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DNA Base Composition and Numerical Analysis of Some Marine and Terrestrial Bacteria

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by

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LIFE

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Bacteria isolated from special environments often possess unusual physiological characteristics which are necessary for their growth and survival in their habitat. These characters vary from extraordinary temperature optimums for growth to the requirement for certain ions found in the environment. A problem facing bacteriologists who attempt to classify these organisms is the taxonomic significance of these characteristics. Most marine bacteria, those which require detectable amounts of Na^+ for growth, which have been isolated so far, are morphologically and physiologically similar to terrestrial species of recognized genera. The requirement for Na^+ by marine bacteria has become the main physiological feature used to distinguish between marine and terrestrial bacteria. Whether or not the requirement for Na^+ by marine bacteria is a characteristic significant enough by itself to warrant generic separation of marine and terrestrial bacteria is the purpose of this thesis. Literature relating to the classification of marine bacteria, be it by their requirement for Na^+, numerical methods of analysis, or by a study of their DNA base compositions, will be reviewed here.

ZoBell and Upham (45) defined marine bacteria as bacteria from the sea which, on initial isolation, required a medium containing seawater. These authors intentionally specified "on initial isolation" since previous work in their laboratory had shown that after prolonged laboratory cultivation certain chitinoclastic bacteria developed the ability to grow in freshwater media (44). Using a chemically defined medium, MacLeod and Onofrey (20) showed that marine bacteria exhibited a specific requirement for Na^+. Leifson (17) reported that his marine isolates could be divided into 3 groups on the basis of their minimum Na^+ requirements: 1) marine strains which required from 0.05 - 0.2 M Na^+; 2) semi-marine strains which required from 0.001 - 0.05 M Na^+; 3) terrestrial strains which required less than 0.001 M Na^+.

Studies on the stability of the Na^+ requirement have been conducted by a number of workers. ZoBell and Michener (43) found that 9 of 12 cultures which initially required seawater were able to grow in a fresh water medium after the cultures had been held for 5 months without transfer. Leifson (17) observed that the numbers of viable semi-marine bacteria in various seawater samples increased while the numbers of viable marine types decreased as the storage time was lengthened. The change in the populations of these seawater samples may be a reflection of the sensitivity of the marine bacteria to prolonged storage and/or of an actual change in their minimum Na^+ requirements.

By plating heavy suspensions of marine bacteria on Trypticase medium prepared without added Na^+, Pratt and Waddell (29) obtained a few colonies of marine bacteria which they concluded arose from mutants no longer requiring Na^+. Several other workers, Stanier (46), MacLeod and Onofrey (21), and Littlewood and Postgate (19), have all reported limited success in growing marine bacteria at lowered salt concentrations.

Most studies on the stability of the Na^+ requirement were conducted using complex media which had a considerable and variable concentration of Na^+. MacLeod and Onofrey (21) reported that a flame photometric analysis of their Trypticase medium prepared without added Na^+ revealed a concentration of 0.028 M Na^+ present as a contaminant. This concentration of Na^+ was sufficient to permit the growth of some marine and all semi-marine bacteria. Using a chemically defined medium, MacLeod and Onofrey (21) attempted to grow marine bacteria at lowered Na^+ levels and to induce non-Na^+ requiring mutants by ultraviolet irradiation. Even after many serial transfers, during which the Na^+ concentration was gradually reduced, all cultures still exhibited a definite requirement for Na^+ even though some did grow faster and at a lower Na^+ concentration than did the parent strains. A total of 14 isolates were obtained following ultraviolet irradiation which were able to grow in a chemically defined medium without added Na^+. The growth of these mutants was stimulated by added Na^+ but this stimulation was easily lost. The non-Na^+ requiring mutants were physiologically similar to their marine parent strains except that they had lost their ability to hydrolyze gelatin and had gained the capacity to reduce nitrates to nitrites.

From these early experiments it appeared that the requirement for Na^+ by marine bacteria was qualitatively specific although its quantitative stability was questionable. Much of the confusion apparent in these studies resulted from the use of media which contained endogeneous Na^+. This fact by itself can explain the growth of many marine bacteria in "Na^+-free" media. Most other recorded changes in the Na^+ requirement of marine bacteria have been the result of the prolonged storage of cultures. Whether the storage of cultures led to a loss of the Na^+ requirement by marine bacteria and/or to a reduction in the minimum Na^+ requirement which then allowed them to grow in a "Na^+-free" medium was not clear.

Sneath (36,37) proposed the use of computers in bacterial classification. He revived Adanson's principles which stated that organisms should be classified by their overall similarity to each other and that the overall similarity was the proportion of characters possessed in common by two organisms. In this system every character was of equal importance in creating taxa while the divisions between taxa were based on correlated features.

Since, in an analysis of this type, every character is of equal weight it is necessary to perform a large number of randomly selected tests (50-100) on each culture. The test results are then encoded using symbols which a computer has been programmed to recognize. These symbols correspond to the test results. The usual symbols are (1) meaning positive,

(0) meaning negative, and (NC) meaning no comparison. The (NC) symbol is used when data are missing, when the test does not apply to the culture under study, or in the handling of quantitative data.

The encoded data are then punched onto cards and fed into a computer which has been programmed to perform a series of simple computations. Every possible combination of strains is compared and the number of characters in which both strains of a pair score (1) and the number in which one scores (1) and the other (0) are counted. The computer does not compare a (0) with a (0), a (1) with a (0), or any combination in which the (NC) symbol appears. Similarity (S) values are then calculated according to the formula: $S = \frac{Ns}{Ns + Nd}$ where Ns is the number of characters positive for both strains and Nd the number of features positive for one but not the other. Once the similarity values are calculated the computer sorts the strains into groups. This is usually done by the highest link method (37). The similarity values are inspected for paired values of 0.99 and then this process is repeated with levels reduced consecutively by 0.1. Each organism is paired at its highest similarity value to any other organism. A number of groups thus arise which join together at the highest similarity level existing between any members of the two groups. The main purpose of this approach is to create taxa which can then be identified objectively and reproducibly.

Several modifications of the original scoring technique proposed by Sneath have occurred. Changes suggested by Beers and Lockhart (1)

facilitated the handling of quantitative data and characters for which there was no logical basis for deciding which was the positive state.

Sokal and Michener (39) devised a scoring technique in which negative matches were counted. In their system $S = \frac{Np + Nn}{Np + Nn + Nd}$ where Np was the number of positive matches, Nn the number of negative matches, and Nd was the number of tests for which one strain was positive and the other negative. Several investigators (9,27) have compared both scoring procedures, one counting and the other disregarding negative matches, on the same set of data and the results were similar. Those codes which included negative matches provided a clearer separation of groups, however. Negative matches are now used in most analyses to calculate similarity values.

Numerical methods of analysis have been used to study the relationship of marine to terrestrial bacteria. Colwell and Gochnauer (4) studied 60 cultures of marine bacteria using approximately 100 characteristics. The data were then compared with similar data for other members of the *Eubacteriales* and the *Pseudomonadales*. Their results suggested that marine bacteria should not be placed in separate genera. Similar results were reported by Hansen *et al.* (10) who, in a computer study of the marine bacterium *Ps. piscicida*, showed a similarity of *Ps. piscicida* to other species of *Pseudomonas* at a level considered adequate for assignment to the genus.

Included in a computer analysis by Colwell and Listen (3) of 40 cultures which contained species of *Xanthomonas* and *Pseudomonas* were 5 cultures isolated from marine sources. Three of these cultures formed a cluster

with the characteristics of a psychrophilic *Pseudomonas aeruginosa*, a fourth joined a mesophilic *Ps. aeruginosa* group, while the fifth organism showed no affinity to any cluster formed in the analysis.

A determinative scheme for the differentiation of gram negative rods found in the marine environment was proposed by Shewan (32). On the basis of flagellation, reaction in Hugh and Leifson medium, and pigmentation, his *Pseudomonas* strains could be divided into 4 groups. Group I contained those organisms which were oxidative in the Hugh and Leifson medium and produced a diffusible fluorescent pigment. Group II strains metabolized glucose oxidatively but did not produce diffusible pigments. Group III organisms gave an alkaline reaction in the glucose medium and liberated no fluorescent pigments, whereas Group IV strains did not change the Hugh and Leifson medium and did not produce diffusible pigments. *Aeromonas* and *Vibrio* species were differentiated from *Pseudomonas* species by their fermentative metabolism of glucose. This scheme was used for the identification of both marine and terrestrial isolates at the generic level. When a comparison of the classification of bacteria derived by using this scheme and that made by numerical taxonomy was made, the results were similar. Studying 60 isolates from marine sources by numerical taxonomy, 3 groups were formed. In general, these groups corresponded to the 4 groups proposed before except that groups III and IV were almost indistinguishable.

In another study, a numerical analysis of 38 isolates from deep sea sediments by Quigley and Colwell (30) revealed 5 groups of which 4 were

identified as species of the genus *Pseudomonas*, one of which was identified as *Ps. fluorescens*. The deep sea strains, with the exception of those identified as *Ps. fluorescens*, when compared with 132 marine and terrestrial strains, clustered separately from all other marine strains.

From the results of these numerical studies it appears that most workers have found similar marine and terrestrial bacteria to be closely related. This is not too surprising since the distinction between these groups is based mainly on the requirement for Na^+ by marine bacteria. In numerical methods of analysis, in which all characters are of equal weight, this one character contributes very little to the formation of groups. In these studies all bacteria isolated from the ocean were considered to be marine. This is not necessarily so since the oceans are continuously contaminated with terrestrial bacteria capable of surviving in the marine habitat. It is important to demonstrate a requirement for Na^+ by these bacteria so that one would be able to distinguish true marine bacteria from those which are transient in the marine environment.

The determination of the DNA base composition has made important contributions to taxonomy since it has shown that related bacteria have similar overall DNA base compositions (11,22). In most instances studies of the DNA base composition have confirmed the present system of classification and also those based on computer studies (5,7,34). Most genera have been characterized by a range of % GC into which their members fit. Species

of *Pseudomonas* have been found to possess GC contents ranging from 57-70% (22), *Caulobacter* from 62-67% (28), and *Vibrio* from 44-50% (33). A catalog of the DNA base composition of many bacteria may be found in Hill (11).

The DNA of terrestrial bacteria has been extensively characterized with respect to its % GC content. The % GC of similar marine and terrestrial bacteria are not always the same. Comparison of the % GC content (43.5) of the DNA of the marine bacterium *Ps. piscicida* with that of the terrestrial pseudomonads (57-70%) showed the marine bacterium to have a much lower % GC content (23). Similar differences in the base ratios of marine bacteria classified as pseudomonads and their terrestrial counterparts have been reported by Leifson and Mandel (18) and Johnson (13).

Sulfate reducing bacteria could be divided into 3 groups based on their DNA base composition (35): group I, 60-62% GC; group II, 54-56% GC; group III, 41-47% GC. Both marine and fresh water strains were found in groups I and II while only marine strains were in group III. With these bacteria it was noticed that a loss of the Na^+ requirement (determined in a complex medium) by one strain had no effect on its DNA base composition. Species of other genera, such as *Caulobacter* and *Vibrio*, appeared relatively homogeneous in respect to their DNA base compositions regardless of whether they were marine or terrestrial (11,28).

It appears then that a requirement for Na^+ is not an indicator of any difference in the DNA base composition between marine and terrestrial bacteria since it is not directly correlated with the DNA base composition in

many genera. Organisms resembling pseudomonads are a major problem in the classification of marine bacteria since they are widely distributed in the marine habitat and show substantial variation, both physiologically and genetically.

This study will concern itself with the classification of marine bacteria. Attempts will be made to correlate the requirement for Na^+ by marine bacteria with other physiological data and this will be compared through the use of numerical taxonomy with similar data determined here for selected terrestrial bacteria. An analysis of the base composition of the DNA from representative marine bacteria will be performed and these results will be contrasted with those presented here and in the literature for various terrestrial bacteria. Based upon the results of these experiments, recommendations concerning the classification of marine bacteria will be made.

CHAPTER 2

BIOCHEMICAL REACTIONS AND IDENTIFICATION OF CULTURES

Genera are generally defined on the basis of a few select morphological and physiological characteristics which have proven to be stable and reliable. The most common, but not all inclusive, list of characteristics used include gram reaction, somatic morphology, flagellation, pigmentation, and O-F glucose reactions. On the basis of these 5 characteristics most of the known terrestrial genera can be distinguished. Marine bacteria have these characteristics in common with comparable terrestrial bacteria and hence can be assigned to one of the defined terrestrial genera. Whether the classification of marine bacteria was based on a few characteristics, such as that of Shewan (32) or on a large number (4,10), as in the numerical approach, marine bacteria have not been clearly differentiated from terrestrial bacteria at the genus level.

The most readily detectable physiological difference between marine and terrestrial bacteria is the requirement for Na^+ by the marine types and this character by itself is used to differentiate between these groups of bacteria. The requirement for Na^+ by marine bacteria is a useful feature in characterizing species but it has not as yet shown itself valuable as a generic characteristic.

In this chapter 89 morphological and physiological characters will be determined for each culture in the collection under study. The data will

form the basis for the classification of all cultures and then will be inspected to determine if any correlations exist with the requirement for Na^+ by the marine types. The data will then be encoded and used as the foundation for a study using numerical taxonomy.

MATERIALS AND METHODS

Culture Collection. The culture collection employed in this study consisted of 196 cultures of which 177 were isolated from marine sources as described by Leifson (16). The remainder were obtained from a culture collection maintained at the Department of Microbiology, Loyola University Stritch School of Medicine, and were mainly *Pseudomonas* species. A list of these cultures is recorded in Table 1.

For maintenance and routine growth a broth medium was used consisting of 0.2% Casitone (Difco) and 0.1% yeast extract (Difco) supplemented with the necessary salts. The salts solution was prepared from reagent grade chemicals so that the final concentrations in the complete medium were $MgSO_4 \cdot 7H_2O$, 0.5%; KCl, 0.1%; $CaCl_2 \cdot 2H_2O$, 0.05%; Tris·HCl, 0.05%. The pH was adjusted to 7.5 with 5% KOH. For the marine isolates NaCl was added to 2%. The marine and terrestrial media will be referred to as M and T, respectively.

All cultures were stored at 4 C and transferred every two months.

Determination of Morphological and Physiological Characters

Morphology. All cultures were examined by a gram and a flagella stain (14). The flagellation was determined from cultures grown both in a liquid and on a solid medium and the formation of lateral flagella on solid media was noted. The type of flagellation was recorded as either polar

monotrichous, polar multitrichous, or peritrichous. No distinction was made between lateral or subpolar flagellation and peritrichous flagellation.

Each culture was streaked on the M or T medium which had been solidified with 1.5% agar (Difco) and incubated at 20 C for 1 week. The plates were examined daily for pigmentation and swarming.

Physiology. Acid production from carbohydrates was determined, for the marine isolates, in the MOF medium of Leifson (15). Sixteen carbohydrates and related compounds were tested. The compounds used are recorded in Table 2. Each compound was sterilized by Seitz filtration of a 10 X solution and added to a final concentration of 1%. The media were then dispensed into individual tubes in 3 ml quantities.

With a Pasteur pipette, each tube was inoculated with one drop of a fresh culture of the bacterium to be tested and then stabbed with a straight wire needle to insure growth throughout the medium. Originally each carbohydrate was inoculated in duplicate and one was sealed with 2 cm of melted sterile petrolatum (White) to provide an anaerobic environment. Later this was done only with glucose after it was observed that the organisms metabolized each carbohydrate as they did glucose. Cultures were listed as producing oxidative, fermentative, or no acid from each carbohydrate after incubation for 7 days at 20 C.

The mode of carbohydrate metabolism of the terrestrial cultures was determined in the O-F basal medium of Hugh and Leifson (12). The methods of preparation, inoculation, incubation, and interpretation were the same as for the marine strains.

Each culture was tested for its ability to grow in the T medium, in a synthetic medium containing various organic compounds as the sole source of carbon and energy, and at 37 C. With a Pasteur pipette, one drop of a fresh broth culture was added to 3 ml of sterile distilled water. Using a fresh pipette, one drop of this suspension was transferred to each tube in the test series. This method of inoculation was adopted in order to reduce the level of organic and inorganic contaminants introduced into the test media.

Twenty-nine different organic compounds were tested as a sole carbon and energy source for growth. A list of the compounds used is recorded in Table 2. Each compound was sterilized like the carbohydrates and added aseptically to a mineral base and then dispensed into individual tubes in 3 ml amounts. The carbohydrates were tested at a final concentration of 0.2% while all other compounds were employed at 0.1%.

The mineral base consisted of 3 separate solutions which were added together after autoclaving in order to avoid formation of precipitates. Solution A contained NaCl, 2.0 gm; $MgSO_4 \cdot 7\ H_2O$, 0.5 gm; KCl, 0.5 gm; $(NH_4)_2SO_4$, 0.1 gm; H_2O, 90 ml. Solution B consisted of 0.1% $CaCl_2 \cdot 2H_2O$. Solution C contained 0.2 M potassium phosphate buffer, pH 7.5. To 90 ml of solution A was added 1 ml of B and 2 ml of C and the pH was adjusted to 7.5 with sterile 5% KOH. The final volume was made to 100 ml with a solution of the appropriate test compound. The NaCl was omitted when the medium was used for the testing of terrestrial organisms.

To determine if any organism would grow at 37 C the cultures were divided into groups of 15. These were inoculated and immediately placed in a 37 C incubator to insure that growth would not initiate at room temperature before being placed in the incubator.

Each marine isolate was transferred to a tube of T broth and then incubated for 48 hr at 20 C after which it was examined for turbidity. Any cultures showing evidence of growth were transferred serially at least once.

Extracellular enzyme activity was assayed as measured by the hydrolysis of starch (Difco), agar (Difco), gelatin (Difco), casein (Difco), or Tween 80 (Sargent-Welch, Chicago, Ill.). The appropriate M or T broth served as the base in the preparation of these media.

Tubes of broth containing 8% gelatin were inoculated with a straight wire and incubated for 7 days at 20 C after which they were inspected for liquefaction.

The hydrolysis of agar was determined in the broth base solidified with 1.5% agar. A recession of the bacterial colonies into the medium was taken as evidence of agar digestion.

To the broth base 1.5% agar and 0.2% soluble starch were added for the detection of amylase activity. The melted medium was poured into petri plates and allowed to solidify. Each plate was then divided into quadrants by marking the bottom of the petri plate with a wax crayon. Each quadrant was inoculated with a single streak of a fresh culture. The plates were

incubated for 3 days at 20 C after which several drops of iodine (Gram #2) were placed on each quadrant. The hydrolysis of starch was indicated by the presence of a relatively clear zone surrounding the bacterial growth on the black background of the unhydrolysed starch.

Casein digestion was detected in a medium composed of the broth base plus 1.5% agar and 0.4% skim milk powder (Carnation). The reconstituted skim milk was autoclaved separately as a 4% solution and 10 ml of this was added to 90 ml of the base and the pH was adjusted to 7.5 with 5% KOH. These plates were inoculated and incubated in the same manner as were the starch plates. The digestion of casein was indicated by clear zones surrounding the bacterial growth on the turbid agar medium.

Lipolytic activity was determined in the M or T agar base plus 1% Tween 80. The Tween 80 was autoclaved separately and then added to 1%. The medium was inoculated and incubated as before. The hydrolysis of Tween 80 was indicated by the appearance of a white precipitate surrounding the bacterial growth.

A medium consisting of 1% peptone (Difco), 0.1% yeast extract, and 0.5% glucose, plus the appropriate salts, was used for the detection of acetylmethylcarbinol. Production of indol was assayed in a medium containing 0.5% Casitone, 0.1% yeast extract, plus the appropriate salts. Nitrate reduction was assayed in a nitrate medium (Difco) supplemented with the necessary salts. Small gas vials were included in each tube of nitrate medium for the detection of nitrogen gas.

All cultures were streaked onto an agar plate for the detection of cytochrome oxidase and catalase. To isolated colonies 1 drop of either p-aminodimethylaniline oxalate (Difco) or 3% hydrogen peroxide was added. Catalase was indicated by the formation of bubbles while colonies containing cytochrome oxidase turned pink and then black.

The ability to deaminate phenylalanine was tested on slants of PD agar (Difco) supplemented with the necessary salts. After 3 days incubation at 20 C, two drops of 10% $FeCl_3$ were added to each slant. The formation of a dark green color indicated the deamination of phenylalanine.

RESULTS

The results of the 89 biochemical tests are recorded in Table 3. The results are expressed in a coded form which was used in performing a numerical analysis. For all characters for which there were only two alternatives, positive or negative, the symbol A was assigned positive and B negative. Those characters which had more than 2 possible alternatives were assigned one letter of the alphabet for each alternative. Thus a character such as color of pigment which had 5 possible alternatives was represented by a letter from A-F. In Table 2 is a list of all characters used plus a key to the alphabetic coding of the results.

Based upon gram reactions, somatic morphology, O-F reactions, and flagellation, members of this culture collection were assigned to one of several terrestrial genera, of which *Pseudomonas* was the dominant genus. Other recognized genera included *Vibrio*, *Caulobacter*, *Achromobacter*, *Xanthomonas*, and *Flavobacterium*.

Polarly flagellated gram negative rods which either oxidized or produced no acid from glucose were tentatively assigned to the genus *Pseudomonas* pending the results of the DNA base composition studies. Strains included in this group could be separated into 2 groups based upon the demonstration of a requirement for Na^+ by the marine types. The terrestrial pseudomonads, most of which were fluorescent strains, formed a homogeneous group and were active biochemically. Named species included in this group

were *Ps. aeruginosa, Ps. putida, Ps. fluorescens, Ps. aureofaciens, Ps. viridolivida, Ps. tabaci,* and *Ps. striata.* The fluorescent strains produced acid oxidatively from most of the sugars and sugar alcohols tested (Table 3). These strains were able to grow in a defined medium without added growth factors with any one of several amino acids, carbohydrates, and organic acids serving as sole sources of carbon and energy. All carbohydrates were of the D-configuration except L-rhamnose and L-arabinose while amino acids were of the D-configuration. All strains were able to utilize D-glucarate as a sole carbon and energy source. Those terrestrial strains isolated from marine sources (3A4, 2B1, etc.) were distinct from and less active biochemically than the fluorescent strains.

