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Taxonomy of Marine Bacteria: the Genus *Beneckea*

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One-hundred-and-forty-five isolates of marine origin were submitted to an extensive physiological, nutritional, and morphological characterization. All strains were gram-negative, facultatively anaerobic, straight or curved rods which were motile by means of flagella. Glucose was fermented with the production of acid but no gas. Sodium but no organic growth factors were required. None of the strains were able to denitrify or fix molecular nitrogen. The results of nutritional and physiological tests were submitted to a numerical analysis. On the basis of phenotypic similarity, nine groups were established. These groups could be distinguished from one another by multiple, unrelated, phenotypic traits. Six groups which had deoxyribonucleic acid (DNA) containing 45 to 48 moles per cent guanine plus cytosine (GC) were assigned to a redefined genus *Beneckea*. All of the strains in this genus, when grown in liquid medium, had a single, polar flagellum. When grown on a solid medium, many strains had peritrichous flagella. Two groups were similar to previously described species and were designated *B. alginolytica* and *B. natriegens*. The remaining four groups were designated *B. campbellii*, *B. neptuna*, *B. nereida*, and *B. pelagia*. An additional group of phenotypically similar strains having the properties of the genus *Beneckea* was not included in the numerical analysis. These strains were readily separable from species of this genus and were designated *B. parahaemolytica*. Of the remaining groups, one was identified as *Photobacterium fischeri*. The other group (B-2) which had about 41 moles % GC content in its DNA could not be placed into existing genera.

The main chemoorganotrophic bacterial flora of the sea, capable of growth on laboratory media, is composed of gram-negative, straight and curved rods, which are motile by means of flagella (27, 31, 33, 34, 43). A large body of literature (reviewed by MacLeod; 33, 34) indicates that the growth of these organisms is dependent on the presence of sodium ion. This requirement is specific and is independent of an osmotic function since growth does not occur in a medium in which sodium is replaced by equimolar amounts of other monovalent cations (33, 34). In the case of two species of marine bacteria, it has been shown that their permease systems require sodium for the uptake of exogenous substrates (17). The sodium ion requirement of many or all bacteria of marine origin has led to the suggestion that this property may serve to distinguish marine bacteria from soil and fresh water strains (33, 34). As yet, no comprehensive survey of the sodium ion requirement of gram-negative bacteria from nonmarine sources has been performed. In addition to a specific requirement for sodium, gram-negative marine bacteria require high concentrations of cations for the maintenance of the integrity of the cell wall (8, 15, 33, 34).

A large proportion of the gram-negative bacteria of marine origin ferment glucose with the production of acid but no gas (10, 27, 31, 43) Most of these organisms have been described as polarly flagellated. A few have been reported to have polar flagella when grown in liquid medium and peritrichous flagella when grown on solid medium (30, 31). The taxonomy of these organisms is still poorly understood. Many have been placed in *Vibrio* (3, 27, 47, 58), *Aeromonas* (36), *Pseudomonas* (10, 42), and *Beneckea* (7, 9). Determinations of the guanine plus cytosine (GC) content of the deoxyribonucleic acid (DNA) of these strains have shown that the moles % GC ranges from 39-48 (5, 11, 13, 28, 32). Some of these organisms are pathogenic for marine fish and shellfish (51). One species, *V. parahaemolyticus*, is pathogenic for man, causing an acute gastroenteritis (47). A common prop-

erty of these organisms is the ability to hydrolyze chitin (3). During a study of the chitin-decomposing, bacterial flora present in marine muds, Campbell and Williams (10) isolated gram-negative rods which had either polar or peritrichous flagella and were able to ferment glucose with the production of acid but no gas. The polarly flagellated strains were placed into the genus *Pseudomonas*, the peritrichously flagellated strains into the genus *Achromobacter*. The strains were screened for their ability to utilize a relatively large number of carbon compounds as sole sources of carbon and energy. New species were described and separated on the basis of a few nutritional properties. The new species which had been assigned to the genus *Achromobacter* were subsequently placed into a newly created genus, *Beneckea* (7).

This study describes the properties of a collection of facultatively anaerobic, gram-negative, marine bacteria which are similar to the marine strains which have been assigned to *Vibrio*, *Aeromonas*, *Pseudomonas*, and *Beneckea*. The results of our taxonomic analysis indicate that most of these organisms should be placed into the redefined genus *Beneckea*.

MATERIALS AND METHODS

The majority of the methods used in this study are those of Stanier et al. (57). Only significant modifications as well as additional methods will be described. Unless otherwise stated, all cultures were incubated at 25 C.

Bacteriological media. The artificial sea water (ASW) used in this study consisted of 0.4 M NaCl, 0.1 M $MgSO_4 \cdot 7H_2O$, 0.02 M KCl, and 0.02 M $CaCl_2 \cdot 2H_2O$ (34). The basal medium (BM) contained 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (*p*H 7.5), 190 mM NH_4Cl, 0.33 mM $K_2HPO_4 \cdot 3H_2O$, 0.1 mM $FeSO_4 \cdot 7H_2O$, and half-strength ASW (34). Basal medium agar (BMA) was prepared by separately sterilizing and then mixing equal volumes of double-strength BM and 20 g of Oxoid Ionagar per liter. Yeast extract broth (YEB) was made by supplementing BM with 5 g of Difco Yeast Extract per liter. Yeast extract agar (YEA) was prepared by solidifying YEB with 20 g of Difco Agar per liter. In addition to the above complex media, Difco Marine Agar (MA) was used.

Tests for sodium and growth factor requirements. All of the strains were tested for their ability to grow on a minimal medium in the presence and absence of sodium. Two types of media were used, BM and a similar medium differing only in the replacement of sodium by an equimolar amount of potassium. Cells from a fresh MA slant were inoculated into 50-ml Erlenmeyer flasks containing 10 ml of the media and incubated with aeration for 48 hr. Turbidity was measured in a Klett-Summerson colorimeter using a green filter. The tests were performed in a medium containing 0.2% (v/v) glycerol as well as in a medium containing 0.2% (w/v) potassium lactate as the sole source of carbon and energy.

Fermentation of carbohydrates. The ability of strains to ferment glucose was determined by the use of two different fermentation media. The first (F-1) differed from YEB by containing 100 mM Tris-hydrochloride (*p*H 7.5), 1 g of Oxoid Ionagar per liter, and 10 g of filter-sterilized glucose per liter. Portions (10 ml) of the medium were dispensed into test tubes and inoculated by means of a stab. A control medium lacking glucose was also inoculated. About 5 ml of melted 10% (w/v) Oxoid Ionagar, at 40 C, was added to make an agar plug. The cultures were examined for turbidity and gas production for a period of 6 days. The second fermentation medium (F-2) differed from F-1 by containing 1 g of sodium thioglycolate per liter. The agar plug was omitted. F-2 medium containing glucose as well as a control medium without glucose was inoculated and examined for growth on the surface and throughout the medium at 24 and 48 hr. At the latter time, the *p*H of the culture was determined.

The ability of strains to produce acid from D-ribose, D-gluconate, D-mannitol, and glycerol under anaerobic conditions was tested on fermentation media (F-3) which differed from BM by containing 25 mM Tris-hydrochloride (*p*H 7.5), 0.5 g of Difco Yeast Extract per liter, 1 g of Oxoid Ionagar per liter, 2 ml of a 1.6% (w/v) alcoholic solution of Brom Cresol Purple per liter, and 10 g of the filter-sterilized carbohydrate per liter. Subsequent procedures were identical to those for F-1. The cultures were examined for color change and gas production for a period of 6 days.

Production of 2,3-butyleneglycol. Strains were grown in test tubes containing 10 ml of YEB with 1% (w/v) D-glucose. After a 72-hr incubation, the Voges-Proskauer (VP) test was performed (52). Quantitative assays for acetoin or diacetyl, or both, as well as for 2,3-butyleneglycol (39) were performed on all cultures which gave a positive VP test.

Nitrogen metabolism. Strains were tested for their ability to denitrify (57). The medium used consisted of YEB supplemented with 0.2% (v/v) glycerol, 3 g of $NaNO_3$ per liter, and 1 g of Oxoid Ionagar per liter. Each strain was first grown in the denitrification medium under semianaerobic conditions. After 48 hr of incubation, the cultures were tested for nitrite by the use of the starch-iodine spot test method (52). A loopful of the semianaerobic culture was then inoculated into a test tube containing homologous medium and was sealed with an agar plug. Observations were continued for 8 days. Cultures producing gas were scored positive for denitrification. Nitrogen fixation was tested by inoculating cells from a fresh MA slant into BM from which NH_4Cl was omitted and which contained 0.2% (v/v) glycerol. Observations were continued for 6 days.

Extracellular enzymes. Strains were scored positive for each of the extracellular enzymes only when the zone of reaction extended beyond the limits of growth. The production of gelatinase was determined by inoculating onto YEA containing 50 g of gelatin per liter. After 48 hr of incubation, the plates were flooded with acidic mercuric chloride (52). Amylase production was determined on YEA containing 2 g of starch per liter. After 48 hr of incubation, the plates were flooded with Lugol's Iodine solution (53). Lipase production was

determined on YEA supplemented with 0.01% (v/v) polyethylene sorbitan monooleate (50). The presence of alginase was tested on a medium consisting of YEA overlaid with YEA containing 20 g of sodium alginate per liter (52). The presence of chitinase was tested on a medium consisting of YEA overlaid with YEA containing 10 g of colloidal chitin per liter (6). Observations on the production of lipase, alginase, and chitinase were continued for 6 days.

Hemolysis. The ability to hemolyze human red blood cells (RBC) was tested on a medium containing Trypticase Soy Agar (BBL), 2.5% NaCl, and 5% defibrinated blood (TSB) and on Wagatsuma's medium (WM; reference 37). Plates were incubated at 37 C and examined at 24 and 48 hr.

Utilization of carbon compounds. Strains were screened for their ability to utilize 150 carbon compounds as sole sources of carbon and energy (57). The list of carbon compounds tested is given below. Whenever the heat stability of a compound was in doubt, it was sterilized by filtration. Cellulose and *n*-hexadecane utilization was tested in liquid culture. The remaining carbon compounds were tested by means of replica plating (57). Only four patches were replicated onto each plate of a potentially fermentable carbon compound. A maximum of 16 patches was replicated onto each plate of the remaining carbon compounds. BM or BMA was used in all cases.

Strain 146 failed to grow in BM unless it was supplemented with Difco Vitamin-Free Casamino Acids (CA). The ability of this strain to utilize selected carbon compounds was tested in media containing 0.2% of the tested carbon source and supplemented with 1 g of CA per liter. The media were dispensed in 10-ml portions into 50-ml Erlenmeyer flasks. The cultures were incubated with aeration for 48 hr. Turbidity was read in a Klett-Summerson colorimeter using a green filter. Cultures grown in media containing only CA gave a reading of 90 to 105 Klett units. Cultures were scored positive if they gave a final reading of over 200 Klett units when grown in media containing CA and the tested carbon compound. Those giving a reading of 95 to 120 Klett units were scored negative. Strain 146 was also tested for its ability to grow in BM containing 0.2% (w/v) glucose and each of 21 natural amino acids, tested singly, at a concentration of 0.2 mg/ml.

Chemolithotrophic growth with molecular hydrogen. Strains were tested for their ability to utilize molecular hydrogen by testing for growth on BMA under the conditions described by Stanier et al. (57).

Arginine dihydrolase. Glycerol-grown cells were washed, suspended in half-strength ASW containing 50 mM Tris-hydrochloride (*p*H 7.5), and tested for the presence of a constitutive arginine dihydrolase (57). Ornithine was determined as described by Ratner (44).

Aromatic ring cleavage. Cells were grown on BMA containing 0.1% (w/v) *p*-hydroxybenzoate, harvested in quarter-strength ASW containing 50 mM Tris-hydrochloride (*p*H 7.5), and tested for the mechanism of protocatechuate cleavage (57).

Luminescence. The ability of strains to luminesce was tested by inoculating onto Farghaly's medium (21) supplemented with 5 g of Difco Yeast Extract per liter, 5 g of Difco Tryptone per liter, 10 g of $CaCO_3$ (powder) per liter, and 20 g of Difco agar per liter, adjusted to *p*H 7.2. The cultures were examined for luminescence for a period of 6 days.

Temperature. Growth at different temperatures was determined by using YEB. Test tubes containing 5 ml of the medium were inoculated and incubated with aeration at the appropriate temperature. Observations at 4 C were continued for 2 weeks. Observations at other temperatures were continued for 4 days.

Cell shape, motility, and Gram stain. All strains were inoculated from fresh MA slants into test tubes containing 5 ml of YEB. After 4 to 6 hr of growth and again at 18 to 24 hr, the cultures were examined by means of phase-contrast microscopy to determine cell shape and motility. At the latter time, the Gram stain was performed (52). Strains to be photographed were grown in 125-ml Erlenmeyer flasks containing 20 ml of YEB. When the culture reached a turbidity of 100 to 150 Klett units, the cells were centrifuged and suspended in half-strength ASW containing 50 mM Tris-hydrochloride (*p*H 7.5) to a turbidity of 400 Klett units. Photomicrographs were taken using a Reichert phase-contrast microscope.

