Effect of different bacterial strain in probiotics on the growth performance of Nile Tilapia (<i>Oreochromis niloticus</i>)

T D Sholihuddin¹, M Arief² and H Kencono Najati³, ⁴

¹Study Program of Aquaculture, Campus Banyuwangi, Faculty of Fisheries and Marine, Universitas Airlangga, Indonesia
²Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Indonesia
³Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Campus Banyuwangi, Universitas Airlangga, Indonesia
⁴Corresponding author: hapsari@fpk.unair.ac.id

Abstract. The main purpose of this study was to investigate the effect of different bacterial type in probiotics on the growth performance of Nile tilapia. In this study, commercial probiotics with a different type of bacteria were added to the diet. The first group, as control, was fed with a commercial diet without probiotic. The second group was fed with commercial diet plus probiotic X contained <i>Lactobacillus</i> sp., <i>Bacillus</i> sp. and <i>Pseudomonas</i> sp. The third group was applied commercial diet plus probiotic Y contained <i>Lactobacillus</i> sp., <i>Bacillus</i> sp. and <i>Bifidobacterium</i> sp. Feeding trial was performed for 30 days. The result showed that the growth performance of Nile Tilapia including weight gain (WG) and specific growth rate (SGR) was significantly higher than the control group (P<0.05). The addition of probiotics in the commercial diet also improved feed utilization significantly. The best growth performance was obtained in the third group where the probiotic Y applied. Different bacterial strain in probiotic could give a different effect on the growth performance of Nile Tilapia because of their different action mode in fish. Therefore, the appropriate selection of probiotic strains can lead to specific advantages for species in aquaculture.

1. Introduction

Probiotics are described as living microorganism in sufficient amounts which contribute to the balancing of intestinal microbes and give beneficial effect to the hosts health [1]. The use of probiotics in aquaculture is relatively new when compared to its use in humans, poultry and cattle. In latest years, probiotics become an effective strategy for successful aquaculture due to its advantageous effect on fish health. It could promote growth by helping the digestion system, protect from pathogen, enhance immune system, and improve water quality [2]. Various microorganism, including Gram-positive and Gram-negative bacteria, microalgae, and yeast, has been examined as probiotics in aquaculture [3]. There are various techniques to apply probiotic in aquaculture management, such as supplementation on diet, immersion trough water of tanks or pond, and injection [4].

Probiotic can be applied as a single strain or multiple strains. Previous study proved that multiple strain on probiotic is more effective than a single strain probiotic [5]. The effectivity of mixed strain probiotic to improve growth performance and health status of fish are related to synergistic effect between its constituent strains. [6] displayed that the administration of mixed four probiotics, containing <i>Lactobacillus pentosus</i>, <i>Lactobacillus fermentum</i>, <i>Bacillus subtilis</i>, and <i>Saccharomyces cerevisiae</i>
cerevisiae, on Asian seabass produced the improvement of its growth performance and health status. However Yazici et al., [7] reported that mixed strains probiotic, containing Lactobacillus plantarum and Bacillus subtilis, had no significant effect on growth performance of rainbow trout. This case indicated that different strain of probiotic could give different effect on growth performance and health status of fish. Based on this case, we need to evaluate the effect of different bacterial strain in probiotics on the growth performance of Nile tilapia (Oreochromis niloticus).

2. Material and methods

2.1. Experimental fish collection

The study was conducted at Balai Benih Ikan Kabat, Banyuwangi. A hundred and fifty Nile tilapia seeds (mean weight ± 20 g) were purchased from local fish farm in Genteng, Banyuwangi. Fish were acclimatized for 7 days and fed three times with commercial diet contained 25-27% of protein.

2.2. Experimental diet preparation

The two probiotic were commercially available probiotic products contained different strain of bacteria. Probiotic X contained Lactobacillus sp., Bacillus sp., and Pseudomonas sp. while probiotic Y contained Lactobacillus sp., Bacillus sp., and Bifidobacterium sp. Experimental feeds were prepared by spreading the probiotic as much as 10 ml.kg\(^{-1}\) on commercial diet. The commercial diet without probiotic supplementation was taken as a control diet. The diets were air-dried for 5 minutes prior to a feed.

2.3. Experimental design

A hundred and fifty Nile tilapia seeds were divided into 3 treatment groups. Each treatment was performed in 5 replications and designated with 10 fish per aquarium. Group A was fed with a commercial diet without any probiotic supplementation, group B was fed with a probiotic X supplementation on diet, and group C was fed with a probiotic Y supplementation on diet. The fish were fed at the rate of 4% of total body weight three times daily.

Siphoning the residual feed and fish feces was carried out every morning prior to a morning feed. The water quality, such as temperature, pH and dissolved oxygen, were measured during the maintenance. The water quality was in range of 27-29°C, pH 6-7, and dissolved oxygen 3-4 mg.L\(^{-1}\).

