การติดเชื้อแบคทีเรียและเชื้อราในการเพาะและอนุบาลปลาบึก

Bacterial and Fungal Infection in Mekong Giant Catfish (Pangasianodon gigas Chevey) Hatchery

วัชริยา ภูรีวิโรจน์กุล[1] นนทวิทย์ อารีย์ชน[2] องอาจ เลาหวินิจ[3] และชาญชัย ภู่รักษ์เกียรติ[4]

Watchariya Purivirojkul[1], Nontawith Areechon[2], Ong-ard Lawhavinit[3] and Charnchai Purukkiate[4]

บทคัดย่อ

การศึกษาชนิดของแบคทีเรียและเชื้อราในไข่และลูกปลาบึกที่ได้จากการผสมเทียมระหว่างพ่อแม่พันธุ์ที่เลี้ยงในบ่อดินที่ศูนย์วิจัยและพัฒนาประมงน้ำจืดพะเยา จ.พะเยา ระหว่างเดือนพฤษภาคมถึงเดือนกรกฎาคม 2547 จากตัวอย่างไข่ 150 ฟอง และลูกปลาบึกจำนวน 90 ตัว พบว่าตัวอย่างทั้งหมดมีการติดเชื้อแบคทีเรียและเชื้อรา โดยพบแบคทีเรีย 7 ชนิด ได้แก่ *Aeromonas hydrophila, Pseudomonas fluorescens, Plesiomonas shigelloides, Chromobacterium violaceum, Chryseomonas meningosepticum, Pasteurella haemolytica* และ *Ochrobactrum anthropi* เชื้อรา 3 สกุล ได้แก่ *Saprolegnia sp., Aphanomyces sp.* และเชื้อราในกลุ่มที่สร้าง vesicle การติดเชื้อน่าจะมีสาเหตุส่วนหนึ่งจากน้ำที่นำมาใช้ซึ่งพบว่ามีการปนเปื้อนของแบคทีเรียปริมาณ 2.9 + 1.8 x 10 9cfu/มล. และเชื้อรา 10.2 + 10.3 zoospores/มล. และพบว่าอาหารของลูกปลาบึก ได้แก่ ไรแดง และอาร์ทีเมียก็มีปริมาณแบคทีเรียสูงเช่นเดียวกันโดยเฉพาะ *A. hydrophila* ผลสรุปที่ได้จากการวิจัยครั้งนี้พบว่าน้ำและอาหารที่ใช้ในการเพาะและอนุบาลลูกปลาบึกมีปริมาณแบคทีเรียและเชื้อราในปริมาณที่สูงเป็นสาเหตุที่ทำให้อัตราฟักของไข่และอัตรารอดของลูกปลาต่ำ ดังนั้นหากสามารถควบคุมการปนเปื้อนของเชื้อแบคทีเรียและเชื้อราในน้ำและอาหารมีชีวิตที่ใช้ในขบวนการเพาะและอนุบาลลูกปลาบึกได้ ก็จะทำให้การเพาะขยายพันธุ์ปลาหายากชนิดนี้ประสพความสำเร็จยิ่งขึ้นต่อไป

ABSTRACT

Bacterial and fungal isolation from Mekong giant catfish (Pangasianodon gigas Chevey) hatchery was conducted. Egg and fry were obtained from artificial spawning at Phayao Inland Fisheries Research and Development Center, Phayao province, Thailand during May 2004 to June 2004. Total number of 150 eggs and 90 fries of giant catfish were investigated and 100% were found to be infected with bacteria and fungi. Seven species of bacteria were isolated including *Aeromonas hydrophila, Pseudomonas fluorescens, Plesiomonas shigelloides, Chromobacterium violaceum,*

[1]ภาควิชาสัตววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ จตุจักร กทม. 10900

[1]Department of Zoology, Faculty of Science, Kasetsart University Jatujak, Bangkok THAILAND 10900

[2]ภาควิชาเพาะเลี้ยงสัตว์น้ำ คณะประมง มหาวิทยาลัยเกษตรศาสตร์ จตุจักร กทม. 10900

[2]Department of Aquaculture, Faculty of Fisheries, Kasetsart University Jatujak, Bangkok THAILAND 10900

