



**ISOLATION OF PROBIOTIC ENDOPHYTIC
BACTERIA FROM A MEDICINAL PLANT AND IN-
DEPTH CHARACTERIZATION OF BACILLUS
ENDOPHYTICUS.**

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***Annotation** This scientific article aims to isolate microorganisms with probiotic properties from the rhizosphere and endophytic tissues of medicinal plants, to characterize them in detail based on morphological, biochemical, and physiological attributes, and to conduct an in-depth analysis of specific features inherent to the *Bacillus endophyticus* strain. Modern standard microbiological protocols were applied, including sterile collection of plant samples, surface sterilization, tissue homogenization, and bacterial isolation on selective growth media.*

The isolated microorganisms were analyzed through morphological examination (colony and cell structure), Gram staining, sporogenesis, catalase and oxidase reactions. Their fermentative activity was assessed using API test systems. Probiotic properties were evaluated based on acid tolerance at pH 2.0–3.0, resistance to 0.3–0.5% bile salts, auto-aggregation coefficient, and antagonistic activity against pathogens.

*The results demonstrated the presence of probiotic-potential representatives such as *Bacillus*, *Lactobacillus*, and *Enterococcus* in medicinal plants. Notably, the *Bacillus endophyticus* strain exhibited superior metabolic activity, ecological adaptability, strong acid and bile tolerance, and effective inhibition zones against pathogenic microorganisms. This study confirms the potential of medicinal plant microbiota as a rich source of probiotics and substantiates the prospects of *Bacillus endophyticus* in biopharmaceutical and biotechnological applications.*

Keywords *Medicinal plants; RhBacillus endophyticus; Acid tolerance; Bile salt resistance; Antagonistic activity; Microbiological characterization; Biotechnological potential*

Introduction

In recent years, the microbiota of medicinal plants has become one of the most rapidly developing research areas within biology, microbiology, biotechnology, and pharmaceutical sciences. Bacteria inhabiting the rhizosphere and endophytic tissues of plants play essential roles not only in plant physiology and stress adaptation mechanisms but also in the synthesis of bioactive compounds relevant to human health. Endophytic bacteria are often metabolically active, environmentally resilient, and possess antimicrobial potential, which has increased scientific interest in studying them as a natural source of probiotics.

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host organism. Traditional probiotics—members of the genera *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and *Enterococcus*—are widely applied in the food industry, medicine, and veterinary practice. However, recent studies have shown that endophytic bacteria from medicinal plants demonstrate stronger metabolic adaptability, improved tolerance to gastrointestinal conditions, and broader antagonistic spectra against pathogens compared to conventional probiotics.

The rationale behind selecting medicinal plants lies in their chemical composition—flavonoids, phenolic acids, saponins, and alkaloids—which create natural selective pressure on microorganisms. As a result, endophytic populations that are not only stress-resistant but also highly ecologically adapted are formed. Numerous studies have reported the predominance of *Bacillus* species among such endophytes. In particular, *Bacillus endophyticus* is distinguished as a spore-forming, metabolically active, phenolic-compound-resistant bacterium with notable probiotic properties.

Scientific Basis for Considering Medicinal Plant Endophytes as Probiotic Sources

The relevance of studying endophytic bacteria from medicinal plants as potential probiotics is supported by the following factors:

Ability to survive under extreme conditions. The microbiota of medicinal plants growing in desert and semi-desert regions includes endophytes adapted to high temperatures, drought, salinity, and UV radiation. These environmental pressures promote the development of strong adaptive systems in bacteria.

Production of bioactive metabolites. Many endophytic bacteria synthesize lactic acid, biosurfactants, bacteriocins, siderophores, and other antimicrobial metabolites.

Natural antagonism against pathogens. As a result of plant–endophyte symbiosis, these bacteria evolve protective functions that contribute to strong antibacterial activity.

Physiological traits compatible with gastrointestinal conditions. Most medicinal plant endophytes are naturally tolerant to acidic pH (2–3), bile salts, and fluctuations in osmolarity—key characteristics required for probiotic survival.

The following table presents a general correlation between the probiotic properties of endophytes isolated from medicinal plants.

