jurnal βeta kimia
e-ISSN: 2807-7938 (online) dan p-ISSN: 2807-7962 (print)
Volume 5, Number 2, November 2025
http://ejurnal.undana.ac.id/index.php/jbk

Isolation and Identification of Halotolerant Bacteria in Saline Soils from the Coastal Areas of Oebelo White Sand Beach and Pariti Beach

Yosep Lawa[1,*], Serlina Utami[2], Rut Lede Kore[3], Yantus A. B. Neolaka[4], Arvinda C. Lalang[5], Jacky Anggara Nenohai[6], Maria Anastasia Kutu Woa[7]

[1–7] *Chemistry Education, Faculty of Teacher Training and Education, Nusa Cendana University, Adisucipto Street, Penfui, Kupang, NTT, Indonesia*

**e-mail correspondence: yosep_lawa@staf.undana.ac.id*

ARTICLE INFO

Article history:
Received:
4 December 2024
Revised:
31 October 2025
Accepted:
8 November 2025

Keywords:

Halotolerant bacteria, saline soil

ABSTRACT

A study has been conducted to isolate and identify halotolerant bacteria in saline soils from the coastal areas of Oebelo White Sand Beach and Pariti Beach. The sampling method used was random sampling. During sampling, pH, electrical conductivity (EC), and soil temperature were also measured. Bacterial isolation was performed using a serial dilution technique, resulting in three pure isolates from each beach sand sample. The isolates were cultured on Nutrient Agar (NA) medium and incubated at 37°C for 24 hours. The colonies obtained were small, round, and white. The isolates were then purified using the streak plate method. The halotolerant test was conducted using Tryptic Soy Agar (TSA) medium without added salt. The results showed that the bacterial colonies were halotolerant, as indicated by growth after 24 hours of incubation. Further biochemical tests, including motility, citrate utilization, urease, and sugar hydrolysis, revealed that all isolates from Oebelo White Sand samples showed positive results for all tests, whereas isolates from Pariti Beach exhibited some variations. These findings indicate that the isolates were capable of hydrolyzing urea into ammonia, utilizing citrate as a carbon and energy source, and fermenting sugars present in the medium.

How to cite: Lawa, Y., Utami, S., Kore, R. L., Neolaka, Y. A. B., Lalang, A. C., Nenohai, J. A., Woa, M. A. K. (2025). Isolation and Identification of Halotolerant Bacteria in Saline Soils from the Coastal Areas of Oebelo White Sand Beach and Pariti Beach, 5(2), 41-55. https://doi.org/10.35508/jbk.v5i2.19815

INTRODUCTION

Salinity is one of the major environmental factors that negatively affect plant productivity worldwide. One of the main challenges in plant growth is saline soil, which occurs when the salt concentration in the soil exceeds the tolerance threshold of plants [1]. According to a 2008 report by the Food and Agriculture Organization (FAO), more than 800 million hectares of land and 20% of irrigated agricultural land worldwide are affected by salinity. This problem can arise from improper irrigation practices, excessive fertilizer application, or natural salt accumulation. High soil salinity hinders plants from absorbing water and nutrients efficiently, thus inhibiting overall plant growth [2].

Coastal lands are generally less productive due to their high salt content. Communities living along coastal regions often face difficulties in cultivating crops because the soil medium cannot adequately support plant growth. This is mainly caused by the high salt and lime content in the soil, which makes the land unsuitable for planting, particularly for vegetables [3]. In addition, the limited availability of freshwater for irrigation increases the salinity of coastal soils because seawater nutrients contribute to higher salt levels. Coastal soils tend to have low nutrient content; therefore, appropriate methods are needed to maintain soil fertility and support plant

growth. One promising approach is the use of halotolerant bacteria to enhance nutrient availability and improve plant productivity.

Halotolerant bacteria are bacteria that do not require salt (NaCl) for growth but are capable of surviving and thriving in both low and high salt environments. These bacteria possess adaptive mechanisms that enable them to overcome osmotic stress caused by saline conditions. Halotolerant bacteria are known to act as plant growth promoters and contribute to sustainable agriculture by supporting plant metabolism and assisting in the reclamation of saline soils [4]. They can produce compounds that reduce soil salinity, increase nutrient availability, and protect plants from salt stress [5]. Therefore, isolating halotolerant bacteria is an important step toward enhancing plant growth in saline soils [6].

A study on halotolerant bacteria isolated from coastal saline soils in Patenga, Bangladesh, showed bacterial tolerance at salt concentrations of 2%, 4%, and 6%, with most isolates belonging to the families *Enterobacteriaceae*, *Clostridium*, and *Corynebacterium* [7]. This suggests that halotolerant bacteria could potentially be utilized to transfer salt tolerance genes into plants to increase productivity in saline coastal lands [8]. Their ability to grow in high salt conditions, coupled with simple nutritional requirements and a reduced risk of contamination, makes halotolerant bacteria highly valuable for biotechnological applications [9].

Halotolerant bacteria have been widely used in various biotechnological applications, including food fermentation, polymer production, degradation of toxic compounds, and the synthesis of osmoprotectants and hydrolytic enzymes such as amylase, protease, and nuclease, which are valuable for industrial use [10]. In saline environments, these bacteria can also promote plant growth through phosphate solubilization, production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and nitrogen fixation—mechanisms that improve plant tolerance to salt stress and enhance productivity [11].

