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# ISOLATION AND ANALYSIS OF PLANT GROWTH HORMONE AUXIN – IAA FROM *RHIZOBIUM* SPP.

**Chandani K.Patil, Mr. S.G.Kulkarni**
Department of Biotechnology,Vivekanand College,Kolhapur
Tarabai Park,Kolhapur-416003 Maharashtra,India 2012

*Plant Growth hormone auxin – IIA synthesized by Rhizobium Spp , which is a symbiotic nitrogen fixer present in root nodules of leguminous plants. IIA from Rhizobium isolated by using physiological precurssor Tryptophan. Tryptophan with variying concentraion is used for synthesis of Auxin. The produced IIA can be confirmed by xylene, Kovac's reagent and Salkowaski reagent . Further FTIR analysis of produced IIA is carried out . The two main benefits of this study is that Rhizobium Spp capable of producing Auxin can be used as a Biofertilizer and the synthesized Auxin is applied in Plant Tissue culture.*

**Key words:-** *Auxin, Kovac's reagent , precurssor , pleomorphism, FTIR*

## Introduction:-

Plant hormones are signal molecules acting as a chemical messangers that cantrol plant growth and development. In addition numerous soil bacteria and fungi produce plant growth hormones. This hormones are very simillar to plant hormonesin their physiological activities.Now these micrbial origin plant hormones used in plant tissue culture. It is now well established that there are two sources of phytohormones one as endogenous production by the palnt tissues and exogenous production by associated microorganisms. The ability to synthesize plant growth hormone is wide spread among soil and plant associated bacteria responsible for plant growth promotion & symbiotic association.

## Material and Methods:-

### 1. Isolation of Rhizobium:-

For isolation of Rhizobium root nodules of legumonous plants Trigonella Spp were selected, the root nodules were washed with tap water to remove the soil. The nodules were surface sterolized by deeping in 0.2% HgCl2 solution for 3 to 5 mints, then follwed by washing with D/W. The surface sterilized root nodules were crushed in minimum volume of 0.85% Saline(NaCl) with help of strilized glass rod to obtain uniform suspension.

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The prepared Suspension was subjected for Gram's Staining for presence of Gram negative Bacteriods, as rhizobia are pleomorphic in nature, when they are present in root nodules they looks like chineese letter , the morphological forms resembles to different letters like, L,T,V,Y,X these forms are called as Bacteriods.

A loopfull of suspension was streak inoculated on Congored yeast extract mannitol agar plate. The colony characteristics of well isolated colourless colony were recorded and confirmed for Gram negative short rods . such colourless colony was again restreaked on fresh CRYEMA medium to maintain pure culture on Plate as well as Slant.

2. **Enrichment of Rhizobium And Biosynthesis of Auxin:-**

Sterilized CRYEMA broth with tryptophan was inoculated with isolated rhizobium. The 3 different flasks were prepared cantaining CRYEMA broth with varying concerentrations of tryptophan in each flask as 2mg/ml, 4mg/ml, 6mg/ml . the flasks were incubated at 37$^0$ C for 48 hrs in shaking incubator at 100 rpm.

After incubation broth was centrifuged at 3000 rpm for 10 min and the contentes were filtered through whatman's filter paper . the filterate were stored at 20$^0$ C and used for further studies.

3. **Determination of Auxin biosynthesis:-** The Auxin-IIA produced by Rhizobium was confirmed by Qualitative as well as Quantitative Test.

**i)Qualitative Test by using Xylene & Kovac's reagent:-**

In 3 different test tubes samples obtained from filterate is taken ,followed by addition of xylene to form a separate layer, then addition of Kovac's reagent from the sides of the test tube and observed for formation of pink colour ring.

Xylene extracts the indole which further react with Kovac's reagent to form pink colour.

