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Investigation of Bacteria and Fish Pathogenic Bacteria Found in Freshwater Aquaponic System

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Abstract

Bacteria are very vital organisms in aquaponic system decomposing and converting the toxic components of the fish waste into usable vegetable nutrients; however, some unwanted bacteria could cause fish diseases. The purposes of this research were to compare the amount of bacteria in water from aquaponic catfish cultured in different stocking densities and Denaturing Gradient Gel Electrophoresis (DGGE) of Polymerase Chain Reaction (PCR)-amplified 16S rDNA was applied to characterise the bacterial populations. Three different stocking densities; 80, 100 and 120 fish/m^2 of hybrid catfish were cultured in aquaponic system. Each treatment contained 3 replications; Treatment 1, 360 catfish (80 fish/m^2, without lettuce); Treatment 2, 360 catfish (80 fish/m^2, without lettuce); Treatment 3, 450 catfish (100 fish/m^2); and treatment 4, 540 catfish (120 fish/m^2). This research was conducted for 4 months. Water was sampled two times before fish stocking, it was found that the number of bacteria in the first week were higher than the third week. The average number of bacteria in water from treatment 1, 2, 3 and 4 were $(2.49 \pm 0.40) \times 10^7$, $(2.43 \pm 0.23) \times 10^7$, $(2.48 \pm 2.22) \times 10^7$ and $(2.29 \pm 2.38) \times 10^7$ Colonies forming unit (CFU)/ml respectively. According to four-time samplings after catfish were stocked, the average number of bacteria in water from treatment 1, 2, 3 and 4 were $(1.85 \pm 0.53) \times 10^7$, $(2.73 \pm 0.15) \times 10^7$, $(2.45 \pm 0.30) \times 10^7$ and $(2.24 \pm 0.19) \times 10^7$ CFU/ml respectively. The average number of bacteria in water after lettuce harvest from treatment 1, 2, 3 and 4 were $(0.185 \pm 0.04) \times 10^7$, $(0.26 \pm 0.04) \times 10^7$, $(0.30 \pm 0.01) \times 10^7$ and $(0.29 \pm 0.05) \times 10^7$ CFU/ml respectively. There were no differences in bacterial counts among treatments ($p > 0.05$). In addition, no significant difference was observed in the number of bacteria in filter tanks ($p > 0.05$) and the number were quite similar to the ones in catfish tanks. *Aeromonas hydrophila* and *Flavobacterium columnare* infected catfish at the beginning of the experiment; however, it got better and no more death found after acclimation. The DGGE analysis of PCR-amplified 16S rDNA fragments revealed that bacterial populations in water were dominated by *Aeromonas* spp., *Pseudomonas* spp. and *Staphylococcus* spp. Antibiotic sensitivity test indicated that enrofloxacin might be the best option for bacterial infection treatment. The results indicate that aquaponic system could reduce the wastewater from catfish culture; however, maintaining a good water quality environment for fish culture should take into account.

Keywords: aquaponics, catfish culture, bacterial fish diseases

1. Introduction

Hybrid catfish (female Thai walking catfish, *Clarias macrocephalus* × male African catfish, *C. gariepinus*) are locally cultured in Thailand because of its good taste and rapid growth. However, dense stocking density possibly results in high organic wastes leading to water pollution with nasty smell and disease susceptibility. For this reason, the aquaponic system was carried out to reduce the organic wastes in catfish ponds and use them as nutrients to grow vegetables. A recirculation aquaponic system was applied in tilapia (Graber & Junge, 2009), hybrid catfish (Sikawa & Yakupitiyage, 2010) and African catfish (Endut et al., 2010) cultivations. Aquaponics can provide locally grown vegetables without using pesticides, chemical fertilizers, or antibiotics (Love et al., 2015). Without proper management, low dissolved oxygen, accumulation of toxic amonia and pathogens could occur. As a result, disease surveillance is required.

Bacterial catfish disease is one of the main risk factors causing business failure. The main pathogens are *Aeromonas hydrophila* and *Flavobacterium columnare*. Most bacterial fish diseases are secondary infection. Fish prone to bacterial infection is due to high stocking density, improper transport, polluted water and wound

infection. Decostere et al. (1998) suggested that *F. columnare* resulted in the secondary infection of *A. hydrophila* leading to more mortality. As a result, water management and disease prevention are necessary, especially in aquaponic system since high organic matter and high ammonia could cause fish stress and prone to diseases.

