giosin-type pigments, with a red pigment predominant and identified as cycloprodigiosin. Colonies on agar are initially pink, becoming more intensely red with age, and are smooth and convex with an entire edge. Produce bright blue, purple, or red pigments in liquid media. Produce a polyanionic, autotoxic antibiotic on complex media. Sodium ions are required for growth; the optimal level is about 2.5% NaCl; no growth occurs in the presence of 6% NaCl. Require amino acids for growth. Grow between 2 and 30°C, with optimum growth at about 25°C. Other characteristics are as given in the genus description and in Tables BXII.γ.144 and BXII.γ.146. Strains are also resistant to tetracycline and penicillin, but are susceptible to chloramphenicol. Strains possess a high sensitivity to ultraviolet radiation.

Isolated from seawater collected from fjords of western Norway.

The mol% G + C of the DNA is: 36.5–37.1 (T_m).

Type strain: Nygaard 1977, ATCC 43337, DSM 6059, IAM 14445, NCIMB 13179.

GenBank accession number (16S rRNA): X82138.

9. **Pseudoalteromonas distincta** (Romanenko, Mikhailov, Lysenko and Stapanenko 1995a) Ivanova, Chun, Romanenko, Matte, Mikhailov, Frolova, Huq and Colwell 2000a, 143^{VP} (*Alteromonas distincta* Romanenko, Mikhailov, Lysenko and Stapanenko 1995a, 879.)
di.stinc′ ta. M.L. adj. *distincta* distinct.

Cells are rods, about 0.9 × 2.5 μm. Motile by a single polar flagellum, very rarely by 2–4 polar flagella with 5–7 lateral flagella. Cells do not form capsules. Colonies on marine agar are black-brown, smooth, convex, and circular with entire edges. Produce a diffusible, black-brown, melanin-like pigment that is formed most strongly at low incubation temperatures and on tyrosine and arginine-containing media. Sodium ions are required for growth. Growth factors are not required. Grow between 4 and 35°C; no growth occurs at 40°C. Grow between pH 6.0 and 9.0. Strictly aerobic with a respiratory metabolism. Oxidase and catalase positive. Do not denitrify. Produce lipase and gelatinase, but do not hydrolyze starch, chitin, alginate, carrageenan, or agar. Decompose L-tyrosine and L-arginine. Utilize citrate, but not D-arabinose, D-xylose, D-glucose, D-mannose, L-rhamnose, cellobiose, lactose, maltose, sucrose, D-mannitol, or *N*-acetylglucosamine.

Isolated from a marine sponge near the Komandorskiye Islands, Russian Pacific region.

The mol% G + C of the DNA is: 44 (T_m).

Type strain: ATCC 700518, KMM 638.

GenBank accession number (16S rRNA): AF043742, AF082564.

10. **Pseudoalteromonas elyakovii** (Ivanova, Mikhailov, Kiprianova, Levanova, Garagulya, Frolova and Swetashev 1997) Sawabe, Tanaka, Iqbal, Tajima, Ezura, Ivanova and Christen 2000, 270^{VP} (*Alteromonas elyakovii* Ivanova, Mikhailov, Kiprianova, Levanova, Garagulya, Frolova and Swetashev 1997, 601.)
el.ya.kov′ i.i. M.L. gen. n. *elyakovii* of Ekyakov, named after G.B. Elyakov for his work in microbial biotechnology.

Cells are rods, about 0.5–0.7 × 1.0–1.2 μm. Motile by a single polar flagellum. Cells do not form spores or capsules. Colonies on marine agar are nonpigmented, smooth, convex, and circular, with entire edges. Melanin-like pigments are not formed. Nonbioluminescent. Sodium ions are required for growth. Growth factors are not required. Grow between 4 and 35°C; no growth occurs at 41°C. Grow between pH 7 and 8.5. Strictly aerobic with a respiratory metabolism. Oxidase and catalase positive. Do not denitrify. Produce lipase, amylase, and gelatinase, but do not hydrolyse chitin. Decompose L-tyrosine and L-arginine. Utilize D-ribose, D-xylose, D-fructose, D-galactose, D-glucose, cellobiose, lactose, salicin, adonitol, D-sorbitol, *m*-inositol, ethanol, propionate, butyrate, succinate, lactate, 2-oxoglutarate, glycolate, *p*-hydroxybenzoate, phenylacetate, L-alanine, L-histidine, L-lysine, L-ornithine, L-phenylalanine, and L-threonine, but not arabinose, rhamnose D-mannose, cellobiose, lactose, maltose, sucrose, trehalose, D-gluconate, D-mannitol, acetate, caprylate, valerate, caproate, pelargonate, glutarate, citrate, fumarate, L-malate, pyruvate, glycerate, aconitate, L-asparagine, L-glutamate, or L-proline. Other characteristics as are described by Ivanova et al. (1996b).

Isolated from the coelomic fluid of the mussel *Crenomytilus grayanus*.

The mol% G + C of the DNA is: 39 (T_m).

Type strain: 40MC, ATCC 700519, KMM 162, VKPM B3905.

GenBank accession number (16S rRNA): AF082562.

11. **Pseudoalteromonas espejiana** (Chan, Baumann, Garza and Baumann 1978) Gauthier, Gauthier and Christen 1995a, 760^{VP} (*Alteromonas espejiana* Chan, Baumann, Garza and Baumann 1978, 220.)
es.pe.ji.a′ na. M.L. fem. adj. *espejiana* of Espejo, a Chilean microbiologist who isolated one of the first lipid-containing bacteriophages.

Cells are rod-shaped, 0.2–1.0 × 2.0–3.5 μm. Colonies are nonpigmented, smooth, and convex, with an entire edge. Sodium ions (0.2–0.3 M concentration optimal) required for growth. Amino acids are required for growth. Grow between 10 and 35°C, with optimal growth at about 30°C. No growth at 4°C. Other characteristics are as shown in the genus description and in Tables BXII.γ.144 and BXII.γ.145.

Isolated from seawater.

The mol% G + C of the DNA is: 43–44 (T_m).

Type strain: 261, ATCC 29659, CCUG 16147, DSM 9414, IAM 12640, LMG 2866, NCIMB 2127.

GenBank accession number (16S rRNA): X82143.

12. **Pseudoalteromonas luteoviolacea** (Gauthier 1982) Gauthier, Gauthier and Christen 1995a, 760^{VP} (*Alteromonas luteoviolacea* Gauthier 1982, 85; *Alteromonas luteo-violaceus* [sic] Gauthier 1976c, 147.)
lu.te.o.vi.o.la′ ce.a. L. adj. *luteus* yellow; L. adj. *viola* violet; M.L. adj. *luteoviolacea* yellow-violet.

Cells are rod-shaped, 0.8–1.5 × 2–4 μm. Motile by single polar flagellum, which may or may not be sheathed. Colonies on agar are smooth or waxy and circular convex, with a more or less violet pigment (violacein); edges of colonies may be pigmented pale violet or yellow. Some strains form diffusible brown pigments on complex media. Most strains produce a polyanionic, autotoxic antibiotic on complex media. Sodium ions are required for growth; the optimal level

is about 2–3% NaCl; no growth occurs in the presence of 8% NaCl. May require amino acids for growth. Grow between 10 and 30°C, with optimal growth at about 25°C. Other characteristics are as given in the genus description and in Tables BXII.γ.144 and BXII.γ.146. In addition, strains can form lecithinase and produce acid from trehalose and maltose. Acid from glucose varies between strains. Negative for the following tests: cellulose hydrolysis, methylene blue reduction, β-galactosidase, glycine decarboxylase, asparagine decarboxylase, and alanine decarboxylase. Acid is not produced from D-mannose, D-xylose, D-fructose, lactose, sucrose, D-mannitol, or glycerol. Susceptible to polymyxin B, chloramphenicol, and oleandomycin.

Isolated from surface seawater.

The mol% G + C of the DNA is: 40.9–42.2 (T_m).

Type strain: CH130, ATCC 33492, DSM 6061, NCIMB 1893.

GenBank accession number (16S rRNA): X82144.

13. **Pseudoalteromonas nigrifaciens** (Baumann, Baumann, Bowditch and Beaman 1984a) Gauthier, Gauthier and Christen 1995a, 760[VP] emend. Ivanova, Kiprianova, Mikhailov, Levanova, Garagulya, Gorshkova, Yumoto and Yoshikawa 1996b, 227 (*Alteromonas nigrifaciens* Baumann, Baumann, Bowditch and Beaman 1984a, 146.)
ni.gri.fa′ ci.ens. L. adj. *niger* black; L. v. *facio* to make; M.L. part. adj. *nigrifaciens* blackening.

Cells are straight rods, 0.8–1.2 × 1.8–2.3 μm. Cells may be encapsulated. Colonies are smooth, circular, convex with an entire edge, and nonpigmented. May form a brown-black, melanin-like pigment constitutively at low temperatures. On tyrosine-containing media, melanin-like pigments are formed by all strains. Sodium ions are required for growth. Most strains do not require growth factors. Grow between 4 and 35°C, with optimal growth at about 30°C. Other characteristics are as given in the genus description and in Tables BXII.γ.144 and BXII.γ.145. See Ivanova et al. (1996a) for additional phenotypic information.

Isolated from seawater, mussels and salted food.

The mol% G + C of the DNA is: 38.5–40.2 (T_m).

Type strain: ATCC 19375, DSM 6063, NCIMB 8614.

GenBank accession number (16S rRNA): X82146.

14. **Pseudoalteromonas peptidolytica** Venkateswaran and Kohmoto 2000, 572[VP]
pep.ti.do.lyt′ i.ca. Gr. n. *peptos* a class of organic compounds; Gr. adj. *lytica* dissolving; M.L. fem. adj. *peptidolytica* peptide dissolving.

Gram negative. Cells are rods, 2.0–3.0 × 0.7–0.8 μm. Motile with a polar unsheathed flagellum. Colonies on marine agar are circular, opaque, and yellow-pigmented. Requires Na^+ ions for growth. Grows between 1–10% NaCl. Growth occurs at 15–40°C. Optimum growth temperature is 30°C.

Chemoheterotrophic and strictly aerobic. Catalase and oxidase positive. Produces gelatinase, amylase, lipase (Tween 80), and alginase. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, chitinase, and β-galactosidase are not produced. Does not produce H_2S from thiosulfate. Indole and Voges–Proskauer tests are negative. Does not denitrify. Utilizes glucose, maltose, *N*-acetylglucosamine, succinate, and fumarate as sole carbon sources. Does not utilize mannose, fructose, galactose, lactose, sucrose, salicin, mannitol, glycerol, citrate, DL-lactate, γ-aminobutyrate, sarcosine, putrescine, α-ketoglutarate, sorbitol, DL-malate, acetate, *m*-hydroxybenzoate, or methanol.

Isolated from surface seawater, Yamoto Islands, Sea of Japan.

The mol% G + C of the DNA is: 41–42 (HPLC).

Type strain: F12–50–A1, MBICC F1250A1.

GenBank accession number (16S rRNA): AF007286.

15. **Pseudoalteromonas piscicida** (ex Bein 1954) Gauthier, Gauthier and Christen 1995a, 760 (*"Pseudomonas piscicida"* (Bein 1954) Buck, Meyers and Leifson 1963, 1125; *"Flavobacterium piscicida"* Bein 1954, 115.)
pi.sci.ci′ da. L. n. *piscis* fish; L. suff. *-cida* from L. v. *caedo* to cut or kill; M.L. n. *piscicida* fish killer.

Cells are straight rods, 0.2–0.6 × 1.2–2.0 μm. Fimbriae are present on cells. Possess single, sheathed, polar flagella. Colonies are yellow-orange, translucent, smooth, circular, and convex, with an entire edge and a butyrous consistency. Liquid cultures possess even, moderate turbidity, with a pigmented pellicle.

Cell cultures and filtrates produce an antibiotic active against yeasts. Filtrates and cells also induce neuromuscular toxicity syndromes in a variety of fish and crab species, including minnow (*Gambusia* sp.), schoolmaster (*Lutjans apodus*), sandperch (*Eucinostomus pseudosula*), killifish (*Fundulus similis*), mollie (*Mollieresia latipinna*), and fiddler crabs (*Uca pugnas* and *Uca pugilator*).

Sodium ions required for growth, with cultures growing between 0.5% and 10% NaCl. Use ammonia, but not nitrate as a nitrogen source; require amino acids for growth. Grow between 10 and 37°C; optimal growth is at about 30°C; no growth occurs at 4°C or 40°C. Grow between pH 6.0 and 8.0. Other characteristics are as given in the genus description and in Tables BXII.γ.144 and BXII.γ.146. Strains can also reduce trimethylamine-*N*-oxide to trimethylamine, oxidize gluconate to 2-keto-D-gluconate, coagulate litmus milk, and produce lecithinase. Acid is formed from D-glucose, sucrose, and maltose, but not from lactose, D-xylose, or D-mannitol.

Isolated from an area of dead fish.

The mol% G + C of the DNA is: 43.5–45.5 (Bd).

Type strain: MB-1, ATCC 15057, NCMB1142.

GenBank accession number (16S rRNA): X82215.

16. **Pseudoalteromonas prydzensis** Bowman 1998, 1040[VP]
prydz.en′ sis. M.L. fem. adj. *prydzensis* pertaining to Prydz Bay, Antarctica, the site of isolation.

Cells are rod-shaped, 0.5–0.7 × 1–2.5 μm. Colonies on agar are nonpigmented, translucent, smooth, circular, and convex, with an entire or lobate edge; diffusible pigments are not formed on complex or tyrosine-containing media. Sodium ions are required for growth; the optimal level is about 2–3% and cultures grow at NaCl concentrations up to about 15%. Growth factors are not required. Grow between −2 and 30°C, with optimal growth at about 22–25°C. Other characteristics are as shown in the genus description and in Tables BXII.γ.144 and BXII.γ.146. In addition, the strains can produce acid from L-arabinose, D-glucose, D-mannose, maltose, sucrose, trehalose, and *N*-acetylglucosamine. Acid from cellobiose varies between strains. Do not

hydrolyze dextran, urate or xanthine. Acid is not produced from D-xylose, L-rhamnose, D-fructose, D-galactose, lactose, melibiose, D-raffinose, dextran, adonitol, D-mannitol, *meso*-inositol, D-sorbitol, or glycerol. Resistant to ampicillin and bile salts.

Isolated from sea ice.

The mol% G + C of the DNA is: 38–39 (T_m).

Type strain: MB8–11, ACAM 620.

17. **Pseudoalteromonas rubra** (Gauthier 1976a) Gauthier, Gauthier and Christen 1995a, 760^{VP} (*Alteromonas rubra* Gauthier 1976a, 464.)
rub′ra. L. fem. adj. *rubra* red.

Cells are rod-shaped, 1.0–1.5 × 2–4 μm. Colonies on agar media are smooth, convex with an entire edge, and pigmented bright red. Sodium ions are required for growth. Require amino acids for growth. Grow between 10 and 35°C; optimum growth occurs at about 30°C, and no growth occurs at ≥40°C. Produce a polyanionic, autotoxic antibiotic on complex media. Other characteristics are shown in the genus description and Tables BXII.γ.144 and BXII.γ.146.

Isolated from surface seawater.

The mol% G + C of the DNA is: 46–48 (T_m).

Type strain: ATCC 29570, DSM 6064.

GenBank accession number (16S rRNA): X82147.

18. **Pseudoalteromonas tetraodonis** (Simidu, Kita-Tsukamoto, Yasumoto and Yotsu 1990) Ivanova, Romanenko, Matté, Matté, Lysenko, Simidu, Kita-Tsukamoto, Sawabe, Vysotskii, Frolova, Mikhailov, Christen and Colwell 2001b, 1077^{VP} (*Pseudoalteromonas haloplanktis* subsp. *tetraodonis* Gauthier, Gauthier and Christen 1995a, 760; *Alteromonas tetraodonis* Simidu, Kita-Tsukamoto, Yasumoto and Yotsu 1990, 336.)
tet′ra.o.do.nis. M.L. *tetraodonis* of *Tetraodontidae*, a family of plectognathic fishes.

Cells are rod-shaped, 2.0–3.0 × 1.0 μm. Colonies are non-pigmented, smooth, and convex with an entire edge. Sodium ions are required for growth. Grows between 1–10% NaCl. Growth factors (amino acids) are not required for growth. Grows between 10–35°C, with optimum growth occurring at about 30°C. Optimum pH for growth is 7.5–8.0. Growth occurs between pH 5.5–9.5.

Utilizes glucose, sucrose, maltose, galactose, DL-lactate, valerate, caproate, butyrate, citrate, fumarate, succinate, caprylate, L-glutamate, acetate, pyruvate, ethanol, and L-tyrosine. Does not utilize mannose, fructose, mannitol, L-histidine, L-proline, or DL-malate (Ivanova et al., 2001b). Strains are susceptible to benzylpenicillin, carbenicillin, vancomycin, and tetracycline. Other characteristics are shown in the genus description and in Tables BXII.γ.144 and BXII.γ.145.

The mol% G + C of the DNA is: 41.5 (T_m).

Type strain: GFC, ATCC 51193, DSM 9166, IAM 14160, KMM 458, NCIMB 13177.

GenBank accession number (16S rRNA): X82139; AF214729.

19. **Pseudoalteromonas tunicata** Holström, James, Neilan, White and Kjelleberg 1998, 1210^{VP}
tu.ni.ca′ta. L. fem. n. *tunicata* clothed with a tunic

Gram negative. Cells are rods, 2.0–3.4 μm in length. Motile with a polar sheathed flagellum. Colonies on marine agar are circular, opaque, and dark green. Colonies on trypticase soya agar are non-pigmented. Requires Na+ ions for growth with biomass produced optimally with 1–2% NaCl. Growth occurs at 4–30°C; no growth at 37°C. Optimum growth temperature is 25°C. Optimum pH for growth is 7–8; no growth at pH 5 or 11.

Chemoheterotrophic and strictly aerobic. Catalase, oxidase, and gelatinase positive. Utilizes trehalose, glucose, maltose, and Tween 20 as sole carbon sources. Does not utilize galactose, fructose, xylose, sucrose, lactose, raffinose, melibiose, xylitol, erythritol, glycine, threonine, arabinose, L-asparagine, or L-histidine. Susceptible to erythromycin, rifampicin, gentamycin, tetracycline, ampicillin, neomycin, kanamycin, and nalidixic acid at 100 μg/ml. Susceptible to the vibriostatic agent O/129 at 150 μg/ml.

Isolated from an adult of the tunicate species *Ciona intestinalis* collected from the coast of western Sweden.

The mol% G + C of the DNA is: 42.2 (T_m).

Type strain: Strain DS, CUG 26757.

GenBank accession number (16S rRNA): Z25522.

20. **Pseudoalteromonas undina** (Chan, Baumann, Garza and Baumann 1978) Gauthier, Gauthier and Christen 1995a, 760^{VP} (*Alteromonas undina* Chan, Baumann, Garza and Baumann 1978, 220.)
un.di′na. L. fem. n. *undina* water nymph.

Cells are rod-shaped, 0.7–0.9 × 1.8–3.0 μm. Colonies are nonpigmented, smooth, and convex with an entire edge. Sodium ions are required for growth. Require amino acids for growth. Grow between 4 and 30°C, with optimum growth occurring at about 25°C. No growth at ≥35°C. Other characteristics are as shown in the genus description and in Tables BXII.γ.144 and BXII.γ.145.

Isolated from seawater.

The mol% G + C of the DNA is: 43–44 (T_m).

Type strain: ATCC 29690, DSM 6065, NCIMB 2128.

GenBank accession number (16S rRNA): X82140.

Genus XII. **Psychromonas** *Mountfort, Rainey, Burghardt, Kaspar and Stackebrandt 1998b, 631^{VP} emend. Nogi, Kato and Horikoshi 2002, 1531 (Effective publication: Mountfort, Rainey, Burghardt, Kaspar and Stackebrandt 1998a, 237)*

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Psy.chro.mo′nas. Gr. adj. *psychros* cold; Gr. fem. n. *monas* a unit; N.L. n. *Psychromonas* a cold monad.

Motile Gram-negative rod-shaped or oval cells; polar flagellum. **Grow at temperatures <22°C.** Catalase and oxidase positive. Major quinone Q-8; major fatty acids are $C_{14:1}$, $C_{16:0}$, and $C_{16:1}$. **Facultative anaerobes;** use carbohydrates for energy for growth. **Found in marine or high salinity environments.**

The mol% G + C of the DNA is: 38.1–43.8.

Type species: **Psychromonas antarctica** Mountfort, Rainey, Burghardt, Kaspar and Stackebrandt 1998b, 631 (Effective publication: Mountfort, Rainey, Burghardt, Kaspar and Stackebrandt 1998a, 237.)

Further descriptive information

The genus *Psychromonas* includes organisms adapted to a variety of conditions in marine or other saline aquatic environments, including an Antarctic saline pond (*Psychromonas antarctica*), seawater (*Psychromonas arctica* and *Psychromonas marina*), and deep sea sediments (*Psychromonas kaikoae* and *Psychromonas profunda*). All species are able to grow at minimum temperatures of 0–4°C and exhibit both fermentative and respiratory metabolism (Mountfort et al., 1998a; Kawasaki et al., 2002; Nogi et al., 2002; Groudieva et al., 2003; Xu et al., 2003). Increased pressure enhances the growth of *Psychromonas profunda* (Xu et al., 2003) and is required for the growth of *Psychromonas kaikoae* (Nogi et al., 2002).

Enrichment and Isolation Procedures

Psychromonas antarctica was isolated under anaerobic conditions and maintained in a basal medium emended with 3% NaCl (w/v) and filtered water from the Antarctic salt pond from which the organism was isolated (5%, v/v) (Mountfort et al., 1998a). *Psychromonas arctica* was enriched and isolated on a complex marine medium (Groudieva et al., 2003) containing starch. High pressure incubation was used for the isolation of *Psychromonas kaikoae* (Nogi et al., 2002). Difco Marine Medium 2216 was used for *Psychromonas kaikoae*, *Psychromonas marina*, and *Psychromonas profunda* (Kawasaki et al., 2002; Nogi et al., 2002; Xu et al., 2003).

Differentiation of the genus *Psychromonas* from other genera

Psychromonas species are facultatively anaerobic, whereas members of the genera *Alteromonas*, *Glaciecola*, *Idiomarina*, *Marinobacterium*, *Microbulbifer*, and *Pseudoalteromonas* are strictly aerobic. *Psychromonas* species exhibit both fermentative and respiratory metabolism, whereas *Ferrimonas balearica* and *Alishewanella* species have a strictly respiratory metabolism; in addition, *Alishewanella* species are nonmotile. *Psychromonas* species utilize carbohydrates as energy sources, whereas *Marinobacter* species do not. *Psychromonas* species can be distinguished from *Shewanella* species by the unusual fatty acid profiles of the latter. *Psychromonas* species are ecologically and phenotypically similar to members of the genera *Colwellia* and *Moritella*.

Differentiation of the species of the genus *Psychromonas*

Differential characteristics of *Psychromonas* species are given in Table BXII.γ.147.

List of species of the genus Psychromonas

1. **Psychromonas antarctica** Mountfort, Rainey, Burghardt, Kaspar and Stackebrandt 1998b, 631[VP] (Effective publication: Mountfort, Rainey, Burghardt, Kaspar and Stackebrandt 1998a, 237.)
ant.arc.ti.ca. M.L. gen. n. *antarctica* of Antarctica.

Rods, 1.3 × 2.5–6.0 μm. Grows at NaCl concentrations 0–4% (w/v); 3% NaCl is optimum and protects against killing by freeze-thaw. Killed quickly by temperatures >50°C or by temperatures >30°C for >20 h. Catalase positive; oxidase positive. Ferments glycogen, starch, *N*-acetyl glucosamine, and hexose monosaccharides and disaccharides with the production of CO_2, formate, acetate, ethanol, lactate, and butyrate. Does not hydrolyze esculin. Does not grow on *N*-acetyl muramic acid, alanine, casamino acids, esculin, formate, fumarate, glycerol, isoleucine, lactate, lactose, malate, mannose, putrescine, rhamnose, sorbitol, valine, yeast extract, xylan, or xylose.

TABLE BXII.γ.147. Differential characteristics of *Psychromonas* species[a,b]

Characteristic	*Psychromonas antarctica*	*Psychromonas arctica*	*Psychromonas kaikoae*	*Psychromonas marina*	*Psychromonas profunda*
Temperature range (optimum) (°C)	2–17 (12)	0–25 (20)	4–15 (10)	0–25 (14–16)	2–13 (3–4)
Growth at atmospheric pressure	+	+	−	+	+
Indole production	−	nd	−	−	+
H_2S production	−	nd	−	+	+
Acid produced from:					
Inositol	−	nd	−	−	+
Lactose	−	nd	−	+	+
Rhamnose	−	nd	−	−	+
Trehalose	+	nd	+	−	+
Growth on:					
Alanine	−	+	nd	nd	+[c]
Cellobiose	−	nd	+	+	+[c]
Fumarate	−	+	nd	nd	+
Glycerol	−	+	−	+	+
Mannose	−	+	+	−	−
Xylose	−	−	−	+	+[c]
Hydrolysis of:					
Gelatin	+	−	+	−	−
Starch	+	+	−	+	w

[a]Abbreviations: +, positive; −, negative; nd, not determined; w, weak.

[b]Data from Mountfort et al.(1998a), Kawasaki et al. (2002), Nogi et al. (2002), Groudieva et al. (2003), and Xu et al.(2003).

[c]Extended incubation (up to 4 w) may be required.

Isolated from the anaerobic sediment of a saline pond on the McMurdo Ice Shelf, Antarctica.

The mol% G + C of the DNA is: 42.8 (HPLC).

Type strain: star-1, DSM 10704.

GenBank accession number (16S rRNA): Y14697.

2. **Psychromonas arctica** Groudieva, Grote and Antranikian 2003, 544[VP]

arc′ti.ca. L. fem. adj. *arctica* from the Arctic, referring to the site where the type strain was isolated.

Rods 0.7–1.7 × 1.3–2.6 μm. Aging cultures contain coccoid cells 1.3–1.7 μm in diameter. Forms biofilms by secreting fibrous exopolysaccharides. Optimal NaCl concentration for growth, 2% (w/v); range, 1–7%. Temperature optimum, 20°C; range, 0–25°C. Killed by temperatures >27°C for >4 h. Carbon sources include acetate, alanine, fructose, fumarate, glucose, glycerol, lactose, maltose, mannitol, mannose, pyruvate, succinate, and sucrose. Fermentation products are CO_2, formate, acetate, ethanol, and lactate. No growth on chitin, citrate, gelatin, lactate, malate, propionate, xylan, or xylose. No vitamin requirement.

Isolated from seawater collected off Svalbard, Spitzbergen, Norway.

The mol% G + C of the DNA is: 40.1 (HPLC).

Type strain: Pull 5.3, DSM 14288, KCTC 12111.

GenBank accession number (16S rRNA): AF374385.

3. **Psychromonas kaikoae** Nogi, Kato and Horikoshi 2002, 1531[VP]

kai.ko′ae. N.L. fem. gen. n. *kaikoae* of KAIKO, the unmanned submersible that collected the samples from which the organism was isolated.

Motile rods, 0.8–1.0 × 2.0–4.0 μm; one polar flagellum (unsheathed). NaCl required for growth; optimal concentration 3% (w/v). Catalase and oxidase positive. Optimum growth at 10°C and 50 Mpa; does not grow at atmospheric pressure. Possesses both fermentative and respiratory pathways. Hydrolyzes gelatin. Reduces nitrate to nitrite; nitrite not further reduced. No H_2S, amylase, or indole production. Major quinone Q-8; major fatty acids are $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, and $C_{16:1}$.

Isolated from sediment from the Japan Trench at a depth of 7434 m.

The mol% G + C of the DNA is: 43.8 (method not given).

Type strain: JT7304, ATCC BAA-353, JCM 11054.

GenBank accession number (16S rRNA): AB052160.

4. **Psychromonas marina** Kawasaki, Nogi, Hishinuma, Nodasaka, Matsuyama and Yumoto 2002, 1458[VP]

ma.ri′na. L. adj. *marina* of the sea.

Motile rods, 0.8–1.2 × 1.5–2.0 μm; one polar flagellum. Catalase and oxidase positive. NaCl required for growth; optimum, 3–5% (w/v); maximum, 7%. Temperature range for growth, 0–25°C; no growth at or above 26°C. Ferments D-fructose, D-galactose, D-glucose, D-maltose, D-mannitol, sucrose, and D-xylose with no production of gas; in addition, utilizes *N*-acetyl glucosamine, D-cellobiose, D-galactose, glycerol, and lactose. Does not utilize L-arabinose, *myo*-inositol, D-mannose, melibiose, raffinose, rhamnose, raffinose, sorbitol, or trehalose. No vitamins, amino acids, or organic nitrogen sources required. Reduces nitrate to nitrite; produces H_2S but not indole. Produces β-galactosidase. Hydrolyzes alginic acid, DNA, starch, tributyrin, Tween 20, Tween 40, Tween 60, and Tween 80. Does not hydrolyze gelatin, casein, or chitin. Susceptible to vibriostatic agent O/129. Major fatty acids are $C_{14:1}$, $C_{16:0}$, $C_{16:0\ iso}$, $C_{16:1}$, $C_{18:1}$; also contains $C_{22:6}$.

Isolated from seawater collected in the Okhotsk Sea, Japan.

The mol% G + C of the DNA is: 43.5 (HPLC).

Type strain: 4-22, ATCC BAA-724, JCM 10501, KCTC 12105.

GenBank accession number (16S rRNA): AB023378.

5. **Psychromonas profunda** Xu, Nogi, Kato, Liang, Rüger, Kegel and Glansdorff 2003, 530[VP]

pro.fun′da. L. fem. adj. *profunda* from the deep.

Motile rods, 0.9–1.2 × 2.0–5.5 μm; one polar flagellum (unsheathed). NaCl required for growth. Growth occurs at 2–14°C. Greater than atmospheric pressure enhances growth. Possesses both fermentative and respiratory pathways. Produces acid from both fermentation and oxidation of cellobiose, dulcitol, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, rhamnose, salicin, sucrose, trehalose, and xylose. Hydrolyzes esculin and DNA. Weak hydrolysis of starch; no gelatin hydrolysis. Catalase and oxidase positive. Reduces nitrate to nitrite. Produces indole, H_2S, and β-galactosidase. Susceptible to chloramphenicol, furazolidone, penicillin, polymyxin B, tetracycline, and vibriostatic agent O/129. Major quinone Q-8. Major fatty acids are $C_{14:1}$, $C_{16:0}$, and $C_{16:1}$.

Isolated from Atlantic sediment samples taken at 2770 m depth off the coast of West Africa.

The mol% G + C of the DNA is: 38.1 (HPLC).

Type strain: 2825, JCM 11437.

GenBank accession number (16S rRNA): AJ416756.

Genus XIII. **Shewanella** *MacDonell and Colwell 1986, 355[VP] (Effective publication: MacDonell and Colwell 1985, 180)*

JOHN P. BOWMAN

Shew.a.nel′l.a. M.L. dim. ending *-ella*; *Shewanella* named after James Shewan for his work in fisheries microbiology.

Straight or curved rods, 0.5–0.8 × 0.7–2.0 μm. Endospores and microcysts are not formed. **Motile by a single, unsheathed, polar flagellum.** Gram negative. Colonies are often pale tan to pink-orange, due to cytochrome accumulation. **Oxidase and catalase positive.** Chemoheterotrophic. **Facultatively anaerobic. Oxygen is used as the electron acceptor during aerobic growth. Anaerobic growth is predominantly respiratory**; the oxidation of organic carbon compounds or H_2 is coupled to the reduction of various inorganic and organic electron acceptors, including NO_3^-, NO_2^-, Fe^{3+}, trimethylamine-*N*-oxide, fumarate, various sulfur

compounds, and Mn^{4+}. **May also be fermentative**, producing acid (but usually no gas) from carbohydrates, such as D-glucose and *N*-acetylglucosamine. Constitutive arginine dihydrolase is usually absent. Most strains can form H_2S from thiosulfate. **May require Na^+ ions** for growth. **Most species can grow at 4°C**. Some species are psychrophilic. One species is psychrophilic and barophilic. Major fatty acids are $C_{13:0\ iso}$, $C_{14:0}$, $C_{15:0}$, $C_{15:0\ iso}$, $C_{16:1\ \omega 7c}$, $C_{16:0}$, and $C_{17:1\ \omega 8c}$. Several species can also form significant quantities of the omega-3 fatty acid eicosapentaenoic acid ($C_{20:5\ \omega 3c}$). Isoprenoid quinone components include ubiquinone-8, ubiquinone-7, and menaquinone-7, with smaller amounts of methylmenaquinone-7. Major polyamines are putrescine and cadaverine. Member of the order *Alteromonadales*, family *Alteromonadaceae*, class *Gammaproteobacteria*. Isolated from clinical samples, chilled meats, butter, cutting oils, fresh water, freshwater sediment, estuaries, salt marshes, marine algae, seawater, marine sediment, fish, marine invertebrates, sea ice, marine snow, and abyssal ocean waters.

The mol% G + C of the DNA is: 38–54.

Type species: **Shewanella putrefaciens** (Lee, Gibson and Shewan 1977) MacDonell and Colwell 1986, 355 (Effective publication: MacDonell and Colwell 1985, 180) (*Alteromonas putrefaciens* Lee, Gibson and Shewan 1977, 449.)

Further descriptive information

Morphology Cells are pleomorphic rods, possessing either straight or slightly curved axes. In nutrient media, short filaments and, more rarely, helical forms may develop in older cultures. Cells do not contain poly-β-hydroxybutyrate inclusions or gas vesicles, form endospores or microcysts, or develop cellular appendages, such as prosthecae or spinae. Inclusion bodies of unknown function, possibly consisting of protein, have been found in *S. putrefaciens* (Krause et al., 1996).

Cultural characteristics Colonies on complex nutrient media typically have a pale tan to pink-orange or salmon color, which is due to strong accumulation of cytochrome proteins. This tendency is less pronounced in some species, such as *S. hanedai* and *S. benthica*. *S. colwelliana* and some *S. hanedai* strains form dark brown, melanin-like pigments when growing on complex media or media containing L-tyrosine. Studies on *S. colwelliana* indicate that this pigmentation derives from L-tyrosine, which is catabolized to homogentisate, which in turn is polymerized to form the pigment pyomelanin (Fuqua and Weiner, 1993; Coon et al., 1994; Ruzafa et al., 1994; Kotob et al., 1995).

