



Morphological and Biochemical Characterization of Endophytic Bacteria from Leaves of Tamarind (*Tamarindus indica*)

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ABSTRACT

Endophytes are ubiquitous organisms that live within the host plants without causing any apparent symptom of disease. The aim of the study was to identify the various morphological characteristics of endophytic bacteria from leaves of *Tamarindus indica*. The preliminary identification of the endophytic bacterial isolates was done based on various morphological features of isolated endophytic bacteria from leaves of *Tamarindus indica*. Growth characteristics of endophytic bacteria isolated from *Tamarindus indica* on King's B media showed that colonies were either irregular or circular in shape, either flat elevation on petri plate or of raised elevation, margin of colonies were either undulated or entire, the surface of the growth opaque and white in colour. All the endophytic bacterial isolates from leaves of *Tamarindus indica* were non-haemolytic in nature. Growth characteristic of isolated endophytic bacteria from *Tamarindus indica* in BHI broth showed turbidity and pellicle formation, either they form sediment or ring. The microscopic examination of endophytic bacterial isolates had shown that all endophytic bacterial isolates from *Tamarindus indica* were gram positive rods. Isolated endophytic bacteria were tested for various biochemical tests also. Based on the findings the endophytic bacteria were characterized and providing insights on the nature of microbiome present in the leaves of Tamarind (*Tamarindus indica*).

HIGHLIGHTS

- The colony characteristics of endophytic bacteria isolated from leaves of *Tamarindus indica* were circular in shape with wavy mucoid and soft irregular margin.
- The colonies were entire, the surface of the growth was smooth, opaque and white in colour.

Keywords: Endophytic bacteria, *Tamarindus indica*, morphological characterization

Antimicrobial resistance is one of the major public health problems especially in developing countries like India where relatively easy availability and inappropriate use of antibiotics leads to higher incidence of resistance. Research on antibiotics and other microbial natural products is pivotal in the global fight against the growing problem of antibiotic resistance. It is necessary to find new antibiotics to tackle this problem (Compant *et al.*, 2010; Beneduzi *et al.*, 2013). Global health problems due to drug resistance

among pathogenic microorganisms have necessitates the urgent need for new and effective antimicrobial agents. *Tamarindus indica* Linn is one of the most widespread trees of the Indian subcontinent. Several authors have

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proposed various geographical areas as the origin of the tamarind tree. Tamarind fruit was at first thought to be produced by an Indian palm, as the name tamarind comes from a Persian word '*tamar-i-hind*', meaning 'date of India'. Its name 'amlika' in Sanskrit indicates its ancient presence in the country (Mishra, 1997). It is a large evergreen tree with an exceptionally beautiful spreading crown, and is cultivated throughout the world except in the Himalayas and western dry regions (Rao *et al.*, 1999). In the present study, endophytic bacteria were isolated from the leaves of *Tamarindus indica* and their morphological characterization was done.

MATERIAL AND METHODS

Location and place of work

The proposed work was conducted in the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.).

Collection of leaves

Fresh mature leaves of *Tamarindus indica* were procured from, three different places of Jabalpur viz. Jawaharlal Nehru Krishi Viswa Vidyalaya (J.N.K.V.V), Tropical Forest Research (T.F.R.I.), State Forest Research Institute (S.F.R.I), Jabalpur. Samples were immediately brought to laboratory and were used within 24 hrs and finally processed for isolation of endophytic bacteria.

Sterilization of leaves

The sterilization of leaves and isolation of endophytic bacteria from the leaves was done according to Mahajan *et al.* (2014), with some modifications. The leaves were excised with autoclaved scalpel and forceps in laminar air flow.



Fig. 1: Sterilization of leaves and isolation of endophytic bacteria from *Tamarindus indica*

Sterility check

To confirm that the surface of leaves were effectively sterilized, 1 ml of the sterile distilled water that was used in final rinse of surface sterilization procedures was placed on to nutrient agar media and incubated at 37° C for 24 hrs. Then bacterial growths were observed.

