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Studies on the composition and properties of bacterial populations colonizing the submerged parts of reed-stands of Lake Fertö

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Introduction

It is an accepted statement that bacteria, yeasts and fungi are responsible for the decomposition of organic remainders in the water and bottom mud of lakes and for the self-purification of natural water bodies. Our earlier studies carried out in different regions of the Lake Balaton showed that the reed-stands function as very active and efficient natural biofilters. Epiphytic bacterial communities of particular species composition and biochemical abilities can densely colonize the submerged (subhydric) parts of the reed-stems, filtering the streaming water masses intensively. A lot of observations and data of water analyses clearly showed that during the continuous horizontal movement of the water masses in Lake Fertö, the lake´ s water will be considerably purified after flowing through the widely extended reed-stands of the Hungarian territory.

The aim of our research work was to study the composition and properties of those complex epiphytic microbial populations which contribute to the self-purification of the water in the reed-stands of Hungarian areas of Lake Fertö.

Materials and methods

The sampling was carried out on the 8th of October, 1986. Reed-samples were collected at 4 larger sampling regions, presented in Fig.1. Samples obtained at 2 to 4 different sites within an individual region were bulked and formed a collective regional sample. In all cases, we collected young as well as more than one year old aseptically obtained pieces of reed-stems, then we transported them into the laboratory in sterilized test tubes filled with untreated lake-water. Reed samples were stored at 4°C until processing being started within 24 hours. Pieces of reed were washed three times with sterile tap water to remove organisms adhering loosely. Subsequently scrapes were taken aseptically from the surface of the reed-stems. Dilution series were made from the suspension of the homogenized crust material. Plating was carried out on 3 kinds of media, as follows: nutrient agar, starch-ammonium salt agar and distilled water solidified with 1.5 % of agar. Inoculated plates were incubated at 28° C for 7 to 10 days, then colonies were isolated non-selectively to slants composed of the same media as plates. No satisfactory growth was observed on starch-ammonium salt agar and water agar media, therefore the colony number countings and isolations were carried out only using the inoculated plates of the nutrient agar medium. A total of 1436 isolates were obtained this way. Their cultures were incubated at 28° C for a week. After

this, from our isolates true eubacterial ones were selected and isolates not to be maintainable on laboratory media, were neglected. So we obtained 1106 isolates altogether from the four sampling regions.

Thereupon tentative groupings (on the basis of selected cultural-morphological features of diagnostic value) and selections were done among our isolates obtained from reed-crust matter taken from the reeds of the individual sampling regions. In this manner several groups of similar bacterial isolates were formed in relation of all of the 4 studied regions. Similar isolates were bulked into similarity groups and representative strains were selected from all of them. Solitary isolates which remained outside the groups and represented only rarely occuring types or species were excluded from the further work. At the end 177 representative strains were subjected to detailed feature analyses. (Table 1).

With the representative strains the presence or absence of 99 diagnostic features, such as micromorphological, cultural-physiological and biochemical properties were tested. Table 2 presents the list of the studied characteristics. After the feature-analyses, on the basis of the obtained results, comparisons among all of the representative strains were carried out. For comparison the statistical methods of the Adansonian-type numerical taxonomy were used. Between each pairs of compared strains the similarity index according to Sokal and Mitchener was calculated on the basis of 89 coded features by an IBM PC AT-type computer. The cluster analysis and the construction of a dendrogram were based on more than 15 000 data. The computer program was wirtten by G. Lörinc.

Results

In Fig. 2 we present a dendrogram constructed on the basis of mutual similarity values of the 177 selected representative strains.

The computer created ten (I-X), more or less clearly separated groups of similar organisms involving altogether 114 strains. 11 strains occupied intergroup positions and were placed out of the created ten assemblages. Finally at the right side of the dendrogram a large agglomeration of morphologically relatively different but physiologically similar bacterial strains was formed. In this case further detailed feature analyses would be necessary to clarify the relationship of these organisms and for this reason, at present, we can not consider them as a separate group of related microbes.

