BIODIVERSITAS
Volume 25, Number 3, March 2024
Pages: 964-970

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250308

Isolation and identification of bacterial pathogens causing ice-ice disease in *Eucheuma cottonii* seaweed at Seira Island Waters, Tanimbar Islands District, Maluku, Indonesia

NOVIANTY C. TUHUMURY[1,♥], JACQUELINE M. F. SAHETAPY[2], JOLEN MATAKUPAN[2]
[1]Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Universitas Pattimura. Jl. Chr. Soplanit, Kampus Poka, Ambon, Indonesia. Tel. +62-811-472239, ♥email: noviantytuhumury@gmail.com
[2]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Universitas Pattimura. Jl. Chr. Soplanit, Kampus Poka, Ambon, Indonesia

Manuscript received: 3 September 2023. Revision accepted: 12 March 2024.

Abstract. *Tuhumury NC, Sahetapy JMF, Matakupan J. 2024. Isolation and identification of bacterial pathogens causing ice-ice disease in* Eucheuma cottonii *seaweed at Seira Island Waters, Tanimbar Islands District, Maluku, Indonesia. Biodiversitas 25: 964-970.* Seaweed cultivation has proven to be beneficial and enhances the well-being of communities. Production of seaweed in the waters of Maluku has increased despite facing the problem of ice-ice disease. Therefore, this research aimed to isolate and identify bacteria on the *Eucheuma cottonii* seaweed species affected by ice-ice disease in the waters of Seira Island, Tanimbar Islands District, Maluku. It was conducted in August 2023 at three observation stations, and water quality samples were collected in situ at all three stations, including temperature, salinity, pH, dissolved oxygen (DO), and turbidity. Furthermore, bacterial isolation and identification were carried out at the Laboratory of Marine Aquaculture, Ambon. Bacteria isolation and purification were performed on TCBS media using the streak plate technique, while identification used the API 20E test. The results showed that water quality parameters supported seaweed growth. Four bacteria that caused ice-ice disease in the waters of Seira were identified as *Vibrio alginolyticus, Vibrio fluvalis, Vibrio cholerae*, and *Aeromonas caviae*. Moreover, all four bacteria found were gram-negative and pathogenic. Bacteria belonging to the genera Vibrio and Aeromonas have been proven to cause infections in marine organisms, including seaweed. In conclusion, four types of bacteria were identified in seaweed affected by ice-ice disease.

Keywords: API 20E, bacteria, *Eucheuma cottonii*, ice-ice disease, Seira Island

INTRODUCTION

Seaweed production in Indonesia is crucial in the global industry. As the world's largest producer, Indonesia contributes significantly to meeting the global demand for various types of seaweed (Efendi et al. 2015). According to recent data, the production accounts for 98% of the total Indonesian mariculture production and contributes to 84% of the overall value (Mulyaningrum et al. 2019). Seaweed is a beneficial resource due to its high nutritional value and the presence of bioactive components (Xu et al. 2023). Despite being one of the important marine natural resources in Indonesia, seaweed has not been maximally managed, which stands as a flagship commodity within fisheries and marine industries (Bintang et al. 2019). This is due to the vast potential of Indonesian waters that support the development of seaweed, but it has not been adequately used. The characteristics of the Maluku region, consisting of island seas, strongly support the growth of seaweed (Basir et al. 2017). According to data from the Maluku Maritime and Fisheries Office, seaweed production in the Maluku Province reached 71,928 tons in 2021, with the largest contribution coming from the Tanimbar Islands District at 34,573 tons. However, only 10% of the water in this district is used for seaweed cultivation. This commodity has been proven to enhance the coastal community's economy (Rimmer et al. 2021). Sales of dried seaweed provided livelihoods and fulfilled the needs of cultivators for sustenance and necessities (Larson et al. 2021). Therefore, seaweed can be used for the welfare of the Indonesian people.

Considering the potential and significant economic value of seaweed, efforts to increase production are necessary (Zhang et al. 2022). The success depends on selecting suitable cultivation locations, selecting and ensuring the availability of seedlings, adopting appropriate cultivation methods, maintaining regular care to prevent diseases, and implementing effective harvesting processes (Abdullah et al. 2020). One recurring challenge faced by cultivators is ice-ice disease (Ward et al. 2021). Previous studies showed that seaweed affected by the ice-ice disease is characterized by excessive mucus production and the presence of white spots on the thallus, causing the plant to become brittle (Tahiluddin and Terzi 2021). The onset of this disease is associated with alterations in environmental water conditions, rendering seaweed more vulnerable to bacterial infections (Ward et al. 2020). Changes in water quality parameters include temperature, salinity, and weak currents (Arasamuthu and Edward 2018). Currents are also important in the cleansing process and the removal of particles adhering to seaweed. Moreover, the influence of epiphytes adhering to the thallus affects the growth. Epiphytes, as disruptive or parasitic plants, derive nutrients from seaweed, facilitating bacterial infection (Badraeni et al. 2020).

