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RESEARCH ARTICLE

EFFECT OF ENDOPHYTIC BACTERIAL INOCULATION ON GROWTH AND BIOMASS OF SOYBEAN (*GLYCINE MAX* L.)

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Abstract: Endophytic bacteria from medicinal plants represent a valuable resource for novel bioinoculants for enhancing crop productivity. This study evaluated the effect of selected endophytic bacterial isolates (A4 and A6) from *Adhatoda vasica* on the growth and biomass of soybean (*Glycine max* L.) under pot conditions. Healthy soybean seeds were inoculated with individual isolates, and growth parameters including shoot length, root length, and fresh and dry biomass were recorded at 35 and 60 days after sowing. Inoculated plants exhibited significant improvement in all measured parameters compared to uninoculated controls. Inoculation with isolate A4 resulted in more significant enhancement of overall plant growth. This highly potent isolate was identified via 16S rRNA gene sequencing as *Brevibacillus formosus* strain JFI-4. These findings highlight the potential of selected endophytic bacteria as eco-friendly bioinoculants for sustainable soybean cultivation.

Keywords: *Adhatoda vasica*, Biocontrol, Endophytic bacteria, Plant Growth Promotion (PGP), *Fusarium sp.*

INTRODUCTION

The use of chemical fertilizers in modern agriculture has significantly enhanced the agricultural productivity. However, their uncontrolled application adversely affects environmental sustainability, soil health, and human well-being. In response to these issues, there is a growing concern to develop and adopt more sustainable practices, including the application of biofertilizers and biopesticides. Beneficial microbes, particularly bacteria, are preferable due to their rapid growth, genetic tractability, and diverse metabolic capabilities. Recently, a specific group of microbes known as endophytes has attracted more interest from researchers.

Endophytic bacteria are nothing but group of rhizospheric bacteria that colonize the internal plant tissues. These microbes colonize various parts of the plant, such as roots, stems, and leaves, without inducing any damage to the host (Ting *et al*., 2021). Unlike pathogens, endophytic bacteria coexist with their hosts, forming symbiotic associations (Bhutani *et al*., 2021). This close association facilitates a more direct and efficient exchange of nutrients and metabolic signals that can significantly enhance host plant fitness, stress tolerance, and defense. Endophytic bacteria offer advantages such as nitrogen fixation, phosphate solubilization, siderophore release, and the production of growth-regulating hormones, all of which significantly contribute to plant growth and productivity. Bacterial endophytes isolated from arid land plants produce substantial levels of Indole-3-Acetic Acid (IAA), leading to enhanced shoot and root growth in crop plants through hormonal regulation (Asaf *et al*., 2017). Endophytic bacteria also play an important role in enhancing plant tolerance to various biotic and abiotic stress (Ganie *et al*., 2022; Jan *et al*., 2019). Endophytic bacteria enhance resistance against abiotic stresses by the modulation of plant antioxidant systems and hormone-mediated stress responses (Narayanan & Glick, 2022).

Hence, the selection of the host plant is a critical factor in the discovery of novel endophytic strains with unique beneficial properties. Medicinal plants, mainly acknowledged for their therapeutic properties, are promising reservoirs of such beneficial microorganisms. In this context, *Adhatoda vasica*, a medicinal plant widely used in traditional medicine, is compelling candidate. Its antimicrobial properties suggest that the associated endophytes may produce bioactive compounds important for plant growth and biocontrol.

In spite of such promising findings, the diversity and functional capabilities of endophytic bacteria in different medicinal plant particularly their application as bioformulations, is still underexplored (Inamdar & Deshpande, 2025). The unique biochemical environment within this medicinal plant could be a reservoir for novel bacterial isolates with dual-function capabilities, promoting plant growth and simultaneously providing biological control against pathogens. As of now, only one significant study has been conducted on the isolation of endophytic bacteria

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from *Adhatoda vasica* and the evaluation of their plant growth-promoting (PGP) traits, highlighting a substantial research gap in this area (Vyas & Kaur, 2019). To address this research gap, the present study was undertaken with the aim to demonstrate the efficiency of most promising isolates in promoting plant growth *in vivo* through a pot experiment.

MATERIALS AND METHODS

Selection of Endophytic Bacterial Isolates

Endophytic bacterial isolates used in the present study were selected based on their superior plant growth–promoting potential identified during preliminary investigations. Selected endophytic bacterial strains obtained from *Adhatoda vasica* were evaluated for their effect on soybean growth under experimental conditions.

Preparation of Bacterial Inoculum

The selected endophytic bacterial isolates were cultured individually in nutrient broth and incubated at 28 ± 2 °C for 24–48 h under shaking conditions until reaching the exponential growth phase. The bacterial cell density was adjusted to approximately 10^8 CFU ml^{-1}. For consortium treatment, equal volumes of individual bacterial suspensions were mixed thoroughly prior to application.

