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Aquaculture infection of *Kappaphycus alvarezii* by *Vibrio alginolyticus* causing ice-ice disease

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Abstract. *Prasetyo EN, Permanasari Y, Basyirah A, Fitriya SA, Jadid N, Zulaikah S, Ekawati I, Isdiantoni, Koentjoro MP. 2024. Aquaculture infection of* Kappaphycus alvarezii *by* Vibrio alginolyticus *causing ice-ice disease. Biodiversitas 25: 4086-4094.* The occurrence of ice-ice disease on seaweed (*Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva) tissue is indicated by the presence of bleaching and softening symptoms on thallus, which attracts microbial infection. In addition, it is often caused by unfavorable environmental conditions during specific seasons and is characterized by fragmentation as well as a reduction in biomass. To prevent the disease, several studies have suggested the use of ice-ice disease-resistant seeds. Therefore, the aim of this study was to investigate the resistance level of seaweed seeds to microbial infection, particularly *Vibrio alginolyticus*, which is responsible for ice-ice disease in different aquaculture areas in Sumenep District. Data were collected from four different villages, including Brakas, Padike, Tanjung, and Lobuk, followed by analysis using descriptive qualitative method. *K. alvarezii* seeds collected from four villages were infected with *V. alginolyticus* using the in vitro method. Thallus of seaweed was then observed and water samples were taken every 6 hours for 24 hours post-infection. The results showed that seeds from Brakas had the lowest (11%) infection rate, followed by Tanjung (67%), and Padike and Lobuk (89% each). This indicated that *K. alvarezii* seeds from Brakas possessed a prominent activity against *V. alginolyticus* infection with no sign of chlorosis or tissue softening on thallus.

Keywords: Aquaculture, ice-ice disease, infection, *Kappaphycus alvarezii, Vibrio alginolyticus*

INTRODUCTION

Kappaphycus, a genus of red seaweed (*Rhodophyceae*), is among the most widely cultivated seaweeds in Indonesia due to its high economic value and versatile applications. This seaweed produces carrageenan, a hydrocolloid extensively used as a stabilizer, thickener, gelling agent, and emulsifier in industries such as food, cosmetics, and pharmaceuticals (Rupert et al. 2022). Carrageenan is derived from *Kappaphycus alvarezii* through a water or alkaline extraction process and is a linear polysaccharide composed of over 1000 galactose residues, including ester, potassium, sodium, calcium sulfate, galactose, and the copolymer 3,6-anhydrogalactose (Firdaus et al. 2021). As of 2013, Indonesia's carrageenan production reached 12.5 million tons, with an export capacity of approximately 10,527,500 tons (Salim and Ermawati, 2015). This highlights Indonesia's prominence in the global seaweed industry. However, the cultivation of *K. alvarezii* faces significant challenges, particularly from ice-ice disease (IID), a condition that can reduce seaweed production by up to 70% (Riyaz et al. 2020).

Ice-ice disease severely impacts both the quality and quantity of seaweed crops. This disease is primarily associated with poor environmental conditions, often observed during specific seasons (Ward et al. 2021). Its symptoms include bleaching and softening of infected tissues, typically starting at the base of the thallus, which leads to fragmentation and biomass loss (Tahiluddin and Damsik 2023). Infected seaweed experiences a gradual breakdown of tissue and the detachment of primary branches from cultivation lines, resulting in decreased yield by up to 70%. The disease also reduces carrageenan quality and quantity by 25% to 40%, significantly lowering its market value (Ward et al. 2021). Pathogenic bacteria, particularly *Vibrio* and *Cytophaga-Flavobacterium* groups, have been identified as the primary causative agents of IID (Syafitri et al. 2017; Tuhumury et al. 2024). Additionally, bacterial genera such as *Gammaproteobacteria* (including *Pseudoalteromonas*, *Alteromonas*, and *Stenotrophomonas*) and *Alphaproteobacteria* (such as *Aurantimonas* and *Ochrobactrum*) have been implicated in the disease. Studies have consistently shown that Gram-negative bacteria are predominant in IID-affected tissues (Ward et al. 2021).

To combat IID, most strategies have focused on mitigating environmental stressors that exacerbate the disease (Azizi et al. 2018). One widely adopted approach is the selection of appropriate farming methods. Longline cultivation, which involves growing seaweed in deeper waters, has proven effective in reducing exposure to temperature fluctuations and air during low tides compared to other methods (Kambey et al. 2021). Research has also

revealed that water near the surface (20–50 cm depth) in active seaweed cultivation areas often contains carrageenan-hydrolyzing bacteria, such as *Vibrio*, *Flavobacterium*, and *Pseudomonas*, which also known as potential opportunistic pathogens (Hollants et al. 2013).

Another critical factor influencing seaweed production is the quality of *K. alvarezii* seeds. High-quality seeds play a pivotal role in determining productivity, carrageenan quality, and resistance to diseases like IID (Rahayu et al. 2021). Seed quality is directly linked to resilience against bacterial infections, making it essential to prioritize the use of robust and healthy seeds in cultivation. Improving seed quality can be achieved through genetic enhancement, such as genetic engineering or molecular breeding techniques, which offer the potential to develop strains with greater resistance to pathogens (Tokan et al. 2015; Suryati et al. 2010).