Oebelo White Sand Beach, located in Tanah Merah Village, and Pariti Beach, located in the Sulamu District, are both coastal regions known for their mangrove forests, which stretch approximately 4.5 kilometers. These mangrove ecosystems are influenced by salinity conditions that are essential for the survival of various organisms. Typically, mangrove areas exhibit salinity levels between 11–25‰ and a pH range of 7.5–8.5. The existence of mangrove forests not only supports coastal ecosystems but also provides a habitat for diverse microorganisms, including halotolerant bacteria capable of surviving and reproducing in saline environments. Research on halotolerant bacteria in these regions remains limited despite their significant potential in biotechnology and environmental conservation. Based on these considerations, this study aims to conduct the "Isolation and Identification of Halotolerant Bacteria in Saline Soils from the Coastal Areas of Oebelo White Sand Beach and Pariti Beach" to provide insights into the potential utilization of halotolerant bacteria for environmental and agricultural benefits, particularly in the East Nusa Tenggara (NTT) region.

RESEARCH METHODS

Materials and Equipment

The materials used in this study included soil samples collected from the coastal areas of Oebelo White Sand Beach and Pariti Beach, Nutrient Agar (NA) medium, distilled water (aquadest), 70% alcohol, crystal violet, Lugol's iodine, acetone, Simmon's Citrate Agar, Tryptic Soy Agar (TSA), MIO medium (Motility–Indole–Ornithine), SIM medium, immersion oil, urease medium, and safranin dye. The instruments used included ziplock plastic bags, Petri dishes, test tubes, graduated cylinders, Erlenmeyer flasks, drop pipettes, beakers, inoculating loops, spatulas, microscopes, test tube racks, ovens, autoclaves, laminar air flow cabinets, Bunsen burners or

candles, magnetic stirrers, hot plates, analytical balances, water quality testers, watch glasses, microscope slides, and L-shaped glass rods.

Research Procedures

Sample Collection

Soil samples were collected from the coastal areas of Oebelo White Sand Beach and Pariti Beach using a simple random sampling technique to ensure that the samples were representative of the overall bacterial population. Samples were taken approximately 50 meters from the shoreline, with coordinates of -10.104713° S and 123.727437° E for Oebelo Beach, and -10.028133° S and 123.742801° E for Pariti Beach. Samples were collected from three different points, each weighing approximately 20 grams, using a sterile spatula, then placed in plastic bags and transported to the laboratory for further analysis. The collected soil samples were characterized as sandy loam and were analyzed for texture and pH using standard soil analysis methods.

Preparation of Growth Media

Bacterial growth media were prepared using Nutrient Agar (NA). A total of 6 grams of NA powder was weighed and dissolved in 350 mL of distilled water in an Erlenmeyer flask. The mixture was homogenized using a magnetic stirrer at a temperature of 100–150°C and sterilized in an autoclave at 121°C for 20 minutes [12]

Bacterial Isolation

Bacterial isolation began by weighing 1 gram of soil and placing it into a sterile test tube containing 9 mL of distilled water. Serial dilutions were then performed from 10^{-1} to 10^{-5}. The last three dilution series (10^{-3}, 10^{-4}, and 10^{-5}) were inoculated onto agar plates and incubated at 37°C for 48 hours. The resulting colonies were observed, and isolates were subsequently purified to obtain pure cultures [12].

Preparation of Pure Isolates

Single bacterial colonies were purified using the streak plate method. A single colony was aseptically transferred using an inoculating loop onto the surface of a fresh NA plate and incubated at 37°C for 24 hours. This procedure was repeated several times until pure cultures with uniform colony morphology were obtained [13].

Halotolerance Test

The halotolerance test was performed using Tryptic Soy Agar (TSA) medium without additional NaCl. Pure isolates were inoculated onto TSA plates and incubated at 37°C for 48 hours. The appearance of colony growth on the medium indicated that the isolates were halotolerant bacteria [14].

Macroscopic Observation

Bacterial morphological characterization includes both colony morphology and cell morphology. The macroscopic morphology of bacterial colonies was observed directly on agar media, focusing on colony edge, color, elevation, and shape, to determine distinctive features of each isolate [15].

Microscopic Observation

The Gram test was carried out using the staining method. The bacterial isolate was aseptically taken using an inoculating needle and gently smeared on a glass slide in a thin layer to allow easy observation under a microscope. Gram staining was performed on a 24 hour old bacterial culture grown on TSA (Tryptic Soy Agar) medium. A loopful of the bacterial isolate was spread evenly on a sterile glass slide. Then, 1–2 drops of distilled water were added, and the smear was heat fixed over a Bunsen burner until dry. The prepared smear was then stained with crystal violet for 1 minute and gently rinsed with running distilled water. Next, the slide was treated with Gram's iodine (Lugol) and allowed to stand for approximately 1 minute before being rinsed again with distilled water. The slide was then decolorized drop by drop using acetone–alcohol, rinsed, and counterstained with safranin for about 1–2 minutes, followed by a final rinse. The slide was

air-dried and covered with a cover slip. Immersion oil was added on top of the cover slip, and the slide was observed under a microscope Bacteria identified as Gram-positive appeared purple, while Gram-negative bacteria appeared red. The size and shape of the bacterial cells were also observed to determine whether they were spherical (coccus), rod-shaped (bacillus), or spiral-shaped (spirillum) [13].

Biochemical Tests

Biochemical tests were carried out to identify and characterize the physiological properties of pure bacterial isolates.

Motility Test

One loopful of bacterial isolate was inoculated by stabbing the MIO (Motility–Indole–Ornithine) medium. Kovac's reagent was added, and the culture was incubated at 37°C for 48 hours. This test evaluates bacterial motility, indole production, and ornithine decarboxylase activity simultaneously.

Citrate Utilization Test

A loopful of bacterial culture was streaked onto Simmon's Citrate Agar slants and incubated at 37°C for 24–48 hours. A color change from green to blue indicated the ability to utilize citrate as the sole carbon source.

