https://doi.org/10.33451/florafauna.v31i1pp49-52
FLORA AND FAUNA ISSN 2456 - 9364 (Online)
2025 Vol. 31 No.1 PP 49-52 ISSN 0971 - 6920 (Print)

Antimicrobial activities of bacterial endophytes associated with Pakhangba leiton (*Euphorbia hirta*), an ethnomedicinal plant of Manipur, India

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Received **:** 25.02.2025; ***Accepted*** : 15.04.2025

How to cite : Thokchom S, Ningthoujam DS, Mukherjee S. Antimicrobial activities of bacterial endophytes associated with Pakhangba-leiton (*Euphorbia hirta*), an ethnomedicinal plant of Manipur. *Flora and Fauna* 2025. 31(1) : 49-52.

ABSTRACT

There is potential for isolation of novel bacterial endophytes and purification of secondary metabolites for pharmacological as well as agroactive applications. We have isolated endophytic bacteria from Pakhangba leiton (*Euphorbia hirta*), an ethnomedicinal plant of Manipur. Among these bacterial isolates, 5 (PL19, PL7, PS31, PS22, and PS11) strains exhibited potent antibacterial activities. PL19 and PS11 showed antibacterial activities in both primary and secondary screening.

Figures : 03 References : 18 Table : 01
KEY WORDS : Antimicrobial activities, Ethnomedicinal plants, Manipur

Introduction

Medicinal plants are rich sources of natural compounds and used as therapeutic agents such as *Cannabis sativa* (natural products:Dronabinol and Cannabidiol) and *Capsicum annuum* (natural product: Capsaicin). Many natural products are extracted from medicinal plants such as *Tiotropium* (medicinal plant: *Atropa belladonna*) used in treatment of chronic obstructive pulmonary disease); Galantamine, (medicinal plant: *Galanthus nivalis*), used in treatment of Alzheimer's disease), and Apomorphine(medicinal plant:*Papaver somniferum)*, used in treatment of Parkinson's disease. Bacterial endophytes colonizing medicinal plants' tissue secrete diverse secondary metabolites belonging to tannins,quinones, alkaloids, flavonoids, terpenoids,coumarins *etc.* They have diverse pharmacological activities.Manipur lies in the Indo-Myanmar Biodiversity Hotspot.

Mankind faced risk of severe food crisis in the 21st century and maintaining sustainable crop production is major challenge in agricultural sector. Soil fertility is bound to suffer because of climate change. Rise of pests and fungal pathogens leading to plant diseases may cause sharp decline in crop plants including rice[9]. Use of synthetic fertilizers and fungicides to combat the pathogens may lead to financial constraints on farmers and adverse effects on ecosystem and human health. An alternate approach using bacteria-based bio fertilizers and bioinoculants having plant growth promoting potential by producing phytohormones and siderophores, doing phosphate solubilization and ammonia synthesis can lead to sustained and ecofriendly agriculture.[6]

Discovery of novel bacteria from terrestrial habitats is very limited because of over exploitation of such sources.[14]Therefore microbiologists focused on exploring novel and unique ecosystems from freshwater and marine sources as well as caves, deserts and bacterial endophytes associated with ethomedicinal plants and insects are potential novel sources.[1,2, 17] One such promising niche habitat for discovery of novel bacteria with potential agricultural and biomedical applications is the endosphere of plants, especially, the medicinal plants[8,11].

Endophytes reside in a symbiotic association

ACKNOWLEDGEMENTS : The authors acknowledge Manipur University for providing University fellowship to Ms. Shantirani Thokchom and the necessary infrastructure for the research work.

