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Sustainable Carbon Source Utilization for Indole-3-Acetic Acid Production by Marine *Pseudomonas fluorescens* BCPBMS-1 Using the One-Factor-at-a-Time Approach

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ABSTRACT

Background and Objective: Indole-3 acetic acid (IAA) is a phytohormone that stimulates plant growth and facilitates plant development. Indole-3-acetic acid is a widely occurring endogenous plant hormone that has been shown to enhance root development and morphology. The current study involved the extraction of IAA from marine *Pseudomonas fluorescens* BCPBMS-1. **Materials and Methods:** Indole acetic acid (IAA) production in *P. fluorescens* was screened using Luria broth with L-tryptophan and detected calorimetrically with the Salkowski reagent. Optimization involved testing various factors, including temperature, pH, salinity, incubation time and different carbon and nitrogen sources. The IAA concentration was measured spectrophotometrically at 530 nm using a standard IAA curve. **Results:** The optimized conditions for maximum IAA production are 30°C, pH 6, 10 ppt salinity and 72 hrs of incubation. Glucose and yeast extract were the most effective carbon and nitrogen sources, respectively, while soybean husk was the best low-cost alternative. Mass-scale synthesis under these conditions yielded 0.72 µg/mL IAA using cost-effective substrates and precise parameters. **Conclusion:** Microbes' ability to produce indole-3-acetic acid (IAA) offers an economical replacement for expensive and unreliable chemical IAA synthesis methods. According to this study, soybean husk represents a more economical carbon source for the production of IAA.

KEYWORDS

IAA, time culture parameters, carbon source, marine *Pseudomonas fluorescens*, soybean husk

INTRODUCTION

Over the past few decades, there has been a consistent increase in the world population and the subsequent need for food. As a result, there is a pressing need for advancements in agricultural production. Indole-3 acetic acid (IAA) is a plant hormone that is essential for the growth and development of plants. Indole-3-acetic acid (IAA) is the predominant natural auxin in plants and is known to enhance root growth and shape. This is thought to improve nutrient uptake from the soil[1]. Numerous researchers have paid close attention to the role of IAA in the intricate interplay between the microflora in the

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rhizosphere and the host plant, which is based on a continuous exchange of substances and signals[2-5]. The synthesis of elevated quantities of IAA by rhizosphere bacteria has been proven to inhibit rather than promote root growth[6,7]. This is in contrast to the production of IAA by microorganisms commonly found in the rhizosphere of plants, such as *Pseudomonas* spp. and *Rhizobium* spp. which are frequently linked to their potential to stimulate plant growth[8-11].

Plants and soil microbes in the rhizosphere can interact in ways that are either positive, neutral, variable, or detrimental to plant growth. According to Cartwright *et al.*[12], these organisms' beneficial activities include fixing nitrogen, producing siderophores, chitinase, antibiotics and dissolving phosphate and other nutrients, as well as producing or altering the concentrations of plant hormones such as IAA, gibberellic acid, cytokinin and ethylene. Gram-negative, aerobic, polarly flagellated rods are the organisms that belong to the *Pseudomonas* genus. They are known to grow on basic medium and are aggressive invaders of the rhizosphere of a variety of crop plants[13,14].

Marine *Pseudomonas* spp. is a member of the microorganism community in marine extreme environments[15] and represents untapped novel bioactive substances[16]. Marine isolates of *Pseudomonas* spp. are found in diverse ecosystems, including coastal regions, the deep sea and more extreme environments[17].

Few studies have been undertaken on optimizing marine microbial IAA production, leaving a knowledge gap. Therefore, it is essential to optimize the production medium for IAA from marine *Pseudomonas fluorescens* BCPBMS-1. This technique is the most straightforward to use and greatly aids in the identification of the essential variables that influence IAA production. This study investigated the sustainable utilization of carbon sources for the production of indole-3-acetic acid (IAA) by marine *Pseudomonas fluorescens* BCPBMS-1 using the one-factor-at-a-time approach.