Preparation and sterilization of media

King's B (KB) media (HiMedia), Mueller Hinton media (HiMedia), Blood agar media (HiMedia) and BHI broth (HiMedia) were prepared by adding agar into the distilled water. Hot plate was used for the proper mixing of media and autoclaved at 121° C for 15-20 minutes at 15 lbs.

Inoculation of leaves and isolation of endophytic bacteria

The media were poured into different autoclaved small petri plates and leaves of the plant were embedded in small petri plates. These plates were then incubated at 37° C for 24 hrs. Characterization of the bacteria was done according to its morphology and by Gram's staining. After that a single colony was transferred into BHI broth and incubated at 37° C for 24 hours.

Morphological characterization

Form, elevation, margin, surface, opacity and chromogenesis of isolated endophytic bacterial colonies were noted.

Growth characteristics of endophytic bacteria isolated from *Tamarindus indica* on King's B media showed that 66.67 per cent of isolates were irregular in shape while 33.33 per cent were circular in shape, 73.33 percent showed flat elevation on petri plate while 26.67 per cent were of raised elevation, margin of 73.33 per cent colonies were undulated while 26.67 per cent showed entire, the surface of the growth was smooth for the undulated colonies and 86.67 per cent growth were opaque and white in colour.

Purification of endophytic bacteria

For purification of endophytic bacteria, sub culturing was mainly done by streaking a loop full of BHI broth on the fresh pre solidified blood agar plates and then incubated

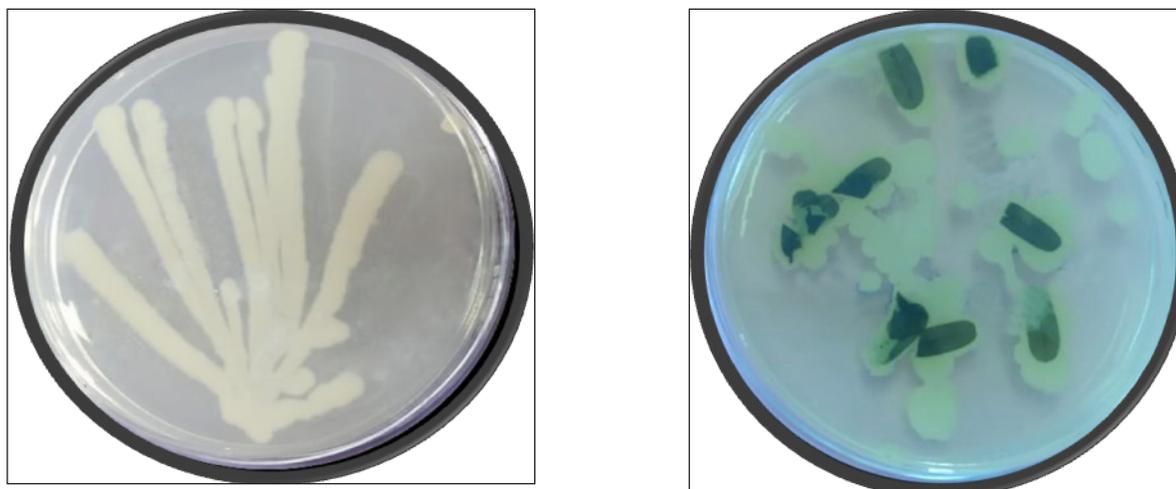


Fig. 2: Growth of Endophytic bacteria from leaves of *Tamarindus indica* on King's B Media

at 37° C for 24 hrs. After incubation the colony was transferred into BHI broth and then incubated at 37° C for 24 hrs and purity was checked by Gram's staining and stored for further work.

