

DIVERSITY OF SEAGRASS IN PULICAT LAKE AND IDENTIFICATION OF ASSOCIATED BACTERIAL ISOLATES

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DOI: [https://doi.org/10.63001/tbs.2026.v21.i04.S.I\(4\).pp69-83](https://doi.org/10.63001/tbs.2026.v21.i04.S.I(4).pp69-83)

KEYWORDS <i>Seagrass, Pulicat Lake, Halophila ovalis, Syringodium isoetifolium, bacterial isolates</i> Received on: 18-08-2026 Accepted on: 20-09-2026 Published on: 07-10-2026	ABSTRACT Background: Seagrasses are marine flowering plants that form productive ecosystems in shallow coastal waters and support diverse epiphytic and endophytic bacterial communities. The present study was undertaken to document the diversity of seagrass species present in Pulicat Lake, India's second-largest brackish water lagoon, and to isolate, identify, and characterize the bacteria associated with them. Materials and Methods: Seagrass species were collected from Pulicat Lake and identified (<i>Halophila ovalis</i> and <i>Syringodium isoetifolium</i>) on the basis of their morphological characteristics. Bacteria associated with the roots, leaves, and rhizosphere of both species were isolated on nutrient agar and Zobell marine agar, yielding 7 isolates from <i>H. ovalis</i> (HO-1 to HO-4) and <i>S. isoetifolium</i> (SI-1 to SI-3). Results: <i>Halophila ovalis</i> and <i>Syringodium isoetifolium</i> were the seagrass species collected from the Pulicat Lake. The associated bacterial isolates were characterized on the basis of colony morphology, biochemical tests, and carbohydrate fermentation patterns. Totally 7 bacterial species were obtained from the different genera. Conclusion: The findings provide baseline data on seagrass and associated bacterial diversity in Pulicat Lake and highlight the lagoon's potential as a reservoir of ecologically and medically significant marine bacteria.
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INTRODUCTION

Seagrasses represent a secondary evolutionary transition of angiosperms from land to sea, which is thought to have occurred between 70 and 100 million years ago [1]. Only about 60 seagrass species have successfully adapted to living underwater, in contrast to the over 250,000 species of terrestrial flowering plants [2]. Seagrass

meadows are one among the most productive and biodiverse ecosystems, despite their tiny number. They are essential to preserving the structural complexity, geographical heterogeneity, and functional stability of coastal ecosystems and provide as habitat for a variety of marine animals [3]. Seagrass meadows are ecologically delicate and extremely susceptible to biological invasion and human disturbance due to their location at

the land-sea interface and their global decrease has garnered growing attention [4].

Seagrasses also sustain a variety of microbial communities, which play an important ecological role in the marine environment [5]. Numerous studies have connected seagrasses to endophytic and rhizospheric bacterial populations, and many of these connections appear to be mutualistic [6]. In these relationships, the host plant provides protection to microbial communities in return for their assistance with processes like nitrogen fixation, growth promotion, pollution cleanup, and disease defense [7]. Many bacteria are antagonistic to pathogens and can produce bioactive compounds that protect the host against disease and predators, according to Bhatnagar and Kim (2010) [8]. Marine bacteria often inhabit a wide diverse range of habitats, from the intertidal zone to the deep sea, hydrothermal vents, and polar waters, exhibiting an incredible capacity to adapt through a number of metabolic techniques [9].

A wide variety of bacteria with potential use in biotechnology, the environment, and medicine can be found in the rhizospheres, leaves, and surrounding sediments of seagrass. In addition to their ecological significance, natural ecosystems

like seagrass meadows are becoming more widely acknowledged as sources of bioactive chemicals that are important for medical and pharmaceutical research. Seagrass-associated organisms have been connected to antioxidant, anti-inflammatory, and enzymatic activities of therapeutic interest. Plant-driven gradients of oxygen and dissolved organic carbon within the seagrass rhizosphere create dynamic microhabitats that select for distinct microbial communities.

The variety of seagrass-associated bacteria in India is still mostly unknown, especially in biologically unique environments like Pulicat Lake, the nation's second-largest brackish water lagoon. Seagrass diversity and the bacterial flora that is linked with it in this ecosystem are not well documented in systematic investigations; which is a lost opportunity for both basic biodiversity assessment and practical research, including drug discovery. Therefore, the goal of the current study was to use morphological, biochemical, and molecular techniques to isolate and identify the bacteria linked to the variety of seagrass species found in Pulicat Lake. It is anticipated that this work would produce baseline data pertinent to the Sustainable Development Goals of the United

Nations, including SDG 14 (Life below Water) and SDG 3 (Good Health and Well-Being).

