

Isolation and Characterization of Halophyte-Associated Endophytes with Plant Growth Promoting Potential in *Suaeda fruticosa* and *Suaeda maritima* from Coringa Wildlife Sanctuary, Andhra Pradesh, India

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Abstract

Halophytes are specialized plants that thrive under extreme saline and tidal conditions where most crop species fail to survive. These plants have evolved unique physiological and biochemical strategies to tolerate high salt stress, often supported by beneficial microbial communities residing within their tissues. Among these, endophytic bacteria play a crucial role by enhancing host growth, nutrient uptake, and stress tolerance without causing any visible harm to the plant. The present study aims to isolate, screen, and characterize halophyte-associated endophytic bacteria from *Suaeda fruticosa* and *S. maritima* collected from Koringa Wildlife Sanctuary, India, and to evaluate their plant growth promoting potential under halophytic conditions.

1. Introduction

Drought and soil salinity are the most severe abiotic stresses limiting agricultural productivity, particularly in arid and semi-arid regions, thereby reducing cultivable land and threatening global food security (Shrivastava & Kumar, 2015). Currently, more than 800 million hectares of land worldwide are affected by salinity, posing a major challenge to sustainable agriculture. Halophytes, which are naturally adapted to saline environments, offer a promising approach for the reclamation of salt-affected soils through revegetation (Breckle et al., 2001; El Shaer, 2010). However, high salinity significantly delays seed germination and reduces biomass production in halophytes, limiting their establishment in highly saline soils (Song et al., 2008).

It is increasingly recognised that plants in natural ecosystems maintain symbiotic associations with endophytic microorganisms highly adapted to their habitats. These endophytes play a crucial role in enhancing host stress tolerance and overall fitness, thereby improving plant survival under extreme environmental conditions (Redman et al., 2011; Rodriguez et al., 2008).

Recent advances in omics technologies have greatly enhanced the discovery and characterization of endophytic microbes. Almost all plant species in natural ecosystems harbour diverse endophytic microbial communities, with more than 16 bacterial phyla and over 200 genera reported to date (Afzal et al., 2014).

The diversity and composition of endophytic communities are influenced by multiple factors, including host species and genotype, plant growth stage, geographic location, environmental conditions, and methodological approaches such as surface sterilization procedures (Hallmann 2001; Hallmann *et al.*, 1997; Shi et al., 2012; Deng *et al.*, 2023).

Even under similar soil conditions, different plant species and cultivars harbour distinct endophytic microbial assemblages, as observed in *Brassica napus*, tomato, and canola grown across varied locations (Rashid *et al.*, 2012). Environmental stresses further shape these communities; for example, plants in petroleum-contaminated sites host endophytes capable of degrading hydrocarbons, highlighting the adaptive nature of plant–microbe associations. Endophytic microbes are non-pathogenic microorganisms that inhabit internal plant tissues and play a vital role in enhancing plant growth, health, and stress tolerance. Advances in omics and molecular techniques have greatly improved the understanding of their diversity, distribution, and functional roles (Singh, 2009). Endophytes are now recognized as universal plant associates, with more than 16 bacterial phyla and over 200 genera reported across plant species. Dominant groups include Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes, which colonize not only roots but also stems, leaves, seeds, fruits, tubers, and ovules.

The composition of endophytic communities is influenced by host genotype, developmental stage, geographic location, and environmental conditions. These microbes adapt to biotic and abiotic stresses in their host environments; for example, plants growing in contaminated soils harbor endophytes capable of degrading pollutants. Understanding the diversity and ecological roles of endophytic microbes is essential for their application in sustainable agriculture, particularly for improving plant growth, stress resistance, and disease management.

Mangroves represent a remarkable ecosystem known for its salt tolerance. Typically found in tropical and subtropical intertidal zones across the globe, they thrive in regions situated between high water levels and near mean sea levels. These ecosystems are prevalent along shores, tidal creeks, brackish waters, estuaries, lagoons, marshes, and mud flats. One such significant mangrove ecosystem is the Godavari mangrove, located in Andhra Pradesh, India. It plays a pivotal role in ensuring livelihood security for coastal communities and ecological stability in the coastal regions.

The genus *Suaeda* (Amaranthaceae) comprises salt-tolerant shrubs and herbs commonly distributed in coastal marshes and mangrove-associated ecosystems. *Suaeda fruticosa* and *Suaeda maritima* are dominant halophytes in the Koringa Wildlife Sanctuary, a protected mangrove ecosystem along the eastern coast of Andhra Pradesh, India. This region is characterized by fluctuating salinity, waterlogging, and nutrient-poor soils, making it an ideal natural laboratory for exploring stress-adapted microbial communities.

Suaeda fruticosa is well suited to saline and coastal habitats, including salt marshes, estuaries, and sandy coastal areas. It is often found in regions with high salinity levels, where many other plant species struggle to survive. This species exhibits a high degree of salt tolerance, allowing it to thrive in environments with elevated salt concentrations in the soil and water. The succulent leaves and salt tolerance of *Suaeda fruticosa* are adaptations that help it cope with the challenging conditions of coastal ecosystems. These adaptations enable it to survive in areas where freshwater is scarce and salinity is a dominant factor. *Suaeda fruticosa* plays a crucial ecological role in stabilizing coastal soils, preventing erosion, and providing habitat and food for various wildlife species.

Suaeda maritima is primarily an annual plant. This adaptation allows it to thrive in dynamic coastal environments where conditions can change rapidly. This versatile plant can be found in a range of habitats, including salt marshes, seashore and coastal areas. It plays a crucial role in the accumulation of mud and contributes to the formation and stability of salt marsh ecosystems. *Suaeda maritima* unique combination of

fleshy, salt tolerant leaves and its adaptability to various coastal habitats make it a notable species in coastal ecosystems.

Endophytic bacteria associated with halophytes are increasingly recognized for their ability to produce plant growth-promoting (PGP) metabolites such as indole-3-acetic acid, siderophores, ammonia, and ACC deaminase, and for their capacity to solubilize phosphate and fix atmospheric nitrogen. These traits contribute significantly to plant vigour, especially under saline and abiotic stress conditions. Moreover, halophyte-derived endophytes often exhibit high salt tolerance, making them promising candidates for application in saline agriculture and environmental restoration programs.

Despite the ecological and biotechnological importance of mangrove-associated halophytes, the endophytic bacterial diversity of *Suaeda* species from the Koringa region remains largely unexplored. Most studies on plant growth-promoting endophytes have focused on crops, while native halophytes from extreme coastal environments have received limited attention. Exploring these microbial communities can reveal novel, stress-resilient bacterial strains with potential agricultural and environmental applications.

This study aims to isolate and characterize endophytic bacteria associated with the halophytes *Suaeda fruticosa* and *Suaeda maritima* from Koringa Wildlife Sanctuary, located in Andhra Pradesh, India, and to assess their plant growth-promoting potential under saline conditions, contributing to sustainable bioinoculant development and improved understanding of plant-microbe interactions in saline ecosystems.

2. Materials and methods

2.1 Study Area

The present study was carried out at the Department of Botany, Andhra University, Visakhapatnam, using plant samples collected from the Coringa Wildlife Sanctuary, located in the East Godavari district of Andhra Pradesh, India, at 16°49'53.0" N latitude and 82°20'12.0" E longitude (16.831389, 82.336667). Salinity levels vary with seasonal conditions in the region. During the monsoon season, the influx of freshwater from the Godavari River reduces salinity to 3-10 PPT. However, in the summer season, salinity in both water and soil increases, with water salinity reaching 30-35 PPT and soil salinity ranging from 3-10 PPT (Sivakumar, et al., 2016).

