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Production of Indole Acetic Acid by *Azotobacter* sp.

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Abstract

Six bacterial isolates were isolated from different rhizospheric soils. These isolates were further tested for the production of IAA in a medium with 0, 1, 2 and 5 mg/ml of tryptophan. A low amount of IAA production was recorded by Azotobacter strain without tryptophan addition. Production of IAA in Azotobacter increased with increase in tryptophan concentration from 1 to 5 mg/ml. In presence of 5 mg/ml of tryptophan, Azotobacter produced high levels of IAA. Production of IAA was further confirmed by 3 isolates of Azotobacter (Azb3, Azb5 Azb7) and subsequent TLC analysis. A specific spot from the extracted IAA preparation was found corresponding with the standard spot of IAA with the same Rf value. Azotobacter isolates (Azb3, Azb5 Azb7) showed inhibitory effects on the growth of root elongation at all concentrations of tryptophan compared to control. On the other hand, high concentration of exogenous tryptophan could exhibit toxic effects on plant growth.

Key Words: Indole acetic acid, tryptophan, *Azotobacter*

Introduction

Plant growth promoting rhizobacteria (PGPR) are soil borne bacteria, which enhance the plant growth directly or indirectly [6]. PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (auxins, gibberellins, and ethylene). Indole acetic acid is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR [1].

Bacteria from rhizospheres are likely to synthesize and release auxins as secondary metabolites because of rich supplies of substrates exuded from the roots compared with non-rhizospheric soils [3].

Plant growth regulators play a critical role in plant growth promotion. Effects of plant growth regulators including IAA on the plant will be concentration dependent. To assess this hypothesis, local isolates of pseudomonas sp. were screened for their intrinsic ability to produce IAA in presence of varying amounts of L-tryptophan and their effect on root elongation of germinating seeds of test plants.

Materials and Methods

These include isolation and biochemical characterization of isolates of Azotobacter. Rhizospheric soils of different crops were collected for the isolation of *Azotobacter.* Strains were isolated on Jensen's medium as per the standard method. Isolates of *Azotobacter* sp. were biochemically characterized for Gram reaction, H_2S production, starch hydrolysis, carbohydrate fermentation, IMVIC tests, and oxidase test as per the standard methods. All the test strains of *Azotobacter* spp. were screened for IAA Production. The test bacterial culture was inoculated in the respective medium with tryptophan (1, 2 & 5 mg/ml) or without tryptophan incubated at 28-30^{o}c temperature for 15 days and cultures were centrifuged at 3000 rpm for 30 min. 2 ml of supernatant was mixed with 4 ml of Solawaski's reagent (50ml, 35% Perchloric acid; 1ml 0.5 feCl3). Development of pink colour indicates IAA production. O.D was read at 530 nm using spectrophotometer, Systronic 106. The level of IAA produced was estimated by a standard IAA graph.

Extraction of crude IAA

Single bacterial colony of 3 isolates of *Azotobacter* spp. (Azb3, Azb5 Azb7) was inoculated in 200 ml of nutrient broth amended with 1 and 5 mg/ml of tryptophan and incubated 28-30 ^{o} c for 1 week on a shaker incubator. Bacterial cells were separated from the supernatant by centrifugation at 10,000 rpm for 30 min. the supernatant was acidified to 2.5 to 3.0 with 1N HCL and extracted twice with ethyl acetate at double the volume of the supernatant. Extracted ethyl acetate fraction was evaporated to dryness in a rotatory evaporator at 40 ^{o} c. The extract was dissolved in 300 ml of methanol and kept at -20^{o}c.

Thin layer chromatography

Ethyl acetate fractions (10-20 ml) were plated on TLC plates (silica gel G f 254,thickness 0.25 mm) and developed in benzene : n-Butanol : acetic acid (70:25:5) spots with Rf values identical authentic IAA were identified under U.V light (254nm) by spraying the plates with Ehmann's reagent.

Effect of Rhizobacteria at different concentration of Tryptophan on Root elongation

Different concentrations of tryptophan (0, 1, 2 and 5 mg/ml) were incorporated in the respective media for *Azotobacter.* One-fifth of each plate was streaked with test

bacteria and plates were incubated for 48-72 hrs at 28-30°c. Surface sterilized seeds were placed 1cm away from the bacterial growth. Ten seeds were placed in each plate. Plates were further incubated for seed germination for 96 hrs at 28-30°c. The roots of the germinated seeds were then measured.

Observation table for root length Root length in Ground nut (in mm)

Tryptophan conc. mg/ml	Control	Azb3	Azb5	Azb7
0	38.88	42.50	42.80	49.88
1	45.53	49.00	47.20	55.25
2	40.24	46.10	45.20	50.09
5	37.88	27.53	29.18	32.95

Root length in Gram seeds (in mm)

Tryptophan conc. mg/ml	Control	Azs 3	Azs 5	Azs 7
0	27.65	31.38	32.38	36.35
1	35.65	36.90	38.00	41.85
2	32.63	31.37	32.00	32.23
5	25.50	25.50	19.15	21.70

Results and discussion

A total of 9 isolates of Azotobacter were isolated from rhizospheric soils and tentatively identified on the basis of biochemical tests and sugar fermentation behaviuor as described in Bergey's manual of determinative bacteriology. These bacterial isolates were screened for their ability to produce plant growth regulator, IAA.

Varying levels of IAA production were recorded with different concentrations of tryptophan i.e….0, 1, 2 and 5mg/ml. nine isolates of *Azotobacter* were able to produce IAA without tryptophan in the range of 2.68 to 10.80 mg/ml. A further increase in IAA production was observed in the presence of tryptophan (1, 2 & 5mg/ml). These isolates varied greatly in their intrinsic ability to produce IAA. On the basis of IAA production level, culture filtrates of Azotobacter (Azb3, Azb5 Azb7) were used to extract IAA for characterization by TLC. The spots of extracts of culture & standard IAA were tested in solvent system benzene: n-butanol: acetic acid (70:25:5). Chromatograms of culture spots and standard IAA, sprayed with Ehmann's reagent showed almost same Rf values. In addition to IAA, other compounds were also detected on TLC plates, which remain to be identified. The effect of *Azotobacter* on root elongation was evaluated at different concentrations of tryptophan (1, 2 & 5 mg/ml) & without tryptophan.

IAA production by test isolates (mg/ml)

Treatments of Tryptophan (mg/ml)	Azb3	Azb 5	Azb 7
0	7.40	10.80	4.40
1	11.53	14.36	7.25
2	19.8	24.80	23.8
5	27.6	32.80	28.9

The root elongation of germinating seeds of groundnut and chana at 1and 2 mg/ml concentration of tryptophan was highest. At 5 mg/ml tryptophan concentration, in both groundnut & Gram, root elongation was decreased which indicated that tryptophan of 5 mg/ml concentration is toxic in the presence of test bacteria. However at a higher concentration of tryptophan, the production of IAA is higher which might exert an adverse effect on plant growth. The findings of the present investigation highlighted that IAA producing bacteria from local soil could be easily isolated & may be exploited after strain improvement for local use. However, further studies using IAA mutant strains of these isolates are needed to explore the exact contribution of IAA production in the promotion of plant growth as well as the contribution of other PGP traits. There are numerous soil microflora involved in the synthesis of Auxins in the pure culture and soil.

Some microorganisms produce Auxins in the presence of a suitable precursor such as L-Tryptophan. They effects of auxins on plant seedlings are concentration dependent i.e….low concentration may stimulate growth while high concentrations may be inhibitory. Different plant seedlings respond differently to variable auxin concentrations and type of microorganisms.

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