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Is *Aeromonas hydrophila* the dominant motile Aeromonas species that causes disease outbreaks in aquaculture production in the Zhejiang Province of China?

M. E. Nielsen[1,*], L. Høi[1], A. S. Schmidt[1], D. Qian[2], T. Shimada[3], J. Y. Shen[2], J. L. Larsen[1]

[1]Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University, Department of Veterinary Microbiology, 4 Stigbøjlen, 1870 Frederiksberg C, Denmark

[2]Zhejiang Institute of Freshwater Fisheries, Huzhou, Zhejiang Province 313001, PR China

[3]National Institute of Infectious Diseases, Tokyo 162-8640, Japan

ABSTRACT: The significance of *Aeromonas hydrophila* in association with disease outbreaks in aquaculture production in the Zhejiang province of China was investigated. Bacteriological examination of moribund fish and crabs resulted in 95 bacterial isolates: 88 bacterial isolates from fish and 7 isolates from crabs. PCR and traditional biochemical methods were used for identification of *A. hydrophila*. Out of 69 motile aeromonads, 35 isolates were identified as *A. hydrophila* by biochemical tests. However, 6 of those were not identified as *A. hydrophila* by a species specific PCR method. Serotyping revealed 2 dominant serotypes (O9 and O97) among *A. hydrophila* isolates. The data presented show that approximately 42% of the motile aeromonads isolated from disease outbreaks among various fish species were *A. hydrophila*. It is noteworthy that *A. hydrophila* accounted for more than 50% of the isolated aeromonands isolated from crucian carp *Carassius carassius* and Wuchang bream *Megalobrama amblycephala* with haemorrhagic septicaemia. Although this species was the most frequently isolated organism from internal organs of diseased fish and crabs in the present study, other motile *Aeromonas* spp. were also found. The PCR assay was useful in preventing misidentification of *A. hydrophila*, which may occur when only phenotypic tests are employed.

KEY WORDS: *Aeromonas hydrophila* · PCR · China · Haemorrhagic septicaemia · Fish diseases

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INTRODUCTION

The aquaculture industry in Asia has evolved dramatically during the last decades, leading to major structural changes and capital-intensive production systems (Shariff 1998). Many Asian countries have increased their aquaculture production significantly, but it has not been without environmental and socioeconomic impacts; among the most important are increasing fish health issues. Fish disease is the major risk factor in commercial aquaculture with millions of dollars lost annually, and in Asia, disease has emerged as a major constraint to the sustainable development of aquaculture (FAO 1997, Shariff 1998). In 'low-income food deficit countries', aquaculture is currently the fastest growing food-producing system and fish protein is a major source of protein for human consumption (FAO 1998). Therefore, understanding the ecology and epidemiology of the infectious agents associated with mass mortalities in aquaculture is important.

Aeromonas hydrophila has been recovered from a wide range of freshwater fish species worldwide (Austin & Adams 1996). Conflicting views have been expressed concerning whether *A. hydrophila* is a

*E-mail: men@kvl.dk

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primary pathogen of freshwater fish or a secondary opportunistic pathogen of compromised or stressed hosts (Jeney & Jeney 1995). *A. hydrophila* has been associated with tail and fin rot, haemorrhagic septicaemia and epizootic ulcerative syndrome (EUS) (Austin & Adams 1996, Roberts 1997). *A. hydrophila* has also been described as the dominant infectious agent of 'fish-bacterial-septicaemia' in freshwater cultured cyprinid fishes, mainly crucian carp *Carassius carassius*, Wuchang bream *Megalobrama amblycephala* and silver carp *Hypophthalmichthys molitrix* in the Zhejiang province and other provinces in the Southeast of China between 1989 to 1993 (Qian et al. 1997). 'Fish-bacterial-septicaemia' occurs each summer in the Zhejiang province and results in significant losses for the fish farmers of the region; from 1989 to 1991 the losses were estimated to have been approximately 2200 tons of fish per year (D. Qian pers. comm.).