One of seaweed species most commonly found in Indonesian seawater is *Eucheuma cottonii*, which is cultivated for food and non-food purposes (Supriyono et al. 2022). *Eucheuma cottonii is* a species that belongs to *Rhodophyceae* class (Sudirman et al. 2018). This species produces carrageenan (Diharmi et al. 2019), an additive used to thicken and preserve food and drinks. Carrageenan is very important for the resistance of seaweed in high salinity environments because it plays a role in maintaining ion balance in cells (Rijoly et al. 2020). The industrial demand for carrageenan, specifically in the food and beverage sector, is quite high in accordance with market demand. Moreover, excessive acidity or alkalinity in the water can affect carrageenan content in seaweed (Mujiyanto et al. 2020). This plant is widely cultivated in Asian, African, and Oceania countries because of the abundance and high economic value. However, ice-ice disease, along with epiphytes, is a major problem in Eucheuma cultivation. The characteristics of seaweed infected with ice-ice are marked by the loss of pigmentation in the thallus, followed by the detachment of seaweed from the cultivation ropes, leading to a biomass decrease. This can have negative impacts on seaweed farming industry (Brakel et al. 2021). Despite the presence of harmful bacteria, studies showed that bacteria associated with *E. cottonii* was a source of antibacterial substances, but investigations on this aspect were still limited (Purnami et al. 2022). Several studies on the isolation and identification of bacteria in *E. cottonii* seaweed infected with ice-ice disease showed the presence of bacteria such as *Vibrio sp., Pseudomonas sp.*, and *Bacillus sp.* (Riyaz et al. 2019; Rahman et al. 2019). Other findings have reported the existence of bacteria such as *Pasteurella* sp., *Edwardsiella* sp., *Plesiomonas* sp., and *Chromobacterium* sp. (Azis et al. 2022). Ice-ice disease in seaweed production results in decreased yields, leading to reduced income. Currently, this harvesting failure occurs among cultivators in the waters of Seira Island, Tanimbar Islands District, Maluku. For the people of Seira Island, seaweed cultivation is the only source of income, hence, harvest failure due to ice-ice disease is causing unrest. A single cultivator was able to produce 1 to 2 tons of dried seaweed in 2022, but the subsequent year experienced a significant decrease (personal communication with seaweed farmer, Mr. Simon). To effectively manage ice-ice disease, it is important to identify the bacterial pathogens responsible for the occurrence in *E. cottonii* cultivation. Therefore, this study was conducted to isolate and identify bacteria in *E. cottonii* seaweed affected by ice-ice disease in the waters of Seira Island, Tanimbar Islands District, Maluku, Indonesia.

MATERIALS AND METHODS

Study area

This study was conducted in the water of Seira Island, Tanimbar Islands District, Maluku, in August 2023 (Figure 1). Meanwhile, water and seaweed samples were collected at three stations, namely Tatun Ngatun cultivation site as Station 1 (St 1), Kalenan Island as Station 2 (St 2), and Ngolin Island as Station 3 (St 3). Each station had two sub-stations as replicates, where data on water quality parameters, including temperature, salinity, dissolved oxygen (DO), pH, and turbidity was collected.

Procedures

Sample handling

Water and seaweed samples collected at each station were placed in sample bottles and plastic bags (Figure 2). Subsequently, the samples were placed in a cool box containing seawater ice for transport to the Laboratory of Marine Aquaculture Fisheries Center, Ambon, Indonesia.

