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Numerical analysis on the composition of bacterial communities of the open water region of Lake Fertö

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Introduction

As it is wellknown the microorganisms play a decisive role in the self-purification, detoxification of the water of rivers and lakes and generally in the decomposition of dead organic material. Regarding these facts the modern water bacteriological studies concentrate more and more on detailed species level analyses of complex bacterial communities investigating many biochemical properties of the isolated strains. Such data can give a deeper insight into the community position of metabolic types of microorganisms which can govern the cycling of elements and the energy flow in the water ecosystems. The physiological - biochemical studies on a great number of isolates obtained from an aquatic milieu can give at the same time much more information about the potential physiological abilities of the members of the analysed complex community than the conventional estimates on the germ-numbers of the so called "physiological groups" (ammonifiers, cellulose decomposers, etc.) During the last ten years numerous lakes and rivers of Hungary were studied on such a basis by the workers of the Microbiological Department of the Eötvös University. Below we present the results of our computer aided numerical analysis carried out on the composition of the planktonic bacterial communities occurring in the open water region of the Hungarian area of Lake Fertö.

Material and methods

Water samples for counting and isolating bacteria were collected at four sampling sites in the open water region of Lake Fertö. The location of these sites are presented in Figure 1. The aseptically collected watersamples were cooled and transported within 24 hours into the laboratory where they were, after serial dilutions plated onto agar media of different composition. The first sampling date was the 21.10.1986. At this time the water contained a considerable amount of suspended mud particles due to the strong disturbance of the lake by wind. The second sampling took place on 04.12.1986 and the third one on 17.03.1987. The water temperatures were 10° C, 2° C and O° C respectively. The plating media were nutrient agar, simple water agar and starch-ammoniumsalt agar. Incubation lasted at 22° C one week. On a non-selective basis a total of 827 bacterial isolates were obtained. The water agar and the starch-ammoniumsalt agar proved to be less available for isolation purposes or for any other investigation. Firstly our isolates were grouped tentatively, on the basis of certain easily identifiable diagnostic properties, such as microscopic cell morphology, cultural properties, etc., into larger groups of similar strains. From every group representative strains were selected for further detailed investigation.

Accordingly, altogether 225 representative strains were involved in the comparison using the tests presented in Table 1. During the test period a lot of strains which belonged mostly to the genus *Flavobacterium* lost their viability and their cultures proved no more to be adequate for complete feature analyses. Altogether 94 such strains were discarded. Accordingly, only the remaining 131 representative strains were compared and clustered to obtain a more precise grouping of similar strains. This comparison was based on 201 coded features. Internationally accepted methods (see Szabo, 1974, etc.) were used to detect the physiological-biochemical abilities of our strains. Numerical statistical methods according to the principles of the Adansonian-taxonomy were employed to evaluate the obtained data. The similarity index of Sokal and Mitchener was calculated between all of the compared pairs of strains. IBM PC XT type computer was used and the programme of clustering was written by P.Töke.

Results

The realtionships and similarities among the 131 representative strains are presented by a dendrogram in Figure 2. Altogether ten groups of similar strains were created by the computer. The smallest group (II) contained only two, the largest one (VII) 37 representative strains. Many strains were placed in intergroup positions. Into the ten groups altogether 104 strains were incorporated, while 27 strains remained ungrouped. There was a close correlation between the numbers of similar random isolates, the numbers of selected representative strains belonging to the individual similarity groups and the frequency of occurrence of selected representative strains belonging to the individual similarity groups and the frequency of occurrence of the members of these groups in the water of the lake. This statement is exceptionally not true concerning the *Flavobacteria* which were common in the lake water but poorly represented among our representative strains because they were very hardly to be cultivated on the laboratory media or anyhow at all.

In Figure 2 it can be seen that group I contains only strains of micrococci and both of the two created subgroups involve local varieties of the species *Micrococcus varians* . Group III is similarly composed out of strains of *Micrococcus* but the majority of them (subgroup IIIa) are belonging to *Micrococcus agilis*. Table 2 presents selected diagnostic features of strains of these two characteristic *Micrococcus* species of Lake Fertö. All of them are Gram-positive, oxidase-negative and catalase-positive cocci, which cannot ferment glucose but are capable of growing at high salt concentrations and high pH values. In Figure 2 the two further groups of the dendrogram the II and the IV ones compose strains of the genus *Flavobacterium*. In accordance with the data in the last edition of Bergey`s Manual (1984) they are catalase-positive, Gram-negative and not fermenting organisms (see Table 3). Their species position remained undetermined.

