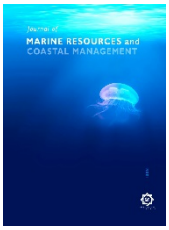
JOURNAL OF MARINE RESOURCES AND COASTAL MANAGEMENT
INDONESIAN JOURNAL OF MARINE LIFE AND UTILIZATION

e-ISSN: 2722-0966
Vol. 3 Issue 1



Morphological characteristics of halophilic bacteria in traditional salt production

Dewi Anugrah Fitri, Eka Nurrahema Ning Asih*, Ary Giri Dwi Kartika, Ninik Agustina, Bahri Fadholi, Kartika Dewi, and Makhfud Efendy
Marine Science Study Program, Faculty of Agriculture, Trunojoyo University, Madura, Indonesia
*E-mail: eka.asih@trunojoyo.ac.id

ABSTRACT

The use of bacteria in improving the quality of salt on a laboratory scale is still minimal. Bacteria are found in seawater as raw materials for various types of salt. One of the bacteria that are tolerant to salinity levels in raw saltwater is halophilic bacteria. Exploration of halophilic bacteria isolates contained in seawater as raw material for salt is an effort to provide initial information on the use of these bacteria in improving the quality and quality of salt. This study aims to determine the morphological characteristics and gram grouping of halophilic bacteria contained in raw water, reservoir water, and evaporator water during the traditional salt production process. The methods used in this study were bacterial isolation, purification, and gram staining test. Morphological characteristics were carried out by visual observation of bacterial colonies formed in Petri dishes, while the gram test of bacteria was carried out by staining pure isolates. Morphological characteristics and groupings of gram bacteria were observed under a CX43RF binocular microscope with a digital camera type MDCE-5C. The results of this study found 2 isolates circular in raw saltwater, 5 isolates in irregular, filamentous and circular shapes in reservoir water, and 3 isolates in circular and filamentous shape in purification water. The bacterial isolates found varied in the form of groups of gram-negative bacteria and groups of positive bacteria, while the predominant form of bacteria was bacilli. The results of this study are expected to be initial information that can be used as a reference to improve the quality and quantity of salt production.

Keywords: characterization, morphology, gram test, halophilic bacteria, salt production

INTRODUCTION

Salt is composed of chemical compounds consisting of sodium chloride (NaCl) and impurities such as $CaSO_4$, $MgSO_4$, $MgCl_2$. The function of salt is to add flavor to food. The utilization of salt in the field of fishery processing is used as the main ingredient for preservatives and improving the texture of meat such as making surimi [1]. According to [2], the use of salt is not only for consumption but on an industrial scale requires salt for pharmaceutical needs.

Salt production is influenced by several factors including the quality and quantity of seawater as raw material for making salt [3]. The increase and success of salt production are closely related to the formation of salt crystals on the crystallization table. The process of forming salt crystals is influenced by many factors, including the level of saturation of raw saltwater which is measured in degrees on the Baume scale (°Be) [4]. The level of raw water saturation is also influenced by rainfall, cloud cover, evaporation, and surface winds [4].

Another aspect that affects the quality of salt is the level of contamination in raw saltwater. The contamination contained in saltwater opzet is influenced by the presence of the source of contamination. The location of water raw adjacent to community settlements can trigger high concentrations of contamination contained in saltwater raw water. One of the contaminants originating from domestic waste in saltwater is *Coliform* bacteria. According to [5], the bacteria that pollute marine waters are dominated by *Escherecia coli, Coliform,* and *Fecal coli bacteria*. *Coliform bacteria* group is a type of bacteria that acts as a bioindicator of organic matter contamination in aquatic areas. The presence of these bacteria in an aquatic area is a marker of the level and type of contamination that accumulates in these waters.

The marine bacteria group is divided into 2 groups, namely pathogenic bacteria, and non-pathogenic bacteria. Several types of seawater bacteria adjacent to the aquaculture location are classified as pathogenic bacteria including *Staphylococcus aureus, Pseudomonas aeruginosa, Pseudomonas pseudomelle, Enterobacter agglomerates, Vibrio Cholera* [6], *and Streptomyces* sp. [7] while the non-pathogenic bacteria group includes *Nitrobacter* sp. and *Bacillus subtilis* [6].

