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Isolation and Identification of Endophytic Bacteria from the Leaves *Bryophyllum Pinnatum*

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ABSTRACT

Endophytes are microorganisms that lives within the plant tissues without causing any disease symptoms to their plant hosts. Endophytic microorganisms inhabiting the medicinal plants synergistically produce pharmaceutically important metabolites in their host plants. This present study aimed to explore and identify the available endophytic bacteria present in the leaf of Bryophyllum pinnatum. The leaves of medicinal plants were collected from a garden in Iyamoh community, Edo State, Nigeria. Method: Six bacterial endophytes were isolated from the leaves of Bryophyllum pinnatum and were identified using vitek 2 compact system. A total of six endophytic bacteria were isolated based on their colony and morphological characteristics. Only two endophytic bacteria isolates were identified by vitek 2 compact system. This study implies that further characterization by molecular techniques of these endophytes will provide accurate characterization at species level in the identification of endophytic bacteria.

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INTRODUCTION

Endophytes, by definition, are microorganisms such as actinomycetes, fungi, and bacteria that invade plant tissues without giving hosts any outward signs of illness and are a rich source of bioactive natural products (Herrera *et al.*, 2022). The word "endophyte" is derived from the Greek word "Endon" which means within and "phyton" which means plants. In 1866, the word "endophyte" was originally proposed by De Bary (Bary, 1866). There are about 300,000 plant species on the earth that harbor endophytes. Endophytic microorganisms live in association with plants for at least a portion of their life cycle, forming symbiotic interactions, modulating both abiotic and biotic stresses, and playing a crucial role in the plant defense system. Endophytes increase the ability of host plants to resist herbivores (Nazir & Rahman, 2018).

They create pharmaceuticals, enzymes, and other bioactive substances with biotechnological value in addition to phytohormones (Parthasarathi *et al.*, 2012). Every plant investigated so far has shown to contain one to more different of endophytes, according to researchers. Endophytes can establish themselves on the stem, roots, petioles, leaf segments, weed inflorescences, fruit, buds, seeds, and dead and hollow hyaline cells (Mukhopadhyay, 2021).

Bryophyllum pinnatum is classified within the family Grassulaceae. It is characterised as an erect, succulent, perennial shrub with a height of approximately 1.5 metres.

This plant species is capable of reproduction through both seed propagation and vegetative means, specifically through the use of leaf bubils. The plant in question is a cultivated species that has been intentionally introduced for its aesthetic qualities.

However, it has since become an invasive species, spreading uncontrollably in the vicinity of agricultural plantations (Ogidi *et al.*, 2019). *Bryophyllum pinnatum*, also referred to as air plant, never die, and miracle leaf, is a widely recognized botanical species. The utilization of this substance can be observed in traditional medicinal practices within tropical regions of Africa, America, India, China, and Australia. The substance is widely recognized for its ability to promote wound healing and facilitate hemostasis. Historically, this substance has been employed for medicinal purposes to address a range of conditions, including anthelmintic, immunosuppressive, hepatoprotective, anti-nociceptive, anti-inflammatory, anti-diabetic, nephroprotective, antioxidant, antimicrobial, analgesic, anticonvulsant, neuropharmacological, and antipyretic effects. In the region of South Eastern Nigeria, there is a traditional practice of utilizing a specific herb to aid in the expulsion of the placenta following childbirth (Ogidi *et al.*, 2019). The plant leaf undergoes a mild exposure to heat, after which the resulting juice is extracted and subsequently applied to the baby's placenta on a daily basis.

In the study conducted by Muhammad *et al.* (2007), it was observed that a mixture of crushed leaves and extracted juice was utilized in combination with palm oil for the application on abscesses. The objective of this study was to isolate and identify endophytic bacteria from the leaf of *B. pinnatum* using vitek 2 system.

Materials and Methods

Materials

Materials used in this research were petri dishes, masking tape, aluminum foil paper, distilled water, 70% ethanol (Bratachem®), Nutrient Broth (NB) (Merck®), Nutrient Agar (NA) (Merck®), sodium hypochlorite, violet crystal, lugol, safranin, ethanol 96% and H₂O.