This study investigates the resilience of *K. alvarezii* seeds from different areas in Sumenep District, East Java, Indonesia, to bacterial infection caused by *Vibrio alginolyticus* in aquaculture settings. By identifying resilient seed strains, this research aims to support the development of high-quality seeds and enhance seaweed production. The findings are expected to contribute to sustainable seaweed farming practices, improving both yield and product quality while addressing the challenges posed by IID. This effort aligns with the broader goal of maintaining Indonesia's position as a leading producer of carrageenan in the global market.

MATERIALS AND METHODS

Time and place

This study was conducted from November 2016 to May 2017 in the Laboratory of Microbiology and Biotechnology, Department of Biology Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia. Seaweed seeds were collected and acclimatized in Sumenep, East Java, Indonesia (Figure 1).

Sampling area

Seaweed seeds of *K. alvarezii* used in this study were collected from 4 different areas in Sumenep, as shown in Figure 1. The sampling areas were located in Raas Sub-district, Guwa-Guwa Village (Figure 1.A1), Tonduk Village (Figure 1.A2), Brakas Village (Figure 1.A3) and Alasmalang Village (Figure 1.A4), which represented the archipelago area of Sumenep District. The sample of seeds representing the mainland area of Sumenep District included Bluto Sub-district, Lobuk Village (Figure 1.D1), Saronggi Sub-district, Tanjung Village (Figure 1.D2) and Dungkek Sub-District, Longos Village (Figure 1.I.B1). Meanwhile, Talango Sub-district, Talango Village (Figure 1.C1), Padike Village (Figure 1.C2), and Cabbiya Village (Figure 1.C3), which represented seeds from small island areas closest to the mainland of Sumenep District.

The seeds of *K. alvarezii* were selected according to the Indonesian National Standard of Kotoni Seaweed Seeds (BSN 2016), which should follow criteria like quantitative requirements, which included age 25 days to 30 days, minimum branching thallus of 3, main thallus diameter at least 0.5 cm and uniform, weight per hill 50 g to 100 g. Qualitative characteristicts included bright and fresh thallus, clean from dirt, attachment of organism and moss, disease-free, without not injured and broken, spiky buds, and proportional shape. Seed packaging was done using the semi-dry method. Seeds were wrapped in newspaper and stored in a cool box to keep them fresh during the trip to acclimation area (Jiksing et al. 2022).

