Identification And Patogenisity Characteristic Of *Vibrio* Sp. Isolated From Eel (*Anguilla* Sp.) Skin Mucus.

Meigy Nelce Mailoa, Beni Setha

Abstract: - The aim of this study was to identification and analyzing patogenisity characteristic of Vibrio sp. Isolated From Eel Anguilla sp. Skin Mucus. The samples were taken from Tambala and Agogo rivers in Tanawangko waters, North Sulawesi, Indonesia,were put into sterile plastics, and then put into a cool box during their trip from the field to the laboratory. Analysis microbiology is of conducted by isolation and identification of *Vibrio* sp. There for the pathogenicity analysis consisted of agglutination and hemolytic tests. From 60 *Vibrio* which has been isolated from the mucus of eel fish, it was found that: *Vibrio* sp (45,0 %) *V. alginolitycus* (23,3 %) *V. cholerae* (21,7%) *V. parahaemolitycus* (6,7%), and *V. vulnificus* (3,3 %). The result of pathogenicity test showed *V. alginolitycus* (Lr7.b1) was α-haemolysis wheles *V. parahaemolitycus* (Lr10.a1) was β-haemolysis. There for the aglutinase test was positive on *V. alginolitycus* (Lr.7.b1) and *V. parahaemolitycus* (Lr10.a1), but agglutination of *V. cholerae.* show negative

Index Terms : Eel,Vibrio,Identification,Pathogenicity

1. INTRODUCTION

Eel fish (*Anguilla* sp.), or the so called "*sogili*" fish is one of potential resources which has not been yet cultivated optimally in North Sulawesi. The taste of eel has not been popularly known and liked by Indonesian people. In contrast, in America, Europe and Asia, eel meat is popular and expensive (Setiawan, 1996)[1]. *Sogili* fish is a catadromous species, in this case an adult fish migrates from river and freshwater to ocean in order to breed, and the larvae go back to freshwater. *Anguilla* sp. is a strong type fish, and is very sensitive to environmental condition. , The best temperature for living is 25-28^{o}C, but it may survive in 23-30^{o}C, and around pH 9 (Utsui,1991)[2].On another hand, *Vibrio* sp. is a type of bacteria which must be avoided from any foodstuffs. *Vibrio cholera* can cause cholera and serious infection to the sufferer in which he may suffer diarrhea symptom for 20-30 times a day and loss of body liquid for about 18 liter. Meanwhile, *V. parahaemolyticus* may cause acute gastroenteritis through food contamination, especially seafoods which are not perfectly prepared.Vibrio species can be isolated and found in mucus of eel skin, however there is still limited data on it. The aim of this research is to give information about the type of *Vibrio* sp. in *Anguilla*'s mucus that may cause diseases to human.

2. MATERIALS AND METHODS

The object was mucus taken from fresh eel skin which were still alive in Tanwangko water of Ranowangko village, Tombadiri sub district. The mucus was taken by using a sterile *ose* needle. The needle was scratched on the surface of eel fishes, and was transfered to alkaline peptone water. Identification of vibrio was done according to the characteristics of morphology, physiology and biochemistry as well, and was compared to Bergeys Manual of Bacteriology (Bauman *et al*.,1984)[3]. While, pathogenicity test was done according to Agglutination test and hemolytic test. Agglutination test used an antigen that look like a whole cell. Antigen (like bacteria's cell) has some antigen determinant on the surface that reacted with antibody in which it created a hank visible to naked eyes. The hank consisted of particles that united with antibody. In agglutination test, microorganism suspension i mixed with antiserum. After 2-3 minute there is a hank appeared and seen through object glass. Then, a reaction of agglutination takes place. Haemolytic test is done by using Blood Agar Plate. Suppose that microorganism experienced haemolysis, a haemolysis zone would be visible on Blood Agar Plate. There are three types of haemolysis: β-haemolysis (no blood cells around the colony), α-haemolysis (blood cells found around the haemolysis zone, or some greenish color appeared around the colony, and δ-haemolysis (non-haemolysis) (Sheena,1985)[4]. The analysis was conducted in the Microbiology Laboratory of Fishery Research, faculty of fishery and maritime, Sam Ratulangi University, North Sulawesi, Indonesia. Mean over the parameter in which all data were put in table or histogram, and pictures (qualitative).

