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4. Isolation and Preliminary Identification of Azotobacter Spp. from Agricultural Soil of Latur Region, Maharashtra

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Abstract

An attempt was made to look for, ecofriendly, indigenous free living N2 fixing Bacteria inhabitant to Agricultural soil of Latur region situated in Maharashtra State of India. Isolates were made from a clay soil in South (AusaTaluka) and western part (Latur Taluka) of Latur District. Latur, where most of the soils are alkaline in nature, and most of the economical crops are grown including Sugarcane, Soybean, Corn, Tomato, Brinjal, Pumpkin, Chiku, Mango, Guava, Marygold. Isolation and Identification of the isolates has been done using selective media and standard methods forAzotobacter spp.

Characterization of the isolates were made including morphological and biochemical tests. The obtained results revealed similarity with the results that had been reported earlier in the literature. AshbyesMannitol Agar Media was used for isolation of Azotobacter. The collected data indicated that Azotobacter isolates from Latur region soils grow best in media with neutral pH (7), tolerate temperatures in the mesophylic range (28-300C).Further studies were stressed to know Azotobacter spp. found in Latur region using various other media viz Phenol Red Broth, Nitrate Broth, Urease Media, TSI Media, SIM Media, Starch Agar and KB002 HiAssorted Biochemical Test Kit etc.The isolates were evaluated for colony and cellular morphology, motility and someother biochemicalcharacters.

Keywords: Azotobacter, Indigenous, Isolates, biochemical identification, Nitrogen Fixing bacteria,

Introduction

Agricultural production has increased in developing and developed countries during the last 3-4 decades due to use of high yielding crop varieties and enhanced consumption of

chemical fertilizers. Dependence on chemical fertilizers for future agricultural growth may lead to loss in soil quality, possibilities of water contamination and unsustainable burden on agricultural System (Rajasekaranet al:2012).Adoption of bio-fertilizer technology is recently advocated to reduce the use of chemical fertilizers (Zarabiet al., 2010). Extensive studies have been conducted in this aspect due to the negative impact of chemical fertilizers on the soil, water, plant, animal and human health (Venuturupalli, 2010). Therefore, attention is focused on bio-fertilizers sources, since they are considereded as eco-friendly and safe to plant, animal and human health. The world, therefore started to find living microorganism and use them as bio-fertilizers to promote plant growth.

Biological nitrogen fixation is the most important process that promotes plant growth and its productivity get increased (Kizilkaya, 2008). It could be non-symbiotic or symbiotic according to the relationship between bacteria and plant.

One of the major groups of free living bacteria that can fix nitrogen non-symbiotically is Azotobacter spp., which is indigenous to the soil (plant rhizosphere), grow well on a nitrogen free media like Ashbyes Mannitol Agar media, Jensens media, Burks Media etc. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of Azotobactercells thereby contributing towards the availability of nitrogen to crop plants.

Isolation, identification, characterization, growth and ability of these bacteria to fix nitrogen have been extensively studied (Breed et al., 1957). However, less information is available regarding their isolation, characterization, and identification of the strains dominating agricultural soil of Latur region.

This study was therefore conducted to isolate Azotobacter sp. from Agricultural soil Latur and their identification was made on the basis of biochemical characterization.

Materials and Methods

Collection of Soil

This study was conducted at the Department of Biotechnology RSM Latur(17°52' North to 18°50' North and 76°18' East to 79°12' East in the Deccan plateau). Soil samples were collected using sterile tools from the rhizosphere (5-6" depth) of Soybean (Glycinemax), Corn (Zea mays), Sugarcane (Saccharumofficinarum); Vegetables –Tomato (Solanumlycopersium), Brinjal(Solanummelongina), Pumpkin (Cucurbitapepo); Fruit crops –Chicku (Manilkarazapota),

Mango (Mangiferaindica), Guava (Psidiumguajava), Coconut (Cocosnucifera); Flower-Marigold (Tagetes) cultivated in Agricultural Soil of Latur and transferred directly to the laboratory and kept refrigerated (4C°) until used. Soil samples were collected in the month of July-August (Rainy season)

Isolationof Rhizobacteria

The Rhizobacteria were isolated using the AshbyesMannitol Agar media as described by (Subbarao, 1977), and (HiMedia Laboratories, 2015). Gram staining was made according to the method described by (Gerhardt, 1985).

