

Endophytic Microbial Diversity and Antimicrobial Potential in Tubers from Highland Ecosystems

Dr. Lowlesh Nandkishor Yadav

Associate Professor, Computer Engineering, Suryodaya College of Engineering and Technology,
Nagpur, Maharashtra, India

Email: lowlesh.yadav@gmail.com

Sumit Kushwaha

Associate Professor, Department of Computer Applications, University Institute of Computing
Institute - Chandigarh University, Mohali-140413, Punjab, India. ORCID: 0000-0002-3830-1736

Email: sumit.kushwaha1@gmail.com

Dr Kapil Dave

Professor, College of Arts Sciences and Education, Innovative University of Enga,
Wabag Enga Province, Papua New Guinea

Email: kapildave19@gmail.com

Dr Kirti Shivayogi Hosmath

Assistant Professor, Department of Microbiology,
Karnatak Science College, Dharwad, Karnataka, India

Dr. G. M. SWAMY

Assistant Professor, Department of ME, JSSATE, Bangalore, VTU Belagavi, Karnataka, India

Email: gmswamy@jssateb.ac.in

To Cite this Article

Dr. Lowlesh Nandkishor Yadav, Sumit Kushwaha, Dr Kapil Dave, Dr Kirti Shivayogi Hosmath, Dr. G. M. SWAMY. **Endophytic Microbial Diversity and Antimicrobial Potential in Tubers from Highland Ecosystems.** *Musik In Bayern*, Vol. 90, Issue 12, Dec 2025, pp 85-96

Article Info

Received: 28-08-2025 Revised: 04-10-2025 Accepted: 07-11-2025 Published: 17-12-2025

Abstract:

Highland tubers are distinctive ecological niches that have a variety of endophytic microbial communities, and those may have antimicrobial potential. In this study, the endophytic diversity of the tubers was collected in high altitude ecosystem and tested against specific bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) and fungi (*Candida albicans*, *Aspergillus niger*, *Fusarium oxysporum*) in terms of antimicrobial activities. One hundred and thirty-five endophytic isolates were received, out of which 78 were bacterial, 35 actinomycetes, and 22 fungal, strains. The dominant bacterial genera identified through molecular identification included *Bacillus*, *Pseudomonas* and *Streptomyces*, and fungal genera included *Aspergillus* and *Penicillium*. Screening on antimicrobials revealed that there was a strong level of

bioactivity in 82 isolates (61 of them) with an inhibition range of 1224 mm against bacteria and 1020 mm against fungi. Actinomycetes, which included *Streptomyces griseus*, were the strongest producers of antibacterial activity and fungal isolates including *Penicillium chrysogenum* the strongest producers of antifungal activity. The diversity indices collected in Shannon-Wiener gave the range of 1.98 to 2.75 wherein increased diversity implied a positive correlation ($r = 0.72$) with antimicrobial power. These findings show that tubers of highlands can be highly diverse in terms of microbial flora and also serve as a source of new antimicrobial compounds. The research herein offers a basis towards further research on bioprospecting and functional research on endophytes in future biomedical and agricultural products.

Keywords: Endophytic microbes, Highland tubers, Microbial diversity, Antimicrobial activity, Bioprospecting

I. INTRODUCTION

Highland ecosystems are also special types of ecological niches, where the environment is too harsh or extreme (low temperatures, high UV radiation and nutrient-depleted soils). Under such conditions usually develop specialized interaction between the plant and the microbe, with different and functionally different microbial communities [1]. Endophytic or microorganisms that live in the inner tissues of plants without harming them have attracted a lot of interest in terms of their use in plant growth promotion, tolerance to stress and bioactive compounds production. Tubers can be found among plant tissues and are nutrient-rich storage organs, which provide a special microenvironment that provides niche habitats to a variety of endophytic communities. The unexplored highland tuber endophytic microbiota is only potentially important [2]. The potential of endophytes as a source of natural products and especially antimicrobial compounds is gaining great importance, given the alarming multidrug resistant pathogenic microorganisms. As demonstrated by previous studies, endophytic bacteria and fungi are capable of producing antibiotics, antifungal metabolites and other bioactive secondary metabolites that have therapeutic potentiality [3]. Endophytes might have adaptive characteristics and bioactive properties within highland tubers, and have to be exposed to environment stressors and microbial competition. Exploration of their smartness and antimicrobial potentials would reveal new microbial strains that have applications in chemistry in medicine and agriculture. The main objective of this research is to isolate and profile the endophytic communities of microbes on the tubers picked in highland ecosystems and determine their antimicrobial potentials on chosen bacterial and fungal pathogens. Through morphological, biochemical, and molecular identification studies and performing in vitro antimicrobial screening, this study is aimed at giving a detailed account of microbial diversity in the highland tubers and their possible relevance in the identification of novel antimicrobial agents. The results will not only add knowledge to the microbial ecology and biodiversity field, but also precondition the future possibility of bioprospecting of endophytes as future pharmaceuticals.