Marine members of this group were decidedly less active in the biochemical tests used in this study (Table 3). Approximately 50% of all strains tested could not grow in a defined medium with any of the compounds tested as a carbon source. When this same medium was supplemented with yeast extract, abundant growth occurred indicating the requirement for some growth factor(s) by these strains. Of all the compounds tested, L-alanine, L-aspartate, L-glutamate, and acetate were the most acceptable sources of carbon and energy. In most instances carbohydrates were poor sources of carbon and energy for the marine strains.

Caulobacter strains were identified primarily on the basis of their ability to form rosettes or stalks. With the exception of strain 1F2, all members had a distinct flagellar wavelength of 0.98 μ. Named species included in this group were *C. intermedius* and *C. sub.* All strains, except

2F25, would not grow in the defined medium with any of the compounds tested as a source of carbon and energy and were presumed to require additional growth factors. Most cultures exhibited very few positive reactions in the tests used in this study and were differentiated from *Pseudomonas*, *Achromobacter*, and *Flavobacterium* cultures mainly by somatic morphology and flagellar wavelengths.

Those cultures which were gram negative rods, motile with polar flagella, and which fermented glucose were assigned to the genus *Vibrio*. Almost all cultures could grow in a defined medium with glucose as the source of carbon and energy. Carbohydrate fermentation patterns were very similar and most cultures were differentiated from each other by their extracellular enzyme activity and their ability to utilize several of the compounds tested as the source of carbon (Table 3).

The peritrichously flagellated organisms were divided into 2 groups based upon pigmentation. All non-pigmented cultures were assigned to the genus *Achromobacter* while the pigmented cultures were placed in the genus *Flavobacterium*. The *Achromobacter* and *Flavobacterium* cultures were physiologically similar to the marine "pseudomonads", being distinguished mainly by their flagellation.

A more detailed morphological and physiological description of individual strains will be presented in the section on numerical taxonomy. A complete record of the results of all tests used is provided in Table 3.

TABLE 1

List of Named Cultures Used in This Study

Pseudomonas aeruginosa	ATCC 10145
Ps. fluorescens	RH 818
Ps. putida	--
Ps. aureofaciens	--
Ps. striata	ATCC 15070
Ps. tabaci	ATCC 15383
Ps. viridolivida	ATCC 19048
Ps. melanogena	ATCC 17806
Caulobacter intermedius	ATCC 15262
C. sub	ATCC 15264
Vibrio alkaligenes	ATCC 14736
V. mercenariae	ATCC 19106
V. mercenariae	ATCC 19107
V. mercenariae	ATCC 19108
V. mercenariae	ATCC 19109
Oceanomonas alginolytica	ATCC 14035
Xanthomonas feronica	--
Commomonas percolans	--

TABLE 2

Characters Used in Numerical Analysis*

1	Flagellation	A	polar monotrichous		
		B	polar multitrichous		
		C	peritrichous		
2	Lateral flagella				
3	Swarming				
4	Aggregation				
5	Pigment Production				
6	Type of Pigment	A	water soluble		
		B	water insoluble		
		C	not produced		
7	Color of Pigment	A	yellow	D	pink
		B	green	E	blue
		C	brown	F	not produced
8	Growth in T medium				
9	37°				
10	5% NaCl				
11	6% NaCl				
12	8% NaCl				
13	10% NaCl				
14	12% NaCl				

* All characters exist in only 2 states except where indicated and for these A = positive and B = negative.

Acid Production from

15	Glucose	24	Mannitol
16	Mannose	25	Inositol
17	Galactose	26	Glycerol
18	Rhamnose	27	Maltose
19	Fructose	28	Sucrose
20	Arabinose	29	Lactose
21	Xylose	30	Inulin
22	Erythritol		
23	Sorbitol		

Type of acid produced from: A - oxidative B - fermentative
C - no acid produced

31	glucose	35	fructose	39	sorbitol	43	maltose
32	mannose	36	arabinose	40	mannitol	44	sucrose
33	galactose	37	xylose	41	inositol	45	lactose
34	rhamnose	38	erythritol	42	glycerol	46	inulin

Hydrolysis of
47 starch
48 gelatin
49 casein
50 Tween 80

51 nitrate, reduction of
52 nitrates, gas produced from
53 H_2S, production of

Presence of
54 oxidase
55 catalase
56 phenylalanine deaminase

57 acetylmethylcarbinol
58 indol

59 morphology A - straight B - curved
60 agar, hydrolysis of

Utilization of as sole carbon source:

61	formate	71	glucose	81	glycerol
62	glycine	72	galactose	82	maltose
63	alanine	73	rhamnose	83	sucrose
64	arginine	74	fructose	84	lactose
65	isoleucine	75	l-arabinose	85	saccharate
66	leucine	76	d-xylose	86	acetate
67	lysine	77	erythritol	87	pyruvate
68	valine	78	sorbitol	88	lactate
69	aspartate	79	mannitol	89	succinate
70	glutamate	80	inositol		

TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
CAULOBACTER																														
C. INTERMEDIUS	A	B	B	A	B	C	F	A	A	B	B	B	B	B	A	B	A	A	B	A	A	B	B	B	B	B	B	B	B	B
C. SUB	A	B	B	A	A	B	A	A	A	B	B	B	B	B	A	B	A	A	B	B	A	B	B	B	B	B	B	A	B	B
DW320	A	B	B	A	B	C	F	A	A	B	B	B	B	B	A	B	A	B	B	B	A	B	B	B	B	B	A	B	B	B
DW102	A	B	B	A	B	C	F	A	A	B	B	B	B	B	A	B	A	B	B	B	A	B	B	B	B	B	A	B	B	B
2F38	A	B	B	A	B	C	F	A	A	B	B	B	B	B	A	A	B	A	B	A	A	B	B	B	B	B	A	B	B	B
3F15	A	B	B	A	B	C	F	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
2F26	A	B	B	A	B	C	F	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B
4-4	A	B	B	A	B	C	F	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
7-5	A	B	B	A	B	C	F	A	A	A	A	A	A	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	C
2F5	A	B	B	A	B	C	F	A	A	A	A	A	A	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	C
2F8	A	B	B	A	A	B	A	A	A	A	A	B	B	A	B	A	B	B	A	A	A	B	B	B	B	A	B	B	B	A
2F25	A	B	B	A	A	B	A	A	A	A	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
1F2	A	B	B	A	B	C	F	B	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	C
PSEUDOMONAS	B	B	B	A	B	C	F	B	B	A	A	A	A	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
PS. FLOURESCENS	B	B	B	B	A	A	B	A	A	A	B	B	B	B	A	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B
PS. PUTIDA	B	B	B	B	A	A	B	A	A	A	B	B	B	B	A	A	A	A	A	A	A	B	A	A	A	A	B	B	B	A
PS. AUREOFACIENS	B	B	B	B	A	A	B	A	A	A	B	B	B	B	A	A	A	B	A	A	A	B	B	A	A	B	A	B	B	A
PS STRIATA	B	B	B	B	A	A	B	A	A	A	B	B	B	B	A	A	A	B	A	A	A	B	B	B	A	B	B	B	B	A
PS. TABACI	B	B	B	B	A	A	B	A	A	A	B	B	B	B	A	A	A	B	A	A	A	A	A	A	A	B	A	B	B	A
PS. VIRIDOLIVIDA	B	B	B	B	A	A	B	A	A	A	B	B	B	B	A	A	A	B	A	A	A	B	A	A	B	A	B	B	B	A
PS. SP. B	B	B	B	B	A	A	B	A	A	A	B	B	B	B	A	A	A	B	A	A	A	B	B	A	B	A	A	B	B	A
PS. SP. C	B	B	B	B	A	A	B	A	A	A	B	B	B	B	A	A	A	B	A	A	A	A	B	A	B	A	B	B	B	A
PS. AERUGINOSA	B	B	B	B	C	F	A	A	A	B	B	B	B	A	A	A	B	A	A	A	A	A	B	A	A	B	B	B	B	A
3L6	A	B	B	B	A	E	A	A	A	B	B	B	A	A	B	B	A	A	A	A	A	B	B	A	B	A	B	B	B	A
	A	B	B	B	A	A	B	A	B	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	A

* Character numbers 1-89 correspond to those found in Table 2.

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
B	B	B	B	B	A	B	A	A	B	A	A	B	B	B	B	B	B	B	B	B	A	C	A	A	C	A	A	C	C	C	C	C	C	C	C	C	B	B	B	B	B	B	B	B	B	B	B	B	A	B
B	B	B	B	B	A	B	A	A	B	B	A	B	B	B	B	B	B	A	B	B	A	C	A	A	C	C	A	C	C	C	C	C	C	A	C	C	B	A	B	B	B	B	B	B	B	B	B	B	A	B
B	B	B	B	B	A	B	A	B	B	B	A	B	B	B	B	B	A	B	B	B	A	C	A	C	C	C	A	C	C	C	C	C	A	C	C	C	B	A	B	B	B	B	B	A	B	B	B	B	A	B
B	B	B	B	B	A	A	B	A	B	A	A	B	B	B	B	B	A	B	B	B	A	A	C	A	C	A	A	C	C	C	C	C	A	C	C	C	B	B	B	B	B	B	B	B	B	B	B	B	A	B
A	A	A	A	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	B	B	B	B	B	B	B	A	A	B	B	B	A	B
A	A	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	B	A	B	B	B	B	B	A	A	B	B	B	A	B
A	A	A	A	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	B	A	B	B	B	B	B	B	A	B	B	B	A	B
A	A	A	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	C	C	C	C	C	A	A	C	C	C	C	C	C	C	C	C	B	A	B	B	B	B	B	B	A	B	B	B	A	B
A	A	A	A	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	C	C	C	C	C	A	A	C	C	C	C	C	C	C	C	C	B	B	B	B	B	B	B	A	A	B	B	B	A	B
A	A	A	B	B	A	B	A	B	B	A	A	B	B	B	B	B	A	B	B	B	A	C	A	C	C	A	A	C	C	C	C	C	A	C	C	C	B	B	B	B	B	B	B	A	A	B	B	B	A	B
A	A	A	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	B	B	B	B	B	B	B	A	A	B	B	B	A	B
A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	B	B	B	B	B	B	B	A	A	B	B	B	A	B
A	A	A	A	B	A	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	A	C	C	C	C	C	A	C	C	C	C	C	C	C	C	C	B	A	B	B	B	B	B	A	A	B	B	B	A	B

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
A	B	B	B	B	A	A	A	B	A	A	A	A	A	A	A	A	B	A	B	B	A	A	A	C	A	A	A	A	A	A	A	A	C	A	C	C	B	B	B	B	A	B	B	A	A	B	B	B	A	B
A	B	B	B	B	A	A	A	A	A	A	A	B	A	A	A	A	B	B	B	B	A	A	A	A	A	A	A	C	A	A	A	A	C	C	C	C	B	A	B	A	A	B	B	A	A	B	B	B	A	B
A	A	B	B	B	A	A	A	B	A	A	A	B	B	A	A	A	B	A	B	B	A	A	A	C	A	A	A	C	C	A	A	A	C	A	C	C	B	A	B	A	B	B	B	A	A	B	B	B	A	B
A	A	B	B	B	A	A	A	B	A	A	A	B	B	B	B	A	B	B	B	B	A	A	A	C	A	A	A	C	C	C	C	A	C	C	C	C	B	B	B	B	A	B	B	A	A	B	B	B	A	B
B	B	B	B	B	A	A	B	A	A	A	A	A	A	A	A	A	B	A	B	B	A	A	C	A	A	A	A	A	A	A	A	A	C	A	C	C	B	B	B	A	A	B	B	A	A	B	B	B	A	B
A	A	A	B	B	A	A	A	B	A	A	A	B	A	A	B	A	B	B	B	B	A	A	A	C	A	A	A	C	A	A	C	A	C	C	C	C	B	B	B	B	A	B	B	A	A	B	B	B	A	B
B	B	B	B	B	A	A	A	B	A	A	A	B	B	A	B	A	B	B	B	B	A	A	A	C	A	A	A	C	C	A	C	A	C	C	C	C	B	B	B	B	A	B	B	A	A	A	B	B	A	B
A	B	B	B	B	A	A	A	B	A	A	A	A	B	A	A	A	B	B	B	B	A	A	A	C	A	A	A	A	C	A	A	A	C	C	C	C	B	A	B	B	B	B	B	B	A	B	B	B	A	B
A	A	B	B	B	A	A	B	B	A	A	A	B	B	A	B	A	B	B	B	B	A	A	C	C	A	A	A	C	C	A	C	A	C	C	C	C	B	A	A	A	A	A	B	A	A	B	B	B	A	B
A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	C	C	C	C	B	B	B	B	B	B	B	A	A	B	B	B	A	B

TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Y – 9	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	A	B	B	B	B	A	B
Y – I 3	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	A	A	A	A	B	A	B
Y – I 5	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	B	B	B	B	B	B
Y B 3	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	B	B	B	B	B	B
Y M 4	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	A	B	B	A	B	B	B
Y M 5	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B
Y M I O	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	A	A	A	B	B	B
S N A I L G	A	A	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B
F L A T C	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	B	B	B	B	B	B
X D 4	A	B	B	B	A	B	B	B	A	A	A	A	A	A	A	B	A	A	A	B	B	B
X O 8	A	A	B	B	A	B	A	B	B	A	B	B	B	B	A	B	B	B	B	B	B	B
X Q 5 O	A	B	B	B	A	B	D	B	B	B	B	B	B	B	A	A	A	B	A	A	A	A
2 F I 9	A	B	B	B	A	B	A	B	A	A	A	A	A	A	A	A	B	A	B	A	A	B
I S 2	A	B	B	B	A	B	A	B	A	A	A	A	B	B	A	A	A	A	A	A	A	B
Y – 9 A	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	A	B
I O 5	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	A	B	B	B
X L 7	A	B	B	B	B	C	F	B	B	A	A	A	B	B	B	B	B	B	B	B	B	B
4 K 2 3	A	B	B	B	A	B	A	B	B	A	A	B	B	B	A	B	A	B	A	B	A	B
3 L I	A	B	B	B	A	B	A	B	B	A	A	B	B	B	A	B	B	B	B	B	B	B
7 L 2	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B
I I L 3	A	B	B	B	B	C	F	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B
2 O 8	A	B	B	B	B	C	F	B	A	A	A	A	A	A	B	B	B	B	A	B	B	B
I T 5	A	B	B	B	B	C	F	B	A	A	A	A	A	A	B	B	B	A	B	B	B	B
I – 9	A	B	B	B	B	C	F	B	A	B	B	B	B	B	A	A	A	A	A	A	A	B
3 – 6	A	B	B	B	B	C	F	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B

* Character numbers 1-89 correspond to those found in Table 2.

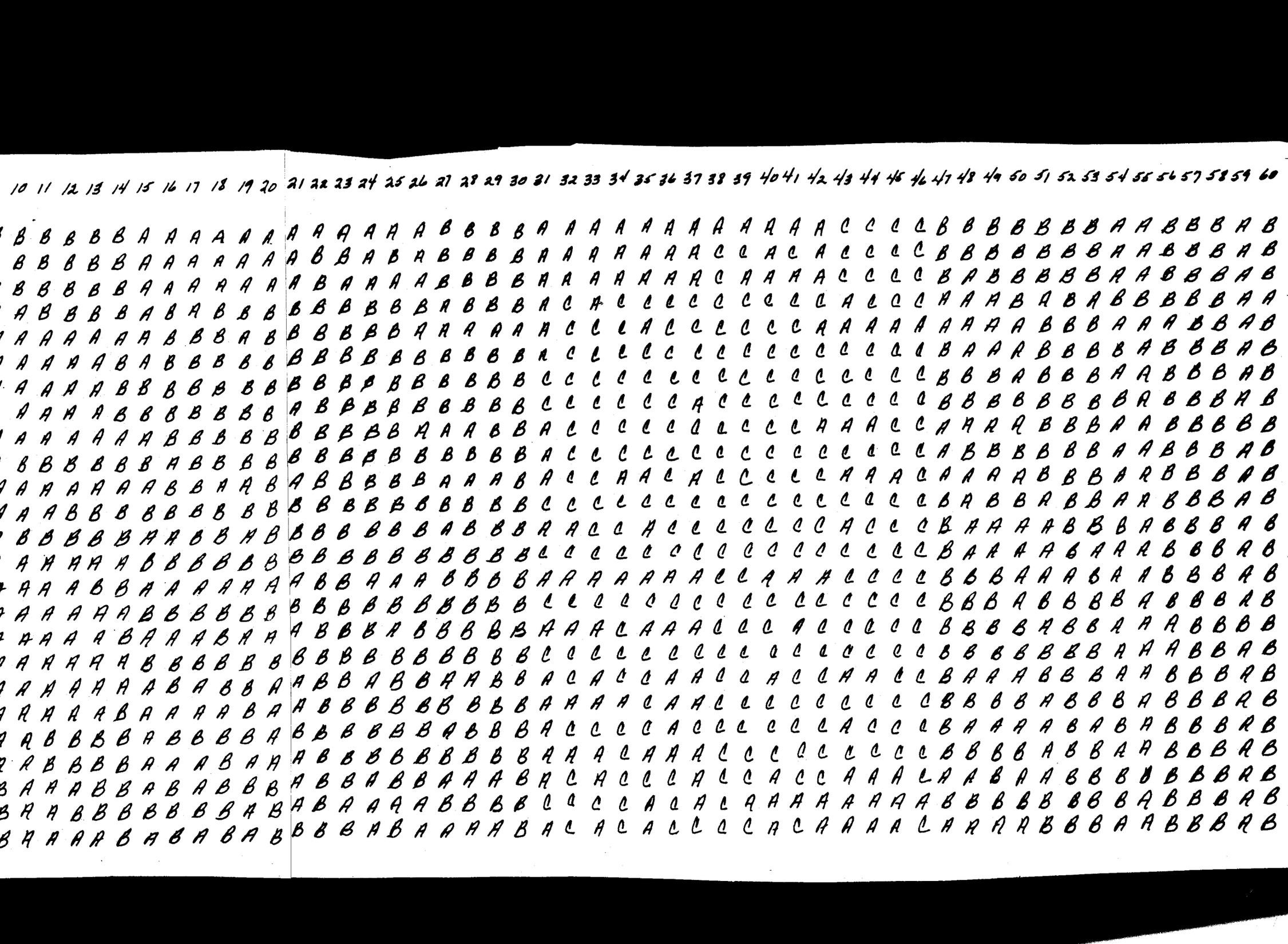


TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
X P 3	A	B	B	B	B	C	F	B	B	A	B	B	B	B	B	A	B	B	A	B	B	B	B	B	B	B	B	B	B
X P I O	A	B	B	B	A	B	C	B	B	A	A	B	B	B	A	B	A	B	B	B	B	B	B	B	B	B	A	B	B
X Q I 7	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	B	A	B	A	B	B	B	B	A	B	B	A	A	B
X Q 5 5	A	B	B	B	B	C	F	B	B	A	A	A	B	B	A	A	B	B	A	B	A	B	B	A	B	A	B	B	B
X R 4	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	A	A	B	A	B	B	B	A	B	A	A	A	A	A
X R I O	A	B	B	B	B	C	F	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
X W I	A	B	B	B	B	C	F	B	A	A	A	A	A	A	R	B	B	B	B	B	B	B	B	B	B	B	B	B	B
I D 4	A	B	B	B	B	C	F	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
I F 2 6	A	B	B	B	B	C	F	B	A	A	A	A	A	B	R	B	B	B	B	B	B	B	B	B	B	B	B	B	B
2 F 2 7	A	B	B	B	B	C	F	B	A	A	A	A	A	B	R	B	B	B	B	B	B	B	B	B	B	B	B	B	B
2 F 2 8	A	B	B	B	B	C	F	B	A	A	A	A	A	B	R	B	A	A	A	A	B	B	A	B	B	A	A	A	A
I G I I	A	B	B	B	B	C	F	B	B	A	A	A	A	B	R	B	A	R	A	B	A	B	B	A	B	B	A	A	A
4 H 7	A	B	B	B	B	C	F	B	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
2 K I O	A	B	B	B	B	C	F	B	B	B	B	B	B	A	A	B	B	A	B	B	B	B	B	B	A	B	A	A	A
4 K I 8	A	B	B	B	B	C	F	B	B	B	B	B	B	A	A	B	B	A	B	B	B	B	B	B	A	B	A	A	A
3 L 3	A	B	B	B	B	C	F	B	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
5 L 2	A	B	B	B	B	C	F	B	B	A	B	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B
7 L 7	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	B	A	B	A	B	A	B	B	A	B	B	A	A	A
7 L 4	A	B	B	B	B	C	F	B	A	A	A	A	A	B	A	B	B	B	A	B	B	B	B	B	B	A	A	A	A
2 N 6	A	B	B	B	B	C	F	B	A	A	A	A	A	A	B	B	B	A	A	B	A	B	B	A	B	B	A	B	B
I O I	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	A	B	B	B	A	B	B	A	B	B	B
I U I	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	A	B	B	B	A	B	B	A	B	B	B
3 - 2 2	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	A	B	B	B	B	B	B	A	A	B	B
7 - 8	A	B	B	B	B	C	F	B	A	A	A	A	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B
I 3 - 8	A	B	B	B	B	C	F	B	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B

* Character numbers 1-89 correspond to those found in Table 2.

