IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Estimation of Auxins by Azospitillum brasilense and Phylogenetic relationship between Azospirillum brasilense and Bradyrhizobium japonicum

Harika Allamsetty
Department of Biochemistry
S.D.M.Siddhartha Mahila Kalasala
Vijayawada, Andhra Pradesh

Abstract: Azospirillum brasilense, a nitrogen- fixing bacterium found in the rhizosphereof various grass species, was investigated to establish the effect on plant growth of growth substances produced by the bacteria. Thin- layer chromatography, high- pressure liquid chromatography and bioassay were used to separate and identify plant growth substances produced by the bacteria in liquid culture. Indole acetic acid and indole lactic acid were produced by A. brasilense from tryptophan. Indole acetic acid production increased with increasing tryptophan concentration from 1 to 100 µg/ml. Indole acetic acid concentration also increased with the age of the culture until bacteria reached the stationary phase. Shaking favoured the production of indole acetic acid, especially in a medium containing nitrogen A Small but biologically significant amount of gibberellin was detected in the culture medium. Also atleast three cytokinin- like substances, equivalent to about 0.001µg of Kinetin per ml, wee present. The morphology of pearl millet roots changed when plants in solution culture were inoculated. The number of lateral roots was increased, and all lateral roots were densely covered with roots hairs. Experiments with pure plant hormones showed that gibberellin causes increased production of lateral roots. Cytokinin stimulated root hair formation, but reduced lateral root production and elongation of the main root. Cobination of indole acetic acid, gibberellin, and kinetin produced changes in root morphology of pearl millet similar to those produced by inoculation with A. brasilense

I. INTRODUCTION

This group of bacteria can take nitrogen from the air and provide it to the plant (nitrogen fixation). Nitrogen fixation is not the major benefit Azospirillum can give to plants. When Azospirillum are placed on the roots of the plants they have the ability to increase the number of root, hairs are important in helping the plant get water from the soil. This benefit the plant derives from its association with Azospirillum helps the plant do better in their drier places. This bacterium lives in soil. It is able to live on its own in soil, or in close association with plants in the rhizosphere .A. brasilense is helpful to plants and important to farmers because it is able to fix nitrogen gas in the air in to nitrogen bound up in amino acids and proteins. Azospirillum is a diazotropic bacterium used as a biofertilizer for a variety of crops like maize, rice, cotton, sugar cane, sorghum, sunflower and vegetables including plantation crops.

Three pathways are known for the conversion of Tryptophan to Auxin. The pathways are via,
Indole pyruvic acid, Tryptoamine, Indole acetamide
Strains of Azospirillium.: Azospirillum lipoferum, A.brasilens, A.amazonense A.doebereineren, A.halopraeferens

Characters Of Azospirillum

It belongs to the kingdom Eubacterium, Family Rhodospirillacea, Genus Azospirillum.It is aerobic, microaeropilic, motile, gram negative, helical/vibroid bacteria, Vibroid 0.6-1.7 cell diameter. Nitrogen fixed only under microaerophilic. It is known tobe plant-associated nitrogen fixers. G+Ccontents of DNA is 64-71. This belongs to the ∝–sub class of proteo bacteria, corresponding to the RNA super family 1V. Azospirillium forms the red (or) pink pigment when grown in the dark under aeropilic conditions and form intracellular poly-β hydroxy butyrate

Characters of Brady rhizobium

It belongs to the Domain Bacteria, Phylum Proto bacteria, Class Alphapro bacteria, Order Rhizobils, and Family Bradyrhizobiaceae. Bradyrhizobium japonicum is a gram negative, rod shaped nitrogen fixing bacteria develops a symbiosis with

the soyabean plantglycine max. It belongs to the family, Rhizobiaceae which includes other nitrogen fixing bacteria that develops symbiosis with legumes.