MATERIALS AND METHODS

Study area: Sponges have been collected from the Gulf of Mannar, India's Southeast Coast (Latitude 9°5'N, Longitude 79°5'E). Shortly after collection, the sponge sample was transferred to a sterile plastic bag and transported frozen to the laboratory for the isolation of bacteria. The samples were collected and study performed in April, 2010.

Screening for indole acetic acid (IAA) production: Screening was done by using Luria broth (HI-Media) supplemented with L-tryptophan. A loop full of *P. fluorescens* was inoculated in 10 mL of Luria broth supplemented with 0.1% of L-tryptophan and incubated for 72 hrs at 30°C. Then, the culture was centrifuged at 10,000Xg for 10 min. and the supernatant was collected. The 1 mL of supernatant was allowed to react with 2 mL of Salkowski reagent (1 mL of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$) at 30°C for 30 min. Pink color development indicated the presence of IAA[4].

Medium optimization in shake-flask cultivation

Experimental design for optimizing IAA production using low-cost substrates by one factor at a time method: The effects of various factors on the production of IAA were tested individually. These factors included temperature (ranging from 20-35°C with a 5°C interval), pH (ranging from 5-8 with a 1-unit interval), salinity (ranging from 2-12 ppt with a 2 ppt interval), the incubation period (ranging from 24-96 hrs with a 24 hrs interval) and different carbon and nitrogen sources. The carbon sources tested were 3% glucose, sucrose and fructose, while the nitrogen sources tested were yeast extract, peptone and beef extract at a concentration of 0.5%. Additionally, economically cheaper sources such as soybean husk, wheat bran and molasses were tested at a concentration of 3%. The experiments were conducted under shaken conditions at 150 rpm.

Estimation of IAA: The *P. fluorescens* culture was centrifuged at 13000Xg for 10 min and the supernatant was collected. The presence of IAA was measured in a spectrophotometer by adding 2 mL of Salkowski reagent to 1 mL of supernatant and incubating for 30 min. The optical density was read at 530 nm. The recorded OD values were plotted in a standard curve prepared from commercially available IAA and their concentration was calculated[18].

RESULTS AND DISCUSSION

Screening for indole acetic acid (IAA) production: The initial development of roots, either through the elongation of primary roots or the multiplication of lateral and adventitious roots, is beneficial for young seedlings. This enables them to firmly anchor themselves to the soil and acquire water and nutrients from their surroundings, thereby improving their chances of survival. Root colonization by bacteria known as "plant growth promoting" bacteria (PGPR) can boost plant growth[19-22]. Microbial synthesis of the plant-growth regulator IAA[23,24] can result in to improved plant growth.

In the present study, *P. fluorescens* which was isolated from the *Callyspongia diffusa* produced indole acetic acid. It was screened by using Luria broth supplemented with L-tryptophan and the addition of the Salkowski reagent. A positive result was indicated by pink color. Bhadbhade *et al.*[11] reported that a higher level of IAA production was produced by *Pseudomonas* spp. Shokri and Emtiazi[25] also reported that indole acetic acid was produced higher level in *Pseudomonas* spp., compared to *Azotobacter* spp.

Optimization by one factor at a time: The results showed that the highest IAA production of 0.39 µg/mL occurred at a temperature of 30°C, indicating that this temperature was the most beneficial for IAA production (Fig. 1). According to Shokri and Emtiazi[25], the ideal temperature for producing IAA was found to be 30°C. A study conducted by Aldesuquy *et al.*[26] revealed that temperatures between 25-30°C were optimal for both the development and synthesis of indole-3-acetic acid (IAA) in *Streptomyces* spp. According to Apine and Jadhav[27], a temperature of 30°C was found to be optimal for the synthesis of IAA by the *Pantoea agglomerans* strain PVM.