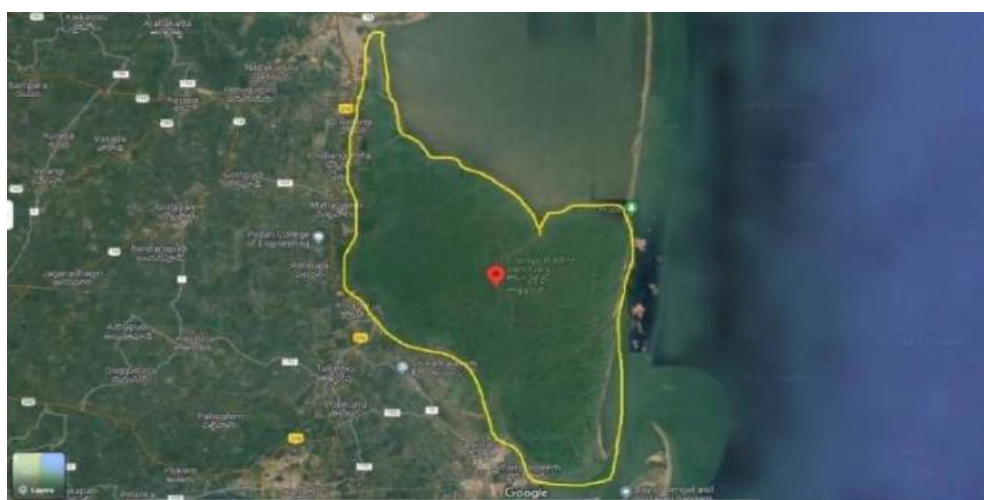


Fig 1. Satellite image of Coringa Wildlife Sanctuary, Andhra Pradesh.

2.2 Collection of Plant Samples

Aerial and root tissue samples of selected *Suaeda* species were gathered from the mangrove zone of Coringa Wildlife Sanctuary. All plant samples were collected in sterile bags and transported immediately to the laboratory in a cold pack for isolation of bacterial endophytes.



Fig 2. Collection of Plant Samples. A. *Suaeda maritima* B. *Suaeda fruticosa*

2.3 Isolation of symbiotic endophytic bacteria from tissue samples

Plant samples were thoroughly washed to remove soil and then surface sterilized using 70% ethanol, followed by 2% sodium hypochlorite, with the process repeated twice to eliminate external contaminants. The sterilized tissues were air-dried under laminar airflow, and the outer layers were removed. Inner tissues were macerated in sterile saline to release endophytic bacteria. The homogenates were serially diluted, and 100 μ L of the highest dilution was spread on Nutrient Agar plates. The plates were incubated at 32°C, and endophytic colonies were observed after 24 and 48 hours.

Nutrient media from Hi-Media and Merck were prepared as per the manufacturers' instructions by dissolving in distilled water and sterilized by autoclaving at 121 °C and 15 psi for 15 minutes before use for bacterial endophyte isolation.

2.4 Phenotypic categorization of endophytic isolates

Phenotypic characterization of endophytic bacterial isolates provides essential information regarding their physical attributes and growth patterns. This data contributes to the detailed understanding of these isolates and helps in their accurate identification and classification. All the endophytic bacterial isolates were cultivated on nutrient agar plates. Detailed records of both cell and colony morphology were documented as per the procedure detailed by Aneja (2006).

Gram staining was conducted to reveal the bacterial morphology including cocci, rods, coccobacilli and spiral shapes, as well as their Gram staining reaction (Gram-positive or Gram-negative) using the Modified Gram's Staining procedure.

2.5 Biochemical categorization of endophytic bacterial isolates

All the endophytic bacterial isolates were subjected to different biochemical test like catalase test, oxidase test, Indole, Methyl Red, Voges Proskauer and Citrate (IMViC) test for identification and classification of the bacterial cultures.

2.5.1 Catalase Test

The catalase test was performed to detect the ability of endophytic bacterial isolates to produce the enzyme catalase, which decomposes hydrogen peroxide into water and oxygen. Fresh bacterial cultures from nutrient agar were placed on a clean glass slide, and 3% hydrogen peroxide was added. The appearance of gas bubbles indicated a positive reaction, while the absence of bubbles indicated a negative result (Aneja, 2006).

2.5.2 Oxidase Test

The oxidase test was performed to detect the presence of cytochrome oxidase, an enzyme involved in the bacterial electron transport chain. Endophytic bacterial isolates were grown on Trypticase Soya Agar, and after incubation, TMPD reagent was applied to the culture. A rapid color change from pink to purple within 15–30 seconds indicated a positive reaction, while no color change indicated a negative result (Aneja, 2006).

2.5.3 Indole Production Test

The indole test was used to determine the ability of endophytic bacterial isolates to metabolize tryptophan and produce indole. Isolates were grown in glucose tryptone broth for 48 hours, after which Kovac's reagent was added. The formation of a red alcoholic layer on the surface indicated a positive result, while the absence of a red ring indicated a negative reaction.

2.5.4. Methyl Red Test

The Methyl Red (MR) test was performed to assess acid production from glucose metabolism by endophytic bacterial isolates. Cultures were grown in glucose phosphate broth at 32°C for 48 hours, after which methyl red indicator was added. A red colour indicated a positive result, while no colour change indicated a negative result.

2.5.5. Voges-Proskauer Test

The Voges-Proskauer (VP) test was conducted to detect acetylmethylcarbinol production from glucose fermentation by endophytic bacterial isolates. Cultures were grown in glucose phosphate broth at 32°C for 48 hours. After incubation, Barritt's reagents A (5% α -naphthol) and B (40% KOH) were added in a 3:1 ratio. Formation of a red ring at the surface indicated a positive result, while no colour change indicated a negative result.

2.5.6. Citrate Utilization Test

The citrate utilization test was used to determine whether endophytic bacterial isolates can use citrate as the sole carbon source. Isolates were inoculated on Simmons' citrate agar containing bromothymol blue and incubated at 32°C for 24–48 hours. A colour changes from green to blue indicated a positive result, while no colour change indicated a negative result.

2.5.7. Amino Acid Decarboxylase Test

The amino acid decarboxylase test was performed to assess the ability of endophytic bacterial isolates to produce decarboxylase enzymes that remove the carboxyl group from amino acids (arginine, lysine, or ornithine), producing amines and carbon dioxide. Cultures were grown in decarboxylase medium with 0.5% of the respective amino acid and incubated. Bromocresol purple was used as a pH indicator: a colour change to purple indicated a positive reaction (enzyme activity), while yellow or no colour change indicated a negative reaction.

2.6. In Vitro Characterization of Enzymatic Activities of Endophytic Bacteria

Endophytic bacterial isolates were tested for enzyme production and plant growth-promoting potential.

2.6.1. Amylase (Starch Hydrolysis): Isolates were grown on starch-containing agar and stained with iodine. Clear zones indicated positive amylase activity, while blue zones indicated no hydrolysis.

2.6.2. Protease (Gelatin Hydrolysis): Isolates were grown on gelatin agar and treated with ammonium sulfate. Clear zones indicated protease activity, while cloudy zones indicated a negative result.