Group V is composed of typical members of *Bacillus sphaericus* . Table 4 informs us about the diagnostic features of our *Bacillus sphaericus* representative strains. The latter were grouped together by the computer into the IX assemblage. Strains of *Bacillus pumilus* isolated from the Fertö`s watersamples proved to be able to grow at 10% NaCl concentration or at more. Group VI involves Actinomycete-

isolates. The largest group, the VII one contains the most characteristic open water bacteria of the lake, namely strains of *Pseudomonas alcaligenes.*

Table 5 presents the summarized characteristics of 37 representative strains of *Pseudomonas alcaligenes* , which is in our opinion the most important member of the Fertö`s planktonic microbiota. These strains can grow at high pH values, show a medium level salt tolerance and do not produce acids from a lot of carbon sources. These features seem to be advantageous for such an organism which is living in a lake characterized by the particular chemistry of Lake Fertö. Interestingly our *Pseudomonas alcaligenes* strains from Lake Fertö have a relatively lower temperature optimum for growth than the typical members of this species, have in general.

Finally at the right side of the dendrogram (Figure 2) near to group IX of *Bacillus pumilus* the strains of *Aeromonas hydrophila* form a separate group (X). The data incorporated in Table 6 corroborate our opinion that the *Aeromonas* strains of Lake Fertö are typical represetative of the species *Aeromonas hydrophila.*

Summary

1. We present a complex planktonic bacterial community from Lake Fertö which is composed of many species and presumable responsibly for the most important biologically catalysed processes in the open water region.

2. The predominant species of this bacterioplankton are *Pseudomonas alcaligenes, Micrococcus varians* , *Micrococcus agilis* and furthermore *Flavobacterium* spp. the isolates of which are very hardly to be cultivated and maintained alive during a long term experiment under laboratory conditions.

3. Among the bacilli which occur in the open water of this lake *Bacillus spaericus* is the most common species.

4. The predominant members of the bacterioplankton are in general aerobic respiratoric organisms which cannot ferment or multiply under anaerobic conditions.

5. From the water-samples of the open water region of Lake Fertö a great number of bacterial strains were isolated which can tolerate high salt concentrations and grow at high pH values; many of them do not produce acids.

6. From our water-samples only one species of the Genus *Aeromonas* was isolated: *Aeromonas hydrophila.* The studied strains of this species showed a very broad spectrum of biochemical abilities.

References

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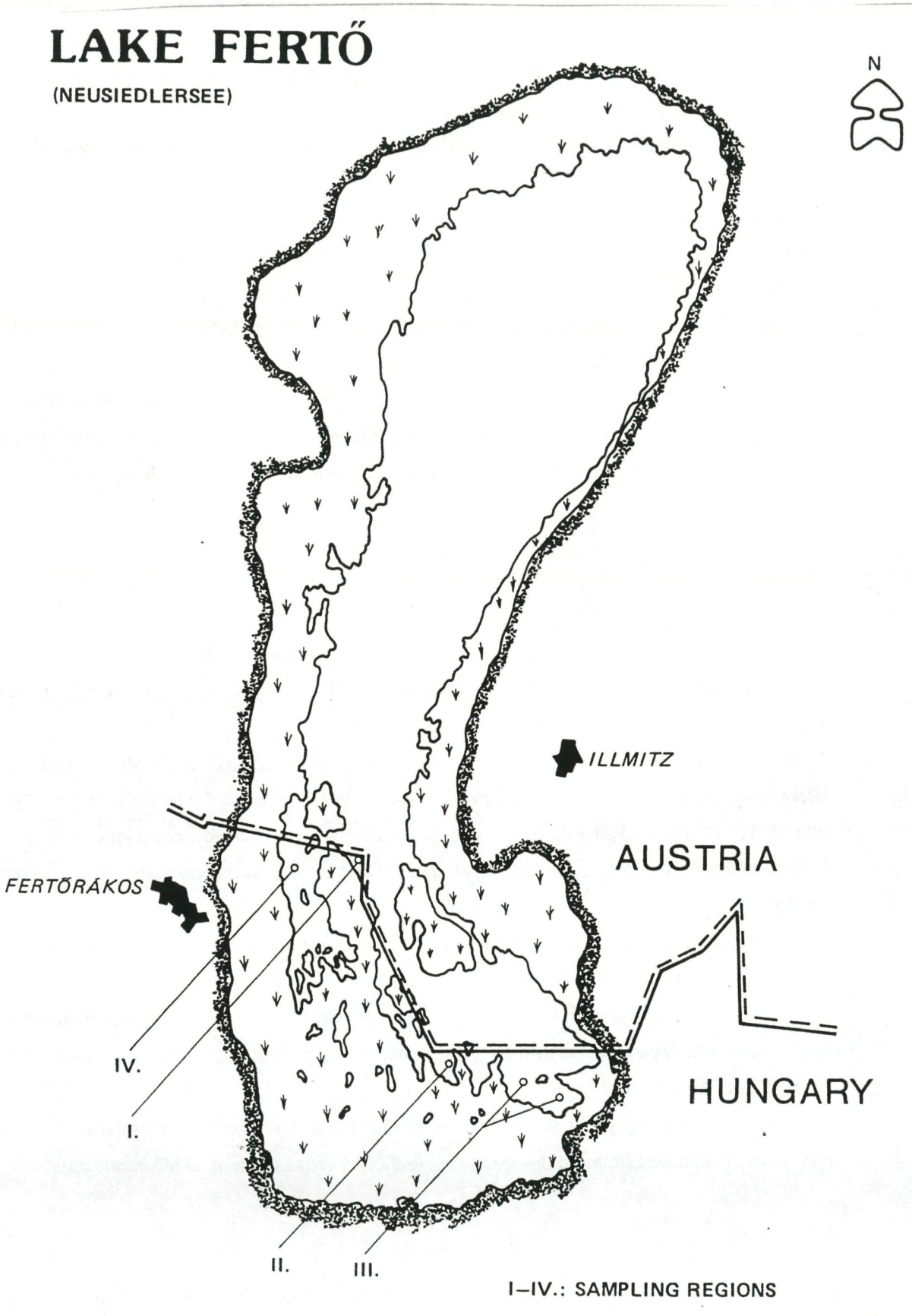

