Identification of Vibrio spp. causing vibriosis in spiny lobsters (Panulirus homarus L.) in Bengkulu marine temporary shelter ponds

R H Wibowo¹*, Sipriyadi¹, W Darwis¹, N Susianti², S P Yudha³, N Rosianti⁴

¹ Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Kandang Limun, Bengkulu 38112, Indonesia.
² Quality Control and Fishery Product Safety Agency (BKIPM) of Bengkulu, Bengkulu, 38213, Indonesia.
³ Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Kandang Limun, Bengkulu 38112, Indonesia.
⁴ Under Graduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, Kandang Limun, Bengkulu 38112, Indonesia.

*Corresponding email: riskyhadiwibowo80@gmail.com

Abstract. Spiny lobster (Panulirus homarus) is one of the export commodities of the Indonesian fisheries subsector and an important component for shrimp fisheries in Indonesia. In the development of lobster cultivation, there are several obstacles, the presence of vibriosis infection caused by the pathogenic Vibrio bacteria. This study aimed to identify Vibrio spp. bacteria in spiny lobsters (P. homarus) reared in the marine cultivation ponds, Bengkulu, Indonesia. Clinical symptoms of lobsters infected with vibriosis are red spots on the uropod, pleopod, and abdominal parts. Bacterial isolation was conducted by isolated some internal organs in spiny lobsters, that are, gills, stomachs, haemolymph, and hepatopancreas. The result showed there are 5 isolates of Vibrio bacteria that coded by IN3, ST2, HA1, HP2, and HP3. These bacteria isolates were identified through their colony morphology and biochemical tests. Characterization on the Thiosulfate Citrate Bile Salt Sucrose (TCBS) medium showed that lobsters were infected with Vibrio species. Based on Bergey's Manual of Determinative Bacteriology, Austin and Austin, the identification results showed that HA1 isolate was identified as Vibrio algynolyticus, IN3 isolate was identified as V. anguillarum, ST2 was identified as V. ordalii, HP2 in first lobster was identified as with V. algynolyticus that mostly in the hepatopancreas, and HP3 was identified as V. splendidus

1. Introduction
Lobsters are massive crustaceans scattered in tropical and subtropic waters. Panulirus lobsters belong to the Palinuridae family and unremarkably brought up as spiny lobsters [1]. One in every of the lobsters of the genus Panulirus unfold across state is Panulirus homarus. Lobster (Panulirus homarus) or spiny lobster is one of the export commodities of the Indonesian fisheries subsector and a vital element for shrimp fisheries in Indonesia. This commodity has to be any developed due to its high trade price and
potential. Lobster cultivation in Indonesia began in early 2000, with the invention of puerulus attachments within the waters of the bay of Lombok. The potential for developing this cultivation is supported by the transfer of data and technology from the lobster cultivator community in Vietnam. Among many lobster species, *P. homarus* has the advantage of being elite as a candidate for cultivated species. This is often concerning market demand, high prices, and convenience of seeds in nature [2]. Lobster cultivation business failure often happens because of the high mortality caused by illness infections [3], one of them is the presence of vibriosis infection. Vibriosis is caused by *Vibrio* bacteria from the *Vibrionaceae* family [4]. Clinical symptoms of lobsters affected by vibriosis are brownish hepatopancreas, brownish red gills [5], and red spots on the pleopod, uropod, and abdominal parts [6]. The presence of vibriosis is very detrimental because it can cause death of more than 80% with floating net cage systems [7]. However, the lack of information about vibriosis disease that infects spiny lobster in Bengkulu makes this research interesting to do. Therefore, this study aimed to provide information about the *Vibrio* bacteria that causes vibriosis in spiny lobster.