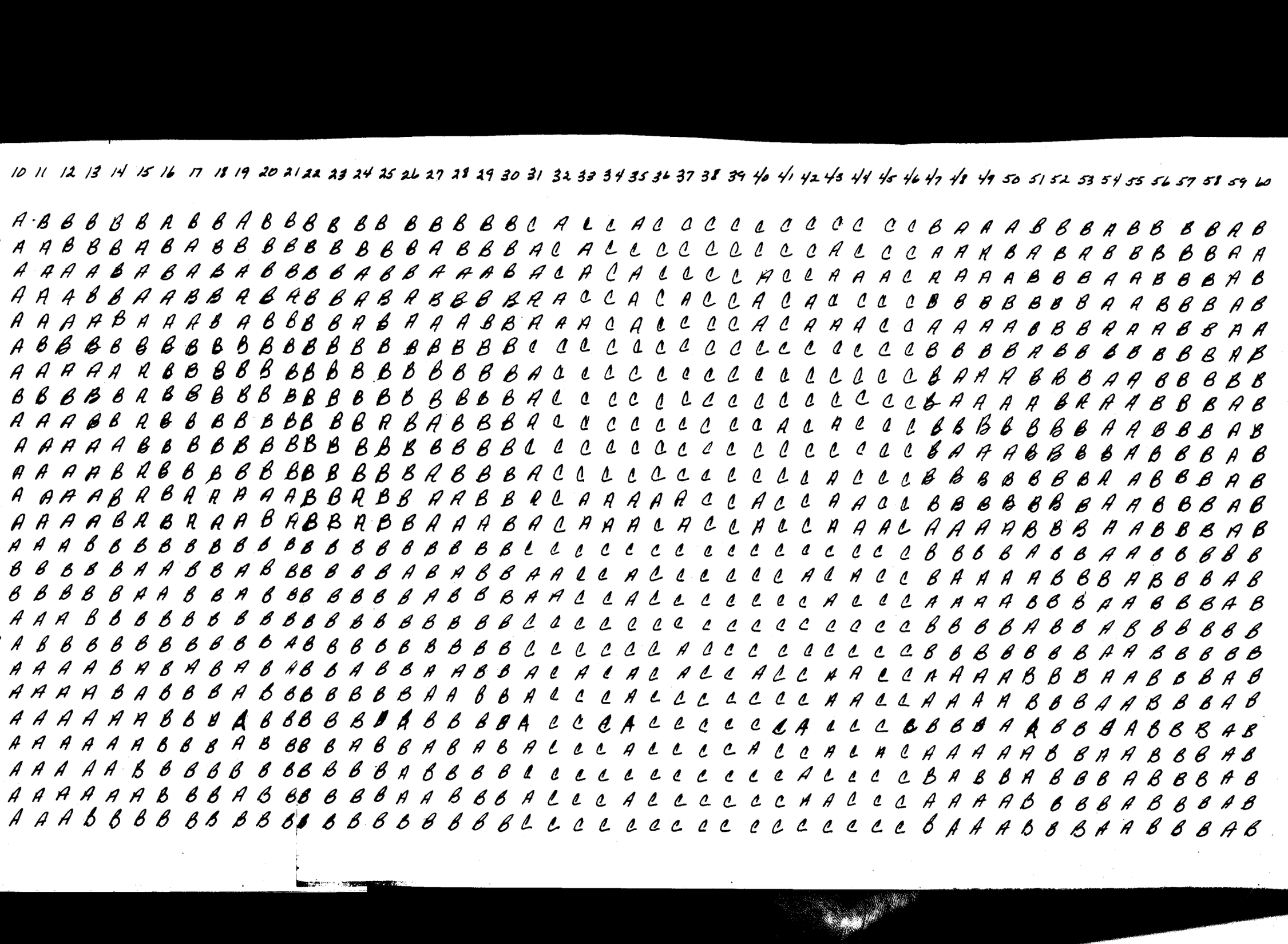

10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Y – 9	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	A	B	B	A	B	B	A
Y – I 3	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	A	A	A	B	B	A	B	B	A	B	B	A
Y – I 5	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	B	B	B	B	B	B	B	A	B	B	B
Y B 3	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B
Y M 4	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	A	B	B	A	B	B	B	B	B	B	A	B
Y M 5	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A
Y M I O	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	A	A	A	B	B	B	B	B	B	B	A
S N A I L G	A	A	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A
F L A T C	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B
X D 4	A	B	B	B	A	B	B	B	A	A	A	A	A	A	B	A	A	A	A	B	B	B	B	B	B	A	A
X O 8	A	A	B	B	A	B	A	B	B	B	A	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	A
X Q 5 O	A	B	B	B	A	B	D	B	B	B	B	B	B	B	A	A	A	B	A	A	A	A	A	A	A	A	A
2 F I 9	A	B	B	B	A	B	A	B	A	A	A	A	A	A	A	A	B	A	B	A	A	B	B	B	B	A	B
I S 2	A	B	B	B	A	B	A	B	A	A	A	A	B	B	A	A	A	A	A	A	A	B	B	A	B	B	A
Y – 9 A	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	A	B	B	A	B	B	A
I O 5	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	A	B	B	B	B	B	B	B	A
X L 7	A	B	B	B	B	C	F	B	B	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A
4 K 2 3	A	B	B	B	A	B	A	B	B	A	A	B	B	B	A	B	A	B	A	B	A	B	B	B	B	B	A
3 L I	A	B	B	B	A	B	A	B	B	A	A	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	A
7 L 2	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A
I I L 3	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B
2 O 8	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	A	B	B	B	B	B	B	B	A
I T 5	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	A	B	B	B	B	A	B	A	A
I – 9	A	B	B	B	B	C	F	B	A	B	B	B	B	B	A	A	A	A	A	A	A	B	B	A	B	A	A
3 – 6	A	B	B	B	B	C	F	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	A

* Character numbers 1-89 correspond to those found in Table 2.

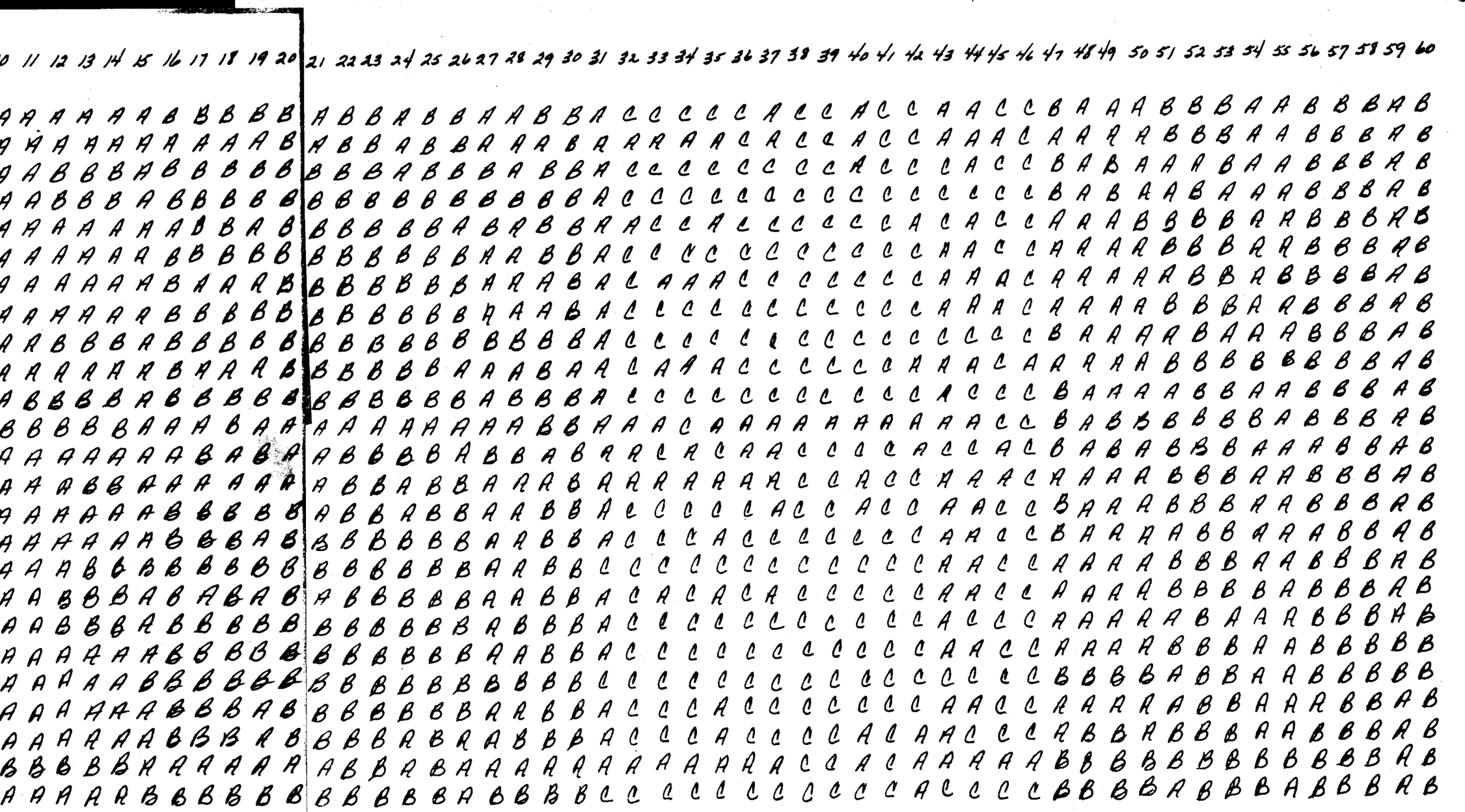

10 11 12 13 14 15 16 17 18 19 20
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
4 - 7	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	A	B	B	B	B	B	B	A	A	B
5 - 5	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B
6 - 1 7	A	B	B	B	B	C	F	B	A	A	A	A	A	A	B	B	B	A	B	B	B	B	B	B	B	A	B	B
7 - 1 5	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	B	A	A	A	A	B	B	B	A	B	B	A	A
1 0 - 4	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	A	A	A
1 1 - 6	A	B	B	B	B	C	F	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B
1 1 - 1 5	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	A	B	B	B	B	B	B	A	A
X A 5	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	A
X B 2	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	B	A	B	A	B	A	B	B	A	B	B	A	A
X C 5	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	B	A	B	A	B	B	B	B	B	B	B	A	A
X D 2	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	A	B	B	A	B	B	B	B	B	B	B	A	A
X E 3	A	A	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	A
X H 2	A	A	B	B	B	C	F	B	B	A	A	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	A	A
X H 5	A	B	B	B	B	C	F	B	B	A	A	A	B	B	A	A	B	B	A	B	A	B	A	A	A	A	A	A
X H 8	A	B	B	B	B	C	F	B	A	A	A	A	B	B	A	A	A	B	A	A	A	B	B	A	A	A	B	A
X L 1	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	A	B	A	B	B	B	B	B	B	A	A	A
VIBRIO																												
X T 7	B	B	B	B	A	B	A	A	A	A	A	A	A	A	A	A	A	B	A	B	B	B	B	A	B	A	A	A
1 G 7	B	B	B	B	A	B	A	A	A	A	A	A	A	A	A	A	A	B	A	B	B	B	B	A	A	A	A	A
X T 8	B	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	B	B	B	A	A	A
X G 3	B	B	B	B	B	C	F	B	A	A	B	B	B	B	A	A	A	B	A	B	B	B	B	B	B	A	A	A
X G 1	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	B	B	A	A	A
X T 1 6	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	B	B	B	A	B	A
1 C 2	A	B	B	B	B	C	F	B	B	A	A	A	B	B	A	A	A	B	A	B	B	B	A	A	B	A	A	A

* Character numbers 1-89 correspond to those found in Table 2.

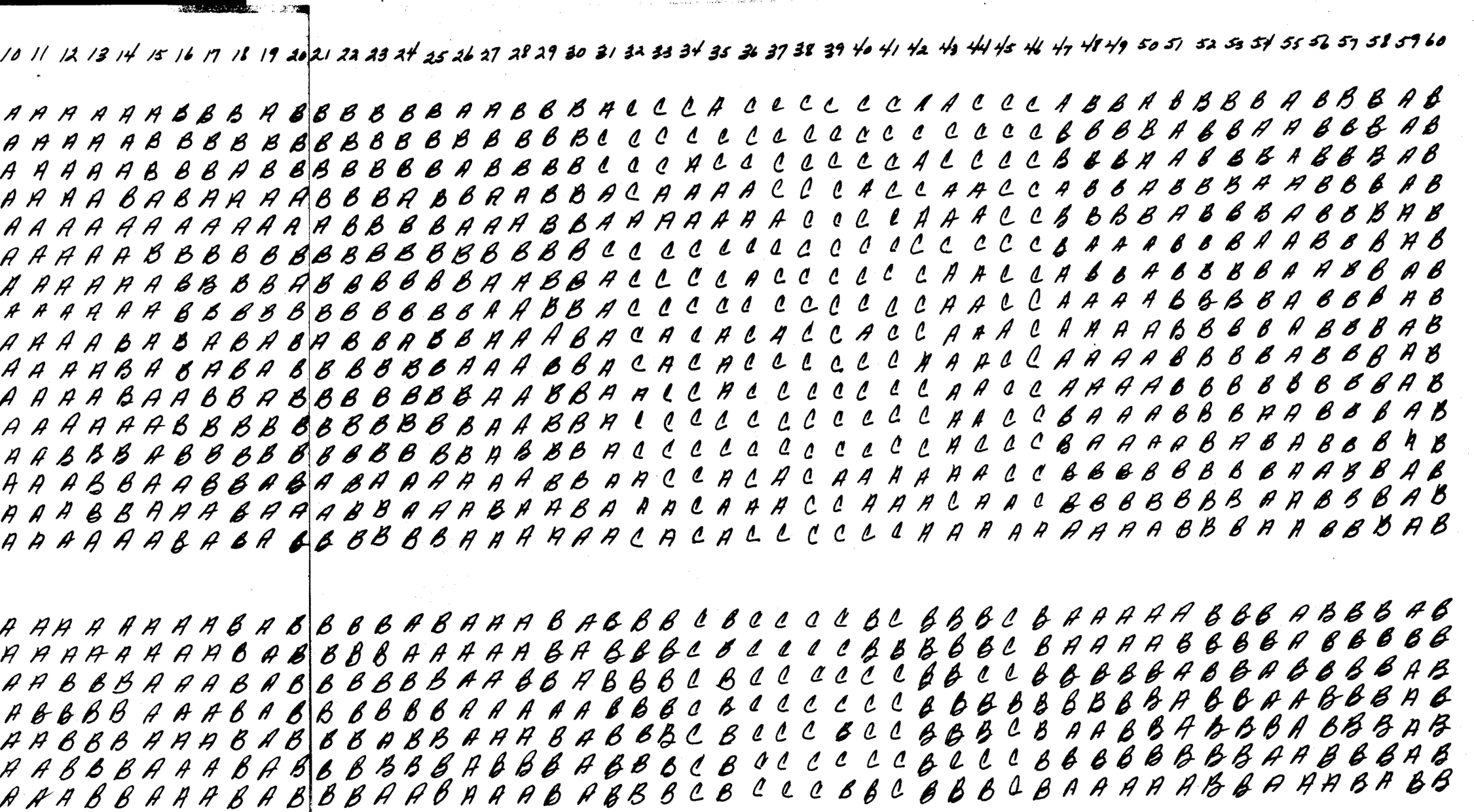


TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
I G I 4	A	B	B	B	B	C	F	B	B	A	A	A	B	B	A	A	A	B	A	B	B	B	A	A	A
4 H I 2	A	A	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	A
7 L 3	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	A	A	B	A	B	B	B	A	A	B
3 – 5	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	A	A	B	A	B	B	B	A	A	B
Y – I 7	A	A	A	B	B	C	F	B	B	A	A	B	B	B	A	B	A	B	A	B	B	B	A	A	B
Y B 7	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	A	A	B	A	B	B	B	A	A	B
Y C 4	A	A	A	B	B	C	F	B	A	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	B
Y F 3	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	B
Y S 3	A	A	B	B	A	B	A	B	A	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	B
Y S C 6	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	A	A	B	A	B	B	B	A	A	B
F L A T D	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	B	B	B
X T I	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	B	B	B
X A I	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	A	B	A	B	B	B	A	A	A
X A 3	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	A	B	A	B	B	B	A	A	A
X A I 2	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	A	B	A	B	B	B	A	A	A
X C I	A	A	B	B	B	C	F	B	B	A	B	B	B	B	A	A	A	B	A	B	B	B	B	B	B
X C 6	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	A
X D I	A	A	A	B	B	C	F	B	A	A	A	A	A	B	A	A	A	B	A	B	B	B	A	A	A
X D 5	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	A
X D 6	A	A	A	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	A
X E I	A	A	A	B	B	C	F	B	B	A	A	A	A	B	A	A	A	B	A	B	B	B	A	A	A
X P 4 6	A	A	A	B	B	C	F	B	A	A	A	A	A	B	A	A	A	B	A	B	B	B	A	A	A
X F I	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	A	B	A	B	B	B	A	A	A
X F 3	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	A	B	A	B	B	B	A	A	A
X F 7	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	A

* Character numbers 1-89 correspond to those found in Table 2.

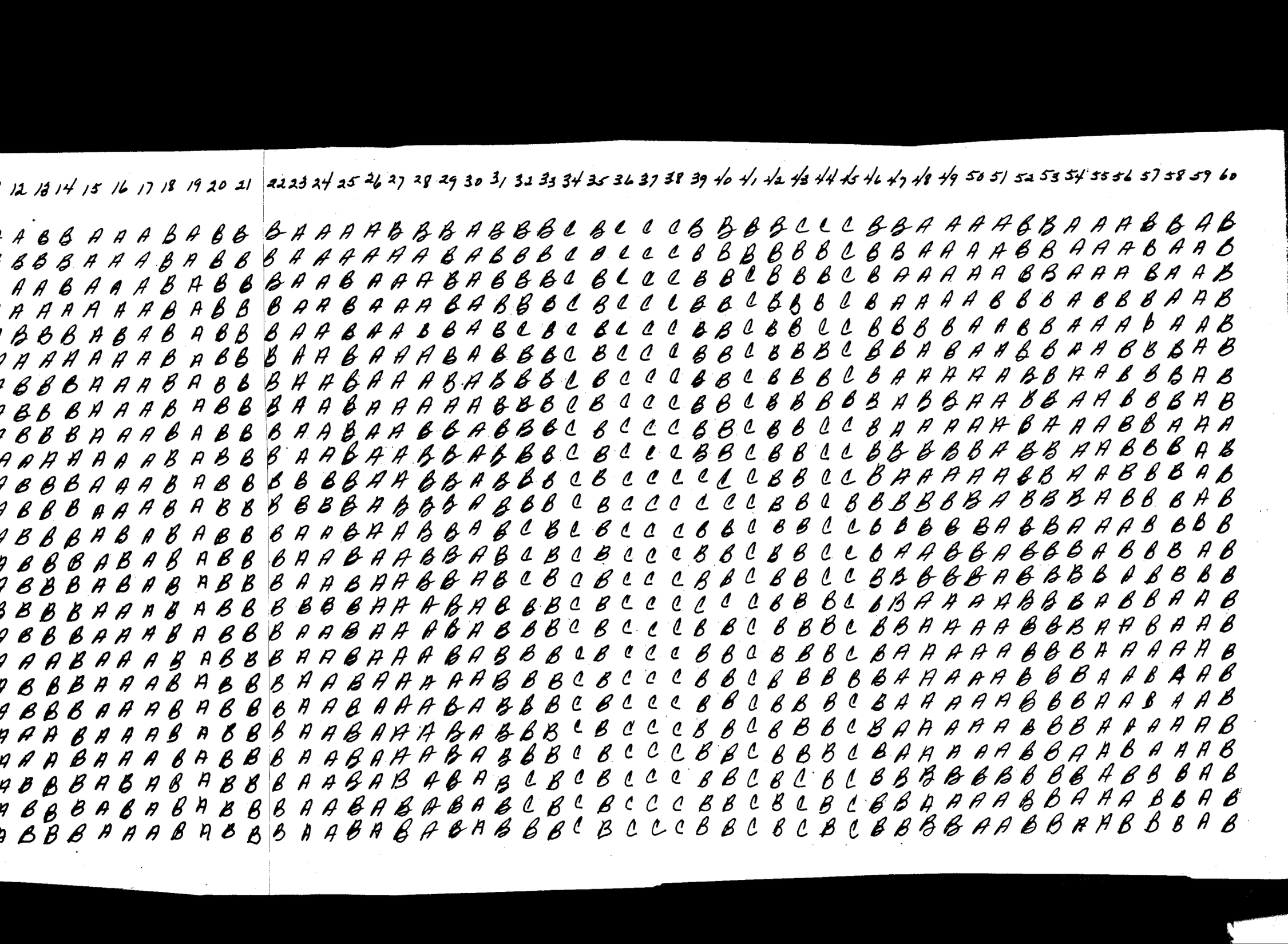


TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
X O 5	A	B	B	B	B	C	F	B	B	A	A	B	B	B	B	A	A	B	A	B	B	B	A	A	B	B	B	B	
X P I	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	B	A	A	A	
X Q I	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	B	A	A	A	
X Q 4	A	B	B	B	B	C	F	B	B	B	B	B	B	B	A	A	A	B	A	B	B	B	A	A	B	B	A		
X Q 5	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	B	A	B	A	B	B	B	A	A	B	A	A		
X Q 7	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	B	B	B	B	B		
X R I 4	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	B	A	A		
X T 6	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	A	A			
X T 2 3	A	B	B	B	B	C	F	B	B	B	B	B	B	B	A	A	A	B	A	B	A	B	B	B	B	A			
X T 2 4	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	A				
X U 5	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	B	A			
X U I 4	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	B	B	B	A			
X V 6	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	B	A	B	B	B	A	A	A	A			
X M 7	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	B	A	B	B	B	B	A	B	B	B			
I 7 L 5	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	A			
2 N 5	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	B	B	A	B	B	B	B	A	B	A	A		
ACHROMOBACTER																													
I I – 8	C	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	A	B	A	B	B	B	B	B	B	A	A		
X D I O	C	B	B	B	B	C	F	B	B	A	B	B	B	A	B	A	B	B	B	B	B	B	B	B	B	A	B		
3 A 5	C	B	B	B	B	C	F	B	A	A	A	A	A	A	A	A	A	B	A	A	A	B	A	A	A	A	A		
2 4 R 3 A	C	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	A	B	B	B	B	B	B	A		
X Q 7 3	C	B	B	B	B	C	F	A	A	B	B	B	B	A	A	A	A	A	A	A	A	B	A	A	A	A	A		
2 M 4	C	A	B	B	B	C	F	A	A	A	A	A	A	A	A	B	A	B	A	B	B	B	B	A	B	A	A		
9 – I 3	C	B	B	B	B	C	F	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B		

* Character numbers 1-89 correspond to those found in Table 2.

