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Morphological and biochemical characterization of endophytic bacteria from selected medicinal plants found at the University of Dar es Salaam, Tanzania

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Keywords

Medicinal flora;
Bacterial endophytes;
Biochemical assay

Abstract

Endophytic bacteria are capable of colonising internal tissues of medicinal plants, and producing secondary metabolites (biochemical compounds) of beneficial importance like the ones produced by their host plants. Though Tanzania harbours a wide species of medicinal plants with endophytic bacteria capable of producing such important bioactive substances, research on these bacteria remains scant, which limit their biotechnological applications. This work was undertaken to isolate and document endophytic bacteria from tropical almond, local avocado, banana and blue snake weed plants found at the University of Dar es Salaam, main campus. The study characterised the isolated endophytes based on morphological descriptors and biochemical assays. Furthermore, Principal coordinate analysis (PCoA), Multiple correspondence analysis (MCA), and Hierarchical cluster analysis (HCA) were utilized in assessing the morphological and biochemical relatedness of the isolated endophytic bacteria. A total of 34 bacterial isolates were obtained from root and leaf samples of four medicinal plants and were tentatively identified as belonging to seven genera: *Bacillus, Lysinibacillus, Brevibacterium, Curtobacterium, Enterobacter, Microbacterium,* and *Pseudomonas.* While PCoA (recovered from morphological data) did not show any grouping of the studied endophytic bacteria, MCA (recovered from biochemical data) and HCA (recovered from both morphological and biochemical dataset) grouped the bacteria into seven and two groups, respectively. This diversity presents opportunities for discovering novel bioactive compounds that could be utilized for various biotechnological applications. Genetic and secondary metabolites profiling of the isolates is proposed to accurately delineate them to the species or strain level.

Introduction

Tanzania harbours a wide range of important medicinal plants, such as tropical almond (*Terminalia catappa*), avocado (*Persea americana*), banana (*Musa acuminata*) and blue snake weed (*Stachytarpheta jamaicensis*) (Schlage et al. 2000). Tropical almond known as ‘mkungu’ in Swahili is planted for various purpose, including shade, edible nuts, woods, ornaments, and medicinal uses. The plant is globally distributed along the coastal areas,

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and known to tolerate high salinity environments (Mohale et al. 2009, Anand et al. 2017). It is a common medicinal plant for treatment of diseases, including dermatitis and hepatitis (Lin et al. 1997). The plant roots are used as remedy for typhoid and dysmenorrhea in Nigeria (Ichôron et al. 2018), the barks are used to treat dysentery in southeast Asia (Terças et al. 2017), and the nuts are used as aphrodisiac in Taiwan (Cock 2015). The leaves, roots and nuts of the tropical almond plant are rich in phytoconstituents including phenols, flavonoids and carotenoids, which have antidiabetic, anti-inflammatory, antioxidant, hepatoprotective, anticancer and antimicrobial effects (Anand et al. 2017).

Avocado (‘parachichi’ in Swahili) is a tropical and subtropical fruit tree plant that is native to Mexico and Central America (Araújo et al. 2018). The plant grows well in tropical and subtropical climates, and prefers temperatures of 20 to 30 °C and an average annual rainfall of about 2000 mm (Patra et al. 2025). In Tanzania, avocado is widely cultivated in Mbeya, Songwe, Iringa, Tanga, Kilimanjaro, Kigoma, Kagera and Arusha regions (Juma et al. 2019). Ground dried avocado fruit is used as a remedy for diarrhoea, asthma, dysentery, and toothaches (Roig 1974), while dried avocado leaves are utilized to manage toothache, diabetes, high blood pressure, malaria, and painful menstruation (Lima et al. 2012). Avocado leaves are endowed with polyphenols, flavonoids, vitamin C, saponins, tannins, alkaloids, phytate, oxalate, and terpenoids, which have antioxidant, anticancer, liver-protective, anti-inflammatory and antidiabetic properties (Juma 2025).

Banana (‘mgomba’ in Swahili) is a giant monocotyledonous herb that was domesticated about 7000 year ago in the Southeast Asia. It is a significant staple food for millions of people in Africa (Li and Ge 2017). The main banana-growing regions in Africa include Central Africa, Western Africa, the Great Rift Valley zone, which includes Tanzania, the Ethiopian Highlands, and the southern uplands of Malawi (De Langhe et al. 2015). Traditionally, banana has been used to treat a wide range of ailments, including cough, bronchitis, dysentery, allergies, and sexually transmitted diseases. It is also used to manage diarrhoea, anaemia, ulcers, fever, intestinal lesions, diabetes, gout, hypertension, and nephritis (Sarma et al. 2021, Ranjha et al. 2022, Bhanushali et al. 2024).

Blue snake weed or ‘kikwayakwaya’ in Swahili, is cultivated for medicinal benefits in South East Asia, Northern, South America, Brazil, Ecuador, Colombia and Venezuela. It grows as weed across African countries including Tanzania. The plant is used for treatment of indigestion, acid reflux, ulcers, constipation, respiratory conditions, and skin infections. It helps soothe an inflamed, irritated respiratory system, providing relief from coughs, colds, flu, bronchitis, and allergies, indigestion, acid reflux, ulcers, constipation, respiratory conditions, and skin infections. It soothes an inflamed, hot respiratory system that is suffering from coughs, colds, flu, bronchitis and allergies (Liew et al. 2016). This plant has been used traditionally as a cure for fever, digestive complications, respiratory conditions, allergies, and dysentery, and promotes menstruation, among others (Sivaranjani et al. 2014). Several phytochemicals are present in this plant, including flavonoids, alkaloids, steroids, phenols and terpenoids (Liew et al. 2016) These phytochemicals possess antiplasmodial, immunomodulatory (Amaechi et al. 2025), anti-diabetic (Idu et al. 2021), anti-cancer (Dewi et al. 2025), antioxidant, anti-arthritic, anti-inflammatory, and bactericidal activities (Zs et al. 2017).