A rapid and accurate method for fish disease diagnosis is needed. Some molecular methods have been widely applied including specific primer PCR (Reischl et al., 2000), universal primer PCR (UPPCR) (Osorio et al., 1999; Peng et al., 2000) and DGGE (Bernard et al., 2001; Kawai et al., 2002; Peng et al., 2004). DGGE provides a rapid method for detecting pathogenic bacteria in fish by PCR-amplified fragments coding for 16S rRNA. The objective of this research was to apply the PCR-DGGE method for determining the bacteria in catfish aquaponic system.

2. Method

2.1 Aquaponic System

A completely randomised design was applied. There were four treatments with triplicate: (1) 360 catfish/tank without lettuce; (2) 360 catfish/tank with lettuce; (3) 450 catfish/tank with lettuce and (4) 540 catfish/tank with lettuce. The individual water treatment tanks were connected to each fish tank. Hybrid catfish were acclimated for a week. Commercial pellet feed (Charoen Pokaphan Co. Ltd, Thailand) containing 34% protein was used to feed catfish until satiation.

2.2 Water Sampling

Water samples were randomly taken from each tank every two weeks. The 10-fold diluted samples were spread uniformly over the surface of Tryptic Soy Agar (TSA) and incubated at 28 °C for 24–36 hours. Colonies were counted and the bacteria were further purified and identified.

2.3 DNA Isolation

DNA was extracted from the water using the commercial DNA isolation Wizard® Genomic DNA Purification kit (Promega, USA).

2.4 Denaturing Gradient Gel Electrophoresis (DGGE)

The extracted genomic DNA was used as target DNA in the PCR to amplify fragments suitable for subsequent DGGE analysis using primer combinations GC-341F, and 907R. DGGE was performed using a Dcode 16/16 cm gel system (BioRad, USA). PCR samples were loaded onto 6% (wt/vol) polyacrylamide gels in 0.5×TAE (20 mM Tris, 10 mM acetate, 0.5 mM Na_2 EDTA (pH 7.4)). The linear gradient of urea and formamide ranged from 30 - 45% denaturant. The electrophoresis was run at 60 °C for 20 hr at 60 V. The gels were stained for 1 hr with a 1:10,000 dilution of SYBR Green II (Molecular Probes, USA) in distilled water before photography.

2.5 Sequencing and Identification of DGGE Fragments

DGGE bands were excised from the gel and sequenced. The 16S rDNA sequence was searched for nucleotide-nucleotide matches in the BLAST database on the NCBI homepage (http://www.ncbi.nlm.nih.gov/BLAST/) to establish strain identity.

3. Results and Discussion

When properly managed, aquaponic systems provide the advantages of both reducing water usage and effluent. However, at the beginning, catfish were infected with *Aeromonas hydrophila* and *Flavobacterium columnare* since fish possibly were stress after transportation. Prevention through quarantine, selection of healthy fingerlings, avoidance of stress on the fish, and water quality management is probably the most effective tool to avoid these bacterial infections. Quarantine and disinfection fish before releasing into the aquaponic ponds could reduce or eliminate pathogens and are important practices of a good biosecurity program. Catfish must be examined for potential pathogens before taking them to the system.

Without suitably controlled, water quality problems in recirculating systems especially toxic levels of ammonia or nitrite could happen. On the other hand, the good water quality could prevent the occurrence of undesired diseases. In addition, low dissolved oxygen can occur during culture period because of too high stocking densities, high feeding rates, insufficient water flow, inadequate aeration, high organic loads in the system leading to large numbers of bacteria. Not only routine monitoring of water parameters is needed, the measurement of total viable bacteria is very important also. Hu et al. (2015) stated that ammonia is firstly oxidized to nitrite by ammonia oxidizing bacteria and then converted to nitrate by nitrite oxidizing bacteria (mainly *Nitrobacter* spp. and *Nitrospira* spp.). Not enough bacteria in this aquaponic system possibly results in

deteriorated water while too high bacteria could make fish prone to diseases. As a result, the amounts of bacteria should be routinely checked.