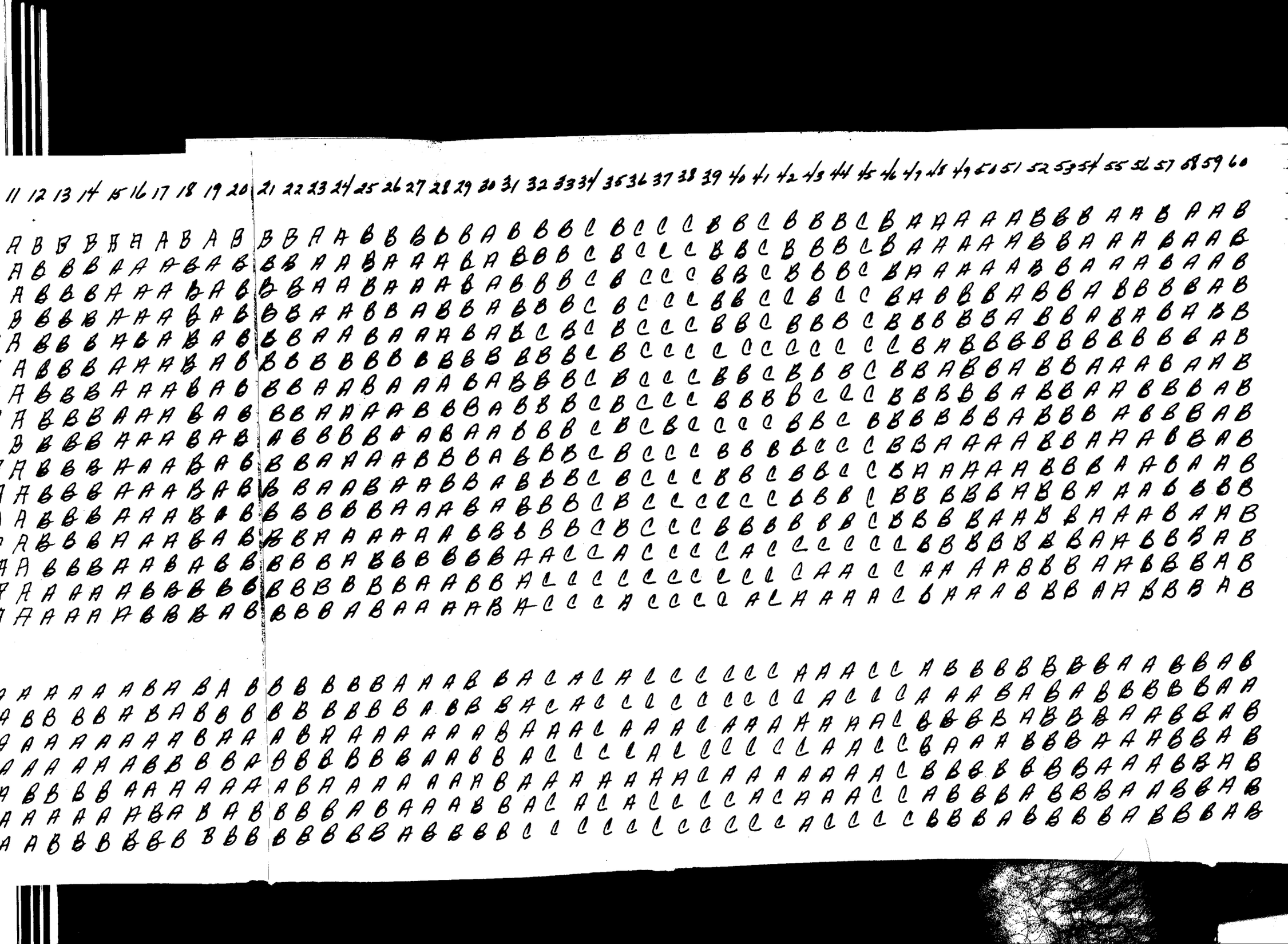


TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1 3 - 2 1	C	B	B	B	B	C	F	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
X O 2	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	A	B	B	A	B	B	B	B	B	B	B	A	A	B
X P 1 8	A	A	B	B	B	C	F	B	B	A	A	A	A	B	B	A	A	B	B	A	B	B	B	B	B	B	A	B	B
X P 2 6	A	B	B	B	B	C	F	B	B	A	A	A	B	B	A	A	B	B	B	B	B	B	B	B	B	B	A	B	B
X P 4 6	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	B	A	A	B	A	A	B	B	B	B	B	A	A	A
X Q 1 9	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	B	B	B	A	B	B	B	B	B	B	A	B	B
X Q 2 0	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	B	A	B	B	A	B	B	B	B	B	A	A	A	B
X Q 5 9	A	B	B	B	A	B	C	B	B	A	A	A	A	A	A	B	A	B	A	B	B	B	B	A	B	B	A	A	A
X S 3	A	B	B	B	B	C	F	B	B	A	A	A	A	B	A	B	A	B	A	B	A	B	B	A	B	B	A	A	A
2 E 2	A	B	B	B	B	C	F	B	B	B	B	B	B	B	A	A	A	B	A	B	B	B	A	A	B	A	B	A	B
2 F 2	A	B	B	B	B	C	F	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	A	B	A	B	B
2 F 1 9	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	B	A	B	A	A	B	B	B	B	A	B	B	B	A
2 H 6	A	B	B	B	B	C	F	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	A	B	B	A	A	A
4 H 6	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	A	A	B	A	B	B	B	B	A	B	B	A	A	A
4 H 8	A	B	B	B	B	C	F	B	B	A	A	A	A	A	A	B	B	B	B	B	B	B	B	B	B	B	A	A	B
4 K 5	A	B	B	B	B	C	F	B	B	B	B	B	B	B	A	A	B	B	A	B	B	B	B	B	A	B	A	A	B
4 K 1 4	A	B	B	B	B	C	F	B	B	A	A	B	B	B	A	A	A	A	A	A	A	B	A	A	A	A	A	A	B
FLAVOBACTERIUM																													
3 F 2	C	B	B	B	A	B	A	A	A	A	A	A	B	A	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B
2 N 1	C	A	B	B	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	B	A
4 - 5	C	B	B	B	A	B	A	A	A	A	A	A	A	A	B	A	B	B	A	A	B	B	B	B	B	B	A	B	B
6 - 2	C	B	B	B	A	B	A	A	A	A	A	A	B	B	A	B	A	A	A	A	B	B	B	B	B	A	B	B	B
9 - 2 1	C	A	B	B	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	B	B	B	B	A	A	A	B
FLAVOBACTERIUM	C	B	B	B	A	B	A	A	A	B	B	B	B	B	A	A	A	A	A	A	A	B	A	A	A	B	A	A	B

* Character numbers 1-89 correspond to those found in Table 2.

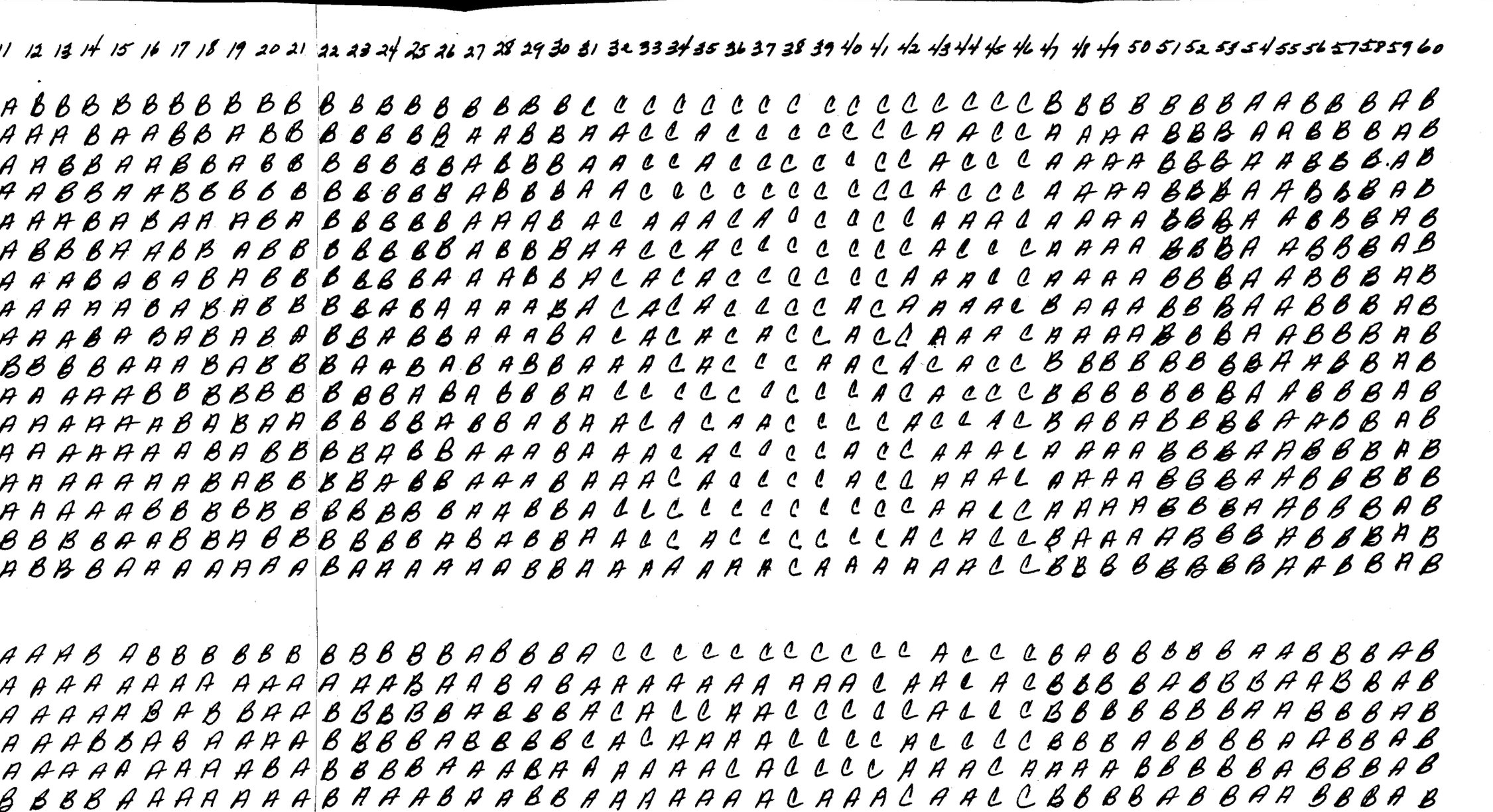


TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
C. PERCOLANS B	B	B	B	B	B	C	F	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
X. FERONICA	A	B	B	B	A	B	A	A	A	A	A	B	B	B	A	A	A	A	B	A	A	B	B	B	B	B	B	B	A

* Character numbers 1-89 correspond to those found in Table 2.

10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
A	A	B	B	B	A	A	A	A	B	A	A	B	B	B	B	B

27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
B	B	B	B	C	C	C	C	C	C	C	C	C	C	C	C	C
B	B	A	B	A	A	A	A	C	A	A	C	C	C	C	C	C

44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
C	C	C	B	A	B	A	A	B	B	A	A	A	B	B	A	B
C	A	C	B	B	B	A	B	B	B	B	A	B	B	B	A	B

TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89
PSEUDOMONAS																													
PS. FLOURESCENS	B	B	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	B	B	B	A	A	A	A			
PS. PUTIDA	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A	A	B	B	B	A	A	A	A			
PS. AUREOFACIENS	B	B	A	A	A	A	A	A	A	A	A	B	A	A	B	B	B	A	A	B	A	B	A	A	A	A			
PS. STRIATA	B	B	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A	A	A	B	B	B	A	A	A	A			
PS. TABACI	B	B	A	B	D	A	A	B	A	A	A	B	A	A	B	A	A	B	R	B	A	B	A	A	B	A			
PS. VIRIDOLIVIDA	B	B	A	A	A	A	A	A	A	A	A	B	A	A	A	B	B	A	B	A	B	B	B	A	A	A			
PS. SP. B	B	B	A	A	A	A	A	A	A	A	A	B	A	A	A	B	B	A	R	B	B	B	A	R	B	R	A		
PS. SP. C	A	B	A	A	B	A	B	B	A	A	A	B	A	A	A	A	A	B	R	B	B	B	A	R	B	R	A		
PS. AERUGINOSA	A	A	A	A	A	A	A	A	A	A	A	B	A	B	B	B	B	A	B	A	B	B	B	A	A	A	A		
3 L 6	B	B	A	B	A	A	A	B	A	A	A	B	A	A	B	A	A	A	A	B	B	B	A	A	A	A	A		
3 M 7	B	B	A	A	A	A	B	B	A	A	A	B	A	A	A	B	A	A	A	B	B	B	A	A	A	A	A		
D W - 6	B	A	B	B	B	B	B	B	B	A	A	A	B	A	B	B	B	A	A	A	A	B	B	B	A	A	A		
3 A 4	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	B		
I 7 L 3	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B			
I R 5	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	B			
I 4 R I	B	B	A	B	B	B	B	A	A	B	B	A	A	B	B	B	B	A	A	B	B	A	A	B	A	A	B		
4 - 9	B	B	A	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B		
ACHROMOBACTER	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B	B	A	B	B	B	A	A	A	A		
D W - 3 I 7	B	A	A	A	A	A	A	A	A	B	A	A	A	B	B	B	A	A	B	A	B	B	A	A	A	A	A		
4 K 2 3	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B		
3 L I	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	B			
7 L 2	B	B	A	B	B	A	B	B	A	A	A	B	B	B	B	B	B	B	B	A	B	A	A	B	A	A	B		
I I L 3	B	B	A	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	B		
2 O 8	B	B	B	B	B	B	B	B	A	B	A	B	B	A	B	B	B	B	B	B	A	A	A	B	B	A	B		
I T 5	B	B	A	B	B	A	B	B	A	A	A	A	B	A	B	B	B	B	A	B	A	B	B	A	B	A	A		

* Character numbers 1-89 correspond to those found in Table 2.

TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89
1 – 9	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	B
3 – 6	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	A	B
4 – 7	B	B	B	B	B	A	B	B	A	A	A	A	B	A	B	B	B	B	B	B	A	A	B	B	B	A	A	B	B
5 – 5	B	B	A	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	B
6 – 1 7	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B
7 – 1 5	B	B	B	B	B	B	B	B	B	B	A	A	B	A	B	B	B	B	B	B	B	A	B	B	B	B	A	B	B
1 0 – 4	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	A	B	B	B	B	A	B	B	B
1 1 – 6	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	B	B
1 1 – 1 5	B	B	A	B	B	B	B	B	A	A	A	A	B	B	A	B	B	B	B	B	B	A	A	B	B	A	A	B	B
X A 5	B	B	A	A	A	A	B	B	A	B	A	A	B	B	B	B	B	B	B	B	B	A	A	B	B	A	A	A	A
X B 2	B	B	A	B	A	B	B	B	A	A	A	A	B	A	B	A	B	B	A	B	A	A	A	B	B	A	A	B	B
X C 5	B	B	A	B	B	A	B	B	A	B	A	B	B	A	B	B	B	B	B	B	A	A	A	B	B	A	A	B	B
X D 2	B	B	A	A	A	A	B	B	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	B	B	A	A	B	A
X E 3	B	B	A	B	A	A	B	B	A	A	A	A	B	B	B	B	B	B	B	B	B	A	A	B	B	A	A	B	A
X H 2	B	B	A	B	A	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	A	B	B	B	A	A	A	B	B
X H 5	B	A	A	A	B	A	B	A	A	A	B	A	B	A	B	A	A	A	A	A	A	A	B	B	A	A	A	B	B
X H 8	B	B	A	A	B	B	B	B	A	A	A	A	B	A	B	B	B	B	A	A	B	A	B	B	A	A	A	B	B
X L 1	B	B	A	A	A	A	B	A	A	A	A	A	B	A	B	B	B	B	B	B	A	A	A	A	B	A	A	A	B
VIBRIO																													
X A 1	B	B	A	B	B	B	B	B	A	A	A	A	B	A	B	B	B	B	A	B	A	A	B	B	B	B	A	A	B
X A 3	B	B	A	B	B	B	B	B	A	B	A	B	B	A	B	B	B	B	A	B	A	A	B	B	B	A	A	B	B
X A 1 2	B	B	A	B	B	B	B	B	A	B	A	A	B	A	B	B	B	A	A	B	B	A	B	B	B	B	A	A	A
X C 1	B	B	A	A	B	B	B	B	A	A	A	A	B	A	B	B	B	B	B	B	A	A	B	B	B	A	A	A	B
X C 6	B	A	A	A	B	B	B	B	A	A	A	A	B	A	B	B	B	B	A	B	B	A	A	B	B	A	A	A	A

* Character numbers 1-89 correspond to those found in Table 2.

TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89
X D 1	B	A	A	A	A	A	B	B	A	A	A	A	B	A	B	B	B	A	A	B	A	A	A	B	B	A	A	A	A
X D 5	B	A	A	A	B	B	B	B	A	A	A	B	B	A	B	B	B	B	A	B	B	A	A	B	B	B	B	A	B
X D 6	B	A	A	A	B	A	B	B	A	A	A	A	B	A	B	B	B	B	A	B	A	A	A	B	B	A	A	A	A
X E 1	B	B	A	A	A	A	B	B	A	A	A	A	B	A	B	B	B	A	A	B	A	A	A	B	B	A	A	A	A
X P 4 6	B	A	A	A	A	A	B	B	A	A	A	A	B	A	B	B	B	A	A	B	A	A	A	B	B	A	A	A	A
X F 1	B	B	B	B	B	B	B	B	A	A	A	A	B	A	B	B	B	A	A	B	A	B	A	B	B	B	B	B	B
X F 3	B	A	A	A	B	B	B	B	A	A	A	A	B	B	A	B	B	B	B	A	B	B	A	A	B	B	A	A	A
X F 7	B	B	B	B	B	B	B	B	A	A	A	A	B	A	B	B	B	B	A	B	A	A	A	B	B	A	B	B	B
X O 5	B	A	B	A	B	B	B	B	A	A	A	A	B	A	B	B	B	B	A	B	A	A	A	B	B	B	B	A	A
X P 1	B	A	A	A	B	B	B	B	A	A	A	A	B	A	B	B	B	B	A	B	A	A	A	B	B	A	A	A	A
X Q 1	B	A	B	A	B	B	B	B	A	A	A	A	B	A	B	B	B	B	A	B	A	A	A	B	B	A	A	A	B
X Q 4	B	B	B	B	B	B	B	B	A	A	A	A	B	B	B	B	B	B	B	B	A	B	B	B	B	B	B	B	B
X Q 5	B	B	A	B	B	B	B	B	A	B	B	A	B	B	B	B	A	B	A	A	A	B	B	B	A	A	B	B	B
X Q 7	B	B	B	B	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
X R 1 4	B	A	A	A	B	B	B	B	A	A	A	A	B	A	B	B	B	A	A	B	A	A	A	B	B	A	A	A	A
X T 6	B	B	A	A	B	B	B	B	A	A	A	A	B	A	B	B	B	B	A	B	A	A	A	B	B	A	A	A	A
X T 2 3	B	B	B	B	B	B	B	B	A	B	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
X T 2 4	B	A	A	A	B	B	B	B	A	A	A	A	B	A	B	B	B	A	A	B	B	A	A	B	B	A	A	A	B
X U 5	B	A	A	B	B	B	B	B	A	A	A	A	B	A	B	B	B	B	A	B	A	A	B	B	B	A	A	A	B
X U 1 4	B	B	B	B	B	B	B	B	A	A	A	A	B	A	B	B	B	B	B	B	B	A	A	B	B	B	B	B	B
X V 6	B	B	B	B	B	B	B	B	A	A	A	A	B	A	B	B	B	A	B	B	A	A	B	B	B	A	A	A	A
ACHROMOBACTER																													
X O 2	B	B	A	A	A	A	B	B	A	A	A	B	B	B	B	B	B	B	B	B	A	A	B	B	A	A	B	A	
X P 1 8	B	B	A	B	B	B	B	B	A	B	A	B	B	B	B	B	B	B	B	B	B	A	B	B	B	A	A	B	A

* Character numbers 1-89 correspond to those found in Table 2.

TABLE 3

Morphological and Physiological Characteristics of the Marine and Terrestrial Bacteria Used in This Study

	61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89
X P 2 6	B B B B B B B B A B B B B B B B B B A A B A A A B A
X P 4 6	B B A B A A B B A B A A B B A B B B B B A A A B A A A B
X Q 1 9	B B A B B B B A A A B B B B B B B B B B A B B B A A B B
X Q 2 0	B B A B A B A A A A B A B B B B B B A A A B B A A B B
X Q 5 9	B B A B A B B B A B A A B A B B B A B A A A B A A B B
X S 3	B B A B B A B B A B A A B A B A B A A B B A A A B A A B B
2 E 2	B B A A B B A B A A A B B B B B B B B B A B B B B A A B B
2 F 2	B B A B B B B B B B B B B B B B B B B B B B B B B B B B
2 F 1 9	B B A B B A B A A A B B B B B B B B B B B B B B B A A B B
2 H 6	B B A B B B B A A A A B A B B B A A B B A A B B A A B B
4 H 6	B B B B B B B B B B A B B B B B B B B B B B B B B A A B B
4 H 8	B B A B B B B B B B B B B B B B B B B B B B B B B B B B
4 K 5	B B B B B B B B A B B B B B B B B B B B B B B B B B B B
4 K 1 4	B B A B B B B B A A B B B B B B B B B B B B B B B B B B
C. PERCOLANS . B	B B B A B B B B A A B B B B B B B B B B B B B A A A A
X. FERONICA	B B A A A A B A A A A B B B A A B B B B B B B B A A A A

* Character numbers 1-89 correspond to those found in Table 2.

CHAPTER 3

RELATION OF Na^+ TO THE GROWTH OF MARINE BACTERIA

In Chapter 2 it was shown that marine bacteria were morphologically and physiologically indistinguishable from comparable terrestrial bacteria at the genus level. The only physiological characteristic which was of any value in differentiating these 2 groups was a requirement for Na^+ by the marine bacteria.

Marine bacteria require Na^+ for growth (20). Tyler *et al.* (42) studied 96 isolates of marine bacteria from Atlantic coastal waters off Florida and found all to require Na^+. Leifson (17) found that his marine bacteria required 0.05 - 0.2 M Na^+ while what he termed semi-marine bacteria required 0.001 - 0.05 M Na^+ for growth.

Since representatives of almost all of the defined terrestrial genera have been isolated from marine sources little taxonomic significance has been attached to the requirement for Na^+ by marine bacteria. Indeed, several workers have reported that this requirement for Na^+ was lost simply by the storage of cultures (44), ultraviolet irradiation of cultures (21), or by spontaneous mutation (29).

In this chapter experiments will be designed to determine the minimum Na^+ requirement of selected marine bacteria and studies regarding its qualitative and quantitative stability will be performed.

