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Research Article

PRELIMINARY STUDIES REGARDING ISOLATION OF *AZOTOBACTER* AND CHARACTERIZATION FOR PLANT GROWTH PROMOTION

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ABSTRACT

Azotobacter is nonsymbiotic free living, nitrogen fixing bacteria that resides in the soil rhizospheres. To reduce the adverse effects of toxic chemical fertilizers and pesticides we are in search of biofertilizers. As these biofertilizers can help for the sustainable growth of crop plants. In the present study an attempt was done to isolate the soil bacteria *Azotobacter* from the crop fields. *Azotobacter* is having many useful characters and these organisms can be used as biofertilizers. After isolation of these bacteria they were examined for the growth promoting traits like IAA production, Phosphate solubilizing ability, Nitrogen fixation and siderophore secretions. This is a preliminary study for knowing the presence of useful *Azotobacter* from the crop fields. So that they can be used for formulating as bioinoculants and can be applied as biofertilizers later.

Keywords: *Azotobacter, Characterization, Plant Growth Promoting Rhizobacteria*

INTRODUCTION

Microorganisms which are omnipresent can grow in diverse areas and in their ability to grow help us a lot. Environmental pollution is increasing at an alarming rate and anthropogenic interference is the prime factor for this. For our selfish attitudes we are destroying our beautiful ecological niches. Increasing demand for food in the world is also forcing farmers to used chemical fertilizers and pesticides. Over exploitation of natural resources is also main concern. All these activities of humans, decreases the quality of environment and mounts pressure on natural resources. Increasing pollutants also leads to hazardous waste accumulation. This must be controlled for our own welfare. Next viable option is sustainable development and bioremediation. Bioremediation helps in degrading hazardous waste and increases soil fertility also with the help of Microorganisms (Gadd, 2001; Kamaludeen *et al.,* 2003). Biofertilizers play a dynamic role in the sustainable Agricultural practices (Mahajan *et al.,* 2003; Husen, 2003).

Soil dwelling microbes plays a major role in cleaning the environmental pollution (Jacques *et al.,* 2008). As microorganisms can adopt to wide range of climatic and geographic conditions, this character makes them as perfect dependable natural resources. So indigenous microorganisms or extraneous ones present in nature can be selected (Prescott *et al.,* 2002). Restricted use of toxic chemicals and pesticides has to be done. In agriculture soil microorganisms that help in Nitrogen fixation (Symbiotic or free living) and phosphate solubilizing organisms has to be identified and used for crop improvement. Plant growth promoting rhizobacteria (PGPR) can colonize and directly enhance plant growth (Tiwari and Thrimurthy, 2007; Babalola, 2010). Many scientists have isolated and multiplied these PGPR from the rhizosphere (Glick, 2012).

*Azotobacter*is one of the most important Nitrogen fixing free living soil bacteria. Its application for wide range of crops is increasing due to their properties like nitrogen fixation, secretion of antifungal agents, vitamins, plant growth regulators, improving soil aggregation etc. (Kloepper, 1982; Boddey *et al.,* 1995; Inamdar *et al.,* 2000; Wedhastri, 2002). As ecofriendly soil bacteria this *Azotobacter* inoculums can support plant growth (Husen, 2003).

MATERIALS AND METHODS

In the present investigation an attempt was done to isolate and characterize the useful *Azotobacter*, isolated from soils of agricultural crops around Ananthapuramu, Andhra Pradesh, India. Rhizosphere soil samples were collected from a depth of 10-15 cm after uprooting carefully, from plantation crops like Banana and Ground nut. Five different soil samples were collected in sterilized polythene zip lock bags, sealed and brought to the laboratory of Sri Krishnadevaraya University for further studies, where it will be stored at 4^0C. Soil sample was sieved with 2mm sieve, air dried for 4 hours and 10gm of this soil sample was used for serial dilution. Serial dilution method was done for the isolation of different bacteria using 10^{-4} to 10^{-9}. Later further isolation of Nitrogen fixing bacteria was done using pour plate, spread plate methods using Jensen's Agar media and Ashby's mannitol agar medium. Plates were incubated at 37 ± 1^0C. Individual colonies were selected and maintained as pure cultures. These pure cultures were maintained on nutrient agar slants and subcultured periodically on the same medium at 37^0C.

