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Full Length Research Paper

Population of bacteria in tilapia (*Oreochromis niloticus*) culture pond deposit in Vietnam

Kulapat R. Dhanasevi

Department of Aquaculture and Aquatic Resources Management, Chulalongkorn University, Bangkok, Thailand.
E-mail: Kulapat2010@chula.ac.th

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Three intensive tilapia ponds were studied to determine the bacterial population during the culture period in Hai Duong province, Vietnam. Bacteria were identified to genus or species level. Total bacteria count in sediment ranged from 6.49 × 10^5 to 1.29 × 10^6 cfu g^{-1}. In total, 11 bacterial genera and 13 species were identified from pond sediments, in which both gram-negative and gram-positive rod-shaped bacteria dominated the population accounting for 52 and 48%, respectively. Both beneficial and harmful bacteria were found in sediments, in which the harmful bacterial species dominated (62.5%) and appeared more at the beginning of culture. The harmful bacteria were *Pseudomonas fluorescens, Pseudomonas* sp., *Vibrio pectenicida, Enterococcus*, sp.HD31, *Enterococcus* sp.HD1, *Vibrio damsella, Aeromonas* sp.HD5, *Vibrio* sp. HD7, *Vibrio* sp.HD331, *Aeromonas* sp.HD361, *Enterococcus* sp.HD371, *Vibrio* sp.V31, *Aeromonas hydrophyla* and *Pseudomonas putida*. In contrast, beneficial bacteria accounted for 20.8% only of total isolates. The beneficial bacteria were *Carnobacterium inhibens, Lactobacillus plantarum, Bacillus cereus, Lactobacillus delbrueckii* and *Bacillus pumilus*; they appeared more in the later stage of culture period.

Key words: Bacteria, *Oreochromis niloticus*, intensive culture pond, sediment, beneficial bacteria, harmful bacteria.

INTRODUCTION

Tilapia is an important cultured species in most of tropical, subtropical and temperate regions and currently second to carps in total production; production of tilapia is 2,505,465 tonnes in 2007 (FAO, 2009). However, the success of intensive culture of this species depends on water quality in pond which is influenced by native microflora. Intensive production usually leads to an increase in disease due to poor water quality and high

stocking densities of species. Tilapia is susceptible to many infectious agents such as bacteria and parasites (Shoemaker et al., 2006). The important pathogen of tilapia is *Streptococcus iniae*, a gram-positive bacteria responsible for significant losses in intensive culture (Perera et al., 1994; Shoemaker et al., 2006).

The organic matter content in pond is normally due to the feed input and fertilizer (Boyd, 1990). The uneaten feed will be broken down into ammonia, nitrite, and nitrate through the processes of mineralization, nitrification and denitrification by different microorganisms.

The activities of algae and microorganism govern the dissolved oxygen (DO) in water. Photosysnthesis of algae produces DO using sunlight, while bacteria consume oxygen for respiration. The microbial processes including aerobic and anaerobic, also effect on the ammonia content and water quality factor such as pH, DO, nitrite (Moriarty, 1997).

In intensive tilapia pond, the bacterial community may contribute to food web. They may be eaten directly by tilapia. Moreover, the nitrogen and phosphorus may be recycled to stimulate primary production through the activity of decomposed microorganism (Moriarty, 1997).

Recently, there are some studies of Hien et al. (2005) about bacterial composition in shrimp pond in Thanh Hoa province, Vietnam. These studies applied the useful native bacteria to produce probiotic to improve water quality in shrimp pond. Bacterial population in intensive aquaculture pond has an important role in improving water quality because of decomposing organic matter and reducing harmful bacteria in sediments.

Studies on bacterial population in aquaculture are important in predicting the possibility of disease outbreaks and providing information for developing an optimal strategy for effective management to improve production and environmental condition. However, no study has been reported on bacterial variation in freshwater tilapia pond sediments, particularly in the north of Vietnam. Recent studies have only focused on the variation of bacteria in tilapia brackish water pond in Saudi Arabia (Al-Harbi and Uddin, 2003, 2005). This study determined the variation of bacterial population in intensive freshwater tilapia pond during the culture period. Research findings can be useful as basis to implement optimal management strategies to improve production.