Endophytes are microbial group capable of colonising inside tissues of plant and live symbiotically (Bilal et al. 2018). They can produce varieties of primary and secondary bioactive compounds similar to the ones produced by medicinal plants. Primary bioactive compounds include proteins, carbohydrates and fats whereas the secondary bioactive compounds include phytohormones, alkaloids, quinols, peptides, polyketones and others (Mercado-Blanco and Bekker 2007). When isolated, endophytes may have wide range of applications in pharmaceutical, agriculture and food industry (Bhoonobtong 2013). Endophytes can be commercially cultured on formulated substrates to efficiently produce bioactive compounds. Compared to traditional plant extracts, this approach offers several advantages: endophytes yield high quantities of metabolites with a high degree of purity, the process is independent of seasonal and weather variations, and it supports forest sustainability and biodiversity by reducing the need to harvest medicinal plants for bioactive compound extraction (Saini et al. 2016).

Endophytic bacteria have been isolated from variety of plant hosts including agriculture crops, plants found in extreme environments, perennial plant and weed plants (Nair and padmavathy 2014). Colonisation of endophytic bacteria into host plants depends on various factors such as endophyte growth rate, motility, extent of root exudation, quorum sensing and ability to adapt to competition to obtain nutrients from plants required for their growth (Compant et al. 2010). Plant specific factors such as age, geographical location, genotype, type of tissue, plant nutrients and climatic conditions also influence types and number of endophytes residing in the host plant (Partida-Martínez and Heil 2011, Penuelas et al. 2012, Shi et al. 2014). Endophytic bacterial communities colonizing agricultural crops are dominated by *Bacillus* and *Pseudomonas* genera (Maela 2020).

Endophytes assist their host plants with nutrient acquisition from the soil, provide resistance against pathogenic microbes, help in stress tolerance and also promote their growth and development by producing phytohormones (Martinez-Klimova et al. 2017, Maela 2020). The secondary metabolites produced by endophytes such as alkaloids, flavonoids, peptides, phenols, quinols, polyketones and terpenoids possess antimicrobial, anticancer, anti-inflammatory and immunosuppressive activity (Ludwig-Müller 2015, Singh et al. 2017). These secondary metabolites can be used to produce drugs with pharmaceutical importance and also produce enzymes of biotechnological interest (Maela 2020). Liu et al. (2016) found that *Promicromonospora sp.*, an endophytic bacterium isolated from *Artemisia annua*, was capable of producing cellulase and proteinase enzymes, which may be of importance to agriculture, pathology, medicine, and ecology. Likewise, Mishra et al. (2018) discovered that the endophytic bacteria namely *Bacillus amyloliquefaciens* (MPE20) and *Pseudomonas fluorescens* (MPE115) modulated the withanolide biosynthetic pathway and tolerance against *Alternaria alternata*, which causes leaf spot disease from *Withania somnifera*.

Although Tanzania is endowed with a wide range of medicinal plants that harbour a variety of endophytic bacteria with potential for biotechnological exploitation in agriculture, food, and pharmaceutical industries, scanty research has been conducted to characterize these endophytic bacteria, thereby limiting their biotechnological applications. Therefore, this research was undertaken to isolate and characterize endophytic bacteria, both morphologically and biochemically, from selected medicinal plants (tropical almond, local avocado, banana and blue snake weed plants) located at the University of Dar es Salaam, main campus.

Materials and Methods

Plant Sample Collection and Bacterial Endophyte Isolation

The study site was the University of Dar es Salaam, Mwalimu Nyerere campus, found in Dar es Salaam, Tanzania. The campus experiences a tropical coastal climate with average temperatures around 32°C and bimodal rainfall totalling about 1,150 mm annually. The soil is predominantly sandy clay and clayey sand, influenced by marine limestone formations. The colonization of endophytic bacteria in the plant species found on this campus is influenced by the soil type i temperature and rainfall. Four medicinal plants included in the present study are tropical almond (*Terminalia catappa*), local avocado (*Persea americana*), banana (*Musa acuminata*) and blue snake weed (*Stachytarpheta jamaicensis*). Collection of the plant roots and leaves, and isolation of endophytic bacteria were carried out following the method by Makuwa and Diamini (2021). For root sampling, five to seven fibrous roots were collected per plant at a depth of approximately 10–15 cm. For leaf sampling, mature, sun-exposed leaves were selected, with five to seven leaves collected per plant. Sampling was conducted from two plants per species, except for avocado, where only one plant was sampled, with all plants spread across scattered plots. Based on morphological characteristics i.e., shape, colour, size, margin, consistency and texture, isolates were selected then sub cultured using streak plate technique until pure colonies were obtained (Figure 1). In order to classify the isolates into Gram positive and negative bacteria, Gram staining Test was carried out following the method by Gephardt et al. (1981). Thereafter, the isolates were stored at 4 °C for biochemical tests.