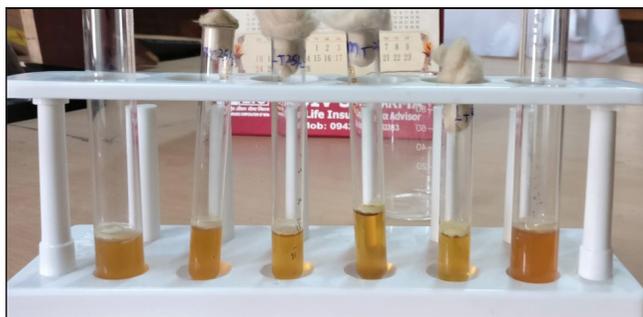


Fig. 3: Growth of Endophytic bacteria from leaves of *Tamarindus indica* in BHI broth

Growth characteristic of isolated endophytic bacteria from *Tamarindus indica* in BHI broth showed characteristics as 33.33 per cent isolates with turbidity and 80 per cent isolates with pellicle formation. Sediment formation was seen in 26.67 per cent isolates and 53.33 per cent isolate showed ring formation.

Growth of Endophytic bacteria from leaves of *Tamarindus indica* on 5 per cent sheep blood agar

Colonies of endophytic bacteria grown on King's B agar were transferred to 5 per cent sheep blood agar plates

and incubated at 37° C for 24 hrs. All the isolates from *Tamarindus indica* were non-haemolytic in nature.



Fig. 4: Growth of Endophytic bacteria from leaves of *Tamarindus indica* on 5 per cent Sheep blood agar

Gram's Staining

The smear was prepared and fixed. Gently flooded the smear with Gram's iodine and allowed to stand for 1 minute. Tilted the slide gently and rinsed with distilled water. Decolorized with 95 percent ethyl alcohol and tilted

Table 1: Growth of Endophytic bacteria from leaves of *Tamarindus indica* on King's B media

Sl. No.	Isolate No.	Form	Elevation	Margin	Surface	Opacity	Chromo-genesis
1	JN-1a	Irregular	Flat	Undulated	Smooth	Opaque	Absent
2	JN-1b	Irregular	Flat	Undulated	Smooth	Opaque	Absent
3	JN-1c	Circular	Flat	Undulated	Smooth	Opaque	Absent
4	JN-1d	Irregular	Raised	Entire	Smooth	Opaque	Absent
5	JN-1e	Irregular	Flat	Undulated	Smooth	Glistening	Absent
6	TF-2a	Irregular	Flat	Undulated	Smooth	Opaque	Absent
7	TF-2b	Circular	Raised	Entire	Smooth	Opaque	Absent
8	TF-2c	Irregular	Flat	Undulated	Smooth	Opaque	Absent
9	TF-2d	Irregular	Flat	Undulated	Smooth	Opaque	Absent
10	TF-2e	Circular	Flat	Entire	Smooth	Opaque	Absent
11	SF-3a	Irregular	Raised	Undulated	Smooth	Opaque	Absent
12	SF-3b	Circular	Flat	Undulated	Smooth	Opaque	Absent
13	SF-3c	Irregular	Flat	Undulated	Smooth	Opaque	Absent
14	SF-3d	Circular	Raised	Entire	Smooth	Glistening	Absent
15	SF-3e	Irregular	Flat	Undulated	Smooth	Opaque	Absent

JN- Jawaharlal Nehru Krishi Viswa Vidyalaya (J.N.K.V.V), TF- Tropical Forest Research (T.F.R.I.), SF-State Forest Research Institute (S.F.R.I), Jabalpur.

Table 2: Growth of endophytic bacteria isolated from leaves of *Tamarindus indica* in BHI broth

Sl. No.	Isolate No.	Turbidity	Pellicle	Sediment	Ring formation
1	JN-1a	Absent	Present	Absent	Present
2	JN-1b	Absent	Present	Absent	Absent
3	JN-1c	Present	Present	Present	Absent
4	JN-1d	Absent	Present	Absent	Present
5	JN-1e	Absent	Present	Absent	Present
6	TF-2a	Present	Present	Absent	Present
7	TF-2b	Absent	Absent	Present	Present
8	TF-2c	Absent	Present	Absent	Absent
9	TF-2d	Absent	Absent	Absent	Absent
10	TF-2e	Present	Present	Absent	Present
11	SF-3a	Absent	Present	Absent	Present
12	SF-3b	Present	Absent	Present	Absent
13	SF-3c	Present	Present	Absent	Present
14	SF-3d	Absent	Present	Absent	Absent
15	SF-3e	Absent	Present	Present	Absent

the slide gently and applied alcohol drop by drop to 10 seconds. Immediately rinsed with distilled water gently flooded with safranin to counter stain and allowed to stand for 1 minute. Tilted the slide gently and rinsed with distilled water, dried the slide. Viewed the smear under 100X microscope.