Several *Shewanella* species require Na^+ for growth, and best growth occurs on seawater media such as marine 2216 agar (Difco Laboratories, Detroit, Michigan). *S. putrefaciens*, *S. baltica*, *S. algae*, and *S. frigidimarina* can grow well on marine agar and most commonly used nutrient media, including nutrient agar (Oxoid Ltd., Basingstoke, UK), plate count agar (Oxoid), triple sugar iron agar (Oxoid), etc. *S. colwelliana* requires amino acids, including L-aspartate and L-glutamate, for growth (Weiner et al., 1988). *S. hanedai* and *S. benthica* strains may also require amino acids and/or vitamins for growth (Baumann et al., 1984d; Bowman et al., 1997b). In defined media, such as basal medium agar (see *Alteromonas*), these requirements can be met by adding yeast extract at concentrations of 0.05–0.1% (w/v). *S. putrefaciens*, *S. baltica*, *S. frigidimarina*, *S. algae*, and *S. gelidimarina* can grow well on defined seawater media containing ammonia as a nitrogen source. The growth requirements for *S. woodyi* are unknown. Some *S. algae* strains and most *S. frigidimarina* strains do not require Na^+ but are stimulated by its presence. Growth occurs between 0.05–1.0 M NaCl, with optimal NaCl concentrations being 0.4–0.5 M. Strains of *S. algae* and *S. frigidimarina* exhibit moderate halotolerance and are able to tolerate Na^+ ion concentrations of up to 1.7–2.0 M.

The physiological diversity of *Shewanella* species is reflected by the wide range of habitats from which they can be isolated. Species may be mesophilic (*S. algae*, *S. colwelliana*, *S. putrefaciens* DNA hybridization group III), psychrotolerant (*S. putrefaciens* group I, *S. baltica*, *S. frigidimarina*, *S. woodyi*), psychrophilic (*S. gelidimarina*, *S. hanedai*), and both psychrophilic and barophilic (*S. benthica*). The majority of *S. algae* strains can grow at 41°C, but no *Shewanella* strains can grow at 45°C or higher.

Energy metabolism *Shewanella* species are chemoheterotrophic facultative anaerobes, with anaerobic growth typically of a respiratory nature; however, some species can also grow fermentatively. These include *S. frigidimarina* and *S. benthica*, both of which can ferment D-glucose, and *S. gelidimarina*, which can ferment *N*-acetylglucosamine and chitin, but not D-glucose (MacDonell and Colwell, 1985; Bowman et al., 1997b). Gas is not produced by these species during fermentation; however, *S. violacea*, a barotolerant species closely related to *S. benthica*, can ferment D-glucose and produce both acid and gas (Nogi et al., 1998b). The end products and relevant biochemistry of this process has not been studied.

Most studies on the diverse modes of anaerobic respiration in *Shewanella* have concentrated on strains of *S. putrefaciens* and *S. algae*; however, all *Shewanella* strains can use nitrate as an electron acceptor for growth (although denitrification is a strain-specific characteristic). Trimethylamine-*N*-oxide (TMAO) is also a common terminal electron acceptor among *Shewanella* species, and its reduction to trimethylamine is usually responsible for the odors associated with *Shewanella* food spoilage (Shewan, 1971). *Shewanella* strains can grow anaerobically by reduction of various sulfur compounds to H_2S, including S^0, thiosulfate, and sulfite (Semple and Westlake, 1987; Perry et al., 1993; Moser and Nealson, 1996), thiosulfate, and sulfite. Anaerobic growth can also occur via reduction of fumarate to succinate, coupled to the oxidation of formate. The fumarate reductase of *S. frigidimarina* NCIMB strain 400 is similar to a fumarate reductase flavoprotein found in *Wolinella succinogenes* (Simon et al., 1998).

Extensive studies have concentrated on the relatively unusual ability of *Shewanella* to facultatively reduce ferric iron, manganese, and other metals. The majority of *Shewanella* strains tested can grow anaerobically by coupling the oxidation of carbon compounds or H_2 (in the presence of a utilizable carbon source) to the reduction of Fe^{3+} to Fe^{2+} (Semple and Westlake, 1987; Lovley and Phillips, 1986; Lovley, 1993) or of Mn^{4+} to insoluble Mn^{3+} (Myers and Nealson, 1988; Caccavo et al., 1996b). Mn^{4+} and Fe^{3+} have redox potentials higher than that of sulfate and are capable of out-competing electron acceptors of lower potential, such as SO_4^{2-} (used for sulfate reduction) and CO_2 (used for methanogenesis) (Nealson and Saffarini, 1994). Dissimilatory metal reduction is believed to be important in terms of metal cycling and mobilization, as well as for its contribution to geomagnetism and sedimentary diagenesis (Nealson and Saffarini, 1994; Lovley, 1997; Pedersen, 1997). Owing to the broad specificity of the *Shewanella* anaerobic reductase enzyme system, *S. putrefaciens* strains can reduce and mobilize toxic and radioactive metallic pollutants, including Co, Cr, Se, Tc, and U (Lovley, 1993; Caccavo et al., 1994, 1996b; Lloyd and McCaskie, 1996; Ganesh

et al., 1997; Gorby et al., 1998). In addition, strains can reductively dehalogenate and transform organic pollutants, such as tetrachloroethane and carbon tetrachloride (Petrovskis et al., 1994; Picardal et al., 1995; Backhus et al., 1997). Studies indicate that the different modes of anaerobic respiration utilize different sets of enzymes. For example, a transposon mutant of *S. putrefaciens* MR-1 lacks fumarate reduction ability, but other reductive pathways are not compromised (Myers and Myers, 1997). Genes for anaerobic respiration by *S. putrefaciens* strain MR-1 appear to be both chromosomal and borne on megaplasmids (Saffarini et al., 1994).

During aerobic and anaerobic growth, *Shewanella* species can utilize a range of organic acids as sole sources of carbon and energy. In general, aromatic and heterocyclic compounds are not utilized, while the degree of utilization of carbohydrates varies considerably among the species, with some strains unable to use any carbohydrates. Glucose is catabolized via the Entner–Doudoroff pathway (Abu et al., 1994; Scott and Nealson, 1994). Most strains can degrade gelatin and form lipases, while certain species form chitinases, but in general do not attack starch, except for *S. colwelliana* and some strains of *S. woodyi* and *S. hanedai*. *S. gelidimarina* appears to be adapted almost specifically to chitin degradation, given its ability to utilize both aerobically and anaerobically only *N*-acetylglucosamine and chitin, out of a wide range of carbohydrates tested.

S. putrefaciens produces a potent iron chelator, putrebactin, that is structurally similar to chelators formed by *Pseudoalteromonas haloplanktis* (bisucaberin) and *Bordetella* spp. (alcaligin) (Ledyard and Butler, 1997). *S. algae* strains have been shown to form 4-epitetrodotoxin, a derivative of tetrodotoxin, which is a potent neurotoxin (Simidu et al., 1987, 1990; Nozue et al., 1992).

Genetic characteristics DNA base composition values for *Shewanella* vary from 38 to 54 mol% (T_m, Bd), and the ranges for individual species are shown in Table BXII.γ.148.

Temperature and pressure-regulated genes have been studied in a number of barotolerant and barophilic *S. benthica* strains (Kato et al., 1997; Chilukuri and Bartlett, 1997) and include a series of cytochrome proteins, including a cytochrome *c* oxidase. A set of PCR-amplified, pressure-regulated gene products have been found to be universal in *S. benthica* strains and have been proposed as a rapid means to identify this particular barophilic species (Li et al., 1998).

S. hanedai and *S. woodyi* have the distinction of being the only known nonfermentative bioluminescent species. Studies of the *luxA* sequence of *S. woodyi* have shown it to have only little homology with the *luxA* nucleotide sequence of *S. hanedai*. Overall, the *S. hanedai luxA* gene shows closer similarity to sequences found in *Vibrio logei* and *Vibrio fischeri* (*Photobacterium fischeri*) (Makemson et al., 1997).

Fatty acid profiles *Shewanella* species have a distinctive fatty acid pattern, as compared with other bacteria of the *Gammaproteobacteria*, in that they are rich in branched and odd-chain-length fatty acids (Wilkinson and Caudwell, 1980; Moule and Wilkinson, 1987; Rosselló-Mora et al., 1995a; Bowman et al., 1997b). Major fatty acids (and their ranges of % composition) include: $C_{13:0\ iso}$ (1–16%), $C_{14:0}$ (1–12%), $C_{15:0\ iso}$ (4–24%), $C_{15:0}$ (1–8%), $C_{16:1\ \omega7c}$ (16–55%), $C_{16:0}$ (5–31%), $C_{17:1\ \omega8c}$ (1–15%), and $C_{18:1\ \omega7c}$ (0–8%). The quantitative proportions of fatty acid components vary considerably among species, but it is difficult to judge with the data available whether these differences would be useful in species differentiation or whether they are merely due to variations in cultivation conditions. *Shewanella* possess both aerobic and anaerobic desaturatase pathways for fatty acid synthesis. *Shewanella* strains grown anaerobically thus form a different fatty acid profile that those grown aerobically (Nichols et al., 1997b; Venkateswaran et al., 1999).

Psychrotolerant and psychrophilic *Shewanella* species have the unusual ability to synthesize eicosapentaenoic acid (EPA, $C_{20:5\ \omega3}$), the levels of which range from 2–22% (Bowman et al., 1997b). Few other bacterial species known to possess EPA, including *Flexibacter polymorphus* (Johns and Perry, 1977) and *Psychroflexus torquis* (Nichols et al., 1997a; Bowman et al. 1998c), which are members of the flavobacteria division. Several studies have detected EPA in unidentified marine bacteria with fatty acid profiles very similar to *Shewanella* (Iwanami et al., 1995, Watanabe et al., 1996; Yazawa, 1996; Jostensen and Landfald, 1997; Yano et al., 1997). Compelling evidence is available that links production of EPA synthesis with cold adaptation. In *S. gelidimarina* ACAM 456, EPA levels decrease markedly when incubation temperatures are above its optimal growth temperature (Nichols et al., 1997b), and there seems to be a correlation between the optimal growth temperatures of *Shewanella* species and their inherent EPA levels (Bowman et al., 1997b). In *S. benthica*, EPA levels increase until the hydrostatic pressure reaches the particular strain's growth pressure optimum (DeLong and Yayanos, 1986). Increased levels of EPA decrease the homeoviscosity of cellular membranes (Nichols et al., 1995), an adaptation important for organisms living in perpetually cold and high-pressure environments. Because omega-3 fatty acid precursors cannot be synthesized *de novo* by most metazoa, it is thought that bacteria and microalgae act as a dietary supply of these lipids (DeLong and Yayanos, 1986). The EPA synthesis gene cluster of *Shewanella* sp. SCRC-2738 has been cloned and sequenced, and weak expression of the genes with EPA production has been conferred to a *Synechococcus* strain (Takeyama et al. 1997). The synthetic pathway of EPA has been partially characterized in the same strain and appears to be derived from an aerobic desaturation pathway (Watanabe et al., 1997). Mesophilic species do not appear to produce EPA, and their fatty acid profiles are similar to those of *Ferrimonas balearica*, another mesophilic, iron-reducing halophilic species isolated from sediment (Rosselló-Mora et al., 1995a). The major polar lipids have been determined in *S. putrefaciens*, *S. baltica*, and *S. algae* and include mostly phosphatidylglycerol and phosphatidylethanolamine, a pattern typical of Gram-negative bacteria (Moule and Wilkinson, 1987).

Lipopolysaccharides (LPS) *S. putrefaciens* possesses R-type lipopolysaccharides, rather than the ladder-type LPS typically found in many other Gram-negative bacteria (Sledjeski and Weiner, 1991). The chemical composition of the LPS varies among various species of *Shewanella* (Moule and Wilkinson, 1989). The lipid fraction of the LPS in *S. putrefaciens* (strains ATCC 8071 and 8073), *S. baltica* (strain ATCC 8072), and *S. algae* (NCIMB 11157) consists of (% range) $C_{13:0\ iso}$ (4–9%), $C_{13:0}$ (6–13%), $C_{10:0\ 3OH}$ (8–13%), $C_{12:0\ 3OH}$ (11–16%), $C_{13:0\ iso\ 3OH}$ (8–14%), $C_{13:0\ 3OH}$ (13–21%), and $C_{14:0\ 3OH}$ (7–9%). In addition, *S. baltica* contains high levels of $C_{12:0\ iso\ 3OH}$ (10%) and $C_{14:0\ iso\ 3OH}$ (8%); these components are much less abundant in the other strains tested (0–2%). The neutral and amino sugar content of the LPS core oligosaccharide of all strains tested contained of galactose and heptose. In addition *S. putrefaciens* ATCC 8071 contains glucose and 3-amino-3,6-dideoxyglucose; both *S. putrefaciens* ATCC 8073 and *S. baltica* contain galactosamine; and *S. algae* contains quinovosamine.

TABLE BXII.γ.148. Phenotypic characteristics which differentiate the species of the genus *Shewanella*[a]

Characteristics	*S. putrefaciens*	*S. algae*	*S. amazonensis*	*S. baltica*	*S. benthica*	*S. colwelliana*	*S. frigidimarina*	*S. gelidimarina*	*S. hanedai*	*S. oneidensis*	*S. violacea*	*S. woodyi*
Bioluminescence	−	−	−	−	−	−	−	−	+	−	−	+
Violet pigment	−	−	−	−	−	−	−	−	−	−	+	−
Growth at 4°C	d	−	+	+	+	−	+	+	+	d	+	+
Growth at 25°C	+	+	+	+	−	+	−	−	d	+	−	+
Growth at 37°C	+	+	+	−	−	−	−	−	−	+	−	−
Barophilic	−	−	−	−	+	−	−	−	−	−	+	−
Requires Na^+ for growth	−	S	+	−	+	+	S	+	+	−	+	+
Tolerates 6% NaCl	−	+	−	−	−	−	+	−	−	−	nd	nd
Fermentation of:												
D-Glucose	−	−	−	−	+	−	+	−	−	−	+	−
D-Glucose (with gas)	−	−	−	−	−	−	−	−	−	−	+	−
N-Acetylglucosamine	−	−	nd	−	+	−	−	+	−	nd	nd	nd
Chitinase	−	−	−	−	+	−	−	+	+	nd	nd	−
Amylase	−	−	−	−	−	+	−	−	−	−	−	d
Lipase	+	+	nd	+	+	−	+	+	+	nd	nd	+
Hemolysis	−	+	+	−	−	−	−	−	−	−	nd	−
Denitrification	d	−	+	−	−	−	d	−	d	−	−	+
Ornithine decarboxylase	+	+	−	+	−	−	d	−	−	−	−	nd
EPA synthesis[b]	−	−	−	−	+	nd	+	+	+	−	+	nd
H_2S production	+	+	+	+	+	−	d	+	+	+	−	nd
Iron reduction	+	+	+	+	d	nd	+	+	−	+	nd	nd
Acid from D-glucose	d	d (weak)	nd	+	+	−	+	−	+	nd	+	nd
Utilization of:												
D-Glucose	d	−	nd	+	+	−	+	−	d	nd	nd	+
Cellobiose	−	−	nd	+	−	−	+	−	−	nd	nd	+
Maltose, sucrose	d	−	−	+	−	−	+	−	−	−	nd	−
N-Acetylglucosamine	d	+	nd	+	+	−	−	+	+	nd	nd	−
D-Gluconate	−	−	nd	+	−	+	d	−	d	nd	nd	nd
Mol% G + C	43–49	54	52	46–47	46–47	46	40–43	48	44–47	45	47	46

[a]For symbols see standard definitions; S, growth is stimulated by the presence of Na^+ ions.

[b]EPA, eicosapentaenoic acid.

Lipids A number of additional substituted lipids have been found in *S. putrefaciens* ATCC 8071, including two glycolipids, identified as β-D-glucopyranosyldiacylglycerol and β-D-glucopyranuronosyldiacylglycerol, and an ornithine amide lipid (Wilkinson, 1968a, 1972; Wilkinson et al., 1973).

Quinones The quinones found in *S. putrefaciens* (*"Pseudomonas rubescens"*), *S. algae*, *S. baltica*, and *S. frigidimarina* are ubiquinone-7 (11–51%), ubiquinone-8 (31–58%), and menaquinone-7 (5–52%), with small quantities of methylmenaquinone-7 (thermoplasmoquinone-7, 0–6%) also present (Itoh et al., 1985; Moule and Wilkinson, 1987; Akagawa-Matsushita et al., 1992a). This pattern distinguishes *Shewanella* spp. from other related bacteria, such as *Alteromonas* and *Pseudoalteromonas*, which contain mostly Q-8 (Akagawa-Matsushita et al., 1992a). Menaquinones appear to play an important role in anaerobic respiratory activity in *Shewanella* (Myers and Myers, 1993).

Polyamines The major polyamines found in *Shewanella* species are putrescine and cadaverine. This pattern differs from those of *Pseudoalteromonas* and *Alteromonas*, which, in addition to putrescine and cadaverine possess hydroxylated polyamine derivatives. *Moritella marina*, by comparison, possesses only cadaverine (Hamana, 1997).

Exopolysaccharides *S. putrefaciens* strain S29 possesses a phosphorylated exopolysaccharide made up of tetrasaccharide–phosphate repeating units consisting of 2-acetamido-2,6-dideoxyglucose and 4-acetamido-4,6-dideoxyglucose (Shashkov et al., 1997).

Pathogenicity *S. algae* and, less often, *S. putrefaciens* have been implicated occasionally in polymicrobial bacteremia and septicemia (Vogel et al., 1997) and are associated with a wide spectrum of clinical syndromes, such as surface and soft-tissue infections, eye infections, pneumonia, arthritis, peritonitis, and empyema (von Graevenitz and Simon, 1970; Debois et al., 1975; Holmes et al., 1975; Kim et al., 1989; Heller et al., 1990; Chen et al., 1991, 1997; Brink et al., 1995; Butt et al., 1997a; Dominguez et al., 1996; Yohe et al., 1997; Levy and Tessier, 1998). Hemolysin production by *S. algae* is believed to be an important factor contributing to the pathogenicity of this species (Khashe and Janda, 1998), but the exact details are yet to be resolved. *Shewanella*-like isolates have been proposed as a possible cause of lesions on some fish species (Subasinghe and Shariff, 1992; Mashima et al., 1997), although goldfish directly inoculated with a number of *Shewanella* strains have failed to exhibit any infection (Decostere et al., 1996).

Ecology *Shewanella* is a ubiquitous group of bacteria that has been isolated from a wide range of habitats. *S. putrefaciens* and its close relative *S. baltica* are among the major spoilers of chilled foods and are frequently isolated from a wide range of dairy, poultry, beef, and seafood products (Shewan, 1971; Lee et al., 1977; Molin and Ternström, 1982, Stenström and Molin, 1990;

Borch et al., 1996; Gram and Huss, 1996; Leroi et al., 1998). *Shewanella* has also been isolated from cutting and lubricating oils, oil brine, seawater, sediment, soil, ponds and lakes, sewage, and subsurface groundwater (Pivnick, 1955; Iizuka and Komagata, 1964b; Semple and Westlake, 1987; Aznar et al., 1992; DiChristina and DeLong, 1993; Höfle and Brettar, 1996; Pedersen et al., 1996a; Ziemke et al., 1997; Bowman et al., 1997a).

The exact isolation sites of *S. putrefaciens senso stricto* are unclear, owing to the species heterogeneity. The most common sites for isolation include various lightly salted food products, the coastal marine environment, freshwater sediments and overlying waters, and water and soil mixed with crude oil and other petroleum products (Semple and Westlake, 1987). A close relative of *S. putrefaciens*, *S. oneidensis* (Venkateswaran et al., 1998) has been isolated from lake sediment. Another close relative, *S. baltica*, appears to occur frequently in coastal marine environments. Genetic analysis of *S. baltica* (described in the study as *S. putrefaciens*) in Baltic Sea waters indicates the population is stable at the clonal level (Ziemke et al., 1997). Populations spike in correspondence with increased sulfur levels in the Baltic Sea and other locations (Nealson et al., 1991; Brettar and Höfle, 1993). *S. algae* comprises the bulk of strains isolated from clinical specimens (Nozue et al., 1992, Vogel et al., 1997), and this species has also been isolated from various saline habitats, including salt marshes (Rosselló-Mora et al., 1994a), oil brine (Semple and Westlake, 1987), surfaces of macroalgae (Simidu et al., 1990), seawater, and occasionally salted food products (Vogel et al., 1997). Most *Shewanella* species appear to be strictly marine in origin. *S. amazonensis* has so far been isolated only from coastal marine muds off the Brazilian coast. The bioluminescent species *S. woodyi* (Makemson et al., 1997), as well as other novel *Shewanella* strains, has been shown to be associated with the ink and reproductive organs of certain squid (*Loligo* spp.) (Leonardo et al., 1999). *S. woodyi* has also been isolated from seawater and organic detritus collected at depths of 200–300 meters in parts of the Mediterranean Sea. *S. colwelliana* has been isolated from cultured oysters and their vicinity in the Chesapeake Bay, United States (Weiner et al., 1988). Studies suggest that *S. colwelliana* can enter a symbiotic arrangement with a host oyster (Bonar et al., 1986).

A host of cold-adapted *Shewanella* species has been isolated from marine ecosystems. *S. frigidimarina* appears to be ubiquitous in the Antarctic marine environment and has been isolated from seawater, ice, sediments, and cyanobacterial mat communities of the southern ocean and continental, marine-derived lakes (Bowman et al., 1997a, b; Rea et al., unpublished). It has also been isolated from the deep sea (DeLong et al., 1997) and the North Sea (Reid and Gordon, 1999), as indicated by 16S rDNA sequencing of isolates. *S. gelidimarina*, by comparison, has a much more restricted distribution and inhabits diatom-rich communities found in coastal Antarctic sea ice (Bowman et al., 1997a). *S. hanedai* has also been isolated from Antarctic sea ice diatom communities, but was first isolated from sediments of the Arctic and Southern Oceans and from coastal areas of Canada (Jensen et al., 1980). Strains of *S. benthica* as well as *S. violacea* have been isolated from several deep-sea sites, including the Marianas Trench at a depth of nearly 11,000 m (Kato et al., 1995, 1996, 1998). This species has been found in water and sediment samples and organic detritus, but is most frequently associated with decaying deep sea invertebrates, including amphipods and holothurians (Deming et al., 1988a; DeLong et al., 1997).

Enrichment and Isolation Procedures

Most *Shewanella* species can be directly isolated from source material onto marine 2216 (Difco Laboratories) or nutrient agar (Oxoid) without prior enrichment. *Shewanella* colonies are often identifiable by their pale tan or pink-salmon color. *S. putrefaciens* and *S. algae* can be isolated semi-selectively from clinical and environmental samples on a medium containing 1% NaCl, 0.1% ox bile salts, and 1% peptone (Nozue et al., 1992). Nonfermentative, H_2S-producing isolates (which are usually positive for ornithine decarboxylase) can be further selected on deoxycholate–hydrogen sulfide–lactose medium (Eiken Chemical Co., Ltd., Tokyo, Japan). Additional selection for *S. algae* includes incubation at 40°C on nutrient agar containing 6% NaCl. The identification of *S. algae* strains can be confirmed by their α-hemolytic activity on sheep blood agar; strains can also be selected and isolated by plating clinical samples onto *Salmonella–Shigella* agar (Nozue et al., 1992). *S. putrefaciens* and *S. algae* strains and several other *Shewanella* species can also be enriched and isolated with iron-, nitrate- or sulfur-reducing media (see Procedures for Testing Special Characters). Alternatively, direct isolation can be performed anaerobically on S^0 reduction agar (Moser and Nealson, 1996) with transfer of growth to nutrient or marine agar.

A brief enrichment of samples in marine 2216 broth at 2°C for 1–2 d is advantageous for the isolation of psychrophilic species such as *S. gelidimarina* (Bowman et al., 1997a). *S. woodyi* and *S. hanedai* produce bioluminescence most effectively on marine agar, rather than on the standard glycerol-containing luminous medium normally used to detect bioluminescent *Vibrio* or *Photobacterium* spp. (Ruby et al., 1980; Hastings and Nealson, 1977).

Isolation of *S. benthica* involves a relatively straightforward method that can also be used to isolate barophilic species belonging to other genera (Sakiyama and Ohwada, 1997); it is similar to methods employed in other studies (DeLong and Yayanos, 1986; DeLong et al., 1997; Kato et al., 1995, 1996, 1998). Small portions of sediment or seawater filters are added to 0.5× marine 2216 broth in small, sterile polyethylene bags (Whirl-Pak, Nasco, USA) and heat sealed. The bags are then incubated at low temperatures (2–4°C) in stainless steel pressure vessels under a hydrostatic pressure equivalent to that at the isolation site. For further details on the use of high-hydrostatic-pressure culture equipment, see Yayanos et al. (1979, 1982). Strains are isolated and purified using marine 2216 broth solidified with 2% SeaPrep low-melting-point agarose (FMW Inc., Maryland, USA) or silica gel (Dietz and Yayanos, 1978) in tubes sealed with silicone rubber double stoppers and pressurized to about 30–70 mPa (300–700 atmospheres). Individual colonies are then cut out of the medium with a scalpel and transferred to fresh marine 2216 broth.

Maintenance Procedures

In general, *Shewanella* species are relatively robust and can be preserved and maintained in various ways in a carbohydrate-free medium in which they grow well, such as nutrient or marine agar. *Shewanella* species can be lyophilized using 20% skim milk as a cryoprotectant. In addition, strains can sustain viability for over 6 months when frozen in liquid nitrogen or cryopreserved at −80°C in broth containing 20–30% glycerol or DMSO. *S. algae* and *S. putrefaciens* strains can be maintained at 15–20°C in semi-solid agar medium containing 0.1% Proteose peptone no. 3 (Difco Laboratories), 0.1% yeast extract, 0.05% phytone, 0.02% sodium thiosulfate, 0.005% sodium sulfite, 0.004% ferric citrate, and 0.3% agar dissolved in 3:1 aged seawater (or artificial sea

salts) and water; pH 7.6. (Simidu et al., 1990). *S. frigidimarina* and *S. gelidimarina* can be stored on marine agar slants or as heavy suspensions in sterile seawater at 2°C for 12 months or longer.

Procedures for Testing Special Characters

Dissimilatory iron reduction is a common property of *Shewanella* species and can be used in their isolation. Iron-reducing medium[1] Lovley and Phillips, 1988) supplemented with 0.05% yeast extract to boost growth can be used to test this property and to enrich for iron reducing strains. A variety of iron electron acceptors can be used, including amorphic ferric oxide, ferric citrate, and ferric pyrophosphate (Lovley and Phillips, 1986, 1988). These electron acceptors can be added separately or in combination. Electron donors that are typically utilized include organic acids, such as lactate, citrate, malate, etc. The addition of H_2 to the headspace can substantially stimulate growth (Caccavo et al., 1992). Samples are suspended in phosphate-buffered saline buffer or seawater and then sparged with nitrogen. The diluted sample is then added to the media by syringe or added under a stream of nitrogen, and vials or tubes are crimp sealed. Reduction of Fe^{3+} to Fe^{2+} is indicated by the change of the rust-colored medium to a clear solution that contains white (smectite or vivianite) or black (FeS_2, pyrite) precipitates. A similar approach can be used with other electron acceptors, such as nitrates[2] and MnO_2[3] (Lovley and Phillips, 1988).

Anaerobic sulfur reduction can be tested using a plate assay developed by Moser and Nealson (1996), which contains polysulfide[4]. Colonies within clearing zones on the sulfur plates can then be transferred to nutrient or marine agar for further purification with aerobic incubation.

1. Iron-reducing medium consists (per liter distilled water): $NaHCO_3$, 2.5 g; $CaCl_2 \cdot 2H_2O$, 0.1g; KCl, 0.1 g; NH_4Cl, 1.5 g; NaH_2PO_4, 0.6 g; carbon source (acetate, lactate, citrate, etc.), 20 mM; trace element solution, 10 ml; and 200 mM amorphic ferric oxide. The medium is prepared with vigorous boiling and gassing with nitrogen to remove oxygen and dispensed into vials or tubes sealed with thick butyl rubber stoppers. Following autoclaving, the medium pH is about 6.7. The trace element solution (Balch et al., 1979) contains (per liter distilled water) nitriloacetate, 1.5 g; $MgSO_4 \cdot 7H_2O$, 3 g; $MnSO_4 \cdot H_2O$, 0.5 g; NaCl, 1 g; $FeSO_4 \cdot 7H_2O$, 0.1 g; $CoCl_2 \cdot 6H_2O$, 0.1 g; $CaCl_2 \cdot .2H_2O$, 0.1 g; $ZnSO_4 \cdot 7H_2O$, 0.1 g; $CuSO_4 \cdot 5H_2O$, 0.01 g; $AlK(SO_4)_2 \cdot 12H_2O$, 0.01 g; H_3BO_3, 0.01 g; $Na_2MoO_4 \cdot 2H_2O$, 0.01 g; $NiSO_4 \cdot 6H_2O$, 0.03 g; Na_2SeO_3, 0.02 g; and $Na_2WO_4 \cdot 2H_2O$, 0.02 g. The nitriloacetate is dissolved in 500 ml distilled water and the pH is adjusted to 6.5 by KOH. The remaining salts are added one at a time, and the volume is brought up to 1 liter. Samples are added using Hungate techniques or in an anaerobic chamber. N_2/CO_2 (80:20) or $N_2/CO_2/H_2$ (80:10:10) can be used as the headspace atmosphere. For enrichment of marine samples, the medium is supplemented with 27 g/l NaCl and 3.7 g/l $MgCl_2$. Amorphic ferric oxide is prepared by neutralizing a 0.4 M $FeCl_3$ solution to pH 7 with NaOH. Ferric citrate can be used instead of amorphic ferric oxide and is added at 20 mM (with $CaCl_2 \cdot 2H_2O$ omitted to prevent precipitation).

2. Nitrate-reducing medium: similar to iron-reducing medium, but with amorphic ferric oxide replaced by 20 mM KNO_3 ($CaCl_2 \cdot 2H_2O$ is omitted to prevent precipitation).

3. Manganese-reducing medium: similar to iron-reducing medium, but with amorphic ferric oxide replaced by 15 mM MnO_2. The latter is prepared by slowly adding 30 mM $MnCl_2$ to 20 mM $KMnO_4$ with constant stirring.

4. For sulfur-reduction plates, the basal medium used is similar to iron-reducing medium, but the amorphic ferric oxide is omitted and the medium is supplemented with 0.5% Casamino acids and 1.5% agar. Immediately before dispensing the medium into plates, 40 mM polysulfide is added from a polysulfide stock (2.25 M total sulfur) prepared by adding 7.2 g sulfur flowers and 24 g $Na_2S \cdot 9H_2O$ to 100 ml boiling water and stirring for 15 min. After adding the polysulfide, the medium is left in air overnight to allow for sufficient precipitation of sulfur globules and to shift the pH back to neutrality. The medium is then inoculated and incubated anaerobically.

The ability to ferment carbohydrates is tested most effectively in the oxidation/fermentation medium of Leifson (1963), to which carbohydrates are added at a concentration of 0.5% (w/v). This medium is more sensitive than the usual Hugh and Leifson medium because it contains phenol red instead of bromothymol blue. Other biochemical and nutritional traits can be tested using standard procedures, provided that the incubation-temperature and Na^+-ion requirements are met.

Differentiation of the genus *Shewanella* from other genera

Characteristics that differentiate *Shewanella* from other members of the *Alteromonadaceae* are shown in Table BXII.γ.135 of the chapter describing the family. Due to the diversity of species, there are only a few properties shared by all the members of the genus, thus resulting in a potential for misidentification. Individually, most species possess sufficiently distinct properties (H_2S production, ornithine decarboxylase activity, psychrophily, etc.) to make them relatively easy to identify, assuming enough tests are employed. The generally nonfermentative nature of *Shewanella* species distinguishes them from members of the *Vibrionaceae*. *S. frigidimarina* is an exception; however, it lacks sheathed flagella and possesses a mol% G + C value lower than those of most *Vibrio* species. There is a possibility that *S. algae* can be confused with *Ferrimonas balearica*, but, unlike the latter, it can lyse sheep's blood, degrade gelatin, and tolerate 10% NaCl and is unable to produce phenylalanine deaminase (Rosselló-Mora et al., 1995a). In ambiguous cases, fatty acid analysis and/or partial 16S rRNA sequencing provides direct identification.

Taxonomic Comments

Phylogenetic trees based on 16S rRNA sequences of several *Shewanella* strains are shown in Fig. BXII.γ.156. Overall, two major groups are delineated within the genus, with *S. algae* and *S. amazonensis* (Venkateswaran et al., 1998), forming relatively distinct branches. One large group includes the psychrotolerant, nonhalophilic species *S. putrefaciens*, *S. baltica*, and *S. frigidimarina*. The second group includes the psychrotolerant and psychrophilic Na^+-requiring species *S. benthica*, *S. hanedai*, *S. gelidimarina*, *S. woodyi*, and *S. pealeana* (Leonardo et al., 1999). Overall, *Shewanella* is phylogenetically most closely related to the genera *Pseudoalteromonas*, *Alteromonas*, *Moritella*, *Ferrimonas*, and *Colwellia*, all of which are members of the family *Alteromonadaceae*.

DNA–DNA hybridization (Owen et al., 1978; Semple et al., 1989), ribotyping, and protein electrophoretic pattern analysis (Vogel et al., 1997) have shown the *S. putrefaciens* complex (not including *S. algae*) to be made up of at least three groups. DNA hybridization data for a variety of *S. putrefaciens* and related strains available in major culture collections are shown in Table BXII.γ.149. Other species, such as *S. algae* (Nozue et al., 1992; Vogel et al., 1997), *S. frigidimarina*, *S. gelidimarina*, and *S. hanedai* (Bowman et al., 1997b), each form genotypically distinct groups (Table BXII.γ.149).