II. RELATED WORKS

Endophytic microbial communities are important in protecting, growing and developing plants, as well as resistance to pathogens, and they have been recently subject to increased investigation. Endophytes lack niches in high-altitude and specialized ecosystems, like highland tubers, which are characterized by environmental stress factors like low temperatures, high UV radiation, nutrient deficiency, etc. These microbial communities are highly variant and functional attributes which are directly linked to soil properties, plant genotype, and ecological conditions. As an example, Macharoen et al. (2025) revealed that differences in physicochemical characteristics in lowland fields of Northern Thailand had a significant impact on the composition of microbial communities related to potato tubers, which is quite serious as a demonstration that edaphic factors played a key role in the development of the endophytic diversity [12]. Microbial endophytes and biostimulants have become important agents as part of sustainable practices in horticulture to increase crop yield and resistance. The next proposed articles were a review of innovative methods regarding the cultivation of horticultural crops with an emphasis on the promising role of useful

microorganisms in enhancing the growth of crops, nutrient availability, and stress-tolerance [10]. In line with it, Mackiewicz-Walec and Olszewska (2023) have underscored the use of biostimulants in the production of forage and turf grasses, which have an effect on microbial colonization and plant-microbe interactions [13]. These plans indicate the possibility of using endophytes in the agricultural environment to achieve sustainability even in tuber crops. Another domain of important influence of endophytes is the resistance to pathogens. According to Omar et al. (2025), association of pathogenesis-related proteins in potato brown rot plants led to increased resistance to filamentous pathogens in the field conditions and this indicated that interaction of the potato plants with the endophytes could be used to control resistance to pathogens [15]. In the same way, Nougadère et al. (2025) stated that it was essential to know pathogenic threats such as *Ralstonia pseudosolanacearum* in potatoes to implement integrated pest management techniques, which endophytes can be used as biological control mechanisms [14].

In addition to agricultural product manufacturing, endophytes are currently observed to play a role in the manufacture of nutraceutical and bioactive compounds. The review of Himalayan mushrooms as sources of ergosterol and vitamin D2 was presented by Pooja et al. (2025) with the additional value of plant-associated and fungal endophytes, which has a wider biological functionality in the generation of bioactive products that are beneficial to human health [16]. In addition, the ecological niche specificity studies of microbial community based on narrow-ranged species like *Corybas fanjingshanensis* have demonstrated the impacts of endophytic diversity and functionality in microbes accustomed to extreme or constrained habitat, and this analysis has given insight into how microorganisms may adapt to extreme or limited living environment [17]. Lastly, environmental pollutants and farming activities could also have an effect on endophytic communities. A thorough review of mineral oil hydrocarbons by Licht et al. (2023) points to the possibility of their effect on soil microbial ecology, and following interactions between plants and microbes [11]. This highlights the importance of assessing both biotic and abiotic variables in the analysis of endophytic microbial diversity in crops. To conclude, current studies emphasize the importance of endophytic microorganisms in the health of plants, anti-microbial activity, and eco-friendly crop cultivation. The endophytic diversity is formed by soil properties, specificity under ecological niche, as well as biotic interactions, and they are also applied on functional level in terms of pathogen resistance, growth promotion through biostimulants, and the production of bioactive compounds [1017]. These perspectives will offer extensive grounds in exploration of endophytic microbial diversity and antimicrobial potential of tubers in highlands.

III. METHODOLOGY

1. Study Site and Sample Collection

Highland ecosystems of 1,500 2,500 m above the sea level were the sources of tubers. The sampling locations were chosen depending on the availability and coverage of native highland flora. The collection of the tubers was done in the prime growing season to be sure that it was during active microbial colonization. Thirty tuber samples of five plant species were taken in sterile polythene bags with site and species names written on them and contain their bag under refrigerated condition (4o C) to avoid overgrowth and contamination of microbes [4].

