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, 10⁵ CFU/ml, 10⁶ CFU/ml and 10⁷ CFU/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 10⁵ CFU/ml, 10⁶ CFU/ml and 10⁷ CFU/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.
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 28-30°C 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.

| 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 | 10³ | 10⁶ | 10⁷ | 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 |
| Page | Event Description | Action 1 | Action 2 | Action 3 |
|------|-------------------|----------|----------|----------|
| 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 |
Table 3. Observation of clinical effect of bacterial infection *Vibrio* sp. on *Kappaphycus* seaweed varieties of green

| Days  | $10^3$                          | $10^6$                          | $10^7$                          | 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            |

Figure 2. Changes of thallus on *Kappaphycus* green varieties
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. 10^5 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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