Int.J.Curr.Microbiol.App.Sci (2021) *10*(05): 52-58

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 05 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1005.009

Isolation, Characterization and Identification of Salt Tolerant Bacterial isolates from Sodic Soil of Ahmednagar, Maharashtra

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Keywords

Salt tolerant bacteria and sodic soil

Article Info

Accepted:
12 April 2021
Available Online:
10 May 2021

A B S T R A C T

Sodic soils are characterized by high pH_s (>8.5),high ESP (>15) and variable electrical conductivity of soil saturation paste. These soils are very less organic carbon and microbial population. The sodic lands contain high concentration of sodium in their soil and difficult to cultivate. The applications of halophilic bacteria include recovery of salt affected soils by directly supporting the growth of vegetation thus indirectly increasing crop yields in salt affected soils. The present study was undertaken during 2018-19 for isolation of salt toerant bacteria from sodic soils that sustain in these soils and their formulation applications can increase nutrient uptake by the plants in sodic soils. The sodic soil collected from salt affected area in Ahemednagar district of Maharashtra was used for isolation of salt tolerant bacteria and these three isolates *viz.*, STB-1, STB-2 and STB-3 were characterised morphological, physiological and biochemically. Out of these, all 3 isolates were white in colony colour and colonies were round, circular in shape. The bacteria were rod shaped and singular or pair habitat. The isolates STB-1 and STB-2 were Gram negative and STB-3 was Gram positive in reaction. All 3 STB isolates were streaked on Nutrient Agar medium plates containing different NaCl concentrations showed STB-1, STB-2 and STB-3 grew prolific at 3 and 5 per cent (slightly salt tolerant) and moderate at 7 per cent salt concentration (moderately salt tolerant). All STB-1 to STB-3 bacterial isolates were characterized biochemically *viz.*, starch hydrolysis, H_2S production, Catalase test, oxidase test, denitrification, Methyl Red test and gelatin liquefaction. All three STB were positive in oxidase and catalase in reaction. Out of 3 bacterial isolates, 2 isolates were positive in starch hydrolysis reaction and 1 was negative in starch hydrolysis, H_2S production, Denitrification, Gelatin liquefaction. Thus, the salt tolerant bacteria were tentatively identified on the basis of morphological, physiological and biochemical characterization. The isolate-1 was found to be *Pseudomonas* spp. (STB-1), isolate-2 was *Azatobacter* spp. (STB-2) and isolate-3 was *Bacillus* spp. (STB-3). *The* consortia of salt tolerant bacteria viz., *Azotobactor sp., Bacillus sp., Pseudomonas sp*. is be useful for decreasing the pH of sodic soil and these consortia useful further for reclamation of Sodic soil.

Introduction

In India, about 6.73 M ha area is salt-affected; sodic and saline soils constitute ~56 per cent (3.77 M ha) and 44 per cent (2.96 M ha) of the total salt-affected area, respectively (Singh *et al.,* (2010). Sodicity is a severe obstacle to sustainable crop production in 11 states. Uttar Pradesh has the largest area (1.35 M ha) under sodic soils constituting nearly 36 per cent of the total. Besides Uttar Pradesh, Gujarat (14.36 %), Maharashtra (11.21 %), Tamil Nadu (9.41 %), Haryana (4.86 %) and Punjab (4.02 %) have high sodicity problem and together represent about 80 per cent of the total sodic lands in India. In Maharashtra, highest sodicity affected area is in Ahmednagar district (265000 ha) followed by Nashik (40000 ha), Aurangabad (31000 ha), Pune (26000 ha) and Solapur (20000 ha).

Sodic soils are characterized by variable electrical conductivity of soil saturation paste (EC_e, mostly < 4 dSm^{-1}), high pH_s (>8.5) and high ESP (>15). These soils are very less organic carbon and microbial population. The sodic lands contain high concentration of sodium in their soil. Sodic soils are characterized by a poor soil structure, they have low infiltration rate, they are poorly aerated and difficult to cultivate. Thus, sodic soils adversely affect the plant growth (Anonymous, 1998).