Urease Test

Bacteria were inoculated into urease medium containing phenol red and incubated at 37°C for 24–48 hours. A color change from yellow to pink indicated urease enzyme activity and ammonia production.

Sugar Hydrolysis Test

A loopful of bacterial isolate was stabbed and streaked onto Triple Sugar Iron Agar (TSIA) medium and incubated at 37°C for 48 hours. A yellow color indicated acid production (fermentation), black coloration indicated H_2S production, and cracks or lifting of the medium indicated gas formation.

RESULTS AND DISCUSSION

Sample Collection

The samples used in this study were sandy soils collected from the coastal areas of Oebelo White Sand Beach and Pariti Beach. The sampling locations are shown in Figures 1a and 1b, and the physicochemical parameters of the samples are presented in Table 1.

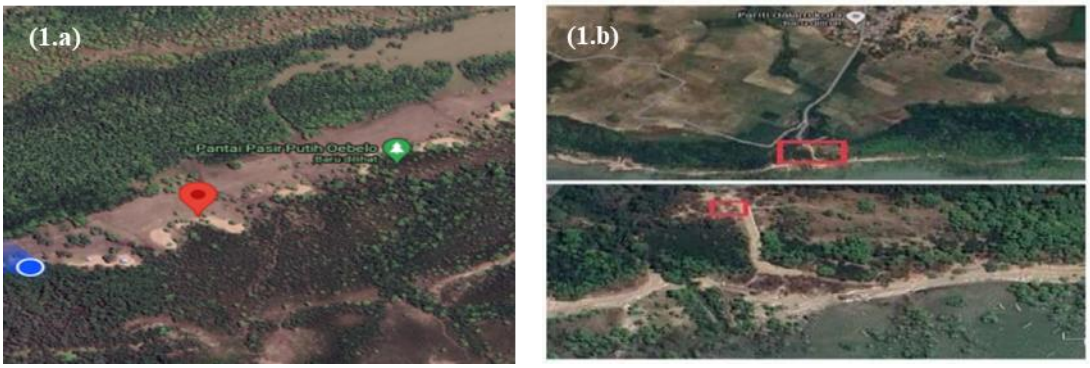


Figure 1a. Sampling Location Map of Oebelo White Beach and **Figure 1b.** Sampling Location Map of Pariti Beach

Table 1. Sample Collection Data

Location	Sample	Coordinates	pH	EC (mS/cm)	Temperature (°C)
Oebelo Beach	O1	-10.104837° S 123.727247° E	8,22	3,81	28
	O2	-10.104859° S 123.727247° E	8,09	3,47	29
	O3	-10.104766° S 123.727436° E	8,40	2,39	30
Pariti Beach	P1	-10.028082° S 123.742835 E	9,00	2,50	31
	P2	- 10.027888° S 123.743638° E	9,80	4,00	30
	P3	-10.027922° S 123.743457° E	8,90	4,00	30

Variations in parameters such as pH, electrical conductivity (EC), and temperature may be attributed to natural environmental fluctuations in temperature and soil water composition. These changes can occur over time and may not remain consistent between field and laboratory conditions. Environmental factors, such as direct sunlight exposure and air contamination, can also influence the properties of field samples.

Bacterial Isolation

The purpose of bacterial isolation was to obtain pure bacterial cultures for morphological characterization. Serial dilutions (10^{-3}, 10^{-4}, and 10^{-5}) were performed to reduce microbial diversity, allowing the growth of dominant species. The results of bacterial isolation from both sampling sites are shown in Table 2.

Table 2. Bacterial Isolation Results

Sample	Dilution			Colony Morphological Characteristics				
	10^{-3}	10^{-4}	10^{-5}	Color	Shape	Margin	Elevation	Size
O1	✓	✓	-	White	Round to irregular	Entire to undulate	Flat to raised	Small–medium
	-	-	✓	White	Round	Spreading	Flat to raised	Small–medium
O2	✓	✓	✓	White	Round to irregular	Entire to undulate	Flat to raised	Small–medium
O3	✓	✓	✓	White	Round to irregular	Entire to undulate	Flat to raised	Small–medium
P1	✓	✓	✓	White	Round	Entire to undulate	Convex to raised	Small–medium
P2	✓	✓	✓	White	Round	Entire to undulate	Convex to raised	Small–medium
P3	✓	✓	✓	White	Round to irregular	Entire to undulate	Convex to raised	Small–medium

Based on Table 2, it can be seen that all samples from several points showed colonies with the same color, namely white, with shapes varying between circular and irregular, entire and

wavy margins, and small to medium sizes. The elevation of the isolates also varied—ranging from flat to raised in the Oebelo white sand samples, and from convex to raised in the Pariti sand samples. A notable difference was observed only at sampling location O1 with a dilution level of 10^{-5}, which showed isolates with circular shapes and spreading edges, likely due to the mixture of microorganisms present in the sample. The observations also indicated that most of the obtained isolates exhibited similar morphological characteristics in terms of margin, elevation, size, and colony color. This may be due to the isolation of pure microorganisms from a single bacterial species, which are genetically similar and therefore exhibit comparable morphological traits. Isolates showing variations in shape (ranging from round to irregular), margin (entire to undulate), size (small to medium), and elevation (flat to raised or convex to raised) suggest morphological diversity among the isolates. This indicates that the isolates originated from different bacterial species with distinct morphological characteristics. Therefore, a purification process is necessary to separate each bacterial type based on its physical characteristics [16].

