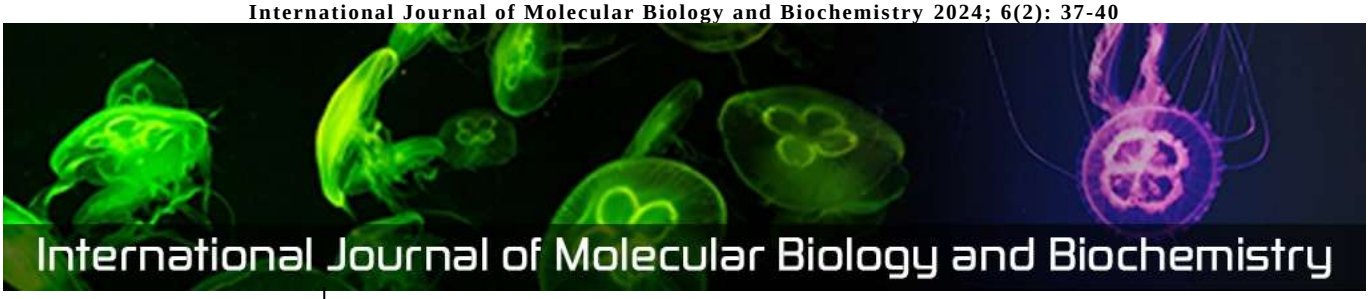

ISSN Print: 2664-6501
ISSN Online: 2664-651X
Impact Factor: RJIF 5.4
IJMBB 2024; 6(2): 37-40
www.biologyjournals.net
Received: 22-06-2024
Accepted: 29-07-2024

Ravi Prakash
Department of Chemistry, B.S.A. College, Mathura, Uttar Pradesh, India

RB Singh
Scienctist 'C' (UGC), Department of Zoology, School of Life Sciences, Dr. Bhimrao Ambedkar University, Khandari Campus, Agra, Uttar Pradesh, India

Corresponding Author:
Ravi Prakash
Department of Chemistry, B.S.A. College, Mathura, Uttar Pradesh, India

Exopolysaccharide production in a mean to tolerate salinity by *Rhizobium meliloti* nodulating *Mucuna pruriens* Linn. plant

Ravi Prakash and RB Singh

DOI: https://doi.org/10.33545/26646501.2024.v6.i2a.75

Abstract
The Rhizobial strains isolated from *Mucuna pruriens* Linn. plants were screened for the sodium chloride tolerance and exopolysaccharide productions in a salinity stressed medium containing up to 800 mM salt. The maximum growth was observed at 200 mM NaCl. Exopolysaccharide production was found to be directly proportional along with increasing NaCl concentration compared to control (Without salt). The nodulation activity of salt adapted Rhizobia with the host plant was limited up to 40 mM *in vivo*. Compared with control, the isolates retained their survival by increasing the EPS secretion thereby tolerating the salinity stress to a limited extent.

Keywords: *Mucuna pruriens*, *Rhizobium meliloti*, exopolysaccharide

Introduction
Salinity is a serious problem of agriculture in arid and semi-arid regions of the world [1]. Since most of the land area in Savannah and arid regions of Australia, Africa, North America, South America, India and Southeast Asia, suffer from high salts, achievements is when both the symbiotic partners in in legume- *Rhizobium symbiosis* resist such stress [2, 3, 4] Excessive soluble salts affect more than $4x10^6$ ha of the potentially arable lands of the world [5] and 6.1 million ha alone in India [6]. Infection of the root hairs to form the nodules by *Rhizobium species* is affected by salinity that decrease the number of nodules per plant and the amount of the nitrogen fixed per unit weight of nodules [7] resulting into decrease yield in the saline soil due to the lack of successful symbiosis [8]. Exopolysaccharide (EPS) is a mucoid substance, which is produced by bacteria that helps to protect the organism from the adverse environmental conditions. In *Rhizobium*, the role of EPS has been suggested in the binding and host specificity, of strain during the early stage of the infection [9, 10, 11, 12] and a large numbers of non-mucoid strains in different Rhizobium species have been isolated by different procedures and those mutants with less EPS usually make ineffective nodules on their test host plant [13].
In this study rhizobial strains have been isolated from the nodules of wildly growing *Mucuna pruriens* Linn. (Velvet bean) plants in the foothills of Himalayas. After morphological, physiological and biochemical characterization, the effect of different salt concentrations on growth and the production of EPS by rhizobia in explanta as well as the nodulation activity *in vivo* reported herein.

