EFFECTIVENESS OF BACILLUS SPP. PRESS ON BLUE GREEN ALGAE (BGA) CAUSING OFF-FLAVOR IN CATFISH CULTIVATION

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Abstrak

Permasalahan yang selalu menjadi keresahan para pelaku usaha budidaya ikan patin yaitu adanya indikasi bau lumpur/tanah/muddy taste atau yang lebih dikenal dengan istilah off-flavours. Off-flavours tersebut diidentifikasi berasal darisenyawa kimia geosmin (GSM) dan 2-Methyl Iso-borneol (MIB) yang diproduksi oleh Cyanophyceae (Blue Green Algae atau BGA) dan Actinomycetes. Senyawa bau tanah ini larut dalam air, masuk kedalam tubuh ikan melalui insang dan diteruskan pada jaringan lemak ikan sehingga lama kelamaan daging ikan terasa bau lumpur/tanah/apak. Probiotik merupakan salah satu upaya untuk menurunkan senyawa penyebab off-flavours. Penggunaan probiotik yang mengandung Bacillus spp. pada pakan dapat mendegradasi bahan organik melalui bioremediasi organ pencernaan ikan dan lingkungan budidaya untuk meminimalisir akumulasi bahan organik. Berkurangnya bahan organik diperairan dalam bentuk total organik berdampak pada persentase BGA penyebab bau tanah didalam perairan terutama untuk jenis Oscillatoria Sp. Dalam penelitian ini kami menggunakan Bacillus spp. dengan perlakukan 10 ml/Kg dan 20 ml/Kg pakan secara tidak langsung dapat menurunkan persentase BGA, populasi Oscillatoria Sp. dan off-flavours dalam daging ikan patin dibandingkan kontrol.

Keywords: akuakultur, bacillus spp, blue green algae, off-flavors, plankton.

Abstract

A problem that often occurred for the actor of catfish cultivation was an indication of smelly mud/dirt/muddy taste (off-flavors). Off-flavors were identified from chemical geosmin compound (GSM) and 2-Methyl Iso-borneol (MIB) produced by Cyanophyceae (Blue Green Algae or BGA) and actinomycetes. This soil odor compounds were soluble in water, into the body of fish through the gill and forwarded to the fatty tissue of fish, so that the fish meat was finally mud-smelly/dirt/musty. Probiotics was an attempt to reduce the compounds that caused off-flavors. The use of probiotics containing Bacillus spp. on feed could degrade organic matter through bioremediation digestive organs and environmental fish farming to minimize the accumulation of organic matter. The reduced of organic matter in waters on total organic odor, has impacted on the percentage of BGA as cause of mud-smelly in waters, especially for species Oscillatoria Sp. In this study, the researchers used Bacillus spp. to treat 10 ml/kg and 20 ml/kg of feed that can indirectly lower the percentage of BGA, Oscillatoria population Sp. and off-flavor in catfish meat as compared to controls.

Keywords: aquaculture, bacillus spp, blue green algae, off-flavors, plankton.

INTRODUCTION

The smell of mud/dirt/muddy taste that is known as *off-flavor* in the catfish meat is associated with the presence of *geosmin* (GSM) and *2-MIB* that are influenced by the plentiful nutrients and waste organic materials in the water that can cause the bloom of plankton because of poor management of water quality [1]. *GSM* and *2-MIB* are a set of tertiary alcohol resulted as enantiomers (+) and (-). *GSM* compounds and *2-MIB* are produced as secondary metabolites of various types of bacteria, such as actinomycetes, Cyanobacteria, Proteobacteria and Fungi [2].

Cyanobacteria or Cyanophyceae can produce odor compounds mud/dirt (*GSM* and *2-MIB*) of the groups/types of plankton such as Oscillatoria, Lyngbya, and Schizothrix [3]. Odor compounds are released when plankton is experiencing exponential growth phase and after death [4].

Some efforts that are carried out to reduce *off-flavor* are difficult, because the

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causes are variety. The substitution of water or using water recirculation (RAS) is often used to reduce the *GSM* and *2-MIB* in catfish cultivation [5], for 10-15 hours with clean water and recirculation of the salmon, but it can reduce the body weight of fish. The diet feed is still used, but it can cause fish stress and the weight of fish declines [6]. Several attempts by the use of probiotics either through feed and water aquaculture, such as the use of Bacillus spp. of some Bacillus species such as B. subtilis, B. polymyxa, B. megaterium and B. laterosporus which can inhibit the growth of harmful bacteria, remodel organic matter into inorganic [7].

