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Preface

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The 3rd International Conference on Fisheries and Marine Sciences (INCOFIMS) Surabaya Indonesia, 10 September 2020

International conference on fisheries and marine sciences (INCOFIMS) is an annual conference organized by Faculty of Fisheries and Marine Universitas Airlangga, Surabaya, Indonesia. The main aim is to provide a sharing platform that enables researchers, academics and practitioners from all over the world to share their most recent findings as well as to propose the best strategies to address issues and challenges which we have been currently facing in aquaculture and fisheries practices worldwide. The 1st INCOFIMS was held successfully offline in Surabaya in 2018, followed by the second in 2019.

The 3rd INCOFIMS was previously scheduled offline in Surabaya on 10th September 2020. However, due to the Covid-19 pandemic and travel restriction for foreigners come into Indonesia as well as traveling within the Indonesian islands, we had the 3rd INCOFIMS in a virtual format with ZOOM on 10 September 2020, and hosted from Faculty of Fisheries and marine, Univesias Airlangga, Surabaya Indonesia. We were unable to postpone the event because INCOFIMS is our annual event and also most of the participants requested to have the conference in the virtual format (online)

The theme in the 3rd INCOFIMS was “challenges and strategies for the development of sustainable aquaculture and fisheries”. Technically, we had the conference divided into 2 (two) sessions in general: (1) keynote speaker session and (2) guest speaker session. In the keynote session, we had 3 (three) keynote speakers delivering a speech which were Prof. Andrew Greig Jeffs from Newzealand, Prof. Mustafa Kamal from Malaysia, and Dr Gunanti Mahasri from Universitas Airlangga. Each keynote speaker had 1.5 hours for giving a presentation using **ZOOM** and 30 minutes for discussion in one virtual room. After the keynote speaker session, we proceeded to the guest speaker session in which all participants were divided into 7 (seven) rooms according to our subtopics for oral and poster presentations:

- Room 1: Aquaculture technology
- Room 2: Fish Nutrition
- Room 3: Fish Diseases
- Room 4: Fisheries Management
- Room 5: Marine sciences,
- Room 6: Aquatic Resource management, and
- Room 7: Fisheries socioeconomics

In this session, every speaker had 15 minutes for presentation and 5 minutes for discussion. Total participants joined in this conference was 225 participants from at least 6 different countries (Australia, New Zealand, Switzerland, Malaysia, Taiwan and Indonesia).

The conference was in general quite successful, acknowledging the number and enthusiasms of participants during the discussion sessions in both the keynote speaker session and guest speaker’s session. We thank all participants and organizing committee for their support to this conference and see you in the 4th INCOFIMS 2021.

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Isolation and Identification Bacillus Bacteria in Tilapia (*Oreochromis niloticus*) Using the Vitek-2 Compact

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Isolation and Identification Bacillus Bacteria in Tilapia (*Oreochromis niloticus*) Using the Vitek-2 Compact

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Abstract. Tilapia, *Oreochromis niloticus*, is fish commodity that plays an important role in the tropic and subtropics country with their economic value for the community and tolerance for salinity. The high demand for tilapia resulted in the need to increase their production through the stocking density method. The purpose of this research is isolation and identification of pathogenic bacteria in tilapia fish using a Vitek-2 compact. The method used is sampling, perform bacterial isolation using TSB and TSA media using streak plate technique with OSE needle loop and cotton swap, identification of bacteria through gram staining, bacterial pathogenitas test, and using the Vitek-2 compact. The results of this study revealed three species of *Bacillus thuringiensis*, *Bacillus mycoides* and *Bacillus cereus*. All bacteria detected in the tilapia cultures were known as putative pathogens in Fish.

1. Introduction

Tilapia is an important species in the cultivation of tropical and subtropical regions. According to Jansen and Mohan [1] in 2015, it is estimated that global production of Tilapia fish reached 6.4 million metric tons (MMT). Tilapia fish cultivation is very profitable because it is economically valuable and supports the nutritional needs of animal proteins. Increased demand for Tilapia fish has resulted in increased production through high-spread solid methods. But the use of high-spread solid methods causes fish to contract pests as well as bacteria.

Tilapia is often stricken by infectious agents such as bacteria and parasites [2]. Some pathogenic bacteria in fish are facultative [3] and are able to survive in water for a long time making their existence difficult to prevent. The inspection of pathogenic bacteria should be completed properly in anticipation of the on anion of pathogens. According to Wirawan *et al.* [4] to avoid widespread disease attacks need to be carried out prevention and control through the diagnosis of diseases in fish by ensuring and identifying the type of disease. Therefore, there needs to be information about the types of pathogenic bacteria that often attack Tilapia fish by performing isolation and identification before handling.