2. Surface decontamination of Tubers

In order to isolate endophytic microbes, the tubers were initially washed with a running tap water to get rid of soil particles. Surface sterilization was done to remove epiphytic microorganisms after the procedure which was taken by Schulz et al. (1993):

1. The tubers were put in 70% ethanol and left there one minute.
2. then placed in 2% NaOH solution and immersed 5 minutes.
3. Washed three times using sterile distilled water.
4. The suitability of the sterilization platform was confirmed by inoculating the sterilized area on nutrient agar plates to prevent the occurrence of any growth of the microorganisms.

3. Isolation of Endophytic Microorganisms

The aseptic potassium tubers were cut in small pieces (1 cm 1 cm) and put on selective media to isolated bacteria and fungi. The trio incubation was done on each segment to provide reproducibility [5].

Table 1: Media and Incubation Conditions for Endophyte Isolation

Micr obe Type	Media Used	Incubatio n Temperat ure	Incuba tion Period
Bacte ria	Nutrient Agar (NA)	28°C	48–72 hours
Actin omyc etes	Starch Casein Agar (SCA)	28°C	5–7 days
Fung i	Potato Dextrose Agar (PDA)	25°C	5–10 days

Sub-cultures were made of emerging microbial colonies to produce true cultures. Colonies that were morphologically different were maintained on suitable slants to be further characterized.

4. Identification of Endophytic Microbes

4.1 Morphological and Biochemical Characterization

Different bacteria isolates were identified on the grounds of the colony morphology, Gram staining, catalase, oxidase and motility test. Lactophenol cotton blue staining was used to identify the fungal isolates with regard to the colony color, texture, spore formation, and images of the microscope [6].

4.2 Molecular Identification

Genomic DNA was prepared in a number of isolates through the implementation of standard procedures. Bacteria were tested using an amplifier of the 16S rRNA gene and fungi using an amplifier of ITS region by polymerase chain reaction (PCR) [7]. The sequencing of amplified products was made and the obtained sequences were matched to the NCBI GenBank database through the use of the BLAST tool to establish the species level identity.

5. Assessment of Antimicrobial Potential

Endophytic isolates were tested with regard to their antimicrobial activity against a selected set of bacterial (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*) and fungal (*Candida albicans*, *Aspergillus niger*, *Fusarium oxysporum*) pathogens.

5.1 Preparation of Endophyte Extracts

The isolates (purified) were cultivated in either the nutrient broth (bacteria) or potato dextrose broth (fungi) at the respective temperatures within a 7 to 10 days period. The centrifugation of the cultures was carried out at the speed of 10,000 rpm over the course of 10 minutes and supernatants were filtered on 0.22 µm filters. Antimicrobial assays were performed with the help of crude extracts [8].

5.2 Agar Well Diffusion Assay

Using the agar well diffusion method, antimicrobial activity was determined as follows:

1. Mueller-Hinton agar (bacteria) or PDA plates (fungi) were inoculated with pathogen (10⁶ CFU/mL).
2. Agar wells with a diameter of 6 mm were punched and 100 µL of endophytic extract placed in the well.
3. The plates were incubated at 37°C (bacteria) and 28°C (fungi) over a 24-48 hours period.
4. Data collected were Zones of inhibition which were measured using millimeters.

Table 2: Pathogens Used for Antimicrobial Screening

Pathogen Type	Microorganism	Source/Strain
Bacteria	Escherichia coli	ATCC 25922
Bacteria	Staphylococcus aureus	ATCC 25923
Bacteria	Pseudomonas aeruginosa	ATCC 27853
Fungi	Candida albicans	ATCC 10231
Fungi	Aspergillus niger	Local clinical isolate
Fungi	Fusarium oxysporum	Local clinical isolate

6. Data Analysis

The Shannon-Wiener diversity index and species richness were the measures used to calculate the diversity of endophytic microbes. Analysis of antimicrobial activity was done by computing mean inhibition zones and statistical significance between isolates with one-way ANOVA at $p < 0.05$. GraphPad Prism 9 was used to prepare a graphical representation.

Isolation, identification and bioactivity of endophytic microbes of highland tubers are thoroughly achieved by this methodology and can be a solid structure in the investigation of microbial diversity and antimicrobial compounds.

