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Taxonomy of Marine Bacteria: *Beneckea parahaemolytica* and *Beneckea alginolytica*

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A collection of 169 strains, including 91 obtained from cases of gastroenteritis and 41 from localized tissue infections and infections of the eye and ear, was submitted to an extensive nutritional, physiological, and morphological characterization. The nutritional and physiological data obtained from these strains, as well as data for strains of other species of the genus *Beneckea*, were submitted to a numerical analysis which grouped the strains into clusters on the basis of phenotypic similarity. Strains from cases of gastroenteritis formed a group of three clusters which linked at a similarity value of 68%. These three clusters could not, however, be separated from each other by universally positive or negative traits, and on the basis of their overall phenotypic similarity were assigned to a single species, *B. parahaemolytica*. The majority of the strains from human, nonenteric sources segregated into two distinct clusters, one designated *B. alginolytica* and the other unassigned with respect to species (group C-2). *B. parahaemolytica*, *B. alginolytica*, and group C-2 could be readily distinguished from one another as well as from the remaining species of the genus *Beneckea* by multiple, unrelated, phenotypic traits. Activities of selected enzymes of glucose and gluconate catabolism in cell-free extracts of *B. parahaemolytica*, *B. alginolytica*, and group C-2 suggested that these organisms utilized glucose primarily via the Embden-Meyerhof pathway and gluconate primarily via the Entner-Doudoroff pathway. Similar results were observed in the other members of the genus *Beneckea*.

Beneckea parahaemolytica and *B. alginolytica* are two pathogenic bacteria of marine origin (references 2, 8, 26, 31, 36; Baumann and Baumann, *in press*). *B. parahaemolytica* has been recognized in Japan as a causative agent of gastroenteritis (26, 27, 36). The establishment of its medical importance by Japanese investigators has been predicated on two factors: the custom of consuming raw fish (sashimi, shirasu, sushi) and the recognition that *B. parahaemolytica* is a marine organism unable to grow on most common laboratory media unless they are supplemented with sodium chloride. The latter factor has been instrumental in the identification of this organism and explains why *B. parahaemolytica* has only recently been implicated in outbreaks of gastroenteritis in other countries (7, 10, 11). The potential health hazard that *B. parahaemolytica* presents in countries other than Japan has been demonstrated by recent outbreaks of gastroenteritis in the United States and in Australia (7, 10). In both outbreaks, cooked seafood appears to have been contaminated with materials from marine sources prior to consumption. Recently, organisms resembling *B. parahaemolytica* have been implicated in localized tissue infections (31) and in a case of gangrene which necessitated the amputation of a leg (24). *B. alginolytica*, unlike *B. parahaemolytica*, does not appear to cause gastroenteritis (27, 36). The pathogenicity of this common marine bacterium is indicated, however, by its isolation from infections of the ear and eye as well as from localized skin infections (31).

B. parahaemolytica was initially assigned to the genus *Pasteurella* (16). Subsequently, this species as well as *B. alginolytica* were placed into the genus *Oceanomonas* (21) and later into *Vibrio* (27). Recent work has suggested that the most reasonable placement of these two species is into *Beneckea* (references 2, 8; Baumann and Baumann, *in press*). The genus *Beneckea* consists of gram-negative, facultatively anaerobic, straight or curved rods of marine origin which

require sodium ion for growth. All are oxidase positive and ferment glucose with the production of acid but no gas. The guanine plus cytosine content of the deoxyribonucleic acid (DNA) ranges from 45 to 48 mol %. All species when grown on liquid medium have a single, sheathed, polar flagellum. *B. parahaemolytica* and *B. alginolytica* as well as several other species of this genus, when grown on solid medium, have unsheathed, peritrichous flagella in addition to the sheathed, polar flagellum. (2, 8; Baumann and Baumann, *in press*).

B. parahaemolytica can be readily identified on the basis of a few phenotypic properties when isolated from cases of gastroenteritis (26, 27). Its identification is simplified since the host selects for the pathogen and eliminates other similar marine bacteria (26, 36). When the source of isolation is sea water or marine animals, however, identification of this organism presents a number of difficulties since it must be differentiated from other common marine bacteria. The major problem has been the general lack of knowledge concerning the taxonomy and phenotypic properties of marine species. Consequently, it has not been known whether the phenotypic traits used for the differentiation of *B. parahaemolytica* from other marine bacteria were in fact valid for this purpose. The most commonly utilized scheme for the identification of *B. parahaemolytica* (6, 7, 15, 22, 26, 30, 36) is that proposed by Sakazaki et al. (27). This scheme purports to distinguish *B. parahaemolytica*, *B. alginolytica*, and a third group of marine bacteria, loosely designated *V. anguillarum*, primarily on the basis of salt tolerance, the Voges-Proskauer reaction, sucrose fermentation, and swarming on complex, solid media. The repeated use of this scheme has led to the implication that all facultatively anaerobic, gram-negative polarly flagellated, marine organisms which ferment glucose with the production of acid but no gas can be subdivided into three species. The fallacy of this unstated assumption has been demonstrated by work on the genus *Beneckea* (2, 8, 9) and the DNA homology studies of Anderson and Ordal (3). Both studies establish the existence of a number of species and groups which cannot be accommodated by Sakazaki's scheme.

