A Comparative analysis of Indole Acetic Acid in *Azospirillum* species and enhancing the growth and yield of varieties of Paddy plants

S.Umadevi* and B. Suhameena,
Department Microbiology and Biochemistry,
Nadar Saraswathi College of Arts and Science, Vadaputhupatti,
Theni – 625531, Tamil Nadu.

Project Code: AS-049

Introduction:

Phytohormones are hormones produced by plants necessary for their growth and development for important crop production. One of the important properties of PGPR organism while screening of biofertilizer from rhizosphere region of soil is Indole acetic acid (IAA) production. Microbes at root surface utilize nutrients and using L- tryptophan act as precursor for IAA production which is a secondary metabolite through different Trp-dependent pathways. IAA increases root uptake for plants, delay leaves abscission and induce flowering and fruiting. In this study we are screening the rhizosphere strains for their IAA production qualitative and quantitatively.

Materials and Methods:

Production of growth promoting substance by the PGPR Test for IAA production:

The isolates were subjected to analysis for the production of IAA Luria agar supplemented with 0.5 mml tryptophan, sodium dodecyl Sulphate and 1% glycerol was prepared and plated. The surface area of the agar medium was divided into squares of 2 cm × 2 cm by marking on the bottom of each plate. The overnight grown culture of each isolate on Luria broth was spotted with sterile tooth pricks in each square. The spotted plates were overlaid immediately with sterile Whatman No. 1 filter paper disc of the size of the inner diameter of the petriplate. The plates were incubated until the colonies reached the size of 0.5 to 2 mm diameter after incubation. The filter paper discs were removed from the plates and treated with Salkowaskis reagent (2% solution of 0.5 M FeCl3 in 35% perchloric acid) by soaking in the reagent taken in a petridish. The filter paper discs were observed for IAA.

Qualitative test for Indole acetic acid (IAA) production:

A quantity of 0.5 ml of the sample was taken in a test tube and 1.5 ml of distilled water was added followed by a 4 ml of Sapler's reagent and incubated in darkness for 1 hr at 280C. The intensity of pink colour developed was read in spectrophotometer at 540 nm. By referring to a standard graph prepared with chemical grade indole 3-acetic acid, the quantity of IAA in the sample was determined and expressed as µg per ml of culture filtrate.

Quantitative estimation of IAA production in isolates by HPLC:

The sample turned to pink were subjected to ethyl acetate extraction and analyzed by HPLC. Bacterial cultures were grown in LB liquid medium containing tryptophan for 3 days. The cells were removed by centrifugation for 10 min at 8000 rpm. The supernatant was separated and its pH was adjusted to 2.8 by HCl.50ml cell free liquid medium was mixed with equal volume of ethyl acetate in a separating funnel. Ethyl acetate fraction was collected and evaporated to dryness and residue was dissolved in 1-2ml methanol. The 20µl samples were analyzed on HPLC

by using methanol: acetic: water (30:1:70) as mobile phase, and UV detector at wave length 260 nm. The IAA were identified and quantified on based of retention time and peak area of standard IAA. The solvent used to separation of IAA were water: acetonitrile [76:24 (v/v)] as a mobile phase. The solvent and sample flow rate was adjusted at 2mL/min, with an average run time of 20 min /sample. The wavelength was used for detection of IAA as 280nm.

Result:

Production of PGPR of paddy field isolates – IAA production by *Azospirillum:*

The paddy field isolate, S2 showed IAA production potential of 28.83 µg per 50 ml of medium, when compared with reference S3 value of 31.84 µg per 50 ml of medium. The lowest amount of IAA production by paddy field isolates S1 of 13.42 µg per 50 ml of medium. Thus the values of IAA production of paddy field isolates ranged from 13.42 to 28.83 µg per 50 ml of medium.

Quantitative estimation of IAA production in isolates by HPLC:

The HPLC peaks in the acidic ethyl acetate extracts were collected individually, and were analyzed by HPLC. They confirmed by HPLC based identification of IAA. Although, IAA was identified quantitative estimates based on the HPLC analysis. The amount of IAA present was *Azospirillum* sp.,The HPLC analyses indicated that all of the *Azospirillum* cultures contained IAA.

Comparative analysis of Indole Acetic Acid:

The Comparative analysis study results, it can be inferred that the efficient strain as bioinoculant increasing plant growth and development was found to be S2 sample followed by S3sample. it was because the soil nature and strain resulted in highest numbers of roots, shoots and weight increased substantially when compared to other sample. Thus the strain *Azospirillum* can have potential for higher rice production, being ecofriendly to farmers.

Conclusion:

Thus screening the rhizosphere soil for diazotrophic bacteria and IAA producers can give beneficial organisms for high crop production. Utilization of such strains could significantly improve the grain yield. Further field experiments are to be carried out to bring it as a commercial biofertilizer in agriculture to benefit farmers. This study is of importance to develop much effective biofertilizers necessary for the hour in pollution scenario also bringing about high crop production.

Guide: **Mrs.S.Umadevi, Assistant Professor, Department of Microbiology and Biochemistry, Nadar Saraswathi College of Arts and Science, Theni – 625531.**

Project Code: **AS-049**