Exploration of indigenous bacteria in an intensive aquaculture system of African catfish (Clarias sp.) in Banyuwangi, Indonesia

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Abstract. Intensive African catfish culture in tarpaulin pond was popular in Banyuwangi, Indonesia since the government supported the fisheries sector. Unfortunately, the failure of African catfish culture still occurred since the waste from fish metabolite process and feed residue decreased the water quality. Bacteria in the water could be the solution to increase the success rate of aquaculture by improving the water quality. This study purpose was to obtained indigenous bacteria in intensive aquaculture system of African catfish to improve water quality. This study successfully isolated bacteria contained with amylase, protease and lipase characteristic. Isolated bacteria in this study were identified as Pseudomonas pseudomallei (97.81%), Bacillus subtilis (95.81%) and Pseudomonas stutzeri (61.21%).

1. Introduction
In 2010, the Government of Banyuwangi Region in East Java launched a program which established 10,000 fish ponds [1]. The program was aimed to increase not only the incomes of the people, but also their nutrition since the fish contain high protein[2]. One of the fish species cultured in Banyuwangi is African catfish (Clarias sp). African catfish have a high economic value, are easy to maintain, and grow fast [3]. There are many systems used for African catfish aquaculture in Banyuwangi, including intensive aquaculture system in tarpaulin pond. Intensive aquaculture system in tarpaulin pond is low-cost and easy to maintain [4].

Unfortunately, the system is still facing challenges, especially water management issues. The issues are related to aquaculture waste including metabolite waste and feed residues, which often leads to failure in the African catfish culture due to decreased water quality [5]. Bacteria have an important role in overcoming aquaculture waste.

Bacteria act as biological control agents that can improve water quality through the degradation of organic materials specifically [6]. The process of degrading the organic materials requires an important role of proteolytic, lipolytic bacteria, and amylolytic bacteria [7, 8, 9]. Proteolytic bacteria is bacteria producing proteinase [7]. Proteolytic bacteria use proteinase to break down proteins into peptides and amino acids, and turn them into energy[9]. Lipolytic bacteria produce lipase [10]. Lipase is an enzyme for catalyzing the hydrolysis reaction of triacylglycerol into fatty acids and glycerol [8]. Amylolytic bacteria are bacteria producing amylase. Amylase can degrade amylum into simpler polymers or monosaccharide sugars, wherein the next monosaccharide will be
changed into energy [7]. Three kinds of bacteria are essential for managing water quality in African catfish culture.

This study aims to obtain the indigenous bacteria for degrading organic materials to improve water quality in the intensive aquaculture system of African catfish in the tarpaulin pond. This study also determines the effectiveness of the indigenous bacteria in degrading organic materials to overcome the waste in the intensive aquaculture system.

2. Methodology
2.1 Material
The study was conducted from April until October 2016. The bacteria samples were collected from the intensive aquaculture system of African catfish in Muncar, Banyuwangi, Indonesia. The water containing the bacteria was put into bottles aseptically and taken using ice box to the microbiology laboratory of Science and Technology Faculty, Airlangga University. Each bottle contained 200 ml of water.

2.2 Method
2.2.1 Isolation
Each sample was homogenized and removed 100 ml of each into collection tubes. The homogenized tubes were then diluted until $10^{-4}$. The 0.01 ml of diluted suspension was inoculated to the Bushnell Haas (BH) media using a poured plate method (10 samples were labeled as A, B, C, D, E, F, G, H, I and J). This study used three kinds of BH media. BH with 1% of fat (olive oil) was used to isolate fat-degrading bacteria. BH media with 1% of amylum was used to isolate the amylum-degrading bacteria. BH media with 2% of skim milk was used to isolate the protein-degrading bacteria. All media were incubated at 37°C for 24-48 hours.

2.2.2 Hydrolysis index test
Inoculated bacteria in media resulted in the growth of bacterial cells appearing as multiple colonies. Colonies of degrading bacteria could be shown by the formation of clear zones around the growing colonies. The potential of hydrolysis of each isolate could be calculated based on the ratio of the diameter of the clear zone to the diameter of the growing bacterial colony. Its value is called the hydrolysis index [11].