The first group involves 14 strains of *Aeromonas*. This group represents altogether 72 reed-surface *Aeromonas* isolates. Aeromonads are common organisms in the epiphytic microbial community of the reed. 9 *Aeromonas* strains which were grouped together into the subgroup I b proved to be typical members of the species *A.sobria*. In our opinion the further members of Group I are mostly extreme variants of this species. The reed-*Aeromonas* population shows a very heterogeneous distribution in the reed-stands of Fertö, because all of our *Aeromonas* isolates were obtained from the reed-crust sample of only a single reed-stand. Table 3 shows some selected diagnostic properties of our *A.sobria* strains.

Group II is composed of 2 *Micrococcus* strains showing a considerable similarity but not complete identity with *M. varians*. Table 4 inform us about these organisms.

Two strains, which represent a minor fraction of the reed-community and belong to Group III. are the members of Enterobacteriaceae and may be identified as a *Shigella* species. This statement is corroborated by the data incorporated in Table 5.

A large group designated as Group IV contains 12 *Pseudomonas* strains, which represent altogether 57 similar reed-isolates. The distribution of these organisms proved not to be uniform too because they occurred only in the reed-stands of the inner lakes. Otherwise they belong to *Pseudomonas alcaligenes* which is a common member of the overall bacterial community of Lake Fertö. The identity of our *Pseudomonas* isolates and *P. alcaligenes* is presented in Table 6.

Group V involves again *Micrococcus* strains which may be considered as local variants of *M. luteus*.

The members of groups VI and VII represent organisms which, in our opinion, can play a subordinate role in the reed-crust communities and remained taxonomically undetermined.

A large group (Group VIII) of red coloured, lipolytic, phosphatase active organisms capable of growing on salts of organic acids and showing *Pseudomonas* diagnostic properties, remained at species level, undetermined. In the future we intend to clarify the biology and taxonomy of these bacteria which are predominant members of the epiphytic microbial populations of the reed in the littoral zone, but do not occur in the reed-stands of the inner regions of Lake Fertö.

In the reed-stands around the open water regions the epiphytic bacterial communities were in every studied case characterized by the presence and very common occurrence of relatively homogeneous populations of a coryneform microorganism. Altogether 4B representative strains of this coryneform, selected from 247 similar isolates, form the largest aggregate of the dendrogram: Group IX. At present we are trying to obtain a deeper insight into the taxonomic relationships of these bacteria.

Finally the last group of taxonomically evaluated assemblages of strains, Group X, comprises flavobacteria. It is a well known fact that flavobacteria, at present, are hardly identifiable on the species level. Our *Flavobacterium* isolates, their characteristic features are presented in Table 7, remained also undetermined.

Summary

1. No significant quantitative differences were detected in the total numbers of bacteria living epiphytically on the surface of submerged parts of reed-stems in different sampling regions of Lake Fertö.

2. The submerged reed-surface bacterial communities are complex assemblages of numerous different species.

3. The species composition of these epiphytic bacterial communities shows considerable regional differences.

4. In general, in the individual local reed-surface bacterial populations, only one or a few species can attain pre-or codominance, and many others remain subordinates.

5. The species diversity is largest in the reed stands of the inner lakes.

6. Surprisingly, in the epiphytic communities on the submerged reed-stems the number of facultatively anaerobic bacteria which can ferment e.g. glucose is very low, and in these milieus obligately aerobic organisms predominate.

7. The very common aerobic bacteria of the reed surface populations are, in general, unable to utilize inorganic N-sources, and cannot decompose many sugars offered to them as sole source of carbon. A maximum NaCl tolerance of 8 percent was detected at a few strains but several isolates showed considerable sensitivity to very low salt concentrations too.

References

Bergey´s Manual of Systematic Bacteriology, Vol. 1.,2. Williams and Wilkins, Baltimore- London 1984.

