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Isolation of microbial community and physicochemical assessment of the seawater

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Abstract

This study explores the microbial diversity of seawater to assess its microbiological quality and evaluate its suitability for public use. Understanding the composition of seawater is essential for identifying ecological dynamics and potential health risks. Key physicochemical parameters such as pH, temperature, electrical conductivity, total dissolved solids, and salinity were analyzed, all aligning with established seawater standards. These parameters offer insights into oceanic processes and climatic patterns. Microbial isolation was conducted using a range of agar media to identify and characterize bacterial species and their metabolic traits. Nutrient agar revealed multiple colony morphologies, indicating the presence of diverse bacterial populations. Eosin Methylene Blue agar facilitated the detection of non-lactose fermenting gram-negative bacteria, while MacConkey agar showed no growth, suggesting the absence of lactose fermenting gram-negative strains. Additional use of urea agar and mannitol salt agar enabled the identification of urease-producing bacteria and Staphylococci species. The findings provide valuable baseline data on microbial presence in seawater, contributing to enhanced understanding of marine microbial ecology and supporting initiatives in environmental monitoring and public health safeguarding.

Keywords: Environmental monitoring; marine microbiology; microbial diversity; public health; seawater quality.

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1. INTRODUCTION

Understanding microbes' diversity and potential uses depends heavily on isolating bacteria from various environmental sources. The coastal marine ecosystem is one type of ecological resource. Because beaches are popular places for recreation and tourism, beachgoers frequently select beaches based on their perception of cleanliness. Even a clean beach might nonetheless be dangerous for people's health because sand can be a source of numerous bacteria. Therefore, saltwater's microbiological quality must be assessed to determine whether or not beaches are safe for public use. Due to the increase in beach enjoyment, it is more important than ever to monitor various microbiological, physical, and chemical indicators of beach quality in coastal management programs (De Giglio et al., 2022). The 2021 revised guidelines of the World Health Organization recommend monitoring the quality of sand in addition to water at recreational beaches, highlighting the importance of comprehensive microbial assessments (Solo-Gabriele et al., 2023).

Following this, numerous countries, including the Philippines, already monitor beach quality in compliance with national and/or international standards due to the global acknowledgment of the problem of saltwater pollution (Migo et al., 2018). An increase in the contamination of beach seawater has been caused by improper waste disposal, the release of raw domestic sewage, the disposal of animal waste, pollution from precipitation and river water, and other factors (Li et al., 2023; Mostafa et al., 2024). Numerous sources contribute to the pathogenic and non-pathogenic microorganisms frequently detected in recreational water (Samarasekera & Abeygunawardena, 2017). Studies have shown that Enterococcus levels at marine beaches can vary regionally and are influenced by environmental factors, emphasizing the need for localized monitoring strategies (Futch et al., 2021).

Beach sand has so far been discovered to include harmful bacteria such as *Vibrio vulnificus, Salmonella, Campylobacter, Pseudomonas aeruginosa*, and *Staphylococcus aureus* (including methicillin-resistant varieties) (De Giglio et al., 2022). Furthermore, *Enterococcus* is recommended as the indicator organism for marine water. *Aeromonas* and *Enterococcus* spp. have been found in numerous investigations. *E. coli, Pseudomonas*, and *Staphylococci* species may infect humans in seawater, beach sand samples, or both (Samarasekera & Abeygunawardena, 2017). Recent research has identified the presence of antibiotic-resistant Enterococcus faecium in beach waters, raising concerns about the potential health risks associated with recreational water use (Silva et al., 2024). Additionally, the occurrence of potentially human pathogenic bacteria such as Aeromonas, Pseudomonas aeruginosa, Staphylococcus, and Vibrio-like organisms in beach sand and adjacent seawater has been documented, underscoring the need for comprehensive microbial assessments (Mudryk et al., 2014).

In this study, the researcher focused on providing baseline data on the microbial presence in the area by isolating bacteria from the seawater of Barangay Liboganon, Tagum City, Davao del Norte. The region's coastal areas are rich in biodiversity, but there is limited knowledge about the microbial communities in these environments.

1.1. Purpose of study

This study pursued the following objectives: first, to determine the physicochemical parameters of seawater, including pH level, temperature, electrical conductivity, total dissolved solids, and salinity; second, to isolate potential bacterial communities present in seawater samples collected from Barangay Liboganon, Tagum City, Davao del Norte.

