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Characterization of Selected Bacteria from the North Arm of the Great Salt Lake

John L. Crane

CHARACTERIZATION OF SELECTED BACTERIA FROM THE NORTH ARM OF THE GREAT SALT LAKE

by

John L. Crane Jr.

A thesis submitted in partial fulfillment
of the requirements for the degree

of

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in

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Approved:

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John L. Crane Jr.

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ABSTRACT

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North Arm of the Great Salt Lake

by

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Utah State University, 1974

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Thirteen bacterial cultures were isolated from the North arm of Great Salt Lake during the months of January and February of 1973. Eleven isolates were gram-negative pleomorphic rods which lysed in hypotonic solution. The remaining two were gram-positive cocci. All isolates and one known strain of Halobacterium salinarium were subjected to examination of morphological, cultural, physiological, and biochemical characteristics. A numerical taxonomic analysis was applied to the compiled characters to compute a coefficient of similarity for each individual isolate as compared to all other isolates. A comparative analysis was included in the similarity computation using characters assembled from those reported in the literature for six taxonomically accepted species of halophilic bacteria. The lake isolates proved to be extreme halophiles with relative high levels of similarity between each other and the known bacteria included in the numerical analysis.

(46 pages)

INTRODUCTION

The harsh environment provided by the high concentration of salts in the water of Great Salt Lake prescribes that the living inhabitants be highly specialized and suited for survival. The living inhabitants are in fact limited to a few specialized arthropods and microorganisms. This study was attempted to provide information about the nature and character of some representative bacteria found in the north arm of the lake.

Great Salt Lake is 110 km long and 45 km wide, and located 41 degrees 15 minutes North, 112 degrees 31 minutes West in the northeastern part of the Basin and Range Province of the Great Basin area. The mean surface area is 4.352×10^5 hectares and the mean volume 1.90×10^{10} m^3. It has a mean surface elevation of 1280 m with a mean depth at this level of 4.4 m and a maximum of 11.5 m. The lake experiences seasonal and cyclic variations in lake level, dependent on inflow and evaporation (Stokes, 1966). The mean dissolved solids is 22 percent during these fluctuations (Handy, 1966).

The lake was divided into two basins in 1957 by a rock fill causeway from Promentory Point to Lakeside. The causeway allows little water exchange thus the division into two basins. The Southern Basin receives 95 percent of the surface inflow and the recharge-deficient Northern Basin has become more saline as a result of greater evaporation in the North and replacement from the South Basin (Adams, 1964).

Thirteen bacteria were isolated from the waters on the North arm of Great Salt Lake. The isolates were phenotypically characterized as to their morphological, cultural, and physiological characters. A numerical taxonomic analysis was applied to the collected data as a means of further characterizing and ordering the lake isolates. Included in the numerical analysis were six known species of halophilic bacteria with as many corresponding characters as were possible to obtain from the literature.

REVIEW OF LITERATURE

The earliest report of the halophilic bacteria is possibly found in a Chinese treatise written about 2700 B.C. Baas-Becking (1931) writing on historical methods of obtaining salt describes the method found in the treatise. The Chinese concentrated salt from sea water collected into evaporation ponds, where the evaporating water would leave the salt behind in increasing concentrations. The index of concentration used was an intense red-coloration which correlated directly with the increasing salt in the water. This coloration presumably was the proliferation of red-pigmented halphilic organisms.

In the late 1800's the reddening of salted fish, thought to be caused by chemical reaction, was found to be due at least partially to microorganisms in the salted products. Thus the initial interest in the halophilic bacteria began with this discovery in the preserved fish industry. Farlow in 1880 described red-colored bacteria of varying shapes and sizes as the cause of the reddened salt-fish, but it was not until the early 1920's that any information on the general bacteriology of the organisms was reported. With the contributions of various workers such as Harrison and Kennedy (1922) it was realized that these bacteria living in a very salty environment were highly specialized organisms.

Following this recognition several descriptions of the bacteria were published, such as that of Gibbons (1936), but it was not until the mid-1950's that much consideration was given to the biochemical basis for the extreme salt

requirement of these bacteria. Gibbons (1956) initiated this research and reported that protein hydrolysis, and indol and hydrogen sulphide production could vary with changes in salt concentration.

Research along this line continued revealing a characteristic picture of these bacteria. They are considered extreme halphiles if they have been shown with certainty to grow best in sodium chloride concentrations greater than 20 percent. Bacteria fitting this character are referred to as either halobacteria or halococci. They are usually colored red to orange and are obligate aerobes.

The halobacteria are pleomorphic rods varying in size and shape. They are gram-negative and lophotrichously flagellated when motile. If exposed to hypotonic saline solution lysis will occur. A minimum of 12-20 percent sodium chloride is required for growth, but a concentration approximate to 25 percent sodium chloride provides optimal growth. Organisms such as these are collected into the genus Halobacterium and assigned to the family Pseudomonadaceae in the 7th edition of "Bergy's Manual" (1957).

The halococci, coccoid in shape are non-motile, non-spore forming, and gram-variable. The variability of the gram stain Brown and Cho (1970) speculate, is due to the gram-positive character of the cell wall, but which lacks muramic acid. The cells tend to occur singly, in pairs, and sarcina-like packets. Lysis is not demonstrated in hypotonic solutions, but at least 12 percent sodium chloride is required for growth, with optimal growth in 20-25 percent concentrations of sodium chloride. In the 7th edition of "Bergy's Manual" (1957), the halococci are placed into the genera Micrococcus and Sarcina in the

family Micrococcaceae. Kocur and Hodgkiss (1973), however, propose they be assigned the genus Halococcus, based on extensive character and taxonomic analysis of twenty-two strains of extreme halophilic cocci.

The character of orange to red pigmentation of the extreme halophiles is due to carotenoids. The presence of these carotenoids, however, has no exact relation to the halphilic character of the bacteria. The significance of the pigmentation is that the carotenoids apparently have the ability to protect the cells from the damaging effects of harsh sunlight; a characteristic of the areas of the earth producing environments suitable for these organisms. Larsen (1962) has shown this in comparative experiments using a carotenoid containing halophile and a colorless mutant artificially produced from the pigmented progenitor. Under simulated conditions of bright sunlight the carotenoid lacking mutant grew at a significantly decreased rate. Under conditions of no simulated sunlight the mutants rate of growth was comparable to that of the carotenoid containing non-mutant.

The nutritional requirement of the extreme halophiles is one of a complex nature. They are generally considered not to use carbohydrates and preferentially metabolize proteins and amino acids for their carbon source. Tomlinson (1972, 1973), however, showed that they utilized many carbohydrates readily, when provided the proper environment and medium. Flannery (1956) pointed out that these bacteria were quite metabolically active, but because they grew so slowly a longer period of incubation was sometimes necessary to demonstrate any change in a particular substrate. Regardless of their

metabolic activity, however, the best growth and cultivation is obtained on a complex medium containing a protein digest. Katznelson and Lockhead (1952) described a medium containing vitamin-free casamino acids, cystine and tryptophan plus the appropriate salts. Kocur and Hodgkiss (1973) utilized a medium devised by Sreenivasan and Venkataraman (1956) in their taxonomic study of the halococci which consisted of sterile skim milk, peptone, glycerol and a salt solution.