Microorganisms that can grow in an environment with high salt content are halophilic bacteria. Halophilic bacteria based on the ability to survive are divided into 3, namely low halophilic bacteria can grow optimally at 2-5% NaCl, medium halophilic types grow optimally at 5-20% NaCl, and extreme halophilic types grow optimally at 20-30% NaCl levels [8]. The presence of halophilic bacteria on the crystallization table can accelerate evaporation and

increase the quality of salt because these bacteria consume organic matter in saltwater [9].

Observations of the presence of halophilic bacteria from raw water, reservoir water, evaporator water to crystallization tables are still rarely carried out. Early identification of morphological characterization and grouping of halophilic bacteria in traditional salt production water is an attempt to find initial information that can be used as a reference for the application of these bacteria in improving the quality and quantity of traditional salt. This is the background of this research being carried out. The purpose of this study was to determine the morphological character of halophilic bacterial isolates and gram grouping of halophilic bacteria in traditional salt production water.

METHODS

Sample Collection

A sampling of salt production water was carried out at the Salt Ponds, Tanjung Village, Pademawu District, Pamekasan Regency (Figure-1) with the coordinates of each location as shown in Table-1.

Water samples were taken starting from raw water, reservoir water, and evaporator water. Samples of salt production water were taken as much as 10 ml using a screw test tube that had been sterilized. A sampling of salt production water is done by inserting a screw test tube with a closed state into the water, then opening the lid of the tube in the water and closing it again with the tube still in the water. The water sample that has been obtained is put into a cool box that has been filled with ice cubes. The samples were then taken to the Biotechnology Laboratory of the Marine Science Study Program, Trunojoyo University, Madura for bacterial isolation.

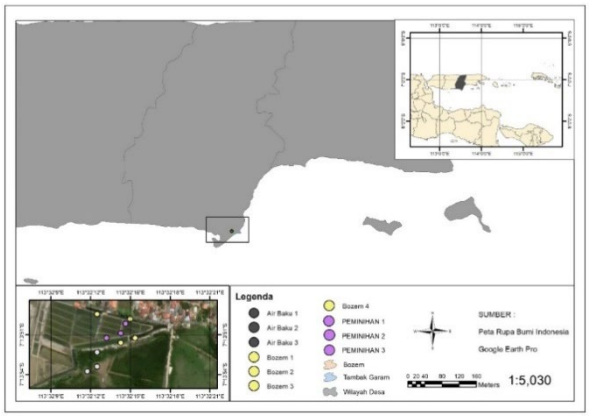


Figure-1. Salt ponds in Tanjung Village, Pademawu District, Pamekasan Regency

Table-1. Coordinate sampling point

No	Coordinate point	Sample Location
1	-7.135386,113.321170	Salt Raw Water
2	-7.134926,113.321252	Water Reservoir
3	-7.135022,113.321453	Evaporator Water

Halophilic Bacteria Isolation

Isolation of halophilic bacteria was carried out to obtain a single isolate [10]. Isolation of halophilic bacteria was carried out using the dilution method [11]. Sample dilutions were carried out periodically starting with 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, $10^{-5,}$ and 10^{-6} sample waters in the form of raw water, reservoir water, and sterilized evaporator water of 9 ml/test tube, as a dilution medium. Samples were taken as much as 1 ml, then put in a dilution of 10^{-1} to 10^{-6}. Samples that have been diluted in serial dilutions 10^{-4}, 10^{-5}, and 10^{-6} were isolated on solid media as much as 50 m per petri dish with a Duplo system. The isolated samples were stored in an incubator at a temperature of 28-30°C for 48 hours.

Bacterial Colony Purification

Purification or what is known as the purification method of bacterial colonies using the scratching method [11]. The purification technique used in this study was quadrant streak (4 strokes in agar plates). Purification of bacterial colonies was carried out by taking 1 loop of bacterial colonies, then scraping on solid media, then the samples were stored in an incubator at a temperature of 28-30°C for 48 hours. Bacterial colonies that had grown during purification were then transferred to an inclined agar medium.