Materials and Methods
Isolation and characterization of rhizobial strains
Rhizobial strains were isolated from the root nodules of *Mucuna pruriens Linn* [14]. The strains were maintained on Yeast Extract Mannitol Agar (YEMA) slants at 4 °C. Total 10 strains were isolated and characterized according to Bergey's Manual of Determinative Bacteriology [15]. Growth on Hofer's alkaline medium (HAM) was tested by taking the log phase culture (10 cells ml-1) of the isolates [16] Catalase and oxidase activities were checked [17, 18] Gelatin and urea hydrolysis were studied [19, 20] The strains were tested for the ability to grow on Glucose Peptone Agar (GPA), medium [21] Citrate utilization [22] growth on 8% KNO [23] were tested.

The reduction of 2,3,5 Triphenyl Tetrazolium chloride (TTC) was checked. Carbon source utilization was determined [23] Cross infectivity [24] were carried out on 11 test plant species. The strains *R. meliloti* (MPR-8) showing the characteristics of the family-Rhizobiaceae [25] were selected for the further study. All the strains have been deposited in the Department of Botany and Microbiology, School of Life Sciences, Dr. Bhimrao Ambedkar University Agra, U.P. (India).

Effect of salt stress on growth

YEM broth (50 ml) was taken in conical flasks with NaCl (0mM to 800 mM). A loopful of log phase inoculums 10^a viable cell/ml was transferred to each flask and incubated at 28±10° C. The survival of the bacterial strain was checked in terms of colony forming unit (cfu ml-') by dilution plate technique after every 8 h up to 48 h.

Effect of salt stress on exopolysaccharide production

The broth culture amended with different NaCl concentrations were taken out after 96 h, 10 ml of each culture was subjected to centrifugation at 10,000 rpm for 45 minutes. The EPS was precipitated from the supernatant by adding 2 volumes of chilled acetone [26]. The extracted EPS was dried at 45±10 °C till constant weight was achieved

Study on symbiotic activity

Gum *Arabic* was used with charcoal to coat the bacterial strains (6x10^o cells per seed) on the seed surface [27] the inoculated seed were sown in the pots containing sterile soil amended with 0, 10, 20, 30, 40 mM NaCl concentration (w/w). Plants were watered with sterile water up to 45 days and uprooted carefully to observe nodulation, plant biomass, nodule weight and plant height. The nodules formed in different treatments were re-used to isolate bacteria to observe the effectivity of the nodules formed [14].

Results and Discussion

All the studied strains were Gram-negative rods, fast growers with average mean generation time of 3.5 h, motile, aerobic and the colonies were circular, convex, semi-translucent, raised and mucilaginous, usually 2-4 mm in diameter within 2-3 days on Yeast Extract Mannitol Agar (YEMA). Pronounced turbidity was observed after 12 h in agitated broth at 28±1 °C. On further incubation a highly viscous solution was formed. All the isolates failed to absorb Congo red dye on YEMA plates. The strains showed no growth on Glucose Peptone Agar (GPA) mediurn and produced acid on YEM agar plates. Growths on Hofer's alkaline medium were positive and were able to utilize urea. The strains showed growth on 8% potassium nitrate but could not utilize citrate. Gelatinase activity was negative. Catalase and oxidase activities were found positive. The strains were able to reduce 2, 3, 5 TTC. Tests on carbon source utilization revealed rhizobial growth on all the C-source tested except starch (Table-1). Cross inoculation studies of the isolates showed nodulation on its host *Mucuna pruriens Linn.*, besides *Medicago sativa* L. and *Trigonella foenum-graecum* L. (Table-2). The morphological, physiological and biochemical studies along with nodulation (Plant infectivity) revealed that the isolated strains belong to *Rhizobium meliloti* [25, 15, 28].