2. Material and method

2.1 Material

The tilapia fish was taken from the freshwater hatchery of terengganu university on November 25, 2019. Samples were taken with a look at clinical symptoms such as pale color, lesions on the skin, decreased appetite, eyes prominently and sluggish motion. The organ samples used in this study were kidneys, liver, brain, intestines and spleen.

2.2 Isolation Bacteria

2.2.1 Isolation

TSB and TSA were used for isolation of *B. cereus*. samples inoculated on TSB media are then inculable at a temperature of 37°C for 24 hours. According to Huda *et al.* [5] bacterial purification was carried out to separate the inoculations consisting of many colonies into pure kolni. After enrichment a loopful was streakes on TSA plate and incubate at 37°C for 24 hours. colonies whose bodies on the media will be characterised.

2.3 Identification of Bacteria

2.3.1. pathogenicity Test

Bacterial identification is carried out with a pathosity test using Blood Agar. this is done to know that bacteria are pathogenic or non-pathogenic. Isolates are taken and distreaked in the blood media so that they are then incubated at a temperature of 37°C for 24 hours [6]. The absence of a clear zone in the blood media to characterize bacteria is pathogenic.

2.3.2. Gram Staining

Gram Staining is done in accordance with Rasool *et al.* [7] using ultra violet crystal line, ocean iodium, 95% ethanol solution and safranin ocean. Discoloration determines the type of gram of bacteria observed. Gram-positive bacteria show violet and negativ colors are purple.

2.3.3. Biochemical Test

Bacterial identification can be done with the AIS (automatic Identification System) using Vitek 2 Compact. Bacillus Identiication Card is used to identify Bacill-type bacteria. The planting process is carried out with 1 ml of saline water solution and inserts a bacterial inokulum with a density of 2.0 McF [8] after the creation of the inokulum is done reading or interpretation of the results using Vitek 2 Compact.

3. Results and discussion

3.1. isolation

Isolation is the transfer of bacteria from the original environment into artificial media so that it is obtained pure breeding [9]. There are colonies that grow in some media that have been isolated. The growing colony in a TSA media showed that there were bacteria.

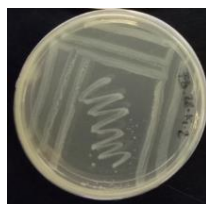


Figure 1. Colonies that grow on TSA media.

3.2. Identification of bacteria

In Figure 2(a) there is a clear zone on the media blood agar. This indicates the arrival of pathogenic bacteria growing in the media. Clear zone can be categorized as β hemolysis According to Vancraynest *et al.* [10] β hemolysis has the potential in dialysis of blood that causes neurotoxic as well as being the cause of death. In addition, in the process of identifying bacteria is done coloring grams to know the type of gram of the bacteria. In Figure 2(b) it appears that there are bacill-shaped purple bacteria on microscopic examination. According to Hamidah *et al.* [11] the color difference in gram staining results is influenced by bacterial cell walls. Gram positive has a cell wall composed of peptidoglicans that is thicker than gram negative bacteria so that it can maintain a purple color.

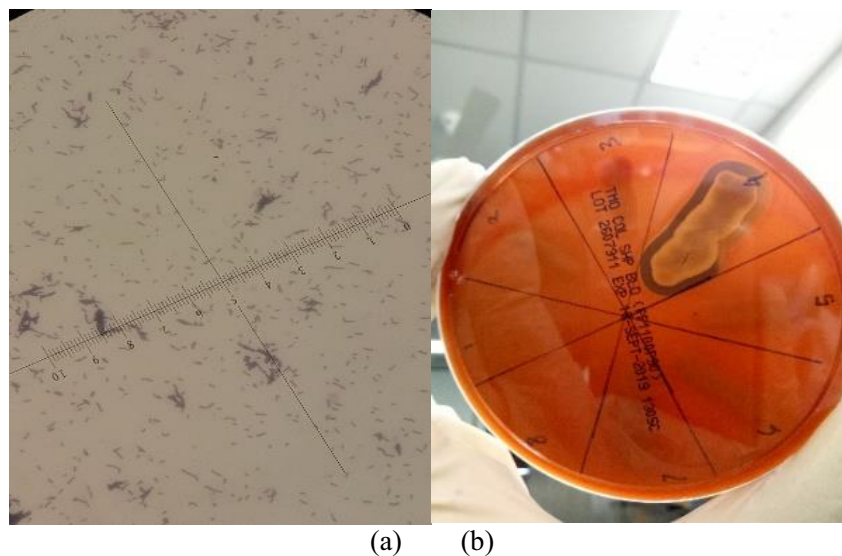


Figure 2. The result of bacterial isolation in tilapia fish (a) clear zone (β hemolysis) in blood order (b) gram positive bacteria. 100x magnification

Biochemical tests are conducted to determine the type of bacteria or the identification of bacteria. Analysis of Vitek 2 Compact results found that two species that bear similarities are *Bacillus Thuringiensis*, *Bacillus cereus* and *Bacillus mycoides*. Examination using vitek 2 compact can be seen on Figure 3.