AIM

To study the diversity of seagrass species present in Pulicat Lake and isolate and identify the bacteria associated with them using morphological and biochemical methods.

OBJECTIVES

- To collect and identify seagrass specimens according to their morphological traits.
- To isolate the bacteria associated with the roots, leaves and rhizosphere of the collected seagrass specimens.
- Identification of bacterial isolates by microscopic examination and colony morphology.
- To characterize the isolates biochemically using assays such as catalase, motility, oxidative-fermentative, IMViC, ONPG, nitrate reduction, and carbohydrate fermentation.

MATERIALS AND METHODS

Study Area and Collection of Seagrass

The second-largest brackish water lagoon in India, Pulicat Lake is located along the coast of Tamil Nadu and Andhra Pradesh

and is home to seagrass beds. Seagrass specimens were manually collected from the intertidal and shallow subtidal zones of Pulicat Lake, together with a sufficient portion of the rhizome and root system, to avoid desiccation during shipping. They were then immediately transferred into sterile polythene bags containing ambient saltwater. Species-level identification was carried out using standard morphological descriptors, such as leaf shape, leaf arrangement, rhizome and root architecture, and growth pattern, in accordance with the Survey Manual for Tropical Marine Resources' field identification protocol and standard taxonomic treatment of seagrasses. [14, 15]. Using this method, two species were identified: Both *Syringodium isoetifolium* and *Halophila ovalis*. On the day of collection, specimens were brought to the lab in aseptic settings and processed right away for bacterial isolation (Figure 1).

Isolation of Bacteria Associated with Seagrass:

Bacterial isolation was carried out using a conventional rhizosphere-and-tissue collection methodology derived from previously described methodologies for marine plant-associated bacteria [10]. Autoclaved filtered seawater (AFS) was used to generate successive dilutions (10^{-3} , 10^{-6} , and 10^{-1}) in

order to isolate bacteria adhering to the root surface and surrounding sediment. The roots were bathed in sterile distilled water to remove adhering soil. Nutrient agar made in 50% filtered seawater, one-tenth strength R2A medium, and Zobell marine agar—the standard medium designed for the culture of marine heterotrophic bacteria—were used to disperse aliquots of each dilution [12].

Roots and leaves were also rinsed many times with sterile distilled water to remove surface contamination before being used to isolate endophytic bacteria. After surface sterilization, a sterile mortar and pestle were used to crush approximately 1.0 g of root and leaf tissue. Aliquots (0.1 ml) were then serially diluted (10^{-3} , 10^{-6} , and 10^{-1}) in AFS and dispersed on the previously mentioned substrate. Before pouring, 50 µg/ml cycloheximide was added to the medium to prevent fungal contamination. Pure colonies were selected and repeatedly restreaked on fresh media until axenic cultures were formed after plates were cultured at 26 °C for around two weeks. For long-term preservation, purified isolates were kept in 15% (v/v) glycerol stocks at -80 °C.

In parallel, both seagrass species' leaves were homogenized with a sterile mortar and pestle after being cleaned of sand,

epiphytes, and debris using sterile distilled water. To obtain isolated colonies, the homogenate was streaked over nutritional agar and Zobell marine agar using the conventional quadrant (four-sector) streaking procedure. The plates were then incubated at 37 °C for 24 hours.

Identification of Bacterial Isolates

Colony Morphology

Based on colony morphology on nutrient agar and Zobell marine agar, the bacterial isolates were first characterized using standard descriptive criteria for bacterial colony characterization. These criteria included shape, size, color, margin, elevation, texture, and opacity.

Biochemical Characterization

Pure isolates were subjected to a standard panel of biochemical tests, which were interpreted in accordance with Bergey's Manual of Determinative Bacteriology [13]. Gram staining, catalase testing, motility testing, oxidative-fermentative (O/F) testing, the IMViC series (indole, methyl red, Voges-Proskauer, and citrate utilization tests), ONPG, and nitrate reduction tests were all included in the panel. The ability to ferment carbohydrates was evaluated using glucose, sucrose, lactose, maltose, and mannitol; in phenol red broth with a favourable outcome noted as acid

production (A) or acid-and-gas production (A&G) in cases where gas was also created.

RESULTS

Diversity of Seagrass in Pulicat Lake:

Based on morphological examination, two seagrass species were identified from Pulicat Lake *Halophila ovalis*, characterized by oval, paddle-shaped leaves arranged in pairs along a creeping rhizome, and *Syringodium isoetifolium*, characterized by cylindrical, needle-like leaves. Both species were found growing in the shallow, brackish waters of the lake, confirming the presence of at least two distinct seagrass taxa in this ecosystem (Figure 2).