2.3 Identification
Isolated indigenous bacteria were identified based on biochemical characterization (lipolytic, amyloytic and proteolytic test). Identification was performed by using Microbact™GNB 12A/B/E, 24E Identification Kits (Thermoscientific). The characterization results were referenced using Bergey's Manual of Determinative Bacteriology and International Journal of Systematic Bacteriology (USB)/International Journal of Systematic and Evolutionary Microbiology (USEM). The determination of species level was also done based on the characterization of the special decomposition properties of each isolate, referring to the latest literature and journals.

2.4 Water quality test
The test was conducted on water samples from catfish pond added with degrading bacteria. Water quality parameters observed were residual protein, fat, carbohydrate, BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) levels. Water parameters were checked at day 0, day 3, day 6 and day 9. 5 mg/ml of bacteria used were Pseudomonas pseudomallei 97.81% (A), Bacillus subtilis 95.81% (B), Pseudomonas stutzeri 61.21% (C), Pseudomonas pseudomallei 97.81% + Pseudomonas stutzeri 97.81% + Pseudomonas stutzeri 61.21% (D). The use of bacteria was based on hydrolytic test results and identification.
3. Results and discussion

3.1 Hydrolysis test

Table 1. Hydrolysis test from isolated bacteria.

| Test       | A  | B  | C  | D  | E  | F  | G  | H  | I  | J  |
|------------|----|----|----|----|----|----|----|----|----|----|
| Proteolytic| 2  | 2.6| -  | 1.666| 1.333| 1.333| 0.25| 0.25| -  | 2.1|
| Amylolitic | 0.25| -  | -  | -  | 0.142| 0.334| -  | 1.5 | -  | -  |
| Lipolytic  | -  | 1  | 1.325| 1  | 1  | 0.818| 3  | -  | 0.5| -  |

The table 1 shows that isolate B was the highest value of proteolytic with 2.6. Isolate G was the highest value of lipolytic with 3. Isolate I was the highest of amylolytic value with 1.5.

3.2 Identification of isolated bacteria

Isolated bacteria chosen as potential waste-degrading aquaculture waste had a huge value of hydrolysis index. Isolated bacteria were B, G and I identified as *Pseudomonas pseudomallei* (97.81%), *Bacillus subtilis* (95.81%) and *Pseudomonas stutzeri* (61.21%).

3.3. Water quality test

3.3.1 Protein level

![Figure 1. Protein level in the water.](image)

Each day, the protein level decreased gradually. At day 3, the highest average protein level was the C treatment of 62 mg/l while the lowest was the B treatment of 54 mg/l. At day 6, the highest protein content was the C treatment of 58 mg/l while the lowest was the B treatment of 40 mg/l. At day 9, the highest protein level was in the C treatment of 39 mg/l, and the smallest was the D treatment of 18 mg/l. The results of the protein content of each bacterial inoculant are shown in figure 1.
3.3.2 Fat level

![Fat level graph](image)

**Figure 2.** Fat level in the water.

This shows that the fat level decreased daily. At day 3, the highest fat level was the C treatment of 29 mg/l while the lowest was the D treatment of 24 mg/l. At day 6, the highest fat level was the C treatment of 21 mg/l while the lowest was the B treatment of 13 mg/l. At day 9, the highest fat level was the C treatment of 12 mg/l while the lowest was the B treatment of 4 mg/l. The data of fat level of each bacterial inoculant are shown in figure 2.

3.3.3 Carbohydrate level

![Carbohydrate level graph](image)

**Figure 3.** Carbohydrate level in the water.
At day 3, the highest carbohydrate level was the A treatment of 20 mg/l while the lowest was the D treatment of 13 mg/l. At day 6, the highest carbohydrate level was the A treatment of 11 mg/l while the lowest was the D treatment of 4 mg/l. At day 9, the highest carbohydrate level was the A treatment of 5 mg/l while the lowest was the D treatment of 2 mg/l. The data of carbohydrate level of each bacterial inoculant are shown in figure 3.

3.3.4 BOD level

![Figure 4. BOD concentration in the water.](image)

The result showed that the BOD level at day 0 had the highest value in each bacterial treatment of 410 mg/l. The level of BOD decreased daily. At day 3, the highest BOD level was the C treatment of 353 mg/l while the lowest was the A treatment of 287 mg/l. At day 6, the highest BOD level was the C treatment of 197 mg/l while the lowest was the D treatment of 111 mg/l. At day 9, the highest BOD level was C treatment of 92 g/l while the lowest was the D treatment of 35 mg/l. The data of BOD level of each bacterial inoculant are shown in figure 4.
3.3.5 COD level

![COD level graph](image)

**Figure 5.** COD level in the water

The study shows that the COD level decreased daily. At day 3, the highest COD level was the A treatment of 349 mg/l while the lowest was D treatment of 334 mg/l. At day 6, the highest COD level was the C treatment of 280 mg/l while the lowest was the D treatment of 229 mg/l. At day 9, the highest COD level was the B treatment of 167 mg/l while the lowest was the D treatment of 110 mg/l. The data of COD level of each bacterial inoculant are shown in figure 5.