2.4. Growth performance

Sampling was taken every 7 days to measure the wet weight. The parameter of growth, including weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR), were calculated using the following formulae:

\[
WG = FW - IW
\]

\[
SGR = \left(\frac{\ln FW - \ln IW}{T}\right) \times 100
\]

\[
FCR = \frac{FI}{WG}
\]

Note FW = final weight (g), IW = initial weight (g), T = duration of feeding (days), WG = weight gain (g), and FI = feed intake (g).

2.5. Statistical analysis

All data were analyzed statistically using SPSS version 20 for Windows. Data are reported as mean ± standard error. The difference between the treatments were determined by one-way analysis of variance (ANOVA) followed by Duncan multiple range test. Significance was accepted at P < 0.05
3. Result and discussion

The use of probiotic in aquaculture has become a great concern in latest years. Some previous study explained that probiotics has many benefit in improvement of aquaculture production through enhancing growth performance, immunity system, and water quality [7,8]. Probiotic can be put to work either as single or mixed strain but the mixed strain probiotic is preferable because it is more effective [5].

In present study, the use of probiotic on diet affected the growth parameters. The growth performance of Nile tilapia seeds after 30 days of feeding has been shown in table 1. The results presented that the use of probiotics on a diet considerably enhanced the growth performance compared to the control group without probiotic. The best growth performance displayed on fish fed with probiotic Y which contained \textit{Lactobacillus} \textit{sp.}, \textit{Bacillus} \textit{sp.}, and \textit{Bifidobacterium} \textit{sp.} The probiotic X contained \textit{Lactobacillus} \textit{sp.}, \textit{Bacillus} \textit{sp.} and \textit{Pseudomonas} \textit{sp.} also produced an improvement on growth performance but not as high as probiotic Y. Whereas, the use of both probiotics did not give significant difference on FCR value. This result revealed that different strain of probiotics on a diet generated different effect on growth performance.

| Table 1. Growth performance of Nile tilapia seeds after 30 days of feeding on experimental diets. |
|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Growth parameter              | Group A                        | Group B                        | Group C                        | P-value                      |
| Weight gain (g)               | 123.42 ± 14.08                 | 172.29 ± 2.92                  | 212.16 ± 11.65                 | < 0.01                       |
| Specific growth rate (%.day⁻¹) | 1.59 ± 0.14                    | 2.07 ± 0.03                   | 2.40 ± 0.09                   | < 0.01                       |
| Feed conversion ratio         | 1.42 ± 0.09                    | 1.17 ± 0.06                   | 1.11 ± 0.06                   | 0.015                        |

\textsuperscript{a} Values are presented as mean ± SE.

\textsuperscript{b} The different superscript notation at the same row indicates a significant difference among the treatment.

\textsuperscript{c} Group A: fish fed with commercial diet; Group B: fish fed with diet supplemented with probiotic X; Group C: fish fed with diet supplemented with probiotic Y.

Both of probiotic used in this study, contained \textit{Lactobacillus} \textit{sp.} and \textit{Bacillus} \textit{sp.} \textit{Lactobacillus} \textit{sp.} and \textit{Bacillus} \textit{sp.} were Gram-positive bacteria belong to lactic acid bacteria (LAB). It is known as the most widely use probiotic due to its ability in balancing of intestinal microbiota and inducing growth rate in different fish species [2]. Previous study has been investigated that these bacteria were naturally found in digestive tract of fish [9–12]. The role of both bacteria for enhancing growth rate on fish were examined due to their ability to produce various digestive enzymes such as protease, amylase, and lipase. [13] evaluated the isolated \textit{Bacillus} \textit{sp.} from digestive tract of \textit{Labeo calbasu} as the potential probiotic candidate. Their study confirmed that the bacteria produce protease, amylase, and lipase. These enzymes can be useful for carbohydrates, protein, and lipid digestion, therefore, the nutrients can be used optimally [14].

