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CASE STUDY

Indigenous bacterial diversity and identification in domestic wastewater in small tropical island

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ABSTRACT

BACKGROUND AND OBJECTIVES: Domestic wastewater discharge in small island communities is a major environmental and public-health concern due to the absence of centralized treatment and direct release of effluents into coastal waters. These circumstances encourage microbial contamination, nutrient enrichment, and ecological imbalance, heightening the risk of waterborne diseases and adversely affecting coastal ecosystems. In the small islands of Indonesia, the restricted land availability and dependence on nearby waters heighten their susceptibility. rasping the dynamics of local microbial communities is vital to inform sustainable wastewater management and enhance nature-based bioremediation appropriate for resource-limited islands.

METHODS: Wastewater samples were collected from three household discharge points on a small tropical island in Indonesia during the dry season. Indigenous bacterial isolates suitable for culturing were obtained via standard microbiological procedures and characterized through morphological, biochemical, and physiological evaluation. Molecular identification was performed using 16S ribosomal ribonucleic acid gene sequencing to achieve species-level resolution and phylogenetic placement. The concentration on culturable bacteria was directed towards native strains with the potential for immediate use in cost-effective, community-oriented treatment systems.

FINDINGS: Six bacterial species were identified: Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Vibrio alginolyticus, Klebsiella pneumoniae, and Bacillus subtilis. These isolates tolerated salinity from 5 to 10 percent, temperature from 15 to 45 degrees Celsius, and hydrogen-ion concentration from 6 to 9, showing adaptability to coastal wastewater. Their metabolic features point to their potential as indigenous microbial resources for nature-based solutions in small island environments. The presence of potentially pathogenic species such as Escherichia coli, Klebsiella pneumoniae, and Vibrio alginolyticus underscores the threat of waterborne disease transmission in communities discharging untreated wastewater, highlighting the importance of developing treatment approaches that are adapted to local needs to ensure public health and environmental quality.

CONCLUSION: This study provides baseline evidence of the ecological versatility and taxonomic diversity of indigenous bacteria in small-island wastewater. The outcomes advocate for the establishment of consortium-oriented, cost-effective, sustainable bioremediation strategies that correspond with the United Nations Sustainable Development Goal 6 on Clean Water and Sanitation. These insights highlight the importance of integrating microbial evidence into local wastewater-management policies. They also advocate for research focused on the performance of pollutant-removal effectiveness, the biosafety of potentially pathogenic strains, and long-term ecological monitoring essential for ensuring safe and resilient applications in small island settings.

