

INVESTIGATING MICROBIAL BIODIVERSITY ALONG MOSHESHKHALI CHANNEL IN BAY OF BENGAL, BANGLADESH



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ABSTRACT

Microbial diversity plays a crucial role in ecosystem functioning and biogeochemical processes. However, bacterial diversity in the Moheshkhali Channel, Bay of Bengal, Bangladesh, remains largely unexplored and this study aimed to investigate the bacterial diversity from seawater and sediment sample. Seawater and sediment samples were collected from the Moheshkhali Channel, and physicochemical parameters, including Environment temperature (35°C) Seawater Temperature (33°C), pH=7, and dissolved oxygen (7.13 mg/L), were measured in situ. Bacteria were isolated using standard microbiological techniques under aseptic conditions and cultured on LB Agar media. A total of six morphologically distinct bacterial isolates were obtained Gram staining confirmed that all isolates are gram positive and majority bacterial strain are rod-shaped bacteria. Biochemical identification using the VITEK® 2 Compact system identified two isolates as *Leuconostoc mesenteroides subsp. cremoris* and *Aerococcus viridans*. The others isolates were unidentified or low-reactive biopatterns. biochemical data show that all bacteria isolates were metabolically different.

KEYWORDS: Bacterial diversity, *Leuconostoc mesenteroides subsp. Cremoris*, *Aerococcus viridans*, Moheshkhali channel

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Introduction

The Moheshkhali Channel is a tidal waterway that separates Moheshkhali Island from the mainland of Cox's Bazar, a district in southeastern Bangladesh (Arafat et al., 2025). This region has received a significant influx of freshwater from river runoff and seasonal rainfall, especially during the monsoon season, creating a favorable condition for microbial growth and diversity (Tamanna Jahan, 2024). Moheshkhali is the unique island in the country which has hills (Uddin et al., 2023). This channel is significant for many reasons such as fish biodiversity, microbial biodiversity, plant diversity, marine aquaculture, and recreational activities. It is a habitat for approximately 35 species of finfish, 10 species of shrimp and 7 species of cephalopods. This channel is susceptible to eutrophication and toxic algae species (i.e. *Dinophysis caudata*, *Alexandrium catenella*, *Gymnodinium coeruleum*, etc.) (Ayshi et al., 2024) (Uddin et al., 2023).

In aquatic environments, Seawater and sediment harbor diverse bacterial communities and play important roles. Seawater is continuously mixing the organic matter and nutrients cycling and sediments provide significant nutrient-rich substrates that help bacterial growth. These ecosystems are interconnected to each other and make an ideal environment for bacterial growth (Zinger et al., 2011). Microbial communities play an important

role in marine ecosystem health, biogeochemical cycles(C,N,S) and sustaining life on earth. They not only regulate ocean health but also as important indicators of environmental change, climate impact, and valuable resources for biotechnological applications because of their metabolic capabilities (Zhang et al., 2024). They regulated carbon and nitrogen cycling across watersheds, driving processes such as organic matter decomposition, primary production, nitrification, and denitrification (Sun et al., 2026). In the marine ecosystem, bacterial communities contribute significantly to primary productivity, food web dynamics, and environmental resilience (Moran, 2015). Marine bacteria have grown various molecular processes to deal with various environmental factors, such as changes in nutrition supply, pH, temperature, salinity and pressure, making them attractive for diverse biotechnological applications (Muriel-Millán et al., 2021). Thus, knowledge of microbial diversity is important for ecological assessment and resource utilization.

Previous study has been conducted on the ecological aspects including fisheries resources, plankton diversity, plant diversity and water quality of the moheshkhali channel (Uddin et al., 2023) (Tamanna Jahan, 2024) (Imran et al., 2020) (Ayshi et al., 2024). These studies have demonstrated the ecological

importance of the channel and highlighted its high biological productivity. While Microbial biodiversity especially bacterial biodiversity, is totally unexplored at from seawater and sediment at Moheshkhali channel. For this reason, the aim of this study was to isolate the bacterial colony from seawater and sediment sample, identify them using biochemical characterization methods and providing baseline data for future microbial and biotechnological research. We hypothesize that bacterial isolates from the Moheshkhali Channel exhibit diverse biochemical traits, reflecting ecological adaptation and potential biotechnological applications.

