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Taxonomy of Aerobic Marine Eubacteria

LINDA BAUMANN, PAUL BAUMANN, M. MANDEL, AND RICHARD D. ALLEN

Department of Microbiology, University of Hawaii, Honolulu, Hawaii 96822, and Department of Biology, The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77025

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Two hundred and eighteen strains of nonfermentative marine bacteria were submitted to an extensive morphological, physiological, and nutritional characterization. All the strains were gram-negative, straight or curved rods which were motile by means of polar or peritrichous flagella. A wide variety of organic substrates served as sole sources of carbon and energy. The strains differed extensively in their nutritional versatility, being able to utilize from 11 to 85 carbon compounds. Some strains had an extracellular amylase, gelatinase, lipase, or chitinase and were able to utilize *n*-hexadecane and to denitrify. None of the strains had a yellow, cell-associated pigment or a constitutive arginine dihydrolase system, nor were they able to hydrolyze cellulose or agar. The results of the physiological and nutritional characterization were submitted to a numerical analysis which clustered the strains into 22 groups on the basis of phenotypic similarities. The majority of these groups were separable by a large number of unrelated phenotypic traits. Analysis of the moles per cent guanine plus cytosine (GC) content in the deoxyribonucleic acid of representative strains indicated that the peritrichously flagellated groups had a GC content of 53.7 to 67.8 moles %; polarly flagellated strains had a GC content of 30.5 to 64.7 moles %. The peritrichously flagellated groups were assigned to the genus *Alcaligenes*. The polarly flagellated groups, which had a GC content of 43.2 to 48.0 moles %, were placed into a newly created genus, *Alteromonas;* groups which had a GC content of 57.8 to 64.7 moles % were placed into the genus *Pseudomonas;* and the remaining groups were left unassigned. Twelve groups were given the following designations: *Alteromonas communis*, *A. vaga*, *A. macleodii*, *A. marinopraesens*, *Pseudomonas doudoroffi*, *P. marina*, *P. nautica*, *Alcaligenes pacificus*, *A. cupidus*, *A. venustus*, and *A. aestus*. The problems of assigning species of aerobic marine bacteria to genera are discussed.

Gram-negative, heterotrophic bacteria which are motile by means of flagella are readily isolated from the ocean and appear to comprise a major component of the bacterial flora of the sea (28, 32, 34, 35, 41). Most or all of these organisms require sodium ion for growth (32, 34, 35, 41). On the basis of their ability to ferment carbohydrates, these strains can be subdivided into two large groups. The fermentative strains have recently been the object of a taxonomic study which has shown that most of these isolates can be placed into the genus *Beneckea* (1, 5). The nonfermentative strains have in the past been assigned to a large number of genera, including *Pseudomonas*, *Achromobacter*, *Alcaligenes*, *Vibrio*, *Flavobacterium* (6, 21, 24, 33, 34, 41, 47, 55) and *Arthrobacter* (7); however, the taxonomy of these isolates is unsatisfactory since generic and specific assignments have usually been made on the basis of an inadequate characterization. The limited number of traits used and their often dubious validity make it difficult if not impossible to assign new strains to previously named species. In addition, type strains are not available for most species, so that further characterization and comparison with new isolates is not possible. The diversity of the aerobic marine bacteria, which is suggested by the number of genera to which they have been assigned, is confirmed by the wide range of the moles per cent guanine plus cytosine (GC) content in their deoxyribonucleic acid (DNA) contents. In a study of 57 marine isolates, Leifson and Mandel (33) found that peritrichously flagellated aerobes had DNA containing 49 to 67 moles % GC and that aerobic, polarly flagellated strains had GC contents of 40 to 67 moles %.

The present study is concerned with the

application of the methods of Stanier et al. (51) to the characterization of 218 nonfermentative, flagellated, marine bacteria, none of which produces a yellow, cell-associated pigment. The results indicate that this collection of strains consisted of a number of well defined species and groups the majority of which could be assigned to *Pseudomonas*, *Alcaligenes*, and a newly created genus, *Alteromonas*.

MATERIALS AND METHODS

The methods as well as the composition of the media used in this study have been previously described (5, 51). Only additional methods or significant modifications will be considered. Unless otherwise stated, all cultures were incubated at 25 C. The abbreviations used are the following: ASW (artificial sea water), BM (basal medium), BMA (basal medium agar), YEB (yeast extract broth), YEA (yeast extract agar), MA (Difco Marine Agar), F-1 (fermentation medium 1), F-2 (fermentation medium 2), PHB (poly-β-hydroxybutyrate), and Tris [tris(hydroxymethyl)aminomethane]. These abbreviations are the same as those used previously (5). The majority of the strains were maintained on MA slants at 20 to 22 C and were transferred every 4 weeks. A few strains, however, rapidly lost their viability and had to be transferred every 2 weeks. Some strains grew poorly on MA and were maintained on BMA containing 0.2% potassium lactate. Tests for sodium and organic growth factor requirements were performed as previously described (5) with a medium containing 0.1% potassium succinate, 0.1% potassium lactate, and 0.1% potassium acetate. Strain 214 grew slowly in BM containing 0.2% D-glucose, potassium succinate, or potassium acetate. The results of the nutritional screening of this strain by replica plating were difficult to interpret since only slight growth occurred on BMA containing the tested carbon compound (5, 51). Growth was improved when the solid medium was supplemented with 10 mg of L-methionine, L-arginine, L-histidine, L-leucine, and L-isoleucine per liter and 20 mg of DL-valine per liter. This medium was devised by J. L. Reichelt in the course of a separate study involving the nutritional screening of growth factor requiring facultative anaerobes of marine origin. Strain 214 did not have growth factor requirements, however, since it could be successively transferred in a minimal medium.

Fluorescin production. The ability to produce fluorescin was tested by inoculating strains onto medium B of King et al. (30), modified by the addition of half-strength ASW. Three strains of *P. fluorescens* gave good pigment production when grown on this medium.

Aromatic ring cleavage. Strains capable of growth on benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, L-tryptophan, or quinate were grown on these compounds and tested for the mechanism of aromatic ring cleavage (5, 51). This test was unsuccessful with benzoate-grown cells of all the strains of group C-3 and strains 168, 177 to 186, 191, and 192 of group G-3. The mechanism of cleavage of diphenolic intermediates by these strains was determined by spectrophotometric methods, using cell-free extracts. Strains 66, 70, 74, and 76 from group C-3 as well as all the strains of group G-3 which were able to grow on benzoate were grown on BM containing 0.15% sodium benzoate. The cells were centrifuged, washed twice in half-strength ASW containing 50 mM Tris-hydrochloride (*p*H 7.5), and suspended in 100 mM Tris-hydrochloride (*p*H 8.0) containing 0.1 mM ethylenediaminetetraacetic acid. Following ultrasonic disruption of the cells, the suspension was centrifuged for 30 min at 30,000 $\times$ *g*, and the supernatant fraction was assayed for protocatechuate 3,4 oxygenase (50), protocatechuate 4,5 oxygenase (53), catechol 1,2 oxygenase (25), and catechol 2,3 oxygenase (14).

Oxidase test. The method described by Stanier et al. (51) was used in this study. In the case of some strains, the oxidase reaction developed slowly, making interpretation difficult. The speed of the color change was greatly accelerated by the addition of a drop of toluene prior to the addition of 1% (w/v) *N,N'*-dimethyl-*p*-phenylenediamine. Addition of toluene did not affect the oxidase reaction of known oxidase-negative strains (*Escherichia coli*, *Acinetobacter calco-aceticus*, and *P. maltophilia*).

Morphology and flagellation. Morphological examination of cells was performed as previously described (5). The medium used was YEB containing 0.1% sodium succinate, 0.1% sodium lactate, and 0.1% sodium acetate. Each strain was grown in this medium, and its flagella were stained by the method of Leifson (5, 31). Strains 51 to 57 (groups B-1 and -2), 139 to 144 (group F-2), 209 to 213 (group I-2), and two representative strains from each of the remaining groups were grown in the above medium, negatively stained, and examined by means of the electron microscope (1). In addition, representative strains were grown on MA and stained for flagella by the Leifson method.

DNA base compositions. The moles per cent GC in the DNA of representative strains was determined from buoyant density measurements in CsCl gradients (36). Reference DNA from bacteriophage 2C (*Bacillus subtilis* host) at a density of 1.742 g/cm^3 was included in each gradient.

Methods of isolation. The enrichment methods used in this study have been previously described (5). The strains were purified by streaking on BMA containing 0.1% of the same carbon compound as used in the enrichment or on BMA containing 0.1% sodium succinate, 0.1% sodium lactate, and 0.1% sodium acetate. In the case of strains obtained by direct isolation, sea water samples were collected aseptically and filtered through 0.45 μm-pore size filters (Millipore Corp.). The filters were placed onto MA or BMA containing 0.1% of the organic substrate. Subsequent purification of strains isolated on defined media was performed by streaking on homologous media. Strains obtained by direct isolation on MA were purified by streaking on BMA containing 0.1% sodium succinate, 0.1% sodium lactate, and 0.1% sodium acetate.

Source of strains examined. The information in

parentheses following each strain number indicates the method of isolation, the carbon compound supporting the initial growth, and the source of sea water. A carbon compound in parentheses indicates that the strain was isolated 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. A carbon compound followed by the letters DI indicates that the strain was obtained by direct isolation in which the designated compound was the sole source of carbon and energy. In the case of some of the strains obtained by direct isolation, MA was the medium supporting the initial growth. When depth (m) of sampling is indicated, the strains were obtained from samples taken at different stations 10 to 35 miles off the coast of Oahu, Hawaii. Strains undesignated with respect to the depth of sampling were obtained from samples of surface water off the immediate coast of Oahu. An asterisk (*) following the carbon compound indicates that the isolated strain was unable to utilize the carbon compound used for its initial isolation. These strains, which were obtained from enrichments, were purified on media which differed in composition from the enrichment medium and probably represented a minority component of the total enrichment flora.

Strain assignment. Strains assigned to *Alteromonas communis* sp. nov. (group A-1) were: 1 (chitin*); 2 (chitin*); 3 (betaine); 4 (chitin*); 5 (betaine, DI); 6 (ethanol); 7 (β-alanine); 8 (DL-β-hydroxybutyrate); 9 (*p*-hydroxybenzoate); 10 (*m*-hydroxybenzoate); 11 [L-(+)-tartrate]; 12 (acetate); 13 (benzoate*); 14 (L-lysine); 15 (citrate); 16 (L-glutamate); 17 (glycerol); 18 (ethanol); 19 (benzoate*); 20 (betaine); 21 (L-glutamate); 22 (*p*-hydroxybenzoate); 23 (L-proline); 24 (L-proline); 25 (butyrate*); 26 (β-alanine); 27 (acetate); 28 (benzoate*); 29 (*m*-hydroxybenzoate); 30 [L-(+)-tartrate]; 31 (L-lysine); 32 (*o*-hydroxybenzoate*); and 33 (citrate).

Strains assigned to *Alteromonas vaga* sp. nov. (group A-2) were: 34 (*m*-hydroxybenzoate); 35 (acetate); 36 (*m*-hydroxybenzoate); 37 (betaine); 38 (*m*-hydroxybenzoate, DI); 39 (L-lysine); 40 (glycerol); 41 (L-glutamate); 42 (*m*-hydroxybenzoate); 43 (sarcosine); 44 (sarcosine); 45 (L-lysine); 46 (*m*-hydroxybenzoate); 47 (L-histidine); 48 (sarcosine); 49 (citrate); and 50 (succinate).

Strains assigned to group B-1 were: 51 (nicotinate, DI); 52 (L-isoleucine); and 53 (L-lysine).

Strains assigned to group B-2 were: 54 (betaine, DI, 10 m); 55 (L-tyrosine, 5 m); 56 (L-tyrosine, DI); 57 (histamine, DI, 300 m).

Strains assigned to group C-1 were: 58 (creatine, DI); and 59 (creatine, DI).

Strains assigned to *Alcaligenes pacificus* sp. nov. (group C-2) were: 60 (L-histidine); 61 (L-lysine); 62 (L-lysine); 63 (laevulinate); 64 (histamine); and 65 (L-glutamate).

Strains assigned to *Pseudomonas doudoroffii* sp. nov. (group C-3) were: 66 (allantoin); 67 (benzoate); 68 (creatine); 69 (creatine, NO_3^-); 70 (allantoin); 71 (allantoin); 72 (allantoin); 73 (allantoin); 74 (creatine); 75 (caprylate*); and 76 (δ-aminovalerate, DI).

Strains assigned to group D-1 were: 77 (L-valine, DI); and 78 (isobutyrate, DI).

Strains assigned to *Alcaligenes cupidus* sp. nov. (group D-2) were: 79 (allantoin); 80 (glycolate); 81 (glycolate); 82 (ethyleneglycol); and 83 (L-mandelate, DI).

Strains assigned to *Alcaligenes venustus* sp. nov. (groups D-3 and D-4) were: 84 (L-histidine); 85 (L-lysine); 86 (*o*-hydroxybenzoate); 87 (*o*-hydroxybenzoate); 88 (L-lysine); 89 (L-lysine); 90 (L-lysine); 91 (L-histidine); 92 (nicotinamide, DI); 93 (nicotinamide); 94 (nicotinamide, DI); 95 (L-histidine); 96 (nicotinamide); and 97 (histamine).

Strains assigned to *Alteromonas macleodii* sp. nov. (group E-1) were: 98 (lactose, DI); 99 (lactose, DI); 100 (lactose, DI); 101 (lactose, DI); 102 (lactose, DI); 103 (caprylate, DI, 5 m); 104 (L-tyrosine, 10 m); 105 (butyrate, DI, 100 m); 106 (chitin*); 107 (lactose, DI); 108 (lactose, DI); 109 (MA, DI, 750 m); 110 (MA, DI, 750 m); 111 (MA, DI, 750 m); 112 (MA, DI, 100 m); 113 (MA, DI, 750 m); 114 (MA, DI, 500 m); 115 (MA, DI, 1100 m); 116 (MA, DI, 750 m); 117 (MA, DI, 1100 m); and 118 (MA, DI, 750 m).

Strains assigned to *Alteromonas marinopraesens* comb. nov. (group E-2) were: 119 (L-lysine*, NO_3^-); 120 (L-serine, NO_3^-); 121 (L-lysine*, NO_3^-); 122 (L-serine); 123 (L-serine); 124 (L-tyrosine); 125 (glycine); 126 (L-histidine); 127 (L-serine, DI); 128 (laevulinate*); 129 (laevulinate*); 130 (L-valine, DI); 131 (L-serine); 132 (laevulinate*); 214 (B-16 of R. A. MacLeod, ATCC 19855); 215 (*Vibrio haloplanktis*, ATCC 14393); and 216 (*Vibrio marinopraesens*, ATCC 19648).

