JIP
Jurnal Ilmiah PLATAX
Universitas Sam Ratulangi
Terakreditasi Ristekdikti No: 28/E/KPT/2019
ISSN : 2302-3589 (Online)
E_mail : jip_platax@unsrat.ac.id

Analysis Of Particle Content In *Kappaphyccus alvarezii* Seaweed In The Waters Of Arakan Village Using Scan Electron Microscopy–Energy Dispersive X-ray Spectroscopy (SEM-EDX)

Sandra Tilaar[1], Stenly Wullur*[1], Esther Angkouw[2]

[1]Marine Science Study Program, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, Indonesia.

[2]Aquaculture Study Program, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado, Indonesia. Corresponding Author: Sandra Tilaar,

*Corresponding author: stenlywullur@unsrat.ac.id

Manuscript received: 15 Oct. 2023. Revision accepted: 21 Nov. 2023.

Abstract

Kappaphycus alvarezii seaweed is widely cultivated in Indonesia and has important economic value. Nevertheless, this seaweed is easily attacked by diseases. This study was to determine the ice-ice triggers in seaweed farming locations. This research study was conducted at Arakan Village, South Minahasa, North Sulawesi. We performed the bacteria isolation on the infected thallus of *Kappaphycus alvarezii* and found *Staphylococcus arlettae* caused the infection. *Staphylococcus aureus* used to be found on the skin and mucous membranes of healthy humans, while *Staphylococcus epidermis* inhabits only the skin of healthy humans. Approximately 30% of the normal healthy population was affected by *Staphylococcus aureus* as it asymptomatically colonizes human hosts. How this *Staphylococcus arlettae* (mainly found in pork farms) infected *Kappaphycus alvarezii* which is in a high salinity environment requires an in-depth study. We completed the experiment by using Scan Electron Microscopy–Energy Dispersive X-ray Spectroscopy (SEM-EDX), analysis on infected thallus *Kappaphycus alvarezii*, and a series of bacteria isolation to observe the morphology and the element contents of *Staphylococcus arlettae* bacteria has successfully attached to the surface of *Kappaphycus alvarezii* and caused an infection. This proves that environmental changes have stimulated pathogenic bacteria *Staphylococcus arlettae* in the area where *Kappaphycus alvarezii* is cultivated. The mechanism of biosorption by microbes that can live in environments that are contaminated with Pb metal is active uptake. This mechanism occurs simultaneously in line with the consumption of metal ions for the growth of microorganisms. *Staphylococcus arlettae* are resistant to heavy metals due to the ability to detoxify the influence of heavy metals in the presence of protein or granular material. From the results of the study using SEM-EDX of algae and bacteria *Staphylococcus arlettae*, it can be concluded that the content of the particle of metal in thallus triggers bacteria to live on the seaweed as an energy source.

Keywords: Bacteria, disease, infection, *Kappaphycus alvarezii*, seaweed, *Staphylococcus arlettae*

***Abbreviations** (if any): All important abbreviations must be defined at their first mention there. Ensure consistency of abbreviations throughout the article.*

***Running title :** Particles in bacteria and seaweed Kappaphycus alvarezii using Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy*

Introduction

Seaweed, the main commodity of cultivation in Indonesia is *Eucheuma cottonii* now known as *Kappaphycus alvarezii.* The first step in the success of seaweed cultivation is the factor of land feasibility (Gerung *and* Ngangi, 2017). The potential of seaweed commodities as a whole is large, but the use of land in this cultivation is not optimal, especially for areas that geographically have the potential for seaweed cultivation (Noor, 2015). *Kappaphycus alvarezii* has the prospect of seaweed cultivation which is very profitable

http://ejournal.unsrat.ac.id/index.php/platax 10.35800/jip.v10i2.51565

if it is developed as a basic material for the production of various needs in the industrial world (Surni, 2014). The success factor of seaweed cultivation, in addition to being determined by the right cultivation method, is necessary to pay attention to good environmental quality, in addition to other factors that influence it, such as the quality of seaweed that will be used in cultivation. The success rate of seaweed farming activities is greatly influenced by environmental factors, including salinity (Ariyanti, *et.al* 2016). However, the main obstacle in seaweed cultivation is the prevalence of *ice-ice* disease causes the seaweed stems to break up and eventually die. The prevalence of *ice-ice* can be caused by water quality conditions in declining waters (Ngangi, 2012).

Seaweed is categorized into poikilothermic organisms where growth and metabolism are strongly influenced by environmental conditions. If the environmental condition such as salinity, temperature, current, and light intensity change then seaweed will be easily stressed (Hurtado, 2013). When stressed, seaweed releases organic substances that cause *thallus* slimy. This condition stimulates the bacteria to grow around it and causes disease, then circumstance decreasing seaweed production (Mendoza, *et al*., 2002 and Lachnit *et al*., 2009).

The results of several studies that the presence of diseases in the cultivation on seaweed are the bacteria. However, infectious information on seaweed *Kappaphycus alvarezii* which are the main commodities in seaweed cultivation, has not been widely known and research. Therefore, this research can provide more data and information for further research, in relation to reducing the level of pathogenicity when cultivating *Kappaphycus alvarezii*. The purpose of this study was analyze the content of particles contained in seaweed that is infected with disease and bacteria that cause disease in seaweed, in Arakan Village, South Minahasa, North Sulawesi .

