The study of live and frozen marine fish in commodities imports using conventional and the Polymerase Chain Reaction (PCR)

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Abstract. Indonesia has big potency of fishery which has entered in export-import market. That export-import activity isn’t balance with quality warranty and the quality assurance. The quality warranty and the quality assurance are important because there found bacteria which have zoonotic characteristic. The method of data collection consisted of primary data and secondary data. Data collection was carried out with active participation, observation, interview and literature. Conventional method was used for bacterial examination on a live fish. The use of this method started from the initial isolation, purification, base test, biochemical test and the last was identification process. Bacteria found in a live fish by using the conventional method are Pasteurella haemolytica, Acinetobacter sp, Aeromonas hydrophila, Pseudomonna maltophilia, Pseudomonas sp, Vibrio sp, Pseudomonas mallei, Plesiomonas sp, Alcaligenes sp and Pseudomonas aeruginosa. Polymerase Chain Reaction (PCR) method was used for bacterial examination in frozen fish. This method had many steps, there were extraction, amplification, electrophoresis, and the last was reading the result with UVdoc machine. Primer which was provided that was used to diagnostic DNA bacteria of Aeromonas salmonicida and Edwardsiella tarda. During the study no bacteria of Aeromonas salmonicida and Edwardsiella tarda found, in frozen fish by PCR method.

1. Introduction
Marine ecosystems are very broad natural ecosystems [1, 2]. This ecosystem is divided into four subsections of the ecosystem—both based on the depth and distance from the beach. The food chain is eating and being eaten that occurs between living things linearly, following a certain trophic level [3, 4, 5]. The food chain can also be interpreted as a biochemical transfer of energy between living things through eating and eating interactions. Phytoplankton is single-celled plankton that acts as a producer like the food chain on land. Phytoplankton is autotrophs because they can make their food through photosynthesis with sunlight [6, 7, 8]. Zooplankton includes small plankton that hovers freely in water [9, 10]. Heterotrophs are dependent on other organisms for survival. In the food chain, including the third to four trophic levels or consumers of levels I to III before predators. There are two types of zooplankton: phytoplankton-eating zooplankton (consumer I) and smaller zooplankton-eating zooplankton (consumers II and III).
Marine fish predators are fish that can hunt, catch, and naturally eat fish. The food chain occupies the highest trophic level in marine ecosystems but has a tiny population. An example of a whale that is able to eat large fish and schools of small fish at once. The last component is the decomposer or decomposer. Decomposer marine ecosystems are known as benthos [11]. This benthos describes living things that have died into smaller components to be reused as food nutrients for phytoplankton [12, 13]. The role of this decomposition commodity forms the basis of Indonesia's natural wealth.

Indonesia has the potential for large fisheries resources and has entered the export-import market. Export-import activities are not balanced with the quality assurance of fishery products. Given that quality assurance is the key to facilitate access to the marketing of fishery products. This was stated by the [14] that fishery products in Indonesia are considered less capable of competing with foreign (imported) fishery products. Indonesian fishery export products are deemed inadequate due to the absence of certification of fishery products' quality. Given the importance of certification for the quality of fishery products, the Ministry of Marine Affairs and Fisheries of Indonesia through the Regulation of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number PER. 15/MEN/2011 article 5a improves the quality of fish imports.

The terms or conditions aim to guarantee the quality of fishery products or products (quality assurance and food safety) and a form of maintaining safety for consumers. The quality and safety assurance system for fishery products refers to the Regulation of the Minister of Marine Affairs and Fisheries Number PER. 01/MEN/2007 [15].

Quality assurance measures can be carried out by checking fishery products that have been imported and are ready to be sold to the broader community. One of the checks carried out is an examination of fishery-produced bacteria. The bacterial examination is carried out by checking these microorganisms can attack fish and affect the quality of fish by reducing the quality of infected fish meat to be attractive to consumers [16]. Some bacteria are pathogenic and capable of attacking humans (zoonoses).

Several cases in the study stated that several strains of A. hydrophila could cause enteropathogenic, especially in children, the elderly, and immunocompromised sufferers, or damage the immune system due to pathogenic infections [16]. Some of the diarrhea symptoms due to A. hydrophila that cause gastroenteritis are closely related to the production of enterotoxins by these bacteria [17]. These bacteria can utilize albumin, casein, fibrinogen, and gelatin as protein substrates; it can be concluded that these bacterial strains are proteolytic so that A. hydrophila has great potential as pathogenic bacteria in fish [16]. Based on this background, a Bacterial Examination Study on Imported Fish Commodities was conducted.