**ii) Confirmation of IIA by TLC using xylene and Kovac's reagent:-**

In this method xylene is used as a mobile phase . 4 Silica gel TLC plates were prepared one for std and other for sample. After spoting the plates were allowed to run in TLC chamber containning xylene. After running the plates were sprayed with kovac's reagent and observed for formation of pink colour spot and R.F.values were calculated.

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| Distance travelled by Solute (Std & samples) | Distance travelled by Solvent:- (xylene)- 10 cm | |
|---|---|---|
| | | R.F. Value |
| Std IIA | 8 cm | 0.8 |
| Sample-1 | 7.8 cm | 0.78 |
| Sample-2 | 8.0 cm | 0.8 |
| Sample-3 | 8.0 cm | 0.8 |

The TLC of the samples and Std IIA showed almost same R.F. values.

**iii) TLC by using Salkowaski's reagent:-**

In this benzene, butanol, acetic acid was used as a mobile phase, the 4 plates one for Std and 3 for samples were spotted separately and allowed to run . After this the plates were sprayed with salkowask's reagent and observed for formation of pink colour spot, R.F.values were calculated.

| Distance travelled by Solute (Std & samples) | Distance travelled by Solvent:- (xylene)- 11.5 cm | |
|---|---|---|
| | | R.F. Value |
| Std IIA | 7.8 cm | 0.67 |
| Sample-1 | 7.8 cm | 0.67 |
| Sample-2 | 7.8 cm | 0.67 |
| Sample-3 | 7.8 cm | 0.67 |

The TLC of the samples and Std IIA showed almost same R.F. values.

**iv)Quntitative Analysis of IIA :-** Clorimetrically the IIA can be estimated by Salkowaski's reagent . IIA react with Salkowaski's reagent to form pink colored complex intensity of color is directly propotional to concentration of IIA . the conc. Of IIA in sample determined by using( std IIA CONC 500µg/ml) Std graph. The conc of IIA is found to be increased in samples with increse in conc. Of tryptophan.

4. **Extraction & Purification of IIA:-** Filterates obtained from broth centrifuged at 10,000 rpm for 30 min ,suparnatant acidified to 2.5to 3.0 by using 0.1N Hcl and made twice with ethyl acetate at double the volume of suparnatant. Extracted ethyl acetate fraction was was evaporated to dryness at 40 C. The extract was disolved in methanol & kept at -20$^0$C ,used for further studies.

5. **FTIR Analysis For detection of IIA:-**

FTIR Can provide useful information about functional groups present in compound for this std IIA & samples were analysed ,results were recorded.

