PRODUCTION, PURIFICATION AND APPLICATION OF PLANT GROWTH HORMONES (IAA AND GIBBERELLIC ACID) BY BACILLUS SUBTILIS

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Abstract

To isolate bacterial strains from chickpea rhizospheric soil and nodules, to characterize and identify bacterial strains by using 16s rRNA gene sequencing. Plant growth promoting rhizobacteria are being preferred nowadays as inoculants for influencing crop via multiple direct or indirect mechanisms but screening to find out the effective PGPR strain is one of the crucial steps. This research is aimed at keeping in view their potential for phosphate solubilisation, indole acetic acid, thin layer chromatography.

Keywords: IAA, GA3, PGPR, FTIR, indole acetic acid, thin layer chromatography.

I. INTRODUCTION

Plant growth promoting bacillus licheniformis (PGPB) are beneficial bacteria which have the ability to colonize the roots and either promote plant growth through direct action or via biological control of plant diseases (Kloepfer and Schroth 1978). They are associated with many plant species and are commonly present in varied environments. Strains with PGPB activity, belonging to genera Bacillus licheniformis, have been reported (Hurek and Reinhold-Hurek 2003). The species of Bacillus are the most extensively studied. These bacteria competitively colonize the roots of plant and can act as biofertilizers and/or antagonists (biopesticides) or simultaneously both.

Gibberellic acid (GA3) is an important member of the gibberellins family and acts as a natural plant growth hormone, controlling many development processes, which is gaining great attention all over the world due to its effective use in agriculture, nurseries, tissue culture, tea gardens, etc. (Davies, 2004; Shukla et al., 2005). Reduction in its production costs could lead to wider applications for a variety of crops (Kumar and Lonsane, 1989; Tudzynski, 1999; Linnemannstons et al., 2002). Industrially GA3 is produced by submerged fermentation (SmF) using the ascomycetous fungus Gibberrella fujikuroi, renamed Fusarium fujikuroi (O’Donnell et al., 1998). The cost of GA3 production using SmF is very high, mainly due to extremely low yield and expensive downstream processing. As plant roots grow through soil they release water-soluble compounds such as amino acids, sugars and organic acids that supply food for the microorganisms. In return, the microorganisms provide nutrients for the plants. All this activity makes the rhizosphere the most dynamic environment in soil.

Rhizosphere microorganisms produce vitamins, antibiotics, plant hormones solubilization of mineral nutrients, synthesis of vitamins, amino acids, auxins, cytokinins and gibberellins which stimulate plant growth and antagonism with potential pathogen of communication molecules that all encourage plant growth. The action and interaction of some growth regulators like auxins regulate most of the physiological activities and growth in plants. Chemically it is Indole acetic acid the ability to synthesize phytohormone is widely distributed among plant associated bacteria. 80% of the bacteria isolated from plant rhizosphere are to produce IAA. (Zakharova Zakharova et al., 1999)

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According to Halda-Alija (Halda-Alija 2003), up to 74% of rhizobacteria identified and tested to produce IAA. The associative nitrogen-fixing bacteria tested produced IAA, especially with tryptophan as a precursor (Pedraza et al., 2004).

The rapid growing population has eventually raised the demand of food production. The researchers emphasized the need to optimized for alternative yield raising techniques without affecting the soil health and environmental quality (Kantar, Hafeez et al.-2007). Bacteria, with a vital role for plant growth known as plant growth promoting rhizobacteria (PGPR) make possible the improved plant growth by colonizing the plant roots. Seeds inoculated with rhizobia, PGPR and PSB (phosphorus solubilization bacteria) have been found out to enhance crop growth and productivity (Dashit, Zhang et al., 1998 and Rudresh DL, Shivaparakash MK et al., 2005).

Siderophores produced by some PGPR scavenge heavy metal micronutrients in the rhizosphere (e.g. iron) starving pathogenic organisms of proper nutrition to mount an attack of the crop. Antibiotic producing PGPR releases compounds that prevent the growth of the pathogens. Bioprotectants are currently being studied by the laboratories of Fernando and Daayf in the Department of Plant Science, University of Manitoba. Biofertilizers-nitrogen fixing bacteria are also available for increasing crop nutrient uptake of nitrogen from nitrogen fixing bacteria associated with roots (Azospirillum). Nitrogen fixing biofertilizers provide only a modest increase in crop nitrogen uptake (at best an increase of 20 Kg N acre-1).

Gibberellic acid is synthesized by Gibberella fujikuroi, Sphaceloma manihoticola, Neurospora crassa, Aspergillus niger, Sphaceloma sp., Rhizobium phaseoli, Azospirillum brasilense, Pseudomonas sp. and Phaeosphaeria sp., whereas IAA is synthesized by Pseudomonas sp., Bacillus sp., Aspergillus niger, Rhizopus, Acetobacter diazotrophicus, Herbaspirillum seropedicae, Rhizobium sp., Azospirillum sp., and Erwinta sp.

Physiological evidence for different Trp-dependent pathways for synthesis in Azospirillum brasilense has been reported (Carreno-Lopez et al., 2000). In Trp dependant pathway, tryptophan is converted to indole-3-acetamide (IAM) by tryptophan-2-monoxygenase and IAM is metabolized to IAA by IAM-hydrolase (Matsukawa et al., 2007).