PHB accumulation. Poly-β-hydroxybutyrate (PHB) accumulation was tested on BM containing 4 g of sodium-DL-β-hydroxybutyrate per liter as the carbon source and 0.2 g of $(NH_4)_2SO_4$ per liter as the sole source of nitrogen. The cultures were examined with a phase-contrast microscope, daily for 4 days (57).

Leifson flagella stain. The flagella of cells grown on liquid and solid media were stained by the method of Leifson (29). Forty per cent (w/v) neutralized Formalin was added to exponentially growing cultures to a final concentration of 6% (w/v). Ten- to 12-hr cultures grown on YEA were harvested in 1 ml of a solution containing 6% (w/v) Formalin, 50 mM Tris-hydrochloride (*p*H 7.5), and half-strength ASW. A bent glass rod was used to facilitate removal of the cells. Cells harvested from either solid or liquid medium were subsequently centrifuged, washed with distilled water, and stained as described by Leifson (29). Great care was taken during the harvesting, centrifugation, and resuspension to carry out these operations as gently as possible since peritrichous flagella were readily removed from cells by mechanical agitation.

DNA base compositions. The moles % GC in the DNA of representative strains was determined from buoyant density measurements in CsCl gradients (35).

Methods of isolation. The strains in our collection were obtained by enrichment methods, direct isolation from sea water, and isolation from marine animals. In the case of enrichment methods, 285 ml of a sea water sample was added to a 1-liter Erlenmeyer flask containing 15 ml of 1 M Tris-hydrochloride (*p*H 7.5), 300 mg of NH_4Cl, 22.5 mg of $K_2HPO_4 \cdot 3H_2O$, 8.4 mg of $FeSO_4 \cdot 7H_2O$, and 0.6 g of a carbon and energy source. The cultures were incubated aerobically, without shaking. Other enrichment cultures contained the above compounds at the same concentrations and also 0.9 g of $NaNO_3$. These cultures were incubated in 300-ml reagent bottles which were tightly stoppered with ground glass stoppers to exclude oxygen. All enrichment cultures were incubated at room temperature

(18 to 22 C) and were examined daily for growth for a period of 6 days. Cultures exhibiting turbidity were streaked on BMA containing 0.2% (v/v) glycerol. A total of four consecutive plates were streaked before a strain was considered pure.

A large number of our strains were obtained by direct isolation using sea water collected aseptically at various depths. The samples were filtered through filters (0.45-μm pore size; Millipore Corp.) which were subsequently placed onto MA. Resulting colonies were purified by streaking on the same medium.

Strains from marine animals were obtained by streaking rectal (R) and gill (G) swabs on TSB. Hemolytic colonies were tested for extracellular amylase production. Only strains which were hemolytic and amylase positive were included in this study.

Source of strains examined. Strains are listed under their species and group assignments. The information in parentheses following each strain number indicates the method of isolation and the source of sea water. Strains obtained by direct isolation (DI) were obtained from samples taken at about latitude 20°30′, longitude 157°30′. In each case, the depth of sampling is indicated. Carbon compounds in parentheses indicate that the strains were obtained by enrichment methods in which the designated carbon compound was the sole source of carbon and energy. Enrichment cultures which were incubated anaerobically with $NaNO_3$ as the terminal electron acceptor are designated by NO_3^- within the parentheses. In the case of strains obtained by enrichment methods, the samples consisted of sea water obtained off the coast of Oahu, Hawaii.

Strains obtained from marine animals were from G or R swabs. With the exception of strains 139 and 140, which were obtained from lobsters, all of the strains were isolated from fish caught off the coast of Oahu.

It should be noted that the strain numbering system (for strains 1–112) is one which groups together strains of high overall phenotypic similarity, irrespective of their origin, and which was established post facto on the basis of a numerical analysis which is discussed later.

Strains assigned to *Beneckea campbellii* (group A): 1(DI, 7.5 m); 2(DI, 300 m); 3(DI, 50 m); 4(DI, 800 m); 5(DI, 800 m); 6(DI, 800 m); 7(DI, 7.5 m); 8(DI, 800 m); 9(DI, 800 m); 10(DI, 800 m); 11(DI, 7.5 m); 12(DI, 800 m); 13 (DI, 7.5 m); 14(DI, 7.5 m); 15(DI, 7.5 m); 16(vibrio 352, of R. M. Johnson); 17(DI, 7.5 m); 18(DI, 800 m); 19(DI, 7.5 m); 20(DI, 7.5 m); 21(DI, 7.5 m); 22(DI, 7.5 m); 23(DI, 7.5 m); 24(DI, 7.5 m); 25(DI, 50 m); 26(DI, 1,300 m); 27(DI, 150 m); 28(DI, 100 m); 29(DI, 350 m); 30(DI, 7.5 m); 31(DI, 1,300 m); 32(DI, 1,300 m); 33(DI, 800 m); 34(DI, 7.5 m); 35(DI, 1,300 m); 36(DI, 7.5 m); 37(DI, 800 m); 38(DI, 800 m); 39(DI, 1,300 m); 40(DI, 800 m); 41(DI, 400 m); 42(DI, 800 m); 43(DI, 800 m); 44(DI, 350 m); 45(DI, 800 m); 46(DI, 1,200 m); 47(DI, 800 m); 48(DI, 50 m); 49(DI, 650 m); 50(DI, 800 m); 51(DI, 600 m); 52(DI, 250 m); 53(DI, 700 m); 54(DI, 1,300 m); 55(DI, 150 m); 56(DI, 150 m); 57(DI, surface); 58(DI, 300 m); 59(DI, 800 m); 60(DI, 100 m).

Strains assigned to *Photobacterium fischeri* (group B-1): 61(DI, 800 m); 62(DI, 800 m); 63(DI, 800 m); 64(DI, 800 m); 65(DI, 600 m); 66(succinate).

Strains assigned to group B-2: 67(DI, 800 m); 68(DI, 750 m); 69(DI, 1,300 m); 70(DI, 7.5 m); 71(DI, 300 m).

Strains assigned to *Beneckea neptuna* (group C): 72(DI, 800 m); 73(D-gluconate, NO_3^-); 74(glycerol, NO_3^-); 75 (lactate); 76(DI, 1,300 m); 77(DI, 700 m); 123(G); 124(R); 125(R); 126(G); 127(G); 128(R); 129(R); 130(R); 131(R); 132(R); 133(R); 134(R); 135(G); 136(R); 137(R); 138(R); 139(R); 140(R); 141(R); 142(R); 143(G).

Strains assigned to *Beneckea nereida* (group D): 78(acetate); 79(D-gluconate); 80(propanol); 81(L-leucine); 82(propanol); 83(D-gluconate); 84(caprylate); 85(succinate, NO_3^-).

Strains assigned to *Beneckea alginolytica* (group E-1): 86(L-glycine); 87(L-histidine); 88(L-α-alanine); 89(sucinate, NO_3^-); 90(succinate); 91(glycine); 92(glycine); 118 (*V. alginolyticus*, ATCC 17748); 119 [*V. alginolyticus*, Center for Disease Control (CDC) strain B3471, isolated from an ear infection in Washington]; 120 (*V. alginolyticus*, CDC strain B3481, isolated from an ear infection in Hawaii); 121 (*V. alginolyticus*, CDC strain B3660, isolated from a wound in Rhode Island); 122 (*V. alginolyticus*, CDC strain B4185, isolated from a blister in Florida).

Strain assigned to group E-2: 93(lactate, NO_3^-).

Strains assigned to group E-3: 94(succinate, NO_3^-); 95(acetate).

Strains assigned to *Beneckea pelagia* (group F): 96(L-arginine); 97(succinate, NO_3^-); 98(glycine); 99(succinate); 100(L-arginine); 101(succinate, NO_3^-); 102(succinate); 103(alginate); 104(alginate); 105(alginate); 106(DI, 1,300 m).

Strains assigned to *Beneckea natriegens* (group G): 107(citrate); 108(benzoate); 109(DL-β-hydroxybutyrate); 110(lactate, NO_3^-); 111(*Pseudomonas natriegens* of W. J. Payne, ATCC 14048); 112(lactate, NO_3^-).

Strains assigned to *Beneckea parahaemolytica*: 113 (*V. parahaemolyticus*. ATCC 17802); 114 (*V. parahaemolyticus*, CDC strain KC821); 115 (*V. parahaemolyticus*, CDC strain KC824); 116 (*V. parahaemolyticus*, CDC strain KC833); 117 (*V. parahaemolyticus*, CDC strain KC830). Strains 113–117 were isolated in Japan from cases of food poisoning.

Other strains: 144 (*Vibrio anguillarum*, ATCC 19264); 145 (*Aeromonas proteolytica*, ATCC 15338); 146 (*Beneckea hyperoptica*, ATCC 15803).

RESULTS

Sodium and organic growth factor requirements. About 45% of the strains obtained by direct isolation and all of the strains obtained by enrichment methods were able to grow in BM to a final turbidity of 150 to 250 Klett units. When sodium was replaced by an equimolar amount of potassium, the medium could not support the growth of our isolates (final turbidity readings, 0 to 10 Klett units). The results indicate that all of our strains, which had no organic growth factor requirements, required sodium ion for growth.

MacLeod (34) has shown the absence of a sodium requirement in two species of nonmarine origin, *P. aeruginosa* and *Escherichia coli*. We have extended these findings by testing additional

strains of soil and fresh water origin for their ability to grow in BM and in a medium in which sodium had been replaced by potassium. The following strains grew to a similar turbidity on both media, indicating the absence of a sodium requirement: *P. aeruginosa* (ATCC 17423 and 17503); *P. fluorescens* biotype A (ATCC 13525), biotype B (ATCC 17815), biotype C (ATCC 17400), biotype D (ATCC 9447), biotype E (ATCC 17416), biotype F (ATCC 17458); *P. putida* biotype A (ATCC 12633), biotype B (ATCC 17484); *P. acidovorans* (ATCC 17479); *Acinetobacter calco-aceticus* (ATCC 11171, 14290, 17902, 17908); *Aeromonas formicans* (ATCC 13137 and 23214) as well as two strains of *Aerobacter aerogenes*. Two strains of *P. stutzeri* (ATCC 17587 and 17588) grew only in BM containing sodium, indicating a requirement for this ion.

Fermentation. Only those isolates which had no organic growth factor requirements, required sodium for growth, and could ferment glucose were used in these studies. The ability to ferment glucose was tested in two media. In F-1 medium, strains which were capable of fermentation produced visible growth but no gas. None of the organisms grew or produced gas in F-1 medium when glucose was omitted. When tested in F-2 medium, strains capable of fermentation produced turbidity throughout the medium and lowered the *p*H to 5.2 to 5.8. Strains which could not ferment glucose grew only at the surface of the medium; the *p*H after 48 hr of incubation was 7.6 to 7.8. In F-2 medium, from which glucose had been omitted, growth occurred only at the surface and the final *p*H was 7.6 to 7.8. These tests showed that about 85% of the strains, which were obtained by direct isolation and which required sodium but no organic growth factors, were capable of fermenting glucose. The facultatively anaerobic strains obtained by direct isolation and by enrichment methods were tested for their ability to produce acid under anaerobic conditions from glycerol, D-mannitol, D-ribose, and D-gluconate. The tests were performed in F-3 medium. Acid production was considered indicative of fermentation. Table 1 lists the strains able to ferment the tested carbohydrates. None of the strains which produced acid made gas. Strain 146 was unable to grow anaerobically in either F-1 or F-2 medium.

Strains 86–93, 118–122, 144 and 145 gave a strong VP reaction, indicating the presence of acetoin, an intermediate of the 2,3-butyleneglycol fermentation. Strains 61, 62, 66–69, 71, and 94 gave a very weak VP reaction. Quantitative assays for acetoin or diacetyl, or both, as well as for 2,3-butyleneglycol were performed on the culture medium of strains which gave a positive VP test (39). Strains 86–93, 118–122, 144, and 145 produced 1.5 to 3.1 μg of acetoin or diacetyl, or both, and 108 to 225 μg of 2,3-butyleneglycol per ml of culture medium. Neither acetoin or diacetyl (or both) nor 2,3-butyleneglycol could be detected in the culture medium of strains 61, 62, 66–69, 71, and 94.

Nitrogen metabolism. None of the strains were able to fix molecular nitrogen or denitrify. In the denitrification medium, under anaerobic conditions, most of the strains were capable of slight growth. Since the majority of the strains produced nitrite from nitrate, this growth was probably due to the utilization of nitrate as an electron acceptor. A medium similar to the denitrification medium but lacking $NaNO_3$ was not able to support growth under anaerobic conditions. The strains which could not reduce nitrate to nitrite were 13, 59, 60, 66–69, and 146.

Range of organic compounds utilized as carbon sources. All of the strains were screened for their ability to utilize a total of 155 carbon compounds. Table 2 lists the substrates not utilized by any of the strains as sole sources of carbon and energy. Table 3 lists the substrates utilized by 15% or less of the strains and gives the positive strains. Table 4 lists the substrates utilized by 90% or more of the strains and gives the negative strains. Table 5 presents the nutritional and physiological traits which are positive for more than 15% but less than 90% of the strains.