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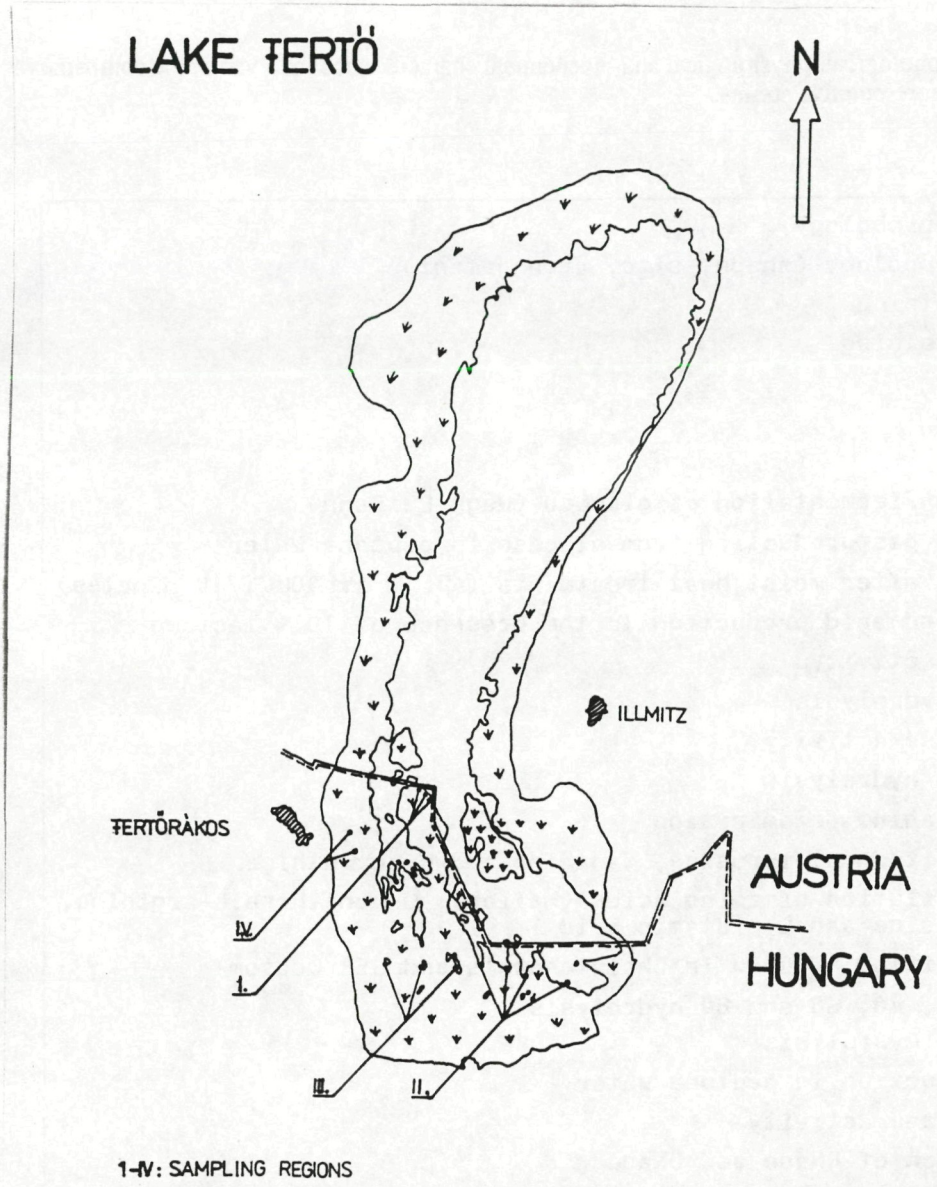

Fig. 1: Distribution of sampling areas in the Hungarian territory of Lake Fertö

Table 1: Total numbers of bacteria in the individual collective regional reed-crust samples, furthermore the numbers of isolates and those of the selected representative strains obtained from samples of the 4 sampling regions.

Sampling region	Total number of bacteria per 1 g homogenized moist reed surface scrape (nutrient-agar was used for plating)	Total number of isolates	Number of isolates wich were tentatively grouped	Number of selected representative strains
I.	2.04×10^8	404	185	37
II.	1.02×10^9	370	246	49
III.	1.14×10^8	326	240	48
IV.	4.7×10^8	336	220	43

Table 2: List of morphological, physiological and biochemical characteristics involved in the comparative studies of representative strains.