Isolation of Bacteria

Totally seven bacterial species were isolated from the two seagrass species; designated HO-1 to HO-4 from *Halophila ovalis* and SI-1 to SI-3 from *Syringodium isoetifolium*. The isolates were subcultured onto Zobell marine agar and nutrient agar and showed variation in colony size, shape, elevation, margin, and colour, indicating the presence of a diverse bacterial community associated with each host.

Microscopic Examination and Colony

Morphology

Microscopic analysis and colony morphology revealed that the seven bacterial

isolates had diverse phenotypic traits. Gram-positive, rod-shaped, spore-forming *Bacillus subtilis* and *Bacillus cereus* were found to produce large, irregular, dry, rough, flat to slightly raised, opaque cream-to-white colonies with irregular or undulating margins, whereas *B. cereus* produced large, flat to slightly raised, opaque greyish-white colonies with a rough or matt surface and irregular margins. Large, smooth, round, convex, moist, translucent to opaque cream or greyish-white colonies with complete edges were generated by *Vibrio alginolyticus*, which appeared as Gram-negative, curved or comma-shaped rods. *Aeromonas hydrophila* generated medium to large, smooth, round, convex, moist, glossy, cream-to-greyish-white colonies with full edges. These cells were rod-shaped and Gram-negative. Gram-negative, rod-shaped, non-motile *Klebsiella pneumoniae* bacteria were found to generate big, smooth, round, convex, extremely mucoid, glossy, greyish-white to cream colonies with complete borders. *Pseudomonas aeruginosa* generated medium to large, smooth to irregular, flat to slightly elevated colonies that were translucent to opaque, with some isolates displaying distinctive blue-green diffusible colouring. *Pseudomonas aeruginosa* was a slender rod-shaped, Gram-negative bacterium. Gram-

negative, rod-shaped *Escherichia coli* bacteria were found to generate medium-sized, smooth, circular, convex, moist, greyish-white to cream-colored colonies with complete borders (Table 1).

Biochemical Characterization

Biochemical characterization demonstrated distinct profiles among the isolates. *Bacillus subtilis* was catalase-positive, oxidase-negative, motile and nitrate-reduction-positive, with positive carbohydrate utilization and generally positive Voges–Proskauer reaction. *Bacillus cereus* was catalase-positive, oxidase-negative, motile and nitrate-reduction-positive, with generally positive Voges–Proskauer and citrate reactions and characteristic haemolytic activity. *Vibrio alginolyticus* was catalase-positive, oxidase-positive, motile and nitrate-reduction-positive, with glucose fermentation and generally negative lactose fermentation. *Aeromonas hydrophila* was catalase-positive, oxidase-positive, motile, indole-positive, methyl red-positive and nitrate-reduction-positive, with glucose fermentation and variable citrate and Voges–Proskauer reactions. *Klebsiella pneumoniae* was catalase-positive, oxidase-negative, non-motile, methyl red-negative, Voges–Proskauer-positive, citrate-positive and urease-positive, and fermented glucose, lactose

and sucrose with acid and gas production. *Pseudomonas aeruginosa* was catalase-positive, oxidase-positive, motile, citrate-positive and nitrate-reduction-positive, showing oxidative rather than fermentative metabolism of glucose and no typical gas production. *Escherichia coli* was catalase-positive, oxidase-negative, motile, methyl red-positive, Voges–Proskauer-negative, indole-positive, citrate-negative and nitrate-reduction-positive, and fermented glucose, lactose and mannitol with production of acid and gas. Overall, the microscopic, colony and biochemical characteristics provided useful presumptive differentiation and supported the identification of the seven bacterial isolates (Table 2&3).

DISCUSSION

The current study aimed to document the diversity of seagrass species found in Pulicat Lake, as well as to identify and characterize the culturable bacterial populations that live there. A total of seven bacterial isolates (HO-1 to HO-4 from *H. ovalis* and SI-1 to SI-3 from *S. isoetifolium*) were recovered and characterized using colony morphology, microscopic examination, and a standard biochemical panel. Two seagrass species, *Halophila ovalis* and *Syringodium isoetifolium*, were identified based on

morphological features. According to previous reports, *H. ovalis* and *S. isoetifolium* are common inhabitants of shallow, brackish, and estuarine waters along the Indian coast [2]. These findings confirm that Pulicat Lake, India's second-largest brackish water lagoon; supports established seagrass beds along with a taxonomically diverse associated bacterial flora. Despite the small global pool of only about 60 recognized seagrass species, the recovery of two morphologically and ecologically distinct species from a single lagoon supports the importance of brackish lagoon systems as reservoirs of marine angiosperm diversity and is generally consistent with the broader ecological literature that describes seagrass meadows as structurally complex habitats that support biodiversity at multiple trophic levels [2, 3].