Strains assigned to *Alcaligenes aestus* sp. nov. (group F-1) were: 133 (*meso*-inositol, DI, 100 m); 134 (*meso*-inositol, DI, 200 m); 135 (creatine, DI, 200 m); 136 (L-valine, DI, 600 m); 137 (adipate, DI, 150 m); and 138 (β-alanine, DI, 600 m).

Strains assigned to *Pseudomonas marina* comb. nov. (group F-2) were: 139 (sarcosine, DI); 140 (D-galactose, DI); 141 (caprylate, DI); 142 (*meso*-inositol, DI); 143 (2,3-butyleneglycol, DI); 144 (sarcosine, DI); and 219 (*Arthrobacter marinus*, ATCC 25374).

Strains assigned to group G-1 were: 145 (*n*-butanol); 146 (*n*-propanol); 147 (malonate*); 148 (L-valine); 149 (*n*-butanol); 150 (D-mandelate); 151 (2,3-butyleneglycol); 152 (histamine); 153 (L-valine, DI); 154 (histamine, DI); and 155 (isobutanol, DI).

Strains assigned to group G-2 were: 156 (laevulinate); 157 (caprylate, NO_3^-); 158 (glycine); 159 (β-alanine, DI); and 160 (β-alanine, DI).

Strains assigned to *Pseudomonas nautica* sp. nov. (group G-3) were: 161 (chitin*, 600 m); 162 (butyrate, NO_3^-); 163 (butyrate, NO_3^-); 164 (butyrate, NO_3^-); 165 (butyrate, NO_3^-); 166 (caprylate, NO_3^-); 167 (valerate, NO_3^-); 168 (acetate, NO_3^-); 169 (butyrate, NO_3^-); 170 (butyrate, NO_3^-); 171 (propyleneglycol); 172 (adipate, DI); 173 (adipate, DI); 174 (butyrate, NO_3^-); 175 (butyrate, NO_3^-); 176 (butyrate, NO_3^-); 177 (adipate); 178 (lactate, NO_3^-); 179 (ace-

tate, NO_3^-); 180 (caprylate, NO_3^-); 181 (MA, DI, 750 m); 182 (MA, DI, 750 m); 183 (MA, DI, 750 m); 184 (MA, DI, 1100 m); 185 (MA, DI, 750 m); 186 (MA, DI, 500 m); 187 (butyrate, DI); 188 (caprylate, NO_3^-); 189 (butyrate, NO_3^-); 190 (butyrate, NO_3^-); 191 (laevulinate, 600 m); 192 (adipate, 600 m); 193 (adipate); and 194 (butyrate, DI).

Strains assigned to group H-1 were: 195 (malonate); 196 (betaine); 197 (sorbitol, DI); and 198 (*meso*-inositol, DI).

Strains assigned to group H-2 were: 199 (succinate, NO_3^-); 200 (propionate, NO_3^-); 201 (acetate, NO_3^-); 202 (malonate*); 203 (succinate); and 204 L-citrulline, DI, 600 m).

Strains assigned to group I-1 were: 205 (L-proline); 206 (β-alanine); 207 (L-leucine); and 208 (L-serine, DI).

Strains assigned to group I-2 were: 209 (creatine, DI); 210 (creatine, DI); 211 (allantoin, DI, 150 m); 212 (betaine, NO_3^-); 213 (sarcosine, DI).

Other strains were: 214, 215, 216 (see group E-2); 217 (*P. nigrifaciens*, ATCC 19375); 218 (*P. perfectomarinus*, ATCC 14405); 219 (see group F-2).

RESULTS

Sodium and organic growth factor requirements. All the strains in our collection were able to grow in BM (which contained 0.2 M sodium) supplemented with 0.1% potassium succinate, 0.1% potassium lactate, and 0.1% potassium acetate, to a final turbidity of 160 to 340 Klett units. When the sodium salts in BM were replaced with equimolar amounts of potassium salts, the medium did not support the growth of our isolates (final turbidity readings 0 to 15 Klett units). These results indicated that the strains had no organic growth factor requirements but required sodium ion for growth.

Fermentation. All the strains were tested for their ability to ferment D-glucose by the use of two media, F-1 and F-2 (5). Strains 67 to 76, which were able to grow on D-fructose but not D-glucose, were also tested on media which differed from F-1 and F-2 in containing D-fructose. Only those strains which were unable to ferment D-glucose or D-fructose were included in this study.

Gram stain, cell shape, and motility. All the cells were gram-negative when stained in exponential phase of growth. Strains 1 to 33 (Fig. 1), 199, 200, and 201 were curved rods; the remaining strains were straight rod (Fig. 2–19). With the exception of strains 142, 191, 192, 218, and 219, all the strains were motile. Strains of groups B-1, B-2 and I-2 had a tendency to aggregate into rosettes (Fig. 5 and 6). The aggregated cells always comprised a minority of the cell population. Nonstalked, rosette-forming marine bacteria have been previously described by Leifson et al. (32). Strains of groups B-2 and I-2 often gave rise to involution forms whose extent and occurrence varied with the phase of growth and the composition of the medium. Involution forms were more frequent in YEB supplemented with 0.1% sodium succinate, 0.1% sodium lactate, and 0.1% sodium acetate than in BM containing the latter three substrates. In general, fewer involution forms were observed in exponential than in early stationary phase of growth.

Pigment production. None of the strains made fluorescin or had a yellow, cell-associated pigment. Strains 68, 70, 71, and 72 (group C-3) produced a soluble, light brown pigment on MA after 6 to 14 days of incubation at 20 to 22 C. No pigment was produced by these strains on a minimal medium. Strains 209, 210, and 213 (group I-2) gave rise to colonies containing a blue-black pigment which was not soluble in water. Pigment production was more intense on BMA containing 0.2% sodium lactate than on YEA. The remaining strains were nonpigmented.

Tests negative for all strains. None of the strains was able to fix molecular nitrogen or grow chemolithotrophically with molecular hydrogen as the sole source of energy and carbon dioxide as the sole source of carbon. None of the strains luminesced or made slime on BMA containing 5% (w/v) sucrose. The following strains were tested for a constitutive arginine dihydrolase system and were found to be negative: 2, 8, 19, 32 (group A-1); 40, 44, 47, 50 (group A-2); 51, 52 (group B-1); 54, 57, (group B-2); 58, 59 (group C-1); 60, 62, 64 (group C-2); 66, 70, 74 (group C-3); 77, 78 (group D-1); 79, 81 (group D-2); 84, 86 (group D-3); 89, 93, 96 (group D-4); 99, 101, 107, 113, 117 (group E-1); 121, 124, 130 (group E-2); 133, 135 (group F-1); 139, 141, 143 (group F-2); 146, 149, 153, 155 (group G-1); 156, 158 (group G-2); 171, 175, 181, 185, 190, 193 (group G-3); 196, 197 (group H-1); 201, 202 (group H-2); 205, 207 (group I-1); 210, 213 (group I-2); and strains 214–219.

Oxidase test. Strains 34 to 50 (group A-2), 79 to 83 (group D-2), and 139 to 144 (group F-2) were oxidase negative. The remaining strains were oxidase positive.

Range of organic compounds utilized. A total of 149 organic compounds were tested for their ability to serve as sole sources of carbon and energy. Of these, 127 were utilized by one or more strains in our collection (Table 1).

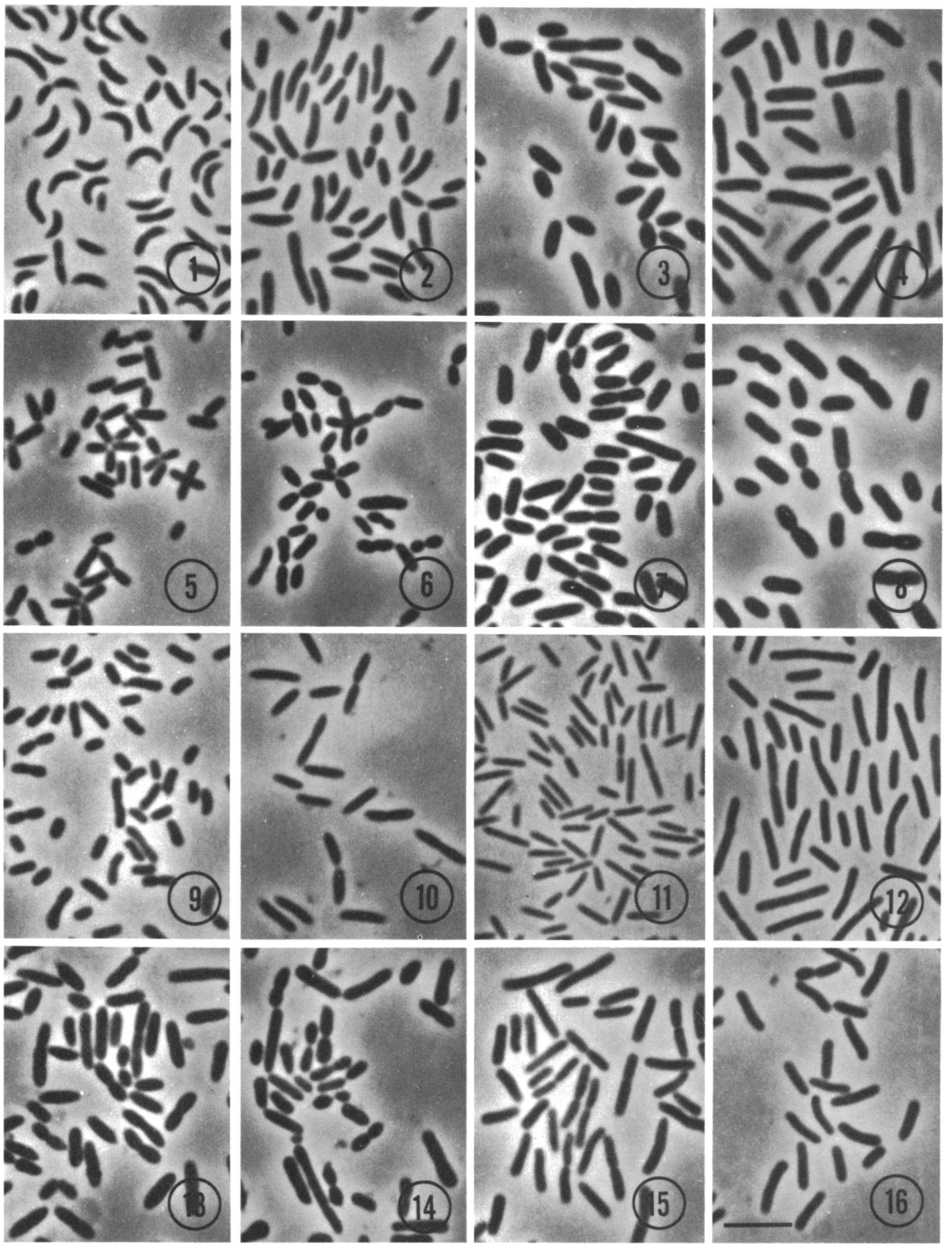

FIG. 1–16. *Phase-contrast micrographs of cells in exponential phase of growth* (× 2,000). *Marker in Fig. 16 represents 5 μm. Fig. 1, Alteromonas communis, strain 8; Fig. 2, A. vaga, strain 47; Fig. 3, A. macleodii, strain 107; Fig. 4, A. marinopraesens, strain 121; Fig. 5, group B-1, strain 51; Fig. 6, group B-2, strain 55; Fig. 7, Pseudomonas doudoroffii, strain 70; Fig. 8, P. marina, strain 140; Fig. 9, group G-1, strain 146; Fig. 10, group G-2, strain 160; Fig. 11, P. nautica, strain 179; Fig. 12, group H-1, strain 197; Fig. 13, group I-1, strain 207; Fig. 14, group I-2, strain 210; Fig. 15, Alcaligenes pacificus, strain 62; and Fig. 16, A. cupidus, strain 79.*

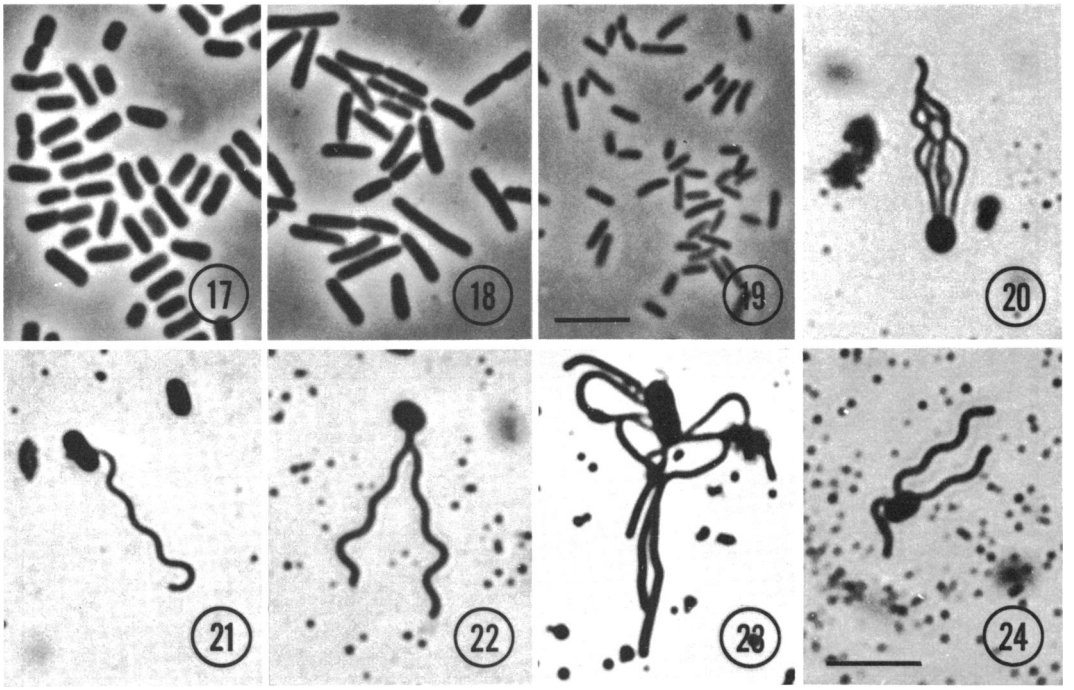

FIG. 17-19. *Phase-contrast micrographs of cells in exponential phase of growth* (× 2,000). *Marker in Fig. 19 represents 5 μm; Fig. 17, Alcaligenes venustus, strain 86; Fig. 18, A. aestus, strain 134; and Fig. 19, group H-2, strain 202.*

FIG. 20-24. *Variation in Leifson flagella stains characteristic of strains in groups B-1, B-2, I-2, and P. marina (group F-2). Photomicrographs illustrating single polar flagella have not been included.* (× 2,500). *Marker in Fig. 24 represents 5 μm; Fig. 20, P. marina, strain 143; Fig. 21, group B-1, strain 52; Fig. 22, group I-2, strain 211; Fig. 23, P. marina, strain 140; and Fig. 24, P. marina, strain 143.*

Only acetate and pyruvate served as universal carbon sources. The compounds which were not utilized by any of the strains were: cellulose, agar, inulin, mucate, formate, oxalate, methanol, isopropanol, geraniol, phenylethanediol, naphthalene, DL-norleucine, DL-α-aminobutyrate, D-tryptophan, *m*-aminobenzoate, *p*-aminobenzoate, methylamine, tryptamine, α-amylamine, 2-amylamine, pantothenate, and guanine.