Total bacterial amounts are shown in Table 1. The highest bacteria were observed at the beginning, before fish stocked. The average amounts of bacteria in water from treatments 1, 2, 3 and 4 were $(2.49 \pm 0.40) \times 10^7$, $(2.43 \pm 0.23) \times 10^7$, $(2.48 \pm 2.22) \times 10^7$ and $(2.29 \pm 2.38) \times 10^7$ CFU/ml respectively. After that, the number of bacteria trended to reduce. However, they were still high due to dense catfish stocked. As reported by Pinkate et al. (2003) who isolated bacteria from tilapia pond and found that the total viable bacteria was only $(7.3 \pm 9.9) \times 10^4 - (8.1 \pm 5.1) \times 10^4$ CFU/ml. In addition, bacterial count in tilapia pond water was $(1.4 \pm 1.5) \times 10^3$ to $(8.6 \pm 2.7) \times 10^3$ CFU/ml (Al-Harbi &Uddin, 2005).

The average number of bacteria in water from treatment 1, 2, 3 and 4 were $)1.85 \pm 0.53) \times 10^7$, $(2.73 \pm 0.15) \times 10^7$, $(2.45 \pm 0.30) \times 10^7$ and $(2.24 \pm 0.19) \times 10^7$ CFU /ml respectively after catfish were stocked. Then, the bacteria trended to decrease. After lettuce harvest, the average number of bacteria in water from treatment 1, 2, 3 and 4 were $(0.185 \pm 0.04) \times 10^7$, $(0.26 \pm 0.04) \times 10^7$, $(0.30 \pm 0.01) \times 10^7$ and $(0.29 \pm 0.05) \times 10^7$ CFU/ml respectively. There were no differences in bacterial counts among treatments ($p > 0.05$). In addition, no significant difference was observed in the amounts of bacterial in filter tanks ($p > 0.05$) and the amounts were quite similar to the ones in catfish tanks.

Recirculating systems are mechanically sophisticated and biologically complex which sometimes fails due to poor water quality leading to fish stress, diseases and off-flavor in poorly managed systems (Masser et al., 1999). As fish are stocked at high density within the recirculation system they are more at risk in becoming stressed and prone to disease. It is necessary to monitor fish health continuously as if a disease outbreak does occur, it can spread extremely rapidly throughout the culture tank (Emperor Aquatics, 2013).

Table 1. Total viable bacteria (CFU/ml) in the water of freshwater fish aquaponic system during the study period

	Bacteria amounts in the water (CFU/ml)			
	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Before fish stocked				
1	$(2.49\pm0.40)\times10^7$	$(2.43\pm0.23)\times10^7$	$(2.48\pm2.22)\times10^7$	$(2.29\pm2.38)\times10^7$
2	$(0.88\pm0.20)\times10^7$	$(0.80\pm0.20)\times10^7$	$(0.70\pm0.51)\times10^7$	$(0.90\pm0.05)\times10^7$
During experiment				
3	$(1.85\pm0.53)\times10^7$	$(2.73\pm0.15)\times10^7$	$(2.45\pm0.30)\times10^7$	$(2.24\pm0.19)\times10^7$
4	$(0.41\pm0.40)\times10^7$	$(0.43\pm0.05)\times10^7$	$(0.44\pm0.14)\times10^7$	$(0.38\pm0.01)\times10^7$
5	$(0.60\pm0.08)\times10^7$	$(0.64\pm0.10)\times10^7$	$(0.69\pm0.02)\times10^7$	$(0.69\pm0.06)\times10^7$
6	$(0.71\pm0.06)\times10^7$	$(0.64\pm0.05)\times10^7$	$(0.49\pm0.08)\times10^7$	$(0.75\pm0.10)\times10^7$
After lettuce harvested	$(0.18\pm0.04)\times10^7$	$(0.26\pm0.04)\times10^7$	$(0.30\pm0.01)\times10^7$	$(0.29\pm0.05)\times10^7$

Note. Treatment 1, 360 catfish/tank without vegetable; Treatment 2, 360 catfish/tank with vegetable; Treatment 3, 450 catfish/tank with vegetable; Treatment 4, 540 catfish/tank with vegetable.