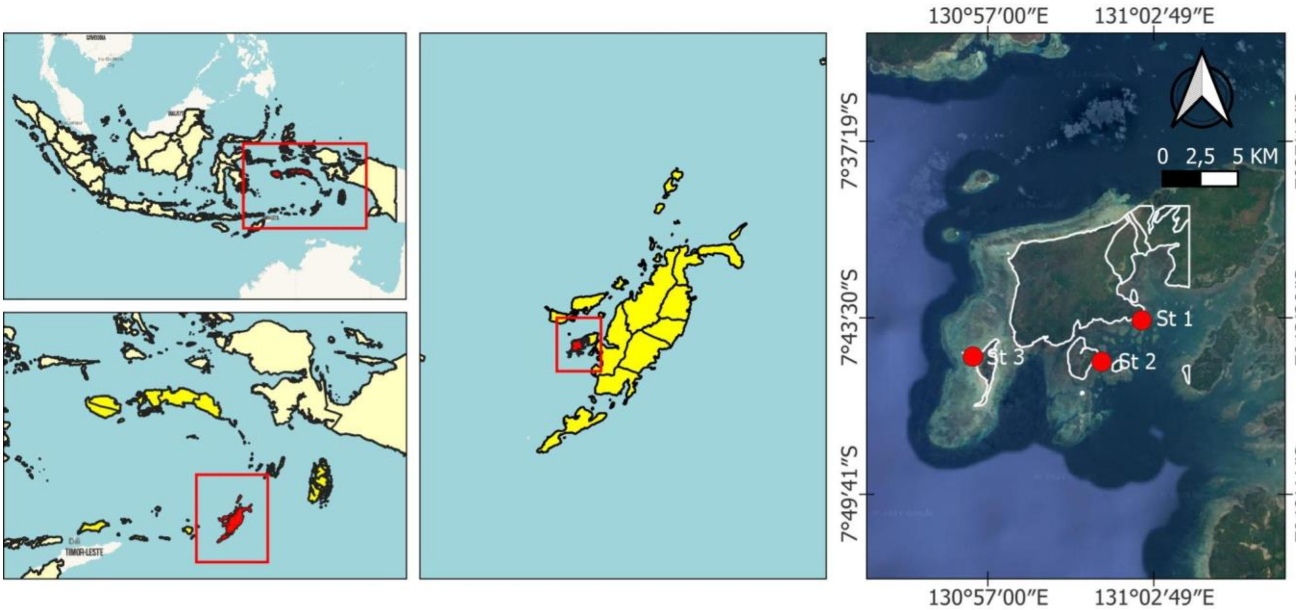

Figure 1. Sampling locations for water and seaweed in the waters of Seira Island, Tanimbar Islands District, Maluku, Indonesia

Measurement of water quality parameters

This study also carried out *in-situ* measurements of water quality parameters that supported seaweed growth, including temperature, salinity, Dissolved Oxygen (DO), pH, and turbidity. Salinity measurement was performed using a refractometer (portable refractometer ATC), while temperature was measured using an oxygen meter (Lutron DO-5510HA) to determine dissolved oxygen levels. The pH and turbidity were measured using a pH meter and turbidity meter (Lutron TU-2016).

Bacterial isolation and identification

The isolation and identification of bacteria in this research focused on parts of seaweed that had been infected with ice-ice disease. Seaweed samples infected with ice-ice disease exhibited white coloration on the thallus (Figure 3). Bacterial isolation was performed by cutting the white-colored thallus parts. Subsequently, grinding was performed using a mortar and pestle, and for water samples, 0.1 mL of seawater sample was taken. Furthermore, the inoculation process was carried out by taking samples using a hypodermic needle, which was then placed into Nutrient Broth (NB) media. The samples were incubated at a temperature of 37°C for 24 hours. Bacterial growth in this process was indicated by turbidity in the media. Subsequently, the bacteria grown on NB media were inoculated into Thiosulfate Citrate Bile Salt Sucrose (TCBS) media as a selective medium aseptically, meaning it was free from contamination with other microorganisms. Bacterial colonies were taken using a hypodermic needle and transferred to TCBS media using the streak plate technique, and incubated at 37°C for 24 hours. After incubation, a purification process was carried out to obtain a pure culture ready for identification.

Biochemical tests

Biochemical tests were conducted to identify bacteria at the species level using the API (Analytical Profile Index) 20E test. The test offers the advantage of rapid and efficient identification of bacteria belonging to the Enterobacteriaceae family based on biochemical properties (Topić Popović et al. 2022; Holmes et al. 1978). The procedure started with the preparation of bacterial suspension by transferring a single isolate colony into a 5 mL API NaCl 0.85% ampule. The bacterial suspension was then pipetted into the wells of the API 20E kit until the bottom of the well was filled. While CIT (Citrate), VP (Voges-Proskauer), and GEL (Gelatin) wells were filled with the bacterial suspension, the ADH (Arginine Dihydrolase), ODC (Ornithine Decarboxylase), LDC (Lysine Decarboxylase), H_2S (Hydrogen Sulfide), and Ure (Urea) wells were sealed with sterile oil. The API 20E kit was incubated in an incubator for 18-48 hours at a temperature of 36°C ± 2°C. After incubation, color changes were observed and recorded. Additional tests were performed requiring extra reagents in the TDA, IND, VP, and GLU wells. TDA and JAMES reagents were added to the TDA and VP wells, while VP1 and VP2 reagents were added to the VP well. NIT1 and NIT2 reagents were added to the GLU well and color changes were observed and recorded on the API 20E result sheet. The data were entered into a computer using specialized software or an API 20E result reading online application (API web).

RESULTS AND DISCUSSION

Water quality parameters

The measurement at the three stations obtained ranges of 26.7-29.7°C, 32-33 ppt, 7.62-7.7, 6.5-8.7 mg/L, and 0.12 to 0.39 ntu for the average water temperature values, salinity, pH, dissolved oxygen and turbidity, respectively (Table 1). The parameter values were still within the established quality standard as evidenced by the water quality parameters supporting seaweed growth. This was outlined in the Indonesian National Standard (SNI) 7673.2:2011 for the production of *E. cottonii* seaweed seedlings (Indonesian National Standard 2010). The standard specifies that suitable water quality conditions for *E. cottonii* growth include temperature between 24 and 32°C, salinity ranging from 28 to 33 ppt, and pH values between 7 and 8.5. Meanwhile, turbidity values at Station 3 were lower than those at the other stations.