Halophilic Bacteria Characterization

Identification of Halophilic Bacteria Morphology

The identification of bacterial morphology includes colony and bacterial cell morphology [12]. Observation of the morphology of bacterial colonies was carried out directly by observing the color, opacity, from, elevation, margins, and the surface of the bacterial colonies. The morphological characteristics of bacterial colonies growing on solid media were determined based on the shape of the bacterial colonies, referring to [13]. Cell morphology observations were carried out microscopically using the Gram stain method.

Halophilic Bacteria Gram Stain

Bacterial gram staining is a microscopic bacterial characterization method used to determine the morphology of bacterial cells and their gram properties [14]. According to [15], some gram-positive bacteria consist of *Bacillus, Lactococcus, Micrococcus, Carnobacterium, Enterococcus, Lactobacillus, and Streptococcus, Weisslla,* while gram-negative bacteria consist of *Aeromonas, Alteromonas, Photorhodobacterium, Pseudomonas, and Vibrio gram-negative bacteria*. The first gram test of bacteria was carried out by preparing the preparations. The preparations were cleaned using alcohol, then heated the ose needle until it turned red. Ose needles that have been hot dripped with distilled water then scratched on the preparations. Heat the ose needle again until it turns red, then take 1 loop of pure isolate and scratch it roundly on the preparation, then dry it by passing it over a Bunsen fire.

The dried preparations formed a circle of isolates, then 1-2 drops of crystal violet were added for 1 minute and then rinsed with distilled water. The isolate was then dripped with 1-2 drops of Lugol for 1 minute then rinsed with distilled water and splashed with acetone alcohol to dissolve the purple color, then 1-2 drops of safranin were added and left for 1 minute then rinsed with distilled

water. The isolates that had finished gram staining were observed under a CX43RF binocular microscope with an MDCE-5C type digital camera.

Data Analysis

The data obtained were processed using Microsoft Excel statistical software (Microsoft Corp, Albuquerque, NM, USA) (IBM). The data obtained were analyzed descriptively and narrated based on the data equipped with references from similar research results.

RESULTS AND DISCUSSIONS

Traditional salt production on Madura Island as the largest salt production center in Indonesia is carried out on ponds along the coast using the *solar evaporation* method [16]. According to [17], the traditional salt production process consists of 6 planning periods, namely the preparation period, the old water treatment period, the soil and water treatment period, the salt table maintenance period, the salt harvest period, and the old water storage period. Traditional salt production carried out by coastal communities in Tanjung Village, Pademawu District, Pamekasan-Madura Regency is generally carried out starting from taking raw water in the sea which is flowed in a holding pond or known as a reservoir. The raw water that has been collected in the reservoir is then channeled to the evaporator pond and ends at the crystallization table.

One of the microorganisms that can live in this traditional salt-producing water environment is a halophilic microorganism such as halophilic bacteria. According to [18] stated that halophilic bacteria can live at high salt levels, namely NaCl levels of 0.5 to 2.5 M, and reduce the risk of contamination in the body by paying attention to the NaCl levels. The morphological characterization of halophilic bacteria was carried out by identification of isolates and gram staining. The identification of isolates aims to see the physical characteristics of the isolates while the morphological characterization is seen from the shape and color of the bacteria using the gram staining method [19]. Identification of isolates started by knowing the shape of the colony, color, and opacity of the colony, margin, elevation, and surface of the colony [20]. The results in this study in raw water samples obtained 2 isolates (Table-2), reservoir water obtained 5 isolates (Table-3), and evaporator water obtained 3 isolates (Table-4).

The identified raw water isolate was bone white, not transparent, circular with raised elevation and entire margin. The difference between the two isolates was that the surface of the colonies on PAB1 isolates was dull while those of PAB2 isolates had glossy surfaces. The reservoir water isolates identified all colonies as bone white and non-transparent, circular in PB2 and PB3 isolates, irregular in PB1 and PB5 isolates, while in PB4 isolates they were filamentous. The elevation of PB1 and PB4 isolates was flat, meaning that the colony was flat when viewed from the side of the cup. The elevation of isolates PB2 and PB3 was raised or convex. The surface of PB4 isolates was rugose or wrinkled. The isolate of the evaporator water was bone white and orange was not transparent. PP1 and PP3 isolates were circular with entire margins, while PP2 isolates were filamentous with filiform margins. The surface of PP2 and PP3 isolates was smooth while PP1 had a rugose or wrinkled surface. The identification results showed that the most common isolates were isolates from reservoir ponds (PB1, PB2, PB3, PB4, and PB5) as many as 5 isolates. This indicates that the bacteria in the reservoir pond can live and adapt well and grow quickly on the prepared bacterial growth media.