[3]ภาควิชาจุลชีววิทยาและวิทยาภูมิคุ้มกัน คณะสัตวแพทย์ศาสตร์ มหาวิทยาลัยเกษตรศาสตร์ จตุจักร กทม. 10900

[3]Department of Microbiology and Immunology, Faculty of Veterinary medicine, Kasetsart University Jatujak, Bangkok 10900

[4]ศูนย์วิจัยและพัฒนาประมงน้ำจืดพะเยา จังหวัดพะเยา 56000

[4]Phayao Inland Fisheries Research and Development Center, Phayao province 56000

Chryseomonas meningosepticum, Pasteurella haemolytica and *Ochrobactrum anthropi.* Three genera of fungi were isolated including *Saprolegnia sp., Aphanomyces sp.* and the vesicle producing fungi. These bacteria and fungi are water borne microorganism which can infect aquatic animals. These pathogens could come from water supply which had high number of bacteria and fungi at 2.9+1.8 x 10 9 cfu/ml and 10.2 + 10.3 zoospores/ml, respectively. Live feed for fry including water flea and brine shrimp also contained high number of bacteria which was dominated by *A. hydrophila.* The results obtained from this study suggested that water supply for giant catfish hatchery and feed could be the source of bacterial and fungal infection that caused low hatching rate of eggs and low survival rate of fry. Establishment of sanitary condition in the hatchery is highly recommended for the improvement of hatching and survival rate of this valuable species.

Key Words : bacteria, fungi, Mekong giant catfish, egg, fry

Email address : fsciwyp@ku.ac.th

INTRODUCTION

Mekong giant catfish *Pangasianodon gigas* is the biggest, scaleless freshwater fish in the world with size measuring up to 3 m in length and weighing more than 300 kg when fully grown. It is a bottom feeder, feeding on algae and other aquatic plants. This giant catfish can only be found in the Mekong River in the region of Chiang Khong, Chiang rai province in Thailand during their spawning period (from the middle of April to the end of May).

The generic name is derived from Pangasius + an (Greek for without) +odon (Greek for tooth) in reference to the toothless state of the adult fish. This fish has the fastest growth rate of any fish in the world reaching 150-200 kg in six years (Rainboth, 1996)

The Mekong giant catfish is in danger of disappearing from Thailand because of the high demand from local market in which the selling price can be more than 500 bath/kg. The population of the Mekong giant catfish in the Mekong river has been decreased rapidly because a lot of adult female fish were caught in spawning season. Moreover, the water level in the Mekong River has dropped to an all time low due to the dams that have been built upstream. Little water is released from the dams during the dry season causing the river to run dry and may force the Mekong giant catfish to find other suitable spawning ground.

Although, in 1983 the Department of Fisheries has been succeeded in artificially breeding the Mekong giant catfish and could produce a lot of fry but the hatching rate and survival rate of fry were very low (in 2003, 120,000 fries hatched from egg but only 30,000 survived after 7 days). This paper reported the study of bacterial and fungal infection in eggs and fry of Mekong giant catfish; bacterial contamination in live feed and water supply in the hatchery.

MATERIALS AND METHODS

Mekong giant catfish egg sampling

Mekong giant catfish eggs used in the study were obtained from artificial spawning at Phayao Inland Fisheries Research and Development Center, Phayao province, Thailand. One hundred eggs were collected for bacteria isolation and 50 eggs for fungi study.

Fry of giant catfish sampling

Giant catfish fry used in the study were obtained from artificial spawning at Phayao Inland Fisheries Research and Development Center, Phayao province, Thailand. Sixty samples were collected for bacteria isolation and 30 samples for fungi study.

Water flea (*Moina macrocopa*) and brine shrimp (*Artemia*) sampling

Water flea and brine shrimp, feed for giant catfish fry used in the study. Forty samples were collected for bacteria isolation and 20 samples for fungi study.

Bacterial isolation

The surface of eggs and the skin of fry were cleaned by 70% ethyl alcohol before grinding by homogenizer and streaked with an inoculating loop on Brain Heart Infusion Agar (BHI Agar) and incubated at 30° C for 18-24 h. Colonies of bacteria were selected from the plate according to their phenotypic differences (color, size, density and shape) and purification on Nutrient Agar (NA).