2. Materials and methods

2.1. Method of collecting data
The data taken in this study are primary data and secondary data obtained through several methods. Primary data is data obtained from the first informant, namely individuals or individuals, such as the results of interviews conducted by research. This primary data can be in the form of notes on interviews, results of direct field observations in the form of notes about situations or events, and data about informants [18]. Sigian and Sugianto [19] state that secondary data is primary data obtained from other parties or primary data that has been further processed and presented by primary data collectors or by other parties, which are generally presented in diagrammatic form. Secondary data is used by researchers to provide additional images or for further processing.

Fish that are tested for bacteria at BBKIPM Soekarno-Hatta aims to maintain fishery products' quality and feasibility. Fish samples tested consisted of live fish and frozen fish, which will be marketed. Samples recorded and coded by the Soekarno-Hatta BBKIPM office are then sent to the Soekarno-Hatta BBKIPM laboratory and the application form. The sample code number is used to make it easier to check the origin of the sample being examined if it is later tested positive for containing HPIK. The Laboratory of the Center for Fish Quarantine (BBKIPM) Soekarno-Hatta has the stages of examining bacteria, as illustrated in the chart.
2.2. *Equipment and room sterilization*
Sterilization is a process of destroying all forms of microorganisms, both vegetative and spore-shaped [20]. Sterilization is one of the essential parts in carrying out conventional and PCR examinations. The Center for Fish Quarantine, Quality Control, and Safety of Fishery Products (BBKIPM) Soekarno-Hatta uses two methods to sterilize the equipment used: ultraviolet light and ultraviolet light by autoclave. Ultraviolet light is used in every laboratory room and starts to turn on when all the workers carry out activities in the laboratory. Sterilization using ultraviolet light also functions to sterilize large equipment that cannot be sterilized using an autoclave.

2.3. *The technique of examining fresh fish bacteria using conventional methods*
The microbiology laboratory is one of the facilities provided by BBKIPM Soekarno-Hatta for testing bacteria using conventional methods. This method is often used for live fish testing. The conventional method consists of several steps, namely isolation, bacterial purification, basic tests and biochemical tests.

2.4. *Isolate bacteria*
Examining bacteria using conventional methods begins with isolation activities on TSA media 0% for freshwater fish samples and 3% for seawater fish samples. Priadie [21] argued that the purpose of carrying out isolation activities was to obtain the desired bacteria by taking the sample and then culturing it using universal or selective media depending on the goals to be achieved. The ose needle is sterilized on top of the bunsen before applying target organ rubbing to kill contaminant bacteria. The isolation activity was carried out by scratching the target organs on the isolation medium, namely Trypticase Soy agar (TSA). The media is then wrapped in paper to avoid contamination and incubated for 24 hours [22].

3. *Result and discussion*
3.1. *Purification*
Purification is a stage for selecting a target colony in accordance with the colony provisions that have been determined in the Work Instructions (IK) of bacteria at BBKIPM Soekarno-Hatta. The target colony in accordance with the provisions of Soekarno Hatta's BBKIPM was isolated by scratching it on the TSA 0% or 3% TSA media then incubating for 24 hours.

3.2. *Basic Test*
The basic test consists of the Gram test, the catalase test, and the oxidase test. This is by the statement of Marlina [23] that biological identification of bacteria consists of Gram testing using a 3% KOH solution, oxidase test using an oxidase test strip, and catalase test. In addition to staining, Gram testing can be done using a 3% KOH solution. The first step in the Gram test is to clean the glass object using alcohol and then burn it over a Bunsen fire. Drop a 3% KOH solution on a glass object and scratch a pure culture on the glass object and stir it several times. Bacteria are stated as Gram-negative if the suspension becomes slimy when the Ose is removed, and bacteria is stated Gram-positive if the suspension is not mucus when the Ose is removed. Gram-negative bacteria have a cell wall structure consisting of thin peptidoglycan and thick lipopolysaccharide (LPS). This content causes the bacteria to produce mucus when given the 3% KOH reagent. Gram-positive bacteria have a cell wall structure that contains thick peptidoglycan. Therefore Gram-positive bacteria do not produce mucus when given 3% KOH reagent [24].