Dundas et al. (1963) further defined the complex nutritional requirement of the extreme halophiles by devising a chemically defined medium for Halobacterium salinarium. The medium consisted of inorganic salts and ten amino acids, four of which (valine, leucine, isoleucine, methionine) proved to be essential. The medium supported good growth of H. salinarium and some of the other strains of halobacteria, but growth was not as rapid as that obtained using a yeast extract medium. The medium proved deficient for the halococci, as they would not grow.

Later Onishi et al. (1965) designed a chemically defined medium which supported all their strains of halobacteria and halococci. They found that growth of Halobacterium cutirubrum on the synthetic medium was as good as that obtained on a complex medium. The defined medium, besides inorganic salts consisted of fifteen amino acids, two nucleotides (adenylic and uridylic acids) and glycerol. Using H. cutirubrum in special tests, it was determined that four of the amino acids (arginine, leucine, lysine, valine) were essential. All the other amino acids were stimulatory. This requirement of so many amino acids is in-

dicative of the nutritional complexity of the extreme halophiles, and indicates an inability to synthesize amino acids readily.

The enzyme systems of the extreme halophiles have been found to be not only tolerant, but dependently adapted to the high salt concentrations of their environment. Baxter and Gibbons (1954, 1956, 1957) demonstrated this finding by testing a number of enzymes to varying sodium chloride concentrations. They showed that the enzymatic activity was good in concentrations that would be found in normal culture conditions and little or no activity was demonstrated in low salt concentrations. Subsequent studies of individual enzymes obtained from extreme halophiles revealed similar findings. Holmes and Halverson (1965a, 1965b) and Cazzulo and Vidal (1972) studying malate dehydrogenase confirmed the halophilic character of this enzyme and found it dependent upon a high salt concentration for its activity. Another enzyme found to be dependent on high salt concentration for its activity is the aspartate transcarbamylase of H. cutirubrum (Norberg et al., 1973). These types of studies revealing the salt dependency of the extreme halophilic enzymes exemplifies the adaptive forces imposed upon them by their environment.

Environmental conditions conducive to the proliferation of extreme halophiles are wherever salt reaches a concentration sufficient for their growth. They have been isolated in salted hides and fish (Anderson, 1954), and in the Dead Sea (Volcani, 1940, as cited by Larsen 1962). Bacteria have also been found in the Great Salt Lake (Daines, 1917; Frederick, 1924; Smith, 1936; and Smith and Zobell, 1937).

Daines (1917) first isolated bacteria found in the Great Salt Lake, but excluded any extreme halophiles by using 2.5 percent sodium chloride for his plate counts. Frederick (1924) followed the work of Daines and made nutritional studies on the bacteria she isolated in the lake. Smith (1936) and later Smith and Zobell (1937) examined the possibility of a bacterial flora indigenous to the lake. Subsequently no studies have been reported on the character of halophilic bacteria that might be found indigenous to the lake.

MATERIALS AND METHODS

The thirteen organisms isolated from Great Salt Lake and one strain of Halobacterium salinarium obtained from Susan Kelley, Texas Woman's University, Denton, Texas were maintained on a basal medium (BM) prepared in two solutions and containing (g/liter): (i) yeast extract (Difco), 10g; tryptone (Difco), 2.5 g; and when required agar (Difco), 20 g; in 500 ml water; (ii) salt solution: NaCl, 220 g; $MgSO_4 \cdot 7H_2O$, 10 g; KCl, 5 g; $CaCl_2 \cdot 6H_2O$, 0.2 g; sodium citrate, 3 g; in 500 ml water. The two solutions were autoclaved separately then aseptically mixed together, and the pH adjusted to 7.0 with 1.0 N NaOH (filter sterilized).

The organisms isolated from Great Salt Lake were obtained from water samples taken aseptically from Rozel Point, located 15 miles Southwest of the Golden Spike National Monument. The sampling was done during the months January and February of 1973. Upon return to the laboratory the samples were thoroughly mixed and 0.1 ml amounts inoculated to individual 16 mm tubes containing 10 ml BM broth, and 60 mm petri plates containing 15-20 ml BM agar. Inoculated agar plates were spread with a sterile bent glass rod to obtain isolated colonies. Three sets of three tube Most Probable Number (MPN) dilution series were inoculated at the same time, and incubated at 4 C, 20 C, and 35 C respectively. Isolated colonies were streaked and subsequently restreaked at least three times to produce pure culture isolates. Upon the appearance of

growth in the broth cultures and likewise in the highest dilution of the MPN, agar plates were streaked and incubated at the temperature of initial growth; isolated colonies were purified as before. After purification of the cultures was accomplished it was determined that of the three temperatures, 35 C was the optimum temperature for growth for all isolates regardless of the temperature of initial isolation. All subsequent incubation was at 35 C.

For morphological, cultural, and physiological studies, cultures were incubated 7 to 28 days at 35 C. Gram staining was done by the method of Dussalt (1955). Colony characteristics, growth in liquid and on agar slants were observed on BM. Desired pH's were obtained after autoclaving by addition of 1.0 N NaOH or 0.1 N HCl (filter sterilized).

The utilization of glucose, arabinose and xylose was determined by using BM agar slants with 1.0 percent (w/v) carbohydrate added and phenol red (.025 g/liter) as the indicator. Citrate utilization was tested on Simmon's citrate agar (Difco) with 22 percent (w/v) NaCl. Catalase was detected by flooding the growth on BM agar plates with 3 percent H_2O_2. The benzidine test for porphyrin (Deibel and Evans, 1959), and the production of oxidase (Gaby and Hadley, 1957) were performed on BM agar growth.

The hydrolysis of starch, gelatin, casein, and chitin was determined using a thin overlay of the polymer in basal medium over a previously solidified layer of BM agar. The starch medium consisted of BM agar with 0.5 percent (w/v) soluble starch added prior to autoclaving. Hydrolysis was determined by flooding plates with Lugol's iodine. The medium for determination of gelatin

hydrolysis consisted of BM agar and 2.0 percent (w/v) gelatin. Hydrolysis was detected by flooding plates with acidified mercuric chloride. The test medium for casein hydrolysis was an overlay of BM agar with 2.0 percent (w/v) sterile skim milk. A clear zone around the colonies was interpreted as hydrolysis of casein. Utilization of chitin was determined with an overlay of BM agar and a heavy suspension of dispersed chitin (Skerman, 1967) added just prior to pouring the plates. Again a clear zone around the colonies was interpreted as hydrolysis.

The presence of Tween-80 splitting enzyme was determined by a modification of Sierra's medium (1957 cited by Skerman, 1969): (g/liter) peptone (Difco), 10 g; NaCl, 250 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; agar, 20 g; and 10 ml Tween-80 added after autoclaving the above solution. Presence of precipitated calcium and fatty acid salts around the colonies was indicative of enzymatic activity.

Nitrate reduction was determined by adding 5 drops of Nitrite A reagent (0.5 g sulfanilic acid; 30 ml glacial acetic acid added to 100 ml water) and 5 drops of Nitrite B reagent (0.1 g alpha-naphtylamine; 100 ml water; 30 ml glacial acetic acid) (Skerman, 1967) to growth on nitrate agar slants. The slants consisted of BM agar plus KNO_3 at a final concentration of 2.5 percent (w/v).