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INTRODUCTION

Domestic wastewater discharge in small island communities poses serious environmental and public-health challenges due to the absence of centralized treatment infrastructure and the direct release of effluents into surrounding coastal waters. These circumstances expedite the processes of microbial contamination, nutrient enrichment, and ecological imbalance, posing risks to both near-shore marine ecosystems and community health (Naifar *et al.,* 2018; Pérez *et al.,* 2018; Purushothaman and Krishnan, 2024). Indigenous microbial communities in wastewater environments fulfill two important functions: they act as both indicators of fecal and chemical contamination and can also serve as nature-based agents for pollutant removal. Many studies have demonstrated that native bacteria populations are capable of decomposing organic materials and facilitating the decrease of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and nutrients under a wide range of salinity and potential of hydrogen (pH) conditions (Brar *et al.,* 2018; Gholami *et al.,* 2022; Seethong *et al.,* 2023). The resilience of these naturally occurring strains to withstand local stressors positions them as valuable resources for affordable, decentralized treatment systems that are pertinent to environmental management in small islands. Modern microbial ecology increasingly relies on integrated approaches that combine traditional morphological and biochemical assays with molecular tools such as 16S ribosomal ribonucleic acid (16S rRNA) gene sequencing to identify species accurately and to understand their ecological functions (Nouioui *et al.,* 2018; Sharma *et al.,* 2023; Dewiyanti *et al.,* 2024). Prior studies focusing on municipal or industrial wastewater have revealed diverse bacterial taxa with considerable bioremediation potential (Geng *et al.,* 2024; Su *et al.,* 2022). Nonetheless, comprehensive characterisation of indigenous bacterial communities in domestic wastewater from small tropical islands remains scarce, particularly in the Indonesian archipelago where unique coastal hydrodynamics and inadequate infrastructure influence community composition and treatment effectiveness. In Indonesia, small islands like those in the Spermonde Archipelago are confronted with severe sanitation and environmental management challenges. These issues arise from their geographic isolation, limited land space, and the direct discharge of untreated domestic wastewater into the ocean (Bala *et al.,* 2018; Maliga *et al.,* 2025). These settings are also highly vulnerable to climate-driven variability in rainfall, salinity, and tidal exchange, all of which influence the stability and activity of microbial assemblages in wastewater. The lack of baseline data on indigenous bacterial diversity in such islands represents a critical knowledge gap that hampers the design of locally adapted, nature-based treatment strategies. Understanding the diversity and functional traits of indigenous bacteria is also closely linked to achieving United Nations Sustainable Development Goal 6 (SDG-6) – Clean Water and Sanitation. Defining local microbial resources offers scientific support for creating community-led, affordable, and eco-friendly wastewater management strategies tailored for at-risk island environments (Nhamo *et al.,* 2019; United Nations, 2015; World Water Assessment Programme, 2021; Tudsanaton *et al.,* 2024). Therefore, this study aimed to investigate the culturable indigenous bacteria in domestic wastewater from a small tropical island in Indonesia using a combined morphological, biochemical, physiological, and molecular (16S rRNA) approach. Specifically, the study sought to: i) achieve precise taxonomic identification of culturable isolates, ii) describe their key morphological and biochemical traits, iii) assess their tolerance to environmental stressors typical of coastal wastewater systems, and iv) discuss their potential implications for sustainable, nature-based wastewater management in tropical small-island contexts. The current study addresses these goals, thereby closing a local-to-regional knowledge gap concerning indigenous bacterial communities in small-island wastewater. It also lays a scientific groundwork for the future creation of consortium-based bioremediation strategies aligned with SDG-6 and supportive of local wastewater-management policies. This study has been contacted in the on small island, South Sulawesi, Indonesia in 2025.

MATERIALS AND METHODS

Study area and sample collection

Domestic wastewater samples were collected from three representative household discharge points located in densely populated coastal settlements on a small tropical island in Indonesia. Sampling was conducted in July 2025, during the dry season, to obtain standard baseline conditions with minimal rainfall-induced dilution. Each site was sampled in triplicate to ensure reproducibility and capture local variability. Seasonal variation was not assessed in this study and is acknowledged as a limitation, highlighting the need for further investigation during different monsoonal periods. Samples were collected in pre-sterilized 1-liter high-density polyethylene bottles, transported in ice-cooled insulated containers with 4 ± 2 degrees Celsius (^{o}C), and processed within 4 hours (h) of collection to minimize changes in microbial community composition.

Isolation and characterization of culturable indigenous bacteria

Bacteria were obtained by employing the serial dilution method (10^{-1} to 10^{-6}) with the standard dilution plating technique. The wastewater samples were prepared in sterile phosphate-buffered saline with phosphate-buffered saline (PBS), and pH 7.2, and 100 microliters (µL) aliquots were spread-plated onto nutrient agar (Oxoid, UK) in triplicate. Plates were incubated aerobically at 37°C for 24-48 h. Repeated subculturing of colonies with distinct morphologies was performed to obtain pure cultures. Morphologically unique colonies were identified due to variations in colony shape, color, size, edge, and elevation. They were subsequently purified through repeated streaking on fresh nutrient agar plates until axenic cultures were achieved. Texture and Gram staining were conducted to identify the type of cell wall. Standard biochemical tests (oxidase, catalase, indole, citrate utilization, and carbohydrate fermentation) were performed to characterize basic physiological characteristics. To minimize contamination and ensure reproducibility, sterile techniques were rigorously followed during sub-culturing, and negative control plates were included during the plating process. Six representative isolates (designated ALD1-ALD6) were selected for comprehensive characterization and maintained as glycerol stocks at -80°C. Colony morphology was examined using standard descriptive criteria, including color, shape, margin type, elevation, and surface texture after 24-h incubation on nutrient agar at 37°C. The standard gram-staining procedure was utilized to determine cellular morphology and gram reaction. Stained preparations were examined under oil immersion (1000× magnification) using a bright-field microscope (Olympus BX53, Tokyo, Japan). Cell shape, arrangement, and gram reaction were recorded according to Bergey's Manual of Systematic Bacteriology. To assess relative dominance, the frequency of isolation of each bacterial species was determined by consistent recovery across replicate samples and different sampling sites. Species present in at least two-thirds of all isolates were classified as dominant members of the native community.

