Microbiology Research Journal International

Volume 34, Issue 11, Page 60-71, 2024; Article no.MRJI.125648
ISSN: 2456-7043, NLM ID: 101726596
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Isolation, Characterization and Molecular Identification of Bacteria Associated with Green and Brown Seaweeds

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ML and SHK conceptualized the research work. Authors ML and BBN searched for resources. Authors ML, SS and SHK supervised the study. Author SS did formal analysis. Author SS Investigated the work. Authors ML, BBN and SHK wrote original draft, reviewed and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/mrji/2024/v34i111499

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/125648

Original Research Article

Received: 22/08/2024
Accepted: 24/10/2024
Published: 30/10/2024

ABSTRACT

Aims: The co-existence of bacterial communities along with macroalgae in marine environment develops a mutualistic relationship resulting in functional advantages for both the groups. The present research studied the composition of epiphytic bacteria associated with green (*Ulva* spp.) and brown seaweeds (*Sargassum* spp. and *Padina* spp.) sampled off Maharashtra coast, India, and their physiological and bioactive properties.

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Cite as: *Saha, Somnath, Sanath H. Kumar, Binaya Bhusan Nayak, and Manjusha Lekshmi. 2024. "Isolation, Characterization and Molecular Identification of Bacteria Associated With Green and Brown Seaweeds". Microbiology Research Journal International 34 (11):60-71. https://doi.org/10.9734/mrji/2024/v34i111499.*

Study Design: Green and brown seaweeds were collected from three locations along Maharashtra coast and analyzed for the associated bacteria.
Place and Duration of Study: Seaweed samples analyzed in this study were from Kelwa, Manori and Ratnagiri along the Maharashtra coast. The analysis was performed during June to September 2021.
Methodology: Bacteria from freshly collected seaweed samples were homogenized in saline, 10-fold serially diluted and plated on Zobell marine agar. Distinct colonies were selected and identified by a series of biochemical tests, followed by partial 16SrRNA gene sequencing. The physiological characteristics of the isolated bacteria were studied by screening for temperature and salinity tolerance, production of protease, lipase, agarose, amylase and biosurfactants.
Results: The total seaweed bacterial count ranged from 4.5 ×10^3 to 2.9 × 10^4 CFU/g. Seventy-seven bacteria were isolated, 44 (57.14%) and 33 (42.85%) isolates from green and brown seaweeds respectively. The 16SrRNA sequencing of 20 representative isolates revealed the dominance of *Bacillus* spp., followed by Vibrio spp. Growth at 0°C was exhibited by all bacteria except *Bacillus tequilensis*, *Bacillus altitudinis*, *Oceanobacillus iheyensis* and one isolate of *Vibrio* spp. A majority of the isolates grew at 45°C. *Vibrio* spp. exhibited protease, amylase, gelatinase, and agarase activities, whereas the biosurfactant activity was commonly associated with *Bacillus* spp.
Conclusion: The results of this study illustrate the occurrence of seaweed-associated beneficial bacteria exhibiting bioactive properties with potential biomedical applications.

Keywords: Seaweed; bacteria; diversity; bioactivities; Ulva; sargassum; Padina.

1. INTRODUCTION

Marine macroalgae/seaweeds are one of the major primary producers in oceanic aquatic food web, dwelling in the coastal intertidal regions (Kumar et al., 2022). Seaweed species predominantly found along the Indian coast belongs to Rhodophyta, followed by Chlorophyta and Phaeophyta. Marine bacteria often live in association with soft-bodied marine organisms, especially seaweeds, which lack structural defence mechanisms (Menaa et al., 2020). In order to adapt to and survive in such extreme unfavourable habitats with space and environment constraints, these marine organisms rely on chemical defence by the production of bioactive secondary metabolites, either by themselves or by associated microflora (Giddings and Newman, 2015). The epiphytic microbial community harboring seaweed surfaces is extremely dynamic and intricate, constituted by bacteria, fungi, diatoms and protozoa (Lachnit et al., 2011). The bacterial population may vary from 10^2 to 10^7 cells cm^{-2} depending on the species, thallus section and season (Armstrong et al., 2000). The metabolite composition, physico-biochemical properties, defence mechanism etc influence the characteristics of the associated epiphytic bacterial community (Kumar et al., 2022). The association of bacteria with seaweeds also depends on several parameters such as temperature, salinity, oxygen, carbon dioxide and pH (Juhman et al., 2020).