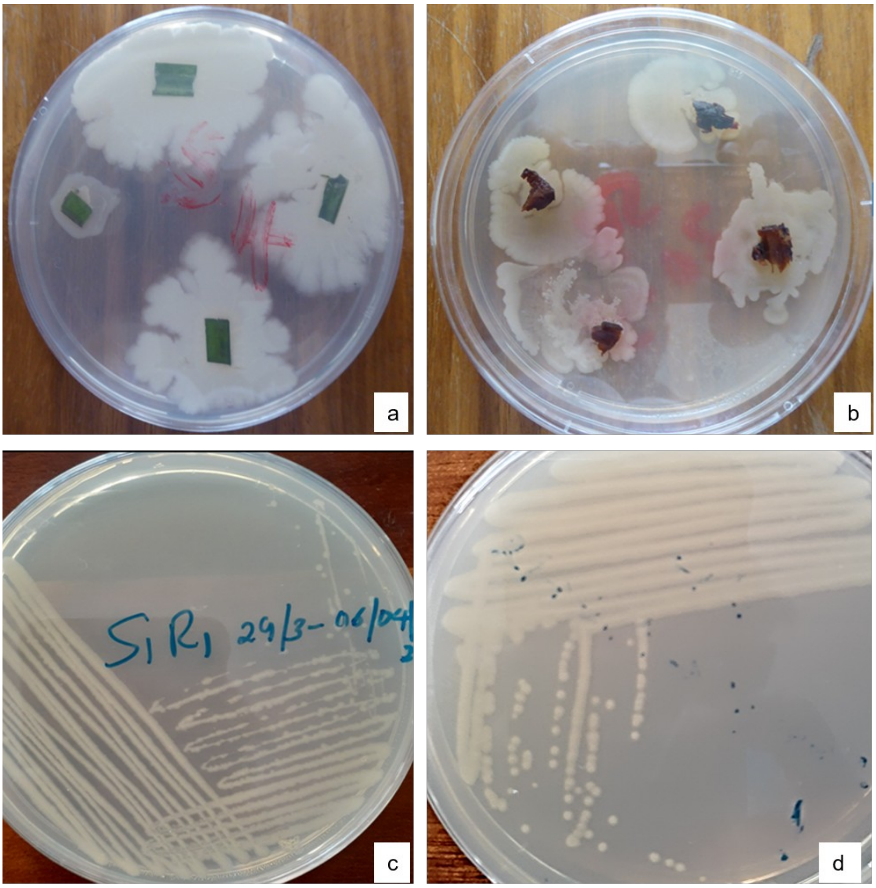

Figure 1. Representative images showing the isolation of endophytic bacteria from leaves (a), roots (b), and streak plating (c and d) to obtain pure bacterial colonies (single colonies)

Biochemical characterization of endophytic bacteria

The biochemical tests involved were catalase, oxidase, methyl red and Voges-Proskauer (VP) tests. While the Catalase and Oxidase tests were carried out following the method by Reiner (2010) and Shields and Cathcart (2010), respectively, the Methyl red and Voges-Proskauer tests was conducted as per Mcdevitt (2009).

Data analysis

Data on morphological (shape, size, colour, margin, consistency, texture, Gram staining) and biochemical traits of the isolates were recorded and organized in Microsoft Excel. To visualize the distribution of endophytic bacteria in leaves and roots for each plant species, heat map was created using the Python Seaborn Version 0.13.0 (Waskom 2021). For displaying the results for the biochemical tests, the radar chart was created in the Python Matplotlib Version 3.5.2 (Hunter 2007). Using XLSTAT software Version 2024.2.2, principal coordinate analysis (PCoA) and multiple correspondence analysis (MCA) were carried out on morphological and biochemical data, respectively, in order to display the morphological and biochemical relatedness of the studied endophytic bacteria, respectively (Addinsoft 2024). The first three principal axes of PCoA were visualized in the Python Matplotlib Version 3.5.2. Similarly, the XLSTAT was employed to perform hierarchical cluster analysis (HCA) on morphological and biochemical dataset to show the relationships among the investigated endophytic bacteria based on their morphological and biochemical features.

Results and Discussion

Number of Isolates from Studied Plants

Thirty-four morphologically distinguished endophytic bacteria were successfully isolated from leaf and root samples of the investigated plants (Figure 2).

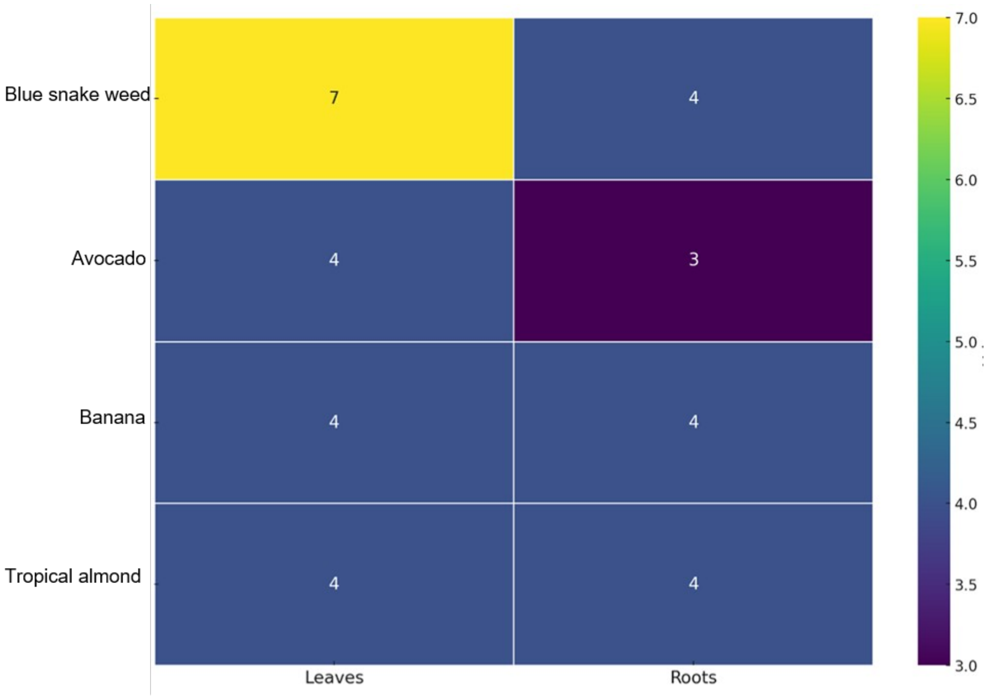

Figure 2: Heatmap visualizing the distribution of endophytic bacteria in leaves and roots for each plant species. The intensity of the colours corresponds to the bacteria count, with darker shades representing higher counts.