Table-2. Identification results of raw water isolates

Sample Code	Isolation Code	Bacterial Morphology				
		Color & Opacity	From	Elevation	Margin	Surface
AB	PAB1	Bone white and not transparent	Circular	Raised	Entire	Dull
	PAB2	Bone white and not transparent	Circular	Raised	Entire	Shiny

Description: AB= Raw Water

Table-3. Identification results of reservoir water isolates

Sample Code	Isolation Code	Bacterial Morphology				
		Color & Opacity	From	Elevation	Margin	Surface
B	PB1	Bone white and not transparent	Irregular	Flat	Undulate	Fine
	PB2	Dense bone white and not transparent	Circular	Raised	Entire	Fine
	PB3	Bone white and not transparent	Circular	Raised	Entire	Smooth and Shiny
	PB4	Bone white and not transparent	Filamentous	Flat	Lobate	Rugose
	PB5	Bone white and not transparent	Irregular	Umbonate	Undulate	Fine

Description: B= Reservoir Water

Table-4. Results of isolate identification

Sample Code	Isolation Code	Bacterial Morphology				
		Color & Opacity	From	Elevation	Margin	Surface
P	PP1	Bone white and not transparent	Circular	Flat	Entire	Rugose
	PP2	Bone white and not transparent	Filamentous	Flat	Filiform	Fine
	PP3	Orange and not transparent	Circular	Raised	Entire	Fine

Description: P = Evaporator Water

According to [21] stated that the colony shape of a bacterium is influenced by age, certain growth conditions, bacterial cell density and density [10]. Variations in the form of bacteria that occur are also influenced by the environment [22], nutritional factors, and temperature (minimum, optimum, and maximum) [23]. The color of the colonies that appear to be different indicates the presence of pigment differences. The pigments found in bacteria include carotenoid pigments, anthocyanins, melanin, tripyrylmethene, and phenazine [24]. Each of these pigments will give a different color. The red and yellow colors of the isolates were due to the presence of carotenoids. Melanin gives brown, black, and orange colors. Tripyrylmethenes is a pigment produced by Serratia marcescens and phenazine which gives yellow-orange, dark orange, and red-orange colors [24].

The identification of bacterial colonies that have been obtained is then purification. Purification was carried out by scratching technique, the identified colonies were scratched on agar medium. The purpose of purification is to separate bacterial colonies into a completely pure isolate [25]. The pure isolate was transferred to a slanted agar which would then be gram tested. Pure isolates can be seen in Figure-2.

The gram test on bacteria is carried out to determine the gram-positive and gram-negative of a bacterium. Gram-positive bacteria because these bacteria bind the crystal violet color strongly while the gram-negative bacteria bind the safranin color strongly [26]. The results of gram staining of pure halophytic bacteria isolated from traditional salt ponds varied, namely groups of gram-negative bacteria and groups of gram-positive bacteria. Groups of gram-negative halophilic bacteria are indicated by a red color indicator seen in bacterial cells, while groups of gram-positive bacteria are indicated by a purple color indicator seen in bacterial cells.

The test results showed that the group of isolates of gram-positive (+) bacteria were isolates PAB1, PB1, PB3, PB6, and PP5 while the group of isolates of gram-negative bacteria (-) were isolated PAB2, PB2, PB8, PP4, and PP9. The pink pigment that appears on bacterial cells is a feature of gram-negative bacteria [27]. The gram-negative bacteria group has a higher percentage of lipid and fat than the gram-positive group [28] because this group of gram-negative bacteria generally has thinner peptidoglycan than the gram-positive group [29].

