Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI - 10.26479/2015.0102.01**

ISOLATION OF *AZOTOBACTER* SPP FROM SALINE SOIL AND ITS APPLICATIONS ON WHEAT (*Tritium aestivum*) PLANT FOR FUTURE USE IN RECLAMATION OF SALINE SOIL WITH WHEAT PLANT

R. B. Nawadkar, D.B. Jadhav, N.R. Shaikh*

Dept. of Microbiology, Yashwantrao Chavan College of Science,

Karad. Dist Satara. (M.S.) India

ABSTRACT

India is agriculture based country & most of Indian economy relies on agriculture. Indian soil is humus rich which ensures better yield of cash crops. But unfortunately many hectares of soil in this region are deposited with salts of sodium and potassium which render the land barren. Nearly 2,069,285 ha of land is saline alkaline & 1,261,266 ha is sodic soil. So the chief enemy of agriculture is saline soil, to reclaim such soil we can use salt tolerant wheat plant along with N_2 fixing diazotroph. Azotobacter is also used as phosphate solubilizer organism. Pot experiment is conducted in washed sand to test N_2 fixing efficiency. The present study deals with the implementation of haloalkalophillic *Azotobacter* in crop improvement. Alkalophilic species of *Azotobacter* were isolated from saline soil from Ratnagiri sea shore and other locations. Enriched samples were prepared from collected saline soil samples, as inoculated on modified Ashbey"s nitrogen free mannitol broth (NaCl 3.5%). The *Tritium aestivum* (wheat) plants were planted in a pot and the *Azotobacter* suspension was applied to it. The inoculum size used was $10^6 10^8$cells/ml & volume used was 10ml per pot containing *Tritium aestivum.* Uninoculated control was run along with the experiment. By applying *Azotobacter spp* suspension. to *Tritium aestivum* (wheat) plant we are successful in improving the dry weight of plant by 38.62%. So we can use haloalkallophillic *Azotobacter* spp to increase the yield of wheat plants to eradicate the problem of scarcity of food and we can reclaim the saline soil.

***Corresponding author: Dr. N.R. Shaikh** Ph.D.
Dept. of Microbiology, Yashwantrao Chavan College of Science, Karad. Dist Satara. (M.S.) India
Email Address: nrsshaikh@gmail.com

1.INTRODUCTION

Nowadays a problem of soil being saline is leading the world to the scarcity of food as the soil remains no more fertile. The saline soil is "The soil containing sufficient soluble salts to adversely affect the growth of most crop plants". In saline soil excess of salts present on soil surface & in the root zone these soils can normally be identified by the presence of white crusts of salts on the surface of land area & poor crop growth in such areas[1] In India the problem has taken serious mode about 9 % of the total cultivated area is affected by salinity. [2]. The problem is acute in the state of Maharashtra, Punjab, Haryana and Uttar Pradesh states of India. In Maharashtra about 34 million hectares is affected by salt, such kind of saline soils are mostly obtained in Satara, Sangali, Kolhapur, Solapur, Ahamadnagar, Dhule districts of Maharashtra state of India. High concentrations of salts have detrimental effects on germination of seeds [3] and plant growth [3].Many investigators have reported retardation of germination and growth of seedlings at high salinity [3]. However plant species differ in their sensitivity or tolerance to salts especially wheat plants are resistant to salts [3]. Microbial bioremediation is suggested by many scientific workers & application of nitrogen fixers have been suggested best strategy for bioremediation of saline soil by many authors. [4] High salt levels can adversely affect plant growth & soil structure. Saline soils contain excessive salts. It is deficient in nitrogen, phosphorous & other trace elements which are required for plant growth. Saline soils contain soluble salts which impairs the soil productivity.

Salinity of soil affects the plant growth in many ways:-

- The harmful effects of saline water irrigation are mainly associated with accumulation of salts in the soil & are manifested through the reduction in availability of water to plants, delayed germination & slow growth rate.[3]
- Excessive salts in the soil cause, early wilting & showed effects similar to those of drought condition.
- Some of the visual symptoms are: plant look stunted, leaves are smaller but thicker & have often dark green in color as compared to plants growing in a salt free area.