Initial studies on the genus *Beneckea* involved an extensive phenotypic characterization of 145 isolates of marine origin (2, 8). By means of a numerical analysis of the data, the strains were clustered into 10 groups which were separable from each other by multiple, unrelated, phenotypic traits. Seven of these groups were given species designations. A subsequent study of 86 authentic strains of *B. parahaemolytica*, obtained from cases of gastroenteritis, indicated that these strains were phenotypically similar and were distinct from the other species of the genus *Beneckea* (Baumann and Baumann, *in press*). An examination of the diagnostic traits previously used for the identification of *B. parahaemolytica* indicated that many of these traits were inadequate for the differentiation of this organism from common species of the genus *Beneckea* (reference 8; Baumann and Baumann, *in press*). This conclusion has been supported by the work of Anderson and Ordal (3). On the basis of DNA homology, studied by in vitro DNA/DNA hybridization methods, these authors were able to subdivide a collection of 80 marine strains (most of which had the properties of the genus *Beneckea*) into at least five groups. One group, which consisted of strains isolated from cases of gastroenteritis (*B. parahaemolytica*), had DNA homologies of 90% or more. A second group consisted of strains of *B. alginolytica* with DNA homologies of over 80%. These two groups had average DNA homologies of 67%, indicating a high degree of genetic similarity. Hybridization between the other groups was on the order of 30% or less, indicating that they were genetically distinct. The study of Anderson and Ordal included 12 strains isolated from marine sources and designated *V. parahaemolyticus* by Baross and Liston (3). Only three of these strains hybridized with the group containing strains isolated from cases of gastroenteritis, pointing to the inadequacy of the traits used for the identification of this organism. The considerable DNA homology between strains of *B. parahaemolytica* and *B. alginolytica* observed by Anderson and Ordal (3) is in agreement with the in vitro DNA/DNA hybridization studies of Hanoaka et al. (17) and contrary to the results obtained by Citarella and Colwell (12). As previously pointed out (3), the strains studied by the latter authors under the designation *V. alginolyticus* were not authentic strains of this species, differing from the type strain in the moles percent guanine plus cytosine contents of their DNA and in their phenotypic properties (12, 13). In addition, it should be mentioned that the studies of Colwell (13) and Citarella and Colwell (12) were primarily directed to the differentiation of *B. parahaemolytica* and *V. cholerae* and did not address themselves to the more important problem of the relation of *B. parahaemolytica* to other common marine bacteria.

The purpose of this work is to extend our previous findings by characterizing additional strains of human pathogens having the properties of the genus *Beneckea* and submitting the data to a numerical analysis. Our results indicate that, on the basis of phenotypic differences, these strains can be placed into the species *B. parahaemolytica*, *B. alginolytica*, and a distinct, undesignated group (C-2). These species and group can be readily separated from the other species and groups of the genus *Beneckea* by multiple, unrelated, phenotypic traits.

MATERIALS AND METHODS

The methods used for the phenotypic characterization of the strains have been previously described (8). The following abbreviations have been used: BM (basal medium), YEB (yeast extract broth), and MA (Difco marine agar). These abbreviations are the same as previously used by Baumann et al. (8). The majority of the methods used for the study of glucose and gluconate catabolism have been previously described (Baumann and Baumann, *in press*) and include assays for the following enzyme activities: glucokinase (EC 2.7.1.2), glucose-6-phosphate (G6P) dehydrogenase (EC 1.1.1.49), 6-phosphogluconate (6PGA) dehydrogenase (EC 1.1.1.44), phosphoglucose isomerase (EC 5.3.1.9), phosphofructokinase (EC 2.7.1.11), fructose-1,6-diphosphate (FDP) aldolase (EC 4.1.2.13), 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase (EC 4.1.2.14), and 6PGA dehydrase (EC 4.2.1.12). The latter two enzymes were assayed jointly as a linked pair with 6PGA as the substrate. Gluconokinase (EC 2.7.1.12) was assayed by coupling 6PGA formation to reduction of nicotinamide adenine dinucleotide phosphate (NADP) by means of excess 6PGA dehydrogenase (Calbiochem). The assay mixture (1 ml) consisted of 50 mM tris (hydroxymethyl)aminomethane-hydrochloride (pH 8.0) containing 0.05 mM ethylenediaminetetraacetic acid, disodium salt; 5 mM $MgCl_2$; 1 mM sodium gluconate; 2 mM adenosine triphosphate; and 0.2 mM NADP. Differential oxidized/reduced cytochrome spectra were measured at room temperature as previously described (29).

Source of strains examined. The strains characterized in this study have numerical designations of 171 to 329. In the strain list, the strain number is followed by the following information enclosed in parentheses: (i) designation under which strain was received, (ii) source of isolation (if known), (iii) geographic source of isolation (if unstated, strain was isolated in Japan), (iv) investigator who isolated strain → investigator from whom strain was received, (v) K followed by a numeral refers to antigen serotype (if known). The following abbreviations have been used: CDC (Center for Disease Control), G (strains isolated from cases of gastroenteritis), A (K. Aiso), D (W. E. DeWitt), F (M. Fishbein), M (Y. Miyamoto), S. (R. Sakazaki), T. (R. M. Twedt), and Z. (H. Zen-Yoji). All other strain numbers refer to the previous work of Baumann et al. (8).

Strains assigned to *B. alginolytica*: 171 (CDC B984, ear, Hawaii); 172 (CDC B3744, ear, N.C.); 173 (CDC B6512, ear, R.I.); 174 (CDC C656, hand wound, Calif.); 175 (CDC B835, eye socket, Calif.); 176 (CDC B6550, skin abscess, Wash.); 177 (CDC B1650, foot wound, Wash.); 178 (CDC A7606, cut on wrist, La.); 179 (CDC B9992, finger, Fla.); 180 (CDC B4364, finger, La.); 181 (CDC B9003, tendon, La.); 182 (CDC B3591, ear, Wash.); 183 (CDC A6670, sputum, Fla.); 184 (CDC B9083, ear, Ga.); 185 (CDC C782, wound, Conn.); 186 (CDC A1539, ear, Conn.); 187 (CDC C205, ear, N.J.); 188 (CDC A3454, ear, N. J.). Strains 86 to 92 and 118 to 122 have been previously described (2, 8).