Assessment of Viable Population

Viablepopulationof rizobacteria were estimated byusing serial dilution method and pour plate technique(Microbiology-Presscott-Harley -5th edition). The bacterial numbers were estimated in terms of Colony Forming Unit (CFU).

Biochemical Tests

The biochemical tests were performed according to (Microbiology- Prescott -Harley -5th edition). The following tests were made: Motility test, Catalase test, Oxidase test, urease test, Nitrate reductase test, Triple Sugar Iron test, Carbohydrate Fermentation test , Starch Hydrolysis test, Citrate utilization State, Ornithine Utilisation Test, Lysine Utilisation Test and Phenylalanine Deaminase Test etc. A pH-Meter was used for the determination of soil PH described by (Gerhardt, 1985)

Table 1: Viable Population of Rizobacteria

Isolates	Viable count of rizobacteria in CFU	Isolates	Viable count of rizobacteria in CFU
LSI-1	1.1×10^6	**LSI-11**	1.4×10^6
LSI-2	1×10^6	**LSI-12**	1.3×10^6
LSI-3	1.3×10^6	**LSI-13**	1.5×10^6
LSI-4	1.2×10	**LSI-14**	$1.4x\ 10^6$
LSI-5	1.3×10^6	**LSI-15**	2.1×10^6
LSI-6	1.4×10^6	**LSI-16**	1.8×10^6
LSI-7	1.2×10^6	**LSI-17**	1×10^6
LSI-8	1.2×10^6	**LSI-18**	1.1×10^6
LSI-9	1.6×10^6	**LSI-19**	1×10^6
LSI-10	1.7×10^6	**LSI-20**	1×10^6

Table 2: Morphological Characteristic of Rizobacterial Isolates

Isolates	Colony Morphology	Gram's Staining Reaction	Isolates	Colony Morphology	Gram's Staining Reaction
LSI-1	Convex, moderate sized, round, translucent, shiny, white in color.	GNB rectangular rods with round end	**LSI-11**	Convex, large sized, irregular, sticky translucent, cream color.	GNB oval rods, paired end to end
LSI-2	Convex, large sized, irregular, sticky translucent, cream color.	GNB oval rods, paired end to end	**LSI-12**	Convex, large sized, irregular, sticky translucent, cream color.	GNB oval rods, paired end to end
LSI-3	Big,irregular, low convex, transluscent, sticky, cream color.	GNB oval rods, paired end to end	**LSI-13**	Convex, large sized, irregular, sticky translucent, cream color.	GNB oval rods, paired end to end
LSI-4	Big, irregular, flat, opaque, cream colored.	GNB oval rods, paired end to end	**LSI-14**	Convex, moderate sized, round, translucent, shiny, whitish	GNB oval rods, paired end to end
LSI-5	Convex, moderate sized, round, translucent, shiny, whitish	GNB rectangular rods with round end single or paired	**LSI-15**	Convex, moderate sized, round, translucent, shiny, whitish	GNB rectangular rods with round end.
LSI-6	Convex, moderate sized, round, translucent, shiny, whitish	GNB rectangular rods with round end single or paired.	**LSI-16**	Convex, moderate sized, round, translucent, shiny, whitish	GNB rectangular rods with round end.
LSI-7	Convex, moderate sized, round, translucent, shiny, whitish	GNB rectangular rods with round end.	**LSI-17**	Convex, moderate sized, round, translucent, shiny, whitish	GNB rectangular rods with round end.
LSI-8	Convex, moderate sized, round, translucent, shiny, whitish	GNB rectangular rods with round end.	**LSI-18**	Convex, moderate sized, round, translucent,	GNB rectangular rods with round end.

				shiny, whitish.	
LSI-9	Convex, moderate sized, round, translucent, shiny, whitish	GNB rectangular rods with round end.	**LSI-19**	Convex, moderate sized, round, translucent, shiny, whitish.	GNB rectangular rods with round end.
LSI-10	Convex, moderate sized, round, translucent, shiny, whitish	GNB rectangular rods with round end.	**LSI-20**	Convex, moderate sized, round, translucent, shiny, whitish.	GNB rectangular rods with round end.

