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# Case report; Identification of Bacteria in Catfish (Pangasius sp.) at the Palembang Fish Quarantine Station

**Madyasta Rarassari[1]*, Marsela Aprilia[1], Raudhatus Sa'adah[1]**
[1]Study Program of Food Technology, Department of Chemical Engineering, Politeknik Negeri Sriwijaya
[2]Study Program of Aquaculture, Department of Fisheries, Faculty of Agriculture, Universutas Sriwijaya

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E-mail addresses:
madyasta.rarassari@polsri.ac.id
*Corresponding author



**ABSTRACT**

One of the causes of disease attacks can be caused by bacteria, two of which are *Edwardsiella ictaluri* and *Streptococcus agalactiae* which often attack catfish. One of the causes of disease attacks can be caused by bacteria, two of which are *Edwardsiella ictaluri* and *Streptococcus agalactiae* which often attack catfish. Disease attacks can be prevented as early as possible by periodically identifying certain bacteria so that it can facilitate appropriate treatment to prevent them. This activity aimed to study bacterial identification techniques in catfish (*Pangasius* sp.) at the Fish Quarantine Station for Quality Control and Safety of Fishery Products in Palembang. The method used in this activity is quantitative by field observation and collecting primary and secondary data. Activities carried out include the preparation of tools and materials. Media creation, test sample preparation, bacterial isolation, bacterial purification, basic tests, biochemical tests, and instrument digestion. The approach was carried out through basic tests and biochemical tests. Data analysis was presented in tabular form and discussed descriptively. Results: There were no bacteria with the characteristics of *Edwardsiella ictaluri* and *Streptococcus agalactiae* found in catfish observed from December 2022 to January 2023.
**Keyword:** *Edwardsiella ictaluri*, catfish, bacterial identification, *Streptococcus agalactiae*

## INTRODUCTION

Catfish (*Pangasius* sp.) is a type of freshwater fish widely cultivated by Indonesians commercially. This fish is a fish that has high economic value. This catfish belongs to the Pangasidae family which is generally found in large rivers such as in Sumatra, Kalimantan and parts of Java. Apart from Indonesia, catfish are also found in many Asian regions such as Vietnam, Thailand and China. There are 14 types of catfish currently available. Among several species of catfish that have been successfully cultivated in hatcheries and rearing on micro, small and medium scale businesses are the catfish (*Pangasius hypophthalmus*) and the Jambal (*Pangasius djambal*). Catfish began to be successfully spawned in Indonesia in 1981, while Jambal in 1997. Aside from that, the Breeding and Technology Research Workshop has bred catfish resulting from crossing female Siamese with male Jambal. Freshwater Fisheries Cultivation produces fish known as Pasupati Catfish (*Pangasius* sp.) (Harmain & Dali, 2017). Various efforts have been made to increase catfish production, one of which is by increasing breeding. However, there are several obstacles in the catfish cultivation process, including disease attacks.

Disease is a condition that deviates from normal conditions. Fish pests and diseases are all organisms that can damage, disturb, and even cause death. One disease that often attacks fish is bacteria. Bacteria are one of the pathogenic agents that cause losses in fish farming businesses. Attacks such as parasites

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and bacteria can affect production levels and the quality of the fish produced (Dewi & Arnie, 2017). Bacteria are a group of organisms that do not have a nuclear envelope. Bacteria belong to the prokaryotic domain and have a microscopic size (Susanti *et al*., 2016). *Edwardsiella ictaluri* and *Streptococcus agalactiae* are bacteria that often attack Catfish. Edwardsiella ictaluri is a bacteria that causes the systemic bacterial disease Enteric *Septicemia of Catfish* (ESC*).*

*Edwardsiella ictaluri* as the main cause of Enteric Septicemia can result in 10 - 50% death in catfish. Infections caused by Edwardsiellosis were the cause of death in zebrafish (Hawke et al., 2013). High mortality can occur in aquaculture organisms if attacked by *Streptacoccus agalactiae* bacteria, where mortality can reach more than 50% (Gustiana *et al*., 2015). *Yersinia* sp. included in a group of bacteria that are initially non-pathogenic but if environmental conditions allow it can result in crop failure (Dali, 2013).

The first step to determining the disease is to diagnose infectious disease. Several things that must be considered in diagnosis are the infectious state, clinical symptoms such as behavior, internal and external characteristics and pathological changes. The disease control system in brackish fish is by using chemicals or antibiotics. In general, farmers often give various types of antibiotics such as ampicillin, tetracycline and disinfectants to fish (A'yunina *et al*., 2019).