Collection of Leaves samples

The leaves of *Bryophyllum pinnatum* were selected and collected from the Garden in a zipper bag in a Garden, Iyamoh community, Edo State, Nigeria. The collected leaves were immediately transferred to the Department of Biological Sciences, Edo state university for authentication before transferring to the laboratory further processing.

Surface Sterilization of Leave samples

The leaves samples were cut into small piece with the aid of sterile knife, The leaves were surface sterilized sequentially using running tap water for 5 minutes, 70% ethanol for 5 minutes, sodium hypochlorite 2.5% for 6 minutes, 70% ethanol for 30 seconds to remove sodium hypochlorite and then rinsed three times using sterile distilled water. To check for sterility, aliquot from last rinse was then inoculated into a nutrient agar plate and incubated at 37°C for 24 hours (Mukhopadhyay, 2021).

Isolation of endophytic bacteria

The sterilized leaves were culture on a nutrient agar media and were incubated at 37°C for 24 hours. After 24 hours of incubation, the grown bacterial colonies were subculture on a fresh N.A using a streak wire loop as described by Duhan *et al.*, 2020.

Identification of bacterial isolates

The endophytic bacteria isolates were identified based on cultural, morphological and biochemical characterization. Cultural identification was done by observing the characteristics of the grown colonies using the following characteristics: shape, color, edge and elevation. The morphological characteristic of the colonies was carried out by Gram staining techniques and automated biochemical tests was carried out for the identification of the isolates using vitek2 compact system. The VITEK 2 system, developed by bioMérieux in Marcy d'Etoile, France, was employed for the purpose of

bacterial identification. This system utilizes test cards specifically designed for the identification of both Gram-negative (GN) and Gram-positive bacteria. The GN identification test card consists of 47 different substrates, while the Gram-positive identification test card contains 43 substrates. Bacterial identification was conducted using a bacterial concentration of 0.5 in McFarland standards. Gram-positive bacteria were subjected to a bacterial load of 6.9×10^6 colony-forming units (CFU)/mL, while Gram-negative bacteria were exposed to a bacterial load of 1.2×10^7 CFU/mL (Hernández-Durán *et al.*, 2017).

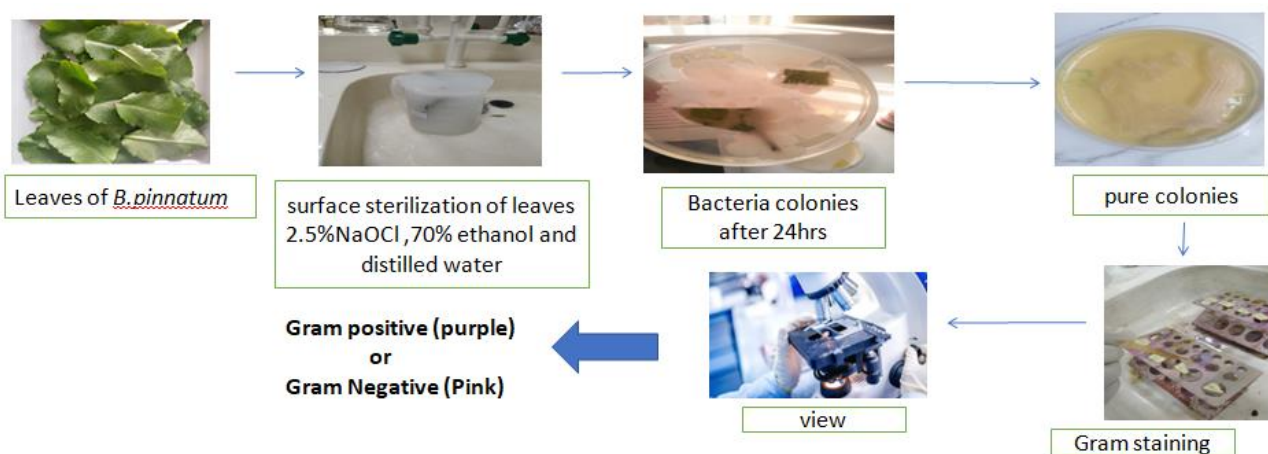


Figure1: Systematic flow chart of the steps involved in isolation of endophytic bacteria

