International Journal of Science, Strategic Management and Technology
Volume **02** Issue **03** March-**2026** | ISSN: **3108-1762 (Online)** | Impact Factor: 3.8
An International, Peer-Reviewed, Open Access Scholarly Journal Indexed in recognized academic databases

Optimization and Molecular Characterization of Indole Acetic Acid Production by Streptomyces Spp. Isolated from Rhizospheric Soil

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https://doi.org/10.55041/ijsmt.v2i3.150

Cite this Article: Singh, D. A. (2026). Optimization and Molecular Characterization of Indole Acetic Acid Production by Streptomyces Spp. Isolated from Rhizospheric Soil. International Journal of Science, Strategic Management and Technology, *02*(03). https://doi.org/10.55041/ijsmt.v2i3.150

Abstract—

Indole-3-acetic acid (IAA) is a vital plant hormone that significantly influences plant growth and development. The present investigation was designed to isolate Streptomyces species from rhizospheric soil and evaluate their ability to synthesize IAA. Selected isolates were screened using the Salkowski colorimetric method. Further, different cultural conditions such as pH, temperature, and L-tryptophan concentration were optimized to enhance IAA production. The most efficient isolate was subjected to molecular identification through 16S rRNA gene sequencing. The findings indicated that optimized conditions resulted in a marked increase in IAA yield. Molecular analysis confirmed the identity of the isolate as a member of Streptomyces. The study suggests the potential application of these strains in sustainable agricultural practices.

Keywords—Streptomyces; IAA; Rhizospheric soil; Optimization; Plant growth promotion

I. Introduction

Microorganisms present in soil ecosystems contribute significantly to plant development by producing growth-promoting substances. Among these, Indole-3-acetic acid (IAA) is one of the most important auxins, which regulates processes such as cell elongation, differentiation, and root formation [1].

The genus Streptomyces is widely distributed in soil and is recognized for its metabolic diversity, including the production of phytohormones and bioactive compounds [2]. The rhizosphere region, being rich in nutrients and root exudates, supports the growth of such beneficial microorganisms.

Environmental and nutritional factors play a crucial role in regulating microbial IAA synthesis. Parameters like pH, temperature, and precursor molecules such as tryptophan directly influence the metabolic pathways involved in IAA production [3]. In addition, molecular identification using 16S rRNA gene sequencing has become a reliable method for bacterial classification [4].

This study aims to isolate IAA-producing Streptomyces spp., optimize the production conditions, and identify the potent strain using molecular techniques.

II. LITERATURE REVIEW

The rhizosphere hosts diverse microbial communities that interact with plant roots and contribute to nutrient cycling and plant health. Streptomyces species, in particular, are well-documented producers of secondary metabolites and plant growth-promoting substances including IAA [2,6]. Previous studies have documented the role of tryptophan as a key precursor in IAA biosynthesis pathways, including the indole-3-pyruvate and tryptamine pathways [3].

Optimization of cultural conditions has been shown to significantly enhance IAA production in various bacterial species. Earlier research reported that pH 7 and moderate temperatures around 28°C are favorable for biosynthesis [1,3]. The colorimetric Salkowski method remains a widely adopted screening tool for IAA detection due to its simplicity and reliability [5]. Molecular identification using 16S rRNA gene sequencing provides high accuracy in classifying bacterial isolates to species level [4,7].

III. MATERIALS AND METHODS

3.1 Collection of Soil Samples

Rhizospheric soil samples were collected from cultivated agricultural land at a depth ranging from 5 to 10 cm using sterile tools.

3.2 Isolation of Streptomyces

Serial dilution technique was employed, and diluted samples were spread on starch casein agar plates. The plates were incubated at 28°C for about one week. Colonies exhibiting typical powdery or chalk-like morphology were selected for further study.

3.3 Screening for IAA Production

Selected isolates were cultured in nutrient broth supplemented with L-tryptophan. After incubation, Salkowski reagent was added, and the development of a pink to reddish coloration indicated IAA production. Quantitative estimation was carried out spectrophotometrically at 530 nm.

3.4 Optimization of Cultural Conditions

To enhance IAA production, the following parameters were tested individually while keeping others constant:

- pH range: 5 to 9
- Temperature: 25°C to 37°C
- L-tryptophan concentration: 0.1% to 1%

3.5 Molecular Identification

Genomic DNA was extracted from the selected high-yielding isolate. The 16S rRNA gene was amplified using universal primers through PCR. The obtained sequence was analyzed using BLAST to determine its similarity with known species.

IV. RESULTS AND DISCUSSION

4.1 Isolation and Screening

A number of Streptomyces isolates were successfully obtained from rhizospheric soil samples. Among them, a few isolates showed the ability to produce IAA, as confirmed by colorimetric analysis. One isolate (designated S-3) demonstrated comparatively higher production.

4.2 Effect of Optimization Parameters

The production of IAA was significantly influenced by cultural conditions. Maximum yield was observed under the following conditions:

Table I: Optimized Cultural Conditions for Maximum IAA Production

Parameter	Optimal Value
pH	7
Temperature	28°C
Tryptophan concentration	0.5%

These findings indicate that neutral pH and moderate temperature favor enzymatic activities associated with IAA biosynthesis. Increased availability of tryptophan enhanced precursor supply, thereby boosting hormone production. Similar observations have been reported in earlier studies [3].

4.3 Molecular Characterization

Sequence analysis of the 16S rRNA gene revealed that the selected isolate showed a high degree of similarity with Streptomyces griseus. This confirms its taxonomic position and supports its potential role in plant growth promotion.

V. CONCLUSION

The present investigation highlights the successful isolation of IAA-producing Streptomyces from rhizospheric soil. Optimization of environmental conditions led to a significant improvement in IAA production. Molecular identification confirmed the strain as a member of Streptomyces. These results emphasize the applicability of such microorganisms as biofertilizers, contributing to eco-friendly and sustainable agriculture.

ACKNOWLEDGMENT

."I sincerely thank my brother Vivek Kumar Singh for his constant support, encouragement, and motivation throughout the completion of this research work."

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