Several types of Bacillus spp. also can produce enzyme, making digestibility of feed nutrients better, improving the health of the digestive tract and producing fish immunostimulant [8]. It is expected that with a good nutrient digestibility, it will be able to reduce the impact of increased organic matter in aquatic cultivation that stimulates abundant growth of BGA. However, the use of probiotics on the prevention or reduction of *off-flavors* is not yet performed, so the smell indication of mud/dirt on catfish become a problem among catfish farmers that urgently needs to explore further.

Several types of Bacillus spp. also can produce enzyme, making digestibility of feed nutrients better, improving the health of the digestive tract and producing fish immunostimulant [8]. It is expected that with a good nutrient digestibility will be able to reduce the impact of increased organic matter in aquatic cultivation that stimulates abundant growth of BGA. However, the use of probiotics on the prevention or reduction of off-flavors is not yet performed, so the smell indication of mud/dirt on catfish become a problem among catfish farmers that urgently needs to explore further.

In this study, we use and test probiotics Mina Pro dominated by B. *licheniformis* and B. *substilis* that play a role in degrading organic compound feed (protein, starch, cellulose, hydrocarbons and order) so as to produce antibiotics, playing a role in nitrification and Denitrification as well as binding nitrogen [9]. These bacteria also serves as heterotrophic and saprophytic bacteria that is *Protista* that behaves unicellular and includes in the microorganisms decomposers, so that it is able to seek the role of Bacillus spp. through feed to reduce off-flavors in catfish culture by emphasizing several water parameters such as the composition of BGA as a producer of GSM and 2-MIB in water. In addition, Bacillus spp. is also expected to be able to stabilize water chemistry as a result of the accumulation of organic matter that spreads pollution.

MATERIAL AND METHOD

This study used a completely randomized design (CRD) as method that involved 2 treatments (probiotic dose of 10 ml/kg of fish feed, 20 ml/kg of fish feed, control) and each comprising three replications. There were 9 pools involved in this study with widely of 400 m2, ± 2 meters water depth, stocking density 20 tails/m2 and size of fish 400g/tail. The probiotics were applied 2 times/week. These were firstly diluted with 200 ml of fresh water per kg of feed as well as to mix it with probiotics spraying evenly on the feed. Furthermore, it needed to stir the feed to the probiotic component that was absorbed into the pores of feed and allowed to stand for 60 minutes in the shade in order that the feed was fermented and finally carried out a feeding.

This study was conducted over 60 days in the CV. Tirta Bumi Agung (TBA), Karangdagangan village District Bandarkedung Mulyo, Jombang, East Java province. This company was led by Ilham Subekti in cooperation with PT. Central Proteinaprima. The cultivation land consisted of ± 4 hectares of 197 plots/fish pond and devided into walking catfish (Greek language: *Clarias*) and catfish (Greek language: *Pengasius*) pond. The test for of fish samples was conducted at the Laboratory of Animal Health Services PT. Central Proteinaprima (CP. Prima) Sidoarjo, East Java.

The tools of this study included pH meter, DO meter, microscopes, Haemositometer, spectrophotometers and TOM testing by titration. The sample test was carried out at the Laboratory of Animal Healt Services PT. Central Proteinaprima. The research material included catfish from the pond CV. TBA, fish feed from PT. Central

Proteinaprima and Mina Pro probiotic species Bacillus spp. from PT. Marindolab Primary.

The checking of the daily water quality involved physical parameters (pH and DO), chemical mingguanparameter (Nitrite and Total Ammonia Nitrogen or TAN, Total Organic Matter or TOM), and biological parameters (plankton and bacteria water). The collection of catfish samples was carried out through random sampling with an interval time (21 days) for organoleptic testing.

The test results of each parameter was then performed through statistical tests (data normality and homogeneity) as a condition for performing Anova. If the result obtained significant, it was then forwarded to further test (Duncan test) to know the differences of each probiotics' treatment applied to decrease the smell of mud/land/muddy taste/*off-flavors* in catfish cultivation.