MATERIALS AND METHODS

Extract Preparation for Bacterial Isolation

Samples of seaweed infected with the disease were taken in the waters of Arakan Village, South Minahasa district, North Sulawesi (Fig.1). The sample was then taken to the Biotechnology and Molecular Laboratory, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University Manado North Sulawesi, for analysis.

First, *thallus* (0.5 mm) of *Kappaphycus alvarezii* was homogenized in 0.1 mL of sterile seawater and crushed with mortar to get the solution. The supernatant solution was placed in a media Nutrient Algae (NA) plate. This sample was incubated at 37°C for 48 hours. Furthermore, the sample was diluted to 10^{-1} µL, 10^{-2} µL, 10^{-3} µL and 10^{-4} µL. The solution of concentration 10^{-3} µL and 10^{-4} µL was inoculated again into media NA plate and incubated at 37˚C for about 48 hours. Growing bacterial isolates were isolated based on their morphological characteristics following the method of identification bacteria by Cappucino *and* Sherman (1998).

The bacterial isolates were regrown in Nutrient Broth media and incubated at 37° C for ± 24 hours. Bacteria that have grown are indicated by the presence of turbidity in the medium as much as 6 ml of centrifuge at 4° C for 5 minutes at a speed of 14,000 rpm (Sari et al., 2013). The supernatant resulting from the centrifuge is then discarded and the pellets that are left behind are bacterial isolates which will continue to the Deoxyribonucleic Acid (DNA) isolation stage.

Bacterial DNA was isolated using DNeasy Blood and Tissue Kit (QIAGEN). DNA isolation was started by adding 180 µl ATL Buffer into 1.5 ml ependorph which already contained DNA pellets. Next, add 20 µl of Proteinase K and vortex for 15 seconds then incubate at 55° C for 1 hour. In the incubation process, bacteria samples are vortexes every 15 minutes. After 1 hour, add 200µl Buffer AL and vortex for 15 seconds. Samples were incubated again at 70° C for 10 minutes. Next, add 200 µl of 100% ethanol and vortex for 15 seconds. The next step is to move the sample

http://ejournal.unsrat.ac.id/index.php/platax 10.35800/jip.v10i2.51565

solution to the DNeasy Mini Spin Column which is above the 2 ml Collection Tube. The sample is put into centrifuge at a speed of 8000 rpm for 1 minute and drain the liquid that is at the bottom. Next, add 500 µl AW 1 and centrifuge for 1 minute at 8000 rpm and then drain the liquid at the bottom. Add 500 µl of AW2 and centrifuge for 4 minutes at 14,000 rpm and after that drain the liquid again at the bottom. After that, move the DNeasy mini spin column to the top of the Elution Tube and add 200µl Buffer AE to the center of the DNeasy mini spin column and then incubate at room temperature for 1 minute. The final step, centrifuge the sample at a speed of 8000 rpm for 1 minute. DNA has been in the elution tube and has been isolated.

Application of Scan Electron Microscopy – Energy Energy-dispersive X-ray Spectroscopy (SEM-EDX)

Analysis for Scan Electron Microscopy, we applied Scan Electron Microscopy (SEM) with SNE 4500M which is integrated with the Energy Dispersive X-ray Spectroscopy (EDX). The SEM-EDX was used to determine the morphology and the composition of particles found in seaweed and bacteria. For the morphological observation process and particle data, seaweed samples were cut into small pieces. In terms of bacterial samples, they were taken from agar plates and affixed to the specimen holder using carbon double types. Previously the sample was vacuumed to remove water content, so that the filament became optimal for analysis.

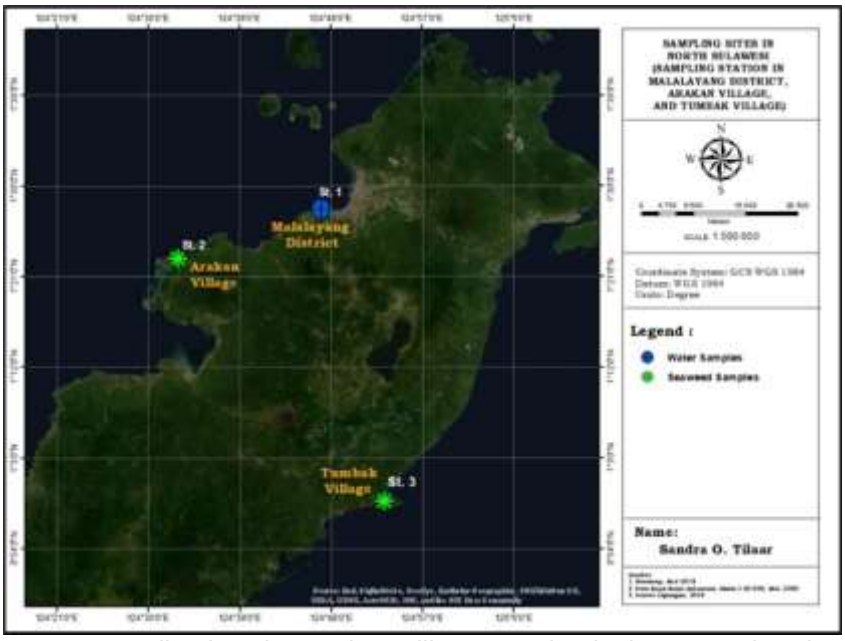

Figure 1. Sampling location Arakan Village, South Minahasa, North Sulawesi

Pengambilan Sampel Zooplankton

Application of Scan Electron Microscopy – Energy Energy-dispersive X-ray Spectroscopy (SEM-EDX)

