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EFFECT OF NUTRIENTS AND ENVIRONMENTAL CONDITIONS ON NITROGEN FIXING ABILITY OF *AZOTOBACTER* ISOLATED FROM DROUGHT AREA.

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ABSTRACT

Azotobacter is free living, diazotrophic bacteria found in soil, which fixes atmospheric nitrogen non-symbiotically. Isolation, characterization of *Azotobacter* from drought area and testing its ability to fix atmospheric nitrogen can be helpful to use it as a biofertilizer in such area. Thus we can enhance the crop yield in these area. Nitrogen fixing ability of isolates is studied using different nutrients like, carbon source, molybdenum, ferrous and at various temperature, pH. NFA of the isolate can be enhanced by adding the nutrients at specific conc.

KEYWORDS: *Azotobacter*, biofertilizer, NFA, drought.

INTRODUCTION

Nitrogen is the major component of all living cells, as it is present in proteins, nucleic acids (DNA, RNA) of every cell. It is one of the important element for growth of plants. Atmospheric nitrogen conc. is 78%, but most of the soil nitrogen is fixed by soil microbes. The nitrogen fixation provides Earth's ecosystems with about 200 million tons nitrogen per year Many soil microorganisms fix nitrogen symbiotically (*Rhizobium*) or individually (*Azotobacter*). The nitrogen from atmosphere is fixed by these microbes in the form of ammonia using the enzyme nitrogenase, and make it available for plants. Water is very important component for plant growth. Water scarcity directly affect the crop yield. Nitrogen fixing bacteria found in drought prone area can be used to improve crop yield in these area. Some nutritional requirement may positively affect the nitrogen fixation rate in drought resistant organisms. Nitrogen fixation rate may be enhanced by selection and breeding in these organisms.

In present study, *Azotobacter* sp. are isolated from drought prone area and studied for their nitrogen fixation ability (NFA). The effect of various nutrients and environmental conditions on NFA is studied. Plant growth promoting properties of *Azotobacter*, along with, nitrogen fixation, production of IAA, gibberline, cytokinins, exopolysaccharide, phosphate solubilization, production of siderophore and antifungal compounds help to enhance the plant growth.

MATERIAL AND METHOD

1. Collection of soil sample: Soil samples are collected from drought prone area of Sangli, (MH), India. These samples were collected from rhizosphere zone of sorghum crop at harvesting stage. Samples at different locations are mixed together in sterile container and brought to lab for study and immediately stored at cool temperature.

2. Isolation of *Azotobacter* from soil samples: For isolation of *Azotobacter*, in nitrogen free mannitol broth 1 g soil sample was added, mixed and incubated at R.T. (28-29^{o}C) for 7 days. Enriched broth was serially diluted up to $10^{-9}$and spreaded on nitrogen free mannitol agar plates and incubated at R.T. for 3-4 days. After incubation, off-white, mucoid, glistening colonies were observed on plates. The isolated colony was purified by sub culturing and confirmed for colony morphology, Gram's nature and motility. The pure cultures were retained on N_2-free Maintenance agar slope.

3. Biochemical characterization
Biochemical characters of isolated organisms were studied as per Bergey's Manual of Systematic Bacteriology Vol.II (2nd edition).

Biochemical characters are as follows:

1) **Sugar fermentation tests**: glucose, maltose, dextrose, mannitol, sucrose, arabinose
2) **Urease test:** Christenson's agar slats
3) **Starch hydrolysis test:** Starch agar plates
4) **Sensitivity to antibacterial agents:** streptomycin (0.2µg/ml), neomycin (1µg/ml), kanamycin

(1µg/ml) and HgCl2 (10µg/ml) N2 free media plates were spread inoculated by isolate and activity of antibacterial agents studied by agar well method.

5) **Catalase test**: This test was done by inoculating isolates on nutrient agar slants. After incubation H_2O_2 solution was applied on slant and observed for bubble formation.
6) **Phosphate solubilization test:** Pikovskaya's (PK) media plates were spot inoculated with isolates and incubated at R.T. for 3 days and observed for clear zone of phosphate solubilization around colony
7) **IAA production:** IAA produced by isolate was determined by the method described by Mathurot (2011). N2 free broth medium containing 0.2% L-tryptophan was inoculated with 1% suspension (10^8CFU ml^{-1}) of isolates and incubated at R.T. for 7 days on rotary shaker. IAA production ability was detected by Salkowski's method. 2ml of supernatant was mixed with 2 drops of ortho-phosphoric acid and 4 ml of Salkowski reagent, kept in dark at R.T. for 30 min. The appearance of pink colouration to solution indicated IAA production.