2.6.3 IAA Production by Endophytic Bacteria

Endophytic bacterial isolates were screened for production of the phytohormone indole-3-acetic acid (IAA) using a colorimetric assay with Salkowski's reagent. Fresh cultures were first activated in nutrient broth, then inoculated into nutrient broth containing 0.2% L-tryptophan and incubated at 32°C for 48 hours. Cultures were centrifuged, and the supernatant was mixed with Salkowski's reagent. IAA production was indicated by a colour change from orange to dark purple, which was quantified spectrophotometrically at 536 nm against an IAA standard curve.

Salkowski's reagent was prepared by combining ferric chloride solution with perchloric acid to yield a pale-yellow solution.

2.6.4 Phosphate Solubilization Assay

Endophytic bacterial isolates were tested for their ability to solubilize phosphate using Pikovskaya's broth. Cultures were first activated in nutrient broth, then inoculated into Pikovskaya's broth and incubated at 32°C for 48 hours. After centrifugation, the supernatant was mixed with phosphate reagent, and the development of blue colour indicated phosphate solubilization. The intensity of the color, measured spectrophotometrically at 680 nm, was compared with a phosphate standard curve to quantify the released phosphate.

The phosphate reagent (Molybdenum Blue Assay) produces a blue complex when orthophosphates react with ammonium molybdate and are reduced by ascorbic acid in the presence of potassium antimony tartrate.

2.7. Molecular Identification Using 16S rRNA Gene

Molecular identification of endophytic bacterial isolates was performed using the 16S rRNA gene, a widely used marker for assessing genetic relatedness and constructing phylogenetic trees. Isolates with >97% 16S rRNA sequence similarity are typically considered the same species (Dighe et al., 2004; Greenwood et al., 2012).

2.7.1 DNA extraction, Storage and Quality check

Bacterial cultures were centrifuged to pellet cells, which were lysed using 200 μ L lysis buffer and heat-treated at 100°C. The DNA was separated from cellular debris by centrifugation, collected, and stored at -20°C. DNA purity and integrity were checked using 1% agarose gel electrophoresis, and the samples were used for subsequent genetic analyses and phylogenetic studies (Godini & Fallahi, 2019; Van de Peer, 2009; Mittreva, 2017).

2.7.2. PCR, Sequencing, and Phylogenetic Analysis

Fragments (~1500 bp) of the 16S rRNA gene were amplified from bacterial DNA using specific primers. The PCR products were sequenced and analyzed using BLAST against the NCBI GenBank database. The top matching sequences were aligned with Clustal W, and a phylogenetic tree along with a distance matrix was constructed using MEGA10 software to determine evolutionary relationships among the isolates.

2.8. Statistical Analysis

All experiments were performed in triplicate, with three samples per replication, and mean values were calculated from at least five observations. Statistical analyses were conducted using IBM SPSS version 25, and the results were presented in tabulated below.

3. Results and discussion

3.1 Collection, isolation and coding of endophytes from samples of *Suaeda fruticosa* and *Suaeda maritima*.

A total of 17 endophytic bacteria were isolated from the two halophytic plant species *Suaeda fruticosa* and *Suaeda maritima* belonging to the family Amaranthaceae. *Suaeda fruticosa* reports a total of 9 isolates, three from aerial parts and six from roots. While *Suaeda maritima* records a total of 8 isolates two from aerial parts and six from roots. Roots generally yielded 12 isolates, 5 isolates from aerial parts, suggesting higher endophytic colonization in roots compared to aerial tissues.

Plant tissues were surface-sterilised, cut into small fragments, and macerated in sterile saline. The resulting suspension was serially diluted, and bacterial endophytes were cultured from higher dilutions (10^{-3} to 10^{-5}) on nutrient agar. Distinct colonies were isolated and purified using repeated quaternary streaking. Pure cultures were sub-cultured, mixed with 20% glycerol for long-term storage at -20°C, and maintained at 4°C for short-term use. Each isolate was assigned a unique code based on the host plant, plant part, and serial number to facilitate easy identification and organisation. The results are cited in Table 1.

Table 1: Coding of endophytic bacterial isolates from *Suaeda maritima* and *Suaeda fruticosa*.

Plant	Locality of Collection	Plant part	No of strains isolated	Coded Strains
<i>Suaeda fruticosa</i>	Coringa Mangrove Wildlife	Aerial parts	3	<i>Suaeda fruticosa</i> Aerial-1 (SFA-1), <i>Suaeda fruticosa</i> Aerial-2 (SFA-2), and <i>Suaeda fruticosa</i> Aerial-3 (SFA-3)
		Root	6	<i>Suaeda fruticosa</i> Root-1 (SFR1), <i>Suaeda</i>

	Sanctuary, East Godavari district, Andhra Pradesh			<i>fruiticosa</i> Root-2 (SFR2), <i>Suaeda fruiticosa</i> Root-3 (SFR3), <i>Suaeda fruiticosa</i> Root-4 (SFR4), <i>Suaeda fruiticosa</i> Root-5 (SFR5), and <i>Suaeda fruiticosa</i> Root-6 (SFR6)
<i>Suaeda maritima</i>		Aerial parts	2	<i>Suaeda maritima</i> Aerial-1 (SMA1), and <i>Suaeda maritima</i> Aerial-2 (SMA2)
		Root	6	<i>Suaeda maritima</i> Root-1 (SMR1), <i>Suaeda maritima</i> Root-2 (SMR2) <i>Suaeda maritima</i> Root-3 (SMR3) <i>Suaeda maritima</i> Root-4 (SMR4) <i>Suaeda maritima</i> Root-5 (SMR5) <i>Suaeda maritima</i> Root-6 (SMR6)

3.2 Colony morphology of endophyte isolates from *Suaeda fruticosa* and *Suaeda maritima*.

The endophytic isolates from *Suaeda maritima* and *Suaeda fruticosa* showed considerable variation in colour, size, shape, surface texture, margin, elevation, and pigment production, indicating a diverse bacterial community. Based on Gram staining, Catalase and Oxidase test, preliminary identification of endophytes was carried out, as presented in Table 2.

Within the *Suaeda maritima* plant, two endophytic bacteria were isolated from aerial samples, while six were obtained from root samples. Preliminary identification indicated that five of these were Gram-positive bacilli, and the remaining three were Gram-negative rods, belonging to the Aeromonas, Vibrio, and Coccobacilli families.

Suaeda fruticosa expressed a total of nine endophytic bacteria, with three from aerial samples and six from root samples. Preliminary analysis categorized six as Gram-positive bacilli and the remaining three as Gram-negative bacteria belonging to the Pseudomonas, Vibrio and Coccus families.

Most colonies were opaque or cloudy, with a few transparent types. Pigment production varied: some isolates showed orange or yellow pigments, while several had no visible pigment. Colony sizes ranged from small to large, and most were round, though some isolates were irregular or swarming, suggesting different growth patterns. The majority of colonies had smooth surfaces with entire margins. Texture varied from dry to moist, indicating differences in exopolysaccharide production or water retention. Most colonies were raised, with occasional flat colonies, reflecting structural diversity among isolates.