Nitrite reduction was tested for in the same manner as above, using BM agar slants and $NaNO_2$ at a final concentration of 0.25 percent (w/v). A red color indicated the presence of nitrite and therefore the absence of nitrite reduction.

The ability to utilize ammonia or nitrate as a sole source of nitrogen was tested for in the following two media: The ammonia medium (i) consisted of the salt solution from the basal medium; glucose, 1.0 percent (w/v); sodium citrate, 0.1 percent (w/v); NH_4Cl, 1.0 percent (w/v); and agar, 2.0 percent (w/v) prepared in slants. The nitrate medium (ii) was prepared as the ammonia medium replacing NH_4Cl with 1.0 percent (w/v) $NaNO_3$. The presence of growth was interpreted as a positive result.

The production of ammonia from peptone was tested in broth and on agar slants. The media were prepared as the basal medium, substituting 4.0 percent (w/v) peptone (Difco) for the tryptone. Brom-Thymol Blue (0.04 percent) was added to the slants to detect the production of ammonia. The presence of ammonia in the broth culture was tested for with Nessler's reagent.

The solubilization of phenyalanine, tyrosine, and xanthine was tested with a heavy suspension of the amino acid in small amounts of BM agar overlayed on BM agar. The amino acid was added and mixed thoroughly just prior to pouring the overlay. Disappearance of the amino acid crystals, suspended in the medium, around colony growth was interpreted as solubilization of the amino acid. Lysine decarboxylase and urease were tested for with Patho Tec test papers (General Diagnostics Division, Warner-Chilchott, Morris Plains, N.J.).

The production of hydrogen sulfide from sodium thiosulfate and cysteine was detected by means of a strip of lead acetate paper inserted into the mouth of a tube containing the test medium. The media were prepared in

slants using BM agar and either 1.0 percent (w/v) cysteine HCl or sodium thiosulfate.

Indol production was determined by Gore's method (Gore, 1921, as cited by Skerman, 1969) by using BM prepared in agar slants.

The methyl-red and Voges-Proskauer reactions were determined by using MR-VP medium (Difco) and the salts of the basal medium. The MR-VP medium and salts were prepared and autoclaved separately then aseptically added together and the pH adjusted to approximately 7.2.

A numerical analysis was applied to compute a percent similarity (%S) of each isolate (OTU) compared with all other isolates and the strain of H. salinarium that was characterized in conjunction with them. Also included were data from "Bergy's Manual" (1957) and other sources for five species of Halobacterium (H. salinarium, H. cutirubrum, H. halobium, H. marismortui, and H. trapanicum) and two species of Halococcus (Sarcina morrhuae and Micrococcus morrhuae) (Kocur and Hodgkiss, 1973). Their comparison with the experimental OTUs was dependent upon what related data were available.

Calculations of %S were made using both positive and negative matches and were computed as follows:

$$\%S = \frac{Nsp}{Nsp + Nd} \times 100 \quad \text{and} \quad \%S = \frac{Nsp + Nsn}{Nsp + Nsn + Nd} \times 100$$

where %S = similarity coefficient, Nsp = number of similar positive matches, Nsn = number of similar negative matches, and Nd = number of dissimilar matches. The experimental data as well as that available for the known

organisms were coded into a form of positive, negative or data missing and then punched onto standard computer cards. The programs were written in Fortran (F. J. Post, personal communication, 1974) and designed to compute similarity coefficients of all the OTUs on the basis of their positive, or positive plus negative, character matches (program SIMCOF) and calculate a median organism (Liston et al., 1963) based on the distribution of positive characters (program MEDORG).

RESULTS

The results presented in Figures 1, 2 and 3 represent a compilation of the characters used to examine and characterize the bacteria isolated from Great Salt Lake. Each bar in the graphs represent a computed percentage of occurrence for a particular character based upon the total number of organisms. The five species of halobacteria and two species of halococci were included with as many characters as were possible to obtain.

The majority of OTUs, 81 percent of the sample, examined were gram-negative rods occurring in variable cell arrangements (Figure 1). Of this majority, the greatest preponderance had a length greater than 4.0 microns (43 percent) and uniform width of .9 microns (91 percent). All OTUs were non-sporeforming and approximately equal in the number of motile and non-motile forms. Only two cocci were isolated from the lake and were found to be gram-positive and non-motile with diameters of .9 microns. These two cocci plus the two recently described (Kocur and Hodgkiss, 1973) coincided closely in morphological character and constituted 19 percent of the computer sample.

Figure 2 shows the various dispositions of colony type, pigmentation and growth in liquid media. Percentages of the total under the major headings may not add up to 100 percent due to the expression of more than one character by an OTU under that heading. Pigment was expressed by all the OTUs but of the 10 percent producing yellow pigment and the 65 percent producing

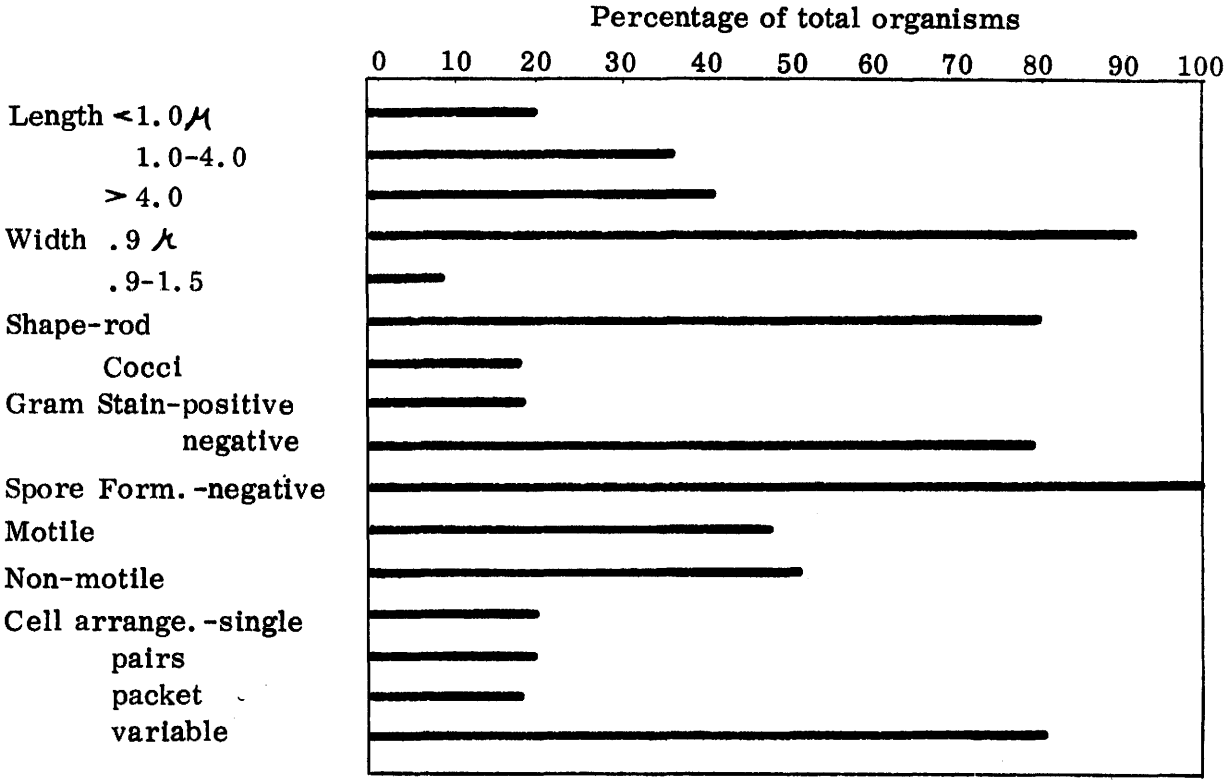

Figure 1. Computed percent occurrence of morphological characteristics.