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3. RESULTS AND DISCUSSION

Isolation and identification of Vibrio

Isolates of bacteria isolated from TCBS media order, are Gram negative, with a rod and rod curved like a comma on the observation under a microscope with magnification 1000 X. (Figure 1).

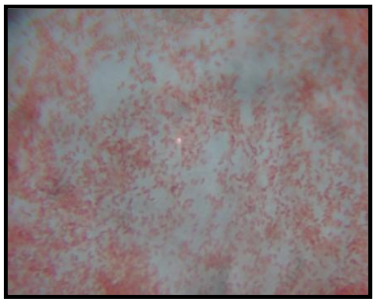


Figure 1. Staining Test Results Isolates Lr7.B1

Gram-negative bacteria are bacteria that bind the primary colors of paint (a solution of crystal violet, the color purple) is not strong. On microscopic observation of bacterial cells are visible red (Fardiaz, 1987)[5]. This is made clear by the difference in phase caused by Gram staining structural differences, the cell wall of Gram positive and negative bacteria, causing differences in the permeability reaction and the addition of dye bleaching solution. Gram-negative bacteria in red due to the complex is soluble when giving a solution of bleaching and then red. Next Ijong (2003)[6], stated that Gram-positive bacteria contain peptidoglycan of thick, rigid and not easily solved, and serve as the basic framework bacteria cells. Furthermore, Gram-negative bacteria have a peptidoglycan is thin, easily broken and wrapped in the cell layer by lipoprotein or lipopolysaccharide. In the present study, biochemical characteristics Vibrio sp that appeared on each isolated bacteria were compared to Bergey's Manual of Bacteriology. There were five species of vibrio bacteria that has been indentified like those on Table 1. Meanwhile, the composition of vibro was shown in Table 2.

Table 1. Biochemistry Characteristics Of *Vibrio* On Eel Skin Mucus**.**

Test	Isolat				
	Lr7.b1	Lr8.a2	Lr10.a1	Lr4.a1	Lr1.a1
Sucrosa	A	AG	-	-	AG
Glucosa	A	AG	AG	A	AG
Maltosa	A	AG	AG	A	AG
Manitol	A	A	AG	A	AG
MR	+	+	+	+	-
VP	+	+	-	-	+
Oxidase	+	+	+	+	+
Catalase	+	+	+	+	+
Motilyti	+	+	+	+	+
Indole	+	+	+	+	+
Citrate	-	+	-	+	-
Species	*V. alginolitycus*	*V. cholera*	*V. parahaemolitycus*	*V. vulnificus*	*Vibrio* sp.

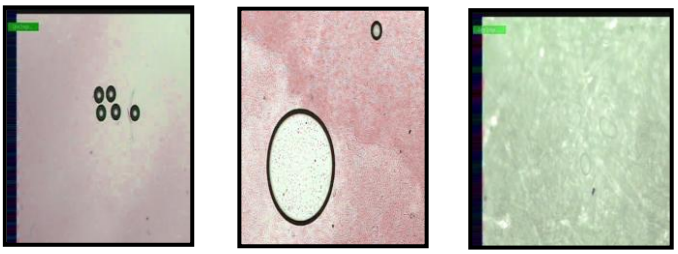
Table 2 shows the dominant species of *V. alginolitycus* and *V. cholera*. Ecologically, these two species can live in water like Tambala river and Agogo river so that they can grow optimally. Commonly, *V. alginollitycus* and *V. chorelae* found in rainy season because they can live in 0% sanility. In contrast, *V. parahaemolitycus* and *V. vulnificus* can survive in water with high level of saltiness, thus their representation in that area is relatively small. Most of *V. parahaemolitycus* found in salty water like sea water, especially in tropical area or in summer of four season countries.