2. Materials and Methods

2.1. Research Material

The tools and materials utilized in this study were beakers glass, analytical scales, oven, trays, spatulas, measuring cylinder, spray bottles, surgical instruments, bunsen burner, micropipette, Erlenmeyer, Petri dishes, inoculation loop, test tubes, test tubes racks, glass objects, incubator, refrigerator, tube, UV-Laminar flow, magnetic stirrer, hotplate, autoclave, stationery, cotton, tissue, label paper, wrapping paper, matches, rubber bands, aluminum foil, and bacteria identification forms, *Panulirus homarus*, alcohol 70%, spritus, absolute alcohol, iodine, paraffin, oxidase paper, H₂O₂ solution (3%), KOH (40%), aquadest, KOH solution (3%), Simmons Citrate Agar media (SCA), Trypticase Soy Agar 2% (TSA), Triple Sugar Iron Agar (TSIA), Thiosulfate Citrate Bile Salts Sucrose media (TCBS), Lysine Iron Agar (LIA), Motility Indole Ornithine media (MIO), MR-VP media, Oxidative-Fermentative media (OF), Urea media, Methyl Red solution, α-naphthol solution, Phenol-Red Broth Base, Kovack's solution, Gelatin, and Sugar test (Glucose, Maltose, Lactose, Mannitol, and Sorbitol).

2.2. Spiny Lobsters (*P. homarus*) Sample Collection

Samples of spiny lobster (*P. homarus*) were collected from Bengkulu Marine Temporary Shelter Ponds. Furthermore, in living conditions, samples were analyzed at the Testing Laboratory, Fish Quarantine Station, Quality Control, and Safety of Fishery Products (SKIPM) Bengkulu.

2.3. Isolation of Target Pathogenic Bacteria from Spiny Lobsters (*P. homarus*) Sample

The body surface of the spiny lobsters were cleaned with iodine using a tissue. Bacteria isolated from the target organs, particularly gills, stomach, hepatopancreas, and hemolymph using an inoculation loop that had been glazed over the bunsen onto the Trypticase Soy Agar media with streak methodology and incubated for 1x24 hours at room temperature. Isolates grown on the media were inoculated and purified.

2.4. Identification of Bacterial Colony Characteristics by Biochemical Test

Identification of bacterial colonies using biochemical tests, namely catalase test, oxidase test, potassium hydroxide test, gelatin test, motility indole ornithine test, lysine iron agar test, simmons citrate test, triple sugar iron agar test, urease test, oxidative-fermentative test, MR-VP test, thiosulfate citrate bile salts sucrose (TCBS) test, carbohydrate tests including glucose, lactose, maltose, mannitol, and sorbitol.

2.5. Identification of Bacterial Isolates from the Target Organs, Gills, Stomach, Hepatopancreas, and Hemolymph of Spiny Lobsters (*P. homarus*)

Identification of the phatogenic bacterial type carried out by adjusting the test results and characteristics of the bacteria using Bacterial identification book from Bergey’s Manual of Determinative Bacteriology 9th Edition [8], Bergey’s Manual of Systematic Bacteriology 2nd Edition Volume 3rd [9].
3. Result and Discussion

3.1. External and Internal Morphology of Spiny Lobster (*P. homarus*)

The external morphology of lobsters consists of a cephalothorax protected by a powerful and spiny cuticle, and a carapace-covered abdomen, and a tail. Barbellate lobsters have nine pairs of batteries consisting of five pairs of walking legs and four pairs of swimming legs (pleopod). The carapace of the spiny lobsters is bluish-gray and there are bright yellow spots. The sample utilized in this study was male spiny lobster and female spiny lobster. Lobster samples taken were more than 20 cm in size which had the characteristics around the uropod, pleopod, and abdomen that there were red spots, then the gills were reddish, and the hepatopancreas was brown (Figure 1). Clinical symptoms of lobsters affected by vibriosis are brownish hepatopancreas, brownish red gills [5], and red spots on the pleopod, uropod, and abdominal parts [6].