RESULTS

Water Quality

pH and Dissolved Oxygen (DO)

The average result of water quality measurement (pH and Dissolved Oxygen) during this study is shown in Table 1 below.

Table 1. The Average Result of the Measurement of pH and DO (mg/L) During the Study

		The Average Result before Treatment			
		pH		DO (mg/L)	
		Morning	Afternoon	Morning	Afternoon
		6.9	7.0	0.20	0.88
Treatment	Week	The Average Result after Treatment			
Control	1	7.0	7.1	0.23	2.77
	2	7.0	7.1	0.22	2.04
	3	7.0	7.1	0.17	2.83
	4	7.1	7.3	0.34	2.65
	5	7.1	7.2	0.24	3.62
	6	7.1	7.3	0.24	2.95
	7	7.1	7.3	0.26	2.67
	Average	7.0±0.05[a]	7.2±0.06[a]	0.24±0.03[a]	2.79±0.45[a]
10 ml/kg of feed	1	7.0	7.0	0.27	2.13
	2	7.0	7.2	0.18	1.65
	3	7.1	7.2	0.15	2.58
	4	7.1	7.3	0.36	2.75
	5	7.0	7.1	0.65	3.76
	6	7.0	7.3	0.29	3.08
	7	7.1	7.2	0.24	1.56
	Average	7.0±0.03[a]	7.2±0.05[a]	0.30±0.05[a]	2.50±0.30[a]
20 ml/kg of feed	1	7.0	7.0	0.26	2.44
	2	6.9	7.1	0.22	2.18
	3	7.0	7.1	0.22	2.41
	4	7.0	7.2	0.24	2.38
	5	7.0	7.2	0.38	3.33
	6	7.1	7.3	0.46	3.08
	7	7.1	7.3	0.30	1.73
	Average	7.0±0.05[a]	7.2±0.08[a]	0.30±0.05[a]	2.51±0.30[a]

The standard of pH for growth of catfish is ranging from 6 to 8.5 [10]. Meanwhile, based on the standard of water quality in Governmental Regulation No. 82 year 2001 (Class II), that the pH is good for freshwater fish farming activities that is ranging from 6-9. In this study, the pH is still at a natural boundary and is feasible for catfish farming activities. The fluctuation value of pH can be affected by a decline in water conditions that have an impact on the occurrence of disease. On the condition that pH is humid, bacteria will grow fast while the existence of fungus will increase in acidic pH [11].

The standard of *Dissolved Oxygen* (DO) for catfish is 3-7 mg/l [10]. The parameters of *Dissolved Oxygen* can be used as an indicator of water freshness [12]. Oxygen plays an

important role in the process of oxidation and reduction of organic and inorganic materials. These oxidation and reduction processes helps to reduce pollution loads in waters naturally [13]. In this study, the checking point of *Dissolved Oxygen* (DO) was carried out at the outlet (bottom of the pond catfish cultivation). *Dissolved Oxygen* (DO) is allegedly generated and tends to be lower (0:17 mg/L). The difference in the middle and the surface of aquaculture ponds were relatively higher (3:50 mg/L).

The value of *Dissolved Oxygen* (DO) when the nitrification process goes well is> 2 mg/L (minimum) and the growth is> 4 mg/L (medium).

Nitrite and Total Ammonia Nitrogen (TAN)

The average result of water quality measurement (Nitrite and Total Ammonia Nitrogen or TAN) during this study is shown in Table 2.

Table 2. The Average Measurement Results of Nitrite and TAN During This Study

The Average Result before Treatment		The Average Result after Treatment					
		Treatment					
		Control		10 ml/kg of feed		20 ml/kg of feed	
Nitrite (mg/L)	TAN (mg/L)	Nitrite (mg/L)	TAN (mg/L)	Nitrite (mg/L)	TAN (mg/L)	Nitrite (mg/L)	TAN (mg/L)
0.31	4.12	0.45±0.20[a]	4.67±1.04[a]	0.15±0.06[a]	3.76±0.83[a]	0.31±0.11[a]	3.91±0.97[a]
Fluctuation or Comparison of Treatment and Control				0.30	0.91	0.14	0.76

The results showed that the average value of Nitrite and TAN has decreased after the application of probiotics that contains Bacillus spp. compared to controls. However, the statistical test indicates that it is not significantly different between the treatment of 10 ml/kg of feed and 20 ml/kg of feed and control.

