e-ISSN: 2637-0611

Available online at
https://journal.uitm.edu.my/ojs/index.php/COS/index

Compendium of Oral Science 12(1) 2025, 96 - 109

Compendium of Oral Science

Antibacterial Activity of Endophytic *Streptomyces* spp. isolated from Medicinal Plants of Dental Importance

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ARTICLE INFO

Article history:
Received 12 April 2024
Revised 22 June 2024
Accepted 25 August 2024
Online first
Published 01 March 2025

Keywords:
endophytes
streptomycete
medicinal plants
antibacterial

DOI:
10.24191/cos.v12i1.5652

ABSTRACT

Wide variations of plant species in Malaysia provide suitable hosts for the isolation of endophytic streptomycetes, which can be potential sources of bioactive compounds with therapeutic applications in dentistry.

Objectives: This study's objectives were to isolate endophytic streptomycetes from medicinal plants and to evaluate their antibacterial activity against *Streptococcus mutans*, a key pathogen responsible for dental caries.

Methods: Endophytic streptomycetes were isolated from seven fresh plants: *Mauritius papeda* (Kaffir lime), *Cosmos caudatus* (Ulam Raja plant), *Lawsonia inermis* (Henna plant), *Piper sarmentosum* (Kadok), *Kaempferia galanga* (Cekur plant), *Ziziphus mauritiana* (Bidara plant), and *Psidium guajava* (Guava plant), using a surface-sterilization method. Different plant parts were placed on five isolation media, namely Water Agar, International Streptomyces Project (ISP) 2, ISP 4, ISP 5, and Tap Water Yeast Agar, and incubated at 37°C for up to one month. Isolates were identified by morphological characteristics and tested for antimicrobial activities through a cross-streak assay.

Results: A total of 38 endophytes were successfully isolated, with 19 identified as streptomycetes. Specifically, seven, two, five, and three endophytes were isolated from stem, root, leaf, and fruit, respectively. Three endophytic streptomycetes exhibited antagonistic activity against *S. mutans*.

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Conclusion: Endophytic streptomycetes demonstrated antibacterial activity against *S. mutans*, indicating their potential for further development in dental applications.

1. INTRODUCTION

Endophytes are organisms that can be found residing inside living tissue of host plants specifically in the tissue beneath the epidermal cell layers. The organisms are symbionts and non-pathogenic bacteria that do not visibly harm the host plant. Approximately 300,000 plant species that exist on the earth are host to one or more endophytes (Strobel & Daisy, 2003). Endophytes are widely found in various parts of a plant such as buds, leaves, stems, bark, fruits, and roots. Some of these endophytes may be producing bioactive substances involved in a host-endophyte relationship as a result of secondary metabolites that may be shown to have applicability in medicine.

The actinomycetes, particularly Streptomyces spp. are generally saprophytic bacteria, soil-dwelling microorganisms that spend most of their life cycle as spores. Endophytic bacteria can be isolated from surface-sterilized plant tissue or extracted from the plant. A total of 131 endophytic actinomycete strains were successfully isolated from surface sterilized banana roots and these isolates belong mainly to Streptomyces (Cao et al, 2005). Furthermore, metabolites produced by 12 endophytic streptomycetes showed antagonistic activity to bacteria. *Bacillus subtilis* and *Escherichia coli* were resistant to the products of all 56 isolated streptomycetes (Cao et al., 2004). The defensive effect could be achieved by root actinomycetes both by acting as competitors and by producing antibiotics and antifungal substances.

In Malaysia, about 2000 medicinal plant species are found to have health-benefit properties (Latif,1997). Research on chemical compounds found in plants has increased rapidly worldwide with numerous studies showing large potential for their use as antimicrobial agents. Thus, medicinal plants such as *Mauritius papeda* (Kaffir lime), *Cosmos caudatus* (Ulam raja plant), *Lawsonia inermis* (Henna plant), *Piper sarmentosum* (Kadok), *Kaempferia galangal* (Cekur plant), *Ziziphus mauritiana* (Bidara plant) and *Psidium guajava* (Guava plant) were taken into consideration for their known therapeutic properties in traditional used and their antimicrobial activity possess in some studies.