Fig 1

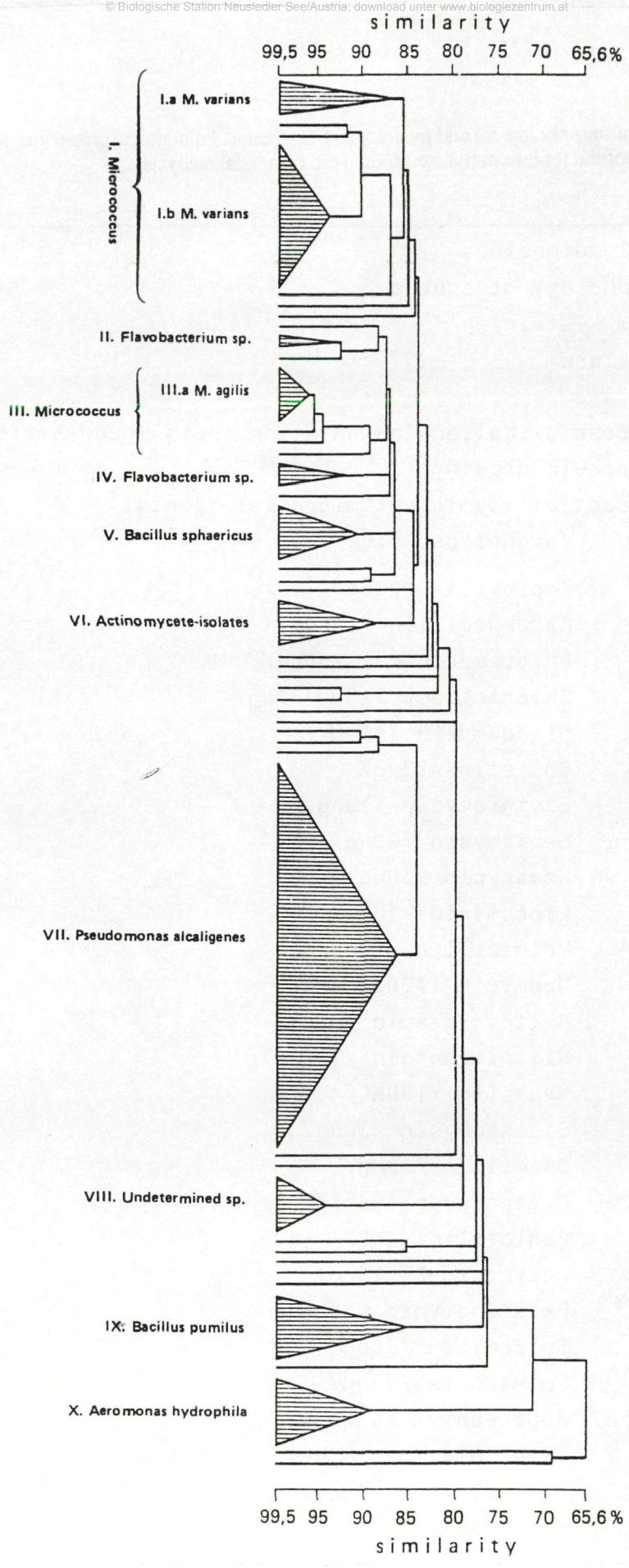

Fig. 2: Dendogram showing the phenetic similarity of 131 bacterial strains isolated from the open water region of Lake Fertö.

Table 1: List of cultural-morphological and pysiological-biochemical diagnostic properties involved in the comparisons of the representative strains and the numerical analysis

1-2.	Cell morphology
3.	Morphology of spores
4.	Gram - staining
5.	Catalase
6.	Oxidase
7-8.	Glucose oxidation-fermentation tests /Hugh-Leifson/
9.	Anaerobic growth
10-63.	Susceptibility to antimicrobial agents: /amount per disk/
	Ampicillin /20µg/
	Carbenicillin /100µg/
	Chloramphenicol /30µg/
	Chlortetracycline /30µg/
	Clindamycin /10µg/
	Colistin /20µg/
	Erythromycin /10µg/
	Gentamycin /20µg/
	Kanamycin /30µg/
	Lincomycin /10µg/
	Methicillin /20µg/
	Neomycin /30µg/
	Nalidixyc-acid /30µg/
	Nitrofurantoin /300µg/
	Nystatin /100NE/
	Oleandomycin /30µg/
	Oxacillin /10µg/
	Oxytetracycline /30µg/
	Penicillin /3NE/
	Polymixin-B /15µg/
	Pristinamycin /10µg/
	Spiramycin /30µg/
	Sumetrolim /25µg/
	Superseptyl /400µg/
	Streptomycine /30µg/
	Tetracycline /30µg/
	Vancomycin /50µg/

Table 1 continued

64.	Aesculin hydrolysis
65.	Arbutin hydrolysis
66.	Arginine dihydrolase
67.	Production of ammonia from peptone
68.	Tyrosine hydrolysis
69.	Casein digestion
70-71.	Production of H_2S from: cysteine and thiosulphate
72.	Indole production in 1% peptone water
73.	Methyl red test
74.	Acetoin from glucose /Voges-Proskauer/
75.	Reduction of NO_3^- to NO_2^-