The nutritional properties of strain 146 were determined in liquid media (described above). Partial results of this screening are presented in Table 5. In addition to the compounds listed, strain 146 was able to utilize D-xylose, D-glucose, D-fructose, maltose, trehalose, melibiose, lactose, succinate, fumarate, DL-lactate, and pyruvate. It was not able to utilize alginate, L-arabinose, DL-β-hydroxybutyrate, glutarate, glycerol, benzoate, *p*-hydroxybenzoate, β-alanine, and L-proline. The data on strain 146 were not included in the calculation of the percentage of strains able to utilize a given carbon compound.

Hemolysis. The results of the tests for hemolysis on TSB are given in Table 5. With a few exceptions, organisms which were hemolytic on TSB were also hemolytic on WM. In general, the zone of hemolysis on WM was less than on TSB. Strain 113 (the proposed type strain of *V. parahaemolyticus*) gave good hemolysis on TSB but no hemolysis on WM. The remaining strains of *V. parahaemolyticus* (strains 114–117) were hemolytic on both media.

Ability to utilize molecular hydrogen. None of the strains tested could grow chemolithotrophically with molecular hydrogen as the source

of energy and CO_2 as the source of carbon.

Arginine dihydrolase. Representative strains were tested for the presence of an arginine dihydrolase system. Arginine consumption, during 2 hr of incubation under anaerobic conditions, was considered evidence for the presence of this enzyme system (57). Many of the tested strains did not consume arginine, whereas others utilized 80 to 95% of the arginine in the incubation mixture. Some strains, however, consumed 10 to 30% of the arginine. Since these organisms are facultative anaerobes, limited growth may occur during the anerobic incubation resulting in incorporation of arginine into cell material. This possibility suggested that production of ornithine may be a better test for the presence of the arginine dihydrolase system. When the concentration of ornithine in the reaction mixtures was determined, it was found that all strains which exhibited a vigorous consumption of arginine produced an equivalent amount of ornithine. Strains which consumed 10 to 30% of the arginine failed to produce ornithine. These results indicate that, in the case of facultative anaerobes, the production of ornithine rather than the consumption of arginine may be a better test for the arginine dihydrolase system. Table 5 lists the strains tested and indicates whether they were positive or negative for this trait.

Aromatic ring cleavage. Only strains 107–112 could utilize *p*-hydroxybenzoate as a sole source of carbon and energy. When grown on this compound, these strains degraded protocatechuate by means of a *meta* cleavage.

Oxidase test and luminescence. Strains 67–71 and strain 146 gave a negative oxidase test. All of the remaining strains were oxidase positive. Only strains 61–66 luminesced when grown on Farghaly's modified medium.

Slime from sucrose. None of the strains were able to make slime on BMA containing 5% (w/v) sucrose.

Temperature. All of the strains grew at 30 C; none grew at 45 C. Table 5 shows the ability of strains to grow at 4, 35, and 40 C.

Cell shape, motility, and Gram stain. All of the strains were gram-negative and motile in liquid cultures. Five strains were straight rods in exponential phase of growth and curved rods in stationary phase. Fifteen strains were curved rods in both exponential and stationary phase. The remaining strains were straight rods. The data for cell shape are presented in Table 5. There is a considerable degree of subjectivity in establishing whether a strain is a curved or a straight rod. The strains which we have designated as curved rods repeatedly showed this attribute when examined in YEB cultures. Photomicrographs of representative morphological types are shown in Fig. 1–13.

TABLE 1. *Fermentation of carbohydrates*

Substrate	Strains
D-Ribose	Negative strains: 7, 8, 11, 13–15, 59, 66, 89, 113, 117, 126, 129, 131, 133, 134, 136, 139, 141–143
D-Glucose	All positive
D-Gluconate	Negative strains: 1–12, 14, 15, 17–24, 26, 27, 30–60, 61–66, 72, 76, 77, 78–85, 94, 95, 96–106, 142, 143
D-Mannitol	Negative strains: 19, 22, 28–34, 38, 42, 51, 59, 60, 61–65, 67–71, 78–83
Glycerol	All negative

PHB accumulation. Strains 78–85, 94, 95, and 107–112 were able to accumulate PHB as an intracellular reserve product. Representative strains containing granules of PHB are shown in Fig. 14–16.

Leifson flagella stain. All of the strains in our collection were stained for flagella after cultivation on solid and liquid media. When grown in liquid medium, strains 1–60 and 72–145 had single, polar flagella (Fig. 17, 21, 23, 25). When grown on solid medium, some of these strains had single, polar flagella, whereas others became peritrichously flagellated (Fig. 18–20, 22, 24). Strains 8, 76, 142, and 143, which were curved rods, had single, polar flagella when grown in liquid medium and peritrichous flagella when grown on solid medium (Fig. 23, 24). The remaining curved rods had single, polar flagella when grown on either medium. Strains 61–66 and 146 had polar tufts of two to six flagella when grown on either solid or liquid medium (Fig. 26). The flagellation of strains grown on solid medium is given in Table 5. Considerable difficulty was experienced with the flagella staining of strains 67–71. Although the specimens stained poorly, it appeared that in liquid medium strains 67, 68 and 70 had a single, polar flagellum (Fig. 27), whereas strains 69 and 71 had 1–3 polar flagella. When grown on solid medium, strains 67 and 69 appeared to be peritrichously flagellated (Fig. 28). Satisfactory flagella stains of strains 69–71 could not be obtained when these organisms were grown on solid medium so that it was not possible to determine whether these strains also undergo a change in flagellation.

TABLE 2. *Substrates not utilized by any of the strains as sole sources of carbon and energy*

Substrate	Substrate
Carbohydrates	Amines
D-Arabinose	Methylamine
D-Fucose	Ethanolamine
Lactose	Benzylamine
Inulin	Histamine
Saccharate	Tryptamine
Mucate	Butylamine
D-Galacturonate	α-Amylamine
Cellulose	2-Amylamine
Fatty acids and dicarboxylic acids	Pentylamine
Formate	Polyalcohols and glycols
Oxalate	Erythritol
Maleate	Adonitol
Adipate	Ethyleneglycol
Pimelate	Propyleneglycol
Suberate	2,3-Butyleneglycol
Azelate	Alcohols
Sebacate	Methanol
Hydroxy acids and miscellaneous organic acids	Isopropanol
D-(−)-Tartrate	*n*-Butanol
meso-Tartrate	Isobutanol
Glycolate	Geraniol
Laevulinate	Non-nitrogenous aromatic and other cyclic compounds
Citraconate	D-Mandelate
Itaconate	L-Mandelate
Mesaconate	Benzoylformate
Aliphatic amino acids	*o*-Hydroxybenzoate
L-Isoleucine	*m*-Hydroxybenzoate
L-Norleucine	Phenylethanediol
L-Valine	Phenol
L-Lysine	Naphthalene
DL-α-Aminobutyrate	Miscellaneous nitrogenous compounds
DL-α-Aminovalerate	Creatine
Amino acids and related compounds containing a ring structure	Pantothenate
L-Phenylalanine	Acetamide
L-Tryptophan	Nicotinate
D-Tryptophan	Nicotinamide
DL-Kynurenine	Trigonelline
Kynurenate	Allantoin
Anthranilate	Adenine
m-Aminobenzoate	Guanine
p-Aminobenzoate	Cytosine
	Thymine
	Uracil
	Paraffin hydrocarbons
	n-Hexadecane

Numerical analysis. With the exception of flagellation, cell shape, PHB accumulation, glucuronic acid utilization, growth at 4 C, arginine dihydrolase, and hemolysis, the data presented in Tables 2–5 for strains 1–112 were submitted to a numerical analysis by E. Szabo (Department of Microbiology, University of Queensland, Brisbane, Australia) with programs written for a General Electric 225 computer. The estimation of similarity between strains was based on the inclusion of both positive and negative characters, using the simple similarity coefficient (*S*) described by Sokal and Sneath (54). The search for clusters of strains within the data was by a Complete-Linkage method (*to be published*). After completion of the numerical analysis, some minor errors were discovered in the transcription of the data. These errors involve changes in a single and usually different character for a few strains and should cause only a slight modifica-

TABLE 3. *Substrates utilized by 15% or less of the strains as sole sources of carbon and energy*

Substrates	Positive strains
D-Xylose	67–69, 71
L-Arabinose	84, 85, 107–113, 117
L-Rhamnose	107–112
Melibiose	107, 109, 110, 112
Salicin	74, 75, 107–112, 123, 125, 132–138
Isobutyrate	78–83, 86–95, 107–112
Isovalerate	78–82, 86–90, 93, 117, 119, 120, 122
Malonate	107–112
Glutarate	78–83, 94, 95, 106–112
L-Tartrate	107
DL-β-Hydroxybutyrate	78–85, 94, 95, 107–112
Sorbitol	144, 145
Inositol	76, 94, 95, 108, 111, 112, 142, 143
Benzoate	107–112
p-Hydroxybenzoate	107–112
Phenylacetate	78, 79
Quinate	76, 77, 103, 104, 106–112
β-Alanine	84, 85, 103, 105, 106, 111, 145
γ-Aminobutyrate	78–83, 94, 103–112
δ-Aminovalerate	78–83, 94, 95, 103–112
Spermine	102, 107–111
Betaine	107–112
Sarcosine	94, 95, 107–112
Hippurate	107–112

TABLE 4. *Substrates utilized by 90% or more of the strains as sole sources of carbon and energy*

Substrate	Negative strains
D-Ribose	None
D-Glucose	None
D-Fructose	4
Trehalose	14–16, 34, 61–64, 67–69
Maltose	59, 66, 70, 71
N-Acetylglucosamine	None
Succinate	None
Fumarate	14–59
DL-Lactate	11, 13, 61–66, 70
Pyruvate	11, 13, 61–66
Glycerol	None
L-Proline	13, 25, 67–71

tion of the *S* values. As seen from Fig. 29, the strains readily separate themselves into groups having *S* values of 68% or less. The major groups have been designated by letters. For reasons to be discussed, further subdivisions were made in major groups B and E. B-1 and B-2 form one cluster at the 74% *S* value; groups E-1, E-2, and E-3 form a cluster at 76%. After completion of the numerical analysis, additional strains were submitted to a phenotypic characterization. The results of this screening indicated that strains 123–143 could be readily placed into group C and strains 118–122 could be placed into group E-1. Strains 113–117 were phenotypically similar (Table 5) and readily separable from the other groups. Table 6 lists some of the traits which are of value in distinguishing the various groups studied. It can be seen that these groups are readily separable on the basis of a large number of unrelated, phenotypic traits. The groups also differ in the extent of their nutritional versatility. Figure 30 gives the range of the organic compounds utilized by each group as sole sources of carbon and energy.

DNA base compositions. The moles % of GC in the DNA of representative strains from each group is given in Table 7. Strains from groups B-1 and B-2 had a GC content of 39 to 41%. Strains 113 and 115 as well as strains from groups A, C, D, E-1, E-2, F, and G had GC contents of 45 to 48%. The two strains from group E-3 had a GC content of 54%. Straight and curved rods belonging to the same group had similar GC contents in their DNA.

Enrichments for chitin decomposers. The isolates of Campbell and Williams (10) were obtained from enrichments for chitin-decomposing bacteria. None of the strains in our collection were obtained by such methods. To characterize the organisms which predominate in enrichment cultures containing chitin, a series of eight enrichment media were prepared and inoculated as previously described. From these enrichment cultures, eight strains were isolated. Nutritional screening on 12 diagnostic media showed that five of these organisms could be placed into group C and three into group F.

Enrichments from soil. The strains used in this study were readily isolated from sea water by enrichment methods. Attempts were made to isolate these organisms from soil by inoculating enrichment media containing full-strength ASW with various soil samples as previously described. From a series of 15 enrichments containing acetate, propionate, butyrate, valerate, ethanol, DL-lactate, glycerol, succinate, citrate, L-α-alanine, and *p*-hydroxybenzoate, 15 strict aerobes were isolated; two were gram-positive and the rest were gram-negative. Ten of the gram-negative organisms were identified as pseudomonads, and three were identified as acinetobacters. None of these strains required sodium ion for growth. These results indicate that the enrichment methods used for the isolation of our strains from samples of marine origin yield a different bacterial flora when soil is used as the source of inoculum.