Test on salinity tolerance showed that the strains were able to tolerate NaCl concentration up to 800 mM in explanta and survival up to 16h at that concentration. Increase in NaCl concentration corresponded to decrease survival percentage up to 800mM. fast growing strains of rhizobia are more tolerant to salinity stress [29, 30] The stress of 950 mM NaCl tolerance in R. melilori strains 629-30 but growth enhancement was recorded up to 300 mM [28]. On the other hand, the rhizobia isolated from arid lands could tolerate the salinity equivalent to sea water [31, 32] reported that strains of *R. meliloti* grew in presence of 0.769 M NaCl was evaluated the effect of salt and pH on *Rhizobium* sp. NBRI 330 and found that the survival limit of the strain was 8 h in a medium containing 32% salt [33].

Exopolysaccharides (EPS) production by rhizobial isolates were found to be increased corresponded with increasing salt concentration of the media. The amount of EPS secretion was obtained minimum at 0 mM and vas maximum at 750 mM NaCl. Further increase in NaCl concentration reduced the secretion of EPS. The production of EPS renders the organism in protection from predation, desiccation and against antibacterial agents [34]. The role of EPS in interaction specifically, with the bost lectins during the initial stages of infection [35] was observed. EPS mutants (Reduced EPS producing strains) also showed reduced colonization and restricted proliferation inside the host roots [36]. Rhizobium inutants deficient in EPS production showed n inability to nodulate and fix nitrogen, thus providing strict correlation between the two, [37, 38] It was shown [26] that 0.4 M and 0.626 M NaCl osmolarity, the visco-elasticity property of *R. meliloti* changes abruptly to typical weak gel system. An induced EPS secretion was reported as salt concentration increased in case of *Rhizobium meliloti* YE2SL [39] Similarly, increased -539) calcium chloride stress (0.3 mM to 3 mM) synthesized. EPS at nearly twice the rate of *Rhizobium meliloti* WSM 419.

Although our strains were highly salt tolerant (800 mM) in explanta but did not establish nodulation at this concentration. The seeds sown in soil containing 40 mM NaCl concentration yield the vegetative growth but on further increase in NaCl could not allow seeds to germinate during pot experiments. *Mucuna pruriens* Linn showed feeble nodulation at 40 mM NaCl. The nodules formed on this concentration were very few, small and devoid of bacteria. Nodulation was decreased by 15%, 45% and 92.5% in 10, 20 and 30 mM NaCl respectively (Table-3). Similarly, the plant dry weight was also Towered by 31, 51, 69.6 and 72.2% respectively in comparison to that of control. The symbiotic activity was reduced with increasing salinity as evidenced by less and small number of nodules in comparison to that of control. The ability of F *Rhizobium sp.* to fix nitrogen is reduced with increasing salinity [7].

The depressive effect of salt stress in directly related to the salt induced decline in dry weight and nitrogen content of the shoot. In our study the nodule number, length of the plant and total biomass decreased in presence of high amount of NaCl treated set of experiment. A 50% reduction in nodule number and weight compared to control in *Soybean* [30] is reported with 26.6 mM NaCl in solution culture. The strain has shown better survival showing growth enhancement upto 200 mM NaCl and after that a declined trend was recorded. On the other hand, EPS production was enhanced upto 750 mM. This was due to excess secretion of EPS by the tolerant strains under defensive mechanisms as under stressed condition the change in morphological and physiological behaviours

cannot be avoided [33] In overall terms salinity affect the survval of *R. meliloti* strains, induce EPS secretion that renders the organism to protect themselves in the adverse saline environment.