Figure 2. Sample Collection and Handling Process

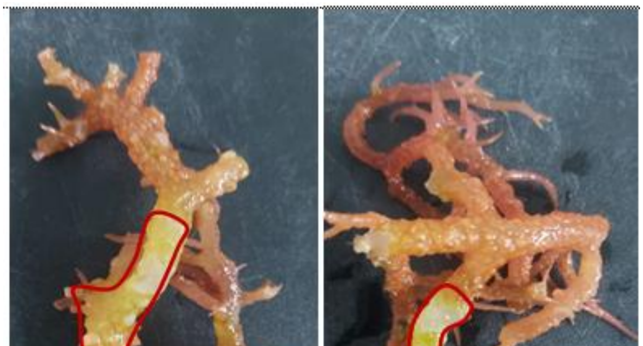

Figure 3. Section of *Eucheuma cottonii* seaweed sample infected with ice-ice disease

Identification of bacteria causing ice-ice disease in seaweed

The API 20E test showed the identification of four types of bacteria in both water and seaweed infected with ice-ice disease, including *Vibrio alginolyticus*, *Vibrio fluvialis*, *Vibrio cholerae*, and *Aeromonas caviae* (Table 2). *Vibrio* bacteria are Gram-negative bacteria commonly found in marine habitats, pathogenic, and can cause disease in the hosts. The colonies on TCBS media spread out, appearing yellow with round colony shapes (Figure 4.A, B, and C). The yellow color arises due to the ability to ferment sucrose from the media. The use of TCBS media, as a selective medium containing thiosulfate, inhibits the growth of other bacteria and supports *Vibrio* growth. *Vibrio fluvialis* and *Vibrio cholerae* were found in seaweed samples, while *Vibrio alginolyticus* were reported in both water and seaweed samples.

Aeromonas bacteria in water samples belong to the Aeromonadaceae family and are Gram-negative with a shiny cream color (Figure 4.D). The isolates exhibited positive reactions in the ONPG (o-nitrophenyl-β-D-galactopyranoside) test with a yellow color, the IND (Indole) test with a pinkish-red color, the GLU (Glucose) fermentation test with a pale-yellow color, and the MAN (Mannitol), SAC (Sucrose) fermentation tests, and AMY (Amygdalin) fermentation tests, all showing a yellow color.

Table 2. Bacteria identified from seaweed and water at the three observation stations

Sample	Station	Sub station	Bacterial species
Seaweed	1	A	*Vibrio alginolyticus*
		B	*Vibrio fluvialis*
	2	A	*Vibrio cholerae*
		B	*Vibrio cholerae*
	3	A	*Vibrio alginolyticus*
		B	*Vibrio alginolyticus*
Water	1	A	*Vibrio alginolyticus*
		B	*Aeromonas caviae*
	2	A	*Aeromonas caviae*
		B	*Aeromonas caviae*
	3	A	*Vibrio alginolyticus*
		B	*Vibrio alginolyticus*

Table 1. The average values of water quality parameters at the three observation stations

Station	Temperature (°C)	Salinity (ppt)	pH	Dissolved Oxygen (mg/L)	Turbidity (ntu)
1	26.7	33	7.7	8.7	0.37
2	27.8	32	7.6	7.9	0.39
3	29.7	33	7.7	6.5	0.12

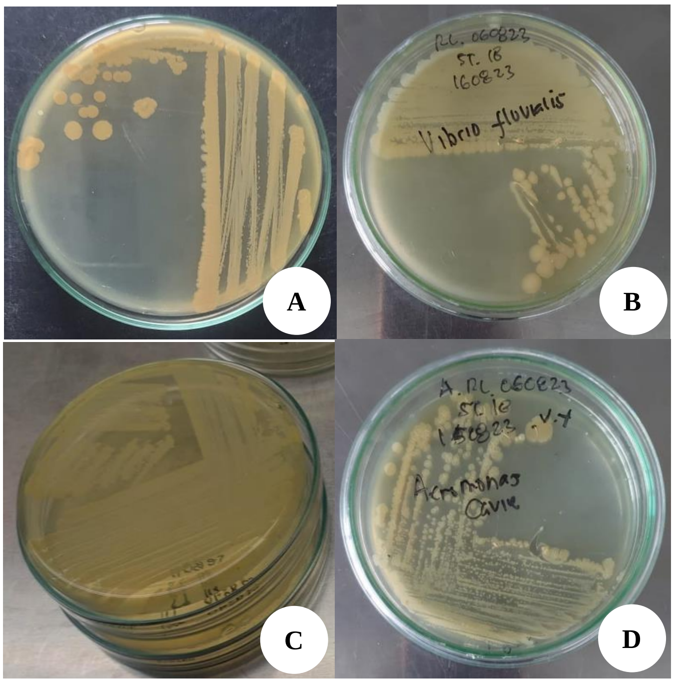

Figure 4. Bacteria species identified in water and seaweed infected with ice-ice disease in the waters of Seira Island. A. *Vibrio alginolyticus*, B. *Vibrio fluvialis*, C. *Vibrio cholerae*, D. *Aeromonas caviae*

Water quality parameters are supporting factors in determining seaweed cultivation locations (Maradhy et al. 2021). The locations at the three observation stations were not close to the mainland, and there were no rivers nearby. The unique aspect of seaweed cultivation in the waters of Seira Island is that the homes of cultivators are situated in the middle of the sea. At Stations 1 and 2, their homes are built in the sea to be close to their cultivation sites. At Station 3, the homes of cultivators were built on land and the waters around Seira Island were not prone to pollution due to the lack of human settlements. The waste generated from daily activities, such as the disposal of wastewater and feces, has the potential to cause pollution. One of the criteria for suitable seaweed cultivation locations is the absence of pollution (Paruch et al. 2019). Therefore, the location of cultivator's homes should not be near the cultivation areas.