76.	Phenylalanine deaminase
77.	Phosphatase production
78.	Hydrolysis of starch
79.	Urea hydrolysis
80.	Hydrolysis of cellulose
81.	Reduction of methylene blue
82.	Proteolytic activity
83.	Gelatine hydrolysis
84-104.	Acid production from different carbon sources:
	L-Arabinose
	Cellobiose
	Dextrin
	Dulcitol
	D-Fructose
	D-Galactose
	D-Glucose
	Glycerol
	Inositol
	Inulin
	Lactose
	Maltose
	D-Mannitol
	D-Mannose
	Raffinose
	Salicin

Table 1 continued

	D-Sorbitol
	L-Sorbose
	Sucrose
	Trehalose
	D-Xylose
105-125.	Utilization of carbon compounds listed above, as sole sources of C.
126-127.	Growth on inorganic N-agar /glucose-/$NH_4/_2HPO_4$; glucose-$NaNO_3$/
128-131.	Tween 20, 40, 60, 80 hydrolysis
132-135.	Salt tolerance, growth in nutrient broth containing 5%, 7.5%, 10% 15% NaCl
136-139.	Growth at: 5°C, 38°C, 42°C, 48°C
140-142.	pH tolerance, growth in nutrient broth at pH: 3, 5, 11
143.	Deoxyribonuclease production
144.	Ribonuclease production
145.	Hydrolysis of tributyrin
146.	Production of lecithinase /egg-yolk/
147.	Citrate utilization /Simmons'/
148.	Growth on MacConkey's agar
149-150.	Growth in the presence of 5% and 10% bile salts
151.	Hydrolysis of hippurate
152.	Reduction of tellurite
153-155.	Tolerance to moist heat treatment (60°C/10'; 70°C/10'; 80°C/10')
156-157.	Litmus milk reaction
158-179.	Utilization of organic acids as sole carbon sources
	Acetate
	Benzoate
	Gluconate
	Lactate
	Malonate
	Mucate
	Oxalate
	Pyruvate
	Salycilate
	Succinate
	Tartrate

