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Development of Phytohormone-Based Bio inoculants from Beneficial Endophytic Microorganisms

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Abstract
The present study investigates the potential of endophytic microorganisms for the production of phytohormones, particularly Indole-3-acetic acid (IAA) and Gibberellic acid (GA3), and their subsequent application as bioinoculants for plant growth promotion. A range of bacterial isolates including Acetobacter diazotrophicus, Azospirillum brasilens, Azoarcus spp, Burkholderia spp, Herbaspirillum spp, and Agrobacterium diazotrophicus were screened for their ability to produce phytohormones. Quantitative estimations of IAA and GA3 were carried out using spectrophotometric assays. Bioformulations were developed based on high-yielding isolates and tested *in vitro* and *in vivo* on maize, wheat, and pea seeds. Results demonstrated significant enhancement in seed germination, shoot elongation, and overall growth, indicating the potential of microbial phytohormones as sustainable alternatives to synthetic growth regulators.

Keywords: Endophytic bacteria, Indole-3-acetic acid, Gibberellic acid, Bioinoculant, Plant growth promotion

Introduction
Plant growth-promoting rhizobacteria (PGPR) and endophytic microorganisms have emerged as promising tools in sustainable agriculture. Endophytic bacteria colonize internal plant tissues without causing disease and facilitate growth by producing phytohormones, nitrogen fixation, nutrient solubilization, and pathogen suppression. Among phytohormones, Indole-3-acetic acid (IAA) and Gibberellic acid (GA3) play critical roles in root initiation, cell elongation, vascular differentiation, and shoot elongation. The objective of this study was to isolate and characterize endophytic microorganisms with phytohormone-producing potential and to develop bioinoculant formulations for application in maize, wheat, and pea crops.

Materials and Methods
Isolation and Identification of Endophytes
Endophytic microorganisms were isolated from sugarcane and other plant sources. Selective media were used for the enrichment and isolation of Acetobacter diazotrophicus, Azospirillum brasilensin, Herbaspirillum spp, Burkholderia spp, Azoarcus spp, and Agrobacterium diazotrophicus. Standard strains were also procured from NCIM and MTCC for comparative studies.

Quantitative Estimation of Indole-3-Acetic Acid (IAA)
Isolates were grown in tryptophan-supplemented broth for 10-12 days at 32°C with shaking. Supernatants were collected, acidified, and extracted using diethyl ether. The residue was dissolved in methanol, and IAA concentration was determined using Salkowski's reagent at 535 nm against a standard curve.

Quantitative Estimation of Gibberellic Acid (GA3)
Bacterial isolates were cultured in selective broth, and supernatants were processed with zinc acetate and potassium ferrocyanide. After treatment with 30% HCl and incubation at 20°C for 75 minutes, GA3 concentration was determined spectrophotometrically at 254 nm using standard calibration curves.

Bioformulation Development
Based on IAA and GA3 production, the top-performing isolates were selected for formulation development. Liquid bioinoculants, endophytic consortia, PGPR-N2 fixing combinations, and compressed cell mass formulations were prepared.

Seed Germination and Pot Assays
Maize, wheat, and pea seeds were treated with bioinoculants at different concentrations (10-100%). Seed germination percentage, shoot and root length, and vigor index were recorded. Pot experiments were conducted to evaluate the effect of GA3 formulations on shoot elongation in maize seedlings.

Results
Quantitative analysis revealed significant variation among isolates in IAA and GA3 production. Azospirillum Brasilens produced the highest IAA (65.16 µg/ml), followed by Azoarcus spp (48.76 µg/ml) and Acetobacter diazotrophicus (44.87 µg/ml). For GA3, Herbaspirillum spp produced the highest concentration (32.4 µg/ml), followed by Burkholderia spp (14.88 µg/ml) and Acetobacter diazotrophicus (12.26 µg/ml).
Seed germination assays demonstrated that bioinoculant treatments enhanced germination rates and seedling vigor compared to control. In wheat, extracts at 0.05-0.1 ml concentrations promoted better germination, while higher doses inhibited growth. In maize, GA3 application led to significant increases in shoot length, with treated plants showing up to 12.7 cm more growth than controls.

Discussion
The results confirm that endophytic bacteria can produce substantial amounts of phytohormones that directly influence plant growth. The positive response of maize, wheat, and pea to microbial phytohormones suggests their potential as eco-friendly alternatives to chemical fertilizers and synthetic hormones. Interestingly, lower concentrations of extracts were more effective in promoting seed germination than higher doses, indicating a threshold level for optimal phytohormonal activity.

Conclusion
This study demonstrates that endophytic microorganisms such as Azospirillum Brasilens, Azoarcus Spp, Acetobacter diazotrophicus, Herbaspirillum spp, and Burkholderia spp are efficient producers of IAA and GA3. Bioinoculant formulations developed from these isolates significantly enhanced seed germination and seedling growth in maize, wheat, and pea. These findings highlight the potential application of microbial phytohormones in sustainable crop production systems.

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