Analysis for Scan Electron Microscopy, we applied Scan Electron Microscopy (SEM) with SNE 4500M which is integrated with the Energy Dispersive X-ray Spectroscopy (EDX). The SEM-EDX was used to determine the morphology and the composition of particles found in seaweed and bacteria. For the morphological observation process and particle data, seaweed samples were cut into small pieces. In terms of bacterial samples, they were taken from agar plates and affixed to the specimen holder using carbon double types. Previously the sample was vacuumed to remove water content, so that the filament became optimal for analysis.

RESULTS AND DISCUSSION

http://ejournal.unsrat.ac.id/index.php/platax 10.35800/jip.v10i2.51565

Results

Isolation and Identification of Bacteria

Seaweed of *Kappaphycus alvarezii* in Indonesia are famous and easy to cultivate with varieties of methods (Parenrengi *and* Sulaeman, 2007; Nursidi *et.al*, 2017; Kasim *and* Mustafa, 2017; Rama *et al*., 2018). In fact, the effort to cultivate *Kappaphycus alvarezii* organically has begun in Boalemo District, Gorontalo Province, Indonesia (Tuiyo, 2016). Although these algae are easy to cultivate and have high economic value, they are easily attacked by diseases. We can see at Figure 2A is fresh and healthy seaweed. Figure 2B is seaweed infected by bacteria. Based on the isolation results from seaweed infected with *ice ice* in Arakan village waters, obtained 3 bacterial isolates. These bacterial isolates were obtained after purification three times. The morphology of each bacterial isolate originating from seaweed can be seen in Figure 3.

Figure 2. *Kappaphycus alvarezii*

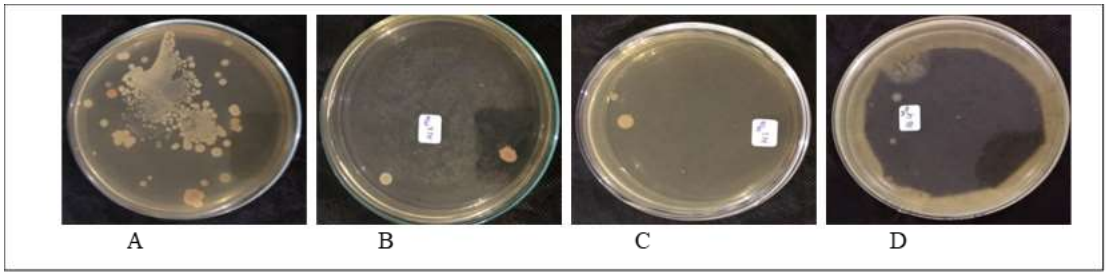

Figure 3. (A) Bacterial colony (B); Bacterial isolation through different dilutions (C) Bacterial Isolation 2nd and (D) Bacterial Isolation 2nd

These bacteria produce hormones such as in dole acetic acid, solubilize phosphate, fix atmospheric nitrogen and synthesize side raphes and anti-microbial products. Concurrently, heavy metal reduction by bacteria may occur directly via enzymatic reduction or complex formation with metabolites (*Cervantes and Campos-Garcia, 2007;* Khan *et al*. 2009). For identification of this isolate, 16SRNA sequencing was carried out. A 1.5 kb 16SRNA fragment was amplified using consensus primers and sequenced. The sequence showed 99% similarity to *Staphylococcus arlettae*. On the basis of the 16SRNA gene sequence analysis, the isolate was identified as *Staphylococcus arlettae* strain LCR34 and submitted with NCBI Gen Bank with accession No.FJ976543.1.

Serial studies in Asian region had been conducted on *Kappaphycus alvarezii* regarding disease infected by several disease agents (Vairappan *et.al*. 2008; Solis *et al*., 2010; Pang *et al*., 2015; Syafitri *et.al*. 2017). Syafitri *et al*. (2017) revealed that on molecular characterization, the disease on this kind of algae mainly by bacteria which comes from the genera of *Alteromonas*, *Bacillus*, *Pseudomonas*, *Pseudoalteromonas*, *Glaciecola*, *Aurantimonas*, and *Rhodococcus*. *Bacillus* was found as agar-digester by forming a clear depression around their colony and/or liquefaction of agar on *Gracilariopsis heteroclite* (Martinez *and* Padilla, 2017). In

http://ejournal.unsrat.ac.id/index.php/platax 10.35800/jip.v10i2.51565

the case of *Kappaphycus alvarezii*, bacterial pathogens belonging to the *Vibrio-Aeromonas* complex and the *Cytophaga-Flavobacterium* complex demonstrate the seaweed-bacteria interaction (Largo *et al*. 2012). We found the new strain of bacteria cause infection to *Kappaphycus alvarezii* which is called *Staphylococcus arlettae.* Studied in several strains on *Staphylococcus arlettae* bacteria showed that these have high remarkable survivability amidst the physicochemical pressures present, both intracellular and externally (Onyango *and* Alreshidi, 2018). *Staphylococcus aureus* normally is found on the skin and mucous membranes of a healthy human, while *Staphylococcus epidermis* inhabits only the skin of healthy humans.