Studies show volatile oil from the *M. papeda* peel exhibits antimicrobial activity against *Bacillus subtilis, Escherichia coli, Salmonella typhimurium,* and *Staphylococcus aureus* (Srisukha et al., 2012). Ethyl acetate extract from *M. papeda* shows broad-spectrum inhibitory activity against Gram-negative, and Gram-positive bacteria, yeast, and mold tested (Chanthaphon et al., 2007). The oil of *K. galangal* possessed marked antimicrobial activity against Gram-positive bacteria with inhibition zones from 12.0-16.0 mm, and 8.0-12.0 mm. against Gram-negative bacteria, whereas it potently inhibited *C. albicans* with an inhibition zone of 31.0 mm (Tewtrakul et al., 2005). Methanol extracts from *K. galangal* showed significant antibacterial activity against E. coli of 15 mm zone of inhibition and *Salmonella* sp. but not *Shigella* sp., *Bacillus* sp., and *Pseudomonas* sp. (Parvez et al., 2005). Rasdi et al (2010) assessed the effects of different *C. caudatus* extracts of n-hexane, diethyl ether, and ethanol against five microbial strains, *B. subtilis, S. aureus, E. coli, Pseudomonas aeruginosa,* and *C. albicans*, and all extracts exhibit significant inhibitory activity against the growth of microbes. These results indicate that *C. caudatus* extracts have highly anti-microbial and anti-fungal properties.

Furthermore, the application of a paste made from dried and powdered leaves of *L. inermis* is a method developed by rural people for burn wound management. Hence the effects of water and chloroform extracts of *L. inermis* leaves against primary invaders of burnt wound was investigated. Muhammad et al. (2005), showed that *L. inermis* leaf extracts possess antibacterial activity when the zone of inhibition is greater than

https://doi.org/10.24191/cos.v12i1.5652

6 mm were recorded in a study. The common part of plants used for *P. sarmentosum* is its leaves. Methanolic leaf extracts of *P. sarmentosum* can provide an alternative to current antibacterial options (Rahman et al., 2014).

The Golden Burden of Disease Study, 2016 estimated that dental caries in permanent teeth being the most prevalent condition assessed affected half the world's population (3.58 billion people worldwide). Based on the Modified Keyes-Jordan Diagram, dental caries results when microbial biofilm (plaque) formed on the tooth surface converts the free sugars contained in foods and drinks into acids that dissolve tooth enamel and dentine over time. Thus, this shows that bacteria are one of the causes of caries.

Key oral pathogens include *Streptococcus mutans* and *Porphyromonas gingivalis*. *S. mutans* is a primary etiological agent in dental caries due to its ability to produce acids that demineralize tooth enamel, while *P. gingivalis* is implicated in periodontal diseases. These pathogens have been extensively studied for their role in oral health, as discussed by Loesche (1986). *S. mutans*, a Gram-positive bacterium, is a primary causative agent of dental caries due to its ability to metabolize sucrose and produce lactic acid, which demineralizes tooth enamel. This cariogenic potential has been extensively documented, as reviewed by Loesche (1986). *P. gingivalis,* on the other hand, is a Gram-negative anaerobic bacterium associated with periodontal diseases. It contributes to tissue destruction through proteolytic enzymes and induces an inflammatory response, further exacerbating periodontal conditions. These pathogens represent targets for novel antimicrobial strategies, including those leveraging endophytic *Streptomyces* spp.

Loesche et al (1986) mentioned that the primary habitats for *S. mutans* are the mouth, pharynx, and intestine. *Streptococcus mutans* and *Streptococcus sobrinus* have a central role in the etiology of dental caries because these can adhere to the enamel salivary pellicle and other plaque bacteria (Davey et al., 2000). Tanzer et al (2001) stated that *mutans streptococci* and *lactobacilli* are strong acid producers and hence cause an acidic environment creating the risk for cavities. The appearance of *S. mutans* in the tooth cavities is usually followed by caries after 6-24 months (Mayooran et al., 2000).

