Journal of Applied Microbiology 2002, **93**, 1034–1041

Phenotypic characteristics and pathogenicity of *Aeromonas* genomospecies isolated from common carp (*Cyprinus carpio* L.)

A. Kozińska[1], M.J. Figueras[2], M.R. Chacon[2] and L. Soler[2]

[1]*Department of Fish Disease, National Veterinary Research Institute, Putawy, Poland and* [2]*Departamento de Ciencias Mèdicas Básicas, Facultad de Medicina y Ciencias de la Salud, Universidad Rovira y Virgili, Reus, Spain*

2002/134: received 25 March 2002, revised 19 August 2002 and accepted 6 September 2002

A. KOZIŃSKA, M.J. FIGUERAS, M.R. CHACON AND L. SOLER. 2002.

Aims: To evaluate the relationship between the genomospecies, phenotypic profile and pathogenicity for carp of 37 motile *Aeromonas* strains.

Methods and Results: *Aeromonas* strains were identified to genomospecies level by the 16S rDNA restriction fragment length polymorphism (RFLP) method and characterized phenotypically by the API 20E and API Zym systems and by conventional tube or plate methods. 16S rDNA RFLP analysis showed that the strains belonged to five species, *Aeromonas bestiarum* (5), *Aerom. salmonicida* (13), *Aerom. veronii* (11), *Aerom. sobria* (6) and *Aerom. encheleia* (2). Most strains of *Aerom. bestiarum* (80%) and *Aerom. salmonicida* (85%) could be separated by growth at 4 and 42°C, autoagglutination after boiling, reaction for lipase (C14) and naphthol-AS-BI-phosphohydrolase. All strains of *Aerom. veronii* corresponded to *Aerom. veronii* biotype *sobria* and could be separated from *Aerom. sobria* by citrate utilization, growth at 37 and 42°C, amygdalin and cellobiose fermentation. All strains of *Aerom. bestiarum* and most strains of *Aerom. salmonicida* (76·9%) and *Aerom. veronii* (63·6%) were pathogenic for carp.

Conclusions: The biochemical identification of carp *Aeromonas* strains is not entirely clear. Some association between *Aeromonas* species, phenotypic profile and specific disease signs was observed.

Significance and Impact of the Study: The results will be useful for ichthyopathology laboratories in the diagnosis of motile aeromonad septicaemia in carp.

INTRODUCTION

Aeromonas spp. are ubiquitous waterborne bacteria responsible for a wide spectrum of diseases among poikilothermic and homoiothermic organisms including humans (Austin and Austin 1987; Khardori and Fainstein 1988).

Two phenotypically distinct groups are well known within the genus *Aeromonas*: (i) psychrophilic and non-motile *Aeromonas salmonicida*, which are relatively homologous phenotypically and (ii) mesophilic and motile aeromonads, which are heterologous. Within the latter group three phenospecies have been recognized, *Aerom. hydrophila*, *Aerom. caviae* and *Aerom. sobria* (Popoff 1984). All these phenospecies are well known as fish pathogens (Toranzo *et al*. 1989; Candan *et al*. 1995; Kozińska 1996; Ogara *et al*. 1998; Austin and Austin 1999).

In recent years, the classification of the genus *Aeromonas* has undergone major changes. Extended DNA–DNA hybridization studies or other molecular techniques and improved phenotypic methods have resulted in the recognition of 14 well-defined genomic species within the genus (Altwegg and Geiss 1989; Janda 1991; Martinez-Murcia *et al*. 1992; Joseph and Carnahan 1994; Esteve *et al*. 1995b; Ali *et al*. 1996; Huys *et al*. 1997a,1997b). These species are as follows: *Aerom. hydrophila*, *Aerom. bestiarum*, *Aerom. salmonicida* (comprising non-motile and psychrophilic as well as motile and mesophilic strains), *Aerom. caviae*, *Aerom.*

Correspondence to: A. Kozińska, National Veterinary Research Institute, Department of Fish Diseases, Al. Partyzantów 57, 24-100 Pulawy, Poland.

media, *Aerom. eucrenophila*, *Aerom. sobria*, *Aerom. veronii* (with two biotypes *sobria* and *veronii*), *Aerom. jandaei*, *Aerom. trota*, *Aerom. schubertii*, *Aerom. encheleia*, *Aerom. allosaccharophila* and *Aerom. popoffii*.