Numerical analysis. With the exception of flagellation, cell shape, PHB accumulation, arginine dihydrolase, mechanisms of aromatic ring cleavage, and reduction of nitrate to nitrite, all the nutritional and physiological data for each strain were submitted to a numerical analysis by Eleanora Szabo (Department of Microbiology, University of Queensland, Brisbane, Australia) with programs for a GE 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 (49). The search for clusters of strains within the data was by a complete-linkage method (*manuscript in preparation*). On the basis of the numerical analysis, the strains were separated into major groups (designated by letters) having S-values of 66% or less (see Fig. 45). Further subdivisions were made at the 68 to 76% S-values. The resulting groups were designated by the major group letter and a numeral. The phenotypic characteristics of the groups, expressed as the number of strains having a given trait, are given in Table 1. From these data it can be seen that the majority of the groups can be distinguished on the basis of a large number of nutritional and physiological traits. Figure 46 gives the range of the number of organic compounds utilized by each group as sole sources of carbon and energy.

Flagellation, PHB accumulation, and mechanism of aromatic ring cleavage. As seen in Fig. 45, flagellation, PHB accumulation, and the mechanism of aromatic ring cleavage, traits which were not included in the numerical analysis, support the groupings es-

TABLE 1. *Morphological, physiological, and nutritional properties of the groups established by numerical analysis*[a]

Trait	A-1[b] 33[c]	A-2 17	B-1 3	B-2 4	C-1 2	C-2 6	C-3 11	D-1 2	D-2 5	D-3 5	D-4 9	E-1 21	E-2[c] 17	F-1 6	F-2[d] 7	G-1 11	G-2 5	G-3 34	H-1 4	H-2 6	I-1 4	I-2 5	217 1	218 1
Straight rods[f]	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	3	+	+	+	–
Flagellation[g]	P	P	P	P	P	Pr	P	P	Pr	Pr	Pr	P	P	Pr	P	P	P	PL	P	P	P	P	P	–
No. of polar flagella[h]	*1*	*1*	4–6	4–6	1		*1–3*	1				1	1		2–5	1	1	1	1	1	1–2	3–6	1	
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	5	+	+	**32**	+	+	+	+	+	–
PHB[i]-Accumulation	–	–	+	+	+	+	+	+	+	+	+	–	–	+	+	+	3	–	+	–	+	+	–	–
Ring cleavage[j]	*m*	*m*	*o*	*o*	*o*	*o*	*o*	*m*	*o*	*o*	*o*	–	–	–	–	*m*	–	*o*	*m*	–	–	*o*	–	–
Oxidase	+	–	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+
Denitrification	–	–	–	1	+	–	–	–	–	–	–	–	–	–	–	–	1	**29**	–	–	–	2	–	+
NO_3^- to NO_2^-[k]	–	–	–	–	–	–	8	–	**4**	+	+	2	6	**5**	–	–	–	3	–	3	+	1	–	–
Amylase	–	–	–	–	–	–	–	–	–	–	–	**19**	1	2	–	–	–	3	–	–	–	–	–	+
Gelatinase	–	–	–	–	–	–	–	–	–	–	–	**20**	+	–	–	–	–	1	–	–	–	2	+	–
Lipase	–	–	–	–	–	–	–	1	–	–	–	+	+	–	–	–	3	+	+	–	–	3	+	–
Alginase	–	–	–	–	–	–	–	–	–	–	–	3	–	–	1	–	–	–	–	–	–	–	–	–
Chitinase	–	–	–	–	–	–	–	–	–	–	–	–	**14**	–	–	–	–	–	–	–	–	–	–	–
D-Ribose	–	5	–	–	–	–	4	–	**4**	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
D-Xylose	8	10	1	3	–	1	–	–	**4**	–	–	4	1	–	–	–	–	–	–	–	–	–	–	–
D-Arabinose	–	2	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
L-Arabinose	11	**15**	–	–	–	–	–	–	+	–	–	–	11	–	–	–	–	–	–	–	–	–	–	–
D-Fucose	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
L-Rhamnose	9	+	2	–	–	–	–	–	2	–	–	1	2	–	–	–	–	–	–	–	–	–	–	–
D-Glucose	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	–	–	–	+	–	–	1	+	+
D-Mannose	**29**	+	+	–	–	–	–	–	+	–	–	–	**15**	–	–	–	–	–	–	–	–	–	–	–
D-Galactose	11	**15**	+	+	–	–	–	+	+	–	–	+	5	3	+	–	–	–	–	–	–	–	+	–
D-Fructose	+	+	+	+	1	+	**10**	+	+	**4**	7	+	11	+	+	–	–	–	+	–	–	–	+	–
Sucrose	–	1	–	–	–	–	–	+	3	**4**	5	+	**15**	3	–	–	–	–	–	–	–	–	–	–
Trehalose	6	3	–	–	–	–	–	+	3	**4**	5	**19**	13	3	–	–	–	–	–	–	–	–	–	–
Maltose	**26**	6	–	–	–	–	–	+	3	**4**	5	+	+	3	–	–	–	–	–	–	–	–	+	+
Cellobiose	–	**14**	–	1	–	–	–	–	3	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Melibiose	–	–	–	–	–	–	–	+	2	–	–	**20**	–	–	–	–	–	–	–	–	–	–	+	–
Lactose	–	–	–	–	–	–	–	–	2	–	–	+	1	–	–	–	–	–	–	–	–	–	+	–
Salicin	–	–	–	1	–	–	–	–	3	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
Gluconate	+	+	2	–	–	+	–	+	+	+	**8**	**18**	1	**5**	+	–	–	–	–	–	–	–	+	–
Saccharate	+	+	1	–	–	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Glucuronate	6	3	1	1	–	–	2	–	+	**4**	–	–	–	–	–	–	–	–	–	–	–	–	–	+

Galacturonate	6	12	−	−	−	−	−	+	+	+	−	**18**	1	−	−	−	−	−	−	−	−	−	−
N-Acetylglucosamine	−	**15**	+	+	−	+	−	+	+	**4**	6	7	**15**	−	−	−	−	−	−	−	−	−	−
Acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Propionate	23	13	+	+	+	+	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Butyrate	−	−	+	1	+	+	−	+	+	+	+	+	6	+	**6**	+	+	+	3	1	+	+	+
Isobutyrate	−	−	+	2	+	+	−	+	+	+	+	+	**14**	+	1	+	+	**31**	−	2	+	+	+
Valerate	−	−	+	+	+	**5**	−	+	+	+	+	+	11	+	**6**	8	+	+	−	1	+	3	+
Isovalerate	−	−	1	−	−	+	−	+	+	+	+	5	1	+	**6**	6	**4**	24	−	−	+	1	−
Caproate	−	10	+	+	+	+	−	+	+	+	+	**19**	2	+	5	7	**4**	+	3	−	2	+	+
Heptanoate	−	10	2	3	−	+	−	+	+	+	4	11	7	+	+	1	**4**	+	2	−	2	−	−
Caprylate	−	10	2	−	−	4	−	+	+	+	**8**	+	**14**	+	**6**	1	2	+	−	−	1	−	−
Pelargonate	−	8	−	−	−	**5**	−	+	+	**4**	+	+	**15**	1	+	2	2	**32**	−	−	−	−	−
Caprate	14	10	−	−	−	4	4	+	+	+	+	+	+	3	+	6	+	**31**	2	−	3	−	−
Malonate	−	−	−	−	−	−	4	+	3	3	1	−	−	1	5	−	−	−	1	−	−	−	−
Succinate	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	**31**	+	+	+	+	+
Maleate	−	−	−	2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Fumarate	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	**31**	+	+	+	+	+
Glutarate	−	−	2	1	+	+	**10**	−	2	+	+	−	−	4	−	6	−	1	−	−	3	1	−
Adipate	−	−	−	−	−	−	−	−	−	2	5	−	−	**5**	−	−	2	11	−	−	−	−	−
Pimelate	−	−	2	2	−	−	−	−	−	1	−	−	−	+	−	−	−	6	−	−	−	−	−
Suberate	−	−	2	2	−	−	−	−	−	−	−	−	−	+	−	−	−	4	−	−	−	−	−
Azelate	−	−	2	2	−	−	−	−	−	−	3	−	−	+	−	−	−	5	−	−	−	−	−
Sebacate	−	−	2	2	−	−	−	−	−	1	−	−	−	+	−	−	−	8	−	−	1	−	−
DL-Malate	+	+	+	+	+	**5**	+	+	+	+	+	−	−	+	+	5	+	**29**	+	−	+	+	−
D-(−)-Tartrate	−	−	−	−	−	−	−	−	1	−	−	−	−	−	−	−	−	−	−	−	−	−	−
L-(+)-Tartrate	**27**	7	−	−	−	−	3	+	3	−	−	−	−	4	2	1	−	−	−	−	−	−	−

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

[b] Denotes group number.

[c] Includes strains 214, 215, and 216.

[d] Includes strain 219.

[e] Denotes number of strains.

[f] + = All strains straight rods; − = all strains curved rods; numbers indicate number of straight rods.

[g] P = Polar; L = some strains have lateral flagella; Pr = peritrichous; − = flagella not detected.

[h] Numbers indicate number of flagella at one pole; italicized number indicates that the flagella often occur at both poles of the cell.

[i] PHB = Poly-β-hydroxybutyrate.

[j] Ring cleavage for strains capable of growth on aromatic compounds.

[k] Does not include strains capable of denitrification.

TABLE 1—*Continued*

Trait	A-1[b] 33[e]	A-2 17	B-1 3	B-2 4	C-1 2	C-2 6	C-3 11	D-1 2	D-2 5	D-3 5	D-4 9	E-1 21	E-2[c] 17	F-1 6	F-2[d] 7	G-1 11	G-2 5	G-3 34	H-1 4	H-2 6	I-1 4	I-2 5	217 1	218 1
meso-Tartrate	2	1	–	–	–	–	–	–	**4**	–	–	–	–	2	–	–	–	–	–	–	–	–	–	–
DL-β-Hydroxybutyrate	+	7	+	+	+	+	+	+	+	+	+	12	7	+	+	+	+	**30**	+	–	+	+	–	+
DL-Lactate	+	**16**	+	+	+	+	+	+	+	+	+	16	2	+	+	+	+	+	+	4	+	+	+	+
Glycolate	–	–	+	–	1	–	**10**	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
DL-Glycerate	**4**	–	2	2	+	**5**	**10**	+	+	–	+	+	–	+	+	–	–	–	–	–	–	–	–	+
Citrate	+	+	+	3	+	+	+	+	+	+	+	–	**15**	3	+	**9**	3	12	+	–	3	–	+	+
α-Ketoglutarate	+	+	+	+	+	+	+	+	+	+	+	–	–	4	+	+	2	–	+	–	2	+	–	+
Pyruvate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aconitate	+	**16**	+	3	1	**5**	+	+	+	**4**	+	–	**15**	–	+	**10**	2	1	3	–	–	–	+	–
Laevulinate	–	–	–	–	–	1	–	–	–	–	–	–	–	3	–	–	2	3	–	–	–	–	–	–
Citraconate	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
Itaconate	1	–	–	1	–	–	–	1	–	–	1	–	–	1	–	1	–	–	–	–	–	–	–	+
Mesaconate	–	–	–	–	–	–	–	+	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	+
Erythritol	–	+	–	–	–	–	–	–	2	1	–	–	–	4	–	–	–	–	–	–	–	–	–	–
Mannitol	+	+	+	+	1	–	–	+	+	**4**	**8**	9	7	+	+	–	–	–	+	–	–	–	–	–
Sorbitol	+	+	1	1	1	–	–	1	**4**	**4**	**8**	–	–	+	–	–	–	–	3	–	–	–	–	–
meso-Inositol	+	+	–	+	–	–	–	+	3	+	+	–	–	2	5	–	–	–	1	–	–	–	–	–
Adonitol	3	10	–	–	–	1	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Glycerol	+	+	+	+	+	+	–	+	+	+	+	+	–	+	+	–	–	–	–	–	–	+	+	+
Ethyleneglycol	–	–	–	–	–	–	–	+	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Propyleneglycol	–	–	–	–	–	–	–	–	–	+	–	5	3	–	–	6	3	8	–	–	2	–	–	+
2,3-Butyleneglycol	–	–	–	–	–	1	2	+	–	+	3	–	–	3	3	8	–	2	–	–	–	–	–	+
Ethanol	+	–	–	–	–	–	**10**	+	**4**	+	+	**17**	**16**	+	5	+	+	22	+	–	+	–	+	+
n-Propanol	**30**	–	–	–	–	–	1	+	**4**	+	+	15	13	+	5	+	+	25	+	–	+	–	+	+
n-Butanol	–	–	–	–	–	–	–	+	3	+	+	–	–	+	1	+	+	27	+	–	3	–	–	+
Isobutanol	–	–	–	–	–	–	–	+	4	+	+	–	–	**5**	–	+	3	19	–	–	+	–	–	+
D-Mandelate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–
L-Mandelate	–	–	–	–	–	–	–	–	1	3	1	–	–	–	–	1	–	–	–	–	–	–	–	–
Benzoylformate	–	–	1	–	–	–	–	–	2	3	1	–	–	–	–	1	–	–	–	–	–	–	–	–
Benzoate	–	–	1	–	1	+	+	+	+	3	2	–	–	–	–	5	–	13	–	–	–	–	–	–
o-Hydroxybenzoate	–	–	–	–	–	–	–	–	1	3	1	–	–	–	–	–	–	–	–	–	–	–	–	–
m-Hydroxybenzoate	+	+	–	–	1	2	–	+	1	1	–	–	–	–	–	7	–	–	–	–	–	–	–	–
p-Hydroxybenzoate	+	+	+	3	+	3	5	+	+	3	2	–	–	–	–	7	–	–	+	–	–	1	–	–
Phenylacetate	9	5	2	–	–	+	–	–	–	+	+	–	–	2	–	**10**	–	–	–	–	–	–	–	–
Quinate	+	+	1	–	–	–	–	+	2	3	1	–	–	–	–	5	–	–	+	–	–	–	–	–
Glycine	–	–	+	+	+	+	+	+	3	–	–	14	10	–	–	–	3	–	–	1	3	–	–	–