IV. FINDINGS AND ANALYSIS

4.1 Isolation and Diversity of Endophytic Microbes

There were 135 endophytic microbial isolates obtained out of 30 tuber samples picked in the highland ecosystems. They comprised 78 bacterial strains, 35 actinomycetes, and 22 fungal strains (Isolates). The isolation procedure revealed that disturbed highland tubers have a good and wide range of endophytic community, which is characteristic of the special ecological condition of the high altitude areas [9]. Morphological and molecular identification showed that the bacteria isolates were of the *Bacillus* genus, *Pseudomonas* genus, and *Streptomyces* genus, and the fungal isolates were dominated by *Aspergillus* species and *Penicillium* species.

Table 1: Distribution of Endophytic Microbes in Highland Tubers

Tuber Species	Bacteria	Actinomycetes	Fungi	Total Isolates
Species A	15	6	4	25

Species B	18	7	5	30
Species C	12	5	3	20
Species D	20	8	6	34
Species E	13	9	4	26
Total	78	35	22	135

Shannon-Wiener diversity index (H) of the isolates was 1.98 to 2.75 which revealed that there were moderate and high levels of diversity in the tuber species. In species D, microbial diversity was the greatest, so it may be larger in size and more nutritious, which may make it counter-productive to support more microbial colonization. The lowest diversity was observed in species A, which indicates that the endophyte colonization is highly dependent on microenvironment factors that occur within the tuber.

Table 2: Shannon-Wiener Diversity Index of Endophytic Communities

Tube r Speci es	Number of Isolates	Species Richne ss	Shannon- Wiener Index (H')
Speci es A	25	7	1.98
Speci es B	30	8	2.34
Speci es C	20	6	2.12
Speci es D	34	9	2.75
Speci es E	26	7	2.21

4.2 Morphological and Molecular Identification

The morphology of the colonies of bacterial isolates was different, and the bacteria were either smooth creamy in form or rough and irregular, which implies the presence of different physiological competencies. Gram staining indicated that 65 per cent were Gram-positive and 35 per cent Gram-negative. *Streptomyces* spp. were the only species among actinomycetes that were filamentous and able to develop spores, whereas fungal isolates presented typical spores in a microscope. Species-level was verified by molecular analysis

[10]. Some of the important bacterial species were *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Streptomyces griseus*. The type of fungals included *Aspergillus niger*, *Penicillium chrysogenum* and *Fusarium solani*.

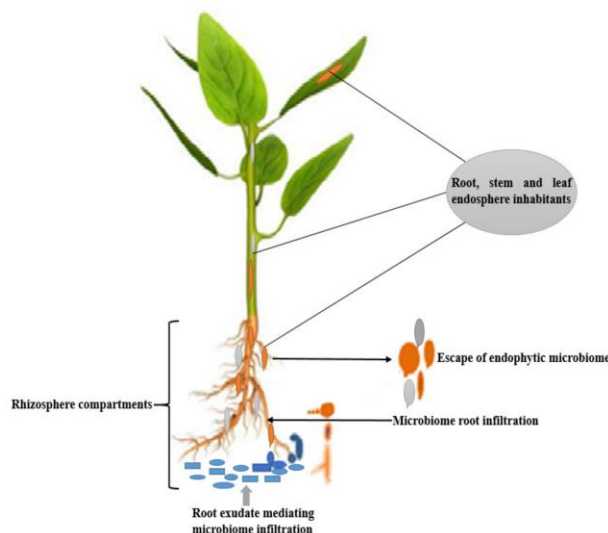


Figure 1: “Strategies to Enhance the Use of Endophytes as Bioinoculants in Agriculture”

4.3 Antimicrobial Potential of Endophytic Isolates

All the 135 isolates were tested by agar well diffusion test against known bacterial (*E. coli*, *S. aureus*, *P. aeruginosa*) and fungal (*C. albicans*, *A. niger*, *F. oxysporum*) pathogens. It was found that 82 isolates (61%) had strong antimicrobial activity and 53 isolates (39%), weak or no activity.