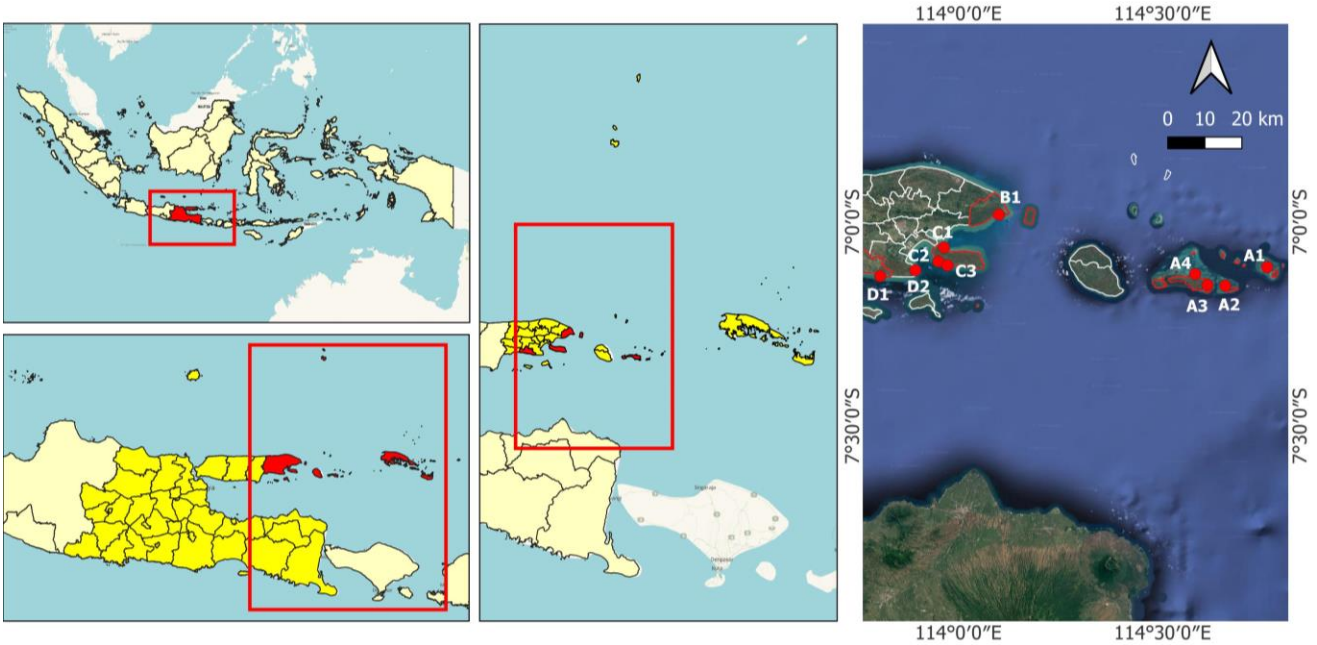

Figure 1. Map of *Kappaphycus alvarezii* sampling area in Sumenep District, East Java, Indonesia. A1: Guwa-guwa, A2: Tonduk, A3: Brakas, A4: Alasmalang, Raas Sub-district; B1: Longos, Dungkek; C1: Talango, C2: Padike, C3: Cabbiya, Talango Sub-district; D1: Lobuk, D2: Sarunggi, Bluto Sub-district, Sumenep, East Java, Indonesia

Collection and maintenance of bacterial isolates

Bacterial isolate of *V. alginolyticus* was obtained from the Brackish Water Cultivation Center (BBAP), Situbondo, East Java, Indonesia. Isolates of *V. alginolyticus* were taken using a sterile inoculating loop and inoculated into seawater complete medium slant and then incubated at 30°C for 24 hours (Isnansetyo et al. 2022).

Acclimatization of *Kappaphycus alvarezii* seeds samples

Healthy seeds of *K. alvarezii* were acclimatized for 28 days using an artificial aquarium with a filter recirculation system (Suryati et al. 2021). In addition, healthy *K. alvarezii* seeds had no spots, were unpeeled, and had strong thallus. Acclimatized *K. alvarezii* seeds had an initial weight of ±10 g with seawater as the acclimatization media of up to 0.56 L per g (Zuldin et al. 2016). Seawater replacement was done twice a week up to 50%. Each artificial aquarium was equipped with a pumping machine and filter for water circulation consisting of (i) physical filters namely cotton and coral; (ii) chemical filters such as activated carbon; and (iii) biological filters including bioball, which were arranged accordingly. Seawater flow in the filter recirculation system was as follows (i) seawater assessed the filter chamber through an outlet channel in the form of a hose; (ii) seawater through physical, chemical, and biological filters in the filter chamber; (iii) seawater entered the filter pump engine room; (iv) seawater was pumped through inlets at the rate of 1200 mL/min for each aquarium.

The observation of environmental factors influencing seaweed maintenance media was conducted in accordance with the methodology outlined by Syahrul et al. (2023). Parameters such as salinity (ranging from 28 to 34 ppt), temperature (between 25 and 35°C), and light intensity were monitored daily. Additionally, pH levels were measured weekly, with values ranging from 6.5 to 8.5.

The parameters of successful seaweed acclimatization included survival rate, daily growth rate, increase in seaweed weight, and thallus length and diameter. Measurements of increased seaweed weight, as well as thallus length and diameter, were performed every week. The measurement of thallus length and diameter was observed from only 1 thallus for each seeded marker. Seaweed weight and thallus length were measured using a digital scale and ruler, respectively, while thallus diameter was measured by sliding range. The increase in seaweed weight and thallus length and diameter was determined using the following formula (Suryati et al. 2021):

Increase in seaweed weight

$$\Delta W = Wt - Wo$$

Where:
Wt : Wet weight of seaweed at time t (gram)
Wo : Previous wet weight or initial (gram)

Increase in length of thallus

$$\Delta L = Lt - Lo$$

Where:
ΔL : Increment of total length or length of thallus (cm)
Lt : Length of thallus at time t (cm)
Lo : Initial length of thallus (cm)

Increase in diameter of thallus

$$\Delta D = Dt - Do$$

Where:
ΔD: Increment of center or center diameter (largest) thallus (mm)
Dt : Center or center diameter (largest) thallus at time t (mm)
Do : Center or initial center diameter (largest) of thallus (mm)

The daily growth rate (α) could be calculated from the weight value obtained during a certain time using the following formula (Lideman et al. 2024):

$$\alpha = ((\ln Wt - \ln Wo) / t) \times 100\%$$

Where:
Wt : Wet weight or biomass at time t (gram)
Wo : Initial wet weight or biomass (gram)
t : Observation time (day)

Survival rate (SR) was the ratio between the total number of individual seaweed alive at the end of the experiment (Ni) and the total number of individual seaweeds used initially (No) (Kasnir et al. 2021).

$$SR = \frac{Ni}{No} \times 100\ \%$$

Where:
SR : Survival rate (% survival rate) (%)
Ni : Total individual seaweed that lives at the end of the experiment (individual)
No : Total individual seaweed that lived at the beginning of the experiment (individual)

Growth curve of *Vibrio alginolyticus* in bacterial acclimatization media

Bacterial isolates of *V. alginolyticus* aged 24 hours were inoculated into a modified SWC medium for bacterial acclimatization. Subsequently, the growth of bacteria was observed every 2 hours. The observation was done using UV-Vis spectrophotometer with a wavelength of 600 nm (Wang et al. 2015), and cell density was calculated using a hemocytometer with calculation formula (Chenoweth and Lorton 2014):

$$\sum \frac{\text{cell}}{\text{ml}} = \frac{N \times Dilution\ factor}{\frac{1}{400} mm^2 \times 80 \times 0.1\ mm} \times \frac{1000\ mm^2}{1\ ml}$$