S. putrefaciens was first described in 1931 as an *Achromobacter* species (Derby and Hammer, 1931), but was transferred to *Pseudomonas* because of its rod-like morphology, motility, and nonfermentative metabolism (Long and Hammer, 1941). Because its mol% G + C values are considerably lower than those of other pseudomonads (Levin, 1972), the species was reclassified as a species of *Alteromonas* (Lee et al., 1981a). Finally, based on 5S rRNA sequences, the species was renamed *S. putrefaciens* and designated the type species of the genus *Shewanella*. The description

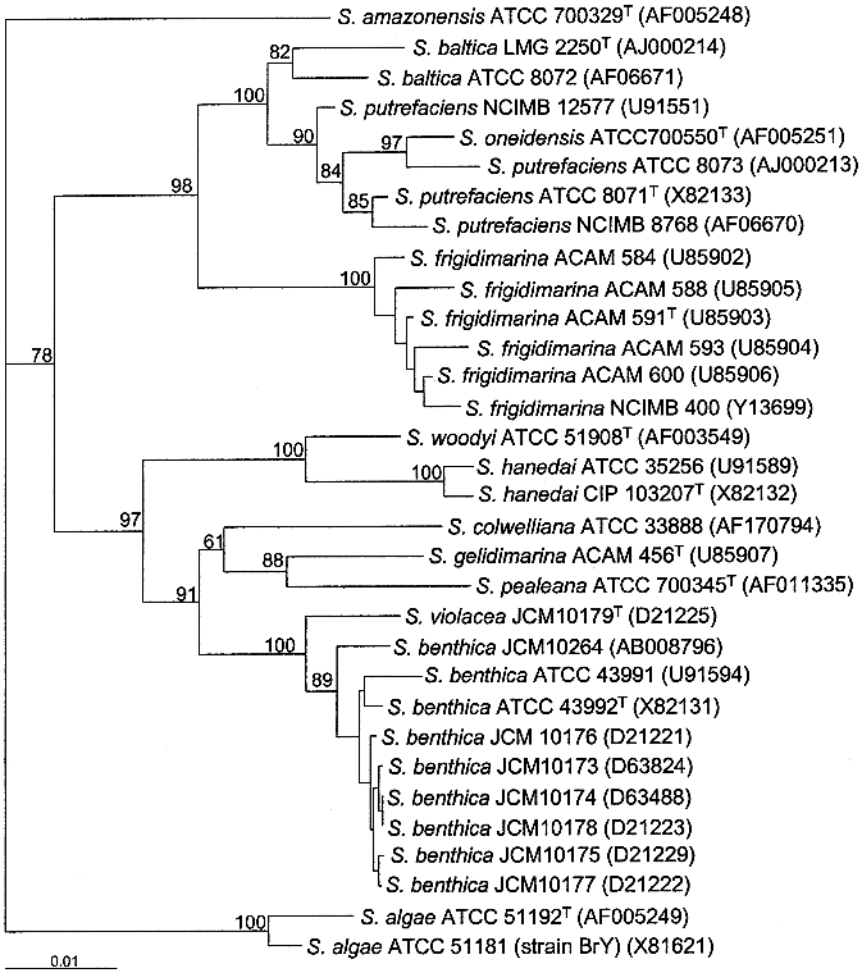

FIGURE BXII.γ.156. Phylogenetic tree of the genus *Shewanella* based on nearly complete 16S rRNA sequences. Species included are those validly published as of 2000. The tree was determined using maximum-likelihood distances and clustered by neighbor-joining. Numbers at branch nodes are bootstrap values (calculated from 250 replicate analyses). Only bootstrap values over 50% are shown. *T* denotes a type strain. Bar = distance of 0.01.

TABLE BXII.γ.149. DNA–DNA relatedness (% hybridization) between *Shewanella putrefaciens* Owen DNA-hybridization groups[a]

	S. putrefaciens				*S. baltica*		*S. algae*
	Group I		Group III		Group II		Group IV
Strain (source)	ATCC 8071T	ATCC 8073	NCTC 10695	CL256/73	NCTC 10735T	ATCC 8072	NCIMB 11157
S. putrefaciens - Group I							
ATCC 8071T (butter)	100		15	34	11–26	27	5
ATCC 19857 (unknown)	86	100	9		41	37	5
NCTC 10695 (cutting oil)		100		32	47		27
S. putrefaciens - Group III							
ATCC 8073 (butter)	10	100		42	38	22	6
CL256/73 (spinal fluid)		29		100	32		40
S. putrefaciens (ungrouped)							
NCIMB 1733 (fish)	9	7				21	6
S. baltica - Group II							
NCTC 10735T (oil brine)		5	39	28	100		13
ATCC 8072 (butter)	12	7		21	58	100	4
NCTC 10736 (fish)			30	31	83		
NCTC 10737 (squid)			32	26	57		
S. algae - Group IV							
NCTC 10738 (feces)	5	4	25	44	13	9	100
NCTC 10762 (pus)			24		9		
NCTC 10763 (bottled blood)			11		27		

[a]Data from Owen et al. (1978); Semple et al. (1989); Bowman et al. (1997b); Ziemke et al. (1998).

of this genus also included descriptions of *S. hanedai* (previously *Alteromonas hanedai*; Jensen et al., 1980) and *S. benthica* (MacDonell and Colwell, 1985). Although *Shewanella* was initially thought to be a member of the *Vibrionaceae* (MacDonell and Colwell, 1985; Farmer, 1992), 16S rRNA sequences have indicated that *Shewanella* is more closely related to the genus *Alteromonas* and its relatives (Kita-Tsukamoto et al., 1993; Gauthier et al., 1995a). *Shewanella colwelliana* was added to the genus following reassessment of its taxonomy using 5S rRNA sequences (Coyne et al., 1989). *S. algae* was created by Simidu et al. (1990) and has been subsequently emended by Nozue et al. (1992). *S. woodyi* (Makemson et al., 1997), *S. frigidimarina*, *S. gelidimarina* (Bowman et al., 1997b), and *S. baltica* (Ziemke et al., 1998) have only recently been described.

The nomenclature of *S. putrefaciens* is still evolving, but at the time of its renaming as *S. putrefaciens* it was a heterogeneous complex of strains. Owen et al. (1978) have demonstrated that *S. putrefaciens* comprises four DNA hybridization groups. In earlier phenotypic studies of *S. putrefaciens*, at least two groups, or "Gilardi" biovars, were observed. Gilardi biovar 2 includes mostly mesophilic clinical strains, which can grow at 42°C and tolerate 6% NaCl, but cannot utilize glucose or maltose. Gilardi biovars 1 and 3 include mostly food and environmental strains, which are psychrotrophic, saccharolytic, and unable to grow in the presence of 6% NaCl (Gilardi, 1972; Riley et al., 1972; Richard et al., 1985; Khashe and Janda, 1998). Subsequently, Nozue et al. (1992) have determined Gilardi biovar 2 to be comprised mainly of strains of *S. algae*, a species that is equivalent to Owen's DNA hybridization group IV. Additional data in the form of ribotypes and protein electrophoretic patterns indicate that strains belonging to DNA groups I, II, and III can be clearly distinguished from *S. algae* (Vogel et al., 1997). This corresponds well with the distinct phylogeny of *S. algae* and *S. putrefaciens* strains (Fig. BXII.γ.156). However, the resolution among DNA groups I, II, and III using the same procedures is quite poor (Vogel et al., 1997). These methods tend to suggest that *S. putrefaciens* is composed of a continuum of a series of genetically different strains. In their study of fish isolates, Stenström and Molin (1990) have claimed that numerical taxonomic analysis of phenotypic data does not correlate well with the hybridization groups of Owen et al. (1978); however, in the light of more recent data, this conclusion may be biased by their isolation of predominantly group II *S. putrefaciens* strains. Phenotypic analyses of *S. putrefaciens* by Semple and Westlake (1987) and Ziemke et al. (1998) suggest that phenotypic resolution of these groups may be possible, although DNA hybridization results (Semple et al., 1989) suggest that they are not necessarily definitive. For the purpose of completeness, phenotypic data differentiating the various DNA groups of *S. putrefaciens* are presented in Table BXII.γ.150. One limitation is that relatively few strains identified as belonging to group III have been examined. Based on 16S rDNA hybridization and the aforementioned phenotypic properties, group II strains have recently been given species status and named *S. baltica* (Ziemke et al., 1998). Overall, *S. baltica* represents a large proportion of isolates conforming to Gilardi biovars 1 and 3. From phylogenetic studies (Fig. BXII.γ.156), it is evident that heterogeneity remains in the vicinity of the type strain of *S. putrefaciens*, particularly among *S. putrefaciens* DNA group III strains (Venkateswaran et al., 1999).

The taxonomy of the remaining species is more clear-cut. Phenotypic differences can readily distinguish these species (Table BXII.γ.148), although data for *S. woodyi* are somewhat limited. DNA hybridization analysis has also not been performed between *S. hanedai* and *S. woodyi*.

Differentiation of the species of the genus *Shewanella*

Phenotypic characteristics that distinguish the different species and genomospecies of *Shewanella* are presented in Tables BXII.γ.148 and BXII.γ.150.

List of species of the genus Shewanella

1. **Shewanella putrefaciens** (Lee, Gibson and Shewan 1977) MacDonell and Colwell 1986, 355^{VP} (Effective publication: MacDonell and Colwell 1985, 180) (*Alteromonas putrefaciens* Lee, Gibson and Shewan 1977, 449.)
pu.tre.fa′ ci.ens. L. v. *putrefacere* to make rotten; L. part. adj. *putrefaciens* making rotten or putrefying.

Cells are rod-shaped, 0.5–1.0 × 1.5–2.0 μm. Colonies on nutrient agar are light tan to salmon pink, opaque, circular, convex with entire edges, and butyrous in consistency. NaCl is usually not required for growth. Can tolerate up to 6% NaCl. Does not require growth factors.

Mesophilic. Growth occurs between 10 and 40°C; optimum, 30–35°C.

Other characteristics are as given in the genus description and in Tables BXII.γ.148 and BXII.γ.150.

Isolated from fish, poultry, chilled meats, machine cutting oils, and freshwater and marine samples.

The current description of *Shewanella putrefaciens* incorporates at least two distinct genomospecies, including group I (*Shewanella putrefaciens stricto sensu*), group III, and probably other groups. Refer to Table BXII.γ.150 for phenotypic differentiation of groups I and III and to Fig. BXII.γ.156 for phylogeny.

The mol% G + C of the DNA is: 44–47 (T_m, Bd).

Type strain: 95, ATCC 8071, DSM 6067, ICPB 352, LMG 2268, NCIMB 10471.

GenBank accession number (16S rRNA): X82133, X81623.

2. **Shewanella algae** Simidu, Kita-Tsukomoto, Yasumoto and Yotsu 1990, 335^{VP} emend. Nozue, Hayashi, Hashimoto, Ezaki, Hamasaki, Ohwada and Terawaki 1992, 633.
al′ gae. L. gen. n. *algae* of an alga.

Cells are straight rods, about 0.8 × 1.6 μm. On marine agar, colonies are pale tan to salmon, circular, convex with an entire edge, and butyrous in consistency. Growth is stimulated by Na^+ ions, and NaCl concentrations of up to 12% are tolerated. Most strains can grow on *Salmonella–Shigella* agar.

Mesophilic. Growth occurs between 10 and 42°C.

Other characteristics are as given in the genus description and presented in Tables BXII.γ.148 and BXII.γ.150. In addition, strains also produce indophenol oxidase, lecithinase, deoxyribonuclease, and acid from D-ribose. Some

TABLE BXII.γ.150. Characteristics differentiating members of the *Shewanella putrefaciens* species complex, *S. baltica*, *S. algae*, and DNA hybridization Group III[a]

Characteristic	*S. putrefaciens*	*S. algae*	*S. baltica*	DNA hybridization Group III
Owen DNA Hybridization Group	I	IV	II	III
Growth at:				
4°C	+	−	+	d
37°C	+	+	−	+
42°C	−	−	−	d
Tolerates 6% NaCl	−	+	−	−
Reduction of sulfite (to H_2S)	+	d	−	−
Acid from:				
D-Glucose	−	d[b]	+	+
Maltose, sucrose	−	−	+	+
Arabinose	+	−	+	+
Growth on *Shigella–Salmonella* agar	+	+	−	−
Christensen's citrate (alkali)	−	−	+	−
Chymotrypsin	+	+	d[b]	+
N-Acetyl-β-glucosaminidase	−	+[b]	d	−
Urease	−	d	d	−
Utilization of:				
α-Cyclodextrin, glucose, maltose	−	−	+	+
Dextrin	−	−	d	+
L-Arabinose	d	−	−	+
Gentobiose, cellobiose, D-gluconate, citrate	−	−	+	−
Glycogen, adipate	−	−	d	−
Tween 80	+	+	d	−
Sucrose	−	−	+	d
N-Acetylgalactosamine	−	+	−	d
N-Acetylglucosamine	d	+	+	+
α-Hydroxybutyrate	−	+	−	−
DL-Malate	d	+	d	d
Caprate	−	+	d	d
Mol% G + C (T_m, Bd)	43–45	52–54	44–48	46–49

[a]For symbols see standard definitions.

[b]Weak reaction.

strains can produce urease and give a positive Simmons citrate test. Alginate is not hydrolyzed. Acid is not formed from D-arabinose, L-arabinose, D-mannose, D-mannitol, *meso*-inositol, L-rhamnose, D-xylose, sucrose, maltose, or lactose.

Several environmental and clinical strains can produce 4-epi-tetrodotoxin.

Isolated from red algae (*Jainia* spp.), salt marsh surface sediments, and human clinical samples, including blood, pus, sputum, feces, and tissue samples.

The species is equivalent to *Shewanella putrefaciens* DNA hybridization group IV of Owen et al. (1978).

The mol% G + C of the DNA is: 52–54 (T_m).

Type strain: OK-1, ACAM 541, ATCC 51192, IAM14159.

GenBank accession number (16S rRNA): AF005249, U91546.

3. **Shewanella amazonensis** Venkateswaran, Dollhopf, Aller, Stackebrandt, Nealson 1998, 971[VP]
a.ma.zo.nen′ sis. M.L. gen. n. *amazonensis* named after the area from the species was isolated.

Cells are rods, 0.4–0.7 × 2–3 μm. Colonies on marine agar are beige to pinkish, circular, convex with entire edges, and butyrous in consistency. No diffusible pigments are formed. NaCl is required for good growth, but cultures grow slowly in the absence of Na^+ ions. For optimal growth, about 1–2% NaCl is required. No growth occurs above 3% NaCl.

Psychrotolerant. Grows between 4 and 40°C; optimum temperature is approximately 37°C.

Other characteristics are as given in the genus description and in Tables BXII.γ.148 and BXII.γ.151. Can couple growth to reduction of S^0 and manganese oxide.

Isolated from oysters.

The mol% G + C of the DNA is: 52 (T_m).

Type strain: SB2B, ATCC 700329.

GenBank accession number (16S rRNA): AF005248.

4. **Shewanella baltica** Ziemke, Höfle, Lalucat and Rosselló-Mora 1998, 184.[VP]
bal′ ti.ca. M.L. fem. adj. *baltica* of the Baltic Sea.

Cells are short, straight rods. On nutrient agar, colonies are pale tan, circular, convex with an entire edge, and butyrous in consistency. Do not require NaCl or organic factors for growth.

Psychrotrophic. Growth occurs between 4 and 30°C. No growth at 37°C.

Other characteristics are as given in the genus description and presented in Tables BXII.γ.148 and BXII.γ.150.

Isolated from brackish water, seawater, oil brine, fish and seafood products, and other food products.

The species is equivalent to *Shewanella putrefaciens* DNA hybridization group II of Owen et al. (1978).

The mol% G + C of the DNA is: 44–47 (T_m).

Type strain: CECT 323, DSM 9439, IAM 1477, LMG 2250, NCTC 10735.

GenBank accession number (16S rRNA): AJ000214.

5. **Shewanella benthica** MacDonell and Colwell 1986, 355[VP]
(Effective publication: MacDonell and Colwell 1985, 180.)
ben′ thi.ca. Gr. n. *benthos* deep sea; L. suff. *-ica* of or pertaining to; M.L. adj. *benthica* pertaining to the deep sea.

TABLE BXII.γ.151. Additional phenotypic characteristics of marine *Shewanella* species[a]

Characteristics	*S. amazonensis*	*S. colwelliana*	*S. frigidimarina*[b]	*S. gelidimarina*[b]	*S. hanedai*[b]	*S. woodyi*
Utilization of:						
D-Ribose	nd	nd	nd	nd	d	nd
D-Mannose	+	nd	−	−	−	−
D-Fructose	+	−	d	−	−	nd
D-Xylose	nd	−	d	−	−	nd
D-Galactose	nd	−	d	−	d	+
Trehalose, isobutyrate	nd	−	+	−	−	nd
D-Mannitol, DL-malate	−	nd	+	−	−	−
Lactose, glycerol, sorbitol	−	nd	−	−	−	−
D-Glucuronate	nd	nd	−	−	−	+
Glycogen, L-phenylalanine	nd	nd	d	−	nd	nd
Acetate	nd	−	+	+	+	+
Propionate	nd	−	+	−	+	+
Butyrate	nd	−	+	+	+	nd
Valerate	nd	−	+	+	−	nd
Caproate, pelargonate	nd	nd	d	−	d	nd
Hepatanoate	nd	nd	−	−	d	nd
Caprate, L-isoleucine, spermine	nd	nd	nd	nd	+	nd
Succinate	+	−	+	−	−	+
DL-Glycerate, glycine	nd	−	nd	nd	+	nd
DL-Lactate	+	−	+	+	−	nd
Pyruvate	nd	nd	+	+	+	nd
Citrate	+	−	d	−	−	−
2-Ketoglutarate	nd	+	−	−	−	+
Fumarate	+	+	+	−	−	nd
Oxaloacetate	nd	nd	+	−	nd	nd
DL-Alanine	nd	−	−	−	+	+
L-Aspartate	nd	nd	−	−	+	nd
L-Glutamate	nd	nd	d	+	+	nd
L-Histidine	−	+	−	−	−	nd
L-Leucine	nd	nd	+	−	d	+
Hydroxy-L-proline, γ-aminobutyrate	nd	nd	d	−	nd	nd
L-Proline	nd	nd	d	−	−	nd
L-Serine	+	nd	−	−	+	+
L-Threonine	nd	−	−	−	−	+
L-Tyrosine	nd	−	−	−	d	nd
Putrescine	−	nd	−	−	+	+

[a]Symbols: +, 90% of strains are positive; d, 10–90% of strains are positive; −, 0–10% of strains are positive; nd, not determined.

[b] *S. frigidimarina*, *S. gelidimarina*, and *S. hanedai* do not utilize DL-arabinose, L-rhamnose, lactose, melibiose, adonitol, *meso*-inositol, D-sorbitol, saccharate, isovalerate, caprylate, adipate, pimelate, azelate, DL-3-hydroxybutyrate, or aconitate. In addition, *S. hanedai* does not utilize D-galacturonate, *i*-erythritol, salicin, ethanol, *n*-propanol, *n*-butanol, propyleneglycol, malonate, glutarate, L-tartrate, benzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, phenylacetate, quinate, β-alanine, L-arginine, L-citrulline, L-lysine, L-ornithine, L-valine, DL-δ-aminovalerate, ethanolamine, betaine, hippurate, sarcosine, or trigollenine.

Cells are slightly curved rods, 0.5 × 1.5–3.0 μm. Colonies on marine agar are off-white to pale tan, translucent, circular, convex with an entire edge, and butyrous in consistency. NaCl is required for growth. No growth occurs with 6% NaCl. Barophilic, growing optimally at 400–600 MPa. Grows slowly at atmospheric pressure.

Psychrophilic. Growth occurs at 0–10°C; optimum temperature, ~5°C at atmospheric pressure.

Other characteristics are given in the genus description and in Table BXII.γ.148.

Isolated from abyssal ocean water, sediments, organic detritus, and deep-sea invertebrates.

The mol% G + C of the DNA is: 47 (T_m).

Type strain: W 145, ATCC 43992, DSM 8812.

GenBank accession number (16S rRNA): X82131.

6. **Shewanella colwelliana** (Weiner, Coyne, Brayton, West and Raiken 1988) Coyne, Pillidge, Sledjeski, Hori, Ortiz-Conde, Muir, Weiner and Colwell 1990, 320[VP] (Effective publication: Coyne, Pillidge, Sledjeski, Hori, Ortiz-Conde, Muir, Weiner and Colwell 1989, 278[VP] (*Alteromonas colwelliana* Weiner, Coyne, Brayton, West and Raiken 1988, 242.)

col.well′ i.a.na. L. gen. n. *colwelliana* named after Rita Colwell for her contributions to marine microbiology.

Cells are straight or slightly curved rods, 0.7–1.5 × 1.0–3.0 μm long. Filamentous and helical cells occur in nutrient-poor media. Colonies on marine agar are circular to irregular, convex with undulate edges, and butyrous in consistency. A dark brown, melanin-like, diffusible pigment may be produced on complex growth media by some strains. NaCl is required for growth; optimal NaCl levels for growth are 2–4%. No growth with 6% NaCl. Amino acids are required for growth.

Mesophilic. Grows between 8 and 30°C; optimum temperature, approximately 25°C. Optimal pH for growth is 7.4–7.8.

Other characteristics are as given in the genus description and in Tables BXII.γ.148 and BXII.γ.151. In addition, phosphatase and deoxyribonuclease are produced. Elastin, casein, albumin, L-tyrosine, alginate, and chondroitin are not degraded. No activity detected for lecithinase or sulfatase. Gluconate oxidation, Voges–Proskauer, and chicken-cell agglutination tests are negative. Acid is not produced from carbohydrates.

Isolated from oysters.

The mol% G + C of the DNA is: 46 (T_m).

Type strain: LST-W, ATCC 39565.

Additional Remarks: ATCC 39565 is not currently available; however, a similar strain (ATCC 33888) is available from Dr. R. Weiner, University of Maryland, USA.

7. **Shewanella frigidimarina** Bowman, McCammon, Nichols, Skerratt, Rea, Nichols and McMeekin 1997b, 1045.[VP]
fri.gid.i.ma.ri′na. L. adj. *frigidus* cold; L. fem. adj. *marina* belonging to the sea; M.L. adj. *frigidimarina* belonging to the cold sea.

Cells are curved or straight rods, 0.5–0.8 × 1.0–2.5 μm. Colonies on marine agar are tan, translucent, circular, convex with entire edges, and butyrous in consistency, but becoming increasingly mucoid with prolonged incubation. NaCl is not required for growth. Can tolerate up to 9% NaCl. Growth factors are not required.

Psychrotrophic. Growth occurs between <0 and 30°C; optimum temperature, ~20–22°C.

Other characteristics are as given in the genus description and in Tables BXII.γ.148 and BXII.γ.151. In addition, strains produce deoxyribonuclease and lecithinase and hydrolyze casein. Strains may degrade L-tyrosine and give a positive Simmons citrate test. Do not hydrolyze alginate, esculin, urea, urate, or xanthine. Acid is produced from cellobiose, maltose, D-mannitol, and sucrose. Strains may produce acid from trehalose, D-galactose, D-mannose, and D-xylose. Acid is not formed from L-arabinose, L-rhamnose, lactose, D-melibiose, D-melezitose, D-raffinose, dextran, *N*-acetylglucosamine, adonitol, *meso*-inositol, D-sorbitol, or glycerol. Resistant to 0.0075% KCN and 5% ox-bile salts.

Isolated from sea ice, seawater, marine sediments, and waters and sediments of saline meromictic lakes.

The mol% G + C of the DNA is: 40–43 (T_m).

Type strain: IC-P1, ACAM 591, DSM 12253.

GenBank accession number (16S rRNA): U85903.

8. **Shewanella gelidimarina** Bowman, McCammon, Nichols, Skerratt, Rea, Nichols and McMeekin 1997b, 1045[VP]
ge.li.di.ma.ri′na. L. adj. *gelidus* ice-cold; L. fem. adj. *marina* belonging to the sea; M.L. adj. *gelidimarina* belonging to the icy sea.

Cells are curved or straight rods, 0.5–0.8 × 1.0–2.5 μm. Colonies on marine agar are light tan, opaque, circular, convex with entire edges, and butyrous in consistency. NaCl is required for growth. Can tolerate up to 6% NaCl. Growth factors are not required.

Psychrophilic. Growth occurs between <0 and 23°C in liquid media; no growth occurs at 20°C on agar media. Optimal temperature, approximately 15–17°C.

Other characteristics are as given in the genus description and in Tables BXII.γ.148 and BXII.γ.151. In addition, strains produce lecithinase and can hydrolyze casein. Strains may produce deoxyribonuclease. Simmons citrate and urease tests are negative. Unable to hydrolyze alginate, esculin, urate, or xanthine. Acid is produced oxidatively and fermentatively from *N*-acetylglucosamine and chitin, but not from other carbohydrates. Resistant to 0.0075% KCN and 5% ox-bile salts.

Isolated from sea-ice diatom blooms.

The mol% G + C of the DNA is: 40–43 (T_m).

Type strain: IC-P6, ACAM 456.

GenBank accession number (16S rRNA): U85907.

9. **Shewanella hanedai** (Jensen, Tebo, Baumann, Mandel and Nealson 1981) MacDonell and Colwell 1986, 355[VP] (Effective publication: MacDonell and Colwell 1985, 180) (*Alteromonas hanedai* Jensen, Tebo, Baumann, Mandel and Nealson 1981, 382.)
han.e′dai. M.L. gen. n. *hanedai* of Haneda; named after Y. Haneda, a Japanese biologist who pioneered studies on bioluminescence.

Cells are rod-shaped, 0.5–1.0 × 1.5–2.0 μm. Colonies on marine agar are light tan, translucent, circular, convex with entire edges, and butyrous in consistency. NaCl is required for growth. Can tolerate up to 6% NaCl. Strains may require yeast extract or vitamins for growth.

Psychrophilic. Growth occurs between 0 and 25°C; optimum temperature, approximately 15°C.

Other characteristics are as given in the genus description and in Tables BXII.γ.148 and BXII.γ.151. In addition, alginate is not hydrolyzed.

Isolated from sediments and seawater of the Arctic Ocean, Southern Ocean, and western coast of Canada.

The mol% G + C of the DNA is: 44–47 (T_m).

Type strain: ATCC 33224, CIP 103207, DSM 6066.

GenBank accession number (16S rRNA): X82132, U91590.

10. **Shewanella oneidensis** Venkateswaran, Moser, Dollhopf, Lies, Saffarini, MacGregor, Ringleberg, White, Nishijima, Sano, Burghardt, Stackebrandt and Nealson 1999, 721[VP]
o.nei.den′sis. M.L. gen. n. *oneidensis* named after Lake Oneida where the species was isolated.

Cells are rods, 0.5–0.6 × 2–3 μm. Colonies on nutrient agar are pinkish beige, circular, smooth, convex with entire edges, and butyrous in consistency. No diffusible pigments are formed. NaCl is not required for growth.

Grows between 4 and 40°C; optimum temperature, ~30°C.

Other characteristics are as given in the genus description and in Table BXII.γ.148. Can couple growth to the reduction of manganese oxide (Nealson and Saffarini, 1994) and S^0 (Moser and Nealson, 1996). Does not form indole, acetoin, alginase, arginine dihydrolase, or lysine decarboxylase. Utilizes D-galactose, but not D-fructose, citrate, D-mannitol, glycerol, D-sorbitol, DL-malate, or DL-lactate. Some strains can utilize lactose, succinate, and citrate.

Isolated from water and sediment of Lake Oneida, New York, USA and from clinical specimens.

The mol% G + C of the DNA is: 45 (T_m).

Type strain: MR-1, ATCC 700550.

GenBank accession number (16S rRNA): AF005251.

11. **Shewanella pealeana** Leonardo, Moser, Barbieri, Branstner, MacGregor, Paster, Stackebrandt and Nealson 1999, 1349[VP]
peal′le.ana. M.L. adj. *pealeana* from *peale or pealei* the species name of the squid; *Loligo pealei* from which the bacterial species was isolated.

Gram negative. Cells are rods, 2.0–3.0 × 0.4–0.6 μm. Motile with a single polar flagellum. Colonies on marine agar are circular, opaque, and salmon-colored. Requires Na^+ ions for growth with biomass produced optimally at 0.5 M NaCl. Also requires choline chloride as an essential growth factor in minimal media. Grows between 0.125–0.75

M NaCl. Growth occurs at 4–30°C. Optimum growth temperature is 25°C. Grows between pH 6–8. Optimum pH for growth is 7.0.

Chemoheterotrophic facultative anaerobe. Can grow anaerobically using nitrate, fumarate, iron, manganese, TMAO, thiosulfate, and elemental sulfur as alternative electron acceptors with lactate acting as the carbon source. Catalase, oxidase, and lipase positive. Amylase and gelatinase negative. Glucose, galactose, lactate, acetate, pyruvate, citrate, succinate, glutamate, Casamino acids, yeast extract, and peptone are used aerobically as energy sources. Fructose, glycerol, sorbitol, arabinose, formate, and ethanol are not utilized.

Isolated from the accessory nidamental glands of female adults of the squid species *Loligo pealei*.

The mol% G + C of the DNA is: 45.0 (HPLC).

Type strain: ANG-SQ1, ATCC 700345.

GenBank accession number (16S rRNA): AF011335.

12. **Shewanella violacea** Nogi, Kato and Horikoshi 1999, 341[VP] (Effective publication: Nogi, Kato and Horikoshi 1998b, 337.)
vi.o.la′ ce.a. L. gen. n. *violacea* of violet.

Cells are straight or slightly curved rods, 0.8–1.0 × 2–4 µm. Colonies on marine agar are circular, smooth, convex with entire edges, and butyrous in consistency. After 2–3 d, colonies are nonpigmented; after more than 7 d, colonies appear violet. NaCl is required for growth; optimal levels for growth are 2–3%. No growth with 6% NaCl.

Psychrophilic. Grows optimally between 4–10°C. Barophilic. Optimal pressure for growth is 30 MPa.

Other characteristics are as given in the genus description and in Table BXII.γ.148. Acid is produced from cellobiose and D-galactose, but not from DL-arabinose, D-fructose, glycerol, inositol, lactose, maltose, D-mannitol, D-mannose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, or D-xylose.

Isolated from the Ryukyu Trench, northwest Pacific Ocean, at a depth of 5110 m.

The mol% G + C of the DNA is: 47 (T_m).

Type strain: DSS12, JCM 10179.

GenBank accession number (16S rRNA): D21225.

13. **Shewanella woodyi** Makemson, Fulayfil, Landry, Van Ert, Wimpee, Widder and Case 1997, 1039[VP]
wood′ y.i. M.L. gen. n. *woodyi* of Woody, in honor of the American biologist J. Woodland Hastings.

Cells are rod-shaped, 0.4–1.0 × 1.4–2.0 µm. Colonies on marine agar are pink-orange due to the accumulation of cytochromes. NaCl is required for growth. Growth factors are not required.

Psychrophilic. Growth occurs between 4 and 25°C; optimum temperature, 20–25°C; no growth at 30°C.

Other characteristics are as given in the genus description and in Tables BXII.γ.148 and BXII.γ.151.

Isolated from squid ink, seawater and marine snow (collected from the Alboran Sea).

The mol% G + C of the DNA is: 39 (by measurement of the relative binding of DNA-binding-fluorescent dyes bisbenzimide and chromomycin A3).

Type strain: MS32, ATCC 51908, DSM 12036.

GenBank accession number (16S rRNA): AF003549.

Order XI. **"Vibrionales"**

Vib.ri.o.na′ les. M.L. masc. n. *Vibrio* type genus of the order; *-ales* ending to denote order; M.L. fem. pl. n. *Vibrionales* the order of bacteria whose circumscription is based on the genus *Vibrio*.

Description is the same as for the family *Vibrionaceae*.

Type genus: **Vibrio** Pacini 1854, 411.

Family I. **Vibrionaceae** Véron 1965, 5245[AL]

J.J. Farmer III and J. Michael Janda

Vib.ri.o.na′ ce.ae. M.L. masc. n. *Vibrio* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Vibrionaceae* the family of bacteria whose circumscription is based on the genus *Vibrio*.

Gram-negative straight or curved rods. Motile by means of polar flagella. Additional lateral flagella may be produced when grown on solid media; these differ in wavelength and antigenicity from the polar flagellum and may number from a few to over 100 flagella/cell. Do not form endospores or microcysts. Chemoorganotrophs. Facultative anaerobes, having both a respiratory and a fermentative metabolism. Oxygen is a universal electron acceptor. Do not denitrify. Most strains: are oxidase positive, reduce nitrate to nitrite, ferment D-glucose and utilize it as a sole or principal source of carbon and energy, grow in minimal media with D-glucose or other compounds as the sole source of carbon and energy and use NH_4^+ as the sole nitrogen source. A few species require vitamins and amino acids. Ferment and utilize a wide variety of simple and complex carbohydrates and utilize a wide variety of other carbon sources. Most species require Na^+ or a seawater base for growth and require 0.5–3% NaCl for optimum growth. Several species are bioluminescent; other species include a few bioluminescent strains. Primarily aquatic. Found in fresh, brackish, and sea water, often in association with aquatic animals and plants. Several species are pathogenic for humans.

M NaCl. Growth occurs at 4–30°C. Optimum growth temperature is 25°C. Grows between pH 6–8. Optimum pH for growth is 7.0.

Chemoheterotrophic facultative anaerobe. Can grow anaerobically using nitrate, fumarate, iron, manganese, TMAO, thiosulfate, and elemental sulfur as alternative electron acceptors with lactate acting as the carbon source. Catalase, oxidase, and lipase positive. Amylase and gelatinase negative. Glucose, galactose, lactate, acetate, pyruvate, citrate, succinate, glutamate, Casamino acids, yeast extract, and peptone are used aerobically as energy sources. Fructose, glycerol, sorbitol, arabinose, formate, and ethanol are not utilized.

Isolated from the accessory nidamental glands of female adults of the squid species *Loligo pealei*.

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Cells are straight or slightly curved rods, 0.8–1.0 × 2–4 µm. Colonies on marine agar are circular, smooth, convex with entire edges, and butyrous in consistency. After 2–3 d, colonies are nonpigmented; after more than 7 d, colonies appear violet. NaCl is required for growth; optimal levels for growth are 2–3%. No growth with 6% NaCl.

Psychrophilic. Grows optimally between 4–10°C. Barophilic. Optimal pressure for growth is 30 MPa.

Other characteristics are as given in the genus description and in Table BXII.γ.148. Acid is produced from cellobiose and D-galactose, but not from DL-arabinose, D-fructose, glycerol, inositol, lactose, maltose, D-mannitol, D-mannose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, or D-xylose.

Isolated from the Ryukyu Trench, northwest Pacific Ocean, at a depth of 5110 m.

The mol% G + C of the DNA is: 47 (T_m).

Type strain: DSS12, JCM 10179.

GenBank accession number (16S rRNA): D21225.

13. **Shewanella woodyi** Makemson, Fulayfil, Landry, Van Ert, Wimpee, Widder and Case 1997, 1039[VP]
wood′ y.i. M.L. gen. n. *woodyi* of Woody, in honor of the American biologist J. Woodland Hastings.

Cells are rod-shaped, 0.4–1.0 × 1.4–2.0 µm. Colonies on marine agar are pink-orange due to the accumulation of cytochromes. NaCl is required for growth. Growth factors are not required.

Psychrophilic. Growth occurs between 4 and 25°C; optimum temperature, 20–25°C; no growth at 30°C.

Other characteristics are as given in the genus description and in Tables BXII.γ.148 and BXII.γ.151.

Isolated from squid ink, seawater and marine snow (collected from the Alboran Sea).

The mol% G + C of the DNA is: 39 (by measurement of the relative binding of DNA-binding-fluorescent dyes bisbenzimide and chromomycin A3).

Type strain: MS32, ATCC 51908, DSM 12036.

GenBank accession number (16S rRNA): AF003549.

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Description is the same as for the family *Vibrionaceae*.

Type genus: **Vibrio** Pacini 1854, 411.

Family I. **Vibrionaceae** Véron 1965, 5245[AL]

J.J. Farmer III and J. Michael Janda

Vib.ri.o.na′ ce.ae. M.L. masc. n. *Vibrio* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Vibrionaceae* the family of bacteria whose circumscription is based on the genus *Vibrio*.