The highest number of endophytic bacteria was isolated from the leaves of the blue snake weed (seven isolates) compared to its roots (four isolates). This suggests that the bacterial population in the leaves of the blue snake weed could be more diverse. In contrast, avocado displayed nearly balanced bacterial distribution between leaves (four isolates) and roots (three isolates), indicating a relatively equal bacterial colonization in both organs. With four bacterial isolates from both leaves and roots, banana and tropical almond displayed equal distributions, suggesting that the plant species did not vary in the bacterial colonization of these two plant components. Soil composition, host plant species/genotype, age, physiology, leaf/root architecture and nutrients along with biogeography, microbe-microbe interactions and plant-microbe interactions and the unique microenvironment inside each plant organ may be responsible for the differences in bacterial distribution among various plants and organs investigated (García-Guzmán and Heil 2014, Chen et al. 2020, Galindo-Castañeda et al. 2024). The higher diversity of endophytic bacteria in the blue snake weed leaves may suggest a wealth of beneficial bacteria for a range of uses. The findings of this study are comparable to Hakizimana et al. (2011) who successfully isolated 8 endophytic bacteria from *P. americana* and observed that 76.1% of isolates were *Bacillus* species from the roots. Contrary to that, Cordero et al. (2022) isolated 34 endophytic bacteria from *P. americana* of which only 10 isolates (29.41%) were *Bacillus* species.

Morphological and biochemical diversity of endophytes

The endophytic bacteria isolated from the medicinal plants exhibited a wide variation in phenotypic traits, including colony colour, size, margin, consistency, and texture, as well as a range of biochemical characteristics (Table 1). For example, the isolates *TR 3* and *TR 4* both from the roots of the same plant exhibited variation in their morphology with TR 3 being small, yellow and featuring a flat, mucoid, regular shape with short rods while TR 4 was small and translucent white, also flat and mucoid, but characterized by single rods that contained spores. The differences in these morphological features may be ascribed to the differences in bacterial genera/species, the environmental growth conditions and/or the adaptation to plant organs.

The growth forms of all isolates were rods with various types: single rods, rod diploids, short rods, rod chains and rod clusters. The single rods may indicate bacteria that are more independent in the plant tissue, while the rod clusters and chains may indicate bacteria that form colonies with close cell-to-cell relationships. Some endophytic bacteria may be able to survive in hostile environments by producing spores, as indicated by the presence of spores in isolate TR 4. The capacity of producing spores may aid the bacteria in surviving stressful conditions like drought or nutrient shortage in the plant environment (Hanaka et al. 2021). Zeigler and Perkins (2021) highlighted that all members of the genus *Bacillus* possess the capability of making dormant endospores aerobically when confronted with unfavourable growth conditions.

A wide variation in consistency among the isolates was noticed including creamy, mucoid and slimy (Table 1). The mucoid colony forming bacteria (SL 2 and AL 3) produce extracellular polysaccharides that can form biofilms for shielding the bacteria from environmental stress (Limoli et al. 2015). Slimy colonies (like SL 3) can be a sign of biofilm development that assist the bacteria to survive inside the plant tissues (Rajkumar et al. 2016).

Gram-positive bacteria were the most common endophyte group in the four medicinal plants as indicated by the fact that 31 isolates (91% of the all isolates) were Gram-positive (Table 1). This suggests that Gram-positive bacteria had superior ability to adapt to the internal environment of the investigated plants compared to their counterpart's Gram-negative bacteria that had a 9% abundance. The thick peptidoglycan layer found in the cell wall of Gram-positive bacteria may provide them more tolerance to environmental stresses, including defense systems found in plants (Lavigne et al. 2021). Several beneficial functions including plant growth promotion and defense against pathogens are linked to Gram-positive bacteria that also help plants withstand environmental stresses (Ryan et al. 2008, Lal and Tabacchioni 2009, Ek-Ramos et al. 2019). The higher abundance of Gram-positive bacteria observed in the present study concurs with Szymańska et al. (2016) who observed a greater percentage of Gram-positive endophytic bacteria (77.3%) in the roots of the halophyte *Salicornia europaea* sampled from the saline site.

Table 1: Morphological and biochemical characteristics of endophytic bacteria and their tentative genera identification