The isolates obtained were dominated by the form of bacilli as many as 9 isolates and other forms identified, namely coccus form as many as 1 isolate. Marine bacteria have 75-85% character, there are flagella and rod-shaped. Coccus bacteria have mucus so that the cells are connected to form a solid surface. This has an impact on bacteria to form a surface layer so that bacteria can live in symbiosis [26]. The results of gram staining of Gram-negative and Gram-positive bacteria can be seen in Figure-3.

Extreme red halophilic bacteria function as absorbers of sunlight so that the temperature in old water will increase and cause the evaporation rate to be high [30]. Halophilic bacteria are red as the initial formation of salt crystal nuclei. The benefit of the presence of halophilic bacteria in old water acts as an oxidizer of dissolved organic particles. According to [31] the use of extreme red halophilic bacteria can produce salt with a NaCl purity of 94.15% to 9.6%, and also the addition of halophilic bacteria can affect the physical changes of the salt.

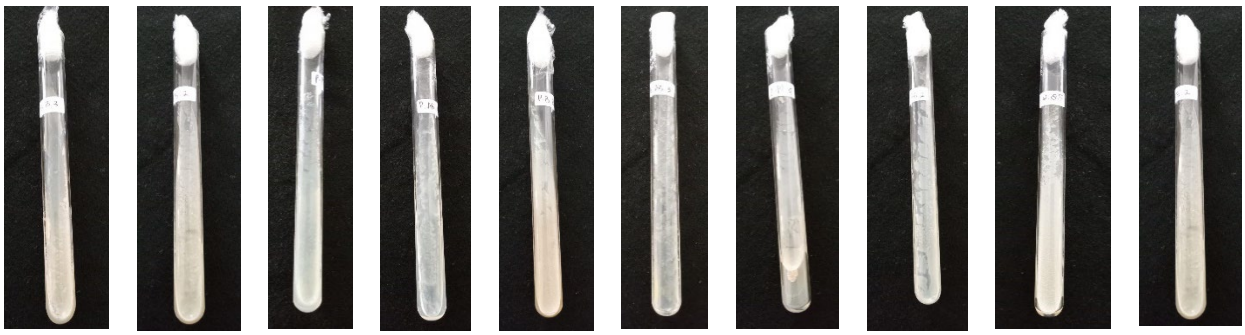
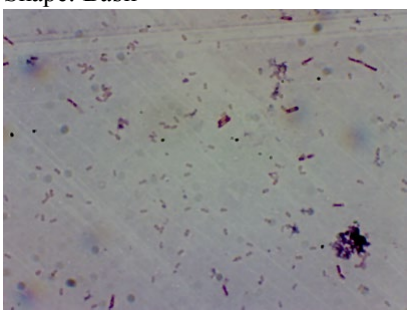
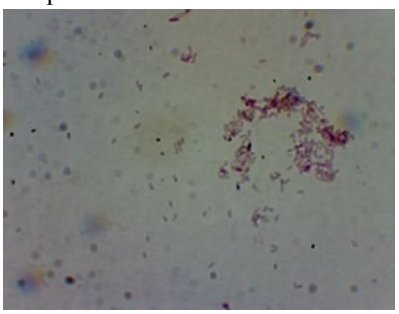


Figure-2. Pure halophilic bacteria isolates from traditional salt production water

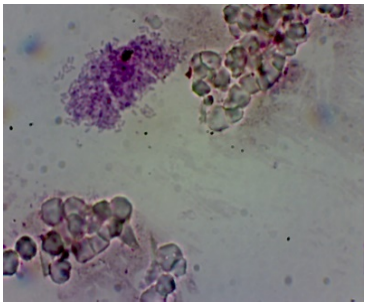
Isolation Code: PAB1
Grams: +
Shape: Basil



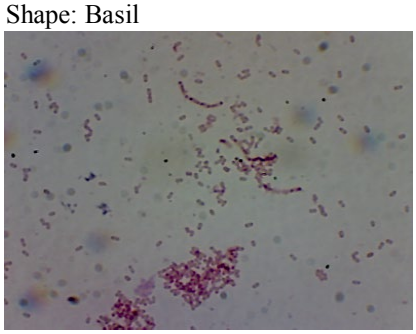
Isolation Code: PAB2
Grams: -
Shape: Basil



