JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2011. Vol. **5**(1), p. 349-353

Screening of Native Bacteria Isolated from Tea Garden Soil of South Assam for their Abiotic Stress Tolerance

Pratibha Huidrom*, Bibhas Rajkumar and G.D. Sharma

Department of Life Science, Microbiology Laboratory,
Assam University, Silchar - 788 011, India.

(Received: 16 November 2010; accepted: 24 December 2010)

Native bacterial isolates of tea garden soils were evaluated for their ability to withstand abiotic stress (salt, pH and temperature). Composition of 23 bacterial species was *Azotobacter* 11, *Burkholderia* 6, *Enterobacter* 1 and *Rhizobium* 5. Growth of each bacterial isolate on selective broth (Yeast Extract Mannitol broth for *Rhizobium*, Ashby's for *Azotobacter* and Luria broth for *Burkholderia* and *Enterobacter*) having variable range of pH (2.5-10.0), different concentration of NaCl (0.1%- 4.5%) and temperature range of 15 ºC to 70 ºC were recorded at 580 nm using spectrophotometer after incubation at 28± 2ºC for 48 hours. Survival of bacterial isolates under variable stress of temperature was also selected using thermal death point (TDP) process. A total of 14 bacterial isolates were screened on the basis of their ability to withstand such extreme conditions. Such tolerant bacterial strains could be exploited as good PGPR (plant growth promoting rhizobacteria) in adverse conditions.

Key words: Abiotic stress tolerance, Bacteria, Tea garden soil.

Tea (*Camellia sinensis*) is an important agricultural crop for South Assam. Tea industry has a very important position in India's economy since the popularity of this non-alcoholic beverage has spread far and wide. However, tea is a highly chemical input based crop and therefore tea garden soils are polluted with chemicals and pesticides.

Rhizospheric bacteria play a vital role in improving soil fertility. In a spoonful of soil, there are thousands of beneficial soil microbes. Among them, there are groups that can fix atmospheric nitrogen, which can be easily available to plant for utilization. Some bacteria can thrive on extreme environments.[1]

Tea garden soils of South Assam are acidic (pH 4.5-5.5) in nature[2]. The leguminous shade trees (*Albizia* and *Delberzia*) of tea gardens are subject to severe environmental stress. The liberal use of synthetic fertilizers and pesticides has led to a global concern for environmental pollution as well as harmful side effects created by their excessive use in tea plantation[3]. Heavy input of chemical fertilizers and pesticides cause disturbance in the plant-microbe interactions[4]. Among the various environmental conditions, salinity, temperature extremes and pH stress are probably the most problem causing[5]. Screening of stress tolerant strains of rhizospheric bacteria may be useful to enhance the nodulation and nitrogen fixing ability of leguminous plants under stress conditions.

* To whom all correspondence should be addressed.
Mob: +91-9401281423, 9435072390
E-mail: pratibha_drom@yahoo.co.in

The ability of legume hosts to grow and survive in saline conditions is improved when they are inoculated with salt tolerant strains of rhizobia[6,7]. (Zou et.al., 1995; Hashen et.al., 1998; Shamseldin and Werner, 2005).

This study aims at selection of efficient and effective stress tolerant bacterial strains which could help in maintenance of a balanced ecosystem by improving soil fertility and thereby enhancing the production of tea.

MATERIAL AND METHODS

Study site

The study was conducted at five tea estates of Silchar, South Assam (longitude 92.51 ºE and latitude 24.5 ºN). The Barak Valley region covers an area of 6922 km2. The average altitude of the area above sea level is about 22 metres. The average annual rainfall of the study area is 2800 mm. The mean maximum temperature ranges from 25.4°C (January) to 32.6°C (August) and the mean minimum temperature ranges from 11°C (January) to 25°C (August). The average humidity of the place varies from a maximum of 97.5% to a minimum of 47.5 %.