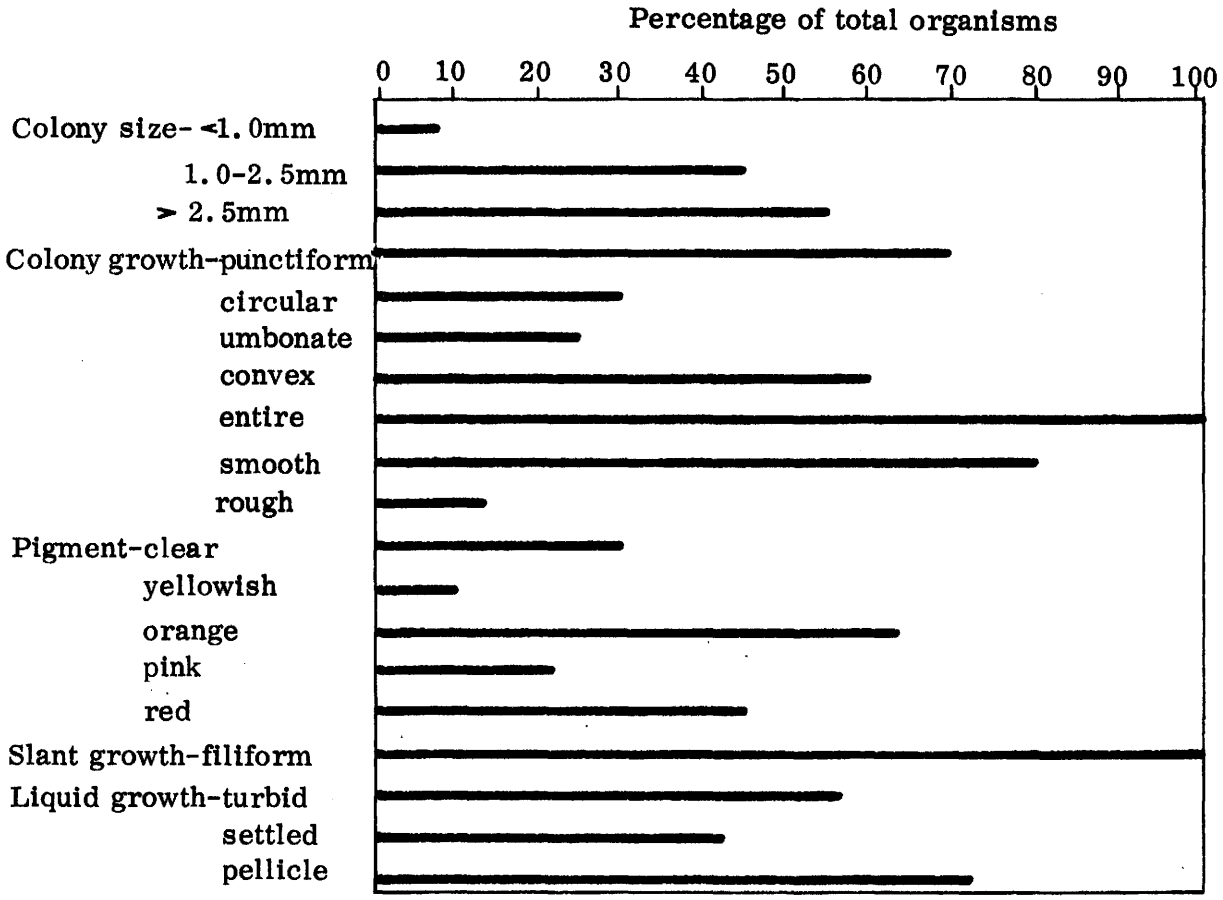

Figure 2. Computed percent occurrence of colony characters, pigment production, and growth phenomena.

orange pigment a few colonies would appear to be clear and were recorded as clear to yellow or orange. In coding the data a character of clear to orange was coded as two characters and scored appropriately. This yielded 30 percent of the OTUs with a clear character for pigmentation in the figure. Similarly, 70 percent of the sample produced a pellicle in liquid growth as well as other characters of liquid growth. One hundred percent of the OTUs produced a filiform type of growth on agar slants.

Figure 3 shows the various physiological and biochemical characters examined in the study. One hundred percent of the organisms have a salt requirement of 15 percent (w/v) or greater NaCl. Of the 100 percent that grow well at pH 7, 86 percent show growth at pH 6 and 43 percent at pH 8. Growth was not found at pH 9 or pH 5 or lower and are not indicated in the figure. The biochemical activity of the organisms as a group is relatively high, given the proper environment and incubation time. The hydrolysis of all the large polymers tested, except starch (not shown in Figure 3), was evidenced by some members of the group in each test. Seventy-six percent were able to hydrolyse gelatin and 58 percent hydrolysed casein. Tween-80 was hydrolysed by 50 percent of the tested OTUs. A significant number of the lake isolates, 43 percent were able to hydrolyse chitin.

Nitrate was reduced to nitrite by 71 percent of the OTUs. Reduction of nitrite as well as production of gas (not shown in Figure 3) was not evidenced by any of the isolates using the technique employed in this study. It has recently been found, however, that several more isolates demonstrated the ability to

