Typing of Clinical and Environmental *Aeromonas* sp. Strains by Random Amplified Polymorphic DNA PCR, Repetitive Extragenic Palindromic PCR, and Enterobacterial Repetitive Intergenic Consensus Sequence PCR

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A collection of 120 strains isolated from stool specimens collected from humans suffering from gastroenteritis and from environmental samples were analyzed by random amplified polymorphic DNA PCR (RAPD), repetitive extragenic palindromic PCR (REP-PCR), and enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR). Species of *Aeromonoas hydrophila*, *A. bestiarum*, *A. salmonicida*, *A. caviae*, *A. media*, and *A. veronii* revealed clonal structure. There was no dominant clone causing gastroenteritis in humans. Moreover, there was no genetic similarity between clinical and environmental strains of *Aeromonas* sp. isolated from different geographical areas as well as from the same geographical area. Some clones colonized specific ecosystems, e.g., drinking water distribution systems. RAPD and ERIC-PCR methods had the same discriminatory power and proved to be useful for epidemiological investigation and population genetic analysis of *Aeromonas* spp., whereas REP-PCR was less effective for differentiating the isolates of *Aeromonas* spp.

Bacteria of *Aeromonas* sp. are gram-negative, straight cells (rod-shaped to coccoid) with rounded ends. They are oxidase and catalase positive, reduce nitrate to nitrite, and ferment D-glucose. These bacteria are widely spread in the environment, especially in surface water and sewage; they also occur in untreated and treated drinking water (1, 2, 4, 18). In humans, *Aeromonas* spp. are responsible for gastroenteritis, chronic diarrhea, wound infections, respiratory tract infections, peritonitis, urinary tract infections, and septicemia (2, 17). Among *Aeromonas*-associated infections of humans *A. hydrophila*, *A. caviae*, and *A. veronii* are the predominating species, whereas *A. eucrenophila*, *A. popoffii*, (2), and *A. culicicola* (28) have never been found in clinical samples. Some *Aeromonas* species are associated with a wide variety of diseases in cold- and warm-blooded animals, including fish, frogs, water buffaloes, reptiles, birds, and cattle (16, 28). *A. veronii*, *A. hydrophila*, and *A. salmonicida* are capable of causing septicemia in freshwater and marine fish (3, 9). Clinical and environmental *Aeromonas* sp. isolates secrete many extracellular products, such as hemolysins, enterotoxins, and proteases. Studies conducted by Kühn et al. (22) showed that some isolates of a given species produce virulence factors more frequently than others. These findings indicate that the virulence within the genus *Aeromonas* might be a clonal property and only some clones may be responsible for progressive disease. However, there have been no studies that would have determined clonal structure within *Aeromonas* spp. and the spread of specific clones in human population and in the environment. Our study was undertaken to recognize the clonal relatedness of strains derived from diarrheal stool spec-

imens collected from humans living in different geographical areas. We also compared genetic similarities of clones recovered from stool and the environment. In addition, we attempted to determine the genetic relationship of *Aeromonas* strains isolated from healthy and dead fish of the species *Rutilus rutilus*. Moreover, we evaluated random amplified polymorphic DNA PCR (RAPD), repetitive extragenic palindromic sequence PCR (REP-PCR), and enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR) PCR methods for fingerprinting of *Aeromonas* spp. isolates.

MATERIALS AND METHODS

Bacterial strains. One hundred and twenty strains of *Aeromonas* spp. were used in this study. Their geographical origin and sources are listed in Tables 1 to 3. Forty type and reference strains representing all recognized *Aeromonas* species were included in the study. Identification of the strains was performed on the basis of their phenotypic properties and DNA-DNA hybridization according to the method of Kaznowski (19).

Isolation of DNA. Colonies of *Aeromonas* spp. grown on Trypticase soy agar at 37°C for 24 h were scraped off and suspended in 0.85% NaCl. After centrifugation, the pellet was resuspended in a lysis buffer and incubated after adding protease K. The DNA was extracted using the NucleoSpin C + T kit (Macherey-Nagel, Düren, Germany) according to the procedure established by the manufacturer. The quality and quantity of the DNA were determined spectrophotometrically at 260 nm.