A wide range of *Aeromonas* species have been found in association with diseased or healthy fish (Martinez-Murcia *et al.* 1992; Sugita *et al.* 1994, 1995; Esteve *et al.* 1995a; Esteve *et al.* 1995b; Huys *et al.* 1996), but only some of them have been documented as fish pathogens (Torres *et al.* 1993; Esteve *et al.* 1995a). Since the changes in *Aeromonas* classification, the importance of recently described species in fish pathology is mostly unknown. DNA–DNA hybridization techniques are used to accurately identify isolates to the genomospecies level, but are not available in routine ichthyopathology laboratories. For this reason, most of these laboratories identify motile *Aeromonas* as members of the '*Aerom. hydrophila*' complex, which includes *Aerom. hydrophila*, *Aerom. bestiarum* and the motile biogroup *Aerom. salmonicida*; the '*Aerom. caviae*' complex, which includes *Aerom. caviae*, *Aerom. media* and *Aerom. eucrenophila*; and the '*Aerom. sobria* 'complex, which includes *Aerom. sobria*, *Aerom. veronii*, *Aerom. jandaei* and *Aerom. trota* (Janda 2001).

Recently, 16S rDNA restriction fragment length polymorphism (RFLP) methods have been developed for the identification of all known *Aeromonas* species (Borrel *et al.* 1997; Figueras *et al.* 2000). Moreover, several schemes for the phenotypic identification of *Aeromonas* genomospecies from clinical and environmental sources have been proposed (Altwegg *et al.* 1990; Abbot *et al.* 1992; Oakey *et al.* 1996; Brunner and Stolle 1997; Borrel *et al.* 1998). The identification of some of the genomic species mentioned above is still difficult because of some discrepancies between the DNA homology and phenotypic characters of various *Aeromonas* species.

There has been only a limited amount of investigation into the association of specific biochemical characters with specific *Aeromonas* species originating from fish (Austin *et al.* 1989; Esteve 1995; Noterdaeme *et al.* 1996; Carson *et al.* 2001; González *et al.* 2001). In the present study, numerous strains from common carp (*Cyprinus carpio* L.) were identified by 16S rDNA RFLP analysis and characterized phenotypically. The relationship between the genomospecies, phenotypic profile and pathogenicity for carp of these strains was evaluated.

MATERIALS AND METHODS

Bacterial strains

Thirty-four strains of motile aeromonads isolated from healthy or diseased carp and two strains from tank water were used in this study. All strains were obtained from carp farms in Poland during the last 10 years. Bacteria were isolated by inoculating plates of R-S agar (Shotts and Rimler 1973) with fish skin and kidney or water samples and incubating the cultures at 28°C for 24 h. Yellow colonies were then selected and identified to the genus *Aeromonas* with a panel of tests according to Popoff (1984). All strains were Gram-negative rods, positive for oxidase, catalase, motility, glucose oxidation/fermentation (O/F test), 0/129 resistance and growth in nutrient broth without but not with 6% NaCl. Additionally, one strain previously identified biochemically as non-pigmented *Chromobacterium violaceum*-like (Kozińska and Antychowicz 1996) was used.

Identification to the genomospecies level

All strains were identified by the 16S rDNA RFLP technique as previously described (Borrel *et al.* 1997; Figueras *et al.* 2000). In brief, the method consists of the digestion of the complete polymerase chain reaction-amplified 16S rDNA gene using two endonucleases (*Alu*I and *Mbo*I) simultaneously, which enables the identification of *Aerom. hydrophila*, *Aerom. caviae*, *Aerom. media*, *Aerom. veronii*, *Aerom. jandaei*, *Aerom. eucrenophila*, *Aerom. sobria*, *Aerom. trota*, *Aerom. allosaccharophila* and *Aerom. schubertii*. However, *Aerom. bestiarum*, *Aerom. salmonicida*, *Aerom. popofii* and *Aerom. encheleia* exhibit the same RFLP pattern after this digestion (Borrel *et al.* 1997). To identify *Aerom. popofii* and *Aerom. encheleia*, further digestion with the enzyme *Nar*I followed by *Hae*III and *Alu*NI is necessary while for *Aerom. bestiarum* and *Aerom. salmonicida Nar*I followed by *Pst*I is required (Figueras *et al.* 2000).