Endophytes have potential impacts on oral flora, including both pathogenic and beneficial microbes. Studies have shown that endophytic metabolites can inhibit biofilm formation by pathogenic bacteria without significantly disrupting beneficial microbes. This dual action is crucial for maintaining oral homeostasis (Strobel and Daisy, 2003).

Endophytes can exert selective antibacterial effects that may help maintain a balanced oral microbiome. While their metabolites can inhibit biofilm formation and reduce the growth of pathogenic bacteria such as Streptococcus mutans, they may also spare or have minimal impact on beneficial oral microbes. This selective action is particularly important for oral health as it helps preserve normal flora while targeting harmful pathogens. Strobel and Daisy (2003) noted that endophytic metabolites often exhibit specificity, which can be advantageous for developing targeted oral therapeutics. Such specificity minimizes the risk of dysbiosis and supports the concept of precision antimicrobial therapy in dentistry.

https://doi.org/10.24191/cos.v12i1.5652

2. MATERIALS AND METHOD

2.1 Collection of the material

Fresh seven medicinal plants namely, *Mauritius papeda* (Kaffir lime), *Cosmos caudatus* (Ulam raja plant), *Lawsonia inermis* (Henna plant), *Piper sarmentosum* (Kadok), *Kaempferia galangal* (Cekur plant), *Ziziphus mauritiana* (Bidara plant) and *Psidium guajava* (Guava plant) were collected from a credible source with Global Positioning System (GPS) coordinate as stated, Ulam Raja (3.2106128,101. 7526205), Kaffir Lime (3.2160916,101.7492935), Kadok (3.1253367,101.7418860), Henna Plant (3.1251302,101.7415832) and Cekur (3.2270418,101.7478646). Different samples were gathered from different parts of each plant such as from Kaffir Lime (leaf, stem, fruits, and root), Henna plant (leaf, stem, and root), Ulam Raja, leaf, flower, stem, and root, Cekur, root and leaf, Kadok leaf, stem, and root sample were collected, placed in paper bags and were taken to the laboratory and processed within 4 hours.

2.2 Isolation of endophytes

Plant samples were washed thoroughly with tap water to remove soil and organic matter. It was then sonicated for 60 seconds and air-dried. The samples were prepared in two sets. All first sample sets were crushed and stored in sterile distilled water. The second set was surface-sterilized by sequential immersion in 70% (v/v) ethyl alcohol for 60 seconds, 3.125% sodium hypochlorite solution for 6 minutes, and 70% (v/v) ethyl alcohol for 30 seconds. Then, samples were rinsed in sterile distilled water twice to remove surface sterilization agents. Each sample (root, stem, fruit) was divided into small fragments and sectioned into small pieces with a sterile scalpel. Other samples (leaf and flower) were crushed aseptically using a sterile mortar and pestle in 5 mL sterile phosphate buffer. Prepared samples of each plant were placed onto five isolation media (Water Agar, Internationalized Streptomyces Project (ISP) 2, ISP 4, ISP 5, and Tap Water Yeast Agar). Each media was supplemented with 50 mg/L nalidixic acid and 100 mg/L nystatin as antibacterial and antifungal agents. Plates were incubated at 37 °C for up to 8 weeks.

2.3 Identification of endophytes

The isolated strains were purified and identified as different groups based on their cultural and morphological characteristics on ISP2 media. Characterization of the actinomycetes was based on the observation of their mycelial structure, spore mass color, diffusible pigment color, and sporophore and spore chain, according to Bergey's manual of systematic bacteriology. The isolates were stained by Gram stain for the configuration of the spore chain.

2.4 Antimicrobial activities

(i) Preparation of bioassay plates

Screening of endophytic actinomycetes was done by cross streak method on ISP 2 media. The isolates were streaked on the media as a straight line in one side corner of the petri dish plate and incubated at 37°C for 14 days.

(ii) Antimicrobial assay

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Test bacteria Streptococcus mutans were incubated overnight in ISP 2 media, at 37°C. The next day, S. mutans were streaked at a right angle perpendicular to actinomycetes isolates and incubated at 37°C for 24 hours.