The problem that often occurs in fish farming is that there are many cases of fish being attacked by bacteria caused by various factors such as hosts, pathogens and the environment. So it is necessary to prevent disease-causing factors that can attack these fish (Aldo & Munir, 2020). However, diseases that attack catfish often occur due to improper management in the cultivation system. One of the most common problems in cultivating fish, one of which is catfish, is the presence of infectious diseases that attack the fish (A'yunin et al., 2020).

The Fish Quarantine Station for Quality Control and Safety of Fishery Products, Palembang is one of the technician implementation units of the Ministry of Maritime Affairs and Fisheries whose main task is to prevent the entry and spread of quarantine fish pests and diseases outside the territory of the Republic of Indonesia, ensuring quality control and safety of fishery products, implement a quality management system and prevent the spread of illegal fishing for fishery products.

## MATERIAL AND METHOD

### *Site sample*

Sample observations were conducted between December 2022 and January 2023. Sempel fish is a checking fish for the Fish Quarantine Station for Quality Control and Safety of Palembang Fishery Products for early detection of bacteria in catfish.

### *Equipment and tool*

The tools used in this bacterial identification technique are autoclave, Bunsen, petri dish, erlenmeyer, object glass, tube needle, microscope, surgical equipment, dropper pipette, analytical balance, and UV laminar flow-hood. Meanwhile, the ingredients used are distilled water, 70% alcohol, Brain Heart Infusion Agar, 3% KOH solution, 40% KOH solution, MR-VP solution, gelatin medium, Triple Sugar Iron Agar, Lysine Iron Agar candy, medium Oxidative/Fermentative, Motility Indole Ornithine media, Tryptic Soy Agar slants, 4% TSA media, NaCl, liquid paraffin, peptone, cytochrome oxidase reagent, Simon's citrate agar, urea agar, 3% $H_2O_2$ catalase reagent, and gram staining reagent.

### *Preparation sample*

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Before use, laboratory equipment and materials are sterilized first. Sterilization of laboratory equipment and materials is usually carried out using physical methods. Sterilization is carried out by dry heating using an oven at a temperature of 130°C for 30 minutes, wet heating using an autoclave at a temperature of 121°C for 15 minutes. Apart from that, sterilization is carried out by lighting and spraying alcohol directly. The method used in this activity is quantitative by collecting field observation data and primary and secondary data. Activities carried out include preparation of tools and materials. Media creation, test sample preparation, bacterial isolation, bacterial purification, basic tests, biochemical tests and instrument digestion.

## RESULT AND DISCUSSION

The results of the observations and tests carried out can be obtained which are then compared with the literature presented in Table 1. **There are three basic tests**: the gram stain test, the oxidase test, and the catalase test. This test determines whether a biochemical test will be carried out. The basic test involves stealing bacterial colonies from catfish samples and isolating and purifying them. The target organs are the liver and kidneys. The goal of the Gram test procedure is to determine whether the bacteria are Gram-positive or Gram-negative. If the bacterial cells are purple, the bacteria are gram positive, while if they are red, the bacteria are gram negative. This is because the cell walls of gram-positive bacteria are made of thicker peptidoglycan than gram-negative bacteria.

The thicker peptidoglycan is able to maintain its crystalline purple color even when exposed to a bleach solution. Gram positives have purple cell walls because they retain the purple color of crystal violet. This is due to complex ribonucleic proteins forming after bleaching, which can maintain the basic color. Apart from that, there are phosphoric ester elements in gram-positive bacteria. Gram positive bacteria have cell walls consisting of two layers, namely thick peptidoglycan and an inner membrane. This peptidoglycan layer can bind crystal violet dye. The gram staining results for the first sample were negative, the second sample was positive, and the third sample was negative.

**Table 1**. Basic Test Results

| Sample n- | Measurement date | Characteristics | | | Identification |
|---|---|---|---|---|---|
| | | Gram strain | Oxidase test | Catalase test | |
| 1 | 20/12/2022 | - | + | + | *E. ictaluri* (-) |
| 2 | 22/12/2022 | + | - | + | *S. agalactiae* (-) |
| 3 | 29/12/2022 | - | - | - | |

Note (-): this type of bacteria was not identified

The oxidase test aims to determine the presence or absence of oxidase enzyme in the bacteria. Oxidase testing uses filter paper that has been soaked with a solution of N, N, Nl, Nl, -Tetramethyl – p – Phenylenediamine dihydrochloride. If the colony turns purplish blue on the filter paper in a short time or approximately 10 seconds, it shows a positive oxidase reaction. The filter paper does not change color as a result of the negative oxidase reaction. This is in accordance with the statement of Wadjdjy & Setiadi (2019) which states that positive results of the oxidase test are indicated by the appearance of a purplish blue color on the filter paper. The oxidase test for the first sample was positive, the second sample was positive, and the third sample was negative.