Colony morphology
Cell morphology (shape, size, arrangement)
Gram staining
Spore staining
Motility
Catalase
Oxidase
Oxidation/fermentation of glucose (Hugh-Leifson)
Acid and gas production from glucose in peptone water
Survival after moist heat treatments (60, 80 and 100 ^{0}C/10 minutes)
Growth and acid production in the presence of 10 % lactose
Casease activity
Starch hydrolysis
Gelatinase activity
Aesculin hydrolysis
Phenylalanine dezamination
Decomposition of tyrosine, xanthine and hypoxanthine
Decarboxilation of amino acids (Møller): L-ornithine,L-arginine, L-lysine and L-glutamic acid
Decomposition of urea in Christensen's and SSR medium
Tween 20, 40, 60 and 80 hydrolysis
Arginine hydrolysis
NH_3 production in peptone water
Phosphatase activity
Production of RNase and DNase
Acid and gas production from glucose (NH_4-salt+yeast extract)
Reduction of NO_3^- to NO_2^-, to NH_3 and to N_2 and/or N_2O
Indole production in peptone water, nutrient- and tryptone broth
H_2S production from cycteine and thiosulfate
Growth (acid or alkali reaction) in MacConkey's agar
Growth in the presence of 1,3,4,5,6,7 and 8 % NaCl
Utilization of organic acids as sole carbon sources (citrate, acetate, malonate, oxalate, succinate, benzoate, pyruvate, tartarate and lactate)
Inorganic N-source utilization (NO_3^- and NH_4^+ salts)
Acid and gas production on TSI agar
Growth and acid production on different carbohydrates (glucose, maltose, lactose, mannitol, inositol, sucrose, dulcitol and xylose)
pH in MR - VP broth
Acetoin from glucose (Voges-Proskauer)
Litmus milk reaction