(iii) Percentage of inhibition

The percentage inhibition was measured and recorded as total inhibition of growth (TIG), growth retardation (GR), and no inhibition (NI). Results were tabulated. Percentage of inhibition(%) were calculated as:

$$Percentage\ inhibition\ (\%) = \frac{Length\ of\ inhibition}{Total\ length\ of\ streaking} \times 100$$

3. RESULTS

3.1 Isolation of endophytic streptomycetes

A total of 38 endophytic streptomycetes species were isolated from the root, stem, leaf, root, fruits, and flower of seven medicinal plants from different mediums. The presence of endophytic streptomycetes in various parts of different medicinal plants is shown in Table 1. It is observed that *Piper sarmentosum* (Kadok) yielded a maximum number of isolates, a total of 17 isolates, 13 from the root, 1 from the stem, and 3 from the leaf. The host-endophyte relationship varies from host to host and endophyte to endophyte. The number of isolates from root, stem, leaves, and fruit from different isolation mediums is presented in Table 2. Isolates from ISP 2, ISP 5, WA, and TWYE made up of 18, 2, 15, and 3 in total, respectively.

Table 1. Isolates of endophytic streptomycetes from the root, stem, leaf, fruit, and flower of seven medicinal plants.

No.	Medicinal plants	Root	Stem	Leaf	Fruit	Flower	Total
		Number of isolates					
1	Mauritius papeda (Kaffir lime)	-	-	1	5	-	6
2	Cosmos caudatus (Ulam raja plant)	-	-	-	-	-	-
3	Lawsonia inermis (Henna plant)	2	3	1	-	-	6
4	Piper sarmentosum (Kadok)	13	1	3	-	-	17
5	Kaempferia galangal (Cekur plant)	-	-	-	-	-	-
6	Ziziphus mauritiana (Bidara plant)	4	-	1	-	-	5
7	Psidium guajava (Guava plant)	4	-	-	-	-	4

3.2 Identification of isolates

A total of 35 isolates were identified by Gram staining. The Gram staining done on the prepared slide culture found that all 35 isolates were Gram-positive bacteria as shown in (Table 3). 19 isolates exhibit filamentous gram-positive features. A total of six, six, three, and one isolates are rod, coccus, diplococcus, and streptococcus in shape, respectively.

3.3 Percentage of inhibition

Antibacterial activity test of the isolated endophytic Streptomyces spp. against *Streptococcus mutans* which the antagonistic activity of the isolates was done by cross streak method (Figure 1). Four isolates exhibit inhibition against *S. mutans* as shown in Table 4.

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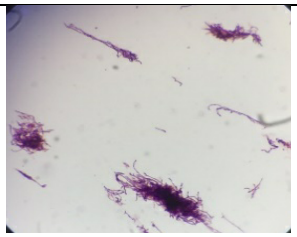
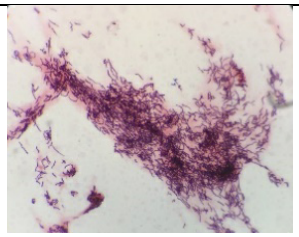
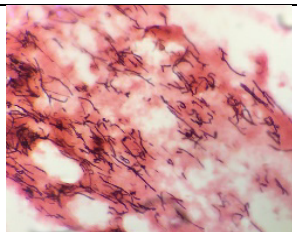
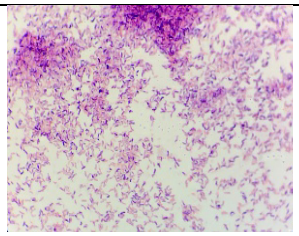
Table 2. Isolates of endophytic streptomycetes spp. and type of isolation medium.