L-α-Alanine	+	+	+	+	+	+	+	+	+	+	+	**19**	+	+	+	+	+	5	+	3	+	+	+	+
D-α-Alanine	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	8	+	1	+	2	–	+
β-Alanine	**32**	8	+	+	+	4	**10**	–	**4**	+	3	–	–	1	+	7	2	–	+	–	+	+	–	–
L-Serine	**31**	12	+	+	+	+	+	–	+	–	2	9	+	–	+	–	2	–	–	–	2	**4**	+	–
L-Threonine	+	**14**	+	+	+	–	8	–	1	–	–	–	1	–	–	–	–	–	–	–	2	+	–	–
L-Leucine	–	–	+	+	+	**5**	–	+	**4**	+	**8**	15	6	3	**6**	–	1	1	–	–	2	+	–	+
L-Isoleucine	–	–	2	–	+	–	–	1	2	+	2	13	–	+	4	–	1	2	–	–	2	1	–	–
L-Valine	–	–	+	–	+	–	–	+	–	+	6	11	1	2	–	2	1	–	–	–	3	–	–	–
L-Aspartate	7	**14**	+	3	+	+	+	–	+	+	+	9	11	**5**	+	–	2	–	–	1	3	2	–	+
L-Glutamate	+	+	+	+	+	+	+	1	+	+	+	15	**14**	+	+	**9**	**4**	16	1	–	2	+	+	+
L-Lysine	**29**	13	+	1	+	+	–	–	+	+	+	1	–	+	2	–	–	–	–	–	3	3	–	–
L-Arginine	+	**14**	+	3	+	+	+	–	+	+	7	8	9	+	5	2	–	–	2	–	+	+	–	–
L-Ornithine	+	+	+	3	+	+	+	–	3	+	4	–	1	4	5	**10**	–	–	–	–	+	**4**	–	–
L-Citrulline	22	2	2	3	+	–	–	–	–	–	–	–	2	–	–	–	–	–	–	2	+	3	–	–
γ-Aminobutyrate	+	**16**	2	3	+	**5**	+	1	**4**	3	**8**	–	–	**5**	+	3	2	–	+	–	+	+	–	–
δ-Aminovalerate	–	–	–	–	+	+	+	–	**4**	+	+	–	–	–	–	3	2	–	–	–	+	–	–	–
L-Histidine	**30**	**15**	+	+	+	+	+	–	**4**	+	7	–	8	–	–	–	–	–	–	–	–	**4**	–	–
L-Proline	+	+	+	+	+	+	+	1	+	+	+	1	9	+	+	+	**4**	**28**	+	+	+	+	–	–
L-Tyrosine	–	–	+	+	+	+	–	+	+	+	+	**18**	**15**	–	+	+	–	–	–	–	–	**4**	+	+
L-Phenylalanine	9	3	+	+	+	+	–	–	2	+	**8**	–	1	–	1	+	2	–	–	–	1	**4**	–	–
L-Tryptophan	–	–	–	–	–	–	–	–	–	3	3	–	–	–	–	3	–	–	–	–	–	–	–	–
DL-Kynurenine	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–
Kynurenate	–	–	–	–	–	+	7	+	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Anthranilate	–	–	–	–	–	+	–	–	1	3	7	–	–	–	–	–	–	–	–	–	–	–	–	–
Ethanolamine	21	–	+	+	+	–	–	–	–	+	**8**	–	–	–	–	–	–	–	1	–	2	**4**	–	+
Benzylamine	–	–	–	–	1	**5**	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Putrescine	+	**15**	+	+	+	+	+	1	+	+	+	–	–	–	–	**9**	3	2	+	–	+	+	–	–
Spermine	**31**	12	2	3	2	+	5	–	2	–	–	–	–	–	–	–	–	1	3	–	+	**4**	–	–
Histamine	–	–	+	1	+	+	1	–	3	–	1	–	–	–	–	6	–	–	–	–	+	2	–	–
Butylamine	–	–	–	–	+	+	–	1	–	1	7	–	–	–	–	3	2	–	–	–	2	1	–	–
Pentylamine	–	–	–	–	–	1	–	–	2	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–
Betaine	+	**16**	+	+	+	+	+	+	+	+	+	–	–	–	5	–	–	–	+	–	+	+	–	–
Sarcosine	+	+	+	+	+	+	+	+	+	+	+	–	–	–	**6**	–	–	–	–	–	+	+	–	–
Creatine	–	–	+	+	+	–	+	+	1	–	–	–	–	1	–	–	–	–	–	–	2	+	–	–
Hippurate	–	–	1	–	1	4	8	+	**4**	3	2	–	1	–	–	–	–	–	–	–	–	–	–	–
Acetamide	–	–	–	–	–	–	–	–	–	2	1	–	–	–	–	–	–	–	–	–	–	–	–	–
Nicotinate	–	–	+	–	–	2	–	–	–	+	+	–	–	–	–	–	–	–	1	–	–	–	–	–
Nicotinamide	–	–	–	–	–	–	–	–	–	2	4	–	–	–	–	–	–	–	–	–	–	–	–	–

TABLE 1—*Continued*

Trait	A-1[b] 33[a]	A-2 17	B-1 3	B-2 4	C-1 2	C-2 6	C-3 11	D-1 2	D-2 5	D-3 5	D-4 9	E-1 21	E-2[c] 17	F-1 6	F-2[d] 7	G-1 11	G-2 5	G-3 34	H-1 4	H-2 6	I-1 4	I-2 5	217 1	218 1
Trigonelline	2	12	+	3	+	1	−	−	−	−	−	−	−	−	−	2	1	4	3	−	+	2	−	−
Allantoin	−	−	+	−	+	**5**	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Adenine	−	−	−	−	−	3	**8**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	**1**	−	−
Cytosine	−	−	−	−	−	−	−	−	−	−	−	−	−	**1**	−	−	−	−	−	−	**1**	−	−	−
Thymine	−	−	**1**	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Uracil	−	−	**1**	−	+	−	−	−	−	−	−	−	−	**1**	−	−	−	**28**	−	−	**1**	−	−	−
n-Hexadecane	−	−	−	−	−	−	−	−	−	−	−	−	**2**	−	**6**	−	−	−	−	−	−	−	−	−
4 C	−	−	−	−	−	−	−	−	−	+	+	−	**15**	+	+	+	+	+	+	+	−	**2**	−	−
35 C	+	+	+	**3**	+	+	+	+	+	+	+	+	−	+	**1**	+	+	**29**	+	+	−	−	−	+
40 C	+	−	**2**	−	+	+	+	+	**3**	+	+	**15**	−	+	−	**6**	**2**	**17**	−	−	−	−	−	+
45 C	−	−	−	−	−	**5**	**3**	−	−	−	−	−	−	**1**	−	−	−	−	−	−	−	−	−	−

tablished on the basis of nutritional similarities. Groups C-2; D-2, -3, -4; and F-1 were peritrichous (see Fig. 40–43). The remaining groups had polar flagella (see Fig. 25, 27–39, 44). Some strains of group G-3 had lateral flagella of a shorter wave length in addition to the single polar flagellum (see Fig. 37). When representative strains were examined by means of the electron microscope, no evidence was found for the presence of sheathed flagella of the type observed in *Beneckea* (1). All the strains in groups B-1, -2; C-1, -2, -3; D-1, -2, -3, -4; F-1, -2; G-1; H-1; I-1, -2; and strains 158 to 160 of group G-2 accumulated PHB as an intracellular reserve product. The remaining strains were negative for this trait. In groups B-1, -2; C-1, -2, -3; D-2, -3, -4; G-3; and I-2, strains capable of growth on aromatic compounds gave an *o* cleavage of diphenolic intermediates. Strains of groups A-1, -2; D-1; G-1; and H-1 gave a *m* cleavage.

Interpretation of the results of Leifson flagella staining. The flagellation of the majority of the groups studied could be unambiguously determined by the Leifson staining method; this was confirmed by electron microscopic examination of representative strains. The results for groups B-1, -2; F-2; and I-2 were, however, difficult to interpret since preparations of each strain stained by the Leifson method contained cells which had single polar flagella, polar tufts, single lateral, lateral tufts, and, rarely, degenerately peritrichous or possible peritrichous flagellation (Fig. 20–24). Examination with the electron microscope showed that these strains had polar tufts (Fig. 25, 27, 28). This type of flagellation was observed in all preparations where the origins of the flagella were detectable. The flagella of these groups appeared to be pliant and tended to curl and bend toward the cell, providing arrangements which when stained by the Leifson method could give rise to misinterpretations (Fig. 25). In rare cases, lateral or subpolar tufts or bundles were observed (Fig. 26) which could have given rise to the Leifson preparations seen in Fig. 21 and 22. In such cases, the origins of the flagella were not evident, suggesting that they could have been polar flagella which had bent toward the pole opposite their origin. The frequent occurrence of cells with one flagellum in Leifson preparations could be explained by the tendency of the flagella to form single bundles (Fig. 26, 27). In some cells, the insertions of the flagella appeared to be close together (Fig. 25, 27) while in others their origins appeared to be more dispersed (Fig. 20, 28). Leifson flagella

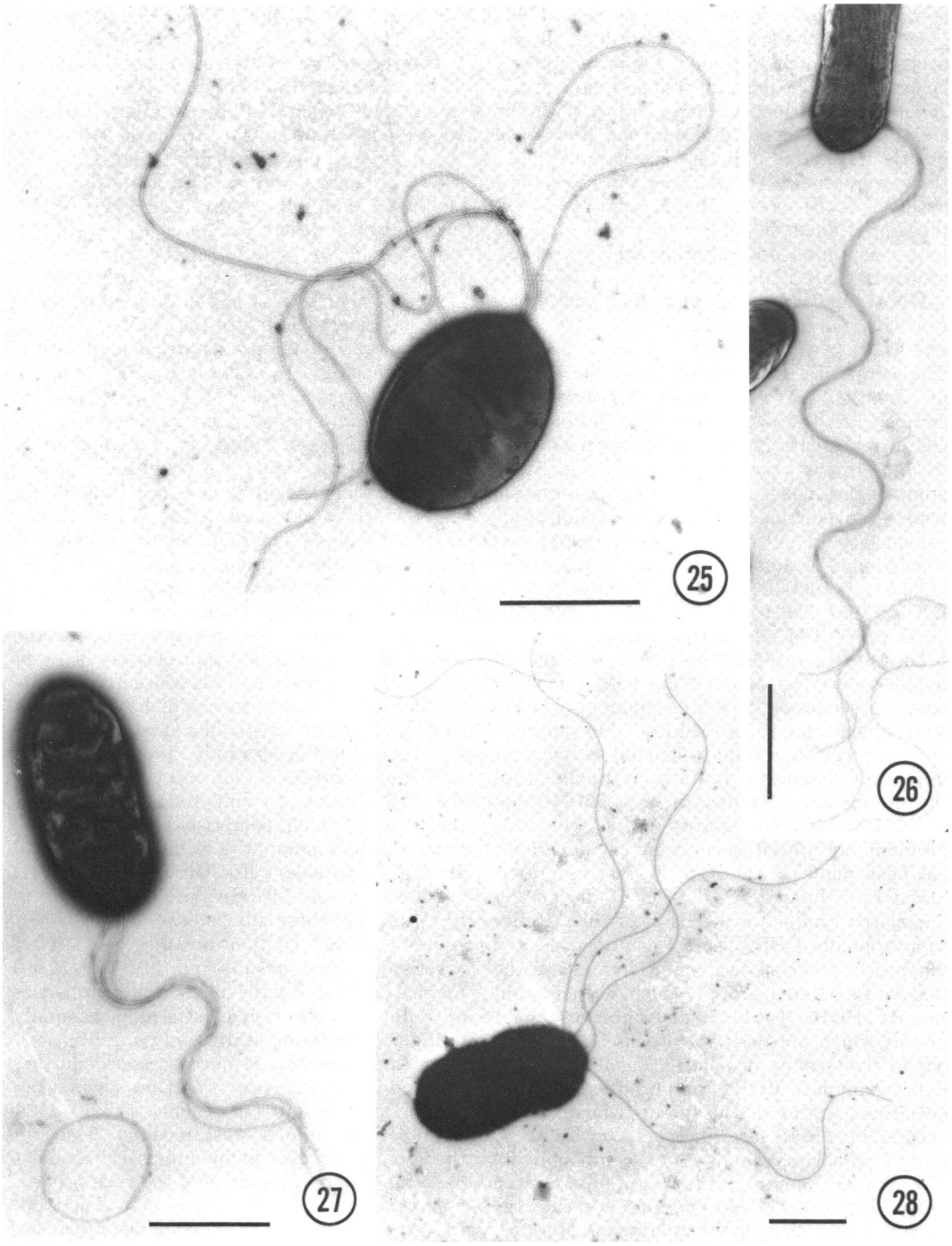

FIG. 25–28. *Electron micrographs of strains illustrating types of flagellar arrangements found in groups B-1, B-2, I-2, and P. marina (group F-2) some of which may lead to misinterpretations when stained by the Leifson method. Negatively stained. Marker represents 1 μm. Fig. 25, group B-1, strain 52, illustrates the tendency of flagella to curl and bend backwards (× 21,000); Fig. 26, group B-2, strain 54, rare cell giving the appearance of subpolar insertion of a bundle of flagella (× 17,000); Fig. 27, group I-2, strain 212, typical preparation showing bundle of polar flagella (× 18,500); Fig. 28, P. marina, strain 143 (group F-2), a polar tuft in which the flagella appear to be inserted at dispersed points (× 11,000).*

staining of the two strains in group D-1 was complicated by the occurrence of lateral projections which were of variable length and lacked the wave pattern characteristic of flagella. Examination of these strains with the electron microscope showed that they were polarly flagellated and that the lateral projections were similar to the tubular projections observed in *Beneckea* (1). These results stress the importance of confirming results of the Leifson staining procedure by an electron microscopic examination.

DNA base compositions. The moles per cent GC content in the DNA of representative strains from each group are given in Table 2. The composite values for each group are given in Table 3. In the peritrichously flagellated groups; C-2; D-2, -3, -4; and F-1; the moles % GC of representative strains ranged from 53.7 to 67.8. In the case of the polarly flagellated groups, the moles % GC of representative strains ranged from 30.5 to 64.7. The latter organisms could be arranged into three groups consisting of strains having moles % GC values of 30.5 (group H-2), 43.2 to 48.0 (groups A-1, -2; E-1, -2) and 52.0 to 64.7 (groups B-1, -2; C-1, -3; D-1; F-2; G-1, -2, -3; H-1; I-1, -2).