Gram's staining of Endophytic bacterial isolated from leaves *Tamarindus indica*

Endophytic bacteria isolated from leaves of *Tamarindus*

indica from Jawaharlal Nehru Krishi Viswa Vidyalaya JN- (J.N.K.V.V), Tropical Forest Research TF-(T.F.R.I.), State Forest Research Institute SF-(S.F.R.I) Jabalpur, showed gram positive reaction and were found to be rods.

Biochemical characterization

The endophytic bacteria isolated from *Tamarindus indica* had shown positive reaction to Catalase test, Coagulase test, Voges-Proskauer (VP), orthonitrophenyl β -galactoside (ONPG), Urease, Arginine utilization and

Sugar fermentation tests were done. Jawaharlal Nehru Krishi Viswa Vidyalaya JN- (J.N.K.V.V), Tropical Forest Research TF-(T.F.R.I.), State Forest Research Institute SF- (S.F.R.I) Jabalpur.

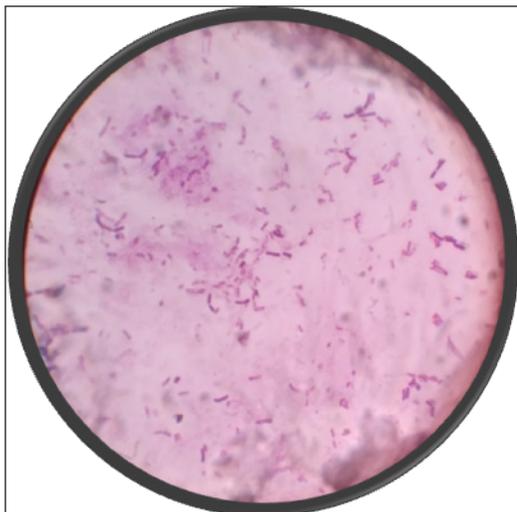


Fig. 5: Gram's staining of Endophytic bacterial isolated from leaves *Tamarindus indica*



Fig. 7: Coagulase test reaction of isolated Endophytic bacteria from leaves of *Tamarindus indica*

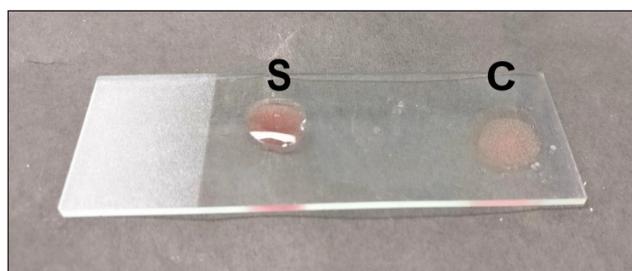


Fig. 8: Coagulase test reaction of isolated Endophytic bacteria from leaves of *Tamarindus indica*



Fig. 6: VP (1), ONPG (2), Urease (3), Arginine utilization (4) and Sugar fermentation tests (5, 6, 7) of Endophy bacteria isolated from leaf *Tamarindus indica*

The endophytic bacteria isolated from *Tamarindus indica* had shown positive reaction to catalase, Vogasproskaur's (VP) Orthro-Nitrophenyl- β -galactoside (ONPG), urease and negative reaction to coagulase, arginine utilization test.

