Effects of Probiotic Dosage on Water Quality, Total Count of *Aeromonas* spp. and *Pseudomonas* spp. in Eel *Anguilla bicolor* Cultivation

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Abstract. This study was carried out to evaluate the effects of probiotic dosage on water quality, total number of *Aeromonas* spp. and *Pseudomonas* spp., in eel (*Anguilla* sp.) cultivation. Experiment was conducted by using fiber glass tanks (1x1 m²) with 800 L water volume. Eel fingerling with 50 g average body weight were stocked at a density of 20 eel/tank in triplicates. Observation was conducted on total number of bacteria and water quality. The results indicated that the total bacterial count increased gradually by the time of cultivation with highest density of 1.49x10⁷ cfu/mL. Probiotics at a dose of 40 mL/tank weekly increased the total count of bacterial cells in water. Probiotic application was able to decrease the total number of both *Aeromonas* spp. from 1.95 x 10⁵ to 5.44 x 10⁴ cfu/mL, and *Pseudomonas* spp. from 3.57 x 10⁴ to 8.1 x 10³ cfu/mL. Water quality during this study was not significantly affected by the application of probiotic. This result suggested that the application of proper dose probiotics suppresses *Aeromonas* spp. and *Pseudomonas* spp. cells density in water, and might decrease the bacterial infections in eel culture.

1 Introduction

Eel (*Anguilla* sp.) has economic value, high prices and very popular in Japan, Europe, America, Korea and Taiwan. In Indonesia, eel also is a popular food that the aquaculture still depends on the availability of eel seed (elver/glass eel) from capture [1]. Elver are available along the coast or estuary of the southern coast of Java Island, including Yogyakarta Special Region. Eel cultivation can be carried out starting from the supply of glass eel; larvae nursery with up to 2 g; grow out with up to 120-200 g for market size. The optimal water qualities of eel aquaculture in accordance with temperatures 28-33 °C [2]; dissolved oxygen (DO) 1-10 mg/L [3]; pH 6.5-8.0 [4]; and free CO₂ < 25 mg/L are crucial in the eel culture.

Petrogrow is one of probiotic products is considered as a supplement contains with microorganisms which are given through various ways to balance the digestive microflora, so that it is beneficial for eel. *Petrogrow* also useful to support the environment by improving water quality and inhibits the growth of pathogens in water. *Petrogrow* is a liquid that contains beneficial microorganisms including *Bacillus subtilis*, *Lactobacillus* sp., *Pantoea*

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sp., and *Bacillus* sp. The purpose of this research was to study the application of *Petrogrow* probiotics on water quality and the number of total bacteria of *Aeromonas* spp. and *Pseudomonas* spp.

2 Research Methods

The fiberglass tanks of 1x1 m² with water depth of 80 cm were used to culture eel with an average weight of 50 g. Each tank was equipped with continuous aeration. Eel fingerlings were stocked at a stocking density of 20 eel/tank, and cultured for 5 months. The experiment was designed in a completely random design in triplicates. The probiotic (*PetroGrow*, *Petrokimia Kayaku*) was applied at dosages of 0, 10, 50, 100 ml/m³ water. The eel was fed with feed paste (39.8 % protein) at feeding rate of 3 % total body weight. Water change was carried out weekly by 35 % of total water volume. The total count of bacteria, *Aeromonas* spp. and *Pseudomonas* spp. were observed monthly by total plate count method on Tryptic Soy Agar (TSA) medium for total count of bacteria, and on GSP agar medium for total count of *Aeromonas* spp. and *Pseudomonas* spp. The water quality parameters observed including temperature, turbidity, pH, and dissolved O₂ were measured with Water Quality Checker, while organic matter and free carbon dioxide (CO₂⁼) were measured by titration method.

3 Result and Discussion

Total cells counts of *Aeromonas* spp. and *Pseudomonas* spp. subsequently are presented in Tables 1, 2, and 3. The total number of bacterial cells showed all bacteria including beneficial and pathogenic bacteria especially *Aeromonas* spp. and *Pseudomonas* spp.