Collection of samples and isolation of bacteria

Rhizospheric soils as well as root nodules of shade tree (*Albizia* sp.) of tea garden were collected from a depth of 10 cm in sterilized plastic bags and brought to the laboratory and kept at 10ºC till further processing of these samples. Nodules were washed in sterilized distilled water for several times. Following serial dilution agar plating technique[8] (Somasegaran and Hoben, 1993) using YEMA (Yeast Extract Mannitol Agar) medium containing 0.005% congo red dye[9] (Vincent, 1970), bacterial isolation was carried out. The plates were

Table 1. Effect of temperature on the growth of bacterial isolates

Identity of bacterial isolates	Range of temperature								
	Control	15ºC	25 ºC	28 ºC	35 ºC	45 ºC	55 ºC	65ºC	70ºC
AULS- B1a	+++	+	++	+++	+++	++	-	-	-
AULS- B1b	+++	+	++	+++	+++	++	-	-	-
AULS-B1c	+++	++	+++	+++	++	+	+	+	-
AULS-B3	+++	+	++	+++	+++	+	+	+	+
AULS-B3a	+++	++	++	+++	+++	+	+	+	+
AULS-B3b	+++	+	++	+++	+++	++	+	+	+
AULS-E4	+++	++	++	+++	++	+	+	+	+
AULS-R1	+++	++	+++	+++	+	-	-	-	-
AULS-R2	+++	+	++	+++	++	+	+	+	-
AULS-R3	+++	+	++	+++	+	+	-	-	-
AULS-R4	+++	+	++	+++	++	+	-	-	-
AULS-R5	+++	+	+++	+++	++	+	-	-	-
AULS-A1	+++	++	+++	+++	+	-	-	-	-
AULS-A2	+++	+	+++	+++	+	-	-	-	-
AULS-A3	+++	+	+++	+++	+	-	-	-	-
AULS-A4	+++	+	+++	+++	+	-	-	-	-
AULS-A5	+++	+	+++	+++	+	-	-	-	-
AULS-A6	+++	++	+++	+++	+	+	-	-	-
AULS-A7	+++	+	+++	+++	++	-	-	-	-
AULS-A8	+++	+	+++	+++	+	-	-	-	-
AULS-A9	+++	+	+++	+++	++	-	-	-	-
AULS-A10	+++	+	+++	+++	++	-	-	-	-
AULS-A11	+++	+	+++	+++	++	-	-	-	-

(+++; luxuriant growth, ++; moderate growth, +; Scanty growth)
Growth recorded by Thermal Death Point (TDP) Procedure
* Data indicate mean value of three replicates

incubated at 28± 2ºC for 48-72 hours. Pure cultures were obtained with further sub-culturing. The other rhizobacterial isolates were isolated from rhizospheric soil by serial dilution method and subsequent plating on selective media[10].

Stress tolerant studies

Tubes of LB (Luria broth) having variable concentration (1-4.5%) of salt (sodium chloride) and variable range of pH (2.5-10) were used. These tubes were inoculated with pure bacterial culture suspensions and incubated at 28± 2ºC for 48 hours. Their growth was measured as optical density (OD) at 580 nm using spectrophotometer (Systronics). The pure bacterial isolates were also studied for temperature stress by thermal death point process using different temperatures (15ºC- 70ºC)[10]. Mean value of triplicate OD reading was taken and mean±S.E against different concentration of NaCl was represented on a graph (Fig. 1 & 2)

RESULTS AND DISCUSSION

Isolation and authentication

23 bacterial species were isolated and identified following their morphological, biochemical and molecular characterization[11,12]. Accession numbers GU391260, GU569895, GU479029, GU569896, GU569897 and GU569898 are some of the band sequences submitted to the NCBI database. These belong to the genera of *Burkholderia*, *Enterobacter* and *Rhizobium*. *Azotobacter* was characterized by morphological and biochemical methods. Amongst these, 11 were *Azotobacter*, 6 *Burkholderia*, 1 *Enterobacter* and