Where:
N : Number of calculated cells
1/400 : Small box area
80 : Σ small box
1/10 : Height of Haemacytometer
1000 mm²/mL: Conversion form to unit mL

Ice-ice disease bacterial infection in *Kappaphycus alvarezii* seeds

Bacterial infection of ice-ice disease was done through aquaculture in an acclimatization aquarium container of *K. alvarezii* seaweed seeds. *V. alginolyticus* bacteria, which had the highest pathogenicity level on *K. alvarezii* seaweed

thallus, was infected into an aquarium container containing medium acclimatization of *K. alvarezii* seeds with a density of 10^6 cells/mL (Aris et al. 2011). Thallus was observed and a water sample was taken every 6 hours for 24 hours post-infection.

Data analysis

Qualitative data on *V. alginolyticus* bacterial infection were obtained based on the occurrence of ice-ice disease on *K. alvarezii* seaweed seeds and descriptively analyzed.

RESULTS AND DISCUSSION

***Kappaphycus alvarezii* seeds selection**

The selection of *K. alvarezii* sampling area was based on superior potential data of Sumenep District information. These areas, as shown in Figure 1, were the nursery and seaweed agribusiness cultivation centers. Moreover, the selection of seaweed seeds was conducted based on the basic ecosystem type of aquaculture pond water. The water base in Raas Sub-district was the main ecosystem of coral reef and seagrass, water base in Dungkek Sub-district was sand. Meanwhile, Dead rocks and mud deposits dominated the water of the Bluto sub-district, while sand composed the water base in the Saronggi sub-district, which was relatively close to the mouth river. Conversely, the sampling area in the Talango Sub-district was dominated by sand and dead coral rocks but far from the mouth river.

Seeds obtained from 10 areas (Figure 1) were screened based on Indonesian National Standard (SNI) of Seaweed Seeds. As it turned out, only 4 seeds obtained from Brakas, Lobuk, Tanjung, and Padike could be further processed to the acclimatization stage. This seed selection aids in obtaining a high quality-plants with optimum acclimatization results and satisfactory growth (Jiksing et al. 2022).

Acclimatization of *Kappaphycus alvarezii* seeds

The selected seeds of *K. alvarezii* were acclimatized in an aquarium with a filter recirculation system for 28 days (Figure 2). The average daily temperature and light intensity of media in *K. alvarezii* acclimatization aquarium are shown in Figure 3. This figure depicted that the average daily temperature of media in the aquarium ranged from 26°C to 29°C. The optimum temperature range for the growth of *K. alvarezii* seaweed is around 25°C to 30°C (Khotijah et al. 2020). The average daily light intensity exposure to the aquarium was around 1234 to 3255 lux, which was lower compared to the sea level of 5000 lux. Low-light intensity provided an optimal temperature for growth compared to high-light intensity because the latter tended to inhibit seaweed growth due to increased temperature (Lutfiati et al. 2022).

Another environmental factor that influenced seaweed growth and morphology was salinity. The average daily salinity of media in an aquarium during the acclimation process is shown in Figure 4. The average daily salinity of media in aquarium was approximately 30 to 38 ppt. The value for the cultivation of *K. alvarezii* is in the optimum range of 28 to 34 ppt (Basir et al. 2017). Seaweed could also grow in the water with a salinity of 30 to 37 ppt (Kasnir et al. 2021). The increasing water salinity helped to reduce the evaporation of seawater in the aquarium. Moreover, in equilibrium conditions, salt solution reached a high temperature (Jeppesen et al. 2023). The decrease in water salinity, which occurred every week, was due to the dilution used to maintain the salinity and volume of seawater in the aquarium. After 15 days, salinity was more stable at 30 to 31 ppt, this was possible due to the microclimate conditions at the acclimation area, which were stable with no change in weather. Fluctuating microclimates affected the maintenance of media in the aquarium.

