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CHARACTERIZATION OF HALOTOLERANT PLANT GROWTH PROMOTING *BACILLUS MEGATERIUM* ISOLATED FROM LONAR SODA LAKE

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ABSTRACT

Twelve halotolerant bacteria were isolated from the composite soil sample collected from the vicinity of Lonar Soda Lake. Of these, highest salt tolerating gram positive rod shaped motile organism was selected and designated as ADL5.The isolate was identified as *Bacillus megaterium* using cultural, microscopic and biochemical characterization. Isolate ADL5 showed luxuriant growth at 3 % NaCl at pH 9 and temperature 30^{o}C and produced indole acetic acid and ammonia under salinity stress , it also have phosphate solubilization and nitrogen fixation ability.

Key words: PGPB, IAA, phosphate solubilization, nitrogen fixation

INTRODUCTION

Salinity of soil is a major abiotic stress which adversely affects the plant growth and yield. Throughout the world about 800 million hector of land is affected by salinity (Rojas et.al 2012). The direct effect of salinity on plant growth involves the loss of control on nutrient uptake which causes nutrient imbalance (Munns 2002). The previous studies showed that the plant required the additional supply of nutrients to maximize the adverse effect of stress under environmental stress. One approach to solve the salt stress problem and minimize the crop loss is use of halotolerant plant growth-promoting bacteria (PGPB) (Nakbanpote et. al 2014, Bharti et. al 2013, Awasthy et. al. 2011). Many gram positive and gram negative plant growth promoting bacteria have been reported earlier which promote the plant growth by different mechanism like production of plant growth regulators (IAA production, cytokinins, giberrelic acid), asymbiotic nitrogen fixation, ammonia prodution and solubilization of phosphate (Adesomoye et. al 2008,Tipre et al 2015). In the present study we have selected Lonar Soda Lake (19^{o} 58′ N and 76^{o} 31′E) which is highly saline and alkaline in nature for the isolation of halotolerant plant growth promoting bacteria to overcome the problem of salinity. (Rathod and Pathak 2014, Hingole and Pathak 2013, Joshi et. al 2008).

MATERIAL AND METHODS

Isolation of halotolerant bacteria

Soil samples were collected from the bank of Lonar Lake (*Latitude* 19° 58', Longitude 76° 36') in pre-sterilized cotton bags. Composite soil samples were diluted and soil aliquots were spread on nutrient agar plates (pH 9.0) with 2% NaCl concentration. Plates were incubated at 30^{o} C for 48 h, well-isolated colonies were selected, purified, and maintained on nutrient agar slants (Pathak and Rathod 2013, Sonalkar et. al 2015). These isolated colonies were further screened for salt and pH tolerance, for this purpose nutrient broth supplemented with various concentrations of NaCl (ranging from 0-5%) and pH (ranging from 5 to 10) was used for inoculation (Pathak and Sardar 2012, Pathak et.al 2015a, 2015b). Incubation was carried out at 30°C, 120 rpm for 48 h and growth was determined by measuring absorbance at 600 nm The isolate showing high salt and pH tolerance was selected for its morphological and biochemical characterization (Pathak and Rathod 2016,Pathak et. al 2014, Sneath et. al 1986).

Screening of selected isolate for plant growth promoting traits at 3% NaCl

Indole acetic acid (IAA) production by selected isolate was checked in yeast extract mannitol broth supplemented with 10 mgl^{-1} of L-tryptophan. The media was incubated in the dark on an orbital shaker at 200rpm for 72 h 30^{o} C. After incubation, the medium was centrifuged at 3,000 rpm for 20 min and the supernatant was used for quantitative IAA production using Salkowsky's reagent method (Sarwer and Kremer 1995). P-solubilization activity was tested on Pikovaskaya's agar medium containing 2% tricalcium phosphate (Maheshwari et. al. 2012). The appearance of clear halo zone around bacterial colonies after incubation for 24–48 h at 28 °C was observed. Nitrogen fixation ability of the isolate was determined by inoculating selecting isolate on nitrogen free medium containing 0.005% bromothymol blue (Nakbanpote et al 2013). Plates were incubated at 30^{o}C for 48 h and observed for change in colour of medium from greenish to blue. Ammonia production was detected by adding Nessler's reagent (0.5 ml/tube) to 24-h old bacterium grown in peptone water (Sengupta et. al 2015).

RESULTS AND DISCUSSION

Isolation, characterization and identification of halotolerant bacteria

Total 12 colonies were appeared on nutrient agar plate after incubation of 48 h. Out of the 12 colonies, 5 distinct colonies were selected and designated as ADL1 to ADL5. Among these ADL5 showed luxuriant growth and highest salt and pH tolerance was selected for further studies. Morphological and biochemical characters of ADL5 are given in Table 1.