No	Medicinal Herbs	Part	Isolation medium				
			ISP2	ISP4	ISP5	WA	TWYA
1	Piper sarmentosum (Kadok)	Root	4	-	1	7	1
2	Piper sarmentosum (Kadok)	Leaf	2	-	-	1	-
3	Piper sarmentosum (Kadok)	Stem	-	-	-	1	-
4	Mauritius papeda (Kaffir lime)	Fruit	1	-	-	2	2
5	Mauritius papeda (Kaffir lime)	Leaf	-	-	-	1	-
6	Lawsonia inermis (Henna plant)	Root	2	-	-	-	-
7	Lawsonia inermis (Henna plant)	Leaf	-	-	1	-	-
8	Lawsonia inermis (Henna plant)	Stem	-	-	-	3	-
9	Ziziphus mauritiana (Bidara plant)	Root	4	-	-	-	-
10	Ziziphus mauritiana (Bidara plant)	Leaf	1	-	-	-	-
11	Psidium guajava (Guava plant)	Root	4	-	-	-	-
	Total		18	-	2	15	3

Table 3. Gram Stain isolation identification.

PLATES	FINDINGS	PLATES	FINDINGS
1	 - Gram-positive - Network of branching filamentous shape	2	 - Gram-positive - Filamentous shape
3	 - Gram-positive - Filamentous shape	4	 - Gram-positive - Single rod

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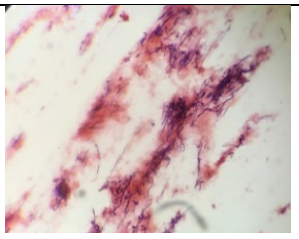
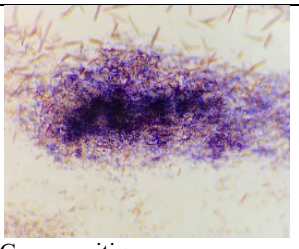
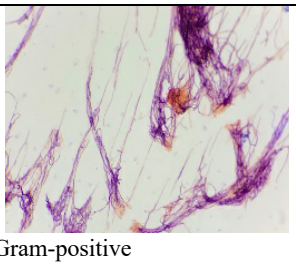
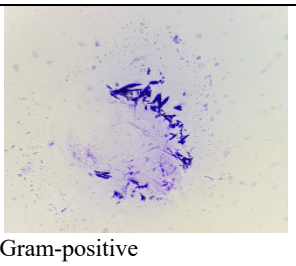
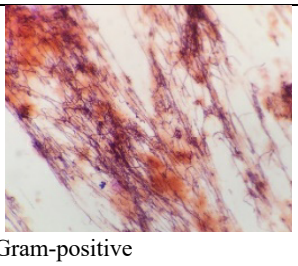
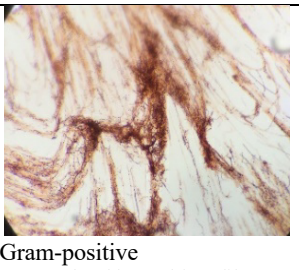
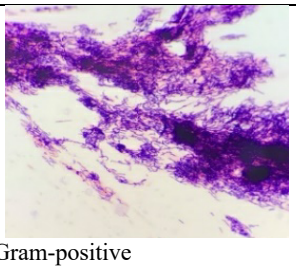
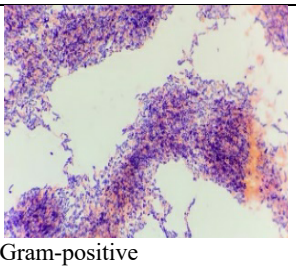
5	 - Gram-positive - Filamentous shape	**6**	 - Gram-positive - Cocci shape
7	 - Gram-positive - Network of branching filamentous shape	**8**	 - Gram-positive - Single rod
9	 - Gram-positive - Network of branching filamentous shape	**10**	 - Gram-positive - Network of branching filamentous shape
11	 - Gram-positive - Filamentous shape	**12**	 - Gram-positive - Diplococcus shape

