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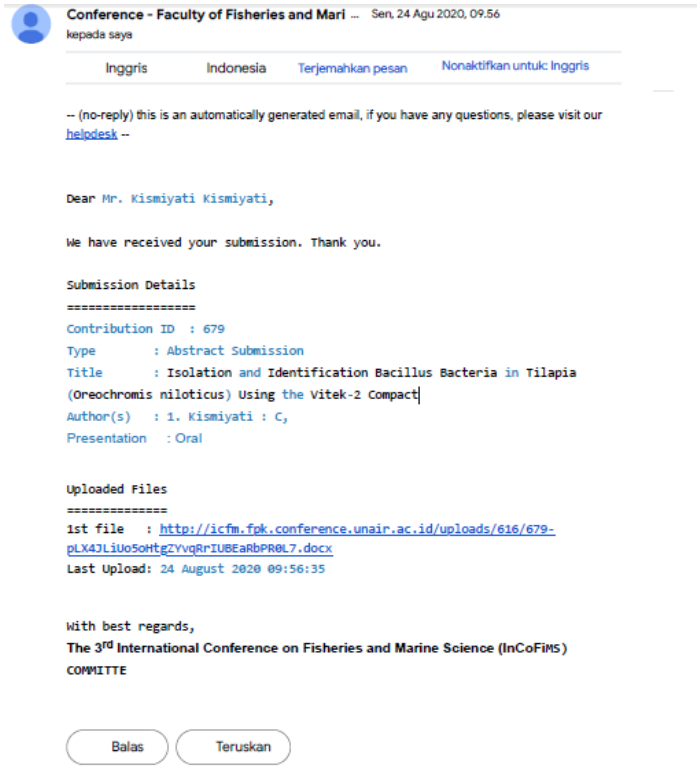

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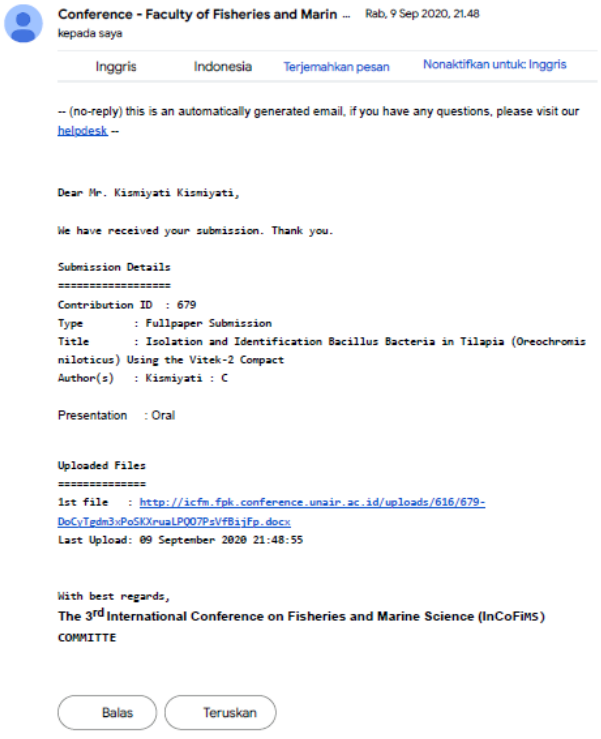