MATERIALS AND METHODS

Relation of Na^+ Concentration to Growth. Since the concentration of NaCl has a direct effect on the growth of marine bacteria (20) experiments were designed to detect both the minimum and maximum levels of NaCl which permitted visible growth. A nutrient medium consisting of 2% Casitone and 1% yeast extract was prepared and passed through a mixed bed deionizer (Deeman Deionizer, Crystal Research Laboratories, Hartford, Conn.). Three amino acids, DL-alanine, L-aspartic acid, and L-glutamic acid, were then added to a concentration of 0.3%. This base was then diluted 1:10 with the appropriate salts solution and the pH adjusted to 7.5 with 5% KOH. This medium was used in all experiments related to the effect of Na^+ on the growth of marine bacteria and will be referred to as deionized T medium.

For NaCl tolerance studies concentrations of 5, 6, 8, 10, and 12% NaCl were added to the deionized base. This was dispensed into individual tubes in 3 ml amounts and inoculated as described for the T medium. The highest concentration showing turbidity after 7 days incubation at 20 C was considered the maximum level of NaCl allowing growth.

The minimum Na^+ requirement of the marine bacteria was determined in the deionized base containing 2% NaCl. Using the Na^+ free deionized base as the diluent, doubling dilutions of this base were made and these were dispensed into individual tubes and inoculated as described for the T medium. The lowest concentration of NaCl permitting visible growth after 7 days incubation at 20 C was taken as the minimum Na^+ requirement of the culture.

Stability of the Minimum Na^+ Requirement. In order to determine the stability of the minimum Na^+ requirement, 9 cultures of marine bacteria were selected for study. Each culture was inoculated into a tube of T, deionized T, and a synthetic T broth containing glucose as the carbon source. All tubes were incubated at 20 C for up to 2 months during which they were inspected for turbidity. If growth occurred in any medium this culture was serially transferred at least once and its minimum Na^+ requirement determined. These same cultures were then transferred to a M medium and to a medium containing the maximum level of NaCl that the culture would grow in. The cultures were serially transferred twice and their minimum Na^+ requirements determined and compared with those previously found.

Effects of Storage. The effect of prolonged storage on the minimum Na^+ requirement of 9 cultures of marine bacteria was studied. The cultures were grown for 24 hr in the M medium, centrifuged and resuspended in 8 ml of the sterile marine salt mixture, pH 7.5. The cultures were stored at 4 C and 0.1 ml samples were withdrawn every 2 weeks. These samples were inoculated into tubes of T, deionized T, synthetic T, and M broth and incubated at 20 C for up to 1 month. If growth occurred in any of the T broths it was serially transferred. After several such passages the minimum Na^+ requirement of the cultures was determined.

Effects of Ultraviolet Irradiation. Nine cultures of marine bacteria were exposed to ultraviolet irradiation in an attempt to induce mutants

capable of growing without added Na^+. The age of the cultures, type of growth medium, and concentrations of nutrients were studied as variables in these experiments.

Bacterial cells were harvested by centrifugation, resuspended in 10 ml of distilled water, and transferred to empty petri dishes containing a sterile metal pin used as a stirring bar. The plates were then placed on top of a magnetic stirrer. The suspensions were then exposed to an ultraviolet lamp (type 30600, Hanova Chem. & Manuf. Co., Newark, N.J.) for a total of 30, 60, or 120 secs at a distance of 6 in. Preliminary experiments indicated that under these conditions, after 60 sec exposure, there were less than 0.1% survivors. Samples of 0.1 ml were withdrawn and, with a bent glass rod, spread over the entire surface of either a T, deionized T, or synthetic T agar medium containing glucose as the carbon source. The plates were incubated at 20 C for up to 1 month and any colonies which arose were transferred to a tube of deionized T broth and subjected to a morphological analysis.

RESULTS

Determination of Minimum and Maximum Na^+ Levels. The minimum Na^+ requirement of 27 cultures of marine bacteria was determined and contrasted to the maximum levels of NaCl allowing growth in order to establish a range of concentrations in which marine bacteria would grow (Table 4). The organisms examined were all gram-negative rods, motile with a single polar flagellum and could be divided into 2 groups based on their metabolism of glucose. Group I contained those strains which either oxidized or produced no acid from glucose. Members of this group were variable in their minimum Na^+ requirements ranging from 0.003 - 0.172 M with the majority of strains starting to grow in the 0.022 - 0.043 M range. More than one-half of the organisms were able to grow in 2.06 M NaCl with the rest growing well in concentrations of from 0.086 - 1.72 M. Three strains, XP3, 2K10 and 162, grew only in a narrow range of NaCl concentrations (Table 4). Terrestrial pseudomonads, which did not require Na^+, grew at slightly lower maximal NaCl concentrations than did the marine types (Table 3). Most terrestrial pseudomonads grew in NaCl concentrations ranging from 0.69 - 1.03 M, but occasional strains were able to grow in 2.06 M NaCl.

Group II organisms all fermented glucose anaerogenically and grew in a narrow range of NaCl concentrations (Table 4). The most common types had a minimum Na^+ requirement of 0.086 M while the minimum Na^+ requirement ranged from 0.003 (XD1) to 0.172 M (XT15). Most cultures would not grow

in a broth with the NaCl concentration above 1.03 M and this concentration was considerably lower than that found for the oxidative marine types (Table 4).

From these results it appeared that the oxidative marine bacteria were heterogeneous in their minimum Na^+ requirements, ranging from 0.003 - 0.172 M, with the majority growing at 0.022 M. The maximum concentrations of NaCl in which these bacteria could grow was of little diagnostic value. The fermentative bacteria grew only in a narrow range of NaCl concentrations, the majority growing in from 0.086 - 1.03 M. The most common minimum Na^+ requirement of the fermentative bacteria (0.086 M) was substantially higher than that found for the oxidative bacteria (0.022 M). None of the named terrestrial bacteria studied here was found to require Na^+.

Stability and Variability of the Minimum Na^+ Requirement. Nine cultures of marine bacteria were selected for the study of the stability and variability of their minimum Na^+ requirements. The cultures used, with the exception of Snail I, were gram-negative rods, motile with a single polar flagellum, and contained both oxidative and fermentative types. Snail I was a gram-negative *Spirillum* sp., motile with polar multitrichous flagella, and was used in these experiments because of its distinctive morphology.

All cultures were stored in the marine salts mixture at 4 C for periods of up to 9 months and their ability to grow in the T, deionized T, or synthetic glucose broth was determined. From Table 5 it appeared that as the

length of storage time increased the time necessary for the production of visible growth in the T broth decreased. In those instances where growth did occur, there was a marked reduction in the time, ranging from 20-70%. Not all cultures were able to grow in the T medium after storage and those that did were able to grow in the T medium, without first being stored, after direct inoculation and a period of incubation ranging from 7-35 days. In no instance was any culture able to grow in the synthetic or deionized T medium.

The determination of the minimum Na^+ requirements of those cultures able to grow in the T medium revealed that these bacteria now exhibited a much lower requirement than when grown in an M broth. The cultures originally had Na^+ requirements ranging from 0.003 - 0.043 M, but after growth in the T broth, the requirements dropped sharply, the range becoming 0.0008 - 0.006 M. These reductions ranged from 50% (XW1) to 94% (XD4) and appeared only in those organisms requiring 0.043 M Na^+ or less. Passage of these T grown cultures in an M broth led to a reversion back to the original minimum Na^+ requirements (Table 5). No change in the minimum Na^+ requirement was observed when the cultures were grown in an M broth containing NaCl concentrations near the maximum in which each culture would grow.

Since the minimum Na^+ requirement was shown to be quantitatively variable, attempts were made to induce the formation of mutants capable of growing without Na^+ by the exposure of cultures to ultraviolet irradiation. Cultures selected for these experiments were grown both in the M and T

broths and stored for varying periods. The ability to grow in the absence of Na^+ was assayed on either a deionized T agar or a glucose salts agar medium containing from 0.2 - 5% glucose as the sole carbon source. All cultures, except 3 - 6 and Snail I, were able to use glucose as a sole carbon source.

The results of experiments using cultures grown and stored in an M broth for a period of 1 day and then exposed to ultraviolet irradiation are recorded in Table 6. The results when the storage time was increased to 1, 4, or 8 weeks were the same. The CFU's/ml were determined for 1 day old cultures and were considerably less for the older cultures. Regardless of the age of the culture, in no case could any mutants capable of multiplying without Na^+ be isolated.

No organisms were isolated capable of growing in the absence of Na^+, following ultraviolet irradiation, when the cultures were grown in a T broth or an M broth containing the maximum NaCl concentration in which each culture would grow (Table 7). Growth in the T medium, which was shown to reduce the Na^+ requirement, did not enhance the desired mutagenic effect of ultraviolet irradiation. From these results it appeared that the Na^+ requirement of marine bacteria was qualitatively stable and that neither the type of growth medium nor the age of the cultures facilitated the induction of non-Na^+ requiring mutants by ultraviolet irradiation.

TABLE 4

Minimum and Maximum NaCl Concentrations Permitting Growth of Selected Marine Bacteria

Strain	A	B	C	Strain	A	B	C
XP3	O	.172	0.86	3-22	O	.022	2.06
4H8	O	.022	2.06	3-6	O	.022	2.06
11-6	-	.022	2.06	11L3	-	.022	2.06
13-8	-	.003	1.38	XG4	F	.172	0.67
1G2	-	.086	0.69	3-5	F	.011	2.06
XD4	O	.043	2.06	XA1	F	.086	1.03
XL1	O	.022	2.06	XC1	F	.086	0.86
XW1	O	.011	2.06	XD1	F	.003	1.72
XH2	O	.022	1.03	XP1	F	.086	1.03
2F27	-	.011	2.06	4H12	F	.086	1.03
7-15	O	.003	1.72	YC4	F	.011	1.03
5L2	-	.086	1.38	XT15	F	.172	1.03
5-5	-	.006	2.06	XT6	F	.086	1.03
2K10	-	.172	1.38				

A - Type of acid production from carbohydrates

B - Minimum Na^+ requirement (m/l)

C - Maximum NaCl tolerance up to 2.06 M

O - oxidative

F - fermentative

\- no acid produced

TABLE 5

Effect of Storage on the Growth Response in T Medium and the Minimum Na^+ Requirement of Selected Marine Bacteria

Strain	Min. Na^+	Months of Storage				Min. Na^+ [B]	Min. Na^+ [C]	Min. Na^+ [D]
		0	3	6	9			
XA1	.086 M	-	-	-	-	-	-	.172M
XC1	.086	-	-	-	-	-	-	.086
XD1	.003	4[A]	4	3	2	.0008 M	.003 M	.001
XD4	.043	14	12	6	4	.003	.043	.043
XL1	.022	5	-	-	4	.006	.022	.022
XP1	.086	-	-	-	-	-	-	.086
XW1	.011	20	16	10	8	.006	.011	.022
Snail I	.043	-	-	-	-	-	-	-
3-6	.043	-	-	-	-	-	-	.043

A - Days of incubation at 20 C before growth occurred in a T medium.

B - Minimum Na^+ requirement of strains grown in T broth.

C - Minimum Na^+ requirement of T adapted strains when grown in M broth.

D - Minimum Na^+ requirement of strains grown at their maximum levels of NaCl tolerance.

TABLE 6

Effect of Ultraviolet Irradiation of Fresh and Stored Cultures on the Induction of Non-Na^+ Requiring Mutants of Marine Bacteria

Strain[b]	CFU/ml	Glucose Concentration 0.2%	1%	5%	Deionized T
XA1	1.6×10^8	0[a]	0	0	0
XC1	4.3×10^7	0	0	0	0
XD1	-	0	0	0	0
XD4	3.4×10^8	0	0	0	0
XL1	2.9×10^6	0	0	0	0
XP1	2.1×10^7	0	0	0	0
XW1	1.9×10^7	0	0	0	0
Snail I	7.8×10^6	ND[c]	ND	ND	0
3-6	1.2×10^7	ND	ND	ND	0

[a]CFU per ml on either a Na^+ free glucose salts medium or on deionized T agar after irradiation for 0, 30, 60, and 120 sec.

[b]All cultures used were grown and stored in an M broth for periods of 1 day, 1 week, 4 weeks, and 8 weeks.

[c]ND - not done.

TABLE 7

Influence of Growth Medium on the Effect of Ultraviolet Irradiation on the Stability of the Na^+ Requirement of Marine Bacteria

Strain	Growth Medium A	Growth Medium B	CFU/ml	Glucose Concentration 0.2%	Glucose Concentration 1%	Glucose Concentration 5%	Deionized T
XD1	T_{10}	-	-	0[C]	0	0	0
XD4	T_1	-	3.7×10^6	0	0	0	0
XD4	T_4	-	8.9×10^6	0	0	0	0
XD4	T_7	-	2.1×10^7	0	0	0	0
XL1	T_7	-	5.4×10^7	0	0	0	0
XW1	T_2	-	6.4×10^5	0	0	0	0
XA1	-	1.03	3.9×10^7	0	0	0	0
XC1	-	0.86	6.5×10^6	0	0	0	0
XD1	-	1.72	-	0	0	0	0
XD4	-	2.06	3.4×10^7	0	0	0	0
XL1	-	2.06	9.6×10^6	0	0	0	0
XP1	-	1.03	7.3×10^6	0	0	0	0
XW1	-	2.06	8.9×10^5	0	0	0	0
3-6	-	2.06	1.8×10^7	ND	ND	ND	0

A - Number of serial transfers in a T medium.

B - Concentration of NaCl (M/l) in pre-experiment growth medium.

C - CFU's per ml on either a Na^+ free glucose salts medium or on deionized T agar after 0, 30, 60 and 120 sec. irradiation.

DISCUSSION

The minimum Na^+ requirements of selected marine bacteria were determined and studied with respect to their qualitative and quantitative stability.

Leifson (17) reported that his marine isolates could be divided into 3 groups based on their minimum Na^+ requirements: 1) marine strains which required from 0.05 - 0.02 M Na^+; 2) semi-marine strains which required from 0.001 - 0.05 M Na^+; 3) terrestrial strains which required less than 0.001 M Na^+. From the results presented in Table 4 it appeared that the majority of cultures studied in this thesis were semi-marine with only 4 (XP3, 1G2, 5L2, 2K10) cultures definitely being marine. These were not the expected results since the majority of cultures isolated from seawater are marine not semi-marine (17). Leifson, however, reported that the minimum Na^+ requirements he determined were the amounts necessary for prompt growth while those values reported in this thesis were determined after 7 days incubation. This extended incubation period, and also when the cultures were stored, enabled the marine bacteria to grow at lower Na^+ concentrations. Similar results were reported by MacLeod and Onofrey (21) who found that after a long incubation period their cultures grew at almost one-tenth of their optimal Na^+ concentration. This difference in techniques probably led to the lower minimum Na^+ requirements found in this thesis. These low values did not permit any distinction to be made between marine

and semi-marine bacteria although no difficulty was encountered in differentiating marine from terrestrial bacteria.

In Tables 5 and 6 it was shown that either storage or serial transfer of marine bacteria in a T medium led to a reduction, but never a loss, of their minimum Na^+ requirement. This reduction was reversible simply by growth in a M medium. This was important since it has been reported that prolonged storage of cultures could lead to a loss of the Na^+ requirement by marine bacteria (44). The observed "loss" can now be explained as a temporary reduction in the minimum Na^+ requirement which allowed the cultures to grow in a "Na^+ free" medium. That this can happen is evidenced by the report of MacLeod and Onofrey (21) who found that their Trypticase medium contained 0.028 M Na^+ present as a contaminant. From the results presented in Table 4 most cultures had minimum Na^+ requirements of 0.022 - 0.043 M Na^+ which are low enough to allow most of them to grow in this medium. After long periods of storage the minimum Na^+ requirement dropped still further (Table 5) making it more likely that these bacteria could grow in a Trypticase medium.

No non-Na^+ requiring mutants of marine bacteria could be isolated following the exposure of cultures to ultraviolet irradiation. MacLeod and Onofrey (21) did manage to isolate 14 non-Na^+ requiring mutants of marine bacteria. They stated, however, that this was done only with great difficulty indicating the stability of this requirement. Non-Na^+ requiring mutants of marine bacteria were desired to determine if the loss of the

requirement for Na^+ by marine bacteria had any influence on their DNA base composition. Unfortunately no such mutants were isolated and the cultures of MacLeod and Onofrey were no longer available for study.

The results presented in this chapter indicate that the minimum Na^+ requirements of marine bacteria were qualitatively stable although they were quantitatively variable. Procedures such as prolonged storage or serial transfer in a T broth were shown to reduce the minimum Na^+ requirement but never to completely eliminate it.

CHAPTER 4

NUMERICAL TAXONOMY OF MARINE AND TERRESTRIAL BACTERIA

In Chapter 2 it was shown that no distinction could be made at the genus level between comparable marine and terrestrial bacteria on the basis of a few selected morphological and physiological characteristics. A classification was attempted, therefore, based on the analysis of a large number of characteristics using the technique of numerical taxonomy (36,37).

Numerical methods of analysis have generally confirmed the present system of classification (8,9,38). Several workers, however, have not been able to differentiate between such physiologically diverse genera as *Vibrio* and *Pseudomonas* (13) or *Aeromonas* and *Pseudomonas* (31). The main physiological characteristic used to distinguish between these genera is the method of acid production from glucose. In conventional taxonomic studies this characteristic is extremely important in defining groups while in numerical studies this characteristic has little importance since all characters are of equal weight. In the analysis performed in this thesis each carbohydrate will be scored not only as to whether acid was produced but also as to the method of acid production (oxidative vs. fermentative). Thus 2 bacteria which produced acid from glucose, one oxidative and the other fermentative, would be 50% similar to each other instead of 100% if only acid production was scored. The scoring of carbohydrate characters in this manner ensures that all members of any groups formed in this analysis will have a similar method of carbohydrate metabolism.

MATERIALS AND METHODS

The results of the morphological and physiological tests determined in Chapter 2 were used as the basis of a numerical analysis of 196 cultures of marine and terrestrial bacteria. A list of the characters used are recorded in Table 2 and the encoded results are in Table 3.

The data were encoded using a multiple symbol code which consisted of letters of the alphabet from A-F. For those characters which had only 2 alternatives, positive or negative, A was arbitrarily assigned positive and B negative. Those characters which had more than 2 alternatives were assigned a different letter for each possible alternative. These characters are listed in Table 2 along with the symbols used and their meaning. For example, the type of acid produced from glucose had 3 possible alternatives: 1) oxidative, 2) fermentative, 3) no acid produced. This character was alphabetically encoded as A meaning oxidative, B fermentative, and C meaning no acid produced.

The encoded data was recorded on 80 column data processing layout sheets and sent to Dr. W. R. Lockhart, Iowa State University, Ames, Iowa, where the actual computations were performed. Similarity (S) values were calculated according to the formula $S = \frac{Ns}{Ns + Nd}$, where Ns is the number of characters in which a given pair of strains are alike and Nd is the number of characters in which they differ. A similarity was scored when 2 organisms possessed identical alphabetic code symbols for a given character and a difference was scored when they exhibited 2 different symbols.

Since the only requirement for a similarity was identical code symbols, negative matches contributed to the calculation of S values. The S values were then multiplied by 100 in order to be expressed as percent similarity.

Once the similarity values were calculated between every possible combination of strains, the computer was then instructed to sort the strains into groups. In this analysis group formation was achieved by use of the highest link method (36,37). The similarity values were inspected for paired values of 99% and then this process was repeated with S values reduced consecutively by 1%. Each organism was paired at its highest similarity value to any other organism. A number of groups of related strains were thus created which joined together at the highest similarity level existing between any members of the groups. The groups thus formed were represented in the form of a dendrogram.

In this analysis all comparisons were counted so that (Ns + Nd) was always equal to the total number of characters. This meant that every character had to be determined for each strain. Since data on the ability to use various organic compounds as a sole source of carbon and energy were not determined for approximately one-half of the marine bacteria it was necessary to divide the culture collection into 2 parts, each of which was analyzed separately. The first section contained all 196 cultures for which 60 characters were determined while the second consisted of 88 cultures and 89 characters. The second section contained only those cultures able to grow in a synthetic medium with at least one of the compounds tested as

the sole carbon and energy source. The growth response to these compounds represented the additional 29 characters. After all similarity values had been calculated and groups formed, the 2 analyses were compared to determine if the additional 29 characters had any effect on the structure of the groups formed.

RESULTS

The results of the computer analysis of 196 strains of bacteria based on 60 characters is shown in Figure 4. This Figure is composed of 3 parts which must be placed end to end to form the complete dendrogram. At the 84% S level 5 groups could be distinguished, all of which merged together at 80% S.

The first group was composed of 4 strains. This group contained both marine and terrestrial bacteria which produced acid oxidatively from carbohydrates and were motile with either polar monotrichous (XH5, 4K14) or peritrichous flagella (XQ73, 3A5).

Group 2 was also small, containing only 4 strains. All cultures in this group formed rosettes and showed good stalk formation. These cultures were terrestrial, neither requiring Na^+ nor able to grow in 5% NaCl. Most cultures produced acid oxidatively from at least 5 carbohydrates (Table 3). Included in this group were 2 named *Caulobacter* species plus 2 other *Caulobacter* strains isolated from distilled water.

The third group consisted entirely of terrestrial pseudomonads, most of which produced a green fluorescent pigment. All cultures produced acid oxidatively from glucose and were motile with polar multitrichous flagella with the exception of cultures 17L3, 3L6, and 3M7, which were polar monotrichous. Cultures 17L3 and 8-2 did not produce fluorescent pigments. *Ps. aeruginosa* and *Ps.* sp. C were not sufficiently related to any of the members to be included in this group.

Group 4 was the largest of the groups formed and contained the majority of the aerobic marine cultures. Several terrestrial cultures were also found here, namely *V. alkaligenes, Ps. melanogena, Commomonas peroolans* B and several marine and terrestrial *Caulobacter* cultures (4-4, 7-5, 2F5, 2F25, 3F15, 2F38, 2F26). Only those cultures which oxidized glucose or produced no acid from it are included in this group. All cultures were gram negative rods, the majority of which were motile with a single polar flagellum. Cultures 2M4, 11-8, XD10, 4-5, and 3F2 were peritrichously flagellated while cultures 3A6, XW7, 1F2, 2N5, 17L5, and *C. peroolans* B were lophotrichously flagellated.

