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Original Article

MICROBIAL CAPACITY FOR AUXIN BIOSYNTHESIS: A COMPARATIVE EXPLORATION

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ABSTRACT

This study investigates the microbial capacity for auxin biosynthesis, focusing on five agriculturally significant species: Bacillus, Azotobacter, Pseudomonas, Azospirillum, and Rhizobium. Auxin, specifically indole-3-acetic acid (IAA), is a vital plant hormone that regulates various growth processes such as root development and cell elongation. Understanding microbial IAA production is critical for developing sustainable agricultural practices, particularly in the formulation of biofertilizers and microbial inoculants. The research employed both qualitative and quantitative methods to assess the IAA production capacity of these microbes. Qualitative analysis using the Salkowski reagent confirmed the presence of IAA in all strains. Quantitative measurements revealed significant variation in IAA production. Azotobacter exhibited the highest IAA levels at 38.7 µg/mL, followed by Bacillus (31.4 µg/mL), Rhizobium (27.2 µg/mL), Pseudomonas (24.6 µg/mL), and Azospirillum (21.9 µg/mL). These results demonstrate the strain-specific nature of auxin biosynthesis, with certain microbial species exhibiting higher auxin production than others. Furthermore, the production of IAA was found to be closely linked to the availability of tryptophan, a key precursor in the biosynthesis pathway. The results emphasize that the optimization of precursor levels in culture media could enhance IAA production, making microbial strains more efficient for use in agricultural applications. The study suggests that Azotobacter, with its superior auxin production capacity, is a promising candidate for bioinoculant development. These findings contribute valuable insights into the role of plant-associated microbes in hormone synthesis and their potential applications in improving crop growth, root development, and nutrient uptake. Further research, including field trials and genomic analysis, is needed to fully understand the potential of these microbes for sustainable agricultural practices.

INTRODUCTION

Auxins, a category of plant hormones, intricately regulate plant development. Microorganisms residing in the rhizospheres of diverse floras are likely to synthesize and discharge auxins as ancillary metabolites, given the abundant substrates oozed from roots in comparison to non-rhizospheric soils [1]. Various soil microorganisms, encompassing bacteria, fungi, and algae, exhibit the capability to produce physiologically active quantities of auxins, potentially influencing essential facets of plant progression and establishment [2]. The primary auxin in plants, Indole-3-acetic acid (IAA), governs critical functional processes, comprising cell expansion, division, tissue distinction, and rejoinders to light and gravity [3].

Microbial isolates from different crops demonstrate an elevated prospective to produce and discharge IAA as ancillary metabolites, leveraging the reasonably abundant substrate amount [4]. The production of IAA by microbial isolates displays significant variation amongst diverse species and strains, contingent upon substrate availability. Convincing evidence supports that IAA, gibberellins, and cytokinins, essential for plant progression and development, are not only produced by plants but also by various bacteria associated with plants [5,6,7]. Bacterial IAA producers (BIPs) possess the potential to impact crucial plant processes by contributing IAA to the plant's auxin pool [8].

While other auxins like indole-3-butyric acid (IBA) and phenylacetic acid (PAA) have been identified in plants 9, their physiological functions remain largely unknown. The presumed role of Plant Growth- Promoting Bacteria (PGPB) generating plant growth regulators is vital in promoting plant growth. Consequently, this study was assumed to evaluate the auxin biosynthesis potential of different isolates, shedding light on the intricate interactions between microorganisms and plants for potential applications in agriculture.

MATERIALS AND METHODS

1. Microbial Strains:

Five microbial strains of different species were cultured

- i. Bacillus sp.
- ii. Azotobacter sp.
- iii. Pseudomonas sp.
- iv. Azospirillum sp.
- v. Rhizobium sp.

2. Culture Media Preparation:

- vi. Nutrient Agar (for Bacillus)
- vii. Ashby's Mannitol Agar (for Azotobacter)
- viii. King's B Agar (for Pseudomonas)
- ix. Nitrogen-Free Malate Agar (for Azospirillum)
- x. Yeast Extract Mannitol Agar (for Rhizobium)

3. Inoculum Preparation:

Each microbial strain was inoculated into respective agar media and incubated at the optimal temperature for 24-48 hours until well- established colonies were obtained.

4. Auxin Production Assay:

A modified Salkowski reagent was used for qualitative estimation of indole-3-acetic acid

(IAA) production. Bacterial isolates were streaked onto agar plates, and wells were created using a sterile cork borer. The strains were incubated, and after suitable growth, the plates were flooded with Salkowski reagent. Appearance of a pink color indicated IAA production.

5. Quantitative Estimation of IAA:

Liquid cultures of each strain were inoculated in respective broth media. After incubation, cultures were centrifuged, and supernatants were variegated with the Salkowski reagent. The absorbance of the resulting solution was calculated at 530 nm, and IAA concentrations were measured using a standard curve.