Materials and Methods

Study Area and Sample Collection

Seawater and Sediment samples were aseptically collected from the Moheshkhali Channel, Cox's Bazar, Bangladesh on 18 November 2024. Seawater sample were collected 0-20 cm below from the surface and Sediment samples were collected from the upper layer (0-5 cm depth), and stored in 250 ml sterile bottles (Matyar et al., 2008). All samples were transported to the laboratory in an ice box and stored at -40°C until further analysis.

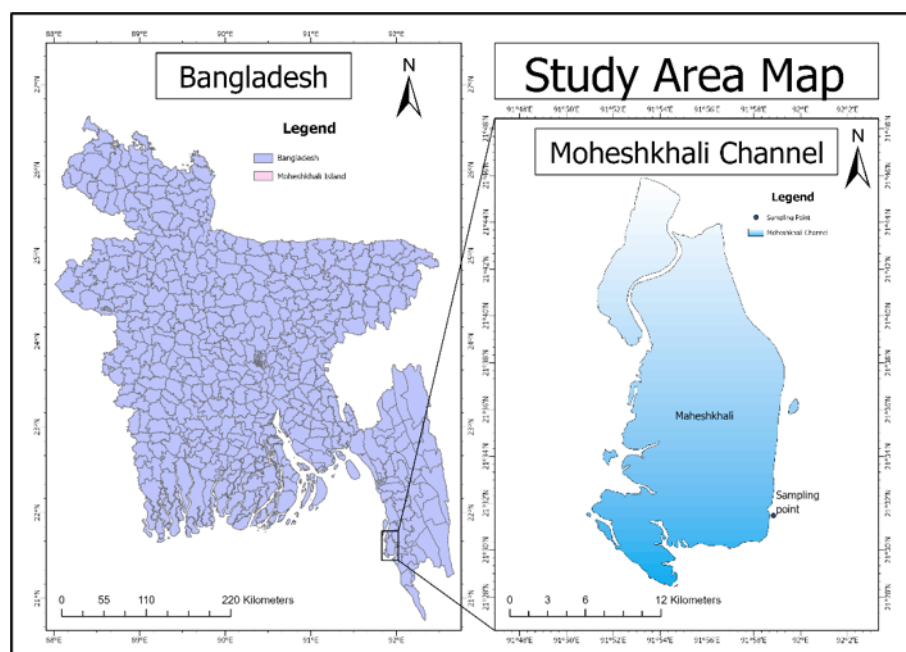


Figure 1. Study Area Map

Physicochemical Analysis

Environmental parameters including air temperature, water temperature, pH, and dissolved oxygen (DO) were measured in situ using portable digital multiparameter instruments.

Bacteria Isolation

Bacteria were isolated from sediment and seawater samples using the spread plate technique and maintain aseptic condition to prevent unwanted contamination. For seawater samples, 1 mL of sample was serially diluted in sterile distilled water, and tenfold serial dilutions were prepared up to 10^{-5} and for sediment samples, 1 g of sample was homogenized in 9 mL of sterile distilled water to prepare the initial suspension, followed by tenfold serial dilutions were prepared up to 10^{-5} . Then 100 μl of selected dilutions were spread onto LB agar media plates for bacterial isolation. Bacterial culture plates were incubated at 37°C for 2-6 days for bacterial growth. After the bacteria grow from the seawater and sediment samples, morphologically distinct colonies were selected for repeated streaking on fresh LB agar plates until pure bacterial colony were obtained

(Matyar et al., 2008). For long-term preservation, bacterial isolates were maintained as 20% glycerol stock at -20°C .

Morphological and Gram Staining Analysis

Purified bacterial isolated were characterized based on their colony morphology such as shape, size, color, elevation and margin (Breakwell et al., 2007). Gram staining was performed and bacterial cells were observed under microscope (Gram Stain Protocols, 2019).