TABLE 5. *Traits present in more than 15 but less than 90% of the strains*

Strain	Flagellation[a]	Morphology[b]	Amylase	Gelatinase	Lipase	Alginase	Chitinase	D-Mannose	D-Galactose	Sucrose	Cellobiose	D-Gluconate	D-Glucuronate	Acetate	Propionate	Butyrate	Valerate	Caproate	Heptanoate	Caprylate	Pelargonate	Caprate	DL-Malate	DL-Glycerate	Citrate	α-Ketoglutarate	Aconitate	D-Mannitol	Ethanol	Propanol	Glycine	L-α-Alanine	D-α-Alanine	L-Serine	L-Threonine	L-Leucine	L-Aspartate	L-Glutamate	L-Arginine	L-Ornithine	L-Citrulline	L-Histidine	L-Tyrosine	Putrescine	Arginine dihydrolase[c]	Growth at 4 C	Growth at 35 C	Growth at 40 C	Hemolysis at 37 C[d]
1, 2	m	*c*	+	+	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	−	+	+	+	+	−	−	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−	x
3	m	c	+	+	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	±	+	+	+	+	+	−	−	+	+	+	+	+	−	±	+	−	−	−	−	−	−	n	−	−	−	x
4	m	*c*	+	+	+	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−	x
5	m	*c*	+	+	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	±	−	+	+	+	+	−	−	+	+	+	+	+	−	+	−	−	−	−	+	−	−	n	−	−	−	x
6	m	*c*	+	+	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	−	+	+	+	+	−	−	−	+	+	±	+	−	+	−	−	−	−	+	−	−	−	−	−	−	x
7	p	s	+	+	+	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	+	+	+	+	+	−	+	±	−	−	−	±	−	−	−	−	−	−	x
8	p	c	+	+	+	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	±	±	±	−	+	−	±	−	−	−	−	−	−	−	n	−	−	−	x
9	p	s	+	+	+	−	+	−	−	−	+	−	−	±	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	±	+	+	+	+	−	+	−	−	−	−	+	+	−	n	−	−	−	+
10	m	c	+	+	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	+	+	+	±	+	−	+	+	−	−	−	−	+	−	−	−	+	−	x
11	p	s	+	+	+	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	n	−	−	−	x
12	m	c	+	+	+	−	+	−	−	−	−	−	−	±	−	−	−	−	−	−	−	+	+	+	−	−	+	+	+	−	−	−	±	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	x
13	p	s	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	n	−	−	−	x
14	p	s	−	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+	−	−	−	+	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	x
15	p	s	−	+	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	±	−	+	+	+	+	−	−	−	+	−	±	−	−	−	−	−	−	−	−	−	−	n	−	−	−	+
16	m	c	+	+	+	−	+	+	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	±	±	±	+	−	−	−	±	−	±	−	−	±	±	−	−	−	−	−	−	−	−	−	−	x
17	p	s	+	+	+	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	+	−	±	−	−	−	−	−	−	−	−	−	−	n	−	+	−	+
18	p	s	+	+	+	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+	+	±	+	+	+	+	−	−	−	+	+	−	+	−	−	−	−	−	−	−	−	−	n	−	+	−	+
19	p	s	+	+	+	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	+	−	+
20	p	s	+	+	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	±	+	+	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	n	−	+	−	+
21	p	s	+	+	+	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	±	+	−	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	n	−	+	−	+
22	p	s	+	+	+	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	n	−	+	−	+
23	p	s	+	+	+	−	+	+	−	−	+	−	−	−	+	−	−	−	−	−	−	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	−	−	−	−	−	±	−	−	n	−	+	−	+
24	p	s	+	+	+	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+	+	−	−	−	+	+	−	+	−	−	−	−	−	−	+	−	−	−	−	+	−	+
25	p	s	+	+	+	−	+	+	−	−	+	+	+	−	−	−	−	−	−	−	−	+	−	−	+	+	+	+	−	−	−	+	−	+	+	−	−	−	−	−	−	−	−	−	n	−	+	−	+

[a] Flagellation of strains grown on solid medium. m, Monotrichous (polar); p, peritrichous; l, lophotrichous (polar); *, see text for discussion of flagellation.

[b] Morphology of strains in liquid medium. c, Curved rods in exponential and stationary phase; *c*, straight rods in exponential and curved rods in stationary phase; s, straight rods in exponential and stationary phase.

[c] n, Not tested.

[d] Tested on TSB. x, No growth.

TABLE 5—*Continued*

26	p	s	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
27	p	s	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	+	-	-	-	+	+	±	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
28	p	s	+	+	+	-	+	+	-	-	-	+	-	+	+	-	-	-	-	+	-	+	-	-	+	+	+	-	-	-	-	+	+	±	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
29	p	s	+	+	+	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	±	-	n	-	+	-	+
30	p	s	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
31	p	s	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	±	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+
32	p	s	+	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	+	±	+	+	-	-	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	±	-	n	-	+	-	+
33	p	s	+	+	+	-	+	+	-	-	-	-	-	±	+	-	-	-	-	+	±	-	-	-	+	+	+	-	-	-	-	+	+	+	±	-	-	-	-	-	-	-	±	-	-	-	+	-	+
34	p	s	+	+	+	-	+	+	-	-	-	-	-	±	+	-	-	-	+	±	+	+	-	+	+	+	+	-	-	-	-	+	+	±	+	-	-	-	-	-	-	-	-	-	n	-	+	-	+
35	p	s	+	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	+	+	+	±	-	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
36	p	s	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
37	p	s	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	+
38	p	s	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
39	p	s	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	±	-	+	+	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	±	-	n	-	+	-	+
40	p	s	+	+	+	-	+	+	-	-	+	-	-	-	+	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-
41	p	s	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	±	-	+	+	±	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
42	p	s	+	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	±	-	+	+	-	+	-	±	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
43	p	s	+	+	+	-	+	+	-	-	+	-	-	+	+	-	-	-	-	+	±	±	+	±	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
44	p	s	+	+	+	-	+	+	-	-	+	-	-	+	+	-	-	-	-	±	-	+	+	+	+	+	+	+	-	-	-	±	+	-	±	-	-	-	-	-	-	-	±	-	-	-	+	-	+
45	p	s	+	+	+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	±	-	+	+	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	±	-	n	-	+	-	+
46	p	s	+	+	+	-	+	-	-	-	+	-	-	+	+	-	-	-	-	+	-	+	+	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	n	-	+	-	+
47	p	s	+	+	+	-	+	-	-	-	+	-	-	-	+	-	-	-	-	+	-	+	+	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	±	-	n	-	+	-	+
48	p	s	+	+	+	-	+	-	-	-	+	-	-	+	+	-	-	-	-	±	-	+	+	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	±	-	-	-	+	-	+
49	p	s	+	+	+	-	+	-	-	-	±	-	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	±	-	n	-	+	-	+
50	p	s	+	+	+	-	+	+	-	-	-	-	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
51	p	s	+	+	+	-	+	-	-	-	-	-	-	±	+	-	-	-	-	+	-	+	-	+	+	+	+	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
52	p	s	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	±	-	+	-	+	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	n	-	+	-	+
53	p	s	+	+	+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	+	-	+	-	-	+	+	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+
54	p	s	+	+	+	-	+	+	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
55	p	s	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	+	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	n	-	+	-	+
56	p	s	+	+	+	-	+	-	-	-	-	-	-	+	+	-	-	-	-	+	-	+	+	-	-	+	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	±	-	-	-	+	-	+
57	p	s	+	+	+	-	+	-	-	-	+	-	-	+	+	-	-	-	-	±	-	+	-	-	-	+	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	±	-	n	-	+	-	+
58	p	s	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	n	-	+	-	+
59	p	s	+	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	±	-	-	+	-	-	-	-	-	-	+	-	-	n	-	-	-	+
60	p	s	+	+	+	-	+	-	-	-	+	-	-	-	+	-	-	-	-	+	-	+	+	+	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+	+	-	-	-	+	-	+

TABLE 5—*Continued*

Strain	Flagellation[a]	Morphology[a]	Amylase	Gelatinase	Lipase	Alginase	Chitinase	D-Mannose	D-Galactose	Sucrose	Cellobiose	D-Gluconate	D-Glucuronate	Acetate	Propionate	Butyrate	Valerate	Caproate	Heptanoate	Caprylate	Pelargonate	Caprate	DL-Malate	DL-Glycerate	Citrate	α-Ketoglutarate	Aconitate	D-Mannitol	Ethanol	Propanol	Glycine	L-α-Alanine	D-α-Alanine	L-Serine	L-Threonine	L-Leucine	L-Asparate	L-Glutamate	L-Arginine	L-Ornithine	L-Citrulline	L-Histidine	L-Tyrosine	Putrescine	Arginine dihydrolase[c]	Growth at 4 C	Growth at 35 C	Growth at 40 C	Hemolysis at 37 C[d]
61	1	s	−	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×
62	1	s	−	+	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×
63	1	s	−	+	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×
64	1	s	−	+	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×
65	1	s	−	+	+	−	−	+	+	+	−	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
66	1	s	−	+	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
67	*	s	−	−	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×
68	*	s	−	−	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×
69	*	s	−	−	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×
70	*	s	−	−	+	−	−	+	+	+	+	+	−	+	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×
71	*	s	−	−	+	−	−	+	+	+	+	+	−	+	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	×
72	p	s	−	−	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
73	p	s	−	−	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
74	p	s	−	−	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
75	p	s	−	−	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
76	p	c	−	−	+	−	−	+	+	+	+	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
77	m	c	−	−	+	−	−	+	+	+	−	+	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
78	m	s	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
79	m	s	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
80	m	s	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
81	m	s	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
82	m	s	−	−	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
83	m	s	−	−	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
84	m	s	−	−	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
85	m	c	−	−	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
86, 87	p	s	−	−	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
88	p	s	−	−	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+
89	p	s	−	−	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+

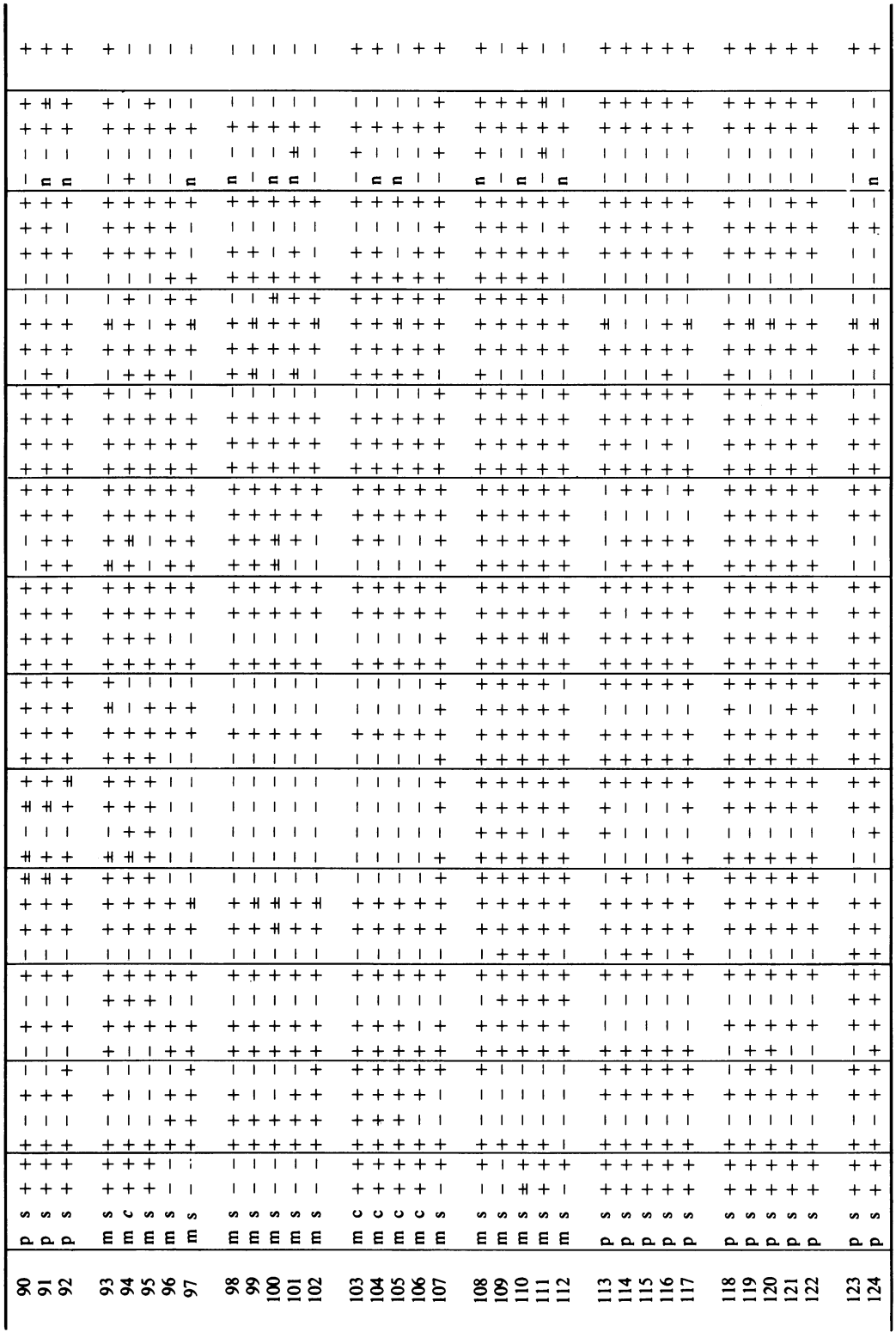

TABLE 5—*Continued*

Strain	Flagellation[a]	Morphology[b]	Amylase	Gelatinase	Lipase	Alginase	Chitinase	D-Mannose	D-Galactose	Sucrose	Cellobiose	D-Gluconate	D-Glucuronate	Acetate	Propionate	Butyrate	Valerate	Caproate	Heptanoate	Caprylate	Pelargonate	Caprate	DL-Malate	DL-Glycerate	Citrate	α-Ketoglutarate	Aconitate	D-Mannitol	Ethanol	Propanol	Glycine	L-α-Alanine	D-α-Alanine	L-Serine	L-Threonine	L-Leucine	L-Aspartate	L-Glutamate	L-Arginine	L-Ornithine	L-Citrulline	L-Histidine	L-Tyrosine	Putrescine	Arginine dihydrolase[c]	Growth at 4 C	Growth at 35 C	Growth at 40 C	Hemolysis at 37 C[d]
125	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	−	−	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
126	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	+	−	+
127	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
128	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	−	−	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
129	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	+	−	+
130	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
131	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
132	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
133	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	−	−	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
134	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
135	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
136	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
137	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
138	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	−	−	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
139	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	−	−	+	−	+
140	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
141	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
142	p	c	+	+	+	−	+	+	+	+	−	+	+	+	+	−	−	−	−	−	+	−	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
143	p	c	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
144	m	c	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
145	p	s	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+
146	l	s	+	−	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	+	−	−	−	−	u	−	+	−	+