olate	Colour	Size	Margin	Cons	Texture	G	Shape	C	O	M	V	Possi
L 1	White	Large	Circular	Creamy	Flat	+	Single rod	+	+	+	+	*Bacillu*
L 2	Pale yellow shiny	Small	Circular	Mucoid	Convex	+	Short single rod	+	+	-	-	*Microb*
L 3	Pale yellow	Large	Lobate	Slimy	Raised	-	Rod cluster	+	+	-	-	*Pseudo*
L 4	Yellow	Small	Circular	Creamy	Raised	-	Short rod	+	-	-	+	*Enterob*
L 5	Cream	Large	Lobate	Slimy	Raised	+	Single rod	+	+	-	+	*Bacillu*
L 8	White	Large	Regular	Creamy	Flat	+	Rod diploid	+	+	-	+	*Bacillu*
L 9	Pale yellow	Small	Regular	Mucoid	Raised	+	Short rods	+	-	-	-	*Curtob*
R 1	Cream	Large	Lobate	Creamy	Raised	+	Single rods	+	+	-	+	*Bacillu*
R 2	Shiny white	Small	Regular	Creamy	Flat	+	Chain rods	+	+	-	+	*Bacillu*
R 3	White	Small	Regular	Creamy	Flat	+	Rod cluster	+	+	-	+	*Bacillu*
R 4	White	Small	Regular	Creamy	Flat	+	Rod	+	+	-	-	*Brevib*
L 1	White	Large	Lobate	Creamy	Flat	+	Single cluster	+	-	-	+	*Bacillu*
L 2	White	Small	Irregular	Creamy	Flat	+	Rod cluster	+	+	+	-	*Bacillu*
L 3	Shiny gold	Small	Regular	Mucoid	Convex	+	Short rods in cluster	+	+	-	-	*Microb*
L 4	Pale yellow	Small	Regular	Creamy	Flat	+	Short rods	+	+	-	-	*Microb*
R 1	Cream	Small	Regular	Mucoid	Convex	+	Short rods	+	-	-	+	*Bacillu*
R 2	Pale yellow	Large	Regular	Mucoid	Convex	+	Short rods	+	-	-	+	*Bacillu*
R 3	Translucent white	Small	Regular	Mucoid	Convex	+	Rods in cluster	+	-	-	+	*Bacillu* *Lysinib*
L 1	Shiny gold	Small	Regular	Mucoid	Convex	+	Single Rod	+	-	+	+	*Bacillu*
L 2	White	Large	Regular	Mucoid	Raised	+	Short rods in cluster	+	-	+	-	*Bacillu*
L 3	White	Small	Regular	Mucoid	Convex	+	Short rods	+	-	+	-	*Bacillu* *Paenib*
L 4	Translucent white	Small	Regular	Mucoid	Flat	-	Short rods	+	+	-	-	*Pseudo* *Enterob*
R 1	White	Large	Irregular	Slimy	Raised	+	Rod diploid	+	+	-	-	*Bacillu*
R 2	Pale yellow	Large	Lobate	Slimy	Raised	+	Rod enclosed	+	+	+	+	*Bacillu*
R 3	Gold	Small	Regular	Mucoid	Convex	+	Short rods clustered	+	-	-	+	*Bacillu* *Microb*
R 4	Pale yellow	Small	Regular	Creamy	Flat	+	Rod single	+	+	-	+	*Bacillu*
L 1	White	Large	Irregular	Creamy	Flat	+	Rods, cluster	+	+	+	-	*Bacillu*
L 3	Translucent	Large	Irregular	Mucoid	Flat	+	Short rods	+	-	-	-	*Bacillu* *Curtob*
L 4	White	Large	Regular	Creamy	Flat	+	Rods in chain	+	+	+	-	*Bacillu*
L 5	Pale yellow	Large	Regular	Creamy	Flat	+	Single rods	+	+	+	-	*Bacillu*
R 1	Gold	Small	Regular	Creamy	Flat	+	Short rods	+	+	-	-	*Microb*
R 2	Cream	Small	Regular	Creamy	Flat	+	Rods in chain	+	+	-	+	*Bacillu*
R 3	Yellow	Small	Regular	Mucoid	Flat	+	Short rods	+	-	-	+	*Bacillu*
R 4	Translucent white	Small	Regular	Mucoid	Flat	+	Rods single, spores	+	+	+	-	*Bacillu*

Key:

- Cons = Consistency; G = Gram staining; C = Catalase test; O = Oxidase test; M = Methyl red test; V = Voges-Proskauer.

- First letter (in sample names) stands for a host plant species: S = blue snake weed (*Stachytarpheta jamaicensis*); A = avocado (*Persea americana)*; B = banana (*Musa acuminata*); T = tropical almond (*Terminalia catappa*).
- Second letter in sample names stands for the host plant organs; R = root; L = leaf.

In the present study, four biochemical tests i.e.; catalase, oxidase, methyl red and Voges Proskauer tests, were used to assess the biochemical traits of the endophytic bacteria, and classify them based on these traits. The results showed that all the bacterial isolates tested positive for the catalase test (Table 1; Figure 3), indicating that they were capable of breaking down hydrogen peroxide (a free radical species generated during aerobic respiration) into water and oxygen.

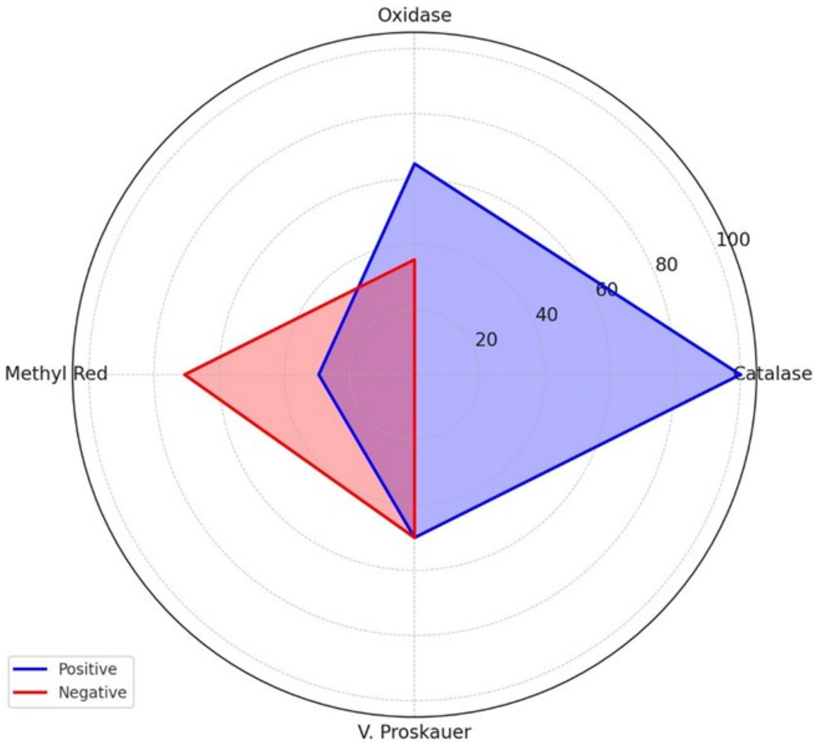

Figure 3: The radar chart displaying the results for the catalase, oxidase, methyl red and Voges-Proskauer (V. Proskauer) tests among the isolates with the blue and red areas representing the percentages for the positive and negative results, respectively.