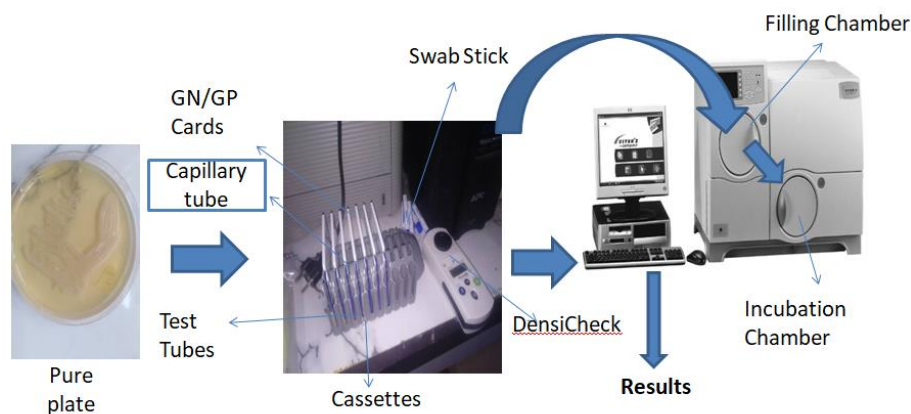


Figure 2: systematic flow chart of the steps involved in biochemical tests of endophytic bacteria

RESULTS AND DISCUSSION

The isolation of endophytic bacteria from the leaves of *B. pinnatum* aimed to explore the distribution and identity of endophytic bacteria. Based on cultural, morphological and biochemical characteristics of the isolated endophytic bacteria from the leaf of *B. pinnatum*, A total of six (6) endophytic bacteria were isolated from the leaf of *B. pinnatum* collected from a Garden, Iyamoh communities, Edo State,

Nigeria as showed in (Table 1). The isolates were denoted as LF1 – LF2. There was no any growth on the sterility plates which indicates that the colonies around the cultured leaves are actual endophytes and not epiphytes. Out of the six bacterial isolates, two were Gram positive rod bacteria while others were Gram negative rod bacteria (Table 1). Out of the six endophytic bacteria isolates, only 2 were identified by vitek 2 compact systems as presented in table 2.

Table1. Cultural and Morphological Characteristic of Endophytic Bacterial Isolates

PARAMETERS	LF1	LF2	LF3	LF4	LF5	LF6
CULTURAL						
Shape	Circular	Circular	Round	Round	Circular	Circular
Colour	Creamy	White	White	Creamy	Creamy	Creamy
Size	Medium	Large	Small	Large	Small	Small
Elevation	Raised	Convex	Raised	Convex	Flat	Flat
Transparency	Opaque	Transparent	Opaque	Opaque	Transparent	Opaque
MORPHOLOGICAL						
Gram stain	GPR	GPR	GNR	GNR	GNR	GNR

Table 2: Vitek 2 Biochemical Identification of Endophytic Bacteria Isolated from the Leaf of *B. pinnatum*.

S/N	Endophytic bacteria isolates	Gram Reaction	Probability Percentage (%)	Probable organism
1.	LF1	GPR	00	Unidentified
2.	LF2	GPR	00	Unidentified
3.	LF3	GNR	94	<i>Serratia ficaria</i>
4.	LF4	GNR	00	Unidentified
5.	LF5	GNR	86	<i>Sphingomonas paucimobilis</i>
6.	LF6	GNR	00	Unidentified