The taxonomy of the genus *Aeromonas* has been revised, and new motile, mesophilic species have been identified: *A. allosaccharophila*, *A. veronii* biogroups *sobria* and *veronii* and *A. encheleia* have been reported as fish pathogens (Toranzo et al. 1989, Paniagua et al. 1990, Joseph & Carnahan 1994, Esteve et al. 1995). Still, *A. hydrophila* is regarded as the predominant fish pathogen within the mesophilic aeromonads, although its importance may have been overestimated in the past.

Thus, the purpose of the present study was to estimate the actual significance of *Aeromonas hydrophila* in disease outbreaks in fish and crab farms reported to The Zhejiang Institute of Freshwater Fisheries, Huzhou, China, supplementing conventional biochemical identification schemes with a molecular method.

MATERIAL AND METHODS

Bacterial isolates. A total of 95 bacterial isolates were isolated from moribund fish and crabs from 6 fish farms and 2 crab farms in the Zhejiang and Jiangsu Province in China during the summer of 1999 (Table 1). The sampled ponds all received water from local canals and their size varied from 1000 to 100 000 square meters. The construction and the management varied considerably between the farms. Seven fish farms consisted of excavated earth ponds with or without concrete walls. One farm was a dammed lake, and was the only farm using a polyculture farming system. These 8 farms had recently reported disease problems to The Zhejiang Institute of Freshwater Fisheries at the time when the field study was initiated. In each farm diseased crabs and fish were sampled. The main criteria for selection of individuals for further investigations were: (1) whether clear signs of disease were present and (2) whether it was practically possible to both watch and catch moribund crabs and fish with a hand net from the bank of the farm pond or a boat. Fish and crabs were caught and killed. From each fish, samples were collected from the kidney, liver, spleen, brain, gills and heart, as well as subcutaneous muscle tissue. From each crab samples were collected from kidney, liver, brain, gills, heart and muscle tissue. The surfaces of the organs were disinfected with 96% alcohol and an incision made with a sterile scalpel. A sample was taken using a mini-tip culturette collection and transport system (Becton Dickinson Microbiology Systems, Sparks, MD). The samples were stored in the mini-tip culturettes, which contained a modified Stuart's bacterial transport medium and kept at room tempera-

Table 1. Source of *Aeromonas hydrophila* isolates and total bacterial isolates from moribund fish in Chinese fish farms during the summer of 1999

Name of fish farm and location	Sampling date (mo/d/yr)	Fish species	No. of *A. hydrophila* isolates/total no. of isolates
Xiangshan Fish Farm,	5/21/99	Yellow headed catfish	0/2
Zhejiang Province	8/8/99	Large mouth buffalo[a]	0/2
	8/11/99	Large mouth buffalo[a]	1/7
Shiaoxin Crab Farm,	7/15/99	Rice eels	0/1
Zhejiang Province	8/12/99	River crab	0/4
	8/17/99	River crab	0/1
Ziff Eel Farm,	7/12/99	Rice eels	1/2
Zhejiang Province	8/11/99	Rice eels	0/4
		Large mouth buffalo[a]	0/7
	8/13/99	Grass carp	0/1
	8/16/99	Wuchang bream	3/6
		Crusian carp	1/2
		Japanese eel	0/5
	8/17/99	Black carp	0/3
Ziff Fish Farm,	8/11/99	Wuchang bream	0/4
Zhejiang Province		Curimbata[b]	0/1
Dai Xi Fish Farm,	8/11/99	Wuchang bream	7/10
Zhejiang Province		Crusian carp	2/4
Shangai Fish Farm,	8/20/99	Wuchang bream	3/6
Dai Feng,		Crusian carp	1/5
Jiangsu Province		Silver carp	0/1
		Japanese eel	0/5
Mai Feng Village,	8/21/99	Wuchang bream	5/5
Zhejiang Province		Crusian carp	4/4
Tongxiang crab farm,	8/17/99	River crab	0/2
Zhejiang Province			