Materials And Methods

Test tubes
Pipettes
Conical flasks
Petri plates
Eppendoffs
Micropipettes
Vials

Instruments

Weigh balance
PHmeter
Autoclave
Laminar air flow chamber
Micro centrifuge
Incubator
Orbital shaker
Spectrophotometer
Transilluminator

I. Specific malate media for Azospirillum(1000ml):-

Carbon source (sucrose (or) glucose)	…. 4 g
K2HPO4	…. 0.5 g
MgSO4.	…. 0.2 g
Nacl	…. 0.1
Yeast extract	…. 0.5 g
FecL3. 6HO	…. 0.015 g
DL-Malic acid	…. 5 g
KOH	…. 4.8 g
Agar	…. 20 g

P^H adjusted to 7.0 with 0.1 N KOH

II. Rojo Congo (congo red): 15ml of a 1:400 aq solution of congo red

III. Specific Yema Media for Bradyrhizobium (1000ml):-

KHPO	…. 0.5 g
MgSO.7HO	…. 0.2 g
Nacl	…. 0.1 g
Mannitol	…. 10 g
Yeast extract	…. 1 g
Agar	…. 15 g
Distilled water	…. 1 liter
P^H	…. 6.8

IV. Reagents for Isolation of Genomic DNA:

Extraction buffer	
100 m M Tris Hcl p 9	-1.825/500ml
40 m M EDTA	- 5.92 /500ml
10/SDS	-10g in 100ml
Benzyl chloride	-3ml
3 M sodium acetate p 5	-3ml
Isopropanol	-0.86 g/10ml22

For this study secondary data has been collected. From the website of KSE the monthly stock prices for the sample firms are obtained from Jan 2010 to Dec 2014. And from the website of SBP the data for the macroeconomic variables are collected for the period of five years. The time series monthly data is collected on stock prices for sample firmsand relative macroeconomic variables for the period of 5 years. The data collection period is ranging from January 2010 to Dec 2014. Monthly prices of KSE - 100 Index is taken from yahoo finance.

I . PROCEDURE

Isolation of Azospirillum Species

The species of azospirillum species are abundant at the vicinity of paddyfields etc. The soil was taken from the fields and the azospirillum species are isolated by using nitrogen free cultures media and congo red medium was used to distinguish between the rhizobia and other bacteria. The crops include corn (Zea mays), sorghum, rice (oriza sativa) 9or) the roots of these plants were collected and treated with sterile distilled water or 1% chloamines T (4), are placed in enrichment nitrogen free broth medium. These enrichment cultures were incubated at 37°C for 72 hours, White, dense, undultating, diffuse pellicles of azospirillum colonies are easilty observed. First streak small azospirillum colonies among the contaminants, after then 1 to 4mm below the surface are observed. When observed in microscope under high power objective lens, fat droplets and active movements of azospirillum species are observed. The positive cultures are serially diluted to 10fold in sterile tap water to 10^{-4} to $10^{-5.}$ Loopfulls of the dilution were straked on plates of RC medium, which were incubated at 37°Cfor 72hours. Light pink and colorless colonies were observed after 48hours. After 72 hours, the light pink colonies become scarlet. Small scarlet colonies were in the streak, incubating the presence of azospirillum species among the contaminants. The microscopic examination of wet mounts of scarlet colonies reveal rods resembling azospirillum cells. The colonies were streaked on the RC medium to check the purity of the isolates. Uniformity of he colony colour was observed The species were identified on the basis of the capacity to use glucose as the sole carbon source for growth in nitrogen free broth medium.The colonies that are observed in the RC medium are incubate at 37°C for 96 hours have the following characteristics

Scarlet colour

Abundant growth

Dry consistency, CC

Diameter of 1.5 to 2mm

Round or irregular form, undulate edge and

Rugose surface with ridges radiating from the center.