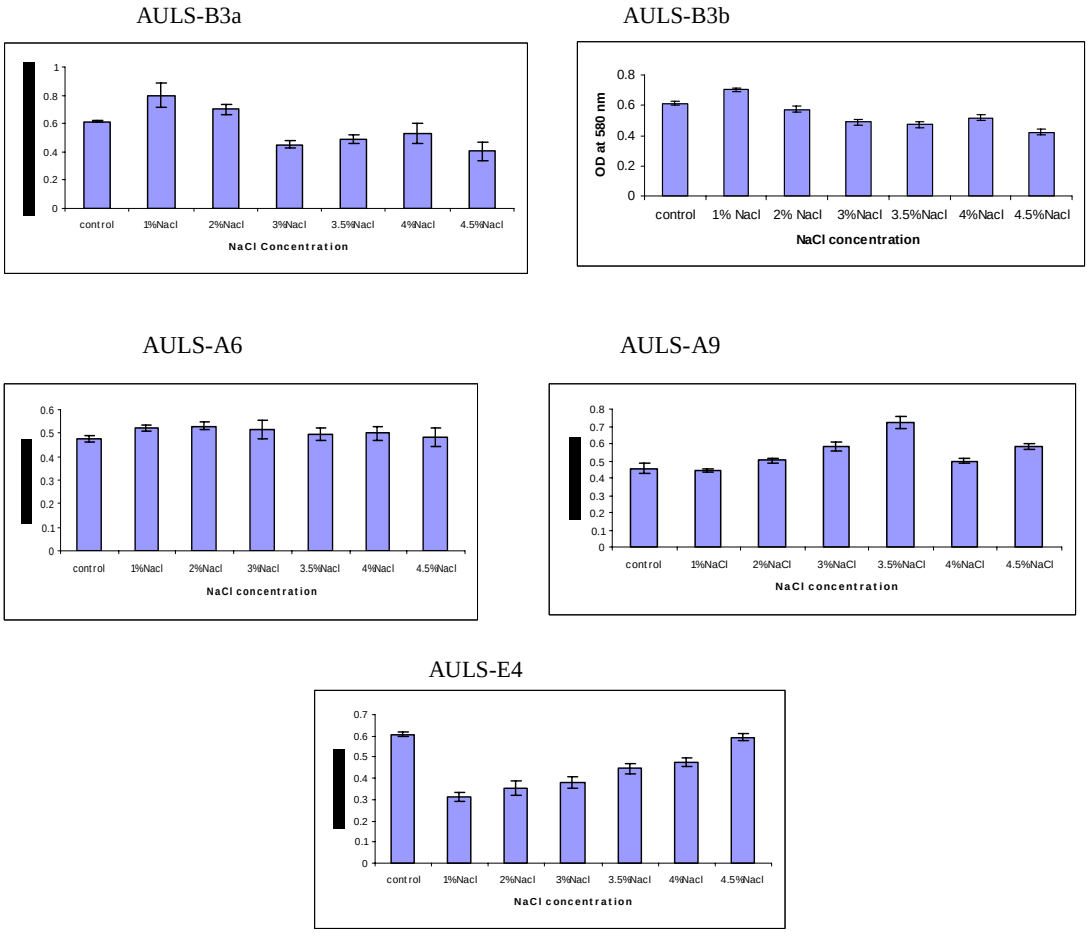

Fig. 1. Graphs showing tolerance of different concentration of NaCl by five rhizobacterial isolates. Error bars are SEM

5 *Rhizobium*. Nomenclature of the isolates was carried out representing the initials of the University, Department, Generic name and isolate number (numeric figure). For example, AULS-R2 indicates Assam University Life Science Rhizobium 2.