Table 1 continued

180-182.	Amino acid decarboxylases /Møller/: /arginine, lysine, ornithine decarboxylases/
183-190.	Utilization of amino acids as carbon and nitrogen sources:
	L-Arginine
	L-Cystine
	L-Glycine
	L-Histidine
	L-Ornithine
	L-Serin
	L-Threonin
	L-Tyrosine
191-198.	Utilization of amino acids as nitrogen sources
199.	Production of chitinase
200.	Decomposition of xanthine
201.	Decomposition of hypoxanthine

Table 2: Selected diagnostic properties of *Micrococcus varians* and *M. agilis* representative strains isolated from the Lake Fertö.

	I.a group M.varians		I.b group M.varians		III.a group M.agilis	
	Number of strains 3		14		7	
Gram-staining	+/1/	±/2/		±/14/		±/7/
Motility		-/3/		-/14/	+/5/	±/2/
Hugh-Leifson test:						
glucose oxidative		-/3/		-/14/		-/7/
glucose fermentative		-/3/		-/14/		-/7/
Production of:						
Oxidase		-/3/		-/14/		-/7/
Catalase	+/3/		+/14/		+/7/	
Urease		-/3/	+/14/		+/7/	
Phosphatase		-/3/	+/10/	-/4/		-/7/
Voges-Proskauer reaction		-/3/		-/14/		-/7/
Indole formation		-/3/		-/14/		-/7/
Nitrate reduced to nitrite		-/3/		-/14/		-/7/
Acid from:						
D-glucose		-/3/	+/9/	-/5/		-/7/
D-xylose	+/3/			-/14/		-/7/
Lactose		-/3/		-/14/		-/7/
Maltose		-/3/	+/3/	-/11/		-/7/
Hydrolysis of:						
Aesculin		-/3/		-/14/		-/7/
Tween 80		-/3/	+/14/			-/7/
Starch	+/3/		+/8/	-/6/		-/7/
Gelatine	+/1/	-/2/	+/14/		+/7/	
Growth:						
in 5% addes NaCl	+/3/		+/12/	-/2/	+/7/	
in 7.5% added NaCl	+/2/	-/1/	+/6/	-/8/	+/7/	
in 10% added NaCl	+/2/	-/1/	+/2/	-/12/	+/7/	
at pH 3	+/3/			-/14/	+/1/	-/6/
at pH 5	+/3/		+/1/	-/13/	+/1/	-/6/
at pH 11	+/3/		+/14/		+/7/	
at 5°C	+/3/		+/14/		+/6/	-/1/
at 38°C		-/3/	+/14/			-/7/
at 48°C		-/3/		-/14/		-/7/

Table 3: Selected diagnostic properties of Flavobacterium spp representative strains isolated from Lake Fertö

	II. group *Flavobacterium* sp.		IV. group *Flavobacterium* sp.	
	Number of strains			
	2		3	
Production of yellow pigm.	+/2/		+/3/	
Gram-staining		-/2/		-/3/
Hugh-Leifson test:				
glucose oxidative		-/2/		-/3/
glucose fermentative		-/2/		-/3/
Production of:				
Oxidase	+/1/	-/1/	+/3/	
Catalase	+/2/		+/3/	
Urease		-/2/		-/3/
Phosphatase	+/2/		+/3/	
Deoxyribonuclease		-/2/		-/3/
Indole formation		-/2/		-/3/
Acid from: D-glucose		-/2/		-/3/
L-arabinose		-/2/		-/3/
D-xylose		-/2/		-/3/
D-mannitol		-/2/		-/3/
Hydrolysis of:				
Aesculin		-/2/	+/3/	
Starch	+/1/	-/1/	+/3/	
Casein	+/2/		+/3/	
Tributyrin		-/2/	+/3/	
Gelatine	+/1/	-/1/	+/3/	
Growth:				
in 5% added NaCl		-/2/		-/3/
in 7.5% added NaCl		-/2/		-/3/
in 10% added NaCl		-/2/		-/3/
at pH 3		-/2/		-/3/
at pH 5		-/2/		-/3/
at pH 9		-/2/	+/1/	-/2/
at pH 11		-/2/		-/3/
at 5°C	+/2/		+/1/	-/2/
at 38°C		-/2/		-/3/
at 48°C		-/2/		-/3/

Table 4: Selected diagnostic properties of *Bacillus sphaericus* and *B. pumilus* representative strains isolated from Lake Fertö.