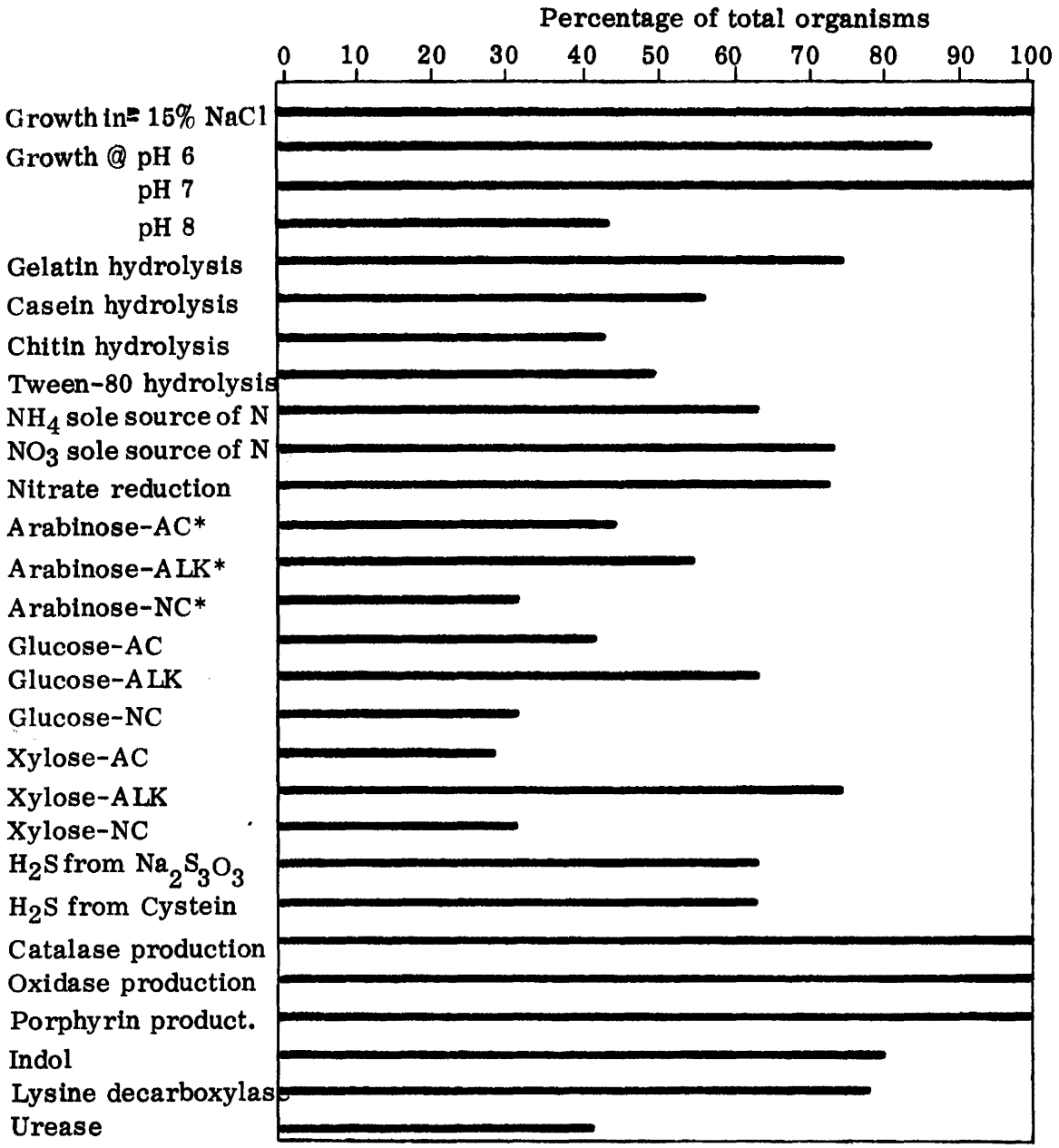

Figure 3. Computed percent occurrence of physiologic and biochemical characters. *AC, acid reaction; ALK, alkaline reaction; NC, no change.

reduce nitrite and four have been shown to produce nitrogen gas (F. J. Post, personal communication 1974). Sixty-four percent of the isolates were capable of utilizing ammonia and 71 percent were able to use nitrate as a sole source of nitrogen.

The metabolism of the three carbohydrates by the lake isolates was varied (Figure 3). The OTUs from the lake uniformly produced either acid or alkaline reactions, whereas the usual reported reaction for the known halophilic bacteria is no change. Gas production was not evident in the test media used, but acid or alkaline changes were definite. The deviation of percent above 100 for each particular sugar is due to the absence of data under the categories of acid or alkaline reactions for the known halophiles.

Sixty-seven percent of the OTUs produced H_2S from sodium thiosulfate and/or cysteine. However, it was recently found that a few of the isolates produce H_2S while growing on the basal medium alone (F. J. Post, personal communication 1974). Consequently, it is questionable whether the H_2S produced came from the break down of cysteine, sodium thiosulfate and/or certain constituents of the basal medium. In view of this the general production of H_2S might be a more appropriate category to use at this time in characterizing the lake isolates.

Indol production was evidenced by 81 percent of the OTUs. Lysine decarboxylase was produced by 80 percent of the lake isolates and urease by 40 percent. All OTUs were catalase, oxidase and porphyrin positive.

A diagonal matrix of similarity coefficients (%S) was computed and is presented in Figure 4. Negative matches were included in the computations that are based upon the characters examined for each OTU. The matrix is a random representation of the similarity coefficients and rearrangement or clustering of the position or order of the member OTUs is needed to yield meaning to the matrix. Figure 5 is the rearranged cluster matrix, partially plotted with four differentially shaded symbols representing four numerical ranges (80 - 100%S). It was found in the rearranged matrix that all the member OTUs became united at the 72%S level, but groups or clusters appeared only at levels of 80 - 100%S. In this range six groups or clusters are found.

Using these data dengrograms were constructed (Figures 6 and 7) which illustrate the relative relatedness of the member OTUs. The levels at which the branches join together represent the highest similarity coefficient linking a pair or group of OTUs.

Figure 6 was constructed using the order of OTUs arrived at in the cluster matrix (Figure 5). At a level of 85%S the six groups of OTUs found in the cluster matrix are discernible, and are listed in Table 1. Lowering the level to 80%S reduces the number of groups to four by increasing the number of OTUs related in a particular group. The four groups obtained at 80%S are listed in Table 2.

The dendrogram in Figure 7 was constructed from the same data, but slightly rearranged to provide a different visualization of the groups. OTUs 002, 005, and 003 were shifted to the left and 001 and 014 were shifted to the right of 016. Placement of 002, 005, and 003 is essentially arbitrary as they are not

	Organism no.																				
	01	02	03	04	05	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22
02	69																				
03	67	70																			
04	69	75	61																		
05	58	67	66	77																	
07	64	70	69	73	69																
08	70	77	72	70	63	94															
09	63	75	64	69	70	80	86														
10	78	72	67	81	61	77	80	69													
11	72	66	64	72	64	80	83	84	75												
12	50	63	52	59	55	70	77	78	63	66											
13	52	64	50	58	53	69	75	77	61	64	98										
14	80	61	63	77	66	63	59	52	70	64	39	41									
15	80	67	75	73	69	69	69	61	80	67	48	47	78								
16	77	64	70	73	61	59	61	50	75	52	63	64	80	86							
17	62	68	74	65	65	62	65	53	68	50	35	35	65	68	79						
18	73	68	58	75	63	65	65	58	85	60	43	40	68	78	77	87					
19	77	67	62	72	56	74	74	62	69	69	41	38	72	69	75	69	80				
20	70	68	63	65	53	75	75	68	73	63	48	45	60	65	72	72	82	94			
21	53	45	43	39	31	49	49	39	45	47	63	65	47	41	49	42	53	56	55		
22	59	44	38	41	36	49	46	46	41	51	69	72	51	41	44	29	48	52	52	95	

Figure 4. Matrix of percent similarity between OTUs. Data for cultures 16-20 taken from "Bergy's Manual" (1957) other sources. Data for cultures 21 and 22 taken from Kocur and Hodgkiss (1973).