Strains assigned to *B. parahaemolytica*: 189 (K34, M → T); 190 (12042, G, Md., D, K30); 191 (K28, G, S → T); 192 (K8, A → T); 193 (K4, A → T); 194 (K39, M → T); 195 (8213, G, M, K7); 196 (8490, G, M, K7); 197 (8188, G, M, K7); 198 (8911, G, M, K29); 199 (9222, G, M, K7); 200 (9328, G, M, K7); 201 (128, G, S); 202 (CDC P28); 203 (8926, G, M, K7); 204 (8958, G, M, K29); 205 (9302, G, M, K29); 206 (8917, G, M, K29); 207 (K40, M → T); 208 (8813, G, M, K38); 209 (8485, G, M. K38); 210 (8824, G, M, K38); 211 (8447, G, M, K38); 212 (127, G, S); 213 (CDC P8); 214 (8065, G, M, K38); 215 (8360, G, M, K38); 216 (124, G, S); 217 (T41, crab salad, Md., F); 218 (8215, G, M. K7); 219 (8333, G, M, K7); 220 (8476, G, M, K7); 221 (8487, G, M, K7); 222 (8679, G, M, K7); 223 (9317, G, M, K7); 224 (9374, G, M, K7); 225 (8066, G, M, K7); 226 (8080, G, M, K7); 227 (8181, G, M, K7); 228 (CDC P22) 229 (126 G, S); 230 (4203, crab salad, Md., D); 231 (10981, G, Md., D, K33); 232 (CDC P2); 233 (CDC P23); 234 (CDC P29); 235 (8982, G, M, K10); 236 (9036, G, M, K29); 237 (9297, G, M, K5); 238 (9380, G, M, K11); 239 (9414, G, M, K8); 240 (125, G, S); 241 (2882, steamed crab, Md., D); 242 (9373, G, M, K5); 243 (9423, G, M, K8); 244 (K45, M → T); 245 (9325, G, M, K7); 246 (9201, G, M, K7); 247 (8363, G, M, K7); 248 (CDC P3); 249 (9143, G, M, K12); 250 (CDC A5704, lesion on foot, S.C.); 251 (135, G, S); 252 (K15, G, S → T); 253 (129, G, S); 254 (131, G, S); 255 (136, G, S); 256 (8657, G, Md., D, K11); 257 (8659, G, Md., D, K11); 258 (8302, G, M. K11); 259 (8455, G, M, K15); 260 (8853, G, M, K8); 261 (133, G, S); 262 (138, G, S); 263 (134, G, S); 264 (130, G, S); 265 (CDC A1334, wound, Va.); 266 (CDC B9004, muscle, La.); 267 (132, G, S); 268 (137, G, S); 269 (140, G, S); 270 (141, G, S); 271 (142, G, S); 272 (143, G, S); 273 (8700, G, Md., D, K11); 274 (8658, G, Md., D, K11); 275 (3525, steamed crab, Md., D, K30); 276 (10734, G, Md., F, K11); 277 (11883, G, Md., D, K11); 278 (11759, G, Md., D, K11); 279 (11590, G, Md., D, K11); 280 (CDC P6); 281 (CDC P4); 282 (8343, G, M, K11); 283 (3AC41, crab, Md., F, K28); 284 (139, G, S); 285 (CDC P21); 286 (8828, G, M, K8); 287 (9364, G, M, K3); 288 (CDC P26); 289 (CDC P1); 290 (CDC A8633, ear, N.Y.); 291 (K41, M → T); 292 (CDC 9235); 293 (8049, G, M, K11); 294 (K25, food, S → T); 295 (9170, G, M, K12); 296 (11587, G, Md., D); 297 (K12, G, S → T); 298 (CDC P9); 299 (CDC P10); 300 (8192, G, M, K43); 301 (8940, G, M,

K29); 302 (8279, G, M, K36); 303 (8961, G, M, K44); 304 (K19, A → T); 305 (8219, G, M, K7); 306 (K18, A → T); 307 (K20, G, S → T); 308 (K32, Z → T); 309 (K31, M → T); 310 (K4, G, S → T); 311 (T2194 (K6), A → T); 312 (HP838 (K7), Z → T); 313 (K42, M → T); 314 (CDC A4871, ete, Fla.); 315 (K3, G, S → T). Strains 113 to 117 have been previously described (2, 8).

Strains assigned to group C-2: 316 (CDC A3308, blood, Ark,); 317 (CDC A8974 A[1], blood, Tex.); 318 (CDC A8814, leg, Tex.); 319 (CDC B9943, blood, La.); 320 (CDC A6614, blood, Fla.); 321 (CDC A3490, leg ulcer, Wis.); 322 (CDC A6546, blood, Alaska); 323 (CDC B2828, blood, Alaska); 324 (CDC B9629, blood, Fla.); 325 (CDC B8867, finger, La.); 326 (CDC B3721, leg wound, S.C.); 327 (CDC B308, blood, N.C.); 328 (CDC A1402, corneal ulcer, Va.); 329 (CDC B3547, leg wound, R.I.).

Strains assigned to group C-1 (72 to 75, 123 to 141) and group C-3 (76, 77, 142, 143) have been previously described (2, 8).

RESULTS

Common physiological properties. All strains fermented glucose with the production of acid but no gas, were oxidase positive, reduced nitrate to nitrite, and were unable to denitrify. All strains grew in BM (which contained 0.2 M sodium ion) with 0.2% glycerol as the sole source of carbon and energy. None grew when the sodium salts in BM were replaced by an equimolar concentration of potassium, indicating that sodium ion but no organic growth factors were required for growth. All strains were able to grow on YEB at 30, 35, and 40 C but not at 4 C. Strains 171 to 188 gave a positive Voges-Proskauer reaction; the remaining strains were negative. Fifteen representative strains of *B. alginolytica*, 20 strains of *B. parahaemolytica*, and 5 strains of group C-2 were tested for the presence of a constitutive arginine dihydrolase system (8) and were found to be negative for this trait. None of the strains produced a soluble or a cell-associated pigment.