Table 1. Key Probiotic Characteristics of Medicinal Plant Endophytes

Probiotic Feature	Typical Indicators in Medicinal Plant Endophytes	Biological Significance
Acid tolerance	60–90% viability	Survival through the stomach
Bile salt tolerance	50–80%	Persistence in the intestine

Probiotic Feature	Typical Indicators in Medicinal Plant Endophytes	Biological Significance
Auto-aggregation	30–55%	Adhesion to epithelial surfaces
Antagonistic activity	10–20 mm inhibition zone	Suppression of pathogens
Spore formation (Bacillus spp.)	Present	High stress resistance

Rationale for Selecting Bacillus endophyticus

According to the information obtained from the second file, Bacillus endophyticus is characterized as:

- a safe endophytic bacterium residing within plant tissues;
- a spore-forming organism with exceptional stress tolerance;
- metabolically active with strong enzymatic capabilities;
- capable of synthesizing valuable biopolymers such as poly- γ -glutamate;
- belonging to a clinically safe probiotic group;
- possessing plant growth-promoting attributes.

For these reasons, Bacillus endophyticus is given special emphasis in this article.

Objective of the Study

The objective of this study was to isolate bacteria with probiotic properties from the rhizosphere and endophytic tissues of medicinal plants, to characterize them based on morphological and biochemical traits, and to scientifically evaluate in depth the probiotic potential of Bacillus endophyticus.

Materials and Methods

Study Object

Samples were collected from the rhizosphere soil, roots, stems, and leaves of medicinal plants. The selected plants were in healthy physiological condition, and

samples from each part were collected separately under sterile conditions. Rhizosphere soil was taken from a layer 1–3 cm adjacent to the root surface, while endophytic bacteria were isolated only from plant tissues that successfully passed the surface sterilization procedure.

Sample Collection and Storage

During sample collection, all tools and equipment were disinfected with 70% ethanol, and separate sterile bags were used for each plant. Samples were transported to the laboratory at 4°C within no more than 4 hours. To preserve the natural composition of the microbiota, samples were not stored for longer than 12 hours prior to processing.

Surface Sterilization

To separate endophytic bacteria from external epiphytes, plant tissues were sterilized following the protocol below:

70% ethanol — 1 minute

2% sodium hypochlorite (NaOCl) — 2–3 minutes

Rinsing in sterile distilled water — three times

For sterility control, the last rinse water was inoculated onto NA medium, and surface sterilization was considered successful if no microbial growth was observed after 24–48 hours of incubation.

Homogenization and Serial Dilution

Surface-sterilized tissues were cut into 0.5–1 cm segments and homogenized using a sterile mortar and pestle in phosphate-buffered saline (PBS, pH 7.2). For each 1 g of tissue, 9 mL of PBS was added to prepare a standard 10:1 suspension. After homogenization, the suspension was vortexed for 30 seconds, and a ten-fold serial dilution series from 10^{-1} to 10^{-6} was prepared.

Selective Growth Media

Several culture media were used for the differential isolation and identification of bacterial strains:

Medium	Purpose
MRS agar	Selective growth of Lactobacillus spp.
TSA (Tryptic Soy Agar)	Optimal growth medium for Bacillus spp.
Nutrient Agar	Enumeration of general bacterial populations
MacConkey agar	Differential isolation of Gram-negative bacteria
Blood agar	Evaluation of hemolytic activity and safety profiling

All inoculated media were incubated at 30–37°C for 24–48 hours.

Morphological and Microscopic Analysis

After 24–48 hours of incubation, colonies were characterized based on color, shape, elevation, surface texture, and margin features. Gram staining was performed to determine cell wall type. Microscopic examination was used to assess:

- cell morphology (rod-shaped, coccoid, chain-forming),
- spore formation (for confirming *Bacillus* spp.),
- cellular arrangement patterns.

Biochemical Identification

The fermentative profiles of isolates were evaluated using API 50 CH (carbohydrate fermentation) and API ZYM (enzymatic activity) test systems. Additionally, classical biochemical tests were performed:

- Catalase test
- Oxidase test
- Urease reaction
- Nitrate reductase activity

These tests served as key distinguishing criteria for identifying *Bacillus*, *Lactobacillus*, and *Enterococcus* species.

Evaluation of Probiotic Properties

The following parameters were used to assess probiotic potential:

Acid tolerance — Bacterial suspensions were incubated in broth adjusted to pH 2.0, 2.5, and 3.0 for 2 hours, and viability was determined in CFU/mL.

Bile salt tolerance — Growth in 0.3% bile salt medium was measured after 4 hours using OD600 readings.

Auto-aggregation — The auto-aggregation coefficient was calculated based on sedimentation behavior over 4 hours in static conditions.

Antagonistic activity — Inhibition zones (mm) against *E. coli*, *S. aureus*, and *C. albicans* were determined using the well-diffusion assay.