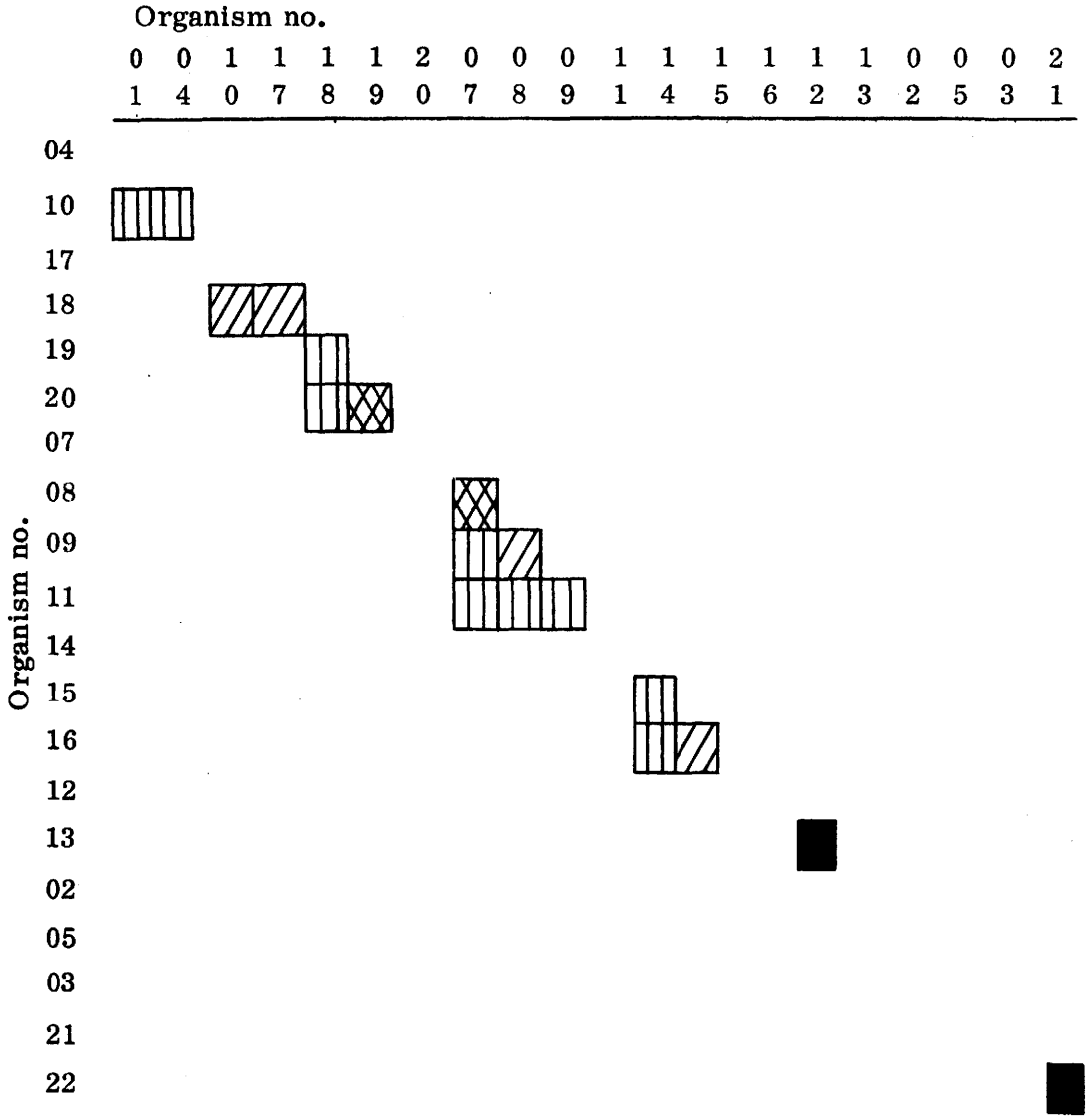

Figure 5. Clustered matrix of percent similarities of sorted OTUs from Figure 4. The range of 80-100 percent was divided into four 5 percent ranges and plotted as shown in the key.

Key:

95-100%

90-95%

85-90%

80-85%

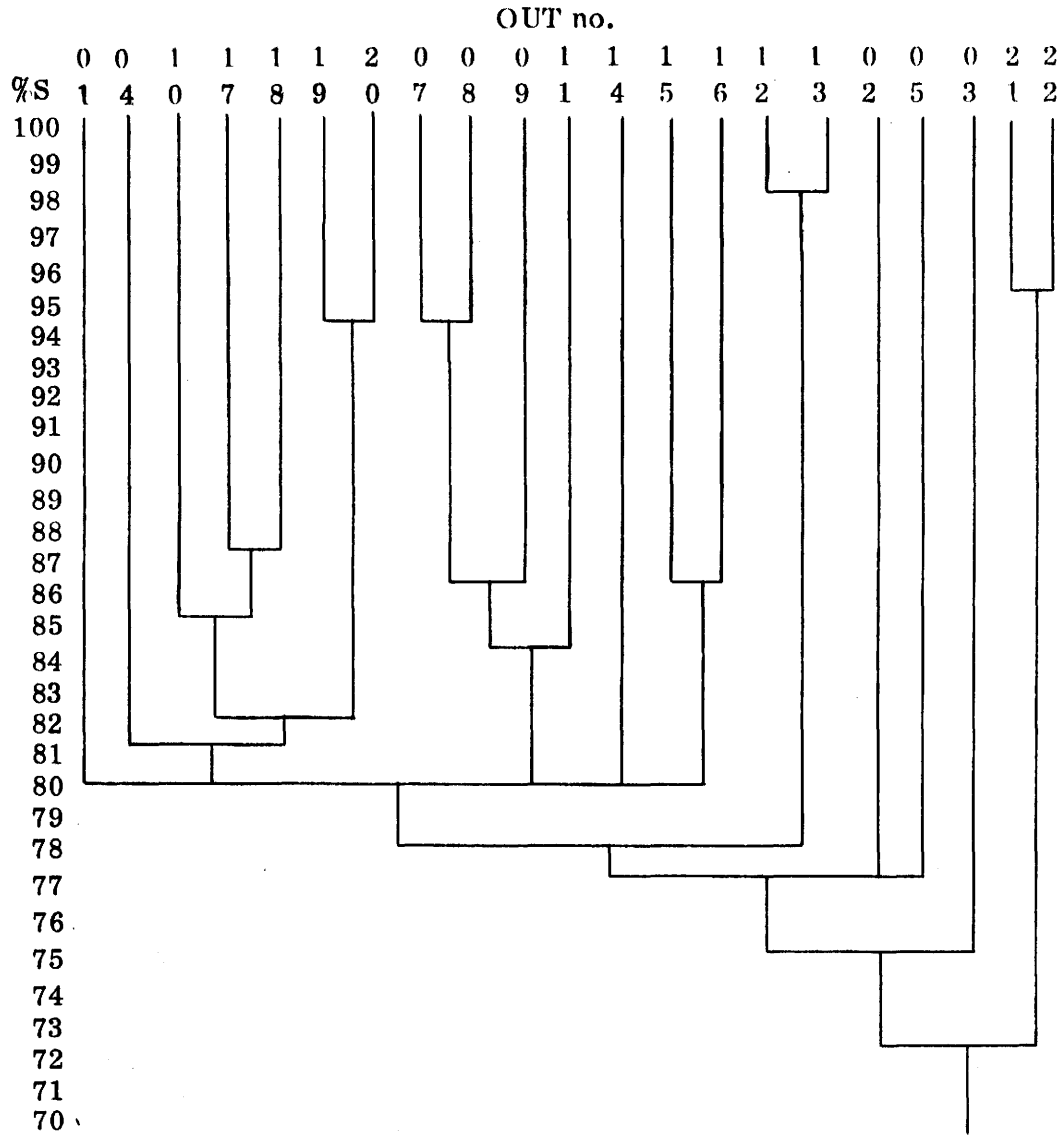

Figure 6. Computer generated dendrogram constructed from percent similarities of sorted OTUs, negative matches included.

