Growth Performance and Survival Rate of Catfish (*Pangasius sp*) with the Application of the Nitrifying and Denitrifying Bacteria

**Y Yosmaniar**, T Sumiati, M Mulyasari

1 Research Institute for Freshwater Aquaculture and Fisheries Extension, Bogor, West Java, Indonesia

*yosmaniar@yahoo.com*

**Abstract.** Nitrifying and denitrifying bacteria can be used as bioremediation agents in aquaculture. The purpose of this experiment is to evaluate the optimal growth and survival performances of catfish rearing with the application of nitrifying and denitrifying bacteria. A completely randomized design was performed with the following treatments: A) nitrifying and denitrifying bacteria NP2-DP1; B) nitrifying and denitrifying bacteria NP2-DP2; C) commercial bacteria and D) without bacterial isolate (control), each with 3 replications. Twelve containers (34 x 34 x 45 cm) were used with a volume of 20 L equipped with aeration. The catfish used (*Pangasius sp*) has a body weight of 8.33 g ± 0.1 and stocking density of 20 fish/container reared within 30 days. Feed was applied to the fish at 3% of their body weight for three times a day at 08.00 am, 12.00 and 15.00 pm. Inoculation of bacteria on day 10th and 20th, that is 10^8 cfu/mL. The parameters measured were growth rate, survival rate, and water quality. Sampling was carried out every 10 days. The results showed that the application of NP2 and DP1 was the optimal to increase the growth and survival of catfish.

1. **Introduction**

Catfish is one of the potential freshwater fishery commodities to be developed because it is in great demand by the people of Indonesia, being one of the mainstays in increasing aquaculture productivity and even having the opportunity to be exported. This can be seen by the increase in catfish production from 319,966 tons in 2017 to 391,151 tons in 2018, which increased by 22.25% [1]. However, along with the catfish farming business that continues to grow and is carried out intensively, there are problems faced. One of them is the accumulation of organic matter in the cultivation media derived from the metabolism of fish, feed, feces and other sources. High fish density causes high accumulation of organic waste in cultivation media which can reduce water quality. Dissolved oxygen levels in water will decrease and vice versa nitrogen compounds will increase. The high nitrogen compounds such as ammonia (NH₃), nitrate (NO₃) and nitrite (NO₂) in the rearing media affect the health and appetite of fish so that it can inhibit the growth and survival of fish. In addition, nitrogen compounds such as ammonia, nitrite and nitrate in high concentrations are toxic so that they can threaten the survival of reared animals.
One of the efforts to control nitrogen levels in water is by using bioremediation technology. Bioremediation is the process of using microorganisms to clean chemical pollution by reducing the concentration and/or toxicity of chemical compounds and restoring natural conditions that are environmentally friendly because they use the enzymatic activity of microorganisms by utilizing pollutants as a source of nutrition [2]. According to [3] bioremediation bacteria are able to improve the water quality of the maintenance media for the Siamese catfish Pangasius hypophthalmus. This technique only uses less energy and does not involve many natural resources, so it is cheaper and more sustainable than physical-chemical treatment [4].

The use of groups of nitrifying and denitrifying bacteria is one of the bioremediation techniques that can be applied to reduce the concentration of nitrogen compounds in fish culture media. Nitrifying bacteria are a group of bacteria capable of compiling nitrate compounds from ammonia compounds which generally take place aerobically in the soil [5]. Meanwhile, denitrifying bacteria are a group of bacteria that have the ability to carry out a reaction to reduce nitrate compounds (NO3-) to free nitrogen compounds (N2) [5].

The application of nitrifying and denitrifying bacteria has been carried out to overcome nitrogen waste from fish farming, as reported by [6]. According to [6], a consortium of nitrifying, denitrifying, heterotrophic and anoxygenic photosynthetic bacteria can work well to maintain the water quality of tiger shrimp ponds so that they are below the pollution threshold and are able to decompose toxic compounds. However, research related to the effect of using nitrifying and denitrifying bacteria in the maintenance of catfish is still very limited. Therefore, it is necessary to conduct research to determine the effect of the application of these nitrifying and denitrifying bacteria on the growth and survival of catfish.