In this study, different pH levels were used to see how they impacted the production of IAA. At pH 6, the most was made (0.4 µg/mL) and at pH 8, the least was made (0.12 µg/mL) (Fig. 2). According to Huddedar *et al.*[28] *Acinetobacter baumannii* A16 and *Acinetobacter* A18 strains can make the most IAA when the pH is 7.0. A study by Aldesuquy *et al.*[26] observed that highest amount of IAA was produced when the pH was 7.0. Mandal *et al.*[29] reported that the highest level of IAA production came at pH 7.2. According to Yurekli *et al.*[30] the production of IAA is suited to slightly acidic conditions with a pH of 7.5 or higher.

In the present observation, 10 ppt salinity showed the maximum production of 0.41 µg mL and at 2 ppt salinity only 0.26 µg/mL IAA was produced (Fig. 3). Ravikumar *et al.*[31] reported that *A. brasiliense* isolated from mangrove roots produced the highest level of IAA at a concentration of 1% NaCl. According to Sachdev *et al.*[32], the optimal NaCl concentration for IAA production in *Klebsiella* spp., is 0.5 w/v.

Regarding incubation time, maximum production was observed at 72 hrs and the minimum was observed at 24 hrs (Fig. 1-3). Castillo *et al.*[33] observed IAA accumulation in *Azospirillum* and *Arthrobacter* spp., during the stationary phase. The current investigation supported this finding as well. Chung and Tzeng[34] found that fungi *U. maydis* produced IAA after 3 days of incubation.

Shokri and Emtiazi[25] similarly found that 72 hrs was the best incubation time for IAA production. Lin and Xu[35] observed the same thing in *Streptomyces* spp. The decrease in IAA synthesis after 72 hrs could be attributed to the release of IAA-degrading enzymes such as IAA oxidase and peroxidase, as previously observed in *Rhizobium* spp., isolated from *Cajanus cajan*[36,37].