The inorganic nitrogen consists of ammonia (NH3-), ammonium (NH4 +), nitrite (NO 2), and nitrogen (N2). Biologically, the process of the metabolism result of cultivation biota can be converted into Total Ammonia Nitrogen (TAN), nitrite, and nitrate (NO3). The increase in the rate of organic material degradation can be achieved if the sediment is under the aerobic conditions. In this study, the oxygen in the water is still relatively high for the process of nitrogen reform, so that the TAN and Nitrite are quite low. Bacillus spp. in the feed can degrade organic matter through the digestive organs of bioremediation and environmental fish farming to minimize the accumulation of organic matter in the water.

Plankton

The average percentage of plankton during this study is shown in Table 3 below.

Table 3. The average result of the amount of BGA plankton during this study

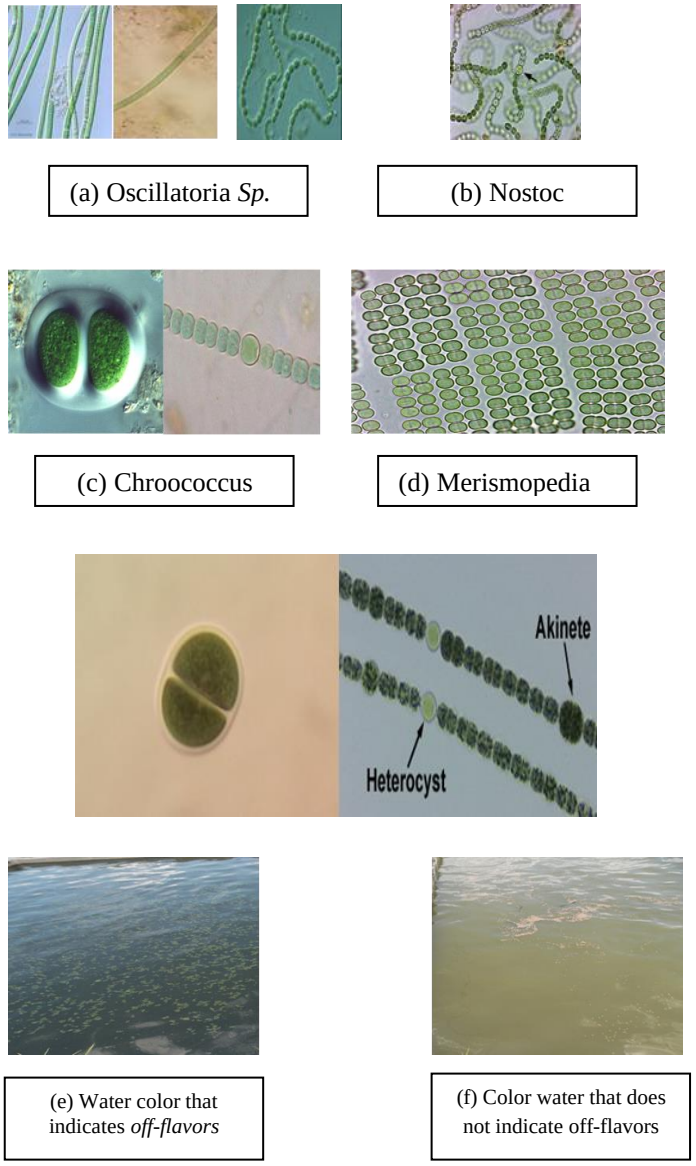
Blue Green Algae	The Average Result before Treatment (x 10^4 cells/ml)	The Average Result after Treatment (x 10^4 cells/ml)		
		Control	10 ml/kg of feed	20 ml/kg of feed
Anabaena	3	8±1.9[a]	8±1.7[a]	6±1.3[a]
Chroococcus	5	18±3.0[b]	9±2.0[a]	9±1.7[a]
Merismopedia	73	85±9.0[b]	50±12.0[a]	98±9.7[b]
Oscillatoria	169	161±24.3[a]	137±23.0[a]	135±13.9[a]