Table 2. The Composition Of *Vibrio* On Eel Skin Mucus

Genus/species	Isolates	Total (%)
Vibrio sp.	Lr1.a1, Lr2.a1, Lr3.a1, Lr5.a1, Lr7.a1, Lr1.b1, Lr3.b1,	45,0
	Lr6b1, Lr10.b1, Lr1.a2, Lr2.a2, Lr3.a2, Lr4.a2, Lr9.a2,	
	Lr1b2, Lr2.b2, Lr3.b2, Lr4.b2, Lr6.b2, Lr7.b2, Lr8.b2,	
	Lr9.b2, Lr5.a3, Lr6.a3, Lr3.b3, Lr4.b3, Lr6.b3.	
V. vulnificus	Lr4.a1,Lr4.b1.	3,3
V. alginolitycus	Lr6a1, Lr8.a1, Lr9.a1, Lr2.b1, Lr7.b1, Lr8.b1, Lr5.b2,	23,3
	Lr2.a3, Lr4.a3, Lr10.a3, Lr7.b3, Lr8.b3, Lr9.b3, Lr10.b3	
V. parahaemolitycus	Lr10.a1,Lr5.a2,Lr6.a2,Lr7.a2	6,7
V. cholera	Lr5.b1, Lr9.b1, Lr8.a2, L10.a2, L10.b2, Lr1.a3, Lr3.a3,	21,7
	Lr7.a3, Lr2.a3, Lr9.a3, Lr1.b3, Lr2.b3, Lr5.b3	

Patogenicity Characteristics *Vibrio* sp.

Human body can react to antigen, and create a specific antibody in response to that antigen. This concept basically applies to bacteria, virus, fungus, or parasite when one want to diagnose some types of infections. According to Lay (1994)[7], serologic reaction is done to know the response of the body towards infectious diseases because its reaction is specific. One of serologic techniques which is popularly used is agglutination reaction. According to the agglutination test, there were three isolates taken among 60 isolated bacteria: Lr.7b1(*V. alginlyticus*), Lr.10. a1(*V. parahaemolitycus*), and Lr.8.a2 (*V. cholerae*). The result of agglutination test can be seen in Figure 2.

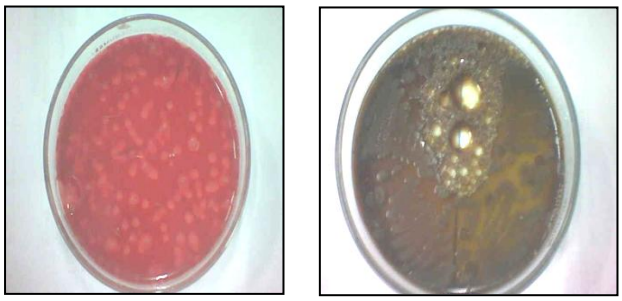


V. alginollyticus (Isolat Lr.7b1) ***V. parahaemolitycus*** (Isolat Lr.10.a1) ***V. chorelae*** (Isolat Lr.8.a2)

Figure 2. The Result Of Agglutination Test

V. alginlyticus and *V. parahaemolitycus* showed positive agglutination in which there was a hank on the blood cell that indicated by a black hole. This suggested that the reaction between antigen and antibody creates a complex hank of antigen-antibody. In contrast, *V. cholera* gave a negative response. The results showed that *V. alginolitycus* and *V. parahaemolitycus* could be easily caused infections because it gave the way agglutination reaction occurred. *V. alginlyticus* is a non-pathogenic strain, while *V.*

parahaemolitycus is pathogenic (Fardiaz, 1983)[8]. According to Geo *et al (*2003)[9] *V. alginolitycus* causes infection on eyes, ears, or wound after having contact with sea water. Extra-intestinal infection of *V. parahaemolitycus* has been reported in many countries such as Asia, Australia, Europe and North America. It can influence the ears, eyes, and wounds of swimmers (Kiiyuki, 1990)[10]. Till now, regarding the symptom of the infection, it is still not clear whether it is caused by the production of enterotoxin or the invasive of bacteria. After that, *V. cholera* gave response of non-agglutination because it cannot create agglutination reaction. Although, this strain was non-agglutination, it could create diarrhea symptom smaller than agglutinated of *V. cholerae*, and it was able to cause ear infection as it was usually found in small wounds on skin or other parts of body. How the *Vibrio* can poison has not been known clearly, but it has hemolisyn ability which is believed to cause gastroenteritis. Agar Medium at high salt level was made by Wagatzuma to examine the level of *Vibrio* hemolytic. The part which was positive by Kaganawa test would show β-hemolytic, marked by the presence of a colony with transparent area around it. While, α-hemolytic showed negative by Kaganawa reaction in which the colony appeared with the sign of colorless. The result of examination of five isolated bacteria, Lr.1.a1 (*Vibrio* sp), Lr.b.1 (*V. alginolitycus*) gave response by α-haemolysis (Kanagawa-negative), while Lr.10.a1 (*V. parahaemolitycus*), Lr.4 a1 (*V. vulnificus*) and Lr8.a2 (*V. cholerae*) showed β-haemolysis (Kanagawa-positive). The examination results were shown in the following Figure 3.