Diagnostic tables. The groups established by the numerical analysis are with a few exceptions separable by a large number of unrelated phenotypic traits (Table 1). For purposes of identification, a table restricted to the most important diagnostic traits is desirable. If all the groups are included in one table, it becomes unwieldy and confusing. To avoid this problem, an initial separation of the groups has been made on the basis of flagellation followed by a further subdivision of the polarly flagellated groups on the basis of their ability to accumulate PHB. Table 4 contains selected diagnostic traits of use in differentiating the polarly flagellated groups which do not accumulate PHB; Table 5 distinguishes those groups which are positive for this trait. Three out of five strains in polarly flagellated group G-2 accumulate PHB. For purposes of differentiation, strains of this group which do not accumulate PHB are included in Table 4; strains which accumulate this compound are included in Table 5. Table 6 contains selected diagnostic traits of use in differentiating the peritrichously flagellated groups, all of which accumulate PHB. In this table, groups D-3 and D-4 have been combined into one column. If necessary, additional diagnostic traits for the differentiation of the groups may be obtained from Table 1.

DISCUSSION

Interpretation of the numerical analysis. The aerobic marine bacteria characterized in this study were submitted to a numerical analysis which grouped the strains according to their phenotypic similarities (see Fig. 45). These groupings were supported by the mode of flagellation, ability to accumulate PHB, mechanism of aromatic ring cleavage (see Fig. 45), and, in the majority of the cases, by the moles per cent GC content in the DNA of representative strains (Table 3), traits which were not included in the numerical analysis. The majority of the groups designated in Fig. 45 are separable by a large number of unrelated, phenotypic traits (Table 1). If they are combined into larger clusters encompassing strains of a lesser similarity, the number of diagnostic traits is greatly reduced. In most cases, this has the effect of eliminating all the traits which can be used for the differentiation of the clusters. These observations indicate that clusters having their highest linkage at an S-value between 68 and 76% are readily identifiable taxonomic units and suggest that they deserve the status of species. Many of the groups do not segregate into clusters of lower S-value having a similar GC content and mode of flagellation, properties which are usually considered characteristic of a single genus (Fig. 45; Table 3). For example, the cluster consisting of groups C-1, C-2, and C-3 is linked at the 70% S-value. In spite of this similarity, the strains which comprise group C-1 have polar flagella, group C-2 is peritrichous, and group C-3 has polar tufts. Groups H-1 and H-2 are linked at the 74% S-value, yet representative strains of these groups have a moles % GC content in their DNA of about 55 and 30, respectively. These results indicate that a numerical analysis of the phenotypic properties of a diverse collection of strains is generally useful for clustering strains into species; however, these species are not necessarily further grouped into higher taxonomic units which can be assigned the status of genera.

Choice of generic designations. Many of the groups established by numerical analysis have the general properties of the genera *Pseudomonas* (51) and *Alcaligenes* (15). The well-characterized species of these genera are of soil and fresh water origin. A large number of studies indicate that marine bacteria differ from most soil and fresh water forms in requiring sodium ion for growth (5; 34, 35). In the case of a few marine strains which have been

TABLE 2. *CsCl buoyant density determinations and guanine plus cytosine (GC) contents of DNA molecules of species and groups of aerobic marine bacteria*

Species and/or group	Strain	^{P}CsCl (gKm^{-3})[a]	GC (moles %)	Species and/or group	Strain	^{P}CsCl (gKm^{-3})[a]	GC (moles %)
H-2	200	1.690	30.6	G-1	146	1.7155	56.6
	201	1.6915	32.1		148	1.7145	55.6
	202	1.690	30.6		150	1.715	56.1
	203	1.688	28.6		153	1.7145	55.6
					155	1.714	55.1
Alteromonas communis (A-1)	1	1.705	45.9				
	8	1.707	48.0	G-2	156	1.7145	55.6
	19	1.706	46.9		157	1.7145	55.6
	24	1.7055	46.4		159	1.7115	52.6
	32	1.707	48.0		160	1.7105	51.5
Alteromonas vaga (A-2)	35	1.707	48.0	*Pseudomonas nautica* (G-3)	164	1.7203	61.5
	40	1.7083	49.3		170	1.716	57.1
	42	1.7055	46.4		179	1.718	59.2
	47	1.706	46.9		185	1.7165	57.7
	49	1.707	48.0		189	1.716	57.1
					193	1.719	60.2
Alteromonas macleodii (E-1)	101	1.705	45.9				
	104	1.704	44.9	H-1	195	1.713	54.1
	107	1.705	45.9		196	1.7135	54.6
	111	1.704	44.9		197	1.715	56.1
	113	1.7055	46.4		198	1.7135	54.6
Alteromonas marinopraesens (E-2)	121	1.702	42.9	I-1	205	1.7155	56.6
	125	1.703	43.9		206	1.715	56.1
	130	1.7035	44.4		207	1.7155	56.6
	214	1.701	41.8		208	1.715	56.1
	215	1.702	42.9				
	216	1.702	42.9	I-2	209	1.720	61.2
					210	1.720	61.2
B-1	51	1.7225	63.8		211	1.720	61.2
	52	1.722	63.3		212	1.7223	63.6
	53	1.7215	62.8				
				Alcaligenes pacificus (C-2)	60	1.7266	68.0
B-2	54	1.7185	59.7		62	1.7265	67.9
	55	1.7175	58.7		63	1.726	67.3
	56	1.7175	58.7		64	1.7265	67.9
	57	1.7214	62.7				
				Alcaligenes cupidus (D-2)	79	1.7195	60.7
C-1	58	1.7225	63.8		81	1.721	62.2
	59	1.7225	63.8		82	1.719	60.2
Pseudomonas doudoroffii (C-3)	66	1.7145	55.6	*Alcaligenes venustus* (D-3, 4)	84	1.713	54.1
	70	1.7185	59.7		85	1.712	53.1
	71	1.7175	58.7		86	1.713	54.1
	74	1.718	59.2		88	1.712	53.1
	75	1.7175	58.7		89	1.712	53.1
					90	1.713	54.1
D-1	77	1.723	64.3		93	1.7135	54.6
	78	1.7235	64.8		94	1.7115	52.6
					95	1.713	54.1
Pseudomonas marina (F-2)	139	1.7215	62.8				
	140	1.722	63.3	*Alcaligenes aestus* (F-1)	133	1.716	57.1
	143	1.722	63.3		134	1.7165	57.7
	144	1.721	62.2		135	1.7165	57.7
	219	1.722	63.3		136	1.7165	57.7
					217	1.702	42.9
					218	1.7205	61.7

[a] Mean of two determinations; strains 1, 40, 60, 164, 212, 214, three determinations; strains 57 and 66, four determinations.

TABLE 3. *Summary of CsCl buoyant density determinations and guanine plus cytosine (GC) contents of DNA molecules of species and groups of aerobic marine bacteria*

Species and/or group	^{P}CsCl (gKm^{-3}) + σ	GC (moles %)
H-2	1.6899 ± 0.0015 (8)[a]	30.5 ± 1.5
Alteromonas communis (A-1)	1.7060 ± 0.0010 (11)	46.9 ± 1.0
A. vaga (A-2)	1.7070 ± 0.0015 (11)	48.0 ± 1.5
A. macleodii (E-1)	1.7047 ± 0.0008 (10)	45.6 ± 0.8
A. marinopraesens (E-2)	1.7023 ± 0.0010 (13)	43.2 ± 1.0
B-1	1.7220 ± 0.0006 (6)	63.3 ± 0.6
B-2 [54, 55, 56][b]	1.7178 ± 0.0008 (6)	59.0 ± 0.8
[57]	1.7214 ± 0.0008 (4)	62.7 ± 0.8
C-1	1.7225 ± 0.0006 (4)	63.8 ± 0.6
Pseudomonas doudoroffii (C-3) [70, 71, 74, 75]	1.7179 ± 0.0010 (8)	59.1 ± 1.0
[66]	1.7145 ± 0.0010 (4)	55.6 ± 1.0
D-1	1.7234 ± 0.0005 (4)	64.7 ± 0.5
Pseudomonas marina (F-2)	1.7217 ± 0.0005 (10)	63.0 ± 0.5
G-1	1.7147 ± 0.0007 (10)	55.8 ± 0.7
G-2 [156, 157]	1.7145 ± 0.0006 (4)	55.6 ± 0.6
[159, 160]	1.7110 ± 0.0008 (4)	52.0 ± 0.8
Pseudomonas nautica (G-3) [164, 193]	1.7198 ± 0.0011 (5)	61.0 ± 1.1
[170, 179, 185, 189]	1.7166 ± 0.0012 (8)	57.8 ± 1.2
H-1	1.7138 ± 0.0009 (8)	54.9 ± 0.9
I-1	1.7153 ± 0.0006 (8)	56.4 ± 0.6
I-2 [209, 210, 211]	1.7200 ± 0.0000 (6)	61.2 ± 0.0
[212]	1.7223 ± 0.0006 (3)	63.6 ± 0.6
Alcaligenes paci-ficus (C-2)	1.7264 ± 0.0005 (8)	67.8 ± 0.5
A. cupidus (D-2)	1.7198 ± 0.0011 (6)	61.0 ± 1.1
A. venustus (D-3, 4)	1.7126 ± 0.0008 (9)	53.7 ± 0.8
A. aestus (F-1)	1.7165 ± 0.0005 (8)	57.4 ± 0.5

[a] Number of determinations.
[b] Strain numbers.

studied in detail, sodium was found to be necessary for the preservation of the integrity of the cell wall and for the activity of permease systems (12, 17–19, 22, 23, 34, 35). In addition, there is considerable evidence that soil and fresh water bacteria, unlike marine organisms, are unable to survive in sea water (26, 29, 34, 42). The ability of marine strains to survive in the ocean may indicate the possession of attributes lacking in organisms of soil and fresh water origin. Since the differences between marine and soil and fresh water bacteria have, as yet, not been properly elucidated, it seems unreasonable to exclude marine strains from genera such as *Pseudomonas* and *Alcaligenes* solely on the basis of their marine origin. It should, however, be kept in mind that additional studies may support generic separations of marine and soil and fresh water bacteria.

On the basis of general physiological and structural properties and the moles per cent GC content in their DNA, we have placed the majority of our groups into three genera: *Pseudomonas* (polarly flagellated groups B-1, -2; C-1, -3; D-1; F-2; G-3; I-2), *Alcaligenes* (peritrichously flagellated groups C-2; D-2, -3, -4; F-1), and a newly created genus *Alteromonas* (polarly flagellated groups A-1, -2; E-1, -2). Groups G-1, G-2, H-1, and I-1, although similar to *Pseudomonas*, have not been assigned to this genus for reasons which will be discussed. Although the above three genera are defined in part on the basis of physiological and structural properties (15, 51), two traits are of key importance in their definitions: the mode of flagellation and their moles per cent GC content. When phenotypic comparisons are made among the groups which have been placed into *Pseudomonas*, *Alcaligenes*, and *Alteromonas*, no additional traits are obtained which can be used for generic differentiations.

The genus *Pseudomonas*, as defined by Stanier et al. (51), is composed of gram-negative, polarly flagellated rods which have a respiratory metabolism. The organisms which comprise this genus have a moles % GC content in their DNA of 57 to 70 (36, 51). Stanier et al. recognized that the limits of the moles per cent GC content characteristic of this genus could not be set pending further study. Recent evidence from in vitro DNA hybridization studies has indicated that the species encompassed by the genus *Pseudomonas* form at least three genetically distinct groups (3, 39, 40, 44). No major phenotypic traits were found which would adequately reflect this genetic distinction and justify a generic separation on phenotypic grounds. The polarly flagellated organisms in our collection, all of which have the structural and physiological properties characteristic of the genus *Pseudomonas*, range from 30.5 to 64.7 moles % in their GC content. Discontinuities within the GC range spanned allow these organisms to be subdivided into three groups containing 30.5, 43.2 to 48.0, and 52.0 to 64.7 moles % GC (Table 3). Since the majority of the members of the latter group have a GC content in the range of the genus *Pseudomonas* (51), the moles % GC characteristic of this genus could be lowered from 57 to 52 to include these isolates. Since one of the authors (M. Mandel) is opposed to widening the moles per cent GC range of the genus *Pseudomonas*, and since, with the exception of

TABLE 4. *Some distinguishing properties of the polarly flagellated species and groups which do not accumulate PHB*[a]

Trait	*A. communis* A-1 33[b]	*A. vaga* A-2 17	*A. macleodii* E-1 21	*A. marinopraesens* E-2[c] 17	217 1	G-2[d] 2	*P. nautica* G-3[e] 34	H-2 6
Straight rods[f]	–	+	+	+	+	+	+	3
Brown-black pigment	–	–	–	–	+	–	–	–
Ring cleavage[g]	*m*	*m*	–	–	–	–	*o*	–
Oxidase	+	–	+	+	+	+	+	+
Denitrification	–	–	–	–	–	1	**29**	–
Amylase	–	–	**19**	1	–	–	3	–
Gelatinase	–	–	**20**	+	+	–	1	–
Lipase	–	–	+	+	+	+	+	–
Chitinase	–	–	–	**14**	–	–	–	–
D-Glucose	+	+	+	+	+	–	–	–
D-Mannose	**29**	+	–	**15**	–	–	–	–
Cellobiose	–	**14**	+	–	–	–	–	–
Melibiose	–	–	**20**	–	+	–	–	–
Salicin	–	–	+	–	–	–	–	–
Saccharate	+	+	–	–	–	–	–	–
N-Acetylglucosamine	–	**15**	7	**15**	–	–	–	–
Butyrate	–	–	+	6	+	+	+	1
Caprate	14	10	+	+	–	+	**31**	–
Succinate	+	+	–	+	+	+	**31**	+
DL-Malate	+	+	–	–	–	+	**29**	–
DL-β-Hydroxybutyrate	+	7	12	7	–	+	**30**	–
DL-Glycerate	4	–	+	–	–	–	–	–
α-Ketoglutarate	+	+	–	–	–	–	–	–
Erythritol	–	+	–	–	–	–	–	–
Sorbitol	+	+	–	–	–	–	–	–
Glycerol	+	+	+	–	+	–	–	–
Ethanol	+	–	**17**	**16**	+	+	22	–
m-Hydroxybenzoate	+	+	–	–	–	–	–	–
γ-Aminobutyrate	+	**16**	–	–	–	–	–	–
L-Tyrosine	–	–	**18**	**15**	+	–	–	–
n-Hexadecane	–	–	–	–	–	–	**28**	–
4 C	–	–	–	2	+	–	–	–
40 C	+	–	15	–	–	+	**29**	–

[a] + = All strains positive; – = all strains negative; numbers indicate number of positive strains; boldface numbers indicate that the number represents 80% or more of the strains.
[b] Denotes number of strains.
[c] Includes strains 214, 215, and 216.
[d] Includes strains 156 and 157 which do not accumulate PHB.
[e] Some strains have lateral flagella; two strains of this group are nonmotile.
[f] + = All straight rods; – = all curved rods; numbers indicate number of straight rods.
[g] Ring cleavage for strains capable of growth on aromatic compounds.

group G-1, none of the remaining groups contains sufficient strains to merit a species designation, we propose to leave these groups (which have a moles % GC range of 52.0 to 56.4) unassigned with respect to genus.

