Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [1] January 2023: 339-343.

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Isolation And Characterization Of Rhizobia From The Root Nodule Of Soybean Plant.

Rahul S Wakde[1*] and Anil P Narsinge[2].
Department Of Microbiology, Yogeshwari Mahavidyalaya, Ambajogai, Maharashtra, India.

** Corresponding author: E-mail: rahulwakde0510@gmail.com*

ABSTRACT

Nitrogen is present large amount in air but is in gaseous non usable form. Only microbes can fix it into usable form through its activity, nitrogen is essential element for plant growth and development which is supplied by Rhizobia that are present symbiotic association to produces nodule in root of plants. Biological nitrogen fixation could help to increases soil quality, agricultural productivity, food nutritional quality and security. In present study soybean root nodule were used for the isolation, morphological characterization and biochemical characterization of Rhizobium strains. Plant samples of soybean were collected from fields of surrounding area of ambajogai, maharashtra. On the basis of morphological characterization on YEMA medium and biochemical test of selected colony indicated that presence of Rhizobium. In gram staining reaction of selected colony were found gram-negative and rod shaped.Biochemical characterization of Rhizobium strain showed that glucose peptone agar test was positive, starch utilization test was negative, citrate utilization test was negative reaction, catalase test was found positive result and lipase test was negative reaction. Rhizobium isolated in this study could find potential application for development of biofertilizer for sustainable agriculture practices to replacechemical fertilizers.

Keywords : *Soybean, Root Nodule, Rhizobium Isolate, Morphological Characterization, Biochemical Characterization.*

Received 12.11.2022 Revised 23.11.2022 Accepted 20.12.2022

INTRODUCTION:

Agriculture plays a crucial importance role in the growth and survival of nations; therefore, maintaining its quantity and quality is essential for feeding the population and economic exports. Modern agriculture involves usage of pesticides and chemical fertilizers. Continuous application of chemical fertilization leads to the decay of soil quality and fertility and might lead to the collection of heavy metals in plant tissues[13]. The application of bioinoculants containing N-fixing bacteria and P-solubilizing bacteria have proven to improve leaf chlorophyll, plant nutrient uptake, and yield of crop in which the use of N and P fertilizer was able to be minimized by 50%[1]. Living organisms may affect their host plant by one or more mechanisms such as nitrogen fixation, production of growth-promoting substance or organic acids, enhancing nutrients uptake, or protection against pathogens [12].

Biofertilizers are likely called as bioinoculants as they are the preparations containing living or latent cells of microorganisms that facilitate crop plants uptake of nutrients by their interactions within the rhizosphere and some produces root nodules once applied through seed or soil [7]. Practically, formulation determines potential success of inoculants. For application these bioinoculants have to be put in carrier, either liquid or solid based, along with osmoprotectant, sticking agents, nutrients.[8]. The beneficial microbes play an important role in increasing soil fertility[11]. Diazotrophic organisms are those have ability that fix atmospheric nitrogen gas into more usable form such as ammonia. The use of microbial inoculants for their potential to replace chemical fertilizers and pesticides in agricultural systems, and improve environmental[5]. Plant-aiding microorganisms, often referred to as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), Bacteria can contribute to plant growth via N2- fixation and solubilization of low mobile nutrients. Biological N2-fixation is carried out by various symbiotic and non-symbiotic bacteria[4]. Biofertilizer play important role in plant growth such as they improve the sugar, protein and chlorophyll content, ultimately yield of crop is increases without any harmful effect in environment.

MATERIALS AND METHODS

The experiments were carried out at the Department of Microbiology, yogeshwari Mahavidyalaya, ambajogai (MH)

Collection Of Sample:
Plant sample of soybean were collected from fields of surrounding area of ambajogai and taken from the field to the laboratory for the isolation of *Rhizobium* strains.


Fig 1: Soybean uprooted plant.

Isolation Of Efficient *Rhizobium* Bacteria:
The collected uproot plant sample of soybean are brought into the laboratory for the isolation of *Rhizobium* bacteria[figure 2A and 2B]. Soybean plant were washed with water so that to remove the soil particals. Nodules from the plants are picked with sterile forceps. These nodules were washed under running tap water so that soil particles were fully removed and nodules were wash again in distilled water[3]. For surface sterilization nodules were dipped in 0.1% mercuric chloride solution for 30 s, and than in 70% ethanol for another 30s and wash with distilled water. [10].