![Image of spiny lobster](image1.png)

Figure 1. External morphology of Spiny Lobster (*Panulirus homarus*), wherein (a) full body, (b) abdomen, (c) pleopod, uropod, and telson, and (d) gills and hepatopancreas.

| Bacterial Morphology | Bacterial Isolates |
|----------------------|--------------------|
| Color                | HA1 | IN3 | US2 | HP2 | HP3 |
| Yellow               | Yellow | Yellow | Yellow | Yellow | Yellow |
| Margin               | Entire | Entire | Entire | Entire | Entire |
| Elevation            | Convex | Convex | Convex | Convex | Convex |
| Form                 | Circular | Circular | Circular | Circular | Circular |

![Image of bacterial colonies](image2.png)

Table 1. Identification of Bacterial Colony Morphology on TCBS media
Figure 2. Bacterial isolates from spiny lobster organs on the Thiosulfate citrate bile salts sucrose (TCBS) agar and incubated at 25°C for 1x24 hours, wherein (a) HA1, (b) IN3, (c) US2, (d) HP2, and (e) HP3 in Spiny Lobster (Panulirus homarus).

| Biochemical Test                | Isolated Code |
|--------------------------------|---------------|
|                                | HA1 | IN3 | US2 | HP2 | HP3 |
| Catalase Test                  | +   | +   | +   | +   | +   |
| Potassium hydroxide Test       | +   | +   | +   | +   | +   |
| Motility                       | +   | +   | +   | +   | +   |
| MIO Test                       | +   | +   | -   | +   | -   |
| Ornithine                      | +   | -   | -   | +   | -   |
| Gelatin Hydrolysis Test        | +   | +   | +   | +   | +   |
| MR Test                        | MR  | +   | +   | -   | +   |
| Glucose                        | +   | +   | +   | +   | +   |
| Lactose                        | -   | -   | -   | -   | -   |
| Carbohydrate Tests             |     |     |     |     |     |
| Maltose                        | +   | +   | +   | +   | +   |
| Mannitol                       | +   | +   | +   | +   | +   |
| Sorbitol                       | -   | -   | -   | -   | -   |
| Oxidative-Fermentative Test    | F   | F   | F   | F   | F   |
| Slunt/Butt                     | A/A | A/K | A/K | A/K | A/K |
| Triple Sugar Iron Agar Test    | H2S | -   | -   | -   | -   |
| Gas                            | +   | -   | -   | -   | -   |
Biochemical Test | Isolated Code
--- | ---
| HA1 | IN3 | US2 | HP2 | HP3
Simmons Citrate Agar Test | + | + | + | + | +
Lysine Iron Agar Test | + | - | - | + | +
| Lysine Decarboxylase | + | - | - | - | -
| Lysine Deaminase | - | - | - | - | -
Urease Test | + | - | - | - | -
Thiosulfate citrate bile salts sucrose (TCBS) Agar | Yellow | Yellow | Yellow | Yellow | Yellow

Based on the biochemical tests in the table 2, the results of the 3% potassium hydroxide (KOH) test, the five isolated isolates formed mucus when dropped with 3% KOH solution. This shows that the five bacterial isolates were Gram negative bacteria. According to [10] that the KOH test can be used to determine Gram positive bacteria have thick cell walls and thin fat, while Gram negative bacteria have thin walls and thick fat. 3% KOH solution will react with the lipid bilayer layer (fat) and the strong alkalinity will make the cell walls of Gram negative bacteria break. Bacterial cells that burst will release genetic material (DNA). DNA molecules are sticky strings (similar to mucus) so they will secrete mucus when removed with a needle. Meanwhile, Gram-positive bacterial cells will not form mucus [11].

In the catalase test, the five isolates formed air bubbles when dropped with H₂O₂ solution. This shows that the five bacterial isolates have catalase enzymes that can decompose H₂O₂ solutions into water and oxygen bubbles. The catalase test aims to determine the ability of bacteria to degrade hydrogen peroxide through the production of the enzyme catalase [12].