GNB- Gram Negative Bacteria

Table 3: Biochemical Characterization of Isolated Rizobacterial spp. according to Bergey's Manual(1981)

Isolates	LA	SU	GL	ML	AR	GA	MN	SO	AL	CI	LY	OR	PH	NR	OX	CA	AM	UR
LSI-1	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	+	+
LSI-2	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	+	+
LSI-3	+	+	+	+	-	+	+	+	-	-	-	-	-	+	+	+	+	+
LSI-4	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+
LSI-5	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	-	+
LSI-6	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	-	+
LSI-7	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+
LSI-8	+	+	+	+	-	+	-	+	-	-	-	-	-	+	+	+	+	+
LSI-9	+	+	+	+	-	+	-	-	-	-	+	-	-	+	+	+	+	+
LSI-10	+	+	+	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+
LSI-11	+	+	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+	+
LSI-12	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	+	+
LSI-13	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	+	+
LSI-14	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+	-	+
LSI-15	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	+
LSI-16	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
LSI-17	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+
LSI-18	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+
LSI-	+	+	+	+	-	-	+	-	-	-	-	-	-	-	+	+	+	+

19																		
LSI-20	+	+	+	-	-	+	-	+	-	-	-	-	-	-	+	+	+	+

LSI-2-Soybean1 **LSI-12**-Guava2 **LA**-Lactose **CI**-Citrate utilisation

LSI-3- Sugarcane1 **LSI-13**-Coconut1 **SU**-Sucrose **LY**-Lysine utilisation

LSI-4- Sugarcane2 **LSI-14**- Coconut2 **GL**-Glucose **OR**-Ornithine utilisation

LSI-5-Corn1 **LSI-15**–Tomato **ML**-Maltose **PH**-Phenylalanine Deamination

LSI-6-Corn2 **LSI-16**-Brinjal **AR**-Arabinose **NR**-Nitrate Reductase

LSI-7-Chicku1 **LSI-17**-Pumpkin1 **GA**-Galactose **OX**-Oxidase

LSI-8-Chicku2 **LSI-18**-Pumpkin2 **MN**-Mannitol **CA**-Catalase

LSI-9 Mango1 **LSI-19**-Marygold1 **SO**-Sorbitol **AM**-Amylase

LSI-10 Mango2 **LSI-20**-Marygold2 **AL**-Aldonitol **UR**- Urease

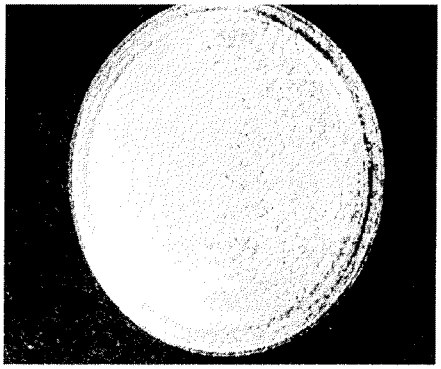

Plate 1 Azotobacter on Ashbyes Media (spread plate)

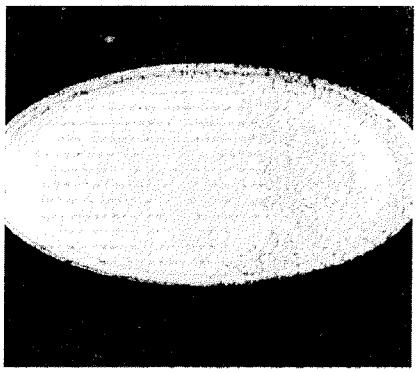

Plate 2 Azotobacter on Ashbyes Media(stricking plate)

