Isolation and identification of fish import consumption bacteria in a fish quarantine center, focusing on the quality control and safety of fishery products at Tanjung Priok, Jakarta

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Abstract. Several bacteria were found that were not classified as DIQP I or II bacteria. Every imported fish that enters the territory of Indonesia has to go through a quarantine process first. This is because the imported fish may contain the identified bacteria. This study aims to isolate and identify bacteria in several types of imported fish and to find out whether the bacteria identified are a Disease Inducing Quarantine Pest (DIQP) I or II. The bacterial identification was conducted using biochemical and molecular biology tests, including *Polymerase Chain Reaction* (PCR). The fish samples analyzed were 69 tails consisting of 10 imported fish species (Mackerel, Salmon, Tuna, Swordfish, Marlin, Black Cod, Oil Fish, Yellow Tail, Pacific Saury, Flounder fish). The results of the isolation and identification of the imported fish samples through the biochemical tests identified 16 types of bacteria, dominated by *Aeromonas caviae, Pseudomonas aeruginosa, Proteus morganii, Proteus vulgaris,* and *Vibrio fluvialis*. The results of the test with the *Polymerase Chain Reaction* obtained all negative test samples for *Aeromonas salmonicida*. The bacteria found in imported fish are therefore not classified as DIQP I or DIQP II.

1. Introduction

There has been an increased volume of fishery product imports by 10%, but the increase in import volume is not in line with the increase in frequency, which actually decreased by 5.25% in 2016, compared to 2015. Indonesian fishery product imports are used in order to meet the needs of organizing international events (exhibitions, embassy events and so on) [1] and also to supply the several types of fish needed by markets in Indonesia.

For every fishery import activity in a country, there is the usual initial process which is quarantine. The quarantine process aims to examine certain health and quality standards so then the fish can proceed to the next step. Imports can be interpreted as activities to enter goods from one country (abroad) into the customs territory of another country [2]. This can be represented by the interests of the two companies between the two countries, which are different. There are certain regulations, suppliers and other acts involved as the recipient country.

Increasing the flow of fishery commodity traffic (Export-Import) can also have a negative impact on the sustainability of fishery resources. The high demand for fishery commodities causes

uncontrolled fishery development, thus ignoring the carrying capacity of the surrounding environment. This results in a decrease in fishery productivity, and this can even lead to crop failure due to the decreased environmental quality and disease attacks. Harvest failure due to disease attacks is more common than the other factors [3].

Fish disease is caused by pathogenic microorganisms (parasites, bacteria, viruses, and fungi), feed, and environmental conditions that do not support the fish's life. Diseases caused by bacteria, in addition to causing mass death in fish, can also interfere with the quality of the fish by reducing the quality of the meat so then they are not favored by consumers; this can potentially disrupt human health [4].

According to [5], bacteria are the most numerous and widespread organisms, more so than other living things. Bacteria are made up of hundreds of thousands of species that live on land, in the ocean and in extreme places. Bacteria are beneficial, but there are also harmful bacteria. Bacteria can be identified by their biochemical reactions. Bacteria are isolated in bacterial media; this is to help learn of the nature of a colony. The characteristics of a colony are the properties that have something to do with their form, composition, surface, lacing and so on [6].

The control and supervision of quarantine fish disease pests (DIQP) and the quality of imported fishery products are the responsibility of Jakarta II Fish Quarantine Center, to protect - in the context of protecting domestic consumers - and to obtain quality products that are guaranteed in quality and health. In this case, the Jakarta II Fish Quarantine Center is required to play an important role in protecting the security of citizens who consume imported products and to protect the existing biological resources of the fisheries in Indonesia from the entry and distribution of certain DIQPs from abroad that are likely to be carried along with imported fisheries of this type of *invasive alien species* (threat to biodiversity) [1].

Quarantine Fish Disease Pests (DIQP) are all fish pests and diseases that do not yet exist and/or have existed only in certain areas in the territory of the Republic of Indonesia, which is a relatively fast time can become endemic and harm the socio-economy or public health [7]. DIQP is divided into two Group I DIQP and Group II DIQP.