Table 2: Colony morphology of endophytes obtained from halophytic *Suaeda maritima* and *Suaeda fruticosa*

Strain	Colour	Size	Shape	Surface	Texture	Margin	Elevation	Pigment
<i>Suaeda maritima</i>								
SMA1	Cloudy	Small	Round	Smooth	Moist	Entire	Raised	Orange
SMA2	Opaque	Medium	Round	Smooth	Dry	Entire	Raised	Orange
SMR1	Opaque	Small	Round	Shiny Smooth	Dry	Entire	Raised	Orange
SMR2	Transparent	Small	Swarming	Smooth	Moist	Entire	Flat	---
SMR3	Cloudy	Medium	Round	Smooth	Dry	Undulate	Raised	---
SMR4	Opaque	Medium	Round	Smooth	Moist	Entire	Raised	---
SMR5	Cloudy	Medium	Round	Shiny Smooth	Moist	Entire	Raised	---
SMR6	Opaque	Small	Round	Smooth	Dry	Entire	Raised	---
<i>Suaeda fruticosa</i>								
SFA1	Opaque	Small	Irregular	Smooth	Dry	Entire	Raised	---
SFA2	Opaque	Small	Round	Smooth	Dry	Entire	Raised	---
SFA3	Cloudy	Small	Round	Shiny Smooth	Moist	Entire	Flat	Yellow
SFR1	Cloudy	Small	Round	Smooth	Moist	Entire	Raised	---
SFR2	Opaque	Large	Irregular	Smooth	Dry	Entire	Raised	
SFR3	Opaque	Small	Round	Smooth	Moist	Entire	Raised	Light Yellow
SFR4	Transparent	Small	Round	Smooth	Dry	Entire	Raised	Yellow
SFR5	Cloudy	Large	Irregular	Smooth	Dry	Undulate	Raised	---
SFR6	Cloudy	Small	Irregular	Smooth	Dry	Entire	Raised	---

3.3 Bacterial cell morphology of isolates from *Suaeda fruticosa* and *Suaeda maritima*.

The cell morphology of the purified endophytic bacterial isolates was examined and categorized following the procedures described in the Materials and Methods section. Young bacterial cultures were subjected to dry smear preparation followed by Gram staining for preliminary identification. The stained smears were observed under a microscope at 100× magnification. Observations were recorded based on Gram reaction, with purple colouration indicating Gram-positive bacteria and pink colouration indicating Gram-negative bacteria. Additional characteristics such as cell shape, cell size, cell arrangement, and motility were also noted. The recorded observations are presented in Table 3.

In *Suaeda maritima*, a total of eight endophytic bacterial isolates were obtained, including two from aerial tissues and six from root tissues, indicating greater colonization in roots. Preliminary identification showed that five isolates were Gram-positive bacilli, while three were Gram-negative forms belonging to *Aeromonas*, *Vibrio*, and coccobacilli types.

Similarly, *Suaeda fruticosa* yielded nine endophytic bacterial isolates, with three from aerial tissues and six from roots. Among these, six isolates were identified as Gram-positive bacilli, whereas the remaining three were Gram-negative bacteria related to *Pseudomonas*, *Vibrio*, and coccoid forms. Overall, Gram-positive bacilli predominated in both species, with roots harbouring a higher diversity of endophytic bacteria.

Table 3. Bacterial cell morphology of isolates from *Suaeda maritima* and *Suaeda fruticosa*.

Strain	Gram's reaction	Shape	Size	Arrangement	Endospore	Shape and position of the Spore	Motility	Preliminary Identification
<i>Suaeda maritima</i>								
SMA1	Negative	Rods	Small	Individual cells	Negative	--	Positive	<i>Aeromonas</i> sp.
SMA2	Positive	Rods	Small	Diplobacilli	Negative	--	Positive	Bacillaceae
SMR1	Positive	Rods	Medium	Chains of 2–4	Positive	Oval/Centre	Negative	Bacillaceae
SMR2	Negative	Rods	Small	Individual cells	Negative	--	Positive	Vibrionaceae
SMR3	Positive	Rods	Medium	Diplobacilli	Positive	Oval/Centre	Positive	Bacillaceae
SMR4	Positive	Rods	Small	Individual cells	Negative	--	Positive	Bacillaceae
SMR5	Positive	Rods	Medium	Diplobacilli	Positive	Oval/Terminal	Negative	Bacillaceae
SMR6	Negative	Coccus	Small	Individual cells	Negative	--	Positive	Cocco bacilli
<i>Suaeda fruticosa</i>								
SFA1	Positive	Rods	Small	Individual cells	Negative	--	Positive	Bacillaceae
SFA2	Positive	Rods	Medium	Diplobacilli	Positive	Oval/Centre	Negative	Bacillaceae
SFA3	Negative	Rods	Very small	Individual cells	Negative	--	Positive	<i>Pseudomonas</i> sp.
SFR1	Positive	Rods	Medium	Diplobacilli	Positive	Oval/Centre	Negative	Bacillaceae
SFR2	Positive	Rods	Medium	Individual cells	Positive	Oval/Centre	Negative	Bacillaceae
SFR3	Positive	Rods	Medium	Individual cells	Positive	Oval/Terminal	Negative	Bacillaceae
SFR4	Negative	Rods	Small	Diplobacilli	Negative	--	Positive	Vibrionaceae
SFR5	Negative	Coccus	Small	Individual cells	Negative	--	Positive	Coccus
SFR6	Positive	Rods	Large	Chains of 2–4	Positive	Oval/Centre	Negative	Bacillaceae

3.4 Salt Tolerance

Sodium chloride plays a significant role in bacterial growth by influencing osmotic balance, cellular metabolism, and adaptation to saline environments. It is particularly important in the growth and survival of halophilic and halotolerant microorganisms. The study of salt tolerance in bacteria provides valuable insights into microbial adaptation mechanisms and their potential survival in extreme environmental conditions.

The salt tolerance of the isolated endophytic bacterial strains was evaluated by culturing them on nutrient agar medium supplemented with varying concentrations of sodium chloride. The inoculated plates were incubated at 32°C for 24–48 hours, after which bacterial growth was observed and recorded. The ability of the isolates to grow under elevated salinity conditions indicated their halotolerant or halophytic nature, suggesting their adaptation to saline host environments.

The endophytic bacterial isolates obtained from the halophytic plants *Suaeda maritima* and *Suaeda fruticosa* exhibited varying degrees of tolerance to sodium chloride concentrations ranging from 0% to 14%, indicating their adaptation to saline environments.

Among the isolates from *Suaeda maritima*, most strains demonstrated good growth at moderate salinity levels (3–8% NaCl). Strains SMA1 and SMR6 showed maximum growth (+++) at 3% NaCl and were able to tolerate salinity up to 12%, indicating strong halotolerant characteristics. Several isolates, including SMR2, SMR4, and SMR5, exhibited consistent growth up to 10% NaCl, while SMR3 displayed limited salt tolerance and failed to grow beyond 6% NaCl. Growth was absent in almost all isolates at 14% NaCl, suggesting that extremely high salinity inhibited bacterial proliferation. The results are presented in Table 4.

Similarly, isolates from *Suaeda fruticosa* also exhibited moderate to high salt tolerance. Most strains showed optimal growth at 3% NaCl, with moderate growth maintained up to 8% NaCl. Strains SFA2 and SFR3 demonstrated comparatively higher tolerance, surviving up to 12% NaCl, whereas several other isolates failed to grow beyond 10% NaCl. No isolate from this species was able to tolerate 14% NaCl concentration.

