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**Original Research Article**

# Isolation, characterization and salt tolerance activity of *Rhizobium* sp. from root nodules of some legumes

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**A B S T R A C T**

*Rhizobium* acts as a primary symbiotic fixer of nitrogen. The present study was focused on isolation, biochemical and salt tolerance activity of *Rhizobium* sp. from root nodules of three leguminous plants namely Soybean, *Trigonella* and Groundnut. About three isolates (R1, R2 and R3) were obtained and maintained on Yeast Extract Mannitol Agar medium (YEMA). For salt tolerance activity, YEM broths were supplemented with different concentration of NaCl (0.2, 0.4, 0.6, 0.8 and 1.2%). The isolated *Rhizobium* sp. were rod shaped, gram negative mucous producing. Among these three isolates, R3 isolate was grown into the YEM broth supplemented with 0.8 % NaCl. Therefore, R3 isolate may be beneficial bacteria to improve growth and development of leguminous plants under salinity condition.

**Keywords**

Rhizobium sp., Salt tolerance, legume

## Introduction

Nitrogen is one of the most essential nutrients required for growth and development of plants. The nitrogen can be provided for plants in the form of chemical fertilizers and biological fixation. Among these two, biological nitrogen fixation is the less costly and ecofriendly. According to Franche *et al.,* (2009) leguminous plants fix atmospheric nitrogen into soil with the help of nitrogen fixing micro-organisms. *Rhizobium* acts as primary symbiotic fixer of nitrogen by infecting root of leguminous plants. Kiers *et al.,* (2003) state that, *Rhizobium* bacteria stimulates the growth of leguminous plants by fixing atmospheric nitrogen into soil by symbiotically interaction.

Several environmental stresses may affect the nitrogen fixation in plants. It includes salinity, water stress, soil pH, temperature and heavy metals (Kucuk and Kivanc 2008). Waraporn Payakapong (2006) reported that, nearly 40% of total world land is affect due to salinity. Most of the leguminous plants are more sensitive to salinity and they require slightly acidic

soil for $N_2$ fixation. According to Zahran, (1999) salt stress directly affects symbiosis than free living *Rhizobia*. Thus, the isolated salt tolerance *Rhizobia* (R3) would be the highly important inoculums to improve the growth and development of the leguminous plants under saline environment.

## Materials and Methods

In the present study, three leguminous species namely Soybean, *Trigonella* and Groundnut were collected from different area in Sangli District (MS), India. The bacteria were isolated from root nodules as per the method described by Somasegaran and Hoben (1994). The morphologically healthy and undamaged root nodules were immersed in 95% ethanol for 5-10 sec. then it was transferred to 3% (v/v) sodium hypochloride solution for 2-4 min. and wash thoroughly with distilled water. *Rhizobium* sp. was obtained by streaking the crushed root nodules on YEMA (pH 7) media plates incubated at 28°C.

### Morphological characteristics

After 48 hrs of incubation, colony morphology of isolates was recorded on YEMA plates. The observed colonies were characterized as per method described by Aneja (2003), it include colour, size, shape, margin, elevation, opacity and consistency.

### Biochemical characterization

The different biochemical characterizations were done namely, Catalase test, Indol test, Methyl red test, Vogus Proskar test, Citrate utilization test, Gelatinase test, Nitrate reductase test, Sugar fermentation test, $H_2S$ production test as described by Lowe (1962).

### Salt tolerance study

A bacterial suspension was prepared from the pure isolates of *Rhizobium* and further inoculated into freshly prepared YEM broth supplemented with different concentration of NaCl (0.2, 0.4, 0.6, 0.8 and 1.2%) for salt tolerance study. These flasks were incubated at $26\pm2^0$C for 10 days. After completion of $10^{th}$ day incubation, the absorbance was measured at 540nm using a spectrophotometer (Mansah *et al.*, 2006).

## Results and Discussion

The colonies of *Rhizobium* sp. were observed on YEMA plates after completion of two days incubation at $30^0$C. Three isolate were obtained designated as R1, R2 and R3. These three isolates were rod shaped and gram negative in nature.

The conformations of this *Rhizobium* sp. were done by specific biochemical tests viz. Catalase, Indol test, Methyl red test, Vogus Proskar test, Citrate test, Gelatinase test, Sugar fermentation test.

In the present investigation it was found that, samples gives positive results for Catalase and Motility tests while, negative for Methyl red test, Vogus Proskar test, Indol test, Citrate utilization test, Hydrogen sulphide production (Table-1). Our result shows close conformity with findings of Shahzad *et al.*, (2012).

These results conformed the isolated bacterial strains are *Rhizobium* sp. Previously, Erum (2008), Singh (2008) and Shahzad *et al.*, (2012) mentioned that sugar tests are positive during isolation and characterization of *Rhizobium meliloti*.