Extended phenotypic studies

All strains were tested for 20 characteristics by the API 20E system, 19 characteristics by the API Zym system (bio Mérieux, MarcyL'Etoile, France) and 26 other phenotypic characters. Selected biochemical tests were carried out according to Abbot *et al.* (1992) and Carson *et al.* (2001) enabling discrimination of *Aeromonas* genomospecies. These tests included gas production from glucose, aesculin hydrolysis, salicin fermentation, gluconate oxidation, elastase production, H_2S production from cysteine, DL-lactate utilization, phenylalanine deaminase, acid production from D-mannose, cellobiose, glycerol, lactose, resistance to cephalothin and ampicillin. Moreover, all strains were characterized for the fermentation of xylose, trehalose, maltose, fructose and raffinose, growth at 4, 37 and 42°C, agglutination in brain heart infusion (BHI) broth before and after boiling (1 h at 100°C) and haemolysis activity (on trypticase soy broth supplemented with 5% (v/v) horse blood) Additionally, ornithine decarboxylase, lysine decarboxylase, arginine dihydrolase, citrate utilization (Simmons) and fermentation of D-mannitol and L-arabinose were

performed using conventional tube tests to confirm the results obtained by the API 20E tests. Bacterial strains were incubated at 27°C and the results read off after 24 h for API 20E, API Zym, haemolysis and autoagglutination or after 2–7 d for the remaining tests.

Pathogenicity assays

Common carp (*C. carpio* L.) of 150 g average weight were obtained from a fish farm in Poland and certified as disease free. The fish were maintained in 100-l aquaria with aerated fresh water at 16°C for 2 weeks and fed commercial pellets (Carp K30; Aqua Pasze, Olsztyn, Poland) in order to adapt to laboratory conditions. All fish were anaesthetized with MS-222 (Sigma, St. Louis, MO, USA) (80 mg l^{-1}) and then injected subcutaneously with 0·1 ml of a suspension containing 10^6 bacterial cells in phosphate-buffered saline (PBS). Ten fish were injected per strain and ten other fish were injected with 0·1 ml of sterile PBS as controls. The fish were then replaced into the aquaria and held under the same conditions as before injection. The morbidity and death of the fish were monitored daily for 7 d and the virulence level of the strains estimated and grouped as follows: more than six fish with disease symptoms and mortality of five to 10 fish (strongly virulent); more than six fish with disease symptoms and mortality of one to four fish (virulent); more than four fish with disease symptoms and without mortality (weakly virulent) and one to four fish with slight pathological signs and without mortality or neither pathological signs nor mortality noted (avirulent). Recently dead fish and any survivors after 7 d were tested bacteriologically for the presence of injected bacterium in damaged fish tissues.

RESULTS

Most *Aeromonas* strains (92%) could be subdivided into two phenogroups (phenospecies) according to Popoff's criteria. The first group, '*Aerom. hydrophila*' complex, comprised 14 strains which were positive for arabinose and salicin fermentation and aesculin hydrolysis. The second group, '*Aerom. sobria*' complex, comprised 19 strains which were negative for the former two tests and revealed variable results for aesculin hydrolysis. Three remaining strains of *Aeromonas* spp. could not be classified to phenospecies by these criteria.

