Bacteria population on *Eucheuma denticulatum* (Rhodophyta) thallus infected by ice-ice disease cultivated on horizontal net cage

[1]Tuti A. Darma, [1]Ma'ruf Kasim, [1]Ruslaini, [1]Indriyani Nur, [1]Abdul Rahman, [1]Wellem Muskita, [1]Muhaimin Hamzah, [1]Abdul M. Balubi, [1]Rahmat S. Patadjai, [2]Wardha Jalil

[1] Faculty of fisheries and Marine Sciences, Halu Oleo University, Kendari, Southeast Sulawesi, Indonesia; [2] Faculty of Fisheries, Dayanu Iksanuddin University, Betoambari, Kota Baubau, Southeast Sulawesi, Indonesia. Corresponding author: M. Kasim, marufkasim@hotmail.com

Abstract. Ice-ice is one of the seaweed diseases. The population of bacteria in seaweed affected by ice-ice is estimated to be quite high. This research aims to identify the amount of bacteria population on seaweed (*E. denticulatum*) infected by ice-ice disease. This research used Complete Random Design with three treatments and three repetitions. Bacteria population analysis used a media of Tryctic Soy Agar (TSA) and solution of Butterfield's Phosphate Buffered (BFP) with agar spread technique. The samples were part of thallus that had ice-ice disease, compared to healthy thallus which was close to ice-ice and healthy thallus sample which was far from ice-ice, each sample measured 25 g in weight. The results show that highest average bacteria population on thallus with ice-ice (TS) was 8.73 x 108 CFU mL^{-1} (log: 7.94) and the lowest on healthy thallus far from ice-ice (TJ) was 1.03 x 107 CFU mL^{-1} (log: 7.01). Water quality parameter measurement results during the research showed water transparency of 6.28 m, temperature 29^{o}C, depth 7.32 m, current velocity 11.56 m, salinity 33 ppt, dissolved oxygen (DO) 7.8 mg L^{-1}, nitrate 0.0104 mg L^{-1} and phosphate 0.0042 mg L^{-1}. There was no significant correlation evidenced between environmental conditions and ice-ice disease.
Key Words: seaweed, cultivation, growth rate, healthy thallus.

Introduction. Seaweed is commonly cultivated and traded as a raw material for various products that have high economic value (Kasim et al 2016; Yahya 2013). Seaweed cultivation development has been performed, however there are many problems faced. The main problem which is commonly found in cultivation is pest attack and ice-ice disease (Alibon et al 2019; Kasim et al 2017). Seaweed disease is known as ice-ice. Ice-ice disease does not only attack *Kappaphycus alvarezii*, but also *Eucheuma denticulatum*. *E. denticulatum* could be attacked by ice-ice disease especially caused by environmental changes such as current, temperature, salinity level, and water transparency in the cultivation site (Arasamuthu & Edward 2018; Budiyanto et al 2019; Kasim et al 2018; Tisera & Naguit 2009). The aggression happens in an extended period of time. This disease occurs with a sign of red fleck/spots on some thallus and eventually becomes pale yellow and gradually the thallus becomes white, and then the thallus become fragile and break easily in the end (KKP 2005). The broken thallus causes weight loss of the seaweed. Ice-ice disease on one thallus of seaweed could damage other thallus seaweed in very short time if there is no prevention. Negative impact that commonly happens on carrageenan industry is the spread of ice-ice disease that attack seaweed in the cultivation location (Mendoza et al 2002). Impact of ice-ice disease infection towards seaweed varies between 10 and 100%, so that usually it causes harvest failure and profit loss to the farmers (Khan & Satam 2003). An early harvest activity of seaweed is the only way to handle it for the cultivation farmers which is usually done to anticipate bigger material loss from the ice-ice disease. The effort to reduce material loss, the harvest has to be conducted as soon as possible if the disease occurs (Trono 1993; KKP 2005). Ice-ice disease occurs because of environmental change that is not suitable with optimal condition to support the growth of seaweed, therefore it generate body endurance loss of the seaweed (Tokan et al 2015; Trono 1974). Occurrence of ice-ice disease is possibly because a particular pathogen bacteria attack especially when the seaweed is in a weak condition (stress) because of significant water quality parameter changes (Arasamuthu & Edward 2018). This shows that the bacteria attack which causes this disease is secondary attack that effectively generates disease only when the growth is not optimal (abnormal) Uyenco et al 1981.

Consistent efforts have been done in order to prevent ice-ice disease. One of them is the utilization of cultivation technology (Tokan et al 2015). Seaweed cultivation uses simple system with longline and floating cage method which is very common and highly advanced by farmers (Kasim et al 2016; Kasim et al 2019). However, the cultivation method yet is not able to overcome the problem of seaweed cultivation (Kasim et al 2017; Yulianto 2003). A method to prevent ice-ice disease is using floating cage cultivation technology with the purpose to increase cultivation protection from pest and to prevent the ice-ice disease occurence (Kasim et al 2016, 2019). Besides, the usages of herbs material like *Saliara* sp. which have antibacterial compound has been used to prevent bacterial attack (Jaya 2014). Bacteria population on seaweed infected by ice-ice disease is unknown, so the occurrence of bacterial population is very important to be identified for a basis to analyzing the causes and its aggression. Based on this statement, research of bacterial population on seaweed (*E. denticulatum*) which has ice-ice disease with the floating cage technology as a new developing technology in seaweed cultivation is very important to be conducted.

The present study aims to determine bacterial populations in healthy and ice-ice infected seaweed thallus.

Material and Method. The present research was conducted from January to March 2014 at two station in Tanjung Tiram seaweed cultivation areas, South Konawe district, Southeast Sulawesi Province. Advanced research to analysis of bacteria population was performed at Laboratory of Fish Quarantine Station, Quality Control and Safety of Fishery Products, Kendari, Indonesia. Analysis of water quality parameters was performed in field and Laboratory of Faculty of Fishery and Marine Science, Halu Oleo University.