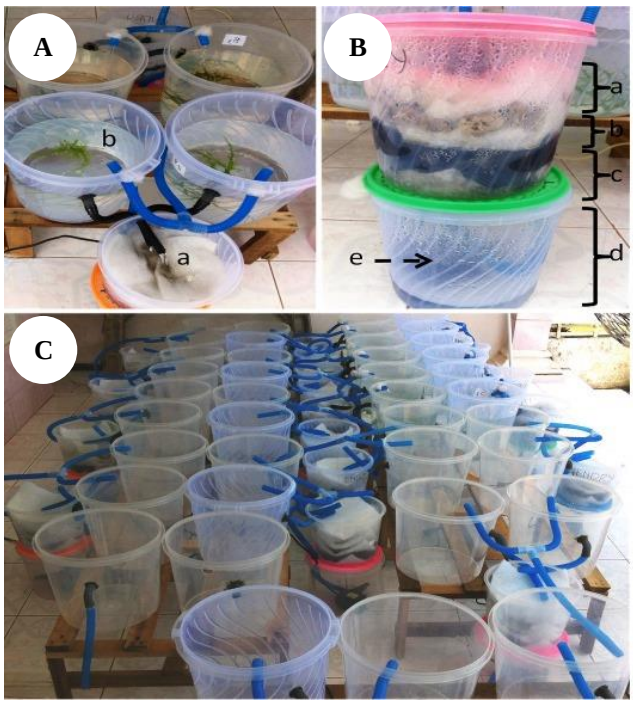

Figure 2. Acclimatization of *Kappaphycus alvarezii* seeds in aquariums. A. *K. alvarezii* seeds in aquarium (a: filter, b: plastic container); B. Filter (a: cotton and coral, b: activated carbon, c: bioball, d: filtered water container and e: pump); C. Aquarium circuit system

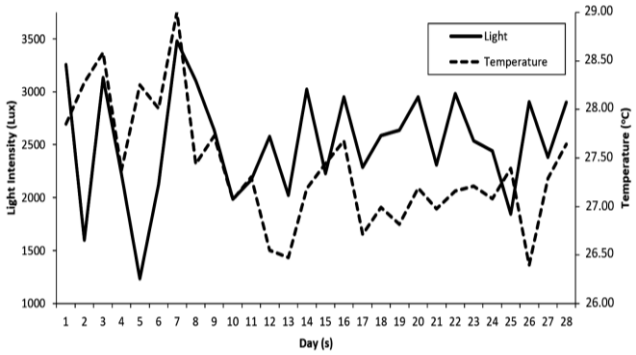

Figure 3. The average of daily light intensity and temperature during acclimatization

During acclimatization, the average pH of seawater in aquarium was stable at 7. This value is in the recommended range of optimal seaweed growth at 6 to 9 (Bui et al. 2018). Any change in seawater pH could alter the equilibrium of the carbonate system and the concentration of inorganic carbon species (Naga 2022), which could affect seaweed growth as seaweed photosynthesis depends on the inorganic carbon source.

The growth of *K alvarezii* seeds during acclimatization is shown in Figure 5. In Figure 5.B, thallus appeared to be longer and more complete after acclimatization with intermittent irregular dichotomous or bifurcated continuous branching forms for all 4 accessions of *K. alvarezii* seeds. Furthermore, there was no significant change in seaweed color before and after acclimatization. As for the morphological appearance, *K. alvarezii* seaweed seeds from Brakas, particularly Raas Sub-district, had a brownish-red color, which was different from the other 3 seeds. The samples from Padike, Lobuk, and Tanjung had green color. This was possibly affected by environmental factors and chromatic adaptation process, including adjustment of pigment proportions with various lighting qualities (Paransa et al. 2020)

During the acclimation process, the survival rate of all seaweed reached up to 100%, from the number of 36 stocked seeds that managed to survive completely. This result was consistent with the study conducted by (Marisca et al. 2013), where seeds could survive well in artificial aquarium containers equipped with a filter recirculation system.

The growth rate parameters of *K. alvarezii* are presented in Figure 6. Figures 6.A and 6.B showed the highest growth rate, which was displayed by seeds from Padike of up to 0.034% per day with an average weight gain of 16.74 g. Seeds from Brakas possessed the lowest percentage of growth rate (with only 0.012% per day) and average weight increase of 4.39 g. The successful acclimatization was also indicated by the increase in thallus length and diameter. Seeds obtained from Padike had the largest average thallus length during the acclimation process (Figure 6.C), reaching up to 15.4 cm after 4 weeks. The smallest average thallus length was observed from seeds obtained from Brakas at 9.4 cm. However, thallus length was not proportional to diameter in this study. Figure 6.D indicated that seeds from Brakas had the largest average diameter value, of 0.5 cm. Meanwhile, seeds from Tanjung Village had the smallest average diameter value of 0.38 cm.

The increasing growth rate parameters of *K. alvarezii* seeds are shown in Table 1. This table indicated that the rate of weight gain was directly proportional to the rate of thallus length increase. The highest growth rate and longest thallus length were observed in seeds obtained from Padike, with an increase of 4.11 g/week and 1.12 cm/week, respectively. This may be possible because seeds of Padike had several branches and small nodules that potentially promoted growth until the end of the acclimatization process. Meanwhile, the lowest growth rate and shortest thallus length were observed in seeds obtained from Brakas, with a weight of 1.00 g/week and a height of 0.28 cm/week. As for thallus diameter, the largest diameter was displayed by seeds obtained from Brakas with a diameter of 0.002 cm/week. The other seeds, obtained from Padike, Tanjung, and Lobuk, grew to approximately the same level, with a diameter of 0.001 cm/week. All *K. alvarezii* seeds obtained from different areas grew to a certain level every week, with a different growth rate.

The growth variance was influenced by the characteristics of various seaweed thallus. Every seaweed thallus had different potential in regulating thallus growth, including thallus length and diameter, as well as thallus weight. In addition, thallus weight is affected by the number of seaweed branches or thallus, which vary between each other (Marisca et al. 2013). The length and diameter were measured to check whether the growth is the result of cell division and enlargement occurring in meristem tissue, the embryonic tissue with high cell division ability (Fadilah 2016).