Several studies have reported the incidence of seaweed-associated bacteria such as *Pseudoalteromonas* sp. (Sánchez Hinojosa et al., 2018), *Pseudomonas* spp. (Kaur et al., 2023), *Bacillus* sp. (Shanoona et al. 2024), (Souza et al., 2011), *Vibrio* spp. (Naik et al., 2019), *Pseudovibrio* spp. (Penesyan et al., 2011), *Streptomyces* sp., *Staphylococcus* sp. (Braña et al., 2015) etc. The bacterial communities thriving on seaweed surfaces utilize the nutrients produced by the hosts in the form of organic matter. They in turn produce numerous enzymes such as amylases, agarases, phosphatases, ureases, esterases, β-galactosidases, cellulases and lipases which help in assimilating the seaweed-produced compounds. The microorganisms protect their macroalgal hosts from the harmful entities present in the environment by secreting bioactive substances, which also regulate the morphogenesis of marine organisms and help them to survive under variable environmental conditions as they lack cell-based immune system (Azanza et al., 2013). Seaweed-associated bacteria are diverse in their composition and act as a rich source of beneficial bioactive secondary metabolites (Albakosh et al., 2016). Seaweed-microbes derived bioactive substances such as enzymes, peptides, polysaccharides, phenolic compounds etc. are known for their biological properties such as antimicrobial, antisettlement, antifouling, antiprotozoan, antiparasitic, and antitumor

activities (Variem et al., 2021; Lee et al., 2013) and are useful for a variety of applications.

Over the years, there has been an increasing demand for new therapeutic drugs from natural products effective against multidrug resistant pathogens. Seaweeds are one of the potential sources of such drugs which are being exploited along with other useful compounds that have potential applications in biotechnology (Ren et al., 2022). Epiphytic marine bacteria are also reported to produce enzymes like proteases (Comba González et al., 2018; Ren et al., 2022; Sánchez Hinojosa et al., 2018).

Oil spill in the ocean is one of the major problems, which can negatively affect the physiology and biochemistry of marine life. Polyaromatic hydrocarbons can cause sub-lethal injury or death of fish larvae and fish eggs (Al-Majed et al., 2014; Langangen et al., 2017). Biosurfactants are amphiphilic substances produced by living surfaces, largely on microbial cell surfaces or excreted extracellular hydrophobic and hydrophilic moieties. The presence of these two groups in the same molecule, helps in reducing surface tension at the interface between air and water and demonstrate emulsifying activity (Cunha et al., 2004). Due to important benefits including low toxicity, biodegradability, substrate specificity and the general interest in natural goods which are environmentally safe, biosurfactants have gained prominence for environmental purposes (Banat et al., 2000). The present study analysed the bacterial composition of green and brown seaweeds, characterized the physiological properties of the isolated bacteria and evaluated their bioactivity.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Four seaweed samples comprising two each of green and brown seaweeds were collected from Kelwa beach (19°36'41.7"N 72°43'45.7"E), Manori beach (19°12'37.9"N 72°46'51.0"E), and Ratnagiri beach (16º55'34.38" N 73º15'57.89"E), along Maharashtra coast. The samples were collected manually and transported aseptically to the laboratory in chilled condition in sterile sampling containers for bacteriological analysis within six hours of collection.