Seaweed sample collection. Samples of seaweed thallus were collected from seaweed cultivation area in field research, particularly from inside a horizontal net cage. Horizontal net cages were used in other to avoid herbivorous fish to graze samples. There 9 horizontal net cages were used. The sampling was done randomly. Seaweed thallus samples consisted of 3 treatments: the part of thallus with ice-ice (TS), part of healthy thallus close to the ice-ice (TD) and part of healthy thallus far from ice-ice (TJ). For every treatment there were 3 times repetition, therefore it becomes TS1, TD1, TJ1; TS2, TD2, TJ2; and TS3, TD3, TJ3, with 25 g for each sample.

Bacteria population. For the sterilization of the equipment we used an aseptic agent. The sterilized equipment was utilized during seaweed bacteria population observation.

Each part of the thallus was blended together with 255 mL BFP solution. After that, thallus and BFP solution were homogenized for ±2 minutes. The preparation of dissolving solution used BFP 9 mL per test tube. After that the bacteria dilution was done periodically until dilution step 5 with 100 µL in every square root of dilution.

Bacteria plantation was done using spread method. After that, bacteria incubation process was done for 1x24 hours with storage cup upside down. Bacteria that grew on TSA media was manually calculated with simplo and duplo repetition and marked with a marker in the calculated colony. The dilution was counted only until the growth colony resulted around 30-300 colonies (Gobel 2008).

Bacteria population on seaweed thallus were analyzed as the part of thallus that got ice-ice disease (TS), part of healthy thallus close with the ice-ice (TJ), and part of healthy thallus far from the ice-ice (TD). Calculation of bacteria colony amount is to determine bacteria population in every sample. Bacteria density was calculated according to Lay (1994):

$$N_{tot} \text{ CFU/mL} = T/Q \times 1/S \times 1/V$$

Where:

N_{tot} - Total bacteria colony/mL
T - Total of bacteria in petri dish
Q - Total of petri dish used
S - Dilution used
V - Inoculated volume (mL)

Water quality parameter measurement. Environmental parameters measurement was done once during the research. Measurements were performed in the field, simultaneously with the seaweed collection. Parameters recorded in the research field consisted of water transparency, temperature, depth, current velocity, and salinity. While nitrate, phosphate, and dissolved oxygen was analyzed at the laboratory.

Statistical analysis. To identify the impact of different bacteria population treatment towards observed variable, therefore variable analysis (ANOVA) was conducted with confidence level of 95%. If the analysis results showed significant differences, it was continues with Duncan Test to see the differences between treatments. Analysis was performed using computer program SPSS ver. 24.0.

Results and Discussion

Ice-ice disease. The ice-ice disease outbreak on *E. denticulatum* occurred in March, on the sixth week of the growing period. Basically ice-ice occurs on the edge of the thallus. The edge of thallus is a young part of the thallus that grows on seaweed. Part of the thallus that has ice-ice got physical characteristics which are slimy thallus surface, friable, and white colored. Healthy thallus, whether close or far from ice-ice infection have the same characteristics of brown color, intact (not broken), not slimy and have a high amount of branches. One of the factors that cause the occurrence of ice-ice disease is the inappropriate environmental conditions, which makes the seaweed vulnerable to ice-ice disease triggered by some bacteria. Based on our observations, thallus that has ice-ice disease white was colored and appeared translucent and was slimy. It is assumed that white coloration of the thallus was caused by the loss of pigment due to environment change and less supportive for the growth of seaweed. While slimes that are produced on thallus is the result of organic substance freed (carrageenan) which triggers the occurrence of pathogen bacteria growth. Ice-ice disease is commonly marked by whitening process on the root of thallus, middle, and edge, begins with color change of thallus to become crystal white or transparent (KKP 2004). Ice-ice infection attacks on the root of thallus, stem, and edge of young thallus, causes tissue turning to white and part of thallus that turns into transparent white (Lundsor 2001; Santoso & Nugraha 2008). Healthy seaweed thallus condition is completely different from infected seaweed thallus. Healthy thallus condition includes brown color and not slimy, so that bacterial infection triggers on the seaweed does not happen. Steps of the occurrence of ice-ice disease on seaweed begin with drastic environment change. Parameter such as temperature, salinity, and water current velocity, which less supportive in a long term, causes seaweed stress. Stress caused by sudden environment condition change such as salinity, water temperature, and light intensity, becomes main triggers of the ice-ice disease occurrence on seaweed (Arasamuthu & Edward 2018; KKP 2005). The second step is that stressed seaweed shows color change from brown to white. It is assumed that pigment from the thallus has disappears caused by the environment condition, so there is no photosynthesis process. Seaweed thallus which loses its pigment and shows symptom of ice-ice disease has low carrageenan content between 0.5-25% (Hayashi et al 2010). The third step is the occurrence of slime on thallus with ice-ice disease. It is assumed that the slime comes from organic substance (carrageenan) coming out from the broken thallus cell wall caused by whitening thallus process. Organic substance release triggers for pathogen bacteria to grow and develop, so that the chance for ice-ice disease to occur is higher in combination with fluctuating environmental conditions. In a stressed condition seaweed (*Gacilaria*, *Eucheuma*, and *Kappaphycus*) will release organic substance that causes slimy thallus and stimulate bacteria to excessively grow around it (Mendoza et al 2002; KKP 2005). The fourth step is that thallus becomes soft and broken. It is assumed that before the thallus is fragile and broken, young tissue (meristem) on thallus that has ice-ice could trigger pathogen bacterial infection which worsens the seaweed condition. Bad environment condition continuously causes the thallus to break and the decrease of seaweed weight. Plant tissue on the part which got disease becomes soft and broken, while the infected branch part will crack and fractured fall to the ocean, so it causes plant weight loss (Amiluddin 2007).

Part of thallus which relatively got ice-ice disease is the edge (young thallus) compared to the root part (old thallus). Edge of the thallus is vulnerable because it is a young tissue that always performs cell division and it is categorized as vulnerable towards environmental changes. It is also assumed that immune system from the edge part of the thallus is lacking, in addition with bad environment condition makes the thallus susceptible to disease (Arasamuthu & Edward 2018). The scar on thallus represents a potential spot for pathogen bacteria installation. Some ways to seaweed get infected by bacteria of ice-ice disease are traces of cuts (cuttings for seed) and scars due to extremely tight seed bond, so that bacteria enters from thallus pores (Amiluddin 2007). Ice-ice infection attacks on the root of thallus, stem and edge of young thallus, causes tissue whitening (Largo 2002; Santoso & Nugraha 2008).