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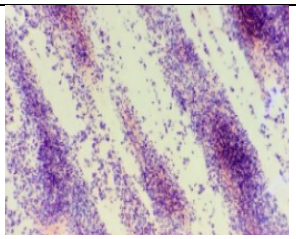
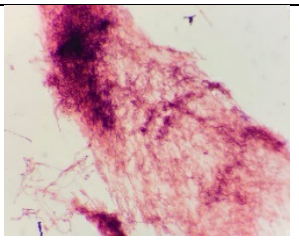
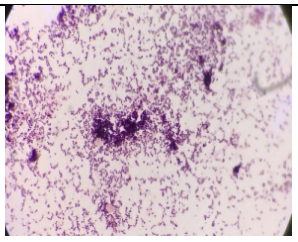
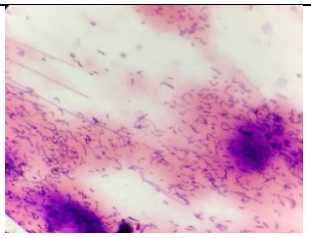
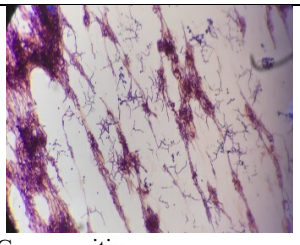
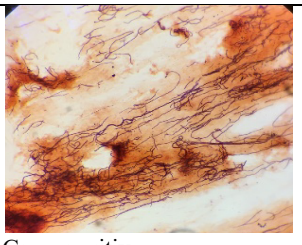
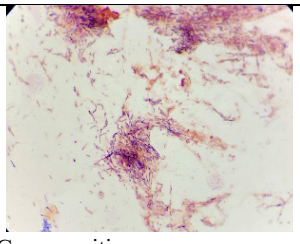
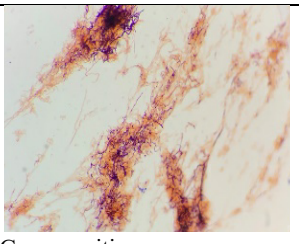
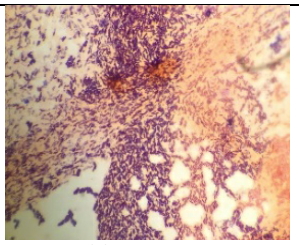
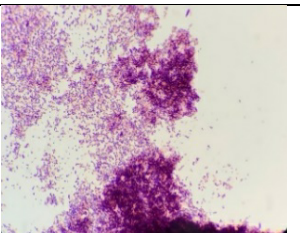
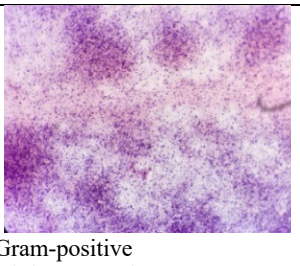
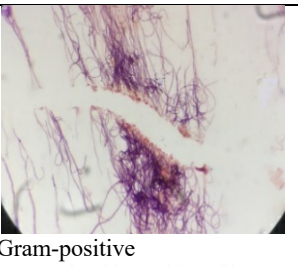
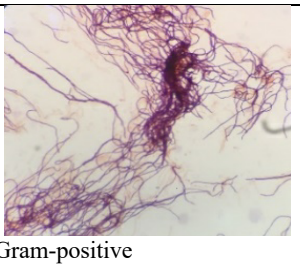
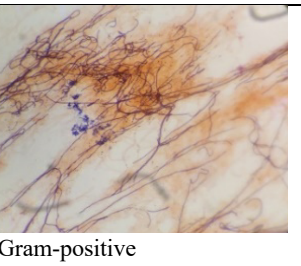
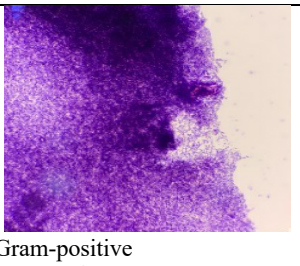
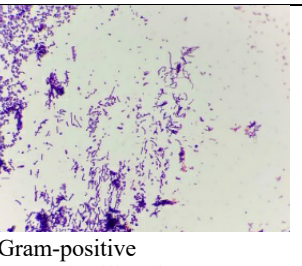
13	 - Gram-positive - Coccus shape	**14**	 - Gram-positive - Network of branching filamentous shape
15	 - Gram-positive - Streptococcus shape	**16**	 - Gram-positive - Filamentous shape
17	 - Gram-positive - Network of branching filamentous shape	**18**	 - Gram-positive - Network of branching filamentous shape
19	 - Gram-positive - Single rod shape	**20**	 - Gram-positive - Filamentous shape

https://doi.org/10.24191/cos.v12i1.5652

21	 - Gram-positive - Palisaded rod arrangement	**22**	 - Gram-positive - Diplococcus shape
23	 - Gram-positive - Diplococcus shape	**24**	 - Gram-positive - Network of branching filamentous shape
25	 - Gram-positive - Network of branching filamentous shape	**26**	 - Gram-positive - Network of branching filamentous shape
27	 - Gram-positive - Coccus shape	**28**	 - Gram-positive - Streptobacillus shape

