Infection *Vibrio* sp. Bacteria on Kappaphycus Seaweed Varieties Brown and Green

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Abstract. Disease in seaweed or ice-ice, until today is still a major problem in the cultivation of seaweed. Changes in extreme environmental conditions is a trigger factor of ice-ice, which can result in seaweed susceptible to infection with pathogenic microorganisms, such as bacteria *Vibrio* sp. This research aims to determine the bacteria *Vibrio* sp. infection in seaweed *Kappaphycus* varieties of brown and green. *Vibrio* sp. bacteria isolated in the infected seaweed thallus ice-ice, grown on TCBS media, purification, gram staining and biochemical tests. *Vibrio* sp. infected to seaweed *Kappaphycus* brown and green varieties in containers controlled by different density, 10^5 CFU/ml, 10^6 CFU/ml and 10^7CFU/ml. Observations were made to change clinical effect in thallus seaweed for 14 days of observation. The results obtained show that the levels of infection bacteria *Vibrio* sp. higher in seaweed *Kappaphycus* green varieties both in density 10^5 CFU/ml, 10^6 CFU/ml and 10^7CFU/ml, when compared with varieties brown.

1. Introduction
Diseases of seaweed or ice-ice, until now still a major problem in seaweed cultivation, this attack it can cause damage to the seaweed thallus, so often farmers are in cultivation. Changes in extreme environmental conditions are the trigger factors of ice - ice disease, which can store seaweed vulnerable to infection of pathogenic microorganisms, is bacteria. The results of several studies indicate the presence of diseases in the cultivation in the sea, both fish and shrimp as well as on seaweed are the bacteria *Vibrio* sp. *Vibrio* sp. in fish and shrimp both cultivated and living in nature, has been widely known. However, infectious information directly on seaweed in controlled containers has not been widely known and published, especially in *Kappaphycus* brown and green varieties of seaweed which are the main commodities in seaweed cultivation, so this research can serve as preliminary data for further research to reduce the level of pathogenicity of *Vibrio* sp.

2. Methodology
This research was conducted in June-August 2016, and are analyzed in the microbiology laboratory, Tual State Fisheries Polytechnic. The equipment that is used consist of tube, petri dish, beaker glass, objek glass, cover glass, hotplate stirrer, needles Ose, autoclave, incubator, microscope, battery aerator, and aquarium.

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Materials; seaweed, media Thiosulfate Citrate Bile Salt Sucrose (TCBS), gram staining materials (violet crystal, lugol iodine, safranin) alcohol 95%, paper oxidase, Media OF (Oxidase-Fermentation), H_2O_2 3%, aluminum foil, sandpaper, aqua, cotton, tissue, and tape.

The cultivation of seaweed begins with the filling of seawater in aquarium, installation of rope for binding of seaweed and aeration installation. Before the experiment, seaweed acclimatized for 5 days. *Kappaphycus* seaweed, brown and green varieties are placed on different aquariums, which consist of 3 treats with 2 replications and as controls. The treatments were infected with different bacterial densities of 10^5 CFU/ml, 10^6 CFU/ml and 10^7 CFU/ml by submersion method.

The samples bacteria taken from thallus of seaweed that is exposed ice-ice. By scratching the ose needle on the ice-ice section, recast on the TCBS medium and incubated in the incubator at 28^0C - 30^0C for 24-48 hours. The reconstituted bacteria, subsequently grown into Nutrient Agar medium, were scrawled on Nutrient Agar Slant, tested by gram staining, and identified by biochemical and Bergey's Manual of Determinative Bacteriology [4].

3. Results and Discussions

Gram staining on bacterial isolates showed that the colonies obtained were gram-negative bacteria because of the binding of safranin colors on the cell walls of these bacteria. [8] States that gram-negative pathogenic bacteria, its outer walls play an important role in infecting and damaging the hosted host. The results of identification of bacteria are presented in Table 1.