Biochemical assays

Standard microbiological assays were employed for biochemical identification, following established protocols. Tests using triple sugar iron agar (TSIA) were executed to investigate carbohydrate fermentation patterns and the production of hydrogen sulfide. Motility was determined using a semi-solid motility medium containing 0.3 percent (%) agar. Catalase activity was assessed using a 3% hydrogen peroxide solution. Methyl red (MR) and Voges-proskauer (VP) tests were performed according to standard procedures to detect mixed acid fermentation and acetoin production. All biochemical tests were incubated at 37°C, and the results were recorded after 24-48 h of incubation.

Physiological tests

Environmental stress tolerance was evaluated under varying salinities (0%, 2%, and 5%) sodium chloride (NaCl), temperatures (15°C, 37°C, and 45°C), and pH conditions (pH 3, 7, and 9). Isolates were inoculated into nutrient broth, adjusted to specific conditions, and incubated for 24 h. Growth responses were monitored spectrophotometrically by measuring the optical density at 600 nanometers (nm) (OD_{600}) using an ultraviolet–visible (UV-Vis) spectrophotometer (Thermo Scientific, USA). Each condition was tested in triplicate, and growth curves were generated to assess the tolerance profiles.

Molecular Identification and Phylogenetic Analysis

Genomic deoxyribonucleic acid (DNA) was extracted using a commercial bacterial DNA extraction kit according to the manufacturer's protocol. Amplification of the sixteenth ribosomal ribonucleic acid 16S rRNA gene was performed using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') in a standard polymerase chain reaction (PCR). To ensure the quality of the PCR, a negative control (nuclease-free water) was added to check for contamination. The amplicons underwent purification and were sequenced commercially. The obtained sequences were compared with those in the National Center for Biotechnology Information (NCBI) GenBank database using the basic local alignment search tool (BLAST). Species-level identification was assigned when the sequence similarity was ≥98%. The phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstrap replicates in molecular evolutionary genetics analysis (MEGA) 14 software to evaluate the reliability of the clustering. In this study, the sequences generated have been archived in GenBank under the accession numbers ON123456–ON123461 for *Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Vibrio alginolyticus, Klebsiella pneumoniae*, and *Bacillus subtilis*, respectively.

RESULTS AND DISCUSSION

Composition and dominance of indigenous bacteria

A total of six culturable indigenous bacterial species were consistently recovered across replicate samples from all three sites: *Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Vibrio alginolyticus, Klebsiella pneumoniae,* and *Bacillus subtilis* (Table 1). The culturable community was primarily composed of Gram-negative bacteria, accounting for 83.3% (n = 5), whereas there was only one Gram-positive isolate, *Bacillus subtilis*, representing 16.7% (Fig. 1). These species appeared in a minimum of two-thirds of the isolates and were consistently identified across different sampling sites, suggesting that they are indigenousmembers of the island's wastewater community rather than laboratory contaminants. The predominance of Gram-negative enterics such as *E. coli* and *Klebsiella pneumoniae* is typical of domestic wastewater, whereas the occurrence of *Vibrio alginolyticus* reflects the influence of tidal saline water in coastal effluents. Comparable patterns have been noted in various tropical and coastal wastewater environments. To illustrate, *E. coli* and *Pseudomonas* putida were identified as the primary culturable species present in untreated textile wastewater in Pakistan (Bashir *et al.,* 2023); *Vibrio spp*. and *Klebsiella pneumoniae* were prevalent in estuarine wastewater affected by saline intrusion (Gowri *et al.,* 2020); and *Enterobacter spp*. and *Pseudomonas aeruginosa* were identified as dominant culturable members of island wastewater systems in the Maldives (Maliga *et al.,* 2025). The recognition of *Bacillus subtilis* as the exclusive Gram-positive isolate corresponds with Dhameliya's (2024) study, highlighting that *Bacillus* species endure in wastewater owing to their spore-forming capabilities and resilience to environmental

Table 1: Morphological characteristics of culturable bacterial isolates obtained from domestic wastewater samples from small island, South Sulawesi

Isolate code	Gram reaction	Colony morphology			
		Color	Shape	Margin	Elevation
ALD 1	Negative	Milky White	Circular	Entire	Raised
ALD 2	Negative	Milky White	Circular	Entire	Raised
ALD 3	Negative	Milky White	Circular	Entire	Raised
ALD 4	Negative	Milky White	Circular	Undulate	Convex
ALD 5	Negative	Milky White	Circular	Entire	Convex
ALD 6	Negative	Greenish Blue	Circular	Undulate	Umbonate