Isolation of Bradyrhizobium species**:**

The species of the bradyrhizobium sps are abundant at the vicinity of legume fields. The soil was taken from the fields and the bradyrhizobium sps are isolated by using Yema media was used to distinguish between azospirillum and other bacteria.The crops include legume plants or the roots of these plants were collected and treated with sterile distilled water or 1% chloramines- T 94) are placed in yema medium.These enrichment cultures were incubated at 37°C for a week. First streak shows *small* bradyrhizobium colonies among the contaminants, after then 1 to 4mm below the surface are observed. When observed in microscope under high power objective lens, active movements of bradyrhizobium sps are observed. The positive cultures are serially diluted to 10 fold in sterile tap water to 10^{-4} tp 10^{-5}. Loop full of the dilution were streaked on plates of yema medium, which were incubated at 37°C for a week. *White coloured colonies were observed after a week.* The microscopic examination of wet mounts of white colonies reveals rods resembling bradyrhizobium sps. The colonies were streaked on the Yema medium to check the purity of the isolates. Uniformity of the colony colour was observed.The sps were identified on the basis of the capacity to use glucose as sole carbon source for growth in yema medium.

Introduction:

Production and Estimation of Auxins

Among the plant hormones today, auxin (from Greek term, auxien to increase) was the first phytohormone recognized and chemically detected in the 19th century. A number of indole compounds and phenyl acetic derivatives have been reported with auxin activity. Among these, indole-3-aceticacid(IAA) is considered the most physiologically active auxin in plants. Auxins are involved in a variety of diverse plant growth and developmental responses ; however their mechanism of action are not fuly understood. Today several synthetic auxins are used in commercial application. For increase, indole- 3 butyricacid(IBA) and naphthaleneacetic acid(NAA) are used commercially for the auxin literature focuses on IAA, it is the primary auxin.Biosynthesis of quxin is not limited to higher plants. Microorganisms are also very active auxin producers. Many rhizosphere microflora have the capacity to synthesize auxin in vitro in the presence or absence of physiological precursors.

Physiological Action of Auxin On Plants:

Like other phytohormones, quxins are synthesized endogenously by plants, and their hormonal effects have been elucidated largely from exogenous application. Many functions attributed to auxins overlap with the function of other phytohormones particularly ethylene. The auxin – ethylene interactions is now well recognized the influence of quxin on plant growth and development is concentration dependent (i.e; a low concentration may be stimulatory where as high concentration is often inhibitory).

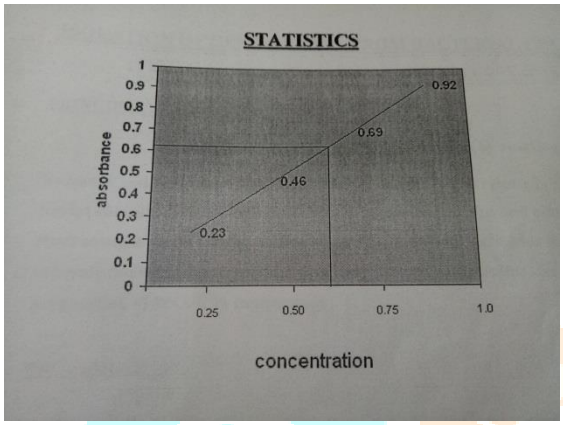
Production And Estimation of Auxin:-

Wild and mutated strains of azospirillum were grown on nitrogen free bromothymol blue medium for 5-7 days.

The culture was centrifuged at 4000rpm for 30minutes and the supernatant was brought to P^H to 2.8 with 1N HCL.

Then it was extracted with diethyl ether. The Ether phase was dried and dissolved in 2ml ethanol. Auxin present in ethanol extract was estimated by using salpers reagent (1ml of 0.5ml ferric chloride in 50ml of 35% oer chloric acid). The colour intensity was read at 540nm.

Sample In Orbital Shaker



Graph showing concentration of Auxins

RESULT

The estimated amount of Auxins present in the sample is 0.63mg/ml from the standard concentrations.

