Application of moderately haloalkaliphilic nonsymbiotic diazotrophs of Lonar lake to saline soils

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Abstract- India is an agriculture based country and most of the Indian economy relies on agriculture. Western Maharashtrian soil is rich in humus which ensures better yield to the cash crops. Asymbiotic nitrogen fixing microorganisms play an important role in converting atmospheric nitrogen to ammonia in such soils to increase its fertility. But unfortunately many hectares of soil in this region are deposited with salts which render the land barren. Most of the agricultural land in India has become barren due to accumulation of excessive salts. Predominant nitrogen fixing bacteria in salt deposited soils find it difficult to fix atmospheric nitrogen due to high pH and salinity. Keeping this drastic situation in view, it was thought to isolate moderately haloalkaliphilic nonsymbiotic diazotrophs from Lonar Lake which is rich in salts and alkalinity. Lonar Lake is saline and alkaline lake formed due to meteorite impact some 52,000 years ago. Primary studies were carried to apply the same isolated diazotrophs into such salt deposited barren soil by pot experiments. Six nonsymbiotic diazotrophs isolated from Lonar Lake were applied on saline soils during pot experiments conducted in the polyhouse. Physical characters of the plants grown in pots, morphological characteristics, pesticide resistance potential and tolerance of the obtained isolates to various NaCl concentrations are presented in this paper.
Keywords: - agriculture, nonsymbiotic, diazotrophs, meteorite, haloalkaliphilic.

Introduction

India is an agriculture based country and most of the Indian economy relies on agriculture. Western Maharashtrian soil is rich in humus which ensures better yield to the cash crops. But unfortunately many hectors of soil in this region are deposited with salts which render the land barren. Nonsymbiotic nitrogen fixing bacteria fix atmospheric nitrogen independently and convert it into ammonia which increases soil fertility and plant productivity [1]. Salinity in soil is developed due to accumulation of excessive salts and predominant nitrogen fixing bacteria in salt deposited soils find it difficult to fix nitrogen due to high pH and salinity [1]. Keeping this drastic situation in view it was thought to apply haloalkaliphilic nonsymbiotic diazotrophs from Lonar Lake to saline soils. Lonar Crater situated in the village Lonar (Lat. 19058', Long. 76034') in Buldhana District of Maharashtra State, India is the only one in the world formed by meteorite impact in basaltic rock. Formed some 52,000 years ago, it is today a closed basin lake that is saline and alkaline, rich in carbonates and bicarbonates [2]. Photograph 1: A view from the eastern rim of hypervelocity meteorite impact Lonar Crater Lake. Primary pot culture studies were carried out by applying moderately haloalkaliphilic nitrogen fixing bacteria from Lonar Lake to saline soils. Pesticide resistance potential of the isolates and tolerance of the same to various NaCl concentrations was also studied [3].

Materials and Methods

Halotolerant nonsymbiotic nitrogen fixers from Lonar lake soils were cultivated on modified Ashby's nitrogen free mannitol solid medium (pH 10.5 and NaCl-4%) for morphological and cultural characters and in liquid medium for pot experiments. Gram staining was performed by Hucker and Conn's modified Gram staining method and motility by hanging drop method. The inoculum size used was 108 cells / ml and volume used was 100 ml per pot containing 5 seeds of *Triticum aestivum L*. Soil pH and salinity was measured before experiment was carried out. The experiment was carried out in polyethylene pouches using five replicates in each pouch. Soil was sterilized at 1600C in a hot air oven for two hours each day for three consecutive days. The soil was then filled in pouches so as to fill about two third the capacity. The seeds of *Triticum aestivum L*. were washed with water, surface sterilized in H2O2 for 4-5 min. and then washed with sterile distilled water. The seeds were sowed about 1-2 cm. below the surface of the soil in the pouches. Fresh suspensions of moderately haloalkaliphilic Nitrogen fixers cultivated in Ashby's nitrogen free mannitol broth were centrifuged and the cell mass suspended in sterile saline was poured into the respective pouches. Uninoculated control was also run along with the experiment. After 15 days of cultivation, the plants were removed from the pouches. Shoot length and root length of the grown plant was measured. The roots were cut and removed. The plants (Shoots) were then dried at 700C in a hot air oven till constant weight measured. Pesticides may adversely affect the growth of nitrogen fixing bacteria. Hence, four commonly used chemical pesticides such as Endosulfon, Thiomethaxan, M-45 and Glycophosphate were used to test the effect of pesticides on the obtained isolates [4]. Different concentrations of every pesticide were prepared

and added in Ashby's nitrogen free solid medium after sterilization. Plates were poured and loopful of the each culture was inoculated in each solidified plate containing increasing concentration of the respective pesticide. Plates were incubated at room temperature for 48 hours and appearance of growth was checked and reported. Effect of salt (NaCl) concentration on the isolates was seen by inoculating loopful culture of each isolate on Ashby's nitrogen free Agar containing variable concentrations of NaCl on separate plates ranging from 1% - 20% respectively. The plates were incubated at room temperature for 48-72 hours. Presence of growth was observed and reported.