Aquatic microorganisms not only affect the water quality but are also associated with the fish physiological status, diseases and postharvest quality (Al-Harbi & Uddin, 2005). According to the biochemical tests and API 20 E strip kits used in the identification, there was a prevalence of *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Plesiomonas shigelloides*, *Escherichia coli*, *Acinetobacter baumannii*, *Salmonella* sp., *Staphylococcus* sp., *Micrococcus* sp. and unidentified bacteria. However, some bacteria are not able to grow on TSA. Subsequently, water samples were randomly investigated for the bacteria population by DGGE. A comparison of the DGGE analysis of the 16S rDNA fragments recovered from four different catfish tanks showed somewhat differences in the profile of the different samples (Figure 1). Nevertheless, some bands with similar positions were present in different samples.

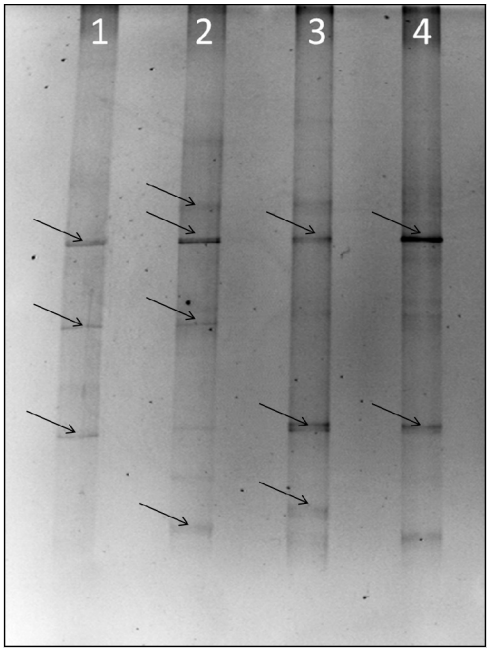

Figure 1. DGGE profiles of 16S rDNA bacterial fragments from water in catfish aquaponic ponds. Lanes 1, 2, 3 and 4 were bands of PCR-amplified 16S rDNA bacterial fragments from water in catfish aquaponic ponds 1, 2, 3 and 4 respectively

Excision of the twelve DGGE bands was re-amplified, and then sequence determination was carried out. The phylogenetic relationship between the bacteria represented by different bands was drawn. The dendogram shows the affiliation and the phylogenetic relationship of the sequences from DGGE bands with sequences from already sequenced bacteria in the database (Figure 2). The possible bacteria were *Pseudomonas* spp., *Staphylococcus* spp. and *Aeromonas* spp. It demonstrated a high degree of homology in the bacteria community. Bacteria that seem to increase in number in recirculating systems include *Aeromonas* spp., *Vibrio* spp., *Mycobacterium* spp., *Streptococcus* spp. and *Flavobacterium columnare* (Yanong, 2013). In addition, microbial communities associated with filter materials in the recirculating water system should be analysed. A limitation of this study was the the lack of information on energy cost and cost benefit analysis.

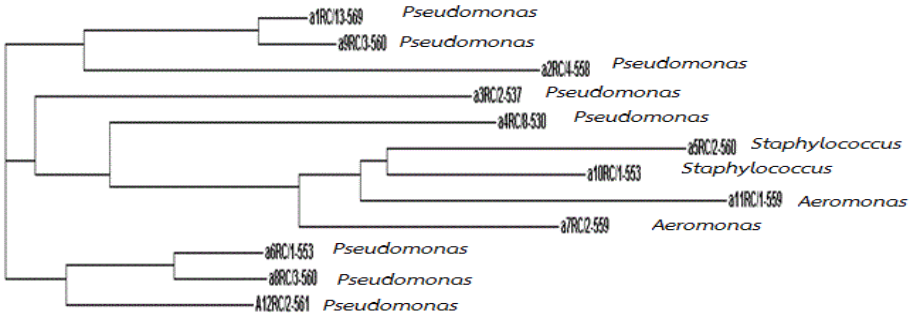

Figure 2. Phylogenetic tree of bacteria in the water from aquaponic tanks

4. Conclusion

Fish aquaponic system offers an environmentally friendly mean. However, the feasibility in term of economy and efficacy must be investigated. The water management must be closely monitored. Fish should be in the healthy status because some bacteria are opportunistic pathogens. In this study, the Denaturing Gradient Gel Electrophoresis (DGGE) of PCR-amplified 16S rDNA could be applied to identify the bacterial population.

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