Gram stain, morphology, and flagellation. All strains were gram-negative and motile in liquid culture. Strains 316, 317, and 319 to 329 were curved rods, as were occasional cells of strains 310 and 311. All the remaining strains were straight rods. When grown on MA and stained by the Leifson method (8, 19), strains 207, 230, 297, 308, 310, 311, 313, 316 to 320, and 322 to 329 were found to have a single, polar flagellum; all the remaining strains were peritrichous. Thirty of the latter strains were found to have single, polar flagella when grown in YEB. Strains 310, 316, and 327 were grown in YEB, negatively stained (2), and examined by means of the electron microscope. Each was found to have a single, sheathed, polar flagellum. Strains representative of *B. parahaemolytica* and *B. alginolytica* have been previously examined by electron microscopy (reference 2; Baumann and Baumann, *in press*).

Cytochrome spectra. Differential oxidized/reduced cytochrome spectra were determined for two representative strains of each species of the genus *Beneckea* (8). The results of all the determinations were qualitatively similar. The principal bands were as follows: Soret band, 428 to 430 nm; β-bands, 523 and 530 nm; α-bands, 552 and 560 nm. These results are consistent with the presence of cytochromes of the *b* and *c* types (29). No bands characteristic of cytochromes of the *a* type were detected.

Extracellular enzymes. All strains had an extracellular amylase, gelatinase, and lipase; none had an alginase. With the exception of strains 193, 248, 308, and 313, all strains had an extracellular chitinase.

Range of organic compounds utilized. Strains 171 to 329 were tested for their ability to utilize 94 organic compounds as sole sources of carbon and energy. The results of this screening are given in Table 1. None of the strains were able to utilize D-xylose, D-arabinose, D-fucose, L-rhamnose, lactose, inulin, salicin, saccharate, mucate, galacturonate, isobutyrate, malonate, glutarate, sebacate, D-(−)-tartrate, L-(+)-tartrate, *meso*-tartrate, DL-β-hydroxybutyrate, erythritol, sorbitol, inositol, adonitol, 2,3-butyleneglycol, butanol, benzoate, *p*-hydroxybenzoate, phenylacetate, quinate, β-alanine, L-isoleucine, L-valine, L-lysine, γ-aminobutyrate, δ-aminovalerate, L-phenylalanine, L-tryptophan, anthranilate, spermine, betaine, sarcosine, creatine, and hippurate. None of the strains hydrolyzed agar.

Numerical analysis. With the exception of flagellation, cell shape, arginine dihydrolase, and poly-β-hydroxybutyrate (PHB) accumulation, the data for strains 171 to 329 as well as for 30 representative strains of *B. campbellii* and all the remaining strains of species of the genus *Beneckea* (8, 9) were submitted to a numerical analysis as previously described (8). 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 (28). The results of the numerical analysis are presented in Fig. 1.

Glucose and gluconate catabolism. Cell-free extracts of strains of *B. parahaemolytica*, *B. natriegens*, and group C-3, grown on either

TABLE 1. *Substrates utilized by strains 171 to 329 as sole sources of carbon and energy*

Substrate	No. of positive strains[a]	Positive or negative strains[b]	Substrate	No. of positive strains[a]	Positive or negative strains[b]
D-Ribose	+		DL-Lactate	+	
L-Arabinose	89	(+): 189-194, 207, 208, 212-217, 225-244, 248-283, 290-300, 304, 306-310, 312, 313	DL-Glycerate	157	(−): 283, 326
			Citrate	158	(−): 291
			α-Ketoglutarate	157	(−): 308, 309
			Pyruvate	+	
D-Glucose	+		Aconitate	158	(−): 291
D-Mannose	146	(+): 174-178, 182, 183, 189-314, 316-328	Mannitol	153	(+): 171-322, 326
			Glycerol	+	
D-Galactose	139	(+): 177, 178, 189-303, 305, 306, 308, 309, 312-329	Ethanol	118	(+): 171-175, 189, 190, 193-195, 197-250, 252-260, 263-305, 310, 314
D-Fructose	+				
Sucrose	18	(+): 171-188			
Trehalose	157	(−): 309, 326	n-Propanol	124	(+): 171-175, 179-181, 189, 190, 193, 194, 197-215, 217-306, 308, 310, 315
Maltose	158	(−): 315			
Cellobiose	22	(+): 208-215, 316-329			
Melibiose	9	(+): 195-201, 206, 248	Glycine	104	(+): 171-175, 177, 179-188, 195-206, 209, 210, 212-248, 253-262, 265-287, 293, 299, 300, 308
Gluconate	+				
Glucuronate	93	(+): 193, 195-250, 265, 290, 291, 293-305, 307-312, 316-329			
N-acetylglucosamine	+		L-α-Alanine	145	(+): 171-191, 195-289, 293-305, 309, 310, 316-329
Acetate	155	(−): 309, 311, 312, 315			
Propionate	+		D-α-Alanine	+	
Butyrate	119	(+): 171-188, 191-193, 195-243, 248-287, 289, 291-295, 301-303	L-Serine	131	(+): 171-227, 229-248, 250-291, 294-296, 300-303, 305, 306, 308, 310, 312
Valerate	21	(+): 171-177, 179, 180, 182, 184-187, 194, 249, 250, 252, 265, 266, 303	L-Threonine	155	(−): 264, 310, 311, 314
			L-Leucine	142	(+): 171-311, 315
Isovalerate	114	(+): 171-179, 182-188, 191, 192, 194-206, 216, 218-243, 247-300, 302, 303	L-Aspartate	158	(−): 313
			L-Glutamate	157	(−): 308, 315
			L-Arginine	147	(+): 171-187, 188-247, 249-289, 291-297, 299-305, 310, 313, 316-329
Caproate	28	(+): 177, 194, 195, 197-201, 223, 224, 240-243, 251-264			
			L-Ornithine	3	(+): 217, 306, 312
			L-Citrulline	2	(+): 194, 252
Heptanoate	132	(+): 171-180, 182-186, 188, 190-245, 247-306	L-Histidine	145	(+): 171-302, 304, 305, 312-320, 325, 326
Caprylate	150	(+): 171-307, 316-324, 326-329	L-Proline	158	(−): 307
			L-Tyrosine	155	(−): 175, 305, 310, 315
Pelargonate	133	(+): 171-192, 195-305	Putrescine	122	(+): 171-175, 179-194, 197-200, 202-217, 229-246, 248-303, 308-311, 313-315
Caprate	152	(+): 171-307, 314, 316-329			
Succinate	+				
Fumarate	+				
DL-Malate	151	(+): 171-191, 194-310, 312, 313, 316-326			