The physiologically most active auxin in plants is Indole acetic acid (IAA) which is known to stimulate rapid cell elongation.
Selective 4 rhizobial cultures were inoculated in YEMB amended with 5mM tryptophan. 1% inoculum of O.D. 600 1.0 was used for inoculation. The inoculated broth was incubated at 28°C for 24 hrs. After 24 hrs of incubation, broth was centrifuged at 7000 rpm for 10 minutes. pH of broth brought to 3.0. 4:1 aliquots of liquid portion of centrifuged sample were extracted three times with ethyl acetate. The organic phase was concentrated to dryness and then diluted with 0.5 ml methanol. This solution along with the standard IAA was applied on silica gel G plate and TLC was run by using a solvent system chloroform:Ethyl acetate/Formic acid in 5:3:2 proportion and developed by using Salkowski reagent. Red colour spots were developed. Rf value of the standard and IAA produced by the selected isolates was calculated.

2.4. Effect of Carbon sources on IAA production
IAA production was studied by replacing mannitol from YEB by glucose, sucrose, lactose, arabinose, xylose and mannitol 1% w/v. supplemented with 2.5 mg/ml of tryptophan. IAA production was studied by using Salkowski reagent after 24, 48 and 72 hrs. Cultures selected for the study are RG and RH i.e. Bacillus licheniformis isolated from groundnut and chickpea respectively.

2.5. Effect of pH on IAA production
To study the extent of IAA produced by the different isolates at different pH, YEMB with 2.5 mg/ml of tryptophan is adjusted to different pH as 5, 6, 7, 8 and 9. Media were inoculated with 1% inoculum of O.D. 600 and incubated at 28°C for 24 hrs. IAA production was studied by using Salkowski reagent after 24hrs.

2.6. Characterization of IAA production
To determine the amounts of IAA produced by each isolate, a colorimetric technique was performed with Van Urk Salkowski reagent using the Salkowski’s method (Ehmann, 1977). The isolates were grown in yeast malt dextrose broth (YMD broth) (Himedia, India) and incubated at 28°C for 4 days. The broth was centrifuged after incubation. Supernatant was reserved and 1ml was mixed with 2ml of Salkowski’s reagent (2% 0.5 FeCl in 35% HClO solution) and kept in the dark. The optical density (OD) was recorded at 530 nm after 30 min and 120 min.

IAA production was compared in YMD and LB media. YMD medium was compared with and without tryptophan.
2.9. FTIR

Infrared spectrum of the purified compound was obtained using Fourier Transform Infrared (FTIR) spectrophotometer (Shimadzu FTIR 8400) and the spectra was recorded in the nujol mull using KBr cells and expressed in wave number (cm$^{-1}$).

2.10. Method of seed treatments:

Bio control agents were grown in respective medium on rotating shaker (150rpm) for 2 days and centrifuged at 10,000 rpm for 5min the pellet was mixed with sterile carboxyl methyl cellulose (CMC) suspension. *Oryza sativa* surface sterilized with sodium hypochlorite solution were placed in CMC cell suspension and air dried inside laminar air flow chamber (jagadeesh, 2000).The bio coated seeds were transplanted into the field.

2.11. Shoot length and root length (cm)

Three plants were randomly selected for recording the root length and shoot length of *oryza sativa* plants. They were measured by using centimeter scale.

2.12. Fresh and dry weight (mg/g dry wt.)

Three plant samples were randomly selected at experimental pot. They were separated into root and shoot. Their fresh weight was taken by using an electrical single pan balance. The fresh plant materials were kept in a hot air oven at 80°C for 24 hr and then their dry weight were also determined.

3. RESULT & DISCUSSION

Figure 1. Paper Chromatography was measured in sample movements. IAA standard = 0.63; Sample 1 = 0.22; Sample 2 = 0.55

Figure 2. IAA colour changed for red colour formed. This colour changed in present for chlorophyll. The gibberellic acid standard value = 0.129

Figure 3. The IAA spray plant in promoting the hormones good condition for present in chlorophyll.

4. DISCUSSION

PGPR mediated phosphate solubilisation through biosynthesis of organic acids that create acidification conditions in the media. PGPPR solubilised more inorganic phosphate through gene modification followed by their expression in specific bacterial strain. Phytase genes have been cloned from number of PGPR. In our study significance drop in pH of broth culture IAA which was observed comparatively low without tryptophan.

5. SUMMARY

Modern biotechnology has resulted in a resurgence of interest in the production of new therapeutic agents using botanical sources. With nearly 500 biotechnology products approved or in development globally and with production capacity limited, the need for efficient means of therapeutic protein production is apparent. Through genetic engineering, plants can now be used to produce pharmacologically active proteins, including mammalian antibodies, blood product substitutes, vac-cines, hormones, cytokines and a variety of other therapeutic agents. Efficient biopharmaceutical

Growth hormone production in plants involves the proper selection of host plant and gene expression system, including a decision as to whether a food crop or a non-food crop is more appropriate. Product safety issues relevant to patients, pharmaceutical workers, and the general public must be addressed, and proper regulation and regulatory oversight must be in place prior to commercial plant-based biopharmaceutical production. Plant production of pharmaceuticals holds great potential, and may become an important production system for a variety of new biopharmaceutical products.

5. REFERANCE


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