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Isolation and characterization of *Rhizobium* sp. from a collar rot tolerant groundnut (*Arachis hypogaea* L.) variety

Shalvi Pandey & Dipjyoti Chakraborty*

Department of Bioscience and Biotechnology, Banasthali Vidyapith, Rajasthan 304 022, India

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Abstract

Groundnut, a major pulse crop, is prone to fungal diseases especially collar rot at seed germination and early seedling stage. In the present study, symbiotic bacteria was isolated and characterised from a collar rot resistant groundnut variety, Mallika and identified as *Rhizobium pongamiae* following biochemical and molecular characterization (NCBI Acc. No. MK770416). The bacterial isolate on cross inoculation, may be useful in improving crop productivity and enhancing collar rot disease tolerance in susceptible crops.

Keywords: *Arachis hypogaea*; *Aspergillus niger*; collar rot; crop legume; *Rhizobium pongamiae*

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*Correspondence

Dipjyoti Chakraborty

✉ cdipjyoti@banasthali.in**Indexing:** Plant Science Today is covered by Scopus, Web of Science, BIOSIS Previews, ESCI, CAS, AGRIS, UGC-CARE, CABI, Google Scholar, etc. Full list at <http://www.plantsciencetoday.online>

Introduction

Groundnut belonging to Fabaceae family (earlier Leguminosae), is commonly known as peanut, monkey nut or goober. It is an important crop for the tropical and sub-tropical regions. Groundnut, as a major pulse crop, plays an important role in Indian economy and is also grown as an oil seed crop (1). Groundnut seeds contain 22-30% of protein and almost 44-56% of oil. Approximately 9.5-19% carbohydrates and different types of minerals such as Mg, K, Ca, P and vitamins (K, E and B group) are also present in groundnut seeds (2).

Nowadays, for the higher yields of crops, agriculture community highly depends on the different types of pesticides and agro-chemicals (3,

4). These harmful agro-chemicals causes various problems including health issues, environmental pollution, and disturbance in biological communities etc. As an alternative, symbiotic rhizobacteria have the ability to fix the atmospheric nitrogen, improve soil fertility and can increase productivity of legume crops like groundnut when used as inoculants (5). Bio-fertilizers are capable to enhance the soil quality, provide protection to the plants from the different types of stresses either biotic or abiotic (6). Collar rot disease in groundnut is a serious issue, the causal organism being *Aspergillus niger*. Different types of soil factors and environmental factors also facilitate this disease.

The present study focused on the isolation and characterization of *Rhizobium* sp. from a collar

rot resistance groundnut variety (Mallika) which may suppress or inhibit *A. niger* when cross inoculated on collar rot susceptible cultivars.

Materials and Methods

Plant material

The plants of *Arachis hypogaea* cv. Mallika (ICHG-00440) were collected from the Krishi Vigyan Kendra, Banasthali Vidyapith, Rajasthan.

Root nodule collection and microbial isolation

Nodules collected from roots were thoroughly washed with tap water. For the surface sterilization, nodules dipped were momentarily in 95% ethanol, washed and dipped in sodium hypo-chloride solution for 1-2 min and after that again washed thoroughly with autoclaved water. Root nodules were crushed in minimum amount of sterile water and streaked on petri plates containing Yeast Extract Mannitol Agar (YEMA) medium (7).

Biochemical characterization

Congo red test

YEMA media plates were prepared by adding Congo red dye (10 ml of 0.25%). Bacterial strain was streaked on it and incubated at 28 ± 2 °C (8).

Bromothymol blue test

YEMA media plates were prepared containing BTB dye (5 ml of 0.50%), bacterial isolate was streaked on it and incubated at 28 ± 2 °C (8).

Alfa-ketolactose test

The bacterial isolate was streaked on lactose medium and incubated at 28 ± 2 °C for 5 to 7 days. Benedict solution was poured over the medium with bacterial culture and kept at room temperature for 2 h (9).

Catalase test

Catalase activity was observed by the following method of McLeod and Gordon, 1923. Bacterial colonies were picked in inoculation loop and dipped in hydrogen peroxide. Appearance of water bubbles within 5-8 sec confirms oxygen evolution by bacteria (10).

Indole test

Production of indole acetic acid was observed in inoculated tryptophan broth (2 g/l). After 7 days of incubation (at 30 °C), Kovac's reagent (1 ml) was added to the bacterial culture taken in a test tube and observed for 10-15 min for colored ring formation (11).

HCN production

Nutrient Agar plate was prepared containing 4.4 g/l glycine and the selected bacterial strain was streaked on it. After that, sterile filter paper which was dipped in picric acid and sodium

carbonate solution was kept over the inoculated region. Plate was incubated for 2-5 days at 30 °C. Formation of pale yellow color indicates low HCN production and dark brown color indicates high HCN production (8).

Molecular characterization

The bacterial DNA was isolated by using GenElute Bacterial Genomic DNA Kit (Sigma, catalogue number- NA2110-1KT). PCR amplification was done with the help of universal 16S rRNA gene primers fdl (AGAGTTTGATCCTGGCTCAG) and rd1 (AAGGAGGTGATCCAGCC) (12). The 16s rRNA gene primer set, 63f (5'-AGGCCTAACACATGCAAGTC-3') and Rhiz-1244r (5'-CTCGCTGCCACTGTAC-3') specific to *Rhizobium* sp. was also used (13). To design the primers, the conserved regions of the 16S rRNA genes are used and are capable of amplifying nearly full-length 16S rDNA.