[a] A total of 159 strains were tested; +, indicates all strains were positive.
[b] +, Indicates positive strains; −, indicates negative strains.

glucose or gluconate, had similar activities of the following enzymes: phosphofructokinase, 6PGA dehydrogenase, glucokinase. G6P dehydrogenase, FDP aldolase, and phosphoglucose isomerase (Table 2). The activities of the first two enzymes were also similar in cell-free extracts of representative strains of the remaining species and groups of *Beneckea* (Table 2). When grown on gluconate, representative strains of all the species and groups tested had a gluconokinase; the activity of this enzyme was not detected in cell-free extracts of glucose-grown cells (Table 2). With the exception of strain 118 of *B. alginolytica*, all the gluconate-

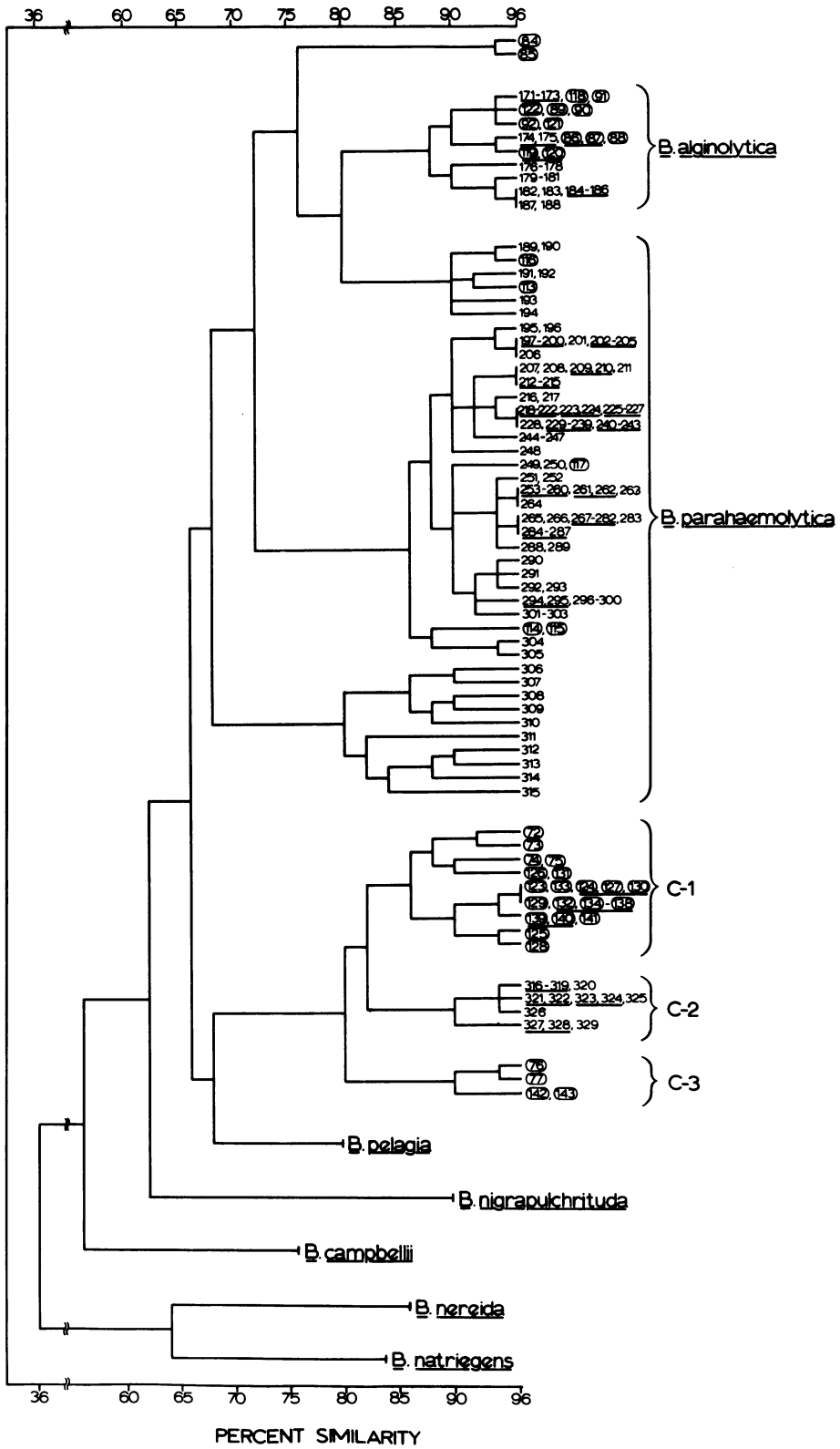

FIG. 1. *Numerical analysis of strains. Circled strain numbers refer to previously characterized strains (2, 8, 9). Strains which were identical in their nutritional properties are denoted by an underline.*

grown strains had an increase in the combined activities of 6PGA dehydrase and KDPG aldolase when compared to the activities of these enzymes in cell-free extracts of glucose-grown cells (Table 2). In strain 118, the combined activities of these two enzymes were similar in both glucose- and gluconate-grown cells.