Gram-negative straight or curved rods. Motile by means of polar flagella. Additional lateral flagella may be produced when grown on solid media; these differ in wavelength and antigenicity from the polar flagellum and may number from a few to over 100 flagella/cell. Do not form endospores or microcysts. Chemoorganotrophs. Facultative anaerobes, having both a respiratory and a fermentative metabolism. Oxygen is a universal electron acceptor. Do not denitrify. Most strains: are oxidase positive, reduce nitrate to nitrite, ferment D-glucose and utilize it as a sole or principal source of carbon and energy, grow in minimal media with D-glucose or other compounds as the sole source of carbon and energy and use NH_4^+ as the sole nitrogen source. A few species require vitamins and amino acids. Ferment and utilize a wide variety of simple and complex carbohydrates and utilize a wide variety of other carbon sources. Most species require Na^+ or a seawater base for growth and require 0.5–3% NaCl for optimum growth. Several species are bioluminescent; other species include a few bioluminescent strains. Primarily aquatic. Found in fresh, brackish, and sea water, often in association with aquatic animals and plants. Several species are pathogenic for humans.

Other species are pathogenic for fish, eels, and other aquatic animals. The mol% G + C of the DNA is 38–51%. The family is classified in the phylum *Proteobacteria* in the class *Gammaproteobacteria*.

Type genus: **Vibrio** Pacini 1854, 411.

Historical overview A history of the family *Vibrionaceae* as it has appeared in *Bergey's Manual* is given in Table BXII.γ.152. Related families include *Enterobacteriaceae*, *Aeromonadaceae*, and *Pasteurellaceae*. The family *Vibrionaceae* has undergone intense study since the first edition of *Bergey's Manual of Systematic Bacteriology* (Krieg and Holt, 1984) was published in 1984. In their chapter on the family *Vibrionaceae* in that edition, Baumann and Schubert (1984) included the genera *Vibrio*, *Photobacterium*, *Aeromonas*, and *Plesiomonas*. These are the same four genera included in the original classification of the family proposed by Véron almost twenty years earlier (Véron, 1965). In this *Manual* *Aeromonas* and *Plesiomonas* are classified in other families (Table BXII.γ.152). For practical identification schemes, it is still useful to consider *Aeromonas* and *Plesiomonas* together with other oxidase positive genera of fermentative bacteria such as *Vibrio* and *Photobacterium* (Table BXII.γ.153). A detailed history of changes in the classification of *Vibrio* and related genera that occurred as new methods were introduced has been given by Farmer (1992). These methods include examinations of the structure, function, and regulation of proteins; comparison of mol% G + C content; DNA–DNA hybridization; rRNA–DNA hybridization; 5S rRNA cataloging and sequence comparisons; and 16S rRNA gene sequence comparisons (Fig. BXII.γ.157).

The family *Vibrionaceae* presently includes three genera:

Genus 1. *Vibrio* (the type genus)
Genus II. *Photobacterium*
Genus III. *Salinivibrio*

The type strain of the type and only species of the genus *Allomonas*, *Allomonas enterica*, is very closely related to *Vibrio fluvialis* by DNA–DNA hybridization studies and phenotypic analysis (Kalina et al., 1984). *Allomonas* and *Allomonas enterica* are not described separately in this edition of the *Manual*; the reader is referred to the description of *V. fluvialis*. The two species of the genus *Listonella*, *Listonella anguillarum* (the type species) and *Listonella pelagia*, are included in the genus *Vibrio* in this edition of the *Manual* as *Vibrio anguillarum* and *Vibrio pelagius*.*

Further descriptive information

Habitats The ecological niches of members of the family *Vibrionaceae* have been described by Campbell (1957); Baumann and Baumann (1981a); Sakazaki and Balows (1981); and Simidu and Tsukamoto (1985)). In humans, some vibrios cause diarrhea, wound infections, and occasionally other extraintestinal infections. In aquatic animals, vibrios cause wound and generalized infections. Many vibrios and related organisms are also widely distributed in aquatic environments. Many factors govern the distribution of these organisms, but the most important probably include: particular human, animal or plant hosts; inorganic nutrients and carbon sources available; temperature; salinity; dissolved oxygen; and depth below the surface for the species that are found in the ocean (Simidu and Tsukamoto, 1985). A few species are adapted to particular hosts. For example, *Vibrio cholerae* serogroup O1 is adapted to humans and is the cause of cholera, a life-threatening diarrheal disease. Recent studies have shown that the ecology of this organism is more complex than originally thought. *Photobacterium leiognathi* is usually isolated from fish in shallow tropical water, and *P. phosphoreum* is usually found in the luminous organs of fish that live at depths of 200–1200 meters (Hastings and Nealson, 1981).

Isolation Most members of the *Vibrionaceae* grow well on ordinary complex media. Samples are spread onto solid medium or diluted in an enrichment broth. NaCl concentrations of 0.5–0.85% satisfy the requirements of most species, although a few require greater concentrations of NaCl. Incubation temperatures are also important. A few species grow only at temperatures <25°C; others grow at 25°C but not at 35–37°C. General and selective media for *Vibrionaceae* are described in the chapter on the genus *Vibrio*.

Identification Methods for the isolation and identification of *Vibrio* spp. from clinical specimens and non-clinical samples are discussed in detail in the chapter describing the genus *Vibrio*. Assignment of non-clinical isolates to a species can be problematic because over 50 species of *Vibrio* and *Photobacterium* must be considered and because comparative data for these organisms are sparse relative to data available for clinically important species.

The US Centers for Disease Control and Prevention maintain computer programs and databases for the identification of isolates subjected to a battery of 45–60 phenotypic tests; for details contact the *Vibrio* Laboratory at the CDC.

These alternatives to phenotypic methods are now being used routinely and have proven extremely useful in a research setting. It will be important to evaluate the sensitivity and specificity, and to understand the advantages and disadvantages of these methods. In the United States, the reporting of cultures from human specimens is subject to specific government regulations (the Clinical Laboratory Improvement Amendments of 1988), which has limited the application of these approaches in clinical and public health laboratories.

Acknowledgments

We dedicate this chapter to M. Véron for giving us the name *Vibrionaceae* and for all his contributions to our understanding of the family, its organisms, and their close and distant relatives.

Further Reading

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Brenner, D.J., G.R. Fanning, F.W. Hickmann-Brenner, J.V. Lee, A.G. Stei-

Editorial Note: Readers are advised that *Listonella pelagia* and *Listonella anguillarum* are valid names currently in use in the literature regarding fish pathology.

TABLE BXII.γ.152. Different classifications of *Vibrio*, *Photobacterium*, *Aeromonas*, *Plesiomonas*, and related organisms in five editions of *Bergey's Manual*[a] (1957–2001)

Edition	Classification
2004 *Bergey's Manual of Systematic Bacteriology*, 2nd Ed. (See the Revised Road Map by Garrity et al., in Volume 2(A) of this *Manual*.)	Phylum BXII. *Proteobacteria* phyl. nov. Class III. *Gammaproteobacteria* class. nov. Order VIII. *Oceanospirillales* ord. nov. Family IV. *Halomonadaceae* Genus I. *Halomonas* (19 species) Order X. *Alteromonadales* ord. nov. Family I. *Alteromonadaceae* Genus III. *Colwellia* (7 species) Genus X. *Moritella* (4 species) Genus XII. *Shewanella* (13 species) Order XI. *"Vibrionales"* Family I. *Vibrionaceae* Genus I. *Vibrio* (Type genus; 44 species) Genus II. *Photobacterium* (6 species) Genus III. *Salinivibrio* (1 species) Order XII. *Aeromonadales* ord. nov. Family I. *Aeromonadaceae* Genus I. *Aeromonas* (Type genus) (14 species) Order XIII. *"Enterobacteriales"* Family I. *Enterobacteriaceae* Genus XXVII. *Plesiomonas* (1 species)
1994 *Bergey's Manual of Determinative Bacteriology*, 9th Ed. (Holt et al., 1994)	Group 4: Gram-negative aerobic/microaerophilic rods and cocci Genus *Halomonas* Group 5: Facultative anaerobic Gram-negative rods Subgroup 2, Family *Vibrionaceae* Genus *Vibrio* (Type genus) Genus *Aeromonas* Genus *Enhydrobacter* Genus *Photobacterium* Genus *Plesiomonas*
1984 *Bergey's Manual of Systematic Bacteriology*, 1st Ed. (Krieg and Holt, 1984)	Section 5: Facultative anaerobic Gram-negative rods Family II. *Vibrionaceae* Genus I. *Vibrio* (Type genus) Genus II. *Photobacterium* Genus III. *Aeromonas* Genus IV. *Plesiomonas*
1974 *Bergey's Manual of Determinative Bacteriology*, 8th Ed. (Buchanan and Gibbons, 1974)	Part VIII. Gram-negative facultatively anaerobic rods Family II. *Vibrionaceae* Genus I. *Vibrio* (Type genus) Genus II. *Aeromonas* Genus III. *Plesiomonas* Genus IV. *Photobacterium* Genus V. *Lucibacterium*
1957 *Bergey's Manual of Determinative Bacteriology*, 7th Ed. (Breed et al., 1957)	Class II. *Schizomycetes* Order I. *Pseudomonadales* Family IV. *Pseudomonadaceae* Genus IV. *Aeromonas*[a] Genus V. *Photobacterium*[a] Genus IX. *Alginomonas*[a] Family VII. *Spirillaceae* Genus I. *Vibrio*[a] Order IV. *Eubacteriales* Family III. *Achromobacteriaceae* Genus IV. *Agarbacterium*[a] Genus V. *Beneckea*[a] Family IV. *Enterobacteriaceae* Genus V. *Alginobacter*[a]

[a]These seven genera contain species or strains that (probably) would be classified in the family *Vibrionaceae* as defined by Véron (1965). The genus *Plesiomonas* is not listed because it was not named until 1962 (Habs and Schubert, 1962). *"Pseudomonas shigelloides"* Bader 1954 (now *Plesiomonas shigelloides*) was not listed in the 1957 edition.

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TABLE BXII.γ.153. Properties of the four genera originally classified in the family *Vibrionaceae* by Véron (1965); comparison with *Enterobacteriaceae*

Test or property[a]	*Vibrio*	*Photobacterium*	*Aeromonas*	*Plesiomonas*	*Enterobacteriaceae*
Associated with diarrhea and extra-intestinal infections in humans	+		+	+	+
Mol% G + C content of DNA	38–51	40–44	57–63	51	38–60
Sheathed polar flagellum	+				
Peritrichous flagella when grown in liquid media					+
Accumulate poly-β-hydroxybutyrate, but do not utilize β-hydroxybutyrate		+			
Na^+ is required for growth or stimulates growth	+	+			
Sensitive to the vibriostatic compound 0/129	+	+		+	
Lipase production	+	d	+		d
D-Mannitol fermentation	+		+		+
Enterobacterial common antigen (eca)				+	+

[a]Symbols: +, trait is present; d, trait varies among species; blank, data not available. These are properties of most species of the genera and most strains of the species, but there are exceptions. Adapted from Baumann and Schubert (1984).

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Genus I. **Vibrio** *Pacini 1854, 411*[AL]

J.J. Farmer III, J. Michael Janda, Frances W. Brenner, Daniel N. Cameron and Karen M. Birkhead

Vib′ ri.o. L. v. *vibrio* move rapidly back and forth, vibrate; M.L. masc. n. *Vibrio* the vibrating, darting organism.

Small, straight, slightly curved, curved, or comma-shaped rods, 0.5–0.8 × 1.4–2.6 μm. Involution forms often occur in old cultures and are formed under adverse growth conditions. Do not form endospores or microcysts. Gram negative. In liquid media, motile by monotrichous or multitrichous **polar flagella enclosed in a sheath** continuous with the outer membrane of the cell wall. **On solid media, some species synthesize numerous lateral flagella** with a wavelength shorter than that of the sheathed polar flagellum. **Facultative anaerobes** capable of both **fermentative and respiratory metabolism**. Molecular oxygen is a universal electron acceptor. Most do not denitrify or fix molecular nitrogen. All are chemoorganotrophs; most are able to **grow in a mineral medium containing D-glucose as the sole carbon source and NH^+ as the sole nitrogen source**. A few strains have organic growth factor requirements. **Na_4^+ stimulates growth of all species and is an absolute requirement for most**; the minimal concentration necessary for optimal growth ranges from 5 to 700 mM (0.029–4.1%). Most species grow well in media containing a seawater base. **All ferment D-glucose producing acid but rarely gas; several species produce acetoin and acetyl methyl carbinol (positive Voges–Proskauer test)**. Most ferment and utilize D-fructose, maltose, and glycerol; are **oxidase positive and reduce nitrate to nitrite**. Several grow at 4°C; all grow at 20°C; most grow at 30°C; many grow at 35–37°C. **A few species are bioluminescent**, as are a few strains of normally nonluminescent species. **Primarily aquatic**; species distribution is usually dependent on Na^+ and nutrient content of the water as well as its temperature. Very common in marine and estuarine environments and on the surfaces and in the intestinal contents of marine animals. Species with a low Na^+ requirement are also found in freshwater habitats. Twelve species occur in human clinical specimens; **11 of these are apparently pathogenic for humans, causing diarrhea or extraintestinal infections. Several species cause diseases of other vertebrates and invertebrates**.

The mol% G + C of the DNA is: 38–51.

Type species: **Vibrio cholerae** Pacini 1854, 411.

Further descriptive information

Cell morphology The cell morphology of *Vibrio cholerae* and related organisms has been studied extensively since *V. cholerae* was first depicted by Robert Koch. Examples of cell morphology, including cytoplasmic inclusions and cell surface features such as tubular appendages and "blebs", are shown in the section below describing flagella and in figures accompanying the species descriptions. Vibrios commonly appear as small, straight, slightly curved, curved or comma-shaped rods 0.5–0.8 × 1.4–2.6 μm in size. The amount of curvature is generally more pronounced in early stationary phase in liquid media than during exponential growth (Baumann et al., 1984b). However, considerable pleomorphism can occur. Some marine vibrios naturally have significant morphologic variation when grown in broth, and irregularly shaped bacilli co-exist with "involuted cells" that resemble L-forms (Onarheim et al., 1994). This phenomenon often occurs in stationary phase cultures (Farmer and Hickman-Brenner, 1992). Other halophilic species, including those associated with human disease, may form spherical or thin elongated forms in response to growth conditions containing suboptimal electrolyte (NaCl) concentrations. When vibrios are deprived of nutrients, a series of morphologic changes occurs over 1–7 days. Early events in this morphogenesis include a decreasing cell diameter

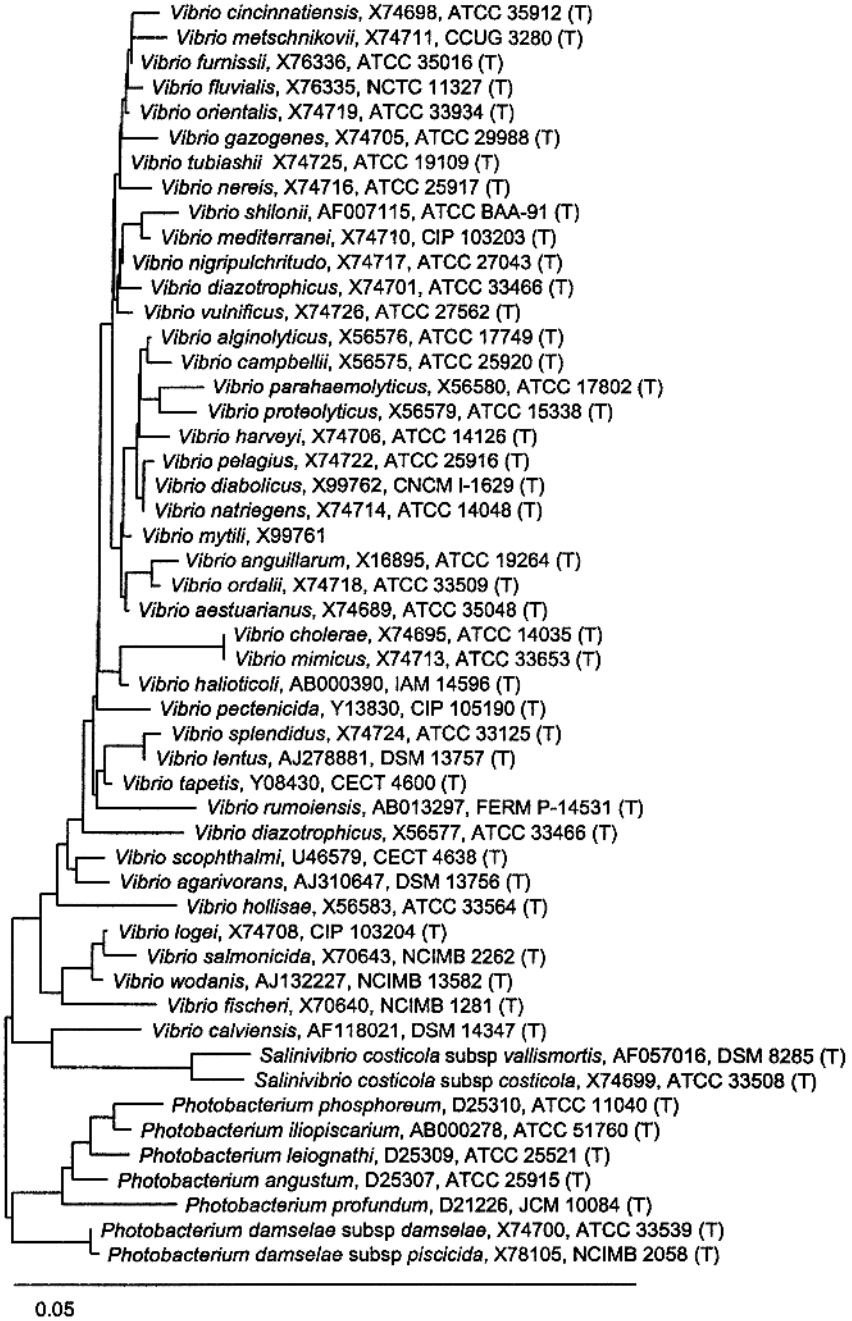

FIGURE BXII.γ.157. Relationship of most of the species of *Vibrio* and relatives based on 16S rRNA gene sequences.* Only the type strain of each species was included. The distances in the tree were calculated using 1101 positions (the least-squares method, Jukes-Cantor model). (Courtesy T. Lilburn of the Ribosomal Database Project.)

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*. *Vibrio pelagius* and *Vibrio anguillarum* are synonyms of *Listonella pelagia* and *Listonella anguillarum*, respectively.

and volume (15–67%) and a conversion of rods into coccal forms called spherical ultramicrocells (Holmquist and Kjelleberg, 1993; Kondo et al., 1994; Nelson et al., 1997). As the length of nutrient starvation increases, cytoplasmic inclusions and granules disappear, cell cultivability decreases, and the nuclear region becomes compressed (Hood et al., 1986). There are also noticeable differences in the integrity of the outer membrane and cell wall. Some changes may be linked to specific nutrient starvation (for example, nitrogen starvation produces long filaments and phosphorus starvation produces swollen large rods), whereas others occur regardless of the type of nutritional stress (Holmquist and Kjelleberg, 1993). "Non-culturable" *V. cholerae* O1 strains produced in response to nutrient deprivation display a number of ultrastructural changes, which include an undulating outer membrane, a surface layer of fine fibers, and a thicker peptidoglycan layer (Kondo et al., 1994).

Poly-β-hydroxybutyrate granules (PHB) can be found in a number of *Vibrio* species, including *V. cholerae* O1 and O139 and *V. harveyi* (Hood et al., 1986; Sun et al., 1994; Finkelstein et al., 1997). In *V. cholerae*, accumulation of PHB appears to be related to colonial opacity and growth on glycerol-containing media (Finkelstein et al., 1997). In *V. harveyi*, PHB accumulation is dependent on cell density and is controlled by the autoinducer, *N*-(3-hydroxybutanoyl) homoserine lactone (Sun et al., 1994). Other kinds of granules can be found in vibrios, including electron dense lipoid particles and electron translucent inclusions of unknown composition (Sun et al., 1994; Finkelstein et al., 1997).

Cell wall composition Vibrios contain the same three lipopolysaccharide (LPS) elements found in other Gram-negative bacteria: lipid A, core polysaccharide, and an O polysaccharide side chain that determines serological specificity. The most extensive work on biochemical characterization of *Vibrio* LPS has been done on *V. cholerae*. The lipid A portion consists of a β(1′-6)-linked glucosamine disaccharide backbone with two phosphoryl groups (Janda, 1998). Pyrophosphorylethanolamine is linked to one of these phosphoryl groups at the C-1 position of the reducing sugar, and a phosphate group ester is bound to the nonreducing glucosamine residue (Manning et al., 1994). Three fatty acids are ester linked at hydroxyl positions to this disaccharide backbone: tetradecanoic acid ($C_{14:0}$), hexadecanoic acid ($C_{16:0}$), and 3-hydroxydodecanoic acid ($C_{12:0\ 3OH}$). A fourth, 3-hydroxytetradecanoic acid ($C_{14:0\ 3OH}$), is connected to the backbone by an amide bond.

The core oligosaccharide region of *V. cholerae* contains KDO (keto-3-deoxy-D-mannose-octulosonic acid), D-glucose, heptose (L-glycero-D-manno-heptose), D-fructose, and ethanolamine phosphate (Manning et al., 1994). KDO, a normal constituent of the core oligosaccharide of enteric LPS, was originally thought to be absent in *Vibrio* species. However, when conventional periodate-thiobarbituric acid tests were replaced by strong acid hydrolysates, KDO was detected in *Vibrio* (Janda, 1998). The KDO molecule of *V. cholerae* differs in several aspects from those of enteric bacteria such as *Escherichia coli* and the genus *Salmonella*: only a single KDO molecule has been detected in the core oligosaccharide of *V. cholerae*, and the KDO moiety is phosphorylated at the C4 position (Kondo et al., 1990; Manning et al., 1994). The C5 position binds to a distal portion of the core region (heptose) similar to the KDO-C5 binding of L-glycero-D-manno-heptose (Janda, 1998). The other sugars form the remaining portion of the core oligosaccharide region and often contain additional sugar substitutions at various positions.

The *O*-polysaccharide side chain of *V. cholerae* O1 is a homopolymer of D-perosamine (4,6-dideoxy-D-mannose) approximately 17–18 units in length (Manning et al., 1994; Knirel et al., 1997). The amino groups of perosamine units are commonly acetylated with 3-deoxy-L-glycero-tetronic acid. Another compound, quinovosamine, is thought to be a "capping sugar" on either the distal or the proximal end of the O antigen (Manning et al., 1994). An unusual sugar, 4-amino-4,6-dideoxy-2-*O*-methylmannose is present only in the LPS of serogroup Ogawa and may have a role in serological specificity (Itoh et al., 1994).

The LPS composition of *V. cholerae* O139—a second serotype capable of causing pandemic cholera—is remarkably similar to that of O1 (Hisatsune et al., 1993; Isshiki et al., 1996). The lipid A moieties of O1 and O139, including fatty acid substitutions, appear to be identical (Hisatsune et al., 1993). The core oligosaccharide region contains two subtle differences: the presence of 2-aminoethyl phosphate, which is the *O*-acetyl group, and the presence of a second fructose molecule (Knirel et al., 1997). The most profound differences between O-groups 1 and 139 occur in the *O*-polysaccharide side chain. Unlike serogroup O1, which has long *O*-polysaccharide side chains, *V. cholerae* O139 has a short chain LPS similar to "SR strains" (Knirel et al., 1997). These truncated side chains migrate with the core oligosaccharide-lipid A fraction in LPS SDS-PAGE gels (Waldor et al., 1994). Classic "ladder-like" profiles of silver stained LPS side chains in SDS-PAGE gels are absent in O139 strains (Hisatsune et al., 1993; Nandy et al., 1995). Perosamine, the main component of the O1 side chain, is also absent in O139 strains (Hisatsune et al., 1993); instead, the unique sugar colitose (3,6-dideoxy-L-galactose)—which is not found in any other *Vibrio* species—is the main side chain subunit in O139 strains (Hisatsune et al., 1993). The abbreviated *O*-polysaccharide side chain of *V. cholerae* O139 appears to be a hexasaccharide containing colitose residues and a cyclic phosphate group (Knirel et al., 1997). The LPS of other *Vibrio* species is similar in many aspects to that of O139. KDO-phosphate has been detected in *V. parahaemolyticus* by gas chromatography-mass spectrometry analysis (Janda, 1998). The O-polysaccharide side chains of *Vibrio* species produce only a single fast-migrating band on silver-stained SDS-PAGE gels (Amaro et al., 1992; Iguchi et al., 1995). This result suggests that the side chains are short; a chain length of ≤10 monosaccharides has been proposed for *V. parahaemolyticus* (Iguchi et al., 1995). Some species however (e.g., *V. vulnificus*) may exhibit ladder-like patterns by immunoblotting with whole cell antisera; this result suggests that the LPS *O*-polysaccharide side chains are acidic. Lambert et al. (1983) studied the cellular fatty acids of most of the *Vibrionaceae* and postulated that differences among the *Vibrio* species might prove useful for identification.

Flagella Two types of flagella are synthesized by vibrios in different environments. In liquid culture, swimmer cells predominate due to production of a single sheathed polar flagellum in most species (Figs. BXII.γ.158 and BXII.γ.159). The sheath is an extension of the outer membrane (Fig. BXII.γ.160). The polar flagella are 24–30 nm in diameter with a central core 14–16 nm in thickness with a wavelength of 1.4–1.8 μm (Baumann et al., 1984b; Janda, 1998). Some *Vibrio* species (e.g., *V. harveyi*, *V. fischeri*, *V. logei*, and *V. salmonicida*) produce tufts (3–12) of polar flagella (Fig. BXII.γ.161) with a wavelength of approximately 3.6 μm (Baumann et al., 1984b; Ishimaru et al., 1996). Polar flagella provide chemotactic motility in liquid media and derive their energy from the sodium membrane potential (McCarter, 1995). In some marine vibrios (e. g., *V. anguillarum*), the polar flagellum appears critical for disease production in estuarine fish (Milton et al., 1996; O' Toole et al., 1996). When vibrios come into contact with solid surfaces, a series of morphogenetic changes are initiated that result in the conversion of swimmer cells into swarmer cells in some marine species such as *V. parahaemolyticus*, *V. alginolyticus*, *V. diabolicus*, and *V. pectenicida* (Raguénès et al., 1997a; Lambert et al., 1998). During this process, cell septation ceases, the cells elongate from 1 to 30 μm, and numerous lateral flagella are formed (Fig. BXII.γ.162) (McCarter and Silverman, 1990). These lateral flagella, 14–15 nm in diameter with a wavelength of 0.9 μm, are distinct from polar flagella. They are unsheathed, have a different protein subunit composition, and are internally driven by the protonmotive force (Baumann et al., 1984b; McCarter, 1995). Formation of lateral flagella permits swarmer

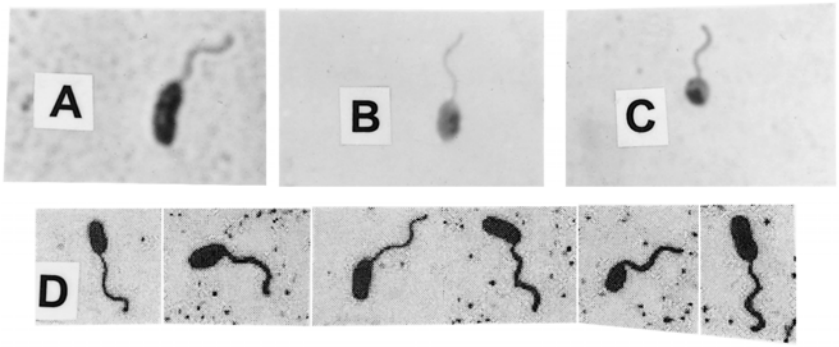

FIGURE BXII.γ.158. Leifson flagella stain of *Vibrio cholerae.* (Source: CDC archive, courtesy of Ed Ewing.)

FIGURE BXII.γ.159. Electron micrograph of *Vibrio alginolyticus* grown in liquid medium. Note the sheathed polar flagellum and absence of peritrichous flagella. Shadowed preparation. × 13,000. (Reproduced with permission from C. Golten and W.A. Scheffers, Netherlands Journal of Sea Research *9:* 351–364, 1975, ©Netherlands Institute for Sea Research.)

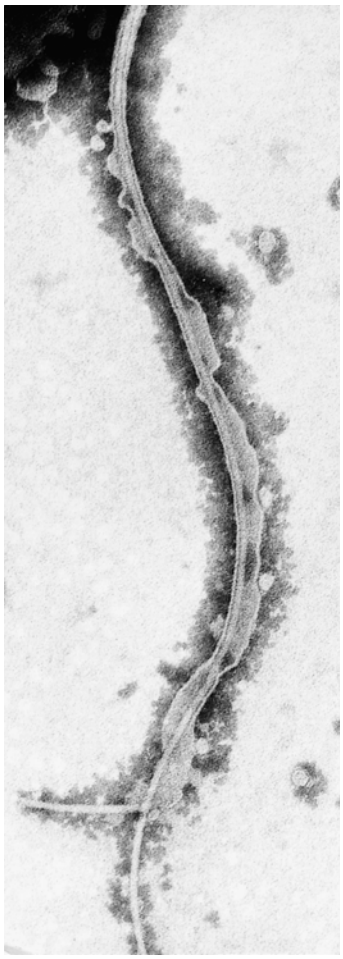

FIGURE BXII.γ.160. Electron micrograph of a polar flagellum of *Vibrio alginolyticus.* Note that the sheath has partially disintegrated exposing the inner core. Negatively stained preparation. × 30,000. (Courtesy of R.D. Allen.)

migration across solid surfaces and results in progressive spreading of the bacterial colony (McCarter and Silverman, 1990), a phenomenon called swarming. Swarming in many vibrio species is dependent upon a number of factors including agar concentration, media composition, iron availability, temperature, and relative viscosity (Baumann et al., 1984b; McCarter and Silverman, 1990). The microscopic morphology of vibrio cells removed from different concentric zones of swarming has been studied in some *Vibrio* strains (Sar and Rosenberg, 1989). Innermost zones consist of irregular cells including bent rods that progressively evolve into short rods and then into large rods with bundles of detached flagella (Fig.BXII.γ.163), whereas cells in

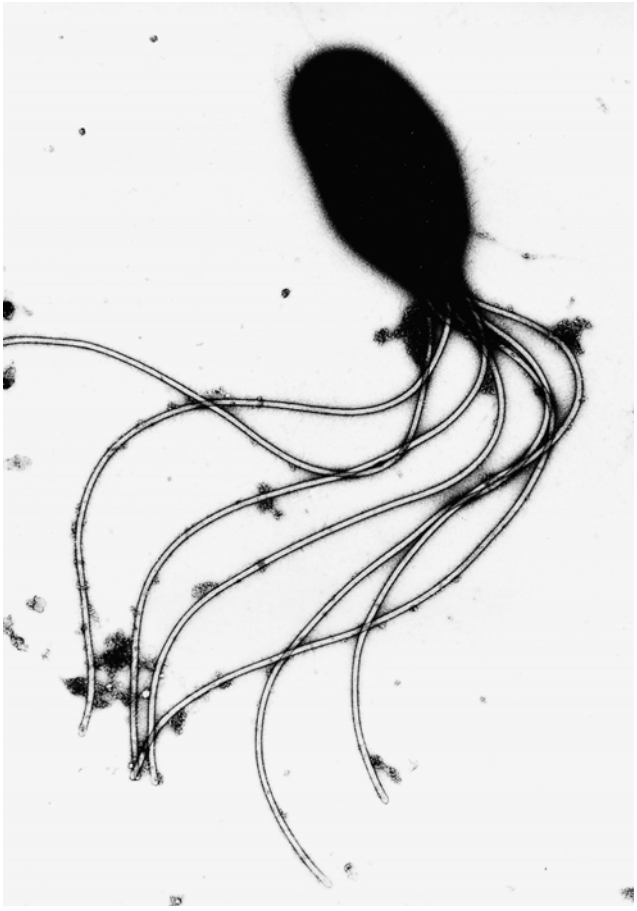

FIGURE BXII.γ.161. Electron micrograph of *Vibrio fischeri*. Note the tufts of sheathed polar flagella. Negatively stained preparation. × 23,000. (Reproduced with permission from: J.L. Reichelt and P. Baumann, Archives of Mikrobiology *94:* 283–330, 1973, ©Springer-Verlag, Berlin.)

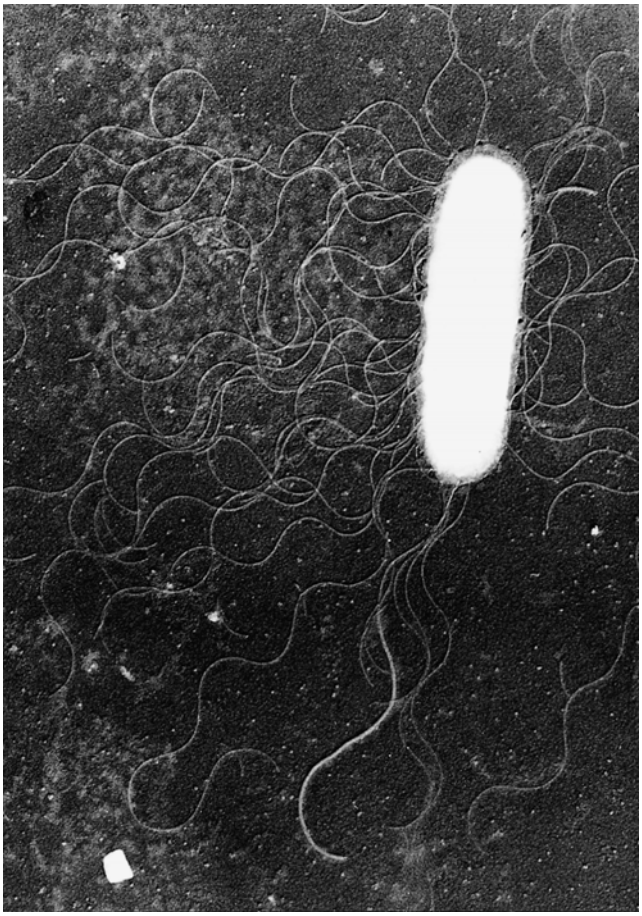

FIGURE BXII.γ.162. Electron micrograph of *Vibrio alginolyticus* grown on solid medium. Note the thick, sheathed flagellum and numerous unsheathed lateral flagella. Shadowed preparation. × 18,000. (Reproduced with permission from W.E. de Boer et al., Netherlands Journal of Sea Research *9:* 197–213, 1975, ©Netherlands Institute for Sea Research.)

the outermost circles of swarming colonies consist of long filamentous forms.