The strains on the left-hand side of Group 4 did not form any distinct clusters. In this section were found most of the terrestrial strains plus some of those which had previously been identified as *Caulobacter* species and several peritrichously flagellated strains. These strains showed little organization, each member or pair being distinct from another at 87% S.

Several rosette forming organisms (2F26, 2F38, and 3F15) identified as *Caulobacter* species formed a small cluster near the center of Group 4. This cluster became numerically united at 95% S. To the left of this group was another pair, 4-4 and 7-5, which were related at 93% S. This group was related to strains 1F2 and 2F25 at 88% S.

The peritrichously flagellated strains were represented by cultures 4-5, 2M4, 11-8, XD10 and 3F2. Of these, only 2M4 and 11-8 formed a pair and were related to each other at 90% S. The rest of the strains were not

very similar to each other and were scattered throughout the left-hand side of the group.

In this analysis most groups were formed primarily by their reactions on carbohydrates. Of a total of 60 characters used, 32 represented acid production from carbohydrates. This represented over 50% of the characters used and was large enough to mask any differences represented by only 1 or 2 characters. That this is the case is revealed by inspection of this group where members of several genera (*Caulobacter*, *Pseudomonas*, *Commomonas*, *Achromobacter*) could be found. Most of these genera could not be distinguished from each other by acid production from sugars but they could easily be differentiated on the basis of somatic morphology and flagellation.

Near the center of Group 4 was a well-defined group which consisted of a number of highly related clusters of strains. All members of this group became united at 93% S and were separated into sub-groups at 94.5% S. Four strains (1R5, 6-17, 2F25, XW1) were attached to the edge of this group. Of these, 2F25 was a *Caulobacter* species while the rest were marine types physiologically comparable to *Pseudomonas*.

The first sub-group consisted of strains 1R4 and 3A4. These strains did not produce acid from carbohydrates and they hydrolyzed Tween 80 but not gelatin, casein, or starch.

Strains 3-22 and 3-6 formed the second small cluster. These strains only produced acid from glycerol. They could not hydrolyze gelatin, casein, starch, or Tween 80, but they could reduce nitrate.

The third sub-group was composed of 4 strains (2K10, 5L2, 11L3, 5-5). Members of this group did not produce acid from carbohydrates but could reduce nitrate. All were marine strains and did not hydrolyze gelatin, casein, or Tween 80.

Sub-group 4 consisted of 3 strains (13-8, 11-6, 2F27). All were marine bacteria which did not produce acid from any of the carbohydrates tested. Gelatin, casein and Tween 80 were hydrolyzed while nitrate was not reduced.

To the right of this last sub-group was another large group and was represented by cultures XL7-3L3. This group consisted entirely of marine bacteria and was divisible into 3 small subgroups.

The first sub-group contained 5 strains (17L5-YM5). These organisms produced acid from glucose, maltose and sucrose. They were active enzymatically, being able to hydrolyze starch, gelatin, casein, and Tween 80. Two additional strains were attached to the left-hand edge of the group. These strains were similar to other members of the group with XE3 forming lateral flagella and not being able to hydrolyze starch and Snail G able to produce acid from lactose. These strains joined the group at a level of 93% S.

The second sub-group consisted of 2 pairs of organisms which were related to each other at 93% S. Pair X02 and XD2 produced acid from several carbohydrates, namely glucose, mannose, fructose, maltose, and sucrose. They also hydrolyzed starch, gelatin, casein, and Tween 80. They could not grow at 37 C nor could they reduce nitrate or deaminate phenylalanine.

Pair 105 and 208 produced acid from glucose, fructose, maltose and sucrose, but not mannose. They grew at 37 C, deaminated phenylalanine and hydrolyzed gelatin, casein, and Tween 80. These strains also reduced nitrate.

The third sub-group was composed of 3 strains which merged together at 95% S and which were related to a fourth strain at 92% S. Strains 3L3, XQ19 and XP18 were all marine bacteria which oxidized glucose, maltose, fructose, and mannose. They hydrolyzed starch, gelatin, casein, and Tween 80, but could not reduce nitrate.

To the right of the last group was another cluster made up of 11 strains. This cluster showed 3 internal sub-groups which could be distinguished at the 94% S level. All strains were marine bacteria which produced acid from glucose and hydrolyzed starch, gelatin, casein and Tween 80. These strains were oxidase and catalase positive but did not reduce nitrate. The three small sub-groups could be differentiated from each other by their patterns of acid production from carbohydrates.

The next cluster, Group 5, contained all of the fermentative types. Members of this group were all gram-negative rods motile with a single polar flagellum. They fermented glucose, mannose, galactose, fructose, sorbitol, mannitol, glycerol, maltose, sucrose and inulin anaerogenically. Most strains reduced nitrate and were oxidase and catalase positive. All members of this group became numerically united at 87% S. Inside of this large group existed 4 smaller clusters which were distinct at 94.5% S.

Strains XA1 and XA12 are representatives of sub-group 1 which merged together at 94% S. Three other strains (Y-17, XQ5, XA3) were attached to the edge of this group. These organisms were all marine types unable to grow at 37 C or to hydrolyze gelatin, casein, Tween 80 or starch.

The second sub-group was composed of 3 marine strains, 2 of which were related at 94% S. Strains of this cluster (1G14, XT24, XT1) were very active on carbohydrates, could deaminate phenylalanine and hydrolyze gelatin, casein, and Tween 80.

The third sub-group contained 3 marine strains, all of which swarmed on agar and formed lateral curly flagella. These organisms were very active metabolically, being able to hydrolyze starch, gelatin, casein, and Tween 80. Phenylalanine was deaminated, indol was produced, and the V-P test was positive for all members of this group.

Sub-group 4 was the largest of the groups formed in the fermentative cluster. This sub-group contained 9 strains (XU5 - 1C2) all of which were indol positive and could deaminate phenylalanine. Starch, gelatin, casein, and Tween 80 were all metabolized as were a wide variety of carbohydrates. Members of this group neither swarmed nor formed lateral flagella on solid media. This sub-group was related to sub-group 3 at approximately 93% S.

A numerical analysis of 88 strains of bacteria based on 89 physiological characters is presented in Figure 5. The strains used in this study were capable of using at least one of the compounds tested as a sole

carbon and energy source. The additional 29 characters determined for this group were based upon their growth response to these compounds (Table 3).

After analysis, the collection could be divided into 4 distinct groups which joined together at a value of 75% S.

Group 1 was composed entirely of fluorescent pseudomonads. These strains were very active metabolically, being able to grow with a variety of carbohydrates, amino acids, or organic acids as a sole carbon source (Table 3). All strains were rather loosely related to each other with *Ps. aeruginosa* and *Pseudomonas* sp. C being distinct from each other and the rest of the fluorescent strains.

The second group was relatively small, being composed of only 5 strains. Two of the strains, 17L3 and 4-9, grew in the T medium, while the rest were strictly marine. This group was metabolically active as judged by acid production from carbohydrates as compared to Group 3, but not as active as Group 1 strains. Members of this cluster, however, did not utilize carbohydrates as carbon sources. They grew with a restricted number of compounds tested as sole carbon and energy sources with alanine, aspartate, glutamate, and acetate being most commonly used.

Organisms in Group 3 were marine bacteria which were characterized by their generally weak metabolic activity. Two terrestrial strains, *C. peroolans* B and *Achromobacter* sp., were attached to the periphery of this largely marine group.

Two small sub-groups could be seen inside of Group 3. The first contained 7 strains which became numerically united at S = 92%. Of these

only 3A4 and 1R5 grew in the T medium. Members of this sub-group produced no acid from carbohydrates and did not use any of the sugars as a carbon source. All strains were gram-negative rods motile with a single polar flagellum and showed little extracellular enzyme activity with the exception of strain 11-6.

The second sub-group consisted of 5 strains, all of which were marine bacteria. These organisms were morphologically identical to the first sub-group but they could both produce acid from and use glucose as a carbon source.

Group 4 strains were gram-negative polar monotrichously flagellated rods which both fermented glucose anaerogenically and utilized it as an energy source. This cluster appeared rather homogeneous with no sharp demarcations between groups of strains. Two small sub-groups could be discerned, however.

The first sub-group contained 4 strains, XA1 - XQ5. Members of this cluster were curved rods which grew in a medium with acetate or pyruvate as the carbon source.

The second sub-group contained 12 strains, XC6 - XO5, which utilized glucose as an energy source and which were indol and phenylalanine deaminase positive. Several strains formed lateral flagella (XD1, XD6, XE1 and XP46) and swarmed on the surface of agar plates.

One strain labeled *X. feronioa* did not join any of the groups formed and it showed a higher similarity to two marine strains (XH5 and XH8) than it did to any of the terrestrial organisms.

From the results of the computer analysis presented in Figures 4 and 5 it appeared that certain of the groups formed corresponded to recognized genera. These groups could be distinguished from each other at 84.5% S with sub-groups which may correspond to individual species indicated at 94% S.

Of the 5 groups formed in Figure 4, at least 3 could be positively identified. Group 2 was made up entirely of *Caulobacter* strains. However, not all stalked bacteria fell into this group. This cluster contained only those strains which produced acid from a number of carbohydrates. Those strains which did not produce acid or did so from a limited number of sugars were found mixed into Group 4.

The fluorescent pseudomonads comprised Group 3 in its entirety. *Ps. aeruginosa* appeared to be only marginally related to other members of the fluorescent group. No other fluorescent pigment-producing strains were found in these diagrams, indicating a rather close relationship amongst these strains.

Group 5 contained only the fermentative bacteria which correspond to the genus *Vibrio* morphologically and physiologically.

Group 1 was not recovered as a distinct group in Figure 5. This was probably due to the fact that in Figure 4 Group 1 contained both marine "pseudomonads" and *Achromobacter* strains.

Identification of strains within Group 4 revealed the presence of a variety of genera. In this group were found *Caulobacter, Pseudomonas,*

Achromobacter and *Commomonas* species which were both marine and terrestrial. The relationship amongst members of this group were obscured by the generally weak biochemical activity of the strains included here. The almost complete lack of acid production from carbohydrates coupled with the use of the highest link method of group formation undoubtedly brought many of these diverse morphological types into one group. On the left-hand side of this group, however, were located most of the terrestrial bacteria, the *Caulobacter* and the *Achromobacter* strains. On the right-hand side of this group were all of the aerobic marine bacteria.

A comparison of the structure of Figures 4 and 5 was made to determine if the additional features included in Figure 5 influenced the construction of the groups formed. In both Figures 4 and 5, Group 4 appeared essentially unchanged except for minor internal rearrangement. Near the center of Figure 4 was another group (11L3-1R5) which also could be found in Figure 5. On the left of both Figures the same strains (2N1-XH8) are found to be closely related to none of the other organisms. A more detailed inspection of the figures revealed that essentially all of the groups formed in Figure 4 were found in Figure 5. From these observations it seemed that the additional features did not alter the results. These extra characters have, however, provided slightly lower and probably more accurate S values than in Figure 4.

Figure 4

Dendrogram STC01 Based on 60 Characters

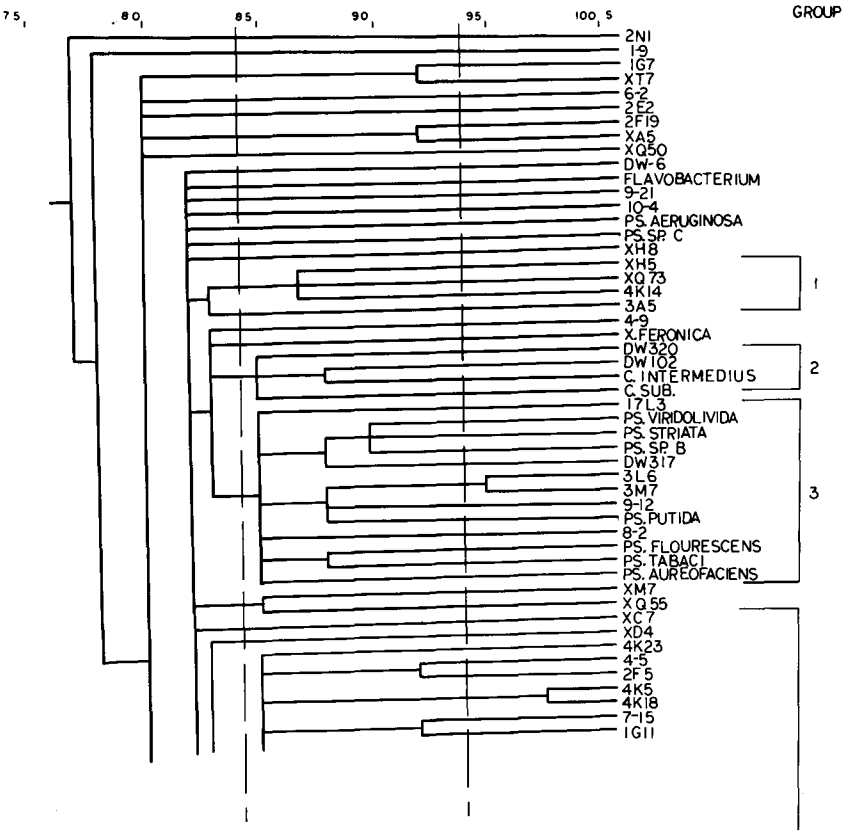


Figure 4 (continued)

Dendrogram ST001 Based on 60 Characters

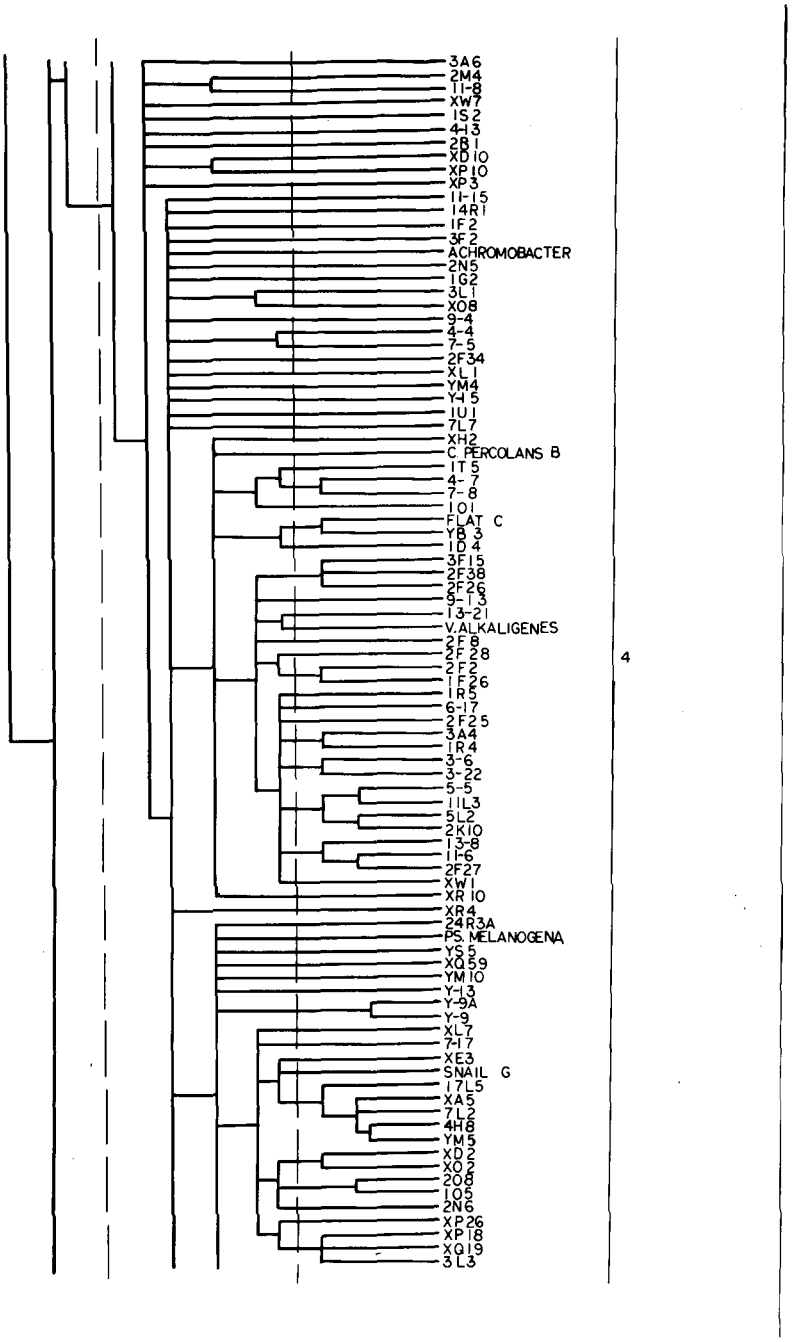
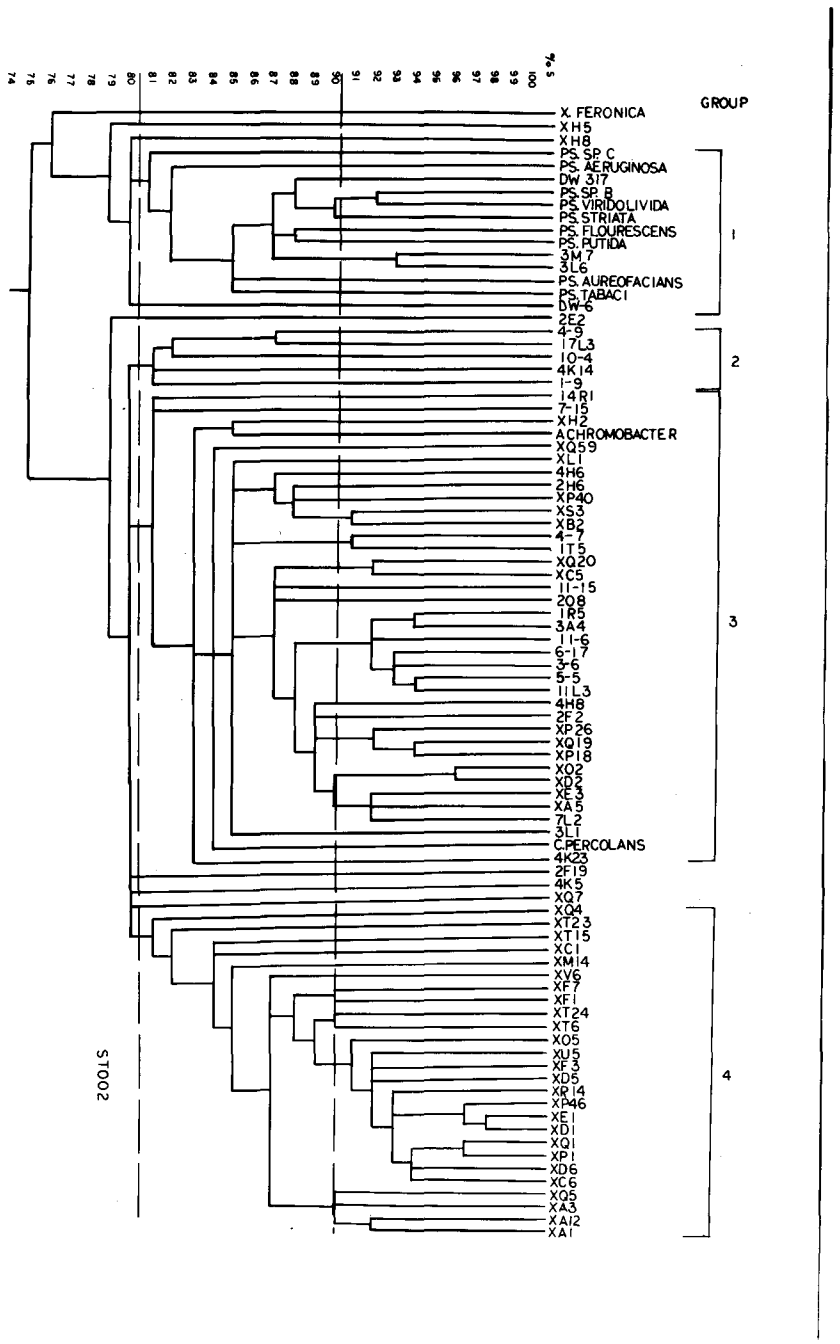


Figure 4 (continued)

Dendrogram ST001 Based on 60 Characters

Figure 5

Dendrogram ST002 Based on 89 Characters



DISCUSSION

A classification based on the numerical analysis of 196 cultures of marine and terrestrial bacteria showed that the marine bacteria were similar to comparable terrestrial bacteria (Figures 4 and 5). Similar results were also reported by Hansen *et al.* (10) and Colwell and Gochnauer (4). Each major physiological group which was recognized in Chapter 1 was recovered as a distinct cluster in this numerical analysis (Figures 4 and 5) although those groups based upon morphological characteristics could not be clearly recognized. The genera *Caulobacter*, *Pseudomonas*, *Achromobacter*, and *Flavobacterium*, which were physiologically but not morphologically similar, were clustered together at what was proposed as a generic level (84% S) of similarity (Figure 4). Members of the genus *Vibrio*, which were physiologically distinct from all other strains studied, were recovered as a separate homogeneous group as were the fluorescent pseudomonads (Figures 4 and 5). The fluorescent pseudomonads clustered separately from all other pseudomonads studied (Figures 4 and 5). This was probably due to their nutritional versatility and biochemical activity when compared to the other pseudomonads (Table 3). Marine and terrestrial *Pseudomonas*, *Caulobacter*, and *Achromobacter* strains all clustered together in Figures 4 and 5 indicating that these bacteria were similar to each other.

The scoring of the carbohydrate characters based not only as to whether or not acid was produced but also as to the method of acid production (oxidative vs. fermentative) led to the formation of groups whose members

had a similar method of carbohydrate metabolism. This was a distinct improvement over those numerical analyses which did not use this characteristic. Johnson (13) found both *Vibrio* and *Pseudomonas* species in the same groups since he assigned only one character to the type of acid produced from carbohydrates. Rhodes (31) found 2 of his *Aeromonas* isolates in the same group as *Ps. fluorescens* since he did not use the method of acid production from carbohydrates as a character in his analysis. Nigel de Silva and Holtz (26) found that their *Corynebacterium* strains could not be distinguished from the *Microbacterium* strains studied. They too did not include the method of acid production as a taxonomic character. Since the taxonomic significance of oxidative vs. fermentative metabolism has been generally recognized (12) this character should be recorded for each carbohydrate used when performing a numerical analysis of a heterogeneous collection of bacteria.