16S rDNA restriction fragment length polymorphism analysis

Of 14 strains from the phenotypic '*Aerom. hydrophila*' complex, 10 were assigned to *Aerom. salmonicida* and four to *Aerom. bestiarum* by 16S rDNA RFLP analysis. No strain was classified as *Aerom. hydrophila* (*sensu stricto*). Of 19 phenotypic '*Aerom. sobria*' strains, six exhibited the RFLP pattern of *Aerom. sobria* (*sensu stricto*), nine the RFLP pattern of *Aerom. veronii* and two the RFLP pattern of *Aerom. encheleia* and *Aerom. salmonicida*. Three different *Aeromonas* spp. strains were identified as *Aerom. bestiarum*, *Aerom. salmonicida* and *Aerom. veronii*. One strain, previously identified phenotypically as *Ch. violaceum*-like, showed the RFLP pattern of *Aerom. veronii* (Table 1).

Phenotypic analysis

All strains tested were invariably positive for maltose and fructose fermentation and resistant to ampicillin and 0/129. All strains were positive, by API 20E, for ortho-nitrophenylo-galactosidase (ONPG), gelatinase and D-glucose and, by API Zym, for alkaline phosphatase, esterase, esterase lipase, leucine arylamidase and acid phosphatase. Moreover, D-mannitol fermentation and arginine dihydrolase were positive for each strain by API 20E as well as conventional tube tests. Invariably, negative results were observed for hydrogen sulphide, urease, inositol (all by API 20E), α-chymotrypsin, α-galac- tosidase, β-glucuronidase, α-mannosidase, α-fucosidase (all by API Zym), ornithine decarboxylase and lactose fermentation. Only one strain (*Aerom. salmonicida*) was negative for indole production (by API 20E) and positive for the fermentation of

Table 1 Phenotypic and genotypic identification of strains used in this study

	% identity by 16S rDNA RFLP				
Phenospecies	*Aerom. bestiarum*	*Aerom. salmonicida*	*Aerom. sobria*	*Aerom. veronii*	*Aerom. encheleia*
Aerom. hydrophila	80·0 (4)	76·9 (10)			
Aerom. sobria		15·4 (2)	100 (6)	81·8 (9)	100·0 (2)
Aeromonas spp.	20·0 (1)	7·7 (1)		9·1 (1)	
Chromobacterium violaceum-like				9·1 (1)	

Numbers in parentheses = number of strains.
RFLP, Restriction fragment length polymorphism.

Table 2 Phenotypic properties for the separation of motile *Aeromonas* species isolated from carp

Character	% of positive strains				
	Aerom. bestiarum (5)*	*Aerom. salmonicida* (13)	*Aerom. veronii* (11)	*Aerom. sobria* (6)	*Aerom. encheleia* (2)
API 20E					
Citrate utilization	0	46	64	17	0
Tryptophan deaminase	0	0	9	0	0
Voges–Proskauer reaction	100	92	56	50	0
Sorbitol	40	62	0	50	0
Rhamnose	60	62	0	0	0
Amygdalin	80	92	0	100	100
Arabinose	100	69	0	17	0
Nitrate reduction to nitrite	100	100	91	100	100
Nitrite reduction	0	0	9	0	0
API ZYM					
Lipase (C14)	20	92	64 (w)	67 (w)	0
Trypsin	80	92	18	17	100
Naphthol-AS-BI-phosphohydrolase	0	77	100	100	50
α-glucosidase	20	15	18	67	0
β-glucosidase	80	85	0	0	0
Other characters					
Acid production from:					
salicin	60	69	0	17	0
cellobiose	60	62	18	83	50
Aesculin hydrolysis	100	69	0	33	50
Elastase production	80	77	0	0	0
β-haemolysis (after 24 h)	100	100	73	33	100
Growth at (°C):					
4	100	23	36	0	0
37	100	100	91	17	100
42	80	23	73	17	0
Autoagglutination	0	15	45	17	0
Agglutination after boiling	20	69	100	83	100
Resistance to cephalothin	100	92	9	17	100

*Number of strains.
w, Weakly positive reaction.

xylose and two strains (one each of *Aerom. bestiarum* and *Aerom. veronii*) were negative for the fermentation of trehalose.