Effect of selected isolates on growth of soybean *(Glycine max)* by pot experiments

Based on the *in vitro* PGP screening results, two promising isolates, A4 and A6, were selected to evaluate their effect on the growth of soybean (*Glycine max*) under greenhouse conditions. The pot experiment was conducted in a randomized complete block design with 3 replications. Bacterial inoculum was prepared by growing isolates in Nutrient Broth at 28°C for 48 hours to a cell density of approximately 10^8 CFU/ml. Soybean seeds were surface-sterilized with 1% NaOCl for 2 minutes, rinsed with sterile water, and then soaked in the respective bacterial suspension for 30 minutes. Control seeds were treated with un-inoculated sterile Nutrient Broth. Five treated seeds were sown per pot. Pots were watered as required. After 35 days of sowing, plants were carefully uprooted, and growth parameters including root length, shoot length, and total dry biomass were recorded.

Molecular identification of potential isolate

The isolate showing significant growth promotion *in vivo* (A4) was identified by 16S rRNA gene sequence analysis. The obtained sequence was then compared with existing sequence in the National Center for Biotechnology Information (NCBI) database using BLASTn. A phylogenetic tree was constructed using MEGA software. The 16S rRNA gene sequence of the isolate A4 was submitted to the GeneBank database to obtain an accession number.

Statistical Analysis

All experiments were performed in triplicate, and the data are presented as the mean ± standard deviation (SD). The experimental data obtained in the study was analyzed by one-way analysis of variance (ANOVA) using Microsoft excel.

RESULT

Effect of selected isolates on growth of soybean *(Glycine max)* by pot experiments

Inoculation of soybean with selected endophytic bacterial isolates significantly improved plant growth compared to the uninoculated control. Both individual isolates enhanced shoot and root development as well as biomass accumulation at 35 and 60 days after sowing (DAS).

The enhanced growth response of soybean plants inoculated with isolates A4 and A6 may be attributed to their ability to express multiple plant growth–promoting traits, including nutrient solubilization and phytohormone production, as evidenced in preliminary studies. These observations were further corroborated by the in vivo evaluation conducted on soybean plants. At 35 days after sowing, inoculation with isolate A4 significantly enhanced both shoot length (13 cm) and root length (17.2 cm) compared to the uninoculated control (12 cm). Furthermore, isolate A4 considerably increased the fresh weight (1.76 g) and dry weight (0.410 g) of shoots, as well as the fresh weight (1.960 g) and dry weight (0.242 g) of roots.

While isolate A6 also displayed multiple PGP traits in vitro, its effect on soybean growth parameters was less pronounced compared to A4. Plants treated with A6 showed a slight decrease in shoot length (10.5 cm) and a notable reduction in root length (10.0 cm) compared to the control. However, A6 did show a marginal improvement in shoot fresh weight (1.280 g) and dry weight (0.233 g) compared to the control, with similar trends observed in root fresh weight (0.623 g) and dry weight (0.129 g).

Table 1. Effect of bacterial isolates on growth parameters of soyabean on 35 DAS (Days After Sowing)

Sr. no.	Treatment	Shoot length (cm)	Root length(cm)	Shoot weight (g)		Root weight (g)	
				Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
1	Control	11	12	1.06	0.245	0.489	0.109
2	A4	13	17.2	1.76	0.410	1.960	0.242
3	A6	11	10.0	1.280	0.233	0.623	0.129

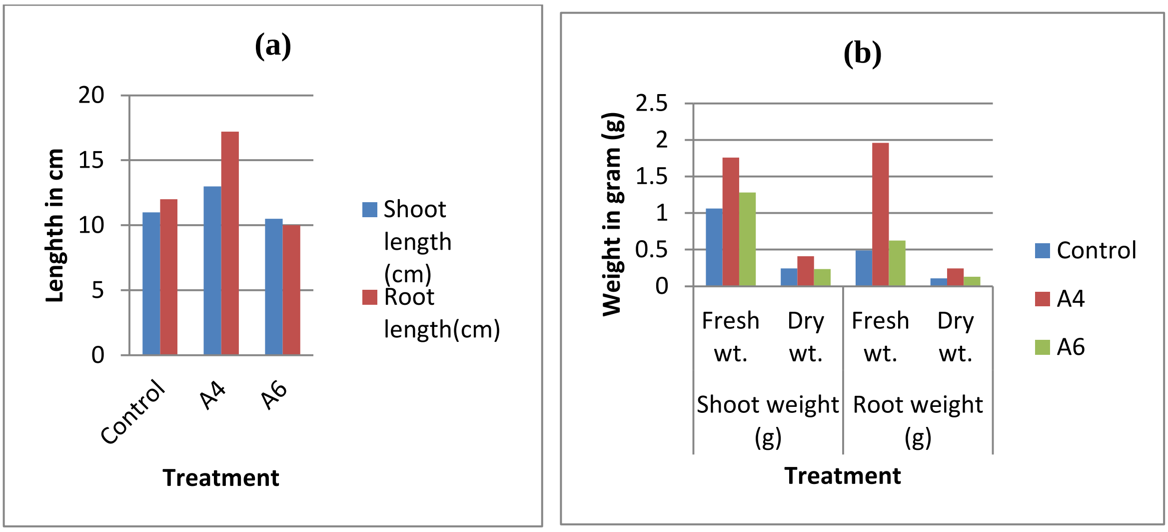

Fig. 1. Effect of endophytic bacterial isolates on (a) shoot length and root length and (b) fresh and dry weight of shoot and root of soybean on 35 DAS