α-Haemolysis (Isolat Lr.7.b1) **β-Haemolysis** (Isolat Lr.8.a2)

Figure 3. The Result Of Haemolysis Test

Sakazaki *et al*. (1974) as it was cited by Fardiaz (1983)[8], presumed that the ability of Kanagawa-positive strain to proliferate is faster than Kanagawa-negative one in digestive system that was being a very important factor which determined its virulence characteristics, while the production of enterotoxin by Kanagawa-positive or Kanagawa-negative determined its pathogenicity. Apart from that, Kanagawa-positive strain was far more enduring than Kanagawa-negative strain. Kanagawa-positive was shown in the Figure 4.

Figure 4. Kanagawa-Positives (Isolate Lr.10.A1)

Karsinah *et al*. (1994)[11] stated that *V. cholerae* in normal circumstance was only pathogen for human being since it does no invasive. This bacteria never comes into blood circulation system, but stay permanently in intestines, while *V. cholerae* produces Kanagawa-positive haemolysis. This was emphasized by Fardiaz (1983)[8] that enterotoxin has not been isolated from *V. parahaemolitycus* although it produces enterotoxin. This toxin might take a role in determining its patogenicity. Isolat of *V. parahaemolitycus* showed that 95% hemolytic test of kanagawa was positive (Fardiaz, 1983)[8]. Is it was mention in Anonymous (2005)[12], the determinant of pathogenicity of *V. parahaemolitycus* has not been known exactly. Another test on *V. parahaemolitycus* showed pathogenicity determinant, namely the ability to stick on hospes cell. This means that *V. parahaemolitycus* which gave positive results on Kanagawa test would be easier to stick on hospes cell than the one which gave negative result on Kaganawa test. This was accentuated by Teramoto (1969) , Zen-Yoji *et al*. (1970), Honda *et al*. (1987) cited by Kiiyukia 1990)[10] that the one which has been considered pathogen, has been reported as food poisoning contained strain of Kanagawa-negative. TDH production from the result of Kanagawa-negative isolat conducted through an accurate research put away the doubt that Kanagawa-negative also determined the pathogenicity of this organism (Honda *et al*. 1998 cited by Kiiyukia, 1990)[10]. *V. vulnificus* could cause infection of bacteria and gastroenteritis (Geo *et al*., 2003)[9]. *V. vulnificus* could cause blood poisoning and inflammation on digestive system where many of poisoned cases found in the United States because of daily consumption of sea food such as oyster.

4. CONCLUSION

From 60 isolated bacteria, it was found that *Vibrio* sp. (45.0%), *V. alginolyticus* (23.3%), *V. cholerae* (21.7%) and *V. parahaemolyticus* (6.7%), *V. vulnificus* (3.3%). Pathogenicity test showed positive haemolysis, α-haemolysis on *V. alginolitycus* (Lr.7.b1), and β-haemolysis on *V. parahaemolyticus* (Lr.10.a1). On another hand, agglutination test shown various results, such as: positive test on *V. alginolitycus* (Lr.7.b1) and *V. parahaemolyticus* (Lr.101), and negative test on *V. cholerae* (Lr.8.a2).

5. RECOMMENDATION

It is important to do further research on *Vibrio* sp. and its pothogenicity on *Anguilla* sp., not only on its skin mucus but also on the meat because it is the part the people consume. Also, it is important to do research on quality of river water since it may contaminate the eel.

6. REFERENCES

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