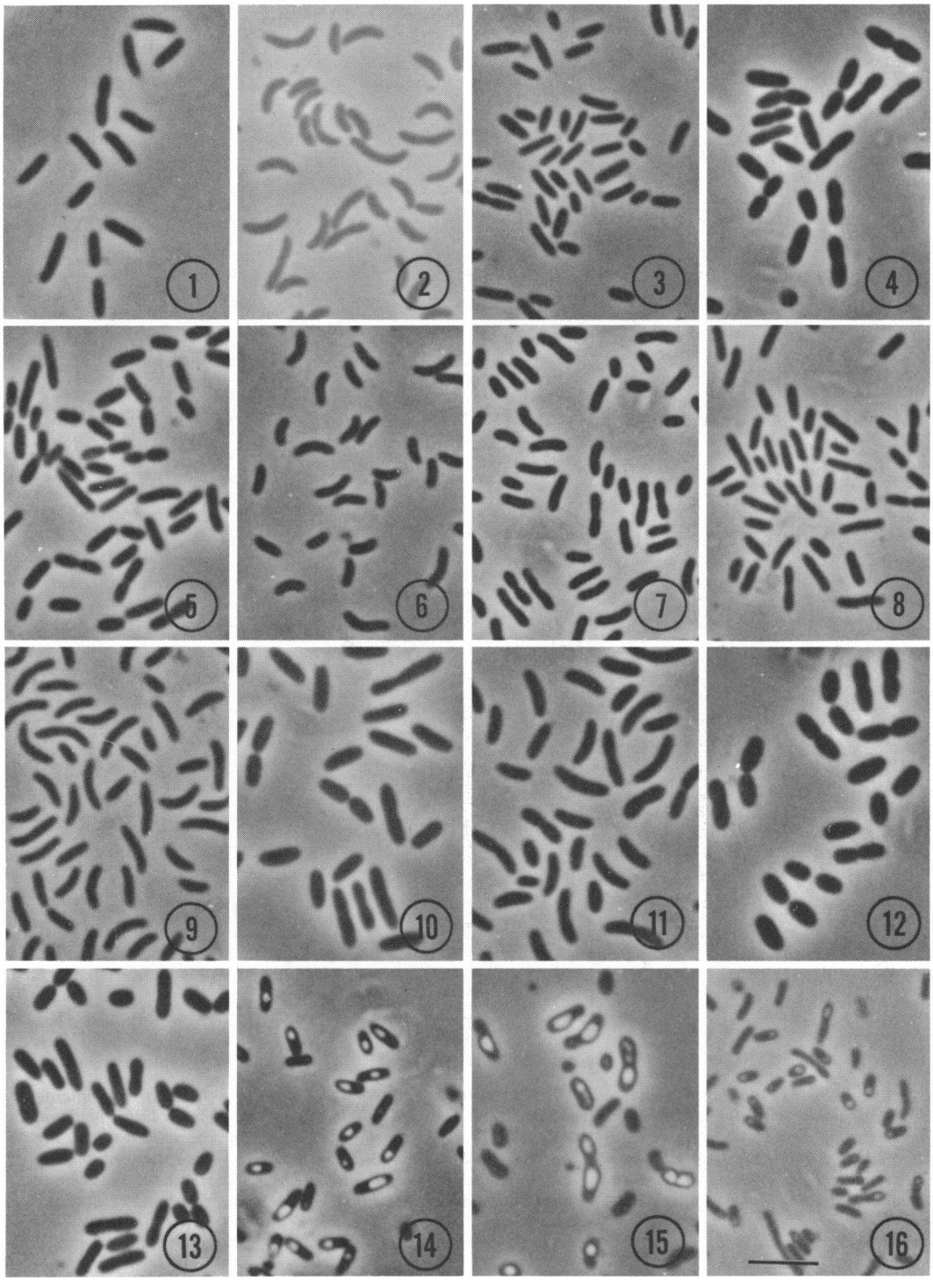

FIG. 1–16. *Phase-contrast micrographs. ×2,000. Marker in Fig. 16 represents 5 μm.* Fig. 1–13. *Cells in exponential phase of growth.* Fig. 14–16. *Cells containing granules of PHB. Fig. 1, B. campbellii, strain 48; Fig. 2. B. campbellii, strain 4; Fig. 3, P. fischeri, strain 60; Fig. 4, group B-2, strain 68; Fig. 5, B. neptuna, strain 73; Fig. 6, B. neptuna, strain 77; Fig. 7, B. nereida, strain 82; Fig. 8, B. alginolytica, strain 118; Fig. 9, group E-3, strain 94; Fig. 10, B. pelagia, strain 116; Fig. 11, B. pelagia, strain 103; Fig. 12, B. natriegens, strain 109; Fig. 13, B. parahaemolytica, strain 113; Fig. 14, B. nereida, strain 78; Fig. 15, group E-3, strain 94; Fig. 16, B. natriegens, strain 109*

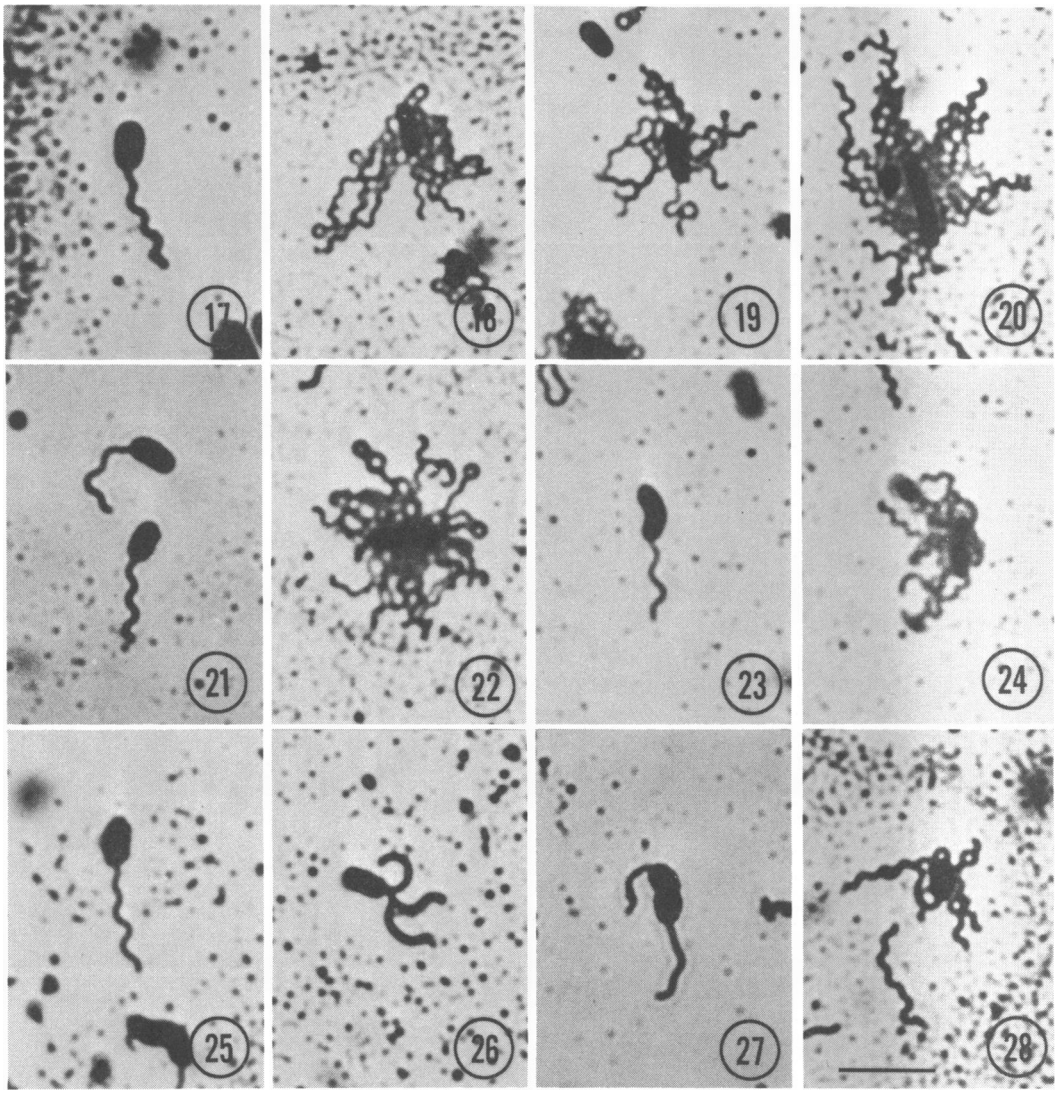

FIG. 17-28. *Leifson flagella stains. ×2,500. Marker in Fig. 28 represents 5 μm. Fig. 17, This photomicrograph is representative of all the species of the genus Beneckea when growing in exponential phase in liquid medium. The actual strain used was B. alginolytica, strain 92. Fig. 18, B. campbellii, strain 34, grown on solid medium; Fig. 19, B. neptuna, strain 74, grown on solid medium; Fig. 20, B. alginolytica, strain 87, grown on solid medium; Fig. 21, B. parahaemolytica, strain 115, grown in liquid medium; Fig. 22, B. parahaemolytica, strain 117, grown on solid medium; Fig. 23, B. neptuna, strain 142, grown in liquid medium; Fig. 24, B. neptuna, strain 142, grown on solid medium; Fig. 25, group E-3, strain 95, grown in liquid medium; Fig. 26, P. fischeri, strain 60, grown in liquid medium; Fig. 27, group B-2, strain 69, grown in liquid medium; Fig. 28, group B-2, strain 69, grown on solid medium.*

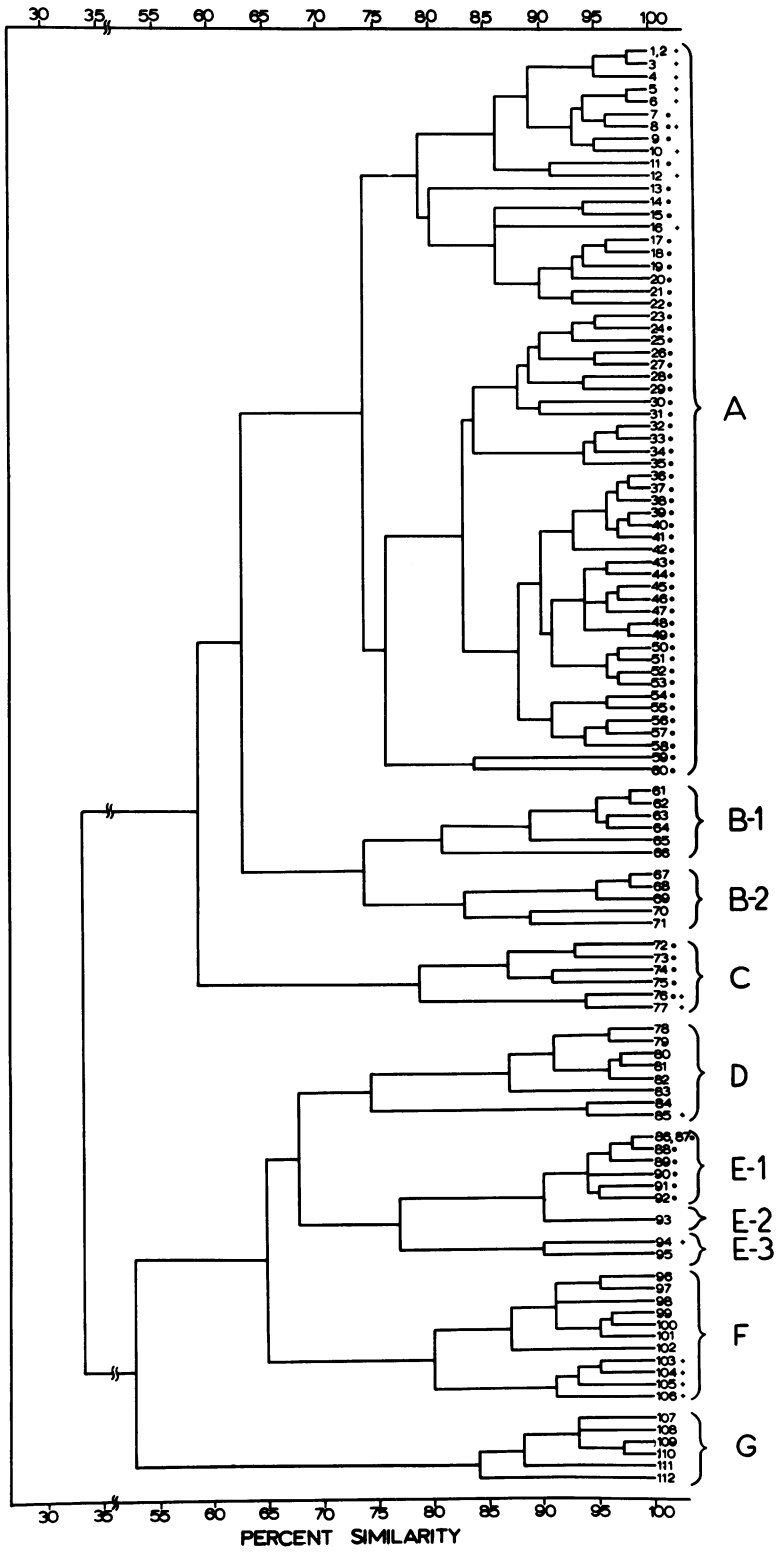

FIG. 29. *Numerical analysis of strains. With the exception of group B-2, (•) indicates that the strain has peritrichous flagella when grown on solid medium. The remaining strains have polar flagella. The symbol (+) indicates that the strain is a curved rod. All other strains are straight rods.*