[a]Imported from the US; [b]imported from Brazil

ture in an air-conditioned car. The samples were streaked on tryptic soy agar (TSA; Difco, Detroit, MI) plates within 10 h after collection and incubated at 37°C for 18 to 24 h. Following incubation, 1 typical colony (entire circular, convex, white to greyish, semitranslucent, size 2 to 3 mm, haemolytic) was selected from each plate with a pure culture and streaked onto TSA plates and stored at 15°C for a maximum of 10 d. Upon arrival in Denmark, the isolates were streaked on Blood Agar (BA; Difco, supplemented with 5% citrated calf blood) and incubated for 18 to 24 h at 37°C. The isolates were preliminary grouped according to colony morphology, haemolysis, and pigmentation before they were stored at –80°C in 15% glycerol until further characterization. The type strain of *Aeromonas hydrophila* ATCC 7966 (American Type Culture Collection) was included in the phenotypic characterization.

Phenotypic characterization. Isolates were classified as *Aeromonas hydrophila* according to their reactions in the following conventional tests: Gram stain, oxidase, catalase, motility, haemolysis on BA, arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase, indole, Voges-Proskauer, citrate (Simmon's), starch, urea, triple sugar iron (TSI), aesculin, arabinose, cellobiose, glucose (acid and gas production), sucrose and salicin (Barrow & Feltham 1993, Holt et al. 1994). Each substrate was incubated at 37°C and reactions read after 24 and 48 h.

Identification of *Aeromonas hydrophila* by PCR. For the isolation of genomic DNA, each isolate was grown at 37°C for 24 h on BA. A loopful (approximately 10 µg) of bacterial cells was resuspended in 1 ml phosphate-buffered saline (PBS, pH 7.5) in Eppendorf tubes followed by centrifugation at 5000 × *g* for 3 min. The pellets were resuspended in 100 µl 1:10 TE buffer (10 mM TRIS: 1 mM EDTA, pH 7.6) and placed in a 100°C water bath for 10 min. The cell lysates were placed on ice and 900 µl cold 1:10 TE buffer added. The cell lysates were stored at –20°C until the PCR analyses were done. The PCR-assay described by Dorsch et al. (1994) was modified as follows: Ready-to-go-PCR beads (Amersham Pharmacia Biotech, Uppsala, Sweden) were suspended in 23 µl distilled water and 1 µl of cell lysate and 0.5 µl of each of the 2 primer solutions (forward: 5'-GAAAGGTTGATGCCTAATACGTA-3'; reverse: 5'-CGTGCTGGCAACAAAGGACAG-3'; 10 pmol μl^{-1}) were added (DNA Technology, Aarhus, Denmark). Following initial denaturation (5 min at 94°C), the program consisted of 28 cycles with annealing at 60°C for 30 s, extension at 72°C for 45 s and denaturation at 94°C for 30 s, and a final extension step at 72°C for 7 min (Gene Amp PCR System 9700, Perkin Elmer, Foster City, CA). Aliquots of 8 µl PCR products were electrophoresed in 1.5% agarose (SeaKem, Medinova, Rockland, MA) gel for 3 h at 120 V, stained with ethidium bromide and visualized by UV light. A 100 bp ladder was used as a size standard (Amersham Pharmacia Biotech). *Aeromonas hydrophila* (ATCC 7966, HG1) (American Type Culture Collection, Hybridization Group), *A. bestiarum* (ATCC 51108, HG2), *A. caviae* (ATCC 15468, HG4), *A. sobria* (ATCC 35941, HG7), *A. jandaei* (ATCC 49568, HG9), *A. veronii* biogroup *veronii* (ATCC 35624, HG10), *A. veronii* biogroup *sobria* (ATCC 9071, HG8), *A. schubertii* (ATCC 43700, HG12), *A. allosaccharophila* (ATCC 51208, HG15) and *A. trota* (ATCC 49657, HG14) and 2 *A. hydrophila* strains (LMG 3740, LMG 3760; unknown HGs) (LMG, Laboratorium voor Microbiologie Universiteit Gent) isolated from fish and a human case of osteomyelitis, respectively, were also tested to ensure the specificity of the PCR. Distilled water was used as negative control.