Based on the results of the percentage of total plankton in Figure 3, it shows that the percentage of Green Algae (GA) has increased in the application of 10 ml/kg probiotics of feed and 20 ml/kg of feed than the control. Meanwhile, the BGA percentage has decreased as compared with the control. The decrease of BGA plankton average percentage is at 10 ml/kg (52%) and 20 ml/kg of feed (36%). The amount of BGA plankton is empirically lower when compared with control (56%) and prior to treatment (71%). Thus, the use of probiotic Bacillus spp. through the feed with the application (2 times/week for 60 days) can affect the composition of the plankton, especially the number of BGA plankton producer of GSM and 2-MIB in aquaculture water.

Plankton, from the group of Cyanophyceae (Blue Green Algae) is main cause of *off-flavors* of fish meat producers *GSM* and *2-MIB* [14]. In observation of the BGA, the type of Oscillatoria sp. in this study experiences a decrease in treatment when compared to control. Number of drops 10 ml/kg of feed ($137x10^4$ cells/ml), treatment of 20 ml/kg of feed ($135x10^4$ cells/ml) is lower than the control after treatment ($161x10^4$ cells/ml), and control group before treatment ($169x10^4$ cells/ml).

Some types of plankton from Cyanophyceae or BGA has been identified positive (+) as a major producer that produces *GSM* and *2-MIB* dominated by species Oscillatoria Sp. and Lyngbya Sp [3]. Some researchers add Schizothrix species, Anabaena, and Chroococcus as well as other types of BGA. The BGA type that has been found during the investigation is allegedly as a producer of *GSM* and *2-MIB*. In addition to the dominance Plankton Sp Oscillatoria for research, aquaculture pond water colors also indicate *off-flavors* and not *off-flavors* in Figure 1 BGA type found during the investigation allegedly as a producer of GSM and 2-MIB. In addition to the dominance of Plankton Sp.

Oscillatoria in this study, the water color of aquaculture pond also indicates *off-flavors* and not *off-flavors* in Figure 1.



(a) Oscillatoria *Sp.* (b) Nostoc (c) Chroococcus (d) Merismopedia (e) Water color that indicates *off-flavors* (f) Color water that does not indicate off-flavors

Figure 1 (a); (b); (c); (d); (e); (f). The type of BGA that is found during the investigation allegedly as a producer of *GSM* and *2-MIB* as well as the color of aquaculture pond indicates muddy and not muddy.

Based on the results of statistical tests (ANOVA), the significance of the values organoleptic testing is >0.05 (0.866). It means that the statistical tests (ANOVA) is not significantly different in odor reduction of off-flavors land. However, from the results obtained during the study, there showed a downward trend. The observation on catfish meat (back, tail and stomach) is allegedly often found muddy smell/taste as shown in Figure 2.

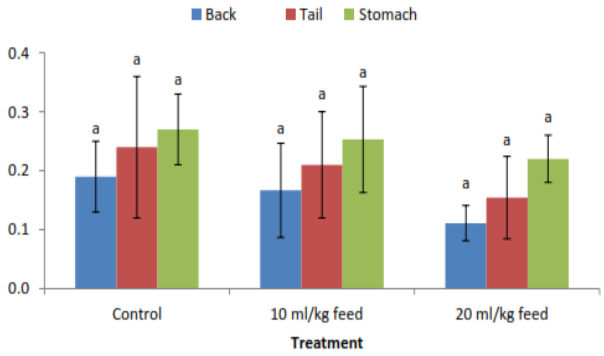


Figure 2. The average result of the catfish meat that often indicates muddy taste

DISCUSSION

Based on the average result of muddy taste test on catfish meat that indicates off-flavors, it appears that the application of probiotics through the feed indicated in the treatment of 10 ml/kg of feed and 20 ml/kg of feed has decreased in part (bac, tail and stomach) is lower when compared to control. Indirectly, probiotic Bacillus spp. is able to act as decomposers bacteria through bioremediation of waters environment, so that it can compete with the microorganism *GSM* and *2-MIB*.