TABLE 6. *Some distinguishing properties of the species and groups studied*[a]

Property	*B. parahaemolytica* (5 strains)	*B. campbellii*, group A (60 strains)	*P. fischeri*, group B-1 (6 strains)	Group B-2 (5 strains)	*B. neptuna*, group C (27 strains)[b]	*B. nereida*, group D (8 strains)	*B. alginolytica*, group E-1 (12 strains)[c]	Group E-3 (2 strains)	*B. pelagia*, group F (11 strains)	*B. natriegens*, group G (6 strains)
Peritrichous[d]	+	**51**	—[e]	—[e]	**26**	−	+	−	−	−
Single polar[d]	−	9	—[e]	—[e]	1	+	−	+	+	+
Straight rods	+	**50**	+	+	**23**	**7**	+	1	7	+
Curved rods	−	10	−	−	4	1	−	1	4	−
PHB[f] Accumulation	−	−	−	−	−	+	−	+	−	+
Arginine dihydrolase	−	−	−	−	1	+	−	1	−	−
Oxidase	+	+	+	−	+	+	+	+	+	+
Production of 2,3-butyleneglycol	−	−	−	−	−	−	+	−	−	−
Growth at 40 C	+	−	−	−	−	**7**	+	1	−	**5**
Amylase	+	**57**	−	−	+	2	+	+	4	2
Lipase	+	+	**5**	2	+	−	+	+	+	**5**
Alginase	−	−	−	−	3	−	−	−	**10**	−
Chitinase	+	**59**	+	2	+	**6**	+	−	**9**	−
Hemolysis at 37 C	+	45	−	1	**25**	−	+	−	3	3
D-Xylose	−	−	−	**4**	−	−	−	−	−	−
D-Galactose	+	−	+	+	**24**	2	2	−	+	+
Sucrose	−	−	−	3	+	+	+	+	+	+
Cellobiose	−	20	**5**	−	**23**	−	−	+	−	4
Salicin	−	−	−	−	11	−	−	−	−	+
D-Gluconate	+	5	−	+	**26**	+	+	+	+	+
D-Glucuronate	3	2	−	−	**23**	−	−	−	−	3
Valerate	1	−	−	−	−	+	+	+	−	+
Caprate	+	**51**	−	−	**24**	+	+	+	+	+
DL-β-Hydroxybutyrate	−	−	−	−	−	+	−	+	−	+
DL-Glycerate	+	24	4	−	**25**	2	+	−	−	**5**
α-Ketoglutarate	+	**57**	−	−	**26**	+	+	+	−	+
Pyruvate	+	**58**	−	+	+	+	+	+	+	+
Propanol	**4**	−	−	−	−	+	**11**	1	8	+
Glycine	−	10	−	−	**23**	**7**	+	+	+	+
L-α-Alanine	3	**48**	−	+	+	+	+	+	+	+
D-α-Alanine	+	**54**	−	−	**26**	+	+	+	+	+
L-Serine	3	22	−	+	+	3	+	+	+	+
L-Threonine	+	**52**	−	−	+	+	+	+	+	+
L-Leucine	+	−	−	−	2	**6**	+	1	−	**5**
L-Citrulline	−	−	−	−	−	+	−	−	+	**5**
L-Histidine	+	6	−	−	−	**6**	+	+	7	+
L-Proline	+	**58**	+	−	+	+	+	+	+	+
Putrescine	+	−	−	−	−	**6**	**10**	+	+	+
Sarcosine	−	−	−	−	−	−	−	+	−	+
L-Rhamnose	−	−	−	−	−	−	−	−	−	+
Benzoate	−	−	−	−	−	−	−	−	−	+
p-Hydroxybenzoate	−	−	−	−	−	−	−	−	−	+
Betaine	−	−	−	−	−	−	−	−	−	+
Hippurate	−	−	−	−	−	−	−	−	−	+

[a] +, All strains are positive; −, all strains are negative; numbers indicate number of positive strains; boldface numbers indicate that the number represents 80% or more of the strains.

[b] Includes strains 123–143.

[c] Includes strains 118–122.

[d] Flagellation of strains grown on solid medium.

[e] See text for discussion of flagellation.

[f] Poly-β-hydroxybutyric acid.

DISCUSSION

Choice of generic designation. Many of the strains studied have properties in common with three genera of gram-negative bacteria, *Vibrio*, *Aeromonas*, and *Beneckea*. The genus *Vibrio* is defined in Bergey's Manual on the basis of cell curvature and polar flagellation (7). It includes strict aerobes, strict anaerobes, and facultative anaerobes and encompasses a large number of diverse physiological types. In general usage, the genus *Vibrio* has usually been restricted to polarly flagellated, oxidase positive, curved rods which are facultatively anaerobic and ferment glucose with the production of acid but no gas. The high degree of subjectivity inherent in the determination of cell shape as well as the fact that this property is often altered upon prolonged cultivation have led to the suggestion that the genus *Vibrio* be expanded to include straight rods (16, 22). In addition, it has been suggested that the genus be restricted to organisms which have about 40 to 50 moles % GC in their DNA (12, 49, 56). The genus *Vibrio*, as currently defined, consists of at least two ecologically distinct groups: straight and curved rods of marine origin and *V. cholerae* and related strains which are inhabitants of fresh water and the human intestine. This ecological difference is reflected in the requirement for high concentrations of sodium or sea water by the marine strains (12, 40, 47, 58) and the lack of such a requirement in *V. cholerae* and related fresh water organisms (12, 47). As shown by MacLeod (33, 34) such a requirement may reflect differences in the cell walls and permease systems of organisms of marine and fresh water origin. The ecological distinction between marine and fresh water strains may, therefore, reflect a profound biochemical dissimilarity between these organisms.

The genus *Aeromonas* consists of oxidase positive, facultatively anaerobic, polarly flagellated or nonmotile, straight rods which ferment glucose with or without the production of gas (18, 19, 41). One species carries out a 2,3-butyleneglycol fermentation. Members of this genus are common inhabitants of fresh water. Some are pathogenic for fresh water fish. If *Vibrio* is defined to include straight and curved rods, it becomes difficult to differentiate the straight rods from species of *Aeromonas* which do not produce gas during the fermentation of glucose (16, 22). The sole reliable criterion for differentiation is a molecular one; members of the genus *Aeromonas* have 50 to 60 moles % GC in their DNA (24, 56).

The genus *Beneckea* is composed of peritrichously flagellated, facultatively anaerobic, straight rods of marine origin which decompose chitin and ferment glucose with the production of acid but no gas (7). With one exception, the described species were isolated by Campbell and Williams (10) in the course of a study of chitin-decomposing bacteria of marine origin. In addition to these strains, other chitin-decomposing organisms were obtained which had all the properties of the genus *Beneckea* but differed in their flagellation. These strains, which were polarly flagellated, were placed into the genus *Pseudomonas*, an assignment currently unsatisfactory due to the suggested restriction of this genus to organisms having only a respiratory metabolism (56, 57).

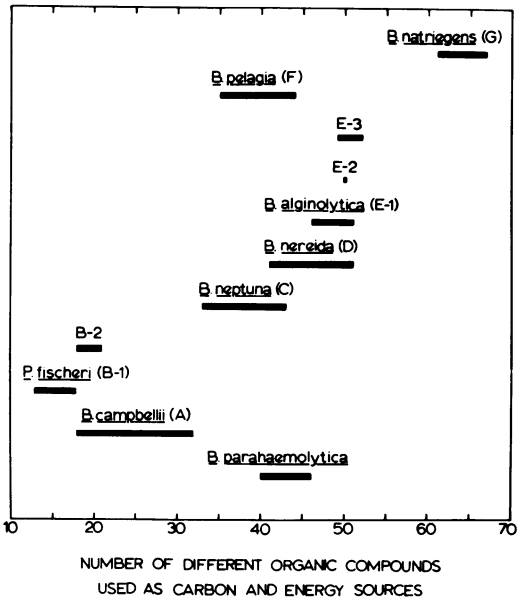

FIG. 30. *Nutritional versatility of the species and groups studied.*

The strains which were used in this study have a number of properties in common. All are of marine origin, require sodium ion for growth, and ferment glucose with the production of acid but no gas. Strains 113–117 as well as groups A, C, D, E-1, E-2, F, and G consist of straight and curved rods which have a single, sheathed (2), polar flagellum when grown in liquid medium. All are oxidase positive, and the moles % GC of the DNA of representative strains falls in the range of 45 to 48%. The strains which comprise these groups share a large number of nutritional and physiological properties (Tables 1–5). Although all strains have a single, sheathed, polar flagellum when cultivated in liquid medium, some synthesize unsheathed (2), peritrichous flagella when grown on solid medium. This prop-

TABLE 7. *CsCl buoyant density and guanine plus cytosine (GC) contents of DNA molecules of species of the genus Beneckea and other marine bacteria*

Species and/or group	Strain	Cell shape[a]	CsCl density (g/cm³)[b]	GC content (moles %)	Mean CsCl density and GC content ± σ
B. campbellii (A)	2	cr	1.705	45.9	
	8	cr	1.7048	45.7	1.7054 ± 0.0011
	19	sr	1.7053	46.2	46.3 ± 1.1% (14)[c]
	40	sr	1.7063	47.2	
B. neptuna (C)	72	sr	1.7055	46.4	
	74	sr	1.7055	46.4	1.7058 ± 0.0008
	76	cr	1.7065	47.4	46.8 ± 0.8% (6)
B. nereida (D)	80	sr	1.7055	46.4	
	82	sr	1.706	46.9	1.7060 ± 0.0009
	85	cr	1.7065	47.4	46.9 ± 0.9% (6)
B. alginolytica (E-1)	90	sr	1.7055	46.4	1.7053 ± 0.0010
	118	sr	1.705	45.9	46.2 ± 1.0% (4)
E-2	93	sr	1.7045	45.4	
B. pelagia (F)	99	sr	1.7055	46.4	
	101	sr	1.7055	46.4	1.7055 ± 0.0006
	103	cr	1.7055	46.4	46.4 ± 0.6% (6)
B. natriegens (G)	109	sr	1.7055	46.4	1.7055 ± 0.0006
	111	sr	1.7055	46.4	46.4 ± 0.6% (4)
B. parahaemolytica	113	sr	1.7055	46.4	1.7055 ± 0.0006
	115	sr	1.7055	46.4	46.4 ± 0.6% (4)
P. fischeri (B-1)	61	sr	1.6995	40.3	1.6990 ± 0.0008
	64	sr	1.6985	39.3	39.8 ± 0.8% (4)
Group B-2	68	sr	1.700	40.8	1.7003 ± 0.0010
	70	sr	1.7005	41.3	41.1 ± 1.0% (4)
Group E-3	94	cr	1.7125	53.6	1.7125 ± 0.0006
	95	sr	1.7125	53.6	53.6 ± 0.6% (4)
	114	cr	1.7045	45.4	
	145	sr	1.7095	50.5	

[a] cr, Curved rod; sr, straight rod.
[b] Mean of two determinations; strains 8 and 40, four analyses; strains 2 and 19, 3 analyses each.
[c] Number of analyses for group.

erty is present in many straight as well as curved rods. In spite of the differences in cell curvature and flagellation on solid medium, the members of groups A, C, D, E-1, E-2, F, and G as well as strains 113–117 have a large number of similarities suggesting their placement into a single genus.