TABLE-1 : Antibacterial activity of bioactive bacterial isolates by Kirby Bauer method

S. No.	Bacterial isolates	Zone of Inhibition (mm)		
		Micrococcus luteus (MTCC 106)	*Bacillus subtilis* (MTCC 121)	*Escherichia coli* (MTCC 739)
1	PL19	23	-	12.1
2	PL7	29.1	11	-
3	PS31	-	-	23
4	PS11	20	-	-
5	PS22	-	13.1	28

inside the tissues of medicinal plants and don't cause any negative effects on the host plant and secrete bioactive secondary metabolites[3,7]Bioactive metabolites belong to alkaloids, steroids, terpenoids, tetralones,benzopyranones, phenolic acids, quinones, isocoumarins, lignans,xanthoneschinones, flavonoids, and other compounds[18] having applications as antiparasitic agents, antioxidants, agrochemicals, antibiotics, immunosuppressants.[15]

Manipur lies in ***the Indo-Burma hotspot-*** in India[4] and hold potential for discovery of bioactive bacteria from various unexplored and underexplored habitats for applications in pharmacological and agricultural sector. Indi-Myanmar Biodiversity Hotspot is a wide reservoir of ethnomedicinal plants having long ethnobotanical history and long-life spans. These ethnomedicinal plants have the potential to harbor novel endophytes[13,15,16].

Materials and Methods

1. **Sampling of the medicinal plant *Euphorbia hirta* (local name- *Pakhangba Leiton*in Manipur)**

   Healthy ***Euphorbia hirta*** plants containing leaves, flowers, stems, and roots were uprooted and sealed in sterile bags (Fig. 1). The samples were subjected to isolation of bacterial endophytes within 96 hrs.

2. **Isolation of the Endophytic Bacteria**

   Isolation of the Endophytic bacteria was carried out according to the protocol with slight modifications[12].Samples (leaves, flowers, stems, and roots) were washed with tap water followed by rinsing with distilled water to remove the dirt and surface soils. The samples were air-dried at RT for 24 hrs. Five steps of surface sterilization were carried out accordingly under sterile conditions. The samples were cut into 1cm pieces. The surface treated samples were inoculated onto five different microbiological media such as Starch Casein Nitrate Agar (SCNA), Nutrient Agar (NA), 2.5% Water Agar (WA), Yeast Malt Agar (YMA), and Tap Water Yeast Extract Agar (TWYE) for

Fig. 1 : The medicinal plant *Euphorbia hirta* (Pakhangba-leiton)

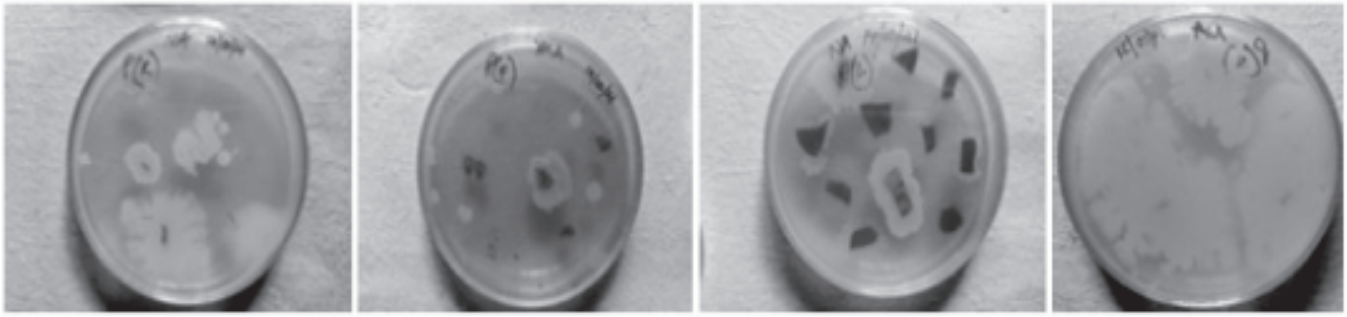

Fig. 2 : Master plates of endophytic bacterial isolates from*Euphorbia hirta* A, B, C, and D represents the master plates of roots, flowers, leaves and stems of *Euphorbia hirta* (Pakhangba leiton) respectively.

isolation of endophytic bacteria (Fig. 2). The master plates were incubated at 3-4 weeks at 30ÚC and sub cultured till axenic cultures were obtained.