2. Materials and methods

The research was conducted at the Fish Health Research Installation in Depok, Research Institution for Freshwater Aquaculture and Fisheries Extension (RIFAIE) for 30 days. The container used in the study was a plastic container measuring 34 x 34 x 45 cm with a volume of 20 L of water equipped with an aeration system. The test fish used was catfish (Pangasius sp) with an average initial weight of 8.33 g ± 0.1 using a stocking density of 20 fish/container. The study was conducted using a completely randomized design (CRD) with 3 treatments and 3 replications. The treatments tested were: A) rearing catfish with the addition of a consortium of nitrifying and denitrifying bacteria (NP2 and DP1); B) rearing catfish with the addition of a consortium of nitrifying and denitrifying bacteria (NP2 and DP2); C) rearing catfish with the addition of commercial bacteria and D) rearing fish without bacteria (control). Feed is given as much as 3% of the weight of the biomass 2 times a day (morning and evening). Bacterial application (treatment) was 10^8 cfu/mL per 20 L on day of 10th and day of 20th. Fish weight, TPC and water quality measurement was carried out on days of 0, 10th, 20th and 30th. Parameters observed were total plate count (TPC), water quality (Temperature, pH, DO, TAN, NO3, NO2), daily growth rate, weight gain and survival of fish. The data was tabulated using Excel 2010 and statistically tested using Anova. Further tests were carried out using Duncan's test at a 95% confidence level with the SPSS ver program. 20.

Nitrifying and denitrifying isolates were grown in specific media according to [7]. The materials used for culture of nitrifying bacteria as much as 1000 mL are: 13.5 g KH₂PO₄; 0.7 g K₂HPO₄; 0.1 g MgCl₂.6H₂O; 0.18 g CaCl₂.2H₂O; 0.1 g NH₄Cl; 0.2 g EDTA and 0.5 g Na₂CO₃; 0.18 g FeCl₃.6H₂O; and 0.5 g of glucose. Meanwhile, the denitrifying bacteria were: 10 g Na-acetate, 5 g KNO₃; 0.2 g KH₂PO₄; 0.9 g K₂HPO₄; 0.1 g CaCl₂.2H₂O; 0.5 g MgSO₄.7H₂O; and 0.2 g EDTA.

3. Results and Discussion

The results of the TPC calculation showed that the log of the highest bacterial count was on day 10 for treatments B, C and D. As for treatment A, the log of the highest number of bacteria was on day 20. Day 10 was the starting point for adding bacteria to the rearing media so that at this time the number of bacteria measured was the highest. However, the number of bacteria in treatments B, C and
D continued to decrease until the end of the study. Different things were seen in treatment A where on day 20 the number of bacteria in treatment A experienced a significant increase although at the end of the study the number of bacteria decreased (Figure 1).

Figure 1. Log of the total plate count (TPC) of bacteria in the treatment: A) a consortium of bacterial isolates NP2-DP1; B) a consortium of NP1-DP1 bacterial isolates; C) Commercial bacteria and D) Control.

Several water quality parameters in catfish rearing containers for all treatments were presented in Table 1. The temperature of water media during catfish rearing ranged from 26.8-32°C. These temperature conditions are still within the normal temperature range for catfish rearing. According to [8] fish can live at a temperature of 25-33°C. Temperature is related to dissolved oxygen levels in the water, if the temperature increases, the dissolved oxygen levels will decrease because with an increase in temperature, fish metabolism will increase so that the level of oxygen consumption is higher. According to [9] the optimal water temperature can affect the rate of respiration and the rate of metabolic rate which greatly affects the appetite of the fish so as to make the fish grow faster.

The pH value of water has a very important role in survival and growth in fish [10]. The pH value of catfish rearing water media in this study ranged from 6.10 to 6.71. This value indicates that the pH at the time of rearing was still slightly below normal levels. The pH of safe water for catfish rearing according to [8] ranges from 6.5 to 9.0 with an optimal pH of around 7-8.5. Dissolved oxygen content is an important water quality parameter for the life of aquatic organisms. The dissolved oxygen content in this study for all treatments ranged from 2.29-4.71 mg/L.