Halophilic microbes are also found to remove salt from saline soils. There are reports that potential salt tolerant bacteria isolated from soil or plant tissues and having plant growth promotion trait, helps to alleviate salt stress by promoting seedling growth and increased biomass of crop plants grown under salinity stress (Arora *et al.,* 2014). The biological conversion of organic matter into organic acids and CO_2 by microorganisms in alkaline sodic soil containing $CaCO_3$ within the soil, freeing Ca^{+2} ions. These Ca^{+2} ions may then exchange with Na+ ions aborbed to clay particles, allowing Na+ ions to be leached down further to the soil profile thus decreasing the ESP% (Lehrsch *et al.,* 1993). Both physical and chemical methods of their reclamation are not cost-effective and also the availability of mineral gypsum or other chemical amendments is a problem. The applications of halophilic bacteria include recovery of salt affected soils by directly supporting the growth of vegetation thus indirectly increasing crop yields in salt affected soils. Hafeez, *et al.,* 2015 reported about halototerant *Azotobacter* spp. and its bio-formulations. The efficiency of N_2 fixing ability of many *Azotobacter chroococcum* isolates was generally better in on saline and slightly saline strains in comparison to saline strains. Suitable management practices for salt affected soils are obviously different than the normal soils. Therefore, present study was undertaken for isolation of salt toerant bacterial isolates from sodic soils that sustain in these soils and their formulation applications can increase nutrient uptake by the plants in sodic soils.

Materials and Methods

The present study was undertaken at Department of Plant Pathology and Agricultural Microbiology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth., Rahuri during the period from 2018-19 to 2019-2020.

The soil sample was collected randomly from salt affected soils of D Block of Post Graduate Institute Farm, Mahatma Phule Krishi Vidyapeeth., Rahuri of Ahmednagar District. The soil sample was kept in polythelene bags and brought to the laboratory for isolation. The sodic soil sample was analyzed for pH_s, EC_e, ESP and OC (Singh *et al.,* 2005, Richards, 1968) and isolation of salt tolerant bacteria.

Isolation, characterization and identification of bacterial isolates

Salt tolerant bacteria were isolated from sodic soil samples on modified nutrient agar medium containing 3, 5 and 7 per cent NaCl, respectively according to their salinity requirements (Kushner, 1993) by serial dilution plate technique. Three isolates of Salt toerant bacteria were isolated from sodic soil collected from D Block of Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth., Rahuri and maintained at Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth., Rahuri, were used in the present investigation.

Characterization of bacterial isolates

Salt tolerant bacterial isolates were tested against NaCl salt of three concentrations. For this, NA medium supplemented with 3, 5 and 7 per cent NaCl were used to differentiate isolated bacteria into slight (tolerance to 3 %, 5 % salt) and moderate (tolerance to 7 % salt) salt tolerant categories, respectively, according to their salinity requirements (Kushner, 1993).

Morphological characterization

All the three isolates were examined for the colony morphology, cell shape and Gram reaction, as per the standard procedures given by Anonymous (1957) and Barthalomew and Mittewer (1950). The bacterial isolates were characterized for their Gram reaction and cell morphology by standard procedures given by Cappuccino and Sherman (1999).

These salt tolerant bacterial isolates were examined for their colony morphology as per standard procedures given by Cappuccino and Sherman (1999) by using nutrient agar media at appropriate NaCl concentration according to the salt requirement of each of bacterial isolates to be characterized.

Biochemical characterization

The isolates were subjected to biochemical characterization by several tests namely Methyl red test, Starch hydrolysis, Gelatin hydrolysis, Hydrogen sulfide production, Catalase test, Oxidase test and Denitrification as per the standard procedures given by Cappuccino and Sherman (1992).