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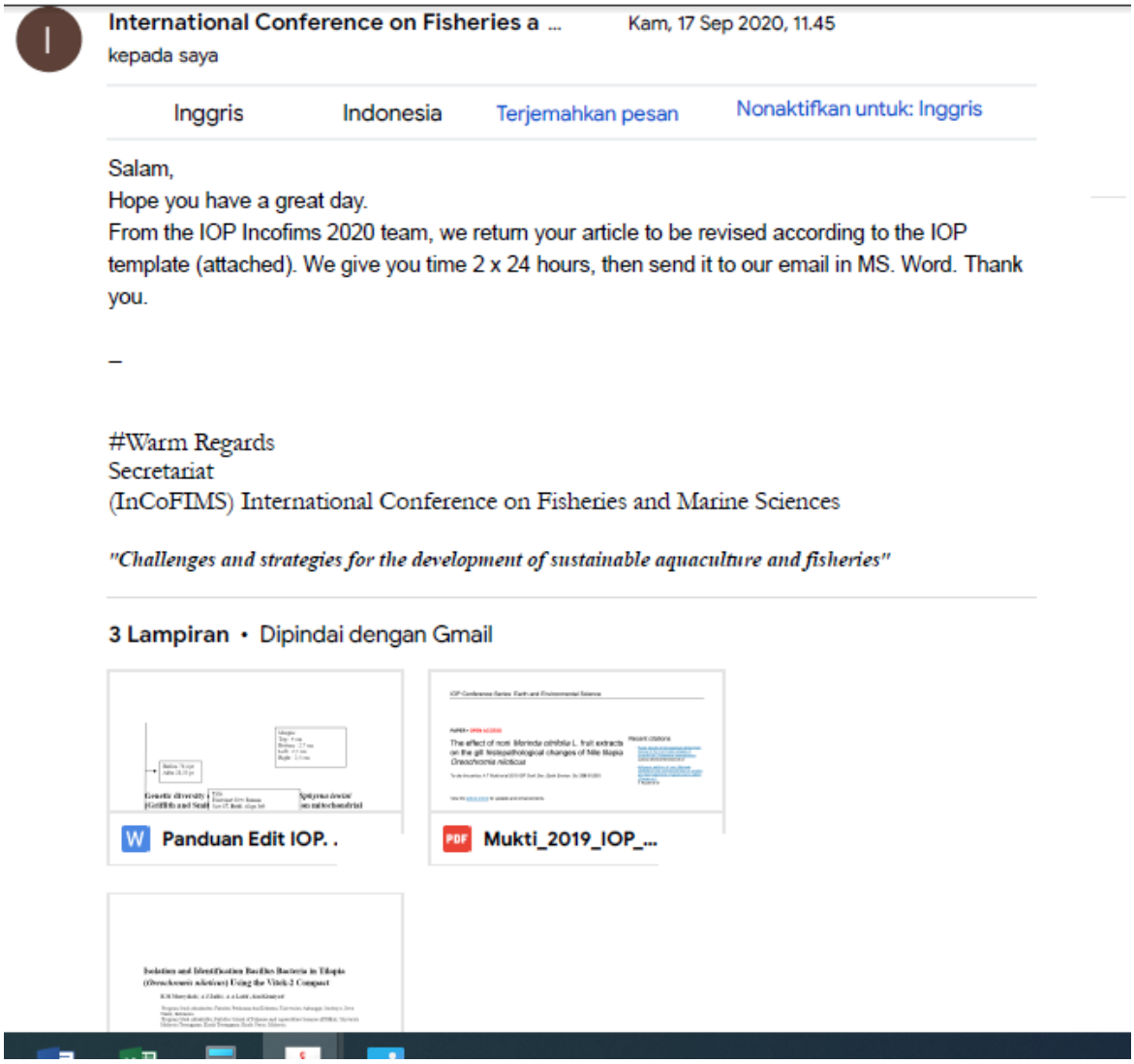

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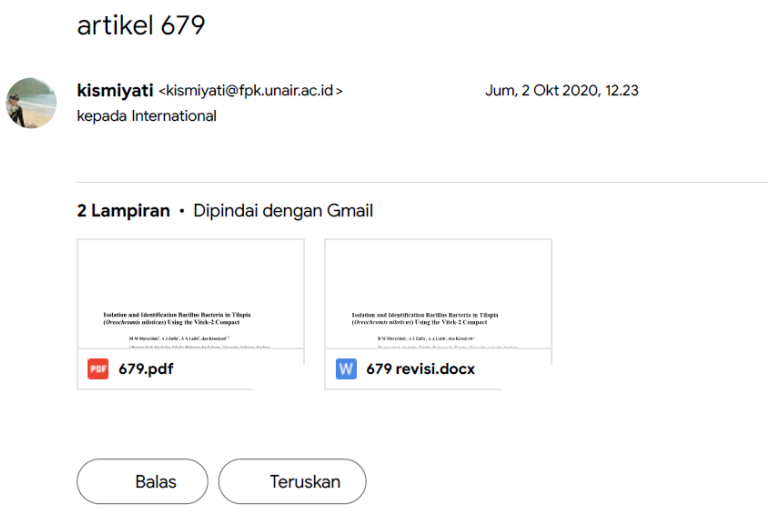