The bacterial isolates from both seagrass species showed significant variation in colony morphology, texture, elevation, and pigmentation, supporting the idea that colony morphology is still a helpful initial method for differentiating bacterial isolates before molecular confirmation. The isolates were assumed to belong to six species based on microscopic and biochemical traits: *Bacillus* (*B. subtilis* and *B. cereus*), *Vibrio*

(*V. alginolyticus*), *Aeromonas* (*A. hydrophila*), *Klebsiella* (*K. pneumoniae*), *Pseudomonas* (*P. aeruginosa*) and *Escherichia* (*E. coli*). *Bacillus*, *Vibrio*, *Pseudomonas*, and related heterotrophic genera consistently dominate culturable isolates recovered from seagrass tissue and rhizosphere sediment; this genus-level spread is consistent with other culture-based surveys of marine plant-associated bacteria [10].

The two *Bacillus* isolates, *B. subtilis* and *B. cereus*, produced dry, rough, spore-forming colonies typical of the genus and were catalase-positive, oxidase-negative, motile, and nitrate-reduction-positive. In keeping with the wider recognition of marine bacteria as a significant source of bioactive natural products, *Bacillus* species are often isolated from plant-associated marine environments and are of particular interest because many strains are known producers of antimicrobial peptides and extracellular enzymes with biotechnological value [9,8]. Gram-negative, oxidase-positive organisms with fermentative glucose metabolism, *Vibrio alginolyticus* and *Aeromonas hydrophila* are well-known residents of brackish and estuarine water environments and have been linked to marine plants in the past.

Klebsiella pneumoniae and *Escherichia coli* are more commonly associated with fecal or anthropogenic contamination than with marine plant surfaces; their presence may reflect genuine rhizosphere colonization, given that brackish lagoons such as Pulicat Lake receive freshwater and agricultural runoff, or low-level contamination during sample handling, a distinction that cannot be made solely based on phenotypic data. *Pseudomonas aeruginosa* is a metabolically diverse organism that is frequently found in marine sediments. It is distinguished by its oxidative glucose metabolism, motility, and, in certain isolates, a distinctive blue-green diffusible pigment. It is also of interest as an opportunistic pathogen and as a producer of bioactive compounds. Overall, the biochemical profiles—catalase activity, oxidase reaction, IMViC results, ONPG hydrolysis, nitrate reduction, and carbohydrate fermentation patterns—were generally consistent with the diagnostic descriptions in Bergey's Manual of Determinative Bacteriology [13]. This supported the validity of the culture-based approach while also highlighting its main drawback: culture-dependent methods are known to underestimate total microbial

diversity in comparison to culture-independent surveys [17].

Vibrio, *Aeromonas*, *Bacillus*, *Pseudomonas*, *Klebsiella*, and *Escherichia* from the rhizosphere, roots, and leaves of *H. ovalis* and *S. isoetifolium* is consistent with growing evidence that seagrasses actively structure a host-associated microbial community distinct from the surrounding water and sediment, which is commonly referred to as the holobiont concept. Although this work did not specifically test for them, the genera found here may have functions including nitrogen fixation, growth stimulation, and pathogen antagonism that have been linked to seagrass-associated bacteria elsewhere [7]. The results also have practical implications for bioprospecting: *Bacillus* and *Pseudomonas* strains obtained here represent priority candidates for future screening against these bioactivity endpoints, and seagrass surfaces and rhizospheres are increasingly recognized globally as sources of antimicrobial, antioxidant, and enzyme-inhibitory secondary metabolites [10, 11]. As one of the first attempts to integrate seagrass diversity assessment with systematic bacterial characterization in Pulicat Lake, the study makes a valuable contribution. It establishes a baseline for future, more comprehensive

surveys and supports the larger research agenda that links coastal biodiversity assessment to SDG 3 (good health and well-being) and SDG 14 (life below water).

CONCLUSION

The present study provides baseline documentation of seagrass diversity in Pulicat Lake and characterizes the bacterial communities associated with two seagrass species, *Halophila ovalis* and *Syringodium isoetifolium*. The key findings are summarized below:

- Two seagrass species, *Halophila ovalis* and *Syringodium isoetifolium*, were identified in Pulicat Lake on the basis of morphological characteristics.
- Seven bacterial isolates (HO-1 to HO-4 and SI-1 to SI-3) were obtained from the two seagrass species and showed distinct colony morphology.
- Biochemical characterization revealed physiological differences between isolates from the two host species.
- The findings establish Pulicat Lake as a habitat supporting diverse seagrass-associated bacterial communities, warranting further investigation into their ecological roles and biotechnological potential.

