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Screening of Haloalkalophillic Rhizobium spp. isolated from saline soils of Gujarat

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Abstracts:

HalophillicRhozobiumspp have been isolated from saline soils, organisms tolerated a higher salt concentration (upto 5% NaCl), also grow at pH 4 and 4.5 level in the laboratory conditions. In the soil adjusted to pH 4-7, all the strains are also utilizing different carbohydrates as a sole carbon source and were found to be more resistant to many antibiotics (streptomycinsulphate, chloramphinicol, penicillin, tetracycline).

Key words: HalophillicRhozobium spp., saline soils, acid tolerance, salt tolerance

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Introduction:

Soil salinity is a significant problem facing agricultural production in many areas and soil infertility in these areas is often due to the presence of large concentrations of salt. Most leguminous plants require a neutral or slightly acidic soil for growth, especially when they depend on symbiotic N_2 fixation and as well more sensitive to salinity than their *Rhizobial*counterparts and consequently, the symbiosis being more sensitive to salt stress than free-living rhizobia. The strategies employed in the last few years to reduce the effect of salt stress on legume production have been focused on a selection of host genotypes that are tolerant to high salt conditions. Thus, an increase of tolerance to salinity of *Rhizobial*bacteria might constitute another approach to improve plant productivity under symbiosis.Few gram negative bacteria, known as rhizobia has been isolated from the saline soils samples. *Rhizobium spp.* can tolerate up to 500 mM of NaCl. It has been found out that some species of rhizobia adapt to saline conditions through the intracellular accumulation of low-

molecular-weight organic solutes called osmolytes, such as glutamate, trehalose, glycine betaine and polyamines, or an accumulation of K^+.

Materials & Methods:

Cultural Characteristics and pH Changes: The isolated gram negative strains were characterized on the basis of morphological and physiological characters. Cultures were examined for cell morphology and gram reaction after 1 to 2 days of growth in yeast extract mannitol liquid medium. Colony purity and morphology were examined using cultures that were grown for three to five days on Yeast Extract Mannitol Agar (YEMA) containing 0 to 25 mg of BromoThymol blue L-1. Change of the agar medium to yellow is indicative of acid production.

Test for High Acidity and Salt Tolerance: Gram reaction, cell shape, acid test, catalase and oxidase activity of these strains were studied. The capability of the *rhizobial*strains to grow in acidic media was tested by their being streaked on YEM agar plates of pH adjusted to 4, 4.5, 5.0, 5.5, 6.0, and 7.0 . The ability of the isolates to grow in different concentrations of salt was tested by streaking isolates on YEM media containing 0.5%, 1%, 2%, 3%, 3.5%, 4%, and 5% (w/v) NaCl.The soil was adjusted to 4, 4.5, 5, 5.5, 6 or 7 using CaCO3. After 15 days remaining at 28°C, 10 ml of distilled sterilized water was added to each one of the tube and 0.1 ml of the suspension streaked on Petri dishes containing yeast extract mannitol agar medium. The appearance of colonies after a lapse of three day time would indicate that the isolate had survived at a particular pH in the soil.

Carbohydrate Utilization: The basal medium was used with different carbohydrates (Cellulose; Fructose; Galactose; Glucose; Lactose; Starch; Sucrose; Arabinose; Acetate; Citrate; Succinate; Oxaloacetic acid) (1% w/v) substituted for mannitol. The medium was solidified with purified agar. Inocula were prepared by diluting the 24 hours young cultures with distilled sterilized water to a density of 10^6 cells per ml then inoculating the surface of carbohydrate containing agar plates. Triplicate plates of each carbohydrate were incubated at 28°C for 7 days and scored for growth.

Intrinsic Antibiotic Resistance: The antibiotics (mg l-1) used were: chloramphenicol (2, 20), sterptomycinsulphate (2.5, 10), Penicillin (20, 30) and Tetracycline (10, 20). Stock solution of the antibiotics was prepared immediately before use in sterile distilled water. Appropriate quantities of the antibiotic stock solutions mentioned above were added to molten YEMA at 48°C, mixed thoroughly and then poured on petri dishes. Isolates were grown in YEM broth for 48 hours. A portion of each culture was diluted in sterile distilled

water to prepare for solutions so that 0.1 ml of each isolate inoculated on a Petri dish would finally contain about 107 CFU/ml of the culture.