4. Discussion

This study identified the bacteria containing amylase, proteinase and lipase enzymes (table 1). The bacteria live in the water and act as biological control agents that can improve water quality [6]. The results of the isolation of organic degradation bacteria taken from intensive aquaculture system in African catfish (*Clarias* sp) in the tarpaulin pond showed 3 potential bacteria: *Pseudomonas pseudomallei*, *Bassilus subtilis*, and *Pseudomonas stutzeri*.

*Pseudomonas* and *Bassilus* are proteolytic bacteria [7]. Proteolytic bacteria are bacteria that produce extracellular proteinase enzymes, which are protein-soluble enzymes produced in cells and then released out of cells[12]. The aquaculture waste present in the water will be absorbed by bacterial cells and decomposed into amino acids by intracellular protease for protein synthesis or ultimately deaminated through intermediary metabolic pathways [13]. The protease was an extracellular enzyme released by microbes, which serves to hydrolyze peptide bonds in proteins to produce simpler peptides or also produce amino acids by peptidase [14]. Reduced levels of protein at day 3 to day 9 show the role of *Pseudomonas* and *Bassilus* as proteolytic bacteria to degrade the protein content in the waters (figure 1).

*Pseudomonas* and *Bassilus* are not only identified as proteolytic bacteria but also as lipolytic bacteria [15, 10]. Lipolytic bacteria are lipase-producing bacteria [10]. The ability of the bacteria in degrading fat is related to the bacteria’s ability to produce lipase enzymes. The lipase enzyme serves to catalyze triglycerides into diglycerides and fatty acids [15]. Reduced levels of fat on day 3 to day 9 show that *Pseudomonas* and *Bacillus* as lipolytic bacteria can break down the protein content in the waters (figure 2).

*Pseudomonas* and *Bassilus* have other roles as amylolytic bacteria [13, 16]. Amylolytic bacteria are enzyme-producing amylase bacteria, enzymes that break down amylum into simpler polymers or
monosaccharide sugars, wherein the next monosaccharide will be degraded once more into energy[7]. Reduced carbohydrate levels at day 3 to day 9 show that *Pseudomonas* and *Bacillus* as amylolytic bacteria can degrade carbohydrate levels in the waters (figure 3).

Bacteria can perform a bioremediation process in the water. Bioremediation is a process that utilizes microorganisms, including bacteria, to transform harmful substances into non-toxic products[15]. Bioremediation is done by bacteria to activate metabolism system [11]. Metabolism is a process of chemical transformation and the formation of energy for bacterial life and proliferate. Bacterial metabolism is divided into two, aerobic and anaerobic. The basis of the reaction in aerobic metabolism[11]. Aerobic metabolism requires $O_2$ to produce energy. Reduced levels of BOD and COD at day 3 to day 9 show that *Pseudomonas* and *Bacillus* could reduce BOD and COD level in waters (figure 4 and figure 5).

Study of the water quality test of in vitro showed that the treatment using inoculant of 3 species of bacteria was more effective. This is indicated by the decreasing protein, fat, carbohydrate, BOD and COD levels occurring in the D treatment (figure 1, figure 3, figure 4 and figure 5). Bacterial concentrations are faster in degrading activity than only one species. The addition of combining inoculant bacteria stimulates the process of decomposition of organic materials into minerals more efficiently because it takes a shorter time [18].

5. Conclusion

In conclusion, we found successfully isolated bacteria contained with amylase, protease and lipase characteristic from intensive aquaculture system of African catfish in Muncar, Banyuwangi, Indonesia. Founded bacteria in this study were identified as *Pseudomonas pseudomallei* (97.81 %), *Bacillus subtilis* (95.81 %) and *Pseudomonas stutzeri* (61.21 %). Water quality test of protein, fat, carbohydrate, BOD and COD showed decrease level of each aspect by adding isolated bacteria. The combination bacteria showed faster degradating activity than individual bacteria.

6. References

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