According to SNI 7673.2:2011, an appropriate seaweed cultivation location should be sheltered from waves. The protection of the locations at the three stations can be considered satisfactory since they are situated between Seira and Yamdena Island. However, among the western monsoon and the transitional phase, specifically spanning from April to early June, alterations in water quality manifest due to the influence of prevailing winds and waves. This phenomenon is noteworthy since the three stations are strategically situated to the south of Seira Island. Waves and winds influence the movement of currents, stirring up bottom sediments, and increasing the turbidity. Meanwhile, water turbidity is closely related to cloudiness caused by the abundance of organic and mineral particles such as sand, microorganisms, decaying plants, and animals, as well as human waste discharge (Liang et al. 2022). Murky waters also obstruct the penetration of sunlight, hindering optimal photosynthesis in seaweed. The turbidity level in the waters of Seira Island is low and conducive to seaweed growth. However, strong waves and currents during the transitional season can lead to high turbidity, resulting in harvest failures.

The results of the measurement of temperature and salinity parameters in this study showed values that are suitable and feasible for seaweed growth. Changes in environmental conditions shown through changes in both water quality parameters can trigger the emergence of ice-ice disease in seaweed. Seaweed is a marine plant that undergoes photosynthesis throughout its entire body. Moreover, water temperature directly affects the physiological processes of seaweed, such as photosynthesis and nutrient absorption. Optimal photosynthesis occurs under high solar intensity and at optimum temperatures. Similarly, salinity affects seaweed growth and its changes lead to alterations in osmotic pressure, impacting nutrient absorption in the water (Balasubramaniam et al. 2023). Increased salinity can cause shrinkage of stomata which plays a role in absorbing CO_2 and nutrients, thereby inhibiting growth. The growth rate is also influenced by the concentration of dissolved oxygen in the process of photosynthesis and respiration. Previous studies showed the dissolved oxygen content that was beneficial for maintaining seaweed was in the range of 6.4-6.8 mg/L for the rainy season and 5.6-6.4 mg/L for the dry season (Aris and Labenua 2020).

Gram-negative bacteria are identified in almost all ice-ice disease-causing bacteria. *Vibrio* is primarily found in the sea, although *Vibrio cholerae* is also found in freshwater (Newman 2022). *Vibrio alginolyticus* isolated from seaweed was found in the waters of Kutuh Beach, Bali (Saraswati and Darmasetiyawana 2016) and Southeast Maluku. This bacterium was also reported in the samples and seaweed in the waters of Southeast Maluku (Erbabley and Kelabora 2018). Seaweed ecosystems can be negatively affected by the presence of *Vibrio alginolyticus*. Due to the pathogenic nature of *Vibrio alginolyticus*, seaweed populations can experience reduced growth, increased susceptibility to diseases, and population declines. Furthermore, *Vibrio alginolyticus* has indirect impacts on other organisms in the seaweed ecosystem, (Sampaio et al. 2022), leading to shifts in ecological dynamics. Besides seaweed (Sugumaran et al. 2022), *Vibrio* sp. causes ice-ice disease in fish and shrimp (Sarjito et al. 2022). Contamination of the bacteria in seafood leads to extra-intestinal infections in humans due to the consumption of raw or undercooked seafood products (Cao et al. 2018). Contamination of *Vibrio alginolyticus* in fish results in acute gastroenteritis (intestinal and stomach infections) through the gills, skin, and gastrointestinal tract (Hao et al. 2023). In addition, waste generated from daily farming activities is directly discharged into the sea, leading to the emergence of pathogenic bacteria. *Aeromonas caviae,* a species of the genus *Aeromonas,* has been found in seaweed, raising concerns about potential risks to public health and the ecosystem. The presence of *Aeromonas* bacteria is quite high in wastewater (Fernández-Bravo and Figueras 2020). Water serves as a medium for the spread of *Aeromonas* bacteria, which is suspected to infect seaweed during processing (Løvdal et al. 2021). These bacteria thrive in cold temperatures, posing a danger to seaweed products and other seafood. Furthermore, when *Aeromonas caviae* thrive in the seaweed ecosystem, it may have negative impacts on the health of other organisms and disrupt ecological balance. Seaweed as a natural host for Vibrios provides an ideal environment for the survival and growth of *Vibrio cholerae*. This bacterium is found free-living in marine environments and is known to have elevated levels in association with various eukaryotic hosts, including seaweeds (Kechker et al. 2017). Furthermore, *Vibrio cholerae* infect seaweed and pose a pathogenic threat to humans. There have been reported infection cases in humans who consumed raw seaweed after one month. A woman ate fresh, raw seaweed from the Philippines containing *V. cholerae* while on holiday and became infected one month later. Seaweed is known to be a suitable habitat for *Vibrio fluvialis*, a species of Vibrio bacteria. This was supported by a research that showed Vibrio species often colonize seaweed surfaces (Løvdal et al. 2021). Research also showed that *Vibrio fluvialis* was one of the most common bacteria found in surface waters in the world, emphasizing its adaptability to a variety of aquatic habitats (Igbinosa and Okoh 2010). The presence of

Vibrio fluvialis in seaweed ecosystems has a significant impact, which can cause population decline, as well as affect biodiversity and ecological balance (Fernández et al. 2020).