The results of the enzyme analyses were supported by respirometry experiments. Cells grown on succinate, glucose, or gluconate oxidized glucose at a linear rate without a lag; only gluconate-grown cells oxidized gluconate without a lag. *B. natriegens* differed from the other strains in that succinate- or glucose-grown cells of this species oxidized gluconate after a lag of 30 min. Succinate- or glucose-grown cells of the remaining strains failed to oxidize gluconate within the time period of the experiment (60 min).

Eagon and Wang (14) demonstrated by means of radiorespirometry, enzyme assays, and respirometry that *B. natriegens*, strain 111, catabolized glucose via a constitutive Embden-Meyerhof pathway. Gluconate utilization was by means of an inducible Entner-Doudoroff pathway (glucose-grown cells, unlike gluconate-grown cells, had neither a gluconokinase nor a 6PGA dehydrase and KDPG aldolase). The results of the enzyme assays presented in Table 2 and the respirometry experiments are consistent with the extension of these conclusions to *B. parahaemolytica*, *B. alginolytica*, and group C-2 as well as the remaining species and groups of *Beneckea*.

DISCUSSION

B. parahaemolytica. The three clusters which contain strains 189 to 315 (Fig. 1) consist of 132 isolates which include all the strains from cases of gastroenteritis (91 strains), 6 strains from suspected seafoods, and 5 out of 41 strains isolated from nonenteric infections (strains 250, 265, 266, 290, and 314). The specific source of the remaining 30 strains was not available, although it is known that they were isolated in Japan from cases of gastroenteritis, suspected seafoods, or sea water (31). The two clusters formed by strains 189 to 305 include the proposed type strain of *B. parahaemolytica* (strain 113) as well as four strains

TABLE 2. *Specific activities of selected enzymes of glucose and gluconate catabolism in cell-free extracts of species and groups of Beneckea*[a]

Enzyme	Species or group and strain no.																	
	B. parahaemolytica 113		*B. natriegens* 107		Group C-1 130		*B. alginolytica* 118		*B. pelagia* 101		*B. nigrapulchrituda* 158		*B. campbellii* 28		*B. nereida* 82		Group C-2 324	
	Glucose	Gluconate	Glucose	Gluconate	Glucose	Gluconate	Glucose	Gluconate	Glucose	Gluconate	Glucose	Gluconate	Glucose	Gluconate	Glucose	Gluconate	Glucose	Gluconate
Gluconokinase	<1	139	<1	316	<1	215	<1	168	<1	93	<1	107	<1	199	<1	161	<1	260
6PGA dehydrase and KDPG aldolase[b]	31	94	55	122	15	54	94	105	12	55	11	43	39	62	21	57	17	188
Phosphofructokinase	105	84	118	97	150	172	101	100	95	83	93	86	152	135	118	132	192	186
6PGA dehydrogenase	59	54	67	78	91	88	61	57	79	70	82	73	88	84	62	58	47	46
Glucokinase	75	82	61	72	106	109												
G6P dehydrogenase	84	84	66	74	102	110												
FDP aldolase	98	73	85	101	68	62												
Phosphoglucose isomerase	508	570	430	506	481	412												

[a] Specific activities are expressed as nanomoles of substrate utilized per minute per milligram of protein.
[b] Assayed jointly with 6PGA as the substrate.

(114 to 117) which have been previously characterized (8). One hundred and twenty-five strains were peritrichously flagellated when grown on solid medium; the remaining seven strains (207, 230, 297, 308, 310, 311, and 313) were polarly flagellated. The latter strains were dispersed throughout the two clusters consisting of strains 195 to 315. A considerable number of isolates were identical for all the nutritional traits tested (Fig. 1). It is of interest that many of these identical strains had different geographical origins. For example, nutritionally identical strains 229 to 239, 240 to 243, 253 to 260, and 267 to 282 consisted of isolates originating from both Maryland and Japan.

Although the relatively low S values linking the three clusters comprised of strains 189 to 315 (68 and 72%) suggest considerable phenotypic heterogeneity, no universally positive or negative traits could be found to differentiate these clusters. A comparable situation was observed in *B. campbellii* where 60 strains segregated into two clusters which, although linked at 74%, were not separable by any universally positive or negative traits (8). The low linkage between the cluster consisting of strains 306 to 315 and the two clusters containing strains 189 to 305 may in part be accounted for by the lower nutritional versatility of the former strains. Strains 189 to 194 and 195 to 305 were able to utilize between 37 to 43 and between 36 to 46 compounds, respectively, as sole sources of carbon and energy, whereas strains 306 to 315 utilized between 27 to 36 carbon compounds. The presence of isolates from cases of gastroenteritis in each of the three clusters as well as their overall phenotypic similarity suggests that they should be recognized as one species. Future work may, however, support the subdivision of this species into several biotypes.