Identification and characterization

Isolated colonies, identification was done using morphological and biochemical test. Morphological characterization was done using standard Gram staining, endospore staining and motility test. Biochemical characterization was done using catalase test carbon utilization test, oxidase test, methyl Red test, Voges Proskauer (VP) Test, Hydrogen sulfide test (H_2S), Hydrogen cyanide production, Nitrate reduction test, Phosphate solubilization test, Siderophores production, Indole acetic acid Production test (IAA). Ammonia production, Urease test and Citrate utilization tests were conducted using standard procedures (Nath *et al.,*2015; Donate-Correa *et al.,*2005; Gopalakrishnan, 2011; Cappucino and Sherman, 1992; Salkowski's method). Optical density was done to measure the growth rate of *Azotobacter* in Nutrient broth, using 550 wavelengths.

RESULTS AND DISCUSSION

Soil sample was subjected to serial dilution and inoculated on Ashby's medium, using pour plate and spread plate methods and colonies formed were observed. *Azotobacter* colonies observed were margin entire, circular, milky white, smooth colonies with glistening appearance. Bacterial growth was observed as slimy colonies and aged cultures produced brown to black pigmentation. Oxidase, catalase, H_2S production, indole test etc we found to be positive. Biochemical tests and their results are tabulated in Table-1. Evidences are there for the *Azotobacter* producing catalase and reductase enzymes was proved (Nath*et al.,* 2015) and in this process grows rapidly. Bubble formation was observed,while performing catalase test using Hydrogen peroxide 3% v/v (H_2O_2) (Table-2). Results of Optical density test using Spectrophotometer are tabulated in Table-3.

IAA production, carbon utilization test by *Azotobacter* were also confirmed by earlier researchers (Wedhastri, 2002; Jimenez *et al.,* 2011). Siderophore production was detected when tested using Chrome-azurol S agar plate. Orange colour halo zone around the bacterial colony was observed indicating the production of siderophore. Nitrogen fixing activity was tested using acetylene reduction assay and was found to be positive (Hardy *et al.,* 1973). Phosphate solubilizing activity of *Azotobacter* was checked and found to be positive (Ponmurugan and Gopi, 2006). With all these tests and morphological and cultural characters isolated organism was found to be *Azotobacter sp* based on the methods of microbiological methods and identification using Bergey's manual of Determinative Bacteriology. Azotobacter was found to be present in all collected samples. *Azotobacter* is a free living nitrogen fixing bacteria. It is flagellated and have thick walled micro cysts and when provided suitable energy source they can fix nitrogen and helps in phosphate solubilization. So this can be used as a biofertilizer to enhance the crop production after converting this into useable form.