Scan Electron Microscopy–Energy Dispersive X-ray spectroscopy (SEM-EDX) Analysis of *Kappaphycus alvarezii*

Structure morphology of *Kappaphycus alvarezii* infected by bacteria is shown in Figure 4. The particle content is shown in Figure 5. Tissues are showing signs of decomposition and bright color. The dominant particle content were Si, Mg, Al and Ca. Mineral contents for healthy algae are K, Ca, P, S, Mn, Fe, Cu, Zn, Ni, Br, Na, Mg and Al, with the higher amount of Na, K, Ca and Mg for macro minerals (Ruperez, 2002; Tillaivasan, 2019). Thus, infected seaweed causes its mineral content to decrease.

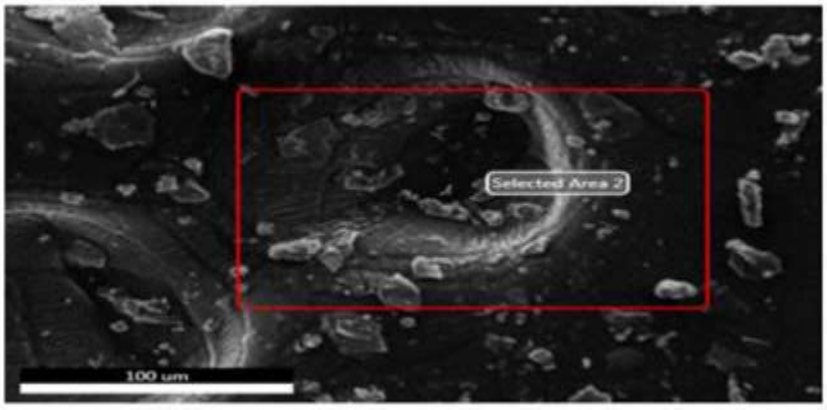

Figure 4. Morphological structure of *Kappaphycus alvarezii* was infected by Bacterial

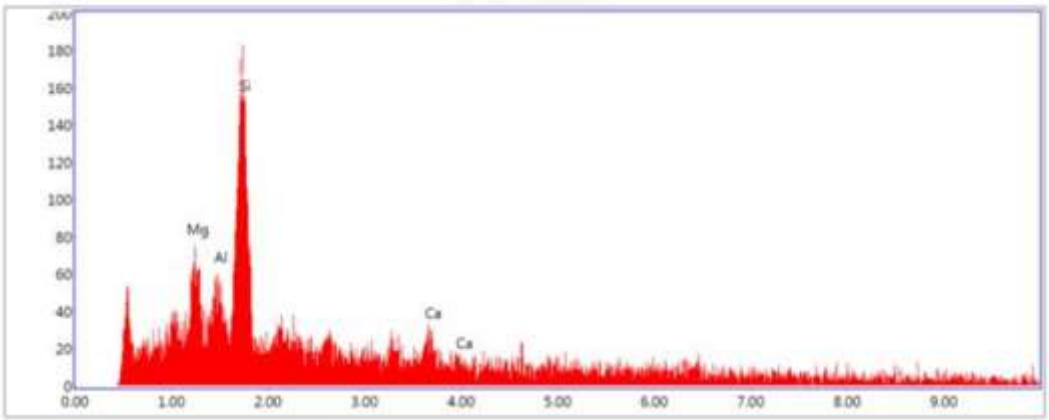

Figure 5. Particle composition in *Kappaphycus alvarezii* was infected by Bacterial

Scan Electron Microscopy– Energy Dispersive X-ray spectroscopy (SEM-EDX) Analysis of Bacterial Isolates.

Results of analysis SEM-EDX on bacteria isolation are shown on Figure 5-8. This research study, *Staphylococcus arlettae* bacteria has successfully attached to the surface of *Kappaphycus alvarezii* and caused infection. The mineral content of the bacteria increase in showing Figure 6-8.

http://ejournal.unsrat.ac.id/index.php/platax 10.35800/jip.v10i2.51565

This result proves that there have been environmental changes that have stimulated pathogenic bacteria *Staphylococcus arlettae* in the area where *Kappaphycus alvarezii* was cultivated. This phenomenon is known as dysbiosis (Fernandes *et al*., 2012; Zozaya-Valdes *et al*., 2015; Egan and Gardiner, 2016). Currently, Syafitri *et.al*. (2017) conducted a study *Kappaphycus alvarezii* in Karimunjawa Island, North Java Sea, Indonesia. They found that *Alteromonas macleodii*, *Pseudoalteromonas issachenkonii* and *Aurantimonas coralicida* caused pathogenity in *Kappaphycus alvarezii.*

Bacteria *Staphylococus arlettae* are able to grow in metal-contaminated media and have the ability to accumulate metals in their cells wall. Metal ions are positively charged, so they are electrostatically bound to the cell surface. The interaction between metal ions and bacterial cells wall shows the role of carboxyl groups in peptidoglycan and phosphoryl groups in the secondary polymers of the teikic and theicuronic acids (Solikha & Dwianita, 2013). The mechanism of biosorbtion by microbes that can live in environments that are contaminated with Pb metal and this is active uptake. This mechanism occurs simultaneously in line with the consumption of metal ions for the growth of microorganisms (intracellular accumulation of metal ions) (Fakhrudin *et al*., 2008). Bacteria are resistant to heavy metals due to the ability to detoxify the influence of heavy metals in the presence of protein or granular material (Adi & Nana, 2010).