Table 1. Results of *Vibrio* sp. biochemical testing

Characteristic	Result
Gram staining	Gram-negative
Oxidase	+
Catalase	+
Oksidase/Fermentation	**F**
Motility	+

The results of biochemical testing of colonies obtained according to the characteristics of the bacteria *Vibrio* sp. According [5] for the characteristics of a biochemical test of bacteria *Vibrio* sp. is a positive oxidase and catalase test, fermentative oxidative/fermentative test, motile motility test, and negative gram staining. The results of observation of changes in the effect of bacterial infection *Vibrio* sp. on *Kappaphycus* brown seaweed varieties are presented in Figures 1 and Table 2, while for green varieties in Figures 2 and Table 3.

Figure 1. Changes of thallus on *Kappaphycus* brown varieties

Table 2. Observation of clinical effect of bacterial infection *Vibrio* sp. on *Kappaphycus* seaweed varieties of brown

Days	*Vibrio* sp. Density			
	10^5	10^6	10^7	Control
1	Has not seen any changes	Has not seen any changes	Has not seen any changes	Has not seen any changes
2	Has not seen any changes	Has not seen any changes	Has not seen any changes	Has not seen any changes
3	Has not seen any changes	Has not seen any changes	Has not seen any changes	Has not seen any changes
4	Has not seen any changes	Has not seen any changes	Has not seen any changes	Has not seen any changes
5	The color of the thallus begins to change	Has not seen any changes	Has not seen any changes	Has not seen any changes
6	The color of the thallus becomes slightly younger than the original color	Has not seen any changes	Has not seen any changes	Has not seen any changes
7	Thallus began to release mucus	The color of the thallus begins to change	Has not seen any changes	Has not seen any changes

8	Color differences in some branches began to be seen clearly	The color of the thallus becomes slightly younger than the original color	The color of the thallus begins to change	Has not seen any changes
9	The former part of the fault on the thallus color begins to change	Thallus began to release mucus	The color of the thallus begins to change	Has not seen any changes
10	Part of the color at the end of the thallus color begins to change	Color differences in some branches began to be seen clearly	The color of the thallus becomes slightly younger than the original color	The color of the thallus begins to change
11	At the tip of the thallus, there are white spots	The former part of the fault on the thallus color begins to change	Thallus began to release mucus	The color of the thallus becomes slightly younger than the original color
12	White spots on the end of the thallus occur almost in all parts of the branch	Part of the color at the end of the thallus color begins to change	Color differences in some branches began to be seen clearly	Thallus began to release mucus
13	White spots begin to widen	At the tip of the thallus, there are white spots	The former part of the fault on the thallus color begins to change	Color differences in some branches began to be seen clearly
14	The tip of the thallus changes color to white	White spots on the end of the thallus occur almost in all parts of the branch	The color of the thallus becomes slightly younger than the original color	The surface of the thallus begins to shrink

Figure 2. Changes of thallus on *Kappaphycus* green varieties

Table 3. Observation of clinical effect of bacterial infection *Vibrio* sp. on *Kappaphycus* seaweed varieties of green

Days	*Vibrio* sp. Density			
	10^5	10^6	10^7	Control
1	Has not seen any changes	Has not seen any changes	Has not seen any changes	Has not seen any changes
2	The color of the thallus begins to change	Has not seen any changes	Has not seen any changes	Has not seen any changes
3	The color of the thallus becomes slightly younger than the original color	The color of the thallus begins to change	Has not seen any changes	Has not seen any changes
4	Thallus began to release mucus	The color of the thallus becomes slightly younger than the original color	The color of the thallus begins to change	Has not seen any changes
5	Color differences in some branches began to be seen clearly	Thallus began to release mucus	The color of the thallus becomes slightly younger than the original color	Has not seen any changes
6	The former part	Color differences in	Thallus began to	Has not seen any