Table 1. Morphological and biochemical characteristics of ADL5

Morphology		**Biochemistry**	
Colony shape	Irregular	Sucrose	+
Size (mm)	2	Mannitol	+
Margin	Entire	Indole test	+
Surface	Dull	MR test	−
Colour	yellow	VP test	−
Consistency	Dry	Citrate test	+
Microscopic features		**Enzyme profile**	
Cell shape	Rod	Catalase	+
Cell size (µm)	2.5×1.5	Oxidase	+

Flagellar arrangement	Peritrichous	Amylase	+
		Gelatinase	+
Gram	Positive	Urease	−
Biochemistry		Protease	+
Melliboise	+	Cellulase	−
Rhamnose	−	**Physiological attributes**	
Inositol	+	Temperature growth range (◦C)	20−50
Ribose	+	Optimum temperature (◦C)	30
Raffinose	+	pH growth range	7−11
Trehalose	−	Optimum pH	9
Mannose	−	NaCl tolerance (%)	0−5
Adonitol	−	NaCl required for optimum growth (%)	3
Lactose	+	Time for optimum growth (h)	24
Arabinose	+	**Antibiotic sensitivity**	
Cellobiose	+	Penicillin	R
Galactose	+	Streptomycin	S
Xylose	+	Tetracyclin	R
Sorbitol	−	Chloramphenicol	R
Fructose	+	**Identified as**	***B.megaterium***
Dextrose	+		

+ Positive, **−** Negative, **R** Resistant, **S** Sensitive**.**

ADL5 have tolerated 0-5% of NaCl concentration and showed optimum growth at 3% NaCl (Fig 1). ADL5 showed growth in the presence of 7-11 pH but failed to grow at pH 4-6 and showed optimum growth at pH 9.0 (Figure 2). On the basis of morphological and biochemical characterization ADL5 was identified as *Bacillus megaterium.*

Figure 1. Effect of NaCl concentration on growth

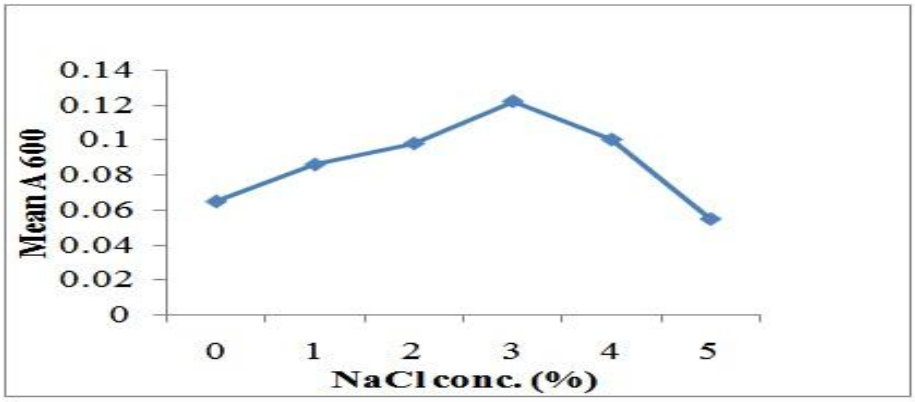
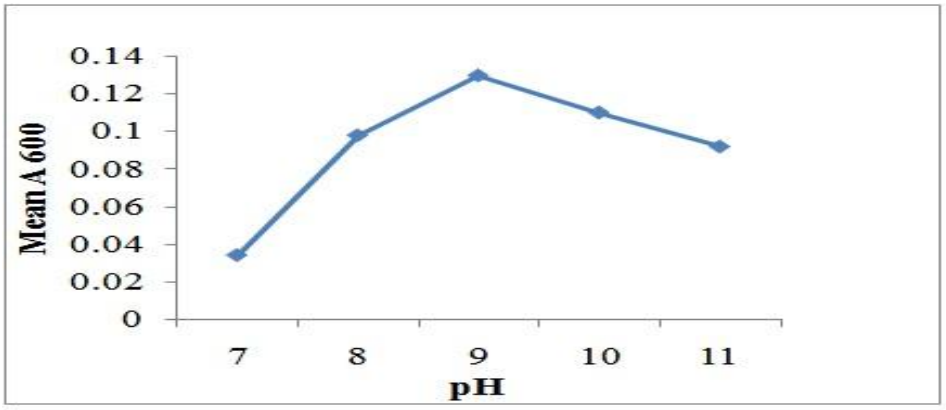


Figure 2. Effect of pH on growth



Screening of selected isolate for plant growth promoting traits at 3% NaCl

The selected ADL5 strain was screened for the plant growth promoting traits at 3% NaCl. Screening results are depicted in Table 2. Addition of Salkowski's reagent to culture supernatant of yeast extract mannitol medium showed pink coloration after incubation of 30 min which indicate IAA production by ADL5. It also has the phosphate solubilization, nitrogen fixation and ammonia production ability under saline condition. *nifH g*ene in *Bacillus megaterium* was reported by Ding et. al (2014) from rhizospheres of plant in China.

Table 2. Plant growth promoting traits of ADL5

	IAA production	**Phosphate solubilization**	**Ammonia production**	**Nitrogen fixation**
ADL5	+	+	+	+

+ Positive result, − Negative result.

CONCLUSION

An efficient halotolerant and alkaliphilic plant growth promoting bacteria was isolates from the Lonar soda lake and identified as *B. megaterium* It may be used as PGPR for enhancing plant growth under saline stress and for reclamation of saline soil to maximize the agricultural benefits.

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