The catalase test aims to determine the ability of bacteria to produce the catalase

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enzyme. Catalase testing uses $H_2O_2$ 3% reagent. Hydrogen peroxide is toxic to cells because it inactivates cellular enzymes. A positive test is indicated by the formation of air bubbles around the colony culture. According to Dwinanti *et al*. (2014), positive results were obtained in the test due to the breakdown of $H_2O_2$ into $O_2$. Catalase testing for the first sample was positive, the second sample was positive, and the third sample was negative.

Table 1 shows the results of Catfish samples that entered SKIPM from December to January showing negative results for the specified target bacteria. This is because the samples entered into SKIPM have implemented good CKIB. The fish samples tested did not show that they were attacked by the bacteria *Edwardsiella ictaluri* and *Streptococcus agalactiae* because the test results did not match the test results for the targeted bacteria. The test results that show that the sample is *Edwardsiella ictaluri* bacteria are negative gram staining, negative oxidase test and positive catalase test, and for *Streptococcus agalactiae* bacteria it is positive gram staining, negative oxidase test and negative catalase test (Faridah *et al*., 2018).

**Biochemical Test**

The results of observations and tests carried out using Yersinia ruckeri bacterial culture media can be obtained which are then compared with the literature and are presented in Table 2.

**Table 2**. Biochemical Test Results

| Testing | Result |
|---|---|
| Form colonies | Round |
| Colony Color | White |
| Grams | - |
| TSIA | K/M, gas (-), $H_2S$ (-) |
| Motility | - |
| Indol | - |
| O/F | F |
| Citrate | - |
| MR | + |
| VP | - |
| TSA NaCl 4% | + |
| Urea | - |
| Gelatin hydrolysis | - |
| Ornithine decarboxylase | + |
| Testing | Result |
| Lysine decarboxylase | - |
| Adonitol, Arabinosa | - |
| Cellobiose | - |
| Dulcitol | - |
| Fructose | + |
| Galactose | + |
| Glucose | + |
| Inositol, Lactose | - |
| Maltose, Mannitol | + |
| Mannose | + |
| Melibiosis | - |
| Raffinosa | - |
| Rhamnosa, Saccharose | - |
| Salicin, Sorbitol | - |
| Trehalose | + |
| Xylose | - |

The biochemical test is a further test if the Catfish sample tested is the target bacteria *Edwardsiella ictaluri* and *Streptococcus agalactiae*. Biochemical tests consist of TSIA test, motility test, indole test, fermentative oxidative test, citrate test, MR-VP test, 4% NaCl test, urea test, gelatin test, ornithin test, lysine test, and sugar test. Biochemical testing does not use the bacteria *Edwardsiella ictaluri* and *Streptococcus agalactiae*. This is because in basic testing the Catfish samples used did not contain the targeted bacteria. Therefore, biochemical testing is conducted using Yersinia ruckeri bacterial culture. Based on Table 4.2. The results obtained were round colonies, white in color, gram negative staining test. The test results are in accordance with the statement (Dali, 2013) that Yersinia is a gram negative bacteria and is round or rod shaped.

Microorganisms were tested to determine their ability to utilize the sugar found in the TSIA test through a series of biochemical tests. These sugars are glucose, lactose and sucrose. TSIA media is a slanted medium, colored red in a test tube. The media used has two parts, namely slant and butt. An acid

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reaction occurs when the color of the media changes to yellow. Alkaline (wet) reaction if no color change occurs. The formation of $H_2S$ is marked by the appearance of black color on the butt. This is in accordance with the research results of Kosasi *et al.* (2019) positive $H_2S$ test results indicate the presence of black deposits at the bottom of the media used. In the TSIA test, if the $H_2S$ results produce a dark black color so that you cannot see the results of the color change in the pierced media, then the way to anticipate this is by checking periodically after incubation for 18 hours or more.

The formation of gas is characterized by the rise of the media's base or the splitting of the media. The way to overcome excessive gas formation is to loosen the test tube cover. Positive results if the color changes to yellow and negative results if there is no color change on the media. Test results showed yellow butts, red slants, negative gases, and negative $H_2S$ levels.

Bacterial motility testing can be observed from bacterial growth in liquid media. According to Panjaitan *et al.* (2020), bacterial motility testing was carried out to determine the movement of bacteria in the media. If bacteria move, they are motile, while bacteria that are still are called non motile. The indole test aims to find out whether bacteria have the tryptophanase enzyme so that the bacteria can oxidize the amino acid tryptophan to form indole. The result is positive if a red ring forms on the peptone medium, while the result is negative if no red ring forms. The test results showed that the bacteria were non motile and the indole test was negative.

