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Isolation and characterization of bacterial endophytes in neem and aloevera

N Alekhya, Bh Sarvani, A Vijay Gopal and Y Bindiya
Department of Microbiology, Acharya N G Ranga Agricultural University,
Agricultural College, Bapatla-522101, Andhra Pradesh, India

ABSTRACT

Neem and Aloevera have various medicinal properties in agriculture which act as plant growth promoters, natural pesticides, soil conditioners. In this study, Endophytic bacteria associated with Neem(*Azadirachta indica)* and Aloevera(*Aloe vera barbadensis)* were isolated to assess their plant growth promoting traits, biotic and abiotic stress tolerance activities. About 101 endophytic bacteria were isolated from different plant tissues of neem and aloevera which include leaf, branches, terminals and roots. Further cultural characterization was done for the endophytic bacterial isolates based on colony shape, opacity, margin, form, pigmentation and colony elevation.

Keywords: *Biotic and Abiotic stress, Cultural characterization. Endophytic bacteria and Plant growth promotion*

Endophytes are microorganisms that invade healthy plant cells and complete their life cycle without causing sickness. They establish symbiotic relationship with the plant, often residing within the intercellular spaces or within the plant cells. Endophytes can be found in various parts of the plant, including leaves, stems, roots and seeds. (Houlden *et al*., 2008).

Endophytes produce chemicals that promote plant development and maintain a healthy relationship (Das and varma, 2009). Endophytes control infections, promote plant growth and yield, remove contaminants and supplies phosphate after solubilization and also helpful in supply of assimilable nitrogen for plants (Santoyo *et al*., 2016).

These endophytes are often present in the target organism, which can be explored as biocontrol agents (Compant *et al*., 2005). The association of these endophytes with plant enhances plant growth (Khamwan *et al*., 2018) and increases protection against biotic and abiotic stresses and also stimulates the synthesis of bioactive compounds (Alvin *et al*., 2014). Endophytic bacteria such as *Pseudomonas* (Ali *et al*., 2014), *Pantoea, Bacillus* (Andreolli *et al*., 2016), *Serratia, Enterobacter* (Otieno *et al*., 2013), *Azospirillum* (Hungria *et al*., 2018) and *Paraburkholderia* (Bernabeu *et al*., 2018) are beneficial for plant growth.

Endophytic bacteria show direct physiological effects on plant growth and development such as nitrogen fixation (Joe *et al*., 2016); phosphate, zinc, potassium solubilization (Andreolli *et al*., 2016); production of ammonia (Yaish *et al*., 2015); siderophores (Abbamondi *et al*., 2016); phytohormones (De Melo Pereira *et al*., 2012); hydrolytic enzymes (Joe *et al*., 2016); Exopolysaccharides (EPS) and HCN production(Kumar *et al*., 2011).

Medicinal plants are abundant in several essential bioactive compounds, including secondary metabolites (SMs), polysaccharides, starch and other carbohydrates. They are frequently employed to make clinical, therapeutic medications and their raw materials for drug manufacturing .

Azadirachta indica (neem) is a divine tree mostly grown in the Indian subcontinent. It belongs to Meliaceae and is usually referred to as neem. (Upma *et al*., 2011). Different plant parts of neem tree are known for various pharmacological activities such as antibacterial, antiviral, anti-inflammatory activity, anti-carcinogenic activity and parasitic disease (Abdel- Ghaffar 2012).

Aloe vera (*Aloe barbadensis)* is well known for its medicinal uses traditionally and has substantial commercial value (Zapata *et al*., 2013). It is widely

used in the food industry, cosmetics and tonics. It also have enormous therapeutic potential and about 75 active components discovered (Bjorklund *et al*., 2018). Endophytic bacteria from Aloe vera including *Pseudomonas hibiscicola*, *Macrococcus caseolyticus*, *Enterobacter ludwigii* and *Bacillus anthracis* were reported to produce bioactive compounds of medical importance with antimicrobial activity(Akinsanya *et al*., 2015).

