



## Isolation and morphological characterisation of endophytic bacteria isolated from the stem of *Leptadenia Pyrotechnica*

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### Abstract

The objective of this study was to isolate endophytic bacteria from the stem of *Leptadenia pyrotechnica*, their identification and investigation of their morphological characterisation. Fresh stem of *Leptadenia pyrotechnica* was procured from the department of botany, university of Rajasthan.

Endophytes are harmless organisms that reside in the tissues of their hosts. Their tight interaction causes them to develop secondary metabolites. It is thought that the bioactive substances found in endophytes cause less damage to normal cells. It is important to investigate endophytes and related compounds. The isolates' cultural traits on nutrient agar petriplates and slants differed greatly. On nutrient agar petriplates, the bacterial colonies had a creamy white appearance, a circular shape, and uneven or complete colony edges. The isolates processed different sources of carbohydrates. One isolate were tested gram negative and fifteen gram positive.

*Leptadenia pyrotechnica* is a remarkable desert shrub whose structural adaptations ensure survival in harsh, arid environments, while its extensive ethno botanical use and rich phytochemical composition reinforce its significant therapeutic and ecological value. Whether viewed through the lens of traditional medicine, environmental restoration, or drug discovery, it stands as a plant of considerable promise.

Our findings especially the strong Gram-positive dominance and unusual prevalence of *Streptococcus* largely align with patterns seen in desert plant endophytes, reinforcing the adaptability and environmental resilience of such communities. The disparity compared to more diverse medicinal plant systems highlights the uniqueness of *L. pyrotechnica*'s micro biome. The endophytes having circular forms 68.75%, entire margins 25%, convex elevation 37.50% large size character 81.25% predominated among all the isolates on nutrient agar plates. The endophytes having bacterial growth on nutrient agar slant moderate growth 68.75%, opaque optical character 25%, mucoid consistency 37.50%, filliform form 81.25% predominated among all the isolates.

**Keywords:** *Leptadenia pyrotechnica*, Endophytes, Host, Secondary metabolites, Bioactive substance etc

### Introduction

Microorganisms linked with plants known as endophytes live in their hosts' living tissues in a symbiotic relationship without causing any obvious harm (Tewari V. *et. al.* 2016) [17]. The ability of endophytes to produce a variety of secondary metabolites is made possible by their close relationship with their host plant. Because they do not affect the eukaryotic host system, it is believed that bioactive chemicals originating from endophytes will have a less harmful effect on normal cells. Consequently, it is important to investigate endophytes and related bioactive substances from their host plants (de Carvalho C.R. *et. al.* 2022).

*Leptadenia pyrotechnica* (Forssk.) Decne. is a leafless, perennial shrub belonging to the family Apocynaceae (formerly Asclepiadaceae), and is widely distributed across arid regions from Senegal through North Africa and the Arabian Peninsula to India (Schmelzer, G. H.; *et. al.* 2008) [15]. It is a shrub with many branches that is frequently upright and leafless. It thrives in hot, dry sandy areas with non-saline, rather deep sand. It is also referred to as markh, blazing bush, Khip, Khimp, and kheep (Masrahi Y. S. 2015, Norton, J. *et. al.* 2009, Vilchez J. I. *et. al.*, 2017, Booth F. E. *et. al.*, 1988, Najla A. *et. al.*, 2023) [4, 11, 12, 13, 18].

This species has a great deal of economic and medicinal value. *Leptadenia pyrotechnica* serves numerous traditional

roles across its native range. In the UAE, its whole plant, seeds, and flowers are used to treat conditions such as rheumatism, sciatica, backache, kidney stones, and have fumigant or anthelmintic properties; its dried seed hairs historically served as tinder hence the name "pyrotechnica". In parts of India, especially Rajasthan, infusions of the whole plant serve against uterine prolapse, stomach disorders, and constipation; and in the central Sahara, it's employed in treating fevers, kidney disorders, urinary ailments, and coughs (Sarjono R. P. *et. al.*, 2019) [14].

The plant is rich in diverse bioactive compounds—more than 200 phytochemicals have been recorded—including flavonoids, phenolic acids, steroidal and pregnane glycosides, alkaloids, fatty acids, terpenes, sterols, and more (Singh A. k. *et. al.*, 2017) [16].

### Methodology

#### Collection of plant Material

Fresh stems of *Leptadenia pyrotechnica* (photo shown in Fig 1) were collected from the Department of Botany, university of Rajasthan, Jaipur. Sample were immediately brought to the laboratory and were used within 24 hrs and finally processed for isolation of endophytic bacteria.