There are many techniques used for saline soil reclamation, such as physical, chemical & biological methods. Microbiological activities involve nitrogen fixation, phosphate solubilization, transformation of many elements etc. which help to increase plant yield. As we know Nitrogen (N), Phosphorous (P) & Potassium (K) are the most important plant nutrients. [5,6] The soil fertility depends on its chemical composition but also, on the qualitative & quantitative nature of microorganisms inhabiting it. [5] Plants growth can be promoted by plant growth promoting rhizobacteria (PGPR) in many ways, like by secreting IAA [14], siderophores [13], phosphate solubalization (14), nitrogen fixation [6]. Today, emphasis is put on plant growth regulating bacteria that are capable of increasing the rate of plant growth by the secretion of vitamins, amino acids, auxins & by fixing atmospheric nitrogen by *Azotobacter* & *Azospirillum* that increases root development & plant growth in association with *Anabena*. [6]. The *Azotobacter* is itself a multitasking organism. It fixes the nitrogen non-symbiotically, degrades cellulose, phosphates and most importantly it degrades lignin also in trace amounts [11]. Seed inoculation of wheat varieties with N_2 fixing & phytohormone secreting *Azotobacter* showed enormous increase in yield. So, in India along with repeated inoculation of *Azotobacter*, reclaimed saline soils can be used to increase the yield of total wheat per annum [9]. Hence present study was carried out on reclamation of saline soil by applying moderately haloalkalophillic, non-symbiotic nitrogen fixers (i.e. *Azotobacter*) having phosphate solubilizing property.

2. MATERIALS AND METHODS

a) Isolation and Cultivation of nitrogen fixers:-

a) Enrichment: - Enriched samples were prepared from collected saline soil samples, as inoculated on modified Ashbey‟s nitrogen free mannitol broth [8] **(NaCl 3.5%)**. They were cultivated at room temperature 28^0C ($\pm 2^0$C) for 48-72 hours. They were purified on the Ashbey‟s nitrogen free mannitol agar.[7]

b) Various enzymatic activities :-

Nitrate reduction test was performed and the reduction of nitrate to nitrite was detected by adding few drops of sulphanilic acid followed by α-napthyl amine.[5] Phosphatase activity was performed. To check the cellulase activity The growth of isolates on cellulose agar was determined [3]. Along with these tests the catalase and oxidase test was also performed.

c) Studies on physiological characteristics of isolates:-

Effect of Temperature, pH, NaCl, KCl, Na_2CO_3 and $NaHCO_3$, concentration was studied using different parameters of all these physiological characteristics.

d) Pot experiment:-

Coarse sand first sieved, by 8 mesh sieve, washed it for 10 to 15 times so as to remove the organic content of the sand.

Sand sterilization:-

Sand was sterilized for pot experiment by tyndalization that is fractional sterilization.

a) Pot experiments conducted in polyhouse:-

The experiment was carried out in polythene pouches using five or seven replicates. After sand sterilization on three consecutive days the sand was then filled in pouches so as to fill about two-third capacity. The seeds of *Tritium aestivum,* market trade name Ajit-102 were washed with water, surface sterilized in 30% H_2O_2 for 4-5min & then washed with sterile distilled water. The seed were sowed about 1-2cm below the surface of the sand in the pouches. Fresh suspension of *Azotobacter* cultivated in Ashbey‟s nitrogen free mannitol broth, after 5-7 days incubation were added at the same time of sowing in respective pouches. The inoculum size used was $10^6 10^8$cells/ml & volume used was 10ml per pot containing *Tritium aestivum*. Uninoculated control was run along with the experiment. For sowing the seeds distilled water is used & then onwards Arnon‟s mineral mixture [10] is used. After 26 days of cultivation the plants were removed from the pouches & shoot length & root length was measured. The plants were then dried at 55^0C in hot air oven till constant weight measured. Root & shoot length, dry mass of plant was compared with control & concluded the results.