Catalase testing uses bactident catalase, which is a reagent of hydrogen peroxide (H₂O₂). Hydrogen peroxide (H₂O₂) reagent is toxic to cells because it inactivates enzymes in bacterial cells [22]. The catalase test’s initial step is to clean the glass object using alcohol and burn it over a Bunsen fire. A pure culture is scratched on a glass object and then given bactident catalase. Positive results if the bacteria produce bubbles, and it is said to be negative if the bacteria do not produce bubbles. The
ability of bacteria to produce bubbles proves that these bacteria can produce the catalase enzyme to break down hydrogen peroxide (H₂O₂) into H₂O and O₂. The oxidase test uses bactident oxidase. This test aims to determine the presence of cytochrome oxidase found in certain microorganisms. Bacteria are taken using sterile Ose then smeared on bactident oxidase, if the paper is purple, then the bacteria is declared oxidase-positive, and if the paper is yellow, then it is declared oxidase negative because it is unable to form cytochrome oxidase.

3.3. Biochemical Test

The conventional method of examining bacteria is a method used to culture bacteria on specific media [25]. Gardenia et al. [16] explained that the examination used conventional methods based on the characteristics of the biochemical test. The biochemical test used at BBKIPM Soekarno Hatta consists of two stages, namely:

1. The first stage of biochemical testing

The first stage aims to see whether the examined bacteria belong to the HPIK group of dangerous bacteria genus. At this stage, it consists of several tests based on the media used, including:

a. TSIA (Triple Sugar Iron Agar) test

The TSIA test uses solid media to differentiate bacterial properties biochemically. The use of this medium is to distinguish bacteria belonging to the Enterobacteriaceae family in their ability to ferment carbohydrates to form acids, gases, and H₂S [26]. The test step is carried out by inserting a pure culture into the media's right part and scratching it on its oblique part. The yellow color indicates that the bacteria can ferment glucose, sucrose, and lactose. The red color shows that glucose, sucrose, and lactose are not fermented. The reaction in this media is as follows: acid butt, which is yellow on the upright side, acid slant, yellow on the oblique part, Gas in Butt, which is gas in the upright part, No H₂S, i.e., no H₂S is formed.

b. SIM (Sulfide Indole Motility) Test

SIM test is used to detect the ability of bacteria to form indoles and motiles. The test was carried out by inserting a pure culture into the media. During the study, biochemical tests on SIM media showed that the red ring was found after Kovacs and the media looked cloudy. This proves that the bacteria tested are motile and able to form indoles. The statement of Chusniati et al. [26] supports these results stated that the formation of indoles is indicated by the presence of a red ring on the top layer of the media after the addition of Kovacs reagent and motility, which is indicated by the spread of bacterial growth on the puncture site or like an inverted pine tree or the media looks cloudy.

c. OF Test (Oxidative/Fermentative Test)

The OF test aims to determine the metabolic ability of bacteria to metabolize in aerobic and anaerobic media. This media's characteristics are that it contains red peptone, semi-solid, and high carbohydrate concentration [24]. The test step is to insert a pure bacterial culture using a sterile needle in two test tubes containing O/F media. One of the tubes has a surface covered with sterile liquid paraffin 1cm thick. The results obtained during the study were that the bacteria were fermentative and oxidative. Fermentative indicates that the bacteria can ferment glucose in standardized and non-standardized media to turn yellow. Meanwhile, oxidation indicates that bacteria are only able to ferment glucose in non-standardized media [27].

d. Ornithin Motility Indole (MIO) Test

MIO test using MIO (Motility Indole Ornithin) media. MIO is a purple semi-solid medium. The MIO test aims to determine the movement of bacteria (motility), to determine the enzymatic ability of bacteria to carboxylase ornithine into amine (Ornithine) form, and to determine the production of indole from tryptophan (Indol). The test step is to take a pure culture of bacteria with a sterile needle,
inoculate it into the MIO by prick, then incubate. For indole testing, it is necessary to add a few drops of Kovacs reagent to the media. The results that can be read from this media are positive ornithine decarboxylase if the media's anaerobic area is gray, purple, blue, and negative ornithine decarboxylase the anaerobic area of the media changes color to yellow.

e. Glucose Test
The glucose test is one of the first stages of the confectionery test. The test step was carried out by inserting a pure culture into the sugar medium. In a study conducted in a microbiology laboratory, the results of the glucose test medium turned yellow. There was also a test on different samples of the media that did not change color. Bacteria that can degrade glucose and produce organic acids cause the media to turn yellow [28].