MATERIALS AND METHODS

Pond information

Three intensive tilapia ponds were selected for this study in Hai Duong province, Vietnam. The area of ponds ranges from 3000 to 5000 m^2 with an average depth of 2.0 m. Ponds were constructed 7 years ago. All ponds are used for intensive tilapia culture using commercial feed. Tilapia stocking density is 6 fish per m^2. Pond water is exchanged once or twice per month. The culture period is 6 months. Probiotic was applied only once a month in Pond 1.

Sample collection

Water and sediment samples were collected from the three intensive tilapia ponds at different sites such as near inlet, outlet, center of each pond and every month starting from the third month of culture. Water samples were collected in sterile glass bottles (250 ml) sub-surface and sediment samples (20 to 25 g) were collected from the top 10 cm of pond bottom using grab and kept in polyethylene bags during transport to the laboratory. The samples were stored with ice and transferred to the laboratory for analysis at the Institute of Biotechnology (Hanoi, Vietnam).

Analysis of samples

Viable count for enumeration of cell

Sediment samples in each pond were pooled, weighed and pulverized with a mortar and pestle.

Dilution method: 1 g sediment sample mixed with 9 ml of sterile distilled water and shaken well. It was assumed that the bacteria were evenly distributed between solid and liquid. Use the pipette to remove 1 ml and delivered into the next dilution tube. Discarded the pipette, and continued for the required number of dilutions (10-, 100-, 1000-fold dilution). Pipetted 0.1 ml of each dilution of sediment samples into center of a plate agar. A separate plate for each dilution was used. Spread each inoculum using sterile bent glass rod over the plate and incubated for 24 to 48 h and then observed the growth. The number of colonies on a plate were counted and calculated.

Cultivation and isolation of bacteria

Sediment samples from three ponds were analyzed to isolate the bacterial strains. The bacteria were divided into different types based on the morphological characteristics such as colony form, size and color. After recording the above characteristics, 3 to 5 representatives of each colony type were streaked on MPA plates to purify the isolates.

Identification of bacteria

All the purified isolates were observed for cell shape, motility, spores, and encapsulation through gram staining and microscopy after incubating at 28 to 30°C for 24 to 48 h. Simultaneously, the commercial API 50CHB and API 50CHL system (Biomerieux, France) method were used to classify the gram-positive bacteria; API 20NE was used for identifying gram-negative bacteria.

Identification of beneficial and harmful bacteria in sediment

Bacteria were classified into beneficial and harmful bacteria based on the following papers:

1) Role and functions of beneficial microorganisms in sustainable aquaculture (Zhou et al., 2009).
2) Bacteria from fish and other aquatic animals (Buller, 2004).

Physiochemical environmental factors

Water temperature and DO were measured by thermometer and DO test kits *in situ* during water sampling.

Table 1. Mean ± standard error of physicochemical parameters of tilapia pond water at different culture month.

Culture month	Temperature (°C)	pH	DO (mg/L)
3rd	25.5 ± 0.29[a]	6.5 ± 0.15[a]	4.9 ± 0.35[a]
4th	22.5 ± 0.28[b]	6.63 ± 0.14[a]	5.43 ± 0.29[a]
5th	18.8 ± 0.43[c]	7.06 ± 0.17[a]	4.96 ± 0.08[a]
6th	14.7 ± 0.44[d]	7.1 ± 0.2[a]	4.46 ± 0.14[a]

Table 2. Bacterial quantity, mean ± standard error (cfu/g) in tilapia pond sediment.

Pond (cfu/g)	Quantity of bacteria
Pond 1	$1.29 \pm 0.68 \times 10^{6a}$
Pond 2	$6.49 \pm 1.33 \times 10^{5a}$
Pond 3	$1.18 \pm 0.39 \times 10^{6a}$

Data analysis

Statistical analysis was performed using Excel and SPSS 13.0. Data are presented as the mean ± S.E. The bacterial population in sediments at different culture months was tested for significant differences ($p < 0.05$) using one-way and two-way analysis of variance (ANOVA), followed by multiple comparisons of means using SPSS version 13.0.

RESULTS

Physicochemical parameters

Mean values for physicochemical parameters of water for all the three ponds at different culture months are shown in Table 1. The difference in pH and DO was not significant ($p > 0.05$) during the culture months, however, water temperature was significantly different ($p < 0.05$) among the culture months; water temperature was 25.5°C in the third culture month which declined to 14.7°C in the sixth month because it was already winter. Pond water pH was slightly acidic (varied from 6.5 to 7.1). DO range from 4.46 to 5.43 mg L^{-1} during the culture period.