Media Thiosulfate Citrate Bile Salt Sucrose (TCBS) is a selective medium for the isolation of growth of bacteria of the genus *Vibrio* sp. TCBS media has a composition consisting of bile salts so that it can inhibit the growth of non-target bacteria. Sodium chloride plays a role in optimizing the development of halophiles, sodium sulfate as a source of sulfur, and ferric citrate which is used to detect the presence of H₂S [13]. Based on observations, the five bacterial isolates that have been isolated belong to the genus *Vibrio* because they can grow on TCBS media. the five isolates consisting of HA1, IN3, US2, HP2, HP3 showed yellow colonies on TCBS media. According to [14] that the color change in TCBS media is caused by bacteria that can ferment sucrose and lower the pH so that the media becomes acidic.

The gelatin test aims to determine the ability of bacteria to produce gelatinase enzymes in hydrolyzing gelatin into amino acids [14]. Results Based on the gelatin test, the five isolates showed positive results which were indicated by agar media after being put in the refrigerator at 4 °C for 30 minutes. This occurs in gelatin media consisting of nutrient broth and gelatin concentration which produces a hard medium and works as a substrate for the gelatinase enzyme. Gelatin in the media will be hydrolyzed by the gelatinase enzyme into amino acids so that when put in the refrigerator, the media cannot become hard again because gelatin has been hydrolyzed by bacteria [12].

In the Methyl-Red (MR) test showed four bacterial isolates coded by HA1, IN3, US2, and HP3 showed positive result. However isolate code by HP2 showed negative result. According to [12] that the MR test is to see the ability of bacteria to oxidize glucose by producing acid at high concentrations. A positive result will indicate a change in color to red, while a yellow-brown color indicates a negative result.

The media for the motility test used semi-solid MIO media. In MIO media, motility, indole production, and ornithine decarboxylation can be observed in bacteria [15]. The test results on the five isolates showed positive results. This is indicated by the growth that spreads around the area where the puncture was made. The ornithine test aims to determine the ability of bacteria to break down ornithine
(an amino acid) into amino acids. In the ornithine test, bacterial isolates with codes HA1 and HP2 showed positive results which were indicated by the media remaining purple. Meanwhile, bacterial isolates with codes IN3, US2, and HP3 showed negative results which were marked by a color change in the media, at the bottom of the media the color changed from purple to yellow. According to [16] that a positive test result is indicated if the media is purple and a negative result if the media turns yellow or yellowish. The indole observations were made after observing the motility and ornithine decarboxylation observations. The indole test aims to identify bacteria that produce indole by using the tryptophanase enzyme. The tryptophanase enzyme functions to break down tryptophan to produce indole, pyruvic acid, and ammonia [17]. Based on the results of the Indole test, it was found that the isolate HA1, IN3, and HP2 showed positive results indicated by the presence of a cherry red ring at the top of the media when it was dropped with Kovac's reagent. The cherry color is produced because indole reacts with p-dimethylaminobenzaldehyde [18]. While the bacterial isolates US2 and HP3 showed negative results which were indicated by the absence of color changes in the media after being dropped with Kovac's reagent.

In the oxidative-fermentative test, five isolates showed positive result, the color of media change from blue to yellow, both in the tube with paraffin or not. According to [19] that the principle of the oxidative/fermentative test of the media covered with paraffin changes color from blue to yellow, so bacteria can utilize carbohydrates in anaerobic conditions and are said to be fermentative.

In the Triple Sugar Iron Agar test, HA1 isolates showed yellow on the slant (oblique part) and yellow on the but (stem part) that showed the bacteria are acidic (acidic). Through the tryptophanase enzyme. However four isolate are IN3, US2, HP2, and HP3 showed red on oblique part and yellow on the stem part. Ornithine test results, bacterial isolates from HA1 and HP3 showed positive test results, while bacterial isolates from IN3, US2, and HP2 showed negative test results. According to [12] that the tryptophanase enzyme and have the ornithine decarboxylase enzyme. Five isolates did not produce H2S. Bacterial isolates from HA1 produced gas, and bacterial isolates from IN3, US2, HP2, and HP3 had no gas.

