

**CHARACTERIZATION OF SALT TOLERANT BACTERIA
ISOLATED FROM THE RHIZOSPHERE OF LEPTOCHLOA
FUSCA AND ATRITPLEX RHOCODOIDAES**

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ABSTRACT

Bacteria from the rhizosphere of the Leptochloa fusca (Lf) and Atriplex rhogodoideas (Ar) were isolated at 0.5 M NaCl Plates. Apparently 20 different purified colonies from each plant species were picked and streaked on 0.8 M salt concentration. The survivors were subsequently streaked on 1M salt concentration. Only 4 from Lf and 3 from Ar could manage to grow at 1M concentration. Slight variation was observed in colonial morphology of these strains. Except for Lf-5b all strains were Gram-negative rods. Lf-5b was Gram-positive and pleiomorphic. Lf- 5b and Ar-5b, were non-motile, while rest of the isolates were motile. Catalase and oxidase enzymes were present in Lf-2a, Lf-4a and Ar-5b while Lf-4b was positive for oxidase only. All but Lf-2b and Af-5b could ferment glucose and m mannitol. Slight variation in the 20 biochemical characters of these isolates was observed. According to Bergey's classification these isolates fall in four groups. Lf-2b and Ar-5b belong to Gram-negative aerobic rods; Lf-4a and Ar-5a to Gram-negative facultative anaerobic rods; Ar-3b to Gram-negative anaerobic rods; and Lf-5b to Gram-positive anaerobic bacteria.

Introduction

Cellular growth is generally inhibited by extreme environmental salinity. However, halophilic organisms have the ability to tolerate wide range of salt stress. Bacteria offer an excellent model system for understanding the biochemistry and genetics (at molecular level) of osmoregulation. Internal osmolarity in salt tolerant bacteria is maintained by inorganic ions and amino acids (Measures, 1975; Milner *et al.*, 1987; Anderson *et al.*, 1988). Interest in halophilic bacteria has recently been increased and bacteria from hypersaline water and soils have been isolated (Quesada *et al.*, 1984; Moral *et al.*, 1988). Halophilic bacteria belonging to genera *Pseudomonas*, *Alteromonas*, *Alcaligenes*, *Nbri*, *Flavobacterium* and *Acinetobacter* have been isolated from saltern water (Rodriguez *et al.*, 1985; Moral *et al.*, 1988). While Gram-positive cocci of the genera *Planococcus* and *Sporosarcina* have been found (Ventosa *et al.*, 1983) in hypersaline soil, *Delevalia halophila* was also identified from hypersaline soil (Quesada *et al.*, 1984).

A number of bacteria thrive in the rhizosphere of plants by obtaining their requirements from the plant exudates (Lynch, 1983). Nitrogen-fixing bacteria and many diazotrophs, which furnish the nitrogen requirement of plants, have been isolated from the roots of different plants (Malik and Zafar, 1985; Bilal and Malik, 1987; Reinhold *et*

al., 1987; Zafar *et al.*, 1988). This study is aimed to isolate salt tolerant bacteria from the rhizosphere of halophytes which like diazotrophs may contribute in developing resistance of plant in stress environment. Further, salt tolerant bacteria may provide means for understanding the genet is basis of osmoregulation.

Material and Method

Atriplex rhogodoidaes, and *Leptochloa fusca* from saline patches around Lahore were brought to the laboratory in polythene bags. In order to remove soil from the roots they were first washed with tap water and then with distilled water. Root samples free from soil were cut into pieces of 1 cm which were incubated in lest tubes containing salt nutrient agar with 0.5 M NaCl at 30°C for 24 hours. Four replicates for each plant pieces were incubated. Having observed bacterial growth, colonies from each master tube were streaked on nutrient agar plates containing 0.5 M NaCl. Initially colonies were purified on 0.5 M salt. Purified colonies were transferred to 0.8M and those which could grow at this concentration were subsequently transferred to 0.8M sail agar. Only those colonies which could grow at IM salt concentration were characterized

Morphology was observed under sterio and light microscope. Gram staining, catalase, oxidasc and OF tests were performed according to Gerhardt (1981), QTS-20 strips for various biochemical tests were obtained from DESTO Laboratories, Karachi,

RESULTS

Apparently 20 different colonies from each plant species were picked and purified on nutrient agar supplemented with 0.5M NaCl. Isolates form *Leptochloa fusca* and *Atriplex rhtogodoidaes* were disignated as Lf and Ar respectively. Purified strains (on 0.5M salt) were then streaked on 0.8 M salt nutrient agar plates and incubated for 48 hours at 30°C. Only 8 colonies form Lf and 7 from Ar were able to grow at 0.8 M concentration. Strains capable of growing at 0.8 M were further grown on 1M salt concentration for 48 hours at 30°C. Only 4 from Lf and 3 from Ar strains were able to grow at 1M salt concentration. Growth was slow at 1 M salt concentration compared with the growth at 0.5 and 0.8 M NaCl.

