
Root	GGRI1
	GGRI2
	GGRI3
Seed	GGSI1
	GGSI2
	GGSI3

Table 3: Morphological characteristics of endophytic bacterial isolates

Isolates	Cultural characters of broth (Clouding of broth)	Colony characters in agar media
GGNI1	Heavy	Round whitish green colonies
GGNI2	Slight	Dirty white slimy glister colonies
GGNI3	Slight	White flat wrinkled colonies
GGNI4	No	White dotted colonies
GGRI1	No	Yellow raised glister colonies
GGRI2	No	White powdery dotted colonies
GGRI3	Slight	White dotted raise colonies
GGSI2	Heavy	White dotted colonies
GGSI2	Slight	Pinkish white slimy colonies
GGSI3	Heavy	White dotted colonies

Table 4: Microscopic Characteristics of endophytic bacterial isolates

Plant part	Isolates	Gram reaction	Cell shape	Motility	Endospore formation
Nodules	GGNI1	-	Rod	+	+
	GGNI2	+	Rod	+	+
	GGNI3	-	Short rod	-	+
	GGNI4	-	Rod	+	-
Roots	GGRI1	-	Rod	+	-
	GGRI2	-	Rod	+	-
	GGRI3	+	Short rod	-	-
Seeds	GGSI1	-	Rod	+	-
	GGSI2	-	Bacilli	-	-
	GGSI3	-	Rod	-	+

Table 5: Biochemical characteristics of endophytic bacterial isolates

Plant part	Isolates	Oxidase test	Indole Production test	H_2S production test	Catalase test	Citrate utilization	MR test
Nodule	GGNI1	+	-	-	+	+	-
	GGNI2	+	-	+	+	+	+
	GGNI3	+	-	+	+	+	+
	GGNI4	-	-	+	+	+	+
Root	GGRI1	-	-	-	+	+	+
	GGRI2	-	-	+	+	+	+
	GGRI3	-	-	+	+	+	+
Seed	GGSI1	-	-	+	+	+	+
	GGSI2	+	-	+	+	+	+
	GGSI3	-	-	+	+	+	+

Table 6: Biochemical characteristics of endophytic bacterial isolates

Plant part	Isolates	VP test	Amylase production	Urease test	Phosphate solubilization test	Gelatin utilization	Casein production
Nodule	GGNI1	-	+	-	+	+	+
	GGNI2	-	-	+	+	-	+
	GGNI3	-	+	+	+	-	+
	GGNI4	-	+	+	+	-	+
Root	GGRI1	-	-	+	+	+	+
	GGRI2	-	+	-	+	+	+
	GGRI3	-	-	+	+	-	+
Seed	GGSI1	-	+	+	+	+	+
	GGSI2	-	-	+	+	-	+
	GGSI3	-	-	-	+	-	+

Table 7: Identification of endophytic bacteria

GGNI1	*Pseudomonas* sp.
GGRI2	*Enterobacter hormaechei*
GGSI1	*Enterobacter* sp.

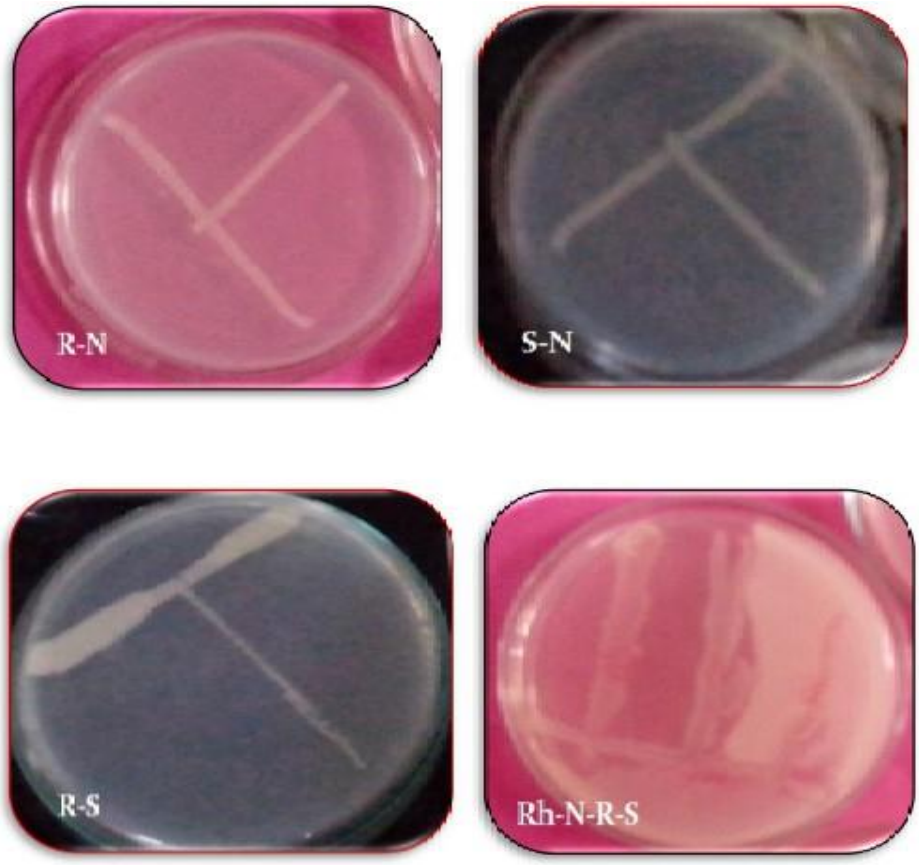

Plate 1: Compatability test between endophytic bacteria and *Bradyrhizobium* sp.