Bacterial population in pond sediment

Quantitative data

The quantity of aerobic bacteria in freshwater tilapia pond sediment in Hai Duong is shown in Table 2. Each number is the average value of viable colonies which grew on duplicate agar plates for each sample. The quantity of bacteria in sediments range from 6.49×10^5 to 1.29×10^6 cfu g^{-1} in the three ponds but bacteria count were not significantly different among the ponds ($p > 0.05$).

Although probiotic was used in Pond 1 to improve water quality during the culture period, the quantity of bacteria did not show any significant difference. This is probably due to the ineffectiveness of the kind of probiotic or the frequency of its application (only once a month).

Bacterial composition in pond sediments

Bacteria in tilapia pond sediment were isolated and identified at genus or species level. The composition of bacterial flora is given and showing the beneficial and harmful bacterial flora which appeared in three studied ponds in Table 3.

In this study, 11 bacterial genera and 13 species were identified from sediment, in which, both gram-negative and gram-positive rod-shaped bacteria dominated all the populations (accounting for 52 and 48%, respectively). Ten bacterial species were common in all three studied ponds: *Pseudomonas fluorescens, Carnobacterium inhibens, Vibrio damsella, Lactobacillus plantarum, Pseudomonas putida, Aeromonas hydrophyla, Granulicatella* sp.V34, *Lactobacillus delbrueckii*, *Enterococcus* sp.HD371, *Pseudomonas* sp., comprising 41.6% of the total isolates. The bacterial species appeared in two of three ponds: *Vagococcus fluvialis*, *Granulicatella* sp.V1, *Aeromonas* sp.HD5, *Vibrio* sp.V31, *Bacillus cereus*, and *Bacillus pumilus* (account for 25% of total isolates). Eight bacterial species which were present in only one of the three ponds were *Aeromonas popoffii*, *Aeromonas* sp.HD361, *Enterococcus* sp.HD31, *Enterococcus* sp.HD1, *Gemella haemolysans*, *Vibrio* sp.HD7, *Vibrio* sp.HD331, *Vibrio pectenicida*.

Beneficial and harmful bacteria in pond sediments

Bacterial species in this study were classified into

Table 3. Bacterial population in intensive tilapia pond sediments in Hai Duong province.

Pond	Bacteria	Name of species	Gram	Presence during culture month				Beneficial/Harmful
				3rd	4th	5th	6th	
1,2,3	HD1.1	*Pseudomonas fluorescens*	-	x				H
1,3	HD1.2	*Vagococcus fluvialis*	+	x				Environmental organism
1	HD2.1	*Aeromonas popoffii*	-	x				Environmental organism
1,2, 3	HD 2.2, HD (2), HD3.5.1	*Pseudomonas* sp.	-	x	x	x		H
1	HD2.3	*Vibrio pectenicida*	-	x				H
2	HD3.1	*Enterococcus sp.HD31*	+	x				H
2,3	V1	*Granulicatella sp.V1*	+	x				H
1,2, 3	V4	*Carnobacterium inhibens*	+	x	x			B
1	HD (1)	*Enterococcus sp.HD1*	+		x			H
3	HD (3)	*Gemella haemolysans*	+		x			H
1,2, 3	HD (4)	*Vibrio damsella*	-		x	x		H
1,3	HD (5)	*Aeromonas* sp.HD5	-		x			H
2	HD (7)	*Vibrio* sp.HD7	-		x			H
1	HD3.3.1	*Vibrio* sp.HD331	-			x		H
1,2, 3	HD3.4.1	*Lactobacillus plantarum*	-			x		B
2	HD 3.6.1	*Aeromonas* sp.HD361	-			x		H
1,2,3	HD3.7.1	*Enterococcus* sp.HD371	+			x		H
1,2	V3.1	*Vibrio* sp.V31	-			x		H
1,2,3	V3.3	*Aeromonas hydrophyla*	-			x		H
1,2,3	V3.4	*Granulicatella* sp.V34	+			x		H
2,3	HD4.2.1	*Bacillus cereus*	+				x	B
1,2,3	HD4.4.1	*Lactobacillus delbrueckii*	+				x	B
1,2,3	HD4.5.1	*Pseudomonas putida*	-				x	H
2,3	HD4.6.1	*Bacillus pumilus*	+				x	B
		Unidentified rod bacteria	+				x	
			-				x	

beneficial and harmful bacteria based on several papers reviewed for this study (Table 3). Zhou et al. (2009) classified bacteria into beneficial, harmless, harmful and pathogenic bacteria. The bacterial population in the intestines of animals will be normal when the health of animals is well, and beneficial bacteria will maintain the balance of intestinal microecosystem.