B. alginolytica. Twenty-two of the thirty strains in this species (Fig. 1) were obtained from localized tissue infections as well as from infections of the eye and ear; seven were obtained from enrichment cultures with sea water as the inoculum. The proposed type strain of this species (strain 118) and strains 86 to 92 and 119 to 123 were characterized in a previous study (2, 8). All the strains were peritrichously flagellated when grown on solid medium. *B. alginolytica* was able to utilize between 39 to 44 compounds as sole sources of carbon and energy, a nutritional versatility similar to that of *B. parahaemolytica* (the nutritional versatility of the former species has been incorrectly stated in Fig. 30 of Baumann et al. [8]). *B. alginolytica* and *B. parahaemolytica* share a large number of phenotypic traits (Table 1, 3). This phenotypic similarity is reflected in the considerable DNA homology between the type strains of these two species (3).

Group C-2. The 14 strains (316 to 329, Fig. 1) which comprise this group were obtained from human, nonenteric sources. Twelve of the strains were curved rods which had a single, polar flagellum when grown on solid medium. Strain 318 was a polarly flagellated, straight rod; strain 321 was a peritrichously flagellated, curved rod. Strains of group C-2 were able to utilize 31 to 35 compounds as sole sources of carbon and energy. In our initial study of the genus *Beneckea*, one cluster consisting of seven strains was designated *B. neptuna* (strains 72 to 77) (8). As noted previously, this cluster exhibited some phenotypic heterogeneity in that strains 76 and 77 linked to strains 72 to 75 at an S value of 78% and were separable from the latter strains on the basis of several phenotypic traits. Additional strains (123 to 143) were characterized but not included in the numerical analysis. Two of these strains (142, 143) were similar to strains 76 and 77; the remaining strains (123 to 141) resembled strains 72 to 75. As seen from Fig. 1, a numerical analysis has confirmed these observations (groups C1, C-3). Although group C-2 is linked to groups C-1 and C-3 at S values of 82 and 80%, respectively, these three groups can be readily distinguished from one another, as well as from *B. parahaemolytica* and *B. alginolytica*, on the basis of their phenotypes (Table 3). Recent work has indicated that strains of *B. neptuna* resemble *Beneckea* (*Photobacterium*) *harveyi* in their phenotypic properties (Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 59, 1972). The taxonomy of these groups (C-1, C-2, C-3) cannot be formalized pending further study of their relationship to *B. harveyi*. Nevertheless, it is apparent that the phenotypically similar strains which make up group C-2 represent a group of marine, nonenteric, human pathogens which deserves a taxonomic status distinct from *B. parahaemolytica* and *B. alginolytica*.

In our previous work on the genus *Beneckea* (8) the species name *B. nereida* was given to two clusters (strains 84 and 85 and 78 to 83) which linked at an S value of 74% and differed by 13 phenotypic traits. Their provisional inclusion under one species designation was justified by the distinctive properties which they shared, such as the ability to accumulate PHB and the presence of a constitutive arginine dihydrolase system. The linkage of these two

TABLE 3. *Selected traits of use in differentiating B. parahaemolytica, B. alginolytica, and groups C-2, C-1, and C-3 from one another as well as from the remaining species of the genus Beneckea*[a]

Trait	Species or group (total no. of strains)											
	B. parahaemolytica (132)	*B. alginolytica* (30)	Group C-2 (14)	Group C-1 (23)	Group C-3 (4)	*B. pelagia* (11)	*B. nigrapulchrituda* (14)	*B. nigracampbellii* (60)	*B. nereida* (6)	*B. natriegens* (6)	Strains 84, 85 (2)	Group *B* E-3[b] (2)
Peritrichous[c]	**125**	+	1	+	3	–	–	**51**	–	–	–	–
Curved rods[d]	2	–	**13**	–	+	4	–[e]	10	–	–	1	1
PHB[f]-accumulation	–	–	–	–	–	–	7	–	+	+	+	+
Voges-Proskauer reaction	–	+	–	–	–	–	–	–	–	–	–	–
Growth at 40 C	+	+	+	–	–	–	–	–	**5**	**5**	+	1
Lipase	+	+	+	+	+	+	+	+	–	**5**	–	+
D-Galactose	**128**	4	+	**20**	+	+	+	–	–	+	+	–
Sucrose	–	+	–	+	+	+	–	–	+	+	+	+
DL-β-Hydroxybutyrate	–	–	–	–	–	–	+	–	+	+	+	+
p-Hydroxybenzoate	–	–	–	–	–	–	–	–	–	+	–	–
Glycine	88	**28**	–	**19**	+	+	+	10	+	+	1	+
L-Serine	**117**	+	–	+	+	+	+	22	2	+	1	+
L-Leucine	**129**	+	–	–	2	–	–	–	+	**5**	–	1
L-Arginine	**119**	**29**	+	**19**	+	+	–	–	+	+	+	1
L-Histidine	**125**	+	7	–	–	7	**13**	6	**5**	+	1	+
Putrescine	**112**	**25**	–	–	–	+	–	–	+	+	–	+
L-Arabinose	92	–	–	–	–	–	–	–	–	+	+	–
Cellobiose	8	–	+	+	–							
Glucuronate	82	–	+	+	–							
Butyrate	103	+	–	–	–							
Pelargonate	**120**	+	–	**20**	–							
n-Propanol	**120**	19	–	–	–							
Strains from cases of gastroenteritis	91	–	–									
Strains from non-enteric infections	5	22	14									

[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] A group unassigned with respect to genus (1).

[c] Number of strains which are peritrichous when grown on solid medium; all other strains have single polar flagella.

[d] All other strains are straight rods.

[e] Straight rods during exponential phase becoming curved during stationary phase.