	of the fault on the thallus color begins to change	some branches began to be seen clearly	release mucus	changes
7	At the tip of the thallus, there are white spots	The former part of the fault on the thallus color begins to change	Color differences in some branches began to be seen clearly	Has not seen any changes
8	White spots on the end of the thallus occur almost in all parts of the branch	At the tip of the thallus, there are white spots	The former part of the fault on the thallus color begins to change	Has not seen any changes
9	White spots begin to widen	White spots on the end of the thallus occur almost in all parts of the branch	At the tip of the thallus, there are white spots	The color of the thallus begins to change
10	The tip of the thallus changes color to white	White spots begin to widen	White spots on the end of the thallus occur almost in all parts of the branch	The color of the thallus becomes slightly younger than the original color
11	The white color begins to turn pale and yellowish	The tip of the thallus changes color to white	White spots begin to widen	Thallus began to release mucus
12	Thallus had a break	The white color begins to turn pale and yellowish	The tip of the thallus changes color to white	Color differences in some branches began to be seen clearly
13	Thallus becomes easily broken when touched	Thallus had a break	The white color begins to turn pale and yellowish	The surface of the thallus begins to shrink
14	Thallus broke all over the branch	Thallus becomes easily broken when touched	Thallus had a break	The color of the thallus becomes pale

In Figures 1 and 2, it shows that the alteration of the *Kappaphycus* seaweed varieties of green varieties due to bacterial infection of *Vibrio* sp. Very different when compared with brown varieties, where almost all parts of the thallus experience bleaching, fracturing and easily broken when touched.

The dominant ice-ice disease strikes seaweed *Kappaphycus alvarezii* cultivated with the early clinical effects such as increased mucus production, rough thallus surface, wilted thallus, white spots, and bleaching of the tip of the thallus. A more severe attack of ice-ice may cause the thallus to become porous, and eventually, the infected thallus becomes fractured [2].

[11] suggested that after observation, the effect of an attack of ice-ice disease on seaweed started from the middle or tip of the thallus where the color change from yellowish brown faded to a pale white, if touched it felt slimy and two to three days later part is covered with a kind of white powder. When this part is touched or subjected to a strong enough water flow, the powder is released and the skin (epidermis) is peeled off, even visible in the tissue (cortex) and soft-looking which eventually breaks easily.

The mechanism of bacterial pathogenicity to seaweed thallus can be seen in the morphological changes of the thallus, especially the normal color changes (green and brown) to white (chlorosis). Color change (depigmentation) occurs due to the infection of pathogenic bacteria cause ice-ice disease in seaweed thallus. The change is increasing along with the increase of bacterial activity time in secreting its virulence factors to the host (seaweed thallus) [1].

Clinical effect observation results (Table 2 and Table 3) showed that in the infected with bacterial density Vibrio sp. 10^7 CFU/ml could have an impact on seaweed thallus even though it is very small and takes a long time when compared with *Vibrio* sp infection. At a density of 106 CFU/ml and 10^5 CFU/ml. [9] the minimum threshold of the presence of *Vibrio* sp. In the water is 10^4 CFU/ml, while the minimum limit of common bacteria is 10^6 CFU/ml.

Infection of *Vibrio* sp. at a density of 10^5 CFU/ml gives a very significant effect on seaweed *Kappaphycus* green varieties, which on the first day after infection, have shown clinical symptoms, i.e. changes in color in the thallus. The result of observation (day 14) also showed a very high degree of pathogenicity, in which there was a fault in all branches of the thallus. These results were very different in the brown *Kappaphycus* seaweed varieties where on the 6th day new infections showed clinical symptoms and outcomes after observation (day 14), only showed the effect of discoloration to white.

These results show that *Kappaphycus* brown seaweed varieties are more resistant to bacterial infections of *Vibrio* sp. when compared to green varieties. The endurance ability of Kappaphycus brown varieties to *Vibrio* sp. infection is thought to be due to brown varieties having higher antibacterial compounds than green varieties. [6] States that brown seaweed is one group of seaweeds that have the highest antioxidant activity when compared with red and green seaweed. Brown seaweed contains three types of hydrocolloids, namely: agar, alginate, and carrageenan [3]. Phenolic compounds are molecules that are known to act as brown algae defenses [7]. Algae activity can be used as an antiviral, antibacterial and antifungal effect on some pathogens [11].

4. Conclusions

Level of infection (pathogenicity) of bacteria *Vibrio* sp. higher in the Kappaphycus varieties of green varieties when compared with the brown varieties. Further research is needed to determine the content of brown seaweed, which is more resistant to *Vibrio* sp. and testing with other types of bacteria.

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