The purpose of this study was to isolate and identify the bacteria in several types of imported fish, and to find out if the bacteria identified are included in DIQP I or II in the Fish Quarantine Center, Quality Control and Safety of Fishery Products, Tanjung Priok, Jakarta.

2. Materials and methods

2.1 Place and time

This study was carried out in the Bacteriology Laboratory of the Fish Quarantine Center, Fishery Product Quality and Safety Control in Tanjung Priok, Jakarta. This study was conducted for one month, namely from the 18th December, 2017 to 18th January, 2018.

2.2 Study material

The fish samples analyzed totaled 69 tails consisting of 10 imported fish species (Mackerel, Salmon, Tuna, Sword Fish, Marlin, Black Cod, Fish Oil, Yellow Tail, Pacific Saury, Flounder Fish), which came from Norway, Chile, Micronesia, Malaysia, Netherlands, United States, Ghana, Taiwan, Korea and Japan.

We used TCBS Medium (Thiosulfate Citrate Sucrose Bile Salt), TSIA Medium (Triple Sugar Iron Agar), 2% TSA Medium, 4% TSA Medium, BGA Medium (Brilliant Green Agar), MIO Medium (Motility Indole Ornithine), 3% KOH, Kovac's Reagent, MR Reagent, Cytochrome Oxidase, H_2O_2 3 %, Media O / F, Gelatine, Media LIA (Agar Lysine Iron), MR / VP Medium, Urease Medium, Citrate Medium, Aesculine Medium, Novobiocin, Adonitol, Arabinose, Dulcitol, Galactose, Glucose, Lactose, Inositol, Maltose, Rhamnose , Sucrose, Sorbitol and D-xylose.

2.3 Study tools

We used petri discs, ose, matches, Bunsen, trays, knives, Tube reaction, Rack reaction, Laminary flow, markers, autoclaves, incubators, drop pipettes, slide objects, and microscopes.

2.4 Work procedures

2.4.1 Equipment and media bacteria identification preparation for fisheries product

The preparation of the isolation media is one of the stages of the bacterial identification process. The media that needed to be prepared for the initial isolation stage of the bacteria was one medium TSA 2% (Trypticase Soya Agar), one BGA medium 2% (Bismuth Green Agar), and one medium TCBS (Thiosulfate Citrate Bile Salt Sucrose) for one sample of fish that was to be examined. For the stages of media bacterial purification that needed to be prepared for, we needed one TSIA 2% (Triple Sugar Iron Agar) medium, and one 4% TSA medium (Trypticase Soy Agar) for one sample of fish to be examined. For the next stage, namely the biochemical test, the media needed in the biochemical tests was one MH (Mueller Hinton) media, one MIO media tube (Motile Indole Ornithine), one LIA (Lysine Iron Agar) media tube, one pair of O / F media tubes, one Gelatin media tube, one MR / VP media tube, one Urea media tube, one Kanamycin Aesculine medium, one Citrate medium and one Sugar media tube package for one type of bacteria to be tested. The tools needed were Petri discs, ose, matches, Bunsen, trays, knives, Tube reaction, Rack reaction, Laminar flow, markers, autoclaves, incubators, Drop pipettes, slide objects, and microscopes.

2.4.2 Isolation from wounds and initial isolation

The main step that needed to be done was to observe the fish organoleptically (externally) and through proper. If the sample of the fish to be tested was still frozen, then the following steps must be taken until the sample until softens. The meat was pierced by the needle on the head, body, or tail, especially in meat that looked damaged or injured. However, if the fish had a good appearance, then the researcher stuck the needle nto the kidney part of the fish.

Before scraping the insulation material into the culture medium, it must be ensured that the oil that was used was already in a sterile state by heating the ose until it glowed or was reddish over the Bunsen fire. After being burnt, the ose was not directly used to take the insulation material but instead, it must be left for a while until it does not glow. After the isolate was taken, it can be scratched into the bacterial isolation media, TSA media 2%, BGA media 2%, and TCBS media. This isolation activity was carried out under a biosafety laminar flow. After the isolation activity was finished, the bacterial isolation media was put into an incubator at 37 °C and this incubation process took 24 hours. This time was needed to find out whether or not bacteria grew on the media used.