Table 1. The increased growth rate of seaweed parameters

Area	Parameters		
	Weight (g/week)	Length of thallus (cm/week)	Diameter of thallus (cm/week)
Brakas	1.00	0.28	0.002
Padike	4.11	1.12	0.001
Tanjung	3.06	0.80	0.001
Lobuk	1.88	0.48	0.001

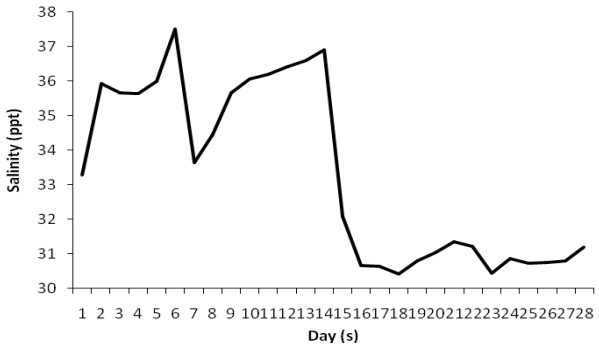

Figure 4. The average of daily salinity during acclimatization

Figure 5. Acclimatization of *Kappaphycus alvarezii*. A. Week 0 before acclimatization; B. Week 4 after acclimatization). The red circle shows the growth of thallus

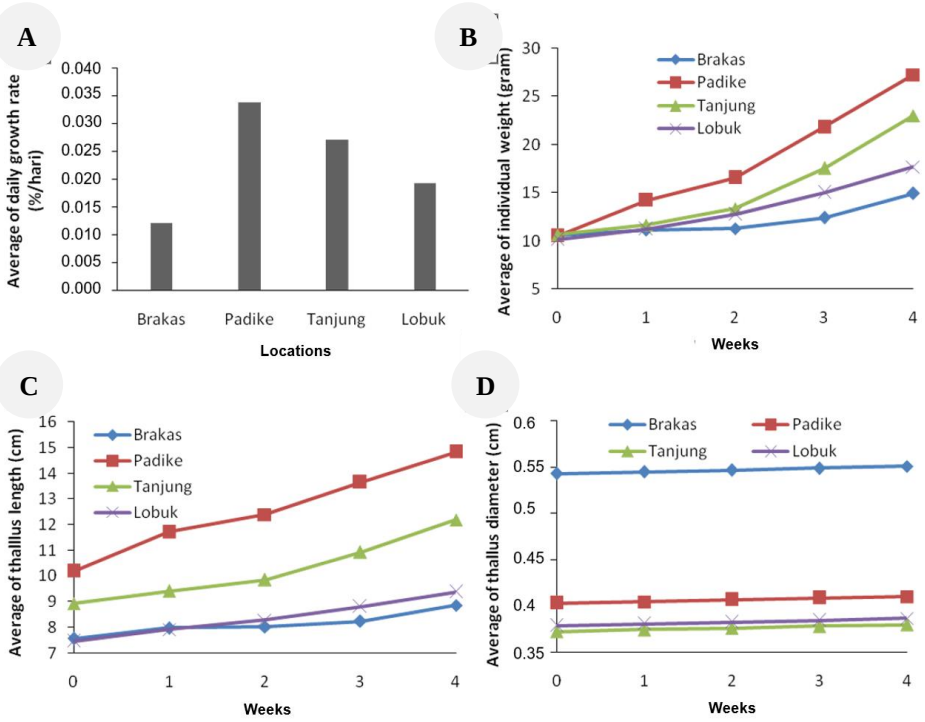

Figure 6. *Kappaphycus alvarezii* growth parameters during acclimatization. A. Average daily growth rate; B. Average individual weight; C. Average thallus length; D. Average thallus diameter

According to Machida et al. (2013), meristem tissue existed in several different parts of the plant, including end meristem, intercalar and lateral. In the present study, each part was responsible for a different growth area. Meanwhile, end meristem tissue produced new cells at the end of seaweed thallus and control thallus length, lateral meristem, and intercalary system affected thallus diameter and branch segments, respectively.

Acclimatization of *Vibrio alginolyticus* bacteria

The growth curve of *V. alginolyticus* ice-ice disease bacteria used for infecting *K. alvarezii* seeds is presented in Figure 7.

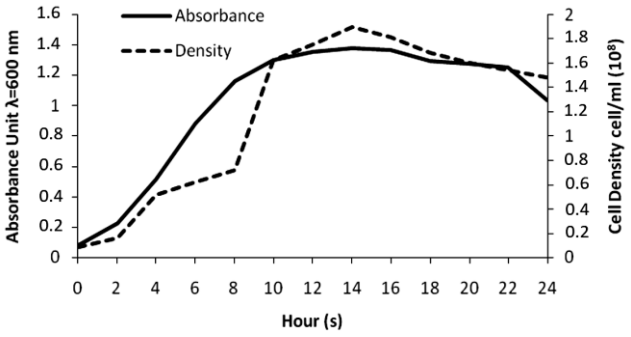

Figure 7. Growth curve of *Vibrio alginolyticus* during acclimatization in modified SWC medium

Symptoms of ice-ice disease by *Vibrio alginolyticus*

The result of *V. algonoliticus* infection in seeds of *K. alvarezii* was obtained after the acclimatization process, as shown in Figure 8. The result was presented by the percentage of infected seeds in 24 hours under aquaculture conditions.