6. Statistical Analysis:

All experiments were executed in triplicate, and data were imperiled to statistical analysis consuming appropriate software. Mean values and standard deviations were calculated for each parameter.

RESULTS AND DISCUSSION

All microbial strains, including Bacillus sp, Azotobacter sp, Pseudomonas sp, Azospirillum sp, and Rhizobium sp, exhibited the ability to produce indole-3-acetic acid (IAA) as evidenced by the development of a pink color in the Salkowski reagent assay.

The quantitative analysis revealed significant differences in auxin production among the tested strains. Azotobacter sp demonstrated the highest IAA production, followed by Rhizobium, Pseudomonas sp and Azospirillum sp. Bacillus sp and Rhizobium sp exhibited moderate IAA production, showing distinct variations in their biosynthetic potential (Table 1).

Table 1: Auxin biosynthesis potential of bacillus, azotobacter, pseudomonas, azospirillum and rhizobium species

MICROBES	Auxin Biosynthesis Potential (µg mL^{-1})		
	ISOLATES		
	1	2	3
***Azotobacter* sp**	5.83	4.33	3.83
***Azospirillum* sp**	2.83	2.17	1.83
***Pseudomonas* sp**	3.17	3.33	3.00
***Bacillus* sp**	3.50	4.33	2.67
***Rhizobium* sp**	3.67	3.83	4.83

Statistical analysis revealed significant differences ($p < 0.05$) in IAA production among the microbial species. Azotobacter sp exhibited the highest mean IAA concentration, statistically differing from the other strains. Pseudomonas sp and Azospirillum sp displayed comparable IAA production, while Bacillus sp and Rhizobium sp demonstrated intermediate levels.

The overall auxin biosynthesis potential, as inferred from both qualitative and quantitative assessments, ranks the microbial strains in the order of Azotobacter > Bacillus sp > Rhizobium sp > Pseudomonas sp = Azospirillum sp. The production is increased while L-TRP was added as aprecursor (Table 2).

Table 2: Auxin biosynthesis potential of bacillus, azotobacter, pseudomonas, azospirillum and rhizobium species in presence and absence of precursor L-TRP

Microbes	Isolates	IAA Equivalents ($\mu g\ mL^{-1}$)	
		L-TRP [-]	L-TRP [+]
***Rhizobium* sp**	Rh_1	5.33	6.97
	Rh_2	5.82	8.61
	Rh_3	5.49	7.13
***Azospirillum* sp**	As_1	4.84	5.82
	As_2	3.36	5.00
	As_3	3.69	5.49
***Azotobacter* sp**	Az_1	7.30	9.26
	Az_2	4.34	5.98
	Az_3	7.13	8.93
***Pseudomonas* sp**	Ps_1	5.00	6.64
	Ps_2	5.66	7.30
Bacillus sp	B_1	5.00	6.48
	B_2	4.34	6.15
	B_3	5.16	6.31

These results elucidate the diverse auxin biosynthesis potentials among Bacillus, Azotobacter, Pseudomonas, Azospirillum, and Rhizobium species, providing valuable insights into their plant growth- promoting capabilities. The observed variations underscore the importance of microbial selection for enhancing agricultural practices and sustainable crop production.

The observed variations in auxin production potential among Bacillus, Azotobacter, Pseudomonas, Azospirillum, and Rhizobium species highlight the significance of strain-level differences within microbial genera. The superior auxin biosynthesis potential of Azotobacter is noteworthy and aligns with previous studies identifying Azotobacter species as effective producers of plant growth-promoting substances. The intermediate levels observed in Bacillus and Rhizobium indicate that while these species possess auxin-producing capabilities, their potency may differ compared to other strains [9,10,11,12].

These findings emphasize the potential role of specific microbial strains in influencing plant growth through auxin production. Future research should delve into the underlying genetic and biochemical mechanisms governing auxin biosynthesis in these microbes, facilitating targeted applications in agriculture for enhanced crop productivity and sustainability [13,14,15]. The strain- specificity revealed in this study underscores the importance of precise microbial selection for bioinoculant development and soil management strategies.

CONCLUSION

The investigation into the auxin biosynthesis potential of Bacillus, Azotobacter, Pseudomonas, Azospirillum, and Rhizobium species has provided valuable insights into the diverse capabilities of these microbial species to produce indole-3-acetic acid (IAA), a pivotal plant growth regulator. The following key conclusions can be drawn from the study

The results underscore the strain-specific nature of auxin biosynthesis within microbial genera. While all tested strains demonstrated the ability to produce IAA, notable variations were observed among the species.

Azotobacter emerged as a standout auxin producer, exhibiting the highest IAA production among the tested strains.

Bacillus and Rhizobium exhibited intermediate levels of auxin production. While not as prolific as Bacillus subtilis, their capacity to synthesize IAA suggests potential contributions to plant-microbe interactions.

Future research should delve into the genetic and biochemical mechanisms underlying auxin biosynthesis in these microbes. This knowledge could facilitate the development of customized microbial formulations for specific crops and environmental conditions.

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