Percent increase in dry weight is calculated by formula,

$$\% \text{ increase in dry weight} = \frac{W_T - W_C}{W_C} \times 100$$

Where,

W_T: - Average dry weight of plants in test pot.

W_C: - Average dry weight of plants in control pot

Chemical analysis of soil:-

Here two parameters were studied as soil pH & soil salinity. **a)**

Measurement of soil pH:-

Here the soil pH was measured by using the electrodes.

b) Determination of salinity of soil:- Method: - Saturated

paste method [12]

The saturated paste method has long been recommended for assaying soil salinity in relation to plant growth.

Table no. 1:-Table showing relationship among soil salinity texture & conductivity.

Soil texture	**Degree of salinity (ms/cm)**				
	Non-saline	Slightly saline	Moderately saline	Strongly saline	Very strongly saline
All soil textures	0-2.0	2.1-4.0	4.1-8.0	8.1-16.0	16.1

Reading of conductivity meter was compared with this table & salinity of soil was measured & decided the type of soil.

3. RESULT AND DISCUSSION

a) Codes for *Azotobacter* isolates.

Table no.2:- *Azotobacter* isolates

Sr. no.	**Codes for isolates**
1	R_1
2	R_2
3	R_3
4	R_4
5	R_5
6	R_7
7	R_8

b) Results of enzymatic properties :- Results of enzymatic properties as presented in table 4.2

Table no.3:- Results of enzymatic properties

Sr. no.	Characteristics of isolates	Isolate code						
		R_1	R_2	R_3	R_4	R_5	R_7	R_8
1	Nitrate reduction test	+	+	+	+	+	+	-
2	Oxidase test	+	-	-	-	-	+	+
3	Catalase test	+	+	+	+	+	+	+
4	Phosphatase test	+	+	+	+	+	+	-
5	Celluase test	+	+	+	+	+	+	+

c) Results of physiological characteristics:- 1) Effect of temperature on growth of *Azotobacter* isolates:- Results of effect of temperature as presented in table 4.3

Table no.4:- Effect of temperature on growth of *Azotobacter* isolates

Sr. no.	Temperature	Isolate code						
		R_1	R_2	R_3	R_4	R_5	R_7	R_8
1	Room temperature	+	+	+	+	+	+	+
2	28^0C	+	+	+	+	+	+	+
3	37^0C	+	+	+	+	+	+	+
4	40^0C	+	+	+	-	-	+	-
5	55^0C	-	-	-	-	-	-	-

2) Effect of pH on growth of *Azotobacter* isolates:- Results of effect of temperature as presented in table 4.4

Table no.5:- Effect of pH on growth of *Azotobacter* isolates

Sr. no.	pH	Isolate code						
		R_1	R_2	R_3	R_4	R_5	R_7	R_8
1	5	+	+	+	+	+	+	+
2	6	+	+	+	+	+	+	+
3	7	+	+	+	+	+	+	+
4	8	+	+	+	+	+	+	+
5	9	+	+	+	+	+	+	+
6	10	+	+	+	+	+	+	+
7	11	+	+	+	-	+	+	+
8	12	+	+	-	-	+	+	-
9	13	-	-	-	-	-	-	-

3) Effect of NaCl concentration on growth of *Azotobacter* isolates:- Results of effect of NaCl concentration as presented in table 4.5

Table no.6:-Effect of NaCl conc. on growth of *Azotobacter* isolates

Sr. no.	NaCl concentration	Isolate code						
		R_1	R_2	R_3	R_4	R_5	R_7	R_8
1	1 %	+	+	+	+	+	+	+
2	2 %	+	+	+	+	+	+	+
3	3 %	+	+	+	+	+	+	+
4	4 %	+	+	+	+	+	+	+
5	5 %	+	-	+	+	+	+	+
6	6 %	+	-	+	+	-	+	-
7	7 %	-	-	+	-	-	-	-
8	8 %	-	-	-	-	-	-	-

4) Effect of KCl concentration on growth of *Azotobacter* isolates:- Results of effect of KCl concentration as presented in table 4.6