In the Simmons citrate test, five bacterial isolates showed positive results as indicated by the change in the media from green to bluish green. The citrate test aims to detect the ability of bacteria to utilize citrate as a carbon source as energy. The five bacterial isolates were inoculated on media containing sodium nitrate, pH indicator Bromthymol Blue, and inorganic ammonium which was used as a nitrogen source. Utilization of citrate involves the enzyme citrate permease which breaks down citrate into oxaloacetic and acetic acids. Oxaloacetic acid will be converted into pyruvic acid and CO₂. The results of the metabolism of sodium citrate will produce Na₂CO₃ and NH₃ which causes the pH of the media to become alkaline. This is what causes the media to change from green to blue [17].

The carbohydrate tests aims to test the ability of bacteria to ferment several types of sugar [17]. The types of sugar used are glucose, maltose, lactose, mannitol, and sorbitol. Based on the results of the study, the five isolates were able to ferment glucose, maltose, and mannitol which was characterized by a change in the media from red to yellow. However, the five isolates could not ferment lactose and sorbitol which was characterized by no color change in the media. The color change in the media is due to the formation of acid resulting from sugar fermentation, causing red phenol to react and change color to yellow [12].

In the urease test, bacterial isolates from HA1 showed positive results, while bacterial isolates from IN3, US2, HP2, and HP3 showed negative results. According to [20] that urease test aims to determine the ability of bacteria to convert urea into ammonia.

In the Lysine Iron Agar Test, three isolates from HA1, HP2, and HP3 showed the positive results of lysine decarboxylase which is characterized by a change in the medium from brown to purple, while bacterial isolates from IN3 and US2 showed negative results. According to [21] that lysine decarboxylase test that showed positive results were purple (purple) isolates on all parts both on the bottom of the media and the slanted part of the media and able to deaminase lysine showed a faded or yellow color change.
Based on Bergey’s Manual of Determinative Bacteriology, bacterial isolate from HA1 (Haemolymph) have close relationship with Vibrio carchariae. V. carchariae is Gram-negative, motile, fermentative, which produce indole, catalase, ornithine and lysine decarboxylase, and can degrade gelatin. V. carchariae can produce acid from glucose, mannitol, maltose, but not sorbitol [22]. Vibrio carchariae generally lives in sea and brackish water, especially shallow air and seasons where the air temperature becomes high [23], it is also found in aquatic habitats, some in sea water, estuarine environments and in association with marine animals [22].

Bacteria isolate from IN3 (gills) have close relationship with Vibrio anguillarum. V. anguillarum have round, raised, entire, and shiny colony, fermentative, Gram-negative rods, which motile by single polar flagella, can produce catalase, and indole, but not lysine or ornithine decarboxylase. V. anguillarum can produce acid from glucose, maltose, and mannitol [24]. Vibriosis due to V. anguillarum is one of the most important bacterial diseases of fish throughout the world. Recently, other Vibrio species taxonomically related to V. anguillarum, and considered environmental strains without pathogenic importance, have been associated with the disease in marine fish and shellfish [25].

Bacteria isolate from US2 have relationship with Vibrio ordalii. V. ordalii is Gram-negative, motile, fermentative, which produce catalase and gelatinase, but not H2S, indole, lysine decarboxylase, ornithine decarboxylase. V. ordalii can produce acid from glucose, sucrose, and maltose, but not sorbitol [22].

Bacteria isolate from HP2 have close relationship with Vibrio alginolyticus. V. alginolyticus is Gram negative, motile, fermentative, which produce indole, catalase, lysine decarboxylase, ornithine decarboxylase, gelatinase, but not H2S, urease, and arginine dehydroylase. V. alginolyticus can produce acid from glucose, maltose, mannitol, but not sorbitol [22]. V. alginolyticus is mainly found in marine estuaries, coastal and aquatic environments [26] with worldwide distribution. It may exist as free-living, a parasite or associated with surfaces of organisms such as marine vertebrates/invertebrates and flora, and even humans [27]. Vibrio alginolyticus has been found to cause varied infections and inflammation in both humans and animals [28] such as otitis, ocular infections, intracranial infection, peritonitis and osteomyelitis among others [29].

