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Production of Plant Growth Promoting Indole Acetic Acid by Azotobacter from Saline Belt of Vidarbha Region

KEYWORDS: Azotobacter, saline soil, IAA

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ABSTRACT *The alluvial vally of Purna river and its tributaries occupy parts of Amravati, Akola and Buldhana districts of Maharashtra State. The Purna alluvial deposits cover an area of 6200 sq.km of which 3000 sq.km is characterized by saline soil possessing pH range 7.5 to 9.5. Nearly 45 isolates of Azotobacter were isolated and identified from saline soil of different villages belonging to four Tahasils of Buldhana district of state Maharashtra. These isolates were identified on the basis of Morphology, Biochemical and Cultural characteristics and were studied for production of plant growth promoting hormone IAA, which was detected by TLC method and estimated by colorimetry. It has been noted that highest concentration of IAA (40 µg/ml) was produced by Azotobacter beijerinkii followed by 13 to21 µg/ml by Azotobacter chrococcum. These strains of Azotobacter can be the excellent plant growth promoting bacteria in the saline soil of this region, and for growth and better agricultural products.*

Introduction

In Vidarbha region Purna river, a tributary of the Tapi river originates from Southern slopes of Gawilgarh hill of Satpura range near Baitul in Madhya Pradesh. The alluvial vally of Purna and its tributaries occupy parts of districts of Amravati, Akola and Buldhana. The purna alluvial deposits cover an area of 6200sq.km of which 3000 sq. km is characterized by saline zone (Adyalkar, 1975). This saline zone ranges in pH from 7.5 to 9.5. the highest salinity was observed in five Tahasils such as Sangrampur, Shegaon, Jalgaon jamod, Nandura and Malkapur of Buldhana district.

The use of biologically active products or microbial inoculants of bacteria, algae and fungi as a source of biofertilizer has become hope for most of the countries as far as economical and environmental zone.The inoculation of plant rhizosphere with *Azotobacter* has a beneficial role as far as crop yields are concerned. *Azotobacter* also produces fungistatic compounds, vitamins of B group like nicotinic acid, pantothenic acid, biotic and plant growth promoters such as auxins, giberllin and cytokinins. Hence study was carried out to screen the *Azotobacter* from saline soils of Buldhana district for production of plant growth promoting Indole Acetic Acid.

Materials & Method

Saline soil samples were collected from the villages of five Tahasils such as Sangrampur, Shegaon, Jalgaon jamod, Nandura and Malkapur of Buldhana district.One gram of each soil was serialy diluted, plated on sterilized petri plate poured with Jenson's medium (Composition: Sucrose 20.0 gm, Agar 15.0, Calcium carbonate 2.0, Dipotassium Phosphate 1.0, Magnesium sulphate 0.5, sodium chloride 0.5, Ferrous sulphate 0.1, Sodium molybdate .005, P^H 6.8 $\pm$ 0.2 at 25^0C, Distilled Water 1000 ml).

Plates were incubated at 28 ^{0}C to30 ^{0}C for 24 hrs. Colonies were identified on the basis of morphology (shape, Gram staining and motility),biochemical characteristics such as sugar fermentation(Mannitol, Glycerol, Sorbitol Rhamnose), H_2S production and enzyme production such as oxidase and nitrate reductase. Azotobacter species were grown separately on 100ml Waksmann 77 liquid medium without calcium carbonate and supplemented with 0.005 M concentration of tryptophan in different conical flasks. Different strains of Azotobacter were inoculated and incubated at 28 ^{0}C on rotary shaker (120 strokes/minute) for about 10 days and centrifuge at 3000 rpm for 10 minutes. Methanol extraction of IAA was made from the crude culture filtrate of Azotobacter and after drying and evaporation of extract dry powder of IAA was prepared.

Detection of IAA

Prepared dry powder containing IAA was dissolved in distilled water and solution was made for detection using TLC. The silica gel and plaster of paris (2:1)TLC plates were preapared for detection of IAA on Chromatography plates by spraying Ehrilch reagent, pink to gray or yellow coloured indicates the presence of IAA on the TLC plates.

Colorimetric estimation of IAA

Colorimetric method was used in the determination of IAA in the crude extract. The methanol extracted filterate culture of Azotobacter was directly used for estimation of IAA, 1ml of methanol extract were mixed with 2.0 ml of Salpers reagent and tubes were incubated in the dark room for about 1 hour, and optical density was recorded at 535 nm, standard graph of IAA was made with the known concentration in the range of 10-180 µg/ml and the concentration of unknown sample was determined from the standard graph.

PROTOCOL FOR DETECTION OF STANDARD INDOLE –3- ACETIC ACID

Reagent	Blank	1	2	3	4	5	6	7	8	9	10
Standard IAA in ml(100 µg/ml)		0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
Salpers reagent (ml)	2	2	2	2	2	2	2	2	2	2	
		Incubated for 60 minutes									
Distilled Water (ml)	3	2.9	2.8	2.7	2.6	2.5	2.4	2.3	2.2	2.1	2

Optical density measured at 535 nm

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Results & Discussion

Total 99 soil samples were analysed for the isolation of *Azotobacter* from saline soils of four tahasils of Buldhana district. 46 isolates of *Azotobacter* were recovered and identified on the basis of morphological, biochemical and enzymatic study. These isolates were found belonging to species such as *Azotobacter beijarinckii. A. chroococcum*, *A. vinelandii* and *A. nigricans* .as shown in Table 1.