Morphological characters have been presented in Table 1. Colonies of Lf-2a, Lf-4a and Lf-4b were round with entire margin and raised (Lf-2b) or convex (Lf-4a, Lf-4b).Colonies of Lf-5b, Ar-3b, Ar-5a and Ar-5b were irregular with lobate (Lf-5b, Ar-5a) margin and raised elevation. Size of these colonies varied from 0.5 to 2 mm after 48 hours of incubation at 30°C (Table 1). Isolates from Lf were pale yellow while that of Ar were yellow in colour. All isolates were rod shaped except Lf-5b which were pleiomorphic. Rods of Lf-2b and Ar-3b occurred either singly of in groups of two while single rods and short chains both were observed in Ar-5a. Except Lf-5b and Ar- 5b, cells of other isolates were motile (Krieg and Holt. 1984).

Table 1: Morphological characterization of the salt tolerant bacterial strains isolated from the rhizosphere of *Leptochloa fusca* and *Atriplex rhogodoideaes*

Strain	Colonial morphology ⁺					Cell morphology		
	Shape	Margin	Elevation	Size mm	Colour	Shape	Size (u)	Motility
Lf-2b	rounded	entire	raised	0.5-7	pale yellow	rods	4.1 x 0.4	+
Lf-4a	rounded	entire	convex	0.8-1	pale yellow	rods	1.6 x 0.3	+
Lf-4b	rounded	entire	convex	0.8-1	pale yellow	rods	1.6 x 0.3	+
Lf-5b	irregular	lobate	raised	0.5-0.6	pale yellow	pleiomorphic rods	-	-
Ar-3b	irregular	undulate	raised	1.8-2	yellow	short rods	1.5 x 0.5	+
Ar-5a	irregular	undulate	raised	2	yellow	rods	2.6 x 0.5	+
Ar-5b	irregular	lobate	raised	1.5	yellow	rods	3.1 x 0.5	-

⁺ after 48 hours of incubation at 30°C.

With the exception of Lf-5b, all strains were Gram-negative only Lf-2b Lf-4a and Ar-5b) had catalase enzyme activities (table 2). Results of oxidase test revealed that Lf-2b, Lf-4a, Lf-4b, Ar-5a were aerobic while Lf-5b and Ar-3b lacked oxidase, system. OF test showed positive results for Lf-4a, Lf-4b, Lf-5b, Ar-5a, where only acid production was observed. Lf-2b and Ar-3b did not ferment glucose and mannitol, thus belonging to "aerobic rod" group as per Bergey's manual of Systematic Bacteriology (Krieg and Holt, 1984). When considering result of oxides and OF test together of other isolates it appeared that Lf-4a Lf-4b. Ar-5a fall in Gram-negative facultative "anaerobic" and Ar-3b in "Gram-negative anaerobic rod". Lf-5b belongs to Gram-positive anaerobic rods. For other biochemical characters. some Harlot, existed in some cases (Table 2). All isolates from *Leptochloa fusca* showed negative results for ONPG, citrate, to alon etc. lysine, arginine, ornithine, H₂SO₄.I, urea, indole, glucose, rhamnase, sorbitol and positive result for TDA, V.P and arabinose. For remaining tests results were variable for different isolates, For gelatin Lf-4a, and Lf-5b; for mannitol isolates Lf-4a and Lf-4b; for nitrate and maltose Lf-2b and Lf-5b; and for sucrose only Lf-2b gave positive results. While isolates from *Atriplex rhogodoideaes* showed positive results only for ONPG, nitrate and arabinose, for other tests negative results were observed for all the isolates except for Ar-3b which could liquefy gelatin.