Purification of Isolates

The selection of purified microbial colonies was based on differences in colony morphological appearance, including color, elevation, and surface texture observed in the pure isolates. Bacterial isolate purification was carried out by transferring the bacteria using the streak plate method, followed by incubation on NA (Nutrient Agar) medium. This technique aims to examine species diversity within the sample, separate individual species from mixed cultures to obtain pure cultures, and study the colony characteristics of each species. The results of the bacterial isolate purification are presented in Table 3.

Table 3. Purified Isolate Characteristics

Sample	Dilution			Colony Morphological Characteristics				
	10^{-3}	10^{-4}	10^{-5}	Color	Shape	Margin	Elevation	Size
O1	-	✓	-	White	Round	Entire	Flat	Small
O2	✓	-	-	White	Round	Entire	Flat	Small
O3	-	-	✓	White	Round	Entire	Flat	Small
P1	-	-	✓	White	Round	Entire	Flat	Small
P2	-	✓	-	White	Round	Entire	Flat	Small
P3	✓	-	-	White	Round	Entire	Flat	Small

Based on Table 3, the colonies that grew appeared homogeneous in size, shape, margin, elevation, and color. This indicates that the obtained colonies were pure, resulting from an effective isolation process. The dilution performed reduced the number of microorganisms in the sample, allowing only the most dominant microorganisms to grow and develop. The results of the isolate purification can be seen in Figures 2a and 2b.

O1 O2 O3

Figure 2a. Purification Results of Isolates from Oebelo White Sand Samples

P1 P2 P3

Figure 2b. Purification Results of Isolates from Pariti Sand Samples

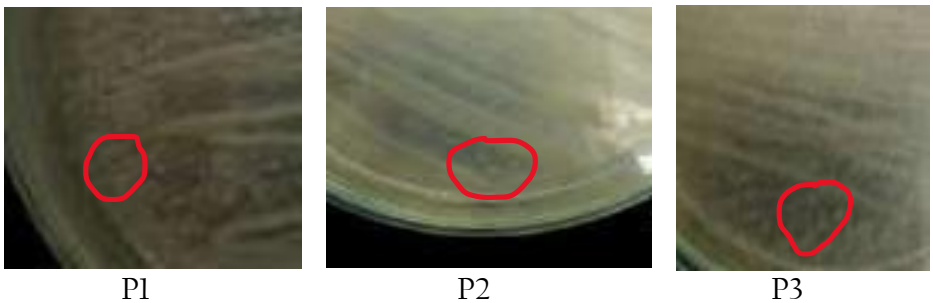
However, the purified isolates are not necessarily classified as halotolerant bacteria. The determination of halotolerance can be made by assessing the ability of the isolates to grow under saline conditions. If an isolate is able to grow on media with a high salt concentration, it can be confirmed that the purified isolate belongs to the category of halotolerant bacteria.

Halotolerance Test

The halotolerance test was carried out using TSA (Tryptic Soy Agar) medium, which aims to confirm or classify halophilic bacteria. The halotolerance test is used to differentiate between halophilic and halotolerant bacteria based on their ability to grow in the presence or absence of salt [14]. The results of the halotolerance test are presented in Figures 3a and 3b.

O1 O2 O3

Figure 3a. Halotolerance Test Results for Oebelo White Sand Samples



P1 P2 P3

Figure3b. Halotolerance Test Results for Pariti Sand Samples

Based on Figures 3a and 3b, the red-circled areas indicate that the halotolerant bacteria found exhibited similar phenotypic characteristics. These characteristics show that the halotolerant bacteria displayed uniform features, namely circular to irregular colony shapes and a white coloration. The growth of halotolerant bacteria on TSA medium across all dilution series indicates that TSA is effective in supporting the growth of halotolerant bacteria isolated from saline coastal soil samples of Oebelo White Sand and Pariti Sand. TSA provides sufficient

nutrients and favorable environmental conditions for the isolation and cultivation of halotolerant bacteria [17]. Halotolerant bacteria are microorganisms capable of growing in media containing up to 25% NaCl, while also being able to grow well in media without NaCl. This indicates that the isolates obtained from growth on media with both high and moderate salt concentrations are classified as halotolerant bacteria [18].

Macroscopic Observation

The colony morphology of bacteria can be determined by macroscopic observation of several parameters. These parameters include colony shape (circular, rhizoid, or irregular), color, size, margin (entire, lobate, undulate, serrated, or filamentous), and colony elevation (flat, raised, convex, or umbonate) [19]. The results of the macroscopic observation of halotolerant bacteria are presented in Table 4.

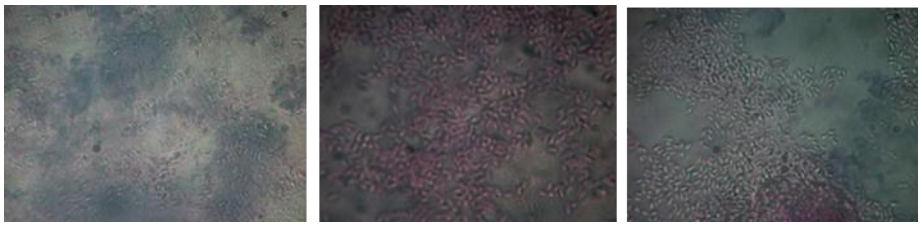
Table 4. Macroscopic Characteristics of Halotolerant Bacteria

Sample	Dilution	Colony Morphological Characteristics				
		Color	Shape	Margin	Elevation	Texture
O1	10^{-4}	White	Round to irregular	Entire	Flat	Mucoid
O2	10^{-3}	White	Round to irregular	Undulate	Flat	Mucoid
O3	10^{-5}	White	Round to irregular	Undulate	Flat	Mucoid
P1	10^{-5}	White	Round to irregular	Undulate	Flat	Mucoid
P2	10^{-4}	White	Round	Undulate	Flat	Mucoid
P3	10^{-3}	White	Round	Undulate	Flat	Mucoid