Determination of nitrogen fixing ability of isolate (NFA)

Nitrogen estimation was done by Kjeldahl's method as below-

Digestion: In Kjeldahl's tube 1ml suspension of isolates was added, 2g catalytic mixture, 10 ml conc. H_2SO_4, the tubes were placed on digestion block and digestion was started by adjusting digestion program. It requires about 2.30 hrs. to complete digestion. After completion of digestion the unit was allowed to cool.

Distillation: Distillation of digested sample was done by using 4% Boric acid, 40% NaOH & mixed indicator. The distillate was collected in a flask.

Titration: Distillate was titrated against 0.02N H_2SO_4

Formula:

$$\%N = 14 * N * X * 100\backslash Wt.of\ sample * 1000$$

=14*0.02*X*100\1000

=0.28*X\1000

Determination of nitrogen fixing ability of isolate (NFA) at various moisture levels

For this, sterile soil/peat can be used as filling material to maintain moisture. Soil was sieved, dried in oven for 5 days. Soil was sterilized by autoclaving & oven dried for 3 days.

Sterility testing of sterile soil

Dried sterile soil (1g) was added in 9 ml sterile d/w, mixed well. This soil suspension was inoculated on NA and incubated at 37^0C for 5days. Intermittantly plates were observed for any growth of soil microbes. There was no growth upto 5 days, indicating that the soil was sterile.

For NFA, 5g of sterile soil was taken in pre-weighed sterile petri plate, 1 ml of isolate suspension and 1 ml liquid media was added and moisture maintained at 10%, 20%, 30%, 40% with sterile d/w. All contents were mixed well using sterile spatula and all plates were incubated at R.T. for 12 days. After every 24 hrs interval moisture was maintained at appropriate level using sterile d/w. After completion of incubation, nitrogen fixed by isolates at various moisture levels was measured by Kjeldahl's method.

Effect of various nutrients on NFA

1) NFA at various carbon sources

Azotobacter can utilize variety of carbon sources for its growth and nitrogen fixation. Various carbohydrates were used such as glucose, lactose, mannitol, maltose, sucrose and fructose. Nitrogen free media was prepared which contain each of these sugars separately as a soul source of carbon. Other ingredients remain same. These media were inoculated using standard suspension of the isolate and incubated at RT in shaker incubator at 180rpm for 7days. After incubation, fixed nitrogen was estimated by Kjeldhal's method and percentage nitrogen fixed by isolate using specific sugar was determined.

2) NFA at various pH

NFA of isolate **at various pH** was studied by maintaining different pH (5, 7, 9, 11) in media, & fixed nitrogen was estimated.

3) NFA at various Na_2MoO_4 conc

Nitrogen free media containing various conc. of Na_2MoO_4 (0.0001%, 0.0005%, 0.0010%, 0.0015%, 0.002%, 0.0025%) was inoculated by isolate and incubated at RT in shaker incubator at 180rpm for 7days. After incubation, fixed nitrogen was estimated by Kjeldhal's method and percentage nitrogen fixed by isolate using specific conc. of Na_2MoO_4 was determined.

4) NFA at various Temperature

Temperature used for NFA was 10^0C, R.T., 37^0C, 50^0C.

5) NFA at various $FeSO_4$ concentration

Different $FeSO_4$ concentrations used were 0.001, 0.005, 0.010, 0.015, 0.020, 0.025% and other media components were remain same.

RESULT AND DISCUSSION

Character/Test	Observation
Color of colony	Off white
Gram nature	Gram negative
Motility	Motile
Glucose	+
Maltose	+
Dextrose	+
Mannitol	+
Sucrose	+
Arabinose	+
Urease	+
Starch hydrolysis	+
Antibacterial activity	
1)Streptomycin (0.2µg/ml)	+
2)Neomycin (1µg/ml)	+
3)Kanamycin(1µg/ml)	+
4)HgCl2 (10µg/ml)	+
Catalase	+
Phosphate solubilization	+
IAA	+

Nitrogen Fixing Ability (NFA):% Nitrogen fixed by isolates in broth

Nitrogen fixing ability of all isolates was studied by Kjeldhal's method. It was found that isolate VW2b fix 0.1988% nitrogen in broth.

NFA using various carbon sources

IsolateVW2b fixes nitrogen at highest rate utilizing sucrose as a sole source of carbon, 0.0448%N, Fructose is also utilized and fixes 0.0420% N. From the study it was found that sucrose gives more nitrogen fixation by isolateVW2b, but other sugars also gives reliable results and no any carbon source gives drastic fall in nitrogen fixation.