Molecular identification of potential isolate

The most promising plant growth-promoting isolate, A4, was selected for molecular identification through 16S rRNA gene sequence analysis. The partial 16S rRNA gene sequence was deposited in the GenBank database under accession number PQ857179. A subsequent BLAST analysis revealed a high degree of similarity to *Brevibacillus formosus* strain, thereby identifying isolate A4 as *Brevibacillus formosus* strain JFI-4. To further confirm its taxonomic position, a phylogenetic tree was constructed, which showed that isolate A4 clusters closely with *Brevibacillus formosus* and related species (Fig. 2).

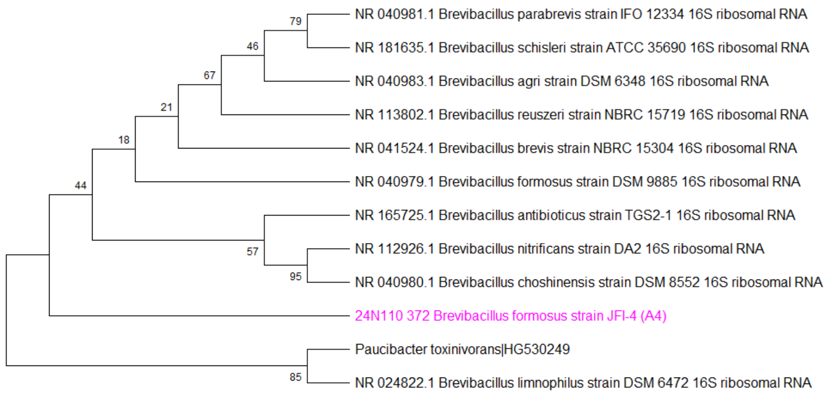

Fig. 2. Phylogenetic analysis of *Brevibacillus formosus* strain JFI-4 (A4) based on 16S rRNA gene sequences

DISCUSSION

The significant improvement in soybean growth due to endophytic bacterial inoculation may be attributed to enhanced nutrient availability, stimulation of root development, and improved physiological efficiency of the plants. Endophytes are known to facilitate plant growth by producing phytohormones, solubilizing nutrients, and promoting root system architecture, which collectively increase biomass accumulation.

The *in vivo* pot experiment provided the ultimate validation of our *in vitro* screening. The results clearly demonstrated that the multifunctional PGP isolate A4 showed significant plant growth promotion in soybean. The substantial increases in shoot and root length, as well as fresh and dry biomass, confirm its efficacy as a bioinoculant. In contrast, isolate A6 exhibiting strong phosphate solubilization and other PGP traits *in vitro*, failed to promote root growth *in vivo*. These findings showed that *in vitro* potential of isolates does not always translate to *in vivo* growth enhancement. The complex and competitive rhizosphere environment, host-microbe specificity, or potential production of inhibitory compounds under specific conditions may explain this inconsistency (Afzal *et al.*, 2019). The superior performance of A4 in the pot experiment strongly suggests its ability to successfully colonize the soybean rhizosphere, and effectively exert its PGP effects.

Molecular identification by 16S rRNA gene sequencing identified the most promising isolate, A4, as *Brevibacillus formosus* strain JFI-4. Its ability to form resilient endospores provides a major advantage for commercial formulation, ensuring a long shelf-life and stability under harsh environmental conditions (Kalboush *et al.*, 2024; Shelke *et al.*, 2023).

In conclusion, this study successfully isolated a potent, multi-trait endophytic bacterium, *Brevibacillus formosus* strain JFI-4, from the medicinal plant *Adhatoda vasica*. This isolate demonstrated significant potential of endophytic bacteria in promoting soybean growth under greenhouse conditions. These findings underscore the value of exploring medicinal plants as reservoirs for potent bio-inoculants and confirm *Brevibacillus formosus* strain JFI-4 as an excellent candidate for the development of a sustainable biofertilizer and biocontrol agent. Future studies should focus on field trials to confirm its effectiveness under diverse agronomic conditions and the specific molecular mechanisms underlying its beneficial effects.

Statements and Declarations

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Author Contributions

Samarin Faruk Inamdar: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – Original draft.

Seema N. Deshpande: Supervision, Guidance in experimental design, Resources, Final approval of manuscript, Project administration.

Rahul Shelke: Data validation, Laboratory assistance, Writing – Review and editing.

Data Availability

The 16S rRNA gene sequence generated and analysed during the study have been deposited in the NCBI GeneBank database under the accession number PQ857179.

URL: https://www.ncbi.nlm.nih.gov/nuccore/PQ857179.1/

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