CONCLUSION

Endophytic bacteria were isolated from different parts of greengram plant through serial dilution method and ten different endophytic bacteria were selected based on their colony morphology and they were subjected to different microscopic and biochemical tests. Under microscopic identification endophytic bacterial isolates were tested for Gram reaction, motility test, endospore formation. Eight isolates were Gram negative and two isolates were Gram positive. Six isolates were motile and four isolates were non motile and four isolates were endospore formers and six isolates were non endospore formers.

Isolates showed different results (positive and negative) for different biochemical tests. All isolates showed positive result for catalase test, citrate utilization, phosphate solubilization, casein utilization and all isolates showed negative result for indole production test and VP test. Out of ten, four isolates showed positive result for oxidase test, eight isolates showed positive result for H_2S production test and same pattern followed for methyl red test. Five isolates showed positive result for amylase production test, seven endophytes showed positive result for urease test and four isolates showed positive result for gelatin utilization test.

REFERENCES

Aneja, K. R. (2006). Experiments in Microbiology, Plant Pathology and Biotechnology. 4th Edition. New Delhi, pp. 245-275.

Anu, R. (2012). Microbial endophytes of crop plants and their role in plant growth promotion. *Ph. D. thesis*. University of Agricultural sciences, Bangalore.

Elbeltagy, A., Nishioka, K., Suzuki, H., Sato, T., Yu, S., Morisaki, H., Mitsui, H., & Minamisawa, K. (2000). Isolationand characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. *J. Soil Sci. and Plant Nutrition*. *46*, 617-629.

Gyaneshwar, P., James, E. K., Natarajan, M., Reddy, P. M., Hurek, R. B., & Ladha, J. K., (2001). Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. *J. Bacteriol*., *183*, 2634-2645.

Hung, P. Q., & Annapurna., (2004). Isolation and characterization of endophytic bacteria in soybean (*Glycine* sp.). *J. Omonrice. 12*, 92-101.

Justin, T. C., & Christopher, M. M. F., (2003). Isolation and identification of actinobacteria from surface-sterilized wheat roots. *Appl. Environ. Microbiol*., *69*, 5603-5608.

Kumaresan, V., & Suryanarayanan, T., (2001). Occurrence and distribution of endophytic bacteria in a legume community. *J. Bacteriol., 105*, 1388-1391

Manoharan, M. J., Shalini, D., Abitha, B., & Tongmin, S., (2016). Isolation of phosphate solubilizing endophytic bacteria from *Phyllanthus amarus* Schum and Thonn: Evaluation of plant growth promotion and antioxidant activity under salt stress. *J. App. Res. Med. Arom. Plnts., 32*, 89-95.

Miche, L., & Balandreau, J., (2001). Effects of rice seed surface sterilization with hypochlorite on inoculated on inoculated *Burkholderia vietnamiensis. J. Appl. Environ. Microbiol., 67*, 3046-3052.

Pereira, P., Ibanez, F., Rosenblueth, M., Etcheverrry, M., & Martinez-romero, M., (2011). Analysis of the bacterial diversity associated with the roots of maize (*Zea mays L*.) through culture-dependent and culture independent methods. ISRN *Ecol*., doi:10.5402/2011/938546.

Petrini, O. (1991). Fungal endophytes of tree leaves, in *Microbial Ecology of Leaves*, editions Andrews, J. H. And Hirano, S. S., *J. Springer-Verlag* 179–197.

Rangaswami, G. (1975), Diseases of Crop Plants in India. Prentice Hall (P) Ltd., New Delhi, 520.

Sanjay, A., Purvi, N. P., Meghna, J. V., & Rao, G. G., (2014). Isolation and characterization of endophytic bacteria colonizing halophyte and other salt tolerant plant species from coastal Gujarat. *African J. Microbiol. Res., 81*, 779-1788.

Son, H. J., Park, G. T., Cha, M. S., & Heo, M. S. (2006), Solubilization of insoluble inorganic phosphates by a novel salt- and pH tolerant *Pantoea agglomerans R-42* isolated from soybean rhizosphere. *Bioresearch Technol., 97*, 204-210.

Stoltzfus, J. R. R., Malarvithi, P. P., Ladha, J. K., & DE, B. F. J. (1997). Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. *J. Plant Soil. 194*, 2536.

Struz, A. V., Christie, B. R., Matheson, B. G., & Nowak, J. (1997). Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *J. Biol. Fertil. Soil*. *25*, 13-19.