Catalase positivity is common among aerobic and facultative anaerobic bacteria, suggesting that all the isolates were capable of surviving in environments with molecular oxygen as they possessed mechanisms to shield their bodies from harmful hydrogen peroxide generated from aerobic respiration. The universal catalase activity among the isolates points to a common metabolic trait among the endophytic bacteria from the different species and/or organs of the investigated medicinal plants.

The oxidase enzyme (cytochrome c oxidase) involved in the electron transport chain was detected (oxidase positive) in the majority of the isolates (64.70%; Figure 3) indicating that many of these isolates respire aerobically. Nonetheless, the fact that 35.29%

of the bacteria were oxidase-negative indicates that the isolates' respiratory systems varied, with some depending on fermentation or anaerobic respiration. Presence of both negative and positive oxidase endophytic bacteria in this study correlates to the findings by Lui and Jurtshuk (1986) who performed oxidase test in 342 bacillus strains and found that 68% of the thermophilic group were oxidase positive.

The methyl red test was employed to assess the capability of the isolates to produce stable acids from glucose fermentation. The results indicated that 29.41 and 70.58 % of the isolates tested positive and negative, respectively (Figure 3). This indicates that the minority (29.41%: methyl red positive) of the studied endophytes produced significant amounts of stable acidic by-products from glucose metabolism while the majority (70.58%: methyl red negative) did not produce stable acids as they utilized alternative metabolic pathways such as butanediol fermentation instead of mixed acid fermentation (Van Houdt et al. 2007). Contrary to the present study, Kumar et al. (2020) noticed that 83.7% of the 27 isolates evaluated in their study were methyl red positive, with the majority of them being from the genus Bacillus.

The Voges-Proskauer test detects the production of acetoin, a precursor in the butanediol fermentation pathway. Half of the isolates (50%; Figure 3) tested positive, which agrees with the observation that many bacteria might be using butanediol fermentation rather than producing stable acids. The even split between positive and negative results suggests that there was a diverse range of fermentative abilities among the isolates with some producing neutral by-products rather than acidic ones.

The morphological and biochemical characteristics identified these endophytic bacteria as belonging to *Bacillus* (21), *Lysinibacillus or Bacillus* (2), *Brevibacterium* (1), *Bacillus* or *Curtobacterium* (1), *Curtobacterium* (1), *Enterobacter* (1), *Microbacterium* (4), *Bacillus or Microbacterium* (1), *Pseudomonas* (1) and *Pseudomonas* or *Enterobacter* (1) genera (Table 1). The highest frequency of *Bacillus* in the identified endophytic bacteria points to its critical role in plant-microbe relationships and marks it as an excellent option for biotechnological usage.

Morphological and biochemical relatedness of endophytic bacteria

The first three principal axes of the 3D PCoA biplot accounted for 39.37% of the total variability in the dataset but did not reveal clear separation among bacterial endophytes isolated from different host plant species or organs (leaves and roots) (Figure 4). Isolates from leaves (marked red) and roots (marked blue) were intermixed, with no distinct clusters based on plant species. Distinct shapes—circles, diamonds, rectangles, and triangles—representing bacterial isolates from different tree species were scattered across the plot, indicating no clear grouping based on host species. The admixture observed could be due to the similar environmental conditions such as temperature, atmospheric pressure and humidity across the investigated plants that might have led to similar selective pressures on the studied endophytic bacteria resulting in convergent evolution of the studied traits.

