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COMPARATIVE STUDIES ON *AEROMONAS* STRAINS ISOLATED FROM LAKES BALATON (HUNGARY) AND FERTŐ/NEUSIEDLERSEE (HUNGARY)

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Ecological and comparative taxonomic investigations were carried out on 49 *Aeromonas* strains isolated from water samples of two moderately alkaline lakes of Hungary, Lake Balaton and Lake Fertő/Neusiedlersee together with 3 authentic strains of *Aeromonas hydrophila*. Five phena were created at greater than 92% similarity value using the UPGMA method with the Jaccard coefficient. Strains isolated from Lake Balaton were determined as *A. hydrophila*, while strains originated from Lake Fertő were identified as *A. hydrophila* and *A. sobria*. The Fertő isolates of *A. hydrophila* grew only at higher salt concentration (5% NaCl). This might be an adaptation to the higher salt contents in the water of Lake Fertő. However, no specific differences were detected in their behaviour against alkaline pH values. The wide range of their degradative enzymes indicate that aeromonads can play an important role in nutrient cycling.

Keywords: *Aeromonas*, microbial ecology

Introduction

Members of the genus *Aeromonas* are widely distributed in aquatic environments. Their occurrence ranges from surface freshwater to sewage, they can even be found in drinking water [1, 2]. They exhibit a marked seasonal cycle: the densities of *Aeromonas* spp. are highest during the summer months and lowest in winter. Monfort and Baleux [3] have shown that temperature could be the most important determining factor responsible for their particular frequency of occurrence.

The ubiquity of aeromonads indicates versatile adaptive mechanisms. They possess the ability to produce a wide variety of enzymes associated with pathogenicity and environmental adaptability. Their most intensively studied and known enzymes

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include β-lactamases (B metallo-β-lactamases, C cephalosporinase, D penicillinase), lipases, haemolytic enterotoxins (α-haemolysin, aerolysin), nucleases, proteases, chitinases and amylases [4]. These capabilities to decompose a wide range of biopolymers could contribute significantly to nutrient cycling in freshwaters as well as to the great adaptability of these organisms to environmental changes.

Aeromonads are known to be opportunistic pathogens of humans [5] and can cause diseases in both warm-blooded and cold-blooded animals [6].

The taxonomy of the genus *Aeromonas* is rapidly developing. Since Popoff [7] has described 4 species based on phenotypic characteristics: *A. hydrophila*, *A. caviae*, *A. sobria*, *A. salmonicida*, several new species have been described: *A. media* [8], *A. veronii* [9], *A. euchrenophila* [10], *A. shubertii* [11], *A. jandaei* [12], *A. trota* [13], *A. allosaccharophila* [14], *A. encheleia* [15], *A. bestiarium* [16]. Working methods proposed for *Aeromonas* taxonomy include multilocus enzyme electrophoresis, ribotyping, colorimetric DNA-DNA hybridization in microplates and AFLP fingerprinting [17]. However, other studies have shown that certain phenotypic characteristics have a very good correlation with, for example, DNA hybridization groups [15, 17, 18, 19] and thus can be used in taxonomy as well.

Lake Balaton being the largest of its kind in Central Europe, the water of which is slightly alkaline, whereas Lake Fertő has a much higher pH, and has a higher salt concentration (Table I). Both lakes are shallow compared to the large surface distribution of their waterbody and in addition Lake Fertő has a significant reed coverage (85% of the Hungarian part) [20].

Several authors have described the presence of aeromonads in Lake Balaton [21, 22, 23, 24], however until now only Borsodi et al. have published species information about Lake Fertő's planktonic bacterial communities [25].

Both lakes are in focus of recreational activities, thus water quality in this case is of prime importance. The present study was undertaken to compare *Aeromonas* strains isolated from Lake Balaton and Lake Fertő/Neusiedlersee based on phenotypic characteristics using numerical analytical methods.