2. MATERIALS AND METHOD

2.1. Research design

This research used a quantitative descriptive research design to analyze the microbial occurrence in seawater and define its physicochemical parameters. The descriptive design offered an objective and systematic summary of visible environmental conditions without studying cause-and-effect or relational variables (Creswell & Creswell, 2017).

Study site. Figure 1 presents Liboganon, a barangay in Tagum City from the province of Davao del Norte. Liboganon is situated at approximately 7.3484, 125.7756, on the island of Mindanao. Elevation at these coordinates is estimated at 6.3 meters or 20.7 feet above mean sea level. Consent was sought from the barangay officials before obtaining the samples. Black volcanic sand beach facing Davao Gulf, warm tropical waters, and an area free from the usual tourist beach crowd.

Figure 1
Study site

2.2. Data collection

2.2.1. Sampling of seawater

Early morning sampling was carried out in low sunshine conditions with no beach bathers to reduce external contamination and obtain representative samples. Seawater (1 L) was randomly collected from each of three sites about 2.5 m from the beach line with a 20 m gap between locations to account for spatial variation (De Giglio et al., 2022). Sterile Whirl-pack sampling bags were utilized to obtain the water samples in order to avoid sampling contamination.

Sampling bags were put into the water at 30 cm to prevent the water layer from being exposed to ultraviolet radiation, thus preventing surface conditions from affecting the collected samples. The water samples collected were transported to the laboratory in a cool box right away to provide a controlled temperature while in transport (Samarasekera & Abeygunawardena, 2017). Water samples were labeled and tagged for traceability to the source point and recordkeeping. Water samples were held at 4°C for preservation while in transport and to prevent potential degradation (Soto-Varela et al., 2021).

2.2.2. *Physicochemical parameters of seawater*

The multi-functional water quality tester EZ-990 was used to measure the physicochemical parameters of the seawater sample. The seawater sample was collected and set up for measurement. In situ direct measurements were done right at the sampling site to ensure real-time data acquisition and potentially avoid any changes to the sample while in transit. The tester was utilized to measure the pH of a seawater sample and to obtain its acidity and alkalinity values. The same device was used to measure temperature, indicating the thermal state of the water. Electrical conductivity (EC), representing the water's ability to conduct ions and its ionic content, was noted as well. The last parameter, total dissolved solids (TDS), indicating the concentration of dissolved substances, was also assessed. Finally, salinity, indicating the sodium content of the seawater, was recorded.

To maintain uniformity and accuracy in the collected data, all measurements were taken using the same apparatus. The measured values of pH, temperature, electrical conductivity, total dissolved solids, and salinity were recorded for analysis and interpretation (De Giglio et al., 2022).

2.2.3. Media preparation and isolation of bacteria

The collected water samples were transported to the microbiology laboratory at Davao Oriental State University, located in Mati City, Davao Oriental, Philippines, for assessment. Five culture media were prepared in induplicate to allow growth and isolation of microorganisms from designated target site, which included Nutrient agar, Eosin Methylene Blue (EMB) Agar, MacConkey Agar, Urea Agar Base (Christensen) (Autoclaved), and Mannitol Salt Agar.

After the culture media were prepared for use in the laboratory, the various water samples were inoculated onto these culture media, which facilitated the growth of a specific microorganism and the development of colonies. The inoculated culture media with the water samples were placed in an incubator for 72 hours, during which we assume the microorganisms to develop and for colonies to be seen. The presence of the laboratory facilities at the University dictated the selection of a 72-hour incubation period. Following the 72-hour incubation period, the cultures were inspected, and the colonies of bacteria that had developed were analyzed (Pelczar et al., 2001).

2.3. Data analysis

Quantitative results from the EZ-990 tester were tabulated and averaged. Microbial colony counts were summarized by media type and sampling site. Results were interpreted in terms of environmental quality and potential microbial contamination. Secondary sources were used to identify the isolated bacterial communities in the study site, such as the book of Madigan et al. (2018)

2.4. Ethical considerations

The study followed environmental sampling and microbiological analysis ethical practices. Previous permission was secured from Liboganon, Tagum City's barangay authorities, to guarantee community involvement and respect for the local authority. Sampling involved non-invasive methods under contained conditions, with minimal environmental impact and risk of contamination.

No human or animal subjects were involved—thus not subject to institutional ethical consideration—yet the essential ethical considerations of transparency, accountability, and scientific integrity were adhered to. These are procedures in keeping with environmental research standards and with general standards of responsible conduct of research (Israel & Hay, 2006; NIEHS, 2023; Resnik, 2018).