Bacterial Identification

Phenotypical characteristics, Gram strain, oxidase production, oxidative/ fermentative utilization of glucose (OF medium), motility and growth on MacConky agar were determined for every selected isolate. Only Gram negative were found and they were further characterized by biochemical typing using API 20E kit (Biomérieux). Tests were performed according to the instructions of the manufacturer, incubated at 30° C for 24 h. Bacterial identification was performed by using the APILAB Plus identification software (Biomérieux).

Isolation of fungi

The samples were washed 2 times before culture on the Gy plus antibiotic medium (1% glucose, 0.25% yeast extract and 50 ug/ml of ampicillin and streptomycin). The cultured medium were incubated at 25 ºC for 2 days and purified by cutting the marginal colony to put on the new medium.

Identification of fungi

The pure culture of fungi were studied to the morphology of asexual zoospore reproduction in sterilized tap water. The genus of isolated fungi were identified by the method belong to Willoughby (1985)

Number of bacteria and fungi from rearing water and stock water

Water samples were collected from egg hatching water, fry rearing water and stock water (holding in 500 ton cement tanks obtained from Phayao reservior). Water samples (10 ml/pond) were taken from approximately 5 cm below the water surface in sterile glass bottles. A tenth ml of each sample were spreaded on NA agar and incubated at 30 ^{o}C for a day. The number of colony were counted and calculated for the total number of bacteria as cfu/ml. Another 0.1 ml of each sample were spreaded on Gy plus antibiotic medium and incubated at 25 ^{o}C for 2 days. The number of fungi colony were reported as unit of zoospores per ml.

Water qualities in Mekong giant catfish hatchery

Water analysis consisting of dissolved oxygen (mg/l), pH, temperature (C^{o}), alkalinity (ppm as $CaCO_3$), hardness (ppm as $CaCO_3$), total ammonia nitrogen (ppm) and nitrite nitrogen (ppm) from water using for Mekong giant catfish egg hatching, fry nursing and stock water were conducted.

RESULTS AND DISCUSSIONS

Bacterial Isolation

The identification of bacteria isolated from egg, fry of Mekong giant catfish, water flea, brine shrimp, egg hatching water, fry rearing water and stock water as displayed in Table 1, showed a high incidence of *Aeromonas hydrophila* in all samples, followed by *Plesiomonas shigelloides* and *Pasteurella haemolytica.*

The results from Table 1 showed that bacteria isolated from eggs and fry dominated by *A. hydrophila* were also found in water used in each step of the hatchery. Moreover, *A. hydrophila* could be found in water flea and brine shrimp which were feed of Mekong giant catfish fry. So, sources of these pathogenic bacteria should be from water and live feed. Although, brine shrimp were cultured in salt water (5 ppt) but *A. hydrophila* could be found in water which salinity up to 10 ppt (Hazen *et al*., 1978; Kaper *et al*., 1979)

All of eleven species of bacteria including *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Plesiomonas shigelloides*, *Chromobacterium violaceum*, *Chryseomonas meningosepticum*, *Pasteurella haemolytica*, *Ochrobactrum anthropi*, *Morganella morganii*, *Vibrio cholerae*, *Vibrio alginolyticus* and *Chryseomonas luteda* were found in water and soil (Austin and Adum, 1996; Austin and Allen, 1982; Bennish, 1994; Durán and Menck, 2001; Jeppesen, 1995; Khardori and Fainstein, 1988; Krovacek *et al*., 2000; de Mondino *et al*., 1995; Reidl and Klose, 2002; Rutala *et al*.,1982). When host (eggs, fishes, water fleas and brine shrimps) had a wound, these pathogens could attach on the host and distribute in host body by blood circulation system

Some bacteria, found in this study, can be pathogenic in other animals or human such as *Pasteurella haemolytica* which is the principal pathogen in the bovine respiratory disease complex, a

major disease of economic importance in the cattle industry. *Vibrio cholerae* infection in human normally starts with the oral ingestion of food or water contaminated with *V. cholerae*. But the infectious dose was determined to be fairly high ranging from 10^6 to 10^{11} colony-forming units (Bennish, 1994)