RAPD typing. The RAPD method involves the use of short random sequence primers, usually 9 to 10 nucleotides long, and low-stringency primer annealing conditions to amplify arbitrary fragments of template DNA. The single primer anneals anywhere on the genome where a near-complementary sequence exists, and if two priming sites are sufficiently close, PCR then amplifies the fragment between them (27). The following primers were used for RAPD typing: OPB-1 5′-GTTTCGCTCC-3′ (27), OPB-6 5′-TGCTCTGCCC-3′ (27), OPB-7 5′-GGT GACGCAG-3′ (27), AP3 5′-TCACGATGCA-3′ (35), AP5 5′-TCACGCTGC G-3′ (35), AK1 5′-ATCACTATGA-3′, AK2 5′-GATCCTGCAG-3′, and AK3 5′-TAAGGTTCGG-3′. RAPD PCR mixtures were prepared as previously described (27). The reaction mixture consisted of 4 µl of 10× reaction buffer [750 mM Tris-HCl (pH 8.8), 200 mM (NH₄)₂SO₄, 0.1% Tween 20], a 250 µM concentration of each deoxynucleoside triphosphate (dNTP), 50 pmol of a primer,

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TABLE 1. Strains of *Aeromonas* spp. isolated from humans

Genospecies	HG	Strain no.[a]	Source of isolation
A. hydrophila	1	RK 70363, RK 226254, RK 217215	Human stool, Hong Kong
		SK 3, SK 6, SK 7, SK 12	Human stool, Thailand
		ATCC 49140	Human
		LMG 13656	Feces, Switzerland
A. bestiarum	2	LMG 13448	Feces, Switzerland
A. salmonicida	3	LMG 13450	Feces, Switzerland
A. caviae	4	AK 375, AK 376, AK 377, AK 378, AK 379, AK 380, AK 383, AK 384, AK 385, AK 386, AK 388, AK 390, AK 393	Human stool, Poland
		RK 25447, RK 27611, RK 65541, RK 66942, RK 77620, RK 217455, RK 220132	Human stool, Hong Kong
		SK 4, SK 9, SK 11	Human stool, Thailand
		LMG 13454	Human, Germany
A. media	5A	LMG 13461	Feces, Switzerland
A. veronii biotype sobria	8/10	AK 382, AK 387, AK 389, AK 391, AK 392	Human stool, Poland
		RK 43939, RK 66113, RK 77343	Human stool, Hong Kong
		SK 2, SK 14, SK 24	Human stool, Thailand
A. veronii biotype veronii	10/8	ATCC 35624[T]	Sputum
A. jandaei	9	ATCC 49568[T]	Human stool
		LMG 13065	Human stool, Switzerland
Aeromonas sp.	11	ATCC 35941	Abscess
A. schubertii	12	ATCC 43700[T]	Abscess
		LMG 13473	Human skin, United States
Aeromonas sp.	13	LMG 17321[T]	Human (leg wound), United States
A. trota	14	ATCC 49657[T]	Human stool
		LMG 13081	Human stool, Thailand
A. enteropelogenes	14	ATCC 49803[T]	Human stool
A. allosaccharophila	15	LMG 14021	Human stool, United States
Aeromonas sp.	ND	RK 61871	Human stool, Hong Kong

[a] Abbreviations: AK, Culture Collection of Department of Microbiology, A. Mickiewicz University, Poznań, Poland; RK, strains obtained from R. Kong, Hong Kong University; SK, strains obtained from S. Korbsrisate, Department of Immunology, Mahidol University, Bangkok, Thailand; ATCC, American Type Culture Collection, Manassas, Va.; LMG, Culture Collection, Laboratorium voor Microbiologie, Universiteit Gent, Ghent, Belgium; ND, isolate not included in any *Aeromonas* sp. HG.

3.75 mM MgCl$_2$, 35 ng of template DNA, and 2 U of *Taq* polymerase (MBI Fermentas) made up to 40 µl with sterile distilled water. The reaction mixture was denatured for 1 min at 94°C and then subjected to 25 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 36°C, and extension for 2 min at 72°C and 15 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 36°C, and extension for 3 min at 72°C with a final extension for 2 min at 72°C.

REP-PCR typing. The REP-PCR method uses primers complementary to REP elements of bacterial genomic DNA (36). PCR amplification of template genomic DNA results in products of different sizes, presumably reflecting the distance and orientation of endogenous repeats. The following primers were used for REP-PCR: Rep1R-I 5′-(inosine)(inosine)(inosine)(ino-sine)CG (inosine)CG(inosine)CATC(inosine)GGC-3′, and Rep2-I 5′-(inosine)CG(ino-sine)CTTATC(inosine)GGCCTAC-3′ (36). REP-PCR analysis was performed

in accordance with previous description (36). The reaction mixture consisted of 4 µl of 10× reaction buffer [750 mM Tris-HCl (pH 8.8), 200 mM (NH$_4$)$_2$SO$_4$, 0.1% Tween 20], a 250 µM concentration of each dNTP, 50 pmol of primer Rep1R-I, 50 pmol of primer Rep2-I, 3.75 mM MgCl$_2$, 100 ng of template DNA, and 2 U of *Taq* polymerase (MBI Fermentas) made up to 25 µl with sterile distilled water. The reaction mixture was denatured for 7 min at 95°C and then subjected to 30 cycles of denaturation for 30 s at 90°C, annealing for 1 min 40°C, extension for 8 min at 65°C, and a final extension for 16 min at 65°C.