MAC (Mac Conkey agar) is a selective medium for the isolation and identification of Gram-negative bacteria. The test is used to distinguish bacteria capable of fermenting lactose (fermenters). The test step was started by scratching the pure culture on MAC media and then incubated. The MAC test results, namely the growth of colonies in red, are also some samples with clear colonies. These colonies' growth negates that lactose fermenters will produce red colonies, and bacteria that are lactose non-fermenters will form transparent or colorless colonies [29].

f. TCBS (Thiosulphate Citrate Bile-Salt Sucrose Agar) test
The TCBS test uses green agar and contains sucrose. Testing on this medium was carried out by etching a pure culture and then incubated. Bacteria that grow on TCBS media will form colonies by forming different colors. The results obtained during the study were the media turned yellow, while the color remained green. Bacteria can ferment sucrose, which causes the media to turn yellow and vice versa, non-fermenters will not change the color of the media [26].

g. Eosin Methilene Blue Agar (LEVINE - EMBA) Test
EMBA media is a selective medium for differentiating Enterobacteriaceae groups. This medium contains eosin and methylene, which are used to inhibit Gram-positive bacteria's growth so that it allows Gram-negative bacteria to grow [29]. The test step on this media is by scratching a pure culture on the EMBA medium. Bacteria that grow on this media will form colonies and colony colors that vary depending on the species. Eosin Methylene Blue Agar also contains lactose and sucrose. Lactose fermenters bacteria will cause the color of the media to turn yellow, while non-lactose fermenters will not change the media's color.

h. Sensitivity Test
Sensitivity test using MHA media. This test aims to determine the sensitivity or sensitivity of a bacterium to antibiotics. The antibiotic used is Novobiocin 30mg. The initial step of testing the sensitivity is by rubbing the pure culture on MHA media and then applying the 30mg Novobiocin antibiotic. Indicators of inhibition of microorganism growth by antibiotics are a clear area (zone of inhibition) around microorganisms' growth [30].

2. The second stage of biochemical testing
This second stage is used when the first stage of the biochemistry test shows the dangerous HPIK group of bacteria, then re-tested to determine the bacterial species. The test consisted of:

a. Citrat Test
The citrate test aims to determine the ability of bacteria to utilize citrate as a carbon source. This biochemical test was initiated by etching the pure culture on the medium in a zigzag manner and then incubated. The results of the study on the Citrate test media were that the media did not change color. The media does not change color because bacteria do not use carbon as a metabolic source. Bacteria
that utilize carbon in the media will produce sodium carbonate, alkaline causing the media to turn blue, which is the bacteria [31].

b. Sugar Test
This test is useful for determining bacteria's ability to degrade sugars and produce organic acids that come from each type of sugar [28]. This process causes the pH to become acidic so that the media turns yellow. The initial step of testing the sugar is by inserting the pure culture into the sugar medium and then incubating it. The reaction results will be the same in the glucose test; namely, if the bacteria can degrade sugar and produce organic acids, the media will turn yellow. In contrast, if the bacteria cannot degrade sugar and are unable to produce organic acids, the media will remain colored.

c. Urea Test
The urea test aims to determine which bacteria have the urease enzyme. The test step was started by infusing the pure culture on the media and then incubated. Certain bacteria can hydrolyze urea and form ammonia by causing a red color due to the phenol red indicator. The formation of ammonia causes the pH value to become alkaline so that the urea test has a light color on the media and means a positive test [30].

e. Lysine Iron Agar (LIA) Test
The LIA test aims to determine bacteria's ability to decarboxylase lysine contained in agar or media [31]. The lysine decarboxylase reaction or the anaerobic alkaline reaction will neutralize the acid formed from glucose fermentation. LIA media is purple and placed in the tube. The test was started by infusing the pure culture into the media and then incubated. Some of the reactions or results that will occur in this media, namely: a) Lysine deaminase if a red color is formed on the top of the media, b) Positive lysine decarboxylase if there is no color change in the media, and c) Negative lysine dekaboxylase if the butt area changes to yellow, H₂S if a black color is formed on the media.

f. MR (Methyl Red) test - VP (Voges Proskauer)
MR test was performed to determine the presence of mixed acid fermentation. Some bacteria can ferment glucose and produce various kinds of acidic products to lower the pH of the media [30]. The addition of an indicator in the form of methyl red can show a change in pH to acid, which is indicated by a red section on the media's surface without shaking.