MATERIAL AND METHODS

The neem plant samples i.e., neem leaf, stem, branches, twigs and aloevera plant samples i.e., roots, leaves were surface sterilizated as given by Tejera *et al*., 2006. Where the leaves, stems, branches, twigs were washed under ethanol of 75% concentration for 1 min, followed by Sodium Hypochlorite 0.5% for 30 sec and then washed three times with sterilized distilled water. Those sterilized parts were cut into small pieces by sterilized knife and scissors. For isolation of endophytic bacteria different tissues were soaked separately in sterile phosphate buffered saline (PBS) solution of pH 7.0 for 15 min for equilibrating osmotic pressure and halting any passive diffusion of sterilizing agent into the roots (Suman *et al*., 2001). Then sap collected for serial dilution (10^{-6} ,10^{-7}) by grinding samples under mortar & pistle. The aloevera roots (0.5 g) were dissected into 1cm pieces and surface-sterilized for 40 sec with 70% ethanol and 20 min with 2.5% Sodium Hypochlorite. To remove the disinfectant, sections were rinsed three to four times in sterile distilled water samples were then dried with sterile filter paper and subsequently ground with 1 mL 0.9% sodium chloride with a sterile mortar. Samples (100 ìL) of tissue extracts and their different dilutions were spread plated on nutrient agar media in triplicate. Aliquots (100 ìL) of the last washing water were plated as sterility controls, which never showed to be contaminated and incubated at 28 ° C for 72 hrs. Cultural characterization of isolated bacterial colonies was done based on bergey's manual of systematic bacteriology which include colony shape, opacity, margin, form, pigmentation, elevation.

RESULTS AND DISCUSSION

About 101 endophytic bacterial isolates were isolated from plant samples of Neem and Alovera. The plant samples taken for isolation of endophytic bacteria include from neem leaves, terminals, branches were taken and from aloevera roots and leaves. They were named according to the plant sample from which isolates were isolated where 44 endophytic bacterial isolates were isolated from leaves (NLEB), 15 from branches (NBE), 10 from terminals of neem (NTE), 18 from roots (AREB) and 12 from leaves (ALEB) of Aloevera. The cultural characterstics observed in endophytic bacteria isolated from neem leaves where 37 isolates observed as round shape, 10 were irregular, 28 were transparent, 16 were opaque, 32 were circular, 5 were rhizoid, 7 were filiform, 18 isolates were smooth, 20 were undulated, 15 were curled, 5 were erose, 35 were white to creamy white, 30 were flat, 14 were raised (Table 1). Similar results were found by singh *et al*., 2017 where 50 bacterial endophytes were isolated from leaves of neem tree. On cultural characterization 10 were circular in form remaining were irregular, 21 were showed flat elevation and remaining were raised, 8 were showed entire margins remaining were showed undulated margins and 2 were showed transparent, remaining were showed opaque in opacity. The cultural characterstics of endophytic bacterial isolates from branches of neem where 10 were round, 5 were irregular, 12 were circular, 3 were rhizoid, 11 were transparent and five were convex (Table 2). Similar results found by Lekatompessy *et al*., 2021 where 19 bacterial endophytes were isolated from branches of neem tree. On cultural characterization of these endophytic bacterial isolates showed different colored colonies from milky white to yellow and with round colony shape. The cultural characterstics of endophytic bacteria from terminals of neem where 10 were round, 3 were transparent, 7 were opaque, 3 were rhizoid, 2 were filiform, 4 were circular, 3 withs smooth margins and 3 with curled margins (Table 3). The cultural characterstics of endophytic bacteria from roots of aloevera where 10 were round, 8 were irregular, 11 were transparent, 7 were opaque, 12 were circular in form and 3 were rhizoid, 5 were with undulated margins, 1 were erose, 17 were with white colonies, 12 were flat, 6 were raised(Table 4). Similar results were found by Swati *et al*., 2022 where 19 bacterial endophytes were isolated from roots of aloevera. On cultural characterizazation of these bacterial endophytes all isolates were round in shape, 5 were flat, 6 were convex, 2 wre raised in elevation, the pigmentation varied from white to yellow. The cultural characterstics of endophytic bacteria from leaves of