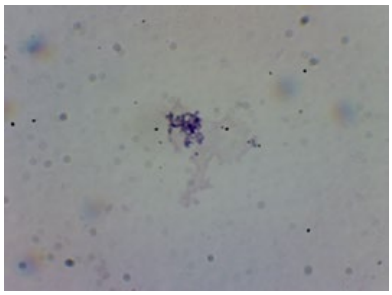
Isolation Code: PB1
Grams: +
Shape: Basil



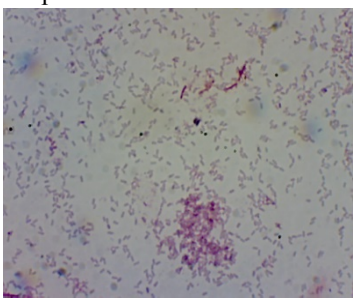
Isolation Code: PB2
Grams: -
Shape: Basil



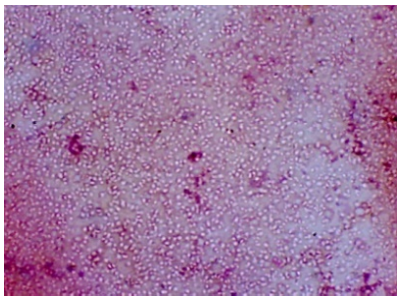
Isolation Code: PB3
Grams: +
Shape: Basil



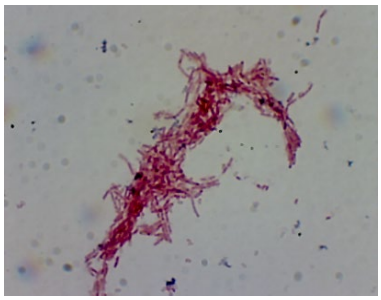
Isolation Code: PB4
Grams: +
Shape: Basil



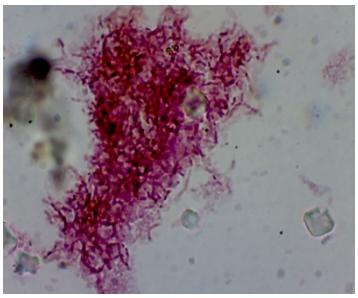
Isolation Code: PB5
Grams: +
Shape: Coccus



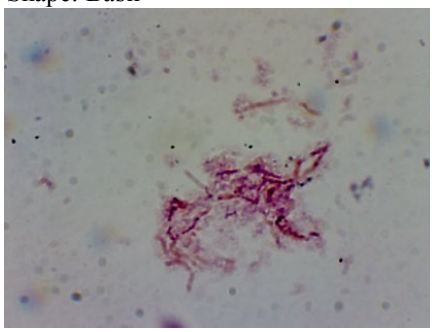
Isolation Code: PP1
Grams: -
Shape: Basil



Isolation Code: PP2
Grams: -
Shape: Basil



Isolation Code: PP3
Grams: -
Shape: Basil



Information

- PAB1 = raw water isolate 1
- PAB2 = raw water isolate 2
- PB1 = reservoir isolate 1
- PB2 = reservoir isolate 2
- PB3 = reservoir isolate 3
- PB4 = reservoir isolate 4
- PB5 = reservoir isolate 5
- PP1 = evaporator isolate 1
- PP2 = evaporator isolate 2
- PP3 = evaporator isolate 3

Figure-3. Gram-positive and gram-negative isolate bacteria

CONCLUSIONS

The morphological characterization of halophilic bacteria was carried out by identification of isolates and gram staining. The identification of bacterial colonies that have been obtained is then purification and transferred to a slanted agar. The results of pure isolates obtained in raw water samples were 2 isolates, reservoir water got 5 isolates and evaporator water 3 isolates. The pure isolates in the gram test mostly have gram negatives which are marked in red. The composition of the gram bacteria group in the salt-produced water sample consisted of gram-negative bacteria and gram-positive bacteria with the morphology of the halophilic bacteria dominated by bacilli.

ACKNOWLEDGMENT

The authors thank to the Institute for Research and Community Service, Universitas Trunojoyo Madura through the UTM 2021 Independent Research Grant Program with research contract number: 3217/UN46.4.1/PT.01.03./2021.