Results revealed that *K. alvarezii* seeds obtained from Brakas displayed the lowest (11%) infection percentage. Only 1 out of 9 seeds exhibited the symptoms of ice-ice disease after being infected with *V. alginolyticus* bacteria. Meanwhile, seeds from Tanjung possessed 67% infection (with 6 out of 9 seeds infected). The highest infection percentage (with a value of 89%) was observed in Padike and Lobuk seeds, with most seeds being infected (Figure 8). The number of bacteria in control aquarium without any infection of *V. alginolyticus* was stable from T-0 to T-24, which is shown in Figure 9.

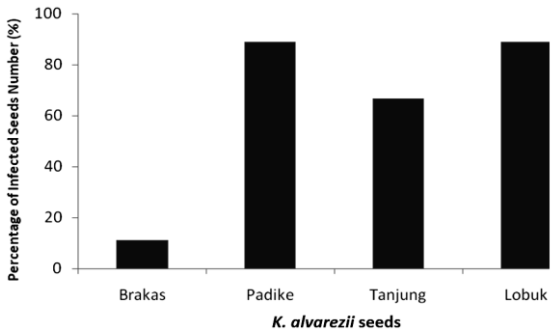

Figure 8. Percentage of infected *K.alvarezii* seeds by ice-ice disease *Vibrio alginolyticus* bacteria after 24 hours

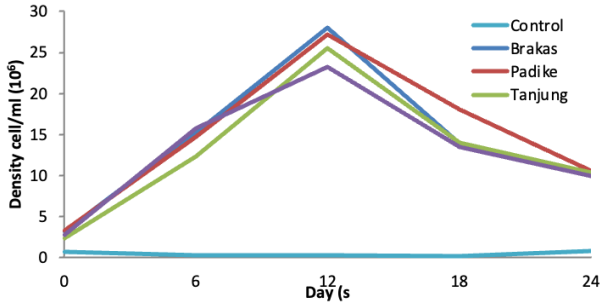

Figure 9. Bacteria cell density in seaweed *Kappaphycus alvarezii* control aquarium after infection

No symptoms of ice-ice disease were detected from the control seeds without treatment of *V. alginolyticus* infection, in all 4 repetitions. This happened when bacterial density of control seeds was lower than the average, with a density of 0.17 to 0.77 x 10^6 cells/mL, while bacterial density of treated seeds ranged from 2.9 to 26×10^6 cells/mL. Previous studies conducted by Darma et al. (2021) showed bacterial density of ice-ice disease bacteria on infected *K. alvarezii* thallus was 10 to 100 times higher than a healthy thallus. At T-0, bacterial density of control was 0.72×10^6 cells/mL and decreased to 0.27×10^6 cells/mL at T-6 hour. Subsequently, there was a slight increase of bacterial density at T-12 hour from the initial point to 0.3×10^6 cells/mL and decreased to 0.17×10^6 cells/mL at T-18 hours. Bacterial density at T-24 hour increased by up to 0.77×10^6 cells/mL (Figure 9). Bacterial density in *K. alvarezii* seaweed seeds aquarium increased after infection of *V. alginolyticus*, compared to the initial density at T-0 hour, with an average density of 2.9 x 10^6 cells/mL. Bacterial density continuously increased until the T-12 hour, with an average of 26 x 10^6 cells/mL, and decreased until T-24 hour, with an average of 10.26×10^6 cells/mL. Ice-ice symptoms appeared as red spots after T-12 hours of infection. Results of the current study showed a significant instance of thallus color change (chlorosis), which started at 12 hours post-infection and the capability to invade *K. alvarezii* thallus with a density of 10^6 cells/mL. As shown in Figure 9, bacterial density in three *K. alvarezii* seeds post-infection reached the highest average value of up to 26×10^6 cells/mL at T-12 hour. *K. alvarezii* seeds visualization after *V. alginolyticus* infection for 24 hours can be seen in Figure 10.

Suspicious infected seeds exhibited ice-ice disease symptoms indicated by the presence of red spots on thallus, which could gradually turn white (Figure 10). The change in seaweed color is caused by the failure of thallus to perform optimum photosynthesis (Riyaz et al. 2021). As carrageenan degrading bacteria, *Vibrio* could infect seaweed tissue (Naik and Dubey 2017) with their hydrolytic activity, resulting in bleaching of thallus during ice-ice infection (Aris and Labenua 2020). During infection, bacteria penetrated the inner part of the seaweed thallus. The invasion promoted degradation and pigment damage to plastid-containing cells, which resulted in bleaching of the infected thallus section (Aris and Labenua 2020) by hydrolytic activity coming from karagenase and cellulase enzyme (Syafitri et al. 2017).