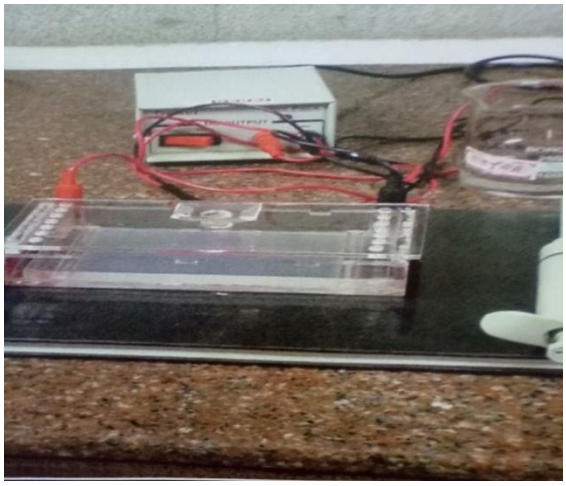
.

Isolation of Genomic DNA from Bacterial cells

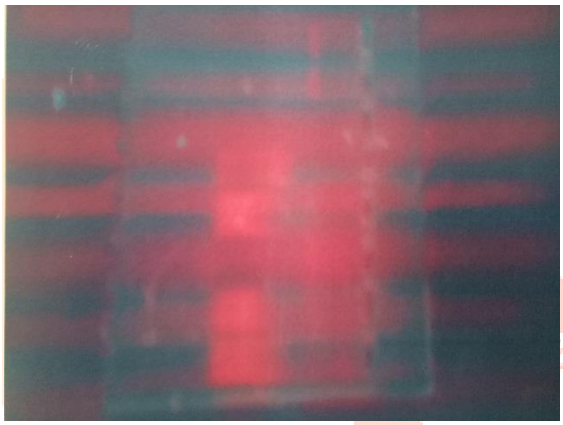
Benzyl chloride destroys cell wall of bacteria by reacting with hydroxyl residue is polysaccharides. Thus genomic DNA is released, further benzyl chloride denatures the proteins and extracted proteins and other cell debris from aqueous phase leaving nucleic acids. EDTA and SDS also aids in the lysis of cell wall. Sodium acetate provides necessary ionic. Concentration for the preparation of DNA with isopropanol.

Procedure:

50Ml of Malate medium was inoculated with a single colony of Azospirillum cells and was agitated overnight at 37ºC in an orbital shaker that was set at 210rpm. 1g of bacterial pellet was harvested by centrifugation at 5000rpm for 2min in centrifuge tube. To the pellet 5mk of extraction buffer. 1ml of SDS and 3ml of benzyl chloride were added. Then the mixture was vortexed to suspend the cells into solution. The contents were incubated at 50^0C for 30minutes. To maintain the cells in suspension state the contents were mixed by inverting the tubes and then the tube was kept on ice for 15minutes. Then centrifugation was carried out at 4ºC for 5minutes. The cell debris and bacterial chromosomal DNA pelleted out. The supernatant was transferred to a fresh tube and equal volume of isopropanol was added and mixed gently and left for 5 minutes The mixture was spun at 10,000rpm for 5 minutes to pellet the plasmid DNA. The supernatant was discarded, the pellet was washed with 70% ethanol for about 3-4 minutes. Etanol was discarded and the tubes were kept inverted to drain etanol. Then the samples were allowed to dry for about 10minutes. The pellet was then resuspended in 20ml T.E buffer and allowed to dissolve completely. 2ml of DNA sample corresponding to each species (Azospirillum, Rhizobium) were loaded in 0.8% agarose gel and was electrophorosed at 50V for 2hours. The quality and quantity of DNA was recorded using U.V transilluminator.



Electrophoretic unit



Phylogenetic Relationship Between Two species by observing the bands Flourescent Bands of Genomic DNA

RESULTS

Azospirillum and Rhizobium have the capacity to synthesize auxins and the florescent bands are observed U.V transilluminator corresponding to DNA.