ERIC-PCR typing. The ERIC-PCR method utilizes primers complementary to ERIC sequences of bacterial genomic DNA (36). The following primers were used for ERIC-PCR: ERIC 1R (5′-ATG TAA GCT CCT GGG GAT TCA C-3′) and ERIC 2 (5′-AAG TAA GTG ACT GGG GTG AGC G-3′) (36). ERIC-PCR was performed as described previously (36). The reaction mixture consisted of 4

TABLE 2. Strains of *Aeromonas* spp. isolated from animals

Genospecies	HG	Strain no.[a]	Source of isolation
A. bestiarum	2	ATCC 51108[T]	Diseased fish
A. salmonicida	3	LMG 3780[T]	Atlantic salmon (*Salmo salar*)
A. caviae	4	ATCC 15468[T]	Guinea pig
A. media	5A	CDC 0862-83	Fish
A. sobria	7	CIP 7433[T], LMG 13469	Fish
A. veronii biotype sobria	8/10	AK 156, AK 160, AK 164, AK 180	Dead fish (*Rutilus rutilus*)
		AK 165, AK 167, AK 176	Healthy fish (*Rutilus rutilus*)
		CDC 0437-84	Fish
		LMG 3785	Frog
A. allosaccharophila	15	CECT 4199[T]	Diseased eel
A. encheleia	16	CECT 4342[T]	Fish
A. culicicola	18	MTCC 3249[T]	Midgut of *Culex quinquefasciatus*, India

[a] Abbreviations: AK, Culture Collection of Department of Microbiology, A. Mickiewicz University, Poznań, Poland; ATCC, American Type Culture Collection, Manassas, Va.; LMG, Culture Collection, Laboratorium voor Microbiologie Universiteit, Gent, Ghent, Belgium; CECT, Coleccion Espanola de Cultivos Tipo, Universitad de Valencia, Valencia, Spain; CIP, Collection bacterienne de l'Institut Pasteur, Paris, France; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; MTCC, Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India.

TABLE 3. Strains of *Aeromonas* spp. isolated from environmental sources

Genospecies	HG	Strain no.[a]	Source of isolation
A. hydrophila	1	AK 44	Lake water
		ATCC 7966[T]	Canned milk
A. bestiarum	2	AK 1, AK 41	Lake water
		AK 115	Drinking water, aluminum rolling mill in Konin, Poland
		ATCC 23213	River water
		ATCC 23211	Drinking water distribution system
		ATCC 13444	Surface water
A. salmonicida	3	AK 46	Lake water
		AK 106, AK 117, AK 125	Rolling mill emulsion
		AK 400	Sea water
		AK 409, AK 410	Drinking water, Poznań
		CDC 0434-84	Freshwater
A. caviae	4	AK 104, AK 126	Drinking water, aluminum rolling mill in Konin, Poland
		AK 220, AK 232	Rolling mill emulsion
		AK 404, AK 405	River water
		AK 406, AK 407	Sea water
A. media	5A	AK 42	Lake water
		AK 403	Sea water
	5B	ATCC 33907[T]	Fresh water
A. eucrenophila	6	AK 65	Lake water
		ATCC 23309[T]	Freshwater
		LMG 13687	Urban well, Germany
A. veronii biotype sobria	8/10	AK 12, AK 59	Lake water
		AK 100, AK 102, AK 120	Drinking water, aluminum rolling mill in Konin, Poland
		AK 279	Sewage
		AK 411, AK 412, AK 413	Drinking water, Poznań
A. ichthiosmia	8/10	ATCC 49904	Surface water
A. encheleia	16	LMG 13691	Infiltration well, Germany
A. popoffii	17	LMG 17541[T]	Drinking water, Belgium
		LMG 17542	Drinking water production plant, Belgium

[a] Abbreviations: AK, Culture Collection of Department of Microbiology, A. Mickiewicz University, Poznań, Poland; ATCC, American Type Culture Collection, Manassas, Va.; LMG, Culture Collection, Laboratorium voor Microbiologie, Universiteit Gent, Ghent, Belgium; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.

μl of 10× reaction buffer [750 mM Tris-HCl (pH 8.8), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20], a 250 μM concentration of each dNTP, 50 pmol of primer ERIC 1R, 50 pmol of primer ERIC 2, 3.75 mM $MgCl_2$, 100 ng of template DNA, and 2 U of *Taq* polymerase (MBI Fermentas) made up to 25 μl with sterile distilled water. The reaction mixture was denatured for 7 min at 95°C and then subjected to 30 cycles of denaturation for 30 s at 90°C, annealing for 1 min at 52°C, extension for 8 min at 65°C, and a final extension for 16 min at 65°C.