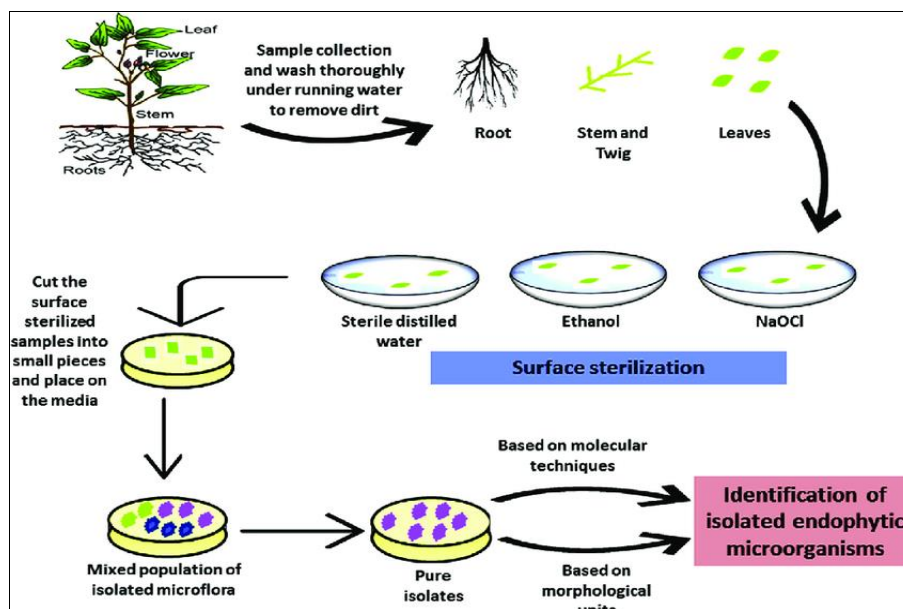


**Fig 1:** Stem of *Leptadenia pyrotechnica*

**Sterilization of stems**

Running water was used to thoroughly wash the plant sections in order to get rid of any associated trash and extra epiphytes. Plant tissues were then surface sterilized by immersion in 95% (v/v) ethanol for 10 seconds, 5% (v/v) sodium hypochlorite aqueous solution for 2-3 minutes, and 70% (v/v) ethanol for 2 minutes. In order to eliminate any remaining sterilants, the plant tissues were rinsed three times with autoclaved distilled water before being dried on aluminium foil to complete the surface sterilization. The procedure of surface sterilization was completed by rinsing the plant tissues three times with autoclaved distilled water

and then drying them on aluminium foil. Every stage of the sterilizing process was carried out in an aseptic environment. Plant explants that had been surface sterilized were divided into 2-centimeter segments using a sterile knife, and each segment was then put on nutrient agar media. Following sealing, plates were incubated. Incubators were used to incubate each plate in duplicate for 48 hours at 37 °C. Endophytic bacteria were seen to develop after incubation. The colonies were chosen on the basis of differences in size, colour, and morphology. Following two NA subcultures, they were kept in storage at 4 °C (Dragojević M. *et. al.*2023) [8].



**Fig 2:** Isolation and Identification procedure of endophytic bacteria

**Sterility check**

1 ml of the sterile distilled water used in the last rinse of surface sterilization processes was placed onto nutritional agar media and cultured for 24 hours at 37 ° C to verify that the stems' surfaces had been successfully sterilized. 24

hours later, bacterial growth was seen. Furthermore, stems that had been surface sterilized were rolled out on nutrient agar plates, incubated for 24 hours at 37°C, and examined for any signs of microbial growth.

### Preparation of Media

To prepare nutrient agar from ready-to-use powdered medium, follow the manufacturer's guidelines or standard laboratory protocols: begin by dissolving the specified amount of powder commonly 28 g per litre in approximately 800–900 mL of distilled water, using gentle heating and stirring until fully dissolved. Once dissolved, adjust the pH typically to  $7.4 \pm 0.2$  and then sterilize the solution by autoclaving at  $121^\circ\text{C}$  for 15 minutes.

### Inoculation of stems

The media was transferred to various autoclaved petri dishes. After autoclaving, cool the media to approximately  $45\text{--}50^\circ\text{C}$  before pouring it aseptically into sterile Petri dishes, allowing it to solidify before sealing and storing (typically at  $2\text{--}8^\circ\text{C}$  until use) and stems of the plants were embedded in petriplates.