Results:

Effect of pH on Growth of Rhizobial strains on Laboratory Media and in Soil Conditions:

In the soil environment the condition is highly different. All the native rhizobial strains were able to survive well in the various soils adjusted to pH 4 up to 7, while the two exotic strains were unable to survive pHs up to 5.5 of the soil. Any of the rhizobial strains tested showed significant differences in their ability to utilize different carbohydrates as a sole carbon source. Of all the carbohydrates tested, only three (acetate, cellulose, succinate) were completely not utilized by all the rhizobial isolates.

Table: 1 Some morphological and physiological features of rhizoabial strains and effect of the different concentration of sodium chloride on their growth

Isolate No.	Gram Reaction	cell shape	Acid test	Catalase activity	Oxidase activity	Different pH Concentration					
						3	5.5	6	7	8	9
Rh1	Negative	Short rods	Yellow	Positive	negative	+	+	+	+	+	+
Rh2	Negative	Short rods	Yellow	Positive	negative	+	+	+	+	+	+
Rh5	Negative	Short rods	Yellow	Positive	negative	+	+	+	+	+	+
Rh8	Negative	Short rods	Yellow	Positive	negative	+	+	+	+	+	+
Rh11	Negative	Short rods	Yellow	Positive	negative	+	+	+	+	+	+
Rh12	Negative	Short rods	Yellow	Positive	negative	+	+	+	+	+	+
Rh18	Negative	Short rods	Yellow	Positive	negative	+	+	+	+	+	+

T**able-2: Effect of different pH of soil and lab media on growth of rhizobial strains**

Isolate No.	Different salt concentration of labmedia (%)						Different pH value of soil					
	1	2.5	5	8	10	15	4	4.5	5	5.5	6	7
Rh1	+	+	-	+	-	-	+	+	+	+	+	+
Rh2	+	+	-	-	+	-	+	+	+	+	+	+
Rh5	+	+	+	+	+	+	+	+	+	+	+	+
Rh8	+	-	-	-	-	-	+	+	+	+	+	+
Rh11	+	+	+	+	+	-	+	+	+	+	+	+
Rh12	+	+	-	-	-	-	+	+	+	+	+	+
Rh18	+	+	-	-	-	-	+	+	+	+	+	+

Table-3: Ability of isolates to utilize different carbohydrates as a sole carbon source

Isolate No.	cellulose	fructose	galactose	glucose	lactose	starch	sucrose	arabinose	acetate	citrate	succinate	OAA
Rh1	-	+	+	+	+	+	+	+	-	-	-	+
Rh2	-	+	+	+	+	+	+	+	-	+	-	-
Rh5	-	+	+	+	+	+	+	+	-	-	-	+
Rh8	-	+	+	+	+	+	+	+	-	+	-	-
Rh11	-	+	+	+	+	+	+	+	-	+	-	-
Rh12	-	+	+	+	+	+	+	+	-	-	-	-
Rh18	-	+	+	+	+	+	+	+	-	-	-	-

Table-4: Effect of different concentration of antibiotics on growth of isolates

Isolate No.	Different concentration of antibiotics (mg/L)							
	Streptomycin sulphate		Chloramphinicol		Penicillin		Tetracycline	
	2.5	10	2	20	20	30	10	20
Rh1	-	-	+	+	+	+	-	-
Rh2	-	-	+	+	-	-	-	-
Rh5	+	+	+	+	+	+	+	+
Rh8	+	+	+	+	+	+	+	+
Rh11	-	-	+	+	-	-	-	-
Rh12	-	-	+	+	+	+	-	-
Rh18	-	-	+	+	+	+	-	-

DISCUSSION:

Growth of the five native *rhizobial*strains are not much affected as the concentration of the salt increases from 0.5 to 5% as compared with that of exotic *rhizobial*strains, which were unable to grow in the sodium chloride concentrations above 2%. This ability of growth of the native *rhizobial*strains in high concentrations of sodium chloride solution can give high competitive value in the rhizosphere to survive and nodulate the host plants in harsh environmental conditions particularly at high concentrations of salt in the soil. This finding is in line with the report of Saraf and Dhandhukia (2005), who found that *Sinorhizobiummeliloti*growth was not completely inhibited by 5% of sodium chloride concentration. Rabie and Alamadini (2005) also stated that the growth of *Rhizobium* was not