Results and Discussion

Isolation of salt tolerant bacteria from sodic soil

The sodic soil of D block, Instructional Farm of Post Graduate Institute, MPKV., Rahuri was used for the isolation of salt tolerant bacteria. The soil sample was collected from salt affected areas and brought to laboratory and subjected for initial chemical analysis and isolation of the salt tolerant bacteria. The initial chemical analysis values of sodic soil are pH_s 8.61, EC_e 3.12 dSm^{-1}, Organic Carbon 0.28% and ESP 15.99%. The isolation resulted that three bacterial isolates of distinct colony types from sodic soil were isolated by using modified Nutrient Agar media and designated as STB-1, STB-2 and STB-3. Arora *et al.*, (2016) isolated two halophilic bacteria from sodic soils from Indo-Gangetic plains of Uttar Pradesh. The bacterial isolates were characterized, screened for salt tolerance.

Morphological, physiological and biochemical characterization of isolates

Salt Tolerant Bacterial isolates isolated from sodic soil were characterized and studied for their morphological, biochemical and physiological characteristics.

Morphological characters

The isolates of salt tolerant bacteria STB-1, STB-2 and STB-3 were studied for colony colour, Cell shape and Gram reaction (Table

1). Out of these, all 3 isolates colonies were round, circular in shape. Two isolates colonies were white and one in light green colony. The bacteria were rod shaped and singular or pair habitat. The isolates STB-1 and STB-2 were Gram negative in reaction and isolate STB-3 was Gram positive in reaction. (Hensyl, 1994) The studies of morphological characters of Holt *et al.,* 1994; Hensyl, 1994 which were conformity with the present study.

Physiological characterization of Salt Tolerant Bacteria

All the three isolates were tested physiologically on the basis of NaCl concentration and temperature and results represented in Table 2, 3.

Testing of Isolates to different concentration of NaCl

All 3 isolates were streaked on Nutrient Agar medium plates containing different NaCl concentrations showed isolate-1 (STB-1), isolate-2 (STB-2), isolate-3 (STB-3) grew prolific at 3 and 5 per cent *i.e.* slightly salt tolerant and moderate at 7 per cent salt concentration *i.e.* moderately salt tolerant (Table 2). The earlier research workers confirmed the halophilic nature on various media containing different concentration of NaCl salt. Yoon *et al.,* 2005 reported that slightly halophilic nature was determined in presence of NaCl concentration 2-3 per cent. The moderately halophilic nature in presence of NaCl concentration 0-10 per cent was studied by the Ramya *et al.,* 2012.

Testing of Isolates at different temperatures

All the three bacterial isolates showed full growth at 37°C whereas STB-1 isolate grows at 4°C and no growth observed at 41°C. STB-2 and STB-3 showed full growth at 37^0C and moderate growth at 41°C. The colony growth representation in given Table 3.

Saribay (2003) and Jadhav *et al.,* (2010) reported that the highest density of *Azotobacter* spp. occurs around 28^0C whereas another study found that the optimum temperature for growth of *Azotobacter* spp. was around 30^0C, which is found similar to present investigation. Rama *et al.,* 2012 isolated salt tolerant bacteria were Gram Positive, in reaction, non- motile, spore foring and grows optimum temperature at 35°C.

Biochemical characterization of Salt Tolerant Bacteria

All the STB-1 to STB-3 bacterial isolates were tested for biochemical characterization *viz.,* starch hydrolysis, H_2S production, Catalase test, oxidase test, denitrification, Methyl Red test and gelatin liquefaction. All three STB were positive in oxidase and catalase in reaction.

Out of 3 STB bacterial isolates, 2 isolates were positive and one was negative in starch hydrolysis, H_2S production, Denitrification, Gelatin liquefaction (Table 4). The results in conformity with the biochemical test of Perk *et al.,* 2006.

Identification of Salt tolerant bacteria

The salt tolerant bacteria were tentatively identified on the basis of morphological, physiological and biochemical characterization. The isolate-1 was found to be *Pseudomonas* spp. (STB-1), isolate-2 was *Azatobacter* spp. (STB-2) and isolate-3 was *Bacillus* spp. (STB-3).