Macroscopic observations presented in Table 4 show that the isolates obtained exhibited morphological characteristics consistent with those of halotolerant bacteria. This finding aligns with the study by Salam (2021) [20], which reported that halotolerant bacteria isolated from salted eggs formed colonies with irregular shapes, wavy margins, white coloration, convex surfaces, and mucoid textures. Similarly, halotolerant bacteria isolated from salt pond waters displayed consistent morphological traits, including circular to irregular colony shapes, white coloration, flat surfaces, and mucoid textures [14]. Therefore, the halotolerant bacteria isolated from the coastal areas of Oebelo White Sand and Pariti Beach demonstrated morphological colony characteristics similar to those of halotolerant bacteria found in salt pond environments.

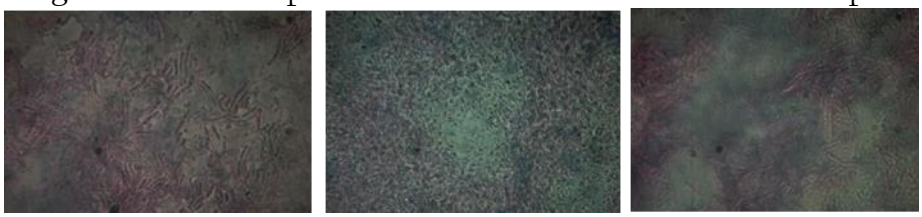
Microscopic Observation

Bacterial colony morphology was also observed microscopically to determine the characteristics of the isolates based on differences in the cell wall structures of Gram-positive and Gram-negative bacteria [13]. Microscopic observations were performed using Gram staining to examine cell shape and color. Bacteria that appeared red or pink were classified as Gram-negative, as they absorbed the safranin dye. In contrast, bacteria that appeared purple or violet were classified as Gram-positive, as they retained the crystal violet dye [21]. The microscopic observations of bacterial cells were conducted using a microscope at magnifications ranging from 400× to 1000×. The results of the microscopic observation of halotolerant bacteria are presented in Figures 4a and 4b.



O1 O2 O3

Figure 4a. Microscopic Observation of Oebelo White Sand Samples



P1 P2 P3

Figure 4b. Microscopic Observation of Pariti Sand Samples

Based on Figures 4a and 4b, the halotolerant bacteria appeared purple or violet, indicating that they belong to the Gram-positive bacterial group. This is due to the lower lipid content in halotolerant bacteria, which causes their cell walls to dehydrate more easily when treated with alcohol. The dehydration of the cell wall reduces pore size and permeability, preventing the crystal violet dye—the primary stain—from being washed out of the cells, resulting in the cells retaining the purple color. In addition to the color change, the bacteria were observed to have a bacillus (rod-shaped) morphology [22]. Gram-positive halotolerant bacteria of the genus Bacillus or Halomonas are heterotrophic bacteria that play a crucial role in maintaining ecosystem balance and providing nutrients for other organisms in extreme environments. These halotolerant bacteria are also capable of adapting to and surviving in environments with high salinity levels [23].

Biochemical Tests

Motility Test

The motility test was conducted to observe the movement of bacteria. Bacterial motility can be identified by the presence of turbidity around the stab line in the medium. The motility test was performed using MIO (Motility, Indole, and Ornithine) medium. A positive motility result is indicated by a whitish spread around the inoculation site, signifying bacterial movement and the presence of flagella (locomotory structures). Halotolerant bacteria possess flagella that enable active movement in environments with high salinity levels [24]. The indole test aims to determine the production of indole from the degradation of the amino acid tryptophan by the enzyme tryptophanase. Tryptophan is converted into indole, which is subsequently oxidized and deaminated into 3-indoleacetic acid (IAA) [25]. The indole test in halotolerant bacteria is important for assessing their potential to promote plant growth by enhancing root density, length, and surface area [26]. The ornithine decarboxylase test is used to determine the ability of bacteria to decarboxylate ornithine through the activity of the enzyme ornithine decarboxylase. This test assists in bacterial identification based on their ability to produce putrescine, which serves as a positive indicator of ornithine decarboxylase enzyme activity [27]. The results of the motility test are presented in Figures 5a and 5b, as well as in Table 5.

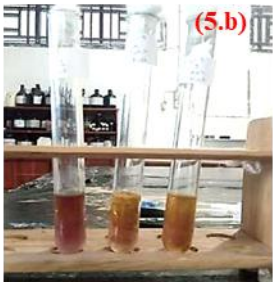


Figure 5a. Motility Test of Oebelo White Sand Samples and **Figure 5b.** Motility Test of Pariti Sand Samples

Table 5. Motility Test

Sample	Dilution	Motility Test		
		Motility	Indole	Ornithine
O1	10^{-4}	-	-	+
O2	10^{-3}	+	+	-
O3	10^{-5}	+	+	-
P1	10^{-5}	-	-	-
P2	10^{-4}	+	-	+
P3	10^{-3}	-	-	+

Based on Table 5 and Figures 5a and 5b, the samples that tested positive for motility were O2, O3, and P2. A positive motility result indicates that the halotolerant bacteria possess flagella, enabling them to move actively in environments with high salinity levels. The samples that tested positive for indole production were O2 and O3, indicating that these halotolerant bacteria are capable of producing enzymes necessary for metabolic activity, even under conditions with high NaCl concentrations. The samples that tested positive for ornithine decarboxylase were O1, P2, and P3, demonstrating that these halotolerant bacteria can degrade the carboxyl group of ornithine and activate the enzyme ornithine decarboxylase, which converts ornithine into putrescine. Negative results in the motility and indole tests may be attributed to suboptimal incubation time, limited nutrient availability, inappropriate pH, or low carbon sources in the medium. Negative results in the ornithine test could be due to genetic regulation or environmental conditions that were not conducive to enzyme activation, thereby preventing the conversion of ornithine into putrescine.