Table 4: Salt tolerance by endophytes isolated from halophytic *Suaeda maritima* and *Suaeda fruticosa*.

Strain	0%	3%	6%	8%	10%	12%	14%
<i>Suaeda maritima</i>							
SMA1	–	+++	++	++	+	–	–
SMA2	–	+	+	+	+	–	–
SMR1	–	+	+	+	+	–	–
SMR2	+	+	+	+	+	–	–
SMR3	–	+	+	–	–	–	–
SMR4	+	+	+	+	+	–	–

SMR5	+	+	+	+	+	—	—
SMR6	+	+++	++	++	+	+	—
<i>Suaeda fruticosa</i>							
SFA1	+	++	++	+	—	—	—
SFA2	—	++	+	++	+	+	—
SFA3	—	++	+	+	—	—	—
SFR1	—	++	+	+	—	—	—
SFR2	—	++	+	+	—	—	—
SFR3	—	++	++	++	+	+	—
SFR4	+	++	+	+	+	—	—
SFR5	+	++	+	+	—	—	—
SFR6	+	++	+	+	—	—	—

3.5 Biochemical categorization of endophytic bacterial isolates

The biochemical analysis of endophytic bacteria from *Suaeda maritima* and *Suaeda fruticosa* showed clear variation in their metabolic behavior. Most of the isolates were catalase positive, indicating their ability to survive oxidative stress, which is common in saline habitats. Oxidase reactions were variable, reflecting differences in their respiratory activity.

In the IMViC tests, most strains showed negative reactions for indole, methyl red, and Voges–Proskauer, while citrate utilization was observed only in a few isolates. This suggests that many of the bacteria follow simpler or non-fermentative metabolic pathways.

The amino acid decarboxylase tests revealed additional diversity, with some strains showing positive reactions for lysine, arginine, and ornithine. This indicates their potential role in nitrogen metabolism and adaptation to stressful conditions.

Table 5: Biochemical categorization of endophytic bacterial isolates from halophytic *Suaeda maritima* and *Suaeda fruticosa*.

Strain	KOH	Catalase	Oxidase	IMViC test				Amino acid decarboxylase test		
				Indole	Methyl Red	Voges Proskauer	Citrate	Lysine	Arginine	Ornithine
<i>Suaeda maritima</i>										
SMA1	+	+	+	−	−	−	−	+	+	+
SMA2	−	+	−	−	−	−	−	−	−	−
SMR1	−	++	+	−	+	−	+	−	+	−
SMR2	+	+	−	+	+	−	+	+	−	+
SMR3	−	+	−	−	−	−	−	−	−	−
SMR4	−	+	+	−	−	−	+	−	+	−
SMR5	−	+	−	−	++	−	+	+	++	+
SMR6	+	+	−	−	+	−	−	+	++	+
<i>Suaeda fruticosa</i>										
SFA1	−	+	+	−	−	−	−	−	−	−
SFA2	−	++	−	−	−	−	−	−	−	−
SFA3	+	+	−	−	−	−	−	−	+	−
SFR1	−	++	−	−	−	−	+	−	+	−
SFR2	−	+	−	−	−	−	−	−	−	−
SFR3	−	+	+	−	−	−	−	−	−	−
SFR4	−	++	D+	−	+	−	+	+	−	+
SFR5	+	+	+	−	+	−	+	−	+	−
SFR6	−	++	D+	−	−	−	−	−	+	−

*Note: ‘+’ indicates normal growth; ‘++’ indicates luxuriant growth; ‘D+’ indicates Delayed growth; ‘—’ indicates No growth

3.6 Preliminary identification of endophytes

The endophytic bacterial isolates obtained from *Suaeda maritima* and *Suaeda fruticosa* exhibited diverse cultural, morphological, Gram’s reaction, and biochemical characteristics. In both plants, most of the isolates were Gram-positive and mainly belonged to the family Bacillaceae, with *Bacillus* spp. being the most frequently recorded genus, indicating their strong association with halophytic plant tissues. Along with these, a few Gram-negative bacteria were also identified. In *S. maritima*, isolates were placed under Enterobacteriaceae (*Aeromonas* sp. and cocco-bacilli forms) and Vibrionaceae (*Vibrio neocaledonicus*), while in *S. fruticosa*,

members of Pseudomonadaceae (*Pseudomonas* sp.), Enterobacteriaceae, and Vibrionaceae (*Vibrio natriegens*) were recorded. The results are represented in Table 6 below.

Table 6: Preliminary identification of endophytes from different halophytic *Suaeda maritima* and *Suaeda fruticosa*.

Strain	Gram's reaction	Preliminary Identification	
		Family	Genus
<i>Suaeda maritima</i>			
SMA1	Negative	Enterobacteriaceae	Aeromonas sp.
SMA2	Positive	Bacillaceae	Bacillus sp.
SMR1	Positive	Bacillaceae	Bacillus sp.
SMR2	Negative	Vibrionaceae	Vibrio neocaledonicus
SMR3	Positive	Bacillaceae	Bacillus sp.
SMR4	Positive	Bacillaceae	Bacillus sp.
SMR5	Positive	Bacillaceae	Bacillus sp.
SMR6	Negative	Enterobacteriaceae	Cocco bacilli
SFA1	Positive	Bacillaceae	Bacillus sp.
<i>Suaeda fruticosa</i>			
SFA2	Positive	Bacillaceae	Bacillus sp.
SFA3	Negative	Pseudomonadaceae	Pseudomonas sp.
SFR1	Positive	Bacillaceae	Bacillus sp.
SFR2	Positive	Bacillaceae	Bacillus sp.
SFR3	Positive	Bacillaceae	Bacillus sp.
SFR4		Vibrionaceae	Vibrio natriegens
SFR5	Negative	Enterobacteriaceae	Coccus
SFR6	Positive	Bacillaceae	Bacillus sp.

3.7 Plant growth promoting property and enzymatic activity of endophytic bacterial isolates.

The endophytic bacteria isolated from *Suaeda maritima* and *Suaeda fruticosa* showed clear differences in their ability to support plant growth and produce useful enzymes. While a few strains did not show any activity, most of them displayed at least one beneficial trait.

IAA production varied widely, but two strains, SMR2 and SFR4, stood out with very high levels, suggesting strong potential to promote plant growth. Many isolates were also able to solubilize phosphate, which can help plants absorb nutrients more efficiently, with SFR3 showing the highest activity.

Enzyme production (amylase and protease) was moderate across most strains, with a few showing stronger activity, especially SFR2 and SFR3. Overall, the results indicate that these halophytic plants host a diverse group of endophytic bacteria, and some of them could be useful for improving plant growth and soil health under saline conditions. The results are tabulated below.

Table 7: Plant growth promoting property and enzymatic activity of endophytic bacterial isolates from *Suaeda maritima* and *Suaeda fruticosa*.