This work contributes to a better understanding of seagrass and associated

microbial diversity in Indian brackish water ecosystems and aligns with global efforts under SDG 3 (Good Health and Well-Being) and SDG 14 (Life below Water).

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FIGURES AND TABLES



Figure 1: Collection of Seagrasses from Pulicat Lake

**Figure 2: Identification of Seagrasses****Table 1: Microscopic examination and morphological characterization**

Isolate Code	Bacterial species	Gram reaction	Cell morphology	Arrangement	Spore	Motility	Colony morphology on Marine Agar / suitable medium
HO-1	<i>Bacillus subtilis</i>	Gram-positive	Large, straight rods	Single/pairs/chains	Endospore-forming	+	Large, opaque, dry/rough, irregular margins; cream to white
HO-2	<i>Bacillus cereus</i>	Gram-positive	Large rods	Single/pairs/chains	Endospore-forming	+	Large, dull/opaque, irregular,

							spreading colonies; grey-white
HO-3	<i>Vibrio alginolyticus</i>	Gram-negative	Curved/comma-shaped rods	Single or pairs	—	+	Smooth, circular, convex, moist colonies; cream to translucent
HO-4	<i>Aeromonas hydrophila</i>	Gram-negative	Straight rods	Single/pairs	—	+	Smooth, circular, convex, moist/mucoid; cream to pale yellow
SI-1	<i>Klebsiella pneumoniae</i>	Gram-negative	Short rods	Single/pairs	—	—	Large, smooth, glistening, highly mucoid, convex colonies
SI-2	<i>Pseudomonas aeruginosa</i>	Gram-negative	Slender straight rods	Single/pairs	—	+	Smooth to slightly irregular; grey-green; some strains produce blue-green pigment
SI-3	<i>Escherichia coli</i>	Gram-negative	Straight rods	Single/pairs	—	+	Smooth, circular, convex, moist, grey-white colonies

*HO- *Halophila ovalis*; SI- *Syringodium isoetifolium*; Colony appearance is medium- and strain-dependent.

Table 2: Biochemical characterization

Iso lat e Co de	Species	Cata lase	Oxid ase	Indole	MR	VP	Cit rat e	Ure ase	Nitrat e reduct ion	O/F glucose
HO-1	<i>Bacillus subtilis</i>	+	+/V	-/V	V	+	+/V	-	+	Oxidative/ fermentative
HO-2	<i>Bacillus cereus</i>	+	+	-	V	+	+	-	+	Fermentative
HO-3	<i>Vibrio alginolyticus</i>	+	+	-/V	V	V	V	-	V	Fermentative
HO-4	<i>Aeromonas hydrophila</i>	+	+	+/V	+/V	-/V	+/V	-	+	Fermentative
SI-1	<i>Klebsiella pneumoniae</i>	+	-	-	-	+	+	+	+	Fermentative
SI-2	<i>Pseudomonas aeruginosa</i>	+	+	-	-	-	+	-	+/V	Oxidative; non-fermentative
SI-3	<i>Escherichia coli</i>	+	-	+	+	-	-	-	+	Fermentative

*HO- *Halophila ovalis*; SI- *Syringodium isoetifolium*; + = positive reaction; - = negative reaction; V = variable among strains/species; A/G = acid and gas production.

Table 3: Carbohydrate utilization/fermentation

Isolate Code	Species	Glucose	Lactose	Sucrose	Maltose	Mannitol
HO-1	<i>Bacillus subtilis</i>	+	V	+/V	+	+
HO-2	<i>Bacillus cereus</i>	+	-/V	+/V	+	+

HO-3	<i>Vibrio alginolyticus</i>	+	–	+	+	+
HO-4	<i>Aeromonas hydrophila</i>	+	–	+ / V	+	+
SI-1	<i>Klebsiella pneumoniae</i>	A/G	A/G	A/G	A/G	A/G
SI-2	<i>Pseudomonas aeruginosa</i>	Oxidative/non-fermentative	–	–	–	– / V
SI-3	<i>Escherichia coli</i>	A/G	A/G	– / V	A/G	A/G

*HO- *Halophila ovalis*; SI- *Syringodium isoetifolium*; + = positive reaction; – = negative reaction; V = variable among strains/species; A/G = acid and gas production.