'C' source	% N
Glucose	0.0392
Lactose	0.0392
Mannitol	0.0364
Maltose	0.0364
Sucrose	0.0448
Fructose	0.0420

NFA of VW2b using various Na_2MoO_4 conc

Nitrogen fixing ability of isolateVW2b was found highest at 0.0005% of Na_2MoO_4 i.e. 0.0644%N. There was decreased % N fixed by isolate at increasing conc. of sodium molybdate. This component plays important role in growth as well as nitrogen fixing ability of *Azotobacter.*

Conc. of Na_2MoO_4 (%)	% N
0.0001	0.0504
0.0005	0.0644
0.0010	0. 0588
0.0015	0.0560
0.0020	0.0504
0.0025	0.042

NFA of VW2b using various $FeSO_4$ conc.

FeSO4 is also important component for growth of *Azotobacter*. Isolate VW2b fix highest nitrogen at 0.015% $FeSO_4$ in media which is 0.056% N. At higher conc. of $FeSO_4$, nitrogen fixation rate is lowered, but at lower conc. of $FeSO_4$, the NFA is quite elevated. This indicates that lower conc. of $FeSO_4$ is essential for nitrogen fixation by the isolate.

Conc. of $FeSO_4$ (%)	% N
0.001	0.036
0.005	0.042
0.010	0. 039
0.015	0.056
0.020	0.014
0.025	0.014

NFA of VW2b using various Temperatures

The highest nitrogen fixation was observed at R.T. (about 30 ^{o} C) which was 0.0224%N. At elevated temperature nitrogen fixation was decreased.

Temperature	% N
10^{o}C	0.0196
R.T.	0.0224
37^{o}C	0.0196
50^{o}C	0.0168

NFA of VW2b using various pH

Effect of pH on nitrogen fixation was studied by using various pH such as 5, 7, 9, 11 separately and found that highest percentage of nitrogen fixed by isolateVW2b was at pH 7. At pH 5 and 11 % N fixed was almost same with slight difference, 0.0252% and 0.0208% respectively. Lowest % N was fixed at pH 9, which was 0.0196.

pH	% N
5	0.0252
7	0.0336
9	0.0196
11	0.0208

CONCLUSION

From above studies it is found that Azotobacter isolated from drought area having ability to synthsize various plant growth promoting components like, IAA production, phosphate solubilization which help the plant for growth. It fixes atmospheric nitrogen using sucrose as a carbon source, 0.0448% N; Na2MoO4 conc. (0.0005%)

0.0644% N; FeSO4 (0.015), 0.056%N and at pH 7, 0.0336%N;R.T0.0224%N.

Thus it can be concluded that isolate obtained from drought prone area can enhance the plant growth if used as a biofertilizer after mass production.

REFERENCES

1. Brenner, D.J., Kriege, N.R., Staley, J.T. Garrity, G.M., Bergey's Manual of Systematic Bacteriology, 2nd Edition, vol.2 Part B, Springer- Verlag, New York., 2005.
2. Charles Then et al, Production of Alginate by *Azotobacter vinelandii* in semi-industrial scale using batch and fed-batch cultivation systems *Journal of Advanced Scientific Research*, 2012; 3(4): 45-50.
3. Chengsheng Zhanga, Fanyu Konga Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants, *Applied Soil Ecology*, 2014; 82: 18–25.
4. Dean Burk, Hans Lineweaver, and C. Kenneth Horner, The specific influence of acidity on the mechanism of nitrogen fixation by *Azotobacter Journal of bacteriology*, 1933; XXVII(4).
5. Fauzia Y. Hafeeza, Sumera Yasmina, Dini Arianib, Mehboob-ur-Rahmana, Yusuf Zafara, Kauser A. Malika, Plant growth-promoting bacteria as biofertilizer**,** *Agron. Sustain. Dev.*, 2006; *26: 143–150*.
6. M Robinson, J.Riov, Sharoni Indole-3-Acetic Acid Biosynthesis in *Colletotrichum gloeosporioides* f. sp. *Aeschynomene*, *Applied and Environmental Microbiology*, 1998; 5030–5032: 64(12).
7. Sandeep Upadhyay, Isolation, characterization, N2 fixing efficiency of *Azotobacter* strains and their effect on finger millet [*Eleusine Coracana* Gaertn.] and soil quality. June, 2010 *Thesis*
8. Vikram Patil, 2011 Production of Indole Acetic Acid by *Azotobacter* sp., *Recent Research in Science and Technology*, 2010; 3(12): 14-16.