Fimbriae Fimbriae are produced by a number of pathogenic vibrios such as *V. cholerae* O1 and non-O1, *V. parahaemolyticus,* and *V. vulnificus* (Hall et al., 1988; Honda et al., 1988; Gander and LaRocco, 1989; Nakasone and Iwanaga, 1990). Several different morphologic types of fimbriae have been described in *V. cholerae* O1. These include both wavy pili 3 nm in diameter and rigid filaments 5–6 nm wide and 180–800 nm in length (Hall et al., 1988). The most important of these pili is composed of the protein TcpA; these pili are 5–6 nm wide and form bundles of parallel undulating filaments up to 15 μm long (Hall et al., 1988). TcpA formation is coregulated with cholera toxin expression and is a key determinant of *in vivo* colonization. The gene encoding TcpA appears to reside on a pathogenicity island.

Capsules Capsules have been detected surrounding cells of strains of *V. cholerae* O139 and *V. vulnificus* strains with a variety of staining techniques such as uranyl acetate, polycationic ferritin, and ruthenium red (Janda, 1998). The *V. vulnificus* polysaccharide capsule is 60 nm thick and has a low electron density (Amako et al., 1984; Hayat et al., 1993). The carbohydrate composition of the capsule of *V. vulnificus* varies from strain to strain. Sugars detected in different isolates include α-*N*-acetyl quinovosamine, α-*N*-acetyl galactosamine uronic acid, rhamnosamine, and fucosamine (Hayat et al., 1993).

Colonial morphology Most vibrios grow well on a variety of media, including protein-based agars and marine and seawater media, if sufficient Na^+ is present (Baumann et al., 1984b; Farmer and Hickman-Brenner, 1992). On most selective media, vibrios appear as smooth, buff-to-cream-colored colonies 2–5 mm in diameter, with an entire margin after overnight incubation (Baumann et al., 1984b; Janda, 1998). Some species tend to produce grayish colonies, particularly on blood agar. Considerable variation in colonial morphology has been reported for some species, and this is best demonstrated by observing colonies with a dissecting microscope at 10–25 magnification with oblique lighting. *V. cholerae* strains can have several different colonial morphologies (smooth, rough, and rugose forms) in response to different growth conditions. Rugose colonies are often chlorine-resistant. They are usually found in older cultures and are composed of an amorphous intercellular matrix of aggregated bacteria and exopolysaccharide material (Morris et al., 1996). Formation of rugose colonies can be enhanced by growth in enrichment broths such as alkaline peptone water (APW) or by picking the growth that has migrated up the sides of a culture tube. They can largely be avoided by picking a smooth colony and freezing it. In addition to these colony types, several path-

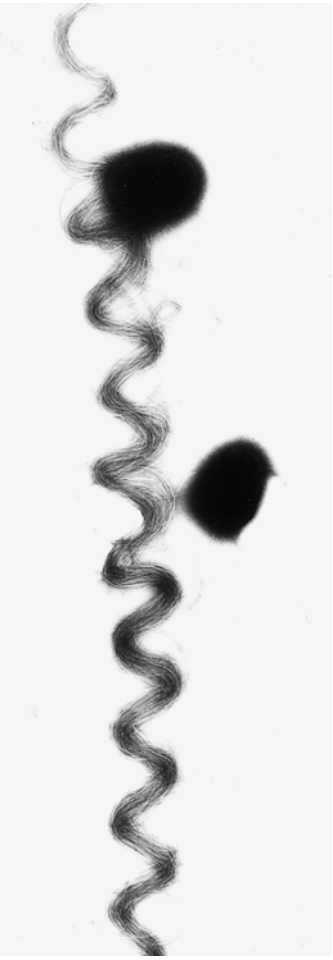

FIGURE BXII.γ.163. Large bundle of flagella in a culture of *Vibrio harveyi*. These bundles are frequently observed in cultures grown on solid medium. Negatively stained preparation. × 13,000. (Courtesy of R.D. Allen.)

ogenic vibrios, including *V. cholerae* and *V. vulnificus*, produce opaque and translucent varieties of smooth colonies on common media such as heart infusion and meat extract agars (Simpson et al., 1987; Finkelstein et al., 1992, 1997). Cells from colonies of these different morphologies differ from each other in a number of characteristics, including encapsulation, cell surface composition, cellular metabolism, and ability to survive under adverse conditions.

Pigmentation Several *Vibrio* species produce pigmented colonies. *V. nigripulchritudo* produces an insoluble blue-black pigment that accumulates in a crystalline form within the colonies (Baumann et al., 1984b). Other *Vibrio* species also produce blue-black crystals under various growth conditions, but typically do not produce the characteristic blue-black colonies of *V. nigripulchritudo*. Similar blue-black colonies are produced by a few strains of *Kluyvera* (Farmer et al., 1981a). Other pigmented species include *V. gazogenes* (red) and *V. fischeri* and *V. logei* (yellow-orange). A few strains of *V. cholerae* produce a brown diffusible melanin-like pigment (Ivins and Holmes, 1980).

Life cycles The marine environment is the natural habitat of vibrios, and the life cycle of these organisms is probably quite complex. A model for the life of *V. cholerae* in Gulf Coast estuaries has been proposed (Hood et al., 1984). Depending on nutrient scarcity and the density/availability of particulate matter, *V. cholerae* can exist in several states. An epibiotic form attached to plankton predominates during periods of relatively high nutrient/particulate matter concentrations; this form changes to a microvibrio form (small rounded cells) during times of nutrient and particulate deprivation (Hood et al., 1984; Janda, 1998). This latter form may be analogous to the "viable but nonculturable state" (Colwell, 1984) that has been described for many *Vibrio* species including *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. anguillarum*, *V. campbellii*, *V. harveyi*, and *V. fischeri* during colder seasons of the year (Oliver, 1995). The microvibrio and "nonculturable" stages may be dormant phases for vibrios in winter from which subsequent blooms are triggered in response to increasing temperatures in spring and summer. However, it is still unclear whether any dormant state actually exists and whether blooms are due to the growth of a small number of cultivable cells (present at all times) or the actual resuscitation of dormant cells (Ravel et al., 1995; Bogosian et al., 1998).

Nutrition and growth conditions *Vibrio* species vary in their nutrition and growth requirements. The most important feature is that Na^+ is required for or stimulates growth. Minimum concentrations of Na^+ required for optimal growth (Fig. BXII.γ.164) range from 5–15 mM (0.029–0.087%) for *V. cholerae* and *V. metschnikovii* to 600–700 mM (3.5–4.1%) for *Salinivibrio costicola* (Baumann et al., 1984b). Most species grow well in solid or liquid media containing 0.5–2% NaCl. Some species (*Photobacterium iliopiscarium*) form bacterial aggregates in broth culture containing 2% NaCl (Onarheim et al., 1994).

The "salt requirement" of a strain will often depend on the test conditions. The main variables are temperature, the growth medium used prior to testing, the suspending medium, and the testing medium. All *Vibrio* species except *V. cholerae* and *V. mimicus* have an absolute requirement for Na^+ (Fig. BXII.γ.164). In some instances, this requirement may be partially offset by concentrations of Mg^{2+} or Ca^{2+} similar to those normally present in seawater (Baumann et al., 1984b). However, most species exhibit a specific requirement for Na^+ (Pujalte and Garay, 1986; Borrego et al., 1996). The range and optimum concentrations of NaCl supporting growth of some of the more recently described *Vibrio* species are listed in Table BXII.γ.154. No single medium or NaCl concentration is optimal for the recovery or growth of all *Vibrio* species. Many vibrios will grow in mildly alkaline conditions. Although most species prefer a pH range of 7–8 (Raguénès et al., 1997a), some species, including *V. cholerae* and *V. metschnikovii*, will even grow at a pH of 10 (Baumann et al., 1984b).

Vibrios also vary in their temperature requirements for growth. Almost all *Vibrio* species grow well at 18–22°C. Some will grow at 0–4°C, whereas others can grow at temperatures up to 45°C. The temperature at which vibrios can grow is also dependent upon other factors including the composition of the medium and the NaCl concentration (Onarheim et al., 1994).

Most *Vibrio* species do not require specific organic growth factors such as vitamins or amino acids, although amino acid supplementation may be required to revive some strains stored for prolonged periods (Baumann et al., 1984b). Complex nutrients are required to induce growth of some species (Baumann et al., 1984b; Raguénès et al., 1997a). Such required supplements include yeast extract (for *V. anguillarum*, *Moritella marina*, and *V. logei*) and a seawater base (for *V. diabolicus*).

Vibrios use a variety of compounds as carbon and energy

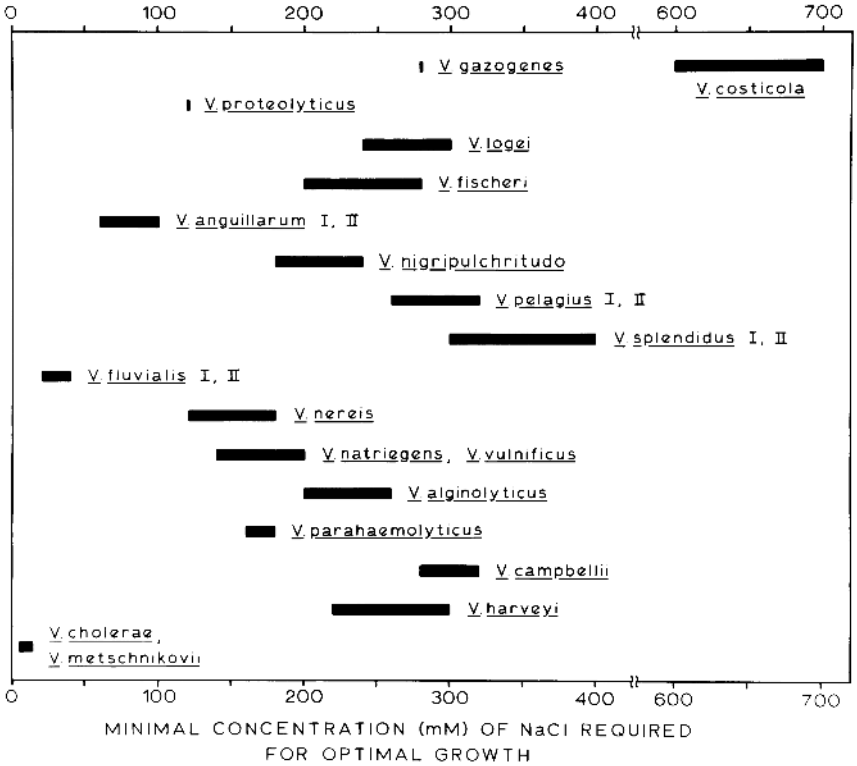

FIGURE BXII.γ.164. Effect of Na^+ (NaCl) on the growth of *Vibrio* species. Data from Baumann et al., 1980b.

TABLE BXII.γ.154. Growth temperatures, electrolyte optima, and nutritional versatility of 10 recently described *Vibrio* species and *Photobacterium iliopiscarium*[a]

Characteristic	*V. diabolicus*	*V. halioticoli*	*V. ichthyoenteri*	*V. mediterranei*	*V. mytili*	*V. navarrensis*	*V. penaeicida*	*V. scophthalmi*	*V. tapetis*	*V. trachuri*	*Photobacterium iliopiscarium*
Temperature range, °C	20–45	10–30	15–30	18–35	10-37	10–42	20–30	22–35	4–22		4–22
Temperature optima, °C	35–40				~28	30–37		22–30	18	~30	~20
NaCl range (%)	1–5		1–6	1.5–6	1–10	0.5-7	1–5	0.5–5	1–3	3–7	0.5–4
NaCl optima (%)	2.5–3.0	3						1–3			2
Nutritional versatility[b]	15/50	7/35	1/13	29/47	43/95	24/87	6/15	5/49	8/47		6/40

[a]Blank space, data not available. Data compiled from Urdaci et al. (1991); Pujalte et al. (1993); Onarheim et al. (1994); Iwamoto et al. (1995); Borrego et al. (1996); Ishimaru et al. (1996); Cerdà-Cuéllar et al. (1997); Sawabe et al. (1998b).

[b]Nutritional versatility is the number of carbon compounds used as a sole energy source/number of compounds tested.

sources. Some of the newly described environmental species (*V. ichthyoenteri*, *V. scophthalmi*) and related organisms (*Photobacterium iliopiscarium*) can only utilize a small number of substrates (<10 compounds) as sole carbon or energy sources (Table BXII.γ.154), while other species utilize over 40 compounds. The carbohydrates utilized include polysaccharides, monosaccharides, disaccharides, and polyhydroxyl alcohols. Vibrios produce a variety of extracellular enzymes that degrade DNA, RNA, proteins, lipids, lecithin, mucin, chitin, agar, alginic acid, starch, and related compounds. They also produce biologically active molecules such as hemolysins, and other toxins.

Physiology and metabolism Vibrios and photobacteria are chemoorganotrophic, facultatively anaerobic bacteria that produce several different acids during fermentation. Common metabolic end products of D-glucose metabolism include formic, acetic, lactic, succinic, and pyruvic acids (Baumann et al., 1984b). Most vibrios ferment sugars without visible gas production ("anaerogenic" fermentation), although some species such as *V. furnissii*, *V. gazogenes*, *V. mytili*, *Photobacterium iliopiscarium*, and a few strains of *Vibrio damsela* produce CO_2 and H_2 as fermentation products. D-Glucose is transported into cells and phosphorylated to glucose-6-phosphate by the phosphoenolpyruvate:carbohydrate phosphotransferase (PTS) system (Postma et al., 1993). A number of other sugars are transported by the glucose–PTS system in *Vibrio* species including trehalose, D-fructose, D-mannose, and D-mannitol (Postma et al., 1993; Sarker et al., 1994). In *V. parahaemolyticus* the glucose–PTS is constitutive or partially inducible. A second mechanism for glucose transport in *V. parahaemolyticus* has been recently identified (Sarker et al., 1994). This Na^+/glucose symport system is stimulated by Na^+ and to a lesser extent by Li^+. D-Galactose, α-D-fucose, and methyl-α-glucosidase also appear to be transported by this mechanism, whereas D-glucose partially represses this Na^+/glucose symport

system. A number of aromatic compounds are degraded by the *meta*-cleavage of the intermediate protocatechuate by the α-ketoacid pathway (Baumann et al., 1984b). All *Vibrio* species except *V. metschnikovii* and *V. gazogenes* are oxidase positive; possession of this trait is correlated with the presence of cytochrome *c* systems (Baumann et al., 1984b). Cytochromes *b*, *c*, *d*, *o*, and a_1 occur in *V. cholerae*, *V. anguillarum*, *V. natriegens*, and *V. alginolyticus*. A number of different *c*-type cytochromes (*c4*, *c5*) have been shown to be present in some species based upon data from *N*-terminal amino acid sequencing of proteins and spectral analysis (Petushkov and Lee, 1997). *V. metschnikovii*, which is oxidase negative, lacks *c*-type cytochromes (Baumann et al., 1984b). *V. alginolyticus* possesses an ubiquinol oxidase of the cytochrome *bo*-type, which may function as a proton pump in the respiratory chain (Miyoshi-Akiyama et al., 1993).

Vibrios can acquire iron for growth by producing iron chelating molecules termed siderophores. Many *Vibrio* species including *V. cholerae*, *V. anguillarum*, *V. parahaemolyticus*, *V. fluvialis*, and *V. mediterranei* produce catechol-like siderophores called vibriobactins (Amaro et al., 1990; Guerinot, 1994). Less often, some *V. cholerae* non-O1, *V. parahaemolyticus*, and *V. fluvialis* strains produce a second hydroxymate-type siderophore. In *V. mimicus* and *V. hollisae* this hydroxymate-type siderophore has been identified as aerobactin (Okujo and Yamamoto, 1994). In *V. anguillarum*, a siderophore, called anguibactin is produced; anguibactin has both catechol and hydroxymate moieties (Guerinot, 1994). In addition to these iron chelators, vibrios can acquire iron by non-siderophore-dependent mechanisms. Ferric citrate can be actively transported into the cell by some vibrios via a non-inducible energy-dependent system (Mazoy et al., 1997). A number of other compounds can also serve as sole iron sources; these compounds include ferrichrome, hemoglobin, and saturated ferritin and transferrin (Simpson and Oliver, 1987; Fouz et al., 1994; Guerinot, 1994).

Several *Vibrio* species have a constitutive arginine-dihydrolase system. All *Vibrio* species produce a single iron-containing superoxide dismutase (Baumann et al., 1984b). The first cysteine protease in a *Vibrio* species has recently been identified in a luminous strain of *V. harveyi* isolated from a diseased tiger prawn (Liu et al., 1997c).

Bioluminescence A striking property of some *Vibrio* strains is the ability to emit yellow to blue-green light in response to certain environmental triggers. Bioluminescent marine species include *V. fischeri*, *V. harveyi*, *V. logei*, and *V. splendidus*. Light is produced as a consequence of the oxidation of the reduced flavin mononucleotide $FMNH_2$ and long-chain aliphatic aldehydes (Hastings and Nealson, 1977). The enzyme catalyzing this reaction is luciferase, an 80,000 MW heterodimer composed of an α (42,000 MW) and a β (38,000 MW) subunit; the α subunit contains the site of catalytic activity (Baumann et al., 1984b). Considerable *N*-terminal amino acid sequence homology exists (57–71%) between the α and β subunits of different luminescent species. At least 35–40% homology also exists between heterologous subunits; this fact suggests that these sequences originally arose from the duplication of a common ancestral gene (Baumann et al., 1984b).

Bioluminescence in marine *Vibrio* species is dependent upon quorum sensing (Stevens and Greenberg, 1997). Quorum sensing involves the ability of bacterial species to recognize relative population densities in their immediate environment and to induce specific cell functions once certain bacterial concentrations have been achieved (Stevens and Greenberg, 1997). In seawater, the concentrations of *V. fischeri* are $<10^2$ cells/ml and bioluminescence is not induced (Kolibachuk and Greenberg, 1993). However, *V. fischeri* also exists in symbiotic associations with marine animals such as the bobtail sepiolid squid. This symbiotic association may be regulated by ADP-ribosyltransferases halovibrin α and β, which could act as signaling molecules in the symbiosis between *V. fischeri* and the squid (Reich et al., 1997). In the light organ of the squid (Fig. BXII.γ.165), population sizes of *V. fischeri* may reach 10^{10}–10^{11} cells/ml and luminescence is induced (Kolibachuk and Greenberg, 1993; Ruby and Lee, 1998).

In vivo, luminescence is triggered when autoinducers reach critical intracellular levels. *N*-(3-oxohexanoyl) homoserine lactone and *N*-(3-hydroxybutanoyl) homoserine lactone are the autoinducers for *V. fischeri* and *V. harveyi* respectively (Sun et al., 1994). The cells are freely permeable to these compounds. At high cell densities, greater concentrations of autoinducers accumulate intracellularly and trigger the expression of specific luminescence genes (Kolibachuk and Greenberg, 1993). Two genes activated in this process are *luxI* and *luxR* (Stevens and Greenberg, 1997). *luxI* encodes an autoinducer synthetase and *luxR* encodes an autoinducer-dependent activator of luminescence genes (Stevens and Greenberg, 1997). *luxR* activates transcription from the promoter of the *lux* operon, which contains seven genes. LuxR is a 250-amino acid polypeptide containing two separate domains. The N-terminal region of LuxR binds the autoinducer; this binding then allows the C-terminal region to activate transcription of the *lux* operon (Stevens and Greenberg, 1997).

Genetics Recent studies employing pulsed-field gel electrophoresis (PFGE), representational difference analysis, and restriction fragment length polymorphism indicate that there are major differences in genome size and composition between *V. cholerae* El Tor, O1, and O139 (Choudhury et al., 1994; Calia et al., 1998). The estimated genome size of *V. cholerae* O1 (3.0 Mb) is at least 500 Kb larger than that of the El Tor biotype (Choudhury et al., 1994). Recent construction of the physical map of a classical biotype strain, *V. cholerae* 395 (Ogawa), by PFGE of genomic DNA digested with I-*CeuI*, *SfiI*, and *NotI* indicates a substantially larger chromosome (4.0 Mb) than previously estimated for this biotype (Trucksis et al., 1998).

Furthermore, it appears that both the classical and El Tor biotypes of *V. cholerae* O1 contain two unique and circular megareplicons rather than a single chromosome (Trucksis et al., 1998). Generation of novel or emerging pandemic strains of *V. cholerae* may occur *in vivo* by transduction. The chromosomal genes for cholera toxin are located on the pathogenicity island CTXΦ, which actually represents the genome of a transducing filamentous bacteriophage (Waldor and Mekalanos, 1996; Mekalanos et al., 1997). It is postulated that new strains may arise as a result of recombination in the intestinal tract of humans simultaneously coinfected with both toxigenic and nontoxigenic strains of *V. cholerae*. A second bacteriophage, K139, belongs to the kappa family of bacteriophages and can be found in a number of *V. cholerae* isolates, including O139 (Mekalanos et al., 1997; Calia et al., 1998). This bacteriophage carries a *glo* gene that encodes for a G-related protein of unknown function. Other large segments of *V. cholerae* chromosomal DNA may be involved in genetic exchange between strains. The pathogenicity island VPI, which houses the structural gene for the toxin coregulated pilus, may have arisen in *V. cholerae* by horizontal gene transfer

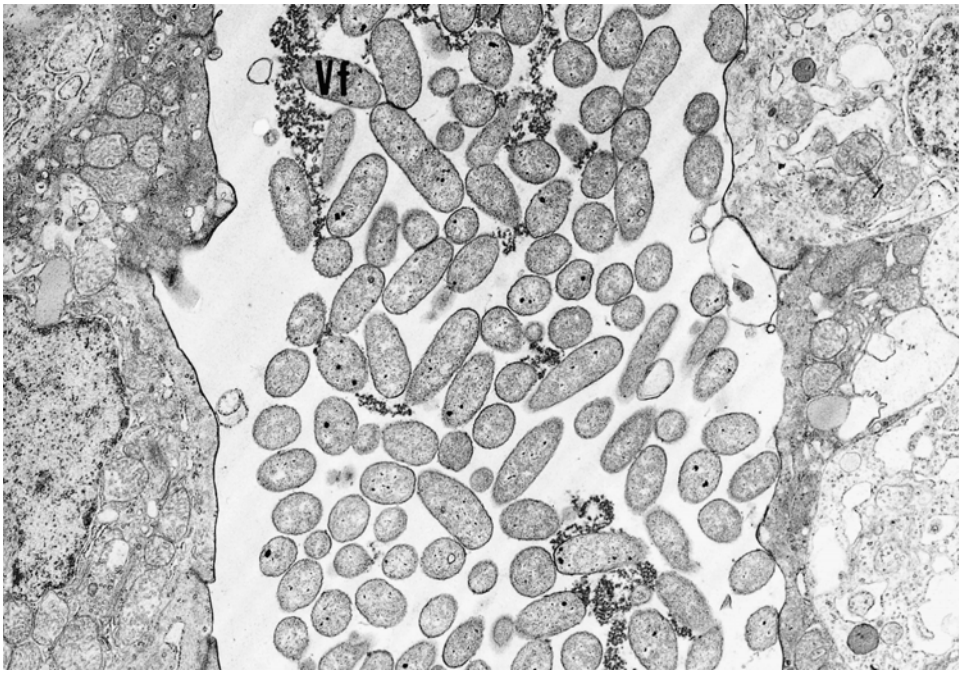

FIGURE BXII.γ.165. Ultrathin section through the luminous organ of *Monocentris japonicus* showing many cells of *Vibrio fischeri*. (× 9000). (Courtesy of K.H. Nealson and B.M. Tebo.)

from a heterologous species and may be transmissible (Mekalanos et al., 1997). A filamentous bacteriophage, designated VPIϕ, carries the VPI pathogenicity island (Karaolis et al., 1999). VPIϕ can be transferred between some *V. cholerae* strains, and the toxin coregulated pilin subunit of the pathogenicity island, TcpA, appears to be a coat protein of VPIϕ. Thus, it appears that the VPIϕ encodes for a protein (toxin coregulated pilus) that serves as a surface receptor for entry of a second phage, CTXΦ, into the bacterial cell. These findings suggest a sequential infection process of the bacterial cells by the two bacteriophages, a scenario which may lead to the emergence of new pathogenic strains of *V. cholerae*.

A 62-Kb chromosomal region, the SXT element, specifies resistance to streptomycin, trimethoprim, and sulfamethoxazole and can be transferred between *V. cholerae* strains by an unknown cell-dependent mechanism (Mekalanos et al., 1997). Transformation of *V. cholerae* strains with plasmids is very inefficient, presumably due to the large amounts of extracellular deoxyribonuclease (DNase) produced. However, transformation of DNase-negative strains using electroporation has been successful (Marcus et al., 1990). Conjugal mating in *V. cholerae*, which is mediated by sex factor P, is another potential mechanism of genetic exchange (Baumann et al., 1984b).

Filamentous phages have been isolated from a number of *V. cholerae* O139 strains (Honma et al., 1997; Shimodori et al., 1997; Jouravleva et al., 1998). These phages contain either single- or double-stranded DNA molecules ranging in size from 6.3 to 9.3 Kb. An open reading frame in one of these filamentous phages codes for a potential protein of 384 amino acid residues that has significant homology to the *zot* gene of *V. cholerae* (Honma et al., 1997). This finding suggests that such filamentous phage might play important roles in the horizontal transmission of genetic information between O1 and O139 strains (Jouravleva et al., 1998).

Current information on the genetics of other *Vibrio* species, although limited, suggests that gene transfer occurs among phylogenetically distant species. The thermostable direct hemolysin of *V. parahaemolyticus* has been detected in a number of vibrios including *V. cholerae* non-O1, *V. mimicus*, and *V. hollisae* (Nishibuchi et al., 1996). In the case of *V. cholerae* and *V. mimicus*, horizontal gene transfer from *V. parahaemolyticus* to these species appears to have occurred recently and to have been mediated by insertion sequence-like elements flanking the *tdh* genes. The *V. hollisae tdh* gene, exhibits significant sequence divergence from *tdh* genes of other *Vibrio* species; this finding suggests an earlier stable transfer of this hemolysin to the former group. Analysis of six *Salinivibrio costicola* strains indicates that the genome size varies from 2.1 to 2.6 Mb and that the DNA is highly methylated (Mellado et al., 1997). DNA methylation in *Salinivibrio costicola* might be affected by salt concentrations present in growth medium.

Mutants Chemical mutagens have been used *in vivo* and *in vitro* to generate mutants of both pathogenic and non-pathogenic *Vibrio* species. Compounds used include *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine, ethylmethane sulfonate, and hydroxylamine (Baumann et al., 1984b; Iida et al., 1995). The Kappa-type lysogenic bacteriophages VcA-1, VcA-2, and VcA-3 have been exploited to generate "random" mutations in the chromosome of *V. cholerae* (Guidolin and Manning, 1987). Insertion of these bacteriophages into bacterial DNA inactivates the genes into which the prophage has been inserted. This process is analogous to that seen with bacteriophage phage Mu. Transposons have been successfully used to construct isogenic mutants in vibrios. The Tn*phoA* system has been used to produce *V. cholerae* mutants in chromosomal iron-regulated genes expressed on the cell surface and gene fusions for important colonization factors such as TcpA (Taylor et al., 1987; Goldberg et al., 1990). However, many strains

of some species such as *V. vulnificus* are refractory to transposon mutagenesis.

Plasmids P plasmids are conjugative extrachromosomal elements in *V. cholerae* that are capable of self-transfer from P^+ to P^- cells (Guidolin and Manning, 1987). Stable integration of P into the host cell DNA does not occur. P plasmids are approximately 68 Kb in size; the exact function of these plasmids is presently unknown. Possession of P plasmids has been linked to attenuated virulence in *V. cholerae* and is associated with decreased TCP pilus production (Bartowsky et al., 1990).

In a study of over 100 *V. cholerae* O1 strains, only 2% were found to contain plasmids (Newland et al., 1984). In contrast, 21–33% of the strains of *Vibrio cholerae* non-O1 examined contained extrachromosomal elements (Newland et al., 1984; Barja et al., 1990; Dalsgaard et al., 1995). Most plasmids are of low molecular weight (2.5 to 22 Kb) (Barja et al., 1990; Dalsgaard et al., 1995). The function of these small plasmids is not known, although some large plasmids (>170 Kb) have been detected which carry genes encoding antibiotic resistance determinants and genes for cytotoxin production (Barja et al., 1990). *V. parahaemolyticus* and *V. vulnificus* infrequently harbor plasmids (Twedt et al., 1981; Davidson and Oliver, 1986). Strains of *Vibrio damsela*, *V. metschnikovii*, and *V. furnissii* commonly contain plasmids (Dalsgaard et al., 1996; 1997; Pedersen et al., 1997). Large plasmids of 190–200 kb have been detected in both *Vibrio damsela* and *V. metschnikovii*, but their function is unknown. Plasmids have also been detected in *V. fischeri*, *V. salmonicida*, *V. ordalii*, and *V. anguillarum* (Giles et al., 1995; Pedersen et al., 1996c). Most strains of *V. anguillarum* serogroup O1 have a 65–67-Kb plasmid named pJM1; this plasmid is a major virulence factor in hemorrhagic septicemia disease in salmonid fish (Litwin and Calderwood, 1993). pJM1 encodes several proteins associated with iron acquisition, including the siderophore anguibactin and an outer membrane receptor protein OM2. Plasmidless derivatives have reduced virulence. A 32-Kb cryptic plasmid is found in most *V. ordalii* strains, although some restriction fragment length polymorphism exists (Pedersen et al., 1996b). A few *Salinivibrio costicola* strains carry multiple plasmids ranging in size from 3–21 Kb up to a megaplasmid of undetermined mass; however, most strains of *S. costicola* do not harbor extrachromosomal elements (Mellado et al., 1997).

Phages and phage typing Temperate bacteriophages active against many *V. cholerae* strains serve as the basis for several phage-typing schemes. Only the scheme of Murkerjee has been used widely to type the classical biovar of *V. cholerae* (Baumann et al., 1984b). A typing system based on lytic phages originally developed by Murkerjee and Basu for *Vibrio cholerae* El Tor has been expanded to provide greater sensitivity (Chattopadhyay et al., 1993); the modified scheme incorporates five new phages as well as modifications of the protocol. Of 1000 *V. cholerae* O1 biogroup El Tor strains tested, >99% could be placed in one of 146 different phage types.

V. cholerae O139 isolates have a capsule and thus they are not usually lysed by choleraphages active against *Vibrio cholerae* O1. Albert et al. (1996) isolated an O139-specific bacteriophage named JA1 that lysed 119 of 122 *V. cholerae* O139 strains. Two Tn*phoA* mutants of *V. cholerae* O139 that lacked the O antigen and capsular polysaccharide were not lysed by phage JA1; this result suggests that the capsule is the receptor for this choleraphage (Albert et al., 1996). A typing scheme for *V. cholerae* O139 strains has been devised based upon the responses of strains to five lytic phages (Chakrabarti et al., 1997); over 60% of the isolates fell into only one of eight phage patterns.

Bacteriocins and bdellovibrios Little recent work has been done on vibriocins. *Enterococcus faecalis*, *Enterococcus faecium*, and *Enterococcus durans* produce substances that are active *in vitro* against *V. cholerae* O1 and non-O1 (Simonetta et al., 1997). The activity is abolished by pre-treatment with proteases, but is resistant to 100°C for 30 min.

A number of *Vibrio* species serve as hosts for predatory marine *Bdellovibrio* strains (Baumann et al., 1984b; see the chapter describing the genus *Bdellovibrio*). The ability of bdellovibrios to lyse *V. parahaemolyticus* strains is temperature dependent; this finding suggests one possible explanation for the seasonal life cycle of this halophile (Joseph et al., 1982).

Antigenic structure As in the *Enterobacteriaceae*, the antigenic structure of *Vibrio* species is defined on the major classes of bacterial antigens: somatic (O), capsular (K), and flagellar (H), which are associated with the bacterial lipopolysaccharide, extracellular layer or capsule, and flagellum respectively (Janda, 1998). Within *V. cholerae* there are over 180 defined O antigens, but serotypes O1 and O139 contain all strains that have caused all large epidemics and pandemics of cholera (Shimada et al., 1994b; Yamai et al., 1997). Strains of *Vibrio cholerae* O1 can be further differentiated into two subtypes designated Ogawa and Inaba, which have antigenic formulas of the AB and AC type, respectively (Table BXII.γ.155; Shimada et al., 1994b). A third, unstable, antigenic type, Hikojima, with serofactors A, B, and C, has also been described (Janda, 1998). The "Hakata" group of *V. cholerae* possesses the C (Inaba) factor but not B (Ogawa) or A (common major O1 antigen) factors and has been designated as serogroup O140 by Shimada et al. (1994b). O serogroups 22 and 155 also share antigenic factors with O139 strains (Shimada et al., 1994b). The flagellar antigens of *V. mimicus* are identical or very similar to those of *V. cholerae*, and most *V. mimicus* strains fall into recognized O-groups of *V. cholerae*.

V. parahaemolyticus strains are differentiated serologically based on O and K (capsular) antigens. At present, 13 O antigens and more than 60 K antigens are recognized (Hisatsune et al., 1993; Janda, 1998). Compositional analysis of the lipopolysaccharide component of *V. parahaemolyticus* serogroups suggests that the side chain consists of no more than 10 monosaccharides (Iguchi et al., 1995). All serogroups produce R-type lipopolysaccharide. Carbohydrate analysis of Sephadex G-50 fractions indicates that glucose, L-glycero-D-manno-heptose, and D-glucuronic acid are common to all 13 serogroups (Iguchi et al., 1995). Serogroup-

TABLE BXII.γ.155. Antigenic serofactors of *Vibrio cholerae* O1: subtypes Ogawa, Inaba, and Hikojima

Serotype O1 subtype	O factors present in culture	Agglutination in absorbed serum:	
		Ogawa[a]	Inaba[a]
Ogawa	A, B	+	−
Inaba	A, C	−	+
Hikojima[b]	A, B, C	+	+

[a]The specific O factor sera are prepared by absorption. For example, an Ogawa antiserum is prepared by injecting an Ogawa culture, and then absorbing the resulting antiserum with an Inaba culture, which removes the antibodies to O serofactor A, leaving antibodies to O serofactor B.

[b]Most authors do not recognize subtype Hikojima and report cultures as either Inaba or Ogawa, based on which serum causes the fastest and strongest agglutination.

related sugars include fucose, arabinose, D-glycero-D-manno-heptose, D-glucosamine, D-galactosamine, 3-amino-3,6-dideoxyglucose, 3-amino-3,6-dideoxygalactose, 2-keto-3-deoxy-D-threo-hexonic acid, and 2-keto-3-deoxyoctonic acid (Iguchi et al., 1995).