DISCUSSION:

1. Auxin constitute a class of phytohormones that play important roles in the coordination of plant growth and development.
2. Indole-3- acetic acid (IAA), the most abundant naturally occurring auxin, has been implicated in regulating a variety of developmental and cellular processes such as cell extension, cell division, vascular differentiation, rootformation, apicaldominance.
3. Regulation of these processes by auxins is believed to involve auxin induced changes in gene expression.
4. A number of plant genes that are transcriptionally induced by quxin, and that may play roles in one or more of these processes. The signal transduction pathways leading to the quxin mediated gene induction in plants.
5. Besides plants, many soil and rhizosphere bacteria, including phytopathogenic, epiphytic and plant growth stimulating bacteria produce IAA.
6. Electrophoresis mainly involves the separating of DNA particles according to their molecular weight.
7. This is done on separating the fragments according to their size. The shorter fragment will move fast and reach first, the larger fragments remain or travel slowly. This technique mainly involves the identification of unknown DNA fragments by comparing with the standard molecular markers.
8. This technique can also be used to establish the phylogenetic relationship between two species.
9. This technique can also be used to establish the phylogenetic relationship between two species.

SUMMARY

Bacteria of the genus Azospirillum are extensively studied for their plant – growth promoting effect following inoculation. Physiological and biochemical studies of these diazotrophic bacteria are now benefiting from recent breakthroughs and cloning of Azospirillum genes involved in N_2 fixation, plant interaction, and phytohormone production have given new life to many research projects on azospirillum. The finding that Azospirillum genes can complement will certainly trigger the exploration of new areas in rhizosphere biology. In order to know the relationship between the closely related species of nitrogen fixing bacteria we opted for this method. Applying this method we got the bands according to this species. By observing them we established a

phylogenetic relationship between these two species. Both these two species have similar genes present for production of nitrogen in plants actng as nitrogen fixing agents. As they contain similar genes we got the similar bands in the running process.

CONCLUSION

The Auxin production by the microorganism such as azospirillum and rhizobium can be known by this method. In order to know whether a particular organism produces auxins or not the isolation process used above is preferred commonly. From the results obtained, we came to the conclusion that the azospirillum and rhizobium have the capacity to produce auxins.

BIBILIOGRAPHY

- Bashan, Y.1986. Enhancement of wheat root colonization and plant development by azospirillum. Appl. Environ. Microbial 51:1067-1072.
- KRIEG, N.R. 1984 Bergeys manual of systematic bacteriology.Vol.1 william and William, Balyimore.
- SCHNEIDER,E.A. and WEGHTMAN,F.197. Metabolism of auxins in higher plants. Plant physiology 25:487-513
- Charles R. Lovell, Michael J. Friez, john W. Longshore, and Christopher E. Bagwell
- Jordan 1982, gen.nov. (Type genus of the family Bradyrhizobiaceae Garrity etal.2006).
- Bakanchikova, T. I., E.V.Lobanok, L.K. Pavlova- Ivanova,
- Redkina, Z.A. Nagapetyan, and A. N. Majsuryan. 1993. Inhibition of tumour formation process in dicotyledonous plants by Azospirillum brasilense strains. Mikrobiologiya 62:515-523.
- Bashan, Y and G. Holguin. 1997. Azospirillum- Plant relationships: Environmental and physiological advances (1990-1996) can.J, Microbiol. 43:103-121
- Beringer, J.E. 1974.R factor transfer in Rhizobium leguminosarum. J.Gen. Microbiol. 84:188-198.

I. ACKNOWLEDGMENT

I Profoundly thank Fr. Emmanuel S.J., my Principal from the bottom of my heart for his constant guidance, support and encouragement. I thank A. Amarendra, research scholar, sastra university for his timely advice and guidance.