3. RESULTS

The obtained results in Table 1 provide valuable insights into the composition and characteristics of the analyzed seawater sample. The slightly alkaline pH of 7.55 corresponds well with the natural alkalinity of seawater. This pH range is essential for marine organisms and their intricate biochemical processes, as slight variations can have significant ecological consequences (Kulthanan et al., 2013).

Table 1

Physicochemical parameters of the sample seawater

Parameter	Values
pH	7.55 pH
Temperature	31.6 °C
Electrical Conductivity (EC)	19344 µS/cm
Total Dissolved Solids (TDS)	9708.33 ppm
Salinity	1.23 %

The temperature value of 31.6°C suggests that the sample originates from a region with relatively warm surface waters. Temperature conditions marine ecosystems, and plays a role in the distribution of nutrients, species, and ocean currents (Laffoley & Baxter, 2016). The very high electrical conductivity (EC) value of 19344 µS/cm is a direct indicator of dissolved minerals and salts in seawater. This is a characteristic of marine settings as it helps to differentiate between marine and freshwater environments.

The Total Dissolved Solids (TDS) concentration value of 9708.33 ppm reinforces the presence of dissolved substances with variable properties/functions, as it is an indicator of seawater reacting with geological processes and biological processes contributing to its composition (Rusydi, 2018). The salinity value of 1.23% is similar to that of seawater, substantiating the analysis. Salinity is a fundamental seawater characteristic that plays an important role in seawater density, circulation patterns, and the distribution of marine organisms (Schmidt et al., 2018). Together, the results helped to describe a seawater sample based on its alkalinity, temperature, salinity, and high mineral content. The characteristics of the seawater sample are critical to the study of ocean processes, marine ecosystems, and geophysical system interactions on the planet.

3.2. Isolation of seawater bacteria

Table 2 shows bacterial isolation using seawater as the medium and different types of agar for culturing and separating bacterial colonies. Bacterial colony growth patterns and characteristics observed on different types of agar reveal information about the diversity and bacterial strain preference of the isolated bacteria.

In nutrient agar, colonies ranged in color and type of growth, such as clear white colonies with irregular, undulated, and lobate borders. Some colonies demonstrated a rhizoid type of growth, while others were opaque white or yellow and of various shapes and edges. Nutrient agar gives a generic-purpose medium containing the required nutrients for bacterial growth. The variation in the color of the colonies implies that there could be a combination of bacterial strains with different metabolic properties in the seawater sample. The white and yellow transparent colonies may signify the existence of various types of bacteria with differing pigment production or metabolic activities (Luz et al., 2018).

Table 2

Observations of culture media after 72 hours of incubation

Specimen	Nutrient agar	EMB Agar	MacConkey Agar	Urea Agar Base (Christensen) (Autoclaved)	Mannitol Salt Agar
Replicate 1	Translucent white color with irregular, undulated and lobate edges, displaying a rhizoid growth pattern, and appearing opaque white in certain areas	Rhizoid shape, undulated margin, and translucent white colonies	No growth	Filamentous, undulated, and translucent characteristics, accompanied by a subtle growth of pale yellow and milky white hues	Opaque yellow shade with a complete, circular shape and margin, as well as a translucent white appearance with an entirely circular and smooth margin
Replicate 2	One colony presents a translucent yellow hue with a rhizoid shape and lobate margin, a second colony displays an opaque yellow color	Irregular shape, undulated margin with translucent to transparent white color	No growth	Translucent white with an irregular shape and undulated margin	It has a punctiform appearance with opaque yellow, irregularly shaped and lobate margin

	with a circular shape and complete margin, while the third colony exhibits an opaque white appearance with a circular shape and entire margin.				
Replicate 3	Irregular shape, undulated margin, and white translucent colonies	No growth	No growth	A filamentous shape characterized by an opaque white color, filiform margin, and an irregular, undulating, and translucent surface	Opaque yellow shade with a complete, circular shape and margin, as well as a translucent white appearance with an entirely circular and smooth margin

4. DISCUSSION

On EMB Agar (Eosin Methylene Blue Agar), the colonies were rhizoid in shape with undulating margins and a translucent to transparent white appearance. There was also no growth in one replicate. EMB agar is a selective medium for gram-negative bacteria and differentiates between lactose fermenters and non-fermenters. Lack of growth in one of the replicates could imply that conditions offered by the EMB agar were not suitable for the development of certain strains of bacteria. The occurrence of translucent to transparent white colonies indicates the dominance of gram-negative bacteria that are not lactose-fermenters (Lal & Cheeptham, 2007; Madigan et al., 2018).