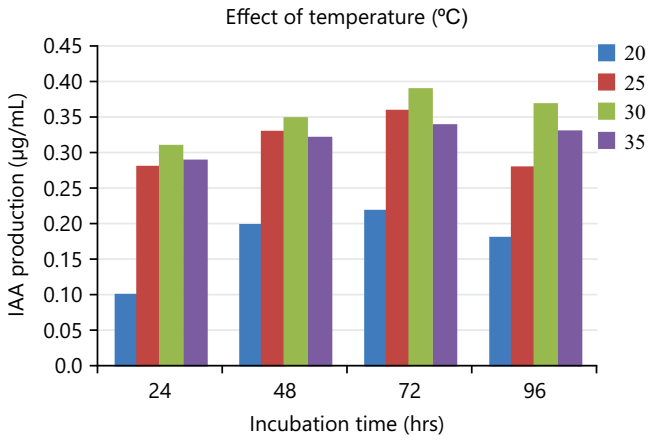

Fig. 1: IAA production at different temperatures

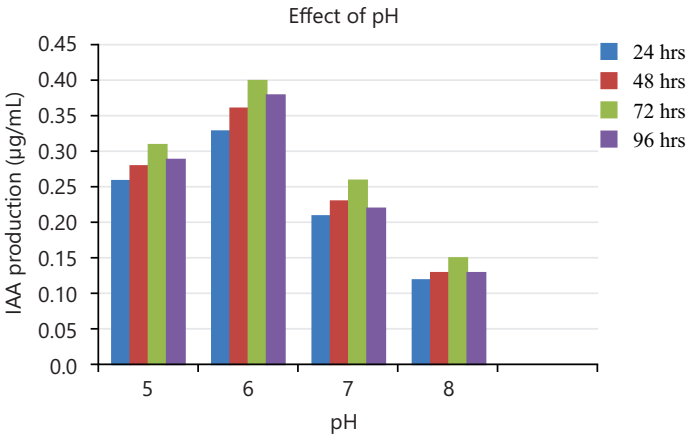

Fig. 2: IAA production at different pH

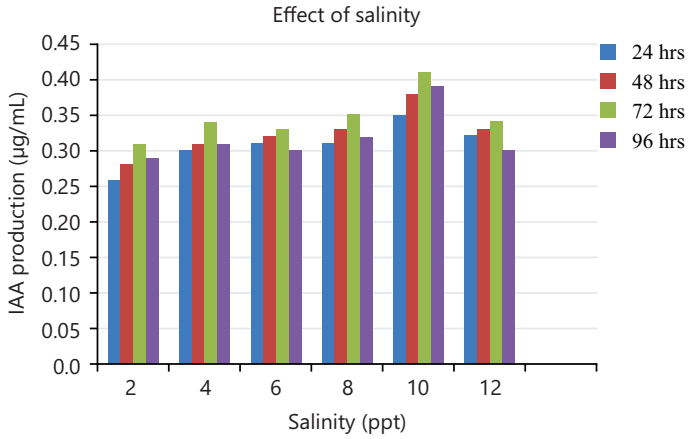

Fig. 3: IAA production at different salinity

Glucose exhibited the highest production (0.42 µg/mL) among the carbon sources utilized, as seen in Fig. 4. The highest production of IAA was reported while using a 3% glucose concentration, as determined using a one-factor-at-a-time approach in the current study. The high IAA synthesis in the glucose-containing medium may be attributed to the more efficient use of this easily utilizable glucose in comparison to other sources[38]. *Rhizobium* spp. derived from *Cajanus caja*n similarly exhibited the highest production of indole-3-acetic acid (IAA) in a medium containing glucose, as previously documented by Datta and Basu[36]. In their study, Narayana *et al.*[38] found that the highest generation of IAA occurred when 1% glucose was employed as the carbon source, using a one-factor-at-a-time method.