Electrophoresis and computer analysis. The amplification products were electrophoresed in 1.5% agarose gel in Tris-borate buffer (0.089 M Tris, 0.089 M H_3BO_3, 0.002 M EDTA). Gene Ruler 100-bp DNA Ladder Plus (MBI Fermentas) was used as a molecular size standard. The gels were stained with ethidium bromide, visualized on a UV light transilluminator, and documented with V.99 Bio-Print system (Vilber Lourmat, Torcy, France). Computer analyses was carried out using GelCompar II (version 3.0; Applied Maths, Kortrijk, Belgium) software. Similarity between fingerprints was calculated with the Dice coefficient. Cluster analysis was performed using the unweighted pair-group method with average linkages (UPGMA).

RESULTS

RAPD fingerprinting. Eight primers were used for RAPD fingerprinting of 30 *Aeromonas* sp. strains representing all hybridization groups. PCR with some primers did not result in amplification products for all isolates. The results obtained with primers AK1 and AK2 revealed poor reproducibility. The best results were achieved with the AP5 primer (34). The AP5 primer was used for the typing of 120 *Aeromonas* sp. strains. The fingerprints of these isolates consisted of 2 to 17 bands ranging from 100 to 3,500 bp (Fig. 1). The bands in each of the patterns produced by the RAPD method were analyzed by applying the Dice coefficient. The similarities of the same strains from different agarose gels ranged from 98 to 100%.

Numerical analysis of RAPD profiles revealed three clusters at the 90% similarity level. Strains within these clusters were considered to be genetically related. The first cluster consisted of three isolates of *A. veronii* biotype sobria (AK 100, AK 102, and AK 120) cultured from drinking water collected from a local industrial water distribution system in Konin, Poland. These isolates shared 98% similarity. The isolates AK 100 and AK 102 appeared to be identical; they shared the following bands: 3,500, 2,440, 2,000, 1,770, 1,580, 1,320, 1,180, 1,060, 840, 740, 580, 540, 450, and 390 bp. The RAPD profile of AK 120 was identical with those of AK 100 and AK 102 except for a single faint band at 2,000 bp. The second cluster was composed of three isolates of *A. salmonicida*, AK 106, AK 117, and AK 125, isolated from aluminum rolling emulsion. The same RAPD pattern was obtained for each of these isolates, showing expansion of a single clone colonizing aluminum cooling fluid. PCR products of these isolates showed fragments with common bands of 3,600, 3,500, 3,100, 2,100, 1,570, 1,330, 1,090, 910, 710, 600, and 440 bp. The third cluster comprised three isolates of *A. veronii* biotype sobria (AK 411, AK 413, AK 412) that originated from drinking water from a city distribution system. The RAPD patterns of these isolates were identical; isolates had fragments with common bands of 2,650, 2,350, 1,970, 1,750, 1,590, 1,220, 1,030, 900, 500, and 240 bp.

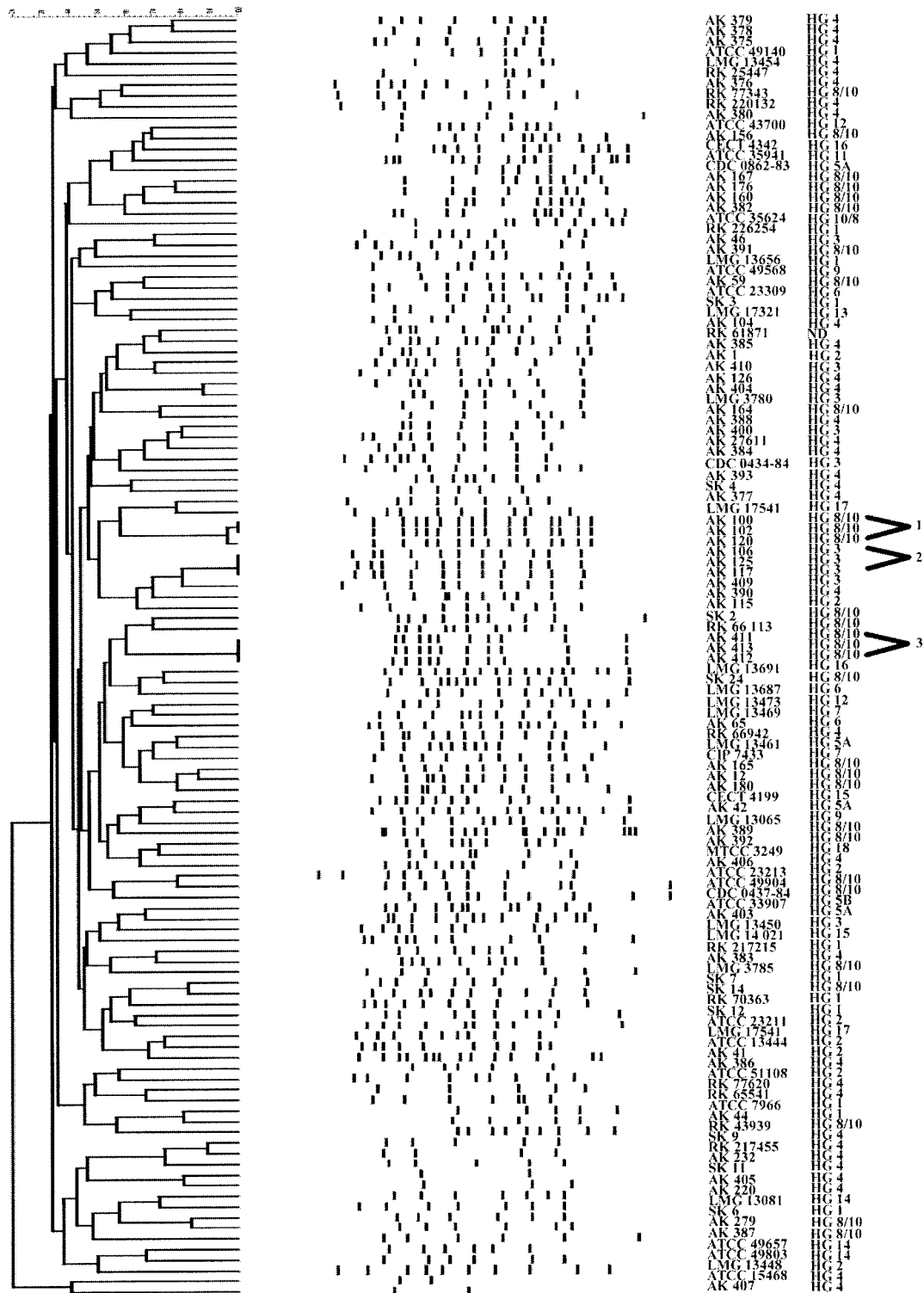The RAPD patterns of all remaining strains were diverse,