Based on observations, the largest harvest failure occurred at cultivation station 3 on Ngolin Island. Even though the turbidity value at this station was low, station 3 was more exposed, allowing for changes in water quality during transitional seasons. Information obtained indicated that farmers did not know the cause of the white discoloration of seaweed. The results showed that the seedlings used to date were obtained in 2006. The success of seaweed cultivation and its production is influenced by the choice of seedlings (Jiksing et al. 2022). The cultivation process affected by ice-ice disease produces low-quality seedlings, with a reduced level of production. Therefore, new seedlings that are free from and resistant to ice-ice disease are needed. Based on the observations, it can be concluded that the water quality at all three locations was suitable for the cultivation of *E. cottonii* seaweed. The results of bacterial identification showed 4 types of bacteria causing ice-ice disease in *E. cottonii* seaweed in Seira waters, namely *Vibrio alginolyticus, Vibrio fluvialis, Vibrio cholerae, and Aeromonas caviae*. Future studies need to conduct a similar investigation using molecular detection methods through genomic DNA analysis based on PCR techniques. The use of 16S-rRNA gene has been employed as a systematic parameter and marker for bacterial species identification.

ACKNOWLEDGEMENTS

This regular fundamental research was funded by the Ministry of Education, Culture, Research, and Technology, Indonesia for the 2023 fiscal year. The authors are grateful to seaweed farmers in the waters of Seira Island who helped with field data collection and provided information for the completion of this research.

REFERENCES

Abdullah N, Wibowo ES, Irfan M, Muchdar F, Malan S. 2020. Seaweed *kappaphycus alvarezii* cultivation using longline method in Kastela waters, Ternate Island, Indonesia. Aquac Aquarium Conserv Legislation 13 (4): 2336-2342.

Arasamuthu A, Edward JK. 2018. Occurrence of ice-ice disease in seaweed *Kappaphycus alvarezii* at Gulf of Mannar and Palk Bay, Southeastern India. Indian J Geo-Mar Sci 47 (6): 1208-1216.

Aris M, Labenua R. 2020. Evaluation of land suitability of *Kappaphycus alvarezii* cultivation in the dry and rainy season. IOP Conf Ser: Earth Environ Sci 584: 012025. DOI: 10.1088/1755-1315/584/1/012025.

Azis, Achmad M, Indarjo A, Salim G, Rozi. 2022. Identification of bacteria in seaweed (*Kapapphycus alvarezii*) affected by the ice-ice disease in Amal and Tanjung Tarakan waters, Indonesia. AACL Bioflux 15 (6): 3061-3068.

Badraeni, Syamsuddin R, Haryati, Samawi F. 2020. Weeds, epiphytes and ice-ice disease on green-strained *Kappaphycus alvarezii* (doty) in Takalar Waters, South Sulawesi in different seasons and locations of cultivation. Plant Arch 20: 2327-2332.

Balasubramaniam T, Shen G, Esmaeili N, Zhang H. 2023. Plants' response mechanisms to salinity stress. Plants 12 (12): 2253. DOI: 10.3390/plants12122253.

Basir AP, Abukena L, Amiludin M. 2017. The growth of seaweed (*Kappaphycus Alvarezii*) cultivated with long line and off bottom method on Tita Banda Neira Maluku coastal area. J Fish Mar Res 1 (1): 20-23. DOI: 10.21776/ub.jfmr.2017.001.01.4.

Bintang AS, Huang WC, Asmara R. 2019. Forecasting of Indonesia seaweed export: A comparison of fuzzy time series with and without markov chain. Agric Soc Econ J 19 (3): 155-164. DOI: 10.21776/ub.agrise.2019.019.3.4.

Brakel J, Sibonga RC, Dumilag RV, Montalescot V, Campbell I, Cottier-Cook EJ, Ward G, Le Masson V, Liu T, Msuya FE, Brodie J. 2021. Exploring, harnessing and conserving marine genetic resources towards a sustainable seaweed aquaculture. Plants People Planet 3 (4): 337-349. DOI: 10.1002/ppp3.10190.

Cao J, Zhang J, Ma L, Li L, Zhang W, Li J. 2018. Identification of fish source *Vibrio alginolyticus* and evaluation of its bacterial ghosts vaccine immune effects. Microbiologyopen 7 (3): e00576. DOI: 10.1002/mbo3.576.