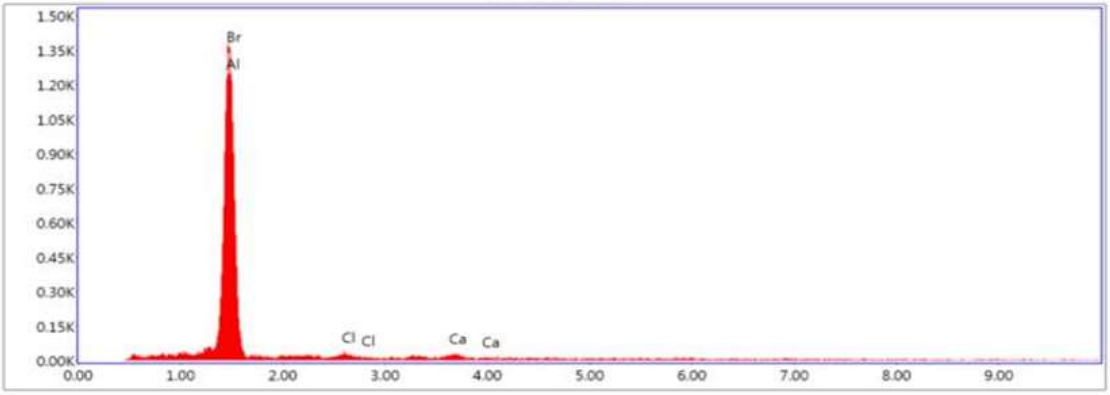

Figure 6. Element content on Bacteria Isolation 1 *Kappaphycus alvarezii*

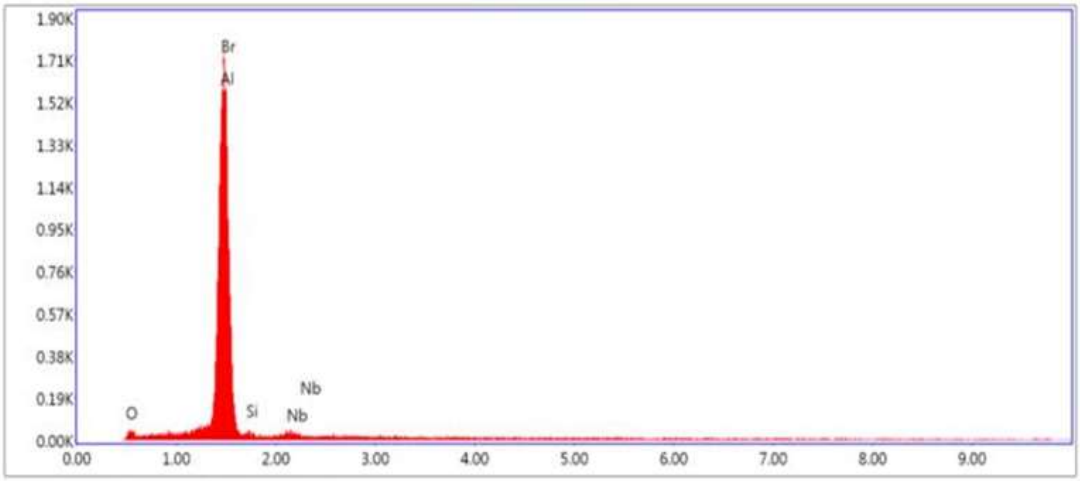

Figure 7. Element content on Bacteria Isolation 2 *Kappaphycus alvarezii*

http://ejournal.unsrat.ac.id/index.php/platax 10.35800/jip.v10i2.51565

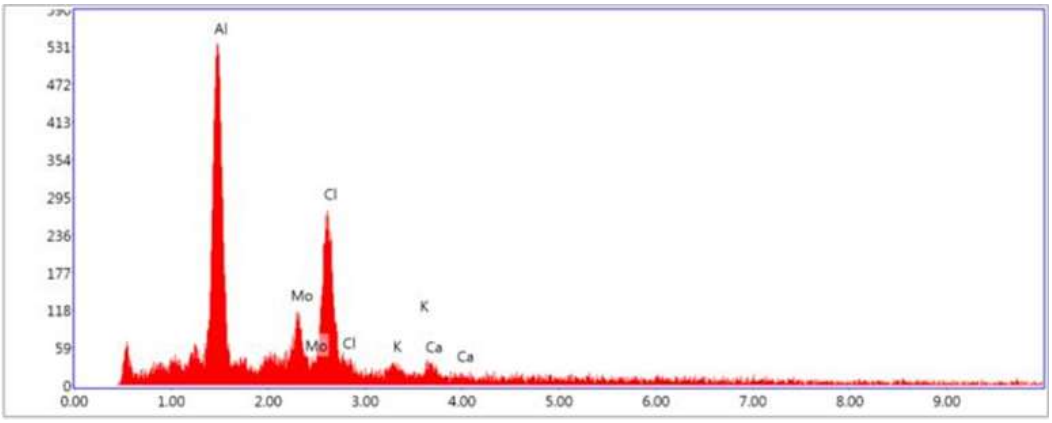

Figure 8. Element content on Bacteria Isolation 3 *Kappaphycus alvarezii*

Discussion

Metal enters to seaweed influenced by the compounds that exist on the seaweed, where the compound is able to absorb metal ions into the cells. The entry of metallic elements into plants resulting in compounding, between metals and proteins and polysaccharides that subsequently able to penetrate the cells wall and enter into the cytoplasm (Sukma Qumain *et.al.*2016). *Ice-ice* is an effect of growing old seaweed and nutritional deficiencies, characterized by the occurrence of red spots/patches in some of the old *thallus* being pale yellow and eventually gradually become white and eventually become crushed or fall (Runtuboy, 2004). From the results of the study using SEM-EDX on algae and bacteria, it can be concluded the presence of metal content that causes damage to the *thallus* triggers bacteria to live on the seaweed *thallus*. The morphological change of *Kappaphycus alvarezii* can result from the inability of biosorption and seaweed growth ability (Bambang Yulianto *et al*. 2006). *Kappaphycus alvarezii* undergoes morphological changes due to bacterial infection. The symptoms of *ice-ice* disease are generally characterized by bleaching at the base of *thallus*, and young *thallus* end, which begins with a change of the color of *thallus* to clear white or transparent, as well as being pale and on some branches to rot (Largo *et.al*.1995). *Thallus* becomes fragile and easily broke and symptoms shown are slow growth. When seaweed is stressed it will facilitate pathogenic infections. On a stressful state, seaweed will liberate the organic substance that causes *thallus* to slid and stimulate bacteria to grow abundantly.

From the results of the study using SEM-EDX on algae and bacteria, it can be concluded that the presence of metal content causes damage to the *thallus* triggers bacteria to live in the seaweed *thallus*. *Staphylococcus arllettae* is a type of Gram-positive bacteria, from the genus *Staphylococcus* bacteria. These bacteria are commonly found from isolated textile plant waste, and can be degraded color (Francisco. E. *et.al*, 2009). Metal particles on seaweed are suspected to trigger the emergence of *Staphylococcus arllettae* bacteria. The damage of the seaweed *thallus* does not always result from changes in weather, temperature or salinity. Water quality could also result in damages of the *thallus* then stimulate the occurrence of bacteria. Since bacteria are good bioaccumulation for metals, *Staphylococcus arllettae* can absorb metal, such as Arsen. The trigger of *Kappaphycuss alvarezii* disease in Arakan Village is caused by the *Staphylococcus arlettae* bacteria. These bacteria use seaweed as a host and reduce weight on the seaweed, so that the seaweed metabolism is inhibited.