Table 1. Cultural characterization of endophytic bacteria from leaves of neem

S.no	Isolate Code	Colony Shape	Opacity	Form	Margin	Pigmentation	Elevation
1	NLEB1	Round	Transparent	Circular	Smooth	Creamy White	Flat
2	NLEB2	Irregular	Transparent	Irregular	Curled	White	Flat
3	NLEB3	Irregular	Opaque	Rhizoid	Undulate	White	Raised
4	NLEB4	Round	Transparent	Circular	Smooth	White	Flat
5	NLEB5	Irregular	Transparent	Circular	Undulate	Creamy White	Flat
6	NLEB6	Round	Opaque	Circular	Undulate	White	Flat
7	NLEB7	Irregular	Opaque	Circular	Erose	White	Flat
8	NLEB8	Round	Transparent	Circular	Smooth	White	Flat
9	NLEB9	Irregular	Transparent	Circular	Erose	Creamy White	Flat
10	NLEB10	Irregular	Opaque	Rhizoid	Undulate	White	Raised
11	NLEB11	Round	Opaque	Circular	Erose	White	Flat
12	NLEB12	Round	Opaque	Circular	Smooth	White	Flat
13	NLEB13	Irregular	Transparent	Rhizoid	Undulate	White	Flat
14	NLEB14	Round	Opaque	Circular	Erose	White	Raised
15	NLEB15	Round	Transparent	Circular	Lobate	White	Raised
16	NLEB16	Round	Transparent	Circular	Smooth	White	Flat
17	NLEB17	Irregular	Opaque	Irregular	Undulate	White	Flat
18	NLEB18	Round	Opaque	Circular	Smooth	White	Flat
19	NLEB19	Round	Transparent	Circular	Curled	Creamy White	Flat
20	NLEB20	Irregular	Opaque	Circular	Smooth	White	Raised
21	NLEB21	Round	Opaque	Circular	Curled	Creamy White	Raised
22	NLEB22	Round	Opaque	Circular	Smooth	White	Flat
23	NLEB23	Round	Transparent	Circular	Lobate	White	Flat
24	NLEB24	Round	Transparent	Rhizoid	Smooth	White	Flat
25	NLEB25	Round	Opaque	Circular	Undulate	White	Flat
26	NLEB26	Round	Opaque	Circular	Undulate	White	Raised
27	NLEB27	Irregular	Transparent	Rhizoid	Undulate	White	Flat
28	NLEB28	Irregular	Transparent	Circular	Smooth	White	Raised
29	NLEB29	Round	Opaque	Circular	Smooth	White	Raised
30	NLEB30	Round	Opaque	Filiform	Smooth	White	Raised
31	NLEB31	Round	Transparent	Rhizoid	Undulate	White	Flat
32	NLEB32	Round	Transparent	Rhizoid	Undulate	White	Raised
33	NLEB33	Irregular	Opaque	Circular	Smooth	White	Flat
34	NLEB34	Irregular	Opaque	Circular	Smooth	White	Flat
35	NLEB35	Irregular	Opaque	Rhizoid	Undulate	White	Raised
36	NLEB36	Round	Transparent	Circular	Curled	White	Raised
37	NLEB37	Round	Transparent	Circular	Erose	White	Flat
38	NLEB38	Irregular	Transparent	Circular	Undulate	White	Flat
39	NLEB39	Irregular	Opaque	Circular	Smooth	Creamy White	Convex
40	NLEB40	Irregular	Opaque	Rhizoid	Smooth	White	Flat
41	NLEB41	Round	Opaque	Circular	Smooth	White	Flat
42	NLEB42	Round	Transparent	Circular	Smooth	White	Raised
43	NLEB43	Round	Transparent	Filiform	Curled	White	Flat
44	NLEB44	Irregular	Opaque	Circular	Curled	White	Raised
45	NLEB45	Irregular	Opaque	Rhizoid	Smooth	White	Flat
46	NLEB46	Round	Opaque	Circular	Curled	White	Convex

Table 2. Cultural characterization of endophytic bacteria from branches of neem

S.No	Isolate Code	Colony Shape	Opacity	Form	Margin	Pigmentation	Elevation
1	NBE1	Round	Opaque	Circular	Smooth	White	Raised
2	NBE2	Round	Transparent	Rhizoid	Curled	White	Raised
3	NBE3	Round	Opaque	Circular	Smooth	White	Flat
4	NBE4	Round	Transparent	Rhizoid	Erose	White	Flat
5	NBE5	Round	Transparent	Circular	Smooth	White	Flat
6	NBE6	Round	Transparent	Circular	Smooth	White	Flat
7	NBE7	Round	Transparent	Circular	Smooth	White	Flat
8	NBE8	Irregular	Transparent	Circular	Smooth	White	Flat
9	NBE9	Round	Opaque	Circular	Smooth	White	Flat
10	NBE10	Round	Opaque	Rhizoid	Undulate	White	Raised
11	NBE11	Round	Transparent	Circular	Smooth	White	Convex
12	NBE12	Irregular	Transparent	Circular	Smooth	White	Flat
13	NBE12	Irregular	Transparent	Circular	Smooth	White	Raised
14	NBE13	Irregular	Transparent	Circular	Curled	White	Flat
15	NBE14	Irregular	Transparent	Circular	Smooth	White	Flat