Aerobic polarly flagellated, marine strains having a GC content of 40 to 50 moles % in their DNA have been previously isolated but poorly characterized (9, 33, 55). Some authors have placed these strains in the genus *Vibrio* (9, 55), a placement which is unsatisfactory since current usage restricts this genus to facultative anaerobes (20). Since our isolates having a GC range of 43.2 to 48.0 cannot be assigned to existing genera, we propose the creation of a new genus *Alteromonas* (from the

TABLE 5. *Some distinguishing properties of the polarly flagellated species and groups which accumulate PHB*[a]

Trait	B-1 3[b]	B-2 4	C-1 2	*P. doudoroffii* C-3 11	D-1 2	*P. marina* F-2[c] 7	G-1 11	G-2[d] 3	H-1 4	I-1 4	I-2 5
Number of polar flagella[e]	4–6	4–6	1	*1–3*	1	2–5	1	1	1	1–2	3–6
Ring cleavage[f]	*o*	*o*	*o*	*o*	*m*	–	*m*	–	*m*	–	*o*
Oxidase	+	+	+	+	+	–	+	+	+	+	+
D-Ribose	–	–	–	4	–	+	–	–	–	–	–
D-Glucose	+	+	+	–	+	+	–	–	+	–	1
D-Mannose	+	–	–	–	–	–	–	–	–	–	–
D-Galactose	+	+	–	–	+	+	–	–	–	–	–
D-Fructose	+	+	1	**10**	+	+	–	–	+	–	–
Sucrose	–	–	–	–	+	–	–	–	–	–	–
Gluconate	2	–	–	–	+	+	–	–	–	–	–
N-Acetylglucosamine	+	+	–	–	+	–	–	–	–	–	–
Valerate	+	+	+	–	+	**6**	8	+	–	+	3
Pelargonate	–	–	–	–	+	+	2	–	–	–	–
Glycolate	+	–	1	**10**	+	–	–	–	–	–	–
DL-Glycerate	2	2	+	**10**	+	+	–	–	–	–	–
Aconitate	+	3	1	+	+	+	**10**	2	3	–	–
Mannitol	+	+	1	–	+	+	–	–	+	–	–
meso-Inositol	–	+	–	–	+	5	–	–	1	–	–
Glycerol	+	+	+	–	+	+	–	–	–	–	+
Ethanol	–	–	–	**10**	+	5	+	+	+	+	–
Phenylacetate	2	–	–	–	–	–	**10**	–	–	–	–
Quinate	1	–	–	–	+	–	5	–	+	–	–
Glycine	+	+	+	+	+	–	–	+	–	3	–
L-Threonine	+	+	+	8	–	–	–	–	–	2	+
L-Valine	+	–	+	–	+	–	2	–	–	3	–
L-Ornithine	+	3	+	+	–	5	**10**	–	–	+	**4**
δ-Aminovalerate	–	–	+	+	–	–	3	2	–	+	–
L-Histidine	+	+	+	+	–	–	–	–	–	–	**4**
L-Tyrosine	+	+	+	–	+	+	+	–	–	–	**4**
Ethanolamine	+	+	+	–	–	–	–	–	1	2	**4**
Sarcosine	+	+	+	+	+	**6**	–	–	–	+	+
Nicotinate	+	–	–	–	–	–	–	–	1	–	–
Allantoin	+	–	+	+	+	–	–	–	–	–	–
4 C	–	–	–	–	–	**6**	–	–	–	–	–

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

[b] Denotes no. of strains.

[c] Includes strain 219; two strains of this group are non-motile.

[d] Includes only strains 158, 159, and 160 which accumulate PHB.

[e] Numbers indicate number of flagella at one pole; italicized number indicates that the flagella often occur at both poles of the cell.

[f] Ring cleavage for strains capable of growth on aromatic compounds.

Latin, alter, meaning another) to accommodate these strains. The following is a definition of this genus.

Alteromonas gen. nov. consists of nonspore-forming, gram-negative, straight or curved rods which are motile by means of unsheathed, polar flagella. None accumulates PHB as an intracellular reserve product. The moles % GC content of the DNA ranges from 43 to 48. All are chemoorganotrophic, strict aerobes which are unable to denitrify, luminesce, fix molecular nitrogen, or grow with molecular hydrogen as the sole source of energy and carbon dioxide as the sole source of carbon. None has a con-

stitutive arginine dihydrolase. All strains are capable of growth on a minimal medium containing ASW with D-glucose as the sole source of carbon and energy and ammonia as the sole source of nitrogen. Sodium ion but no organic growth factors are required for growth. Most strains are able to utilize a variety of organic compounds as sole sources of carbon and energy, including carbohydrates, monocarboxylic fatty acids, and amino acids. Some species may produce an extracellular amylase, lipase, gelatinase, or chitinase. Two species utilize *m*-hydroxybenzoate, *p*-hydroxybenzoate, and quinate. These compounds are degraded by means of a *m* cleavage of protocatechuate. None of the strains hydrolyzes agar or utilizes cellulose, formate, C_5-C_{10} dicarboxylic acids, benzoate, or *n*-hexadecane. The strains which comprise this genus are common inhabitants of the open sea and coastal waters. *A. macleodii* has been designated the type species of the genus *Alteromonas*.

A comparison of the above definition of *Alteromonas* with the definition of the genus *Pseudomonas* (36, 51) indicates that these two genera are separable solely on the basis of CG content. It should be stressed that the GC range designated as characteristic of both genera are separable solely on the basis of GC nuity in the GC composition of the groups assigned to *Alteromonas* and *Pseudomonas* suggests that further study may reveal phenotypic differences between these groups which may be of use for generic separations. It may then become possible to consider the generic assignments of groups which have a GC content intermediate between *Alteromonas* and *Pseudomonas* and to establish to which (if either) of these genera they belong.

The peritrichously flagellated groups characterized in this study have a moles % GC content of 53.7 to 67.8. Physiologically these organisms resemble the genus *Pseudomonas* and are distinguishable from this genus solely on the basis of their flagellation. Their peritrichous flagellation and respiratory metabolism suggest their placement in the genus *Achromobacter* or *Alcaligenes* (*Bergey's Manual*, 7th ed.). Both of these genera are poorly studied, and their validity has been repeatedly questioned (11, 15, 37). Davis et al. (15) have characterized two species of gram-negative, strictly aerobic, degenerately peritrichous, hydrogen-utilizing bacteria which, except for their flagellation, were similar to the members of the genus *Pseudomonas* (51). By a process of elimination, these authors concluded that these species should be assigned to the genus *Alcaligenes*. We shall follow their example and assign our peritrichously flagellated strains to this genus. Two strains designated *Alcaligenes faecalis* (ATCC 8750 and 8455) have moles % GC contents in their DNA of 54.8 (10) and 57.1 (4), respectively, which is within the range of our peritrichously flagellated, marine isolates. An extensive study of the gram-negative, aerobic, peritrichously flagellated organisms of soil and fresh water origin is necessary before the validity of the genus *Alcaligenes* can be established and the reasonableness of our assignment of marine strains into this genus assessed.

The placement of strains into genera on the basis of flagellation presents difficulties in practice as well as in principle. As discussed in the results, flagella stains are sometimes difficult to perform and interpret. Furthermore, the existence of nonmotile strains necessitates placement by means of other phenotypic traits. The major failing of this trait is that a

TABLE 6. *Some distinguishing properties of the peritrichously flagellated species (Alcaligenes)*[a]

Trait	*A. paci-ficus* C-2 6[b]	*A. cupi-dus* D-2 5	*A. venus-tus* D-3, 4 14	*A. aestus* F-1 6
Ring cleavage[c]	*o*	*o*	*o*	−
Oxidase	+	−	+	+
L-Arabinose	−	+	−	−
D-Mannose	−	+	−	−
Saccharate	−	+	−	−
Suberate	−	−	−	+
Sebacate	−	−	1	+
Glycolate	−	+	−	−
Aconitate	**5**	+	**13**	−
Mannitol	−	+	**12**	+
δ-Aminovalerate	+	**4**	+	−
L-Histidine	+	**4**	**12**	−
L-Tyrosine	+	+	+	−
DL-Kynurenine	+	−	−	−
Ethanolamine	−	−	**13**	−
Benzylamine	**5**	−	−	−
Putrescine	+	+	+	−
Sarcosine	+	+	+	−
Allantoin	**5**	+	+	−
4 C	−	−	+	−

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

[b] Denotes number of strains.

[c] Ring cleavage for strains capable of growth on aromatic compounds.

clear-cut distinction between polar and peritrichous flagellation does not always exist. In the case of group G-3 (which we have assigned to *Pseudomonas*) some strains occasionally have one to three lateral flagella of a shorter wave length than the polar flagellum (Fig. 37). A similar situation is found in *Pseudomonas stutzeri*, *P. mendocina* (40), and *Chromobacterium* (48). Palleroni et al. (40) characterized *P. stutzeri* and *P. mendocina* as polarly flagellated since the different wave lengths of these flagella suggested that these species are polarly flagellated organisms which have an additional and distinct genetic determinant for the synthesis of lateral flagella. A more striking case suggesting the existence of two sets of genes for the synthesis of flagella is found in the genus *Beneckea* (1, 5). Species of this genus have a sheathed, polar flagellum when grown in liquid medium. On solid medium, some species synthesize a large number of unsheathed flagella in addition to the single, sheathed, polar flagellum. Such strains appear to be peritrichously flagellated. The main difference between the flagellation of these species of *Beneckea* and the flagellation of *P. stutzeri* and *P. mendocina* is the number of "lateral" flagella. The artificial distinction which is imposed on these organisms by attempts to describe them by the terms "polar" and "peritrichous" could perhaps be avoided by recognizing a third category, such as Leifson's "mixed flagellation" (31), to accommodate organisms whose flagellation is governed by two distinct genetic determinants. The recognition of such flagellation is complicated by the possibility that flagella synthesized by two distinct genetic determinants could have the same wave length, in which case the organism would be characterized as "peritrichous" or "degenerately peritrichous" (40). Recent evidence from studies in comparative enzymology indicates that differences in flagellation may not always justify a generic separation. Species of the polarly flagellated genus *Aeromonas* resemble the enterics and not the pseudomonads in their enzymes of tryptophan biosynthesis (13), control of biosynthesis of amino acids of the aspartate family (8), and allosteric control of 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthetase (27). In addition, the β-galactosidase of *Aeromonas formicans* is similar to that of *Escherichia coli* (45).

Choice of specific designations. The groups which we have assigned to *Pseudomonas* and *Alcaligenes* are phenotypically distinct from the well characterized species of these genera (2, 3, 16, 40, 43, 46, 51). Although most of the groups are sufficiently different phenotypically to deserve distinct species designations, we have only given specific names to 12 groups each of which has five or more strains and can be readily identified by means of simple nutritional, physiological, and morphological tests. Groups G-1 and H-2 meet these criteria but have not been given specific designations since we have not assigned these groups to genera. In the case of the remaining eight groups, it is our opinion that additional strains should be isolated and characterized before these groups are given species names. The following discussion will consider the taxonomy and selected properties of the species and groups studied. Their complete phenotypes are given in Table 1 and their nutritional versatility in Fig. 46. Diagnostic traits of use in distinguishing these groups and species are given in Tables 4 to 6.

Alteromonas communis sp. nov. (group A-1) and A. vaga sp. nov. (group A-2). The 50 strains which comprise these two species are motile by means of single polar flagella inserted at one or both poles (Fig. 29, 30). *A. communis* is a curved rod (Fig. 1) and oxidase positive, whereas *A. vaga* is a straight rod (Fig. 2) and oxidase negative. Neither species accumulates PHB as an intracellular reserve product. The GC content in the DNA of five representative strains of *A. communis* and five representative strains of *A. vaga* is 46.9 $\pm$ 1.0 and 48.0 $\pm$ 1.5 moles %, respectively (Table 2, 3). All the strains of groups A-1 and A-2 metabolize *m*-hydroxybenzoate, *p*-hydroxybenzoate, and quinate by means of a *m* cleavage of the intermediate protocatechuate. None utilizes benzoate or *o*-hydroxybenzoate. Although all the isolates are able to utilize acetate, non utilizes butyrate or valerate (Table 1). Properties of use in distinguishing these two species are given in Table 4. Strains 8 (ATCC 27118) and 40 (ATCC 27119) have been designated the type strains of *A. communis* and *A. vaga*, respectively.