Table.1 Isolation of salt tolerant bacterial isolates from sodic soils

Location	Isolate No.	Colony colour	Shape of colony/ Forms	Gram Reaction
D Block, PGI, MPKV,Rahuri Dist. Ahmednagar	Isolate-1 (STB-1)	Light green	Round colonies, Rod shape bacteria	–ve
	Isolate-2 (STB-2)	White	Round colonies, rod shape bacteria	–ve
	Isolate-3 (STB-3)	White	Circular colony, rod shape bacteria	+ ve

Table.2 Growth of bacterial isolates at different NaCl Concentrations

Salt Concentration(W/V)	Isolate-1 (STB-1)	Isolate-2 (STB-2)	Isolate-3 (STB-3)
3 %	+++	+++	+++
5 %	+++	+++	+++
7 %	++	++	++

+++ = Full growth ++ = Moderate growth + = Poor growth - = No Growth

Table.3 Growth of isolates at different temperatures

Temperature	Isolate-1 (STB-1)	Isolate-2 (STB-2)	Isolate-3 (STB-3)
4^0C	++	-	-
37^0C (Ambient temperature)	+++	+++	+++
41^0C	-	++	++

+++ = Full growth ++ = Moderate growth + = Poor growth - = No Growth

Table.4 Biochemical characterization of salt tolerant bacterial isolates

Isolate Test	Isolate-1 (STB-1)	Isolate -2 (STB-2)	Isolate-3 (STB-3)
Starch hydrolysis	-	+	+
H_2S Production	+	+	-
Catalase	+	+	+
Oxidase	+	+	+
Denitrification	-	+	+
Gelatin liquefaction	+	-	+
Methyl Red	-	+	-

STB-2 Azotobacter is grown on media with different NaCl concentrations, the results indicated that STB-2 *Azotobacter* showed moderate to full growth on 3, 5 and 7 per cent concentrations of salt.

Ravikumar *et al.,* (2004) reported that some types of *Azotobacter* isolated from mangrove sediments *viz., A. chroococcum, A. berijerinkii, A. vivelandii* can survive upto 3 % NaCl concentrations. Several research workers also have isolated salt tolerant *Pseudomonas* from various sources like saline infested zone of wheat rhizosphere (Upadhyay *et al.,* 2009; Zahir *et al.,* 2009).

Ahmed Gaber *et al.,* (2016) screened one bacterial isolate as indole acetic acid (IAA) producer on the basis of IAA colorimetric assay under 2 per cent of NaCl in the medium. Based on morphological, physiological and biochemical characteristics presented in Bergey's Manual of Systematic Bacteriology and on 16SrRNA homology, this isolate identified as *Pseudomonas fluorescens.*

Truper *et al.,* (1991) isolated whole range of halophilic bacteria of Gram positive rods and cocci, e.g. *Bacillus, Micrococcus* and *Salinicoccus*. Arora *et al.,* (2016) screended for salt tolerance of bacterial isolates.

The salt tolerant bacteria were isolated from sodic soil. The microbial population is less in sodic soil as compared to normal soil. On the basis of morphological, physiological and biochemical charactaristics of salt tolerant bacteria, they were identified as *Pseudomonas sp., Azotobactor sp., Bacillus sp.*

The consortia of salt tolerant bacteria viz., *Azotobactor sp., Bacillus sp., Pseudomonas sp.* is be useful for decreasing the pH of sodic soil and these consortia useful further for reclamation of sodic soil.

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How to cite this article:

Patole, A. S., R. B. Sonawane, R. T. Gaikwad and Navale, A. M. 2021. Isolation, Characterization and Identification of Salt Tolerant Bacterial isolates from Sodic Soil of Ahmednagar, Maharashtra. *Int.J.Curr.Microbiol.App.Sci.* 10(05): 52-58.
doi: https://doi.org/10.20546/ijcmas.2021.1005.009