World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.com/
Research Article

Production and extraction of Indole acetic acid by using efficient strain of *Rhizobium* isolated from maize

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Received: 03-03-2014 / Revised: 18-03-2014 / Accepted: 24-03-2014

ABSTRACT

Rhizobium species were isolated from the rhizosphere soil of maize plants and studied for their ability to produce Indole acetic acid. *Rhizobium*, a nitrogen fixing bacteria can live in the rhizosphere soil of non-leguminous plants, exist freely and entraps atmospheric nitrogen and converts the unreactive nitrogen molecule to ammonia, a form that is readily utilize by plants. Rhizosphere soil from maize plants were collected from Lalgudi Taluk, Trichy District and identified based on their morphological and biochemical characters. The efficient strains were used for the production of Indole acetic acid by using thin layer chromatography.

Key words: *Rhizobium*, Phyto hormones, Maize, Indole acetic acid.

INTRODUCTION

Phytohormones are organic substances which are naturally produced in plants, control the growth (or) other physiological functions at a site remote from its place of production and active in extremely minute quantities. Similarly Philips (1971) defined growth hormone as "Substances which are synthesized in particular cells and which are transferred to other cells where in extremely small quantities influence developmental process". However, the term hormone is quite popular and widely used. It is meant for an organic substance synthesized in one tissue and migrates to another tissue of the plant where in very minute quantity affects the growth. The common hormones are auxins, gibberllins, cytokines, ethylene, dormin, florigen, etc.Among the phytohormones, indole acetic acid was the first plant hormone discovered by Darwin (1880) causing a bending in coleoptiles of the canary grass (*Phalaris canariensis*) coleoptiles.Went (1926-1928), confirmed that the isolated active material from the celeoptiles was responsible for including phototrophic response in the celeoptiles. IAA was isolated from a plant which was later confirmed [5,6].

Auxins occur in all plants,most importantly indole-3-acetic acid. Many plants also contain indole-3-aceticaldehyde, indole-3-butyric acid,indole-3-acetonitrile and indole metabolic derivatives such as 2-hydroxyindole-3-acetic acid and ethyl indole-3-acetate with similar cell elongation action.The principle auxin in higher plants is Indole-3-Acetic Acid (IAA).This natural auxin is synthesized by the plant itself from the amino acid tryptophan. There are several other naturally occurring auxins in higher plants, although IAA is by far the most important.

Rhizosphere is a rich niche of microbes and should be explored for obtaining potential plant growth promoting rhizobacteria (PGPR),which can be useful in developing bio-inoculants for enhancement of growth and yield of crop plants.*Rhizobium* species have been found to be present in the rhizosphere and known to exhibit important PGP traits like nitrogen fixation.Although *Rhizobium* has been considered as a model system for intensive study on nitrogen fixation mechanisms. *Rhizobium* species have been isolated and studied for IAA production from rhizosphere of maize.

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MATERIAL AND METHODS

Sample collection: The rhizospheresoil from maize plants were collected from Viduthalaipuram in Lalgudi taluk, Trichy District.

Isolation of *Rhizobium* from rhizosphere soil: Decimal dilutions were prepared by suspending 1g in 10ml of sterilized dis H_2O. For isolation of *Rhizobium* strains aliquot from 10^{-3}, 10^{-4}were used to inoculate on YEMA medium in petriplates. The cultures were incubated at 30^0C for 24 hrs.

Identification of *Rhizobium*

Colony and cell morphology: *Rhizobium* strains were identified by colony morphology and gram staining.

Biochemical test: Production of indole was noticed in inoculated tryptophan broth after 7 days of incubation by adding Kovac's reagent. The reduction of methyl red and voges-prouskauer reaction examined in glucose phosphate broth by adding methyl red and **α-** naptholsolution with KOH respectively. Citrate utilization was observed by using Simmon's Citrate medium with bromothymol blue in basal medium. Liquefication of gelatin was tested in 10% gelatin agar medium [7]. Hydrolysis of starch was examined on starch with nutrient agar& iodine solution. Production of hydrogen sulphide gas examined by method described [8]. Production of ammonia from urea was examined by Christensen urea agar with phenol red as an indicator. Fermentation of carbohydrates was tested by adding 10% Andrade's indicator in the basal medium containing peptone water and 2% sugar. The Gram's staining technique was followed [9,10,11]. Catalase activity was observed by stirring the culture in a drop of hydrogen peroxide (10% by w/v), while oxidase activity was tested according to Kovac's(1956).

CRYEMA test: A 2.5 ml of congored dye was mixed with a litre of YEMA medium to prepare CRYEMA medium. Bacterial colonies of the YEMA medium were streaked on the CRYEMA medium and the petridishes were incubated at 28 ± 2^0C for 5-7 days.

Glucose peptone agar test (GPA test): Rhizobial colonies from YEMA medium were transferred to GPA medium in a petridish by replica plating and observed for white colour colonies.