Serotyping. Fourteen *Aeromonas hydrophila* isolates were serotyped using the serotyping system of mesophilic *Aeromonas* spp. by Dr T. Shimada (Sakazaki & Shimada 1984, Shimada & Kosako 1991). The isolates were selected to represent different fish farms, sampling dates, and fish species. Cell suspensions of each isolate were heated at 100°C for 1 h followed by washing and resuspension in saline to ensure optimal O-agglutinability with the polyclonal rabbit antisera. The system covers 97 O-antigens within the group of mesophilic *Aeromonas* spp., which is generally accepted to include *A. hydrophila*, *A. sobria* and *A. caviae* (Popoff & Veron 1976). The use of the O45-O97 antisera has not previously been described.

Lipopolysaccharide (LPS) extraction and polyacrylamide gel electrophoresis. The purpose of analysing the LPS profiles of the isolates was to establish if there were any correlation between the O-serogrouping and the LPS profiles. LPS was extracted from selected *Aeromonas hydrophila* isolates by a modification of the procedure of Hitchcock et al. (1983). Cells from each isolate tested were grown on BA at 37°C for 24 h and cells were harvested with 1 ml PBS, transferred to an Eppendorf tube and centrifuged at 13 000 × *g* for 10 min. The pellets were weighed and resuspended with a volume of PBS that ensured equal cell densities in all samples. The samples were incubated at 60°C for 20 min and centrifuged at 13 000 × *g* for 10 min. The pellet was discharged and 100 µl supernatant from each sample was placed in a 100°C water bath for 10 min. The heated suspension was cooled to room temperature on ice. Subsequently, 20 µl Proteinase K solution (Sigma, 2.5 mg ml^{-1}) was added to each sample followed by incubation at 60°C for 1 h. An aliquot of 62 µl of each sample was mixed with 13 µl ultrapure water and 25 µl NuPage sample buffer (NOVEX, San Diego, CA) and incubated at 70°C for 10 min. Finally, the samples were loaded on sodium dodecyl sulfate-polyacryl-

amide gels (10% [wt/vol]) and run for 1.5 h at 125 V. LPS profiles were visualized with silver staining (Silver Stain Kit, Bio-Rad, Richmond, VA) (Tsai & Frash 1982).

RESULTS

Isolation and identification of *Aeromonas hydrophila*

Pure cultures were obtained from all samples, and 88 bacterial isolates were obtained from individual fish and 7 isolates from individual crabs. The majority of these isolates (69/95) belonged to the motile aeromonads. A total of 35 isolates were identified as *Aeromonas hydrophila* by biochemical methods and of these, 29 isolates were identified as *A. hydrophila* by PCR analysis (Table 2). Six presumptive *A. hydrophila* isolates were not identified as *A. hydrophila* by PCR although their biochemical characteristics resembled those of the type strain ATCC 7966 (Table 2). The 6 isolates were positive in cellobiose fermentation, which is a test that is negative for both the type strain, and the 29 isolates identified as *A. hydrophila* by PCR. Variations in fermentation of sugars were also seen within the group identified as *A. hydrophila* by PCR when compared to the type strain ATCC 7966; 27 of 29 strains were positive in arabinose and a single strain was negative in sucrose fermentation. This illustrates that correct identification of *A. hydrophila* in our study required that the isolate was negative in cellobiose fermentation whereas fermentation of the other sugars tested could vary. Five sucrose-positive isolates gave the unexpected color combination red/yellow in TSI. The TSI reaction was read after both 24 and 48 h after inoculation since a yellow/yellow reaction in TSI is known to be able to change spontaneously to red/yellow. However, the colour combination red/yellow could not be explained. All *A. hydrophila* isolates in our study were lysine decarboxylase negative in repeated testing.