Seaweed cultivation using longline method is more vulnerable towards ice-ice disease compared to horizontal net cage method. Horizontal net cage method for cultivation could minimize the occurrence of ice-ice disease on cultivated seaweed (Kasim et al 2017). Cultivated seaweed with longline method cannot avoid a strong current flow, while horizontal net cage method could absorb strong current so it can minimize ropes erosion towards seaweed thallus. The occurrence of ice-ice disease in horizontal net cage could be due to environment factor changes like temperature and salinity (Kasim & Mustafa 2017; Kasim et al 2020).

Bacteria population. Our research results show that the highest average bacteria population occurred on part of thallus with ice-ice (TS) with 8.73 x 108 CFU mL^{-1} (log: 7.94), followed by part of healthy thallus close to ice-ice (TD) with 1.64 x 107 CFU mL^{-1} (log: 7.22) and the average of lowest population occurred on healthy thallus far from ice-ice (TJ) with 1.03 x 107 CFU mL^{-1} (log: 7.01) (Figure 1).

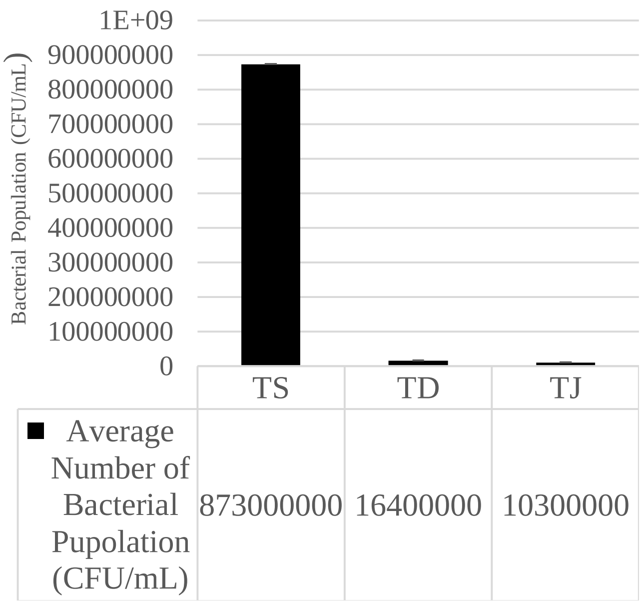


Figure 1. The average value of the bacterial population in seaweed (*Eucheuma denticulatum*). TS - part of thallus by *ice-ice*, TD - part of healthy thallus close to infected *ice-ice*, TJ - part of healthy thallus far from *ice-ice* infected spots.

Variants analysis result shows that the average bacteria population of seaweed *E. denticulatum* is significantly different (Sig.<0.05) (Table 1), therefore it was continued with Duncan test. Based on the result of Duncan test, concerning bacteria population, the part of healthy thallus close to ice-ice (TD) was not significantly different from the healthy thallus far from ice-ice (TJ), but the healthy thallus close to ice-ice (TD) and part of healthy thallus far from ice-ice (TJ) showed significant differences toward part of thallus with ice-ice (TS) (Table 2).

Table 1

Results of variants analysis on bacteria population on *Eucheuma denticulatum*

	Sum of squares	*df*	*Mean square*	*F*	*Sig.*
Between groups	3.339E16	2	1.670E16	6.631	0.030
Within groups	1.511E16	6	2.518E15		
Total	4.850E16	8			

Table 2

Results of Duncan analysis on bacteria population on *Eucheuma denticulatum*

Bacteria population	*N*	*Subset for alpha = 0.05*	
		1	*2*
TJ	3	4.0348E7	
TD	3	4.7381E7	
TS	3		1.7293E8
Sig.		0.869	1.000

Means for groups in homogeneous subsets are displayed.
Uses harmonic mean sample size = 3,000.

Thallus part that had ice-ice signs (TS1, TS2, and TS3) showed an approximately bacteria population of 9.28 x 107 to 2.62 x 106 CFU mL^{-1}, respectively. It is assumed that bacterium on thallus which has ice-ice disease is *Vibrio* sp. (Table 3).

Table 3

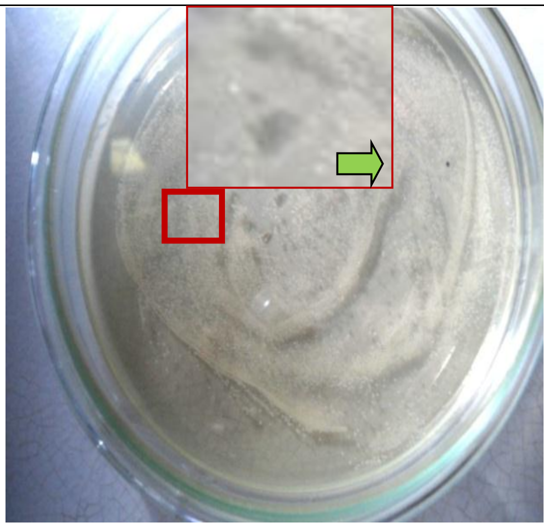
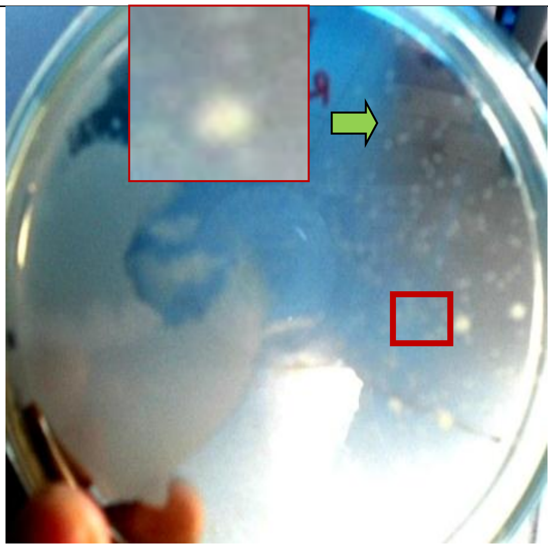
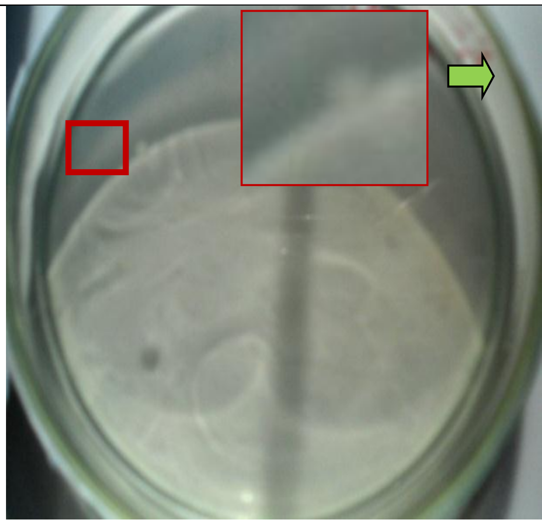
Calculation of bacteria population from each dilution