Salt tolerance

Tolerance to NaCl stress explains the bacterial ability to tolerate the stress and also their ability to adapt to the environmental change. In the current study, decreased growth of bacterial isolates with increasing salt concentration was registered. Increasing salt concentrations may have a detrimental effect on bacterial populations because of direct toxicity as well as through osmotic stress[13] (Nagales *et al.*, (2002) and Thrall *et al.*,[14] (2008). At 4% NaCl concentration the value of OD above 0.4 could be observed for five isolates (AULS-B3a, AULS-B3b, AULS-E4, AULS-A6 & AULS-A9). Hashem et.al, (1998) reported three rhizobial isolates of *Leucaena* showing tolerance to > 3% NaCl[15].

pH tolerance

Since tea garden soils of South Assam are acidic in nature most of the isolates showed good growth at pH 4.5 & pH 5. However, Rodrigues et.al, (2006)[16] quoted that the pH 6.5 – 7.0 is the most optimum pH for the growth of root nodulating bacteria. On the other hand, Harwani (2006) reported that a few of the rhizobial isolates from

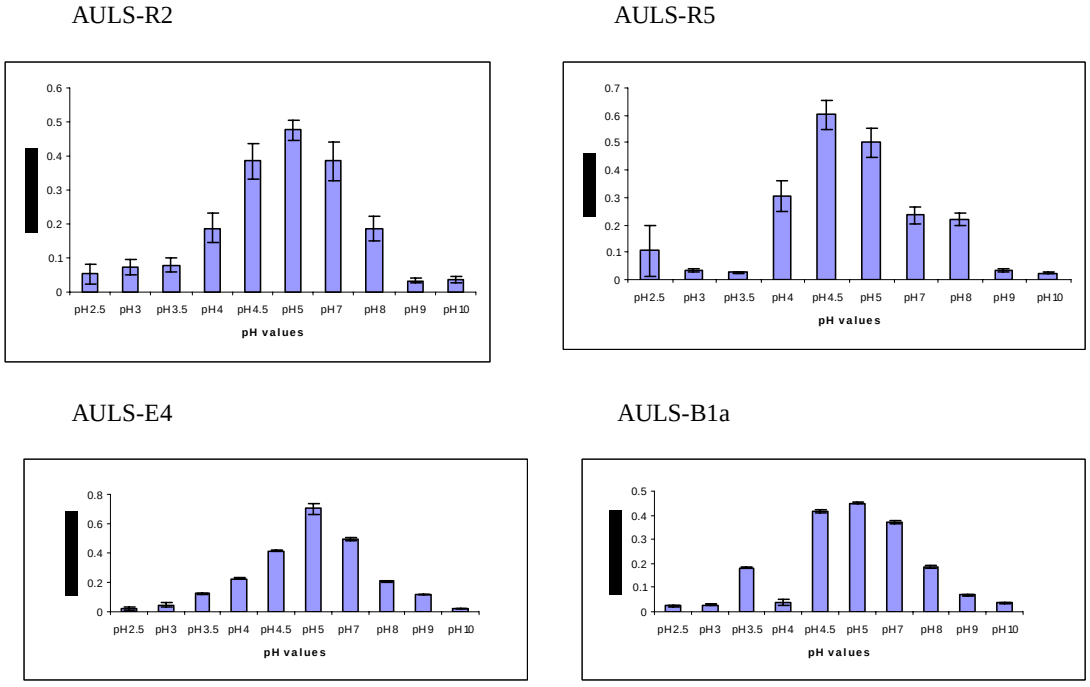

Fig. 2: Graphs showing tolerance of pH extremes by four rhizobacterial isolates. Error bars are SEM

Haroti region of Rajasthan were able to grow at pH 4.5. Isolates AULS-R2, AULS-R5, AULS-E4 and AULS-B1a showed good growths (OD>0.400) at pH 4.5-5 (range). At pH 10 OD values approx. 0.04 were observed for some isolates (AULS-B3, AULS-B3a and AULS-A1)

Temperature tolerance

At temperatures ranging from 25-35°C, majority of the isolates showed luxuriant growth. At 15°C, a few of the isolates (AULS-B1c, AULS-B3a, AULS-E4, AULS-R1, AULS-A1 and AULS-A6) exhibited moderate growth while remaining showed scanty or no growth. With increase in temperature there showed a gradual decline in the growth pattern. Growth and survival of rhizobacteria in soils are adversely affected by high soil temperatures (Meghvansi, 2006). In the present study three isolates viz. AULS- B1a, AULS- B1b and AULS-B3b showed moderate growth at 45°C. (Table 1)

CONCLUSION

This study highlights the importance of some beneficial soil bacteria, which can be utilized as potential PGPRs. Inoculation of stress tolerant strains of rhizospheric bacteria may enhance soil fertility and help in maintaining a balanced plant-microbe interaction.