The result of salt tolerance activity is

shown in Fig-2. The optical densities of all isolates were increased up to 0.6% NaCl. But, there were decrease the optical densities with increasing salt concentration. However, the increased optical density was obtained in R3 isolate at 0.8% NaCl. Thus the growths of R3 at 0.8% NaCl indicate that the R3 isolate have salt tolerant ability. Therefore, R3 isolate may be beneficial to improve growth and development of leguminous plants under salinity condition. Sugar fermentation test results showed that samples positive to glucose, sucrose, lactose, mannitol, maltose, D- xylose and L- arabinose (Table-2).

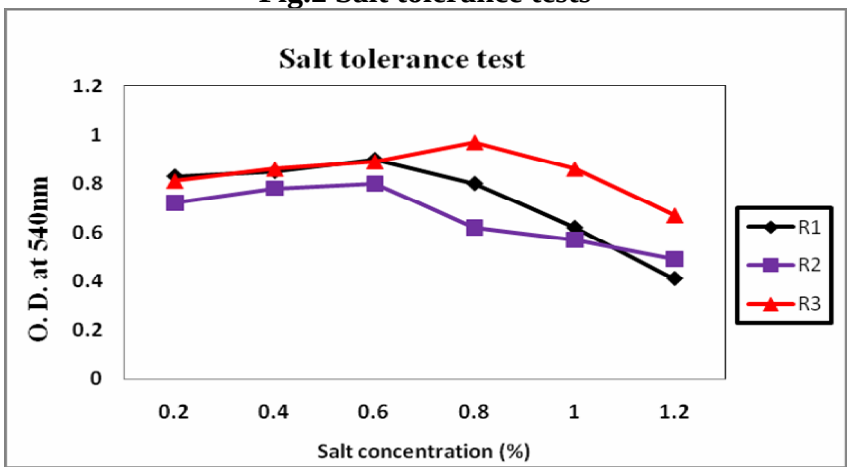
**Table.1** Biochemical characterization of *Rhizobium* sp. (R1, R2 and R3)

| Sr. No. | Biochemical tests | Results | | |
|---|---|---|---|---|
| | | R1 | R2 | R3 |
| 1. | Motility | Positive | Positive | Positive |
| 2. | Catalase test | Positive | Positive | Positive |
| 3. | Methyl Red test | Negative | Negative | Negative |
| 4. | Voges-Proskauer test | Negative | Negative | Negative |
| 5. | Indol test | Negative | Negative | Negative |
| 6. | Hydrogen sulphide production | Negative | Negative | Negative |
| 7. | Urea hydrolysis test | Negative | Negative | Negative |
| 8. | Citrate utilization test | Negative | Negative | Negative |

**Table-2 Sugar fermentation tests**

| Sr. No. | Sugars | Results |
|---|---|---|
| 1 | Glucose | Positive |
| 2 | Sucrose | Positive |
| 3 | Lactose | Positive |
| 4 | Mannitol | Positive |
| 5 | Maltose | Positive |
| 6 | D- Xylose | Positive |
| 7 | L- Arabinose | Positive |

**Fig.2 Salt tolerance tests**



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## References

Aneja, K. R. 2003. Experiments in microbiology plant pathology and Biotechnology, 4th edition, New age International Publishers, New Delhi, India.

Erum, S and B. Asghari 2008. Variation in phytohormone production in Rhizobium strains at different altitudes of northern areas of Pakistan, Pak. *Int. J. Agri. Biol.*10: 536–40.

Franche, C., Lindstrom, K. and Elmerich, C. 2009. Nitrogen fixing bacteria associated with leguminous and non-leguminous plants. *Plant Soil.* 321: 35-59.

Kiers, E. T., R. A. Rousseau, S. A. West and Denison R. F. 2003. Host sanctions and the legume–rhizobium mutualism. *Nature* 425: 79-81.

Kucuk, C. M., M. Kivanç and Kinaci, E. 2006. Characterization of *Rhizobium* Sp. Isolated from Bean. *Turk J. Biol.* 30: 127-132.

Lowe, G. H. 1962. The rapid detection of lactose fermentatation in paracolon organism by demonstration of 6- D-galactosidase. *J. Med. Lab. Technol.* 19: 21-25.

Shahzad, F., M. Shafee, F. Abbas, S. Babar, M. M. Tariq and Ahmad Z. 2012. Isolation and biochemical characterization of *Rhizobium meliloti* from root nodules of Alfalfa (Medico sativa). *J. Animal Plant Sci.* 22(2): 522-524.

Singh, B., R. Kaur, and Singh, K. 2008. Characterization of *Rhizobium* strain isolated from the roots of *Trigonella foenumgraecum* (fenugreek). *Afr. J. Biotechnol.* 7(20): 3671-3676.

Somasegaran, P., and Hoben, H. J. 1994. Handbook for Rhizobia: Methods in Legume-*Rhizobium* Technology. Springer-Verlag, New York.

Waraporn Payakapong 2006. Identification of two clusters of genes involved in salt tolerance in *Sinorhizobium* sp. strain BL3. *Symbiosis.* 41:47-53.

Zaharan, H., 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiology and Molecular Reviews.* 63:968-989.