The parameters used were gram staining, colony shape, growth manifestation, colony surface, colony elevation, border type, optical detail, and consistency. The experiment on morphological characterisation was run twice. The Gram stain test was conducted using the procedure outlined by Tortora *et al.* (2017). To ascertain the morphological traits (gram stain response, culture purity, and shape) of the isolates' pure colonies, the gram staining technique was used.

A common tool for comprehending the function of microorganisms in their ecological habitat is the selective culture medium. sixteen distinct endophytic bacterial strains that were obtained from the stem of *Leptadenia pyrotechnica*.

### Purification of endophytic bacteria

The primary method of subculturing for the purification of endophytic bacteria was streaking a loop full of nutrient agar plates.

### Observation

A total of 16 isolates were obtained from different stem samples of *Leptadenia pyrotechnica*. For identification of bacterial colonies raised on Nutrient Agar Media, bacterial endophyte had morphological characteristic like white colour, round shaped colonies, convex elevation, abundance of growth on Nutrient agar medium (Cappuccino J C *et al.* 1992) [5].

Plant associated bacteria are classified and identified mostly based on their physical and cultural traits. Both the environment and the genetic makeup of the organism have an impact on the phenotypic of the microorganism. The

capacity of an organism to endure, adapt, and acclimate to a variety of climatic circumstances is shown by phenotypic diversity.

### Cultural Characterization of Bacterial Isolates on Nutrient Agar Petriplates

The isolates exhibited wide morphological variation when grown on petriplates containing Nutrient agar. The colonies of all the isolates were small to medium in size and all produced creamy white pigmentation. The shape of the colony growing on nutrient agar medium in petriplates indicates that all the isolates produced circular colonies. The endophytes having circular forms 10 out of 16 (68.75%), entire margins 4 out of 16 (25%), convex elevation 6 out of 16 (37.50%) large size character 13 out of 16 (81.25%) predominated among all the isolates.

### Cultural characterization of bacterial isolates on nutrient agar slants

The 16 isolates were cultured on Nutrient agar slants as per the guidelines of Cappuccino and Sherman (1992) [5] (El-Fitany R. A. *et al.* 2022) [7, 9]. The cultural characteristics of the isolates for a single straight line of inoculation on the surface of Nutrient agar slants were evaluated by observing abundance of growth, optical character, consistency and form. The isolates varied widely in terms of amount of growth, optical character, consistency and their form on Nutrient agar slants. The amount of growth among the isolates varied widely, few endophytic bacterial isolates showed abundant growth while others showed slight and moderate growth. The endophytes having bacterial growth on nutrient agar slant moderate growth 8 out of 16 (68.75%), opaque optical character 13 out of 16 (25%), mucoid consistency 6 out of 16 (37.50%) filliform form 9 out of 16 (81.25%) predominated among all the isolates.

### Cultural characterization of bacterial isolates in nutrient broth

The 16 isolates were cultured on Nutrient Broth. Nutrient broth, essentially a liquid medium used to cultivate picky microorganisms for stock cultures, is composed of peptone and beef extract. Agar serves as a solidifying agent and facilitates the growth of bacteria that are not picky eaters. It is employed in the process of colony development.

### Gram's staining

Among the endophytes, Gram negative bacteria predominated; 1 out of 16 isolates (6.25%) were Gram negative.

**Table 1:** The physical attributes of endophytic bacteria that are present in *Leptadenia pyrotechnica*

Plate No.	Bacterial Growth on Nutrient Agar Plate				Bacterial Growth on Nutrient Agar Slant				Bacterial Growth on Nutrient Broth	Gram, s Staining	Result Species
	Size	Form	Margin	Elevation	Abundance of Growth	Optical Character	Consistency	Form			
JPR 1	Younger cultures being larger & older colonies shrinking	Circular	Undulate or fimbriate	Flate	Moderate	Opaque	Dry	Echumulate	Pellicle	Gram' s (+) ve	Bacillus
JPR 2	Large	Irragular	Undulate	Flate	Large	Opaque	Mucoid	Filiform	Uniform fine turbidity	Gram (+) ve	Pseudomonas