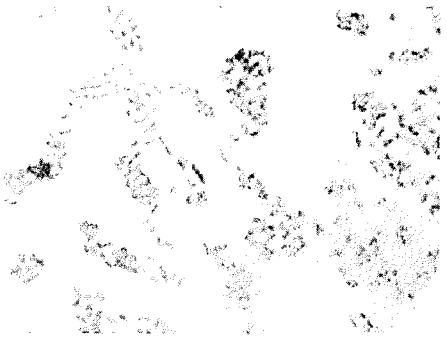

Plate3 Gram staining of Azotobacter

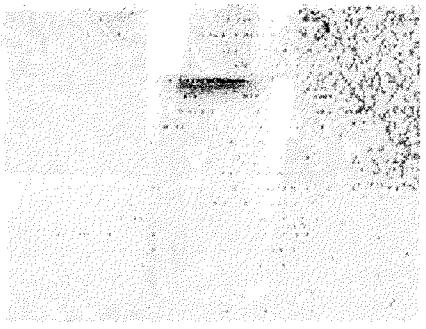

Plate 4 Motility of Azotobacter By SIM media (+ve)

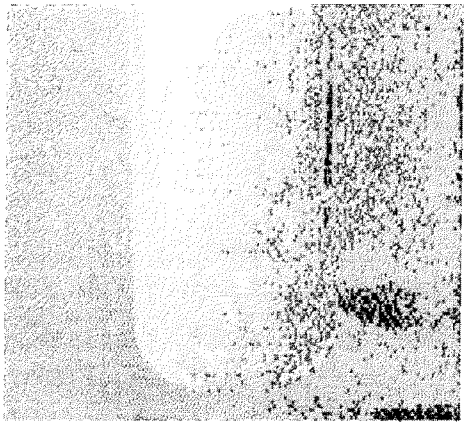

Plate 5 Catalase Test of Azotobacter (+ve)

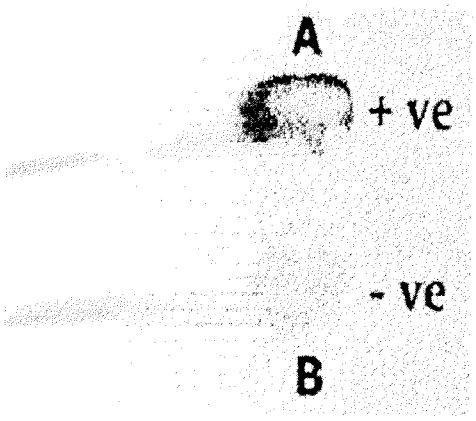

Plate 6 Oxidase Test of Azotobacter (+ve)

Results and Discussions

20 soil samples from different plant sources including cereals viz soybean (Glycinemax), corn (Zea mays), cash crop viz sugarcane (Saccharumofficinarum); Vegetables –Tomato (Solanumlycopersium), Brinjal(Solanummelongina), pumpkin (Cucurbitapepo); Fruit crops Chicku(Manilkarazapota), Mango (Mangiferaindica), Guava (Psidiumguajava), Coconut (Cocosnucifera); Flower-Marigold (Tagetes) etc. were collected from different area of Latur region. From this Azotobacter were isolated andidentified on the basis of Morphological, Cultural and Biochemical Characters.

The isolated Azotobacter spp.were cultured on AshbyesMannitol Agar media. This medium is selective to growth, and suitable for identification and characterization of Azotobacterspp, according to Subbarao (1977), and HiMedia Laboratories (2015). The colony morphology of most of the isolated Azotobacterspp showed that colonies were, raised(convex), moderate sized, round, shiny and whitish (shown in Table 2).

This result agrees with the findings of (Alsamowal M.M. et.alJune,2016).