[f] PHB, Poly-β-hydroxybutyrate.

clusters has been altered by the inclusion of strains of *B. parahaemolytica* in the numerical analysis. As seen from Fig. 1, strains 84 and 85 are now linked to *B. alginolytica* and the cluster consisting of strains 189 to 194 of *B. parahaemolytica* at the 76% S value. These two strains can, however, be readily distinguished from both of these species as well as from the remaining species and groups of the genus *Beneckea* (8, 9). It is probable that if additional strains similar to strains 84 and 85 were to be included in a numerical analysis, the linkage of the composite cluster to *B. parahaemolytica* and *B. alginolytica* would occur at a lower S value. The taxonomic treatment of strains 84 and 85 should await the isolation and characterization of additional strains.

Diagnostic traits. Selected traits of use in

differentiating *B. parahaemolytica*, *B. alginolytica*, and group C-2 from one another as well as from other species of the genus *Beneckea* are given in Table 3. A separation of *B. parahaemolytica* and *B. alginolytica* can be made on the basis of the ability of the latter to utilize sucrose and give a positive Voges-Proskauer reaction. Other traits of use are the ability of most strains of *B. parahaemolytica* to utilize L-arabinose and glucuronate. The differential value of these traits has been previously indicated (references 8, 25, 27; Baumann and Baumann, *in press*). Two additional traits which have been proposed for the differentiation of these two species are the ability of *B. alginolytica* to swarm on complex, solid media and grow in the presence of 10% sodium chloride (25, 27). The latter trait has been shown to be of no diagnostic value by various investigators (references 13, 31, 32; Baumann and Baumann, *in press*). The ability to swarm on solid medium is a trait which is greatly influenced by the composition and water content of the medium (reference 31; Baumann and Baumann, *in press*). Although generally useful as a differential character, this trait should be used with caution since it does not always provide a clear-cut differentiation between these two species.

As a consequence of the extensive phenotypic characterization of a number of species of the genus *Beneckea* (2, 8, 9), it has become possible to evaluate some of the traits previously used for the differentiation of *B. parahaemolytica* from other common marine bacteria. Of the previously proposed traits (25, 26, 27, 31), the inability to utilize sucrose and the ability to grow at 40 C and utilize L-arabinose have proven to be the most valid. As an individual trait, the inability to utilize sucrose distinguishes *B. parahaemolytica* from the majority of the species and groups of *Beneckea* but not from *B. campbellii*, *B. nigrapulchrituda*, and group C-2; when considered in conjunction with the ability to grow at 40 C, the only other known member of *Beneckea* which cannot be distinguished from *B. parahaemolytica* is group C-2. With the exception of *B. parahaemolytica*, none of the characterized species and groups of *Beneckea* combine the inability to utilize sucrose with the ability to utilize L-arabinose. However, the latter trait is of limited use since it is found in only 70% of the strains of *B. parahaemolytica* characterized in this study. Another trait which has been claimed to be unique to this species is hemolysis on a special medium (Wagatsuma medium) (20). Our previous results have indicated that this property is of no diagnostic value since strains of several species of *Beneckea* are also positive for this trait (8). This finding is consistent with the results of Baross and Liston (6) who found that strains able to ferment sucrose were hemolytic when tested on this medium. The results of our studies have provided a considerable number of traits which are useful for the differentiation of *B. parahaemolytica* and *B. alginolytica* from other members of the genus *Beneckea* (Table 3). The application of these traits should allow a more rigorous identification of these two species and group C-2 than has been previously possible. The proposed methods of identification are relatively simple and can be adapted easily for routine use. Suspected strains should be initially tested for the general properties of the genus *Beneckea* (8), followed by a selection of diagnostic traits from Table 3.

During the past few years, a considerable number of reports have been published alleging the isolation of *B. parahaemolytica* from sea water and marine animals (1, 4–6, 15, 18, 22, 30, 33–35). Although in many cases the diagnostic traits used were not adequate for the differentiation of these isolates from other *Beneckea* species, the arabinose-positive, sucrose-negative isolates of Aldová et al. (1), Thomson and Trenholm (30), Vanderzant et al. (33), Ward (34), and Yasunaga (35) are suggestive of *B. parahaemolytica*. The possession of two diagnostic traits, however, cannot be considered adequate for the identification of a species. The unequivocal isolation of *B. parahaemolytica* from marine sources has so far been shown only by in vitro DNA/DNA hybridization experiments. As mentioned previously, a high DNA homology was observed between authentic strains of this species and 3 out of 12 isolates designated *V. parahaemolyticus* by Baross and Liston (3). In addition, Staley and Colwell (Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 58, 1972) have shown that a strain isolated from a healthy crab hybridized with a strain of *B. parahaemolytica* from an enteric source. It should be stressed that the results of our studies do not disprove claims that sucrose-negative isolates from marine sources are *B. parahaemolytica*. Our results do indicate, however, that these claims should not be regarded as proven. Strains isolated from marine sources and having diagnostic properties characteristic of *B. parahaemolytica* should only be considered putative members of this species, since it is possible that the diagnostic traits

given in Table 3 may not be adequate for the differentiation of *B. parahaemolytica* from hitherto uncharacterized species of marine bacteria. The development of a reliable pathogenicity test is important for the verification of the identity of such strains. In addition, it should be noted that most of our nonpathogenic isolates were obtained from the vicinity of Oahu, Hawaii, and probably cannot be regarded as representative of the total bacterial flora of the ocean.

The results of these studies indicate that *B. parahaemolytica*, *B. alginolytica*, and group C-2 are phenotypically distinct from each other and readily distinguishable from the other species and groups of the genus *Beneckea*. These conclusions are primarily based on an extensive nutritional analysis. The validity of such an approach to the taxonomy of nutritionally versatile bacteria has been amply demonstrated in the case of the genus *Pseudomonas*. In a recent publication on the taxonomy of this genus, excellent agreement was found between a taxonomy based on an intuitive analysis of the nutritional data, a numerical analysis of the same data, and groupings based on DNA homology (23).

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