Fungal isolation from eggs and fry

Three genera of fungi were found in this study consisting of *Saprolegnia* sp., *Aphanomyces* sp. and the vesicle producing fungi. These three genera were reported to be found in aquaculture of many regions in the world. *Aphanomyces* sp. especially *Aphanomyces invadans* (also called *A. invaderis* and *A. piscicida*) (Lilley *et al*., 1997) is a pathogenic water mold that causes mycotic granulomatosis in warm freshwater fish. *A. piscicida* invades the muscle by extending the hyphae (Miyazaki and Egusa, 1972; Miyazaki and Egusa, 1973a; Miyazaki and Egusa, 1973b; Miyazaki and Egusa, 1973c). Chinabut *et al*. (1995) reported *A. invadans* infection in snakehead fish in Asia.

Table 1 Percent of bacterial found in eggs, fry of Mekong giant catfish, water fleas, brine shrimps, egg hatching water, fry rearing water and stock water

Bacterial species	Percent of bacterial found						
	Egg	Fry	Water flea	Brine shrimp	Egg hatching water	Fry rearing water	Stock water
(Number of samples)	(100)	(60)	(40)	(40)	(12)	(12)	(12)
Aeromonas hydrophila	50	66	60	80	80	50	60
Pseudomonas fluorescens	25	0	0	0	0	0	0
Plesiomonas shigelloides	15	16	0	0	10	10	20
Chromobacterium violaceum	15	0	0	0	0	0	0
Chryseomonas meningosepticum	15	0	0	0	0	0	0
Pasteurella haemolytica	10	16	0	0	30	20	10
Ochrobactrum anthropi	0	20	0	0	0	0	0
Morganella morganii	0	0	100	0	0	0	0
Vibrio cholerae	0	0	0	30	0	0	0
Vibrio alginolyticus	0	0	0	50	0	0	0
Chryseomonas luteda	0	0	0	0	0	10	0
Not-Identified	0	10	0	0	0	10	10

Saprolegniasis is a widespread mycotic infection in freshwater aquaculture caused by *Saprolegnia* sp. which is a waterborne fungi. This fungi infection on fish and fish eggs causes problem among cultured fish that causes low hatching and survival rate (Ghittino, 1983; Bruno and Poppe, 1996). *Saprolegnia* sp. produces large numbers of infectious asexual zoospores at low temperatures (Bly *et al*., 1992). Noble and Summerfelt (1996) reported *Saprolegnia* sp. infection in rainbow trout culture.

Figure 1 Asexual reproduction of fungi 1.1 *Aphanomyces* sp. 1.2 *Saprolegnia* sp. (40X)

Fungi in water flea and brine shrimp

Twenty five percents of water flea samples infected by fungi were found in this experiment (5 samples from 20 samples). Fifteen percents of brine shrimp samples infected by fungi were found in this experiment (3 samples from 20 samples).

Number of bacteria and fungi from water sample

Number of total fungi in hatching water, fry rearing water and stock water were 90.7 $\pm$ 60.8, 11.4 $\pm$ 14.6 and 12 $\pm$ 13 zoospores per ml, respectively. Number of total bacteria in hatching water, fry rearing water and stock water were 2.22 $\pm$ 0.89 x 10^9, 5.5 $\pm$ 4.95 x 10^9 and 2.9 $\pm$ 1.8 x 10^9 cfu per ml, respectively (Table 2).

Bacteria which was found in hatching water, fry rearing water and stock water were *Aeromonas hydrophila, Plesiomonas shigelloides, Pasteurella haemolytica* and *Chryseomonas luteda*.

Table 2 Number of bacteria and fungi from water sample

Source	Number of bacteria	Number of fungi
Egg hatching water	2.22 $\pm$ 0.89 x 10^9	90.7 $\pm$ 60.8
Fry rearing water	5.5 $\pm$ 4.95 x 10^9	11.4 $\pm$ 14.6
Stock water	2.9 $\pm$ 1.8 x 10^9	12 $\pm$ 13

Fungi which was found in hatching water, fry rearing water and stock water were *Saprolegnia* sp., *Aphanomyces* sp. and the vesicle producing fungi

All of bacteria and fungi species were similar with bacteria and fungi which were found in eggs and fry of Mekong giant catfish.