2.2 Isolation of Seaweed-associated Bacteria

The bacterial strains associated with seaweeds were isolated by the following procedure. The seaweeds were rinsed with 1% saline solution to remove the surface-attached bacteria. Ten-grams of rinsed seaweeds was macerated with 90 ml of physiological saline. The macerated seaweeds were serially diluted up to 10^{-5} dilution and were spread on pre-dried ZoBell Marine Agar (ZMA) plates in duplicate. The seaweed rinse water (saline) and seawater collected from the same location were also plated on ZMA to determine the bacterial composition. The plates were incubated at 37°C for 16-24 hours following which different bacterial colonies were selected based on their colony morphology and color. The selected colonies were picked and purified by re-streaking on ZMA plates. The purified isolates were maintained on Luria Bertani (LB) agar plates supplemented with 1.5% salt, and further stored in glycerol and soft agar stock with 1.5% salt at -20°C for further analysis.

2.3 Molecular Identification of Isolated Bacteria

The bacteria isolated from seaweeds were identified by molecular characterization using universal primers for the amplification of 16SrRNA gene (Roy et al., 2024). Crude DNA lysates were prepared by heating the suspension obtained by mixing a loopful of overnight grown pure bacterial culture in 100 µL of 1x TE buffer at 98ºC for 10 minutes in dry bath followed by rapid cooling on ice. The lysate was then centrifuged at 12,000 rpm for 1 minute. Two µl of the supernatant containing DNA was used as template for PCR, along with 3µl of PCR buffer, 2µl of DNTP mix, and 2µl each of forward and reverse primers 27F (5′-AGAGTTTGA TCCTGGCTCAG-3′) and 1492R (5’-GGTTACCTTGTTACGACTT-3’) respectively. The PCR reaction was set up with one cycle of initial denaturation at 95°C for 4 min, followed by 30 cycles each of denaturation at 95°C, annealing at 50°C, extension at 72°C for 1 min, and 1 cycle of final extension at 72°C for 4 min in a SimpliAmp thermal cycler (Applied Biosystems). The amplicons of 1465 bp were visualized on 1% agarose gel by electrophoresis, using a UV transilluminator and photographed using gel documentation system (BioRad). The PCR products were purified using GeneJet PCR Purification Kit (Thermo Fisher Scientific) and sequenced by Agri-genome Labs Ltd. (Kochi,

India). The sequences were subjected to BLAST analysis against sequences in the GenBank (NCBI), and aligned using homologous sequence single nucleotide-nucleotide alignment tool (Banat et al., 2000). The sequences are available at NCBI GenBank with accession numbers from MZ976788 to MZ976807.

2.4 Characterization of Seaweed Bacterial Isolates

2.4.1 Determination of salinity and temperature tolerance

The bacterial isolates obtained from seaweeds were subjected to Gram's staining and catalase tests, followed by salinity and temperature tolerance tests. For salinity tolerance study, the isolates were grown in 1% tryptone broth in varying NaCl concentrations such as 0%, 3%, 6%, 8%, and 11%, and incubated at 37°C, and for temperature tolerance study, the isolates grown in 1% tryptone broth with 1.5% NaCl were incubated at 0°C, 8°C, 30°C, 37°C, 45°C, for seven days. Tubes showing turbidity or visible growth were recorded as positive whereas tubes with no turbidity were taken as negative. Positive tubes were further confirmed by plating on ZMA.

2.4.2 Determination of bioactivity of bacterial isolates

The isolates were screened for the production of extracellular enzymes protease, lipase, amylase, gelatinase and agarose as described previously (Hmani et al. 2023).

Protease test: The isolates were spot inoculated onto LB agar plates supplemented with milk in 9:1 ratio and incubated at 37°C for 24 hours. The development of a clear zone surrounding the colonies indicated positive result due to the production of protease enzyme, while no zone indicated negative result.

Lipase test: Freshly grown bacterial cultures were spot inoculated on ZMA plates with 0.5% tributyrin oil and incubated at 37°C for 24 hours. A clear zone of hydrolysis around the colonies due to lipase production indicated positive result, while no clear zone was recorded as negative result.

Amylase test: The isolates were spot inoculated onto LB agar plates supplemented with 1% soluble starch and incubated at 37°C for 24-48 hours. Following incubation, the plates were flooded with gram's iodine solution. Amylase producing or starch degrading organisms produced a halo zone around them. The colour of the zones depend on the degree of hydrolysis of the starch. Complete hydrolysis produces colourless zones, whereas reddish brown zones are formed by the production of dextrin.