Table 1. Water quality (temperature, pH, DO, TAN, NO₂ and NO₃) of catfish rearing media for 30 days in the following treatment: A) consortium of bacterial isolates NP2-DP1; B) a consortium of NP1-DP1 bacterial isolates; C) Commercial bacteria and D) Control.

| Parameters | Unit | Treatments |
|------------|------|------------|
|            |      | A          | B          | C          | D          |
| Temperature | °C   | 26.3 - 27.7 | 25.9 - 27.9 | 26.0 - 27.8 | 26.4 - 27.8 |
| pH         |      | 6.50 - 6.66 | 6.50 - 6.66 | 6.60 - 6.70 | 6.10 - 6.71 |
| DO         | mg/L | 2.73 - 4.71 | 2.29 - 4.19 | 2.31 - 3.7  | 2.61 - 3.08 |
| Ammonia    | mg/L | 0.001 - 0.021 | 0.002 - 0.019 | 0.002 - 0.025 | 0.02 - 0.15 |
| Nitrite    | mg/L | 0.001 - 0.015 | 0.001 - 0.059 | 0.001 - 0.056 | 0.02 - 0.09 |
| Nitrate    | mg/L | 1.783 - 3.082 | 1.642 - 5.167 | 3.13 - 12.15 | 3.71 - 14.52 |
The ammonia levels in water media of catfish during rearing were presented in Table 1. The ammonia concentration during the study for all treatments was still within the safe limits for fish rearing, which ranged from 0.001 to 0.15 mg/L. Ammonia safe limit which refers to [11] is <0.5 mg/L. Ammonia levels in treatment A, B and C were lower compared to treatment D (control). This indicates that the nitrifying bacteria in treatments A and B, namely isolates NP1 and NP2 were able to reduce ammonia compounds in the water medium. The same thing was seen in the probiotic of treatment C which showed that these bacteria had the ability to reduce ammonia to other compounds such as nitrite and then to nitrate.

Nitrite is an intermediate form of the nitrification and denitrification processes, namely the transition between ammonia and nitrate (nitrification) and between nitrate and hydrogen gas (denitrification) [12]. Nitrite levels in this study for all treatments ranged from 0.001 to 0.09 mg/L. Treatment A produced the lowest nitrite content (0.001-0.015 mg/L), followed by treatment C (0.001-0.056 mg/L) and treatment B (0.001-0.059 mg/L). Meanwhile, treatment D produced the highest nitrite content, which was 0.02-0.09 mg/L exceeds the safe limit of nitrite levels for fish rearing. The safe limit for nitrite levels for fish according to [11] is < 0.06 mg/L. This indicated that the bacterial isolates added in treatments A, B, C were able to reduce nitrite compounds to nitrates in the catfish rearing media so that the levels were lower than those in treatment D.

The concentration of nitrate during the study for all treatments ranged from 1.78-14.52 mg/L. The safe nitrate concentration for fisheries refers to [11] is < 10 mg/L. The highest nitrate level was found in treatment D (3.71-14.52 mg/L) while the lowest was found in treatment A (1.783-3.082 mg/L) followed by treatment B (1.647-5.167 mg/L). The lower nitrate levels in treatments A and B were thought because the denitrifying bacteria DP2 and DP1 were able to reduce nitrate compounds and convert them into intermediate compounds such as nitrite and nitrous oxide which would then be converted into N2.

The results showed that the addition of NP2-DP1 isolates (treatment A) in catfish rearing significantly affected the daily growth rate, weight growth and survival of catfish when compared to controls (p<0.05). The daily growth rate of catfish in treatment A had the highest value of 1.32%/day, which was significantly different from other treatments (p<0.05) (Figure 2). While the lowest daily growth rate was the control, which was 0.618%.

**Figure 2.** Daily growth rate, weight growth and survival rate of catfish in the following treatments: A) a consortium of NP2-DP1 isolates; B) a consortium of NP1-DP1 isolates; C) Commercial bacteria and D) Control.

In line with the daily growth rate, the weight growth value in treatment A showed different results from other treatments. Weight growth in treatment A resulted in a higher value and significantly different than the other treatments (p<0.05) (Figure 3). The highest weight gain of catfish found in treatment A was 12.36 grams, while the lowest was found in treatment D or control, which was 10.03 grams.
Figure 3. Weight growth of catfish in the following treatments: A) a consortium of NP2-DP1 isolates; B) a consortium of NP1-DP1 isolates; C) Commercial bacteria and D) Control.

The survival of catfish in treatment A also showed the highest value compared to other treatments. Survival rates in treatment A were significantly different from treatment D or control (p<0.05) but not significantly different from treatments B and C (p>0.05) (Figure 4). The highest catfish survival was found in treatment A, which was 95.56%, while the lowest was found in treatment D or control, which was 80%.