The *Aeromonas* strains tested revealed variable results for 39 tests. Of these, 25 tests (Table 2) were found to be useful in separating motile *Aeromonas* genomospecies. Strains belonging to both *Aerom. bestiarum* and *Aerom. salmonicida* could be differentiated from remaining genomospecies on the basis of the reactions for rhamnose, arabinose, salicin, elastase and β-glucosidase. At least three of these tests were positive for individual strains of *Aerom. bestiarum* or *Aerom. salmonicida*. A few other tests could be useful to separate *Aerom. bestiarum* and *Aerom. salmonicida*. Most strains of the former species, as opposed to the latter, were negative for lipase, naphthol-AS-BI-phosphohydrolase and agglutination after boiling but positive for growth ability at 4 and 42°C.

Most strains of *Aerom. veronii* differed from *Aerom. sobria* and *Aerom. encheleia* by positive reactions for citrate utilization (by both API 20E and tube methods), Voges–Proskauer test, growth at 42°C and a failure to ferment amygdalin and cellobiose. Most strains of *Aerom. sobria* differed from those of *Aerom. encheleia* by positive reactions for lipase, α-glucosidase and negative results for trypsin, β-haemolysis, growth at 37°C and resistance to cephalothin.

The strain *Aerom. veronii*, previously identified as *Ch. violaceum*, differed distinctly from other *Aeromonas* species. This strain was positive for tryptophan deaminase and negative for nitrate reduction to nitrite but positive for nitrite reduction (by API 20E tests).

Table 3 Virulence for common carp of motile *Aeromonas* species

Virulence level	% of strains with respective virulence level				
	Aerom. bestiarum	*Aerom. salmonicida*	*Aerom. veronii*	*Aerom. sobria*	*Aerom. encheleia*
Strongly virulent	20 (1)	31 (4)	9 (1)		
Virulent	60 (3)	31 (4)	55 (6)		
Weakly virulent	20 (1)	15 (2)			
Avirulent		23 (3)	36 (4)	100 (6)	100 (2)

Numbers in parentheses = number of strains.

Pathogenicity for carp

There was no death or sign of disease in fish injected with sterile PBS. Table 3 presents the virulence level of *Aeromonas* strains tested in this study. Four strains of *Aerom. bestiarum* were classified as strongly virulent or virulent for carp. These strains caused motile aeromonad septicaemia with intensive external signs including haemorrhages, necrosis and ulcers. Between 10 and 80% of the fish died during the experiment, depending on the infection strain used. Only one *Aerom. bestiarum* strain presented weak virulence for carp and caused no mortality. There were differences in the pathogenicity among individual strains of *Aerom. salmonicida* and *Aerom. veronii*. Ten strains (from a total of 13 tested) of *Aerom. salmonicida* were pathogenic for carp with various levels of virulence. The symptoms caused by these pathogenic strains were similar to those observed in fish infected with *Aerom. bestiarum*. The *Aerom. veroniii* strain previously identified as *Ch. violaceum* was classified as strongly virulent for carp. It caused intensive external as well as internal lesions which presented ascitic fluid in the peritoneal cavity and haemorrhages in the kidney and liver with 100% mortality. Six other strains of this species were classified as virulent and four as avirulent. The symptoms caused by pathogenic strains of *Aerom. veronii* were usually similar to those observed in fish challenged with *Aerom. bestiarum* but, in some fish, there was also a swelling of infected areas and accumulation of red-tinged ascitic fluid in subcutaneous tissue. Pure cultures of injected bacteria were obtained from samples of recently dead fish and also from the damaged skin and kidney of survivors (after 7 d). Of six *Aerom. sobria* strains, only one caused slight clinical signs with local damage of skin in 30% of fish. *Aeromonas encheleia* strains caused neither signs of disease nor mortality.

Strongly virulent, virulent and these avirulent strains formed separate phenotypic groups within *Aerom. salmonicida* as shown in Table 4. Similarly, all virulent strains differed phenotypically from avirulent strains of *Aerom. veronii* (Table 5). However, the strongly virulent strain of *Aerom. veronii* differed markedly from the remaining pathogenic strains of the species.