REFERENCES

[1] Assadad, Lutfi. 2011. The use of salt in fisheries product processing industry. *Squalen: Bulletin of Marine and Fisheries Postharvest and Biotechnology*. 6(1): 26-37. https://doi.org/10.15578/squalen.v6i1.58

[2] Redjeki, S. & Iriani. 2021. Produksi Garam Industri dari Garam Rakyat. *Jurnal Teknik Kimia*. 16(1): 41-44. https://doi.org/10.33005/jurnal_tekkim.v16i1.2846

[3] Batafor, Y. M. J. 2020. Identifikasi Permasalahan Produksi Garam Lokal di Kabupaten Flores Timur. *Jurnal Akuatika Indonesi*a. 5(2): 71–76. https://doi.org/10.24198/jaki.v5i2.27510

[4] Kurniawan, A., Jaziri, A. A., Amin, A. A., Ni'matus S., L. 2019. Indeks Kesesuaian Garam (IKG) untuk Menentukan Kesesuaian Lokasi Produksi Garam: Analisis Lokasi Produksi Garam di Kabupaten Tuban dan Kabupaten Probolinggo. *Journal of Fisheries and Marine Research*. 3(2): 236-244. http://dx.doi.org/10.21776/ub.jfmr.2019.003.02

[5] Tururaja, T. & Mogea, R. 2010. Bakteri Coliform di Perairan Teluk Doreri, Manokwari: Aspek Pencemaran Laut dan Identifikasi Species. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*. 15(1): 47-52. https://doi.org/10.14710/ik.ijms.15.1.47-52

[6] Rahmaningsih, S., Wilis, S., & Mulyana, A. 2012. Bakteri Patogen dari Perairan Pantai dan Kawasan Tambak di Kecamatan Jenu Kabupaten Tuban. *Ekologia: Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup*. 12(1): 1–5. DOI: 10.33751/ekol.v12i1.248

[7] Bahi, M. 2012. Isolasi dan karakterisasi senyawa metabolit sekunder dari bakteri laut *Streptomyces* sp. *Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan*. 1(3): 161–164. DOI: https://doi.org/10.13170/depik.1.3.105

[8] Budiharjo, R., Sarjono, R., & Asy'ari, D. M. 2017. Pengaruh Konsentrasi NaCl terhadap Aktivitas Spesifik Protease Ekstraseluler dan Pertumbuhan Bakteri Halofilik Isolat Bittern Tambak Garam Madura. *Jurnal Kimia Sains dan Aplikasi*. 20(3): 142–145. https://doi.org/10.14710/jksa.20.3.142-145

[9] Marihati, Harihastuti, N., Muryati, Nilawati, Eddy, S., & Danny, H. 2014. Penggunaan Bakteri Halofilik sebagai Biokatalisator untuk Meningkatkan Kualitas dan Produktifitas Garam NaCl di Meja Kristalisasi. *Jurnal Riset Industri.* 8(3): 191-196.

[10] Willey, J. M., Sherwood, L. M. and Woolverton. 2008. *Prescott, Harley, and Klein's Microbiology Seventh Edition.* McGraw-Hill. Newyork.

[11] Waluyo, L. 2007. *Mikrobiologi Umum.* UMM Press. Malang.

[12] Cappuccino, J. G. 2008. *Microbiology: A Laboratory Manual. 10th ed.* Benjamin Cummings. pp 30.

[13] Leboffe, M. J. and B. E. Pierce. 2012. *Brief Microbiology: Laboratory Theory & Application 2nd Edition.* Morton Publishing Company. Englewood.

[14] Wulandari, D. & Purwaningsih, D. 2019. Identifikasi dan Karakterisasi Bakteri Amilolitik pada Umbi *Colocasia esculenta L.* secara Morfologi, Biokimia dan Molekuler. *Jurnal Bioteknologi & Biosains Indonesia.* 6(2): 247-258. https://doi.org/10.29122/jbbi.v6i2.3084

[15] Marzouk, M. S., M. M. Moustafa, and Nermeen, M. Mohamed. 2008. The Influence of Some Probiotic on The Growth Performance and Intestinal Microbial Flora of O. niloticus. *International Symposium on Tilapia in Aquaculture.* pp. 1059-1071.