Materials and methods

The strains included in this study have been isolated from the water of Lake Balaton's Siófoki Bay and Keszthelyi Bay, and from the water of the Hungarian part of Lake Fertő/Neusiedlersee (Madárvárta Bay, Rucás Bay and inner lakes) in the period of 1983 and 1986 during the summer months. Samples were taken aseptically from the top 25 cm of the water. Following a 7–14 day incubation period on 28°C on nutrient agar plates spreading with diluted water samples, strains were isolated: 125 strains

Table I

Water chemical composition data of the two studied lakes

Chemical parameters	Lake Balaton		Lake Fertő/Neusiedlersee	
	minimum	maximum	Minimum	maximum
Na^+ [mg/dm^3]	12	38	165	490
Mg^{2+} [mg/dm^3]	26	52	62	182
Ca^{2+} [mg/dm^3]	28	96	20	104
K^+ [mg/dm^3]	4	8	17	62
HCO_3^- [mg/dm^3]	185	430	486	885
CO_3^{2-} [mg/dm^3]	0	36	0	120
SO_4^{2-} [mg/dm^3]	42	96	240	750
Conductivity [µS/cm]	537	770	1860	2200
Chl-a [mg/m^3]	0.14	8.33	1.4	25.51
pH	8.1	8.7	7.8	10.0

(Sources: [20, 26, 27, 28], Muskó, personal communication.)

from Lake Balaton, 68 strains from Lake Fertő. However, only 32 representative strains from Lake Balaton and 17 representative strains from Lake Fertő were selected for detailed studies. These strains were directly compared with 3 authentic *Aeromonas hydrophila* strains obtained from the Czechoslovak Collection of Microorganisms (CCM-1271, CCM-1150, CCM-2280).

The selected strains were subjected to micromorphological (Gram staining, cell morphology, motility studies) and physiological-biochemical (catalase, oxidase, glucose fermentation test in O/F medium of Hugh and Leifson, acid production from fructose and sucrose, aesculin and arginine hydrolysis, phenylalanine deamination using Report's method, indole and H_2S production, casein, gelatine, Tween 80 and starch hydrolysis, Voges-Proskauer reaction, nitrate reduction, citrate utilisation by Simmon's method, urea hydrolysis and phosphatase activity) tests using the methods described by Cowan and Steel [29], furthermore to environmental tolerance tests (growth in nutrient broth containing 5% and 7 % NaCl, growth at pH 9.0 as well as at 37ºC).

The data obtained were subjected to numerical analysis based on 32 common coded characteristics. Jaccard coefficients were calculated with the help of the SPSS for Windows 6.0 software and a dendrogram was constructed using the UPGMA algorithm. For conventional taxonomic identification of the resulting phena we mainly relied on the Bergey's Manual of Systematic Bacteriology [7].

Results

Out of the investigated 52 strains 49 were clustered by the UPGMA method into 5 phena at S_J values greater than 90% and 3 strains occupied interphenonic positions. The simplified dendrogram obtained is shown in Fig. 1 and the characteristics of the 5 phena are described below.