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# Isolation and Identification Bacillus Bacteria in Tilapia (*Oreochromis niloticus*) Using the Vitek-2 Compact

**R M Mursyidah[1], A J Zulfa[1], A A Laith[2], dan Kismiyati[1,3]**

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[2]Program Studi Akuakultur, Fakultas School of Fisheries and Aquaculture Sciences (FISHA), Universiti Malaysia Terengganu, Kuala Terengganu, Kuala Nerus, Malaysia.

[3]Corresponding author: Kismiyati@fpk.unair.ac.id
**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 aim of this study was to determine the diversity of these bacteria at the genus level. Fish samples were collected and analyzed; each sample was subjected to a macroscopic external and internal observation of organs and tissues. Subsequently, samples were evaluated by microbiological tests using Trypticase Soy Agar (TSA), and conventional biochemical tests aimed at the production of glucose, sucrose, lactose, oxidase, catalase, indole, ornithine and Gram staining. 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. isolasi*

Comment [A1]: do you mean 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.

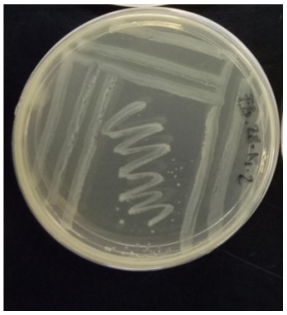

**Figure 1**. Colonies that grow on TSA media.

*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 Bcaillus mycoides. Examination using vitek 2 compact can be seen on Table 1.

**Table 1.** Test results using Vitek 2 Compact on Tilapia infected with pathogenic bacteria naturally

| 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 | - |

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.

## 4. Conclusions and Recommendations

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

**Comment [A2]:** There is not recommendation, please remove it

## 5. References


[1] Jansen, M. D., and C.V. Mohan. (2017). *CGIAR*. FISH-2017-04.

[2] Thanh, N. T. H., L. H. Thuy, G. W. Gallardo, N. H. Thanh. 2014 Vietnam, 6(12):133 – 139.

[3] Nayak S. K. 2010. Probiotics and immunity: A fish Perspective. Fish & Shellfish Immunology, 29: 2-14.

[4] Wirawan, I. K. A., S. A. Suryani and I. W. Arya. 2018. Fakultas Pertanian. Universitas Warmadewa, 23(1)

[5] Huda, C., Salni., Melki 2012 Maspari Journal, 4(1):69-76.

[6] Egwuatu, T.O., Ogunsola, F.T., Okodugha, I.M., Jide, B., Arewa, D.G., and Osenupebi, O.A. 2014 Advance in Microbiology. 4 1237-1241.

[7] Rasool, U.A., Badroo, G.A., Mudasir, M., Fayaz, S., Mustafa, R,. 2017 Int.J.Curr.Microbiol.App.Sci 6(8) :441-447.


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[1] Compagno L J V 1988 *Sharks of the Order Carcharhiniformes* (Princeton: Princeton University Press) p 570. (for book)

[2] Ovenden J, Morgan J T, Street R, Tobin A, Simfendorfer C A, Macbeth W, and Welch D 2011 *Mar Biol.* 158 1497-1509. (for journal)