Serologic typing schemes have been developed by the National Institutes of Health in Tokyo, Japan for several of the more commonly isolated *Vibrio* species. *V. vulnificus* strains can be subdivided into one of 7 O antigen groups (Shimada and Sakazaki, 1984). Most *V. vulnificus* (90%) were typable by this system, with O groups 1 and 4 predominating. Some strains have a heat-labile masking antigen that inhibits O agglutination reactions (Shimada and Sakazaki, 1984). All O antisera contain R antibody. The flagellar antigens of all *V. vulnificus* strains examined are identical. An international typing scheme composed of 35 serotypes has been developed for *V. fluvialis* and *V. furnissii* (Shimada et al., 1991). Some serotypes are apparently identical to those of *V. cholerae* O6, O39, and O41. Serogroup O19 possesses the C (Inaba) factor of *V. cholerae* O1 but not A or B (Shimada et al., 1987). At least 16 serogroups of *V. anguillarum* are presently recognized; O1 and O2 occur worldwide (Grisez and Ollevier, 1995). All strains of *V. ordalii* belong to O-group 2 of *V. anguillarum*. Flagellins from the polar flagella of many *Vibrio* species share common antigenic determinant(s): these species include *V. cholerae*, *V. mimicus*, *V. metschnikovii*, *V. harveyi*, *V. campbellii*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. fischeri*, *V. splendidus*, *V. nigripulchritudo*, and *Salinivibrio costicola* (Baumann et al., 1984b).

Antibiotic sensitivity There are surprisingly few detailed descriptions of the *in vitro* antibiotic susceptibility of halophilic and nonhalophilic *Vibrio* species. Most recent reports highlight only the susceptibility patterns of clinically significant groups (Morris et al., 1985; Clark, 1992; Farmer and Hickman-Brenner, 1992) although some other species, notably *V. harveyi* (now known to be same as the clinically important species *V. carchariae*), have been studied to some extent (French et al., 1989; Liu et al., 1997b). Most studies indicate that most vibrio isolates, regardless of species designation, are susceptible to tetracyclines, aminoglycosides (gentamicin), chloramphenicol, monobactams, carbapenems, quinolones, and nalidixic acid (Table BXII.γ.156) (Clark, 1992; Farmer and Hickman-Brenner, 1992; Janda, 1998). Widespread resistance to sulfa-containing antimicrobials, trimethoprim, penicillin, carbenicillin, ampicillin, and older cephalosporins has been reported and is often linked to specific species or groups (French et al., 1989; Farmer and Hickman-Brenner, 1992). Resistance to newer cephalosporins such as cefotaxime and ceftazidime is less common (French et al., 1989). Cefotaxime and minocycline show a synergistic activity that inhibits the growth of one clinical isolate of *V. vulnificus in vitro* (Chuang et al., 1997). Discrepancies in the literature regarding the susceptibility of *Vibrio* species to various chemotherapeutic agents may be related to test methodology or geographical variation. In a study of 240 strains representing nine *Vibrio* species, about 60% of all isolates expressed a β-lactamase based upon the nitrocefin test; >95% of all *V. alginolyticus* and *V. splendidus* were β-lactamase-positive, whereas <10% of *V. fluvialis* produced this enzyme (French et al., 1989).

Since the late 1970s, increasing drug resistance in *V. cholerae* has been reported. Outbreaks of disease in Bangladesh and Tanzania caused by multiresistant strains of *V. cholerae* O1 El Tor have been reported (Morris et al., 1985; Janda, 1998). These strains carry R factors encoding resistance to many common antimicrobial agents including ampicillin, chloramphenicol, and tetracycline. Drug resistance in these strains has been linked to possession of an IncC plasmid (Janda, 1998). Many isolates (>30%) of *V. cholerae* O1 from an outbreak of cholera in the western hemisphere were resistant to chloramphenicol, doxycycline, tetracycline, and trimethoprim-sulfamethoxazole. Large conjugative plasmids of the IncC group were detected in these strains. An unusual strain of *V. cholerae* O1 El Tor isolated during the same outbreak was resistant to cefotaxime and could transfer this resistance to *E. coli* by conjugation. After an outbreak of cholera in India in the 1990's due to *V. cholerae* O139, resistance of *V. cholerae* O1 strains to co-trimoxazole (trimethoprim-sulfamethoxazole), nalidixic acid, and chloramphenicol increased dramatically compared to O1 strains isolated prior to this epidemic (Mukhopadhyay et al., 1995). These findings suggest rapid emergence of drug resistance in *V. cholerae* facilitated by mobile genetic elements. However, studies have not shown a link between drug resistance in *V. cholerae* (non-O1) and plasmid carriage (Dalsgaard et al., 1995).

Early isolates of *V. cholerae* O139 from India and Bangladesh had different antibiotic susceptibilities (Yamamoto et al., 1995). Most strains were resistant to trimethoprim, sulfamethoxazole, and streptomycin. Six strains (3.5%) carried R factors mediating resistance to tetracycline, ampicillin, chloramphenicol, kanamycin, and gentamicin. Resistance markers were carried on a 200-kb self-transmissible plasmid of incompatibility group C. All plasmids were identical by restriction endonuclease analysis using *Eco*RI and *Sal*I. Most *V. cholerae* O139 strains isolated between 1993 and 1994 carried resistance to co-trimoxazole, furazolidone, and ampicillin (Mukhopadhyay et al., 1995; Albert et al., 1997; Vijayalakshmi et al., 1997). However, beginning in 1995 and continuing into 1996 with the resurgence of *V. cholerae* O139 in India, a change in susceptibility to antimicrobial agents was detected. Unlike previous isolates, strains isolated beginning in 1995 were susceptible to co-trimoxazole but still resistant to ampicillin and furazolidone (Mitra et al., 1996; Mukhopadhyay et al., 1996). These later strains were also resistant to neomycin (95%), streptomycin (85%), and to tetracycline (25%). These data indicate continued temporal shifts in phenotypes and resistance patterns associated with *V. cholerae* O139.

Sensitivity to pteridine compounds, particularly 2,4-diamino-6,7-diisopropylpteridine (commonly known as vibriostatic agent O/129, or simply as O129), varies among vibrios. Intrinsic resistance to O/129 occurs in a number of *Vibrio* species including *V. furnissii*, and *V. fluvialis* (Farmer and Hickman-Brenner, 1992). Recently acquired resistance to pteridine compounds has risen dramatically in previously susceptible species. Studies from Japan and India on *V. cholerae* O1 and non-O1 isolates recovered in Southeast Asia between 1973 and 1983 found only rare strains (1.3–1.8%) resistant to both the 10 μg and 150 μg concentrations of O/129 available in commercial discs (Sundaram and Murthy, 1983; Matsushita et al., 1984). By the early 1990s, widespread resistance (63–83%) to O/129 at both concentrations had been found in water, plankton, and fecal isolates of *V. cholerae* O1 from India, Bangladesh, and Malaysia (Huq et al., 1992; Ramamurthy et al., 1992; Mahalingam et al., 1993). Resistance to O/129 has been associated with concomitant resistance to co-trimoxazole and/or trimethoprim and has been found to be associated with transmissible R plasmids (Matsushita et al., 1984; Ramamurthy et al., 1992; Mahalingam et al., 1993).

TABLE BXII.γ.156. Antibiotic susceptibility patterns of the 12 *Vibrio* species that occur in human clinical specimens*

	Percentage of strains susceptible:[a]											
Antibiotic[b]	*V. cholerae*[c] (480)	*V. alginolyticus* (69)	*V. cincinnatiensis* (14)	*Vibrio damsela* (21)	*V. fluvialis* (25)	*V. furnissii* (9)	*V. harveyi* (2)	*V. hollisae* (34)	*V. metschnikovii* (22)	*V. mimicus* (75)	*V. parahaemolyticus* (144)	*V. vulnificus* (130)
Penicillin G (12–21)	2	0	0	0	0	0	0	97	9	3	0	2
Ampicillin (12–13)	87	0	36	52	32	11	0	100	31	97	12	99
Carbenicillin (18–22)	64	0	7	14	16	0	0	100	27	8	1	54
Cephalothin (15–17)	98	32	100	76	40	0	100	100	100	100	17	65
Colistin (9–10)	4	25	93	76	100	100	0	100	91	61	11	2
Tetracycline (15–18)	98	94	93	86	88	89	100	97	73	100	98	99
Sulfadiazine (13–16)	26	16	36	71	36	11	50	56	5	17	3	28
Chloramphenicol (13–17)	99	100	100	10	88	100	100	100	100	100	100	100
Streptomycin (12–14)	60	54	86	24	84	100	50	100	32	61	17	42
Kanamycin (14–17)	92	62	79	43	88	100	100	100	14	89	37	53
Gentamicin (13–14)	98	100	100	100	100	100	100	100	100	99	97	100
Nalidixic acid (14–18)	99	97	100	100	100	100	100	100	100	99	99	99

[a]Studied at the CDC *Vibrio* Laboratory and done on Mueller–Hinton agar (with no added NaCl) at 35–37°C.

[b]The numbers in parentheses give the zone size range for the category intermediate. For example, (12–21) means that resistant strains have 6- to 11-mm zones, strains of intermediate susceptibility have zones that are 12- to 21-mm, and susceptible strains have zones of 22 mm or larger. These particular break points are the ones established in the early 1970s for each antibiotic, and they have been used in the CDC *Vibrio* Reference Laboratory for almost 30 years for taxonomic studies. They may differ slightly from current break points for use in treating human infections. Therefore, these data should only be used as an aid in identifying cultures.

[c]The number of strains studied is given in parentheses.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

Pathogenicity Several *Vibrio* species cause diarrhea or infections of the gastrointestinal tract (Table BXII.γ.157) and a number of reviews are available (Morris and Black, 1985; Janda et al., 1988; Kaper et al., 1995). Symptoms can range from mild bouts of enteritis to a fulminant secretory diarrhea with vomiting such as cholera gravis, which is associated with some *V. cholerae* O1 infections (Kaper et al., 1995). The two most frequently isolated enteric pathogens of this group are *V. cholerae*, the etiologic agent of pandemic cholera, and *V. parahaemolyticus*, a major cause of foodborne disease, particularly in Japan and South East Asia (Joseph et al., 1982). Epidemiologic and other data strongly suggest that *V. mimicus*, *V. hollisae*, and *V. fluvialis* can cause diarrhea or infections of the gastrointestinal tract (Abbott and Janda, 1994b; Hlady and Klontz, 1996). *Vibrio furnissii* and occasionally other species have been isolated from feces of people with diarrhea, but there is no strong evidence that these other species can actually cause intestinal infections.

V. cholerae O1 is the causative agent of pandemic cholera. Of the seven pandemics recorded since 1817, both the fifth (1881–1896) and sixth (1899–1923) pandemics were attributed to the classical biovar (Kaper et al., 1995). The seventh pandemic, which is ongoing (1961–present), is caused by *V. cholerae* O1 biogroup El Tor. It has been suggested that a second serogroup, O139, has pandemic potential, but O139 is currently restricted to epidemic disease in the Indian subcontinent and South East Asia (Swerdlow and Ries, 1993; Albert, 1994). Despite their similarities in disease presentation, significant evolutionary divergence exists between these groups. Differences in chromosomal structure and DNA sequences of housekeeping genes indicate that the classical and El Tor biotypes are only distantly related to each other on a short-term evolutionary basis (Mekalanos et al., 1997). Representational difference analysis further indicates that *V. cholerae* O139 is more closely related to the El Tor biogroup than the classical and El Tor biogroups are related to each other (Calia et al., 1998).

TABLE BXII.γ.157. Worldwide occurrence of 12 *Vibrio* species in human clinical specimens*

	Occurrence in human clinical specimens:[a]	
Species	Intestinal	Extraintestinal
V. cholerae		
Serogroups O1 and O139	+ + + +	+
Serogroup non-O1	+ +	+ +
V. alginolyticus	+	+ +
V. cincinnatiensis	−	+
V. damsela	−	+ +
V. fluvialis	+ +	−
V. furnissii	+ +	−
V. harveyi (*V. carchariae*)	−	+
V. hollisae	+ +	−
V. metschnikovii	−	+
V. mimicus	+ +	+
V. parahaemolyticus	+ + + +	+
V. vulnificus	+	+ + +

[a]The symbols +, + +, + + +, and + + + + give the relative frequency of each organism in specimens, and apply to the world, rather than to a particular country.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

Infection with *V. cholerae* O1 and O139 can result in gastrointestinal disease of varying severity (mild diarrhea, enteritis, cholera) although most infected persons (60–75%) are clinically asymptomatic (Kaper et al., 1995). Detection and control of such unapparent illnesses can be critical in preventing the spread of sporadic cases to produce outbreaks or epidemics. Two critical regions of DNA on the chromosomes of both *V. cholerae* O1 and O139 are responsible for the pandemic potential of strains of

these biotypes as well as the ability to cause cholera in susceptible populations. Both regions are located in pathogenicity islands (Groisman and Ochman, 1996; Mekalanos et al., 1997). The first island, designated CTXΦ (cholera toxin), is a 7000–9700 bp region encoding at least six genes (Waldor and Mekalanos, 1996; Mekalanos et al., 1997). The most important of these gene products is cholera toxin, an oligomeric protein (MW 84,000) composed of five B subunits (*ctxB*) and one A subunit (*ctxA*). The B subunit binds holotoxin to the cell receptor, whereas the A subunit provides toxigenic activity intracellularly after proteolytic cleavage into 2 peptides, A_1 and A_2. Internal activation of the A_1 peptide results in ADP-ribosyltransferase activity leading to altered ion transport and hypersecretion of water and Cl^- into the lumen of the intestine. A number of excellent reviews on various aspects of the cholera toxin have been published (Spangler, 1992; Kaper et al., 1995; Sears and Kaper, 1996; Scott et al., 1996). The structural genes for the CTX element have recently been shown to reside on a filamentous phage, CTXΦ (Waldor and Mekalanos, 1996). This indicates that toxigenic *V. cholerae* can arise *de novo* by horizontal gene transfer, which presumably occurs in the gastrointestinal tract. Other genes located in this virulence cassette region include an accessory cholera toxin (*ace*), a zonula occludens toxin (*zot*), core encoded pilin (*cep*), and an open reading frame of unknown function (Janda, 1998). An RTX toxin gene cluster (hemolysins/leukotoxins) in *V. cholerae* El Tor is tightly linked to the cholera toxin prophage but is enzymatically independent of the CTX element (Lin et al., 1999). The toxin RtxA exhibits cytotoxic activity against HEp-2 cells. Classic strains that lack this cytotoxic activity contain a deletion in the RTX gene cluster involving *rtxA* (toxin) and *rtxC* (activator).

The second pathogenicity island is designated VPI and is associated with epidemic and pandemic strains of *V. cholerae*. VPI is 39.5 Kb in size and contains two ToxR-regulated genes: a regulator of virulence genes (ToxT) and a gene cluster containing essential colonization factors, including the toxin co-regulated pilus (TCP) (Karaolis et al., 1998). There is some genetic evidence that this island can be transferred from *V. cholerae* O1 to non-O1 strains. The *tcp* gene encodes for a 20.5-kDa protein that forms bundles of long filamentous pili 6–7 nm in diameter (Kaper et al., 1995; Janda, 1998). Both *in vitro* and *in vivo* experimentation indicates that TCP is essential for colonization and, therefore, for infectivity. Volunteers fed mutant strains lacking TCP failed to become colonized with *V. cholerae* O1, and no diarrhea was observed. Codon usage within this pathogenicity island differs from that of the rest of the *V. cholerae* chromosome; this finding suggests that the island may have been acquired by *V. cholerae* by horizontal transfer from an unrelated microorganism (Mekalanos et al., 1997).

V. cholerae O139, an emerging agent of epidemic cholera, is another important cause of diarrhea (Morris, 1995). Like serogroup O1 strains, O139 strains carry the structural genes encoded by the CTX operon and TCP (Hall et al., 1994; Janda, 1998). However, these two groups differ in several ways. The number of copies of *ctxA* gene(s) and their arrangement in O139 strains differ from that of serotype O1. In addition, a maltose-inducible outer-membrane protein, OmpS, is constitutively expressed (Janda, 1998) and O139 strains are encapsulated and lack specific genes (*rfbR*, *rfbS*) involved in O1 antigen synthesis (Johnson et al., 1994). Serotype O139 appears to have resulted from a number of genetic rearrangements in an O1 strain including deletion of the O1 *rfb* region and acquisition by horizontal transfer from a non-O1 strain of a 35 Kb DNA region that encodes the surface polysaccharide (Bik et al., 1995; Comstock et al., 1996). The recent reemergence of *V. cholerae* O139 in late 1996 has been associated with a number of chromosomal (genomic) rearrangements. When compared with O139 strains isolated in 1992, reemergent strains harbor three copies of the CTX genetic element connected by a single direct repeat sequence and carry an additional restriction site in the ribosomal RNA operon (Khetawat et al., 1999).

Isolates of *Vibrio cholerae* belonging to serogroups other than O1 and O139 normally lack cholera toxin genes and have never been found to carry TCP. These isolates can cause sporadic cases of gastroenteritis but have not spread as epidemics or pandemics of diarrheal disease. Pathogenic mechanisms include the production of "non-cholera enterotoxins", most of which are poorly characterized. However, unlike O1 strains, non-O1 strains of *V. cholerae* are commonly involved in invasive disease such as septicemia in immunocompromised hosts (Safrin et al., 1988; Lin et al., 1996).

V. parahaemolyticus has caused numerous cases of gastroenteritis, including many outbreaks. Cases are associated with the consumption of raw or undercooked shellfish such as oysters, shrimp, crabs, and lobster. Early studies suggested an association between human strains causing diarrhea and production of a positive hemolytic reaction on a special medium, Watgatsuma agar. This reaction, termed the Kanagawa phenomenon (KP), was positive for virtually all clinical isolates, whereas most non-clinical strains were "Kanagawa negative". Subsequent studies demonstrated a direct correlation between "Kanagawa positive" strains and the elaboration of a thermostable direct hemolysin (TDH). This hemolysin is enterotoxigenic in rabbit ileal loops and appears to alter ion flux in intestinal cells, thereby causing a secretory response and gastroenteritis (Nishibuchi and Kaper, 1995). Strains classified as "Kanagawa strong positive" have two copies of the TDH gene (*tdh1*, *tdh2x*), whereas strains classified as "Kanagawa weak" have only a single copy. "Kanagawa negative" strains lack this gene (Nishibuchi and Kaper, 1995). In the mid-1980s however, "Kanagawa negative" strains associated with gastroenteritis began to appear from many different geographic areas, including the Maldives (Honda et al., 1987). It was later found that these "Kanagawa negative" strains produced a different hemolysin named "TDH-related hemolysin" and abbreviated as "TRH". The gene for the TDH-related hemolysin, *trh*, has 70–80% homology to *tdh*, and appears to be closely associated with urease expression (Nishibuchi and Kaper, 1995; Suthienkul et al., 1995). Thus, "Kanagawa negative" strains of *V. parahaemolyticus* can apparently also cause gastroenteritis if they have the *trh* gene. The *tdh* gene is also found in several unrelated vibrios, including *V. hollisae*, *V. mimicus*, and *V. cholerae* non-O1 (Nishibuchi et al., 1996). This finding suggests that horizontal transfer of *tdh* genes to other species is a possible common enteropathogenic mechanism.

V. vulnificus is an important pathogen that causes wound infections and septicemia (Hollis et al., 1976; Blake et al., 1979; Tacket et al., 1984; Klontz et al., 1988). Wound infections result from direct inoculation of the organism into traumatized cutaneous surfaces after contact with marine animals or the marine or estuary environment. Septicemia commonly develops in immunodeficient persons with hepatic disease who consume raw or improperly cooked oysters. On rare occasions, a classic secretory diarrhea, typically observed with other vibrio species, has been reported in patients with *V. vulnificus* in their feces (Klontz et al., 1988), but the etiological role of *V. vulnificus* as a cause of

diarrhea is not proved. Other rare complications of *V. vulnificus* infection include meningitis, myositis, endometritis, peritonitis, and ocular disease. Despite intense investigation, the pathogenic mechanisms of *V. vulnificus* are poorly defined. A unique *V. vulnificus* cytotoxin-hemolysin apparently does not play a major role in microbial pathogenesis (Wright and Morris, 1991), although it is a highly sensitive and specific genetic marker for the species *V. vulnificus*. Other postulated virulence factors include a capsule, iron-scavenging systems, and resistance to complement-mediated lysis.

Two distinct biogroups of *Vibrio vulnificus* have also been described, and both can infect humans. Strains of *Vibrio vulnificus* biogroup 2 have caused outbreaks in eels in Taiwan, Japan, and Spain and apparently cause human wound infections (Tison et al., 1982; Amaro and Biosca, 1996). Strains of *Vibrio vulnificus* biogroup 3 have caused outbreaks of severe wound infection in Israel (Bisharat et al., 1999). The infections occurred when people purchased living, pond-raised, "Saint Peter's fish" (*Talapia* spp.) from local vendors and then were inoculated with the organism through an existing skin break or trauma incurred while handling the fish. Interestingly, the halophilic strains of *V. vulnificus* biogroup 3 were able to grow in the "fresh water" inland fishponds. However, these ponds had a high salt content because of the source of the water and evaporation during the hot dry summer months.

Vibrio damsela is a rare but important pathogen of fish and mammals, including humans. Originally described as the causative agent of ulcerative disease in damselfish (Love et al., 1981), *Vibrio damsela* causes wound infections in bottlenose dolphins, leatherback turtles, yellowtail, sea bream, barramundi, turbot, brown shark, and rainbow trout (Pedersen et al., 1997). Damselfish infected with *Vibrio damsela* after scarification of the dermis develop large ulcers within three days, and death follows shortly thereafter (Love et al., 1981). In humans, wound infections due to *Vibrio damsela* are occasionally reported. Illnesses commonly result from traumas sustained in contact with objects in contaminated waters (e.g., a reef), and from penetrating injuries resulting from catfish barbs or stings from stingrays (Morris et al., 1982). A less frequent but serious complication is fulminant *Vibrio damsela* septicemia, which mimics *V. vulnificus* septicemia (Clarridge and Zighelboim-Daum, 1985; Lang, 1992; Shin et al., 1996). A hemolytic cytolysin with phospholipase D activity is postulated to be a virulence factor in wound pathology and subsequent invasive infections (Kreger et al., 1987).

Many non-clinical *Vibrio* species cause serious infections in a variety of marine animals including shellfish and fish. The best studied of these pathogens is *V. anguillarum*, the causative agent of epizootic disease in marine fish and shellfish (Austin and Austin, 1993). The general term vibriosis is used to describe fulminant septicemic infections produced by a number of vibrio species in marine fish. The etiologic agent of the disease may be specific for a single type of aquatic life or may have a very broad host range. Table BXII.γ.158 summarizes the important pathogens of marine animals (Hada et al., 1984; Ishimaru et al., 1995, 1996; Iwamoto et al., 1995; Borrego et al., 1996; Liu et al., 1996a; Yii et al., 1997).

TABLE BXII.γ.158. Diseases in marine fish and invertebrates caused by or associated with *Vibrio* species

Disease(s)	Susceptible Animals	Species
Whitespot disease	Kuruma prawns, tiger prawns	*V. alginolyticus*
Terminal hemorrhagic septicemia	Eels, ayu, rainbow trout, salmonids	*V. anguillarum*
Gastroenteritis	Groupers	*V. harveyi*
Vibriosis (larvae and juveniles)	Kuruma prawns	*V. harveyi*
Intestinal necrosis (larvae)	Flounders	*V. ichthyoenteri*
Brown spot disease	Kuruma prawns	*V. penaeicida*
Brown ring disease	Manila and fine clams	*V. tapetis*
Intestinal hemorrhage, skin discoloration	Japanese horse mackerel	*V. trachuri*
Diseased larvae	Clams, oysters, scallops	*V. pectenicida*, *V. tubiashii*

Ecology Water (and its associated microorganisms, animals and plants) is the natural habitat of most of the nonhalophilic and marine vibrios. Environmental factors regulating vibrio concentrations and distribution in both freshwater and seawater ecosystems include the concentration of organic and inorganic chemicals, pH, temperature, salinity, oxygen tension, and exposure to UV light (Chakraborty et al., 1997). However, salinity (specifically, the concentration of Na^+) is the most important factor governing the environmental distribution of vibrios. Na^+ concentrations required for optimal growth of *Vibrio* spp (Fig. BXII.γ.164) range from a low of 5–15 mM (0.029–0.087%) for *V. cholerae* and *V. metschnikovii* to a high of 600–700 mM (3.5–4.1%) for the halophile *Salinivibrio costicola* (Baumann et al., 1984b). This wide range partially explains why *V. cholerae*, *V. mimicus*, *V. fluvialis*, and *V. furnissii* are recovered from freshwater rivers, streams, and ponds; other species such as *V. vulnificus* prefer moderate salinity and grow in shellfish beds in coastal areas (Kaysner et al., 1987; Chakraborty et al., 1997; Motes et al., 1998). Although salinity is a critical parameter, it does not completely explain the environmental distribution of all vibrios because halophilic species such as *V. parahaemolyticus* can survive in suboptimal Na^+ concentrations. Other *Vibrio* species have been implicated in human infections resulting from apparent contact with freshwater catfish (Lowry et al., 1986; Chakraborty et al., 1997). *V. mimicus*, a nonhalophilic species, is found in aquatic environments where the average salinity is less than 1% (Chakraborty et al., 1997).

Independent of salinity, vibrio densities tend to increase in warmer waters when enough inorganic and organic nutrients are present for growth. Vibrios are typically more common in geographic regions having temperate or tropical climates. Studies of northern Gulf of Mexico and Atlantic coast oysters indicate that large populations of *V. vulnificus* occur in shellfish meats from May through October; populations then decline until late March (Motes et al., 1998). This seasonality is apparent in most *Vibrio* species, with larger populations being observed from late spring to early fall. In one study, *V. fluvialis*, *V. furnissii*, *V. mimicus*, and *V. parahaemolyticus* survived in sea water at 20°C for 15 days without a detectable reduction in cell viability (Munro et al., 1994). Although *V. metschnikovii* and *V. cholerae* numbers initially declined for 2–6 days after inoculation into seawater, a regrowth phase occurred, and populations then rose to initial values. In contrast, *V. vulnificus* survived poorly under identical conditions, with viability being reduced >10,000-fold within the same interval.

The microbial ecology of vibrios is best exemplified by *V. chol-*

erae. *V. cholerae* can survive in a "free living state" in both freshwater and saline environments. It is widely distributed in habitats such as sewage, brackish water, estuaries, and coastal inlets, as well as in polluted streams, rivers, ponds, and lakes. *V. cholerae* can also persist in an epibiotic form associated with various microscopic life (Hood et al., 1984). Common microorganisms associated with *V. cholerae* include cyanobacteria, phytoplankton (diatoms, fresh water algae), and zooplankton (Huq et al., 1995; Chakraborty et al., 1997). *V. cholerae* may attach to the tissues or chitinous exoskeleton of crustaceans and production of the enzyme chitinase might be important in this process. All these combined niches provide a continuous environmental source for the maintenance and dissemination of *V. cholerae* throughout the world.

Transmission of *V. cholerae* O1 and O139 to humans usually occurs through consumption of contaminated water or foods. An outbreak of cholera in South America caused by *V. cholerae* O1 is a grim reminder of the rapidity with which cholera can spread in naive (susceptible) populations. The initial source of this epidemic, which began in 1991, is unknown, but the disease rapidly became waterborne and was quickly disseminated throughout the continent by introduction into water, unwashed fruits and vegetables, raw seafood, and cooked crab (Mujica et al., 1994; Weber et al., 1994). Cholera can be introduced into industrialized countries through the importation of contaminated foods brought by travelers from affected areas (Finelli et al., 1992).

Strains of non-O1 *V. cholerae* greatly outnumber O1 strains in the environment. *V. cholerae* non-O1 has been recovered from birds, amphibians, herbivores, and freshwater fish (Baumann et al., 1984b; Rhodes et al., 1985). A majority of these isolates lacks the classic virulence factors that are present in O1 strains, such as cholera toxin and the toxin co-regulated pilus (Baumann et al., 1984b; Kaysner et al., 1987).

V. parahaemolyticus inhabits inshore coastal areas and estuaries and has only rarely been recovered from pelagic (open ocean) regions (Joseph et al., 1982). It has been isolated from various parts of the water column, sediment, zooplankton, shellfish, and fish. Occasionally, *V. parahaemolyticus* has been recovered from fresh water in Indonesia and India (Chakraborty et al., 1997). It has also been recovered from inland bodies of water in the United States where the Na^+ content is high. Like *V. cholerae*, growth of *V. parahaemolyticus* in the marine environment is favored by warmer temperatures and saline concentrations approximating 12 parts per thousand (ppt) (Joseph et al., 1982). *V. parahaemolyticus* has been isolated from a variety of marine animals including clam, oyster, lobster, scallop, sardine, squid, eel, crab, and shrimp (Joseph et al., 1982). Most outbreaks of gastroenteritis caused by *V. parahaemolyticus* have been linked to the consumption of crabs, shrimp, oysters, and lobsters. In Japan, *V. parahaemolyticus* is a major cause of food poisoning and is associated with the ingestion of raw fish such as sashimi and sushi (Chakraborty et al., 1997). Most strains of *V. parahaemolyticus* isolated from human infections are "Kanagawa positive," and most environmental isolates are "Kanagawa negative." This distinction was originally thought to indicate that "Kanagawa negative" strains do not cause human disease; however, as mentioned previously, it is now known that some strains produce a thermostable-related hemolysin, a virulence factor that has significant homology to the thermostable direct hemolysin responsible for a positive Kanagawa reaction.

V. vulnificus has been isolated from various locales around the world, including Europe and the Pacific and Atlantic coasts of the United States as far north as Washington and Maine, respectively (O'Neill et al., 1990; Veenstra et al., 1994; Chakraborty et al., 1997). *V. vulnificus* is typically found in waters having intermediate salinities (5–25 ppt) and temperatures up to 26°C (Motes et al., 1998); it does not grow at temperatures less than 10°C (Chakraborty et al., 1997). DePaola et al. (1994) isolated *V. vulnificus* from seawater, crustacea, and estuarine fish from U.S. waters in the Gulf of Mexico. The highest concentration of *V. vulnificus* in one study was found in the intestinal contents of bottom-feeding estuarine fish (sea catfish, sheepshead, Atlantic croaker) that consume mollusks and crustacea (DePaola et al., 1994); it is rarely recovered from offshore fish. *V. vulnificus* populations decline as coastal and estuary water temperatures cool in late fall. The presence of *V. vulnificus* in shellfish may result from the constant filtering by these organisms of seawater containing vibrios rather than the active multiplication of *V. vulnificus* in shellfish tissues (Kelly and Dinuzzo, 1985). However, *V. vulnificus* can survive in oysters for 10–14 d when they are held at 2–10°C (Kaysner et al., 1989). In humans, *V. vulnificus* septicemia is almost exclusively associated with the consumption of raw oysters (Klontz et al., 1988). Some indirect evidence suggests that counts of approximately 10^3 bacteria/gm oyster meat are associated with human disease (Jackson et al., 1997). Strains of *Vibrio vulnificus* biotype 2 strains are pathogenic for eels (Amaro et al., 1995).

Few studies have addressed the environmental distribution of other *Vibrio* species. *V. fluvialis* was identified in 40% of 177 samples collected from rivers, ponds, swamp, estuaries, sewage, sediment, and crabs in Louisiana (Nishibuchi et al., 1983). Data on the association of many pathogenic and marine vibrios with aquatic microbiota suggest that they are widely dispersed in the marine environment. *V. fischeri* has developed a symbiotic association with the bobtail sepiolid squid, *Euprymna scolopes* (Ruby and Lee, 1998). *Vibrio damsela* causes ulcerative disease in the damselfish, *Chromis punctipinnis*, as well as in rainbow trout and in a number of other marine animals (Love et al., 1981; Pedersen et al., 1997). Many other vibrios cause disease in crustacea and fish (Table BXII.γ.158).

Enrichment and Isolation Procedures

Much has been written about the isolation of *Vibrio* cultures, and several good reviews and manuals are available (see Further Reading). These topics have also been covered in detail by Farmer and Hickman-Brenner (1992). Two different sets of methods have been used, depending on whether the sample was taken from a human clinical specimen or from the marine environment. Species of *Vibrio* and *Photobacterium* will grow in many microbiological media; however, the low Na^+ content of some media (some have no added NaCl, others have a final concentration of 0.5–0.85%) will prevent growth of some marine species that require a higher concentration (Fig. BXII.γ.164). Only limited data exist on the growth of the species isolated from human diseases on many of the media traditionally used by marine microbiologists. In spite of the differences in Na^+ requirement/tolerance, several media are useful for all types of vibrio work.

There are several good general media for isolation of *Vibrio* and *Photobacterium* (Table BXII.γ.159). Marine agar is a nonselective medium, and almost all *Vibrio* strains will grow on it. Dozens of other media been described and are useful in particular instances, but are not in general use.

Marine agar is probably the most useful general medium for

TABLE BXII.γ.159. General and selective media for the genera *Vibrio*, *Photobacterium*, *Aeromonas*, and *Plesiomonas*[a]

Medium	*Vibrio*	*Photobacterium*	*Aeromonas*	*Plesiomonas*
Media and enrichments often used in clinical microbiology laboratories:				
Sheep blood agar	+ + + +	+ +	+ + + +	+ + + +
MacConkey agar	+ +		+ + + +	+ + + +
Alkaline peptone water	+ + + +	+ +	+ + +	+ + +
Nonselective "marine" media:				
Marine broth	+ + + +	+ + + +	+ +	+ +
Marine agar	+ + + +	+ + + +	+ +	+ +
Photobacterium agar	+ + + +	+ + + +	+ +	+ +
Photobacterium broth	+ + + +	+ + + +	+ +	+ +
Selective media for Vibrio:[b]				
TCBS agar	+ + + +	+ +	no growth	no growth
Vibrio agar	+ + + +			
Selective media for Aeromonas and Plesiomonas:				
Bile salts-brilliant green agar			+ + + +	+ + + +
Dextrin-fuchsin-sulfite medium			+ + + +	
Rimler-Shotts medium			+ + + +	
Aeromonas agar:			+ + + +	
Blood agar plus ampicillin			+ + + +	
DNase agar plus ampicillin and toluidine blue			+ + + +	
MacConkey agar plus ampicillin and Tween 80			+ + + +	
Pril–xylose–ampicillin agar			+ + + +	
Starch–ampicillin agar			+ + + +	

[a]The symbols "+ + + +" to "+" and "−" indicate the general usefulness of the medium and apply to most species in the genera; however, some species and strains may be exceptions and may differ on a particular medium. For example, most strains of *V. hollisae* do not grow well on TCBS agar (see the following chapter). A blank space indicates that data are not available or are contradictory.

[b]Most of the media formulated for *Vibrio* isolation were specifically designed to isolate *V. cholerae*.

Vibrio and *Photobacterium*. It is also useful for the isolation and growth of organisms whose natural habitat is marine or other environments with a high content of Na^+. It is particularly useful for the species of *Vibrio* and *Photobacterium* (Fig. BXII.γ.164) that require Na^+ in amounts higher than the usual content of bacteriological media (0.5–1.0%).