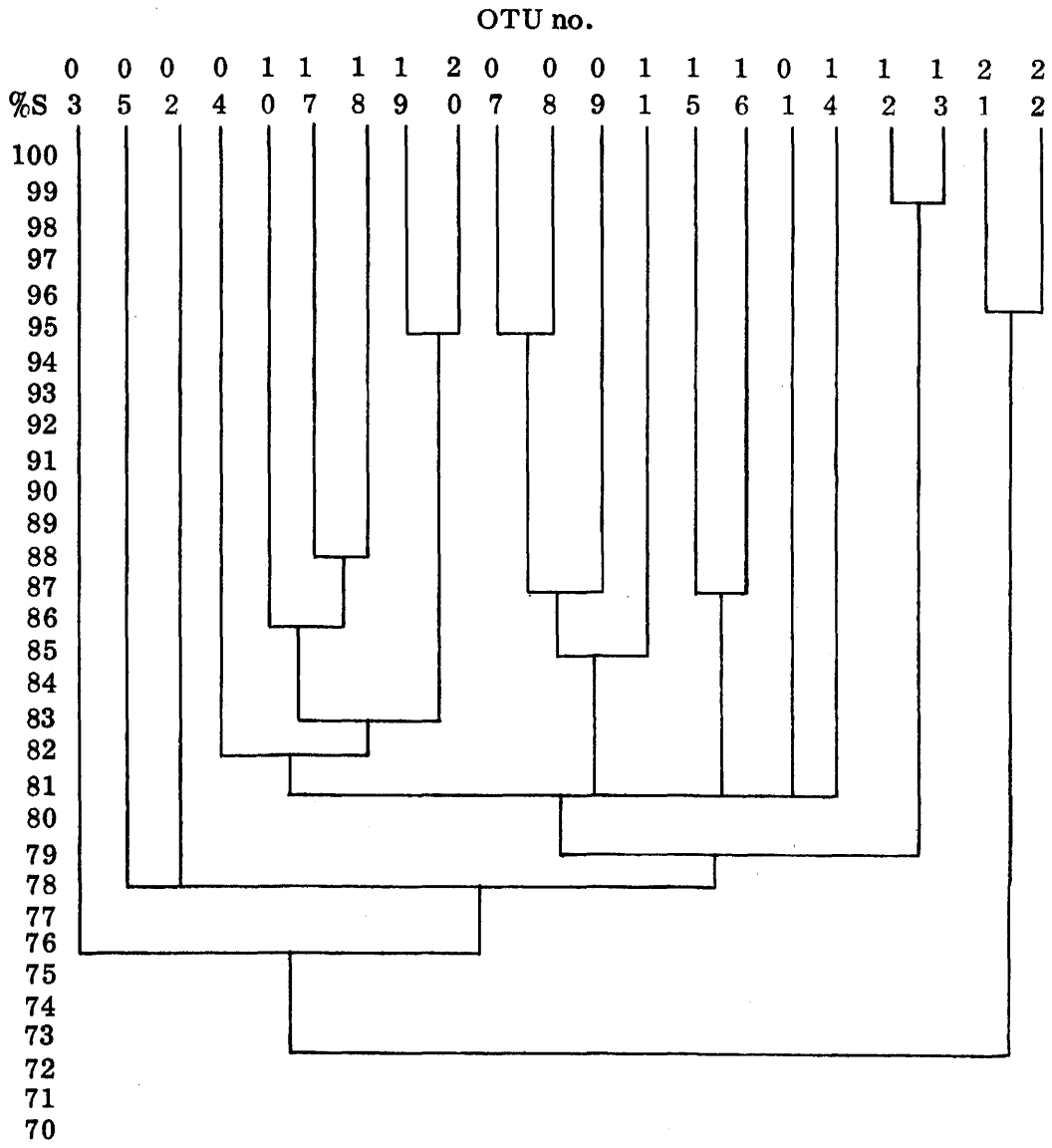

Figure 7. Dendrogram rearranged from Figure 6.

Table 1. Groups of OTUs occurring together at or above the 85%S level in Figures 6 and 7

Groups					
I	II	III	IV	V	VI
010	019	007	015	012	021
	H. marismortui		H. salinarium		S. morrhuae
017	020	008	016	013	022
H. cutirubrum	H. trapanicum		H. salinarium		M. morrhuae
018		009			
H. halobiumun					

Table 2. Groups of OTUs occurring together at or above the 80%S level in Figures 6 and 7

Groups			
A	B	C	D
004	007	012	021
			S. morrhuae
010	008	013	022
			M. morrhuae
017	009		
H. cutirubrum			
018	011		
H. halobium			
019	015		
H. marismortui	H. salinarium		
020	016		
H. trapanicum	H. salinarium		
	001		
	014		

associated with any members of the set above 78%S. At a level of 80%S the majority (excluding 002, 003, 005, 012, 013, 021, and 022) of OTU are tied making it possible to move 002, 003, and 005 without disturbing the order of the rest. The placement of 001 and 014 to the right of 016 is also possible to do without affecting similarity relationships in the order. At 80%S 001 is associated with 014 and 015, also 014 and 016 are tied at 80%S as are 010 and 015. In Figure 6, 001 was placed more proximally to 010 because they share a 78%S level, but 001 is tied at a higher level to 014 and 015 making the adjustment plausible. The 001 and 010 relationship at 78%S is actually insignificant due to the ties between 001, 014, and 015, and the unifying tie between 010 and 015.

DISCUSSION

The purpose of the study was to phenotypically characterize bacteria isolated from Great Salt Lake and then apply a numerical method of taxonomy to organize and separate the members of the lake isolates. A group of known extreme halophiles were also examined in conjunction with the lake isolates as a comparative study. This was limited by the available taxonomic characters existing in the literature that coincided with those used in this study. Any explicit identification as to assigning isolates or groups of isolates to existing taxonomic ranks was not done. However, confirmation of the lake isolates into the group of taxonomically accepted extreme halophiles is evident in their characters of morphology and culture growth. Especially their extreme requirement for high concentrations of salt. All of the lake isolates required a minimum of 15 percent NaCl for maintenance, with optimum growth provided at 20-25 percent NaCl. The numerical analysis revealed that the lake isolates have a relatively high level of relatedness to the known extreme halophiles, with all OTUs being tied together at a 72%S level.

