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RESEARCH ARTICLE

Detection and characterization of *Albizia procera-Rhizobium* for stress tolerance

■ SMRITI KOLHEY, RAKESH PATEL, D. DASH AND T. CHOWDHURY

SUMMARY

A polybag experiment was conducted in glass house of Department of Agricultural Microbiology, College of Agriculture, Raipur, Chhattisgarh during the year 2011-12 taking *Albizia procera* (Safed siris) as test plant for nodulation with the objective to isolate and characterize the *Rhizobium* sp. from *A. procera* nodule. Inoculating effective stress tolerant *A. procera-Rhizobium* will improve nodulation and biomass production, above all for the production of healthy nursery stocks for afforestation programme. *Rhizobium* isolate from nodulated *A. procera* plant was tested for its sensitivity towards salt and acidity tolerance. The *A. procera- Rhizobium* is tolerant upto 30,000 ppm salt concentration whereas maximum growth was seen at 10,000 ppm. The isolate was found good in its growth at pH range of 6.5-7.5 but can tolerate pH 5.0. So it may be useful for tropical acidic rainfed areas of C.G. plain to support the growth of *A. procera* in afforestation programme and wasteland management.

Key Words : Acidity tolerance, *Albizia procera-Rhizobium*, Isolation, Nodulation, Salt concentration

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Albizia procera (Safed siris) is one of the important nitrogen fixing tree (NFT) species belongs to family Fabaceae, sub-family Mimosoideae. This species provides timber for making carts, carriages, small handle tools, supplies excellent fodder during lean periods of summer. It grows best on moist alluvial soils, well-drained loams or clay soils. Its ability to grow on dry, sandy, stony, and shallow soils makes it a useful species for reforestation of difficult sites but in expanding *A. procera* plantation in barren degraded hilly land, low fertility of the area creates major problem.

Planting leguminous trees may be an important option to enrich soil nitrogen as it forms nodules in root with symbiotic association with *Rhizobium* and fix atmospheric N (Chaukiyal *et al.*, 2000). Lots of research has been done on agricultural crops and there is a need to explore on forest legumes as tree- Rhizobia can fix N (upto 300kg N/ha/year) which can enrich soil N status and managing infertile lands. Inoculating effective *A. procera-Rhizobium* will improve nodulation and biomass production, above all for the production of healthy nursery stocks for afforestation programme.

Chhattisgarh region is exposed to extreme dry and hot climate during summer for prolonged period resulting in severe loss of mesophilic crop beneficial microbes in surface soils (Gupta *et al*., 2000). Further due to high temperature, salinity condition may prevail especially in rhizosphere region. Hence, identification of stress tolerant *Rhizobium* from *Albizia procera* is certainly useful in order to formulate those cultures which are able to survive/persist for longer period and work more efficiently in such conditions. So there is a need for searching stress tolerant and effective indigenous *Rhizobium* of Chhattishgarh plain

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
SMRITI KOLHEY, Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, RAIPUR (C.G.) INDIA
Email : smriti.4july@gmail.com

Address of the Co-authors:
RAKESH PATEL, D. DASH AND T. CHOWDHURY, Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, RAIPUR (C.G.) INDIA

which can enhance symbiosis. Therefore, the present study was carried out with the objective to isolate and characterize the *Rhizobium* sp. from *A.procera* nodule.

MATERIAL AND METHODS

Seeds of *Albizia procera* were collected from forest nursery, IGKV, Raipur. These were germinated in tray (soil+ sand,1:1) and then transplanted in polythene bags filled with 5 kg mixture of soil, sand and FYM in 3:1:1 proportion. At two months old, nodule was observed, washed carefully in lab and isolation of *Rhizobium* from nodule was done from fresh nodule of *Albizia procera* (Safed siris) seedling and culture broth was prepared using Yeast extract mannitol agar medium. The isolated *Rhizobium* was multiplied and tested for its tolerance to salt concentration and acidity.

Salt tolerance:

Albizia procera- Rhizobium isolate was inoculated separately on specific agar medium containing 10000, 20000, 30000, 40000, 50000, 60000 and 70000 ppm salt concentration (NaCl). For 10000 ppm 0.5g NaCl was added, for 20000 ppm 1.0g NaCl was added and carried on till 3.5g NaCl for 70000 ppm. Four replications of the plates for each isolate were maintained along with control. After 48 hrs. of incubation, observations for survival and / or growth of inoculums were recorded.

Acidity tolerance:

Yeast extract mannitol agar broth was prepared for inoculation of *Rhizobium* isolate of *Albizia procera*. The pH level of broth was adjusted to 5, 5.5, 6, 6.5, 7, 7.5, 8 and control. The pH was maintained by adding NaOH for higher pH and HCl for lower pH. Each level of pH was inoculated with *Albizia procera – Rhizobium* isolate. After completion of 3 days incubation period, survival of *Rhizobium* were recorded by inoculating on agar plates by streak plate method.