Figure 4. Survival rate of catfish in the following treatments: A) a consortium of NP2-DP1 isolates; B) a consortium of NP1-DP1 isolates; C) Commercial bacteria and D) Control.

The results showed that the bacteria consortium NP2-DP1 was able to reduce ammonia and nitrate from metabolism and feed residues in aquarium water optimally so as to produce water quality that is more conducive to the growth and survival of catfish. NP2-DP1 bacteria are able to synergize in reducing ammonia and nitrate in water so that it has a positive impact on the growth and survival of catfish. According to [13], the application of nitrifying bacteria can increase the ability of oxidation in aquaculture ponds, and vice versa if the nitrification process takes place too high, it can reach undesirable conditions so that it needs to be balanced with the provision of denitrifying bacteria for reduction. High nitrate concentrations can cause cases of Nitrate Toxicity, so it is necessary to reduce it by denitrifying bacteria to nitrogen gas (N2). The addition of these bacterial inoculants can also reduce the content of nitrite compounds in the waters [13].

The results of this study are in line with research [14] which reported that the administration of a consortium of nitrifying and denitrifying bacteria had a positive effect on improving pond water quality conditions, growth, and production of tiger prawns. Meanwhile, [15] reported that the use of a consortium of nitrifying and denitrifying bacteria was able to improve growth performance and feed efficiency of vaname shrimp. According to [16], the right consortium of several types of bacteria will have a positive impact on the environment of the fish rearing media, because the bacterial consortium
is able to utilize the rest of the fish metabolism in the water so that the condition of the water quality of the rearing media remains optimal.

4. Conclusions
Isolates NP2 and DP1 are the best consortium of nitrifying and denitrifying bacteria isolates for increasing the growth and survival rate of catfish.

References
[1] Directorate General of aAquaculture (DJPB) 2019 https://kkp.go.id/djp/artikel/11662-dukung-ekspor-patin-kkp-dorong-industrialisasi-budidaya-patin-berkelanjutan. Downloaded on July 29th, 2021.
[2] Hardani H, Kardiansyah T, Sugesty S 2011 Jurnal selulosa 1(1): 31-41.
[3] Taufik I, Sutrisno, Yuliati P, Supriyadi H, Subandiyah S, Muthalib I 2017 Jurnal penelitian Perikanan Indonesia 7(11): 60-66.
[4] Becker JG and Seagren E 2010 Environmental Microbiology. second Edition. p.177 – 212.
[5] Madigan MT 2009 Brock Biology of Microorganisms Twelfth Edition p. 403–404.
[6] Effidiah 2016 Distilasi 1(1): 57-61.
[7] Rodina G 1972 Methode In Aquatic Microbiology In Rita RC and Machael S eds (University Park Press Baltimore USA) 461 pp.
[8] Ghufran MH and Kordi K 2005 Catfish Farming Biology Hatchery and Enlargement (Yayasan Pustaka Nusatama) Yogyakarta.
[9] Effendi, H 2003 Water Quality Analysis for Aquatic Resources and Environment Managers Jakarta Kanisius.
[10] Tataje DAY Baldisserotto B and Filho EZ 2015 Neotropical Ichthyology 13, 179-186.
[11] Anonymous 2001 The Government Regulation Number 82 2001 President Republic of Indonesia On Water Quality Management And Water Pollution Control (Jakarta: Kementerian Lingkungan Hidup) p 299.
[12] Saputra M A. 2015. Improvement of Water Quality On Enlargement of Giant Prawns With Different Stocking Densities Based On Integrated Multi Trophic Aquaculture Essay Department of Aquaculture. Faculty of Fisheries and Marine Sciences. (Bogor Agricultural University) 22 pp.
[13] Hastuti YP 2011 Jurnal Akuakultur Indonesia 10 (1), 89–98.
[14] Badoeri and Widiyanto 2008 Utilization of nitrification bacteria for remediation and its effect on ammonia and nitrite concentration in shrimp pond. (Oseanografi dan Limnologi di Indonesia). 34(2): 261-278.
[15] Yuniasari D. 2009. The effect of giving nitrifying and denitrifying bacteria and molasses with different ratios of CN on the water quality profile, survival and growth of white shrimp Litopenaeus vannamei Essay Department of Aquaculture. (Faculty of Fisheries and Marine Sciences Bogor Agricultural University) 55 pp.
[16] Nawawi 2013 Jurnal Galung Tropika 2(2): 116-122.