Gelatin liquefaction test: A heavy inoculum of fresh culture was stabbed into 5 ml nutrient gelatin medium. The tubes were incubated at 37°C for seven days. The tubes were chilled in ice every day at 4°C for 30 min with the un-inoculated tube as control. The partial or total liquefaction of the inoculated medium indicated positive result, while complete solidification indicated negative result.

Agarase test: The test isolates were spot inoculated onto modified agar medium plates and incubated at 37°C for 24-48 hours. The plates were then flooded with gram's iodine solution. Clearance zone surrounding the test organisms indicated positive result.

Biosurfactant activity test: The biosurfactant production was tested using the oil displacement method (Morikawa et al. 1993). Crude oil (400 µl) was added to 20 ml distilled water in a petri dish to form a thin oil layer. To the center of the oil layer, 20 µl of culture suspension was gently placed. Displacement of oil and the formation of a clear zone indicates a positive result for biosurfactant activity. A negative control was also maintained with distilled water, in which no oil displacement or clear zone was observed, and Triton X-100 was used as the positive control.

3. RESULTS AND DISCUSSION

Diverse bacterial communities co-exist with seaweeds in the marine environment. Seaweed associated bacteria play important roles in the growth and development of seaweeds by producing growth-promoting and bioactive substances, and other compounds. Many of these bacterial bioactive compounds have beneficial properties that find applications in various important fields (Singh and Reddy, 2014). The present study investigated the isolation and characterization of bacteria that are associated with green and brown seaweeds along the coast of Maharashtra. The bacterial isolates obtained on ZoBell Marine Agar plates were subjected to various assays such as the protease, lipase, gelatinase, agarase and biosurfactant activities in order to determine their physiological and bioactive characteristics.

3.1 Isolation and Identification of Bacteria Associated with Seaweed Samples

In this study, four seaweed samples were screened for the presence of seaweed associated bacteria. The processed seaweeds and ambient seawater plated on ZMA plates showed a bacterial count that ranged from 4.5 × 10^3 to 2.9 × 10^4 CFU/g of seaweed, from both green (*Ulva* sp.) and brown seaweeds (*Padina* sp. and *Sargassum* sp.) From two green seaweeds 44 morphologically different bacterial isolates were obtained, which constituted 57.14% of the total bacteria isolated. The rest 42.85% was constituted by 33 bacterial isolates from two brown seaweeds. Overall, a total of 77 bacterial isolates were obtained from four seaweed samples. Seaweeds are reported to be carriers of beneficial microorganisms, especially the bacteria which possess potential bioactivities (Karthick and Mohanraju, 2018). They provide a favourable habitat to many diverse groups of bacteria and the surface bacterial densities vary from 10^2 to 10^7 cells per cm^2, depending on several factors such as the host species, season and related physico-chemical parameters (Armstrong et al., 2000); (Bengtsson et al., 2010).

In the present study, all the 44 isolates obtained from green seaweed samples were Gram positive, whereas 4 out of the 6 isolates from brown seaweeds were Gram negative and two Gram positive. In a study by Thilakan et al. (2016), out of the 23 isolates from seaweeds belonging to brown and red seaweeds collected from Mandapam, Gulf of Mannar, India, 12 were reported to be Gram-positive and the remaining 11 Gram-negative. Nevertheless, early reports on the bacterial abundance in marine environments indicated that around 95% was constituted by Gram negative bacteria. However, subsequent research findings showed increasing abundance of Gram-positive bacteria from marine samples such as seawater, plankton and macroalgae. The dominance of Gram-positive bacteria in marine samples could be assessed by their specific requirement of sodium for growth and survival, which also confirms them to be species indigenous to marine environment (Rodrigues and de Carvalho, 2022). Five seaweed samples from Mandapam along the southeast coast of India were analysed , which reported surface bacterial counts of *Gracillaria corticata* to be 2.8 X 10^3 CFU per cm^2, *Padina gymnosphora* to be 3.7 X 10^3 per cm^2, *Valoniopsis pachynema* to be 6.2 X 10^3 per cm^2, 5.6 X 103 per cm^2 for *Gelidium pusillum* and 3.2 X 10^3 per cm^2 in the case of *Hypnea musiformis* (JanakiDevi et al., 2013). The surface microbial count of the brown algae *Ascophyllum nodosum* collected from an intertidal area in Nahant, Massachusetts was reported to be >1.1x10^8 microorganisms/ cm^2 (Cundell et al., 1977).