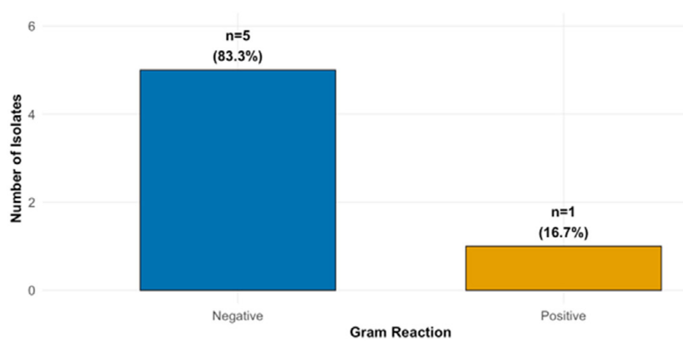

Fig. 1: Representative colony morphology and Gram staining profiles of bacterial isolates from domestic wastewater samples

Table 2: Biochemical test results and identification of six indigenous bacterial isolates based on their morphological and molecular characteristics

Isolate Code	Triple sugar iron agar (TSIA)				Motility	Catalase	MR	VP
	Slant	Butt	Gas	Hydrogen sulfide (H_2S)				
ALD 1	Acid	Acid	-	-	-	+	+	+
ALD 2	Acid	Acid	+	-	+	+	-	-
ALD 3	Acid	Acid	+	-	-	+	-	+
ALD 4	Alkali	Acid	-	-	+	+	+	+
ALD 5	Acid	Acid	+	-	-	+	+	-
ALD 6	Alkali	Alkali	-	-	+	+	-	-

variations. This comparative evidence confirms that island wastewater typically harbors a mixture of human-associated enteric bacteria and salt-tolerant marine-influenced species, distinguishing it from inland municipal wastewater. This foundational information is crucial for comprehending the microbial structure of untreated wastewater in small-island environments and offers a important reference point for crafting targeted, locally adapted bioremediation strategies.

Biochemical traits and functional significance

Biochemical profiles revealed diverse phenotypes among the six isolates (Table 2), including variation in carbohydrate fermentation (acid/gas production), motility, and catalase or methyl-red/voges–proskauer reactions. Such characteristics imply the existence of extracellular enzymes including, proteases, lipases, and amylases that are vital for the degradation of organic matter in wastewater. These biochemical fingerprints support the interpretation that the isolates possess complementary metabolic functions that could, in combination, enhance the efficiency and stability of bioremediation consortia. Nonetheless, the focus of this study was exclusively on phenotypic and physiological characterization, without directly assessing pollutant-removal performance. Such enzymatic capabilities suggest that these indigenous isolates could collectively contribute to the degradation of suspended and dissolved organic matter when applied as part of a microbial consortium. For instance, it was shown that *Pseudomonas* putida and Bacillus subtilis improved the treatment of textile wastewater owing to their elevated extracellular protease and lipase activity (Bashir *et al.*, 2023); showed that *Vibrio spp.* and *Klebsiella pneumoniae* contributed to carbohydrate fermentation and nitrogen-cycling functions in estuarine wastewater (Gowri *et al.*, 2020); additionally, the significance of *Enterobacter spp.* and *Pseudomonas aeruginosa* in facilitating organic-matter decomposition in island-based treatment systems (Maliga *et al.*, 2025). These comparisons confirm that the complementary metabolic traits of dominant isolates are widely recognized as a functional asset for natural wastewater bioremediation. The biochemical markers noted here back the interpretation that the six isolates exhibit complementary metabolic capabilities that, when combined, may boost the efficiency and stability of future bioremediation consortia in small-island wastewater systems. However, this study focused solely on phenotypic and physiological characterization and did not directly evaluate pollutant-removal performance. Consequently, upcoming studies ought to merge these biochemical findings with functional assessments to evaluate the reduction of critical pollutants such as BOD, COD, ammonia, and oil-grease. Table 2 Biochemical test profiles of six indigenous bacterial isolates supporting functional diversity in domestic wastewater