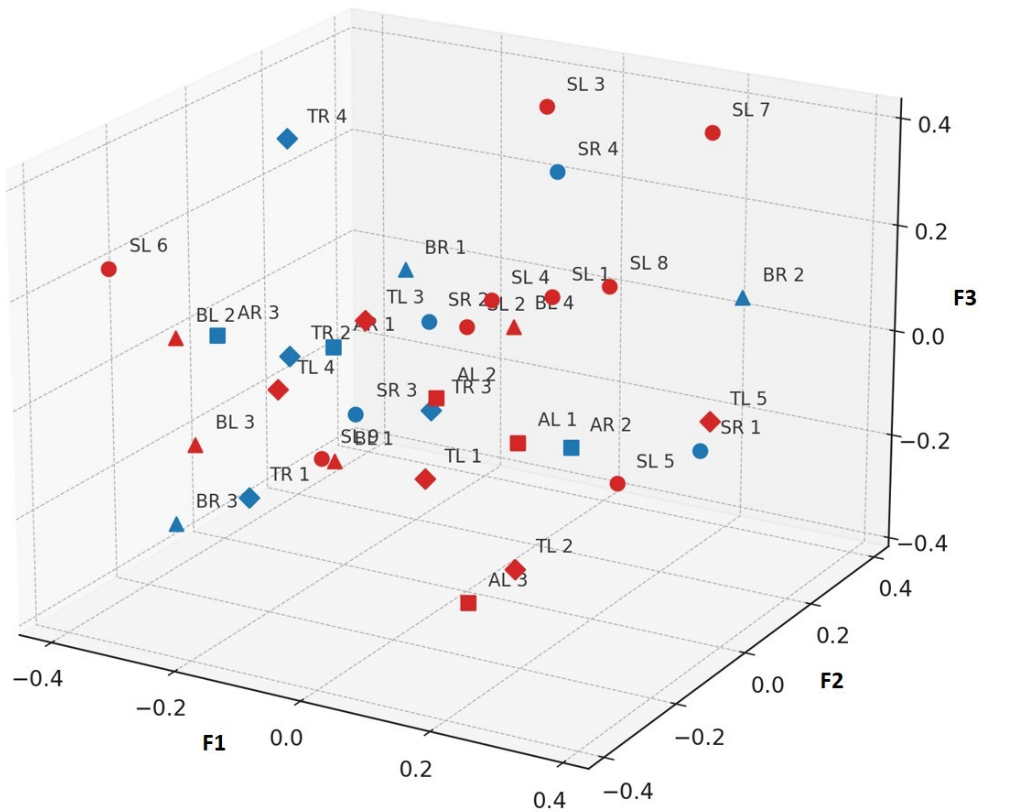

Figure 4 PCoA plot displaying the morphological relatedness of the endophytic bacteria isolated from roots (in blue) and leaves (in red) of the four medicinal plants represented by distinct shapes (circle = blue snake weed; triangle = banana; square = avocado; diamond = tropical almond).

For example, bacteria from the leaves of different plants might face similar challenges, such as ultraviolet exposure or desiccation, leading to similar adaptations/traits and thus the overlapping clusters. Moreover, exchange of genetic material among the isolates residing in different parts of the same plant through horizontal gene transfer, could blur the distinctions between species or environments (leaf versus root). This could lead to bacteria from different parts of the plant sharing similar traits contributing to the observed admixture. Likewise, some endophytic bacteria might be capable of thriving in various environments within the plant (both leaves and roots) and across the different medicinal plant species investigated. These generalist bacteria would not form distinct clusters but would instead be spread across the plot contributing to the observed admixture. Another possible reason is the interactions (example: competition and symbiosis) between various endophytic bacterial species that could influence their traits in ways that make them less distinct. For instance, bacteria from the root might share traits with leaf-associated bacteria due to similar nutrient requirements or other interactions.

The MCA was employed to illustrate the relationships between endophytic bacteria based on their biochemical properties, and to visualize variable associations. MCA categorised the isolate into seven groups irrespective of their origin, with the first two principal axes (F1 and F2) of the biplot accounted for 77.43% of the all variability in the biochemical dataset (Figure 5). The

Voges-Proskauer (V. Proskauer) negative, oxidase positive and methyl red positive bacteria were located to the right of the F1 axis, whereas those which are Voges-Proskauer (V. Proskauer) positive, oxidase negative and methyl red negative were located at the left of the F1 axis. The F2 axis discriminated the isolates based on Methyl Red and Oxidase tests of which the isolates in the lower quadrants typically exhibited Methyl Red Negative and Oxidase positive, whereas those in the higher quadrants were Methyl red positive and Oxidase negative. Since all the isolates exhibited a common catalase activity (catalase positive), the catalase biochemical test results were not employed in delineating the isolates along either axis. No clear separation was noticed among the endophytes with respect to the plant type and/or part (leaves vs. roots), suggesting that the isolated endophytic bacteria shared some biochemical traits regardless of their host plants species/organ.

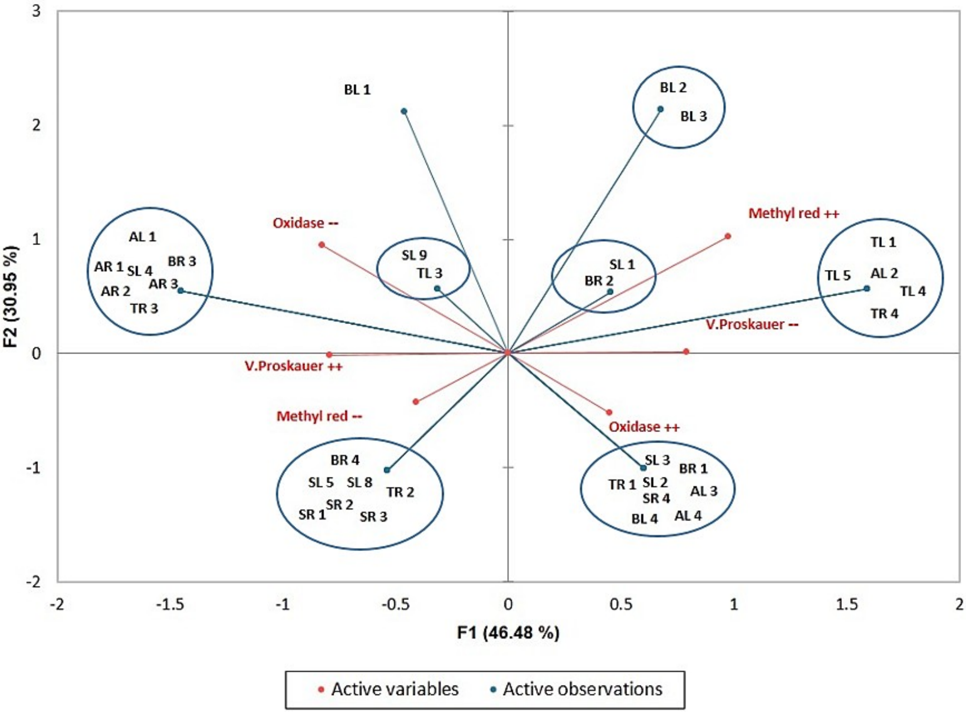

Figure 5: Multiple Correspondence Analysis (MCA) biplot illustrating the relatedness of the endophytic bacterial isolates based on biochemical test outcomes (a positive (++) or negative (--) outcomes).