When the seaweed experiences stress it will facilitate pathogen infection. In a state of stress, the seaweed will release organic substances that cause *thallus* slimy and stimulate bacteria to grow. Bacteria found in the seaweed in Arakan waters are

http://ejournal.unsrat.ac.id/index.php/platax 10.35800/jip.v10i2.51565

pathogenic bacteria that have the potential to cause disease, namely *Staphylococcus arlettae.*

Based on the results it can be concluded that the bacteria that found in *thallus* seaweed are *ice-ice* disease in the Arakan Village, South Minahasa, North Sulawesi is bacteria *Staphylococcus arlettae.* From the SEM-EDX analysis results on seaweed taken in Arakan Village, South Minahasa, North Sulawesi, there are some metal content in *Kappaphycuss alvarezii* seaweed and *Staphylococcus arlettae* bacteria.

Ice-ice disease in seaweed cultivation is one of the big obstacles for cultivators. Regular monitoring of seaweed cultivation by fishermen or by the local government is one way to find out the conditions/problems faced by farmers. The problems faced can be considered in taking preventive action.

ACKNOWLEDGMENTS

The authors are gratefully acknowledged funding by Research and Community Service Sam Ratulangi University, Manado, North Sulawesi, Indonesia.

REFERENCES

Adi, S.E. and D.S. Nana 2010. Reduction of Pb Ion Concentration in Electroplating Wastewater by Bosorption and Stirring Processes. Journal of Chemical Engineering, 5 (1): 1-9

Ariyanti RW, Widowati LL and Rejeki S. 2016. Performa Produksi Rumput Laut Eucheuma cottoni yang Dibudidayakan Menggunakan Metode Longline Vertikal dan Horizontal. Prosiding Seminar Nasional Ke-V Hasil-hasil Penelitian Perikanan dan Kelautan.

Cappucino, J.G., Sherman, N. 1998. Microbiology: A Laboratory Manual. 5th Edition. California: Benjamin/Cummings Science Publishing. p. 94

Egan, S., and Gardiner, M. (2016). Microbial symbiosis: rethinking disease in marine ecosystems. Front. Microbial. 7:991. DOI 10.3389/fmicb.2016.00991

Franciscon Elisangela, Zille Andrea, Dias Guimaro Fabio, Ragagnin de Menezes Cristiano, Durrant Lucia Regina, Cavaco-Paulo Artur. Biodegradation of textile azo dyes by a facultative Staphylococcus arlettae strain VN-11 using a sequential microaerophilic/aerobic process. International Biodeterioration & Biodegradation 2009, 63 (3), 280-288. DOI: 10.1016/j.ibiod.2008.10.003.