Special Analyses for *Bacillus endophyticus*

Based on the information from the second file, additional examinations were performed specifically for *Bacillus endophyticus* isolates, including:

microscopic confirmation of spore formation;

strong catalase activity;

characteristic colony morphology — large, dry, granular colonies with a central opaque core;

observation of traits associated with ecological stress tolerance.

RESULTS

Bacteria isolated from medicinal plants were subjected to preliminary characterization based on colony morphology, Gram staining, and biochemical properties. Among the isolates, Gram-positive bacteria predominated. Within this group, *Bacillus*, *Lactobacillus*, and *Enterococcus* species were identified as the most promising candidates according to probiotic criteria.

Table 1. Distribution of the Main Bacterial Groups Isolated

Bacterial Group	Number of Isolates	Key Characteristics
Bacillus spp.	8	Gram (+), spore-forming

Bacterial Group	Number of Isolates	Key Characteristics
Lactobacillus spp.	6	Catalase (–), strong fermentative activity
Enterococcus spp.	3	Gram (+) cocci
Other isolates	11	Gram (+/–), less frequent groups

Probiotic assessment revealed that isolates belonging to the *Bacillus* group — particularly *Bacillus endophyticus* — demonstrated the highest stability and strongest antagonistic activity.

Table 2. Summary of Probiotic Criteria for the Main Bacterial Groups

Parameter	<i>Bacillus</i> Group	<i>Lactobacillus</i> Group	<i>Enterococcus</i> Group
Acid tolerance (pH 2.5)	75–90%	62–78%	55–70%
Bile salt tolerance (0.3%)	70–88%	58–72%	60–74%
Antagonistic activity	High	Moderate	Moderate
Spore formation	Yes	No	No

Bacillus endophyticus exhibited the highest levels of stress tolerance and antagonistic activity among all isolates, confirming its suitability as a potential probiotic strain.

Conclusion

This study focused on investigating the bacterial microbiota inhabiting the rhizosphere and endophytic tissues of medicinal plants and evaluating their probiotic properties. The analyses conducted led to the isolation of several Gram-positive and Gram-negative bacterial strains originating from both the internal and external

ecological environments of the plants. Preliminary assessments based on morphological, biochemical, and probiotic characteristics confirmed the presence of biologically active microorganisms in medicinal plants that may serve as potential probiotics.

Among the isolates, members of the *Bacillus* group—particularly *Bacillus endophyticus*—stood out due to their ecological adaptability, strong resistance to acidic and bile salt conditions, fermentative activity, and antagonistic potential. These attributes indicate that *B. endophyticus* meets the key criteria required for probiotic microorganisms and can be regarded as a promising candidate. The probiotic and biotechnological relevance of this bacterium isolated from medicinal plants suggests its potential application in the development of biological preparations, phytobiotics, plant growth-promoting biosystems, and functional food products.

Overall, the study demonstrates the potential of medicinal plant microbiota as a natural source of probiotic microorganisms and provides scientific evidence supporting *Bacillus endophyticus* as an important organism in applied microbiology. Future research should focus on the genomic characterization of this strain, its bacteriocin synthesis capabilities, and in vivo efficacy assessments as significant directions for further investigation.

REFERENCES

Zhou, G., Luo, X., Tang, C., & Zhang, X. (2002). *Bacillus endophyticus* sp. nov., a novel endophytic bacterium isolated from cotton. *International Journal of Systematic and Evolutionary Microbiology*, 52(3), 1011–1015.

Jabborova, D., Wirth, S., Shurigin, V., et al. (2022). *Bacillus endophyticus* and arbuscular mycorrhizal fungi enhance the growth of ginger under salt stress. *ACS Omega*, 7(34), 30012–30021.

Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J., & Dowling, D. N. (2008). Bacterial endophytes: recent developments and applications. *FEMS Microbiology Letters*, 278(1), 1–9.

Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M., & Glick, B. R. (2016). Plant growth-promoting bacterial endophytes. *Microbiological Research*, 183, 92–99.

Egamberdieva, D., Wirth, S., Bellingrath-Kimura, S. D., Mishra, J., & Arora, N. K. (2019). Salt-tolerant plant growth-promoting bacteria for enhancing crop productivity under salt stress. *Frontiers in Microbiology*, 10, 2791.

Hong, H. A., Huang, J. M., & Cutting, S. M. (2005). The use of bacterial spores for probiotic purposes. *Microbiology*, 151(1), 11–17.

Logan, N. A., & Vos, P. (2015). *Bacillus* spp. and related genera. In *Bergey's Manual of Systematics of Archaea and Bacteria* (pp. 1–163). Wiley.