Water qualities in Mekong giant catfish hatchery

Water analysis consisting of dissolved oxygen, pH, temperature, alkalinity, hardness, total ammonia nitrogen and nitrite nitrogen of hatching water for giant catfishes egg, fry rearing water and stock water were reported in Table 3. All of water qualities were in normal range for aquaculture.

Table 3 Water quality in Mekong giant catfish hatchery

Water Sample	Dissolved Oxygen (mg/l)	pH	Temperature (C^{o})	Alkalinity (ppm as $CaCO_3$)	Hardness (ppm as $CaCO_3$)	Total Ammonia Nitrogen (ppm)	Nitrite Nitrogen (ppm)
Egg	5.5 $\pm$ 0.1	7.65 $\pm$ 0.13	25.9 $\pm$ 0.26	40 $\pm$ 10	87.5 $\pm$ 2.5	0.14 $\pm$ 0.02	0.04 $\pm$ 0.02
Fry	5.2 $\pm$ 0.2	7.88 $\pm$ 0.03	25.13 $\pm$ 0.12	65 $\pm$ 5	90 $\pm$ 5	0.14 $\pm$ 0.02	0.008$\pm$0.003
Stock	5.0 $\pm$ 0.53	7.75 $\pm$ 0.25	26.67 $\pm$ 0.76	76.67 $\pm$ 5.77	68.33 $\pm$ 17.56	0.13 $\pm$ 0.03	0.01

CONCLUSION

This study clearly showed the heavy infection rate of both eggs and fry of Mekong giant catfish. The most severe problem should be associated with pathogenic *Aeromonas hydrophila* (amongst seven bacteria that were found) which was also found in water supply in hatchery system and in live feed. This could clearly influence the success of the whole hatchery activity of this valuable species.

To control these pathogens, formaldehyde is the most commonly used disinfectants for prophylaxis in the embryonic period and the first stage of larval development (Forneris *et al*., 2003). Although this chemical has been used as an effective fungicide, awareness on safety and impact on the environment has been increased. Marking *et al*. (1994) identified two promising fungicides consisting of hydrogen peroxide and sodium chloride. Noble and Summerfelt (1996) recommended artificial seawater salt 1-1.5% for 1 hr.

To improve the hatchery activity of Mekong giant catfish, sanitation of water supply and live feed is highly recommended.

ACKNOWLEDGEMENTS

This research was supported by The Thailand Research Fund (TRF). We thank Miss Sutida Sopeen and Mr. Metha Katapichat, Fishery Biologist, Phayao Inland Fisheries Research and Development Center for their assistance.

LITERATURE CITED

Austin, B. and C. Adum. 1996. Fish pathogens, pp.198-243. *In* B. Austin, M. Altwegg, P.J. Gosling and S. Joseph (Eds.). The Genus *Aeromonas*. Wiley, Chichester.

Austin, B. and D.A. Allen. 1982. Microbiology of laboratory-hatched brine shrimp (*Artemia*). Aquaculture. 26:369-383.

Bennish, M.L. 1994. Cholera:pathophysiology, clinical features and treatment, pp. 229-255. *In* K.I. Wachsmuth, P.A. Blake and O. Olsik (Eds.). *Vibrio cholerae* and cholera : Molecular to global perspectives. American Society for Microbiology, Washington, DC.

Bly, J.E., L.A. Lawson, D. J. Dale, A.J. Szalai, R.M. Durborow & L.W. Clem. 1992. Winter saprolegniosis in channel catfish. Dis. Aquat. Organ. 13:155-164.

Bruno, D.W. and T.T. Poppe. 1996. A colour atlas of salmonids diseases. Harcourt Bruce & Company, London. 194 p.

Chinabut, S., R.J. Roberts, L.G. Willoughby and M. D. Pearson. 1995. Histopathology of snake heads, *Channa striatus* (Bloth), experimentally infected with specific *Aphanomyces* involved in epizootic ulcerative syndrome (EUS) with other similar fungi. J. Fish. Dis. 20:135-144.

Durán N. and C.F.M. Menck. 2001. *Chromobacterium violaceum.* A review of pharmacological and industrial perspectives. Crit. Rev. microbiol. 27:201-222.