affected by low and moderate levels of salinity. Acidity factors (high Al, low Ca and low PO4) have a direct impact on either *rhizobial*growth,persistence or nodule initiation and nitrogen fixation effectiveness (Covertry and Evans,
1989). Soil acidity limits *Rhizobium* survival and persistence in soils and its subsequent root colonization, infection and nodule activity (Graham *et al*., 1992). There was a greater variability observed in the ability of the *Rhizobium* isolates to grow on laboratory media of low pH and to persist in acid soil conditions of lower pHs. All the *rhizobial*strains were able to persist in the soil adjusted to pH 4 well for fifteen days, while the exotic strains were unable to persist in soil adjusted to pH 5.5, 8 and 9. This result indicates that in soil environment, the effect of acidity could be buffered by the edaphic factors in which the *Rhizobium* is surviving. As a result the *rhizobial*strains that survived in pH 8 of the soil for 15 days are very important candidates as inoculants for the highly acidic soils of most faba bean fields to improve the yield. The ability to grow at an acidic pH would provide these isolates with competitive advantage over other rhizosphere organisms. These observations are in line with the reports of Aurag and Sasson (1992), who indicated that strains of *Rhizobium leguminosarum*bv. *Phaseoli*grew in liquid media of pH 5. Evans *et al.*(1989) have cited critical pH ranges for*Rhizobium* activity as: at pH 7 *Rhizobium* root symbiosis unaffected by pH, at pH range of 7-9 suppression of nod gene occurs, in a pH range between 3 to 6, decreased multiplication and infection of *Rhizobium*occurs. Poor persistence of *Rhizobium leguminosarum*biovar.*viciae*in acidic soils has also been demonstrated and is reflected by a low nodulation score along with poor plant growth (Slattery *et al.*, 2001).

CONCLUSION: Survival, persistence and competitiveness of the *rhizobial*strains are the major factors determining their successful use as inoculants. In present soil inoculated Rhizobial strains are more competitive and resistant towards salinity which increase crop yield (Data not shown) ,the inoculated strains are distinguished from the indigenous *Rhizobia* present in the soil. A large number of methods have been described for these purposes, but because of their complexity most of these methods are of limited applications. Use of intrinsicantibiotics resistance is the simplest and most commonly used method for strain identification. In the present investigation it was found that, 100% of the isolates tested were able to resist chloramphenicol and 28.5% were resistant to the four antibiotics of chloramphenicol, streptomycin sulphate, penicillin and tetracycline while 28.5% were found to be resistant to two antibiotics of: chloramphenicol and penicillin.

REFERENCES

1. Keneni, A., Assefa, F. and Hailemariam, A.2008. Associative Effect of *Rhizobium* andPhosphate Solubilizing Bacteria on Growthand Nutrient Uptake of Faba Bean (*Viciefaba*L).
2. Telaye, A., Bejiga, G. and Berhe, A. 1994.Role of Cool Season Food Legumes andTheir Production Constraints in EthiopianAgriculture, In: "Cool Season FoodLegumes of Ethiopia", Telaye, A. (Ed.).Proceeding of the First National Conference,16-20, December 1993. Addis Ababa,Ethiopia. PP. 3-17.
3. Dibabe, A. 2000. Effect of Fertilizers on theYyield and Nodulation Pattern of Faba Beanon Nitosolosadet North Western Ethiopia.*Ethiopian J. Nat. Resource*, **2**: 237-244.
4. Aurag, J. and Sasson, A.1992. Tolerance of*Rhizobium leguminosarum*by Phaseoli toAcidity and Drought. *World J. Microbiol.Biotechnol***., 8**: 532-535.
5. Adamu, A., Assefa, F., Hailemariam, A. andBekele, E. 2001. Studies of *Rhizobium*Inoculation and Fertilizer Treatments onGrowth and Production of Faba Bean (*Viciafaba*) in Some Yield Depleted and YieldSustained Region of SemienShewa. *SINET,Ethiopia J. Sci*., **24**: 197-211.
6. Breedveld, M. W., Zevenhuizen, L. P. T. M.and Zehnder, A. J. B. 1991. OsmoticallyRegulated Trehalose Accumulation andCyclic Beta (1, 2) Glucan Excretion by*Rhizobium leguminosarum*Biovar. *Trifolii*TA–1. *Arch. Microbiol.,* **156**: 501–506.
7. Cordovilla, M. P., Ligero, F. and Lluch, C.1994. The Effect of Salinity on N2 Fixationand Assimilation in *Viciafaba. J. Exp. Bot.,***45**: 1483–1488.
8. Kucuk, C. and Kivanc, M. 2008. PreliminaryCharacterization of *Rhizobium* StrainsIsolated from Chickpea Nodules. *Afr. J.Biotechnol*., **7(6)**: 772-775.
9. Kucuk, C., Kivanc, M. and Kinaci, E. 2006.Characterization of *Rhizobium* sp. Isolatedfrom Bean. *Turk. J. Biol.*, **30**:127:13210. Covertry D. R. and Evans J. 1989.Symbiotic Nitrogen Fixation and SoilAcidity, In: *"Soil Acidity and Plant Growth*",Robson, A. D. (Ed.). Academic Press,Sydney, PP. 103-136.
10. CSA (Central Statistical Authority), 2002.*Agricultural Samples Survey, 2001/2002*.Main Season Statistical Bulletin No. 114.
11. Evans, J., Hockman, Z., O`Connor, G. E.and Osborne, G. J. 1989. Soil Acidity and*Rhizobium*: Their Effect on Nodulation ofSubterranean Clover on the Slops ofSouthern New South Wales. *Aust. J. Agr.Res.*, **38**: 605-618.
12. El Sheikh, E. A. E. and Wood, M.1989.Response of Chickpea and SoybeanRhizobia to Salt: Osmotic and Specific IonEffects of Salts. *Soil Biol. Biochem*., **21**:889-985.