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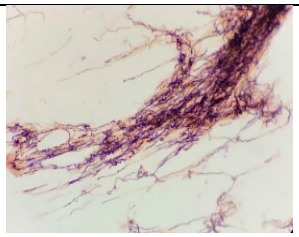
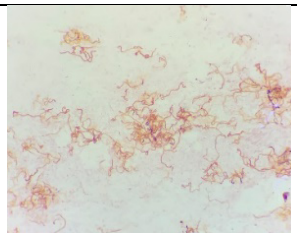
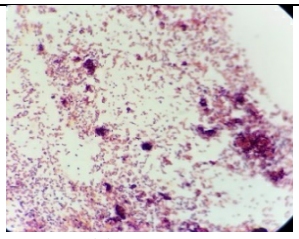
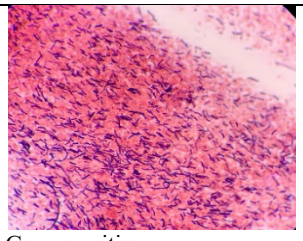
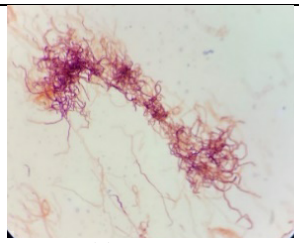
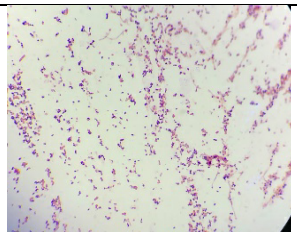
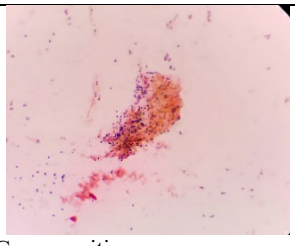
29	 - Gram-positive - Network of branching filamentous shape	**30**	 - Gram-positive - Filamentous shape
31	 - Gram-positive - Coccus shape	**32**	 - Gram-positive - Rod shape
33	 - Gram-positive - Network of branching filamentous shape	**34**	 - Gram-positive - Coccus shape
35	 - Gram-positive - Coccus shape		

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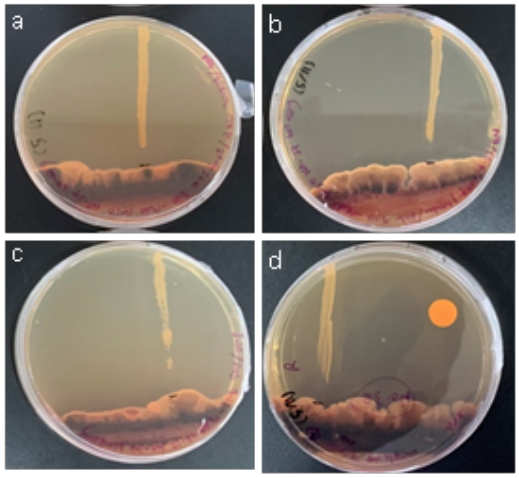

Fig. 1 Antagonistic activity of endophytic streptomycetes by cross-steak method against *Streptococcus mutans*. an endophyte isolated from the root of *Z. mauritiana*. an endophyte isolated from the root of *Z. mauritiana*; b, c & d endophyte isolated from the root of *Psidium guajava* showing significant antagonistic activity.

Table 4. Percentage of Inhibition.