Fernandes, N., Steinberg, P., Rusch, D., Kjelleberg, S., and Thomas, T. (2012). Community structure and functional gene profile of bacteria on healthy and diseased thalli of the red seaweed Delisea Pulchra. PloS One 7:e50854. DOI 10.1371/journal.pone.0050854

Fitrian T. 2015. Hama penyakit *(ice-ice*) pada budidaya rumput laut studi kasus: Maluku Tenggara. Oseana, Volume XL, Nomor 4, Tahun 2015 : 1-10

Gerung GS., Ngangi ELA. 2017. Grand Design Kawasan Pengembangan North Sulawesi Marine Education Center. FPIK Unsrat dan DKP Sulawesi Utara. Manado.

Hurtado, A. Q., 2013. Sosio-Economic Dimensions of seaweed in The Philippines. In; Hishamuda, and D. Valderrama(eds). Social and Economic Dimenssions of seaweed Cultivation: Assessment and Case Studies. Fao FisheriesAnd Aquaculture Technical Paper 580. FAO. pp 91-113

Irmawati Y. and Sudirjo F. 2017. Infection Vibrio sp. Bacteria on Kappaphycus Seaweed Varieties Brown and Green. IOP Conf. Ser.: Earth Environ. Sci. 89012016

Kasim M. and Mustafa A. 2017. Comparison growth of Kappaphycus alvarezii (Rhodophyta, Solieriaceae) cultivation in floating cage and longline in Indonesia. Aquaculture Reports 6, 49-55

Lachnit T, Brummel M, Imhoff JF, Wahl M. 2009. Specific epibacterial communities on macroalgae: phylogeny matters more than habitat. Aquat Biol 5: 181-186

Largo, D.B., Fukami K. and Nishijima T. 2012. Occasional pathogenicbacteria promoting *ice-ice* disease in the carrageenan producing red algae Kappaphycus alvarezii and Eucheuma denticulatum (Solieriaceae, Gigartinales, Rhodophyta). J. Appl. Phycol. 7: 545–554.

Martinez J.N and Padilla P.I.P. 2017. Rotten *thallus* of Red Seaweed, Gracilariopsis heteroclada Zhang et Xia, is Associated with Agar-Digesting Bacillus spp. Journal of Fisheries Sciences.com., 11(4): 034-040

Mendoza, W.G., N.E. Montano, E.T. Ganzon-Fortes and R.D. Villanueva. 2002. Chemical and gelling profile of *ice-ice* infected carrageenan from Kappaphycus striatum (Schmitz) Doty ‘‘sacol’’ strain (Solieriaceae, Gigartinales, Rhodophyta). J. Phycol.14: 409–418.

Ngangi, E.L.A., J. Mudeng, and J. N. Schaduw. 2012. Implementation and Evaluation of Seaweed Cultivation Management Process in the Minahasa Region of North Sulawesi Province. MP3EI Research Report. Unsrat Research Institute. Manado Ngangi, E.L.A., J. Mudeng, and J. N. Schaduw. 2012. Implementation and Evaluation of Seaweed Cultivation Management Process in the Minahasa Region of North Sulawesi Province. MP3EI Research Report. Unsrat Research Institute. Manado.

Noor MN. 2015. Analisis Kesesuaian Perairan Ketapang, Lampung Selatan Sebagai Lahan Budidaya Rumput Laut Kappapycus alvarezii. Journal Maspari. 7(2):91-100.

Nursidi, Syamsu A.A., Niartiningsih, dan Anshary H. 2017. Pengaruh musim dan parameter lingkungan terhadap produksi karagenan Kappaphycus Alvarezii yang dibudidayakan Di Pulau Saugi Sulawesi Selatan. Jurnal Balik Diwa, Vol. 3, No. 2, 47-52

Onyango, L. A., and Alreshidi, M. M. 2018. Adaptive metabolism in Staphylococci: survival and persistence in environmental and clinical settings. J. Pathog., 1-11. doi: 10.1155/2018/1092632

Pang, T., Liu, J., Liu, J., and Li, J. 2015. Observations on pests and diseases affection a eucheumatoid farm in China. Journal of Applied Phycology, 27, 1975– 1984.

Parenrengi A. dan Sulaeman S. 2007. Mengenal rumput laut Kappaphycus alvarezii. Media Akuakultur. Vol. 2, No.1. 142-146

Rama R., La Ode M.A., Wa Iba, Abdul R.N., Armin A. and Yusnaeni Y. 2018. Seaweed Cultivation of Micropropagated Seaweed (Kappaphycus alvarezii) in Bungin Permai Coastal Waters, Tinanggea Sub-District, South Konawe Regency, South East Sulawesi. IOP Conf. Ser.: Earth Environ. Sci.175 012219

Ruperez, P. 2002. Mineral content of edible marine seaweeds. Food Chemistry 79: 23–26.

Runtuboy, N. 2004. Dissemination of Cottoni Seaweed Cultivation (Kappaphycus alvarezii). Annual Report of the Center for Aquaculture Fiscal Year 2003.189-195 p.