Red spots and thallus bleaching occurred in the seeds of Padike, Tanjung, and Lobuk. Meanwhile, other seeds obtained from Brakas (Figure 11) did not exhibit any symptoms of ice-ice disease after *V. alginolyticus* infection. *K. alvarezii* obtained from Brakas (Figure 11.B) showed no symptoms of ice-ice disease with a normal thallus morphology compared to the control seeds (Figure 11.A). Brakas *K. alvarezii* had less branching compared to the other 3 seaweed, which helped to prevent infection. According to Ghazali et al. (2022), the coarse surface of thallus with several branching allowed dirt, epiphytes, parasites, and other microorganisms to easily stick to the surface of thallus and is a good medium for the growing pathogen, making it more vulnerable. In addition to the previous postulation, there were differences in waters and areas for cultivating *K. alvarezii* in Brakas. The area must have a high-water quality, soft water basin, and seagrass beds to support better adaptive structural mechanisms (Cullen-Unsworth and Unsworth 2016).

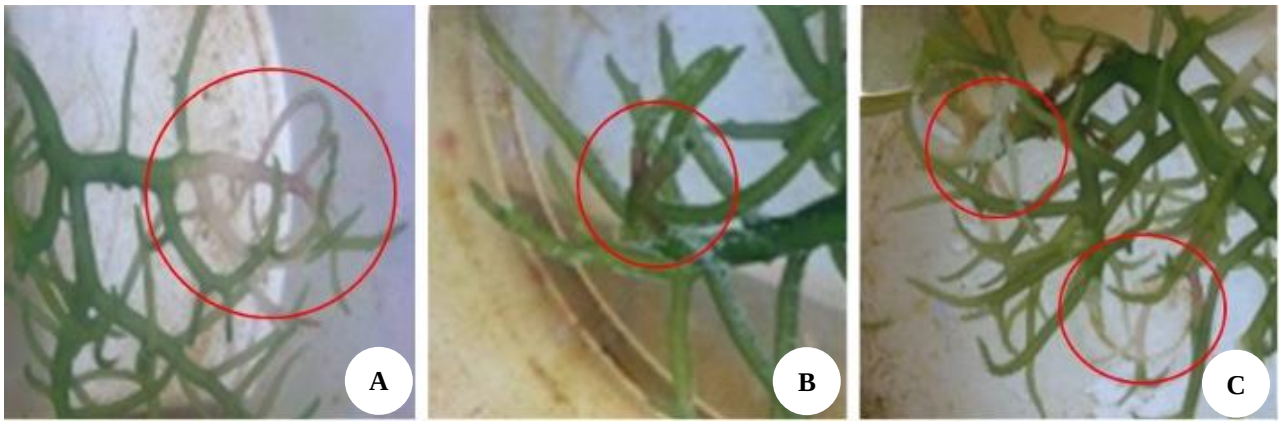

Figure 10. *Kappaphycus alvarezii* seeds after being infected by *Vibrio alginolyticus* for 24 hours. A. Padike Village; B. Tanjung Village; C. Lobuk Village. The red circle shows infected thallus

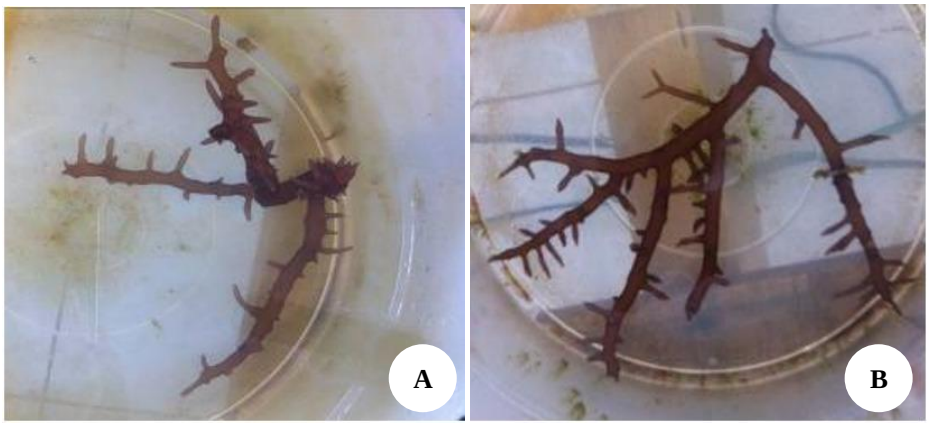

Figure 11. *Kappaphycus alvarezii* seeds from Brakas Village. A. Control without *Vibrio alginolyticus* infection; B. After 24 hours infection of *V. alginolyticus* showed no sign of ice-ice disease

In conclusion, *K. alvarezii* seeds obtained from Brakas Village in the Raas sub-district demonstrated the highest resistance against ice-ice disease (*V. alginolyticus*), with an infection rate of merely 11.11%. It was also observed that *K. alvarezii* seeds sourced from Padike, Lobuk, and Tanjung Villages exhibited considerably high infection rates of 89%, 89%, and 67%, respectively.

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