No.	*Treatment*	*Calculation of each dilution*				*Average (CFU mL^{-1})*
		(2)	*(3)*	*(4)*	*(5)*	
1	TS1	-	-	-	2.62×10^8	2.62×10^8
2	TS2	-	-	-	1.64×10^8	1.64×10^8
3	TS3	-	-	2.16×10^7	1.64×10^8	9.28×10^7
4	TD1	-	2.66×10^6	1.70×10^7	1.28×10^8	4.92×10^7
5	TD2	2.56×10^5	2.24×10^6	2.08×10^7	1.38×10^8	4.03×10^7
6	TD3	-	-	1.92×10^7	8.60×10^7	5.26×10^7
7	TJ1	2.32×10^5	2.34×10^6	2.22×10^7	9.90×10^7	3.09×10^7
8	TJ2	-	-	2.24×10^7	9.80×10^7	6.02×10^7
9	TJ3	-	-	1.98×10^7	4.00×10^7	2.99×10^7

The present research results shows that in the test preparation, there were 3 groups of colonies which gave an overview of bacterial coloni in the ice-ice thallus >300 colony (TS), thallus samples close to the ice-ice (TD) infection, 20-3000 colony and thallus which were far from the ice-ice infection, namely <30 colony (TJ) (Table 4).

Table 4

Bacteria colony of on perpetrates sample analysis

No.	Sample	Figure	Information
1	TS		Too substantial bacteria colony to count (TBUD) (>300 colonies)
2	TD		Countable bacteria colony (30-300 colonies)
3	TJ		Too low bacteria colony to count (TSUD) (<30 colonies)

Thallus part that has ice-ice disease get the highest population amount compared to the healthy thallus part close to ice-ice and healthy thallus part far from ice-ice. It is assumed that thallus which has ice-ice disease is infected with by pathogen bacteria.

While healthy thallus part close or far from ice-ice still got bacteria. It is assumed that bacteria on the healthy thallus were beneficial bacteria. It is because basically bacteria are present in every body of living being. Austin & Austin (2007) stated that every bacterium is a microorganism present in the air, water, soil, and inside of the body of living being. In line with that statement, in the present research bacteria were evidenced on healthy and ill thallus evenly. Based on the research results, TS treatment had the highest bacteria population. It is assumed that thallus which has ice-ice disease is infected with pathogen bacteria. Pathogen bacteria tend to destroy its host (thallus) which is supported by bad water environment condition for seaweed growth. Thallus part which commonly gets the disease is the edge (young) and root thallus (old). That part of the thallus that undergone the cell division process and is categorized as young tissue, has weak immune system, and in unfavorable environment condition cannot prevent bacteria growth. While root part of the thallus is old thallus and it is assumed to possess strong immune system. The occurrence of ropes bond erosion and extreme tightness of ropes bond causes scars on the thallus roots. Scarred thallus condition can be overcome by its immune system for growing well (healthy) and could push a normal bacteria growth. However, in unfavorable environment condition (change of salinity, temperature, water transparency, and drastic water current velocity) thallus immune system activity is lowered so it cannot overcome the bacteria growth. Pathogen bacteria will eventually grow on that area. So the bacteria population in TS treatment was higher. Sutrian (2004) stated that on the edge of the thallus there is initial cell which always split to form a new cell for growing process. Ekawati (2008) stated that little part of thallus is the growing point for seaweed so it has thin epidermis layer which is vulnerable towards bacteria and sensitive to environmental changes. Healthy thallus part has carrageenan and complete cellulose and function very well to protect seaweed wall cell, while thallus that has ice-ice contains low of even none carrageenan and cellulose because it is degraded by pathogen bacteria so the seaweed wall cell is broken (Lundsor 2001).

Pathogen bacteria population on thallus part that has ice-ice besides could cause weight loss of the seaweed; it could also decrease carrageenan level from the thallus. It is assumed that pathogen bacteria could degrade carrageenan which is used as nutrition for the growth. So the pathogen bacteria population increases alongside with the increase of thallus infection (Mendoza et al 2002). Pathogen bacteria could infect seaweed by degrading its chemical component which is carrageenan through polymerization result by carrageen’s enzyme (Vairappan 2006).