Diharmi A, Rusnawati, Irasari N. 2019. Characteristic of carrageenan *Eucheuma cottonii* collected from the coast of Tanjung Medang Village and Jaga Island, Riau. IOP Conf Ser: Earth Environ Sci 404: 012049. DOI: 10.1088/1755-1315/404/1/012049.

Efendi F, Handajani R, Nursalam N. 2015. Searching for the best agarose candidate from genus *Gracilaria*, *Eucheuma*, *Gelidium* and local brands. Asian Pac J Trop Biomed 5 (10): 865-869. DOI: 10.1016/j.apjtb.2015.06.009.

Erbabley NY, Kelabora DM. 2018. Identification of *Kappaphycus alvarezii* seaweed bacteria based on growing season in Southeast Maluku Waters. Akuatika Indonesia 3 (1): 19-25. DOI: 10.24198/jaki.v3i1.23398. [Indonesian]

Fernández-Bravo A, Figueras MJ. 2020. An update on the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. Microorganisms 8 (1): 129. DOI: 10.3390/microorganisms8010129.

Fernández PA, Gaitán-Espitia JD, Leal PP, Schmid M, Revill AT, Hurd CL. 2020. Nitrogen sufficiency enhances thermal tolerance in habitat-forming kelp: Implications for acclimation under thermal stress. Sci Rep 10 (1): 3186. DOI: 10.1038/s41598-020-60104-4.

Purnami PP, Indraningrat AA, Darmayasa IB. 2022. Antibacterial activity screening of bacterial isolates Associated with seaweed *Eucheuma cottonii* from coastal area in Buleleng, Bali. Biotropika: J Trop Biol 10 (2): 132-140. DOI: 10.21776/ub.biotropika.2022.010.02.07.

Hao Y, Zhao Y, Zhang Y, Liu Y, Wang G, He Z, Cao W, Han T, Zhang X, Zhang Z, Wang Y, Gong C, Hou J. 2023. Population response of intestinal microbiota to acute *Vibrio alginolyticus* infection in half-smooth tongue sole (*Cynoglossus semilaevis*). Front Microbiol 14: 1178575. DOI: 10.3389/fmicb.2023.1178575.

Holmes B, Willcox WR, Lapage SP. 1978. Identification of Enterobacteriaceae by the API 20E system. J Clin Pathol 31 (1): 22-30. DOI: 10.1136/jcp.31.1.22.

Igbinosa EO, Okoh AI. 2010. *Vibrio fluvialis*: An unusual enteric pathogen of increasing public health concern. Intl J Environ Res Public Health 7 (10): 3628-3643. DOI: 10.3390/ijerph7103628.

Indonesian National Standard. 2010. Production of kotoni seaweed (*Eucheuma cottonii*) - Part 2: Long-line method. In National Standardization Agency (Issue 2).

Jiksing C, Ongkudon MM, Thien VY, Rodrigues KF, Yong WT. 2022. Recent advances in seaweed seedling production: A review of *eucheumatoids* and other valuable seaweeds. Algae 37 (2): 105-121. DOI: 10.4490/algae.2022.37.5.11.

Kechker P, Ken-Dror S, Laviad-Shitrit S, Arakawa E, Halpern M. 2017. Otitis media caused by *V. cholerae* O100: A case report and review of the literature. Front Microbiol 8: 277089. DOI: 10.3389/fmicb.2017.01619.

Larson S, Stoeckl N, Fachry ME, Mustafa MD, Lapong I, Purnomo AH, Rimmer MA, Paul NA. 2021. Women's well-being and household benefits from seaweed farming in Indonesia. Aquaculture 530: 735711. DOI: 10.1016/j.aquaculture.2020.735711.

Liang Z, Wang W, Liu L, Li G, Xia B. 2022. Influence of commercial-scale seaweed cultivation on water quality: A case study in a typical laver culture area of the Yellow Sea, North China. J Mar Sci Eng 10 (5): 681. DOI: 10.3390/jmse10050681.

Løvdal T, Lunestad BT, Myrmel M, Rosnes JT, Skipnes D. 2021. Microbiological food safety of seaweeds. Foods 10 (11): 2719. DOI: 10.3390/foods10112719.

Maradhy E, Syarief R, Hadi S, Syahbana M, Fedi M. 2021. Evaluation of water suitability for sustainable seaweed (*Kappaphycus alvarezii*) cultivation to support science technopark in North Kalimantan. J Nat Resour Environ Manag 11 (3): 490-503.

Mujiyanto, Syam AR, Wijaya D, Purnamaningtyas SE. 2020. Suitability water quality parameters for seaweed culture at Muara Gembong coastal area, Bekasi District. IOP Conf Ser: Earth Environ Sci 521: 012012. DOI: 10.1088/1755-1315/521/1/012012.

Mulyaningrum SRH, Suwoyo HS, Paena M, Tampangallo BR. 2019. Epiphyte identification on *Kappaphycus alvarezii* seaweed farming area in Arungkeke Waters, Jeneponto and the effect on carrageenan quality. Ilmu Kelautan: Indones J Mar Sci 24 (3): 146-152. DOI: 10.14710/ik.ijms.24.3.146-152.