Bacteria isolate from HP3 (hepatopancreas) have close relationship with Vibrio splendidus. V. splendidus is motile, fermentative, Gram-negative, which produce catalase, lysine decarboxylase, but not H2S, indole, ornithine decarboxylase, degrade gelatin, but not urea, demonstrate positivity for the methyl red test, produce acid from glucose and maltose, but not from sorbitol. V. splendidus can cause disease sign included innaptance, erratic swimming, distended abdomen, stomach, and intestine, which contained clear fluid, haemorrhaging and necrosis in the kidney and liver [30].

Vibriosis can cause symptoms of septicaemia with widespread lesions of the skin, necrosis of the liver, kidneys, and other tissues [31]. Clinical signs of vibrio infection are septimia, haemorrhage on the skin, gills, and tail, ulcers on the skin, haemorrhage on muscle tissue and serosal surfaces [32]. Vibrio are pathogenic when they have reached the amount (quorum) required to express virulence factors [33]. Some Vibrio species can also produce a toxin in the form of anhydrotetrodotoxin. Although this toxin is less toxic than tetrodotoxin, at low pH conditions it is very easily converted to the neurotoxin [34].

Selection and identification of vibrio bacteria that has been carried out by Mancuso et al., Vibrio species consisting of Vibrio alginolyticus, Vibrio anguillarum Vibrio splendidus [35], Vibrio ordalii [36], and Vibrio carchariae have been identified with other Vibrio species. In this study, although it was not stated as the most virulent isolate, it was proven that the five Vibrio species were able to trigger the symptoms of vibriosis.

4. Conclusion
Based on biochemical tests carried out such as oxidase test, catalase test, Simmons Citrate test, Triple Sugar Iron Agar, Oxidative-Fermentative, Motility Indol Ornithine, Lysine Iron Agar, Methyl-Red, urease, gelatin, and Thiosulfate citrate bile salts sucrose (TCBS) test, isolate of HA1 has close relationship with Vibrio carchariae, isolate of HP2 has close relationship with Vibrio alginolyticus, bacteria isolate of IN3 has close relationship with Vibrio anguillarum, US2 has relationship with Vibrio ordalii, and bacteria isolate from HP3 have close relationship with Vibrio splendidus.
Acknowledgment

The author would like to thank to Research and Community Services Centre (LPPM) University of Bengkulu through 2021 Research Grant (Hibah Penelitian Fundamental UNIB) Grant number: 1812/UN30.15 / PG / 2021 dated 22 June 2021 to Risky Hadi Wibowo. The author also thanks to Quality Control and Fishery Product Safety Agency (BKIPM) of Bengkulu, Biology Department, Faculty of Mathematics and Natural Sciences, as well as to all parties who have helped the completion of this research.