FIG. 1. Dendrogram showing genetic relatedness of 120 strains of *Aeromonas* sp. determined by analysis of RAPD fingerprint patterns using Dice similarity coefficient and UPGMA cluster method.

with similarities below 90%, and the strains were considered genetically unrelated. The 13 strains of *A. caviae* isolated from stool specimens collected from Polish children generated 13 clearly distinct patterns. The highest degree of similarity (77%)

was obtained for AK 379 and AK 378. Among five isolates of *A. veronii* biotype sobria that originated from stool specimens collected from Polish children, a great diversity of fingerprints was also observed, and the strains did not form a separate

cluster. The six strains isolated from specimens from patients living in other European countries had six different patterns and did not constitute a group. Strains isolated from stool specimens collected from people living in Asia showed similarities below 90%. The highest similarity (88%) was obtained for two isolates of *A. caviae*: SK 9 and RK 217455. All remaining strains showed similarities below 80%. The RAPD patterns of strains isolated from clinical sources were found to be distinct from those of the strains isolated from environmental sources. It is clear from the dendrogram that there was genetic diversity among strains isolated from healthy and dead fish (*R. rutilus*). The highest degree of similarity (76%) was found between two strains of *A. veronii* biotype sobria: AK 167 and AK 176.

REP-PCR fingerprinting. Among the 120 isolates of *Aeromonas* sp., 25 were not typeable by REP-PCR. This analysis yielded one to nine bands depending on the isolate; the size of the DNA fragments ranged from 100 to 3,500 bp. The majority of the isolates exhibited patterns with small number of REP-PCR products (Fig. 2). The similarities of the DNA patterns of the same strains from different agarose gels ranged from 98 to 100%.

Three clusters were identified among the 95 strains of *Aeromonas* spp. The first cluster was composed of two *A. veronii* biotype sobria isolates (AK 156 and AK 160) isolated from two dead fish (*R. rutilus*) collected from the same lake. The isolates appeared to be identical; they had one fragment of 400 bp. Second group included three isolates of *A. salmonicida*: AK 106, AK 117, and AK 125. They shared two common fragments of 530 and 240 bp. The third cluster consisted of two isolates, AK 100 and AK 102, of *A. veronii* biotype sobria. The REP-PCR patterns of these isolates were identical and consisted of fragments of 390 and 140 bp. The REP-PCR patterns of all remaining strains showed similarities below 90%. Note that none of the clinical strains had the same pattern; however, we did not obtain fingerprints for 13 strains isolated from humans.

ERIC PCR fingerprinting. The fingerprints of *Aeromonas* sp. isolates consisted of 1 to 11 amplification bands, ranging in size from 100 to 3,500 bp (Fig. 3). All strains were typeable by ERIC-PCR. Reproducibility of the DNA patterns from different gels was in the range of 98 to 100%.