Biochemical Identification

Bacterial isolates were identified using the VITEK® 2 Compact system (bioMérieux, France), which analyzes metabolic profiles based on 43 biochemical tests.

Results

The physicochemical characteristics of the water sample collected from the Moheshkhali Channel were measured in situ, and the results are presented in Table 1. The recorded values indicate the environmental condition of the sampling site during the study period.

Table 1. Physicochemical parameters of the sampling site at Moheshkhali Channel

Parameters	Value
Environment Temperature	35°C
Water Temperature	33°C
pH	7.0
DO (Dissolved Oxygen)	7.13 mg/L

A total of six morphologically distinct bacterial colonies were isolated from LB agar media, indicating phenotypic diversity among culturable bacterial populations. The isolates differed in colony characteristics such as size, shape, color, margin and

elevation shown in table 2 and Representative colony morphology of selected isolates is shown in figure 2. These variations suggest the presence of heterogeneous bacterial communities in the study area.

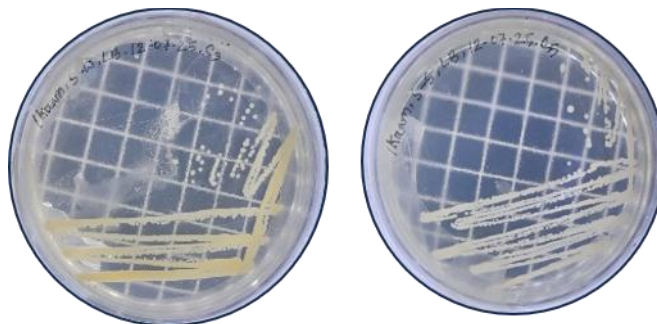


Figure 2. Representative Colony Morphology of purified bacterial isolates on LB agar media showing variation in size, shape and color.

Table 2. Morphological characteristics of bacterial isolates

Sample	Shape	Size	Color	Elevation	Margin
SWC1	Circular	Small	White creamy	Flat	Even
SWC2	Circular	Medium	Yellow	Flat	Even
SWC3	Circular	Small	Yellow	Flat	Even
SWC4	Circular	Small	Yellow	Flat	Even
SSC5	Circular	Medium	White	Flat	Even
SWFC4	Circular	Small	White	Flat	Even

Gram staining analysis revealed that all bacterial isolates were Gram-positive, as indicated by the purple coloration observed under the microscope (Table 3). Morphologically, the majority

of isolates exhibited rod-shaped (bacilli) forms, while one isolate (SWC1) showed coccoid (round) morphology and Representative gram staining image are shown in figure 3.

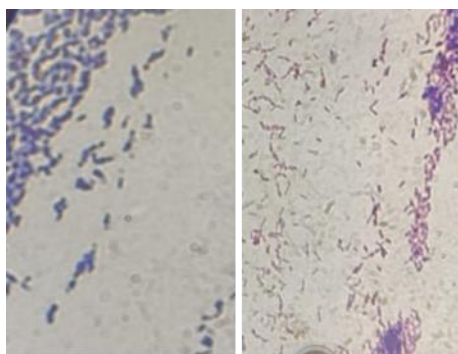


Figure 3. Representative Gram staining bacterial isolates showing gram-positive bacterial cells.

Table 3. Gram staining characteristics of bacterial isolates

Sample	Color	Shape	Gram (P/N)
SWC1	Purple	Round	P
SWC2	Purple	Rod	P
SWC3	Purple	Rod	P
SWC5	Purple	Rod	P
SWFC4	Purple	Rod	P
SSC5	Purple	Rod	P