	V. group Bacillus sphaericus		IX. group Bacillus pumilus	
	Number of strains 6		7	
Gram-staining	+/4/ ±/2/		+/4/ ±/3/	
Sporangium swollen	+/4/			-/3/
Spore shape	spherical/4/		ellipsoidal/3/	
Spore position	terminal/4/		central/3/	
Hugh-Leifson test:				
glucose oxidative		-/6/		-/7/
glucose fermentative		-/6/		-/7/
Production of: Oxidase		-/6/		-/7/
Catalase	+/6/		+/7/	
Voges-Proskauer reaction		-/6/	+/6/	-/1/
Formation of indole		-/6/		-/7/
Nitrate reduced to nitrite		-/6/		-/7/
Acid from: D-glucose		-/6/	+/7/	
L-arabinose		-/6/	+/7/	
D-xylose		-/6/	+/7/	
D-mannitol		-/6/	+/7/	
D-fructose		-/6/	+/7/	
Lactose		-/6/	+/4/	-/3/
Sucrose		-/6/	+/7/	
Hydrolysis of: Casein	+/6/		+/7/	
Gelatine	+/6/		+/7/	
Starch	+/6/			-/7/
Aesculin		-/6/	+/7/	
Growth:				
in 5% added NaCl	+/5/	-/1/	+/7/	
in 7.5% added NaCl	+/2/	-/4/	+/7/	
in 10% added NaCl		-/6/	+/7/	
at pH 3		-/6/	+/3/	-/4/
at pH 5		-/6/	+/3/	-/4/
at pH 11		-/6/	+/6/	-/1/
at 5°C	+/6/			-/7/
at 38°C	+/2/	-/4/		-/7/
at 48°C		-/6/		-/7/

Table 5: Selected diagnostic properties of *Pseudomonas alcaligenes* representative strains isolated from Lake Fertö.

	VII. group Pseudomonas alcaligenes	
	Number of strains 37	
Gram-staining		-/37/
Motility	+/37/	
Number of flagella	1 polar or subpolar /37/	
Hugh-Leifson test:		
glucose oxidative		-/37/
glucose fermentative		-/37/
Production of: Oxidase	+/37/	
Catalase	+/37/	
Utilization of: D-glucose		-/37/
D-fructose		-/37/
Maltose		-/37/
Acetate	+/37/	
Pyruvate	+/37/	
Succinate	+/37/	
Lactate	+/35/	-/2/
Citrate	+/37/	
Hydrolysis of: Gelatine	+/30/	-/7/
Starch		-/37/
Tween 80	+/11/	-/26/
Arginine	+/34/	-/3/
Growth:		
in 5% added NaCl	+/13/	-/24/
in 7.5% added NaCl	+/1/	-/36/
in 10% added NaCl		-/37/
at pH 3	+/7/	-/30/
at pH 5	+/7/	-/30/
at pH 9	+/31/	-/6/
at pH 11	+/31/	-/6/
at 5°C	+/36/	-/1/
at 22°C	+/37/	
at 38°C	+/ 5/	-/32/
at 48°C		-/37/

Table 6: Selected diagnostic properties of *Aeromonas hydrophyla* representative strains isolated from Lake Fertö

	X. group Aeromonas hydrophila		
	Number of strains 7		
Gram-staining			-/7/
Monotrich flagellation	+/7/		
Hugh-Leifson test:			
glucose oxidative	+/7/		
glucose fermentative	+/7/		
Production of: Oxidase	+/7/		
Catalase	+/7/		
H_2S from cysteine	+/7/		
Indole formation	+/6/		-/1/
Voges-Proskauer reaction	+/6/		-/1/
Nitrate reduced to nitrite	+/6/		-/1/
Utilization of: D-glucose	+/7/		
D-fructose	+/7/		
L-arabinose	+/7/		
D-mannitol	+/7/		
D-xylose			-/7/
Sucrose	+/7/		
Succinate	+/7/		
Hydrolysis of: Starch	+/7/		
Aesculin	+/6/	±/1/	
Gelatine	+/7/		
DNA	+/1/		-/6/
RNA	+/4/		-/3/
Tween 80	+/7/		
Growth:			
in 5% added NaCl	+/5/		-/2/
in 7.5% added NaCl			-/7/
in 10% added NaCl			-/7/
at pH 3	+/4/		-/3/
at pH 5	+/4/		-/3/
at pH 11	+/7/		
at 5°C	+/6/		-/1/
at 38°C	+/1/		-/6/
at 48°C			-/7/