The heavy dependence upon carbohydrate reactions (32/64) in the analysis presented in Figure 4 has produced some mixing of strains from several genera. These strains were all physiologically similar, being distinguished from each other only by 1 or 2 morphological features such as the type of flagellation and ability to form stalks. This analysis was not unique in finding physiologically similar but morphologically diverse organisms in the same group. Quigley and Colwell (30) found both peritrichously and lophotrichously (1-3 flagella) flagellated bacteria in the same group. This group was considered sufficiently similar to another group, of which only

63% of the bacteria could be shown to possess flagella, to be considered as a single species. Results such as these probably result from not using enough morphological characters and relying instead almost entirely on physiological data for the construction of taxonomic groups. This is evident even in the analysis presented in this thesis since out of a total of 89 characters, only 8 were based on morphological observations. It should be recognized, then, that similarity values and classifications produced by numerical taxonomy studies depend upon the characteristics determined for each strain. The performance of a numerical analysis does not insure the production of a sound classification.

CHAPTER 5

DNA BASE COMPOSITION OF SELECTED MARINE AND TERRESTRIAL BACTERIA

Taxonomically related bacteria have similar DNA base compositions (11, 22). Recognized species of defined bacterial genera have similar DNA base compositions and this similarity has become an important taxonomic characteristic when used in the classification of bacteria. If marine bacteria are taxonomically related to comparable terrestrial bacteria they should have similar DNA base compositions.

Marine bacteria currently classified as pseudomonads have distinctly lower DNA base compositions than do terrestrial pseudomonads. Mandel (22) found terrestrial pseudomonads to have DNA base compositions of from 57-70% GC while Leifson and Mandel (18) reported that their marine pseudomonads had base compositions ranging from 40-60% GC. Not all marine bacteria have different DNA base compositions than their terrestrial counterparts. Members of the genus *Vibrio* (33) and *Caulobacter* (28) were homogeneous with respect to base compositions regardless of whether the cultures studied were marine or terrestrial.

In this chapter the DNA base composition of selected marine bacteria, representing the genera *Pseudomonas*, *Vibrio*, *Caulobacter*, and *Achromobacter*, will be determined. These results will be compared with published values for terrestrial bacteria and taxonomic recommendations based upon their DNA base compositions will be made.

MATERIALS AND METHODS

Organisms selected for DNA base composition analysis were grown in the M broth at room temperature on a rotary shaker for 24 hr. The cells were collected by centrifugation and the DNA extracted and purified by the method of Marmur (24). The purified DNA was dissolved in a 0.15 M saline plus 0.015 M trisodium citrate solution and stored at 4 C under several drops of chloroform until used.

The base composition of DNA, expressed as % GC, was determined by the thermal denaturation method (25). All DNA solutions were adjusted to a concentration of approximately 20 µg/ml and placed in 3 ml quartz cuvettes (type 6001J, W. H. Kessel Co., Chicago, Ill.). The solutions were then saturated with helium to prevent the formation of bubbles upon heating and the cuvettes stoppered with Teflon plugs. The A_{260} of the solutions was monitored in a Gilford model 2000 recording spectrophotometer equipped with a circulating water bath and a standard temperature probe while the temperature was raised 1 C every 10 min. Further work indicated that gassing of the cuvettes with helium could be eliminated if the temperature was raised slightly faster. A procedure was then adopted in which the temperature was raised 5 degrees every 10 min. Results obtained by this method were identical to those found by the slower heating procedure.

A sharp increase in absorbance occurred in the temperature range in which the DNA became denatured. When no further increase in absorbance occurred while the temperature was raised, the denaturation process was

taken as complete. The absorbance at each temperature was then divided by the absorbance at 25 C and the ratio, the relative absorbance, plotted versus the temperature. That temperature which corresponded to one-half the increase in the relative absorbance was considered the Tm. Using the Tm values found by this method all % GC values were calculated according to the formula $\% \text{ GC} = \frac{\text{Tm} - 69.3}{0.42}\%$

To determine the reliability and reproducibility of this method under the conditions used here two bacterial cultures of known DNA base composition were just studied. Cultures XD1 and 1R4 were used since their % GC values were previously reported (18). DNA from each culture was extracted on 3 separate occasions and its base composition determined. The % GC values calculated here were then compared with those previously reported and also with themselves.

RESULTS

In three independent trials the DNA of culture XD1 showed base compositions of 45.3, 45.6, and 46.0% GC while that from culture 1R4 had 60.1, 60.6, and 60.9% GC. This compared with values of 45.5 and 60.0% GC which were determined by the buoyant density method and reported by Leifson and Mandel (18). From these results it appeared that the method of analysis used here was reliable with the Tm values being reproducible within the 0.5 C range previously proposed (25).

When the % GC of culture XD1 was initially determined it was observed that the values were 4% higher than the published values. The instruments used were then examined for their accuracy. Attempts were made to calibrate the temperature recorded by the temperature probe of the Gilford model 2000 recording spectrophotometer with the actual temperature inside the cuvette chamber. After inserting a National Bureau of Standards thermometer into the cuvette chamber the temperature range from 80 - 95 C was monitored both by use of the thermometer and the machines temperature probe. By this method it was found that the temperature recorded by the probe was 1.7 C higher than the temperature in the chamber. When the Tm values initially calculated for XD1 were reduced by 1.7 C the resulting % GC values were in close agreement with those reported in the literature. All Tm values which were later determined were reduced by this amount.

Approximately 70 cultures of bacteria isolated from marine sources were analyzed with respect to their DNA base compositions by the method

of thermal denaturation. Typical melting curves of purified DNA are shown in Figures 1-3.

The first and largest group studied consisted of 35 polar monotrichously flagellated organisms which were classified as pseudomonads in Chapter 1. The DNA base compositions in this group extended over a large range varying from 41.2 - 60.6% GC (Table 9). Based upon DNA base compositions alone the pseudomonads could be divided into 2 smaller groups. The first group had a % GC of from 41.2 - 48.8 while the second had 51.2 - 60.6% GC. These groups based on DNA base composition could also be distinguished physiologically as will be shown in Chapter 6. Marine bacteria had base compositions extending to both ends of this range while terrestrial organisms only in the upper portion. For example, culture 17L3, which was a terrestrial pseudomonad, had a base composition of 53.6% GC. This value corresponded to those found for the marine group and was slightly lower than the 57-70% GC limits previously proposed for the genus *Pseudomonas* (22).

Three of the pseudomonads studied were motile with polar multitrichous flagella and of these only culture 2N5 required Na^+ and it had a base composition of 46.3% GC. The other 2 strains, 3A6 and 9-12, had base compositions of 54.8 and 60.5% GC respectively (Table 11). The value determined here for culture 2N5 was much lower than that found for most terrestrial pseudomonads (22).

The second group consisted of 22 strains which, in Chapter 1, on the basis of morphological and physiological criteria, had been identified as members of the genus *Vibrio*. The marine strains had DNA base ratios ranging

from 47 - 52.9% GC which were nearly equivalent to those reported in the literature for terrestrial *Vibrio* strains (11,33). In this analysis culture YB2, a terrestrial *Vibrio*, was found to have a GC content of 46.7%. Four strains of the oyster pathogen *V. mercenaria* were also analyzed with respect to their DNA base compositions (Table 11). A % GC of 42.7 was found for ATCC 19106 while ATCC 19105 had 46.2% and ATCC 19107 and 19108 had 47.4%.

One strain of *Oceanomonas alginolytica* ATCC 14035 was studied and found to have a base composition of 45.7% GC (Table 11) which was similar to those values found for the *Vibrio* cultures.

Three cultures identified as *Caulobacter* species exhibited base ratios of from 62.4 - 63.8% GC (Table 11). The one marine strain, 2F25, had a % GC of 62.4, which was similar to those values found for the terrestrial *Caulobacter* strains studied here.

Several cultures identified as *Achromobacter* and *Flavobacterium* strains were found to have base compositions characteristic of their genus. Strains 3A5 and XQ73, which were a marine and a terrestrial *Achromobacter* sp., respectively, had 54.8 and 60.0% GC in their DNA. Two *Flavobacterium* strains, 3F2 and 5F6, were very similar, their DNA's having 61.3 and 63.8% GC (Table 11).

Figure 1

Melting Curve of the DNA from Organism XD1

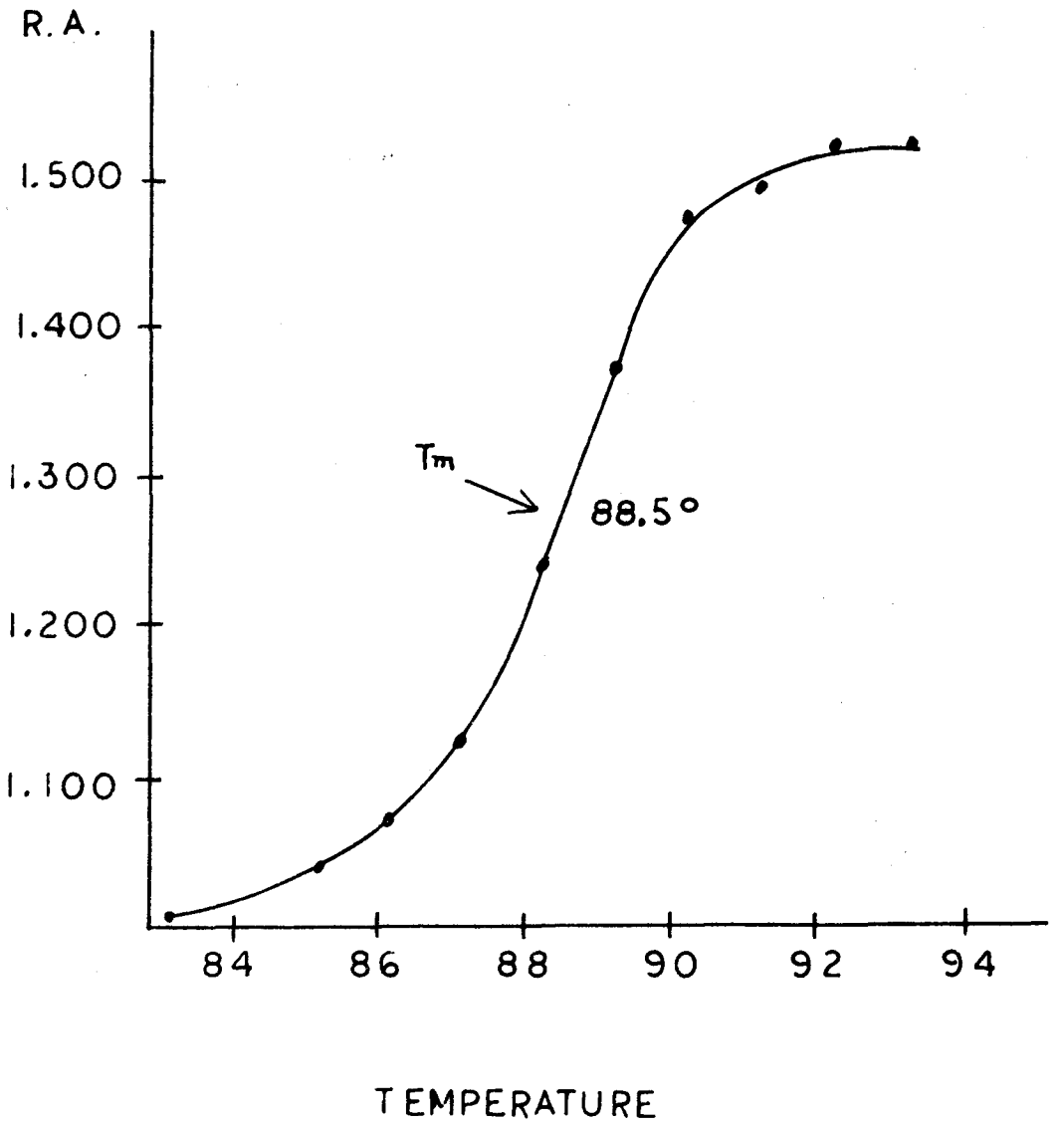


Figure 2

Melting Curve of the DNA from Organism XQ73

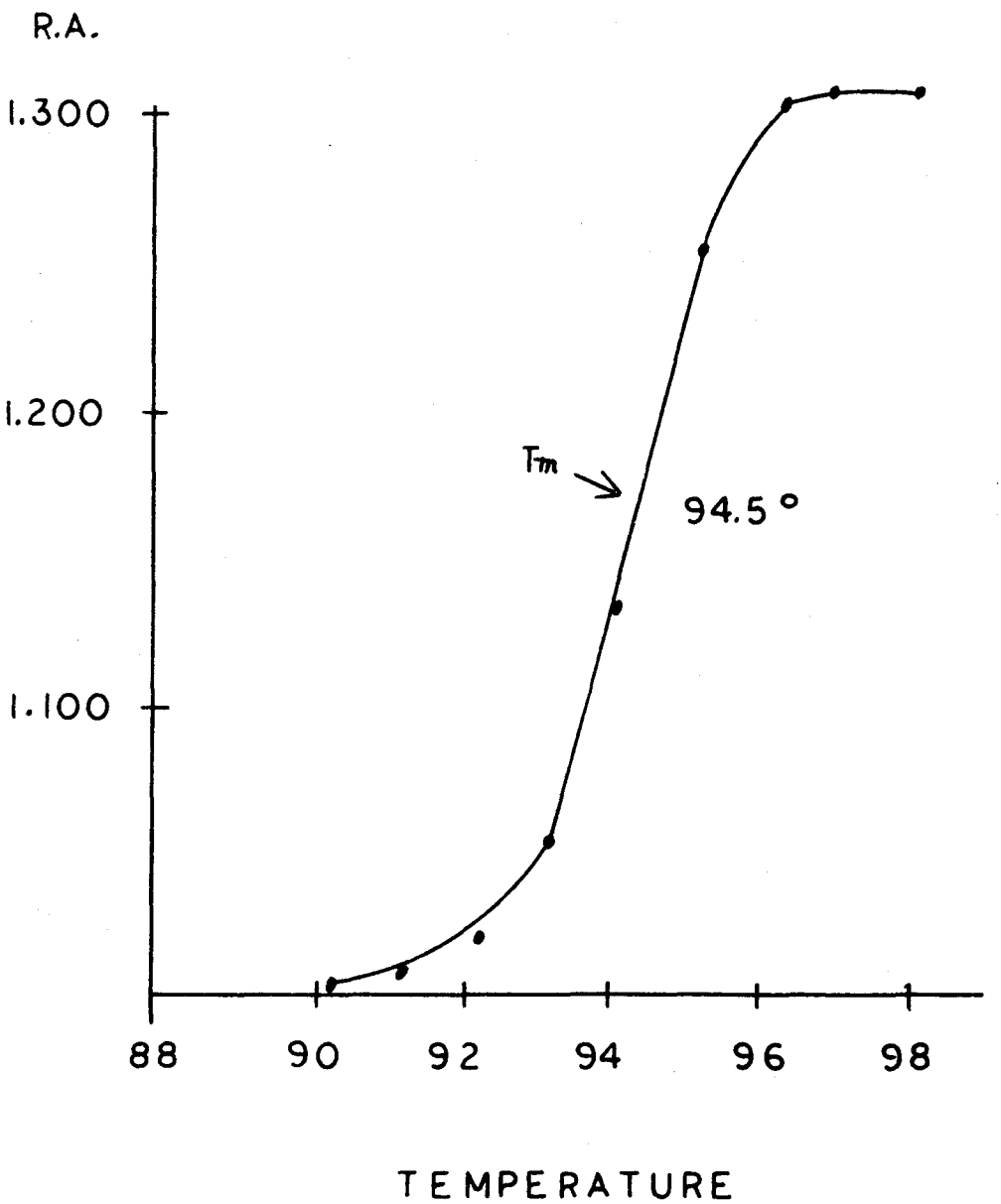


Figure 3

Melting Curve of the DNA from Organism 5L2

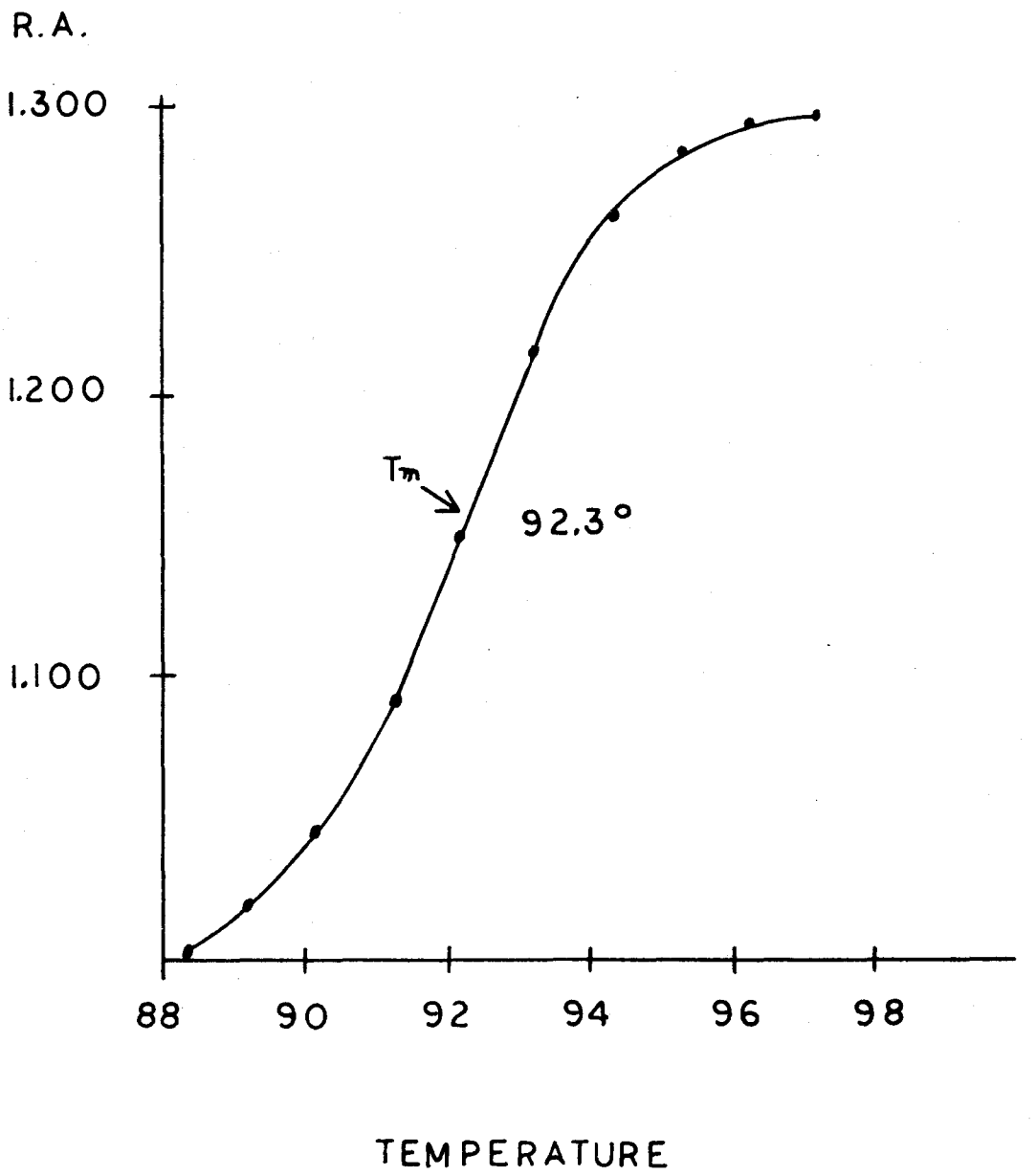


TABLE 9

% GC of Polar Monotrichously Flagellated Marine Bacteria Resembling *Pseudomonas*

Strain	A	Tm	% GC	Strain	A	Tm	% GC
XR4	O	86.6	41.2	SW1	O	89.6	48.3
XP3	O	86.8	41.6	XH2	O	89.7	48.5
XB2	O	87.1	42.3	2F27	-	89.8	48.8
XL3	O	87.2	42.5	11-15	O	90.8	51.2
4H8	O	87.3	42.9	7-15	O	91.5	52.9
Snail G	O	87.4	43.1	1R5	-	91.9	53.8
11-6	-	87.7	43.8	5L2	-	92.3	54.8
Snail D	O	88.2	45.0	5-5	-	92.9	56.2
13-8	-	88.3	45.2	2K10	-	93.1	56.5
XD4	O	88.4	45.5	3-22	O[B]	93.2	56.9
1G2	-	88.5	45.7	3-6	O[B]	94.0	58.8
XL1	O	88.6	45.9	6-17	O	94.0	58.8
105	O	88.6	46.0	11L3	-	94.2	59.3
1S2	O	88.9	46.7	10-15	O	94.7	60.5
2N6	O	89.2	47.3	1R4	-	94.7	60.6
XE3	O	89.6	48.3				

A - Type of acid produced from carbohydrates O - oxidative

B - Glycerol only - - No reaction

TABLE 10

% GC of Polar Monotrichously Flagellated Marine Bacteria Resembling *Vibrio*

Strain	A	Tm	% GC	Strain	A	Tm	% GC
XF1	F	86.5	41.0	XD1	F	88.5	45.6
XF7	F	86.5	41.0	XP1	F	88.7	46.2
Y-17	F	86.6	41.1	1C2	F	88.8	46.3
XF4	F	86.6	41.2	XT23	F	89.5	48.1
3-5	F	87.1	42.3	XM14	F	89.7	48.6
XA3	F	87.1	42.4	4H12	F	89.8	48.8
YC3	F	87.6	43.6	XF7	F	90.3	50.0
XA1	F	87.6	43.6	YC4	F	90.4	50.2
XA12	F	87.7	43.8	XT15	F	90.5	50.5
XF5	F	87.8	44.0	XT6	F	91.5	52.9
XC1	F	88.0	44.5				

A - Type of acid produced from carbohydrates F = Fermentative

TABLE 11

Moles % GC of Bacteria Isolated from Marine Sources

Strain	A	B	C	Tm	% GC	
2N5	M	L	O	88.8	46.3	-
3A6	T	L	O	92.3	54.8	*Pseudomonas* sp.
9-12	T	L	O	94.7	60.5	"
3A5	M	P	O	92.3	54.8	*Achromobacter* sp.
Xq73	T	P	O	94.5	60.0	"
3F2	T	P	O	95.1	61.3	*Flavobacterium* sp.
5F6	T	P	O	96.0	63.6	"
2F25	M	M	-	95.5	62.4	*Caulobacter* spp.
2F8	T	M	O	95.6	62.6	"
2F5	T	M	O	96.1	63.8	"
19105	M	M	F	88.7	46.2	*Vibrio mercenarius*
19106	M	M	F	87.2	42.6	ATCC #'s
19107	M	M	F	89.2	47.4	"
19108	M	M	F	89.2	47.4	"
19109	M	M	F	88.5	45.7	"
YB2	T	M	F	88.8	46.7	*Vibrio* sp.
14035	M	M	F	88.6	46.0	*Oceanomonas alginolytica*
14035	M	M	F	88.5	45.7	

A - Na^+ requirement M = marine T = terrestrial

B - Flagellation M - polar monotrichous L - lophotrichous
P - peritrichous

C - Method of carbohydrate metabolism O - oxidative F - fermentative
- no reaction

DISCUSSION

The results of the DNA base compositions of cultures belonging to the various genera studied in this thesis are in good agreement with those values found in the literature (5,11,18,22,33).