Citrate Test

The citrate test is used to identify the ability of bacteria to utilize citrate as the sole carbon source [28]. This test was conducted to examine halotolerant bacterial isolates originating from salt pond environments. The citrate test demonstrates the capability of halotolerant bacteria to use citrate as a carbon source, which typically yields a consistently positive result, indicated by a color change in the Simmons citrate medium from green to blue [29]. The ability of halotolerant bacteria to utilize citrate as a carbon source is associated with the activity of enzymes that oxidize citrate, producing compounds that render the medium more alkaline and cause a shift in the pH indicator color. The results of the citrate test are presented in Figures 6a and 6b, as well as in Table 6.

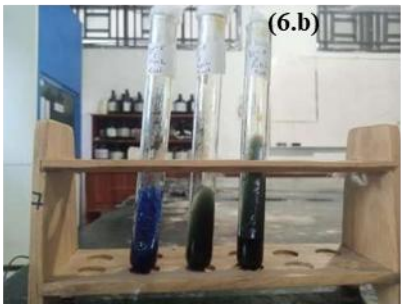


Figure 6a. Citrate Test of Oebelo White Sand Samples and **Figure 6b**. Citrate Test of Pariti Sand Samples

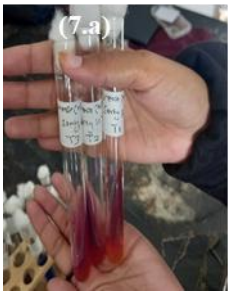
Table 6. Citrate Test Results

Sample	Dilution	Citrate Test
O1	10^{-4}	+
O2	10^{-3}	+
O3	10^{-5}	+
P1	10^{-5}	+
P2	10^{-4}	-
P3	10^{-3}	-

Based on Table 6 and Figures 6a and 6b, the samples that tested positive for the citrate test were O1, O2, O3, and P1, as indicated by the color change of the medium from green to blue. When microorganisms are able to utilize citrate, acids are removed from the culture medium, resulting in an increase in pH and a color change from green to blue. Halotolerant bacteria are capable of utilizing citrate as their sole source of carbon and energy. However, citrate cannot be directly used as a carbon and energy source by halotolerant bacteria; it must first be hydrolyzed into aspartic acid, which can then serve as a carbon and energy source for bacterial metabolism. The hydrolysis of citrate into aspartic acid is catalyzed by the enzyme citrate dehydratase [30].

Urease Test

The urease test is used to detect the presence of the urease enzyme produced by bacteria. Urease is responsible for catalyzing the hydrolysis of urea into ammonia and carbon dioxide. In halotolerant bacteria, the urease enzyme plays an important role in the nitrogen cycle and in adaptation to saline conditions. Urease activity enables these bacteria to break down urea into ammonia and carbon dioxide, which in turn helps neutralize acidity in saline environments and regulate pH levels [31]. The results of the urease test are presented in Figures 7a and 7b, as well as in Table 7.



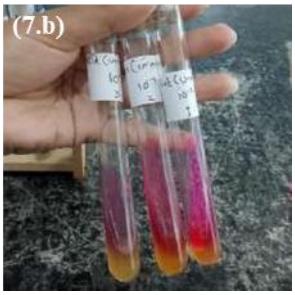


Figure 7a. Urease Test of Oebelo White Sand Samples and **Figure 7b.** Urease Test of Pariti Sand Samples

Table 7. UreaseTest Results

Sample	Dilution	Urease Test
O1	10^{-4}	+
O2	10^{-3}	+
O3	10^{-5}	+
P1	10^{-5}	+
P2	10^{-4}	+
P3	10^{-3}	+

Based on Table 7 and Figures 7a and 7b, all samples tested positive for the urease test, as indicated by the color change of the medium to pink. The presence of ammonia renders the medium alkaline (basic), causing the phenol red indicator in the medium to change color to pink. This color change occurs due to an increase in pH following the hydrolysis of urea. Bacterial cultures that produce the urease enzyme can hydrolyze urea, generating ammonia (NH_3). The release of ammonia increases the pH of the medium, resulting in the characteristic pink coloration. The positive urease results indicate that the halotolerant bacteria are capable of hydrolyzing urea into ammonia. Ammonia (NH_3) plays an essential role in metabolism as a nitrogen source required for the synthesis of amino acids and other nitrogen-containing molecules [14]. Halotolerant bacteria can obtain ammonia from various sources, including the degradation of proteins, amino acids, or other organic nitrogen compounds available in their environment. Therefore, the presence of ammonia in the environment is crucial for supporting the survival and metabolic activity of halotolerant bacteria.

Sugar Hydrolysis Test

The sugar hydrolysis test using TSIA (Triple Sugar Iron Agar) medium aims to observe acid and gas production by bacteria during the oxidation or fermentation of sugars. When bacteria are able to oxidize sugars, they produce acids that lower the pH of the medium. This decrease in pH causes the pH indicator to change color from red to yellow [32]. The results of the sugar hydrolysis test are presented in Figures 8a and 8b, as well as in Table 8.

