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.

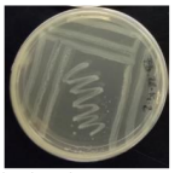
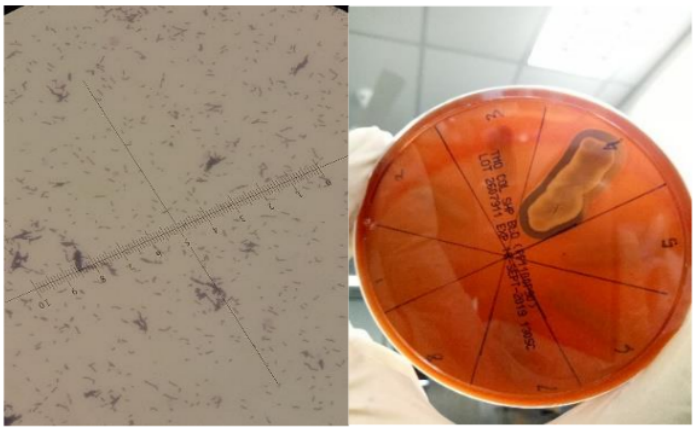


Figure 1. Colonies that grow on TSA media.

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.



(a) (b)

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 Bacaillus 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 Rizhoid Colonies and Toxin Crystal Presence tests. According to Napitupulu *et al.* [12] the Rizhoid Colonies test was used to identify Bacillus mycoides which could distinguish it from B. cereus and B. thuringiensis by producing rizhoid-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	-
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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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RUBRIC: 6TH-8TH SCIENCE ARGUMENT (CER)

CLAIM

Take an arguable position on the scientific topic and develop the essay around that stance.

ADVANCED	The essay introduces a precise, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay develops the claim and counterclaim fairly, distinguishing the claim from alternate or opposing claims.
PROFICIENT	The essay introduces a clear, qualitative and/or quantitative claim based on the scientific topic or text(s), regarding the relationship between dependent and independent variables. The essay effectively acknowledges and distinguishes the claim from alternate or opposing claims.
DEVELOPING	The essay attempts to introduce a qualitative and/or quantitative claim, based on the scientific topic or text(s), but it may be somewhat unclear or not maintained throughout the essay. The essay may not clearly acknowledge or distinguish the claim from alternate or opposing claims.
EMERGING	The essay does not clearly make a claim based on the scientific topic or text(s), or the claim is overly simplistic or vague. The essay does not acknowledge or distinguish counterclaims.

EVIDENCE

Include relevant facts, definitions, and examples to back up the claim.

ADVANCED	The essay supplies sufficient relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
PROFICIENT	The essay supplies relevant, accurate qualitative and/or quantitative data and evidence related to the scientific topic or text(s) to support its claim and counterclaim.
DEVELOPING	The essay supplies some qualitative and/or quantitative data and evidence, but it may not be closely related to the scientific topic or text(s), or the support that is offered relies mostly on summary of the source(s), thereby not effectively supporting the essay's claim and counterclaim.
EMERGING	The essay supplies very little or no data and evidence to support its claim and counterclaim, or the evidence that is provided is not clear or relevant.

REASONING

Explain how or why each piece of evidence supports the claim.

ADVANCED	The essay effectively applies scientific ideas and principles in order to explain how or why the cited evidence supports the claim. The essay demonstrates consistently logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations anticipate the audience's knowledge level and concerns about this scientific topic.

PROFICIENT	The essay applies scientific reasoning in order to explain how or why the cited evidence supports the claim. The essay demonstrates logical reasoning and understanding of the scientific topic and/or text(s). The essay's explanations attempt to anticipate the audience's knowledge level and concerns about this scientific topic.
DEVELOPING	The essay includes some reasoning and understanding of the scientific topic and/or text(s), but it does not effectively apply scientific ideas or principles to explain how or why the evidence supports the claim.
EMERGING	The essay does not demonstrate clear or relevant reasoning to support the claim or to demonstrate an understanding of the scientific topic and/or text(s).

FOCUS

Focus your writing on the prompt and task.

ADVANCED	The essay maintains strong focus on the purpose and task, using the whole essay to support and develop the claim and counterclaims evenly while thoroughly addressing the demands of the prompt.
PROFICIENT	The essay addresses the demands of the prompt and is mostly focused on the purpose and task. The essay may not acknowledge the claim and counterclaims evenly throughout.
DEVELOPING	The essay may not fully address the demands of the prompt or stay focused on the purpose and task. The writing may stray significantly off topic at times, and introduce the writer's bias occasionally, making it difficult to follow the central claim at times.
EMERGING	The essay does not maintain focus on purpose or task.

ORGANIZATION

Organize your writing in a logical sequence.

ADVANCED	The essay incorporates an organizational structure throughout that establishes clear relationships among the claim(s), counterclaims, reasons, and evidence. Effective transitional words and phrases are included to clarify the relationships between and among ideas (i.e. claim and reasons, reasons and evidence, claim and counterclaim) in a way that strengthens the argument. The essay includes an introduction and conclusion that effectively follows from and supports the argument presented.
PROFICIENT	The essay incorporates an organizational structure with clear transitional words and phrases that show the relationship between and among ideas. The essay includes a progression of ideas from beginning to end, including an introduction and concluding statement or section that follows from and supports the argument presented.
DEVELOPING	The essay uses a basic organizational structure and minimal transitional words and phrases, though relationships between and among ideas are not consistently

	clear. The essay moves from beginning to end; however, an introduction and/or conclusion may not be clearly evident.
EMERGING	The essay does not have an organizational structure and may simply offer a series of ideas without any clear transitions or connections. An introduction and conclusion are not evident.

LANGUAGE

Pay close attention to your tone, style, word choice, and sentence structure when writing.

ADVANCED	The essay effectively establishes and maintains a formal style and objective tone and incorporates language that anticipates the reader's knowledge level and concerns. The essay consistently demonstrates a clear command of conventions, while also employing discipline-specific word choices and varied sentence structure.
PROFICIENT	The essay generally establishes and maintains a formal style with few possible exceptions and incorporates language that anticipates the reader's knowledge level and concerns. The essay demonstrates a general command of conventions, while also employing discipline-specific word choices and some variety in sentence structure.
DEVELOPING	The essay does not maintain a formal style consistently and incorporates language that may not show an awareness of the reader's knowledge or concerns. The essay may contain errors in conventions that interfere with meaning. Some attempts at discipline-specific word choices are made, and sentence structure may not vary often.
EMERGING	The essay employs language that is inappropriate for the audience and is not formal in style. The essay may contain pervasive errors in conventions that interfere with meaning, word choice is not discipline-specific, and sentence structures are simplistic and unvaried.