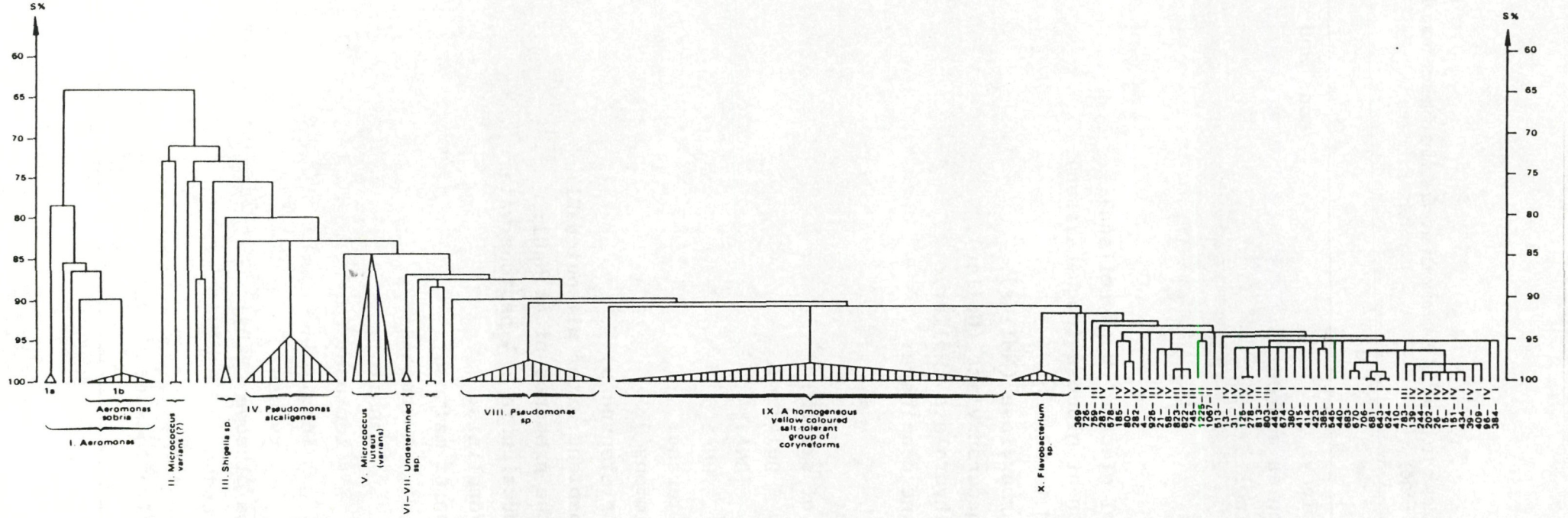

Fig 2: Dendrogram showing the relationships among 177 bacterial strains isolated from epiphytic microbial communities of the subhydric parts of reeds (Lake Fertö, W. Hungary)

Table 3: Selected diagnostic properties of 9 *Aeromonas sobria* strains obtained from the reed-surface region (Lake Fertö)

Cell morphology:	rods and coccobacilli (9)
Rods in singles and pairs:	+(9)
Gram staining:	-(9)
Motility:	+(9)
Catalase:	+(9)
Oxidase:	+(9)
Oxidation of glucose (Hugh-Leifson):	+(9)
Fermentation of glucose (Hugh-Leifson):	+(9)
NO_3^- reduced to NO_2^-:	+(9)
Lysine decarboxylase (Møller):	+(7)
Ornithine decarboxylase (Møller):	-(9)
Arginine dihydrolase (Møller):	+(7)
Phenylalanine deaminase:	-(9)
Urease:	-(9)
Phosphatase:	+(9)
Hydrolysis of starch:	+(9)
gelatin:	+(9)
DNA:	+(9)
RNA:	+(9)
Tween 80 esterase:	+(9)
Citrate (Simmons'):	+(9)
Citrate (Christensen's):	+(9)
Growth in peptone water without NaCl:	+(9)
Growth in the presence of 4% NaCl:	-(9)
Indole production in 1 % peptone water:	+(9)
Aesculin hydrolisis:	-(9)
Fermentation of maltose:	+(9)
sucrose:	+(9)
mannitol:	+(9)
lactose:	-(9)
inositol:	-(9)
xylose:	-(9)
Acetoin from glucose (V-P):	+(9)
Gas from glucose:	+(9)
H_2S from cysteine:	+(9)
Breakdown of malonate:	-(9)

Table 4: Some diagnostic properties of *Micrococcus varians* (?) strains of the reed-surface region

Pigmentation:	yellow
Water soluble exopigment:	-
Cell morphology:	spherical
Cells occur mostly in pairs and tetrads:	+
Gram staining:	+
Motility:	-
Spores are formed:	-
Catalase:	+
Oxidase (Kovács' method):	-
Oxidation of glucose (Hugh-Leifson):	+
Fermentation of glucose (Hugh-Leifson):	-
Aesculin hydrolysis:	+
Arginine dihydrolase:	-
NO_3^- reduced to NO_2^-:	-
Growth in the presence of 6 % NaCl:	+
Inorganic N-source utilization:	+
Citrate (Simmons'):	+
Aerobic formation of acid from lactose:	+
Tween 80 hydrolysis:	-
Starch hydrolysis:	+
Gelatine hydrolysis:	+
Acetoin from glucose (V-P):	+
Urease:	-
Phosphatase:	-
Indole production:	-
Phenylalanine deaminase:	-

Table 5: A comparison of the keycharacteristics of the genus *Shigella* with selected diagnostic properties of strains nos. 811 and 820 of a *Shigella* sp. isolated from reed samples.