Results and Discussion

Isolation and morphological identification of isolated strains

Fourteen bacterial isolates were obtained from *A. hypogaea*, out of which only one strain showed morphological similarity to the *Rhizobium* sp. as mentioned in the Bergey's Manual. The bacterial colony was fast growing, translucent, rod-shaped, mucoid and aerobic in nature. The colony was convex and 2-3 mm in diameter.

Biochemical characterization

Congo red test

Congo red test was done to check the purity of isolated *Rhizobium* sp. as different strains have variable ability to retain the dye as compared to the other bacteria. The bacterial isolate grown on YEMA medium in dark did not retain the dye (Fig. 1A).

Bromothymol test

Growth rate of the isolated bacterial strain was observed. A color change in dye denoted the growth rate among the studied isolates. Fast growing isolates turn dye color from green to yellow and on the other hand slow growing isolates change the dye color from green to yellow. In this study, the isolated *Rhizobium* sp. was fast growing as it turned dye color from green to yellow (Fig. 1B).

Alfa-ketolactose test

Benedict reagent was used in this test. *Agrobacterium* sp. can change the Benedict Reagent's color from blue to yellow but the isolated *Rhizobium* sp. did not change the color (Fig. 1C).

Catalase test

3% hydrogen peroxide was used and when it came in contact with isolated *Rhizobium* sp.

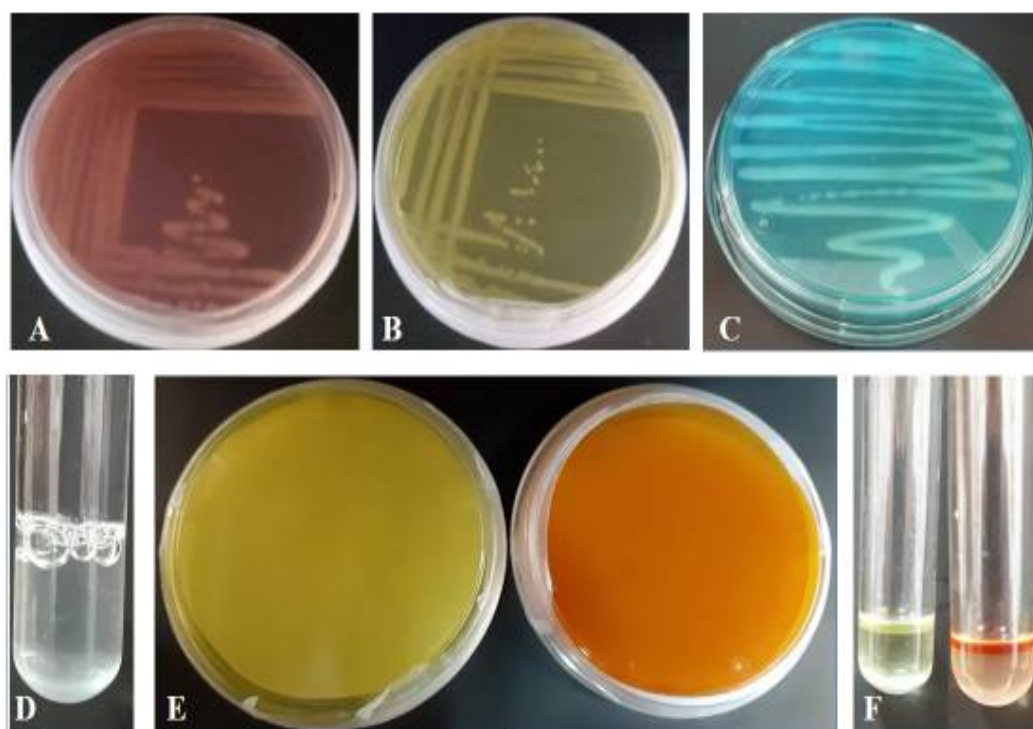


Fig 1. Biochemical Characterization of isolated *Rhizobium* strain (A) Congo red test; (B) Bromothymol test; (C) Alfa-ketolactose test; (D) Catalase test; (E) HCN production; (F) Indole test.

culture, bubble formation occurred. This result show that the isolated *Rhizobium* sp. is aerobic in nature (Fig. 1D).

HCN production

The inoculated *Rhizobium* sp. changed the color from pale yellow to dark brown show the positive result for HCN production (Fig. 1E).

Indole test

A red color ring was formed after the addition of Kovac's Reagent which showed the positive result for high IAA production (Fig. 1F).

Molecular characterization

Universal 16S rRNA gene primers fd1, rd1 and *Rhizobium* specific 16S rRNA gene primers (63f and 1244) were used for PCR amplification were by 1.67 kbp and 1.4 kbp bands were obtained respectively. DNA sequencing was done and EZ Taxon Tool was used to analyse the obtained 16s rRNA gene sequence and the isolate was identified as *Rhizobium pongamiae* strain BVAH-25 and submitted to NCBI (Acc. No. MK770416).

Conclusion

Biotic and abiotic stresses have ability to suppress the growth and adversely affects the productivity of the leguminous plants. Symbiotic interaction between legume plant roots and microorganisms enhance the defence mechanism against these stresses and cross inoculation of selected strains can be used for this purpose (14-16). In the present study, a bacterial isolate was

characterised from the root nodules of *Arachis hypogaea* cv. Mallika and identified on the basis of morphological and molecular characteristics. The isolated *Rhizobium pongamiae* may promote growth and increase tolerance to different types of stresses and also play an important part in sustainable agriculture system.

Competing interest

The authors declare that they have no competing interest.

Author's contributions

SP performed the experiments, designed the manuscript with the collection of references. DC conceptualised the work edited the manuscript.

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