To assess the phenotypic relationships and overall similarity among the bacterial isolates, hierarchical cluster analysis (HCA) was conducted using a combined dataset of morphological and biochemical traits. The dendrogram grouped the endophytic bacterial isolates into two major clusters (marked red and blue) irrespective of their host plant species, plant organ, or their presumed genera (Figure 6). Isolates from different tree species —indicated by the first letter in sample IDs (A = avocado, S = Blue snake weed, B = Banana, T = Tropical almond)—were distributed throughout the dendrogram without forming distinct clusters as per their host plant species. For example, at the top of dendrogram, isolates from avocado (AL3), blue snake weed (SL2), banana (BR1 and BL2), and tropical almond (TL4) were clustered together, suggesting a lack of host-specific phenotypic or biochemical traits.

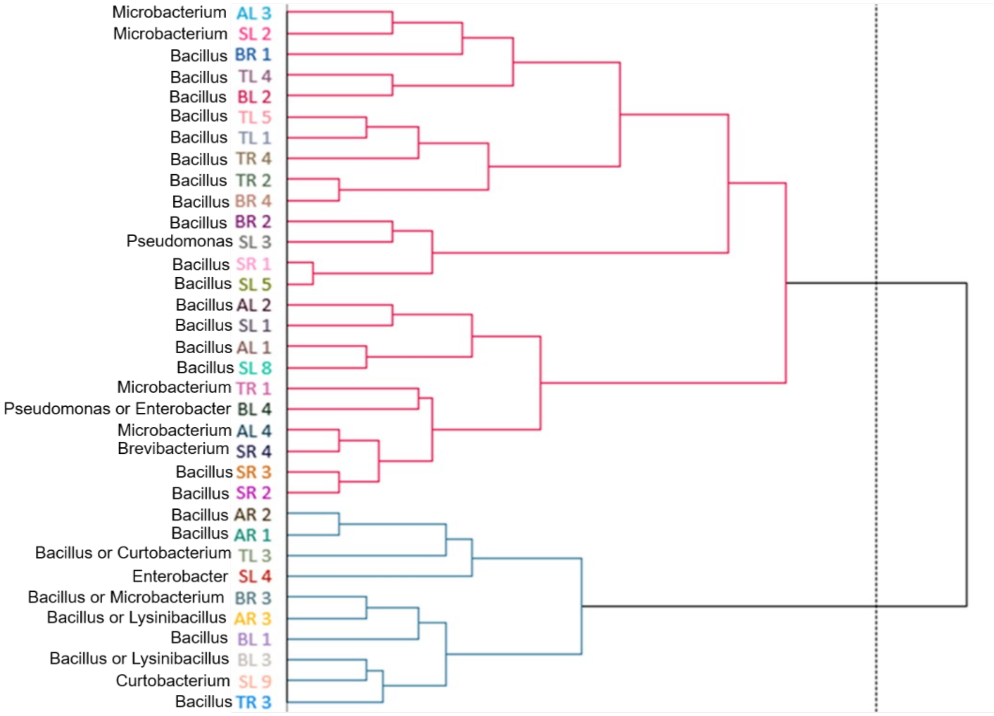

Figure 6: Dendrogram illustrating the hierarchical clustering of endophytic bacteria isolated from different plants and plant parts. The first letter in the sample names indicates the plant species (S = blue snake weed (*Stachytarpheta jamaicensis*); A = avocado (*Persea americana*); B = banana (*Musa acuminata*); T = tropical almond (*Terminalia catappa)* and the second letter signifies the plant organ (L = leaf; and R = root).

Similarly, leaf isolates (denoted by "L" in sample IDs) and root isolates (denoted by "R") were interspersed across the dendrogram. An example for this is root isolates (TR3, AR3 and BR3) that were found to cluster with leaf isolates (SL9, BL3 and BL1) at the bottom of the dendrogram. The mixing of leaf and root isolates across the dendrogram suggests that the endophytes isolated from different plant parts in this study shared overlapping traits, and thus their colonization ability was not exclusive to a specific plant part. Moreover, the presence of isolates of the same genus (such as *Bacillus*) across different clusters on the dendrogram is a reflection of the broad morphological and biochemical diversity that is characteristic of these genera. For example, isolates that were identified as *Microbacterium* (e.g. AL3, SL3, TR1 and AL4) are present in different parts of the dendrogram. Overall, clustering was mainly based on shared phenotypic and biochemical traits, rather than on plant species, tissue origin, or the taxonomy of the isolates, which further underlines the importance of using molecular identification tools such as 16S rRNA gene sequencing for accurate identification and classification.

Conclusions

A total of 34 bacterial isolates were obtained from root and leaf samples of the four medicinal plants and were tentatively identified as belonging to seven genera: *Bacillus*, *Lysinibacillus*, *Brevibacterium*, *Curtobacterium*, *Enterobacter*, *Microbacterium* and *Pseudomonas*. PCoA, MCA and HCA showed that isolates did not form distinctive clusters based on the host plant species, plant organ or their presumed genera. This finding indicates that there was a high overlap of morphological and biochemical characters among the isolates obtained from different plant species and organs. This may suggest that these endophytes are generalists and have functional redundancy to colonize different plant hosts and tissues. The research reveals previously unknown information about trait-based similarities among endophytic bacteria found in medicinal plants within Tanzania. Knowing more about their ecological functions such as nutrient acquisition, plant growth promotion and stress tolerance can be beneficial for sustainable agriculture, food security and pharmaceutical applications. However, their full potential can only be unlocked by conducting molecular characterisation and metabolite profiling to obtain accurate taxonomic identification and identify their biotechnologically relevant functions.

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