2.4.3 Bacteria purification

There were two media needed for the purification isolation, namely 2% TSA media and 2% TSIA media. This activity was carried out 24 hours after the bacteria underwent the incubation process at 37 °C. The method used to purify the bacteria was to take a small amount of bacterial culture that had been grown in the initial isolation medium.

The technique used for purification was streak or scratch like in the initial isolation. The culture taken was the culture that had the most dominant colonies in the isolation medium and the culture grew on top of the streaking. After the culture was scratched on the 2% TSA media and 2% TSIA media, the purification medium was put into an incubator at 37 °C to be incubated. The time required for the incubation process was 24 hours.

2.4.4 Biochemical test and presumptive test

The biochemical test included the OF Test, Indole Test, MRVP Test, Citrate Test, Urea Test, LIA Test, MIO Test (Ornithine), Gelatine Test, Esculin Test, and Sugar Test. The presumptive Test included the Gram Test With KOH 3%, Catalase Test With H_2O_2 and the Oxidase Test. After the test, the sample was put into an incubator at 37 °C to be incubated. The time required for the incubation process was 24 hours.

2.4.5 Bacteria identification preparation for fisheries product

The bacterial identification was carried out on the isolates obtained by referring to Cowan and Steel's Book (1993) Manual for the Identification of Medical Bacteria and Austin and Austin's (2007) Bacterial Fish Pathogen Diseases of Farmed and Wild Fish, Fourth Edition.

The study method used was the survey method, namely by using random sampling. The results from this study were analyzed descriptively.

3. Results and discussion

3.1 Bacteria identified by the biochemical tests at Jakarta II Fish Quarantine Center

Table 1. Results of the biochemical test identification of the imported fisheries products at Fish Quarantine Tanjuk Priok Jakarta, 18 December 2017 – 18 January 2018

Samples of Imported Fisheries Test	Countries of Origin	Results Biochemical Testing	Description
Frozen Atlantic Mackerel	Netherland	*Pseudomonas aeruginosa*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Netherland	*Vibrio fluvialis*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Netherland	*Proteus vulgaris*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Netherland	*Citrobacter freundii*	Not classified as DIQP I or II
Frozen Salmon	Chile	*Proteus vulgaris*	Not classified as DIQP I or II
Frozen Salmon	Chile	*Proteus vulgaris*	Not classified as DIQP I or II
Frozen Big Eye Tuna	Micronesia	*Pseudomonas aeruginosa*	Not classified as DIQP I or II
Frozen Yellow Fin Tuna	Micronesia	*Proteus morganii*	Not classified as DIQP I or II
Frozen Skipjack Tuna	Micronesia	*Vibrio damsela*	Not belonging to DIQP I or II
Frozen Big Eye Tuna	Micronesia	*Citrobacter freundii*	Not classified as DIQP I or II
Frozen Yellow Fin Tuna	Micronesia	*Aeromonas caviae*	Not classified as DIQP I or II
Frozen Skipjack Tuna	Micronesia	*Acinetobacter sp.*	Not classified as DIQP I or II
Frozen Big Eye Tuna	Micronesia	*Proteus sp.*	Not classified as DIQP I or II
Frozen Yellow Fin Tuna	Micronesia	*Enterobacter aerogenes*	Not classified as DIQP I or II
Frozen Skipjack Tuna	Micronesia	*Plesiomonas sp.*	Not classified as DIQP I or II
Frozen Big Eye Tuna	Micronesia	*Proteus rettgeri*	Not classified as DIQP I or II
Frozen Yellow Fin Tuna	Micronesia	*Serratia sp.*	Not classified as DIQP I or II
Frozen Skipjack Tuna	Micronesia	*Pseudomonas sp.*	Not classified as DIQP I or II
Frozen Big Eye Tuna	Micronesia	*Pseudomonas sp.*	Not classified as DIQP I or II
Frozen Yellow Fin Tuna	Micronesia	*Vibrio fluvialis*	Not classified as DIQP I or II
Frozen Skipjack Tuna	Micronesia	*Enterobacter aerogenes*	Not classified as DIQP I or II
Frozen Coho Salmon	Chile	*Aeromonas caviae*	Not classified as DIQP I or II
Frozen Coho Salmon	Chile	*Aeromonas caviae*	No classified as DIQP I or II
Frozen Atlantic Salmon	Chile	*Aeromonas hydrophila*	Not classified as DIQP I or II
Frozen Atlantic Salmon	Chile	*Aeromonas hydrophila*	Not classified as DIQP I or II
Frozen Albacore	Malaysia	*Aeromonas caviae*	Not classified as DIQP I or II