Microscopic observation: Bacterial cells in the CRYEMA medium were stained with carbolfuschin and visualized under a compound microscope. This dye stains the β-polyhydroxy butyrate granules in the *Rhizobium*. The cells of those colonies having β-polyhydroxy butyrate granules were picked up to inoculate on Bacteriocin production media.

Mass cultivation of *Rhizobium:* 500 ml of YEMA broth was prepared in a conical flask.The pure *Rhizobium* colonies from CRYEMA medium was inoculated into YEMA broth.The flask was incubated at 37^0 C for 1 week.

Production of IAA: For production of auxins, test organism was growth in nutrient broth for 72 hrs. At 28 ± 2°C for *Rhizobium* under shake conditions. Supernatant was prepared by centrifugation of cultures at 10,000 rpm for 20 minutes and was stored in deep fridge or 4°C.

Extraction and separation of IAA: IAA were extracted and separated from supernatant by thin layer chromatography.Acidified supernatant extracted with diethyl ether and partitioned with sodium bicarbonate.Extracted and concentrated fraction was dissolved in methanol.Methanol fraction spotted on silica gel-G plates and developed in isoproponal:water(30:20v/v) for 12-14h and sprayed with Salper reagent.

RESULTS AND DISCUSSION

In the present study, Rhizobial strains were isolated from the rhizosphere soil of maize plants.*Rhizobium* found to be having circular colonies with regular borders,convex,whitish pink in colour and glistering.Under light microscope, the isolates were non motile and were Gram negative. They were non sporing forming and aerobic (Table-1).The bacterium showed positive forVoges Proskauer, Citrate utilization, catalase, oxidase, TSI, carbohydrate fermentation (maltose,galactose,arabinose) and negative for Indole production, Methyl red, Urease,gelatin hydrolysis and starch hydrolysis (Table-2).The efficient strains of *Rhizobium* were identified by inoculating on CRYEMA medium and GPA medium.On CRYEMA medium, Rhizobial cells form red colour colonies and on GPA, they forms white, circular, entire raised convex colonies.PHB granules were seen when the Rhizobial cells were stained with Carbol fuschin.The pure Rhizobial colonies were mass cultivated in YEMA broth.The mass cultivated Rhizobial colonies were used for the production of Indole acetic acid.Pink spots corresponding to IAA or IAA like substances were visible when sprayed with Salper reagent.

To study the effect of incubation period on IAA production the YEMA broth containing L-tryptophan was inoculated with *Rhizobium* isolates and incubated at 37 ± on a laboratory shaker at 200

rpm. The amount of IAA produced was estimated for 12 hours until, the *Rhizobium* strains reach their stationary phase of growth, where they produce maximum (IAA) [12].There is firm evidence that plant growth promoting substances like IAA, Gibberllic acid, cytokines are produced by number of rhizosphere microorganisms [13] and proper concentraior of hormons essential to induce successful nodulation [14,15,16]. Rhizobia are known to produce significant levels of IAA both in free living conditions and also symbiotically in nodules [17].In addition to symbiotic nitrogen fixation, rhizobia also produce plant growth regulators (PGRs) including indole acetic acid [18, 19]. Another potential and economic source of indole acetic acid is soil microbiota. Several microorganisms including rhizobia have also been reported to synthesize indole acetic acid [19,20,21,22].

CONCLUSION

The present study was conducted to explore the Rhizosphere of maize for IAA producing *Rhizobium* strains and to study the effect of these strains on plant growth under controlled conditions. The rhizosphere gives support to many active microbial populations capable of exerting beneficial,neutral or detrimental effects on plant growth.Indole acetic acid is one the most physiologically active auxin and a common product of L-Tryptopohan metabolism by several microorganisms inducing plant growth promoting bacteria (PGPR) . Seed inoculation of legumes with these inoculants has been producing dramatic results in the developed as well as developing countries of the world since the beginning of the 20^{th} century.

Table- 1.Cultural & morphological characters of *Rhizobium* isolates

Characters	Observation
Shape	Circular
Color	Whitishpink&Glistering
Elevation	Convex
Margin	Regular
Opacity	Opaque
Motility	Non-motile
Bacterium shape	Rod
Oxygen demand	Aerobic
Spore forming	Non-spore forming
Gram's nature	Gram negative

Table - 2.Biochemical Characters of *Rhizobium* isolates

Test	Results
Indole production	Negative
Methyl Red	Negative
VogesProskauer	Positive
\Citrate utilization	Positive
Catalase	Positive
Oxidase	Positive
TSI	Positive with H_2S Production
Urease	Negative
Carbohydrate fermentation(Maltose)	Positive
Carbohydrate fermentation(Galactose)	Positive
Carbohydrate fermentation(Arabinose)	Positive
Gelatin Hydrolysis	Negative
Starch Hydrolysis	Negative

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