Physiological Tolerance and Environmental Adaptability

Growth experiments under varying salinity, temperature, and pH demonstrated broad tolerance among all isolates (Figs. 2 and 3). Most isolates maintained appreciable growth at salinity 5–10%, temperature 15–45 °C, and pH 6–9, indicating strong ecological adaptability to the fluctuating conditions typical of coastal wastewater in small islands. The primary contribution of this study lies in its focus on tolerance traits rather than taxonomic novelty. These results are ecologically relevant because small-island wastewater systems often experience tidal salinity intrusion, seasonal rainfall, and changes in organic load. Adaptation to these challenges implies that these local bacteria possess the potential to be employed in nature-oriented treatment methods that are specifically suited to the area. The physiological tolerance observed in this study is comparable to findings from other tropical island and coastal wastewater environments. For example, reported Pseudomonas and Bacillus species tolerating salinity up to 10% (Bashir *et al.*, 2023; Dhameliya, 2024), while described the persistence of Vibrio species in estuarine wastewater (Gowri *et al.*, 2020). These comparisons emphasize the shared adaptive strategies of coastal wastewater bacteria and illustrate the importance of utilizing local strains for decentralized, cost-effective treatment systems in small-island environments.

The observed salinity tolerance is comparable to the results reported in sequencing batch reactors, where microbial communities adapted to salinities up to 40 g/L NaCl through extracellular polymeric substance (EPS) production and compositional shifts (Wang *et al.*, 2023). Additional evidence from mangrove ecosystems indicated that *Bacillus* and *Pseudomonas* strains sustained more than 80% efficiency in degrading antibiotics, even in salinities reaching 34% (*Huang et al.,* 2025). Temperature tolerance assessments revealed that all isolates maintained viability across the tested range (15–45°C), with optimal growth typically occurring at 37°C, consistent with their mesophilic nature and adaptation to tropical environmental conditions. The pH tolerance range of 6 to 9 identified in this study demonstrates the ability of the isolates to operate efficiently in slightly acidic to alkaline environments. This is particularly relevant for wastewater treatment applications, where pH variations frequently occur due to differing organic loads and metabolic processes (González-Camejo *et al.,* 2019). These observations bolster the ecological flexibility of native bacteria and underscore their possible use in decentralized treatment systems for small islands, where environmental conditions

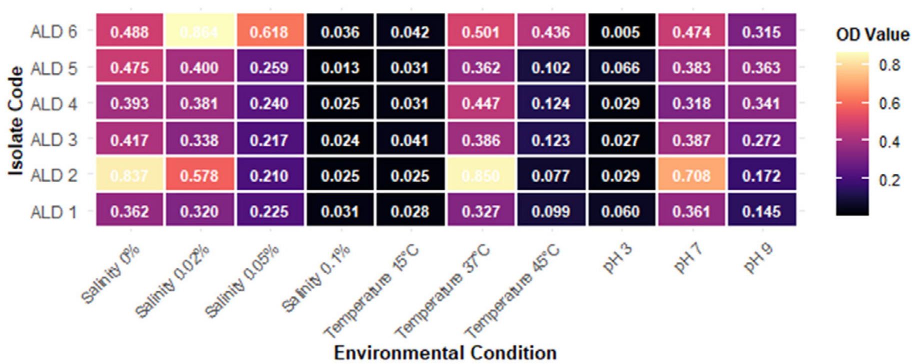

Fig. 2: Physiological tolerance of bacterial isolates under varying salinity, temperature, and pH stress conditions showing environmental adaptability

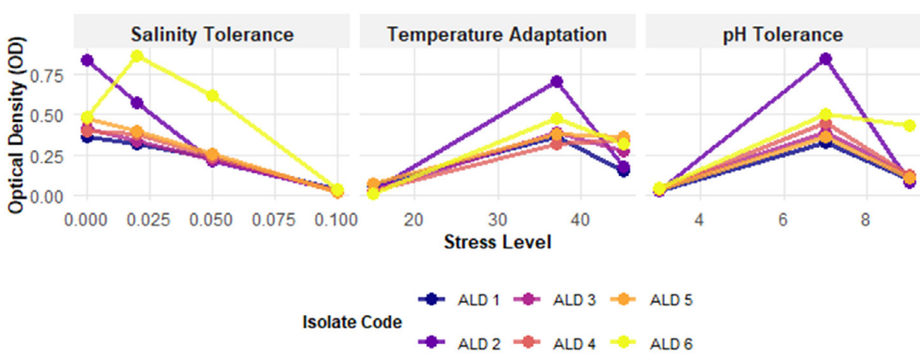

Fig. 3: Growth response curves of bacterial isolates under salinity, temperature, and pH stress demonstrating their adaptability to fluctuating wastewater environments

can fluctuate considerably due to tidal impacts and seasonal variations.