Frozen Wahoo	Malaysia	*Pseudomonas aeruginosa*	Not classified as DIQP I or II
Frozen Sword Fish	Malaysia	*Acinetobacter sp.*	Not classified as DIQP I or II
Frozen Marlin	Malaysia	*Proteus morganii*	Not classified as DIQP I or II
Frozen Mahi - Mahi	Malaysia	*Aeromonas caviae*	Not classified as DIQP I or II
Frozen Tuna	Malaysia	*Proteus vulgaris*	Not classified as DIQP I or II
Frozen Oil Fish	Malaysia	*Pseudomonas aeruginosa*	Not classified as DIQP I or II
Frozen Skipjack Tuna	Malaysian	*Pseudomonas aeruginosa*	Not classified as DIQP I or II
Frozen Coho Salmon	Chile	*Enterobacter aerogenes*	Not classified as DIQP I or II
Frozen Coho Salmon	Chile	*Enterobacter aerogenes*	Not classified as DIQP I or II
Frozen Atlantic Salmon	Chile	*Acinetobacter* sp.	Not classified as DIQP I or II
Frozen Atlantic Salmon	Chile	*Acinetobacter* sp.	Not classified as DIQP I or II
Frozen Black Cod	USA	*Aeromonas caviae*	Not classified as DIQP I or II
Frozen Black Cod	USA	*Aeromonas caviae*	Not classified as DIQP I or II
Frozen Sword Fish	Ghana	*Proteus morganii*	Not classified as DIQP I or II
Frozen Sword Fish	Ghana	*Proteus morganii*	Not classified as DIQP I or II
Frozen Sword Fish	Taiwan	*Acinetobacter sp.*	Not classified as DIQP I or II
Frozen Atka Mackerel	Korea	*Enterogenes aerogenes*	Not classified as DIQP I or II
Frozen Mackerel	Korea	*Pseudomonas aeruginosa*	Not classified as DIQP I or II
Frozen Flounder	Korea	*Proteus rettgeri*	Not classified as DIQP I or II
Frozen Pacific Saury	Korea	*Plesiomonas shigelloides*	Not classified as DIQP I or II
Frozen Spanish Mackerel	Korea	*Proteus morganii*	Not classified as DIQP I or II
Frozen Whole Pollack	Korea	*Vibrio fluvialis*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Norway	*Pseudomonas aeruginosa*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Norway	*Citrobacter freundii*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Norway	*Serratia marcescens*	Not classified as DIQP I or II
Frozen Yellow Tail / Hamachi	Japan	*Enterobacter aerogenes*	Not classified as DIQP I or II
Frozen Mahi - mahi	Malaysia	*Vibrio damsela*	Not classified as DIQP I or II
Frozen Skipjack	Malaysia	*Aeromonas caviae*	Not classified as DIQP I or II
Frozen Sword Phys h	Malaysia	*Aeromonas caviae*	Not classified as DIQP I or II
Frozen Albacore	Malaysia	*Vibrio damsela*	Not classified as DIQP I or II
Frozen Big Eye Tuna	Malaysia	*Vibrio fluvialis*	Not classified as DIQP I or II
Frozen Tuna GG	Malaysia	*Vibrio fluvialis*	Not classified as DIQP I or II
Frozen Oil Fish	Malaysia	*Proteus vulgaris*	Not classified as DIQP I or II
Frozen Marlin	Malaysia	*Vibrio fluvialis*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Norway	*Pseudomonas aeruginosa*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Norway	*Vibrio fluvialis*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Norway	*Proteus vulgaris*	No classified as DIQP I or II
Frozen Atlantic Mackerel	Norway	*Proteus vulgaris*	Not classified as DIQP I or II
Frozen Atlantic Mackerel	Norway	*Pseudomonas aeruginosa*	Not classified as DIQP I or II
Frozen Sword Fish	Taiwan	*Proteus morganii*	Not classified as DIQP I or II