ACKNOWLEDGMENTS

The authors wish to thank the Tea Garden Managers of Silcoorie Tea Estate and Rosekandy TE for their kind cooperation

REFERENCES

1. Delorme *et al*., Phenotypic and genetic diversity of rhizobia. *Soil Sci. Soc. AM.J*, 2003; **67**: 1746-1754
2. Subramaniam B. (ed): Tea in India, New Delhi: Wiley Eastern Ltd, 1995; 27
3. Mazumdar T, Goswami C and Talukdar N.C. Characterization and screening of tea rhizobacteria. *Indian J Biotechnol*, 2007; **6**: 490-494
4. Anonymous. Bugs Interrupted. KnowHow, *The Telegraph*. 2007; 9.
5. Ali S.F. *et al*., Selection of stress tolerant rhizobial isolates of wild legumes growing in dry regions of Rajasthan, India. *ARPN Journal of Agricultural and Biological Science*, 2009; **4(1)**: 13-18
6. Zou N., Dart P.J. and Marcar N.E. Interaction of salinity and rhizobial strain on growth and N_2-fixation by Acacia ampliceps. *Soil Biology and Biochemistry*, 1995; **27**: 409-413.
7. Shamseldin, A. & Werner, D.High salt and high pH tolerance of new isolated Rhizobium etli strains from Egyptian soils. *Current Microbiology*, 2005; **50**: 11-16.
8. Somasegaran P. and Hoben H.J. Collecting nodules and Isolating Rhizobia. In: Handbook of rhizobia: methods in Legume-Rhizobium Technology. Springer, New York; 1994; 13
9. Vincent J.M. A manual for practical study of root nodule bacteria.Blackwell Scientific Publishers, Oxford; 1970; p. 164.
10. Aneja K.R Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International Publishers, New Delhi; 2003; p. 607
11. Cappuccino J.G. and Sherman N. Biochemical activities of microorganisms. In: Microbiology, A Laboratory Manual. The Benjamin / Cummings Publishing Co. California; 1992; pp 125-178.
12. Bharathkumar, S et al., Characterization of the predominant bacterial population of different mangrove rhizosphere soils using 16sRNA gene based single stranded conformation polymorphism (SSCP). *World J Microbiol Biotechnol*, 2008; **24**: 387-394
13. Nagales J., Campos R., Ben-Abdelkhalek H., Olivares J., Lluch C. and Sanjuan J. Rhizobium tropici genes involved in free-living salt tolerance are required for the establishment of efficient nitrogen fixing symbiosis with Phaseolus vulgaris. *Molecular Plant-Microbe Interactions*, 2002; **15:** 225-232
14. Thrall P.H., Bever J.D. and Slattery J.F. Rhizobial mediation of Acacia adaptation to soil salinity: evidence of underlying trade-offs and tests of expected pattern. *Journal of Ecology*, 2008; pp. 1365-2745
15. Hashem F.M., Swelim D.M., Kuykendall L.D., Mohamed A.I., Abdel-Wahab S.M. and Hegazi N.I. Identification and characterization of salt- and thermo-tolerant Leucaena-nodulating Rhizobium strains. *Biology and Fertility of Soils*, 1998; **27:** 335-341.
16. Rodrigues C. S., Laranjo M. and Oliveira S. Effect of Heat and pH Stress in the Growth of Chickpea Mesorhizobia. *Current Microbiology*, 2006. **53(1)**: 1-7.