Bacteria are tested for their oxidative and fermentative properties toward glucose using the O/F test. Fermentative means that the bacteria are anaerobic (can grow without oxygen) while oxidative means that the bacteria are aerobic (can grow in the presence of oxygen). Only tubes without paraffin were affected by yellowing due to oxidative bacteria. Non-reaction when there is no color change in the tubes containing paraffin and those without paraffin. Citrate testing was carried out to determine the ability of bacteria to grow in media that uses a carbon source from citrate.

Positive results if the color changes to blue and negative results if there is no color change. The MR-VP test was carried out to determine the ability of bacteria to produce acid from glucose fermentation (MR) and to determine the ability of bacteria to produce a neutral final product, namely acetyl methycarbinol, from glucone (VP) fermentation using MR and VP reagents (Arwin *et al*., 2016).

MR-VP media is a liquid media. Positive results result from changing the color from yellow to red, while negative results result from keeping the color yellow. The VP test result is positive if after 10-15 minutes the color changes to red and negative if it remains yellow. The test results showed that the OF test was fermentative, the citrate test was negative, the MR test was positive and the VP test was negative.

The 4% NaCl media test was carried out to see the ability of bacteria to grow in high salinity media. Positive results if there are steaks growing on the media and negative results if there are no steaks growing on the media. The urea test aims to determine the ability of bacteria to convert urea into ammonia. Positive results if there is a color change to red or pink on the media and negative results if there is no color change. The gelatin test assesses whether bacteria are capable of producing proteolytic enzymes, such as gelatinase. Positive results if the media is in liquid form and negative results if the media is in solid form. Gelatin will be broken down by microbes that synthesize

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proteolysis enzymes. The gelatin solution is liquid at room temperature and solid when in the refrigerator. The gelatin will remain liquid if it has been hydrolyzed by bacteria (Arwin *et al*., 2016). The results of the 4% NaCl test were positive, the urea test was negative, and the gelatin test was negative.

The ornithin test is carried out to determine the ability of bacteria to break down ornithin (an amino acid) by decarboxylase into amino acids. The ornithin test is the process of decomposing these group from an organic molecule. The ornithin decarboxylase result is positive if the anaerobic area has gray, purple or blue colored media. The ornithin decarboxylase result is negative if the media turns yellow in the anaerobic area.

The LIA test is used to see the ability of a bacterium to break down lysine using decarboxylase and diaminese. The lysine decarboxylase reaction or alkaline aerobic reaction will neutralize the acid formed from glucose fermentation. Lysine is a diamino monocarboxylic acid. Lysine can provide amino acids to other amino acids, but lysine cannot be formed again, meaning that the reamination process cannot occur after lysine undergoes a deamination reaction.

Positive results for lysine decarboxylase if there is no color change in the media. Negative results for lysine decarboxylase if there is a yellow color change on the puncture media and remain purple on the scratch media, which means the bacteria do not have the ability to decarboxylate (Kambey *et al*., 2016). A positive result for lysine diaminese occurs when the color changes to yellow in the puncture media and the color changes to red.

Ornithin test results were positive, but lysine test results were negative. Bacteria were tested on candy media to see if they could break down carbohydrates. Results are positive if the media changes color from white to yellow, and negative if the media does not change color. The color change that occurs indicates that the bacteria form acid from carbohydrate fermentation. During testing the results were found to be adonitol (-), arabinose (-), cellobiose (-), dulcitol (-), fructose (+), galactose (+), glucose (+), inositol (-), lactose (-), maltose (+), mannitol (+), mannose (+), melibiose (-), raffinose (-), rhamnose (-), saccharose (-), salicin (-), sorbitol (-), trehalose (+), and xylose (-). Based on Table 2. The results indicate that the bacteria are *Yersinia ruckeri*. This is because these bacteria have test results that comply with the applicable Indonesian National Standards.

## CONCLUSION

The Catfish observed during December did not contain *Edwardsiella ictaluri* or *Streptococcus agalactiae* bacteria. The results obtained from biochemical tests performed on *Yersinia ruckeri* bacterial culture media were the same as those obtained by the applicable Indonesian National Standards.

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## AUTHORS' CONTRIBUTIONS

The contribution of each author is as follows, MR; collected the data, drafted the manuscript, and designed the table as well as the graph. MA; devised the main conceptual ideas and conducted a critical revision of the article. All authors discussed the results and contributed to the final manuscript.

## CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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