In all populations, the harmful bacterial species dominated (66.6%) including *P. fluorescens, Pseudomonas* sp., *V. pectenicida, Enterococcus* sp. HD31, *Enterococcus* sp.HD1, *V. damsella, Aeromonas* sp.HD5, *Vibrio* sp.HD7*, Vibrio* sp. HD331*, Aeromonas* sp. HD361, *Enterococcus* sp.HD371, *Vibrio* sp.V31, *A. hydrophyla*, and *P. putida.* The beneficial bacterial species accounted for 20.8% only of total isolates. The beneficial bacteria were *C. inhibens, L. plantarum, B. cereus, L. delbrueckii*, and *B. pumilus.* Others were environmental organisms such as *A. popoffii*, and *V. fluvialis*.

DISCUSSION

Physicochemical parameters

The above measured values are suitable for tilapia culture, except temperature in the sixth month of culture because it was in winter. Low temperature possibly affects fish growth and microbial decomposition in pond sediment. Boyd (1990) indicated that a temperature increase of 10°C often doubles the rate of decomposition and oxygen consumption. Uddin and Al-Harbi (2004) reported that water temperature range from 14.5 ± 1.5 to 33.0 ± 2.3°C, pH slightly alkaline and DO from 3.8 ± 0.2 to 6.2 ± 0.3 ppm for tilapia culture system in Saudi Arabia. Slightly alkaline ponds were suitable for fish culture particularly tilapia (Ntengma and Edema, 2008).

Bacterial population in pond sediment

Bacteria counts observed in the present study are lower than the results reported by Al-Harbi and Uddin (2003). They reported that the quantity of bacteria in hybrid tilapia pond sediment ranged from $9.3 \pm 1.1 \times 10^6$ to $1.9 \pm 1.5 \times 10^8$ cfu g^{-1}. Another study of Al-Harbi and Uddin (2005) showed that the bacteria loads in brackish water tilapia pond sediment ranged from $1.2 \pm 3.1 \times 10^6$ to $7.3 \pm 1.1 \times 10^7$ cfu g^{-1}.

Only 17 bacterial species, of which 87% were gram-negative rod-shaped bacteria were found in tilapia pond sediment in the study of Al-Harbi and Uddin (2005), including V*ibrio parahaemolyticus*, *Vibrio carchariae, Vibrio alginolyticus, Vibrio vulnificus, Chryseomonas* sp., *Streptococcus* sp., *Shewanella putrefaciens, Pseudomonas* sp., and *Bacillus* sp.,. These species differed from the identified species in the present study, especially, some pathogenic bacteria such as *V. parahaemolyticus, V. carchariae, V. alginolyticus, V*. *vulnificus, Chryseomonas* sp., and *S. putrefaciens* were not found in the present study due to these species is only common in brackish water environment. Furthermore, *A. hydrophyla* was identified in this study, however, it was not found in sediment in their study. Uddin and Al-Harbi (2004) indicated that *A. hydrophyla* was the dominant bacteria in pond water for all the different seasons. In another study, Al-Harbi and Uddin (2003) reported that the dominant bacteria in pond sediment were *Corynebacterium urealyticum, S. putrefaciens* and *Escherichia coli*, these species were not found in present study, however, *A. hydrophyla* and *P. fluorescens* were also found in this study.