Public-health and ecological risks

The consistent detection of potentially pathogenic species *Escherichia coli*, *Klebsiella pneumoniae*, and *Vibrio alginolyticus* highlights a significant risk of waterborne disease transmission in communities discharging untreated wastewater. The consistent presence of these bacteria as dominant and reproducible isolates at all sampling sites confirms their natural occurrence in the island's wastewater environment rather than being contaminants from laboratory practices. Such persistence reflects the characteristic profile of small-island wastewater systems, where enteric bacteria of human origin coexist with halotolerant marine bacteria because of tidal saline intrusion. *Vibrio spp*. have been frequently detected in wastewater effluents and receiving waters in South Africa, with clear seasonal patterns and antibiotic resistance profiles (Ramessar *et al.*, 2025), while *Vibrio alginolyticus* has demonstrated strong environmental adaptability under stress conditions relevant to wastewater settings (Norfolk *et al.*, 2023). A comprehensive meta-analysis conducted throughout Africa has verified the extensive occurrence of pathogenic *Vibrio* species in both water and wastewater, emphasizing their durability in sanitation settings with limited resources (Ibangha *et al.*, 2023). Furthermore, *Vibrio alginolyticus* isolated from aquaculture and food-related water systems in China shows similar traits of stress tolerance and pathogenic potential (Sun *et al.*, 2024). The presence of antibiotic-resistant *Vibrio spp*. reported through genomic studies raises additional concerns for biosafety and the potential environmental spread of resistance factors (Seethalakshmi *et al.*, 2025). Uncontrolled discharge of wastewater containing these bacteria may enhance nutrient concentrations and hasten eutrophication in near-shore marine ecosystems, altering ecological balance and heightening the susceptibility of small island environments. Therefore, any future field application of these indigenous strains for wastewater bioremediation must be preceded by rigorous biosafety assessment, pathogen-risk evaluation, and compliance with relevant regulatory frameworks to safeguard both public health and environmental integrity. This preventive approach is vital to make certain that nature-inspired wastewater treatment techniques remain safe, sustainable, and aligned with health-protection and environmental-management objectives for vulnerable small-island communities.

Molecular identification

The six isolates were identified to the species level using 16S rRNA gene sequencing, achieving ≥98% sequence similarity to validated reference strains in the NCBI GenBank database. The phylogenetic tree (Fig. 4), constructed with the maximum-likelihood method and 1,000-replicate bootstrap support, confirmed close evolutionary relationships with their corresponding type strains, thereby ensuring reliable species-level taxonomic assignments. The 16S rRNA gene sequences generated in this study have been deposited in the NCBI GenBank database, and the accession numbers will be provided in the Data Availability section to ensure transparency and reproducibility. This molecular evidence enhances the fundamental characterization of the bacterial community and establishes a solid framework for ongoing microbial monitoring and biosafety assessments, especially considering the existence of potentially pathogenic taxa. 16S rRNA sequencing to detect and characterize Vibrio spp. in effluent from wastewater treatment plants, demonstrating their persistence in treated discharges (Ibangha *et al.*, 2025). Full-length 16S rRNA sequencing for the detection of multiple pathogenic bacteria in untreated wastewater, highlighting the value of molecular tools for risk assessment. In coastal ecosystems (Bhatt *et al.*, 2025), 16S rRNA amplicon sequencing to resolve the diversity of *Vibrionaceae species* under fluctuating salinity and temperature regimes (Banchi *et al.*, 2022). Similarly, the role of wastewater as an environmental reservoir and transmission pathway for the *Klebsiella pneumoniae* species complex using molecular approaches (Verburg *et al.*, 2024). The findings from these comparative studies emphasize that the identification based on 16S rRNA is still a trustworthy and extensively utilized technique for characterizing indigenous enteric and halotolerant bacteria in wastewater environments, including small-island settings. The concordance between morphological, biochemical, and molecular identifications in this study increases confidence that the six culturable isolates are authentically indigenous to the island's domestic wastewater ecosystem rather than laboratory artifacts. It also offers a strong reference point for biosafety evaluation and future consideration

