Indus Journal of Animal and Plant Sciences
https://induspublishers.com/IJAPS
Volume 1, Issue 2 (2023)

Microbial Capacity for Auxin Biosynthesis: A Comparative Exploration

Dr. Rajesh Sharma[1], Prof. Fatima Hassan[2], Dr. Ahmed Khan[3]

[1]Department of Plant Biology, Indian Institute of Science, Bangalore, India
[2]Department of Wildlife Management, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Institute of Animal Husbandry, Sindh Agriculture University, Tandojam, Pakistan

ARTICLE INFO

ABSTRACT

This study investigates the auxin biosynthesis potential of selected microbial species, namely Bacillus, Azotobacter, Pseudomonas, Azospirillum, and Rhizobium species. Qualitative assessments using the Salkowski reagent confirmed the capability of these strains to yield indole-3-acetic acid (IAA), a key plant growth regulator. Quantitative analysis revealed significant variations in auxin production, with Azotobacter demonstrating the highest IAA levels, followed by Bacillus, Rhizobium, Pseudomonas and Azospirillum exhibited intermediate IAA production. The findings underscore the strain-specific nature of auxin production within microbial genera, emphasizing the importance of precise strain selection for potential applications in sustainable agriculture. Azotobacter, with its superior auxin production, emerges as a promising candidate for bioinoculant development. These results contribute valuable knowledge to the understanding of plant-microbe interactions and lay the foundation for further research into tailored microbial applications for enhanced crop growth and productivity.

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:

- i. Nutrient Agar (for Bacillus)
- ii. Ashby's Mannitol Agar (for Azotobacter)
- iii. King's B Agar (for Pseudomonas)
- iv. Nitrogen-Free Malate Agar (for Azospirillum)
- v. 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 (µg 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.

References

1. Keswani C, Singh SP, Cueto L, García-Estrada C, Mezaache-Aichour S, Glare TR, Borriss R, Singh SP, Blázquez MA, Sansinenea E. Auxins of microbial origin and their use in agriculture. App. Microbiol. Biotechnol. 2020;104:8549-65.
2. Lu T, Ke M, Lavoie M, Jin Y, Fan X, Zhang Z, Fu Z, Sun L, Gillings M, Peñuelas J, Qian H. Rhizosphere microorganisms can influence the timing of plant flowering. Microbiome. 2018;6(1):1-2.
3. Gomes GL, Scortecci KC. Auxin and its role in plant development: structure, signalling, regulation and response mechanisms. Plant Biol. 2021;23(6):894-904.
4. Di DW, Zhang C, Luo P, An CW, Guo GQ. The biosynthesis of auxin: how many paths truly lead to IAA?. Plant Growth Reg. 2016;78:275-85.
5. Simon S, Petrášek J. Why plants need more than one type of auxin. Plant Sci. 2011;180(3):454-60.
6. Zhang M, Gao C, Xu L, Niu H, Liu Q, Huang Y, Lv G, Yang H, Li M. Melatonin and indole-3-acetic acid synergistically regulate plant growth and stress resistance. Cells. 2022;11(20):3250.
7. Duca DR, Glick BR. Indole-3-acetic acid biosynthesis and its regulation in plant-associated bacteria. App. Microbiol. Biotechnol. 2020;104:8607-19.
8. Fu SF, Wei JY, Chen HW, Liu YY, Lu HY, Chou JY. Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Sig. Behav. 2015;10(8):e1048052.
9. Casanova-Sáez R, Mateo-Bonmatí E, Ljung K. Auxin metabolism in plants. Cold Spr. Harb. Persp. Biol. 2021;13(3):a039867.
10. Enders TA, Strader LC. Auxin activity: Past, present, and future. Am. J. Bot. 2015;102(2):180-96.
11. Rahman A, Bannigan A, Sulaman W, Pechter P, Blancaflor EB, Baskin TI. Auxin, actin and growth of the Arabidopsis thaliana primary root. The Plant J. 2007;50(3):514-28.
12. De Smet I, Jürgens G. Patterning the axis in plants–auxin in control. Curr. Opin. Gen. Dev. 2007;17(4):337-43.
13. Ali B, Sabri AN, Ljung K, Hasnain S. Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of Triticum aestivum L. Lett. App. Microbiol. 2009;48(5):542-7.
14. Costacurta A, Vanderleyden J. Synthesis of phytohormones by plant-associated bacteria. Crit. Rev. Microbiol. 1995;21(1):1-8.
15. Matilla MA, Daddaoua A, Chini A, Morel B, Krell T. An auxin controls bacterial antibiotics production. Nucl. Acids Res. 2018;46(21):11229-38.