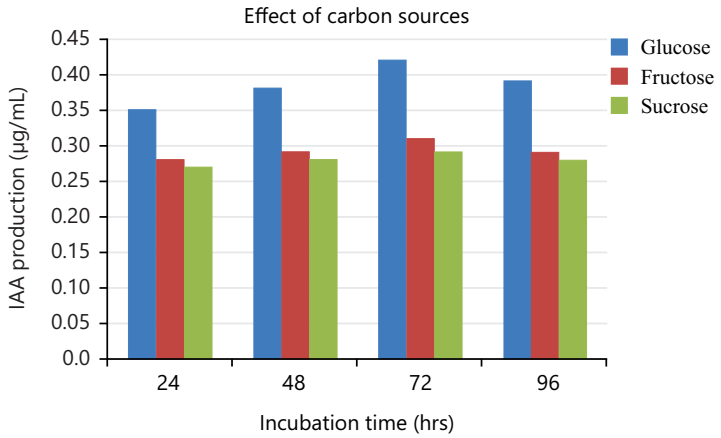

Fig. 4: IAA production using various carbon sources

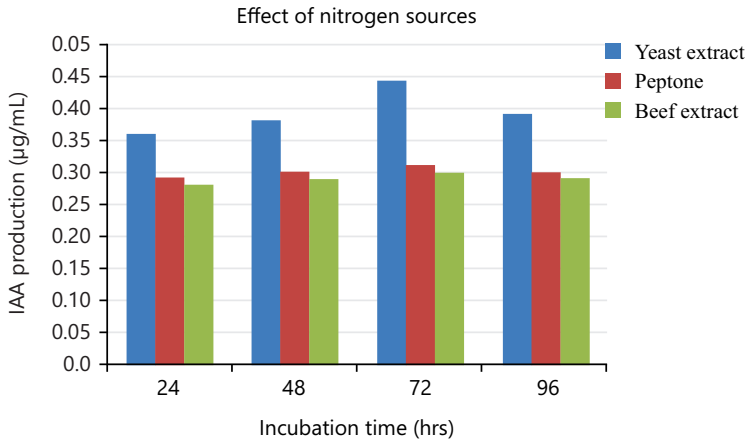

Fig. 5: IAA production using various nitrogen sources

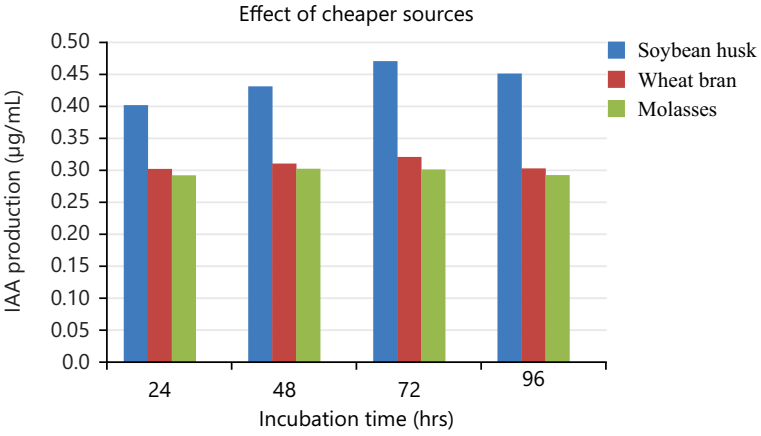

Fig. 6: IAA production using cheaper sources

Among the nitrogen sources tested, yeast extract showed the maximum production of IAA (0.47 µg/mL) and minimum production was observed with beef extract (0.28 µg/mL) (Fig. 5). Narayana *et al.*[38] also observed the highest IAA production from *Streptomyces albidoflavus* in the medium containing yeast extract as a nitrogen source.

Out of the cheaper sources that were evaluated, the highest level of production was reported with soybean husk, which had a concentration of 0.48 µg/mL. The lowest concentration was recorded with molasses at 0.29 µg/mL (Fig. 6). In this investigation, it was determined that soybean husk is the most

cost-effective source. Debebe *et al*.[39] employed soybean supplement to stimulate the production of indole-3-acetic acid (IAA) by *Pseudomonas* spp., Rubio *et al*.[40] found that *Azotobacter* spp., *Enterobacter* spp. and *Pseudomonas* spp. exhibited a preference for soy flour as the substrate for indole-3-acetic acid (IAA) production.

Mass production of IAA: A concentration of 0.72 µg/mL was achieved during large-scale production using the optimized physical as well as chemical parameters and a most cost-effective carbon source (soya bean husk). Nenwani *et al*.[41] documented that the concentration of indole-3-acetic acid (IAA) produced was measured to be 11.45 µg/mL. According to Khakipour *et al*.[42] the *P. fluorescens* strains produced indole-3-acetic acid (IAA) in quantities ranging from 0 to 31.6 mg/L, while *P. putida* produced IAA in quantities ranging from 0 to 24.08 mg/L. According to Leinhos and Vacek[43] *P. fluorescens* produced 1.6-3.3 mg/L of IAA, but Prikryl *et al*.[44] detected auxin production ranging from 0.01-3.93 mg/L. Wu *et al*.[45] reported the observation of a significant generation of indole-3-acetic acid (IAA) by genetically modified *E.coli,* reaching a high concentration of 7 g/L. Nevertheless, to sustain a high level of IAA synthesis, this genetically modified bacterium relied on the presence of an inducer and selective pressure. The technique may not be suited for large-scale industrial production of IAA due to the high expense necessary to maintain stability in bacterial IAA production.

CONCLUSION

This study improved IAA production by marine *Pseudomonas fluorescens* BCPBMS-1 using a one-factor-at-a-time method to investigate formulations that are most suited to economically viable carbon sources for commercial production of IAA. A suitable medium for better IAA production was successfully established and IAA production was increased by adjusting the culture parameters using one factor at a time method. The findings suggest that the most cost-effective carbon source for IAA production is soya bean husk.

SIGNIFICANCE STATEMENT

This study highlights the potential of *Pseudomonas fluorescens* BCPBMS-1 for cost-effective, large-scale production of indole-3-acetic acid (IAA), providing a sustainable alternative to chemical synthesis and conventional microbial processes. The optimization of production parameters establishes a scalable bioprocess framework, promoting environmentally friendly solutions to reduce dependence on harmful agricultural chemicals and enhance sustainable agricultural practices.

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