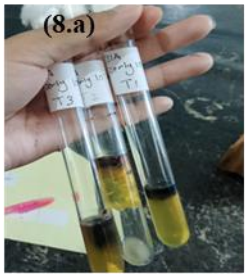


Figure 8a. Sugar Hydrolysis Test of Oebelo White Sand Samples and **Figure 8b.** Sugar Hydrolysis Test of Pariti Sand Samples

Table 8. Sugar Hydrolysis Results

Sample	Dilution	Sugar Hydrolysis Test
O1	10^{-4}	+
O2	10^{-3}	+
O3	10^{-5}	+
P1	10^{-5}	+
P2	10^{-4}	-

P3	10^{-3}	-

Based on Table 8 and Figures 8a and 8b, the samples that tested positive for the sugar hydrolysis test were O1, O2, O3, and P1, as indicated by the color change of the medium to yellow, which signifies acid production. This result indicates that the halotolerant bacteria were able to ferment only one type of sugar, namely glucose. In general, bacteria are capable of fermenting sucrose, glucose, mannitol, and maltose, while only a few isolates can ferment xylose and lactose. In environments with high salt concentrations, such as those commonly inhabited by halotolerant bacteria, the ability to metabolize sugars or carbohydrates plays an important role in enabling these microorganisms to adapt to such extreme conditions. Like most microorganisms, halotolerant bacteria also require sugars or carbohydrates as their primary energy source. Carbohydrates are among the essential nutrients needed by bacteria to maintain their metabolic activities. Sugars such as glucose are one of the most common forms of carbohydrates utilized by bacteria as substrates for glycolysis and fermentation processes [33]. Some samples showed negative results, indicated by the appearance of a black coloration. This occurred due to the production of hydrogen sulfide (H_2S) as a result of sulfur metabolism within the medium, leading to the formation of a black precipitate. Therefore, the black color change is more closely related to hydrogen sulfide production rather than the inability of halotolerant bacteria to metabolize glucose [34].

CONCLUSION

Based on the results and discussion, it can be concluded that bacteria from the coastal soils of Oebelo White Sand and Pariti Beach can be isolated using the serial dilution method, resulting in pure isolates characterized by circular to irregular white colonies with wavy and flat margins, convex surfaces, and mucoid textures. The results showed that all three isolates from the Oebelo White Sand samples tested positive in all assays, while the Pariti Sand samples showed positive results in several tests. Therefore, it can be concluded that the isolates were able to hydrolyze urea into ammonia, utilize citrate as a carbon and energy source, and ferment the sugars present in the medium.

REFERENCES

[1] Ramasamy, K. P., & Mahawar, L. (2023). Coping with salt stress: Interaction of halotolerant bacteria in crop plants: A mini review. *Frontiers in Microbiology*, 14, 1077561.

[2] Nazhifan, S. F., Dewi, K., & Asih, E. N. N. (2023). Bakteri Halofilik dan halotolerant dari air baku tambak garam Universitas Trunojoyo. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 26(1), 67–76.

[3] Firdaus, A. M. (2023). Pemberdayaan Masyarakat Pesisir Melalui Budidaya Tanaman Sistem Hidroponik. *Ininnawa: Jurnal Pengabdian Masyarakat*, 1(2), 238–246.

[4] Reang, L., Bhatt, S., Tomar, R. S., Joshi, K., Padhiyar, S., Vyas, U. M., & Kheni, J. K. (2022). Plant growth-promoting characteristics of halophilic and halotolerant bacteria isolated from coastal regions of Saurashtra, Gujarat. *Scientific Reports*, 12(1), 4699.

[5] Zamanzadeh-Nasrabadi, S. M., Mohammadiapanah, F., Hosseini-Mazinani, M., & Sarikhan, S. (2023). Salinity stress endurance of plants aided by bacterial genes. *Frontiers in Genetics*, 14, 1049608.

[6] Scagel, C. F., Lee, J., & Mitchell, J. N. (2019). Salinity from NaCl alters nutrient and polyphenolic composition in basil leaves. *Industrial Crops and Products*, 127, 119–128.

[7] Rahman, S. S., Siddique, R., & Tabassum, N. (2017). Isolation and identification of halotolerant soil bacteria from the coastal Patenga area. *BMC Research Notes*, 10, 1–6.