[16] Efendy, M., Firman, F. M., Rahmad. F. S., & Heryanto, A. 2012. *Garam Rakyat: Potensi dan Permasalahan.* Universitas Trunojoyo Madura Press. Madura.

[17] Sudarsono, Edi. 2003. *Proses Produksi Garam.* PT. Garam Persero. Sumenep, Madura.

[18] Sabdaningsih, A., & Arina, L. T. 2020. Isolasi dan Karakterisasi Morfologi Bakteri Halofilik dari Bledug Kuwu, Kabupaten Grobogan. *Bioma*. 22(1): 46-52. https://doi.org/10.14710/bioma.22.1.46-52

[19] Wondal, B., Ginting, E. L., Warouw, V., Wullur, S., Olivia, S. T., & Ferfinand, F. T. 2019. Isolasi Bakteri Laut dari Perairan Malalayang Sulawesi Utara. *Jurnal Pesisir dan Laut Tropis.* 7(3): 184–189. https://doi.org/10.35800/jplt.7.3.2019.24448

[20] Cappuccino, J. G., and Welsh, C. T. 2017. *Microbiology: A Laboratory Manual.* Pearson Education. pp. 30-75.

[21] Hidayat, N., M. C. Padaga & S. Suhartini. 2006. *Mikrobiologi Industri.* Penerbit ANDI. Yogyakarta.

[22] Pelczar, M. J. & Chan, E. C. S. 2008. *Dasar-Dasar Mikrobiologi.* Universitas Indonesia Press. Jakarta.

[23] Ilyas, S. 2001. *Mikrobiologi Dasar.* Universitas Sumatera Utara Press. Medan.

[24] Savitri, S. D. N. 2006. Isolasi dan Karakterisasi Bakteri Halotoleran pada Peda Ikan Kembung (*Rastrelliger* sp.). *Skripsi*. Institut Pertanian Bogor. Bogor.

[25] Ayuningrum, D., Rhesi, K., & Meezan, A. A. 2020. Potensi Bakteri Asosiasi Tunikata sebagai Penghasil Senyawa Antibakteri Guna Menghambat Pertumbuhan Bakteri Multidrug Resistant. *Jurnal Pasir Laut*. 4(2): 102–107. https://doi.org/10.14710/pasir%20laut.2020.32807

[26] Wantania, L. L., Ginting, E. L., & Wullur, S. 2016. Isolasi Bakteri Simbion dengan Spons dari Perairan Tongkeina, Sulawesi Utara. *Jurnal LPPM Bidang Sains dan Teknologi*. 3(1): 57-65.

[27] Safrida, Y. D., Yulvizar, C., & Devira, C. N. 2012. Isolasi dan Karakteristik Bakteri Berpotensi Probiotik pada Ikan Kembung (*Rastrelliger* sp.). *Depik*. 1(3): 200-203. https://doi.org/10.13170/depik.1.3.124

[28] Sudarsono A. 2008. Isolasi dan Karakterisasi Bakteri pada Ikan Laut dalam Spesies Ikan Gindara (*Lepidocibium flavobronneum*). *Skripsi*. Institut Pertanian Bogor. Bogor.

[29] Sunatmo, T. I. 2007. *Eksperimen Mikrobiologi dalam Laboratorium*. Penerbit Ardy Agency. Bogor.

[30] Elevi, R., Assa, P., Birbir, M., Ogan, A. and Oren, A. 2004. Characterization Of Extremely Halophilic Archae Isolated From The Ayvalik Saltern, Turkey. *World Journal of Microbiology & Biotechonology*. 20: 719-725. DOI: 10.1007/s11274-004-4515-z

[31] Malik, R. A., Nilawati, Handayani, N. N., Rame, Djayanti, S., Pratiwi, N. I., & Setianingsih, N. I. 2019. Aplikasi Bakteri Halofilik Berwarna Merah Terimmobilisasi dalam Meningkatkan Kualitas Garam dalam Proses Produksi Garam. *Prosiding SNPBS (Seminar Nasional Pendidikan Biologi dan Saintek) IV*. 6(1): 224-231.