3.2 Molecular Confirmation of Isolates by PCR and 16SrRNA Sequencing

Twenty isolates selected based on the colony morphology and bioactivities were sequenced using universal primers for 16SrRNA gene and the results are given in Table 1. Of these, 13 isolates were from two samples of green seaweed *Ulva lactuca* and seven isolates were from brown seaweeds, four from *Padina tetrastomatica* and three from *Sargassum tennerimum.* Among the 13 isolates isolated from green seaweeds 10 belonged to the genera *Bacillus* and other 3 were identified as *Acinetobacter* sp., *Halobacillus blutaparonensis* and *Oceanobacillus iheyensis*. Six isolates of genus *Vibrio* and one isolate of *Psychrobacter* sp. were obtained from brown seaweeds. Several studies report the isolation of different bacterial species from a variety of seaweeds such as *Pseudoalteromonas* sp. from *Himantothallus grandifolius*, *Pantoneura plocamioides* and *Plocamium cartilagineum* (Sánchez Hinojosa et al., 2018), *Pseudomonas* from the red alga *Gracilaria dura* (Gupta et al., 2013), *Bacillus* sp. (Lavin et al., 2013) and *Vibrio* sp. from green algae *Ulva lactuca* (Naik et al., 2019). *Pseudovibrio* sp. was isolated from the surface of the red alga *Delisea pulchra* (Penesyan et al., 2011). Seaweed-associated bacteria perform diverse functions that enhance their survival as well as exert beneficial effects on seaweeds. Many bacteria play important roles in the morphogenesis of the seaweeds (Wichard, 2023); (Singh et al., 2011) and also help in survival of seaweeds in adverse environmental conditions (Chisholm et al., 1996). The isolation of 1624 Gram positive bacteria belonging to diverse groups from the tropical marine sediments collected from the intertidal zones of Republic of Palau in the western Pacific Ocean was reported in a previous study (Gontang et al., 2007). According to Johnson et al. (1991), certain Gram positive bacteria are reported to be selectively attracted by specific species or genera of seaweeds, attach more efficiently to algal exudates on the surfaces and exhibit enhanced growth than Gram negative bacteria. These characteristics could plausibly

facilitate the predominance of Gram positive bacteria being isolated from seaweeds, as reported by the results of the present study as well.

3.3 Salinity and Temperature Tolerances of Seaweed-associated Bacteria

The bacteria whose identities were confirmed through molecular sequencing were subjected to salinity and temperature tolerance tests. As given in Table 2, of the 20 bacteria tested, all except two species of *Vibrio, V. owensii* and *V. neocaledonicus* could grow when no salt was provided in the growth media. *Halobacillus blutaparonensis, Bacillus cereus, B. pumilus, Vibrio alginolyticus, V. harveyi* and one strain of *Vibrio* sp. exhibited growth at all tested concentrations (0%, 3%, 6%, 8% and 11%) of salinity in the media. *Bacillus tequilensis* and *B. altitudinis* showed remarkable inability to grow at higher salt concentrations of 6, 8 and 11%. Majority of the seaweed bacteria tested showed growth at all temperatures of 0°C, 8°C, 30°C, 37°C and 45°C (Table 2). *B. altitudinis* exhibited growth at or near ambient room temperature only, while few species of *Bacillus* and *Oceanobacillus iheyensis* did not grow at 0°C. Overall, 100% of the tested bacteria grew in media with 3% salinity at temperatures of 30°C and 37°C. However, only 35% of them survived in 11% salinity (Fig. 1).