RESULTS AND DISCUSSION

The microscopic examination of endophytic bacterial isolates had shown that all endophytic bacterial isolates from *Tamarindus indica* were gram positive rods and only one type of bacteria was present. The biochemical characterization of endophytic bacterial isolates showed positive reaction to catalase, Vogasproskaur's (VP) Orthro-Nitrophenyl- β -galactoside (ONPG), urease and negative reaction to coagulase, arginine utilization test. The endophytic bacteria isolates were evaluated for the presence of active hydrolytic Khanam and Chandra (2015) had isolated endophytic bacteria from *Beta vulgaris* having morphological characteristics of umbonate elevation, entire margin and red coloured colonies. Soman (2018) had isolated endophytic bacteria from leaves of *Acacia nilotica* and the colonies were circular in shape with raised elevation while endophytic bacteria isolated from the leaves of *Acacia catechu* were irregular in shape, flat elevation on petri plate, entire colony margins, and the surface of the growth was smooth, opaque and white in colour. The endophytic bacteria isolated from the leaves of *Acacia auriculiformis* were irregular in shape, flat elevation on petri plate, undulated colony margin; the surface of the

**Table 3:** Gram's staining of Endophytic bacterial isolated from leaves *Tamarindus indica*

Sl. No.	Isolate No.	Gram's staining	Shape	Types of bacteria
1	JN-1a	Positive	Rod	01
2	JN-1b	Positive	Rod	01
3	JN-1c	Positive	Rod	01
4	JN-1d	Positive	Rod	01
5	JN-1e	Positive	Rod	01
6	TF-2a	Positive	Rod	01
7	TF-2b	Positive	Rod	01
8	TF-2c	Positive	Rod	01
9	TF-2d	Positive	Rod	01
10	TF-2e	Positive	Rod	01
11	SF-3a	Positive	Rod	01
12	SF-3b	Positive	Rod	01
13	SF-3c	Positive	Rod	01
14	SF-3d	Positive	Rod	01
15	SF-3e	Positive	Rod	01

Table 4: Biochemical tests of endophytic bacteria

Sl. No.	Isolate No.	Catalase test	Coagulase test	VP test	ONPG test	Urease test	Arginine utilization test
1	JN-1a	Positive	Negative	Positive	Positive	Positive	Negative
2	JN-1b	Positive	Negative	Positive	Positive	Positive	Negative
3	JN-1c	Positive	Negative	Positive	Positive	Positive	Negative
4	JN-1d	Positive	Negative	Positive	Positive	Positive	Negative
5	JN-1e	Positive	Negative	Positive	Positive	Positive	Negative
6	TF-2a	Positive	Negative	Positive	Positive	Positive	Negative
7	TF-2b	Positive	Negative	Positive	Positive	Positive	Negative
8	TF-2c	Positive	Negative	Positive	Positive	Positive	Negative
9	TF-2d	Positive	Negative	Positive	Positive	Positive	Negative
10	TF-2e	Positive	Negative	Positive	Positive	Positive	Negative
11	SF-3a	Positive	Negative	Positive	Positive	Positive	Negative
12	SF-3b	Positive	Negative	Positive	Positive	Positive	Negative
13	SF-3c	Positive	Negative	Positive	Positive	Positive	Negative
14	SF-3d	Positive	Negative	Positive	Positive	Positive	Negative
15	SF-3e	Positive	Negative	Positive	Positive	Positive	Negative

growth was equally smooth and dull, opaque and white in colour. Baghat *et al.* (2014), who found that the 90 per cent of isolated endophytic bacteria from *Capparis sinaica* were gram positive in nature. Prasad and Dagar (2014) had isolated endophytes from Avacado and Black grapes which were gram positive rods. The coagulase negative reaction suggests that isolates were nonpathogenic in nature. The present findings are very near to the work of Khanam and Chandra (2015).

CONCLUSION

All the endophytic bacterial isolates from leaves of *Tamarindus indica* were having different growth characteristic in King's B media, BHI broth and on Sheep blood agar. The microscopic examination of endophytic bacterial isolates had shown that all endophytic bacterial isolates from *Tamarindus indica* were gram positive rods and only one type of bacteria was present. The biochemical characterization of endophytic bacterial isolates showed

positive reaction to catalase, Vogasproskaur's (VP) Orthro-Nitrophenyl- β -galactoside (ONPG), urease and negative reaction to coagulase, arginine utilization test. The endophytic bacteria isolates were evaluated for the presence of active hydrolytic.

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