The polarly flagellated aerobic marine bacteria could be divided into 2 groups based upon their DNA base compositions (Table 9): 1) those with 41.2 - 48.8% GC; 2) those with 51.2 - 60.6% GC. Similar groups were found by Leifson and Mandel (18) who showed that marine bacteria had DNA base compositions of from 40-50% GC while semi-marine and terrestrial bacteria had base compositions varying from 58-65% GC. In this thesis no distinction, based upon quantitative Na^+ requirements, could be made between marine and semi-marine bacteria (Chapter 3).

Mandel (22) studied many species of the genus *Pseudomonas* and found these to have DNA base compositions ranging from 57-70% GC. The % GC values of these terrestrial bacteria are considerably higher than those found in this thesis for either group of marine bacteria although there is some overlapping of values with the high % GC marine bacteria.

Sueoka (41) has proposed that if 2 bacteria differ by 10% in their DNA base composition they can have very few regions of DNA in common. This is further substantiated by inspection of the limits of % GC which have been proposed for a number of bacterial genera (11). The genus *Corynebacterium* was reported to have DNA base compositions of from 46-58% GC; *Xanthomonas*

from 62-68% GC; *Pseudomonas* from 57-70% GC; *Caulobacter* from 62-67% GC; and *Vibrio* from 43-48% GC. Since genera are composed of similar organisms these values all approximate the 10% GC limits proposed by Sueoka (41).

If the marine "pseudomonads" were to be incorporated into the genus *Pseudomonas* the range of base compositions for this genus would be from 41-70% GC. A range of this magnitude cannot be accepted in view of those posted by other defined genera. It is recommended, therefore, that the marine "pseudomonads" be removed from the genus *Pseudomonas* on the basis of their DNA base compositions and requirement for Na^+.

A genus created for the polarly flagellated aerobic marine bacteria would contain bacteria which have DNA base compositions varying from 41-61% GC. This range is still wider than that found in most other genera. Inspection of the DNA base compositions of those terrestrial pseudomonads (17L3, 3A6) reported in this thesis (Tables 9 and 11) shows these cultures to have base compositions of 53.6 and 54.8 respectively. These values are beneath the lower limits (57% GC) previously proposed (22). There is no question that these bacteria belong in the genus *Pseudomonas* and this, in effect, widens the % GC range of the genus *Pseudomonas* to 53-70% GC which is almost as wide as that found for comparable marine bacteria (41-61% GC).

From the results presented in Tables 10 and 11 it appears that marine members of the genera *Achromobacter*, *Caulobacter*, and *Vibrio* have DNA base compositions nearly equivalent to those reported here and in the literature (11,22,28,34) for terrestrial members of these genera.

The DNA base composition of *Oceanomonas alginolytica* was determined and found to be 45.7% GC (Table 11). This organism was a gram-negative rod, motile with a single polar flagellum, and fermented glucose anaerogenically. Morphologically and physiologically this culture was very similar to the other marine *Vibrios* studied here and its similar DNA base composition (45.7 versus 41-53% GC) indicates that this organism could quite easily be incorporated into the genus *Vibrio*.

CHAPTER 6

CORRELATION OF THE DNA BASE COMPOSITION OF MARINE BACTERIA WITH SELECTED PHYSIOLOGICAL CHARACTERISTICS AND GROUPS FORMED BY NUMERICAL ANALYSIS

Leifson (17) reported that his marine isolates could be divided into 3 groups based on their quantitative Na^+ requirements: 1) marine strains which required from 0.05 - 0.2 M Na^+; 2) semi-marine strains which required from 0.001 - 0.05 M Na^+; 3) terrestrial strains which required less than 0.001 M Na^+. In a later study, Leifson and Mandel (18) reported that the majority of polarly flagellated marine bacteria had DNA base compositions ranging from 40-50% GC while the semi-marine and terrestrial strains had base compositions varying from 52-65% GC.

In Tables 12 and 13 are listed the quantitative Na^+ requirements and DNA base compositions of a number of polarly flagellated bacteria used in this study. All cultures required Na^+. On the basis of their quantitative Na^+ requirements these cultures were almost all semi-marine with only 4 cultures (XP3, 1G2, 5L2, 2K10) definitely being marine. From these results it appeared that there was no correlation between the quantitative Na^+ requirement and the % GC of the bacteria studied here although the cultures could be separated into 2 groups based upon their DNA base composition. These groups, as indicated in Chapter 5, had DNA base compositions of from 41.6 - 48.8 and 52.9 - 59.3. These were roughly the same values observed by Leifson and Mandel (18) to correspond to marine and semi-marine bacteria respectively.

Leifson (17) studied the quantitative Na^+ requirement which his marine isolates needed for prompt growth. In the present study the quantitative Na^+ requirement was determined after 7 days incubation. In Chapter 2 it was shown that extended periods of incubation led to a reduction in the minimum Na^+ requirement. The quantitative Na^+ requirements presented in this thesis were lower than those reported by Leifson (17) and with these values it was difficult to distinguish marine from semi-marine bacteria. It appears, then, that some of the bacteria which were listed as semi-marine in this thesis may really be marine. This indicates that when determining quantitative Na^+ requirements, the technique must be standardized using a short incubation period (48 hr) in order to differentiate marine from semi-marine bacteria.

Even though those groups formed on the basis of DNA base compositions could not be confirmed by quantitative Na^+ requirements other physiological characteristics were found useful in describing them (Tables 12-15). These characters were acid production from carbohydrates, gelatin liquefaction, hydrolysis of casein and Tween 80, and reduction of nitrate. The aerobic cultures, Table 14, which had DNA base ratios from 41.2 - 48.8% GC, were able to produce acid from a variety of carbohydrates and could hydrolyze gelatin, casein, and Tween 80, but could not reduce nitrate. Cultures 11-6, 13-8 and 162 did not produce acid from carbohydrates but they could be recognized by their extracellular enzyme activity. Those cultures with DNA base compositions of from 51.2 - 60.6% GC could not hydrolyze gelatin,

casein, or Tween 80, but they reduced nitrate. Most members in this group did not produce acid or did so from only a few carbohydrates. No such groups could be distinguished in the fermentative bacteria.

DNA base composition of groups formed in the numerical analysis. Correlation of the DNA base compositions of the strains shown in Table 9 with the groups formed in the computer analysis (Figure 4) revealed that most groups which were found to be distinct at what was considered the generic level (84% S) were homogeneous with respect to their base compositions. This lends support to the concept that these groups were indeed separate genera. All strains which were related at 94% S or above had nearly identical base ratios confirming the suggestion that organisms related at this value were members of the same species.

Group 2, *Caulobacter* spp., had DNA base ratios ranging from 62-67% GC as did the fluorescent pseudomonad strains. Strains of Group 5, *Vibrio* spp., exhibited base ratios of from 41-52.9% GC (Table 10). These values were all within the acceptable limits for the respective genera (11,22,28).

Members of Group 4 were slightly more heterogeneous with values ranging from 41.2 - 68% GC. Several of the higher values were due to inclusion of some *Caulobacter* and *Achromobacter* strains in this group. The polarly flagellated marine types ranged from 41.2 - 60.6% GC. This range was found to be wider than that observed in most other genera.

Organisms which were related at 94% S or above had compatible base compositions. This is illustrated clearly in Figure 6 in which the actual

% GC values for individual strains are shown. This group was taken from Figure 4, Group 4.

This group contained 4 sub-groups which were all related at 93% S. Strains 3A4 and 1R5 merged together at 95% S and their base compositions were 61.9 and 60.6 moles % GC respectively. Sub-group 2 strains (3-6, 3-22) had 58.9 and 56.8% GC in their DNA's while the range for sub-group 3 (5-5-2K10) was from 54.8 - 59.3% GC. Members of sub-group 4 had distinctly lower base ratios than any of the other sub-groups. Its members had from 43.7 - 48.8% GC. Four strains which did not join any of these sub-groups exhibited base compositions varying from 48.3 - 62.3% GC. The values presented for this group indicate that a similarity of at least 94% must be shown between 2 strains before they can be considered as one species.

Figure 6

Correlation of DNA Base Composition with Groups Formed in the Numerical Analysis.

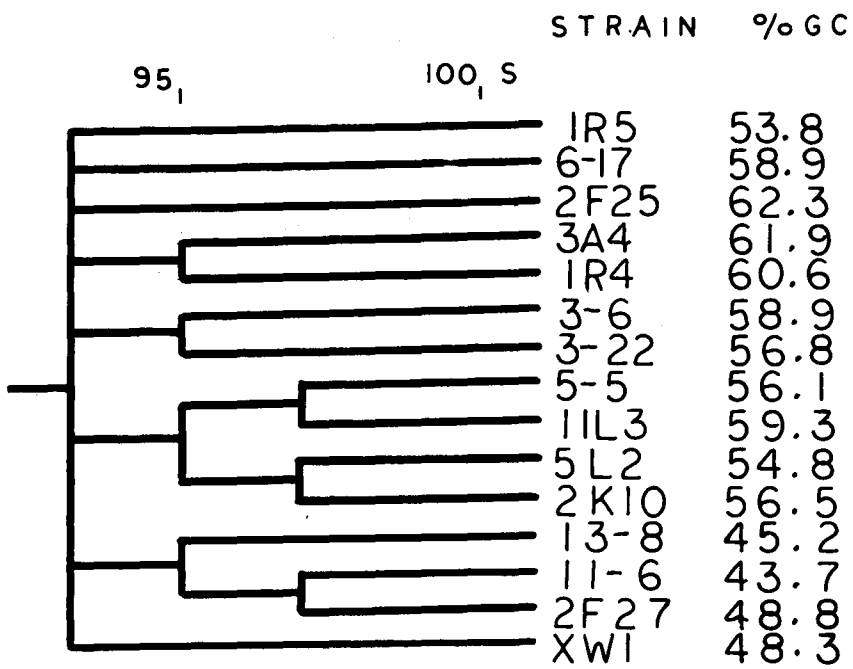


TABLE 12

Correlation of % GC of Marine Bacteria with the Minimum and Maximum NaCl Concentrations Permitting Growth

Strain	A	% GC	Minimum Na^+ (m/l)	Maximum $NaCl^+$ (m/l)
XP3	O	41.6	.176	0.86
4H8	O	42.9	.022	2.06
11-6	-	43.8	.022	2.06
13-8	-	45.2	.003	1.38
1G2	-	45.7	.086	0.69
XD4	O	45.5	.043	2.06
XL1	O	45.9	.022	2.06
XW1	O	48.3	.011	2.06
XH2	O	48.5	.022	1.03
2F27	-	48.8	.011	2.06
7-15	O	52.9	.003	1.72
5L2	-	54.8	.086	1.38
5-5	-	56.2	.006	2.06
2K10	-	56.5	.176	1.38
3-22	O[B]	56.9	.022	2.06
3-6	O[B]	58.8	.022	2.06
11L3	-	59.3	.022	2.06

A - Type of acid produced from carbohydrates
O - oxidative - No reaction

B - Glycerol only

TABLE 13

Correlation of % GC of Fermentative Marine Bacteria with the Minimum and Maximum NaCl Concentrations Permitting Growth

Strain	A	% GC	Minimum Na^+ (m/l)	Maximum Na^+ (m/l)
XF4	F	41.2	.172	0.67
3-5	F	42.3	.011	2.06
XA1	F	43.6	.086	1.03
XC1	F	44.5	.086	0.86
XD1	F	45.6	.003	1.72
XP1	F	46.2	.086	1.03
4H12	F	48.8	.086	1.03
YC4	F	50.2	.011	1.03
XT15	F	50.5	.172	1.03
XT6	F	52.9	.086	1.03

A - Type of acid produced from carbohydrates.

F = Fermentative

TABLE 14

Correlation of % GC of Marine Bacteria with Selected Physiological Characteristics

Strain	% GC	A	Gelatin liquefaction	Casein hydrolysis	Tween 80 hydrolysis	Nitrate reduction
XR4	41.2	O	+	+	+	-
XP3	41.6	O	+	+	+	-
XB2	42.3	O	+	+	+	-
XL3	42.5	O	+	+	+	-
4H8	42.9	O	+	+	+	-
Snail G	43.1	O	+	+	+	-
11-6	43.8	-	+	+	+	-
Snail D	45.0	O	+	+	+	-
13-8	45.2	-	+	+	+	-
XP4	45.5	O	+	+	+	-
1G2	45.7	-	+	+	+	+
XL1	45.9	O	+	+	+	-
105	46.0	O	+	+	+	+
102	46.7	O	+	+	+	-
2N6	47.3	O	+	+	+	-
XE3	48.3	O	+	+	+	-
XW1	48.3	O	+	+	+	-
XH2	48.5	O	+	+	+	+
2F27	48.8	-	+	+	+	-
11-15	51.2	O	-	-	+	-
7-15	52.9	O	-	-	+	-
1R5	53.8	-	-	-	-	-
5L2	54.8	-	-	-	-	+
5-5	56.2	-	-	-	-	+
2K10	56.5	-	-	-	-	+
3-22	56.9	O[B]	+	-	-	+
3-6	58.8	O[B]	-	-	-	+
6-17	58.8	O	-	-	+	+
11L3	59.3	-	-	-	-	+
10-15	60.5	O	-	-	-	+
1R4	60.6	-	-	-	+	-

A - Type of acid produced from carbohydrates
O - oxidative - No reaction

B - Glycerol only

TABLE 15

Correlation of % GC of Fermentative Marine Bacteria with Selected Physiological Characteristics

Strain	% GC	O-F Glucose	Gelatin liquefaction	Casein hydrolysis	Tween 80 hydrolysis	Nitrate reduction
XF1	41.0	F	-	-	-	-
XF7	41.0	F	-	-	+	+
Y-17	41.1	F	-	-	+	+
XF4	41.2	F	-	-	-	+
3-5	42.3	F	+	+	+	-
XA3	42.4	F	+	-	-	+
YC3	43.6	F	-	-	-	+
XA1	43.6	F	-	-	-	+
XA12	43.8	F	-	-	-	+
XF5	44.0	F	-	-	-	+
XC1	44.5	F	+	+	+	+
XD1	45.6	F	+	+	+	+
XP1	46.2	F	+	+	+	+
1C2	46.3	F	+	+	+	+
XT23	48.1	F	-	-	-	+
XN14	48.6	F	-	-	-	+
4H12	48.8	F	+	+	+	+
XF7	50.0	F	+	-	-	-
YC4	50.2	F	+	+	+	+
XT15	50.5	F	-	-	-	+
XT6	52.9	F	-	-	-	+

CHAPTER 7

GENERAL DISCUSSION

The results of this investigation clearly show the relationship of marine to terrestrial bacteria. Based on the morphological and physiological data presented in Chapter 1, marine bacteria were found to be related to comparable terrestrial bacteria at the genus level with the only readily detectable physiological difference between them being a requirement for Na^+ by the marine bacteria.

The minimum Na^+ requirement of marine bacteria was found to be qualitatively stable yet quantitatively variable. No non-Na^+ requiring mutants of marine bacteria could be isolated after use of any of the procedures reported to do so (Chapter 2). Prolonged storage of cultures did, however, reduce the minimum Na^+ requirement of those marine bacteria which originally required 0.043 M Na^+ or less. This reduction was large enough to allow growth in a T medium making it most difficult to distinguish the marine from the terrestrial bacteria which did not require Na^+. This undoubtedly was the same observation reported by ZoBell and Upham (45) only they ascribed the ability of their marine bacteria to grow in a complex terrestrial medium containing low levels of contaminating NaCl to a loss of the Na^+ requirement.

The marine bacteria studied here could not, on the basis of their quantitative Na^+ requirements, be divided into the marine and semi-marine groups of Leifson (17). This was probably due to a difference in techniques

according to which this requirement was assayed (Chapter 2). Most of the values reported in Chapter 2 corresponded to those of the semi-marine group of Leifson with only a few strains belonging to the marine group (Table 4). The polarly flagellated fermentative bacteria had higher quantitative Na^+ requirements than did the aerobic bacteria. This is an interesting observation since the fermentative bacteria are closely associated with marine plant and animal life while the aerobic bacteria are the most common type found in seawater (17).

In the numerical analysis presented in Chapter 3 most of the major physiological groups recognized in Chapter 1 were recovered as distinct clusters. This was achieved through the scoring of carbohydrate characters not only as to whether acid was produced but also as to whether the acid was produced oxidatively or fermentatively. This was an improvement over other numerical analyses in that only bacteria with a similar method of acid production from carbohydrates were included in one group. Although the strains of each group were physiologically homogeneous they were not always morphologically similar. Thus in Figure 4, *Caulobacter* and *Achromobacter* cultures were found mixed with terrestrial pseudomonads. This implies that too much emphasis was placed on such physiological characters as carbohydrate metabolism, in which most of the genera were very similar, and not enough emphasis on morphological characteristics. More characters dealing with morphological aspects, such as flagellation, should have been included in this analysis to help differentiate the genera studied from each other.

From the results of the numerical analysis presented in this study it is obvious that the structure of the various groups formed depends upon the characters used in calculating the similarity values. It is important, then, that the characters used provide an evenly balanced morphological and physiological description since simply performing a numerical analysis does not ensure the production of a good classification.

The results of the DNA base composition analyses of marine members of the genera *Vibrio*, *Caulobacter*, and *Achromobacter* are in good agreement with previously reported values (11,28,33). Since the marine members of these genera are also morphologically and physiologically similar to terrestrial species of these genera there appeared to be no reason for not including both terrestrial and marine strains in each of these genera. The requirement for Na^+ by the marine bacteria should prove useful as a guide in speciation.

The polarly flagellated aerobic marine bacteria were morphologically and physiologically similar to the terrestrial pseudomonads yet they had much lower DNA base compositions (Chapter 4). Since taxonomically related bacteria have similar DNA base compositions it was recommended that these marine bacteria be removed from the genus *Pseudomonas*. The requirement for Na^+ by these bacteria can be used as a characteristic used to separate these marine bacteria from the terrestrial pseudomonads at the genus level.

It was concluded then that the requirement for Na^+ by marine bacteria was a stable characteristic useful in the classification of these organisms.

In the genera *Vibrio*, *Caulobacter*, and *Achromobacter*, it was shown to be helpful in differentiating between species, since it was found that both marine and terrestrial representatives of these genera were very similar. The polarly flagellated aerobic marine bacteria were considered distinct from the terrestrial pseudomonads based on their DNA base compositions. The requirement for Na^+ was found to be a useful taxonomic character in distinguishing them from members of the genus *Pseudomonas*.

SUMMARY

A total of 196 cultures of marine and terrestrial bacteria representing the genera *Pseudomonas*, *Caulobacter*, *Vibrio*, *Achromobacter*, and *Flavobacterium* were studied with respect to their morphology, physiology, and DNA base compositions in an attempt to determine the relationship between marine and terrestrial bacteria.

On the basis of morphology and physiology, marine bacteria were shown to be generically related to comparable terrestrial bacteria.

All marine bacteria were shown to require Na^+ for growth while no terrestrial bacteria required this ion. No distinctions could be made amongst marine bacteria on the basis of their quantitative Na^+ requirement although the polarly flagellated fermentative bacteria required higher Na^+ levels for growth than did the aerobic bacteria (0.086 vs. 0.022). The Na^+ requirement of marine bacteria was found to be qualitatively stable although it was quantitatively variable, being reduced either by growth in a T medium or by periods of storage of up to 6 months.

The relationship between marine and terrestrial bacteria was studied through the use of numerical taxonomy. In this analysis every carbohydrate character was scored not only if acid was produced but also as to the type of acid produced (oxidative vs. fermentative). This was done to insure that all members of each group that was formed had a similar method of carbohydrate metabolism. Five groups were formed, of which 3 could be positively identified. Group 2 corresponded to the genus *Caulobacter* while

Group 3 was composed entirely of fluorescent pseudomonads. Group 5 contained all of the fermentative bacteria identified as *Vibrio* species. Group 4 was mainly composed of polarly flagellated aerobic marine bacteria but also contained some terrestrial *Caulobacter*, *Achromobacter*, and *Pseudomonas* cultures. From these results it was concluded that the polarly flagellated marine bacteria were distinct from the terrestrial fluorescent pseudomonads but similar to the other pseudomonads studied. Marine bacteria identified as *Caulobacter* and *Achromobacter* species were also found to be similar to terrestrial members of these genera. All groups were distinct at the 84% S level and this was taken as a generic level of similarity. A species level of similarity was indicated at 93% S.

Polarly flagellated aerobic marine bacteria could be divided into 2 groups based on their DNA base compositions: 1) 41.6 - 48.8% GC; 2) 52-61% GC. Since these bacteria could be differentiated from the terrestrial pseudomonads by their requirement for Na^+ and DNA base compositions, it was recommended that they be removed from the genus *Pseudomonas*.

Marine members of the genera *Vibrio*, *Caulobacter*, and *Achromobacter*, had DNA base compositions very similar to terrestrial members of these genera and no taxonomic revisions were suggested. *Oceanomonas alginolytica* was shown to be morphologically and physiologically similar to members of the genus *Vibrio*. Its similar DNA base composition suggested that this culture could be reclassified as a member of the genus *Vibrio*.

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APPROVAL SHEET

The dissertation submitted by Ronald T. Stanke has been read and approved by members of the Department of Microbiology.

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that the dissertation is now given final approval with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 27, 1969
Date

Elmer Pearson
Signature of Advisor