Fig 2: A) soybean Root **B) Soybean Root Nodule**

Surface- sterilized nodules were transferred to test tubes containing 5 mL of sterilized distilled water and as per the standard protocol they were crushed with a glass rod to obtain a milky suspension[6]. The crushed nodules were streaked on yeast extract mannitol agar (YEMA) medium plates (HiMedia, Mumbai, India) containing Congo red [2]. 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} MgSO4.7H2O, 0.1 g L^{-1}NaCl, 0.025 g L^{-1}Congo red and 20.0 g L^{-1} agar powder. pH of the culture medium adjusted to 6.8 ± 7.0. The media were placed in autoclave for sterilization and after sterilization YEMA medium were poured into Petri dishes and medium become solidified. This Petri dishes were used forstreaked the bacterial suspension.and plates were placed at 28±2^{0}C for 24-48 h. White, translucent, elevated and mucoid colonies appear than it proved the presence of *Rhizobium.* For the isolation of pure cultures the single colonies were restreaked 2-3 times on fresh YEMA plates[9].
These white and mucoid colonies are picked and inoculated on nutrient agar slants and incubated at 28± 2^{0}C for 24-48 h. After proper growth slants were stored at 4^{0}C in refrigerator for further characterization[3], Gram staining and biochemical tests of the selected isolate were performed [2].

Identification Of Bacterial Culture By Morphological And Biochemical Tests:

Gram Staining:
To study the gram nature prepared the bacterial smear on glass slide and passing over a bunsen burner flame for heat fixing. Smear was stained with crystal violet for one minute. Then, it was rinsed with tap water and immersed in gram's iodine for one minute. Again, rinsed with tap water and blot dried smear was flooded with decolorizing agent such as 95% ethanol for 30 sec.
Slide were rinsed again with water and blot dried. Then the slide were stained with counter stain safranin and slide were rinse with tap water and after air dried slide were examined under microscope using oil immersion [14].

Biochemical Characterization Of *Rhizobium* Bacteria:

Biochemical characterization of isolated bacteria was done on different media as per standard protocol of biochemical test for the confirmation of *Rhizobium* bacteria,The biochemical test are Glucose peptone agar test, Starch utilization test, Triple sugar iron test, Catalase test, Citrate utilization test and Lipase test.

Glucose Peptone Agar (GPA) Utilization Test:

These test were performed by inoculate the isolates on GPA medium to determine can bacteria utilize glucose as sole carbon source for its growth. Incubated *Rhizobium* bacteria show grow thon GPA medium[10].

Starch utilization test

These test were performed to determine the capability of microorganism to use starch as carbon source for its growth. Starch agar media were use to prepare plate and inoculated with isolated *Rhizobium* bacteria and incubate the plate for 24hrs. Bacteria utilize starch or not analyzed by using iodine test. Drops of iodine solution were spread on starch agar petri-plates. Positive test means a clear zone around the colony and remaining media containing starch are react with iodine and formation of blue color. Negative test means no zone around the bacterial colony, only blue color observed[3].

Triple Sugar Iron Agar Test:

Contains three carbohydrates such as glucose, sucrose and lactose and in this medium phenol reduce as an p^h indicator. The test mainly utilized to determine the capability of organism to use this carbohydrate sources[3].

Catalase Test:

This test were performed to identify the ability of organisms to produces the catalase enzyme

The test were performed by using slide method, 24 h old *Rhizobium* bacteria colonies were taken on glass slides and one drop of H_2O_2 (3%) was added. Catalase enzyme detoxifies hydrogen peroxide by breaking it into water and oxygen gas. Gas bubble indicated the test is positive[15].

Citrate Utilization Test:

Citrate agar is used to determine the ability of *Rhizobium* isolate organism to utilize citrate as a carbon energy source. A loopful of culture was streaked on to simmons citrate agar plates and incubated at 37^0Cfor 24hrs[15]. *Rhizobium* isolate cannot grow on the citrate and therefore no change in green colour occurs, test were negative.

Lipase Test:

Lipase test used to determine the ability of organism to produces exoenzyme lipase.

Isolate organism were streak on YEM plates supplemented with 1% (w/v) Tween 80. Plate were Incubated at 37^0C for 24-48 hrs.[15].

RESULTS AND DISCUSSION

Morphological Characterization:

Morphological characteristics of isolates were observed on congo red yeast extract mannitol agar (CRYEMA)medium[figure 3]. The colonies were characterized as round in shape with regular margin. The colonies were 2.3 mm to 2.5in size and show to produce white in color and translucent appearance. The bacteria were motile and gram's stained result is pinkish rod shaped observed under light microscope[figure 4] and hence the isolate were found to be Gram negative bacteria.

Table 1. Morphological Characteristic Of Rhizobial Strain.