RESULTS AND DISCUSSION

Rhizobium isolate from nodulated *A. procera* plant was tested for its sensitivity towards tolerance to salt concentration and acidity. Results revealed that *Albizia procera- Rhizobium* can tolerate salt concentration upto 30,000 ppm with 90 colonies whereas maximum growth was seen at 10,000 ppm with 200 colonies and at control it was 240 colonies (Table 1 and Plate 1).

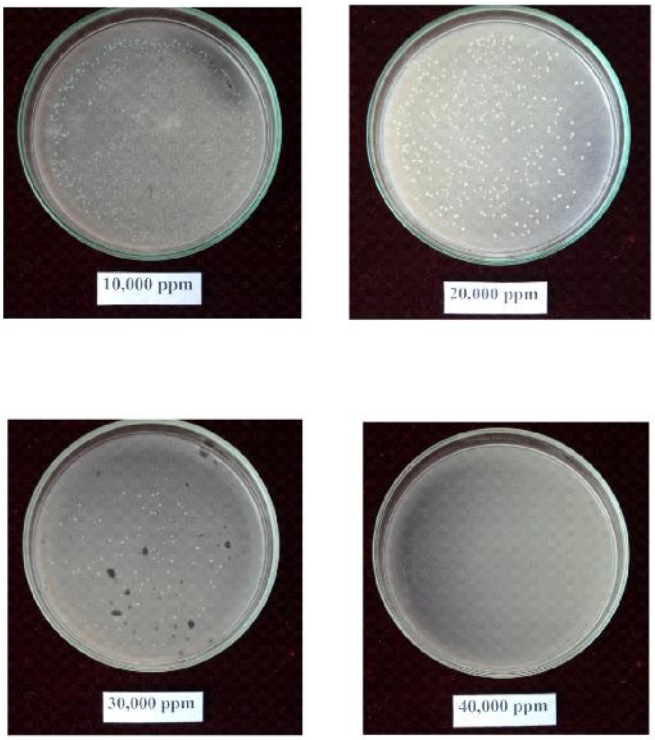

Plate 1 : Effect of salt concentration on *A. procera-Rhizobium*

As concentration of salt increased, growth of *rhizobium* decreased. Increasing salt concentrations caused detrimental effect on *Rhizobium* strains due to osmotic stress (Nagales *et al.*, 2002 and Thrall *et al.*, 2008) and salinity tolerant *Rhizobium* from *Tephrosia purpurea* from Ajmer region were also screened by Ali *et al*. (2009). Gupta *et al*. (2005) also found the *Rhizobium* strains tolerated salt concentration up to 40,000 ppm.

Rhizobia appear to be varying in their growth efficiency under acidic and alkaline conditions. In the current investigations, *Rhizobium* isolate of *A. procera* was the most potent acidity tolerant isolates and survive as low as pH 5.

Table 1 : Characteristics of *Albizia procera- Rhizobium* isolate

Salt tolerance							
10,000	20,000	30,000	40,000	50,000	60,000	70,000	Control
200 colonies	160 colonies	90 colonies	-	-	-	-	240 colonies
+++	++	+	-	-	-	-	+++
pH tolerance							
5	5.5	6	6.5	7	7.5	8	Control
+ +	+ +	+ + +	+ + +	+ + +	+	+	+++

(+++ - Very good growth, ++ - Medium growth, + - Poor growth)

Here pH 6 to 7 was found most suitable pH for its growth and showed survival upto pH 8. Very good growth was seen form 6-7 pH, medium growth at 5 and 5.5 pH and poor growth at 7.5 and 8 pH (Table 1) (Plate 2 and 3).