According to the research results, TD treatment has less bacteria population than TS treatment. It is assumed that bacteria in TD treatment are only a small part that could breed and grow although at a very low rate. It is also assumed that healthy thallus part has immune system in the form of carrageenan and cellulose that can reach the normal bacteria growth and pathogen. Lundsor (2001) stated that carrageenan and complete cellulose on healthy thallus can function well to protect seaweed wall cell from bacteria. TJ treatment had lowest bacteria population amount than TD treatment. It is assumed that thallus could press the bacteria growth. On a healthy condition, thallus produces carrageenan as immune system to avoid pathogen bacterial infection so it can minimalize the chance of disease to occur caused by bacteria. The higher carrageenan level on a thallus in supportive environment condition for seaweed growth and lower pathogen bacteria growth, so serious bacterial infection cannot occur. Lobban & Horrison (1994) stated that the occurrence of infectious disease on seaweed is caused by the imbalance between host, pathogen, and seaweed media environment. Bacteria population on healthy thallus close or far has a stable weight mass. So it is assumed that carrageenan level compound is not decreasing. It is caused by bacteria on healthy thallus part that cannot degrade carrageenan. Carrageenan is assumed as immune system that can protect thallus from bacteria and disease. However, on a less supportive environment condition, thallus immune system decreases, so the thallus is vulnerable towards disease. Trono (1974) stated that the cause of ice-ice is environmental changes which are not suitable for growth and causes a decrease of the seaweed immune system activity. Lundsor (2001) stated that carrageenan and complete cellulose on healthy thallus can function well to protect seaweed wall cell. On thallus part that had ice-ice (TS1, TS2, and

TS3) got an approximately bacteria population of 9.28 x 107 to 2.62 x 106 CFU mL^{-1}, respectively. It is assumed that bacteria on thallus which has ice-ice is *Vibrio* sp. (Arasamuthu & Edward 2018). It is based on the isolation result and bacteria identification from thallus which has ice-ice disease from Ask et al (2003), obtained bacteria from Vibrio genus that dominates seaweed thallus with ice-ice disease.

High bacterial populations on thallus affected by ice-ice disease are assumed because pathogen and also normal bacteria amount increases. Normal bacteria in unfavorable environmental conditions can turn into pathogen bacteria. Pathogen bacteria multiply their number and slowly increase the bacteria population. Bacteria is the main factor on ice-ice disease development, where was found that normal bacteria can become opportunistic pathogen in certain conditions (Largo 2002). Bacteria installations on a healthy thallus begin with the attachment on the thallus surface. Bacteria was able to grow but in small population. The existence of bacteria in health thallus is still in a small population. It is assumed that bacteria are still in normal condition to grow and also thallus (host) has good immune system to push the growth from the bacteria population. Healthy thallus has carrageenan and complete cellulose and function well to protect seaweed wall cell (Lundsor 2001).

Healthy thallus part close to ice-ice (TD) and healthy thallus far from ice-ice (TJ) has a similar bacteria population as healthy thallus close with ice-ice (TD) and healthy thallus far from ice-ice (TJ), and also healthy thallus close to ice-ice (TD) and healthy thallus far from ice-ice (TJ). Distance between healthy thallus close or far does not show difference in bacteria population amount. TD and TJ treatment had small bacteria population. It is assumed that bacteria spread in water body on good environment condition, does not affect the bacteria that attach on healthy thallus (Darma 2014). While bacteria spread in a water body in unfavorable environmental conditions, affect the number of bacteria that attach and grow on thallus with ice-ice disease. Basically bacteria spread horizontally through water media (Santoso & Nugraha 2008)

The movement of bacteria on thallus with ice-ice disease begins with the existence of bacteria on healthy thallus even though before infection by ice-ice disease. Colony of bacteria grows and breeds but in a small population. Unfavorable environmental conditions causes bacteria to be able to breed well but not for the thallus. It is assumed that unsuitable growth conditions for thallus are suitable for bacteria growth. Environmental conditions that affect this status are water current velocity, temperature, salinity, water transparency, and nutrition (Arasamuthu & Edward 2018). Slow current speed causes less nutrition that spread around the edge of thallus (young growth). The edge part of thallus is the part that grows by absorbing nutrients for photosynthesis process. However, thus nutrient is lacking so the thallus is in famished condition. The part of the thallus that starves causes disturbed metabolism. If this condition lasts longer alongside with unfavorable environment could facilitate bacteria installation and growth on the thallus. The impact of hunger (nutrient lack) and accumulation of metabolite leftover combination could cause ice-ice disease. It is assumed that such kind of condition causes those normal bacteria to become pathogen bacteria, because of the suitable living environment for its growth (Neish 2005). From 8 isolates, pathogeny test towards healthy *K. alvarezii* shows that 5 isolates could cause ice-ice disease symptom (Darmayati 2010).

Pathogen bacteria could degrade carrageenan and thallus loses their immune system. Carrageenan degradation result causes thallus to release slime. Slime on thallus triggers pathogen bacteria and other bacteria to flourish. So it favors the thallus ice-ice disease population increase. Lundsor (2001) stated that carrageenan and cellulose content decrease and even disappear because it is degraded by pathogen bacteria so the seaweed wall cell break. KKP (2005) reported that thallus with ice-ice disease will release organic substance that causes thallus to become slimy and stimulate bacteria growth and flourish around it. It also happens to root of thallus (old part). Usually the root of thallus is the place where the cultivation ropes bonds are (hanging ropes). On a healthy thallus, it appears that bacteria grow normally on the surface of the thallus. It is assumed that bad environment condition (strong current speed) with extreme tightness of ropes bond could cause thallus erosion and generates scars. Scars are the way for bacteria to enter into the thallus. Bacteria penetration causes carrageenan degradation in the thallus, and the result is a slimy surface. Besides, pathogen bacteria could also break thallus cell. Scars of thallus caused by tight seed bond could be a way for ice-ice to cause bacterial infection through scars pore (Amiluddin 2007).

Water quality parameters. Water quality parameters measured on seaweed cultivation area during the research can be seen in Table 5.

Table 5

Water quality parameters in *Eucheuma denticulatum* cultivation area during sample collection

No.	*Parameters*	*Units*	*Average value*	*Data comparison*
1	Brightness	m	6.28	>1 (Widodo & Suadi 2006)
2	Temperature	^0C	29	26-32 (Afrianto & Liviawaty 2003)
3	Depth	m	7.32	5-15 (Sulistijo 2007)
4	Current velocity	cm s^{-1}	11.56	20-40 (Indriani & Sumiarsih 2003)
5	Salinity	ppt	33	33-35 (Sadhori 1992)
6	Dissolved oxygen (DO)	mg L^{-1}	7.8	4.5-9.8 (Gerung 2007)
7	Nitrate	mg L^{-1}	0.0104	0.0071-0.0169 (Aslan 2011)
8	Phosphate	mg L^{-1}	0.0042	0.051-1.00 (Alam 2011)