No.	Medicinal herbs	Part	Percentage of Inhibition (%)
1	Ziziphus mauritiana (Bidara plant)	Root	13.11
2	Psidium guajava (Guava plant)	Root	14.55
3	Psidium guajava (Guava plant)	Root	18.64
4	Psidium guajava (Guava plant)	Root	11.32

4. DISCUSSION

The aim of this project was realized with the isolation of 38 endophytic streptomycetes spp. successfully isolated using the surface sterilization method from seven medicinal plants. From the results, ISP 2 media yielded the highest number of streptomycetes spp. of 18 isolates in total. Furthermore, the *Piper sarmentosum* (Kadok) plant exhibits the highest isolation of endophytic streptomycetes spp. from different parts of 17 isolates in total. From a total of seven medicinal plants, the plant part root has the highest occurrence of isolates of 23 in total.

Ikeda et al., 2003 said Streptomyces spp. are filamentous gram-positive bacteria and are not acid-alcohol fast, they are superficially identical and occur in the same habitats as fungi but are not fungi. From our study, a total of 19 isolates exhibit filamentous gram-positive streptomycetes spp. features.

Antagonistic activity of all 38 isolates was observed using a bioassay test. It has been shown that four isolates demonstrate antagonistic activity against *S. mutans*. Three isolates from the root of *Psidium guajava* (Guava plant) and one from the root *Ziziphus mauritiana* (Bidara plant).

Our results show that root isolates exhibited higher antibacterial activity compared to other plant parts. This observation could be attributed to the nutrient-rich microenvironment of the root system, which supports diverse and metabolically active endophytic communities (Nalini & Prakash, 2017). While endophytes target specific pathogens, their impact on the overall oral microbiome requires further investigation (Jannah et al. 2018). A balanced approach is necessary to ensure that beneficial microbes are

https://doi.org/10.24191/cos.v12i1.5652

not adversely affected. This aspect is critical for developing therapeutic agents that are effective yet safe for prolonged use.

The findings of this study emphasize the potential of endophytic *Streptomyces* spp. as a source of antibacterial agents, particularly against pathogens relevant to dental applications such as *S.mutans*. This aligns with research by Jannah et al. (2018), who demonstrated the efficacy of endophytic bacteria-derived compounds in inhibiting *S. mutans*. The antibacterial properties observed in this study are due to the metabolites produced by the endophytic Streptomyces spp., rather than the phytochemicals of the host plants. This aligns with findings by Strobel and Daisy (2003), who highlighted that microbial endophytes produce bioactive metabolites with therapeutic potential independent of their host plant's phytochemical composition.

The antibacterial properties observed in this study can be attributed to secondary metabolites produced by the endophytic Streptomyces spp., including antibiotics and antifungal compounds. In contrast to phytochemicals that are derived from the host plants, these microbial metabolites are produced through specific metabolic processes within the endophytes. Strobel and Daisy (2003) emphasized that endophytes are known to produce unique bioactive compounds not found in the host plants, which can include polyketides, non-ribosomal peptides, and alkaloids. This distinction highlights the independent and significant contribution of endophytes to antibacterial activity. Recent advancements in molecular techniques, such as metagenomics and transcriptomics, have enabled the identification of bioactive compounds and their mechanisms of action. For example, Moussa (2024) reported antimicrobial compounds from bacterial endophytes.

Building on the findings by Moussa (2024), future research should emphasize investigating the synergistic potential of endophytic bacterial metabolites in combination with conventional dental treatments. This synergy could significantly improve therapeutic efficacy, enhance clinical outcomes, and reduce the necessary dosages of standard antimicrobials, thereby mitigating potential side effects and promoting safer treatment protocols.

5. CONCLUSION

For future studies, the evaluation of the antibacterial activity of the successfully isolated endophytic streptomycetes by using minimum inhibitory concentration (MIC) and antibiofilm assay would be rewarding. The next challenge is to characterize the positive isolates using molecular study. The exact mechanism of all isolates and their long-term effects also need to be fully understood for the validity of their efficacy and safety.

ACKNOWLEDGMENTS

We thank the Research Unit, Faculty of Dentistry, UiTM Sg Buloh for good advice and guidance in the isolation process of the species and the support from the Faculty of Dentistry, UiTM Sungai Buloh.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial, or financial conflicts and declare the absence of conflicting interests with the funders.

https://doi.org/10.24191/cos.v12i1.5652

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