Newman SG. 2022. An update on vibriosis, the major bacterial disease shrimp farmers face. Global Aquaculture Advocate 2022: 1-8.

Topić Popović N, Kepec S, Kazazić SP, Strunjak-Perović I, Bojanić K, Čož-Rakovac R. 2022. Identification of environmental aquatic bacteria by mass spectrometry supported by biochemical differentiation. PLoS ONE 17 (6): e0269423. DOI: 10.1371/journal.pone.0269423.

Rahman SA, Sukenda S, Widanarni W, Alimuddin A, Ekasari J. 2019. Isolation and identification of endophytic bacteria from the mangrove leaves of *Avicennia marina* and evaluation of inhibition to bacterium causing ice-ice disease. AACL Bioflux 12 (3): 941-952.

Rijoly SMA, Killay A, Rupilu JA. 2020. Soaking urea fertiliser and concentration levels on *Eucheuma cottonii* seaweed carrageenan. Rumphius: Pattimura Biol J 2 (1): 30-40. [Indoensian]

Rimmer MA, Larson S, Lapong I, Purnomo AH, Pong-masak PR, Swanepoel L, Paul NA. 2021. Seaweed aquaculture in Indonesia contributes to social and economic aspects of livelihoods and community wellbeing. Sustainability 13 (19): 10946. DOI: 10.3390/su131910946.

Riyaz SUM, Nalini S, Kavitha G, Sutha SAD, Inbakandan D. 2019. Characterization and identification of isolated bacteria from ice-ice disease infected seaweed *Kappaphycus alvarezii*. Indian J Geo-Mar Sci 48 (8): 1286-1290.

Sampaio A, Silva V, Poeta P, Aonofriesei F. 2022. *Vibrio* spp.: Life strategies, ecology, and risks in a changing environment. Diversity 14 (2): 97. DOI: 10.3390/d14020097.

Saraswati SA, Darmasetiyawana IMS. 2016. Identification of bacteria on *Euchema spinosum* seaweed with ice-ice disease in Kutuh Beach Waters. J Mar Aquat Sci 2 (1): 11. DOI: 10.24843/jmas.2016.v2.i01.11-15. [Indonesian]

Sarjito S, Amalia R, Sabdaningsih A. 2022. Screening of sponge-associated bacteria to control vibriosis in vannamei shrimp (*Litopenaeus vannamei*). Biodiversitas 23 (10): 5333-5341. DOI: 10.13057/biodiv/d231043.

Sudirman S, Hsu Y-H, He J-L, Kong Z-L. 2018. Dietary polysaccharide-rich extract from *Eucheuma cottonii* modulates the inflammatory response and suppresses colonic injury on dextran sulfate sodiuminduced colitis in mice. PLoS ONE 13 (10): e0205252. DOI: 10.1371/journal.pone.0205252.

Sugumaran R, Padam BS, Yong WTL, Saallah S, Ahmed K, Yusof NA. 2022. A retrospective review of global commercial seaweed production - current challenges, biosecurity and mitigation measures and prospects. Intl J Environ Res Public Health 19 (12): 7087. DOI: 10.3390/ijerph19127087.

Supriyono E, Lideman, Hastuti YP, Arifka AR. 2022. Combination effect of atonic growth regulator with PES (Provasoli Enrich Seawater) on Seaweed (*Eucheuma cottonnii*) growth. IOP Conf Ser: Earth Environ Sci 1033: 012019. DOI: 10.1088/1755-1315/1033/1/012019.

Tahiluddin A, Terzi E. 2021. Ice-ice disease in commercially cultivated seaweeds *Kappaphycus* spp. and *Eucheuma* spp.: A review on the causes, occurrence, and control measures. Mar Sci Technol Bull 10 (3): 234-243. DOI: 10.33714/masteb.917788.

Ward GM, Faisan Jr JP, Cottier-Cook EJ, Gachon C, Hurtado AQ, Lim PE, Matoju I, Msuya FE, Bass D, Brodie J. 2020. A review of reported seaweed diseases and pests in aquaculture in Asia. J World Aquac Soc 51 (4): 815-828. DOI: 10.1111/jwas.12649.

Ward GM, Kambey CSB, Faisan Jr JP, Tan P.-L, Daumich CC, Matoju I, Stentiford GD, Bass D, Lim P-E, Brodie J, Poong S-W. 2021. Ice-ice disease: An environmentally and microbiologically driven syndrome in tropical seaweed aquaculture. Rev Aquac 14 (1): 414-439. DOI: 10.1111/raq.12606.

Xu J, Liao W, Liu Y, Guo Y, Jiang S, Zhao C. 2023. An overview on the nutritional and bioactive components of green seaweeds. Food Prod Process Nutr 5 (1): 5-18. DOI: 10.1186/s43014-023-00132-5.

Zhang L, Liao W, Huang Y, Wen Y, Chu Y, Zhao C. 2022. Global seaweed farming and processing in the past 20 years. Food Prod Process Nutr 4 (23): 1-30. DOI: 10.1186/s43014-022-00103-2.