The ability of 64 percent of the isolates to utilize ammonia and 71 percent able to use nitrate as a sole source of nitrogen is of interest and might be of taxonomic importance. As was stated in the review of literature, Dundas et al. (1963) and Onishi et al. (1965) devised chemically defined media for H. salinarium and H. cutirubrum and found several amino acids to be essential. Many of

the lake isolates appear to be able to produce their amino acids by de novo synthesis unlike the two organisms used by Dundas (1963) and Onishi (1965). The test medium consisted only of the appropriate nitrogen source, glucose, agar and salt solution. In agreement with Onishi (1965) though, the known strain of H. salinarium tested with the lake isolates proved negative for both tests. It would appear that organisms possessing this character would have a selective advantage over those which did not, possibly explaining the predominance of isolates positive for the two tests. It is also possible that organisms such as those used by Dundas (1963) and Onishi (1965) and the strain of H. salinarium used in this study lose some of their synthesizing capabilities after prolonged maintenance on the complex media used with these halophilic organisms.

The hydrolysis of chitin by several isolates is another significant character that might serve as a taxonomic tool. It also implies that these isolates decompose, at least partially, the exoskeletal remains of the arthropodal organisms found in the lake. A process that would be of major importance in nutrient cycling and the lake community.

Examination of the dendrograms (Figures 6 and 7) reveals some possible relationships between the six groups of OTUs tied at a level of 85%S or greater. Of the OTUs which are related at the higher similarity levels, there are two pairs of bacteria which were isolated from the lake. The first pair are OTUs 012 and 013 which are cocci and have a 98 percent similarity. They

are probably the same species of bacteria. The second pair of lake isolates, 007 and 008 are related at a level of 94%S; they too are likely to be the same species. The remaining OTUs found in the 85-100%S levels are comprised mainly of the known and presently accepted species of halobacteria and halococci. OTUs 021 (S. morrhuae) and 022 (M. morrhuae) are related at 95%S. A level that might be interpreted as that which would be shared by the same or similar species of one genus, not two different species of two separate genera. These two halococci in group VI (Table 1) share a similarity of 72%S with the cocci isolated from the lake which are placed in group V. OTUs 019 and 020, the halobacteria H. marismortui and H. trapanicum are tied at a level of 94%S, a high level of similarity for two separate species. A similar occurrence is found with H. cutirubrum and H. halobium (OTUs 017 and 018) which are tied at 87%S. Related to these two OTUs in group I is a lake isolate (010) which is tied to 018 at 85%S indicating it to share a relative high degree of similarity with H. halobium and H. cutirubrum. The remaining known halobacteria H. salinarium 016 and 015 (the strain of H. salinarium tested with the lake isolates) are tied at a similarity level of 85%S.

At a lower similarity level of 80 percent a more encompassing pattern of groups (Table 2) is obtained which include all but three of the OTUs (002, 003 and 005). Group A is comprised of groups I and II of Table 1 combining at 82%S and OTU 004 joining group I at 81%S. The lake isolates, 004 and 010, are most related to H. halobium and H. cutirubrum, but a high level of similarity exists with H. trapanicum and H. marismortui also contained in group A. Group B is

comprised of groups III and IV of Table 1 uniting at 80%S. OTUs 001, 011, and 014 are also included in this group. The OTUs forming Group B have a relative high similarity with the type of halobacteria H. salinarium (OTUs 015 and 016). The isolated cocci from the lake (OTUs 012 and 013) form Group C and S. morrhuae and M. morrhuae (021 and 022) form Group D. OTUs 002, 003 and 005 are not included in any groups as they have similarity coefficients less than 80%S with each other or any other member of the designated groups. They are halobacteria that are tentatively unique from the rest of the OTUs as they share fewer similar characters.

Two different observations might be made from Figures 6 and 7 if certain minimum levels of similarity are chosen that a pair of OTUs could share and still maintain a separate species type designation. If the percent similarity level of 95%S, the level shared by S. morrhuae and M. morrhuae (021 and 022), is designated such a level then all the remaining known and isolated OTUs (except 012 and 013) would be different enough to be labeled separate species. Halobacterium marismortui and H. trapanicum (019 and 020 94%S) would be similar but separate species, as would be OTUs 007 and 008 (94%S). OTU 017 and 018 (H. cutirubrum and H. halobium) would also be separate species. A second observation might be made if 86%S was designated the minimum level. This level is shared by OTUs 015 and 016, the H. salinarium species. At this level H. cutirubrum, H. halobium and probably OTU 010 would be labeled the same species type. A similar situation would be found with H. marismortui and H. trapanicum. OTUs 007, 008, 009 would make up

another species type. The same criteria would apply to OTUs 012 and 013 and S. morrhuae and M. morrhuae. Of the seven known species of bacteria used in the study, six of them would be combined to make four species. Kocur and Hodgkiss (1973) as stated earlier prescribe that only one genus of halococci should be acknowledged. It is possible that a similar situation exists within the genus Halobacterium and some of its members might also be combined into a single species.

SUMMARY

The objectives of this study were to isolate selected bacteria from the North arm of the Great Salt Lake and phenotypically characterize them. The isolation of the bacteria was accomplished by standard field and laboratory procedure. Each isolate and one strain of Halobacterium salinarium were examined for morphology, culture growth, physiology, and biochemical characters. These compiled characteristics were coded and analyzed with a numerical program designed to compute a coefficient of similarity for each individual isolate compared with all other isolates. Six known species of taxonomically accepted halophilic bacteria were included as a comparative analysis, with characters obtained from those reported in the literature.

Thirteen bacteria, eleven pleomorphic rods and two cocci, were isolated from the lake. They all proved to be extreme halophiles requiring 15 percent sodium chloride for maintenance and 20-25 percent sodium chloride for optimum growth. All demonstrated varying pigmentation, and the pleomorphic rods lysed in hypotonic solutions. Reaction of individual isolates to physiological and biochemical tests varied, but as a group proved capable of many metabolic functions. The numerical analysis demonstrated a relative high level of similarity for the entire group, with variations in the degree of similarity being found with individuals or small groups. This analysis of characters indicated that representatives of the halophilic rods isolated from the lake had high degrees of

similarity with the known taxonomically accepted Halobacteria. However, three of the rods as well as the two cocci isolated from the lake had no significant ties with any member or members above the level of similarity uniting the entire group. These three rods and two cocci might be unique, but examination of more characters will be needed to prove this if true.

The physio-biochemical tests examined demonstrated the extensive metabolic capabilities of the lake isolates. Whether representative of the bacterial population in the lake or not, it is apparent that their role in the lake is important. The isolates capable of hydrolysing chitin are necessary in decomposing the exoskeleton of the arthropod populations found in the lake. How much decomposition the bacteria are responsible for requires further research. Similarly, nitrogen cycling in the lake must be affected by the bacteria. High percentages of the lake isolates reduced nitrate and were able to utilize ammonia and nitrate as a sole source of nitrogen. The extent of their role in nitrogen cycling also requires further study.

Added research should be attempted, as a unique situation is provided by the harsh environment of the North arm of Great Salt Lake to study organisms adapted to it. The environment prescribes that the living inhabitants be highly specialized and suited for survival. Therefore, the variety of suited organisms and resulting community members is limited. This situation enables a workable approach to the study of a community ecosystem. Continued research into the aspects of nutrient cycling and breakdown of organic waste by the halophilic

bacteria characterized in this study will provide insight into their function and contribution to the lake community.

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