Strain	Plant Growth Promoting Property		Extracellular enzyme production	
	IAA($\mu\text{g/ml}$)	PSB(ppm)	Amylase (cm)	Protease (cm)
<i>Suaeda maritima</i>				
SMA1	NIL	NIL	NIL	NIL
SMA2	NIL	NIL	NIL	NIL
SMR1	NIL	3.0	1.6	2.0
SMR2	50.0	4.0	1.8	3.0
SMR3	2.6	5.1	1.2	2.0
SMR4	3.6	5.1	0.5	2.4
SMR5	2.3	5.3	1.5	2.0
SMR6	18.0	4.0	0.7	0.6
<i>Suaeda fruticosa</i>				
SFA1	NIL	NIL	NIL	NIL
SFA2	NIL	3.7	0.7	NIL
SFA3	NIL	NIL	NIL	NIL
SFR1	0.7	3.6	1.4	2.2
SFR2	2.7	5.3	2.4	3.4
SFR3	5.8	6.2	1.8	3.8
SFR4	55.0	3.7	1.2	1.7
SFR5	4.7	4.9	2.0	2.0
SFR6	0.7	3.8	0.6	2.0

3.8 Endophytic bacterial isolates showing both IAA production and Phosphate solubilization.

The endophytic bacterial strains were evaluated for their capacity to produce both Indole-3-Acetic acid (IAA) and solubilize phosphate. Among the strains tested, SFR4, isolated from *Suaeda fruticosa*, exhibited notable characteristics with 55 µg/ml of IAA production and 3.70 ppm for phosphate solubilization. Following closely strains SMR2, isolated from *Suaeda maritima*, recorded 50 µg/ml for IAA production and 4.0 ppm for phosphate solubilization. Upon Preliminary identification, strain SFR4 from *Suaeda fruticosa* was identified as *Vibrio natriegenes*. Strains SMR2 and SMR6 from *Suaeda maritima* were identified as *Vibrio neocaledonicus* and *Coccobacilli*, respectively. The results are noted in the Table 8 and Fig.3

Table 8: Endophytic bacterial isolates showing both IAA production and Phosphate solubilization from *Suaeda maritima* and *Suaeda fruticosa*.

Endophytic Bacterial isolates	IAA Production (µg/ml)	Phosphate solubilization (ppm)	Preliminary identification
<i>Suaeda maritima</i>			
SMR2	50.0	4.0	<i>Vibrio neocaledonicus</i>
SMR6	18.0	4.0	<i>Cocco bacilli</i>
<i>Suaeda fruticosa</i>			
SFR4	55.0	3.7	<i>Vibrio natriegenes</i>

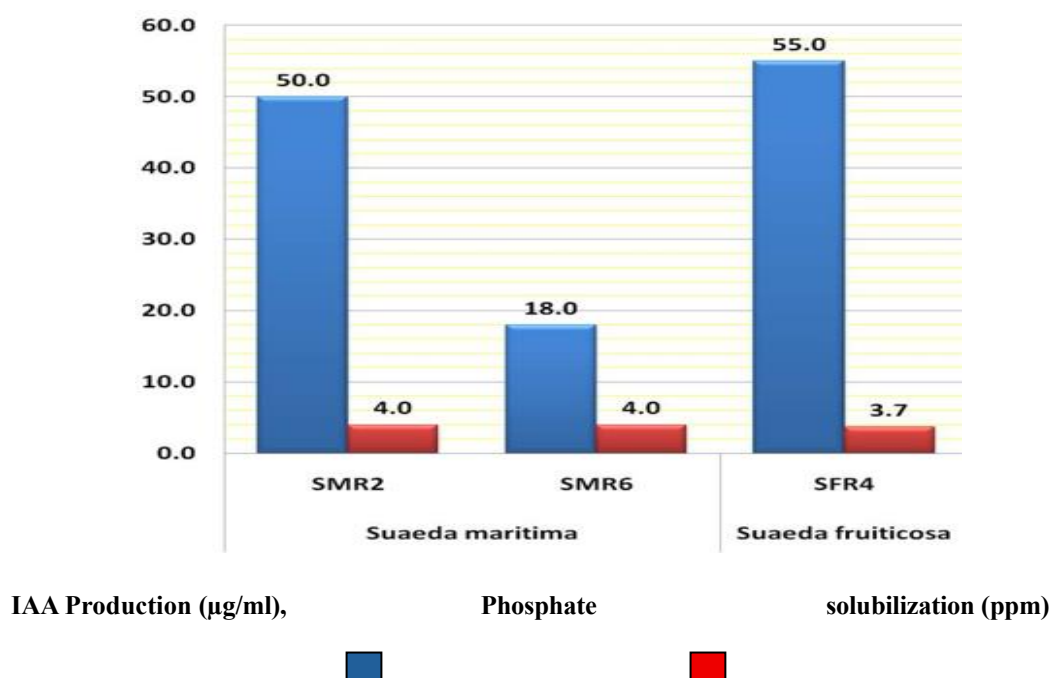


Fig 3: Endophytic isolates recorded both IAA production and Phosphate solubilization characters from *Suaeda maritima* and *Suaeda fruticosa*.

3.9 Potential plant growth promoting endophytes isolates from *Suaeda maritima* and *Suaeda fruticosa*.

Endophytic bacteria isolated from halophytic plants of the genus *Suaeda* showed promising plant growth-promoting (PGP) abilities. The strain SMR2, obtained from *Suaeda maritima*, produced a good level of IAA (50 µg/ml) and also showed phosphate solubilizing activity along with amylase and protease production. It was identified as *Vibrio neocaledonicus*. Another strain, SFR4 from *Suaeda fruticosa*, performed slightly better in IAA production (55 µg/ml) and also showed phosphate solubilization and enzyme activity, and was identified as *Vibrio natriegens*.

Table 9: Potential plant growth promoting endophytes isolates from *Suaeda maritima* and *Suaeda fruticosa*.

Endophytic strain	IAA (µg/ml)	PSB (PP)	Amylase (cm)	Protease (cm)	Identified based on 16S rRNA molecular method
<i>Suaeda maritima</i>					
SMR2	50	4.0	1.8	3.0	<i>Vibrio neocaledonicus</i>
<i>Suaeda fruticosa</i>					
SFR4	55	3.7	1.2	1.7	<i>Vibrio natriegens</i>