Table 2. Identification and characterization of *Aeromonas hydrophila*. V, variable; F, fermentative; y/y/–/–, yellow/yellow/no gas/no H_2S; r/y/+/–, red/yellow/gas/no H_2S

Biochemical tests	Literature (Millership 1996)	Type strain ATCC 7966	Characteristics of isolates identified as *A. hydrophila* by both PCR and biochemical tests						Characteristics of isolates identified as *A. hydrophila* by biochemical tests but negative in PCR		
No. of isolates		1	2	7	3	1	16		1	4	1
Gram stain	–	–	–	–	–	–	–		–	–	–
Motility	+	+	+	+	+	+	+		+	+	+
Hemolysis	+	+	+	+	+	+	+		+	+	+
Oxidase	+	+	+	+	+	+	+		+	+	+
Catalase	+	+	+	+	+	+	+		+	+	+
Hugh & Leifson	F	F	F	F	F	F	F		F	F	F
Decarboxylase:											
Arginine	+	+	+	+	+	+	+		+	+	+
Lysine	V	–	–	–	–	–	–		–	–	–
Ornithine	–	–	–	–	–	–	–		–	–	–
Urea	–	–	–	–	–	–	–		–	–	–
Triple sugar iron	y/y/–/–	y/y/–/–	r/y/+/–	y/y/+/–	y/y/–/–	r/y/–/–	y/y/–/–		y/y/–/–	r/y/+/–	r/y/–/–
Simmon's citrate	+	+	+	+	+	+	+		+	+	+
Indole	+	+	+	+	+	+	+		+	+	+
Voges-Proskauer	+	+	+	+	+	+	+		+	+	+
Aesculin	+	+	+	+	+	+	+		+	+	+
Gas from glucose	+	+	+	+	+	+	+		+	+	+
Acid from:											
Arabinose	V	–	+	+	–	+	+		–	–	–
Cellobiose	V	–	–	–	–	–	–		+	+	+
Sucrose	+	+	+	+	+	–	+		+	+	+
Salicin	+	+	+	+	+	+	+		+	+	+
O-serotype[a] of representative isolates (no. of isolates) LPS profile [I–V]		O1	O9 (1) [III] O97 (1) [II]	O9 (1) [III]	Rough (1) [I] O97 (1) [II] O64 (1) [IV]	O13 (1) [V]	O97 (3) [II] O9 (4) [III]		ND	ND	ND

[a]O-serotyping according to the scheme of Dr T. Shimada, National Institute of Infectious Diseases, Tokyo, Japan; ND, not determined

Occurrence of *Aeromonas hydrophila* and disease signs

Aeromonas hydrophila was isolated from samples from 5 of 6 fish farms and none of the samples from the 2 crab farms (Table 1). A total of 11 fish and crab species were sampled and *A. hydrophila* was isolated from the 4 fish species: large mouth buffalo *Ictiobus cyprinellus*, rice eels *Monopterus albus*, crucian carp *Carassius carassius* and Wuchang bream *Megalobrama amblycephala. A. hydrophila* was not isolated from the following fish and crab species: river crab *Eriocheir sinensis*, yellow headed catfish *Pelteobagrus fulvidraco*, grass carp *Ctenopharyngodon idellus*, Japanese eel *Anguilla japonica*, black carp *Mylopharyngodon piceus*, curimbata *Prochilodus lineatus*, and silver carp *Hypophthalmichthys molitrix*. *A. hydrophila* was isolated from 6% of the samples from large mouth buffalo and 14% of the samples from rice eels. In samples from the 2 fish species Wuchang bream and crucian carp, 58 and 53%, respectively, were positive for *A. hydrophila*. The main signs of the diseased fish were anorexia, exophthalmus, redding due to haemorrhage of the skin and swimming at the surface of the pond. At autopsy, the fish showed a haemorrhagic septicemia with haemorrhage in internal organs, intestine and a red tinged ascitic fluid. The disease signs seen in crabs were less pronounced. Behavioural changes such as low flight reactions and a tendency to stay at the bank near the water surface could be observed.

Serotyping and LPS profiles

The 14 representative isolates belonged to the 4 serogroups O9, O13, O64, and O97 while 1 strain was rough (Table 2). O9 and O97 were the dominant serotypes including 6 and 5 isolates, respectively. Five LPS profiles (I–V) were detected among the 14 isolates (Fig. 1). The LPS profiles correlated with the serogrouping (Table 2). Each serogroup was represented by 1 unique LPS profile.