Among the 120 strains of *Aeromonas* spp. examined, three clusters were identified. The first group included three isolates of *A. veronii* biotype sobria (AK 100, AK 102, and AK 120), which had shown the same DNA profile, consisting of fragments of 1,940, 690, 470, and 220 bp. The second cluster was composed of three *A. salmonicida* isolates: AK 106, AK 117, and AK 125. They shared fragments of 1,760, 780, 650, 550, 340, and 210 bp. The third cluster included three strains of *A. veronii* biotype sobria: AK 411, AK 413, and AK 412. These isolates appeared to be identical and shared the following common bands: 1,800, 1,200, 720, 650, 470, 390, and 300 bp. Figure 3 shows that 18 strains isolated from stool specimens collected from Polish children were distinct, and the highest similarity between two strains was 72% as indicated by the Dice coefficient. The ERIC-PCR patterns of six strains isolated from people living in other European countries were clearly different. Strains isolated from stool specimens collected from people living in Asia exhibited genetic variability as well. The clinical strains could be clearly distinguished from each other

and from environmental isolates. No specific profile was obtained for strains isolated from healthy and dead fish (*R. rutilus*). The highest similarity (72%) was obtained for two strains *A. veronii* biotype sobria, AK 176 and AK 167.

DISCUSSION

Several studies have provided strong evidence that some bacterial epidemic clones can circulate in an infected population for several years and are responsible for outbreaks (5, 15, 30, 32). There have been no studies that would have investigated the relationship among the clones of the pathogenic *Aeromonas* spp. isolated from humans living in different parts of the world. We compared strains isolated from stool specimens collected from humans suffering from gastroenteritis by using RAPD, REP-PCR, and ERIC-PCR methods. The DNA profiles of 18 pathogenic strains isolated from stool specimens collected from Polish children, who were hospitalized in one unit of a hospital in Poznań, Poland, did not form a separate group of related strains. This indicated that the children were not infected with clonally related strains. Our data suggested that the hospital environment was not the source of *Aeromonas* sp. infections of hospitalized patients. This study also revealed that the *Aeromonas* sp. strains recovered from patients in Poland and from different parts of Europe were different clones. We found high genetic diversity among the strains isolated from stool specimens collected from humans living in Asia. None of the clones isolated in Poland showed relatedness to the clones originating from Hong Kong, Thailand, and other areas. Previously, Moyer et al. (26) reported that two *A. hydrophila* and four *A. caviae* strains isolated from stool specimens collected from people living in the same city exhibited genetic variability. Hänninen et al. (13) indicated that strains within *A. veronii* biotype sobria, *A. caviae*, and *A. hydrophila* had a unique ribotype. Those strains were isolated from stool specimens collected from people who had been on the same trip in Morocco. Our experimental results and previous observations provide strong evidence that there have not been predominant clones responsible for *Aeromonas*-associated gastroenteritis.