Marine semisolid medium (marine broth plus 0.4% agar) is a convenient semisolid medium for maintaining "working cultures" of *Vibrio*, *Photobacterium*, or other marine organisms that require added Na^+ and other ions for growth.

TCBS (thiosulfate–citrate–bile salts–sucrose) agar is useful for the isolation of *V. cholerae* and *V. parahaemolyticus* from fecal specimens. It is probably the medium most widely used as a general isolation medium for *Vibrio* strains from human clinical specimens and from the environment (Fig. BXII.γ.166). Some *Vibrio* strains do not grow on TCBS agar or have a low plating efficiency; others are inhibited and grow as small colonies. Details regarding the growth characteristics of *Vibrio* species on TCBS agar are given in Table BXII.γ.160. Enterococci and some other bacteria will sometimes grow on TCBS agar, but most other bacteria are inhibited. Dehydrated TCBS agar is available from several commercial companies.. The exact composition of the medium varies from manufacturer to manufacturer and from lot to lot of the same manufacturer. This variation will influence the medium's selectivity, thus affecting both sensitivity and specificity.

The routine use of TCBS medium for isolation is too expensive for many laboratories that do not isolate vibrios frequently. Kelly (described in Farmer et al., 1985d) found that *Vibrio* isolates obtained on TCBS medium were always obtained on other media also, so long as oxidase-positive colonies were screened.

Alkaline peptone water is probably the most useful liquid medium for isolating and growing vibrios (Colwell 1984; Furniss et al., 1978). This medium is used as an enrichment for *Vibrio cholerae* and other *Vibrio* species, which often grow better than undesired organisms at high pH. Vibrios also tend to grow better than most other organisms at the meniscus. Enrichment in alkaline peptone water is usually followed by spreading onto TCBS

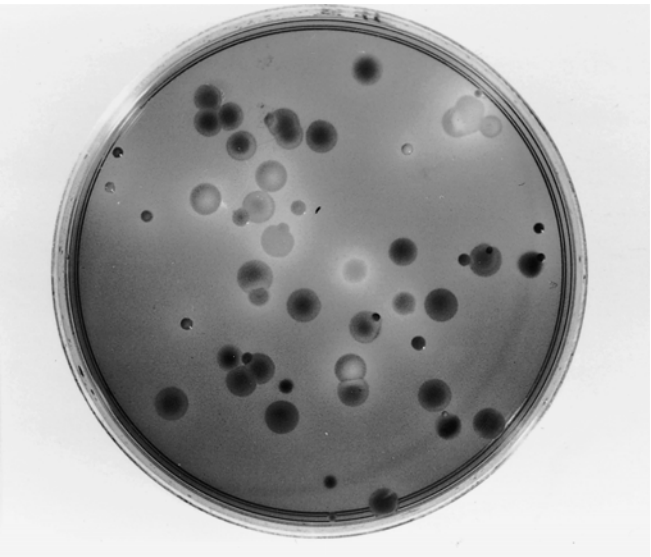

FIGURE BXII.γ.166. Growth of vibrios on TCBS agar. 0.01 ml of seawater collected about 10 m from shore was spread on the agar surface and incubated for 3 days at ambient temperature (about 25°C). Note the many different sizes and shapes of the colonies, which also very considerably in color depending on whether they ferment sucrose. (Reproduced with permission from J.J. Farmer III and F.W. Hickman-Brenner, *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd Edition, Volume III, ©Springer-Verlag, New York, 1992, pp. 2952–3011.)

TABLE BXII.γ.160. Growth of *Vibrio* species on TCBS agar*

Organism	Colony appearance on TCBS (%)		Growth-plating efficiency
	Green	Yellow	
V. cholerae	0[a]	100[a]	Good
V. alginolyticus	0	100	Good
V. cincinnatiensis	0	100	Very poor
V. damsela	95	5	Reduced at 36°C
V. fluvialis	0	100	Good
V. furnissii	0	100	Good
V. harveyi	0	100	Good
V. hollisae	100	0	Very poor
V. metschnikovii	0	100	May be reduced
V. mimicus	100	0	Good
V. parahaemolyticus	99	1	Good
V. vulnificus	90[b]	10[b]	Good
"Marine vibrios"	Variable	Variable	Variable

[a]Percentage of strains that produce green colonies and yellow colonies, respectively.

[b]The original report describing this species gave the percentage positive for sucrose fermentation as 3%. At the CDC *Vibrio* laboratory, about 15% of the strains have been sucrose positive.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

agar or onto a similar plating medium selective for *Vibrio*. The NaCl content of alkaline peptone water is not standardized but is usually 0.5–1%. It can be increased to 2% to enhance the growth of marine vibrios. If the NaCl is omitted ("alkaline peptone water-saltless"), the medium becomes much more selective for *V. cholerae* and *V. mimicus*. The type of peptone used in the medium has also varied depending on several factors including local availability. The formula given below (Formula 1494[2] of the CDC Vibrio Laboratory) is the one used by CDC's *Vibrio* Laboratory for many years. Other formulations may be equally effective.

It is often desirable to use a growth or suspending medium with all of the inorganic constituents of seawater, but without organic compounds or toxic contaminants found in natural sea water. Many artificial types of seawater have been described.

Artificial Sea Water #1 (MacLeod's formula)[3] does not contain several of the ingredients found in formulas for "complete" artificial seawater. Some of the missing components are trace metals. This medium is satisfactory for most strains of *Vibrio* and *Photobacterium*. Artificial Sea Water #2, "Instant Ocean"[4], is a commercial packaged salt mixture available from Aquarium Systems (33208 Lakeland Blvd., Eastlake, Ohio 44094). Stores that have supplies for marine aquariums also sell it. Marine cation solutions[5] are useful alternatives to artificial sea waters, and the *Vibrio* laboratory uses them for making "marine media" used for biochemical testing.

Standard techniques used in clinical microbiology laboratories are suitable for isolating *Vibrio* species from human clinical specimens. Benenson et al. (1964), Hugh and Sakazaki (1972), Morris et al. (1979), World Health Organization (1983), Wachsmuth (1984), and Barua and Greenough (1992)) should be consulted for specialized information. It is important to note that some *Vibrio* cultures do not grow readily on the selective media used for the isolation of enteric pathogens and that most *Vibrio* species require added NaCl for growth (Baumann et al., 1984b; Fig. BXII.γ.164). Many laboratory media have amounts of Na^+ less than 0.5% NaCl.

There are no special procedures for *Vibrio* in regard to the collection, transport, and storage of specimens. Fecal specimens should be obtained within 24 h of the onset of illness and prior to antibiotic treatment. Specimens should be spread on an isolation medium as soon as possible. Vibrios survive longer in the alkaline rice-water stool of cholera patients than in formed stools. *Vibrio* strains are easily killed by desiccation. Samples can be transported in alkaline peptone water or in Cary Blair transport medium, which maintains viability of *Vibrio* cultures for up to 4 weeks. "Buffered glycerol–saline" medium is unsatisfactory. Tellurite–taurocholate–peptone broth has also been used as a transport medium. *Vibrio* strains can survive up to 5 weeks on strips of blotting paper soaked in liquid stool and stored at ambient temperature in airtight plastic bags.

Medically important *Vibrio* strains from fecal specimens grow readily on blood agar. Most strains of *V. cholerae* have a characteristic appearance on blood agar (Fig. BXII.γ.167) and are strongly hemolytic, except for strains of the classical biogroup of *V. cholerae*, which are nonhemolytic. Inclusion of oxidase testing and/or inclusion of TCBS medium in the isolation scheme are recommended for more thorough searches.

Medically important *Vibrio* strains from fecal specimens can grow on blood agar where they may be beta-hemolytic (*V. cholerae* non-O1 and some *V. cholerae* O1 El Tor), alpha-hemolytic (*V. vulnificus* and many others), or nonhemolytic. Vibrios also usually grow on MacConkey agar and produce colorless (lactose-negative) colonies. Colonies grown on blood agar and lactose-negative colonies grown on MacConkey medium should be tested for oxidase activity.

2. Formula 1494 for alkaline peptone water: dissolve peptone (10 g) and sodium chloride (5 g) in 994 ml water. Adjust pH to 8.4 with 1N NaOH (about 6 ml will be required). Dispense into tubes and autoclave at 121°C for 15 min. The final medium will be clear and amber colored.

3. Artificial Sea Water #1, MacLeod's formula, contains (g/l) NaCl (23.38 g), $MgSO_4 \cdot 7H_2O$ (24.65 g), KCl (1.49 g), and $CaCl_2 \cdot 2H_2O$ (2.94 g). A clear, colorless solution with a pH of about 6.6 will result. Dispense into screw cap bottles or tubes and autoclave at 121°C for 15 min. Store at room temperature.

4. Artificial Sea Water #2, "Instant Ocean", is prepared by adding 40 g Instant Ocean to 1 liter of water and stirring until all the salt dissolves. Dispense and autoclave at 121°C for 15 min. Store at room temperature.

5. Marine cations supplement 1558 (Na^+, K^+, Mg^{2+}, and Ca^{2+} (10 X) was modified by the CDC *Vibrio* Laboratory from the "electrolyte supplement" of Furniss et al. (1978)). In 912 ml water, dissolve (in the order listed) NaCl (150 g), KCl (3.7 g), $MgCl_2 \cdot 6H_2O$ (51 g), and $CaCl_2 \cdot 2H_2O$ (7.4 g). Aseptically add one volume to 9 volumes of sterile medium to be supplemented and mix thoroughly. Marine cations supplement 1559 (Na^+, K^+, and Mg^{2+} (10X)) is the same as marine cations supplement 1558 except that it lacks the calcium chloride.

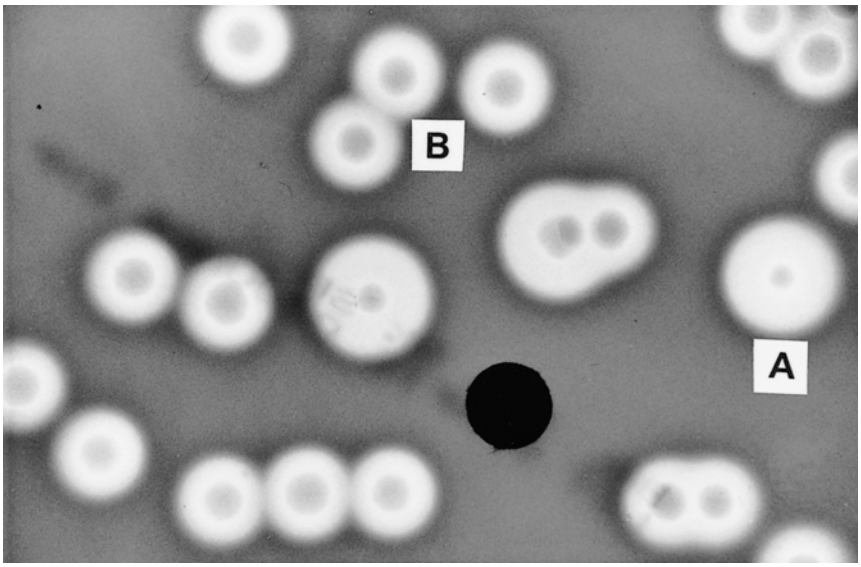

FIGURE BXII.γ.167. Strong hemolytic reaction of the Gulf Coast strain of *Vibrio cholerae* incubated overnight at 36°C on sheep blood agar. Note that the small colony type has a much larger zone of hemolysis *(A)* than the large colony type *(B)*. (Reproduced with permission from J.J. Farmer III and F.W. Hickman-Brenner, *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd Edition, Volume III, ©Springer-Verlag, New York, 1992, pp. 2952–3011.)

Fig. BXII.γ.168 gives a plan for isolating vibrios. Simply testing the oxidase reaction of hemolytic colonies on sheep blood agar plates will detect many *Vibrio* strains as well as many *Aeromonas* strains. Nonhemolytic colonies can also be tested. Procedures for oxidase testing are given in Farmer and Hickman-Brenner (1992).

A protocol adapted from Wachsmuth (1980) and Furniss et al. (1978) for the isolation of *V. cholerae* from stool specimens is shown in Fig. BXII.γ.168. This protocol is also applicable to the isolation of vibrios from other specimens. The use of TCBS agar as the primary plating medium and use of an enrichment step in alkaline peptone water would also enhance recovery of vibrios.

Rapid detection of *Vibrio cholerae* O1 in feces can be accomplished by using a latex bead agglutination test that detects the O1 antigen of *V. cholerae*. Antisera to *V. cholerae* O139 could also be used. A confirmatory culture is needed for the definitive diagnosis of cholera.

Direct detection of *Vibrio* species in feces can be accomplished by microscopic examination. In 1883, Koch described small curved rods that could be observed in the stools of cholera patients but not in the feces of patients without cholera. Later it was observed that sera from convalescent cholera patients stopped the characteristic motility of *V. cholerae* in rice water stools. Later still, antiserum produced in animals was used in this "microscopic vibriocidal assay". The cellular morphology of *V. cholerae* cells varies and typical curved rods, straight rods, short noncurved rods, and "involution forms" can all be seen in the same culture.

Methods for the enrichment of vibrios from seawater have been described by Baumann et al. (1984b). Many of the marine species can be obtained from enrichments involving the addition of 500-ml seawater samples to sterile 2-liter Erlenmeyer flasks containing 25 ml 1 M Tris-HCl (pH 7.5), 0.5 g NH_4Cl, 0.38 g $K_2HPO_4 \cdot 7H_2O$, 14 mg $FeSO_4 \cdot 7H_2O$, and 0.5 g or 0.5 ml of one of a variety of different organic carbon and energy sources. Material from flasks with visible turbidity (generally within 10 days at 20–25°C) is spread onto basal medium agar (BMA; Baumann and Baumann, 1981a) containing 0.1–0.2% (w/v for solids and v/v for liquids) of the homologous carbon and energy source. Suitable organic compounds include sugars, sugar alcohols, sugar acids, tricarboxylic acid cycle intermediates, fatty acids, and amino acids. Strains of marine vibrios have also been obtained by direct isolation from seawater. Seawater is passed through nitrocellulose filters having a pore size of 0.22 or 0.45 μm; the filters are then placed on BMA containing 0.1 or 0.2% of an appropriate carbon and energy source or on yeast extract agar or marine agar. After 2–10 days incubation at 20–25°C, colonies are transferred to homologous medium. Vibrios have also been isolated by plating onto nonselective seawater-based media. For additional information on the isolation of marine vibrios, see Baumann and Baumann (1981a) and Baumann et al. (1984b).

Some strains of *Vibrio* swarm on complex solid media, and isolated colonies cannot be obtained when this occurs. Swarming can be inhibited or greatly reduced by increasing the concentration of agar in complex media to 4% (w/v) (Reichelt and Baumann, 1973b) or by streaking the organism onto a minimal medium such as BMA containing 0.2% (v/v) glycerol (Baumann et al., 1971a).

The isolation of bioluminescent strains of *Vibrio* from seawater does not involve enrichment procedures. Instead, samples are placed directly onto a suitable complex medium and luminescence is detected visually. A solid medium (luminous medium or LM) that promotes luminescence of marine isolates consists of BMA modified by the addition of 0.3% (v/v) glycerol and (in g/l) 5.0 g yeast extract, 5.0 g tryptone, 1.0 g $CaCO_3$, and 20.0 g agar). Because there is strain variation with respect to the intensity and duration of light emission and because some strains will only luminesce as isolated colonies, it is best to examine cultures periodically within 12–36 h of inoculation. A suitable incubation temperature is 15°C; many strains do not produce luminescence

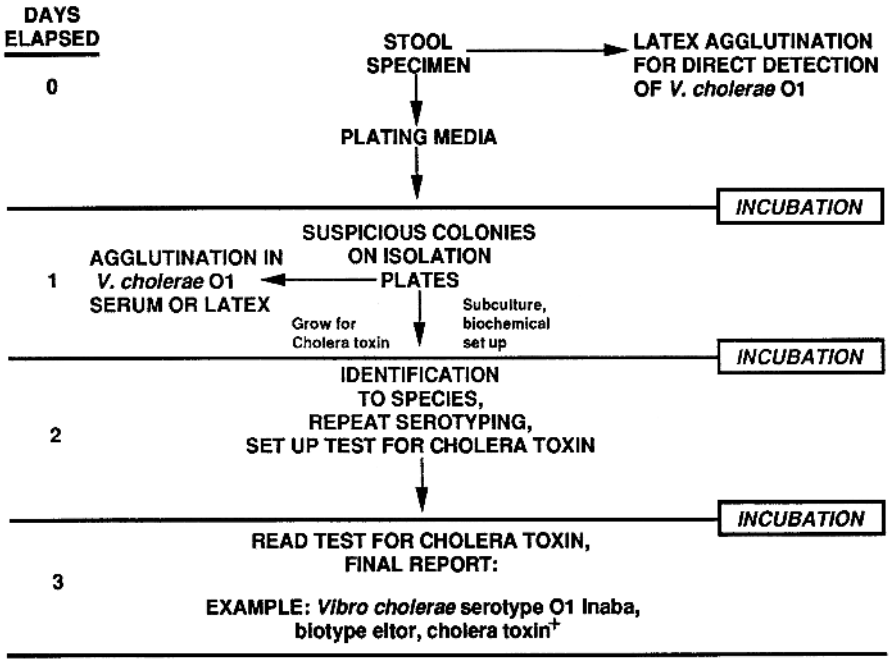

FIGURE BXII.γ.168. Overall plan and special steps for the isolation and identification of *Vibrio cholerae* and other *Vibrio* cultures from feces. (Reproduced with permission from J.J. Farmer III and F.W. Hickman-Brenner, *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd Edition, Volume III, ©Springer-Verlag, New York, 1992, pp. 2952–3011.)

at 20°C or 25°C. Sources of luminous isolates include seawater, the surfaces and intestinal contents of marine animals, luminous organs and, more rarely, freshwater sources. Additional details for the isolation of luminous bacteria are given by Cosenza and Buck (1966), Baumann and Baumann (1981a), Hastings and Nealson (1981), Ruby et al. (1980), and Desmarchelier and Reichelt (1981).

Maintenance Procedures

The following procedures have been used for many years in the Enteric Reference Laboratory, US Centers for Disease Control and Prevention. The methods of two other laboratories with many years of experience in *Vibrio* work have been described in considerable detail and are highly recommended (Furniss et al., 1978; Baumann et al., 1984b).

Strains of *Vibrio* are easy to grow and maintain compared to many other organisms. However, strains will die if allowed to dry on agar plates, in agar slants, or in agar "deeps." Freezing and storage at low temperature is the method of choice for long-term preservation and maintenance. Lyophilization and storage at 4°C are used routinely by the American Type Culture Collection with good results.

If liquid nitrogen storage is not available, *Vibrio* cultures can be stored in a laboratory freezer at −70°C, or even at −10 to −20°C. The lowest temperature available should be used for storage. The culture to be preserved is first inoculated heavily and grown on a marine agar plate for at least 24 h. Cultures of *V. cholerae* and *V. mimicus* can be grown on sheep blood agar instead of marine agar because of their low salt requirement. Growth is removed with a cotton swab and a dense suspension is made in 10% skim milk or other suspending medium. One ml of this suspension is then placed in a sterile plastic freezer vial. The vial is frozen in an alcohol bath that has been kept in the freezer. The vial is stored a divided box that holds 81–100 vials and kept at the lowest temperature available.

For preparation of a "working stock" culture, growth from the top of a single, isolated colony is touched and stabbed to the bottom of 13 × 100-mm screw cap tube of marine semisolid medium. This medium can also be used for *V. cholerae* and *V. mimicus*. Marine semisolid medium is used for the halophilic vibrios. The culture is grown overnight and checked to insure good growth. The tube is sealed by closing the cap tightly. To avoid the problem of drying, two alternative methods are used. First, a small volume (about 0.3 ml) of sterile mineral oil can be added to cover the surface to a depth of about 3 mm above the interface. A second sealing method is to insert a number "000" white rubber stopper into the neck of the screw-cap tube to seal it. After they are sealed, cultures are conveniently stored at room temperature in the dark. Many, but not necessarily all, species of *Vibrio* survive for years in such sealed tubes.

Procedures for Testing Special Characters

Many different procedures have been described for testing the phenotypic characteristics of *Vibrio* strains. The three most important variables are (1) medium composition, (2) addition of Na^+, K^+, Mg^+, and other cations as supplements, and (3) the temperature of incubation. Most clinical and public health laboratories use media and tests designed to identify *Enterobacteriaceae* for *Vibrio* strains. These methods are successful for *V. cholerae* and *V. mimicus* because these species do not require added Na^+. However, most halophilic vibrios require additional Na^+ for growth and expression of various metabolic traits (Fig. BXII.γ.164). In Table BXII.γ.161, results obtained using standard enteric test media are listed first and the percentage of strains positive for each test is given for each species; these data are followed by the percentage of strains positive for the same strains

TABLE BXII.γ.161. The effect of media (especially Na% content) and test conditions on biochemical test results for 10 species of *Vibrio**

Test or property	Percentage positive for:[a]									
	V. cholerae	*V. alginolyticus*	*V. damsela*	*V. fluvialis*	*V. furnissii*	*V. hollisae*	*V. metschnikovii*	*V. mimicus*	*V. parahaemolyticus*	*V. vulnificus*
Indole production:										
Peptone water	86	14	0	0	0	30	22	94	35	39
Peptone water + 1% NaCl	86	24	0	0	0	93	26	98	80	65
Heart infusion + 1% NaCl	97	42	0	13	11	97	17	95	89	94
Methyl red:										
Standard	25	NG	NG	0	0	NG	17	14	NG	NG
Standard + 1% NaCl	99	77	100	96	100	0	96	99	78	79
Voges–Proskauer:										
Standard	74	NG	NG	0	0	NG	26	2	NG	NG
Standard + 1% NaCl	73	8	33	0	0	0	24	1	0	0
Standard + 1% NaCl; Barrit method	93	83	95	0	0	0	96	9	0	0
Arginine dihydrolase:										
Moellers	0	0	81	52	0	0	0	0	0	0
Moellers + 1% NaCl	0	0	95	93	100	0	59	0	0	0
Lysine decarboxylase:										
Moellers	99+	29	0	0	0	0	13	99	63	85
Moellers + 1% NaCl	99+	99	52	0	0	0	36	100	100	99
Ornithine decarboxylase:										
Moellers	99+	3	0	0	0	0	0	99	71	47
Moellers + 1% NaCl	99+	53	0	0	0	0	0	99	89	53
Gelatin hydrolysis:										
Standard	52	45	5	41	56	0	65	60	66	55
Standard + 1% NaCl	62	76	6	85	86	0	38	63	89	75
Esculin hydrolysis:										
Standard	0	0	0	0	0	0	4	0	0	0
Standard + 1% NaCl	2	2	0	8	0	0	59	0	1	39
Nitrate reduction to nitrite:										
Standard	99+	NG	NG	59	33	NG	0	99+	0	2
Standard + 1% NaCl	99+	100	100	100	100	100	0	100	100	100

[a]The number indicates the percentage positive after 48 h of incubation at 36°C; NG, most cultures do not grow in the medium.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

tested in a modified medium with 1% NaCl added. The table also shows other effects of different test conditions; for example, more *Vibrio* species are indole positive when grown in heart infusion broth than when grown in peptone water. More strains are positive for the Voges–Proskauer test when the reagent for detecting acetylmethylcarbinol contains α-naphthol (Barritt method).

Bioluminescence test A bioluminescence test has been described by Baumann et al. (1984b). Several *Vibrio* and *Photobacterium* species usually produce bioluminescence (*V. fischeri*, *V. logei*, *V. orientalis*, *V. splendidus* biogroup 1, *Photobacterium phosphoreum*, and *P. leiognathi*), in contrast to most other *Vibrio* species. Some strains of *V. harveyi* and rare strains of *V. cholerae* and *V. vulnificus* are luminescent (Oliver et al., 1986). Details concerning growth conditions and observation of cultures for bioluminescence have been described by Farmer and Hickman-Brenner (1992).

Carbon assimilation tests Methods for carbon assimilation tests were described by Baumann and Baumann (1981a), who used artificial sea water #1 as a basal medium in their extensive studies to determine the growth of marine bacteria on different substrates. The carbon sources are added to the basal medium at a final concentration of 0.1 to 0.2%.

Susceptibility to vibriostatic compound O/129 Susceptibility to 2,4-diamino-6,7-diisopropyl-pteridine phosphate (vibriostatic compound O/129) is used to differentiate *Vibrio* isolates (usually susceptible) from *Aeromonas* isolates (usually resistant) by means of a disc diffusion assay. This test can also be used to differentiate some *Vibrio* species from each other. Details concerning growth conditions and interpretation of results have been given by Farmer and Hickman-Brenner (1992).

Salt requirement (Na^+ requirement) and tolerance Salt requirement (Na^+ requirement) and tolerance form the basis of useful differential tests for the species of *Vibrio* and *Photobacterium*. In contrast to the halophilic *Vibrio* species, *V. cholerae* and *V. mimicus* are able to grow in 0% NaCl. The data are based on the media and methods given by Farmer and Hickman-Brenner (1992); use of other media may lead to different results.

String test Cells grown on agar medium are suspended in 0.5% sodium deoxycholate. Lysis of the cells is shown by the formation of "strings" of DNA that can be drawn from the suspension with a loop. Isolates that lyse within 60 sec are considered positive. Many *Vibrio* species are positive; *Aeromonas hydrophila* is negative.

Serotyping Serotyping of *Vibrio* was discussed in the section on antigenic structure. Simonson and Siebeling (1988) developed a latex slide agglutination test based on antisera to the flagellin portion of the flagella for the routine identification of several of the *Vibrio* species. *V. cholerae* O1 and O139 antisera are used to confirm isolation of *V. cholerae* O1 or O139 for reporting to health authorities.

Antibiotic susceptibility Clinically important *Vibrio* species can be tested for antibiotic susceptibility using a disk diffusion susceptibility test on Mueller–Hinton agar. However, some marine vibrio species grow poorly on Mueller–Hinton agar, presumably because of their higher requirement for Na^+. Broth dilution susceptibility tests for most *Vibrio* species can usually be done in

unmodified Mueller–Hinton broth, because the broth contains sufficient Na^+ (Hollis et al., 1976).

Antibiotic resistance is relatively rare in *Vibrio* species (Table BXII.γ.156). The term "resistant" is used with the note that the zone size breakpoints that define resistance and susceptibility have been based primarily on isolates of *Enterobacteriaceae* rather than *Vibrio*. In most cases, "resistance" appears to be an intrinsic property of the species rather than a trait acquired through plasmid transfer or mutation. An exception to this generalization is the antibiotic resistance found in some outbreak or imported strains of *V. cholerae* O1 that have become resistant through the acquisition of R factors. Resistance to polymyxin B and colistin can be useful in identifying *V. cholerae* El Tor and *V. vulnificus*. Most other *Vibrio* species are more susceptible.

Differentiation of the genus *Vibrio* from other genera

In this section of the last edition of this *Manual*, Baumann et al. (1984b) stated: "While the number of readily determinable diagnostic traits between these genera is limited, there is little difficulty in their differentiation since the constituent species are generally well defined." We believe that this statement is no longer true and that it is now very difficult to write operational definitions for the genera *Vibrio* and *Photobacterium* that apply to all the species included, and to devise a useful but accurate differential table.

Table BXII.γ.153 in the chapter on *Vibrionaceae* lists the characteristics that distinguish *Vibrio* from *Photobacterium*, *Aeromonas*, *Plesiomonas*, and *Enterobacteriaceae*. However, the reader is warned that some of the *Vibrio* and *Photobacterium* species that have been named recently are incompletely described, and some characteristics have not been determined for all species. Additional data and the eventual subdivision of these two genera into more logical phylogenetic units should make it easier to devise an accurate differential table. Full descriptions and additional information on *Photobacterium*, *Moritella* and *Salinivibrio* can be found in the chapters describing those genera.

Taxonomic Comments

Over the years there has been much speculation on the phylogeny of the genus *Vibrio*. However, only recently has it been possible to do systematic studies that operationally define the evolutionary relatedness of the species in *Vibrio*, *Photobacterium*, and related genera. This topic was introduced in the chapter describing the family *Vibrionaceae*. Many phylogenetic analyses based on the 16S rDNA sequences of the genera *Vibrio* and *Photobacterium* and their relatives have appeared in recent years (Aznar et al., 1994; Cerdà-Cuéllar et al., 1997; Raguénès et al., 1997a; Lambert et al., 1998; Nogi et al., 1998a, b; Sawabe et al., 1998b; Urakawa et al., 1998, 1999a; Yumoto et al., 1999a).

The concept of the genus *Vibrio* itself has changed drastically over the years. For many years organisms were classified in *Vibrio* based on a single morphological property, "curved rods". However, over the years many of these species have been reclassified in other genera with closer phylogenetic relatives. For example, *"Vibrio fetus"* (Smith and Taylor 1919, 301) is now classified in the genus *Campylobacter* (Sebald and Véron, 1963) as *C. fetus* and is the type species for that genus of curved rods, which differ from true *Vibrio* species in being microaerophilic. Similarly, the pink-pigmented methanol-oxidizing bacterium *"Vibrio extorquens"* (Stocks and McCleskey 1964) is now usually classified with other methanol-oxidizing bacteria in the genus *Methylobacterium*. Another species *Vibrio succinogenes* is a microaerophile and is now the type species of the genus *Wolinella*. Today, the trend is to include the term "vibrio" as part of a new genus name to indicate morphologies that include "comma-shaped", "curved", or "slightly curved". Examples include *Bdellovibrio*, *Cellvibrio*, *Halovibrio*, *Micavibrio*, *Vampirovibrio*, *Acetivibrio*, *Anaerovibrio*, *Butyrivibrio*, *Succinivibrio*, *Desulfovibrio*, *"Nitrosovibrio"*, and *Falcivibrio*. These organisms have many different types of metabolism and are found throughout the phylogenetic tree. Clearly, the circumscription of the genus *Vibrio* has evolved over the decades.

Three techniques introduced in the 1960s and 1970s greatly aided our understanding of the phylogeny of the genus *Vibrio*: DNA–DNA hybridization, rRNA–DNA hybridization, and the immunological relationship of enzymes and other proteins (Farmer and Hickman-Brenner, 1992; Baumann et al., 1984b). There is good agreement on many aspects of the classification and phylogeny of *Vibrio*, and the main problem today is how it should be subdivided. Before this issue is discussed, it is helpful to consider the relationships among the species of the genus *Vibrio*.

rRNA–DNA hybridization studies by Baumann and Baumann (1981a), Baumann et al. (1984b), and Fox et al. (1980) of the relationships among *Vibrio*, *Photobacterium*, *Aeromonas*, *Plesiomonas*, and *Enterobacteriaceae* indicated that the genus most closely related to *Vibrio* was *Photobacterium*, and that species of the genera *Pasteurella* and *Aeromonas*, and of the family *Enterobacteriaceae* are more distantly related.

Fig. BXII.γ.169 in this chapter shows that *Vibrio cholerae* is highly related to *Vibrio mimicus* by DNA–DNA hybridization but is much less closely related to the other *Vibrio* species. All other techniques agree that *V. cholerae* and *V. mimicus* are extremely closely related, except that the results of one study that employed 5S rRNA sequencing suggested that *V. cholerae* and *V. mimicus* are separated by a considerable distance and that other *Vibrio* species are closer relatives of *V. cholerae* than *V. mimicus* MacDonell and Colwell (1985).

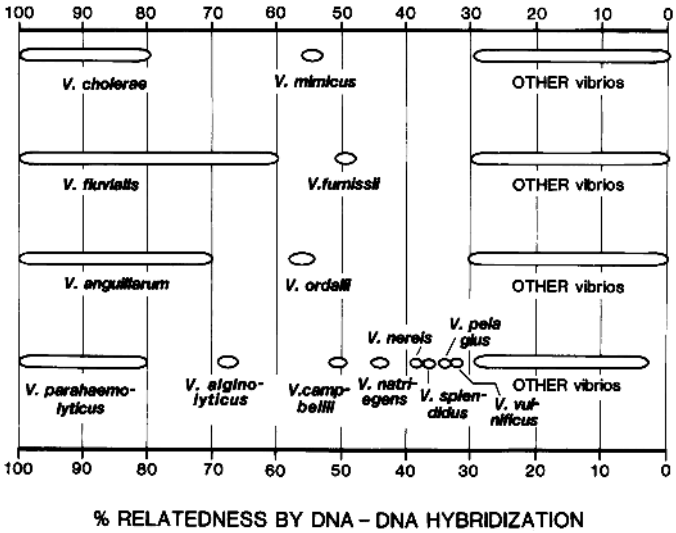

FIGURE BXII.γ.169. Relatedness of several *Vibrio* species by DNA–DNA hybridization. (Conditions: hydroxyapatite, ^{32}P, 60°C). The figure was redrawn from published and unpublished data summarized by Brenner et al. (1983b). (Reproduced with permission from J.J. Farmer III and F.W. Hickman-Brenner, *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd Edition, Volume III, ©Springer-Verlag, New York, 1992, pp. 2952–3011.)

Most of the phylogenetic analyses of the genus *Vibrio* currently being published are based solely on 16S rRNA gene sequences. However, phylogenetic trees rarely agree with each other *in toto*. Inconsistencies can be due to a number of factors, such as failure to use type strains; use of incorrectly identified or mislabeled strains; use of incorrect sequences, including ones obtained from databases; use of incorrect sequence alignments; and use of different methods of calculation in data analysis. For all these reasons data from 16S rRNA sequencing should be examined critically before being used as the basis for any taxonomic proposal.

Subdivision of the genus Over the years reclassifications of the organisms included in the genus *Vibrio* have resulted in proposals of at least 11 new genera (Table BXII.γ.162). Six of these have standing in nomenclature: *Allomonas* (Kalina et al., 1984), *Beneckea* (Campbell, 1957), *Listonella* (MacDonell and Colwell, 1985), *Lucibacterium* (Hendrie et al., 1970), *Moritella* (Urakawa et al., 1998), and *Salinivibrio* (Mellado et al., 1996). Five of the proposed genera are older, do not have standing in nomenclature, and so are written in quotation marks. These are *"Agarbacterium"* (Angst, 1929), *"Alginomonas"* (Thjøtta and Kåss, 1945), *"Alginobacter"* (Thjøtta and Kåss, 1945), *"Alginovibrio"* (Thjøtta and Kåss, 1945) and *"Oceanomonas"* (Miyamoto, et al., 1961).

The genus *Allomonas* was proposed by Kalina et al. (1984) and contained oxidase positive, fermentative organisms that had some properties of *Aeromonas* and some of *Vibrio*. Based on DNA–DNA hybridization experiments, the strains did not appear to be closely related to *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *V. cholerae*, or eight other species in the family *Vibrionaceae*. However, the type strain of *Allomonas enterica* was later found to be highly related to *V. fluvialis* by DNA hybridization and phenotypic analysis (Subcommittee, 1975).