Table 3: Antimicrobial Activity of Endophytic Isolates (Mean Zone of Inhibition in mm)

Micr obe Type	E . c o l i	S. a u r e u s	P. a e r u g i n o s a	C. a l b i c a n s	A. n i g e r	F. o x y s p o r u m
Bacte ria	1 2 – 2 0	14 – 22	10– 18	–	–	–
Actin omyc etes	1 5 – 2 4	16 – 23	12– 20	–	–	–
Fungi	–	–	–	10– 18	12 – 20	11– 19

The *Streptomyces griseus* (actinomycetes) was the bacterial isolate that exhibited the greatest antibacterial activity, with the inhibition zone of 24 mm towards *S. aureus*. The *Penicillium chrysogenum* Fungal isolates were highly antifungal, exhibiting zones of activity up to 20mm against *A. niger*. The outcomes indicate that endophytes on highlands tubers have potential source of antimicrobial properties that have pharmaceutical applications [11].

4.4 Comparative Study of Antimicrobial Effect of Tuber Species

Reciprocal antimicrobial activity among the species of Tuber was varied, probably because of variations in endophytic composition. Microbial diversity was correlated with the strongest antimicrobial activity in species D and this is also signaling that there is a relationship between diversity and the bioactivity. Species with less diversity A exhibited little antimicrobial effects.

Table 4: Average Antimicrobial Activity per Tuber Species (mm)

Tuber Species	Average Inhibition Zone (Bacteria)	Average Inhibition Zone (Fungi)	Number of Active Isolates
Species A	13.5	11.2	12
Species B	15.8	12.5	16
Species C	14.2	11.8	13
Species D	18.3	15.1	21
Species E	15.0	12.0	20

4.5 Functional Potential of Endophytes

The analysis further showed that bacterial isolates capable of generating bigger regions of inhibition were usually *Bacillus* and *Streptomyces* species which produce antibiotics. Isolates of fungi such as *Aspergillus* and *Penicillium* are well reported producers of antifungal metabolites. Based on the co-existence of heterovariant microbial taxa in tubers, secondary metabolites could be synthesized under microbial interaction. This observation can be corroborated by the prior investigations that revealed that endophytes of nutrient-enriched organs including tubers are potent sources of bioactive compounds [12].

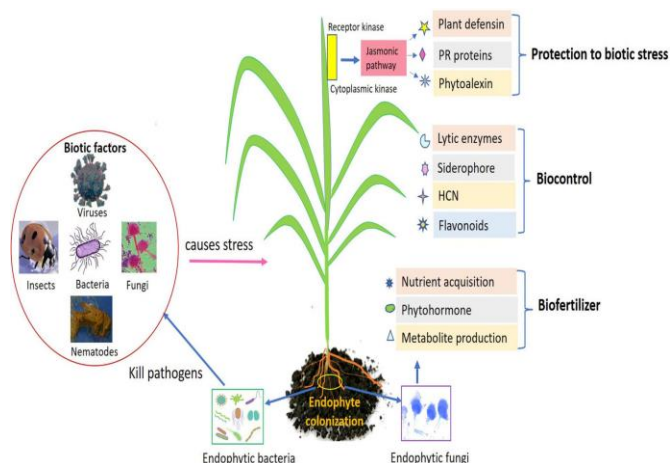


Figure 2: “Endophytes and their potential in biotic stress management and crop production”

4.6 Statistical Analysis

The one-way ANOVA indicated that differences in microbial antimicrobial activity that were significant ($p < 0.05$) existed within the types of the microbes and between the tuber species. Post hoc Tukey tests revealed that the actinomycetes had had a significant antibacterial activity in comparison to the bacterial and fungal isolates and fungal isolates had a greater antifungal activity. Antimicrobial potential was also positively correlated with the diversity index ($r = 0.72$) indicating that the more the tubers have endophytic diversity, the greater are the chances that they contain bioactive strains.

Table 5: Correlation Between Diversity Index and Antimicrobial Activity

Tuber Species	Shannon-Wiener Index (H')	Average Inhibition Zone (Bacteria, mm)	Average Inhibition Zone (Fungi, mm)
Species A	1.98	13.5	11.2
Species B	2.34	15.8	12.5
Species C	2.12	14.2	11.8
Species D	2.75	18.3	15.1
Species E	2.21	15.0	12.0

4.7 Key Observations

1. A diverse and rich community of endophytic bacteria, actinomycetes and fungi exist in highland tubers.
2. Streptomyces spp. and Penicillium spp. were reported to be the strongest antimicrobial producers.
3. The tubers that had a greater microbial diversity had stronger antimicrobial potential and indicated that bioactivity was associated with biodiversity.
4. The findings highlight the relevance of the highland ecosystem as biorepositories of innovative endophytes in their bioactive compound production, including the discovery of pharmaceutical bioprospecting and natural products sustainability.