Table 1: Biochemical Characterization of the strains of Rhiozbia isolated from *Mucuna pruriens* Linn. Plant

	Strains of Rhizobia									
Tests	**MPR1**	**MPR2**	**MPR3**	**MPR4**	**MPR5**	**MPR6**	**MPR7**	**MPR8**	**MPR9**	**MPR 10**
Gram's stain	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Growth on GPA	-	-	-	-	-	-	-	-	-	-
Acid production	+	+	+	+	+	+	+	+	+	+
Growth on HAM	+	+	+	+	+	+	+	+	+	+
Urea hydrolyisis	+	+	+	+	+	+	+	+	+	+
Growth on 8% KNO_3	+	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+
Catalase activity	+	+	+	+	+	+	+	+	+	+
Reduction of 2,3,5 TTC	+	+	+	+	+	+	+	+	+	+
Generation time (h)	3.46	3.6	3.8	3.5	3.45	3.3	3.6	3.54	3.2	3.7
C-Sources utilization										
Dextrose monohydrate	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+
Rhamnose	+	+	+	+	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+	+	+	+	+
D(+) Trehalose	+	+	+	+	+	+	+	+	+	+
Starch	+	+	+	+	+	+	+	+	+	+

-ve = Gram- negative
(+) = Growth occurred
(-) = No growth

Table 2: Cross inoculation studies using the strains from *Mucuna pruriens* Linn. plant

Host Legumes	**Strains of Rhizobia**									
	MPR1	**MPR2**	**MPR3**	**MPR4**	**MPR5**	**MPR6**	**MPR7**	**MPR8**	**MPR9**	**MPR10**
Pisum sativum	-	-	-	-	-	-	-	-	-	-
Vigna mungo	-	-	-	-	-	-	-	-	-	-
Vigna radiata	-	-	-	-	-	-	-	-	-	-
Phaseolus vulgaris	-	-	-	-	-	-	-	-	-	-
Lens culinaris	-	-	-	-	-	-	-	-	-	-
Cicer aerietinum	-	-	-	-	-	-	-	-	-	-
Medicago sativa	+	+	+	+	+	+	-	+	-	+
Mucuna pruricns	+	+	+	+	+	+	+	+	+	+
Trigonella foenum-graecum	+	-	+	+	-	-	+	+	+	+
Arachis hypogaea	-	-	-	-	-	-	-	-	-	-
Trifolium repens	-	-	-	-	-	-	-	-	-	-

(+) Nodulation
(-) No nodulation

Table 3: The effect of salinity on plant growth and nodulation *in vivo* (After 45d) from *Mucuna pruriens* Linn. Plant

Treatment	**Dry wt. (g)***	**Length of shoot (Cm.)***	**Number of Nodules***
0mM (Control)	3.58^4	341	29
10mM	2.448	267	23
20mM	1.719	229	15
30mM	1.087	195	07
40mM	0.779	150	02?#

Values are mean of 3 replicates
small ineffective nodules *Results are significant at 1% level of probability

References

1. Rao DLN, Sharma PC. Int J Exp Biol. 1995;33:500-503.
2. Zahran HH, Rasanon LA, Karsisto M, Lindstrom K. W J Microbial Biotechnol. 1994;10:100-103.
3. Brockwell J. Plant Soil. 1995;174:143-145.
4. Peoples MB. Plant Soil. 1995;174:3-6.
5. Flowers TJ, Trokt PB, Yed NB. Annu Rev Plant Physiol. 1977;28:89-92.
6. Kanwars JS. Agrokem Talajiam. 1969;18:79-81.
7. Lal B, Khanna S. W J Microbiol Biotechnol. 1994;10:637-640.
8. Hafeez FY, Aslam Z, Malik KA. Plant Soil. 1988;106:138-141.
9. Dazzo FB, Brill WJ. J Bacteriol. 1979;137:1362-1365.
10. Dudman WF. The role of surface polysaccharides in natural environments. In: Sutherland I, editor. Surface Carbohydrates of Prokaryotic Cells. New York: Academic Press; c1977. p. 357.