[8] Rohmah, N. S. (2017). Isolasi dan Identifikasi bakteri yang berpotensi sebagai agen bioremediasi timbal (Pb) dari lumpur lapindo. *(Doctoral Dissertation, Universitas Islam Negeri Maulana Malik Ibrahim)*.
[9] Amaliah, P., Mubarik, N. R., & Suryani. (2017). Isolasi dan Seleksi Bakteri Proteolitik Halotoleran dari Inasua dan Karakterisasi Aktivitas Protoasenya. *Bogor Agricultural University*.
[10] Sabdaningsih, A., & Lunggani, A. T. (2020). Isolasi dan Karakterisasi Morfologi Bakteri Halofilik dari Bledug Kuwu,Kabupaten Grobogan. *Bioma: Berkala Ilmiah Biologi*, 22(1), 46–52.
[11] Pallavi, Mishra, R. K., Sahu, P. K., Mishra, V., Jamal, H., Varma, A., & Tripathi, S. (2023). Isolation and characterization of halotolerant plant growth-promoting rhizobacteria from the mangrove region of Sundarbans, India, for enhanced crop productivity. *Frontiers in Plant Science*, 14, 1122347.
[12] Handayani, S. (2018). Isolasi dan Karakterisasi Bakteri dari Tanah pada Lahan Praktek Mahasiswa. *Fakultas Biologi Universitas Medan Area*.
[13] Rohmah, N. S. (2017). Isolasi dan identifikasi bakteri yang berpotensi sebagai agen bioremediasi timbal (Pb) dari lumpur lapindo. (*Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim*).
[14] Nazhifan, S. F., Dewi, K., & Asih, E. N. N. (2023). Bakteri halofilik dan halotoleran dari air baku tambak garam Universitas Trunojoyo Madura. *Jurnal Pengolahan Hasil Perikanan Indonesia*. 26(1), 67-76.
[15] Fitri, D. A., Asih, E. N. N., Kartika, A. G. D., Agustina, N., Fadholi, B., Dewi, K., & Efendy, M. (2022). Morphological characteristics of halophilic bacteria in traditional salt production. *Journal Of Marine Resources And Coastal Management*. 3(1), 1-7.
[16] Romadloni, M. Y., Wibowo, F. A. C., Wahidiah, T., & Pradipta, A. (2024). Isolasi Bakteri Perlarut Fosfat (BPF) pada Hutan Produksi di Kawasan Hutan dengan Tujuan Khusus (KHDTK) Pujon Hill UMM, Kabupaten Malang. *Berita Biologi*, *23*(1), 91-102.
[17] Maryani, M., Monalisa, S. S., & Panjaitan, R. S. (2021). The Effect of Ketapang leaves extracts (Terminalia catappa) in inhibiting the growth of bacteria Edwarsiella tarda on in vitro test. *Jurnal Perikanan dan Kelautan*, *10*(2), 196-208.
[18] Hernández-Canseco, J., Bautista-Cruz, A., Sánchez-Mendoza, S., Aquino-Bolaños, T., & Sánchez-Medina, P. S. (2022). Plant growth-promoting halobacteria and their ability to protect crops from abiotic stress: an eco-friendly alternative for saline soils. *Agronomy*, *12*(4), 804.
[19] Suryani, S., & A'yun, Q. (2022). Isolasi Bakteri Endofit dari Mangrove Sonneratia alba Asal Pondok 2 Pantai Harapan Jaya Muara Gembong, Bekasi. *BIO-SAINS: Jurnal Ilmiah Biologi*, *1*(2), 12-18.
[20] Salam, A. N. (2021). Jumlah dan Morfologi Bakteri Halofilik Dan Halotoleran Pada Telur Asin Komersial di Makassar. *Fisheries Research*, 140(1), 6.
[21] Savitri, S. D. N. (2006). Isolasi dan karakteristik bakteri halotoleran pada peda ikan kembung (Rastrelliger sp.). *Bogor Agricultural University*.
[22] Yusra, Y., Azima, F., Novelina, N., & Periadnadi, P. (2014). Isolasi dan identifikasi mikroflora indigenous dalam budu. *Agritech*, *34*(3), 316-321.
[23] Sutiknowati, L. I. (2018). Keragaman Bakteri Pada Perairan Sabang, Provinsi Aceh. *Majalah Ilmiah Biologis Biosfera: A. Scientific Journal*, *35*(2), 54-62.
[24] Anggraini, R., Aliza, D., & Mellisa, S. (2016). Identifikasi bakteri Aeromonas hydrophila dengan uji mikrobiologi pada ikan lele dumbo (*Clarias gariepinus*) yang dibudidayakan di Kecamatan Baitussalam Kabupaten Aceh Besar. (*Doctoral dissertation, Syiah Kuala University*).
[25] Arwin, M., Ijong, F. G., & Tumbol, R. (2016). Characteristics of Aeromonas hydrophila isolated from tilapia (*Oreochromis niloticus*). *Aquatic science & management*, *4*(2), 52-55.

[26] Lestari, P., Suryadi, Y., Susilowati, D. N., Priyatno, T. P., & Samudra, I. M. (2015). Karakterisasi bakteri penghasil asam indol asetat dan pengaruhnya terhadap vigor benih padi. *Berita Biologi*, *14*(1), 19-28.

[27] Fauziah, S. I., & Ibrahim, M. (2020). Isolasi dan Karakterisasi Bakteri Selulolitik pada Tanah Gambut di Desa Tagagiri Tama Jaya, Kecamatan Pelangiran, Kabupaten Inhil, Riau. *LenteraBio: Berkala Ilmiah Biologi*, *9*(3), 194-203.

[28] Yulianto Ade, P. (2019). Deteksi Fenotipik Escherichia coli Penghasil Extended Spectrum Beta-Lactamases (ESBLS) pada Sampel Makanan di Krian Sidoarjo. *Life Science Journal*, *8*(1), 75-85.

[29] Dewi, K., Asih, E. N. N., Fitri, D. A., & Astutik, S. (2022). Karakterisasi fisiologis isolat bakteri halofilik dari kolam Peminihan tambak garam rakyat di Kabupaten Pamekasan. *Juvenil: Jurnal Ilmiah Kelautan dan Perikanan*, *3*(3), 79-84.

[30] Fallo, G., & Sine, Y. (2016). Isolasi dan uji biokimia bakteri selulolitik asal saluran pencernaan rayap pekerja (*Macrotermes spp.). Bio-Edu: Jurnal Pendidikan Biologi*, *1*(2), 27-29.

[31] Shing, S. (2018). *Characterization of Citrate Dehydrogenase from Citrus sinensis.*

[32] Marzuki, I. (2019). Aplikasi mikrosimbion spons dalam bioremediasi lingkungan.

[33] Manalu, R. T. M., & Febriani, A. (2021). Isolation and characterization of lactic acid bacteria in fermented sweet corn (*Zea mays l*) as antibacterial. *Bioscience*, *5*(2), 141-149.

[34] Sari, R. Identifikasi bakteri asam laktat (BAL) penghasil Bakteriosin dari makanan botok ikan tongkol (*euthynus affinis c*) khas kalimantan barat yang memiliki aktivitas terhadap bakteri patogen. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*, *4*(1).