The absence of three identified species is necessary supplemental test to separate the identified bacteria so that it can be known the species from the bacteria. Analysis Organism and Test to Separate identification results from isolate testing, *Bacillus sp.*, show that it is necessary to retest to identify species of the bacteria by conducting Rhizoid Colonies and Toxin Crystal Presence tests. According to Napitupulu *et al.* [12] the Rhizoid Colonies test was used to identify *Bacillus mycoides* which could distinguish it from *B. cereus* and *B. thuringiensis* by producing rhizoid-shaped colonies. While the Toxin Crystal Presence test is used to identify *Bacillus thuringiensis* which can distinguish it from *B. cereus* and *B. mycoides* by producing crystals. If the rhizoid colonies test results are negative and the Toxin Crystal Presence test is negative then the result of identification of the bacteria is *Bacillus cereus*. Then if the rhizoid colonies test results are negative and the Toxin Crystal Presence test is positive then the result of identifying the bacteria is *Bacillus mycoides*. However, if the rhizoid colonies test results are positive and the Toxin Crystal Presence test is negative then the result of identifying the bacteria is *Bacillus thuringiensis*.

Well	Test	Reaction	Well	Test	Reaction
1	BETA-XYLOSIDASE	-	32	D-MANNOSE	-
3	L-Lysine-ARYLAMIDASE	-	34	D-MELEZITOSE	-
4	L-Aspartate ARYLAMIDASE	-	36	N-ACETYL-D-GLUCOSAMINE	+
5	Leucine-ARYLAMIDASE	-	37	PALATINOSE	-
7	Phenylalanine ARYLAMIDASE	+	39	L-RHAMNOSE	-
8	L-Proline ARYLAMIDASE	-	41	BETA-GLUCOSIDASE	-
9	BETA-GALACTOSIDASE	-	43	BETA-MANNOSIDASE	-
10	L-Pyrrolydonyl-ARYLAMIDASE	+	44	PHOSPHORYL CHOLINE	-
11	ALPHA-GALACTOSIDASE	-	45	PYRUVATE	+
12	Alanine ARYLAMIDASE	+	46	ALPHA-GLUCOSIDASE	+
13	Tyrosine ARYLAMIDASE	+	47	D-TAGATOSE	-
14	BETA-NACETYL-GLUCOSAMINIDASE	+	48	D-TREHALOSE	+
15	Ala-Phe-Pro ARYLAMIDASE	-	50	INULIN	-
18	CYCLODEXTRIN	-	53	D-GLUCOSE	+
19	D- GALACTOSE	-	54	D-RIBOSE	+
21	GLYCOGEN	-	56	PUTRESCINE assimilation	-
22	Myo-INOSITOL	-	58	GROWTH IN 6.5% NaCl	+
24	METHYL-A-D-GLUCOPYRANOSIDE acidification	-	59	KANAMYCIN RESISTANCE	+
25	ELLMAN	+	60	OLEANDOMYCIN RESISTANCE	-
26	METHYL-D-XYLOSIDE	-	61	ESCULIN hydrolysis	+
27	ALPHA-MANNOSIDASE	-	62	TETRAZOLIUM RED	+
29	MALTOTRIOSE	-	63	POLYMIXIN B RESISTANCE	+
30	Glicine ARYLAMIDASE	-			
31	D- MANNITOL	-			
Well	Test	Reaction	Well	Test	Reaction
1	BETA-XYLOSIDASE	-	32	D-MANNOSE	-
3	L-Lysine-ARYLAMIDASE	-	34	D-MELEZITOSE	-
4	L-Aspartate ARYLAMIDASE	-	36	N-ACETYL-D-GLUCOSAMINE	+
5	Leucine-ARYLAMIDASE	-	37	PALATINOSE	-
7	Phenylalanine ARYLAMIDASE	+	39	L-RHAMNOSE	-
8	L-Proline ARYLAMIDASE	-	41	BETA-GLUCOSIDASE	-

Figure 3. The result of biochemical test using Vitek 2 Compact

4. Conclusions

Concluded this *Bacillus* spp., are pathogenic and can naturally infected tilapia fish.

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