High bacteria population on thallus with ice-ice disease infection was affected by drastic environmental changes. Water transparency could affect growth of bacteria. Brightness level during the research was 6.28 m. Light provide energy for photosynthesis process, so the ability of light penetration in certain depth levels really affects water organism vertical distribution (Widodo & Suadi 2006). The main factor that is highly related with light penetration is water transparency. Ideal water transparency is more than 1 m, in this case, photosynthesis process could be performed optimally, and so can produces a high quantity of oxygen. Whereas the measured dissolved oxygen level was 7.8 mg L^{-1}. This value is appropriate with dissolved oxygen (DO) level that is mandatory for the life and growth of *E. denticulatum*, of which range is from 4.5 to 9.8 mg L^{-1}. Water current that flows between small islands groups and the vast seagrass beds highly influences the relative DO concentration in water (Gerung 2007). Bacteria with high DO demand are allegedly located on the surface of thallus (Tisera & Naguit 2009). So the aggression of pathogen bacteria towards thallus is increasing. Some pathogen, especially bacteria, is very motile and could easily attack seaweed surface (Largo et al 1997). Furthermore, aerobe bacteria will be on the surface of the media because bacteria will take oxygen freely from the air, while anaerobe bacteria will be on the bottom, far from surface of experimental media (Largo et al 1999; Ulfa 2004).

Temperature level during the research was 29^oC and had salinity level of 33 ppt. Water temperature is very important for seaweed particularly to help the process of photosynthesis. That recorded temperature was appropriate for seaweed needs. Seaweed grows and breeds well in water with 26-32^oC temperature, water temperature also affects the photosynthesis process. For salinity, on that level seaweed still grow even though with ice-ice disease (Afrianto & Liviawaty 2003). Salinity level that is appropriate for seaweed growth is between 33 and 35 ppt. Temperature and salinity parameter range support seaweed growth, as well as it supports the growth of bacteria that attack the thallus. Pathogen bacteria can grow under low and even high temperature conditions (Sadhori 1992). Ice-ice disease is basically caused by less favorable environmental conditions in the plantation area. Unsupportive factors in cultivation area refer to high or low temperature, high or low salinity, high or low light intensity, and enough nutrition (Largo 2002). Seaweed cultivation is conducted in 5-15 m depth water area. The depth during the research was 7.32 m. The water depth is haigly correlated with light penetration, temperature, and DO content (Sulistijo 2002).

Current speed recorded during the research was 11.56 cm s^{-1}. It shows that the current speed does not support seaweed growth, but in opposite, it supports the pathogen bacteria population growth. Current speed value that is suitable for seaweed cultivation is between 20 and 40 cm s^{-1} (Indriani & Sumiarsih 2003). Some pathogens, especially bacteria, are very motile and easily attack the surface of seaweed. Current speed not only increase nutrition exchange but also prevents pathogen potential which comes from the water around it for the growth of seaweed thallus (Largo et al 1997). When seaweed is stressed because of low salinity, temperature, water movement, and light intensity, will facilitate pathogen infection (Imardjono et al 1989).

During our research, nutrient level was 0.0104 mg L^{-1} nitrate and 0.0042 mg L^{-1} phosphate. Good water nitrate concentration for seaweed growth is 0.0071-0.0169 mg L^{-1} (Aslan 2011). The optimal phosphate concentration for seaweed growth is between 0.051 and 1.00 mg L^{-1}. In case of lower phosphate availability (less than its optimal), the amount will be used as nutrient for photosynthesis process and metabolism. Low nutrition is caused by weak current speed. A lack of nutrition distribution affects the thallus metabolism. Thus, this condition decreases seaweed growth that affects its vulnerability towards ice-ice disease (Alam 2011). In very quiet waters, the impact of nutrient hunger and metabolite leftover accumulation could cause occurrence of ice-ice disease (Neish 2005).

Conclusions. The recorded average bacteria population of *E. denticulatum* on thallus with ice-ice disease (TS) was 8.73 x 108 CFU mL^{-1} (log: 7.94). On healthy thallus close to ice-ice (TD) was 1.64 x 107 CFU mL^{-1} (log: 7.22) and bacteria population average on healthy thallus far from ice-ice (TJ) was 1.03 x 107 CFU mL^{-1} (log: 7.01). Bacteria populations were grown rapidly on thallus infected by ice-ice disease and in a normal range on thallus far from ice-ice infected spots.

Acknowledgements. We are grateful to the Ministry of Research and Technology/BRIN of the Republic Indonesia, for the full research funding of the present research project. Gratitude for Fishery Laboratory, Faculty of Fishery and Marine Science Halu Oleo University for their assistance in sample analysis. Many thanks for LPPM UHO for facilitation of this research.

References

Afrianto E., Liviawaty E., 2003 Sea cultivation and processing. Bharata Press, Jakarta, Indonesia, 84 p.

Alam A. A., 2011 Carrageenan quality seaweed type *Eucheuma spinosum* in the waters of Punaga Village, Takalar Regency. Undergraduate Thesis, Faculty of Fisheries and Marine Sciences, Hasanuddin University, Makassar, Indonesia, 40 p.

Alibon R. D., Gonzale J. M. P., Ordoyo E. A.T., Echem R. T., 2019 Incidence of Ice-ice disease associated with *Kappaphycus alvarezii* in the seaweed farms in Zamboanga Peninsula, Mindanao, Philippines. SSR Institute of International Journal of Life Sciences 5(1):2148-2155.

Amiluddin N. M., 2007 Study of growth and content of carrageenan of *Kappaphycus alvarezii* seaweed affected by Ice-ice disease in the Pari Island Waters of Seribu Islands. Thesis, Graduate School of Bogor Agricultural University, Bogor, Indonesia, 92 p.

Ask E. I., Batibasaga A., Zertuche-Gonzalez J. A., De San M., 2003 Three decade of *Kappaphycus alvarezii* (Rhodophyta) introduction to non-endemic locations. Proceedings of the 17th International Seaweed Symposium, Cape town, 2001. Oxford University Press.

Aslan L. M., 2011 Development strategy for seaweed cultivation in Indonesia. Inaugural speech as professor of Fisheries on January 22, 2011, Faculty of Fisheries and Marine Sciences, Haluoleo University, Kendari, Indonesia, 50 p.