The VP test was conducted to determine the bacteria's ability to produce acetyl methyl carbinol [30]. This can be done by adding reagent alpha naphthol and KOH 40%, shaking it slowly and tilted, and letting it stand for 15-30 minutes. The test is positive if the media turns red because the bacteria can produce acetyl methyl carbinol.

g. Gelatin Test
Gelatin test is used to determine the ability of bacteria to produce the proteolytic enzyme gelatinase. After incubation, this media is tested by placing it in the freezer for five minutes. The reading on this media is that if the media becomes frozen after removing it from the freezer, the result is negative. If the media remains liquid after being placed in the freezer, the result is positive [31].

h. Arginine test
The Arginine test aims to determine the ability of bacteria to hydrolyze arginine in the media. This medium is yellow and has a semi-solid form. The use of this medium is added with paraffin. Testing on this medium begins with inserting a pure culture and then incubating it. This test will get a reaction or result, namely a) a positive response if the media turns red and an adverse reaction if the media does not change color.

3.4. The results of bacterial examination using conventional methods
The biochemical test results were then compared with several reference books used by the Soekarno Hatta KIPM Center to identify bacteria. Ten types of bacteria were identified during the study, namely (Table 1):

| No. | BACTERIA                        |
|-----|--------------------------------|
| 1   | Pasteurella haemolytica         |
| 2   | Acinetobacter sp.               |
| 3   | Aeromonas hydrophilla           |
| 4   | Pseudomonas maltophilia         |
| 5   | Pseudomonas sp.                 |
| 6   | Vibrio sp.                      |
| 7   | Pseudomonas mallei              |
| 8   | Plesiomonas sp.                 |
| 9   | Alcaligenes sp.                 |
| 10  | Pseudomonas aeruginosa          |

The technique of examining frozen fish bacteria was the PCR polymerase chain reaction method. Examination of bacteria can be carried out by conventional methods and by the PCR method; this is by the statement of Whitman [24] that the diagnosis can be carried out by the PCR method. The PCR method is an effective and efficient method of molecular disease detection. Polymerase chain reaction (PCR) is an in vitro DNA synthesis and amplification technique [35]. Frozen fish samples received by the Soekarno-Hatta KIPM Center were tested for bacteria using the PCR method because these fish are fish that rot faster, so it is necessary to test them with a method that does not take a long time. The following is a list of fish tested by the PCR method during the study activity. The PCR method of bacterial examination was initiated by cutting the meat samples, which were carried out in the necropsy room. The meat pieces were placed in a tube containing 70% ethanol and coded according to the sample form. The sample must be checked immediately; if action is not taken immediately, it must be stored in the freezer. The use of ethanol 70% as a cleaning to avoid contamination and maintain the freshness of the sample material to be checked. The use of ethanol 70% as a cleaning to avoid contamination and maintain the freshness of the sample material to be checked. The use of ethanol 70% as a cleaning to avoid contamination and maintain the freshness of the sample material to be checked. The use of ethanol 70% as a cleaning to avoid contamination and maintain the freshness of the sample material to be checked. The stages in the PCR method are extraction, amplification, electrophoresis, and uvidoc. The use of ethanol 70% as a cleaning to avoid contamination and maintain the freshness of the sample material to be checked. The stages in the PCR method are extraction, amplification, electrophoresis, and uvidoc. The use of ethanol 70% as a cleaning to avoid contamination and maintain the freshness of the sample material to be checked. The stages in the PCR method are extraction, amplification, electrophoresis, and uvidoc.