Results and Discussion

Microscopic observations of the six isolates conducted in our laboratory revealed the four isolates to be bacteria and the remaining two to be actinomycetes. The morphological and cultured characters of the isolates are depicted in Table No. 1. Four bacteria and two actinomycetes were used in the pot trial experiments. The results of which are presented in Table No. 2. From Table No. 2 it is evident that as far as dry mass of the plants is concerned the actinomycetes are much superior to the bacteria although the latter are not too far behind. But considering the length of the plants, bacteria, especially isolates B and C are the best. Results of pesticide resistance potential of the isolates to four different chemical pesticides are presented in tables 3-6. The results of six isolates showing growth upto 10% NaCl concentration on Ashby's Nitrogen Free Mannitol Agar are represented in table No. 7.

Conclusion

From dry mass of plants it is concluded that haloalkaliphilic nitrogen fixing bacteria help to improve the plant growth in saline soil. The above mentioned diazotrophs may be used for reclamation of saline soils.

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Table 1- Colonial and Morphological Characters of the Isolates.

Colonial and Morphological Characters	Isolates A →	B	C	W_2	SE - I	E - I
Size (mm.) ↓	1	1	1	3	2	1
Shape	Circular	Circular	Circular	Circular	Circular	Circular
Colour	Colourless	Colourless	Colourless	Colourless	White	White
Margin	Entire	Entire	Entire	Entire	Irregular	Irregular
Elevation	Convex	Raised	Raised	Convex	Flat	Flat
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
Consistency	Mucoid	Mucoid	Mucoid	Mucoid	Dry	Dry
Gram nature	Gram negative Rods	Gram negative Rods	Gram negative Rods	Gram negative Rods	Gram positive Filaments	Gram positive Filaments
Motility	Non motile	Non motile	Non motile	Non motile	Non motile	Non motile

Table 2- Results of pot experiments performed in Polyhouse.

Isolate	Length (cm.) Mean of replicates		Dry mass (gm.)
	Shoot	Root	
Bacteria			
A	8	6	0.010
B	12.5	5	0.009
C	10.2	4.5	0.009
W_2	5.22	4.14	0.008
Actinomycetes			
SE – I	9.68	5.32	0.014
E – I	9.50	3	0.012
Control	4.25	2.5	0.003

Table 3- Table showing pesticide resistance potential of the isolates to different concentrations of Endosulfon.

Sr. No.	Isolate Code	Endosulfon Concentration (mg/100ml)			
		250	500	750	800
1	A	+	+	+	-
2	B	+	+	+	-
3	C	+	+	+	-
4	W2	+	+	+	-
5	E-I	+	+	+	-
6	SE-I	+	+	+	-

Table 4- Table showing pesticide resistance potential of the isolates to different concentrations of Thiomethoxan.

Sr. No.	Isolate Code	Thiomethoxan Concentration (mg/100ml)						
		250	500	750	1000	1250	1750	2000
1	A	+	+	+	+	+	_	_
2	B	+	+	+	+	+	_	_
3	C	+	+	+	+	_	_	_
4	W2	+	+	+	+	_	_	_
5	E-I	+	+	+	+	_	_	_
6	SE-I	+	+	+	+	_	_	_

Table 5-: Table showing pesticide resistance potential of the isolates to different concentrations of M-45.

Sr. No.	Isolate Code	M-45 Concentration (mg/100ml)			
		250	500	750	1000
1	A	+	-	-	-
2	B	+	-	-	-
3	C	+	-	-	-
4	W2	+	-	-	-
5	E-I	+	-	-	-
6	SE-I	+	-	-	-

Table 6- Table showing pesticide resistance potential of the isolates to different concentrations of Glyophosphate.

Sr. No.	Isolate Code	Glyophosphate Concentration (mg/100ml)					
		250	500	750	1000	1250	1750
1	A	+	+	+	+	+	_
2	B	+	+	+	+	+	_
3	C	+	+	+	_	+	_
4	W2	+	+	+	+	+	_
5	E-I	+	+	+	+	_	_
6	SE-I	+	+	+	+	_	_

Table 7- Table showing tolerance of isolates to different NaCl concentrations.

Sr. No.	Isolate Code	NaCl Concentration in %																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	A	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
2	B	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
3	C	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
4	W2	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
5	E-1	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
6	SE-1	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-