There are various abiotic factors such as temperature, salinity, acidification, etc that affect the epiphytic bacterial communities dwelling on seaweeds. In the context of increase in the temperature of marine waters as a result of global warming, it is important to study the survival pattern of bacteria in response to temperature and salinity fluctuations (Düsedau et al., 2023). *Aeromonas hydrophila* was isolated from marine fish samples, whose growth was inhibited at 6% and at 5% the growth of 38% of the isolates was inhibited (Dahdouh et al., 2015). About 80% of bacteria isolated from marine fish and prawns could grow at 1.5-3.5% salt (Surendran et al., 1983). The physiological adaptation of bacterial cells at higher concentrations of NaCl occurs by modifications in the transport and circulation of sodium ions across the cell membrane, and the production of osmoprotectants such as betaine and proline also contribute to the salinity tolerance of such bacteria (Wood et al., 2001).

Table 1. Identification of seaweed-associated bacteria

Isolate No.	Source	Identity
A10	*Ulva lactuca*	*Bacillus tequilensis*
A13	*U. lactuca*	*Bacillus tequilensis*
A19	*U. lactuca*	*Bacillus* sp.
A22	*U. lactuca*	*Bacillus paramycoides*
A30	*U. lactuca*	*Bacillus altitudinis*
B1	*U. lactuca*	*Acinetobacter junii*
B2	*U. lactuca*	*Halobacillus blutaparonensis*
B6	*U. lactuca*	*Bacillus pumilus*
B8	*U. lactuca*	*Bacillus cereus*
B9	*U. lactuca*	*Bacillus pumilus*
B14	*U. lactuca*	*Bacillus subtilis*
B17	*U. lactuca*	*Bacillus cereus*
B24	*U. lactuca*	*Oceanobacillus iheyensis*
C3	*Sargassum tennerimum*	*Vibrio* sp.
C8	*S. tennerimum*	*Vibrio alginolyticus*
C20	*S. tennerimum*	*Vibrio alginolyticus*
D1	*Padina tetrastromatica*	*Vibrio owensii*
D2	*P. tetrastromatica*	*Vibrio neocaledonicus*
D5	*P. tetrastromatica*	*Vibrio harveyi*
D7	*P. tetrastromatica*	*Psychrobacter* sp.

Table 2. Salinity and temperature tolerance of seaweed associated bacteria

	Salinity (%)					Temperature (°C)				
	0	**3**	**6**	**8**	**11**	**0**	**8**	**30**	**37**	**45**
Number of bacteria positive	18	20	14	13	7	15	16	20	20	16

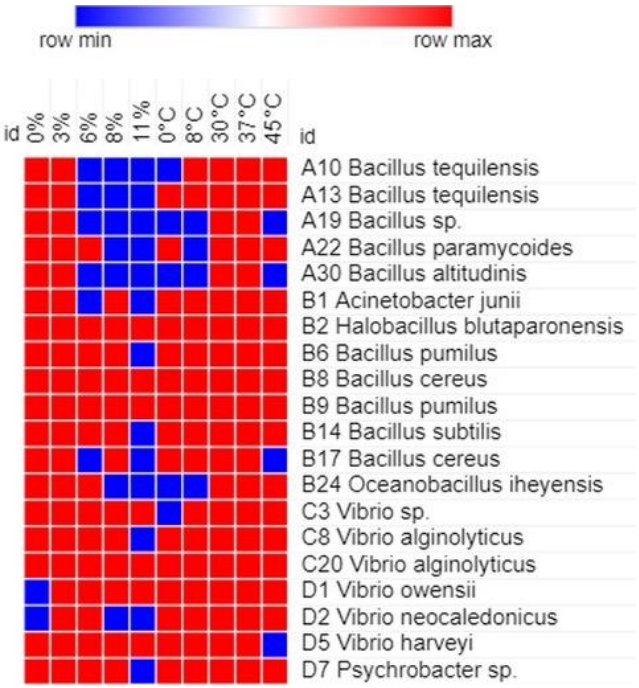

Fig. 1. Salinity and temperature tolerance pattern of seaweed associated bacteria. The parameters are on upper side; right side Y-Side consist of species name. The red and blue boxes indicate the ability to grow in that condition. The heat map was generated using Morpheus (Wood et al. 2001)