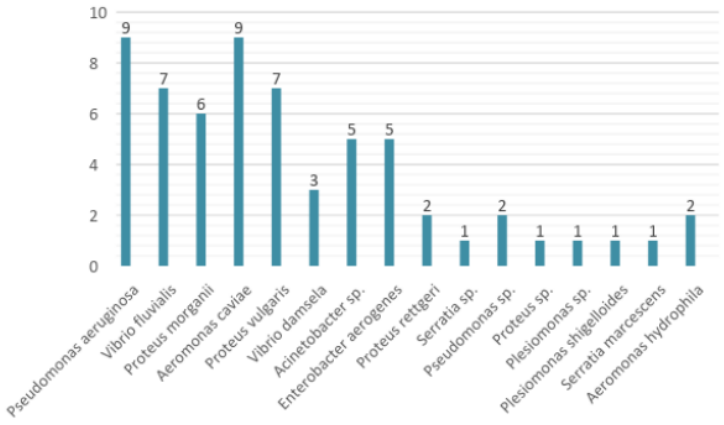

Figure 1. Graphs of the Bacteria Found During the Period 18 December 2017 - 18 January 2018 (Source: BKIPM Jakarta II, 2018)

Figure 1 showed that the results of the bacterial identification related to the imported consumption fish found the presence of: *Vibrio damsela, Acinetobacter* sp., *Enterobacter aerogenes, Proteus rettgeri, Serratia* sp., *Pseudomonas* sp., *Proteus* sp., *Plesiomonas* sp., *Plesiomonas shigelloides, Serratia marcescens, Aeromonas hydrophila* and was dominated by *Pseudomonas aeruginosa, Aeromonas caviae, Vibrio fluvialis, Proteus vulgaris* and *Proteus morganii*.

Table 2. Results of the Test Identification of the Molecular Biology (PCR) related to the Imported Fishery Products That were Tested in Fish Quarantine, in Tanjung Priok, Jakarta

Samples of Fisheries Import	Origin	Result of PCR Test	Description
Salmon(b)	* Makassar	Undetected Aeromonas salmonicida	Not classified DIQP I or II
Frozen Trout	Norway	Not detected by bacteria Aeromonas salmonicida	Not classified as DIQP I or II
Frozen Salmon (a)	* Batam	Not detected bacteria Aeromonas salmonicida	Not classified as DIQP I or II

Remarks: (*) Post-initial loading and unloading of the imported fish before being crossed to Tanjung Priok, Jakarta.

Tables 1 and 2 shows the results of the bacterial identification concerning the imported consumption fish that were analyzed. From these results, it can be seen that all of the imported consumption fish samples that were tested for biochemistry and through PCR did not show positive results for DIQP I and DIQP II bacteria. Biochemical testing has the function of identifying bacteria with certain characteristics. The target organ in this test was the kidney or meat, but the kidney was preferred. The biochemical identification method started with the initial sample isolation, bacterial purification, biochemical testing, and the reading of the test results that refer to Cowan and Steel's book (1993), the *Manual for the Identification of Bacteria*. Biochemical testing is one of the bacterial tests which is still commonly used to identify the characteristics of the bacteria.

Based on the level of the disease, DIQP is divided into two; DIQP class I and DIQP class II. Group I Fish Diseases (DIQP Goal I) are all quarantine fish pests and diseases that cannot be disinfected and/ or cured from the carrier media because the treatment technology has not been controlled, while DIQP

type II are all pests and diseases quarantine fish that can be disinfected and / or cured from the carrier media because the treatment technology has been mastered. Bacteria belonging to DIQP type I, according to KEPMEN NUMBER 80 / KEPMEN-KP / 2015, are unique strains of Vibrio parahaemolyticus, *Xenohaliotis californiensis*, *Nocardia crassostreae*, and *Nocardia asteroides*, while the bacteria belonging to DIQP type II were *Pseudomonas anguilliseptica*, *Aeromonas salmonicida*, and *Edwardsiella ictaluri*.