Note: P=Gram positive bacteria ;N=Gram Negative bacteria

Table 4. Biochemical identification of bacterial isolates using VITEK® 2 system

Isolate Sample	Organism identified	Positive(+) Biochemical Test Result	Probability	Confidence Level
SWC3	<i>Leuconostoc mesenteroides ssp cremoris</i>	dRIB, NC6.5, AGAL, BGAL	91%	Good Identification
SWFC4	<i>Aerococcus viridans</i>	dRIB, OPTO, NC6.5, dMAN, SAL, AGAL, dMNE, SAC, BGAL, AMAN, dMAL, MBdG, dTRE, BACI	86%	Acceptable identification
SWC1	Non or low reactive biopattern	All Test Result are Negative	N/A	Non or low reactive biopattern
SWC5	Non or low reactive biopattern	dRIB	N/A	Non or low reactive biopattern
SWC2	Unidentified Organism	dRIB, OPTO, NC6.5, dSOR, dMAN, SAL, AGAL, dMNE, SAC, BGAL, AMAN, dMAL, MBdG, dTRE, BACI	N/A	Unidentified Organism
SSC5	Unidentified Organism	dRIB, OPTO, NC6.5, dMAN, SAL, BGAR, AGAL, dMNE, SAC, BGAL, AMAN, dMAL, MBdG, dTRE, BACI	N/A	Unidentified Organism

Discussion

In this Study, the measured pH and DO indicate near-neutral and well-oxygenated conditions. Both parameters are favorable for sustaining aquatic life and microbial activity. According to the Environmental Conservation Rules (ECR, 1997) of Bangladesh, the standard pH range for surface water is 6.5–8.5, while the minimum acceptable dissolved oxygen (DO) level is 5.0 mg/L, indicating suitable conditions for aquatic life and microbial activity (The Environment Conservation Rules, 1997 CONTENTS, 1997). Previous studies reported that pH range 7.60–7.92 and DO levels is 7.7–11.7 mg/L (Tamanna Jahan, 2024), as well as pH values of 7.1–8.5 and DO levels of 6.2–6.9 mg/L (Imran et al., 2020). The observed variations may be attributed to differences in environmental conditions such as tidal influence, difference in sampling time and freshwater input. Physicochemical parameters are important because significant variations in these factors directly affect water quality and influence aquatic ecosystem health (Imran et al., 2020). The observed water quality therefore suggests that the environmental conditions were conducive to supporting diverse bacterial communities.

The six bacterial isolates from seawater and sediment are gram-positive bacteria, indicating that gram-positive bacteria may be well adapted in this environment at Moheshkhali Channel; the thick peptidoglycan cell layer of gram-positive bacteria may provide extra protection against environmental stress conditions in coastal ecosystems.

Biochemical profiling revealed that the six bacterial isolates have diverse metabolic capabilities, indicating the adaptability of the isolates to the estuarine environment. Biochemical identification using the VITEK® 2 Compact system showed that two isolates were identified as *Leuconostoc mesenteroides* subsp. *cremoris* and *Aerococcus viridans*, while two isolates remained unidentified and two exhibited non- or low-reactive biopatterns. The identified bacteria have been reported from different environments including clinical, food, and environmental sources (Ezechukwu et al., 2019) (Pedersen et al., 2014). Their presence in seawater and sediment samples suggests wide ecological distribution and adaptability to varying conditions, indicating environmental connectivity rather than habitat specificity. The VITEK® 2 system identifies bacteria based on biochemical reaction patterns compared with a reference database; however, environmental isolates may show low discrimination or no match due to limited database representation, requiring additional confirmation methods (Pincus, 2006).

Conclusion

This study provides baseline information on culturable bacterial diversity in the Moheshkhali Channel, Bay of Bengal, Bangladesh. A total of six morphologically distinct bacterial isolates were obtained. Biochemical identification revealed that two isolates were identified as *Leuconostoc mesenteroides* subsp. *cremoris* and *Aerococcus viridans*. Future studies incorporating molecular techniques are recommended to achieve a more comprehensive understanding of bacterial diversity and to explore their ecological and biotechnological potential in this region.

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Declaration of competing interests

The authors declare no conflict of interest.

Author contributions

Md. Alomgir Hossain: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Kazi Ikram:** Investigation, Methodology, Writing – review & editing; **Sadia Sultana:** Investigation, Writing – review & editing; **Sunzida Akter Eva:** Investigation, Writing – review & editing; **Mohammad Nazir Hossain:** Conceptualization, Funding Acquisition, Methodology, Project Administration, Resources, Supervision, Writing – review & editing.

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