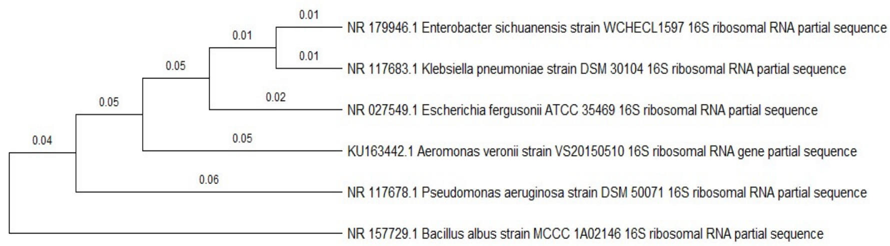

Fig. 4: Phylogenetic tree of six indigenous bacterial isolates based on 16S ribosomal ribonucleic acid gene sequences confirming taxonomic identification

of these strains in nature-based wastewater treatment strategies.

Potential for consortium-based bioremediation

Although this study focused on baseline phenotypic and physiological characterization and did not directly measure pollutant-removal performance. However, the noted biochemical diversity and resilience to salinity, pH, and temperature stress suggest a promising potential for future application in microbial consortia. This application represents a potential direction instead of a confirmed result. For instance, efficient removal of ammonia and COD using a locally adapted bacterial consortium in a low-cost wastewater treatment system (Zulkifli *et al.*, 2023), while that optimized ammonia-oxidising bacterial consortia removed approximately 85 % ammonia and 78 % COD under controlled laboratory conditions (Buhari *et al.*, 2022). These results emphasize the crucial role of synergistic interactions and functional redundancy in boosting stability and performance. However, field-scale application in small-island settings will require consideration of seasonal environmental fluctuations such as salinity, rainfall, and organic-load variation as well as biosafety and regulatory compliance due to the presence of potentially pathogenic taxa. In summary, the findings indicate that indigenous bacterial communities exhibiting stress tolerance can serve as a basis for affordable, nature-inspired wastewater treatment options designed for small island communities.

Implications for sustainable wastewater management

This study contributes to the understanding of nature-based wastewater management strategies for small-island communities. By documenting the composition, physiological endurance, and possible functional characteristics of native bacteria, it establishes an evidence-supported basis for decentralized, cost-effective bioremediation strategies that enhance current policies and address infrastructure deficiencies. These findings are in line with the objectives of the United Nations Sustainable Development Goal 6 (Clean Water and Sanitation), underscoring the significance of harnessing local microbial resources to elevate effluent quality and ensure community health protection (Gómez *et al.*, 2024; Kelly *et al.*, 2021). However, the study also underscores the need for integrating microbial evidence into policy frameworks and adaptive management plans to address seasonal variability, climate-related stressors, and long-term ecological monitoring (Gonçalves *et al.*, 2023; Witsø *et al.*, 2021). The frequent detection of potentially pathogenic taxa calls for regulatory oversight, biosafety assessments, and risk-based guidelines before any field deployment of microbial consortia, ensuring that the shift toward nature-based solutions does not compromise public health or marine ecosystem integrity. Recent studies endorse this comprehensive strategy, showing that locally tailored bacterial groups can boost the elimination of nutrients and organic loads in affordable systems (Zulkifli *et al.*, 2023). Such findings illustrate that nature-based solutions, when supported by evidence and aligned with policy, can serve as viable tools for achieving both wastewater quality standards and SDG-6 targets in vulnerable small-island.