3.10 Properties studies and identification of plant growth promoting endophytes

3.10.1. *Vibrio neocaledonicus*

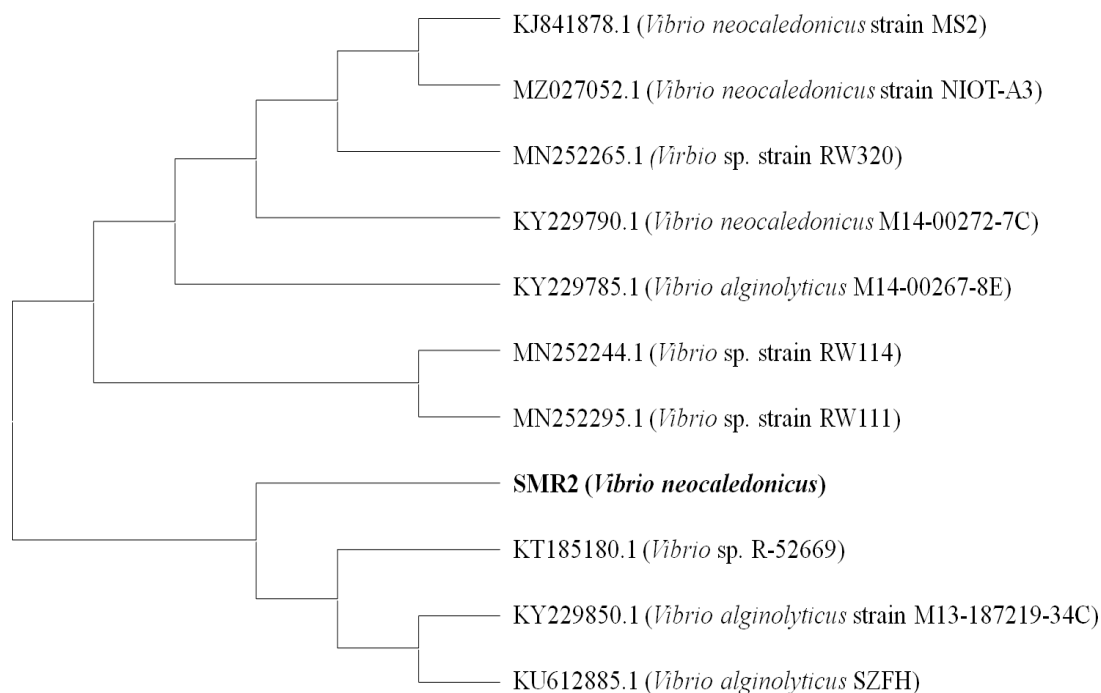
In this study, endophytic strain *Vibrio neocaledonicus* (SMR2) was isolated from *Suaeda maritima* was able to produce plant growth promoting characters like production of IAA production at a concentration of 50 µg/ml, ability to solubilize phosphate at 4.0 ppm. Additionally, this strain displayed the production of extracellular enzymes like amylase and protease at 1.8 and 3.0 amylolytic and proteolytic indices, respectively (Table-10).

3. 10. 2. Molecular characterization of *Vibrio neocaledonicus*

The sequence analysis revealed that endophytic bacterial isolate SMR2 isolated from *Suaeda maritima* showed 99.75 % 16S rRNA gene sequence similarity to *Vibrio neocaledonicus* strain MS2 (Nearest Accession No. KJ841878.1) (Table 11). A phylogenetic tree was constructed using partial 16S rRNA sequences of isolated endophytic bacteria and representative bacterial type strain of related taxa generated by the neighbour-joining method and presented in Fig 4.

Table 10: Sequence producing significant alignment

Description	Max Score	Total Score	Query Cover	E value	Per.Ident	Nearest Accession
<i>Vibrio neocaledonicus</i> strainMS2	2165	2165	99%	0.0	99.75%	KJ841878.1
<i>Vibrio neocaledonicus</i> strainNIOT_A3	2165	2165	99%	0.0	99.75%	MZ027052.1
<i>Vibrio</i> speciesstrainRW114	2163	2163	99%	0.0	99.75%	MN252244.1
<i>Vibrio alginolyticus</i> strain M13-18719-34C	2159	2159	99%	0.0	99.75%	KY229850.1
<i>Vibrio</i> speciesR-52669	2159	2159	99%	0.0	99.66%	KT185180.1
<i>Vibrio</i> speciesstrainRW111	2158	2158	99%	0.0	99.75%	MN252295.1
<i>Vibrio</i> speciesstrainRW320	2158	2158	99%	0.0	99.66%	MN252265.1
<i>Vibrio neocaledonicus</i> M14-00272-7C	2158	2158	99%	0.0	99.58%	KY229790.1
<i>Vibrio alginolyticus</i> M14-00267-8E	2158	2158	99%	0.0	99.66%	KY229785.1
<i>Vibrio alginolyticus</i> strain SZFH	2158	2158	99%	0.0	99.75%	KU612885.1

**Fig 4: Phylogenetic Tree constructed showing the phylogenetic position of the type strain of *Vibrio neocaledonicus* strain MS2**

3.10.3. Phenotypic and biochemical characters of *Vibrio neocaledonicus*

Vibrio neocaledonicus (SMR2) was a Gram-negative, motile bacteria isolated from *Suaeda maritima*. The strain *Vibrio neocaledonicus* (SMR2) can able to tolerate salt concentration upto 10% and not able to growth at high temperature. *Vibrio neocaledonicus* (SMR2) was able to growth on TCBS agar with green colour colony. This strain showed positive reaction to catalase, Indole, Methyl red, Citrate, Lysine & Ornithine decarboxylase test. This strain showed negative reaction to oxidase, Voges Proskauer, and arginine decarboxylase test (Table-11 and Fig 5).

Table 11: Phenotypic and biochemical characters of *Vibrio neocaledonicus*

Characters	SMR2 (<i>Vibrio neocaledonicus</i>)
Cell Morphology	
Gram's reaction	Negative
Shape	Rods
Size	Small
Motility	Positive
Salt Tolerance	
0%	+
3%	+
6%	+
8%	+
10%	+
12%	—
14%	—
Temperature Tolerance	
Growth at 42°C	—
Growth at 52°C	—
Growth at different selective media	
Growth on Bacillus selective media	No colour
Growth on TCBS	Green
Aeromonas selective media	—
King's B media	—

MacConkey Agar	—
Congo Red	—
Hemolysis	—
Biochemical characters	
KOH	+
Catalase	+
Oxidase	—
IMViC test	
Indole	+
Methyl Red	+
Voges Proskauer	—
Citrate	+
Aminoacid decarboxylase test	
Lysine	+
Arginine	—
Ornithine	+
Plant growth promoting characters	
IAA	50.0
PSB	4.0
Amylase	1.8
Protease	3.0
16S rRNA based molecular identification	<i>Vibrio neocaledonicus</i>

Note: '+' indicates normal growth, '++' indicates luxurious growth and '-' indicates no growth

The results obtained are the Mean of three replications

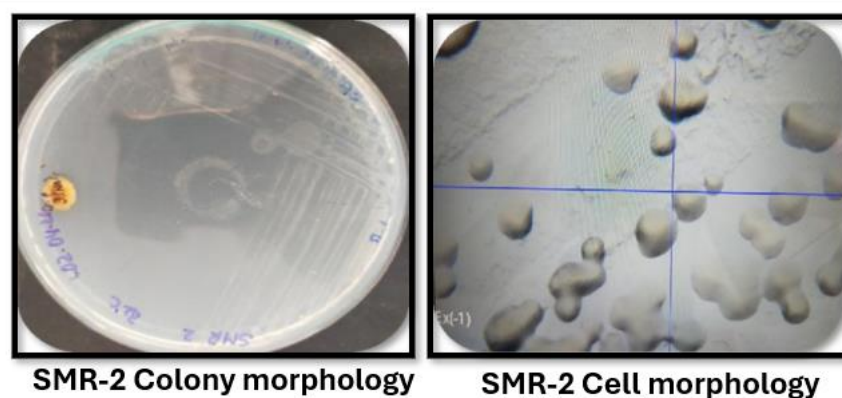


Fig 5. Showing colony and cell morphology of *Vibrio neocaledonicus*

3.10.4. *Vibrio natriegens*

In this study, endophytic strain *Vibrio natriegens* (SFR4) was isolated from *Suaeda fruticosa* has able to produce plant growth promoting characters like production of IAA production at a concentration of 55 µg/ml, ability to solubilize phosphate at 3.7 PPM, additionally, this strain displayed the production of extracellular enzymes like amylase and protease at 1.2 and 1.7 amylolytic and proteolytic index, respectively (Table 12).