From the results of biochemical testing of the 69 fish imported for consumption (consisting of 10 types of fish), we obtained 16 types of bacteria. The results of the analysis of the fish sample of Atlantic Mackerel from the Netherlands and Norway had, in common, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Vibrio fluvialis*. In the previous research, [8] stated that *Proteus vulgaris* is the bacteria most commonly found in Atlantic Mackerel besides the genus *Pseudomonas* and the Vibrionaceae family, which is also found in Atlantic Mackerel. It is a floral marine bacteria commonly found in marine waters [9,10].

The bacteria found in the Malaysian Sword Fish were *Acinetobacter* sp. and *Aeromonas caviae*, then Swordfish from Ghana was host to *Proteus morganii*. The *Swordfish* from Taiwan had *Acinetobacter* sp. and *Proteus morganii*. The identification of the bacteria in the Swordfish was quite varied; this might be due to the different marine waters used for the catchment of Swordfish of the three countries. The common bacteria found in the Swordfish were *Flavobacterium*, *Pseudomonas*, *Moraxella*, and *Acinetobacter* [11].

Skipjack Tuna from Micronesia and Malaysia had the same bacteria, the genus *Pseudomonas*. This indicates that *Pseudomonas* is a genus often found in Skipjack Tuna, besides that the genus *Enterobacter* and *Proteus* can also be found in Skipjack Tuna [12].

The PCR test has several advantages, including speed, specific, and accurate test results. The kidney or meat part of the sample is the organ or tissue that is used as a test material. This identification method starts with the processing of DNA extraction, DNA amplification, electrophoresis, and the interpretation of the results.

Pseudomonas is a bacterium that was found in almost all of the imported consumption fish samples in this field work practice. This is because *Pseudomonas* is one of the types of bacteria that can live in the soil and in the aquatic environment of salt water [13].

The results of the sampling of the imported fish tested by PCR included 3 samples of salmon from Chile. The three samples of salmon tested were negative for *Aeromonas salmonicida*. The PCR test conducted at the Jakarta II Fish Quarantine Center was only used for testing susceptible hosts of bacteria *Aeromonas salmonicida* and *Edwardsiella tarda*, while the samples did not include the susceptible hosts for *Aeromonas salmonicida* and *Edwardsiella tarda*, instead using biochemical bacterial testing. The susceptible host for the bacteria *Edwardsiella tarda* was the tilapia fish and catfish [14]. Based on KEPMEN NUMBER 80 / KEPMEN-KP / 2015, salmon (*Salmonidae*) are one of the susceptible hosts of bacteria *Aeromonas salmonicida*. In addition to class salmon, they are also vulnerable to *Aeromonas salmonicida* namely *Gadus morhua*, *Pomancentrus caeruleus*, *Salvelinus fontinalis*, *Cottus Gobio*, *Clarias sp*, *Cyprinus carpio*, *Anguilla sp*, *Rana sp*, *Osphronemus goramy*, *Sparus aurata*, *Carassius auratus*, *Oreochromis niloticus*, *Hippoglossus stenolepis*, *Esox lucius*, *Chaetodon meyeri*, *Coregonus zenithicus*, *Galaxiidae* and *Scophthalmus maximus*.

4. Conclusion

The results of the isolation and identification of bacteria in imported fish at the Fish Quarantine Center, Quality Control, and Security of the Results of Jakarta II Fisheries, Tanjung Priok, Jakarta - North can be used to conclude that neither DIQP I or DIQP II bacteria were found within the sample. As much as 69 imported fish samples were analyzed and 16 types of bacteria were found. From the 3 samples of imported fish tested using Polymerase Chain Reaction, the results showed that the test samples were negative for *Aeromonas salmonicida*.

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