Several authors suggested the possibility of waterborne *Aeromonas* sp. infection in humans (8, 20, 21, 22, 24, 29, 34). Therefore, one of the purposes of the study was to investigate whether the same clones exist in the environment and in the stool specimens collected from patients with diarrhea. We found that there was no genetic similarity between clinical and environmental clones of *Aeromonas* spp. Previously, Moyer et al. (26) also showed no genetic relationship between clinical and environmental strains. Davin-Regli et al. (7) found that the water sampled in the hospital was not the source of infections of patients at the University Hospital in Marseille, France. The present study revealed the existence of a single clone of *A. veronii* biotype sobria in a city water distribution system for several months. Domination of some clones in the water distribution system could be a result of being a component of biological membranes (6, 11). We identified three other isolates of *A. salmonicida* which showed the expansion of a single clone in a specific environment, i.e., the rolling emulsion. However, we did not note *Aeromonas*-associated infections among humans working in the emulsion's aerosol.
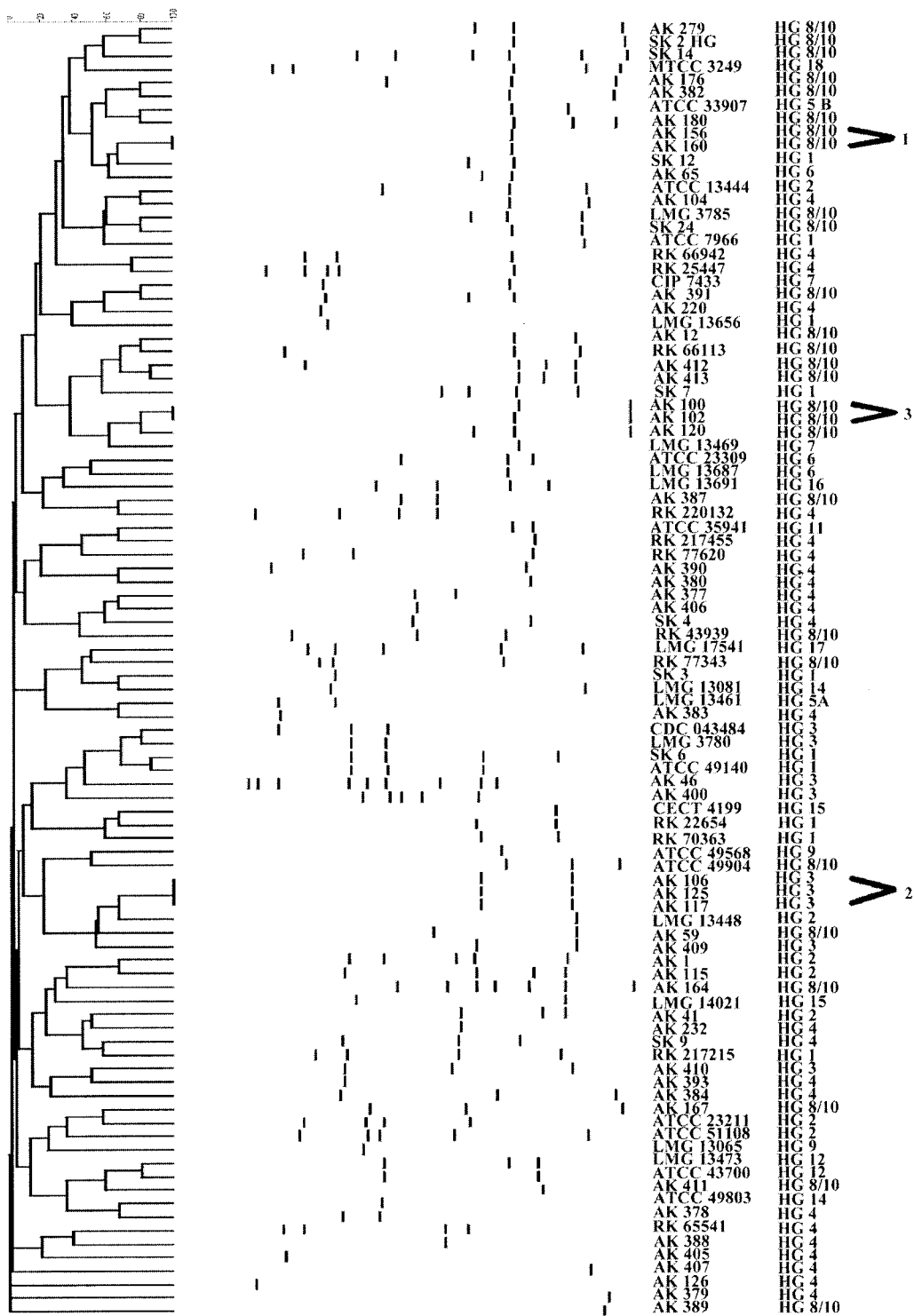
FIG. 2. Dendrogram showing genetic relatedness of 95 strains of *Aeromonas* sp. determined by analysis of REP-PCR fingerprint patterns using Dice similarity coefficient and UPGMA cluster method.

Another area of interest in our study was determination of genetic similarity among strains isolated from healthy and dead fish (*R. rutilus*) that were collected from the same lake. We found that the isolates of *A. veronii* biotype sobria were different clones. Previously, Garcia et al. (10) found genetic heterogeneity among strains of *A. salmonicida* subsp. *salmonicida*