Table 4 Phenotypic differences among *Aeromonas salmonicida* strains showing different virulence levels

Character	Strains		
	Strongly virulent (4) and virulent (4)	Weakly virulent (2)	Avirulent (3)
Acid from:			
rhamnose	+	d	–
arabinose	+	d	[–]
salicin	+	d	–
Aesculin hydrolysis	+	d	–
Elastase production	+	d	[–]

+, All strains positive; d, differences among strains, one strain positive and another negative; –, all strains negative; [–], two strains (67%) negative and one strain (33%) positive. Numbers in parentheses = number of strains.

Table 5 Phenotypic differences among virulent and avirulent *Aeromonas veronii* strains

Character	Strains	
	Virulent (6)	Avirulent (4)
Voges–Proskauer reaction	+	–
Growth at (°C):		
4	–	+
42	+	[–]
Autoagglutination	–	+

+, All strains positive; –, all strains negative; [–], three strains (75%) negative and one strain (25%) positive. Numbers in parentheses = number of strains.

DISCUSSION

The taxonomy of the genus *Aeromonas* has been extended in recent years because of the description of new mesophilic species. These species can be accurately identified by molecular techniques but biochemical systems for the discrimination of genomospecies have recently been developed. Some of these biochemical schemes have been used mainly for the identification of clinical and environmental isolates and have enabled most of the strains (90–99%) to be identified (Abbot *et al*. 1992; Janda *et al*. 1996; Brunner and Stolle 1997; Borrel *et al*. 1998). The protocol described by Carson *et al*. (2001) was useful in the discrimination of numerous strains from farmed and wild salmonid fish. To the authors' knowledge, the present study is the first investigation concerning the phenotypic differentiation of *Aeromonas* carp isolates accurately identified by 16S rDNA RFLP analysis.

Abbot *et al*. (1992) suggested that elastase production, fermentation of D-sorbitol, salicin and lactose are specifically

positive for *Aerom. salmonicida* and D-rhamnose for *Aerom. bestiarum* (formerly HG2). Most strains of *Aerom. bestiarum* examined by Carson *et al*. (2001) were positive for rhamnose and salicin fermentation. In our investigations, most *Aerom. salmonicida* as well as *Aerom. bestiarum* isolates were positive for salicin, rhamnose and elastase and all were negative for lactose. These results are in agreement with those obtained by Noterdaeme *et al*. (1996). Thus, the discrimination of *Aerom. bestiarum* and *Aerom. salmonicida* isolates from carp was very difficult if based on these characteristics. A similar problem has been reported by Figueras *et al*. (2000). These authors identified unequivocally only 18% of *Aerom. bestiarum* and *Aerom. salmonicida* when biochemical tests were applied. Other properties seem to be helpful in separating carp strains of these species. In this study, we found that growth at 4 and 42°C was specific for *Aerom. bestiarum* and positive reactions for lipase (C14), naphthol-AS-BI-phosphohydrolase and agglutination after boiling were specific for most *Aerom. salmonicida* strains. These tests were still not sufficient to correctly identify all these strains; the differentiation of one *Aerom. bestiarum* and two *Aerom. salmonicida* strains was very difficult because of phenotypic homogeneity across both species. This aspect is common to other strains and is currently under investigation (M.J. Figueras, personal communication).

The phenotypic separation of the species *Aerom. sobria*, *Aerom. veronii* and *Aerom. encheleia* was also problematic because of large variations within individual species. The reported biochemical profile of *Aerom. sobria* is usually negative for arginine dihydrolase and the Voges–Proskauer reaction (Abbot *et al*. 1992; Noterdaeme *et al*. 1996; Borrel *et al*. 1998). All *Aerom. sobria* strains tested in this study were arginine positive as previously found by Carson *et al*. (2001). All strains of *Aerom. veronii* corresponded phenotypically to *Aerom. veronii* biotype *sobria* according to the profile proposed by Hickman-Brenner *et al*. (1987). They were positive for arginine and negative for ornithine and aesculin.