3.10.5. Molecular characterization of *Vibrio natriegens*

The sequence analysis revealed that endophytic bacterial isolate SFR4 isolated from *Suaeda fruticosa* showed 99.87 % 16S rRNA gene sequence similarity to *Vibrio natriegens* strain CCUG 16374 (Nearest Accession No. CP016352.1) (Table 12). A phylogenetic tree was constructed using partial 16S rRNA sequences of isolated endophytic bacteria and representative bacterial type strain of related taxa generated by neighbor-joining method and presented in Fig 6.

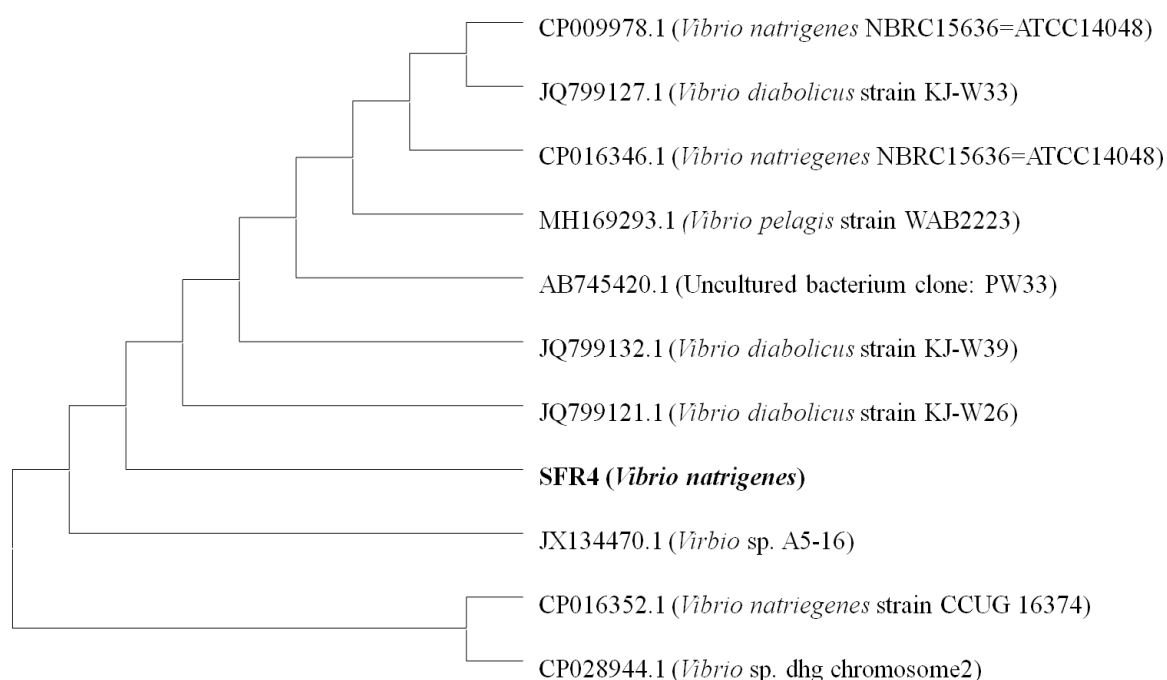


Fig 6: Phylogenetic Tree constructed showing the phylogenetic position of the type strain of *Vibrio natriegens* strain CCUG 16374

3.10.5. Phenotypic and biochemical characters *Vibrio natriegens*

Vibrio natriegens (SFR4) was a Gram-negative, motile bacteria isolated from *Suaeda fruticosa*. The strain *Vibrio natriegens* (SFR4) can tolerate salt concentration up to 10% and not able to growth at high temperature. *Vibrio natriegens* (SFR4) was able to growth on TCBS agar with yellow colour colony and also Aeromonas selective media. This strain showed positive reaction to catalase, oxidase, Methyl red, Citrate, Lysine & Ornithine decarboxylase test. This strain showed negative reaction to Indole, Voges Proskauer, and arginine decarboxylase test (Table 12 and Fig 7).

Table 12: Phenotypic and biochemical characters of *Vibrio natriegens*

Characters	SFR4 (<i>Vibrio natriegens</i>)
Cell Morphology	
Gram's reaction	Negative
Shape	Rods
Size	Small
Motility	Positive
Salt Tolerance	
0%	+
3%	++
6%	+
8%	+
10%	+
12%	—
14%	—
Temperature Tolerance	
Growth at 42°C	—
Growth at 52°C	—
Growth at different selective media	
Growth on Bacillus selective media	No colour
Growth on TCBS	Yellow
Aeromonas selective media	+
King's B media	—
MacConkey Agar	—
Congo Red	—
Hemolysis	—
Biochemical characters	
KOH	—
Catalase	++
Oxidase	D+
IMViC test	

Indole	—
Methyl Red	+
Voges Proskauer	—
Citrate	+
Aminoacid decarboxylase test	
Lysine	+
Arginine	—
Ornithine	+
Plant growth promoting characters	
IAA	55.0
PSB	3.7
Amylase	1.2
Protease	1.7
16S rRNA based molecular identification	<i>Vibrio natriegens</i>

Note: '+' indicates normal growth, '++' indicates luxurious growth and '-' indicates no growth

The results obtained are the Mean of three replications

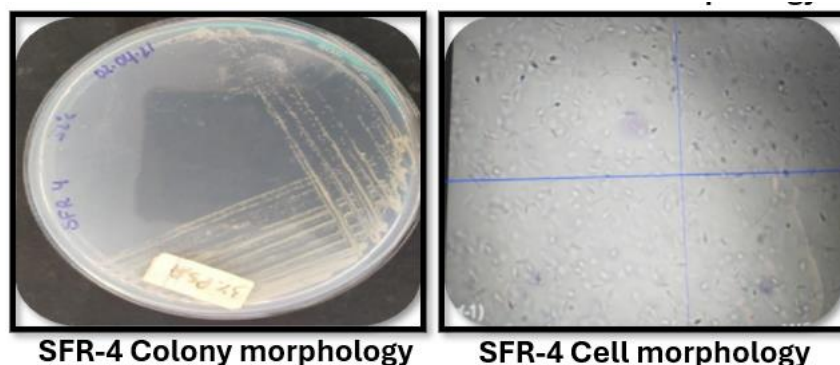


Fig 7. Showing colony and cell morphology of *Vibrio natriegens*

Conclusion

The present study revealed that the halophytic plants *Suaeda fruticosa* and *Suaeda maritima* from the Koringa Wildlife Sanctuary harbour a diverse community of beneficial endophytic bacteria. A total of 17 bacterial endophytes were isolated and characterized, with root tissues showing greater colonization than aerial parts. Most of the isolates belonged to the family Bacillaceae, while a few Gram-negative forms, such as *Vibrio*, *Aeromonas*, and *Pseudomonas*, were also recorded.

The isolated bacteria showed considerable tolerance to saline conditions and exhibited important plant growth-promoting traits, including IAA production, phosphate solubilization, and extracellular enzyme activity. Among the isolates, strains SMR2 (*Vibrio neocaledonicus*) and SFR4 (*Vibrio natriegens*) demonstrated particularly strong plant growth-promoting potential under salt stress conditions.

These findings suggest that halophytic plants growing in mangrove ecosystems serve as valuable reservoirs of stress-adapted beneficial microbes. Such endophytic bacteria may play an important role in improving plant growth and survival in saline environments and could be explored further for the development of eco-friendly biofertilizers and sustainable agricultural practices in alkaline soils.

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