3.5. Extraction
Extraction is one of the most critical steps in determining the amplification process's success using the PCR method. The extraction process aims to obtain template DNA. The initial step of this activity is to take a sample that is then dried on a tissue and then crushed. The crushed sample results were placed on a tube and given a 250 µl lysis buffer solution. Handoyo and Ari [35] explain that the lysis buffer solution is used to damage or break the cell wall. The buffer contained in the solution is used to maintain DNA stability during cell layering [25]. The next stage is the vortex for 20 seconds, which aims to homogenize the crushed sample with a lysis buffer solution, then incubated at 95°C for 10 minutes, which also aims to help to crush the sample cells. The vortex results were centrifuged at 12 rpm for 10 minutes to separate the supernatant and sediment. The centrifuge was taken 100 µl of the supernatant and placed it on a new microtube. The new microtube was added with 200 µl of 95%
ethanol, which was used to precipitate the target DNA so that it was mixed, and then vortex activity was carried out for 20 seconds. The activity was continued with a centrifuge at 12 rpm for another 5 minutes to obtain a precipitate suspected of being pellet-shaped bacterial DNA. Another objective of the multiple centrifugation process is to separate substances based on molecular density by applying a centrifugal force to be at the bottom, while lighter substances will be at the top [36]. The supernatant from the centrifuge is discarded so that only pellets are present and dried. Dry microtubes containing template DNA were added with TE Buffer 80 µl. TE Buffer is used to maintain osmotic pressure [25].

3.7. Amplification
Amplification is a process to multiply nucleic acid chains or segments without changing these nucleic acids [37]. In this process, primers are used to detect the presence of HPIK bacterial DNA. According to Handoyo and Ari [35], a PCR’ success depends on the primer used. The kit used in the detection of bacteria using the PCR method at BBKIPM Soekarno-Hatta is alpha DNA and the primer used is capable of detecting DNA of *A. salmonicida* and *E. tarda* bacteria. A transfer of 2 µl of the template DNA on the microtube was carried out before the amplification process. To the microtube was added 0.25 µl of forwarding primer, 0.25 µl of reverse primer, 12.5 µl of master mix, and 9.5 µl of nucleus free water. The primer is a mold for the acid-base structure of the targeted bacteria. The acid-base composition of *A. salmonicida* primary revers is AGC CTC CAC GCG CTC ACA GC and the first primer is AAG AGG CCC CAT AGT GTG GG. Edwardsiella tarda bacteria has an acid-base structure in the reverse primer, is TAG GGA AGG TGT GAA and on the forward primer is CTC TAG CTT GCC AGT CTT.

3.8. Electrophoresis
Electrophoresis is the stage after amplification, a chemical analysis method based on charged protein molecules’ movement in an electric field or isoelectric point. The movement of molecules in an electric field is influenced by the shape, size, charge size, and chemical properties of the molecule [38]. Wulandhari [39] suggests that in the electrophoresis process, DNA can migrate in a gel in a solid form, which is placed in a buffer solution that is energized by an electric current. The sample used as much as 10 µl was inserted into rose agar. The electrophoresis layout is a marker on the first well, positive control on the second well, negative control on the third well, and samples on the fourth well. Electrophoresis using a power of 150 V, 400 µA for 25 minutes.

3.9. UVdoc
UVdoc is a tool to read the electrophoresis results, which is inserted into agarose, which has been soaked with bromithidium for 15 minutes. Bromithidium attenuation aims to detect nucleic acids [40]. The result of the UVdoc photo shows the appearance of a line on a certain sphere band that has been determined by OIE.

The Soekarno-Hatta Center for Fish Quarantine, Quality Control, and Safety of Fishery Products (BBKIPM) can detect two bacteria species using the PCR method, namely *A. salmonicida* and *E. tarda*. *E. tarda* bacteria will stop at 216 bp, while *A. salmonicida* will stop at 423 bp [41].

3.10. The results of bacterial examination using the PCR method
The study, which took place from January 14 to February 14, 2013, found that the samples examined were not detected to contain harmful bacteria such as *A. salmonicida* and *E. tarda*.