Alteromonas macleodii sp. nov. (group E-1). The 21 strains belonging to this species are straight rods (Fig. 3) which are motile by means of a single polar flagellum originating from one pole (Fig. 31). None of the strains accumulates PHB as an intracellular reserve product or utilize aromatic compounds. The GC content in the DNA of five representative strains is 45.6 $\pm$ 0.8 moles % (Table 2, 3). All or a majority of the strains have an extracellular amylase, gelatinase, and lipase and are able to utilize cellobiose, melibiose, lactose, and salicin. A distinctive property of this spe-

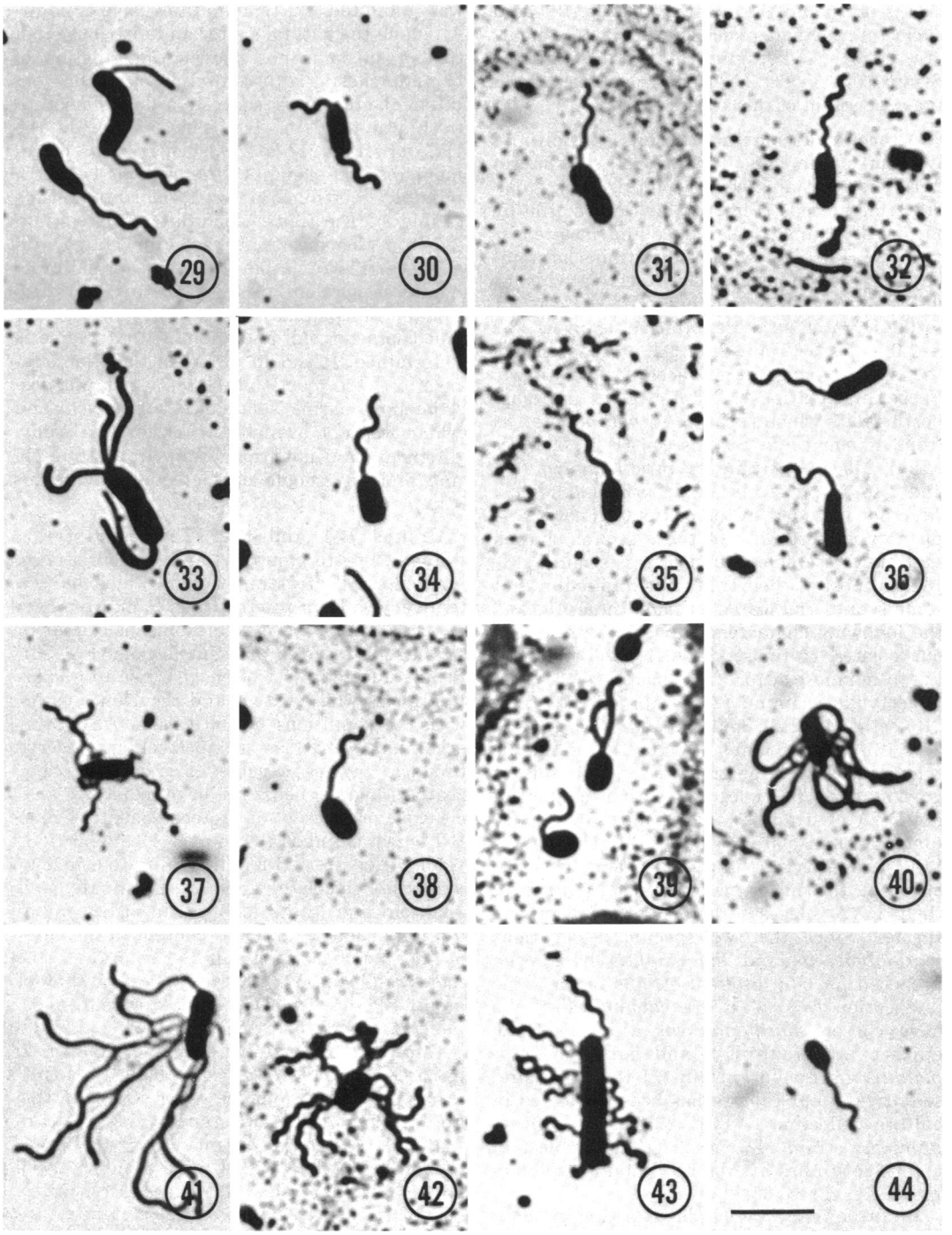

FIG. 29–44. *Leifson flagella stains* (× 2,500). *Marker in Fig. 44 represents 5 μm. Fig. 29, Alteromonas communis, strain 16; Fig. 30, A. vaga, strain 40; Fig. 31, A. macleodii, strain 104; Fig. 32, A. marinopraesens, strain 119; Fig. 33, Pseudomonas doudoroffii, strain 70; Fig. 34, group G-1, strain 153; Fig. 35, group G-2, strain 157; Fig. 36, P. nautica, strain 188; Fig. 37, P. nautica, strain 170; Fig. 38, group H-1, strain 198; Fig. 39, group I-1, strain 207; Fig. 40, Alcaligenes pacificus, strain 64; Fig. 41, A. cupidus, strain 79; Fig. 42, A. venustus, strain 86; Fig. 43, A. aestus, strain 134; Fig. 44, group H-2, strain 202.*

cies is its inability to utilize tricarboxylic acid cycle intermediates such as succinate, fumarate, citrate, α-ketoglutarate, and aconitate. Strain 107 (ATCC 27126) has been designated the type strain of this species.

Alteromonas marinopraesens (ZoBell and Upham) comb. nov. (group E-2) and strain 217. The 17 strains which comprise this group are straight rods (Fig. 4) which are motile by means of a single polar flagellum inserted at one pole (Fig. 32). None of the strains accumulates PHB as an intracellular reserve product or utilizes aromatic compounds. The GC content in the DNA of six representative strains is 43.2 ± 1.0 moles % (Table 2, 3). All or a majority of the strains have an extracellular gelatinase, lipase, and chitinase. Table 4 gives the diagnostic traits which separate *A. marinopraesens* from *A. macleodii*. Strains 214 (B-16 of MacLeod), 215 (type strain of *Vibrio haloplanktis*), and 216 (type strain of *V. marinopraesens*) were not included in the computer analysis. A phenotypic screening of these strains showed that they resembled our isolates (strains 119–132) in most of their traits but differed in that some strains (indicated in parentheses) lacked the following characters universally present in our isolates: chitinase (214–216), ability to utilize D-mannose (215, 216), sucrose (214, 215), *N*-acetylglucosamine (215, 216), citrate (215, 216), ethanol (215), and the ability to grow at 35 C (214, 215). Strains 214 and 216, unlike the remaining strains of group E-2, were able to grow at 4 C. The moles per cent GC content in the DNA of strains 214, 215, and 216 is in the range of our isolates (Table 2, 3). The phenotypic similarity of these strains and the similarity of the moles per cent GC content in their DNA suggests their placement in one species. Since the two specific designations *marinopraesens* and *haloplanktis* have been proposed for two different strains in the same publication (55), both are equally valid. We have chosen *marinopraesens* as the specific epithet for group E-2 and consider *haloplanktis* a synonym. Strain 121 which is representative of our isolates has been deposited in culture collection (ATCC 27127). *A. marinopraesens*, strain 214, has been the subject of extensive studies by MacLeod and his collaborators (22, 23, 34, 35).

In 1940, White (54) isolated a strictly aerobic, polarly flagellated, gram-negative, straight rod from a surface discoloration of salted butter. This organism, which was designated *P. nigrifaciens*, produced a brown-black pigment, required sodium chloride for growth and was able to grow at 4 C. Pigment production was inhibited at temperatures above 25 C. Although the natural source and distribution of this organism is not known, the isolation of bacteria causing a discoloration of butter from different creameries suggests a possible soil or fresh water origin. The moles % GC in the DNA of strain 217 is 42.9, suggesting its placement into the genus *Alteromonas* as well as a possible relationship to *A. marinopraesens* (Table 2). The traits which differentiate strain 217 from this species are given in Tables 1 and 4. It seems inadvisable to make a formal taxonomic assignment of this isolate until additional strains similar to strain 217 are isolated and characterized, and their natural source is determined. It should be noted that the organism of Norton and Jones (38), which was designated as marine isolate of *P. nigrifaciens* and which was not available to us for this study, differs in many nutritional traits from strain 217 and probably should not be equated with this strain.

Groups B-1 and B-2. The three strains which belong to group B-1 and the four strains in group B-2 are straight rods which have a tendency to form rosettes (Fig. 5, 6). Members of these groups are motile by means of four to six flagella inserted at a single pole (Fig. 25). Strains of group B-2 often give rise to involution forms whose extent and occurrence varies with the conditions of cultivation. All strains accumulate PHB as an intracellular reserve product. Strains capable of growth on benzoate, *p*-hydroxybenzoate, or quinate give an *o* cleavage of catechol or protocatechuate. Group B-1 which is linked to group B-2 at the 68% S-value differs from B-2 in its ability to utilize D-mannose, glycolate, L-valine, nicotinate, and allantoin and by its inability to utilize *meso*-inositol (Table 1). The GC content in the DNA of the members of group B-1 is 63.3 ± 0.6 moles % (Table 2, 3). Strains 54, 55, and 56 of group B-2 have a GC content of 59.0 ± 0.8 moles %. Strain 57, which is linked at the 78% S-value to the remaining strains of group B-2, has a GC content of 62.7 ± 0.8 moles %, significantly different from the other strains of this group. Phenotypic differences also indicate that this strain is distinct from the other members of group B-2. Unlike strains 54, 55, and 56, strain 57 is able to denitrify, utilize cellobiose, salicin, glucuronate, glutarate, itaconate, and histamine, and is unable to utilize γ-aminobutyrate or grow at 35 C. Strains 51 and 52 have been deposited in culture collection (ATCC 27120, 27121).

Pseudomonas doudoroffii sp. nov. (group C-3) and group C-1. The 11 strains com-

prising group C-3 are straight rods (Fig. 7) which are motile by means of one to three flagella, usually inserted at both poles of the cell (Fig. 33). All the strains of this group accumulate PHB as an intracellular reserve product. Strains capable of growth on benzoate or *p*-hydroxybenzoate degrade catechol or protocatechuate by means of an *o* cleavage. Ten strains of *P. doudoroffii* are able to utilize D-fructose; none utilize other monosaccharides, disaccharides, sugar alcohols (including glycerol), or C_4-C_9 monocarboxylic fatty acids. The GC content in the DNA of five representative strains of *P. doudoroffii* is 59.1 ± 1.0 moles % (Table 2, 3). Strain 66 which differs from the remaining strains of *P. doudoroffii* by its inability to utilize fructose, glutarate, glycolate, and DL-glycerate also differs significantly in its moles % GC content (55.6 ± 1.0) and probably should not be considered a member of this species. Strain 70 (ATCC 27123) has been designated the type strain of *P. doudoroffii*.

Group C-1 consists of two strains which, like *P. doudoroffii*, are straight rods, accumulate PHB, cleave aromatic compounds (including *m*-hydroxybenzoate) by means of an *o* cleavage, and are unable to utilize disaccharides. Unlike *P. doudoroffii*, the members of group C-1 have a single polar flagellum at one pole and are able to utilize C_4-C_6 monocarboxylic fatty acids, glycerol, L-leucine, L-isoleucine, L-valine, L-lysine, L-tyrosine, L-phenylalanine, ethanolamine, butylamine, and trigonelline (Table 1). In addition, the GC content of strains 58 and 59 (63.8 ± 0.6 moles %) is higher than that of *P. doudoroffii* (Table 2, 3).

Group D-1. The two strains belonging to this group are straight rods which are motile by means of a single polar flagellum. Both strains accumulate PHB as an intracellular reserve product and are able to utilize benzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, and quinate. Cells grown on these aromatic substrates degrade catechol or protocatechuate by means of a *m* cleavage. The GC content of strains 77 and 78 is 64.7 ± 0.5 moles % (Table 2, 3).

Pseudomonas marina (Cobet et al.) comb. nov. (group F-2). The seven strains which comprise this species are straight rods (Fig. 7). Five are motile by means of two to five flagella inserted at one pole (Fig. 20, 28); the remaining two strains are nonmotile. All the strains accumulate PHB as an intracellular reserve product and are oxidase negative. The GC content in the DNA of five representative strains is 63.0 ± 0.5 moles % (Table 2, 3). The analysis of the moles per cent GC content of strain 219 by Cobet et al. (7) is in agreement with our determinations. None of the strains utilizes aromatic compounds or disaccharides; all are able to grow on D-glucose, D-galactose, and D-fructose. Six strains are able to grow at 4 C. Strain 219, designated *Arthrobacter marinus* by Cobet et al. (7), differs from the other members of group F-2 in its ability to grow at 40 C and utilize L-phenylalanine. This strain, like one other strain of group F-2, is nonmotile. We have compared strain 219 with the type strain of *Arthrobacter globiformis* (ATCC 8010). Both strains were grown on YEB, YEA, BM containing 0.2% D-glucose, and BMA containing 0.2% D-glucose. The cells were examined by means of phase-contrast microscopy and stained to determine their Gram reaction in exponential, early stationary, and late stationary phases of growth. On all the media used, the cells of strain 219 were gram-negative, straight rods, usually occurring singly but sometimes in pairs. The rods became shorter in stationary phase but were never coccoid. In all the media tested, A. *globiformis* underwent the cycle of development characteristic of *Arthrobacter* (52). A rudimentary mycelium was observed during exponential and early stationary phases of growth which became segmented and fragmented by late stationary phase. The cells were gram-variable during exponential phase, becoming gram-positive in stationary phase. These results indicate that strain 219 has none of the morphological characteristics of *Arthrobacter*. With the exception of motility, the properties of this strain are those of the genus *Pseudomonas* (51). Its close similarity to motile strains of group F-2 indicates that this group should bear the designation *Pseudomonas marina*. Strain 140 (ATCC 27129), a motile isolate of this species, has been deposited in culture collection.

Pseudomonas nautica sp. nov. (group G-3), groups G-1, G-2, and strain 218. The strains which make up groups G-1, G-2, and G-3 are unable to utilize sugars, sugar acids, and sugar alcohols (Table 1). All are straight rods. Group G-1 consists of 11 strains which are motile by means of a single polar flagellum (Fig. 9, 34) and accumulate PHB as an intracellular reserve product. The GC content in the DNA of five representative strains is 55.8 ± 0.7 moles % (Table 2, 3). Strains capable of growth on benzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, quinate, or L-tryptophan give a *m* cleavage of catechol or protocatechuate. Although this group deserves species status, it has been left undesignated for reasons previously discussed. Strain 146 (ATCC

27130) is representative of this group.

P. nautica (group G-3) consists of 34 straight rods (Fig. 11) of which 32 are motile. All of the motile strains have a single polar flagellum (Fig. 36). Ten strains have one to three lateral flagella in addition to the polar flagellum. The lateral flagella, which are present in a minority of the population, have a distinctively shorter wave length (Fig. 37). None of the members of *P. nautica* is able to accumulate PHB as an intracellular reserve product. Strains 164 and 193 have a moles % GC content in their DNA of 61.0 ± 1.1 (Table 2, 3); strains 170, 179, 185, and 189 have a GC content of 57.8 ± 1.2 moles %, significantly different from that of strains 164 and 193. No phenotypic differences have been found which reflect this difference in GC content. Strains of *P. nautica* which are able to utilize benzoate cleave catechol by means of an *o* cleavage. All or most of the strains of this species have a lipase and are able to denitrify and utilize *n*-hexadecane. Strains of this species are the only isolates in our collection which have the latter trait. Although none of the strains are able to utilize carbohydrates, three strains have an extracellular amylase. Repeated attempts to isolate spontaneous mutants capable of growth on glucose were negative. Strain 179 (ATCC 27132) has been designated the type strain of this species. A highly specific enrichment procedure for *P. nautica* is the enrichment medium for denitrifiers previously described (5) containing 0.2% (w/v) sodium butyrate.