Forneris, G., S. Bellardi, G.B. Palmegiano, M. Saraglia, B. Sicuro, L. Gasco and I. Zoccarato. 2003. The use of ozone in trout hatchery to reduce saprolegniasis incidence. Aquaculture. 221:157-166.

Ghittino, P. 1983. Tecnologia e patologia in acquacoltura: vol. II. Patologia. Tipografia. Bono, Torino. 444 p. (in Italian)

Hazen, T.C., C.B. Fliermans, R.P. Hirsch and G.W. Esch. 1978. Prevalence and distribution of *Aeromonas hydrophila* in the United States. Appl. Envir. Microbiol. 36:731-738.

Jeppesen, C. 1995. Media for *Aeromonas* spp., *Plesiomonas shigelloides* and *Pseudomonas* spp. from food and environment. Int. J. Food Microbio. 26:25-41.

Kaper, J.B., J. Seilder, H. Lockman and R.R.Cowell. 1979. A medium for the presumptive identification of *Aeromonas hydrophila* Enterobacteriaceae. Appl. Envir. Microbiol. 38:1023-1026.

Khardori, N. and V. Fainstein 1988. *Aeromonas* and *Plesiomonas* as etiological agents. Ann. Rev. Microbiol. 42:395-419.

Krovacek, K., L.M. Eriksson, C. González-Rey, J. Rosinsky and I. Ciznar. 2000. Isolation, Biochemical and serological characterisation of *Plesiomonas shigelloides* from freshwater in Northern Europe. Comp. Immunol. Microb. 23:45-51.

Lilley, J.H., D. Hant, R.H. Richards, R. J. Roberts, L. Cerenius and K. Söderhäll. 1997. Pan-Asian Spread of single fungal clone results in large scale fish-kills. Vet. Rec. 140:11-12.

Marking, L.L., J.J. Rach and T.M. Schreier. 1994. Evaluation of antifungal agents for fish culture. Progr. Fish Cult. 56:225-231.

Miyazaki, T. and S. Egusa. 1972. Study on mycotic granulomatosis in fresh water fish I. Mycotic granulomatosis prevalled in gold fish. Fish Pathol. 7:15-25. (in Japanese)

Miyazaki, T. and S. Egusa. 1973a. Study on mycotic granulomatosis in fresh water fish II. Mycotic granulomatosis prevalled in ayu. Fish Pathol. 7:125-133. (in Japanese)

Miyazaki, T. and S. Egusa. 1973b. Study on mycotic granulomatosis in fresh water fish III. Mycotic granulomatosis in bluegill. Fish Pathol. 8:41-43. (in Japanese)

Miyazaki, T. and S. Egusa. 1973c. Study on mycotic granulomatosis in fresh water fish IV. Mycotic granulomatosis in some wild fishes. Fish Pathol. 8:44-47. (in Japanese)

de Mondino, SS., M.P. Nunes and I.D. Ricciardi. 1995. Occurrence of *Plesiomonas shigelloides* in water environments of Rio de Janeiro city. Mem Inst Oswaldo Cruz. 90:1-4.

Narayanan, S.K., T.G. Nagaraja, M.M. Chengappa and G.C. Stewart. 2002. Leukotoxins of gram-negative bacteria. Vet. Microbiol. 84:337–356.

Noble, A.C. and S.T. Summerfelt. 1996. Diseases encountered in rainbow trout cultured in recirculating systems. Ann. Rev. Fish Dis. 6:65-92.

Rainboth, W.J. 1996. Fishes of the Cambodian Mekong. FAO Species Identification Field Guide for Fishery Purposes. *In* FAO, Rome Origin and or Distribution: Endemic to the Mekong basin where it has become rare due to overexploitation. International trade banned (CITES I). 265 pp.

Rutala, W.A., F.A. Sarribbi, C.S. Finch, J.N. MacCormak and G.E. Steomlaris. 1982. Oyster-associated outbreak of diarrhea disease possibly caused by *Plesiomonas shigelloides.* Lancet. I:739.

Reidl, J. and K. E. Klose. 2002. *Vibrio cholerae* and cholera : out of the water and into the host. FEMS Microbiol. Rev. 26:125-139.

Willoughby, L.G. 1985. Rapid preliminary screening of Saprolegnia on fish. J. Fish. Dis. 8:473-476.