3.11. Samples infected with quarantine fish pests (HPIK) in the bacterial class
Samples tested for fish disease pests (HPIK) must be destroyed and not allowed to be traded. These provisions are by the duties and functions of the Fish Quarantine, Quality Control, and Safety of Fishery Products (BKIPM) listed in the Regulation of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number: Per.25/Men/2011. Destruction or rejection aims to protect
consumers from zoonotic bacteria. The bacterial examination results on 14 live fish in the form of ornamental fish were declared infected by bacteria. The number of bacteria identified was ten species of bacteria. The species that have been found are not included in the list of bacterial types rejected by Indonesia [42]. Therefore, 14 live fish were not destroyed. A total of 123 frozen fish were not destroyed because of the results of the PCR examination; all samples were not infected with bacteria.

3.12. Recapitulation of samples examined at the study location

Study activities carried out at BBKIPM Soekarno-Hatta obtained the data that has been attached (attachment 11). The data are bacteria identified during the inspection process using conventional methods or the Polymerase Chain Reaction (PCR) method. The following is a table recapitulation of the number of fish samples received and identified.

| Information          | Sample    | total     | Percentage |
|----------------------|-----------|-----------|------------|
| Number of fish examined | Live fish | 14        | 10, 22%    |
|                      | Frozen fish | 123      | 89.78%     |
| Test result          | Conventional | 14      | 100%       |
|                      | PCR       | 0         | 0%         |

The number of fish examined from 14 January to 14 February 2013 was 137 fish consisting of 14 ornamental fish and 123 frozen fish. The method used in the examination is the conventional method and the PCR method. The number of samples of 14 ornamental fish examined by conventional methods was all positive for bacteria. One hundred twenty-three samples of frozen fish were examined by the PCR method according to primary bacteria; all samples were not infected with *A. salmonicida* and *E. tarda* bacteria. The percentage calculation states that examining bacteria in frozen fish is more than in live fish consisting of 89.78% for the percentage of frozen fish and 10.22% for ornamental fish. The amount of fish examination using the PCR method was not balanced with the results of the fish that were detected to contain bacteria, it is inversely proportional to the study using conventional methods. Conventional methods produce more identified bacteria. Frozen fish is easier to detect as infected by bacteria than live fish, but this is not supported by the data obtained. This can be due to the PCR method using a specific primer or a predetermined type of bacteria so that other bacteria may not be detected. The use of conventional methods is more effective because all types of bacteria in the sample can be identified according to the growing colony. However, this is not supported by the data obtained. This can be due to the PCR method using a specific primer or a predetermined type of bacteria so that other bacteria may not be detected. The use of conventional methods is more effective because, in this method, all types of bacteria in the sample can be identified according to the growing colony. However, this is not supported by the data obtained. This can be due to the PCR method using a specific primer or a predetermined type of bacteria so that other bacteria may not be detected. The use of conventional methods is more effective because, in this method, all types of bacteria in the sample can be identified according to the growing colony.

4. Conclusion

1. The method used in the examination of bacteria at the Soekarno-Hatta Center for Fish Quarantine, Quality Control, and Safety of Fishery Products (BBKIPM) is the conventional method and the Polymerase Chain Reaction (PCR) method.
2. The conventional method is used to examine bacteria in live fish or imported fish commodities. In contrast, the Polymerase Chain Reaction (PCR) method is used to check bacteria on imported fish commodities that have been frozen or frozen fish.
3. The conventional method consists of several steps, namely isolation, purification, basic tests and biochemical tests. The examination showed ten species of bacteria, namely *P. haemolytica,*
Acinetobacter sp., A. hydrophila, P. maltophilia, Pseudomonas sp., Vibrio sp., P. mallei, Plesiomonas sp., Alcaligenes sp. and P. aeruginosa.

4. Bacteria detected by conventional methods consisted of one bacterium, which was HPIK, according to Soekarno Hatta's BBKIPM Work Instructions, namely A. hydrophila. The ten bacteria are not included in the list of bacterial species rejected by Indonesia, according to the Decree of the Minister of Marine Affairs and Fisheries 2010 Number KEP.03/MEN/2010, so that the sample is not destroyed.

5. The Polymerase Chain Reaction (PCR) method has several stages, namely, extraction, amplification, electrophoresis, and photos. The examination was negative. The Soekarno-Hatta Center for Fish Quarantine, Quality Control, and Safety of Fishery Products (BBKIPM) can diagnose bacteria in fish, namely A. salmonicida and E. tarda. A. salmonicida is positive when it is at 423 bp, and E. tarda is positive when it is 216 bp using alpha DNA kits.

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