Sr No	Strain characteristic	Rhizobial strain
1	Shape	Circular
2	Size	2.3-2.5mm
3	Color	White
4	Margine	Entire
5	Opacity	Translucent
6	Bacterium shape	Rod shaped
7	Gram staining	Gram negative
8	Motility	Mobile

Biochemical Characterization:

Biochemical characterization of isolate were performed on the basis of different biochemical tests such as glucose peptone agar test, starch utilization test, triple sugar iron test, catalase test. Citrate utilization test and lipase test. Results of Biochemical characterization explain in table 2.

In the present study, isolate were show positive result for glucose peptone agar test[figure 5]and catalase test[figure8]. Negative result for lipase test[figure 10], starch utilization test[figure 6] and citrate utilization test[figure 9].

Triple Sugar Iron Test:

Rhizobium isolates were subjected to the triple sugar iron (TSI) test by stab method and result were observed that test were positive for fermentation of the sugar, such as glucose, sucrose and lactose[figure 7].

Table 2. Biochemical characterization of the *Rhizobium* isolate from soybean nodule.

Sr No.	Test	Growth of *Rhizobium*
1.	**Glucose peptone agar test**	**+**
2.	**Starch utilization test**	**-**
3.	**Triple sugar iron agar test**	**+**
4.	**Catalase test**	**+**
5.	**Citrate utilization test**	**-**
6.	**Lipasetest**	**-**

["G(-)" =Gram-negative, "-" = No growth observed, "+"=Growth was observed].

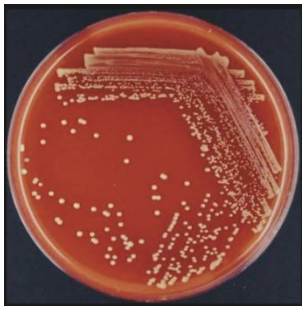

Fig 3: Growth On CRYEMA

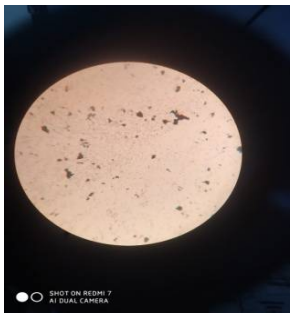

Plate.Fig 4: Gram's Staning Result.

Fig 5: GPA test.

Fig 6: Starch Utilization Test.

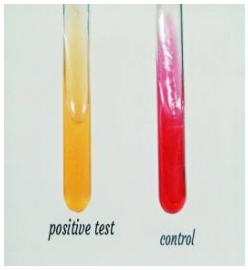

Fig7:Triple Sugar Iron Agar Test.

Fig 8: Catalase test.

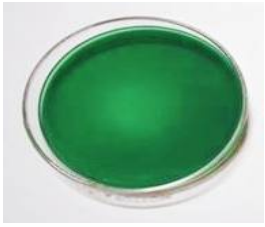

Fig 9: Citrate utilization test.

Fig 10: Lipase test.

DISCUSSION

Isolation and characterization of *Rhizobium* bacteria from soybean nodule which have ability to fix nitrogen and main concerns of this work is to study the soybean nodule bacteria and find out there characteristic.

CONCLUSIONS

This study help to showed the presence of nitrogen fixing bacteria as symbiotic association inroot nodules of leguminous soybean plant. *Rhizobium* help to provided nitrogen mineral for plant growth and development and zero effect on the environment compared to harmful chemical fertilizers. It was found in several research study these *Rhizobium* improve soil quality by nitrogen fixation activity and produces growth hormones and used as biofertilizer.

Therefore, isolation of nitrogen fixing bacteria and study there morphological and biochemical characteristics. Future use of this bacteria might be in preparation of biofertilizer and study there effect on plant growth and development.