Table 3. Bioactivities of seaweed-associated bacteria isolated in this study

	Strain	Protease	Lipase	Amylase	Gelatinase	Agarase	Biosurfactant
1.	*Bacillus tequilensis*	-	-	+	-	-	+
2.	*Bacillus tequilensis*	+	+	+	-	+	+
3.	*Bacillus* sp.	+	-	-	+	-	+
4.	*Bacillus paramycoides*	+	-	-	+	-	+
5.	*Bacillus altitudinis*	-	-	-	-	-	+
6.	*Acinetobacter junii*	-	-	-	-	-	-
7.	*Halobacillus blutaparonensis*	-	-	-	+	-	-
8.	*Bacillus pumilus*	+	-	-	+	-	+
9.	*Bacillus cereus*	-	-	-	-	-	-
10.	*Bacillus pumilus*	+	-	+	+	+	-
11.	*Bacillus subtilis*	+	+	+	+	+	-
12.	*Bacillus cereus*	-	-	-	+	-	-
13.	*Oceanobacillus iheyensis*	-	-	-	-	-	+
14.	*Vibrio* sp.	+	-	+	+	+	+
15.	*Vibrio alginolyticus*	+	+	+	+	+	+
16.	*Vibrio alginolyticus*	+	+	+	+	+	+
17.	*Vibrio owensii*	+	-	+	+	-	+
18.	*Vibrio neocaledonicus*	+	+	+	+	+	+
19.	*Vibrio harveyi*	-	-	+	+	+	+
20.	*Psychrobacter* sp.	+	-	+	+	+	+

The temperature tolerance analysis of the isolates from green seaweeds revealed the mesophilic characteristic of *Bacillus* sp. as all the isolates showed growth at 30°C and 37°C. Except for three species of *Bacillus* and one strain of *V. harveyi*, other isolates grew at 45°C, suggestive of their high temperature tolerance. *B. altitudinis* exhibited growth at or near ambient room temperature only, while few species of *Bacillus* and *Oceanobacillus iheyensis* did not grow at 0°C, indicating their inability to survive at temperatures lower or higher than normal. Whereas in the case of brown seaweeds, all isolates of *Vibrio* sp. grew at all temperatures tested. The survival of bacteria in the extreme environments such as high temperatures could be facilitated by the production of unusual enzymes and polymers (Maugeri et al., 2002).

3.4 Evaluation of Bioactivities of Seaweed-associated Bacteria

The ability of seaweed-associated bacteria to produce commercially useful enzymes such as protease, lipase, amylase, gelatinase and agarase was evaluated, along with their biosurfactant activities (Table 3). Predominantly, gelatinase activity was found in 14 isolates, followed by protease in 12, amylase and biosurfactant activities in 11 isolates each. Nine isolates exhibited agarase production, and four of them were positive for lipase activity. Most of the seaweed-associated Vibrios tested positive for protease, amylase, gelatinase, and agarase activities, whereas biosurfactant activities were expressed by both *Bacillus* sp. and Vibrios. *V. neocaledonicus* isolated from the brown seaweed *Padina* sp. showed positive results for all the bioactivities tested for. This was closely followed by *V. alginolyticus* and *Psychrobacter* sp., which exhibited all activities except lipase. *Acinetobacter junii* and one strain of *B. cereus* isolated from the green seaweed *Ulva* sp. did not show any of the enzymatic or biosurfactant activities. As shown in Table 3, *Halobacillus* sp. showed only gelatinase activity, while *Oceanobacillus* sp. exhibited only biosurfactant activity.