Arasamuthu A., Edward J. K., 2018 Occurrence of Ice-ice disease in seaweed *Kappaphycus alvarezii* at Gulf of Mannar and Palk Bay, Southeastern India. Indian Journal of Geo Marine Sciences 46(6):1208-1216.
Austin B., Austin D. A., 2007 Bacterial fish pathogen: Disease in farmed and wild fish. Springer, Heriot-Watt University, 552 p.
Budiyanto, Kasim M., Abadi S. Y., 2019 Growth and carrageenan content of local and tissue culture seed of *Kappaphycus alvarezii* cultivated in floating cage. AACL Bioflux 12(1):167-178.
Darma T. A., 2014 Study of bacteria population in seaweed (*Eucheuma spinosum*) infected by Ice-ice disease cultivated in floating cage at Tanjung Tiram Coast, Southeast Sulawesi Province. Thesis of the Faculty of Fisheries and Marine Sciences, Halu Oleo University, Indonesia, 113 p.
Darmayati Y., 2010 Pathogenic bacteria causing Ice-ice disease in seaweed *Kappaphyus alvarezii* (Doty, 1986) (*Eucheeuma cotonii*). Bogor Agricultural University University, Bogor, Indonesia, pp. 357-366.
Ekawati C., 2008 Identification of the causes of Ice-ice disease in *Kappaphycus alvarezii* seaweed (Gigartinales, Rhodophyta) with the Long Line Method in Puasana Village, Moramo District, South Konawe Regency. Thesis, Department of Fisheries, Halu Oleo University, Indonesia, 41 p.
Gerung G. S., 2007 Study on the environment and trials cultivation of *Kappaphycus* and *Eucheuma* in Nain Island, Indonesia. Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, 54 p.
Gobel B. R., 2008 General microbiology in practice. Hasanuddin University, Publishing, Makasar, 40 p.
Hayashi L., Nair S., Yokoya, Ostini S., Ricardo T. L., Elisabete P. S., Eurico B., Oliveira C., 2010 Nutrients removed by *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) in integrated cultivation with fishes in re-circulating water. Aquaculture 277:185-191.
Imardjono S., Yuwono S. K., Hermawan A., 1989 The important species of seaweed culture in Indonesia. The Training on Laminaria (Seaweed) Polyculture with Molluscs. Qing Dao. People’s Republic of China 15 June-31 July 1989.
Indriani H., Sumiarsih E., 2003 Seaweed cultivation, processing and marketing. Penebar Swadaya Publishing, Jakarta, Indonesia, 87 p.
Jaya A. M. S., 2014 The use of Tembelekan plant powder (*Lantana camara*) for the prevention of Ice-ice disease in seaweed (*Kappaphycus alvarezii*) cultivated with longline. Thesis, Department of Fisheries, Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari, Indonesia.
Kasim M., Mustafa A., Munier T., 2016 The growth rate of seaweed (*Eucheuma denticulatum*) cultivated in longline and floating cage. AACL Bioflux 9(2):291–299.
Kasim M., Mustafa A., 2017 Comparison growth of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae) cultivation in floating cage and longline in Indonesia. Aquaculture Reports 6:49-55.
Kasim M., Jamil M. R., Irawati, 2017 Occurrence of macro-epiphyte on *Eucheuma spinosum* cultivated on floating cages. AACL Bioflux 10(3):633-639.
Kasim M., Asjan, Effendy I. J., Wanurgayah, Ishak E., 2018 Influence of initial weight of seeds in variation of growth and carrageenan content of *Eucheuma spinosum*. AACL Bioflux 11(4):1155-1163.
Kasim M., Marlia, Abdullah, Balubi A. M., Djalil W., 2019 Succession of epiphyte on thallus of *Kappaphycus alvarezii* (Rhodophyta) in horizontal net cage culture. AACL Bioflux 12(5):1710-1717.
Kasim M., Balubi A. M., Mustafa A., Nurdin R., Patadjai R. S., Jalil W., 2020 Floating cage: A new innovation of seaweed culture. Chapter Book. Emerging Technologies, Environment and Research for Sustainable Aquaculture, IntechOpen. http://dx.doi.org/10.5772/intechopen.82887
Khan S. I., Satam S. B., 2003 Seaweed mariculture. Scope and potential in India. Aquaculture Asia 8(4):26-29.