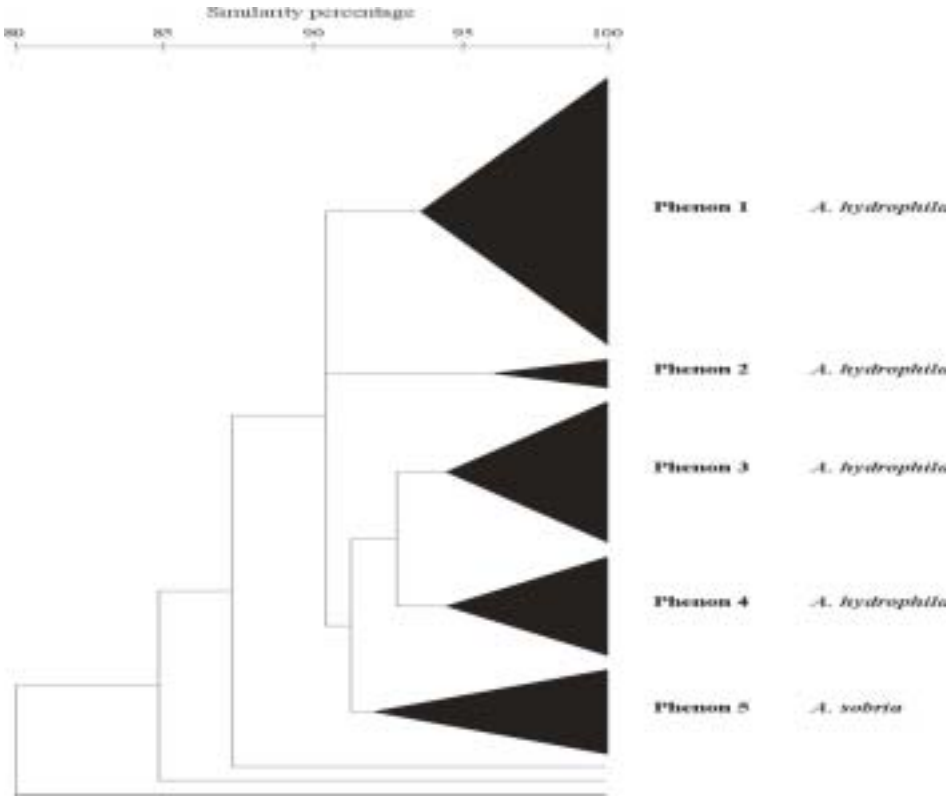

Fig. 1. Dendrogram showing the relationship between the studied strains, based on the S_J/UPGMA clustering algorithm

All investigated strains were Gram negative, short, straight motile rods (1.0–2.5 μm long and 0.3–0.5 μm in diameter), gave a positive test result in oxidase, catalase, glucose fermentation test in O/F medium, H_2S production, casein, gelatine, Tween 80, starch hydrolysis, nitrate reduction, and gave a negative result in growth at 7% NaCl.

Twenty strains were clustered at 93.6% similarity value into Phenon 1. It included besides the type strain CCM-1271 of *Aeromonas hydrophila* strains from Lake Balaton samples. The strains were positive for the production of acid from fructose, arginine hydrolysis, produced indole and acetylmethylcarbinol, possessed urease activity and grew well at pH 9.0. The following tests were negative: acid production from sucrose, growth at 37ºC. They showed variability in the aesculin hydrolysis, phenylalanine deamination, Simmon's citrate and phosphatase tests.

Phenon 2 was formed at 96% and includes 3 *Aeromonas hydrophila* strains from Lake Balaton. They hydrolized arginine, produced indole and acetylmethylcarbinol, utilised citrate, possessed urease activity, grew well at 5% NaCl and pH 9.0. The following tests were negative: acid production form fructose and sucrose, phosphatase activity, growth at 37ºC. They showed variability in the aesculin hydrolysis and phenylalanine deamination.

Phenon 3 was composed of 10 strains from Lake Balaton and an authentic strain CCM-1150 *Aeromonas hydrophila* clustered at a similarity value of 94.5%. These strains showed a positive result in the acid production from sucrose, arginine hydrolysis, produced indole and acetylmethylcarbinol and possessed urease activity. Negative tests included: acid production from fructose, phenylalanine deamination and growth at 37ºC. The following tests gave variable results: aesculin hydrolysis, citrate utilisation, phosphatase activity and growth at pH 9.0 and at 5% NaCl.

Phenon 4 was created also at 94.5% from 7 Fertő strains and an authentic strain CCM-2280 of *Aeromonas hydrophila*. The strains were positive for the production of acid from fructose and sucrose, aesculin and arginine hydrolysis, produced acetylmethylcarbinol, utilized citrate and grew at pH 9.0. The following tests were negative: phenylalanine deamination, urease activity, growth at 37ºC. They showed variability in the production of indole, phosphatase test and in growth at 5% NaCl.

Phenon 5 contained 7 strains isolated from Lake Fertő and have been identified as *Aeromonas sobria* clustered at 92% similarity value. These strains showed a positive result in the acid production from fructose and sucrose, produced indole and acetylmethylcarbinol, utilized citrate, possessed phosphatase activity and grew well at pH 9.0 and 37ºC. Negative tests included: aesculin hydrolysis, urease activity and growth at 5% NaCl. They showed variability in arginine hydrolysis and phenylalanine deamination.