Table 3: Cultural characterization of endophytic bacteria from terminals of neem

S.No	Isolate Code	Colony Shape	Opacity	Form	Margin	Pigmentation	Elevation
1	NTE1	Round	Opaque	Circular	Undulate	White	Flat
2	NTE2	Round	Opaque	Irregular	Undulate	White	Flat
3	NTE3	Round	Transparent	Rhizoid	Curled	White	Convex
4	NTE4	Round	Opaque	Circular	Smooth	White	Raised
5	NTE5	Round	Opaque	Filiform	Smooth	White	Raised
6	NTE6	Round	Transparent	Circular	Curled	White	Flat
7	NTE7	Round	Transparent	Circular	Smooth	White	Flat
8	NTE8	Round	Opaque	Rhizoid	Undulate	White	Flat
9	NTE9	Round	Opaque	Filiform	Curled	White	Raised
10	NTE10	Round	Opaque	Rhizoid	Undulate	White	Flat

aloevera where 6 were circular, rhizoid, transparent, round, smooth, white, flat, 6 were irregular, undulated, opaque, raised, yellow, curled (Table 5). Similar results were found by Missa *et al*., 2023 where 8 bacterial endophytes were isolated from leaves of aloevera. On cultural characterization of these bacterial endophytes 5 were circular, 3 were rhizoid in form, 6 were raised, 2 were convex in elevation and 5 were undulate 3 were entire in margin.

CONCLUSIONS

A total of 101 endophytic bacteria as from neem 70 and from aloevera 31 were isolated. cultural characterization of 101 bacterial endophytes revealed some were round, irregular, rhizoid in form, with varied pigmentation from white to yellow, with undulated, erose, entire margins and with opaque, transparent in opacity.

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Table 4. Cultural characterization of endophytic bacteria from roots of aloevera

S.No	Isolate Code	Colony Shape	Opacity	Form	Margin	Pigmentation	Elevation
1	AREB1	Irregular	Opaque	Rhizoid	Undulate	White	Convex
2	AREB2	Irregular	Transparent	Rhizoid	Curled	White	Flat
3	AREB3	Round	Transparent	Irregular	Curled	White	Convex
4	AREB4	Round	Transparent	Circular	Smooth	White	Flat
5	AREB5	Irregular	Transparent	Circular	Erose	White	Raised
6	AREB6	Round	Transparent	Circular	Smooth	White	Flat
7	AREB7	Irregular	Opaque	Circular	Undulate	White	Flat
8	AREB8	Irregular	Opaque	Rhizoid	Undulate	White	Raised
9	AREB9	Round	Transparent	Circular	Smooth	White	Flat
10	AREB10	Round	Transparent	Rhizoid	Curled	White	Flat
11	AREB11	Irregular	Opaque	Circular	Smooth	White	Raised
12	AREB12	Round	Opaque	Rhizoid	Curled	White	Flat
13	AREB13	Round	Opaque	Circular	Smooth	White	Flat
14	AREB14	Irregular	Opaque	Circular	Curled	White	Flat
15	AREB15	Round	Transparent	Circular	Curled	White	Flat
16	AREB16	Round	Transparent	Circular	Smooth	Yellow	Flat
17	AREB17	Irregular	Transparent	Circular	Undulate	White	Raised
18	AREB18	Round	Transparent	Circular	Undulate	White	Flat

Table 5. Cultural characterization of endophytic bacteria from leaves of aloevera

S.No	Isolate Code	Colony Shape	Opacity	Form	Margin	Pigmentation	Elevation
1	ALEB1	Irregular	Transparent	Circular	Smooth	White	Convex
2	ALEB2	Irregular	Opaque	Circular	Curled	White	Flat
3	ALEB3	Irregular	Opaque	Circular	Undulate	White	Flat
4	ALEB4	Round	Opaque	Circular	Curled	White	Flat
5	ALEB5	Irregular	Transparent	Circular	Curled	White	Raised
6	ALEB6	Round	Opaque	Rhizoid	Smooth	White	Flat
7	ALEB7	Round	Opaque	Circular	Curled	White	Raised
8	ALEB8	Round	Transparent	Circular	Undulate	White	Raised
9	ALEB9	Round	Transparent	Filiform	Curled	White	Flat
10	ALEB10	Irregular	Transparent	Circular	Smooth	White	Raised
11	ALEB11	Irregular	Opaque	Circular	Smooth	White	Flat
12	ALEB12	Round	Opaque	Circular	Curled	White	Raised

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