Strains 113–117 and groups A, C, D, E-1, E-2, F, and G can readily be excluded from the genus *Aeromonas* by the lower GC content of their DNA and, in the case of some strains, by their cell curvature or by their peritrichous flagellation on solid medium, or by both. Group E-1 and the majority of the strains in groups A and C are excluded from the genus *Vibrio* by their peritrichous flagellation. Strains 113–117, which were received under the designation *V. parahaemolyticus*, were assigned to this genus partly on the basis of their polar flagellation (47). Our work has shown that strains 113–117, like the members of groups E-1, A, and C, become peritrichously flagellated when grown on solid medium. Exclusion of these peritrichously flagellated, marine organisms from the genus *Vibrio* is sup-

ported by the lack of hybridization between the DNA molecules of *V. cholerae* and *V. parahaemolyticus* (11) as well as by phenotypic (12) and ecological differences between these two species. The polarly flagellated strains in groups A, C, D, E-2, F, and G could be placed into *Vibrio* if the genus is defined to include straight and curved rods. A generic separation of the polarly and peritrichously flagellated strains would disregard the large number of phenotypic traits shared by these organisms.

Strains 113–117, group E-1, and the majority of the strains in groups A and C can be placed into the genus *Beneckea* on the basis of their peritrichous flagellation and numerous physiological, nutritional, and ecological similarities. The remaining groups and strains are excluded by their polar flagellation and, in the case of group G, by the inability to utilize chitin. The initial placement, by Campbell (7, 10), of phenotypically similar, chitin-utilizing isolates into the genera *Beneckea* and *Pseudomonas* was based on differences in the flagellation of strains grown on solid medium (L. L. Campbell, *personal communication*). Due to the great similarity between our isolates and those of Campbell and Williams (10), it is probable that the peritrichously flagellated strains which were placed into the genus *Beneckea* would have become polarly flagellated when cultivated in liquid medium. The overall similarity between the polar and peritrichous strains suggests their placement into one genus. We propose to redefine the genus *Beneckea* to include strains 113–117 as well as groups A, C, D, E-1, E-2, F, and G.

The genus *Beneckea* consists of nonpigmented, nonsporeforming, gram-negative, straight and curved rods of marine origin which when grown in liquid medium have a single, sheathed, polar flagellum. When grown on solid medium, many strains have unsheathed, peritrichous flagella in addition to the sheathed, polar flagellum. The GC content of the DNA ranges from 45 to 48 moles %. All are chemoorganotrophic, facultative anaerobes which ferment glucose as well as some other pentoses, hexoses, sugar alcohols, and sugar acids with the production of acid but no gas. One species produces 2,3-butyleneglycol as an end product of glucose fermentation. No strains denitrify, fix molecular nitrogen, or luminesce. Most strains reduce nitrates to nitrites. All are oxidase positive. One species has a constitutive arginine dihydrolase system, and two species accumulate PHB as an intracellular reserve product. All strains are capable of growth on a mineral medium containing ASW with glycerol as the sole source of carbon and energy and ammonia as the sole source of nitrogen. Sodium ion is required for growth. Most strains produce an extracellular amylase, gelatinase, lipase, and chitinase and hemolyze RBC. Many strains are able to utilize a variety of organic compounds as sole sources of carbon and energy, including pentoses, hexoses, disaccharides, sugar alcohols, and C_2-C_{10} monocarboxylic fatty acids. One species is able to degrade aromatic compounds via a *meta* cleavage. No strains hydrolyze agar, utilize cellulose, formate, C_6-C_{10} dicarboxylic acids, L-isoleucine, L-valine, L-lysine, aromatic amino acids, purines, pyrimidines, or *n*-hexadecane. The strains which comprise this genus are common inhabitants of the open sea, coastal water, and the gills and gut of marine fish and shellfish. One species is pathogenic for man.

Three groups, B-1, B-2, and E-3, which were established by numerical analysis could not be assigned to the genus *Beneckea*. Group E-3 consists of two strains, one straight and one curved rod. The DNA of each of these isolates contains 54 moles % GC. This is the sole property which excludes these strains from our definition of the genus *Beneckea*. Further work with additional strains is necessary before the taxonomy of these organisms can be resolved. The members of group B-1 luminesce and have 39 to 41 moles % GC in their DNA. On the basis of their morphology, physiology, nutrition, and the GC content of their DNA molecules, these strains were identified as *Photobacterium fischeri* (5, 7, 24–26). The strains belonging to group B-2 share two properties which separate them from the other strains in our collection. They are oxidase negative and have unsheathed (2), polar flagella. The moles % GC in the DNA of representative strains of group B-2 is 41%, which is barely distinguishable from the GC content of members of *P. fischeri*. These organisms do not luminesce, however, and differ from descriptions of luminous bacteria in many traits (7, 55). Other nonluminous marine organisms have been described with DNA base compositions similar to that of the strains in group B-2. *V. marinus* (14), a facultatively anaerobic organism of marine origin which ferments glucose with the production of acid but no gas, has a moles % GC of 40 to 42% in its DNA (13). This organism is different from group B-2 since it is oxidase positive, requires organic growth factors, and cannot grow at 35 C. The taxonomic placement of group B-2 must await further studies on luminous and nonluminous marine bacteria.

Choice of species designations. A strain kept in culture collection and designated *Beneckea hyperoptica* (strain 146) was characterized with respect to its nutrition, physiology, and morphology. The results indicated that this strain differed in numerous traits from the original description of *B. hyperoptica* (7). Unlike the description of *B. hyperoptica* strain 146 was a strict aerobe, required two or more amino acids for growth, failed to reduce nitrates to nitrites, did not hydrolyze starch, and was able to utilize succinate, fumarate, DL-lactate, citrate, and pyruvate. *B. hyperoptica* has been described as peritrichously flagellated. Our examination of this strain indicated that it had two to three flagella, arranged in polar tufts, when grown on either liquid or solid medium. The differences between strain 146 and the original description of *B. hyperoptica* indicate that it is not the original strain of this species.

The isolates of Campbell and Williams (10) have been well characterized with respect to their nutrition. There are 45 nutritional and physiological traits which are common to both of our studies. A comparison between the peritrichous species of Campbell and Williams (10) and the peritrichous, straight rods of groups A, C, and E-1 indicates that they differ in 11 to 21 traits. The polarly flagellated, straight rods of group D and F differ from the polarly flagellated species of Campbell and Williams (10) in 12 to 16 traits. The large number of differences between the species of Campbell and Williams and our isolates does not permit the assignment of their designations to any of our groups.

With a few exceptions, most marine bacteria have been poorly described with respect to physiology and nutrition. The speciation of well characterized strains is consequently difficult unless named strains are available for comparison. Assignment of species names to strains 113–117, group E-1, and group G presents no difficulties, since these groups include named strains. Groups A, C, D, and F do not contain named strains, nor do they have unusual or striking traits (e.g., pigmentation) which could make it possible to relate them to other distinctive marine organisms. We have given these groups new species designations. As seen from Fig. 29, all of our groups link at an *S* value of 68% or less. In addition, all of the groups and subgroups are readily identifiable entities which can be differentiated from each other on the basis of multiple, unrelated, phenotypic traits (Table 6).

Groups, A, C, D, and F, which bear new species designations, appear to have some internal subdivisions. As seen from Fig. 29, straight and curved rods tend to fall into separate clusters. This separation is not clear-cut within the 60 strains comprising group A, since the curved rods are located in a cluster interspersed with straight rods. In groups C, D, and F, which contain fewer strains, the separation of these two morphological types is more pronounced. It must be noted, that in spite of a tendency of straight and curved rods to segregate into different clusters, distinctive traits, some of which were not included in the computer analysis, often span the whole group. In the case of group C, all of the straight rods and three of the four curved rods have peritrichous flagella when grown on solid medium. (Two curved rods, strains 142 and 143, which have been placed into group C were not included in the computer analysis.) In group D, six straight rods are linked to a straight and curved rod at the 74% *S* value. In spite of this low linkage, all of the members of this group accumulate PHB and have a consitutive arginine dihydrolase system. In group F, all of the straight rods and three of the four curved rods have an extracellular alginase. The ability to hydrolyze alginate is restricted to this group and to three other strains of group C. No distinction can be made between the straight and curved rods on the basis of the GC content of their DNA (Table 7). Our results indicate that the straight and curved rods within a group have a large number of traits in common. In view of this, we have assigned to each group a single species designation.

The following discussion will consider the properties and the taxonomy of the groups studied. Selected phenotypic traits of use in distinguishing the various species and groups are presented in Table 6. The morphological, physiological, and nutritional properties of the strains are given in Tables 1–5 and their nutritional versatility in Fig. 30.

Beneckea campbellii sp. nov. (group A). The 60 isolates which comprise this species were obtained by direct isolation from ocean waters some distance from land. Ten of the strains were curved rods (Fig. 2); the remaining strains were straight rods (Fig. 1). When grown in liquid medium, all of the strains had a single, polar flagellum (Fig. 17). When grown on solid medium, the curved rods, with the exception of strain 8, had single, polar flagella; all of the straight rods and one curved rod (strain 8) had peritrichous flagella (Fig. 18). The moles % GC content of the DNA of strains 2 and 8 (curved rods) and strains 19 and 40 (straight rods) was 45.9, 45.7, 46.2, and 47.2, respectively. The seven analyses of the curved rods gave a mean of 45.8 ± 0.7% GC and a like number of analyses of the straight rods yielded 46.8 ± 1.2% GC; these differences

are not highly significant. Strain 40 (ATCC 25920) has been designated the type strain of this species. Although on the basis of the numerical analysis there appeared to be some subdivisions within group A (Fig. 29), no diagnostic traits were found which could unequivocally distinguish these subgroups. For this reason it seemed best to consider group A as one species, even though further study may reveal two or more biotypes. A similar situation has been observed in *Acinetobacter* (4), where 106 strains were placed into two groups by numerical analysis. A satisfactory separation on the basis of universally positive or negative traits could not, however, be obtained.

Photobacterium fischeri (group B-1). Five of the six strains belonging to this species were obtained by direct isolation and one by enrichment methods. All were straight rods (Fig. 3). When grown on either liquid or solid medium, strains of *P. fischeri* had two to six flagella, arranged in polar tufts (Fig. 26). The moles % GC content of the DNA of strains 61 and 64 was 40.3 and 39.3, respectively. Of the species studied, *P. fischeri* was the least nutritionally versatile (Fig. 30). Strain 61 has been deposited in culture collection (ATCC 25918).

Group B-2. The five strains belonging to this group were obtained by direct isolation. All were straight rods (Fig. 4). When grown in liquid medium, members of this group had polar flagella, arranged singly or in tufts of two (Fig. 27). Considerable difficulty was experienced with the flagella staining of strains grown on solid medium. The results indicated that some strains had polar flagella, whereas others became peritrichously flagellated (Fig. 28). The moles % GC of the DNA of strains 68 and 70 was 40.8 and 41.3, respectively. The members of this group were the only strains in our collection which were oxidase negative. Despite the poor nutritional versatility of *P. fischeri* and group B-2, they were readily separable on the basis of many unrelated, phenotypic traits (Table 6). An additional trait not listed in Table 6 was the ability of four of the five strains of group B-2 to grow at 4 C. Strain 68 has been deposited in culture collection (ATCC 25915).

Beneckea neptuna sp. nov. (group C). Of the six strains of this species which were included in the numerical analysis (Fig. 29), three were obtained by direct isolation and three by enrichment methods. Four of the strains were straight rods (Fig. 5); the remaining two were curved rods (Fig. 6). In liquid medium all of the strains had polar flagella (Fig. 17). On solid medium one curved rod (strain 76) and all the straight rods had peritrichous flagella (Fig. 19). The remaining curved rod had a single, polar flagellum. The moles % GC of the DNA of strains 72 and 74 (straight rods) and strain 76 (curved rod) was 46.4, 46.4, and 47.4, respectively. Strain 74 (ATCC 25919) has been designated the type strain of this species.

Strains 123–143 were isolated from marine animals after completion of the numerical analysis. After a phenotypic characterization, these strains could readily be placed into group C. The main difference between strains 123–143 and the strains which were subjected to the numerical analysis was their ability to utilize a greater number of monocarboxylic fatty acids (Table 5). Strains 123–141 were straight rods; strains 142 and 143 were curved rods. All of the strains had single, polar flagella when grown in liquid medium and peritrichous flagella when grown on solid medium. The polar and peritrichous flagella of a curved rod from group C is shown in Figs. 23 and 24, respectively. The straight rods in group C (strains 72–75, 123–141) could be differentiated from the curved rods (strains 76, 77, 142, 143) on the basis of three traits. Unlike the curved rods, the straight rods utilized cellobiose and D-glucuronate and were unable to utilize L-aspartate.

Beneckea nereida sp. nov. (group D). The eight strains comprising this species were isolated by enrichment methods. Seven of the strains were straight rods (Fig. 7), and one was a curved rod. When grown on either solid or liquid medium, all of the strains had a single, polar flagellum (Fig. 17). *B. nereida* was the only species in the genus *Beneckea* which had a constitutive arginine dihydrolase system as well as the ability to accumulate PHB as an intracellular reserve product (Fig. 14). Strains 80 and 82 (straight rods) and strain 85 (curved rod) had a moles % GC content in their DNA of 46.4, 46.9, and 47.4, respectively. Strain 80 (ATCC 25917) has been designated the type strain of this species. Strains 78–83 (straight rods) were linked to strain 84 (straight rod) and strain 85 (curved rod) at the 74% *S* value. These two subgroups were separable by a number of traits. Strains 78–83 utilized butyrate, isobutyrate, glutarate, L-leucine, γ-aminobutyrate, δ-aminovalerate, and putrescine, whereas strains 84 and 85 were unable to utilize these compounds. In addition, strains 84 and 85 had an extracellular amylase and utilized L-arabinose, D-mannose, D-galactose, mannitol, and DL-glycerate. For reasons previously discussed, these organisms were assigned to one species.