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[8] Thomas, K.W., Tam, Z.K., Augustine, F.B. 2001 J Clinical Micro 39(8).
[9] Sabbathini, G. C., S. Pujiyanto, Wijanarka, P. Lisdiyanti 2017 Jur Bio 6(1):59-64
[10] Vancraynest, D., Haesebrouck, F., Deplano, A., Denis, O., Godard, C., Wildemauwe, C. And Hermans, K. (2006) J. Vet. Med. B Infect. Dis. Vet. Public. Health. 53: 418-422
[11] Hamidah, M. N., L. Rianingsih dan Romadhon. 2019. JITP, 1(2)
[12] Napitupulu, H, G., Rumengen, I, F, M., Wullur, S., L, Elvy. 2019. Jurnal Ilmiah Platax (7)1

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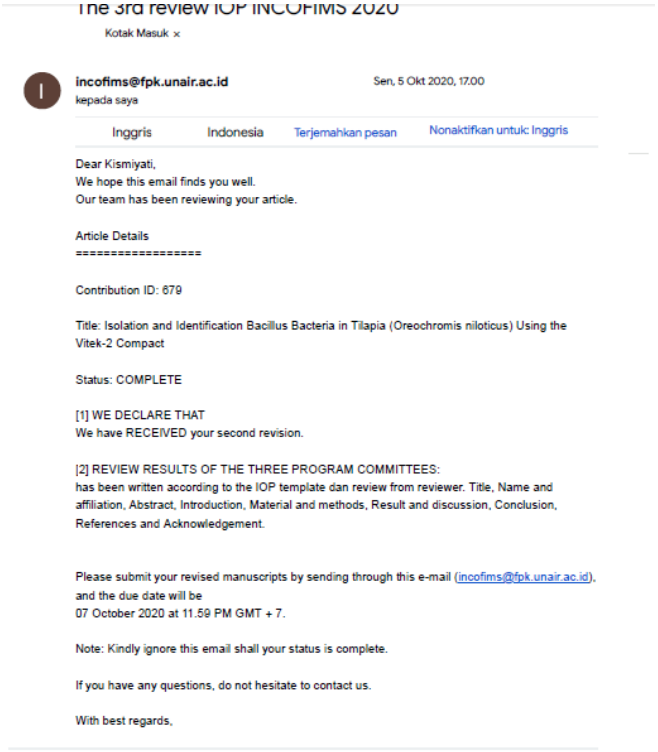

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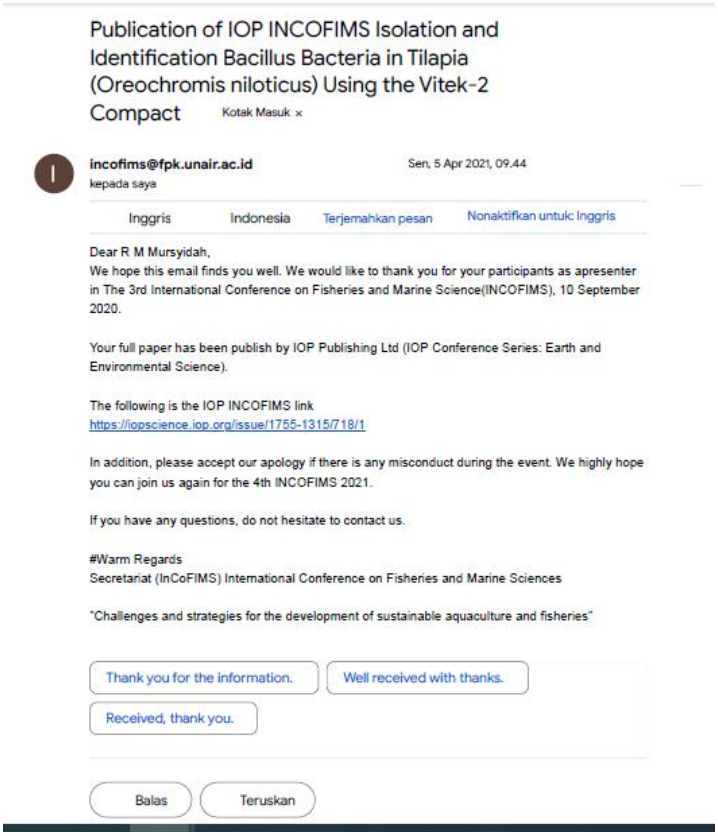

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