Key: LF= leaf

Out of six isolated endophytic bacteria, only two were identified as *Sphingomonas paucimobilis* and *Serratia ficaria* with probability percentage identity of 86 and 94 % respectively while others were not identified. Hernández-Durán *et al.*, 2017 reported better performance of VITEK 2 system in terms of strain identification at both genus and species levels (100%). Rajkumari *et al.*, 2014, also reported similar study of successful identification of 354 isolates out of 445 isolates analyzed. The VITEK 2 system accurately identified 354 isolates, accounting for 79.5% of

the total. However, it failed to identify 48 isolates (10.7%) and exhibited incorrect identification or limited discrimination in 43 isolates (9.6%). These could be that the identities of the unidentified isolates were not among those in the vitek system database or a misguide as the automated method of identification of organisms was considered to be a reliable method. However, in some literature, several plant leave were used as source of endophytic bacterial strains such as *Bacillus amyloliquefaciens*, *Acinetobacter calcoaceticus*

CONCLUSION

From the total endophytic bacteria that were isolated from leaves of *Bryophyllum pinnatum* (LF1-LF6) based on the colony characteristics. As much as two of the bacteria were classified as Gram positive rod and others were Gram negative rod. In conclusion, it is showed that the leaves of *Bryophyllum pinnatum* harbor endophytes of different species.

Reference

- Aransiola, E. F., Daramola, M. O., Iwalewa, E. O., Seluwa, A. M., & Olufowobi, O. O. (2014). Anti-diabetic effect of *Bryophyllum pinnatum* leaves. *International Journal of Biotechnology and Bioengineering*, 8(1), 89-93.
- Duhan, P., Bansal, P., & Rani, S. (2020). Isolation, identification and characterization of endophytic bacteria from medicinal plant *Tinospora cordifolia*. *South African Journal of Botany*, 134, 43-49. <https://doi.org/10.1016/j.sajb.2020.01.047>
- Herrera, H., Fuentes, A., Ortiz, J., Soto, J., da Silva Valadares, R. B., Salas-Eljatib, C., & Arriagada, C. (2022). Root-associated endophytes isolated from juvenile *Ulex europaeus* L.(Fabaceae) plants colonizing rural areas in South-Central Chile. *Plant and Soil*, 474(1-2), 181-193.
- Hernández-Durán, M., López-Jácome, L. E., Colín-Castro, C. A., Cerón-González, G., Ortega-Peña, S., Vanegas-Rodríguez, E. S.,... & Franco-Cendejas, R. (2017). Comparison of the MicroScan WalkAway and VITEK 2 Compact systems for the identification and susceptibility of clinical Gram-positive and Gram-negative bacteria. *Investigación en Discapacidad*, 6(3), 105-114.
- Muhammad, I., & Samoylenko, V. (2007). Antimalarial quassinoids: past, present and future. *Expert Opinion on Drug Discovery*, 2(8), 1065-1084.
- Mukhopadhyay, M. A., Thapa, M. R., & Firoz, A. F. (2021). Potentials of endophytes of *Rhododendron arboreum* for the production of plant growth promoting factors and antimicrobial compounds. *Plant Cell Biotechnology and Molecular Biology*, 1, 108-115.
- Nazir, A., & Rahman, H. A. (2018). Secrets of plants: endophytes. *International Journal of Plant Biology*, 9(1), 7810.
- Ogidi, O. I., Esie, N. G., & Dike, O. G. (2019). Phytochemical, Proximate and Mineral compositions of *Bryophyllum pinnatum* (Never die) Medicinal plant. *Journal of Pharmacognosy and Phytochemistry*, 8(1), 629-635.
- Parthasarathi, S., Sathya, S., Bupesh, G., Samy, R. D., Mohan, M. R., Kumar, G. S., ... & Balakrishnan, K. (2012). Isolation and characterization of antimicrobial compound from marine *Streptomyces hygroscopicus* BDUS 49. *World J Fish Mar Sci*, 4(3), 268-277.
- Rajkumari, N., Mathur, P., Xess, I., & Misra, M. C. (2014). Distribution of different yeasts isolates among trauma patients and comparison of accuracy in identification of yeasts by automated method versus conventional methods for better use in low resource countries. *Indian journal of medical microbiology*, 32(4), 391-397.