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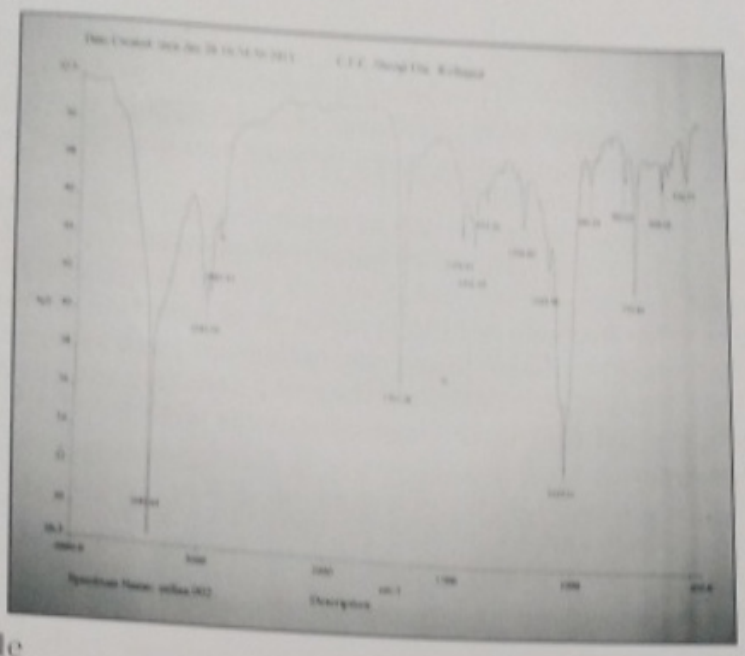
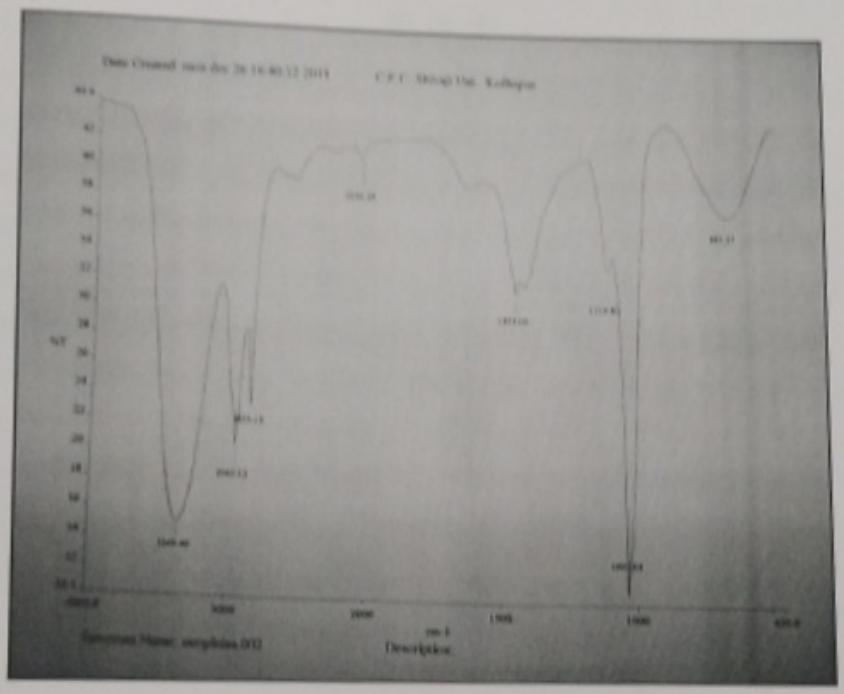
For Std IIA



For Sample



**Result and Discussion :-**

Rhizobium Spp were isolated from the root nodules of Trigonella Spp . The Spp is tested for biosynthesis of auxin in presence of tryptophan. In this study rhizobium show utilization of different concentrations of l-tryptophan for IIA production. As the biological nitrogen fixation palys vital role in increasing the growth of leguminous plants by developing a symbiotic relationship between rhizobia and plant. At the same time the produced IIA responsible for growth of the plant.

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**Refrence: -**


1) Vikram Patil,Production of indole acetic acid by Azotobacter Spp.RRST-Biotechnology recent reaserch in science and technology 2011,3(12): 14-16
2) Muhmmad.Ashfaq Anjum et.al Isolation and Screening of rhizobia for auxin biosynthesis and growth promotion of mung bean seedlings under axenic conditions Soil Environ. 30(1):n18-26,2011
3) M.sridevi and K.V.Mallaiah Production of indole-3-acetic acid by Rhizobium isolates from Sesbania Spp Africal journal of microbiology Reaserch vol.1(7) 125-128 Dec 2007
4) Fankenberger WT.jr.Brunner W (1983) methods of detection of Auxin-Indole acetic acid in soil by high performance liquid chromatography soil soc Ams 47: 237-241
5) Ehmann A 1977 The van Urk Salkowski reagent a sensitive and specific chromogenic reagent for silica gel thin layer chromatographic detection and identification of indole derivatives.
6) Xylene and Kovac's reagent for detection of indole derivatives -Book –Media Preparation by A.M.Deshmukh.
7) Microbial production of Plant hormones- Franken Berger.
8) Biotechnology- V.Kumaresan


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