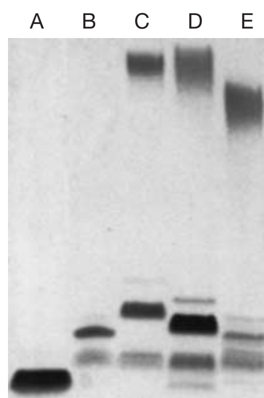

Fig. 1. Silver staining of 5 LPS profiles (I–V) detected among 14 *Aeromonas hydrophila* isolates. Lane: (A) Pattern I; (B) Pattern II; (C) Pattern III; (D) Pattern IV; (E) Pattern V

PCR identification of *Aeromonas hydrophila*

The type strains included in this study comprise all the currently recognized species and hybridization groups of the motile, mesophilic aeromonads except *Aeromonas encheleia*, HG11 and HG13, as the taxonomical status is incomplete (Martinez-Murica et al. 1992). The target sequences of the *A. hydrophila*-specific primers differ only with 2 or 3 nucleotides in relation to some of the *Aeromonas* sp. strains tested (positions 457–476 and 1115–1135 of the *Escherichia coli* numbering system; Dorsch et al. 1994). Despite the few differences of the *A. hydrophila*-specific primers, no amplification with other motile *Aeromonas* type strains than *A. hydrophila* were found. Positive reactions consisted of a 685 bp PCR product (Fig. 2). Larger or smaller amplicons of DNA from non-target regions were not observed under the conditions tested, and no amplification products were obtained from negative controls (Fig. 2). Six of 35 isolates did not give a positive reaction in the PCR assay, even though their biochemical properties fulfilled most criteria typical for *A. hydrophila* (Table 2).

DISCUSSION

This work supports the assumption that *Aeromonas hydrophila* is a common organism associated with dis-

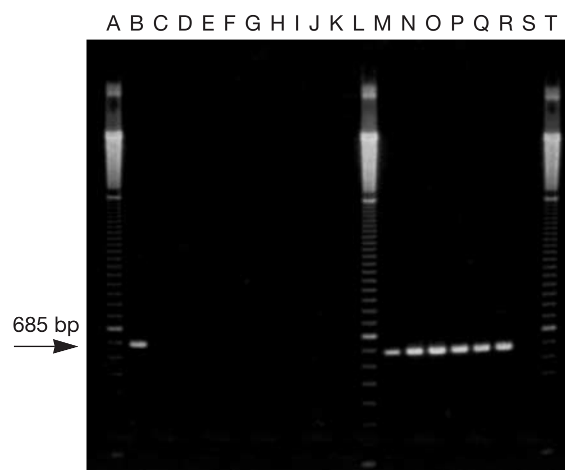

Fig. 2. Agarose gel electrophoresis of PCR-products. Positive reactions produced a 685 bp DNA fragment (arrow). A collection of *Aeromonas* type strains was tested (Lane B–K) along with isolates from water (Lane M, LMG 3740), human osteomyelitis (Lane N, LMG 3760) and from the Chinese fish farms in this study (Lanes O–R). Lane: (S) neg. control; (B) *A. hydrophila* ATCC 7966; (C) *A. bestiarum* ATCC 51108; (D) *A. salmonicida* NCIMB 1102; (E) *A. sobria* ATCC 43979; (F) *A. veronii* biogroup veronii ATCC 35624; (G) *A. caviae* ATCC 15468; (H) *A. trota* ATCC 49657; (I) *A. jandaei* ATCC 49568; (J) *A. schubertii* ATCC 43700; (K) *A. allosaccharophila* ATCC 51208; (A), (L) and (T) 100 bp DNA-ladder

ease outbreaks in aquaculture in the Zhejiang province in China. As few characters differentiate, mesophilic *Aeromonas* spp. and misidentification may occur when precautious means of identification are not applied. In the present study characterization by biochemical methods were supported by the use of PCR. The assay was validated with motile, mesophilic *Aeromonas* sp. type strains. No amplification was seen in any *Aeromonas* strain other than *A. hydrophila* and no amplification products were obtained from negative controls. Thus, it ensured an improved identification of *A. hydrophila* isolated from moribund fish in Chinese aquaculture compared to an identification based solely on phenotypic, biochemical testing.