The beneficial bacteria found in this study mainly belong to *Lactobacillus*, *Bacillus* and *Carnobacterium* genera. These genera are beneficial because they are used in many microbial products to improve water quality to reduce prevalence of fish disease because beneficial bacteria can hinder the growth of harmful bacteria due to the survival competition or increase the health of fish. *Bacillus* sp. could improve water quality because these gram-positive bacteria are better converters of organic matter back to CO_2 than gram-negative bacteria. The high levels of gram-positive bacteria can minimize the buildup of dissolved and particulate organic carbon during the production cycle. Zhou et al. (2009) reported the dominant species in the intestine (>99%) are *Bacillus, Lactobacillus*, and *Bifidobacterium*. The beneficial bacteria in this study can be used to produce probiotic (including beneficial bacteria and *Bacillus* spp.) to restrict the development of pathogens and improve water quality. Dalmin et al. (2001) used probiotic to reduce the *Vibrio* pathogen and enhance beneficial bacilli in the culture leading to improved water quality, survival and growth rate and increase health status of juvenile shrimp *Penaeus monodon*. Qi et al. (2009) used *Bacillus* strains isolated from fish gut or shrimp ponds as an antagonist to *A. hydrophyla* and decrease ammonia level in culture water.

L. plantarum and *L. delbrueckii,* which belong to *Lactobacillus* genus, are important lactic bacteria because they can produce lactic and acetic acids through metabolism and thus lower pH in the intestine and enable beneficial bacteria to be dominant species (Zhou et al., 2009). They can be found in intestine of fish. Many applications of these species are reported due to their fermentative ability as well as their health and nutritional benefits (Rossland et al., 2003). These bacteria were used as microbial feed supplement to improve intestinal microbial balance of fish. *Lactobacillus* genus is also applied to improve water quality and sediment condition.

Beside beneficial bacteria, many harmful bacteria were also found in pond sediment in this study including *A. hydrophyla, P. fluorescens, V. pectenicida, Enterococcus* sp., *V. damsella, Vibrio* sp., and *P. putida*. *A. hydrophyla* is an opportunistic organism, however, pathogenesis and virulence of this species remains unclear. This species can be found in fresh, brackish and coastal waters (Kuijper et al., 1989). Many toxins were produced such as haemolysins and cytotoxins (Chopra et al., 1993). A dominant *A. hydrophyla* was found which is associated with the outbreak of disease in aquaculture of China (Nielsen et al., 2001). Many reports showed that this species was found in tilapia, scallop larvae (*Argopecten purpuratus*) (Riquelme et al., 1996), channel catfish (*Ictalurus punctatus* Rafinesque) (Buller, 2004), European seabass (*Dicentrarchus labrax* Linnaeus), seabass (*Puntazzo puntazzo* Cuvier) (Doukas et al, 1998), blue discus fish (*Symphysodon aequifasciatus* Pellegrin), and walking catfish (*Clarias batrachus* Linnaeus) (Angka et al., 1995) to cause mortality. Moreover, *A. hydrophyla* was also found in the lung and liver of grey seal (*Halichoerus grypus*) (Krovacek et al., 1998) and faeces of human (Brew et al., 1999).

P. fluorescens found in this study is an opportunistic pathogen. It has been found in freshwater ornamental

fish, carp, tilapia, goldfish, seabream, rainbow trout (Kusuda and Yamaoka 1974) and appeared in the gills of cultured salmonids (Rainbow trout, Chinook salmon, Atlantic salmon) (Shotts and Waltman, 1990). Vibriosis is one of the oldest recognized infectious diseases. *V. pectenicida* was identified in this study. It is a pathogenic bacterium and was found in scallop (*Pecten maximus*) larvae, shrimp and prawn.

Results of this study also show the presence of more beneficial bacteria in the later stage of culture period. This demonstrates that these beneficial bacteria can decompose rapidly the organic matter in pond sediment and thus improve water quality in tilapia pond. On the other hand, these bacteria can restrict the development of harmful bacteria which appear mostly at the earlier stage of culture period so that in the later stage, less harmful bacteria were found. From these results, it suggests that culturist should apply some beneficial microorganism in the earlier stage of culture not only to improve water quality but also control the development of pathogenic bacteria to improve the health of fish. However, the beneficial bacteria found in this study also appeared in Ponds 2 and 3 which did not use any probiotic. This suggests that the application of probiotic did not increase the beneficial bacteria in tilapia ponds. The presence of these beneficial bacteria may be due to the frequent water exchange in ponds, resulting in good water quality suitable for beneficial bacteria and cultured fish.

These findings will not only lead to the better control of water quality but also apply the right kind of bacteria or microbial products in ponds. The information on bacterial population may be useful to improve management of intensive tilapia pond. The harmful bacteria appeared in the early stage of culture, thus, more care should be given at this stage and proper pond management such as more water exchange and aeration should be done to enhance the beneficial bacteria.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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