Several studies reported marine microbes associated with macroorganisms to be the true producers of important bioactive chemical entities (Christensen and Martin, 2017). Culturable bacteria (134 no. s) were isolated from red seaweed, *Gracilaria gracilis* from Saldanha Bay and Lüderitz, among which, 70% were positive for agarase activity as well as protease activity (Jaffray et al., 1997). Mohapatra et al. (2003) showed 61% of the bacteria isolated from the marine sources such as sponge and brown seaweed *Sargassum* sp., to possess protease activity. The results of this study are consistent with these previous reports. Out of the 20 seaweed bacterial isolates tested, 70% produced gelatinase enzyme that included eight isolates from green and six from brown seaweeds respectively. Among 208 marine actinomycetes isolated from different marine samples, 116 isolates (55.77%) were positive for the production of gelatinase enzyme (Ramesh and Mathivanan, 2009). Protease activity was shown by six isolates each (60%) from green and brown seaweeds. Vibrios associated with brown seaweeds exhibited amylase and agarase activities, along with few *Bacillus* sp. from green seaweeds. A total of 11 bacteria (55%) turned positive for amylase, nine (45%) for agarase and 4 for lipase activities. Thirty-three representative isolates from 399 bacterial cultures isolated from sea sponges and reported 54.55%, 69.69% and 27.28% to be positive for lipase, protease and agarase activities (Li et al., 2007). These included *Bacillus firmus*, *B*. *vallismortis*, *B*. *cereus*, *B*. *subtilis*, *B*. *anthracis* that were positive for lipase activity and *Alcaligenes* sp. that was positive for protease. Among 60 bacteria isolated from sea anemones, 17 could produce proteolytic exoenzyme and 20 showed lipolytic exoenzyme activity (Du et al., 2010).

Bacteria (53.85%) isolated from marine soilsamples were used to produce amylase enzyme for starch utilization (Ashwini and Sampathkumar, 2015). *Vibrio* sp. was isolated from seawater collected from Sagami Bay in Kanagawa Prefecture, Japan, which could decompose the cell walls of some seaweeds, including *Laminaria* sp. and *Undaria pinnatifida* (Sugano et al., 1993). Alvarado and Leiva, (2017) found 30 pigmented bacteria with agarose activity associated with four macroalgal species (*Adenocystis utricularis*, *Monostroma hariotii*, *Iridaea cordata*, *and Pantoneura plocamioides*), collected from King George Island, Antarctica. One hundred and seventy-two bacterial from three macroalgae (*Himantothallus grandifolius*, *Pantoneura plocamioides and Plocamium cartilagineum*), among which 21 isolates showed agarase production (Sánchez Hinojosa et al., 2018). Twenty-three bacteria were isolated from rocky intertidal zone of Anjuna, Goa, India, among which three isolates (*Vibrio brasiliensis*, *Bacillus subtilis*, *and Pseudomonas aeruginosa*) showed highest agarase activity (Naik et al.,

2019). Out of 163 bacteria isolates, 112 (68.7 %) isolates produced amylases and proteases, while 144 (88.3 %) isolates produced lipases, and 78 (47.9 %) isolates produced all three types of targeted industrial enzymes (Cheng et al., 2020).

Biosurfactant property was exhibited by 11 out of 20 bacteria tested, comprising of four green seaweed and seven brown seaweed associated isolates. Javee et al. (2020) isolated seven potential biosurfactant producers from brown seaweed *Sargassum myriocystum* collected from Tamil Nadu, India, in which one isolate (*Streptomyces* sp. SNJASM6) showed positive result. Forty out of 200 bacteria isolated from oil-spilled seawater exhibited biosurfactant activity (Maneerat, 2005). Of the 45 isolates from marine samples comprising of seawater, sediment and shell collected from the eastern western and southern coast of India, 15 were (6 *Acinetobacter* spp. and 9 other genera) were positive for biosurfactant production (Satpute et al., 2008).

4. CONCLUSION

The mutualistic and symbiotic association between seaweeds and bacteria holds considerable environmental significance. The production of bioactive compounds by such marine bacteria offers a great potential for several applications, especially pharmaceutical and bioremediation of pollutants/wastes. The results of this study indicate the presence of useful bacteria that produce beneficial bioactives which can be further explored for several applications.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

The authors thank the Director & Vice-Chancellor, ICAR-CIFE, Mumbai for support in carrying out this work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/125648