Biochemical tests identified 69 isolates as motile aeromonads and 35 isolates as *Aeromonas hydrophila.* Six of the 35 isolates identified as *A. hydrophila* by biochemical tests could not be identified as *A. hydrophila* by the PCR method and these isolates were assumed to belong to other motile mesophilic *Aeromonas* sp. A possible explanation for this divergence could be that many biochemical identification schemes are based on the analysis of human clinical isolates and that fish isolates may differ in several biochemical characters. Different reaction patterns can also be caused by differences in optimal growth temperatures, incubation at 37°C may not be appropriate for fish isolates from temperate regions (Haenninen et al. 1994). In this study, however, we chose to incubate at 37°C since the Zhejiang province has a subtropical climate.

In the literature, *Aeromonas hydrophila* is usually reported to be lysine decarboxylase positive, and this criterium is frequently included in biochemical identification of this species (Millership 1996, Qian et al. 1997). However, Iqubal et al. (1998) have reported the *A. hydrophila* type strain ATCC 7966 to be lysine decarboxylase negative, which was confirmed in this study.

Aeromonas hydrophila was not isolated from any of the 7 samples obtained from moribund crab. *A. hydrophila* was a minor disease problem in large mouth buffalo and rice eels since the bacterium occurred with low frequencies, 6 and 14% respectively, in these samples. Fish farms producing Wuchang bream and crucian carp were more heavily affected by disease outbreaks associated with *A. hydrophila*; 58 and 53% of the samples, respectively. Thus, *A. hydrophila* appeared to be a minor problem to large mouth buffalo and rice eels while contributing significantly to disease outbreaks among Wuchang bream and crucian carp. However, the diversity among the isolates, according to serotyping and biochemical profile, from moribund fish indicated that the bacteria isolated were secondary invaders in compromised hosts. *A. hydrophila* appears to be an important species among these fish. Furthermore this organism is a well-known opportunistic pathogen in freshwater aquatic habitats with heavily organic load (Austin & Adams 1996). Among the 14 representative *A. hydrophila* isolates the 2 serotypes O9 and O97 were dominant. Furthermore all the identified serotypes were represented by unique LPS profiles.

Aeromonas hydrophila has been associated with EUS, which is a major problem in Southeast Asia, but the etiology of the disease is unclear (Iqubal et al. 1998). However, the gross signs of EUS (ulcerated lesions) are different from the sign seen in the moribund fish in our study (haemorrhage of muscle tissue). Therefore, the recurrent outbreaks of bacterial haemorrhagic septicaemia in Zhejiang Province cannot be classified as EUS.

Two-thirds of the isolates were not identified beyond the genus level, since our study primarily focused on the occurrence of *Aeromonas hydrophila*. Our findings indicate that *A. hydrophila* should still be regarded as contributor to the disease problems in the Zhejiang region although this organism (1) was only retrieved from approximately one-third of the diseased fish investigated, and (2) may be regarded as an opportunistic invader following a primary stressor. However, it is important to understand that Chinese aquaculture differs in many aspects from the advanced aquaculture systems in industrialized countries. Water quality is an essential parameter for effective and disease-free aquaculture systems, but in the fish farms included in the present study there are no means to regulate or improve water quality. *A. hydrophila* is more abundant in waters with a high organic load than in relatively unpolluted water (Jeney & Jeney 1995). The fish ponds in our study all have muddy water with a high organic load, and since *A. hydrophila* is a natural inhabitant of these subtropical aquatic environments the fish are constantly exposed to infection. Disease outbreaks with significant economical losses caused by motile *Aeromonas* spp. are common in the Zhejiang province in the summer (D. Qian pers. comm.) and outbreaks are probably due to higher stress in this period, e.g. because of heavy parasite infections (Nie & Yao 2000), high water temperatures and low oxygen content. In conclusion, *A. hydrophila* was found to be one of the predominant motile *Aeromonas* species recovered from diseased fish with hemorrhagic septicemia, although the etiology of the investigated diseases outbreaks seems to be more complex than initially anticipated.

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