According to the result, probiotic Y significantly generated growth rate higher than probiotic X. This result can be suspected from different strain bacteria on probiotic X and Y wherein probiotic X has \textit{Pseudomonas} \textit{sp.} and probiotic Y has \textit{Bifidobacterium} \textit{sp.} \textit{Pseudomonas} \textit{sp.} and \textit{Bifidobacterium} \textit{sp.} has different character. \textit{Pseudomonas} \textit{sp.} belongs to Gram-negative whereas \textit{Bifidobacterium} \textit{sp.} was a Gram-positive. Both of this bacteria commonly used in aquaculture as probiotics to alleviate numerous diseases. [15] combined \textit{Bifidobacterium} with \textit{Lactobacillus} as probiotic on \textit{Clarias gariepinus} juvenils and verified its ability to enhanced fish growth performance and health. [16] reported that the addition of \textit{Pseudomonads} into tank water could reduce the mortality of rainbow trout infected by \textit{Vibrio anguillarum}. These bacteria have antibacterial activity to protect fish from diseases through some action among others are producing of bacteriocins [17] and competing for iron as the essential elements for microbial growth [18]. Its application as a probiotic is expected to improve the fish health so the fish growth will be optimal.
This study revealed that probiotic contained *Pseudomonas* sp. did not give improvement of growth parameter as high as probiotic contained *Bifidobacterium* sp. The result was corroborated by Abd EL-Rhman et al., [19] that investigated the application of Pseudomonads as a probiotic in Nile tilapia. Their study reported that the addition of Pseudomonas on diet reduced the growth, survival, and feed utilization. Growth reduction in this study may be caused by possibilities pathogenic-version changing of *Pseudomonas* sp. after 30 days of feeding. Contrary to our study, Hai et al., [20] reported that *P. synxantha* and *P. aeruginosa* has been applied to juvenile western king prawns and generated improvement in health, survival, and growth. These cases indicated that Pseudomonads might be applied as probiotic in a certain organism and was not advisable for Nile tilapia.

The enhancement growth on Nile tilapia after feeding with probiotic contained *Bifidobacterium* sp. proved the presence of synergistic interaction between *Bifidobacterium* sp., *Lactobacillus* sp., and *Bacillus* sp. Ouwehand et al., [21] explained that the presence of *Lactobacillus* GG or *Lactobacillus bulgaricus* increase in adhesion of *Bifidobacterium lactis* to intestinal mucosa so the possibility of probiotic strain to colonize raised. Therefore, the appropriate selection of probiotic strains is crucial to give specific advantages for species in aquaculture. Synergistic or antagonistic interaction between two or more strain of probiotic must be evaluated to know their biological effect in fish before it used on mass scale.

4. Conclusion
In conclusion, application of *Bifidobacterium* sp. mixed with *Lactobacillus* sp. and *Bacillus* sp. on Nile tilapia diet improved its growth performance better than *Pseudomonas* sp., The different strain of bacteria in probiotic could give different effect on growth performance because of their different characteristic action on fish guts.

5. Reference

[1] Ibrahem M D 2015 *J. Adv. Res.* 6 765–91
[2] Allameh S K, Noaman V and Nahavandi R 2017 *Adv Tech Clin Microbiol* 1 1–5
[3] Irianto A and Austin B 2002 *J. Fish Dis.* 25 633–42
[4] Michael E T, Amos S O and Hussaini L T 2014 *Fish. Aquac. J.* 5 1–3
[5] Wang Y C, Hu S Y, Chiu C S and Liu C H 2019 *Fish Shellfish Immunol.* 84 1050–8
[6] Lin H L, Shiu Y L, Chiu C S, Huang S L and Liu C H 2017 *Fish Shellfish Immunol.* 60 474–82
[7] Yazici I S, Hisar O, Yilmaz S and Yigit M 2015 *Mar. Sci. Technol. Bull.* 4 21–8
[8] Janakiram P, Veerendra M, Jayasree L and Sivaprasad B 2014 *Int. J. Fish. Aquat. Stud.* 1 199–207
[9] Ghanbari M 2011 *4th Int. Symp. Stock Enhanc. Sea Ranching* 10 152–7
[10] Muthukumar P and Kandeepan C 2015 *Int. J. Curr. Microbiol. Appl. Sci.* 4 607–16
[11] Chen Y, Li J, Xiao P, Zhu W and Mo Z 2016 *Iran. J. Fish. Sci.* 15 701–14
[12] Abraham T, Babu C and Banerjee T 2007 *Bangladesh J. Fish. Res.* 11 65–74
[13] Kavitha M, Raja M and Perumal P 2018 *Aquac. Reports* 11 59–69
[14] Ray A K, Ghosh K and Ringo E 2012 *Aquac. Nutr.* 18 465–92
[15] Ayoola S O, Ajani E K and Fasah O F 2013 *World J. Fish Mar. Sci.* 1 1–8
[16] Spanggaard B, Huber I, Nielsen J, Sick E B, Pipper C B, Martinussen T, Slierendrecht W J and Gram L 2001 *Environ. Microbiol.* 3 755–65
[17] Sarkar A and Mandal S 2016 *Microbiol. Res.* 192 159–71
[18] Smith P and Davey S 1993 *J. Fish Dis.* 16 521–4
[19] Abd EL-Rhman A M, Khattab Y A E and Shalaby A M E 2009 *Fish Shellfish Immunol.* 27 175–80
[20] Hai N Van, Bulter N and Fotedar R 2009 *Aquac. Res.* 40 590–602
[21] Ouwehand A C, Isolauri E, Kirjavainen P V, To’ Ikko’ S and Salminen S J 2000 *Lett. Appl.*
6. **Acknowledgement**

The authors would like to thank Balai Benih Kabat, Banyuwangi for supporting facilities in this study.