REFERENCES

1. Aaronn Avit Ajeng, Rosazlin Abdullah, Marlinda Abdul Malek, Kit Wayne Chew, Yeek-Chia Ho,Tau Chuan Ling, BengFye Lau, Pau Loke Show(2020).The effect of biofertilizers on growth, soilfertility, and nutrients uptake of oilpalmunder Greenhouse Condition. Mdpi.com/ journal/processes, 1-16. *https://doi.org/10.3390/pr8121681*
2. Akbar Hossain, Sunil Kumar Gunri, Manashi Barman, Ayman El Sabagh, Jaime A. Teixeira Da Silva(2019).Isolation, characterization and purification of *Rhizobium* strain to enrich the productivity of groundnut (*Arachis hypogaea* L.) Open Agriculture. 4: 400-409. https://doi.org/10.1515/opag-2019-0040
3. Ankur Tyagi, Vijay Kumar, Purushottam and Akash Tomar (2017). Isolation, identification, biochemical and antibiotic sensitivity characterization of *Rhizobium* strains from *vigna mungo* (l) hepper, *cicer arietinum* l and *vigna radiata* (l) R wilczek in muzaffarnagar, uttar pradesh, india. Int.J.Curr.Microbiol.App. Sci 6(12): 2024-2035. *https://doi.org/10.20546/ijcmas.2017.612.233*
4. Cristian Dal Cortivo, Manuel Ferrari, Giovanna Visioli, Marta Lauro, Flavio Fornasier, GiuseppeBarion, Anna Panozzo, TeofiloVamerali. (2020). Effects of seed-applied biofertilizers on rhizosphere biodiversity and growth of common wheat (Triticum aestivum.) in the field. Frontiers in plant science,Vol 11,article 72, 1-14. https://doi.org/10.3389/fpls.2020.00072
5. Farooq Shahzad, Muhammad Kamran Taj, Ferhat Abbas, Muhammad Shafee, Saeed Ahmed Essote1, Imran Taj And Abdul Manan Achakzai(2019). Microbiological studies on *Rhizobium leguminosarum* isolated from pea (*pisum sativum* l.) Bangladesh j. Bot. 48(4): 1223-1229. https://doi.org/10.3329/bjb.v48i4.49079
6. Mahaveer P. Sharma, Khushboo Srivastava, Sushil K. Sharma(2009).Biochemical characterization and metabolic diversity of soybean rhizobia isolated from malwa region of central india. Plant Soil Environ., *56*, 375–383. https://doi.org/10.17221/247/2009-PSE
7. Murugaragavan Ramasamy, T. Geetha and M. Yuvaraj. Chapter. Role of biofertilizers in plant growth and soil health.Intechopen, 1-11 . DOI: 10.5772/intechopen.87429
8. P. K. Sahu, G. P. Brahmaprakash (2016). Formulations of biofertilizers approaches and advances. Springer India.179-198. https://doi.org/10.1007/978-81-322-2644-4_12
9. Rachael Kapembwa, Alice M. Mweetwa, Munsanda Ngulube, Jones Yengwe(2016). Morphological and biochemical characterization of soybean nodulating rhizobia indigenous to Zambia. Sustainable agriculture research; vol. 5, no. 3; 84-92. DOI: 10 .22004/ag.econ.241748
10. 1Sandeep Prakash Upadhayay, Navneet Pareek, Gaurav Mishra(2015). Isolation and biochemical characterization of *Rhizobium* strains from nodules of lentil and pea in tarai agro-ecosystem, Pantnagar, India. Nusantara Bioscience, Vol. 7, No. 2. 73-76. https://doi.org/10.13057/nusbiosci/n070203
11. Sangeeta S Singh, Kamal Joshi, Sunil Choudhary, Rakesh Nagar, Bindu Nirwan, Neha Sharma, Kuldeep Sharma, Shiwani Bhatnagar, Diksha Bhola, Ajit Varma(2019). Impact of biofertilizer on cropyield of Isabgol(Plantago Ovata) and senna(Cassia Alexandrina). Springer Nature Singapore. 125-131. http://dx.doi.org/10.1007/978-981-13-6790-8_9
12. Shaimaa I. M. Elsayed, A. A. Glala, Aboelfetoh M. Abdalla, Abd El Ghafour A. El-Sayed, Mona A.Darwish(2020). Effect of biofertilizer and organic fertilization on growth, nutrient contents and fresh yield of dill(Anethum graveolens).Elsayed.Bulletin of the national research centre, 44:122.1-10. https://doi.org/10.1186/s42269-020-00375-z
13. Sneha S, Anitha B, Anjum Sahair R, Raghu N, Gopenath T.S, Chandrashekrappa GK and Kanthesh MBasalingappa.(2018). Biofertilizer for crop production and soil fertility. Academia journal of agricultural research 6(8): 299-306. http://dx.doi.org/10.15413/ajar.2018.0130
14. Som Prasad Paudyal, Birendra Kunwar, Niroj Paudel, Bishnu Dev Das(2021). Isolation and characterization of Rhizobia from the root Nodule of some cultivated legume crops. European journal of biological research, 11(3): 294-306. http://dx.doi.org/10.5281/zenodo.4906255
15. Zeenat Wadhwa, Vivek Srivastava, Raj Rani, Tanvi, Kanchan Makkar and Sumit Jangra(2017). Isolation and characterization of *Rhizobium* from chickpea (*cicer arietinum*) *Int.J.Curr.Microbiol.App.Sci 6*(11): 2880-2893. https://doi.org/10.20546/ijcmas.2017.611.340

CITATION OF THIS ARTICLE

R. S. Wakde and A. P Narsinge: Isolation And Characterization Of Rhizobia From The Root Nodule Of Soybean Plant.. Bull. Env. Pharmacol. Life Sci., Spl Issue [1]: 2023:339-343.