Table no.7 :-Effect of KCl conc. on growth of *Azotobacter* isolates

Sr. no.	KCl concentration	Isolate code						
		R_1	R_2	R_3	R_4	R_5	R_7	R_8
1	1 %	+	+	+	+	+	+	+
2	2 %	+	+	+	+	+	+	+
3	3 %	+	+	+	+	+	+	+
4	4 %	+	+	+	+	+	+	+
5	5 %	+	+	+	+	+	+	+
6	6 %	+	+	+	-	+	+	-
7	7 %	-	+	+	-	+	-	-
8	8 %	-	-	-	-	-	-	-

5)Effect of Na_2CO_3 concentration on growth of *Azotobacter* isolates:- Results of effect of Na_2CO_3 concentration as presented in table 4.7

Table no.8:-Effect of Na_2CO_3 conc. on growth of *Azotobacter* isolates

Sr. no.	Na_2CO_3 concentration	Isolate code						
		R_1	R_2	R_3	R_4	R_5	R_7	R_8
1	0.1 %	+	+	+	+	+	+	+
2	0.2 %	+	+	+	+	+	+	+
3	0.3 %	+	+	+	+	+	+	+
4	0.4 %	+	-	+	+	+	+	+
5	0.5 %	+	-	-	-	-	+	+
6	0.6 %	-	-	-	-	-	-	+

7	0.7 %	-	-	-	-	-	-	+
8	0.8 %	-	-	-	-	-	-	-

5) Effect of $NaHCO_3$ concentration on growth of *Azotobacter* isolates:- Results of effect of $NaHCO_3$ concentration as presented in table 4.8

Table no.9:-Effect of $NaHCO_3$ conc. on growth of *Azotobacter* isolates

Sr. no.	$NaHCO_3$ concentration	Isolate code						
		R_1	R_2	R_3	R_4	R_5	R_7	R_8
1	0.1 %	+	+	+	+	+	+	+
2	0.2 %	+	+	+	+	+	+	+
3	0.3 %	+	-	+	+	+	+	+
4	0.4 %	+	-	+	-	-	+	+
5	0.5 %	+	-	-	-	-	+	+
6	0.6 %	+	-	-	-	-	-	+
7	0.7 %	-	-	-	-	-	-	+
8	0.8 %	-	-	-	-	-	-	-

7) Pot experiment result:-

Fig 1. Nitrogen fixation pattern of *Azotobacter* isolates:-

Table no.10:-Nitrogen fixation pattern of *Azotobacter* isolates

Isolate code	Dry weight of wheat plant			% increase in dry weight
	Shoot	Root	Total plant wt. (gms)	
R_1	30	37	0.855	33.17
R_2	32	32	0.890	38.62
R_3	35	40	0.657	27.7
R_4	31	20.3	0.724	12.77
R_5	33	24	0.680	5.91
R_7	33	29	0.715	11.37
R_8	29	22	0.685	6.69
Control	0.28	0.22	0.642	0.00

a. Results of chemical analysis of soil:-

a) Measurement of soil pH:-

Results of pH measurement are as presented in table no.4.16

b) Determination of salinity of soil:-

Results of measurement of soil salinity as presented in table 4.16

Table no.11:- Results of measurement of pH & salinity.

Sr. no	Soil sample	pH	Electrical conductivity (ms/cm)	Soil texture
1	Ratnagiri	8.5	5.2×10^{-3}	Moderately saline & sodic
2	Islampur	7.9	5.1×10^{-3}	Moderately saline
3	Songaon,satara	6.49	4.2×10^{-3}	Moderately saline
4	Songaon,satara	7.2	4.3×10^{-3}	Moderately saline
5	Songaon,satara	7.29	11.2×10^{-3}	Strongly saline

4. CONCLUSION: -

In the present work different *Azotobacter isolates* were obtained from saline soil samples.