References

[1] Holthuis L B 1991 FAO Fisheries Synopsis 13 139-141.
[2] Food and Agriculture Organization of the United Nations (FAO) 2015 Cultured Aquatic Species Information Program Panulirus homarus.
[3] Shields J D 2011 Journal of Invertebrate Pathology 106 79-91.
[4] Chandrakalaka N and Priya S 2017 International Journal of Scientific Research in Science, Engineering and Technology 3 27-33.
[5] Lavilla-Pitogo C R Lio-Po G D Cruz-Lacierda E R Alapide-Tendencia E V and De La Pena L D 2000 Disease of Peneid Shrimps in the Philippines 2nd Edition. Southeast Asian Fisheries Development Center, Philippines.
[6] Ramesh K Natarajan M Sridhar H and Umamaheswari S 2014 Global Journal of Bio-Science and Biotechnology 3 109-114.
[7] Yuasa K Des Roza I Koesharyani F Johnny and K Mahardika 2000 General Remarks On Fish Disease Diagnosis. JICA Booklet, Lolitkanta.
[8] Holt J G, Krieg N R, Sneath P H A, Staley J T and Williams S T 1994 Bergey’s Manual of Determinative Bacteriology. 9th ed. William and Wilkins, USA.
[9] Whitman W Goodfellow M Kampfer P Busse H J Trujilo M Ludwig W Suzuki K and Parte A. 2009 Bergey’s Manual of Systematic Bacteriology 2nd Edition Volume 3rd. Springer, New York.
[10] Halebain S et al 1981 Journal of Clinical Microbiology 13 444-448
[11] Kurnia K Sadi N H and Jumianto S 2015 International Journal of Scientific and Technology 4 83-87.
[12] Cappucino G J and Sherman N 2013 Manual Laboratorium Mikrobiologi, Edisi 8. EGC, Jakarta.
[13] Hikmawati F Susilowati A and Ratna S 2019 Journal of Aquaculture Management and Technology 5 334–339.
[14] Farouque S M Albert M J and Mekalanos J J 2000 Microbiology and Molecular Biology Reviews 62 1301-1314.
[15] MacFaddin J F 2000 Biochemical Tests for Identification of Medical Bacteria 3rd Edition., Lippincott Williams and Wilkins, Philadelphia.
[16] Wahyuni R M Sayuti A Abrar M Erina Hasan M Zainuddin 2018 Jurnal Ilmiah Mahasiswa Veteriner 2 474-487
[17] Hemraj V 2013 Innofare journal of life science 1 1-7.
[18] Rahayu S A and Gumilar M H 2017 Jurnal Sains dan Teknologi Farmasi Indonesia 4 50-56.
[19] Purnamawati, R. 2016. Fish Disease Inspection Methods at Fish Quarantine Center for Quality and Safety Control of Class I Fishery Products Surabaya I. Skripsi. University of Airlangga: Undergraduate Program of Aquaculture.
[20] Ulfia Endang S and Mimien H Preliminary Research. Proceeding Biology Education Conference (ISSN: 2528-5742) 13 1
[21] Haryani Y Chainulfifah dan Rustiana 2012 Jurnal Indonesia Chemia Acta 3 24.
[22] Inglis V Robert R J and Bromage N R 1993 Bacterial Disease of Fish. Back Well Scientific Publication, London.
[23] Kabata Z 1985 *Paracite and Disease of Fish Culture in Tropic*. Taylor and Francis, Philadelphia.
[24] Austin B and Austin D A 2012 *Bacterial Fish Pathogens: Disease in Farmed and Wild Fish*. 4Edth. Springer, USA.
[25] Fouz, B Conchas R. F Bolinches J Romalde J L Barja, J L Toranzo A E. 1990 In: Perkins F O Cheng T C (eds.) *Pathology in marine science*. Academic Press, New York.
[26] Narracci M Acquaviva M I and Cavallo R A 2013 *Environmental Science and Pollution Research* 21 2378–2385.
[27] Chen M X Li H. Y Li G and Zheng, T L 2011 *Brazilian Journal of Microbiology* 42 884–896.
[28] Rubin S J and Tilton R. C 1975 *Journal of Clinical Microbiology* 2 556–558.
[29] Li X C Xiang Z Y Xu X M Yan W H and Ma J M 2009 *Journal of Clinical Microbiology* 47, 3379–3381.
[30] Jensen S Samuelsen O Andersen Torkildsen, L Lambert C Choquet, G Paillard, C and Bergh O 2003 *Disease of Aquatic Organism* 53 25-31.
[31] Wang X H and Leung K Y 2000 *Microbiology* 146 989-998
[32] Irianto A 2005 *Patologi ikan Telostei*. UGM Press, Yogyakarta.
[33] Defoirdt T Boon N Bossier P Verstraete W 2004 *Aquaculture* 240 69-88
[34] Simidu V Naguchi T Hwang D Shida Y Hashimoto 1987 *Applied and Environmental Microbiology* 53 1714-1715
[35] Mancuso M Zaccone R Carella F Maiolino P De V G 2013 Journal of Aquaculture Research and Development 4.
[36] Ruiz P Balado M Fuentes-Monteverde J C Toranzo A E Rodríguez J Jiménez C, Avendaño-