Table 2: Biochemical characters of salt tolerant bacterial strains isolated from the rhizosphere of *Leptochloa fusca* and *Atriplex rhogodoidea*s

Test	Lf-2b	Lf-4a	Lf-4b	Lf-5b	Ar-3b	Ar-5b	Ar-5a
Gram's reaction	-	-	-	-	-	-	-
Catalase reaction	+	+	-	-	-	+	-
Oxidase reaction	+	+	+	-	-	+	+
OF	-	A	A	A	A	-	A
Growth on:							
Mac Conkey's agar	-	-	-	-	-	-	-
ONPG	-	-	-	-	+	+	+
Citrate utilization	-	-	-	-	-	-	-
Malonate	-	-	-	-	-	-	-
Lysine	-	-	-	-	-	-	-
Arginine	-	-	-	-	-	-	-
Ornithine	-	-	-	-	-	-	-
H ₂ S production	-	-	-	-	-	-	-
Urease test	-	-	-	-	-	-	-
TDA	+	+	+	+	-	-	-
Indole	-	-	-	-	-	-	-
VP	+	+	+	+	-	-	-
Nitrate reduction	+	-	-	+	+	+	+
Gelatin liquefaction	-	+	+	+	+	-	-
Sugar fermentation:							
Glucose	-	-	-	-	-	-	-
Nitrate	+	-	-	+	+	+	+
Maltose	+	-	-	+	-	-	-
Sucrose	+	-	-	-	-	-	-
Arabinose	+	+	+	+	+	+	+
Rhamnose	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-

+, positive; -, negative; A, acid production

Discussion

Out of 20 isolates obtained at 0.5 M salt concentration, only fifteen were able to grow at 0.8M NaCl concentration. Only seven of them could tolerate 1M salt concentration in the culture medium. At 0.5M NaCl, growth was faster and within 24 hours rich growth was observed. The growth was slow in 0.8M and 1M salt concentration (it took 48 for growth at 30°C), thereby indicating that cellular growth was generally inhibited or became slow in extremes of environmental salinity. However, few survivals on 1M salt concentration indicated that they acquired the ability to tolerate the extreme stress condition (Could ad Measures, 1977). There are different enzymes and organic/inorganic compounds present in the halophilic bacteria which are osmoprotectant and osmotolerant in nature (Measures, 1975). Thus, proline (Milner *et al*, 1987), glycine betain (Anderson *et al*, 1988), membrane derived oligosaccharides (Kennedy and Rumley, 1988) and glycerol (Reed *et al*, 1978) have been proved to be osmoregulants. Outer membrane proteins have also been involved in osmoregulation (Tsui *et al*, 1988). Colony size of all bacterial strains was larger at 0.5M NaCl than on 0.8 and 1M salt concentration, which could be due to i) change in membrane structure and function, because the phospholipid composition of several bacterial species depends on the salinity of growth medium (Miller, 1965); ii) inhibition of certain gene activity responsible for cellular elongation. There are relatively few genes yet known in the chromosome which are under osmotic control. The *pru^p* and *pro^p* are shown to encode a high affinity transport system in *Salmonella typhimurium* for the osmoprotectant Betaine, which is accumulated to high concentrations in response to osmotic stress (Csonka *et al*, 1985). Mutation was another possibility for salt tolerance by the cells. Isolates from *Leptochloa fusca* and *Amplifier rhogodoidae*s differ slightly from one another. All but Lf-5b were Gram-negative rods. Thus, Gram-negative bacteria are in excess in the rhizosphere of two plant species. Majority of bacteria isolated from the rhizosphere, rhizoplane and histoplane were Gram-negative, except for Bacilli which were Gram-positive. Isolation of bacteria from the rhizosphere is mainly focused on diazotrophs and those which have been isolated from salt tolerant plants are Gram-negative including *Azospirillum* (Reinhold *et al*, 1987), *zooglae* (Bilal and Malik, 1987), *Klebsiella* (Malik and Zafar, 1985) and *Enterobacter zafar et al*, 1988). It appeared that Gram-negative rods which had an adaptability of stress environment were present in the rhizosphere of two halophilic plant species.

The isolates can be divided into four axonomic groups described in Bergey's Manual (Krieg and Holt 1984). Lf-2b and Ar-5b belong to Gram-negative aerobic rods, while Lf-4a, and Ar-5a fall in Gram-negative facultative anaerobic rods. Ar-3h falls in Gram-negative anaerobic rods while Lf-5b is included in Gram-positive anaerobic bacteria. Further studies on the G+C content in DNA, DNA. DNA or DNA. RNA hybridization will ascertain the taxonomic position of this isolates.

Abbreviations used in the text

Lf	Isolates from <i>Leptochloa fusca</i>
Ar	Isolates from <i>Atriplex rhogdoidea</i>
OF	Oxidation Fermentation
ONPG	o-nitrophenyl galctoside
TDA	Tryptophane deamisae
V.P.	Voges Proskauer

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