3. **Bioactivity Screening of the Endophytic Bacteria**

Primary Screening (Cross streak method)

The bacterial isolates were primary screened by Cross streak method against three test bacteria - *Micrococcus luteus* (MTCC 106), *Bacillus subtilis* (MTCC 121), and *Escherichia coli* (MTCC 739)[10] obtained from IMTECH, Chandigarh. Isolates showing inhibition zones of >50% against the bacterial test organisms were considered as potent isolates.

Secondary screening (Kirby Bauer method)

The secondary screening for testing antibacterial activity of the endophytic bacterial isolates was performed against those same bacterial test organisms[4].The absence or presence of inhibition zones were observed around the wells.

Results

Fifteen (15) putative endophytic bacterial isolates were screened for antibacterial activity. Twelve (12) isolates showed antibacterial activity against one or more of the test organisms assayed, of which eight (8) strains (PS10, PL15, PR8, PR21, PR31, PL19, PS31 and PS22) were found to have good antibacterial activity against one or more test organisms in primary screening.

Of 15 isolates screened, five (5) endophytic bacterial isolates (PL19, PL7, PS31, PS11 and PS22) showed antibacterial activity against one or more of the test organisms in secondary screening. All the 5 strains were found to be potent. PL19, PL7 and PS11 showed good activity against *Micrococcus luteus* (sizes of zones of inhibition: 23, 29.1, and 20mm respectively). PS31 and PS22 exhibited potent activity against *Escherichia coli* (zones of inhibition: 23 and 28mm). None of the isolates showed potent activity against *Bacillus subtilis*

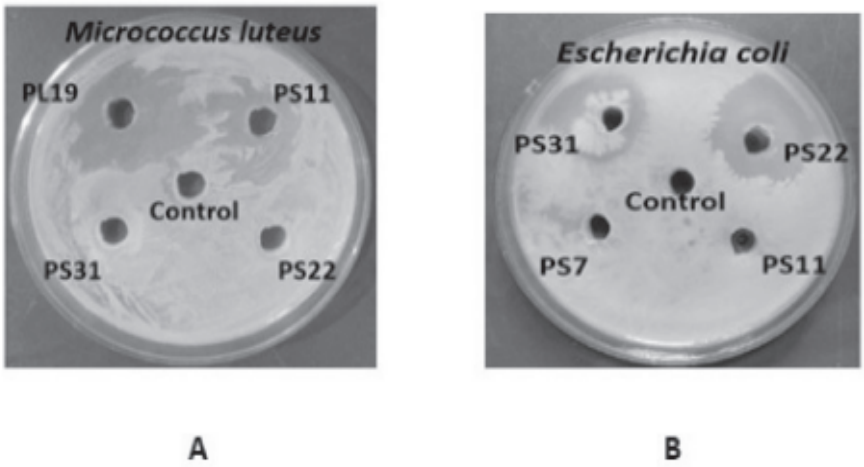

Fig. 3 : Antimicrobial assay by Kirby Bauer method. (A) Endophytic bacterial isolates PL19 and PS11 demonstrates antibacterial activity against *Micrococcus luteus*(MTCC-106). (B) Endophytic bacterial isolates PS22 and PS31 demonstrates antibacterial activity against *Escherichia coli (*MTCC-739). An inhibition zone of 17 mm or above is considered as potent strains.

(MTCC 121).

Conclusion

Among 15 bioactive bacterial isolates screened for antimicrobial activities, 5 (PL19, PL7, PS31, PS22, and PS11) strains exhibited antibacterial activities. PL19 and PS11 were found to be positive in both the primary and secondary screening. Manipur, therefore, holds significant potential for discovery of novel endophytic bacteria for purification ofnovel natural productswith diverse bioactivities.

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