Table 1. Total number of bacterial cells during cultivation with different probiotics dosage

probiotics dosage (mL/800 L water/ week)	Total number of bacterial cells (x 10 ⁵ cfu/mL) in the observation month					
	0	1	2	3	4	Average
0	28.0	29.2	5.8	52.8	20.5	27.3
8	361.2	225.3	25.5	35.9	59.9	141.6
40	367.3	231.8	21.5	61.3	61.9	148.8
80	99.2	156.8	23.2	30.0	32.3	68.3

Remarks: Stock density of eel 20 seeds/container; Feeding dose was only 3%/day

Total bacterial cell densities in water added with doses of probiotic at 0, 8, 40, 80 mL probiotics/800 L every week varied depending on the dose of probiotics, but after 3 months the densities were relatively stable under 62 x 10⁵ cfu / mL. The average number of total bacteria cells at doses of 0, 8, 40, 80 ml probiotic/800 L were 27.3; 141.6; 148.8; 68.3 (10⁵ cfu/mL), respectively. Probiotics at higher doses up to 40 mL of probiotics/800 L increased the total number of bacterial cells in water.

Total *Aeromonas* spp. cell density in water added with doses of probiotic at 0, 8, 40, 80 mL probiotics/800 L every week were 194.7; 128.2; 69.2; 54.4 x 10³ cfu/ml, respectively, while *Pseudomonas* spp. cells densities were 20.0; 8.1; 13.1 x 10³ cfu/ml, respectively.. Probiotics at higher doses suppressed the growth of *Aeromonas* spp. and *Pseudomonas* spp. Water quality during experiment is presented in Table 4. The water quality is still favourable for eel culture.

Table 2. *Aeromonas* spp. cells densities in water during the cultivation of eel at different probiotics dosages

probiotics dosage (mL/800 L water/ week)	Total number of <i>Aeromonas</i> sp. cells (x 10 ³ cfu/mL) in the observation month					
	0	1	2	3	4	Average
0	229.5	229.5	37.7	225.8	251.2	194.7
8	56.9	56.9	85.1	197.2	244.7	128.2
40	71.3	10.5	44.1	108.8	111.3	69.2
80	40.2	16.1	30.6	94	91	54.4

Remarks: Stock density of eel 20 seeds/container; Feeding dose was only 3%/day

Table 3. *Pseudomonas* spp. cells densities in water during the cultivation of eel at various probiotics dosages

Probiotics dosage (mL/800 L water/ week)	Total number of <i>Pseudomonas</i> sp. cells (x 10 ³ cfu/mL) in the observation month					
	0	1	2	3	4	Average
0	44.8	41.5	2.4	7.3	82.5	35.7
8	32.1	21.9	17.0	12.8	16.0	20.0
40	9.7	10.5	2.2	3.1	15.1	8.1
80	16.9	16.1	6.7	2.0	23.6	13.1

Remarks: Stock density of eel 20 seeds/container; Feeding dose was only 3%/day

Table 4. The water quality during the cultivation of eel at various probiotics dosages

Parameter	Unit	Average/ Range	Dosage of Probiotics (mL/800 L water/week)				Standar d optimal
			0	8	40	80	
Temperature	°C	Average	26.6	26.7	26.7	26.7	28-33 ²⁾
		Range	25.5-28.0	25.7-28.0	25.7-28.0	25.7-28.0	
Turbidity	mg/L	Average	12.6	16.1	25.5	12.7	-
		Range	3.0-25.3	3.0-40.0	3.0-56.0	2.0-21.0	
Organic matter	mg/L	Average	19.5	14.1	8.6	8.6	-
		Range	14.5-24.0	12.6-15.2	7.0-10.1	6.3-10.1	
pH	unit	Average	7.2	7.2	7.2	7.3	6.5-8 ¹⁾
		Range	6.9-7.6	7.0-7.4	7.0-7.5	7.0-7.5	
Dissolved O ₂	mg/L	Average	3.4	3.6	3.6	3.7	1-10 ³⁾
		Range	2.4-4.9	2.9-4.9	3.0-4.9	2.8-4.8	
Free CO ₂	mg/L	Average	5.5	5.2	5.1	5.4	< 25 ⁴⁾
		Range	2.6-8.6	2.5-6.5	2.9-6.8	4.3-6.8	

Source: ¹⁾ = Affandi dan Suhendra, 2003; ²⁾ = Lou *et al.*, 2013; ³⁾ = Usui,1974; ⁴⁾ = Saifurridjal dan Sinung Rahardjo, 2012.

4 Conclusions

The results of this study showed that: 1). Probiotics administration at doses up to 40 ml increases the total cell count of bacteria, specifically beneficial bacteria; 2) Addition of probiotics in water at higher doses suppresses the growth of *Aeromonas* spp. and

Pseudomonas spp. 3). Addition of probiotics improve the water quality by reducing the organic matter and increasing DO.

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