Organic Material of *GSM* and *2-MIB* are semi-volatile compounds/volatile terpenoids (secondary metabolites) in the water that can be absorbed by fish and accumulate in the fatty tissue [15]. *GSM* and *2-MIB* are chemical compounds shaped as ion/gas that are rapidly absorbed by the gills of fish. The absorption through the skin is the minor route and mostly stored in the fatty tissue [16]. In addition to the catfish, off-flavors also occurs in other fish, such as Tilapia fish (Greek language: *Oreochromis Niloticus*) and salmon [17].

Off-flavors has become the main reason for the problems in fish products [18]. These compounds are not toxic when concentrated in the water, but it can provide a taste and odor through waters and fish [19]. These compounds can be felt at a very low concentrations by human senses such as for *GSM* 0.9 mg/kg and 0.7 mg/kg for *2-MIB* [20]. The standard of Geosmin compound concentration for freshwater fish is 6-10 mg/kg. The speculation on the origin of off-flavors in catfish meat by Leger (in 1910) is caused by Cyanobacteria. In 1960, Gerber (in 1969) and Gerber and Lechevalier (in 1965) emphasizes that the cause of off-flavors mud odor is *GSM* compound and *2-MIB* that are isolated from cyanobacteria and actinomycetes by using chromatography gas detection.

This research was conducted as a part of efforts to improve the quality and improve the nutrition of catfish meat to meet the supply of catfish both local and non-local area. Some of the complaints by catfish farmers on the problems of smell mud smell/dirt/muddy (off-flavors) that often occur during harvest. Up to now, this case has not found any solution, although some efforts have been carried out, like using water recirculation (RAS), reducing the amount/dose of fish feed or moving the pool to another pool. However, these efforts greatly affect the growth and stress level that can cause fish mortality.

This study conducts an attempt on the application of probiotic Bacillus spp. through feed to lower off-flavors in catfish meat through suppression of BGA composition as a producer of *GSM* compound and *2-MIB* in water and to refine chemical water (nitrite, TAN and TOM). The following is the illustration process of probiotic Bacillus spp. on feed to improve water quality (Nitrite, TAN, TOM) and to press BGA composition in waters.

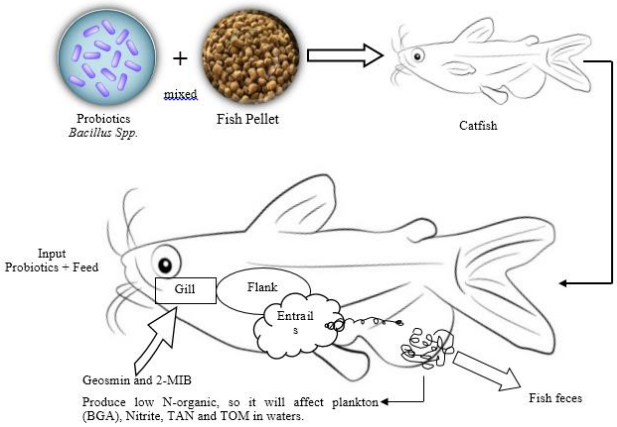


Figure 3. Illustration Probiotics work roses in the feed to improve water quality and reduce the growth of BGA

CONCLUSION

This study has found that the use of probiotic Bacillus spp. in fish feed can lower water chemistry parameters that becomes a pollution indicator, that is nitrite value in treatment with a decrease (0.15 to 0.31 mg/L) as compared to control (0.45 mg/L), TAN (3.76 to 3, 91 mg/L) as compared to control (4.67 mg/L), and TOM (377-471 mg/L) as compared to control (482 mg/L).

In addition, the use of probiotic Bacillus spp. in fish feed can lower the percentage of Blue Green Algae in treatment amounted to 52-36% when compared to control 56%. The population of Oscillatoria Sp. as a producer of *GSM* and *2-MIB* has decreased the number of treatment equally to (137-135x10^4 cells/ml) or ± 15% when compared to controls (161x10^4 cells/ml). Both treatments (10 ml/kg of feed and 20 ml/kg of feed) show a decrease in the BGA typed-Oscillatoria Sp., and the treatment of 20 ml/kg of feed gives an average result lower (better).

Finally, the use of probiotic Bacillus spp. through feed can also provide water condition with lower organic level. In addition, the population of plankton as cause of off-flavors is lower. So, the result of fish organoleptic test has with the treatment of 10 ml/kg of feed and 20 ml/kg of feed gives lower value than the control.

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