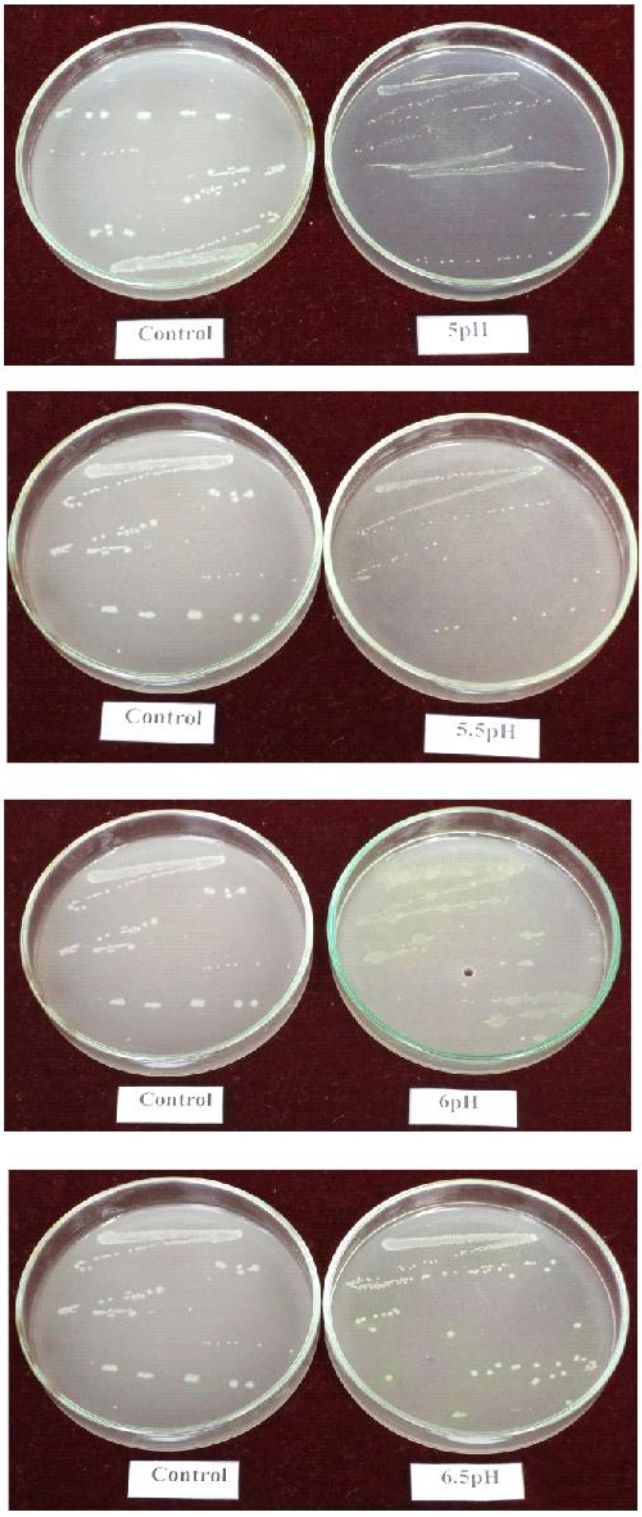

Plate 2 : Effect of pH on *A. procera- Rhizobium*

Harwani (2006) showed that a few of the rhizobial isolates from Haroti region of Rajasthan were able to grow at pH 4.5.These findings are similar to study of Ali *et al*. (2009) and Rodrigues *et al*. (2006) concluded that the pH

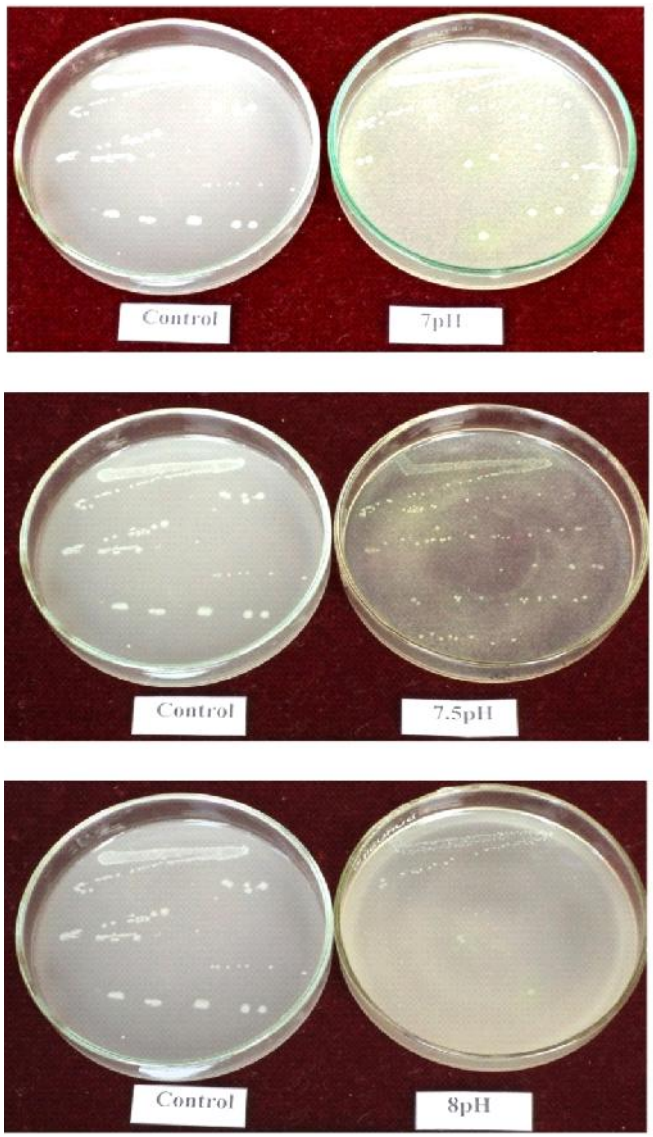

Plate 3 : Effect of pH on *A. procera- Rhizobium*

6.5-7.0 is the most optimum pH for the growth of *Rhizobium* bacteria.

Present result shows that *Albizia procera- Rhizobium* can tolerate salt concentration upto 30,000 ppm whereas maximum growth was seen at 10,000 ppm. The *Albizia procera - Rhizobium* isolate can tolerate acidity upto pH 5.0, so it may be useful for tropical acidic rainfed areas of C.G. plain to support the growth of *Albizia procera* in afforestation programme and wasteland management.

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