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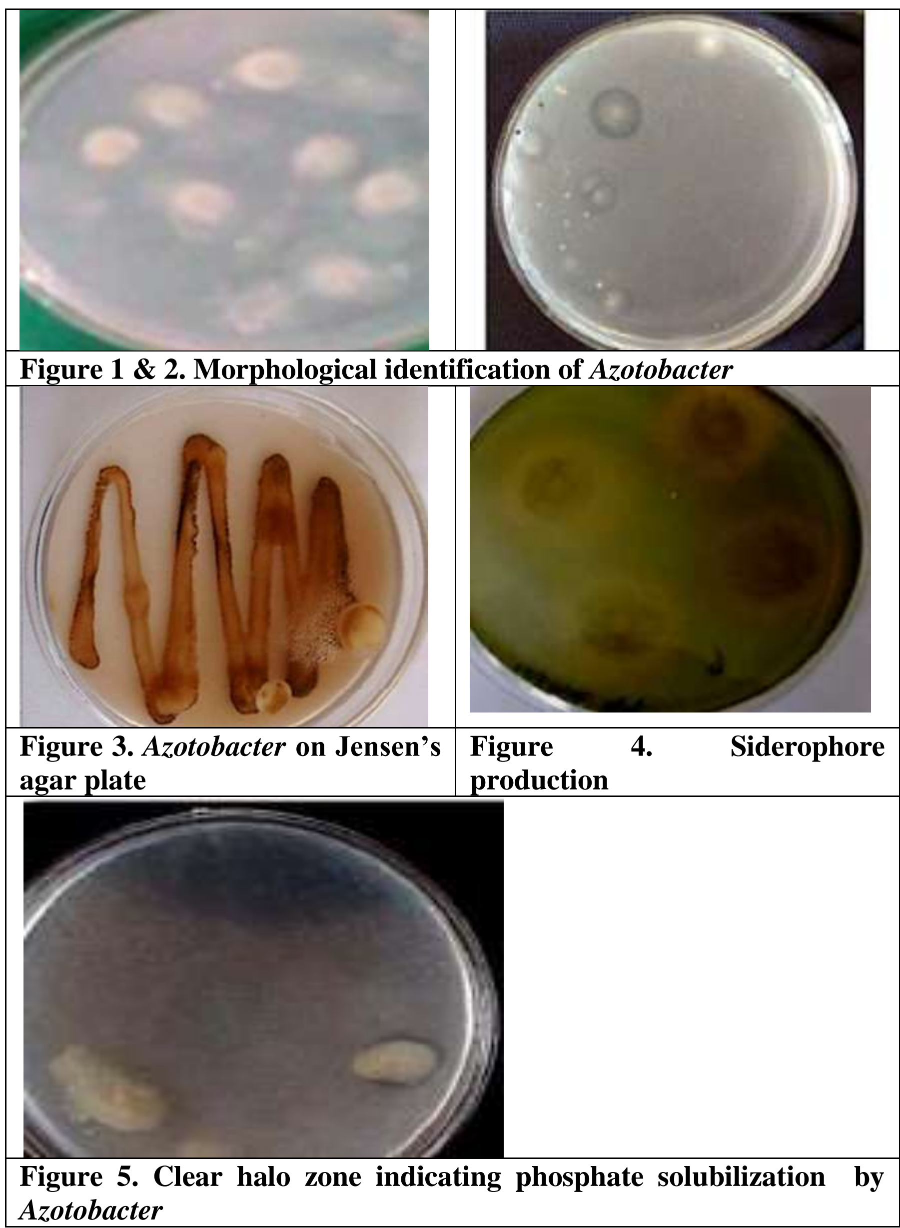

Figure 1 & 2. Morphological identification of *Azotobacter*

Figure 3. *Azotobacter* on Jensen's agar plate

Figure 4. Siderophore production

Figure 5. Clear halo zone indicating phosphate solubilization by *Azotobacter*

Table 1. Morphological and Biochemical tests and identification of characters

S/N	Test	Result
1	Gram staining	Negative, oval shaped
2	Colony characters	White, smooth, glistening, translucent
3	Pigmentation	Brown to black
4	Spore test	-
5	Motility	+
6	Catalase	+
7	Oxidase	+
8	Urease	+
9	H_2S production	+

10	Citrate utilization	+
11	Acetylene reduction	+
12	Hydrogen cyanide	+
13	Nitrate reduction	+
14	Methyl red	-
15	Indole test	+
16	Vogues Proskauer	+

Table 2. Bubbles formation observation during catalase test

S/N	Sample code	Bubble formation
1	AS1	medium
2	AS2	many
3	AS3	many
4	AS4	little
5	AS5	medium

Table 3. Optical density test using Spectrophotometer

S/N	Sample code	Optical density (ABS)
1	AS1	0.837
2	AS2	0.734
3	AS3	0.781
4	AS4	0.914
5	AS5	0.826

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