The isolated bacteria were gram stained and the results showed a gram negative reaction as visuallized by the pink color rods (Table2). This data matched with the findings of Breed et al.1957, Mali, and Bodhankar, (2009).

The cells of isolated bacteria are oval rods and some are rectangular with round end and big in size. This observation noted with the isolates obtained agreed with the findings of (Breed etal., 1957; and Jensen, 1965 and Alsamowal M.M.et.al June, 2016).

The results of biochemical tests that have been made on the isolated bacteria gave a positive results with Glucose, Sucrose and Lactose fermentation test, Catalase, Oxidase and Urease Test. All of these obtained results gave consistent positive reactions with the isolated bacteria (table 3). Isolated bacteria shown variability in results with Mannitol, Galactose, Sorbitol, NitrateReductase ,Starch hydrolysis Test and negative results with Aldonitol and Citrate Utilisation test (shown in Table3)

All the isolates were motile , Gram negative rods, shown positive catalase, oxidase, urease test. These results, therefore confirmed the identity of the isolated bacteria which could be classified as Azotobacterspp as described by (Jensen, 1965).

All isolates shown positive TSI Test that indicated they could utilized all three sugars (Glucose, Sucrose , Lactose) and shown H2S production whereas Carbohydrate fermentation Test show variability in results which could helped in identification of Azotobacter species.

According to Bergey's Manual the species identified would be Azotobacterchroococcum, A.paspalli, A.vinelandii.

Conclusion

All the isolated species are gram negative, motile, catalase, oxidase positive were confirmed to be Azotobacter.Different species of Azotobacter were isolated from test soil samples collected from agricultural soils of Latur region.Results show that those different species would be A. chroococcum, A.vinelandii,andA.paspali.

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References

- Alsamowal M.M. 1 Hadad, M.A2, Mohammed .M.A3 and and G.A.Elhassan4 Volume-2 June, 2016. In vitro study the Characterization of Azotobacter spp. isolates from Sudanese soils. IJRDO - Journal of Agriculture and Research.
- Breed, R., E. G. D. Murray and N. R. Smith. (1957). Bergey's Manual of Determinative Bacteriology, (Seven Editions). Azotobacteraceae. The Williams and Wilkins Company, pp: 283-284.
- Gerhardt, P., (1985). Manual of Methods for General Bacteriology, American Society for Microbiology. Washington, DC 20006, pp: 26-28, 230, 413-418, 423-435.

- HiMedia Laboratories.com. AshbyesMannitol Agar.
- http://himedialabs.com/TD/M706.pdf.
- Jensen, H.L Non-symbiotic nitrogen fixation. In: Soil nitrogen,3rd ED, American Society of Agronomy, Madison,Wisconsin, 1965, pp.436-480.
- Kizilkaya R (2008). Yield response and nitrogen concentration of spring wheat inoculated with Azotobacterchroococcum strains. Ecological Engineering 33 150-156.
- Presscott, Harley (2002). Laboratory Exercises in Microbiology-fifth edition. The Macgrow Hill companies.pp.439-449
- S. Rajasekaran 1, *, K. Sankar Ganesh 1, K. Jayakumar 1, M. Rajesh 1, C. Bhaaskaran 2, P. Sundaramoorthy 3 (2012) Review- Biofertilizers-Current Status of Indian Agriculture. International Journal of Environment and Bioenergy.
- SubbaRao, 1977, Soil Microorganisms and Plant Growth, Oxford and IBH Publishing Co., India.
- Venuturupalli, S. (2010). M.Sc., The use and benefits of bio fertilizer and bio char on Agricultural lands. Department of Chemical and Biological Engineering .Chalmera University of Technology, pp: 9.
- Zarabi, M., I. Alahdadi., G. A. Akbari, and G. A. Akbari, (2010) a study on the effects of different biofertilizer combinations on yield, its components and growth indices of corn (Zea mays L.) under drought stress conditions, African Journal of Agricultural Research Vol. 6(3), pp. 681-685, 4 February, 2011.