13. Graham, P. H., Viteri, S. E., Mackie, F.,Vargus, A. T. and Palacios, A. 1992.Variation in Acid Soil Tolerance amongStrains of *Rhizobium phaseoli*. *Field CropsRes.*, **5**: 121-128.
14. Nogales, J., Campos, R., Ben Abdelkhalek,H., Olivares, J., Liuch, C. and Sanjuan, J.2002. *Rhizobium tropici*Genes Involved inFree Living Salt Tolerance Are Required forthe Establishment of Efficient NitrogenFixing Symbiosis with *Phaseolus vulgaris*.*MPMI,* **15(3)**: 225-232.
15. Jordan, D. C. 1984. Family III.Rhizobiaceae. In: "*BergeyÕs Manual ofSystematic Bacteriology*", Krieg, N. R. andHolt, J. G. (Eds.). Williams and Wilkins,Baltimore, PP. 234-242.
16. Purcino, H. M. A., Festin, P. M. and Elkan,G. H.2000. Identification of EffectiveStrains of *Bradyrhizobium*for *Archispintoi*.*Trop. Agric*., **77**: 226-231.
17. Rabie, G. H. and Almadini, A. M. 2005.Role of Bioinoculants in Development ofSalt-Tolerance of *Viciafaba*Plants underSalinity Stress. *Afr. J. Biotechnol*., **4(3)**:210-222.
18. Saraf, M. and Dhandhukia, P. 2005.Response of *Sinorhizobiummelilotit*o HighSalt Concentration and Effect of AddedOsmotica. *J. Microb. World*, **7**: 250-257.
19. Slattery, J. F, Conventry, D. R. and Slattery,W. J. 2001. *Rhizobial*Ecology as Affectedby the Soil Environment. *Aust. J. Exp.Agric*., **41**: 289-298.
20. Vincent, S. P. 1970. *A Manual for thePractical Study of the Root Nodule Bacteria.*IBP Hand Book, No. 15, Black WellScientific Publications, Oxford.
21. Payakapong, W., Tittabutr, P., Teaumroong,N., Boonkerd, N., Singleton, P. W. andBorthakur, D. 2006. Identification of TwoClusters of Genes Involved in Salt Tolerancein *Sinorhizobium*sp. Strain BL3. Symbiosis,**41**:47-53.
22. Wei, W., Jiang, J. and Yang, S. S. 2004.Mutagenesis and Complementation of *relA*from *Sinorhizobiummeliloti*042BM as aSalt Tolerance Involvement Gene. *Annals.Microbiol.*, **54(3)**: 317-324
23. Zahran, H. H. 1999. *Rhizobium*-legumeSymbiosis and Nitrogen Fixation under Severe Conditions and in an Arid Climate.*Microbiol. Mol. Biol. Rev.,* **63**: 968–989.