isolated from different species of fish. However, they also observed a predominant clone that was responsible for most of the outbreaks of furunculosis in fish. Some authors indicated that water contaminated with *Aeromonas* sp. could be a source of fish disease (2). However, our results revealed that clones of *Aeromonas* sp. involved in fish infections did not show a close
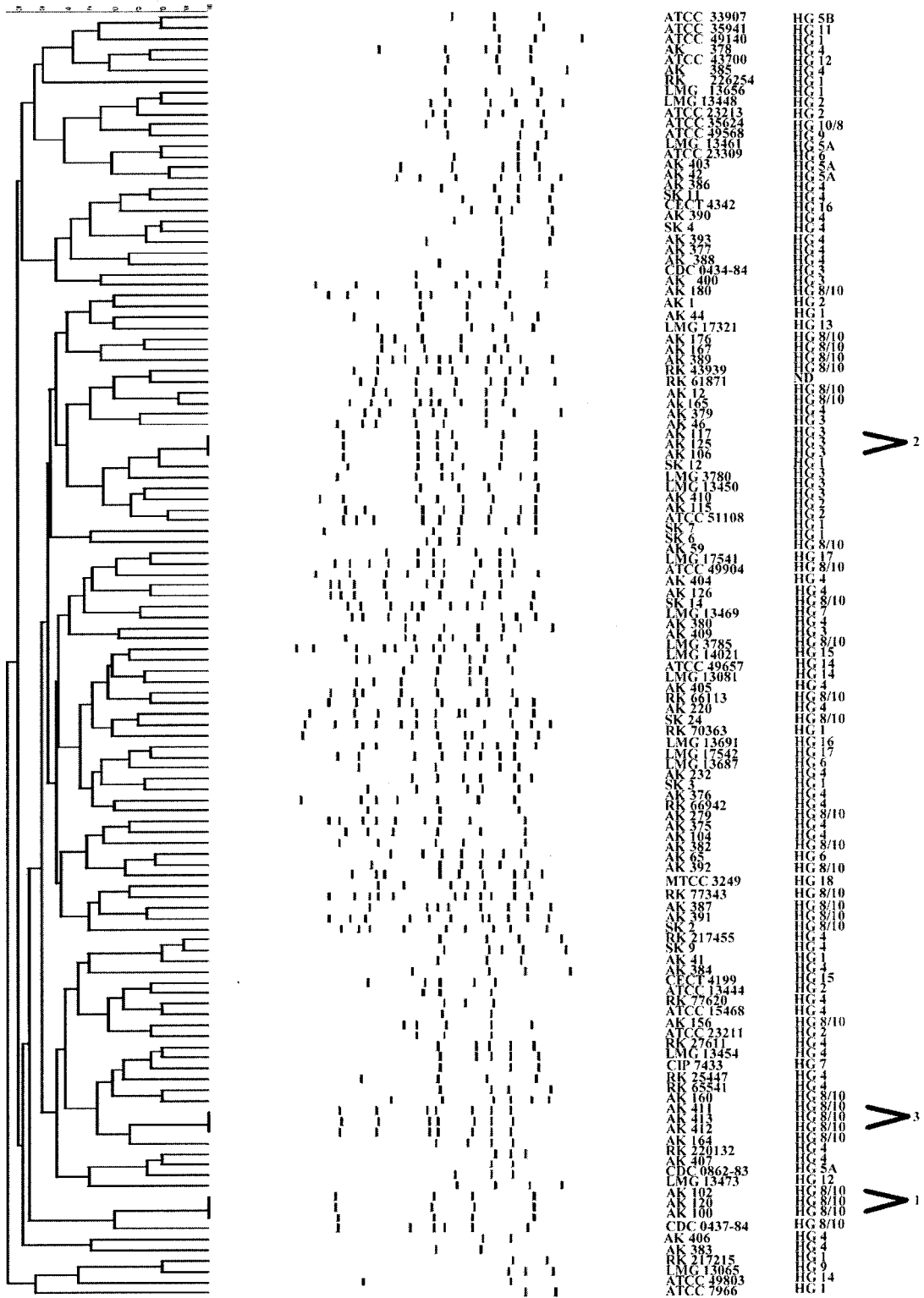
FIG. 3. Dendrogram showing genetic relatedness of 120 strains of *Aeromonas* sp. determined by analysis of ERIC-PCR fingerprint patterns using Dice similarity coefficient and UPGMA cluster method.

genetic similarity with clones isolated from water samples from the lake. The present study revealed the coexistence of many *A. veronii* biotype sobria clones in the water of this recreational lake.

Over the last few years several authors have used molecular typing methods for determination of the clonal structure of several species, e.g., *Neisseria meningitidis* (5), *Corynebacterium diphtheriae* (12, 33), *Streptococcus pneumoniae* (15, 32), *Salmonella enterica* (14), *S. enterica* serovar Enteritidis (23), *Clostridium difficile* (25). We found that species of *A. hydrophila* (HG 1), *A. bestiarum* (HG 2), *A. salmonicida* (HG 3), *A. caviae* (HG 4), *A. media* (HG 5), and *A. veronii* biotype sobria (HG 8/10) had clonal structures. In addition, none of the *A. eucrenophila* (HG 6), *A. sobria* (HG 7), *A. jandaei* (HG 9), *A. schubertii* (HG 12), *A. trota* (HG 14), *A. allosaccharophila* (HG 15), *A. encheleia* (HG 16), and *A. popoffii* (HG 17) strains had similar DNA profiles. Probably these species also possessed a clonal structure, but the number of strains in this study was too low to make a clear conclusion.

Despite the fact that *Aeromonas* species had a clonal structure, we did not find pathogenic clones which circulated among humans living in different geographic areas. In addition we did not determine the reservoir of pathogenic strains in the environment. It is probable that the pathovars of *Aeromonas* spp. do not represent a single evolutionary line but rather that they are composed of several lines. A similar phenomenon was reported for other bacterial species, e.g., most pathogenic *Escherichia coli* strains capable of causing the same disease do not constitute a monophyletic group and do not have a single evolutionary origin (31).

An excellent correlation was obtained between the results of RAPD and ERIC-PCR analyses. All strains with the same RAPD type were found to have the same ERIC type. Our observations demonstrate that RAPD and ERIC-PCR have a good discriminatory power. These methods are useful for distinguishing *Aeromonas* sp. clones and for epidemiological investigation. We do not recommend the use of the REP-PCR analysis for typing of *Aeromonas* sp. strains, because not all strains could be typed by this method. Moreover, the REP-PCR analysis yields a relatively small number of products. This suggests that the repetitive extragenic palindromic sequence may not be widely distributed in *Aeromonas* sp. genome.

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