Discussion

The strains of three clusters identified as *Aeromonas hydrophila* (Phena 1, 2 and 3) were isolated from the water of Lake Balaton while the strains of the other two ones (Phena 4 and 5) identified as *Aeromonas hydrophila* and *Aeromonas sobria* originated from Lake Fertő. The most marked differences among the phena identified as *A. hydrophila* from Lake Balaton were seen in acid production from fructose and sucrose and lack of tolerance to 5% NaCl (Table II). All three Lake Balaton phena contained strains from both sampling sites.

Table II

Characteristics of phena obtained by S_J/UPGMA analysis

PHENA	1	2	3	4	5
	Aeromonas hydrophila				*A. sobria*
No. of strains	20	3	11	8	7
Gram reaction	–	–	–	–	–
Motility	+	+	+	+	+
Oxidase	+	+	+	+	+
Catalase	+	+	+	+	+
D-glucose oxidative-fermentative	+	+	+	+	+
Growth and acid from:					
fructose	+	–	–	88	+
sucrose	–	–	+	+	86
Hydrolysis of:					
aesculin	55	33	64	88	14
arginine	+	+	+	+	43
Phenylalanine deamination	25	33	–	–	75
Indole production	+	+	+	75	86
H_2S from cysteine	+	+	+	+	+
Hydrolysis of:					
casein	+	+	+	+	+
gelatin	+	+	+	+	+
Tween-80	+	+	+	+	+
starch	+	+	+	+	+
Voges-Proskauer	+	+	+	88	86
Nitrate reduction	+	+	+	+	+
Simmons' citrate	65	+	63	+	86
Urease	+	+	+	–	–
Phosphatase	40	–	73	75	86
Growth at:					
pH 9.0	+	+	82	+	+
5% NaCl	–	+	18	75	–
7% NaCl	–	–	–	–	–
37°C	–	–	–	13	+

+, more than 89% of the strains positive;
–, more than 89% of the strains negative;
numbers indicate percentage of positive strains.

Strains of *A. hydrophila* isolated from Lake Fertő (Phenon 4) could be distinguished from the former three phena by their lack of urease activity. The majority of *A. sobria* strains did not hydrolize aesculin and arginine but deaminated phenylalanine compared to *A. hydrophila* strains (Table II). The *A. hydrophila* strains from Lake Fertő came from the open water areas (Rucás Bay and Madárvárta Bay) but the *A. sobria* strains from the water of the inner parts of the lake.

Aeromonas hydrophila and *Aeromonas sobria* are considered to be autochthonous members of freshwaters. This is in accordance with the findings that *A. sobria* and *A. hydrophila* dominate over *A. caviae* in oligosaprobic waters [3]. For the eastern part of Lake Balaton Tóth [24] has shown that *A. hydrophila* can become dominant during the summer period. In comparison, in the more eutrophic western Keszthelyi Bay *A. sobria* was described, with the occasional isolation of *A. caviae* near the inlet of Szent-Imre canal carrying faecal load [23]. Araujo et al. [30] has also shown that the ratio of *A. caviae* to *A. hydrophila* was reduced. These results together with the findings of Fiorentini et al. [31] indicate that *A. hydrophila* and *A. sobria* are characteristic for waters with low levels of faecal pollution, the latter being independent of the total number of aeromonads present.

A characteristic property distinguishing Lake Balaton *A. hydrophila* strains from Lake Fertő ones is the tolerance to 5% NaCl. Strains isolated from Lake Fertő proved to be more tolerant to higher salt concentrations than Lake Balaton isolates. This could be explained by the higher ionic concentrations (especially Na^+, HCO_3^- and SO_4^{2-} ions) and specific conductivity values of the water in Lake Fertő (Table I). Apparently a more alkaline pH value had a much smaller influence. The exhibited tolerance can be an adaptation to the given habitats in which their role can be defined as contributing to the degradation of organic materials due to their degradative enzymes.

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