Largo D. B., 2002 Recent developments in seaweed diseases. University of San Carlos, Philippines, Department of Biology, Proceedings of the National Seaweed Planning Workshop, pp. 35-42.
Largo D. B., Fukami K., Adachi M., Nishijima T., 1997 Direct enumeration of total bacteria from macroalgae by Epifluorescence Microscopy as applied to the fleshy red algae *Kappaphycus alvarezii* (Doty) and *Gracilaria* spp. (Rhodophyta). Journal of Phycology 33:554-557.
Largo D. B., Fukami K., Nishijima T., 1999 Time-dependent attachment mechanism of bacterial pathogen during ice-ice infection in *Kappaphycus alvarezii* (Gigartinales, Rhodophyta). Proceedings of International Seaweed Symposium 16:643-50.
Lay B. W., 1994 Microbial analysis in the laboratory. PT. Raja Grafindo Persada Press. Jakarta, Indonesia, 168 p.
Lobban C. S., Horrison P. J., 1994 Seaweed ecology and physiology. Cambridge University Press, New York.
Lundsor E., 2001 Eucheuma farming on Zanzibar broadcast system: an alternative method for seaweed farming? 2nd Western Indian Ocean Science Association (WIOMSA) Scientific Symposium - Book of Abstracts, p. 29.
Mendoza W. G., Montano N. E., Ganzon-Fortes E. T., Villanueva R. D., 2002 Chemical and gelling of infected *Kappaphycus alvarezii* by ice-ice disease. Journal of Applied Phycology 14:409-418.
Neish I. C., 2005 The Eucheuma seaplant handbook. Vol I. Basic Physiology. Seaplantnet Technical Monograph, 38 p.
Sadhori S. N., 1992 Seaweed cultivation. Balai Pustaka Publishing, Jakarta, Indonesia, 110 p.
Santoso L., Nugraha Y. T., 2008 Ice-ice disease control to increase Indonesian seaweed production. Journal of Fisheries Saintek 3(2):37-43.
Sulistijo, 2002 Seaweed cultivation research (Algae macro/seaweed) in Indonesia. Speech Inauguration of Lead Research Expert in Aquaculture Research Center for Oceanography of the Indonesian Institute of Sciences, Jakarta, Indonesia.
Sutrian Y., 2004 Introduction to anatomy, plants (About cells and tissue). Jakarta: PT Rineka Cipta Publishing, 80 p.
Tisera W. L., Naguit M. A., 2009 Ice-ice disease occurrence in seaweed farms in Bais Bay, Negros Oriental and Zamboanga Del Norte. The Threshold 4:1-16.
Tokan M. K., Dirawan G. D., Imakulata M. M., 2015 Controlling of the Ice-ice disease on seaweed through polyculture cultivation techniques. Asian Journal of Microbiology Biotechnology & Environmental Sciences 17(1):7-15.
Trono Jr. G. C., 1974 Eucheuma farming in the Philippines. University of the Philippines and Natural Science Research Center, Quezon City, Philippines.
Trono Jr. G. C., 1993 Effect of biological, physical and socio- economic factors on the productivity of Eucheuma/Kappaphycus farming industry. In: Calumpong H. P., Menez E. G. (eds), pp. 239-245, Proc. Second RP-USA Phycology Symp./Workshop, Cebu Cityand Dumaguete City, Philippines.
Ulfa U., 2004 Instructions for microbiology practicum. Malang: Malang State Islamic University, 21 p.
Uyenco F. R., Saniel L. S., Jacinto G. S., 1981 The 'Ice-ice' problem in seaweed farming. Proceedings of International Seaweed Symposium 10:625-630.
Vairappan C. S., 2006 Seasonal occurrences of epiphytic algae on the commercially cultivated red alga *Kappaphycus alvarezii* (Solieriaceae, Gigartinales, Rhodophyta). Journal of Applied Phycology 18:611–617.
Widodo, Suadi, 2006 Management of marine fisheries resources. Yogyakarta, Indonesia, 300 p.
Yahya D., 2013 Pattern of small business financing (PPUK). Seaweed cultivation. The basic layout method. Bank of Indonesia, 48 p.
Yulianto K., 2003 Observation of Ice-ice disease and competitor algae phenomenon causing the failure of harvest of *Kappaphycus alvarezii* (C) seaweed (Argadh) in the Seribu Islands, Pari Island in 2000 and 2001. Proceedings of the National Marine Science and Technology Seminar, 119 p.

*** KKP, 2004 Profile of Indonesian seaweed. Ministry of Maritime and Fisheries Affairs. Directorate General of Aquaculture. Jakarta, Indonesia, 25 p.
*** KKP, 2005 Ice-ice disease control instructions in seaweed cultivation. Ministry of Maritime and Fisheries Affairs. Directorate General of Aquaculture. Jakarta, Indonesia, 15 p.

Received: 01 October 2020. Accepted: 18 February 2021. Published online: 25 February 2021.
Authors:
Tuti Ardianti Darma, Alumni Halu Oleo University, Faculty of Fishery and Marine Sciences, Indonesia, Southeast Sulawesi, 93231, Kendari, Andounohu, Kampus Bumi Tridarma UHO, e-mail: tutiadriantidarma2020@gmail.com
Ma'ruf Kasim, Halu Oleo University, Faculty of Fishery and Marine Sciences, Indonesia, Southeast Sulawesi, 93231, Kendari, Andounohu, Kampus Bumi Tridarma UHO, e-mail: marufkasim@uho.ac.id
Ruslaini, Halu Oleo University, Faculty of Fishery and Marine Sciences, Indonesia, Southeast Sulawesi, 93231, Kendari, Andounohu, Kampus Bumi Tridarma UHO, e-mail: ruslaini08@yahoo.co.id
Indriyani Nur, Halu Oleo University, Faculty of Fishery and Marine Sciences, Indonesia, Southeast Sulawesi, 93231, Kendari, Andounohu, Kampus Bumi Tridarma UHO, e-mail: indrinoer@gmail.com
Abdul Rahman, Halu Oleo University, Faculty of Fishery and Marine Sciences, Indonesia, Southeast Sulawesi, 93231, Kendari, Andounohu, Kampus Bumi Tridarma UHO, e-mail: Rahman_uho@yahoo.co.id
Wellem Muskita, Halu Oleo University, Faculty of Fishery and Marine Sciences, Indonesia, Southeast Sulawesi, 93231, Kendari, Andounohu, Kampus Bumi Tridarma UHO, e-mail: wellem_muskita@yahoo.com
Muhaimin Hamzah, Halu Oleo University, Faculty of Fishery and Marine Sciences, Indonesia, Southeast Sulawesi, 93231, Kendari, Andounohu, Kampus Bumi Tridarma UHO, e-mail: imimhmz@gmail.com
Abdul Muis Balubi, Halu Oleo University, Faculty of Fishery and Marine Sciences, Indonesia, Southeast Sulawesi, 93231, Kendari, Andounohu, Kampus Bumi Tridarma UHO, e-mail: ilmibahrain02@gmail.com
Rahmat Sofyan Patadjai, Halu Oleo University, Faculty of Fishery and Marine Sciences, Indonesia, Southeast Sulawesi, 93231, Kendari, Andounohu, Kampus Bumi Tridarma UHO, e-mail: r.sofyanpatadjai@gmail.com
Wardha Jalil, Dayanu Iksanuddin University, Faculty of Fisheries, Indonesia, 93231, Southeast Sulawesi, Kota Baubau, Betoambari, Kampus UNIDAYAN, e-mail: wardha.jalil@yahoo.co.id

How to cite this article:
Darma T. A., Kasim F., Ruslaini, Nur I., Rahman A., Muskita W., Hamzah M., Balubi A. M., Patadjai R. S., Jalil W., 2021 Bacteria population on *Eucheuma denticulatum* (Rhodophyta) thallus infected by ice-ice disease cultivated on horizontal net cage. AACL Bioflux 14(1):399-411.