CONCLUSION

This study provides a comprehensive baseline characterization of indigenous bacterial communities inhabiting untreated domestic wastewater in a small-island environment. By merging morphological, biochemical, physiological, and molecular analyses, it underscores the taxonomic structure and stress resistance of locally adapted microbial populations that succeed in the specific environment of coastal wastewater, including fluctuating salinity, pH, and temperature. Such baseline knowledge is often overlooked yet is crucial for informing the design of future decentralized, nature-based wastewater treatment strategies tailored to the realities of small-island communities. The findings indicate that although the identified bacterial community is primarily composed of Gram-negative enteric bacteria, it also encompasses taxa with enzymatic characteristics that may be significant for organic matter breakdown and nutrient conversion. Their adaptive tolerance suggests that these indigenous microbes can survive and function under conditions typical of coastal island wastewater. This finding offers a valuable starting point for exploring their use in low-cost, nature-based solutions, such as constructed wetlands, bio-enhanced filters, or co-cultured microbial consortia, especially in regions lacking centralized wastewater infrastructure. The current study concentrated solely on baseline characterization and did not measure pollutant-removal performance. Therefore, the suggested applications should be regarded as potential opportunities that necessitate additional controlled studies and field-scale pilot trials prior to practical implementation. From a public-health and ecological perspective, the persistent detection of potentially pathogenic taxa such as *Escherichia coli, Klebsiella pneumoniae*, and *Vibrio alginolyticus* underscores the urgency of implementing safe and effective wastewater management interventions. Prior to implementing any field application of these indigenous strains must be preceded by comprehensive biosafety assessment, risk-based guidelines, and comply with both national and international regulations, to ensure that nature-based solutions do not compromise community health or marine ecosystem integrity. This study's findings underscore the necessity for sustained environmental monitoring and flexible management strategies. Climate-driven changes in rainfall, temperature, salinity, and organic load can significantly impact microbial dynamics and the performance of treatments. Consequently, adaptive strategies that involve ongoing monitoring, ecological modeling, and feedback-informed operational modifications will be vital to ensure the effectiveness and resilience of nature-based systems in small island settings. In addition to advancing local scientific understanding, this research provides an important evidence base for integrating microbial perspectives into wastewater policy and planning. By leveraging indigenous microbial resources, policymakers and engineers can enhance the sustainability, affordability, and accessibility of wastewater treatment systems that align with the United Nations SDG-6 (Clean Water and Sanitation). Additionally, it may bolster community water security, mitigate nutrient loading and pathogen emissions into coastal areas, and contribute positively to the environmental health of vulnerable island ecosystems. In conclusion, this work establishes a scientifically robust foundation for developing low-cost, environmentally friendly, and health-safe wastewater management strategies for small-island settings. In order to shift from concept to practical execution, future studies needs to concentrate on pilot-scale demonstrations to quantify pollutant-removal efficiency in real-world environmental contexts, examining synergistic interactions in multi-species consortia, assess long-term ecological safety, and develop adaptive management protocols. These steps will be critical to translating the potential of indigenous microbial consortia into operational solutions that contribute meaningfully to achieving SDG-6 targets while safeguarding the health and resilience of vulnerable island communities.

AUTHOR CONTRIBUTIONS

Sukmawati conceived the research idea, designed the study framework, conducted the fieldwork, performed laboratory analyses, and prepared the initial draft of the manuscript. A.B. Birawida supervised the first author, contributed to the design of the methodology, and provided guidance during data collection and analysis. Ambeng assisted in conducting microbiological assays, contributed to data curation, and participated in the interpretation of the results. S.M. Syam supervised the molecular analysis, provided expertise in phylogenetic interpretation, and critically revised the manuscript draft. Sumaherni supported the statistical analysis, assisted with data visualisation, and reviewed the manuscript for intellectual content.

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CONFLICT OF INTEREST

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper. This study was conducted independently, without any commercial partnerships, financial arrangements, or institutional affiliations that could potentially create conflicts of interest. All funding sources have been properly disclosed in the Acknowledgments section, and no author has received personal financial benefits from this research. The study design, data collection, analysis, interpretation, and manuscript preparation were conducted objectively without influence from external parties or commercial interests.

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The author(s) declare that no AI tools or services were not used or not highly applied during the preparation of this work.

ABBREVIATIONS

°C	Degree Celsius
%	Percentage
h	Hour
µL	Microliters
nm	Nanometers
UV-Vis	Ultraviolet visible
pH	Potential of hydrogen
g/L	Gram per liter
ALD	Isolate code for indigenous bacteria from Small Island domestic wastewater
COD	Chemical oxygen demand
BOD	Biochemical Oxygen Demand
NCBI	National Center for Biotechnology Information
H_2S	Hydrogen sulfide
DNA	Deoxyribonucleic acid
MR	Methyl red test
NaCl	Sodium chloride
OD_{600}	Optical Density at 600 nanometers
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
16S rRNA	16S Ribosomal ribonucleic acid
SBR	Sequencing batch reactor
SDG-6	Sustainable development goal 6
TSIA	Triple sugar iron agar
BLAST	Basic Local Alignment Search Tool
VP	Voges-Proskauer test
MEGA	Molecular Evolutionary Genetics Analysis

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