11. Dudman WF. The polysaccharides and oligosaccharides of Rhizobium and their role in the infection process. In: Veeger C, Newton WE, editors. Advances in Agricultural Biotechnology. The Hague: Nijhoff/Junk Publishers; c1984. p. 397.
12. Hollingsworth P, Hollingworth R, Dezec F. J Biol Chem. 1989;264:1461-1464.
13. Grey JX, Maagd RA, Rolfe BG, Johnston AWB, Lautenberg BJ. The role of Rhizobium cell surface during symbiosis. In: Verma DPS, editor. Molecular Signals in Plant Microbe Communications. Boca Raton: CRC Press; c1992. p. 359.
14. Vincent JMA. Manual for the practical study of root nodule bacteria. IBD Handbook No. 15. Oxford: Blackwell Publications; c1970.
15. Holt JG, Krieg NR, Sneath PHA, Stanley JJ, Williams SI. Bergey's Manual of Determinative Bacteriology. Baltimore: Williams and Wilkins; c1994.
16. Hofer AW. J Amer Soc Agro. 1935;272:8-10.
17. Graham PH, Parker CA. Plant Soil. 1964;20:383-386.
18. Kobak N. Nature. 1956;178:703-706.
19. Sadowsky MJ, Keyset HH, Bohloot BB. Int J Syst Bacteriol. 1983;33:716-718.
20. Lindstrom K, Lehtomati S. FEMS Microbiol Lett. 1988;50:277-280.
21. Kelzkowska J, Nutman PS, Skinner FA, Vincent JMA. Identification Methods of Microbiologists Part B. 5; c1968.
22. Koser SA. Biotech Agro. 1923;8:493-495.
23. Idrissi MM, Aujjat N, Dessau Y, Filali Maltauf A. J Appl Biotechnol. 1996;80:165-168.
24. Burton JC. Legume inoculant production manual. NifTal Project, MIRCEN, Univ. of Hawaii; c1980.
25. Jordan DC. Rhizobiaceae. In: Bergey's Manual of Systematic Bacteriology, Vol. 1. Baltimore: Williams and Wilkins; c1984. p. 234.
26. Navarini LA, Cesaro S, Siman B. Carbohydr Res. 1992;223:227-230.
27. Gupta CP, Dubey RC, Maheshwar DK. Abstracts of National Symposium on Microbes and Human Welfare. Meerut, India; c2000. p. 38.
28. Arora NK, Kumar V, Maheshwar DK. Symbiosis. 2000;29:121-124.
29. Hua ST, Tsai VY, Lichem GM, Noma AJ. Appl Environ Microbiol. 1982;44:135-138.
30. Singleton PW, Bohlool B. Plant Physiol. 1984;74:72-74.
31. Abd Alla MH, Abdel Wahab AM. Biologia Plantarum. 1995;37:131-134.
32. Kasmem M, Copellano A, Gounot AM. Mircen Journal. 1985;1:63-66.
33. Kulkarni S, Nautiyal CS. Curr Microbiol. 2000;40:221-223.
34. Faizio SA, Uhlinger DJ, Parker JH, Whyte DC. Appl Environ Microbiol. 1981;43(5):1151-1154.
35. Mody B, Meena R, Bindra O, Modi VV. Curr Microbiol. 1990;20:145-148.
36. Garg RK, Sharma PK, Kundu BS. Ind J Microbiol. 1992;36:193-196.
37. Glazebrook J, Walker GC. Meliloti Cell. 1989;56:661-665.
38. Zhan H, Every SB, Lec CC, Omer Leig JA. Proc Natl Acad Sci USA. 1989;86:3055-3057.
39. Ludovicus LD, Zevenbaizen TM, Paolo Fale Schini. Carbohydr Res. 1991;209:203-206.