In MacConkey Agar, no bacterial growth was found in all replicates. MacConkey agar is also selective for gram-negative bacteria and differential for lactose fermentation. Failure to grow on this agar indicates that the seawater sample may not have many lactose-fermenting gram-negative bacteria (Jung & Hoilat, 2022; Madigan et al., 2018).

In Urea Agar Base (Christensen), the colonies had filamentous, undulated, and translucent features, in pale yellowish shades of colors ranging from milky white. A few colonies showed opaque white colors, filiform margins, and undulating, irregular, and translucent surfaces. Urea agar is employed to detect urease activity, a feature of some bacterial species. The growth of filamentous colonies with diverse colors and growth patterns indicates the variety of urease-positive bacterial strains in the seawater sample (Rincon et al., 2006).

For Mannitol Salt Agar, the colonies exhibited distinct levels of opacity, ranging in color from yellow and translucent white, and displayed different colony morphology and margination, including completely circular colony shape and lobate margins. Some also exhibited a punctiform shape. Mannitol salt agar is a differential and selective medium, mostly for *Staphylococci*. The translucent white or yellow colonies suggest staphylococci with diverse metabolic pathways. The differences in colony morphology may have resulted from other staphylococcal species or strains (Jimenez, 2004; Madigan et al., 2018).

In summary, growth observation of bacteria on varied agar types, employing seawater for isolation, indicated that the sample of seawater was a mixed population. The differences in color, morphology, and growth were consistent with the differences in species or strains of bacteria and their metabolic processes. The use of selective and differential agar media was advantageous in isolating and characterizing certain groups of bacteria according to growth and biochemical characteristics.

5. CONCLUSIONS

Assessing the seawater sample provides useful information on its properties and composition. The parameters measured, including pH, temperature, electrical conductivity, total dissolved solids, and salinity, confirm this sample to be typical seawater. The alkaline pH, higher than expected temperature, and heightened mineral content are naturally occurring characteristics of seawater. The results, overall, help understand processes in the ocean substrate, ocean dynamics, and the ecosystem in the ocean environment. For bacterial isolation, the study implemented types of agar to culture and identify the bacterial colonies from the original seawater sample. The observations of growth and colony trait analysis of the bacteria on each agar called to attention information about the isolated bacterial preference and diversity.

Nutrient agar displayed various colony morphologies, colors, and margins, suggesting the simultaneous presence of more than one bacterial species with varying metabolic activities. Furthermore, EMB agar provided evidence of possible lactose-non-fermenting gram-negative bacteria; however, this was based on one replicate of a negative control for growth. The MacConkey agar, which contains lactose and is selective for lactose-fermenting gram-negative bacteria, produced no growth of lactose-fermenting gram-negative bacteria, which suggested they were absent in the sample. In conjunction with previous agar tests, urase production was determined by a distinct filamentous morphology upon growth on urea agar of urease-positive bacteria. Mannitol salt agar displayed variances in staphylococci based on colony morphology and color differences.

Finally, this study provides insights into the valuable microbial dynamics associated with seawater and interactions with three distinct types of agar medium. The findings underscore the importance of a combination of agar to capture variation in bacterial communities in a sea ecosystem. All of this information provides insights into microbial ecology, the diversity of bacteria, and any potential metabolic function of bacteria in a seawater habitat. Future work, including methods such as DNA sequencing and functional analyses of purified strains, will provide additional understanding of the functional roles of these bacteria in marine ecosystems and their contributions to ecological functions.

The conclusion of the study points to a need for further exploration and investigation of the research questions posed. First and foremost, an extensive molecular analysis to confirm the exact species present in the seawater sample through DNA sequencing is important, which may include identifying species already known and possibly unidentified, new strains. Subsequently, biochemical and metabolic assays on isolated strains are critical to clarify the functional potentials and ecological functions of these organisms. Furthermore, the study should be expanded to include longitudinal monitoring to describe seasonal and temporal patterns of bacterial and viral diversity and activity. Other marine systems can provide a basis for comparisons, and comparisons of the variability of bacterial communities can be observed through comparative studies. Finally, collaborative, interdisciplinary studies and educational outreach on the study and consideration for the practical application of findings, the biotechnological application of isolated strains should be embarked on to fully appreciate the impact of the implications of the study.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Approval: The study adheres to the ethical guidelines for conducting research.

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