Beneckea alginolytica (Miyamoto, et al.) comb. nov. (group E-1), groups E-2, E-3, and related

strains. The seven strains of group E-1 which were included in the numerical analysis were isolated by means of enrichment techniques. All were straight rods (Fig. 8). When grown in liquid medium, all of the strains had a single, polar flagellum (Fig. 17); when grown on solid medium, they had peritrichous flagella (Fig. 20). A striking characteristic shared by these organisms was their ability to swarm on complex medium. All members of this species carried out a 2,3-butyleneglycol fermentation. After the completion of the numerical analysis, five additional strains (118–122) which had been obtained from clinical samples were submitted to a phenotypic characterization. These strains were identical to strains used in the numerical analysis, except for their inability to utilize isobutyrate. It is of interest to note that strains isolated from such diverse geographical locations as Hawaii, Washington, Rhode Island, and Florida were almost identical with respect to their phenotypic traits. The moles % GC of the DNA of strains 90 and 118 was 46.4 and 45.9, respectively. Since strain 118, the proposed type strain of *B. alginolyticus* (45), was a typical member of group E-1, we propose to designate this group *B. alginolytica*.

In a study of the organism responsible for an acute gastroenteritis arising from the consumption of raw fish, Sakazaki et al. (47) isolated a large number of bacteria from the feces of patients and from marine fish. Most of these isolates were placed into two biotypes of the species *V. parahaemolyticus*. The first biotype consisted of strains isolated from the stools of patients. These strains were unable to utilize sucrose and gave a negative VP test. The second biotype consisted of strains isolated from marine fish and from sea water. These strains utilized sucrose and gave a positive VP test. Zen-Yoji et al. (60) confirmed and extended these results. In 1968, Sakazaki (45) equated biotype 2 with *Oceanomonas alginolytica* of Miyamoto et al. (38) and designated this biotype *V. alginolyticus*. In addition, Sakazaki showed that one of the distinctive characteristics of this species was its ability to swarm on solid medium. From the poor description of *O. alginolytica*, it is difficult to conclude with certainty that biotype 2 is identical with this species. Although the species name *alginolytica* is unfortunate, since all or most of the strains designated by this name fail to hydrolyze alginate (45), it is legitimate to retain Sakazaki's species designation.

The single isolate belonging to group E-2 (strain 93) differed from *B. alginolytica* in that it had a single, polar flagellum on solid or liquid medium and did not exhibit swarming. In addition, unlike *B. alginolytica*, it had the ability to utilize cellobiose. The moles % GC of strain 93 was 45.4, similar to that of the other species of the genus *Beneckea*. The differences between strain 93 and *B. alginolytica* were not sufficient to justify separation at the species level. However, since petrichous flagellation and the ability to swarm on complex, solid medium are striking traits of *B. alginolytica*, it seems desirable to exclude strain 93 from this species pending further study of isolates similar to this strain.

The type strain of *Aeromonas proteolytica* (strain 145) and the species *B. alginolytica* shared two properties which were not common to the other species of the genus *Beneckea*. Both swarmed on complex, solid medium and produced 2,3-butyleneglycol as an end product of glucose fermentation. Strain 145 was isolated from marine sources and characterized by Merkel et al. (36). Its placement into the genus *Aeromonas* was primarily based on its polar flagellation. Although not stated by the authors, the cells used for flagella staining and electron microscopy were probably grown in liquid medium. Our results showed that strain 145 had a single, sheathed (2), polar flagellum when grown in liquid medium. On solid medium it had unsheathed (2), peritrichous flagella in addition to its sheathed, polar flagellum. Although strain 145 had some nutritional similarities to *B. alginolytica* (Table 5), it was unable to utilize sucrose, C_2-C_7 monocarboxylic fatty acids, and L-leucine. In addition, it differed from *B. alginolytica* in its ability to utilize sorbitol and β-alanine as well as in the possession of a constitutive arginine dihydrolase system. The moles % GC content of the DNA of strain 145 was 50.5, a value which is significantly different from that characteristic of *B. alginolytica*.

Another named strain, the suggested "working type" of *V. anguillarum* (strain 144), resembled *B. alginolytica* and group E-2 in the production of 2,3-butyleneglycol as an end product of glucose fermentation. This strain had a single, sheathed (2), polar flagellum when grown on either solid or liquid medium. The moles % GC of the DNA of strain 144 was 45.4, in the range of the genus *Beneckea*. Strain 144 differed from *B. alginolytica* and group E-2 in its ability to grow at 4 C and utilize sorbitol and in the possession of a constitutive arginine dihydrolase system. Unlike *B. alginolytica* and group E-2, strain 144 was unable to grow at 40 C or utilize DL-glycerate, citrate, aconitate, glycine, L-α-alanine, L-serine, L-threonine, L-leucine, L-arginine, and most of the monocarboxylic fatty acids. Phenotypic differences between strain 144 and *B. alginolytica* have also been observed by Sakazaki et al. (48).

The properties of strains 144 (*V. anguillarum*) and 145 (*A. proteolytica*) suggest that they be placed into the genus *Beneckea*. In the case of strain 145, the only trait excluding this organism from this genus is the somewhat higher moles % GC content of its DNA. The differences between these strains and the species of the genus *Beneckea* indicate that strain 144 and 145 deserve the status of distinct species. The authors are of the opinion that the formal taxonomy of these organisms should await the isolation and characterization of additional strains similar to 144 and 145.

Group E-3, which was related to *B. alginolytica* and group E-2 by 77%, differed from these strains in its ability to utilize caproate, glutarate, DL-β-hydroxybutyrate, inositol, δ-aminovalerate, and sarcosine as well as in its ability to accumulate PHB as an intracellular reserve product (Fig. 15). Unlike *B. alginolytica* and group E-2, strains 94 and 95 (group E-3) did not produce 2,3-butyleneglycol as an end product of glucose fermentation. In addition, these strains were unable to ferment D-gluconate, hydrolyze chitin, or utilize DL-glycerate. Both strains had a single, polar flagellum on either solid or liquid medium (Fig. 25); neither swarmed on complex, solid medium. Strain 94 was a curved rod and had a constitutive arginine dihydrolase system; strain 95 was a straight rod and lacked this enzyme system. The moles % GC of strains 94 and 95 was 53.6%, excluding these isolates from the genus *Beneckea*.

Beneckea pelagia sp. nov. (group F). Of the 11 strains belonging to this species, 10 were isolated by enrichment methods and one by direct isolation. Seven strains were straight rods (Fig. 10) and four were curved rods (Fig. 11). When grown on either solid or liquid medium, all had a single, polar flagellum (Fig. 17). The moles % GC of the DNA of strains 99 and 101 (straight rods) and strain 103 (curved rod) was 46.4 for each. The curved and straight rods clustered in two groups which were linked at the 80% *S* value (Fig. 29). Unlike the straight rods, the curved rods made an extracellular amylase and gelatinase and were able to utilize γ-aminobutyrate and δ-aminovalerate. All of the straight rods and three of the curved rods hydrolyzed alginate and utilized sucrose. Strain 106 was the only isolate which lacked these traits. Strain 99 (ATCC 25916) has been designated the type strain of this species. The ability of most strains of group F to decompose alginate raises the possibility of its relation or identity to some of the organisms placed into the genus *Alginomonas* (7). This genus is poorly defined, and the species descriptions are inadequate. None of the original isolates of *Alginomonas* were available for comparison with our strains, and it was not possible to identify any of the described species with group F. Eller and Payne (20) isolated an alginate-decomposing, gram-negative rod from marine sources which they designated *Alginomonas alginica*. This strain was later redesignated *Agarbacterium alginicum* when it was found to be peritrichously flagellated (1). Since all of the members of group F were polarly flagellated on either solid or liquid medium, the isolate of Eller and Payne appears to be different from *B. pelagia*.

Beneckea natriegens (Payne et al.) comb. nov. (group G). The six strains comprising this species were isolated by enrichment methods. All were rods (Fig. 12) which had a single, polar flagellum when grown on solid or liquid medium (Fig. 17). All strains accumulated PHB as an intracellular reserve product (Fig. 16). The moles % GC of strains 109 and 111 was 46.4. This species was the most nutritionally versatile member of the genus *Beneckea* (Fig. 30) and was the only species in our study which utilized L-rhamnose, benzoate, *p*-hydroxybenzoate, betaine, and hippurate. Cells grown on *p*-hydroxybenzoate cleaved protocatechuate by means of a *meta* cleavage. None of the strains hydrolyzed chitin. Strain 111, the type strain of *Pseudomonas natriegens*, was isolated and characterized by Payne et al. (42). Like all of the strains in our collection, it was a facultative anaerobe. This property excludes it from the genus *Pseudomonas* (57). As seen in Fig. 29 and Table 5, strain 111 is a typical member of group G, indicating that this group should be designated *B. natriegens*.

Beneckea parahaemolytica (Fujino et al.) comb. nov. (strains 113–117). The five strains belonging to this species were obtained after the completion of the numerical analysis. All were isolated from the feces of patients suffering from gastroenteritis contracted from the comsumption of raw marine fish. Strains 113–117 were phenotypically closely related (Tables 1–5) and formed a group which could be readily differentiated from the other species of the genus *Beneckea* (Table 6). All of the strains were straight rods (Fig. 13) which when grown in liquid medium had a single, polar flagellum (Fig. 21). When grown on solid medium, the strains had peritrichous flagella (Fig. 22). The moles % GC content of the DNA of strains 113 and 115 was 46.4. Since strain 113, the proposed type strain of *V. parahaemolyticus*, is representative of the group formed by strains 113–117, we propose to designate this species. *B. parahaemolytica*.

There are at the present no adequate diagnostic criteria for the identification of *B. para-*

haemolytica. The best indication of the presence of this organism is the isolation of a hemolytic salt-requiring, gram-negative, motile rod which ferments glucose with the production of acid but no gas from the stools of patients suffering from gastroenteritis contracted from the consumption of raw marine fish. Additional traits of use are a negative VP test, lack of swarming on solid medium, and the inability to utilize sucrose and cellobiose (45–47, 58, 60). Another trait, which is of limited use since it is not present in all strains of *B. parahaemolytica*, is the ability to utilize arabinose. The identification of this organism is therefore linked to its isolation from clinical or subclinical infections in man. Application of these tests to isolates from sea water or marine animals is not diagnostic for *B. parahaemolytica* since all of these traits (with the exception of arabinose utilization) are present in *B. campbellii*. In several studies, even fewer traits have been used (3, 23) for the identification of these strains, and it is impossible to establish whether the marine isolates studied were *B. parahaemolytica* or other species of the genus *Beneckea*. In a study of the distribution of this organism around the Hawaiian Archipelago, Yasunaga (59) isolated strains having the general properties of the genus *Beneckea*. Most of his isolates were able to utilize arabinose but not sucrose. The only *Beneckea* species which is sucrose negative and arabinose positive is *B. parahaemolytica*, suggesting that at least some of the organisms isolated by Yasunaga (59) belonged to this species.

The traits diagnostic for *B. parahaemolytica* are growth at 40 C; hemolysis of RBC, ability to utilize L-leucine, L-histidine, and putrescine as sole sources of carbon and energy; and the inability to utilize sucrose, cellobiose, DL-β-hydroxybutyrate, glycine, and *p*-hydroxybenzoate. An additional distinctive trait present in some strains is the ability to utilize L-arabinose. The combination of these traits in one strain is unique to *B. parahaemolytica* and distinguishes it from all other species of the genus *Beneckea*, *P. fischeri*, and groups B-2, E-2, and E-3. Other traits of use are the peritrichous flagellation of cells grown on solid medium, a negative VP test, the absence of a constitutive arginine dihydrolase system, and the inability to accumulate PHB. Several workers have established that the abilities to grow at 40 C and hemolyze RBC and the inability to utilize sucrose are present in most or all clinical isolates of *B. parahaemolytica* (45, 47, 58, 60). The remaining diagnostic traits were present in five authentic strains of *B. parahaemolytica* included in our collection. Our study has established the value of these diagnostic traits by showing that they differentiate *B. parahaemolytica* from a large number of otherwise similar marine bacteria.

Our work should be regarded as a beginning effort at phenotypic characterization and speciation of gram-negative, flagellated, facultatively anaerobic, marine bacteria. Although we have established a well defined genus and several well characterized species, part of our taxonomic analysis remains inconclusive. This inconclusiveness is due to our unwillingness to designate species on the basis of one or two strains. It should be stressed that the samples from which our strains were isolated came from a geographically restricted area. This may in part explain our inability to obtain strains similar to 144 and 145 which were isolated from samples obtained at more northern latitudes.

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