Table 1. Characterization of Azotobacter from Saline Soil

Sample no.	Gram staining	Motility	Cyst	Man	Gly	Sorl	Rha	Oxi	H2S	N.R.	Name of organism
NAN-2	Gm-ve Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
NAN-3	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
NAN-4	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
NAN-6	Gm-ve	Non Motile	+	d	-	d	-	+	d	+	A. nigricans
NAN-7	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
NAN-8	Gm-ve	Non Motile	+	d	-	d	-	+	d	+	A. nigricans
NAN-12	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
NAN-14	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SHE-1	Gm-ve	Motile	+	A	d	A	-	+	d	+	A. chroococcum
SHE-3	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SHE-5	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SHE-8	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SHE-14	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
SHE-15	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
SHE-17	Gm-ve	Non Motile	+	d	-	d	-	+	d	+	A. nigricans
SHE-18	Gm-ve	Motile	+	A	d	A	-	+	d	+	A. chroococcum
SHE-19	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
JAL-4	Gm-ve	Motile	+	A	d	A	-	+	d	+	A. chroococcum
JAL-5	Gm-ve	Motile	+	A	d	A	-	+	d	+	A. chroococcum
JAL-9	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
JAL-10	Gm-ve	Motile	+	A	d	A	-	+	d	+	A. chroococcum
JAL-11	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
JAL-13	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
JAL-14	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
JAL-18	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
JAL-24	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
JAL-26	Gm-ve	Motile	+	A	d	A	-	+	d	+	A. chroococcum
JAL-27	Gm-ve	Non Motile	+	d	-	d	-	+	d	+	A. nigricans
JAL-29	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
JAL-31	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SAN-1	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
SAN-2	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SAN-6	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SAN-8	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
SAN-10	Gm-ve	Motile	+	A	d	A	-	+	d	+	A. chroococcum
SAN-12	Gm-ve	Non-Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SAN-13	Gm-ve	Motile	+	A	d	A	-	+	d	+	A. chroococcum
SAN-17	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SAN-19	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SAN-21	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
SAN-22	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SAN-25	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
SAN-28	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii
SAN-29	Gm-ve	Motile	+	A	A	A	A	+	+	+	A. vinelandii
SAN-31	Gm-ve	Non Motile	+	d	d	d	-	+	d	+	A.beijerinekii

Man- Mannitol, Gly- Glycerol, Sorl- Sorbitol, Rha- Rhamnose, Oxi- Oxidase, H2s- Production of H2s, N.R.- Nitrate Reductase

All the four isolates so confirmed were studied for production and estimation of IAA. Indole Acetic Acid production was seen in case of *A. chroococcum, A. vinelandii, A.beijerinekii, A. nigricans.* However highest amount of IAA was generated by *A.beijerinekii,* (40 µg/ml) followed by *A. chroococcum (*21 µg/ml & 13 µg/ml &9.5 µg/ml*),A.nigricans*(10 µg/ml) and *A. vinelandii (*7 µg/ml*).* The results are depicted in Table 2 and fig. 1

Farah Ahmad et.al. (2005) isolated seven *Azotobacter* species from the rhizospheric soil in the vicinity of Aligarh City and found to produce high level (7.2 to 32.8mg/ml) of IAA. Similarly Vikram Patil (2011) also recorded IAA production by *Azotobacter* strains without tryptophan addition,and noted that IAA production increased with the increase in tryptophan concentration from 1 to 5 mg/ml. similarly Edi Husen (2003) isolated fourteen plant growth promoting rhizobacter (PGPR) strains including *Azotobacter vinelandii* Mac 259 and *Bacillus cereus* UW 85 and tested in vitro for production of indole acetic acid. Magda et al.(2012) also found twenty two bacterial isolates from

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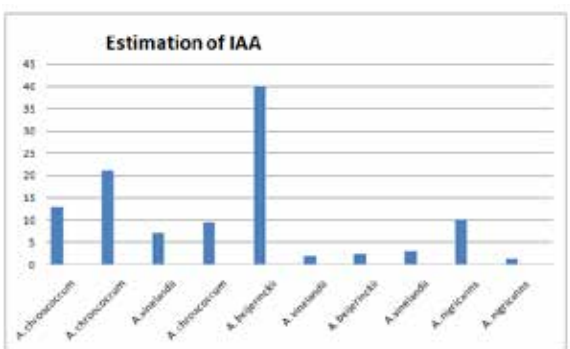
rhizosphere of wheat plants grown in saline soil in western region, Saudi Arabia and showed 17 isolates positive for IAA production. Nitrogen fixation and indole acetic acid production potential of *Pseudomonas* and *Azotobacter* was also deteced in sugarcane growing areas by Muhammad Arsian Ashrat et al. (2011) and suggested inoculation of these organism in sugarcane grown soils.

Table 2:- Detection of IAA (µg/ml) from Azotobacter Spp.

Sr. No.	Code no.	Name of organism	O.D.	Concentration µg/ml
1	SHE-1	A. chroococcum	0.073	13
2	SHE-18	A. chroococcum	0.103	21
3	SHE-14	, A. vinelandii	0.031	7
4	JAL-5	A. chroococcum	0.049	9.5
5	SHE-3	A.beijerinekii	0.210	40
6	JAL-13	, A. vinelandii	0.011	2
7	SHE-5	A.beijerinekii	0.015	2.5
8	JAL-24	, A. vinelandii	0.017	3.0
9	SHE-17	A. nigricans	0.050	10
10	JAL-27	A. nigricans	0.012	1.5

Fig 1: Estimation of IAA



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