Sanz S., Olarte C., Alonso C.A., Hidalgo-Sanz R., Gomez P., Ruiz-Ripa L., Torres C. 2018. Identification of Enterococci, Staphylococci, and Enterobacteriaceae from Slurries and Air in and around Two Pork Farms. J Food Prot (2018) 81 (11): 1776–1782.

Solis M.J.L, Draeger S. and Dela Cruz T.E.E. 2010. Marine-derived fungi from Kappaphycus alvarezii and K. striatum as potential causative agents of ice-ice disease in farmed seaweeds. Botanica Marina 53, 587–594

Sukma Qumain, Dharmawan Agus, Sitoresmi Prabaningtyas, 2016. Comparative Analysis Of Heavy Metal Head (Pb) Comparison In Seaweed

and Jelly at Kupang Village, Jabon, Sidoarjo. Journal-online.um.ac.id

Surni WA. 2014. Pertumbuhan rumput laut (Eucheuma cottonii) Pada kedalaman air laut yang berbeda Di dusun Kotania Desa Eti Kecamatan Seram Barat Kabupaten Seram Bagian Barat. Jurnal Biopendix x, 1 (1), 2014

Syafitri E, Prayitno S B, Ma'ruf W F and Radjasa O K. 2017. Genetic diversity of the causative agent of ice-ice disease of the seaweed Kappaphycus alvarezii from Karimunjawa island, Indonesia. IOP Conf. Ser.: Earth Environ. Sci.55 012044

Thillaivasan M., Kumar K., and Silvakumar K. 2019. Seasonal variation in elemental composition of certain seaweeds from Mandapam and Kilakarai coast, Gulf of Mannar biosphere reserve. World Scientific News, 129, 147-160

Tuiyo R. 2016. Budidaya alga laut (Kappaphycus alvarezii) dalam kantong plastik dengan menggunakan teknologi basningro. UNG Press Gorontalo. 71 hal.

Vairappan C.S, Chong S.C., Hurtado A. Q., Soya F.E., Lhonneur G.B., and Critchley A. 2011. Distribution and symptoms of epiphyte infection in major carrageenophyte-producing farms. J Appl Phycol 20(5):27-33. DOI 10.1007/s10811-007-9299-8

Zozaya-Valdes, E., Egan, S., and Thomas, T. (2015). A comprehensive analysis of the microbial communities of healthy and diseased marine macroalgae and the detection of known and potential bacterial pathogens. Front. Microbiol 6:146. doi: 10.3389/fmicb.2015.00146. 398 hal.

Analysis of particles in bacteria that cause disease in seaweed *Kappaphycus alvarezii* using scanning electron microscopy and energy dispersive X-ray spectroscopy

Comment:
In general this article is good, just add a few additional explanations.

In the discussion section:
-.Clarify the compounds present in the seaweed, explained in detail…
-.metal content found in the percentage mentioned content and compare with the results of other people's research.

-. Need to be explained a little about the bacteria Staphylococcus arlettae that enter the seaweed to damage the thallus

-. The description of each picture is clarified (Fig 1, 3, 4. 5. 6. 7)

Reviewer

Dr. M.Irfan
Email: ifan_fanox@yahoo.co.id

Analysis of particles in bacteria that cause disease in seaweed Kappaphycus alvarezii using scanning electron microscopy and energy dispersive X-ray spectroscopy

In terms of the English used in this article, this article is good enough to convey the meaning correctly and grammatically. However, there are several issues that need to be revised and corrected. For example, the tense that used in this article, the choice of words, the conjunction, repetition, sentence fragments, and grammar are the issues that need to be addressed, changed, and rewrite. It is understandable because the authors are non-native speakers and English is their foreign language (EFL). Therefore, this article need revision in terms of the English used.

There needs a consistency of using the tense in this article, for example, in the first paragraph (Abstract) and in some parts of results where the authors reported what they did and what they found. The past tenses are not used parallelly and consistently with the other verbs: *performed* (instead of *perform*), *was used to* (instead of *is used to*), *cause* (instead of *caused*), *are used* to (instead of w*ere used too*), *have found* (instead of f*ound*). However, the use of present tense is used correctly and grammatically when the authors described their result presented in the Figure, for example, *is shown in Figure* 3…

Repetition is part of the issue in this article. It is not necessary to repeat the same sentences into different part. For example, several sentences mentioned in Abstract are repeated, exactly the same in the other parts (in Introduction in Discussion). The repetition may distract and bore a reader. In terms of the quality of research writing it shows that the study does not have a strong good analysis. Therefore, it is important to avoid the ideas or sentences that are not needed to repeat.

There are several sentence fragments and long sentences are found. In this case, the sentences are incomplete which means there are no main verbs. For example, in the second last paragraph in Abstract, the sentence is too long and it is sentence fragment. It needs a main verb so that the sentence will have meaning and will be complete correctly and grammatically. In other words, the sentence can be shortened by using a conjunction. The use of conjunction is needed in order to link phrases, two words, and clauses together.

To some points this article is potentially to be a better-quality writing in terms of the English used. Some comments on this have been provided. The revision needs to be done. It is important to provide the clarity to the readers about the study that had been conducted, regardless of whether or not English is their first language.

Best Regards,
Reviewer

Dr. Embly Leilany Mumu
Email: embly.mumu@education.wa.edu.au