In our study, *Aerom. veronii* strains could be separated from *Aerom. sobria* and *Aerom. encheleia* by the ability to utilize citrate, growth at 37 and 42°C, negative reactions for amygdaline and cellobiose fermentation. All *Aerom. sobria* strains, in disagreement with the results of Carson *et al*. (2001), were resistant to ampicillin.

Two strains of *Aerom. encheleia* corresponded biochemically to the species described by Esteve *et al*. (1995b) except for the fermentation of rhamnose and lysine decarboxylase. The strains tested in our study were negative for the former and positive for the latter. The separation of *Aerom. encheleia* and *Aerom. sobria* strains was possible by lipase, α-glucosidase (negative for the former species and positive for the latter), β-haemolysis, growth at 37°C and resistance to cephalothin (again positive for the former species and negative for the latter).

Generally, members of the genus *Aeromonas* are considered positive for nitrate reduction to nitrite but negative for nitrite reduction (Popoff 1984). Our study revealed that the strain of *Aerom. veroniii* previously identified as *Ch. violaceum* reduced nitrite by the API 20E test.

In most pathology studies, motile aeromonads isolated from fish have been assigned to Popoff's species or simply reported as motile *Aeromonas* or '*Aerom. hydrophila*' complex (Santos *et al*. 1988; Del Corral *et al*. 1990; Chabot and Thune 1991; Kozińska 1996). Other documented species pathogenic for fish are *Aerom. hydrophila*, *Aerom. jandaei* and *Aerom. veronii* (Esteve *et al*. 1995a; Austin and Austin 1999; Guzman-Murillo *et al*. 2000). *Aeromonas bestiarum* (formerly HG2 *Aerom. hydrophila*) has been recovered from diseased fish (Huys *et al*. 1996). However, negligible information is available about its pathogenicity. There is also no information about the pathogenicity for fish of mesophilic and motile strains of *Aerom. salmonicida* (formerly HG3 *Aerom. hydrophila*). This is the first report to document the pathogenicity of these new species for fish. All *Aerom. bestiarum* and most *Aerom. salmonicida* strains (motile) were isolated from diseased carp and, after experimental infection, caused the disease in fish.

Earlier reports indicated that *Aerom. sobria* strains can be pathogenic for carp (Kozińska 1996) as well as for other fish species (Santos *et al*. 1988; Toranzo *et al*. 1989). The present study showed that all pathogenic strains from the phenotypic '*Aerom. sobria*' group belong, in fact, to *Aerom. veronii*. *Aeromonas sobria* (*sensu stricto*) appeared to be non-pathogenic for carp.

It is important in pathology studies to differentiate the pathogenic from the non-pathogenic strains. The results of our study showed that the taxonomic designation of some species, such as *Aerom. salmonicida* and *Aerom. veronii*, is insufficient to determine their pathogenicity for carp. It was found in previous studies that pathogenic strains of motile aeromonads differ from non-pathogenic strains in their haemolytic, proteolytic and cytotoxic activity, presence of fimbriae and normal serum resistance (Leung *et al*. 1994; Kozińska 1996; Sopińska *et al*. 1997). The present study showed that some other biochemical or cultural properties can be helpful to separate pathogenic and non-pathogenic strains within individual *Aeromonas* species. Only one highly virulent strain of *Aerom. veronii* differed markedly from the remaining pathogenic strains of the species. The strain may be considered as atypical because its biochemical profile is not quite compatible with the genus *Aeromonas* (Kozińska and Antychowicz 1996). Further studies are needed to find an explanation for this.

In conclusion, it seems that biochemical protocols proposed previously to differentiate *Aeromonas* genomospecies will require further revision because of the diversity of these organisms. While some schemes can correctly identify

aeromonads from human specimens and the environment, their use in the detection of species of ichthyopathological importance is limited. Despite some phenotypic differences between *Aeromonas* species isolated from carp, their identification by biochemical methods is not entirely clear and must be regarded as only presumptive. At least three *Aeromonas* species are virulent for carp. Some differences in the lesions observed in infected fish suggest the association of individual species with specific disease symptoms. Further studies are needed to confirm this suggestion.

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