	Strain 811	Strain 820	Shigella (Bergey,1986)
Cell morphology	rods	rods	rods
Gram staining:	-	-	-
Motility:	-	-	-
Catalase:	+	+	+
Oxidase:	-	-	-
Oxidation of glucose (Hugh-Leifson):	+	+	+
Fermentation of glucose (Hugh-Leifson):	+	+	+
Gas production from glucose:	-	-	-
Citrate (Simmons'):	-	-	-
Citrate (Christensen's):	-	-	-
Malonate utilization:	-	-	-
H_2S production on TSI agar:	-	-	-
Indole production:	+	+	d*
Acetoin from glucose (V-P):	+/-*	-	d
Urease (Christensen's):	-	-	-
Phenylalanine deaminase:	-	-	-
Lysine decarboxylase:	-	-	-
Arginine dihidrolase:	-	-	-
Ornithine decarboxilase:	-	-	d
Gelatine liquefaction:	-	-	-
Aesculin hydrolysis:	+	+	-
DNase:	-	-	-
NO_3^- reduced to NO_2^-:	+	+	+
Acid from dulcitol:	-	-	-
inositol:	-	-	-
lactose:	-	-	d
mannitol:	-	-	d
sucrose:	-	-	d
xylose:	+/-	+/-	-

* Symbols: +/-: questionable or weak reactions, d: different results

Table 6: A comparison of the summarized data on the diagnostic properties of our 12 reed-*Pseudomonas* representative strains with the characteristics of *P. alcaligenes* on the basis of the last edition (1986) of Bergey's Manual.

	Our reed-isolates	P.alcaligenes
Cell morphology:	rods (12)	rods
Gram staining:	-(12)	-
Motility:	+(12)	+
Fluorescent pigments:	-(12)	-
Catalase:	+(12)	+
Oxidase:	+(12)	+
Utilization of glucose:	-(12)	-
xylose:	-(12)	-
sucrose:	-(12)	-
maltose:	-(12)	-
mannitol:	-(12)	-
lactose:	-(12)	-
Arginine dihydrolase:	+(12)	+
NO_3^- reduced to NO_2^-:	-(12)	+
Gelatine hydrolysis	+ (9)	d*
Starch hydrolysis	-(12)	-
Tween 80 hydrolysis	+(12)	d
Denitrification:	-(12)	+
Growth on acetate:	+(12)	+
succinate:	+(12)	+
lactate:	+(12)	+
malonate:	-(12)	-
tartarate:	-(12)	-
benzoate:	-(12)	-
citrate:	+(12)	d
pyruvate:	+(12)	+
Decomposition of tyrosine	+(12)	d
Deamination of phenylalanine	-(12)	-

*Symbols: d: different results.

Table 7: A comparison of the sumarized data on the diagnostic properties of our 8 reed-*Flavobacterium* representative strains with the generic properties of *Flavobacterium*.

	Our reed-isolates (8 strains)	Flavobacterium (Bergey, 1986)
Cell morphology:	rods(8)	rods
Gram staining:	-(8)	-
Spores are formed:	-(8)	-
Motility:	-(8)	-
Catalase:	+(8)	+
Oxidase:	+(8)	+
Acid produced aerobically from glucose:	-(8)	d*
lactose:	-(8)	d
mannitol:	-(8)	d
inositol:	-(8)	-
sucrose:	-(8)	d
dulcitol:	-(8)	-
xylose:	-(8)	d
maltose:	+/-(8)*	d
Acid produced from 10 % (w/v) lactose:	-(8)	-
Casein digestion:	+(8)	d
Aesculin hydrolysis:	-(8)	d
Indole production:	-(8)	d
Starch hydrolysis:	-(8)	-
Urease production:	+/-(8)	d
Growth on MacConkey agar:	-(8)	d
Hydrolysis of Tween 20:	+(8)	+
Tween 80:	+(8)	d
Phoshatase production:	+(8)	+
H_2S production:	-(8)	-
Lysine and ornithine decarboxylase:	-(8)	-
Malonate utilization:	-(8)	-
Phenylalanine deaminase:	-(8)	-
Citrate (Christensen's, Simmons'):	-(8)	d
DNase production:	+/-(8)	+
Gelatine hydrolysis:	+(8)	d
NO_3^- reduced to NO_2^-:	-(8)	d
Tyrosine hydrolysis:	+(8)	d

*Symbols: +/-: weak or questionable reactions; d: different results