Group G-2 consists of five strains which are motile by means of a single polar flagellum (Fig. 10, 35). None of the strains utilizes aromatic compounds. The GC content in the DNA of strains 156 and 157 is 55.6 ± 0.6 moles % (Table 2, 3); the GC content of strains 159 and 160 is 52.0 ± 0.8 moles %. This significant difference in GC content correlates with phenotypic differences. Strains 156 and 157, unlike strains 158, 159, and 160, do not accumulate PHB or utilize glycine, are able to grow at 40 C, and utilize caprylate, pelargonate, adipate, and laevulinate. As seen from Table 4, there are no universally positive or negative traits which distinguish strains 156 and 157 from *P. nautica*. The best distinguishing trait is the ability of most strains of *P. nautica* to utilize *n*-hexadecane. The similarity between the phenotypes and the moles per cent GC contents of *P. nautica* and strains 156 and 157 suggests a possible relationship. The taxonomic placement of these two strains cannot be resolved on the basis of the available data.

Strain 218, the type strain of *Pseudomonas perfectomarinus* (ZoBell and Upham), is a nonmotile, curved rod which is able to denitrify and does not accumulate PHB as an intracellular reserve product (Table 1). The moles % GC content (61.7) in the DNA of this strain falls in the range of group G-3 (*P. nautica*) (Table 2, 3). However, numerous phenotypic differences distinguish this strain from *P. nautica*. Unlike group G-3, strain 218 is able to utilize D-glucose, maltose, saccharate, glucuronate, malonate, glycolate, DL-glycerate, α-ketoglutarate, itaconate, mesaconate, glycerol, ethyleneglycol, L-aspartate, L-tyrosine, and ethanolamine and does not have an extracellular lipase. Strain 218 also differs by many traits from the other species and groups having a similar GC content. The original description of this strain (55) stated that it was motile by means of "one to several flagella at each pole". No flagella could be detected in strain 218 by means of the Leifson technique or by electron microscopy. Strain 218 does, however, conform to the original description in being able to denitrify and hydrolyze starch but not gelatin.

Group H-1. The four strains which comprise this group are straight rods (Fig. 12) which are motile by means of a single polar flagellum (Fig. 38). All of the strains accumulate PHB as an intracellular reserve product. The GC content in the DNA of members of this group is 54.9 ± 0.9 moles % (Table 2, 3). Strains which utilize *p*-hydroxybenzoate or quinate cleave protocatechuate by means of a *m* cleavage. All of the strains of group H-1 have a lipase and are able to utilize D-glucose and D-fructose but not other hexoses, disaccharides, or glycerol. Group H-1 is linked to group H-2 at the 74% S-value (Fig. 45). In spite of this close linkage, these two groups differ in 18 phenotypic traits (Table 1) as well as in the moles per cent GC content in their DNA (Table 2, 3). Strain 197 (ATCC 27133) is a typical member of this group.

Groups I-1 and I-2. The strains which belong to these two groups are motile, straight rods (Fig. 13, 14). Group I-1 has one to two polar flagella (Fig. 39); group I-2 has three to six polar flagella (Fig. 27). In both groups, the flagella are inserted at one pole. Members of group I-2 may aggregate into rosettes and have a tendency to give rise to involution forms. The four strains which comprise group I-1 and the five strains of group I-2 are linked at the 76% S-value (Fig. 45). The traits which separate these groups are given in Tables 1 and 5. With the exception of strain 213 (group I-2) none of the members of groups I-1 and I-2 is able to utilize carbohydrates or aromatic com-

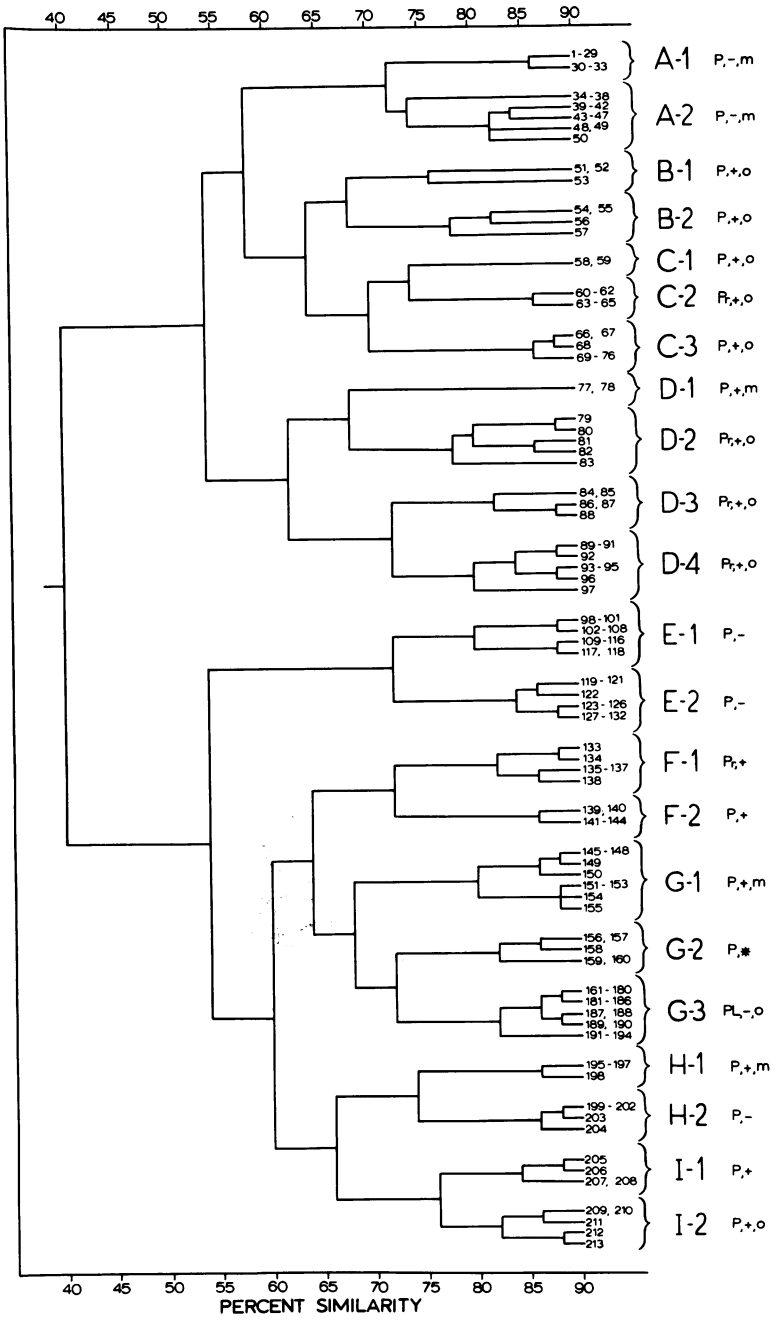

FIG. 45. *Numerical analysis of strains. Symbols apply to all strains within a group: (P) polar flagella; (Pr) peritrichous flagella; (PL) polar flagella, some strains also have one to three lateral flagella of a shorter wave length; (+) accumulate PHB; (*) PHB is accumulated by three out of five strains; (−) do not accumulate PHB; (m) degrade diphenolic intermediates by means of a m cleavage; (o) degrade diphenolic intermediates by means of an o cleavage.*

pounds. Strain 213 is able to utilize D-glucose and *p*-hydroxybenzoate. Cells grown on the latter compound decompose protocatechuate by means of an *o* cleavage. The GC content of the members of group I-1 is 56.4 ± 0.6 moles % (Table 2, 3). In group I-2, significantly dif-

ferent GC contents occur within two sets of strains linking at the 82% S-value. Strains 209, 210, and 211 have a GC content of 61.2 ± 0.0 moles % as opposed to 63.6 ± 0.6 moles % in the DNA of strain 212. Two phenotypic characters, growth at 35 C and the ability to denitrify, also distinguish strain 212 (and 213) from strains 209, 210, and 211. Three members of group I-2 (209, 210, and 213) produce colonies containing a blue-black pigment which is water insoluble. Norton and Jones (38) have described a marine isolate with a similar pigmentation. However, the large number of nutritional differences between their isolate and the pigmented strains of group I-2 suggests that these organisms belong to distinct species. Strains 207 (ATCC 27135) and 210 (ATCC 27136) from groups I-1 and I-2 have been deposited in culture collection.

Alcaligenes pacificus sp. nov. (group C-2). The six strains which make up this species are straight rods (Fig. 15) which are motile by means of peritrichous flagella (Fig. 40). All of the strains accumulate PHB as an intracellular reserve product. The GC content of four representative strains is 67.8 ± 0.5 moles % (Table 2, 3). Strains which are able to utilize benzoate, *m*-hydroxybenzoate, or *p*-hydroxybenzoate degrade catechol or protocatechuate by means of an *o* cleavage. All the strains are able to utilize D-glucose and D-fructose but not other hexoses or disaccharides. Strain 62 (ATCC 27122) has been designated the type strain of this species.

Alcaligenes cupidus sp. nov. (group D-2). The five strains which comprise this species are straight rods (Fig. 16) which are peritrichously flagellated (Fig. 41). All the strains are oxidase negative and accumulate PHB as an intracellular reserve product. The GC content in the DNA of three representative strains is 61.0 ± 1.1 moles % (Table 2, 3). Strains capable of utilizing benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, or quinate cleave catechol or protocatechuate by means of an *o* cleavage. Strains of *A. cupidus* are some of the nutritionally most versatile isolates in our collection (Fig. 46), being able to utilize 69 to 85 different carbon compounds as sole sources of carbon and energy. Strain 79 (ATCC 27124) has been designated the type strain of this species.

Alcaligenes venustus sp. nov. (groups D-3 and D-4). The 14 strains belonging to this species are straight rods (Fig. 17) which are motile by means of peritrichous flagella (Fig. 42). All the strains accumulate PHB as an intracellular reserve product. Strains which are able to utilize benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, quinate, or L-tryptophan cleave catechol or protocatechuate by means of an *o* cleavage. All the strains are able to grow at 4 C. Groups D-3 and D-4, both of which are included in the species *A. venustus*, are linked at the 72% S-value (Fig. 45). These two groups are separable on the basis of three phenotypic traits. Unlike group D-4, group D-3 is able to utilize galacturonate and propyleneglycol and is unable to utilize DL-glycerate (Table 1). The GC contents in the DNA of four representative strains of group D-3 and four representative strains of group D-4 are nearly identical (Table 2, 3); their composite value is 53.7 ± 0.8 moles %. Since phenotypically these two groups are distinguished only by three universally positive or negative traits and since their moles per cent GC content is very similar, they have been combined into a single species. Strain 86 (ATCC 27125) has been designated the type strain of this species.

Alcaligenes aestus sp. nov. (group F-1). The six strains which comprise this species are straight rods (Fig. 18) which are motile by means of peritrichous flagella (Fig. 43). All the strains accumulate PHB as an intracellular reserve product. The GC content in the DNA of four representative strains is 57.4 ± 0.5 moles % (Table 2, 3). None of the strains is able to utilize aromatic compounds. A distinctive property of this species is the ability of all the strains to utilize C_7-C_{10} dicarboxylic acids. Strain 134 (ATCC 27128) has been designated the type strain of this species.

Group H-2. The six strains in this group are motile by means of a single polar flagellum (Fig. 44). Three strains are straight rods (Fig. 19), the remaining are curved rods. None of the strains accumulates PHB as an intracellular reserve product, nor do they utilize aromatic compounds or carbohydrates. The GC content in the DNA of four representative strains is 30.5 ± 1.5 moles %. The large standard deviation is probably due to the large density difference between the sample and reference DNA species used in the CsCl determinations. The members of group H-2 are nutritionally the least versatile strains in our collection (Fig. 46). Although group H-2 is linked to group H-1 at the 74% S-value (Fig. 45), the two groups are separable by a large number of traits (Table 1). Strain 202 (ATCC 27134) is a typical strain of this group. As discussed previously, the properties of group H-2 suggest that these isolates deserve a new generic and specific designation. Additional

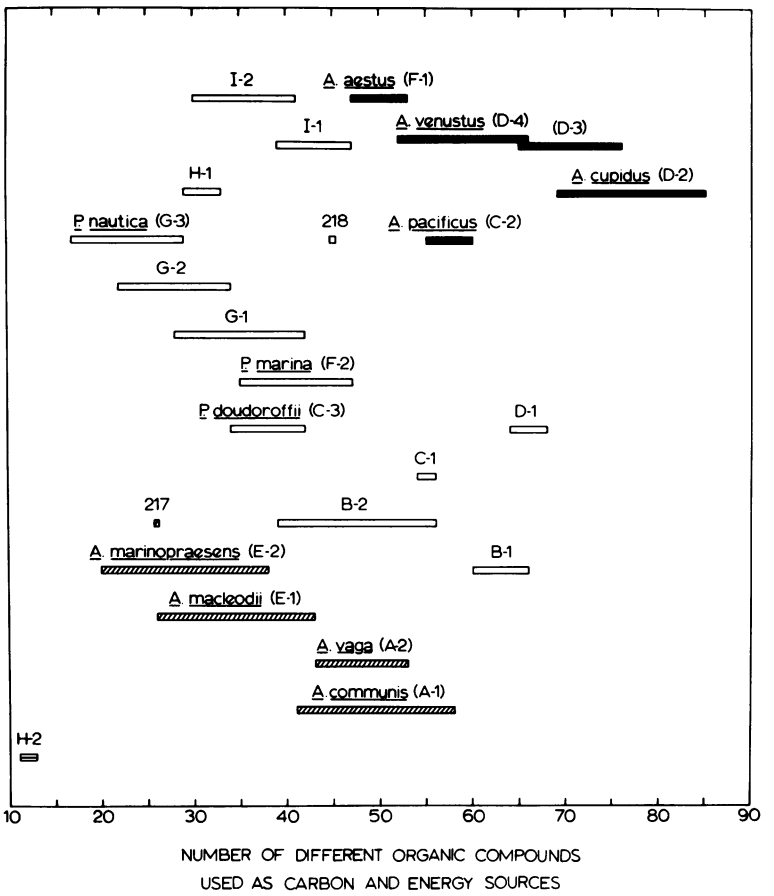

FIG. 46. *Nutritional versatility of the species and groups studied. (crossbarred band) group with GC content of 30.5 moles %; (barred band) Alteromonas; (white band) Pseudomonas and undesignated groups with GC contents of 52.0 to 56.4 moles %; (black band) Alcaligenes.*

strains should, however, be isolated and characterized before a formal taxonomic treatment of this group is attempted.

This study represents a beginning in the taxonomic characterization of gram-negative, flagellated, nonfermentative bacteria of marine origin. Although the described species and groups are well characterized, their generic assignments are in part unsatisfactory due to a dependence on flagellation and moles per cent GC content for generic separations. Further work is necessary in order to obtain adequate generic traits which would allow this large assemblage of organisms to be split into well defined genera. It should be stressed that with a few exceptions our isolates have been obtained from a restricted geographical area and cannot be considered representative of the total heterotrophic, bacterial flora of the sea.

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