JPR 3	Moderate	Circular	Entire	Raised	Moderate	Opaque	Mucoid	Rhizoid	Pellicle	Gram (-) ve	Klebsiella
JPR 4	Small	Oval	Filamentous	Raised	Slight	Translucent	Mucoid	Echinulate	Sediment	Gram (+) ve	Streptococcus
JPR 5	Large	Circular	Lobate	Convex	Slight	Opaque	Smooth Buttery	Filliform	Sediment	Gram's (+) ve	Staphylococcus
JPR 6	Large	Circular	Lobate	Convex	Slight	Opaque	Smooth Buttery	Filliform	Sediment	Gram's (+) ve	Staphylococcus
JPR 7	Large	Circular	Undulate	Flate	Moderate	Opaque	Dry	Echinulate	Pellicle	Gram's (+) ve	Bacillus
JPR 8	Large	Circular	Lobate	Convex	Slight	Opaque	Smooth Buttery	Filliform	Sediment	Gram's (+) ve	Staphylococcus
JPR 9	Small	Oval	Filamentous	Raised	Slight	Translucent	Mucoid	Echinulate	Sediment	Gram (+) ve	Streptococcus
JPR 10	Large	Irregular	Undulate	Flate	Large	Opaque	Mucoid	Filiform	Uniform fine turbidity	Gram (+) ve	Pseudomonas
JPR 11	Large	Circular	Entire	Convex	Moderate	Opaque	Buttery	Filiform	Sediment	Gram (+) ve	Streptococcus
JPR 12	Large	Circular	Undulate	Flate	Moderate	Opaque	Dry	Echinulate	Pellicle	Gram's (+) ve	Bacillus
JPR 13	Large	Circular	Entire	Convex	Moderate	Opaque	Buttery	Filiform	Sediment	Gram (+) ve	Streptococcus
JPR 14	Large	Circular	Entire	Convex	Moderate	Translucent	Buttery	Filiform	Sediment	Gram (+) ve	Streptococcus
JPR 15	Large	Irregular	Undulate	Flate	Large	Opaque	Mucoid	Filiform	Uniform fine turbidity	Gram (+) ve	Pseudomonas
JPR 16	Large	Circular	Undulate	Flate	Moderate	Opaque	Dry	Echinulate	Pellicle	Gram's (+) ve	Bacillus

### Discussion

In a study of endophytic bacteria from neem (*Azadirachta indica*) leaves, isolates predominantly showed irregular colony shapes—84%—while only 16% were circular. Margin characteristics largely favored undulated margins (72%), with only 28% entire. Elevation was mostly flat (64%) with the rest raised (36%) (Agustien A. *et. al.*). Thus, in contrast to our findings, keeep isolates favored irregular shapes and undulated margins over circular, entire ones.

Another study on mangrove-derived endophytes reported mixed colony morphologies: two isolates had entire margins, while other colonies exhibited lobate margins. Some colonies were convex, others flat, and shapes ranged from circular to irregular (Bokhari A. *et. al.* 2019) [2]. This variability contrasts with our study's consistency toward circular and convex characteristics with entire margins.

In studies of endophytes from desert plants, Gram-positive bacteria particularly *Bacillus* tend to dominate. For instance, in a survey of bacterial endophytes from Thar Desert species, *Bacillus* represented 17% of the isolates and was present in all plant types analyzed. The broader Firmicutes phylum (which includes *Bacillus*) consistently showed higher prevalence relative to other groups (Garmana A. N. *et. al.* 2014) [3, 10].

Among 20 selected root endophytes from desert halophytes, 87% produced bright, orange, or yellow colonies, and most were Gram-negative rods, with opaque colonies present (Singh A. K. *et. al.* 2016). Opaque colony morphology in your isolates (81.25%) is consistent with this trend, indicating common morphological traits among desert-derived endophytes.

### Conclusion

16 bacterial endophytes were isolated from *Leptedenia pyrotechnica* and characterized by using morphological techniques. Based on morphological characters, the isolates

were identified as *Bacillus* sp., *Pseudomonas* sp., *Streptococcus* sp., *Staphylococcus* sp., *Klebsiella* sp. *Streptococcus* genus was dominant with five isolates (31.25%). Out of 16 isolates 5 isolates were *Streptococcus* sp. (31.25%), 4 isolates were *Bacillus* sp. (25%), 3 isolates were *pseudomonas* sp. (18.75%), 3 isolates were *Staphylococcus* sp. (18.75%), 1 isolate was *Klebsiella* sp. (6.25%). Morphological trait results showed that isolates were gram-negative and gram positive both bacterias. The Gram's staining of the endophytic bacterial isolates showed that fifteen isolates (JPR1, JPR2, JPR4, JPR5, JPR6, JPR7, JPR8, JPR9, JPR10, JPR11, JPR12, JPR13, JPR14, JPR15, JPR16) were Gram positive whereas one isolates (JPR3) were Gram negative in nature. Thus, in our observation, 93.7% of the isolates were Gram positive whereas 6.25% of the isolates were Gram negative.