1. From different soil samples moderately halo-alkalophilic seven isolates were obtained which can able to grow at 3.5% NaCl concentration.
2. All isolate can able to tolerate 40^0C temperature, upto 10.0 pH, high salt conc. upto 5% & carbonate & bicarbonate upto 0.4% some strains shows more tolerance.
3. Phosphate solubilization & cellulose degradation also shown by these isolates.
4. *Azotobacter* are free living N_2 fixing diazotrophs, and wheat plant is moderately salt tolerant, so combination of both these can be used with minimum use of water(as green manure holds water) and chemical fertilizer, so as to avoid excess of addition of salts to such soils. *Azotobacter* can also degrade the green manure as it is a cellulose degrader and simple sugars will be available for plant growth.
5. Results from present study indicated that yield of wheat can affect significantly by inoculation of *Azotobacter*. This biofertiliser can fix nitrogen, increase phosphorous availability, enhance absorbed elements by plants, produces phytohormone, IAA, Thiamine, Riboflavin, etc. along with this Azotobacter can degrade lignin in trace amount and can fix nitrogen non symbiotically

so this organism is very much useful and economic. Instead of using different organisms we can use *Azotobacter* for phosphate solubilization, lignin degradation, cellulose degradation and as it is a natural nitrogen fixer we can reduce the cost of rearing and cultivation of other organisms. In short we can use *Azotobacter* as a multitasking organism.

REFERENCES: -

1. Arnon D.I. (1938), "Microelements in culture solution", experiment with higher plants, American journal of Botany, 25:322-25.
2. Asuncion, M.J.A. cusaton, M.S, Saubidet, M.I. & Dellepiane, E 1983, Effects of soil inoculation with Azotobacter. Cienica del Suelo, 1(2): 29-35 Biochem Biophys 262:99–110
3. Gangawane L.V. "Rate of nitrogen fixing microorganisms in bioremediation of salt affected soils" In "Fronier‟s in Microbiology & pesticides" Laboratory, Dept. of Botany, Babasaheb Ambedkar, Marathwada university, Aurangabad 431 000 Ms, India.]
4. IS Dahiya; SS Dahiya, J.Aric.Agro.Indus, 1977, 10(1)pp.11-19.
5. Kawai T, Higuchi U, Higuchi T (1988) Degradation mechanisms of phenolic β-1
6. Sambhaji B. Thakar, Kailas D. Sonawane (2013) Mangrove Infoline Database: A Database of Mangrove Plants with Protein Sequence Information Current Bioinformatics. 8.524- 529.
7. Mujeeb-ur-Rahman, "Effects of NaCl Salinity on Wheat (Tritium aestivum L.) Cultivars", World Journal of Agricultural Sciences 4 (3): 398-403, 2008
8. Radwan F.I. 2002 "Response of some Maize cultivation to VA mycorrhizal inoculation, Biofertilization & soil nitrogen application. Alex. J. Ageric Res.,43: 43_56.
9. Somwanshi R.B., Kadu P.P., Tamboli b.D., Patil Y. M. & Bhakare b.D.,"Analysis of plants, Irrigation water & soils" „Determination of soil salinity & Elecrical conductivity‟ –saturated paste method, Richard, 1969. MPKV EXTN Pub. No. 284. Rahuri, Maharashtra. Pp:-229,235.
10. ST Williams; ME Sharpe ; TJ Holt, Bergey‟s manual of systematic bacteriology,Vol.2, The Williams and Wilkins co. Baltimore. 1989.
11. Subba rao N. S., 1999,"Soil Microbiology" 4th Ed. Oxford & IBH Pub. Co. PVT. LTD. New Delhi pp.117-129, 21. substructure model compounds by laccase of Coriolus versicolor. Arch
12. VKumar; N Narula, boil.Fertil.soils, 1999, 28:301-305.

13. www.fao.org/docret/x5871e/x587/e04.html

14. PK Bhagwat, PJ Kasabe, SB Jhample and PB Dandge "Friendly bacteria propping up legumes development in pesticide contaminated soil" Int J Pharm Bio Sci 2013 July; 4(3): (B) 356 – 364

15. Wani PA, Zaidi A, Khan AA, & Khan MS "Effect of phorate on phosphate solubilization and indole acetic acid releasing potentials of rhizospheric microorganisms" Ann. Pl. Protec. Sci. 13 139–144, (2005).