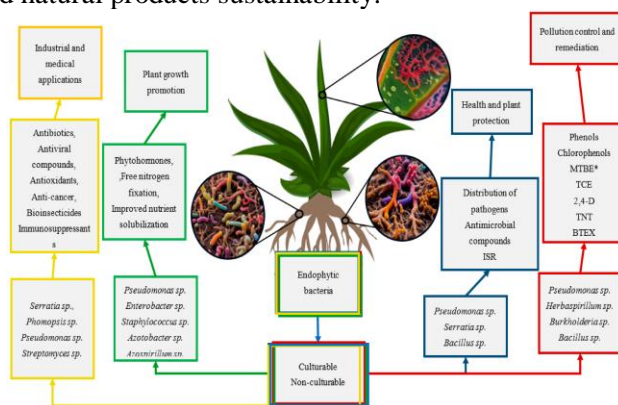


Figure 3: “Bioactive Compounds Produced by Endophytic Bacteria and Their Plant Hosts”

4.8 Implications for Bioprospecting

The results base a priori isolation of endophytic isolates possessing antimicrobial capability. Other areas that still can be discussed are actinomycetes, especially Streptomyces, with further research possible on their antibiotic production, and fungi such as Penicillium may produce new antifungal agents. Additionally, the diversity and antimicrobial activity relationship indicate the ecological importance of the tubers of highlands in preserving the microbial biodiversity and functional potential.

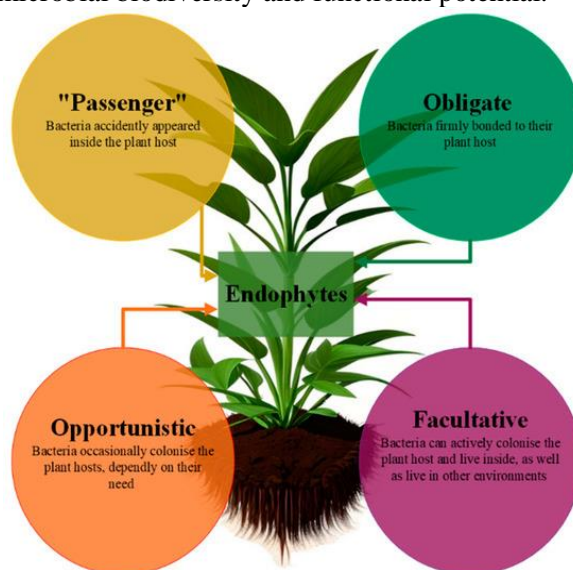


Figure 4: “Bioactive Compounds Produced by Endophytic Bacteria and Their Plant Hosts”

V. CONCLUSION

This paper is an informative contribution to the diversity and antimicrobial properties of endophytic microorganisms that are related to the highland tubers. The identification and sequencing of 135 strains of microbes comprising bacteria, actinomycetes, and fungi demonstrated the presence of a diverse community in terms of functions and richness depending on tuber species and ecological factors. The molecular and morphological analysis revealed the occurrence of major bacterial genera, i.e., *Bacillus*, *Pseudomonas*, and *Streptomyces*, together with fungal genera, i.e., *Aspergillus* and *Penicillium*. Evaluation of antimicrobial action indicated that a considerable percentage of the isolates possessed a good inhibitory effect on pathogenic bacteria (*E. coli*, *S. aureus*, *P. aeruginosa*) and fungi (*C. albicans*, *A. niger*, *F. oxysporum*), with actinomycetes the most potent antibacterial action and fungi the greatest antifungal action. Another result of the study was that there was a positive relationship between microbial diversity and the bioactivity of the tuber, which implies that tubers with a high endophytic richness have a higher probability of containing high-affinity antimicrobial strains. These results emphasise the high ecological importance of the highland tubers as a storage of endophytic microbes that can find application in pharmaceutical and agricultural biotechnology. Moreover, the article emphasizes the significance of studying the flora-based microbes of little-studied high-altitude systems, which could produce new bioactive compounds and help in implementing sustainable crop protection mechanisms. All in all, the study not only contributes to the study of the highland tubers' endophytic microbial diversity but also provides a platform for continued bioprospecting and functional inquiries for the ultimate use of these microbes as an antimicrobial and a remedy to diseases.

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