### References

1. Agustien A.<sup>1\*</sup>, Djamaan A.<sup>2</sup>, Rilda Y.<sup>3</sup> and Nasir N.<sup>1</sup> Isolation and Characterization of Antibacterial Endophytic Bacteria from Mangrove Plants at Kapo-Kapo and Setan Islands, West Sumatra Indonesia. Department of Biology, Andalas University, Kampus Limau Manis, Padang, Indonesia. Faculty of Pharmacy, Andalas University, Kampus Limau, Padang, Indonesia. Department of Chemistry, Andalas University, Kampus Limau Manis, Padang, Indonesia.
2. Bokhari A, Essack M, Lafi F F, Andres-Barrao C, Jalal R, Alamoudi S, Saad M M. Bioprospecting desert plant *Bacillus* endophytic strains for their potential to enhance plant stress tolerance. *Scientific reports*,2019;9(1):18154.
3. Garmana A N, Sukandar E Y, Fidrianny I. Activity of several plant extracts against drug- sensitive and drug resistant microbes. *ProcediaChemistry*,2014;13:16 4–9.

4. Booth F E, Wickens G E. *Non-Timber Uses of Selected Arid Zone Trees and Shrubs in Africa*, Rome, Italy. Food & Agriculture Org, 1988.
5. Cappuccino J C, Sherman N. In *Microbiology A Laboratory Manual*. New York, 1992.
6. de Carvalho CR, Ferreira MC, Amorim SS, da Silva Florindo RH, de Assis C S J, Zani CL, Rosa LH, Bioactive compounds of endophytic fungi associated with medicinal plants. *Recent Advancement in White Biotechnology Through Fungi*,2019:303–361.3.
7. El-Fitiany R A, Khasawneh M A. *Leptadenia Pyrotechnica* Forsk. Decne From Edibility to Drug Discovery A Comprehensive Review. | Published online, 2022,6834-6886
8. Dragojević M, Stankovic N, Djokic L, Raičević V. & Jovičić-Petrović J. Endorhizosphere of indigenous succulent halophytes a valuable resource of plant growth promoting bacteria. *Environmental Microbiome* volume 18, Article number,20 (2023)
9. El-Fitiany R A, Khasawneh M A. *Leptadenia Pyrotechnica* Forsk. Decne: From Edibility to Drug Discovery A Comprehensive Review. Published online, 2022, 6834-6886.
10. Garmana A N, Sukandar E Y, Fidrianny I. Activity of several plant extracts against drug-sensitive and drug-resistant microbes. *Procedia Chemistry*,2014:13:164-169.
11. Masrahi Y S A New Species of *Leptadenia* (Apocynaceae) and Two Other New Records from Southwestern Saudi Arabia. *Saudi J. Biol. Sci*,2015:22(5):631–636. DOI: 10.1016/j.sjbs.2015.02.003.
12. Najla A. Al Shaye. Comparative morpho-anatomical study on *Leptadenia pyrotechnica* (Apocynaceae) growing in the hyper-arid and arid habitats of Saudi Arabia. Published, 2023.
13. Norton J, Majid S A, Allan D, Al Safran M, Böer B, Richer R. *An Illustrated Checklist of the Flora of Qatar*; UK: Browndown Publications Gosport, 2009.
14. Sarjono R P, Putri D L, Budiarti EC, Mulyani SN, Ismiyanto N, Kusriani D, *et al.* Antioxidant and antibacterial activities of secondary metabolite endophytic bacteria from papaya leaf *Carica papaya* L. *IOP Conf. Series: Materials Science and Engineering*,2019:509:012112.
15. Schmelzer GH, Gurib-Fakim A *Medicinal Plants 2*; Protá, 2008, 11.
16. Singh AK, Sharma R K, Sharma V, Singh T, Kumar R, Kumari D. Isolation, morphological identification and *in vitro* antibacterial activity of endophytic bacteria isolated from *Azadirachta indica* (neem) leaves, 2017.PMCID: PMC5465764 PMID: 28620254
17. Tewari V. Some Important Fruit Trees and Shrubs of Hot Arid Regions of Rajasthan State in India, Their Uses and Nutritive Values. *J. Plant Chem. Ecophysiol*,2016:1(1):1004.
18. Vilchez J I, Lally R D, Morcillo R J L. *Advances in PGPR Research*. CABI, Wallingford UK. Biosafety evaluation: a necessary process ensuring the equitable beneficial effects of PGPR, 2017, 50–74. ISBN 9781786140325.