TABLE BXII.γ.162. Alternative classifications that have been proposed that would subdivide the genus *Vibrio*[a]

Genus	Species
Names with standing in the nomenclature:	
Allomonas	*A. enterica*[T]
Beneckea	*B. alginolytica*
	B. campbellii[T]
	B. gazogenes
	B. harveyi
	B. natriegens
	B. nereis (*B. nereida*)[b]
	B. nigripulchritudo (*B. nigripulchritudo*)
	B. parahaemolytica
	B. pelagia
	B. splendida
	B. vulnifica
Listonella	*L. anguillarum*[T] (*L. anguillara*)
	L. damsela
	L. pelagia
Lucibacterium	*L. harveyi*[T]
Moritella	*M. japonica*
	M. marina[T]
	M. viscosa
	M. yayanosii
Photobacterium[c]	
Salinivibrio	*S. costicola*[T]
	S. costicola subsp. *vallismortis*
Names without standing in nomenclature:	
"Listonella ordalii"	
"Agarbacterium"	
"Alginobacter"	
"Alginomonas"	
"Alginovibrio"	
"Oceanomonas"	

[a]The merits and deficiencies of these classifications are discussed in the text.

[b]Names in parentheses are alternative spellings.

[c]See the chapter describing the genus *Photobacterium*.

The genus *Beneckea* was proposed as a new genus in the family *"Achromobacteriaceae"* by Campbell (1957) and was defined as a genus of rod-shaped bacteria that were Gram negative, had peritrichous flagella, fermented carbohydrates, and digested chitin. Baumann et al. (1971a) studied a large group of "marine vibrios" and classified them in a redefined genus *Beneckea* along with *Vibrio alginolyticus* and *Vibrio parahaemolyticus*. However, as additional species of *Beneckea* were described the genus began to become heterogeneous, and Baumann et al. (1980b) concluded that all the *Beneckea* species should be classified in the genus *Vibrio*.

The genus *Listonella* was proposed by MacDonell and Colwell (1985) as new genus of *Vibrionaceae* that included three *Vibrio* species (Table BXII.γ.162): the type species *Listonella anguillarum* (*Vibrio anguillarum*), *Listonella damsela* (*Vibrio damsela*; *Photobacterium damselae*) and *Listonella pelagia* (*Vibrio pelagius*). *Listonella* included organisms fitting the following description: "Curved rods, Gram negative, motile by monotrichous or peritrichous flagella. Chemoorganotrophic, oxidase positive, associated with marine environments. Generally pathogenic for fish or eels. Do not grow at 40°C. Require NaCl for growth. G + C mol% 43–46." *V. anguillarum* is closely related to *V. ordalii* by DNA hybridization (Fig. BXII.γ.169).

The genus *Lucibacterium* was proposed in 1970 by Hendrie et al. (1970); this genus contains a single species, *Lucibacterium harveyi* (= *Vibrio harveyi*). The names *Lucibacterium* and *Lucibacterium harveyi* have standing in nomenclature but are rarely used.

The genus *Moritella* was proposed by Urakawa et al. (1998) based on 16S rRNA gene sequence divergence of 11 strains of *Moritella marina* from *Vibrio cholerae* and other *Vibrio* species. Three additional species, *Moritella japonica* (Nogi et al., 1998a), *Moritella yayanosii* (Nogi and Kato, 1999a), and *M. viscosa* (Benediktsdóttir et al., 2000) have been described.

The genus *Salinivibrio* was proposed by Mellado et al. (1996) based on 16S rRNA gene sequence divergence of six *S. costicola* (= *Vibrio costicola*) strains from *Vibrio cholerae* and other *Vibrio* species; these results were also in agreement with previous phenotypic and genotypic data.

Table BXII.γ.162 provides a summary of previous proposals that subdivided the *Vibrio* as it existed at the time each proposal was made. A successful classification should also be reconcilable with phenotypic data. It should be possible to classify newly discovered and described species into new genera that are limited so as to include only close relatives of the type species. The ideal grouping is illustrated by the pair *Vibrio cholerae* and *Vibrio mimicus* (Fig. BXII.γ.169).

Future of the genus The genus *Vibrio* and many other large heterogeneous genera such as *Pseudomonas* and *Bacillus* will change drastically in circumscription. These genera will eventually be limited to the type species and closely related species. Based on all data available we believe the following changes are likely to occur in the genus *Vibrio*, but it is difficult to determine a time frame for the changes and their acceptance by the scientific community.

The species *Vibrio cholerae* and *Vibrio mimicus* will be the basis of a more limited genus *Vibrio*; any redefinition of the genus will include these two species.

The *Vibrio* species not closely related to *Vibrio cholerae* will form the bases of many new genera, such as the genera *Salinivibrio* and *Moritella*. The figures in this chapter, the chapter describing the family *Vibrionaceae*, and the chapter on *Photobacterium* suggest other possible new genera.

We feel it is logical to include only *Listonella anguillarum* and *V. ordalii* in the genus *Listonella*. These two organisms are closely related by DNA–DNA hybridization (Fig. BXII.γ.169), the immunological relationship of superoxide dismutase (Fig. BXII.γ.170), 16S rDNA sequencing (Fig. BXII.γ.171), and phenotype. *Listonella damsela* and *Listonella pelagia* do not appear to be closely related to *Listonella anguillarum* and *"L. ordalii"*.

Vibrio fluvialis and *Vibrio furnissii* are closely related by DNA–DNA hybridization (Fig. BXII.γ.169), 16S rDNA sequencing (Fig. BXII.γ.171), the immunological relationship of superoxide dismutase (Fig. BXII.γ.170), and phenotype. These two closely related species could be used to define another new genus.

The marine species *Vibrio parahaemolyticus* and *Vibrio alginolyticus* are closely related by DNA–DNA hybridization (Fig. BXII.γ.169), DNA–RNA hybridization, the immunological relationship of superoxide dismutase (Fig. BXII.γ.170), and phenotype. However, data from 16S rRNA gene sequencing (Fig. BXII.γ.171) indicate that *Vibrio parahaemolyticus* is more closely related to other *Vibrio* species than to *Vibrio alginolyticus*. Data from DNA–DNA hybridization studies suggest that *Vibrio campbellii* is the closest relative of this pair. Gradations in digress of relatedness in the "marine vibrio group" will make it difficult to define a new genus.

As originally noted by Baumann and Baumann (1981a) *Vibrio pelagius* biogroups 1 and 2 are closely related and could form the basis of another genus. This close relationship is shown by DNA–DNA hybridization (Brenner et al., unpublished) and by the immunological relationship of superoxide dismutases (Fig. BXII.γ.170).

Vibrio metschnikovii and *Vibrio gazogenes* are unique among *Vibrio* species because they are nitrate negative and oxidase negative. They are distinct from other *Vibrio* species based on the immunological relationship of their superoxide dismutases (Fig. BXII.γ.170) and DNA–DNA hybridization (Fig. BXII.γ.172). In Figure 1 of Cerdà-Cuéllar et al. (1997) they form a tight cluster (B2) based on 16S rDNA sequencing; this cluster also includes *V. cincinnatiensis*. However, *V. metschnikovii* and *V. gazogenes* are less than 30% related by DNA–DNA hybridization (Brenner et al., unpublished). Further data are needed to determine whether *Vibrio metschnikovii* and *Vibrio gazogenes* form the basis of another new genus.

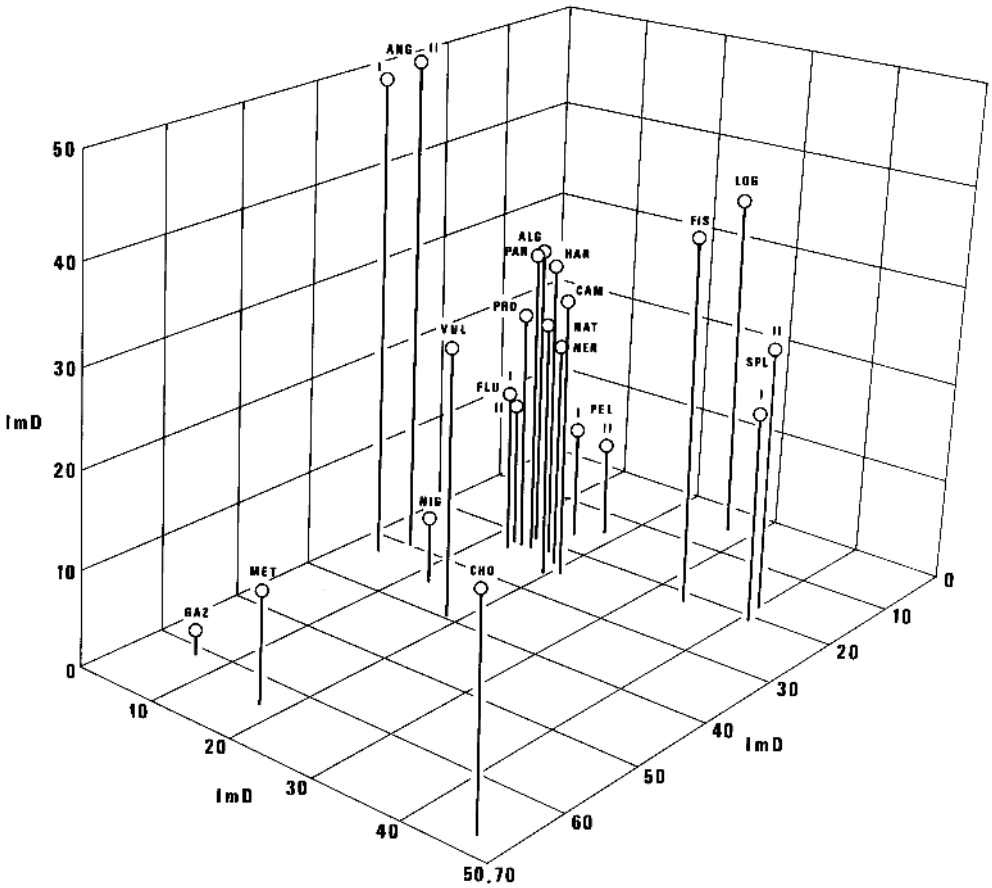

FIGURE BXII.γ.170. Relatedness of *Vibrio* species based on a three-dimensional representation of the immunological relationships of their superoxide dismutase. The spheres represent the relative positions of the species and biogroups. ImD, immunological distance, is a parameter related to percent amino acid sequence difference. *Vibrio* species are designated by the first three letters of their species name. "SPL I" and "SPL II" are *Vibrio splendidus* biogroups 1 and 2. "PEL I" and "PEL II' are *Vibrio pelagius* biogroups 1 and 2. "FLU I" and "FLU II" are *Vibrio fluvialis* biogroups 1 and 2; the latter is now usually classified as *Vibrio furnissii*. "ANG I" and "ANG II" are for *Vibrio anguillarum* biogroups 1 and 2; the latter is now usually classified as *Vibrio ordalii*. Three or four strains from each species or biogroup were used in the analysis; the maximal standard deviation was ± 1.2 ImD. *Salinivibrio costicola* was distant from all other species, and its position is not shown in this figure. (Source: Baumann et al., 1984b; the data were taken from Baumann et al. 1980a, and from S.S. Bang, L. Baumann, and P. Baumann, unpublished).

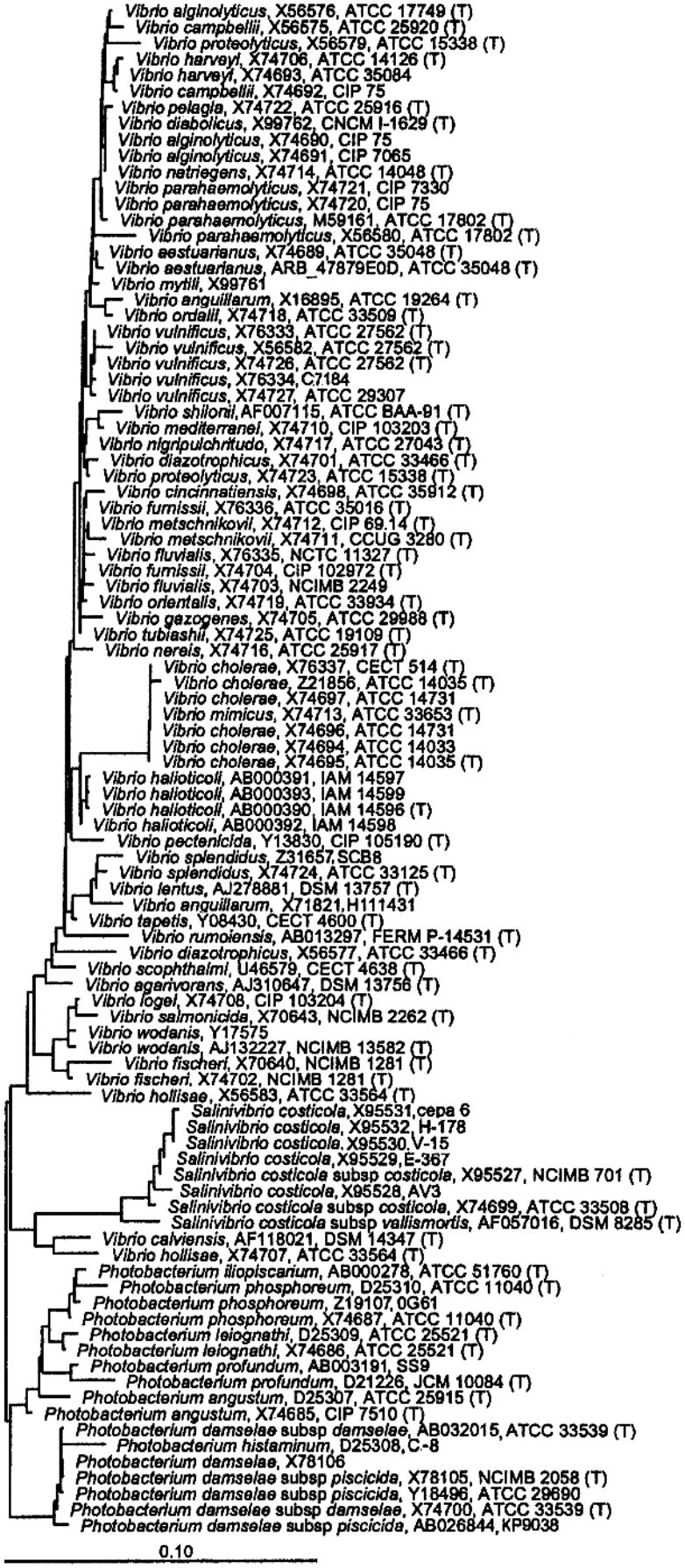

FIGURE BXII.γ.171. Relatedness of *Vibrio* and related species based on 16S rRNA gene sequences.* The distances in the tree were calculated using 1101 positions (the least-squares method, Jukes-Cantor model). (Courtesy T. Lilburn of the Ribosomal Database Project.)

**Editorial Note:* *Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*. *Vibrio pelagius* and *Vibrio anguillarum* are synonyms of *Listonella pelagia* and *Listonella anguillarum*, respectively.

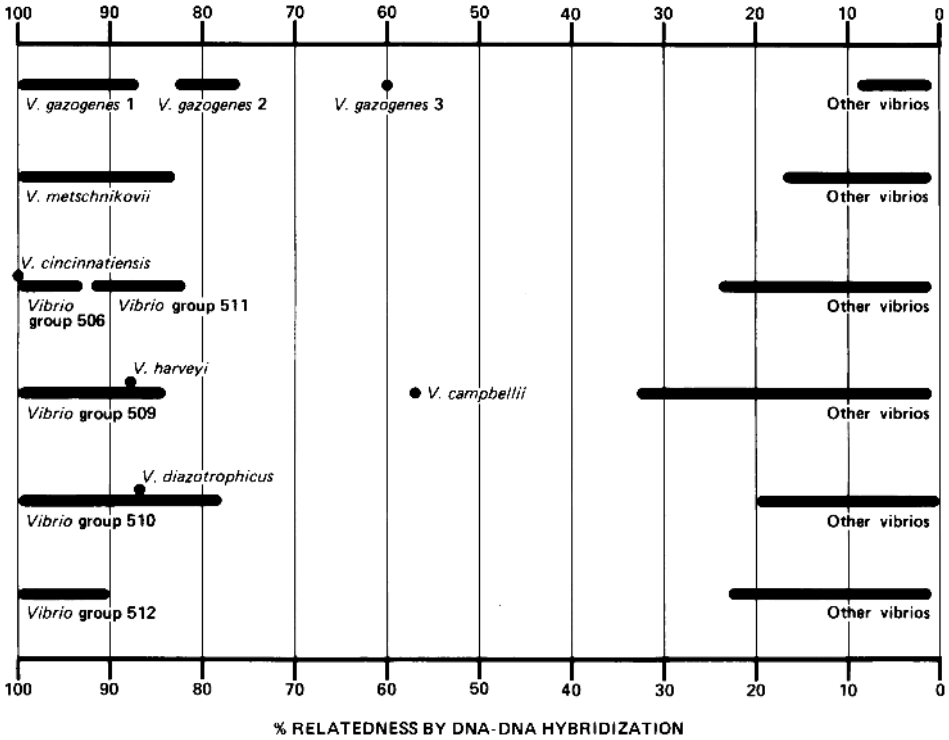

FIGURE BXII.γ.172. DNA relatedness of *Vibrio gazogenes*, *V. metschnikovii*, and five "*Vibrio* groups".

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Differentiation of the species of the genus *Vibrio**

Tables BXII.γ.163 and BXII.γ.164 summarize the characteristics of *Vibrio* spp. and include data on *Salinivibrio*, *Photobacterium*, and *Moritella* spp. for purposes of comparison. Phenotypic characteristics useful for the differentiation of the 12 *Vibrio* species that occur in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.165, and BXII.γ.166. Table BXII.γ.161 gives data on the effects of such factors as basal medium, NaCl concentration, and test method on the results of nine biochemical tests for ten *Vibrio* species.

Species-specific probes have been developed for a number of vibrios. A 17-base pair oligonucleotide probe based on 5S rRNA sequence data is highly specific for the fish pathogens *V. anguillarum* and *V. ordalii* (Ito et al., 1995). However, the limit of detection is 1.5×10^5 viable cells. 16S rRNA gene sequences have also been used to detect *V. anguillarum* (Martínez-Picado et al., 1994). A number of probes designed to detect virulence-associated genes have been described for pathogenic vibrios. A 3.2-Kb probe containing the structural gene for the cytotoxin-hemolysin of *V. vulnificus* is species-specific (Morris et al., 1987). It is highly sensitive (60 viable cells can be detected) and detects all three *Vibrio vulnificus* biogroups. A 0.69-kb probe for the damselysin gene reacts with all hemolytic strains of *Vibrio damsela* but not with nonhemolytic strains or with hemolytic isolates of other vibrios such as *V. cholerae* and *V. fluvialis* (Cutter and Kreger, 1990). A 19-base-pair oligonucleotide probe has been developed that spans an 11-base-pair deletion in the *hlyA* gene in classical biotype strains of *V. cholerae* (Alm and Manning, 1990). This probe detects all O1 El Tor and non-O1 *V. cholerae* strains but not strains of the classical biotype. Two DNA probes (100 and 1000 bp) specific for the chromosomal region that encodes genes for the synthesis of the lipopolysaccharide side chain region of all *V. cholerae* O139 strains detects >99% of O139 strains but not those belonging to other serogroups or species (Nair et al., 1995).

Molecular techniques have also been used to subtype strains of *V. cholerae*, *V. parahaemolyticus*, and some environmental species such as *V. ordalii*. These techniques include pulsed-field gel electrophoresis, restriction fragment length polymorphism, ribotyping, multilocus enzyme electrophoresis, and arbitrarily primed polymerase chain reaction (Koblavi et al., 1990; Faruque et al., 1994; Mahalingam et al., 1994; Evins et al., 1995; Popovic et al., 1995; Kurazono et al., 1996; Pedersen et al., 1996b, c; Okuda et al., 1997).

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

List of species of the genus Vibrio

1. **Vibrio cholerae** Pacini 1854, 411[AL]
chol′ er.ae. Gr. n. *cholera* cholera; M.L. gen. n. *cholerae* of cholera, a severe diarrheal disease.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Fig. BXII.γ.158 shows the polar flagellum; Fig. BXII.γ.173 shows colonies of *V. cholerae* on TCBS agar. The antigenic types of *V. cholerae* are listed in Table BXII.γ.155; the division of *V. cholerae* strains into serogroups is given in Table BXII.γ.167. Characters that differentiate the classical and El Tor biotypes of *V. cholerae* are given in Table BXII.γ.168. Characters that distinguish *V. cholerae* from *V. mimicus* are given in Table BXII.γ.169. Characters that distinguish *V. cholerae* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165 and BXII.γ.166. A few *V. cholerae* strains are bioluminescent, and were formerly classified as *Vibrio albensis* or *Vibrio cholerae* biovar albensis.

V. cholerae causes a diarrhea that has a wide range of severity and symptoms. In severe cholera (cholera gravis), there is severe diarrhea with rice water stools, devastating dehydration and electrolyte imbalance. There can also be cholera that is less severe and even a mild diarrhea with rapid recovery. Current strains that cause pandemic cholera produce cholera toxin and belong to serogroup O1 and to serogroup O139, which emerged recently. Epidemic strains of *V. cholerae* O1 are usually the El Tor biotype, but in some areas of the world, the classical biotype still occurs. The original El Tor strains hemolyzed sheep erythrocytes strongly, but "pandemic" strains isolated in recent years have been very poorly hemolytic or even nonhemolytic. Nontoxigenic strains of *V. cholerae* O1 may be isolated from cases of diarrhea and from the environment in areas where cholera is absent. These strains are usually hemolytic. Fig. BXII.γ.168 shows a procedure for the isolation of *V. cholerae* from feces.

The mol% G + C of the DNA is: 47–49 (T_m, Bd).
Type strain: ATCC 14035, CDC 9061-79, NCTC 8021.
GenBank accession number (16S rRNA): X74695, Z21856.
Additional Remarks: Reference strain, ATCC 14033 (NCTC 8457, CDC 9061-79), is a representative of the El Tor biotype, and was isolated from pilgrims in the El Tor quarantine camp. It was used in the extensive DNA–DNA hybridization studies of Davis et al. (1981a). It is characterized as: serogroup: O1 Inaba, biogroup: El Tor, pathogen classification: Class III, and is listed as nontoxigenic in the 1996 ATCC Catalog.

2. **Vibrio aerogenes** Shieh, Chen and Chiu 2000, 327[VP]
a.e.ro′ ge.nes. Gr. masc. n. *aer* air; Gr. v. *gennanio* to produce; M. L. adj. *aerogenes* gas-producing.

The properties of *V. aerogenes* are given in Table BXII.γ.164. Rods or curved rods 0.6–0.8 × 2–3 μm. Motile by double-sheathed polar flagella. Does not swarm. Not bioluminescent. Facultatively anaerobic; produces organic acids, H_2 and CO_2 during fermentation of carbohydrates. No growth factors required. No PHB granules formed. Grows in media containing 1–7% NaCl; does not grow in 1% or 10% NaCl. The major fatty acid is $C_{12:0}$.

Isolated from sediment in a stand of seagrass, Nanwan Bay, Kenting National Park, Taiwan.

A 16S rDNA sequence analysis that included 35 *Vibrio* species showed that *V. aerogenes* was most closely related to *V. mytili* (Shieh et al., 2000).

The mol% G + C of the DNA is: 46
Type strain: FG1, ATCC 700797, CCRC 17041.
GenBank accession number (16S rRNA): AF124055.

TABLE BXII.γ.163. Biochemical reactions of the named species and unnamed groups of the genera *Vibrio*, *Moritella*, and *Salinivibrio*.*

Characteristic	*Vibrio* (clinical)														*Vibrio* (non-clinical)										
	V. cholerae	*V. alginolyticus*	*V. cincinnatiensis*	*Vibrio damsela*	*V. fluvialis*[a]	*V. furnissii*[a]	*V. harveyi*	*V. hollisae*	*V. metschnikovii*	*V. mimicus*	*V. parahaemolyticus*	*V. vulnificus*	*V. vulnificus* biogroup 2	*V. vulnificus* biogroup 3	*V. aestuarianus*	*V. anguillarum*	*V. campbellii*	*V. diazotrophicus*	*V. fischeri*	*V. gazogenes*	*V. ichthyoenteri*	*V. logei*[b]	*V. mediterranei*	*V. mytili*	*V. natriegens*
Number of strains studied	5	5	2	5	5	5	6	5	5	5	5	5	3	2	3	4	5	5	4	24	2	3	1	1	5
Oxidase (Kovacs)	100	100	100	100	100	100	100	100	0	100	100	100	100	100	100	100	100	100	100	0	100	100	100	100	100
Nitrate reduced to nitrite	100	100	100	100	100	100	100	100	0	100	100	100	100	100	100	100	80	100	75	4	100	100	100	100	100
Indole production	100	80	0	0	60	0	100	100	0	100	100	100	0	100	100	75	80	100	0	0	0	0	100	0	0
Voges–Proskauer	100	80	0	80	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	67	0	0	0	0	20
Lysine decarboxylase (2 d)	100	100	100	60	0	0	83	0	100	100	100	100	100	100	33	0	100	0	50	0	0	100	67	0	0
Arginine dihydrolase (2 d)	0	0	0	100	100	100	0	0	60	0	0	0	0	0	100	75	0	0	0	0	0	0	67	100	0
Ornithine decarboxylase (2 d)	100	40	0	0	0	0	83	0	0	100	100	100	0	100	0	0	20	0	0	0	0	33	0	0	0
Lysine decarboxylase (7 d)	100	100	100	60	0	0	83	0	100	100	100	100	100	100	33	0	100	0	100	0	0	100	67	0	0
Arginine dihydrolase (7 d)	0	0	0	100	100	100	0	0	100	0	0	0	0	0	100	100	0	100	0	0	0	0	67	100	0
Ornithine decarboxylase (7 d)	100	60	0	0	0	0	83	0	0	100	100	100	0	100	0	0	20	0	0	0	0	100	0	0	0
Motility	100	100	100	100	100	100	100	100	60	100	100	100	100	100	100	100	80	100	100	100	100	100	100	100	100
D-Glucose, acid	100	100	100	100	100	100	83	100	100	100	100	100	100	100	100	100	100	100	75	100	100	100	100	100	100
D-Glucose, gas	0	0	0	40	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	96	0	0	0	100	0
Fermentation of:																									
Adonitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Arabinose	0	0	100	0	100	100	0	100	0	0	80	0	0	0	0	75	0	100	0	100	0	0	0	100	100
D-Arabitol	0	0	0	0	80	100	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0	100
Cellobiose	0	100	100	100	20	0	100	0	0	0	0	100	100	0	100	75	100	100	100	100	0	100	100	100	80
Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Galactose	60	20	100	100	100	100	67	100	20	40	60	100	100	100	100	25	0	100	100	88	0	100	100	100	60
D-Galacturonate	0	0	0	0	100	100	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0

[a]The strains for *V. fluvialis* and *V. furnissii* grew in 0% NaCl when incubated at 25°C but not at 36°C.

[b]*V. logei* was tested at 15°C.

[c]Each number is the % positive for the test after 48 h of incubation, except for oxidase and reduction of nitrate to nitrite, which were done only at 24 h. All species except *V. logei* were tested at 25°C. All testing was done in the CDC *Vibrio* Reference Laboratory using standardized "enteric" media supplemented with marine cations (Na^+, K^+, and Mg^{++}). Results in this table may differ from those in the original descriptions of organisms due to variables in media, incubation temperature, reading time, and salt content.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

(*continued*)

TABLE BXII.γ.163. *(cont.)*

Characteristic	*Vibrio* (clinical)														*Vibrio* (non-clinical)										
	V. cholerae	*V. alginolyticus*	*V. cincinnatiensis*	*Vibrio damsela*	*V. fluvialis*[a]	*V. furnissii*[a]	*V. harveyi*	*V. hollisae*	*V. metschnikovii*	*V. mimicus*	*V. parahaemolyticus*	*V. vulnificus*	*V. vulnificus* biogroup 2	*V. vulnificus* biogroup 3	*V. aestuarianus*	*V. anguillarum*	*V. campbellii*	*V. diazotrophicus*	*V. fischeri*	*V. gazogenes*	*V. ichthyoenteri*	*V. logei*[b]	*V. mediterranei*	*V. mytili*	*V. natriegens*
myo-Inositol	0	0	100	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lactose	0	0	0	0	0	0	0	0	20	0	0	0	33	0	67	0	0	60	25	88	0	0	0	100	0
Maltose	100	100	100	100	100	100	100	0	100	100	100	100	100	100	100	75	100	100	100	100	100	100	100	100	100
D-Mannitol	100	100	100	0	100	100	100	0	80	80	80	80	0	0	100	75	40	100	50	96	100	100	100	100	100
D-Mannose	80	100	100	100	80	60	100	100	100	100	100	100	67	100	100	100	100	20	100	100	100	100	100	0	40
Melibiose	0	0	0	0	0	0	40	0	0	0	0	0	0	0	0	0	0	0	0	71	0	0	67	0	60
α-Methyl-D-glucoside	0	0	50	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	60
Raffinose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40
L-Rhamnose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	100
Salicin	0	0	100	0	80	0	0	0	20	0	0	0	0	0	0	0	0	100	75	100	0	0	0	100	100
L-Sorbitol	0	0	0	0	0	0	0	0	40	0	0	0	0	0	0	100	0	0	0	71	0	0	100	0	0
Sucrose	100	100	100	20	100	100	83	0	100	0	0	20	0	0	100	100	0	100	0	100	50	0	100	100	100
Trehalose	80	100	100	40	100	100	100	0	100	100	100	100	100	100	100	75	100	100	0	100	100	67	100	100	100
D-Xylose	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	100	0	0	0	100	0
Growth in:																									
0% NaCl	100	0	0	0	100[c]	100	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.1% NaCl	100	0	100	0	100	100	0	0	80	100	0	40	0	0	0	0	0	100	0	0	0	0	0	0	0
0.2% NaCl	100	0	100	0	100	100	0	0	100	100	0	60	100	50	0	100	0	100	0	0	0	0	0	0	0
0.3% NaCl	100	0	100	40	100	100	0	0	100	100	0	80	100	100	100	100	0	100	0	0	0	0	50	0	0
0.4% NaCl	100	80	100	80	100	100	0	20	100	100	80	100	100	100	100	100	0	100	0	8	0	0	50	100	0
0.5% NaCl	100	80	100	80	100	100	83	100	100	100	80	100	100	100	100	100	60	100	0	29	0	0	67	100	40
1.0% NaCl	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	75	88	50	0	100	100	100
2.0% NaCl	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
3.5% NaCl	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
6.0% NaCl	20	100	100	100	100	100	100	100	100	80	100	100	100	100	100	100	100	100	75	100	100	0	100	100	100
8.0% NaCl	0	100	100	40	80	100	67	100	100	0	100	0	0	0	67	0	60	100	0	100	0	0	33	100	100
10.0% NaCl	0	100	100	0	80	100	0	20	40	0	0	0	0	0	0	0	0	40	0	92	0	0	0	100	20
12.0% NaCl	0	100	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	42	0	0	0	100	0
Swarming	0	100	0	0	0	0	33	0	0	0	80	0	0	0	0	0	0	0	0	0	0	0	100	0	0

[a]The strains for *V. fluvialis* and *V. furnissii* grew in 0% NaCl when incubated at 25°C but not at 36°C.

[b]*V. logei* was tested at 15°C.

[c]Each number is the % positive for the test after 48 h of incubation, except for oxidase and reduction of nitrate to nitrite, which were done only at 24 h. All species except *V. logei* were tested at 25°C. All testing was done in the CDC *Vibrio* Reference Laboratory using standardized "enteric" media supplemented with marine cations (Na^+, K^+, and Mg^{++}). Results in this table may differ from those in the original descriptions of organisms due to variables in media, incubation temperature, reading time, and salt content.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

(*continued*)

TABLE BXII.γ.163. *(cont.)*

Characteristic	*V. nereis*	*V. nigripulchritudo*	*V. ordalii*	*V. orientalis*	*V. pectenicida*	*V. pelagius* biogroup 1	*V. pelagius* biogroup 2	*V. penaeicida*	*V. proteolyticus*	*V. scophthalmi*	*V. splendidus* biogroup 1	*V. splendidus* biogroup 2	*V. tapetis*	*V. trachuri*	*V. tubiashii*	Unnamed *Vibrio* group: *Vibrio* Group 509	*Vibrio* Group 510	*Vibrio* Group 511	*Vibrio* Group 512	Baumann Group E3	*Moritella*: *Moritella marina*	*Photobacterium*: *Photobacterium angustum*	*P. iliopiscarium*	*P. leiognathi*	*P. phosphoreum*	*Salinivibrio*: *Salinivibrio costicola*
Number of strains studied	5	3	1	4	2	4	2	1	1	4	4	2	1	1	4	4	3	2	6	1	1	5	2	5	3	5
Oxidase (Kovacs)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	20	0	60	0 [c]	100
Nitrate reduced to nitrite	100	100	0	100	100	100	100	0	100	100	100	100	100	100	100	100	100	100	0	100	100	40	100	100	33	60
Indole production	60	100	0	100	0	25	100	0	100	0	100	50	0	100	100	100	100	0	0	0	0	0	0	0	0	0
Voges–Proskauer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	60	100	20	67	80
Lysine decarboxylase (2 d)	0	0	0	100	0	0	0	0	100	0	0	0	0	100	0	100	0	0	83	0	0	40	100	60	67	0
Arginine dihydrolase (2 d)	80	0	0	25	0	0	0	0	100	0	25	0	0	0	100	0	0	0	0	100	0	100	100	80	100	40
Ornithine decarboxylase (2 d)	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	75	0	0	100	0	0	0	0	0	0	0
Lysine decarboxylase (7 d)	0	0	0	100	0	0	0	0	100	0	0	0	0	100	0	100	0	100	100	0	0	60	100	60	67	0
Arginine dihydrolase (7 d)	100	0	0	50	0	0	0	0	100	0	75	50	0	0	100	0	0	0	0	100	0	100	100	80	100	60
Ornithine decarboxylase (7 d)	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	100	0	0	100	0	0	0	0	0	0	0
Motility	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	100	50	100	33	80
D-Glucose, acid	100	100	100	100	100	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100	20	0	0
Fermentation of:																										
Adonitol	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Arabinose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0	0	0	0	0	0	0
D-Arabitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cellobiose	0	100	0	100	0	0	0	100	100	0	100	100	0	100	100	100	100	50	67	0	0	0	0	0	0	20
Dulcitol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Galactose	0	33	0	0	0	0	33	0	0	25	0	0	0	0	50	100	100	100	100	0	100	0	100	80	100	20
D-Galacturonate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0

[a]The strains for *V. fluvialis* and *V. furnissii* grew in 0% NaCl when incubated at 25°C but not at 36°C.

[b]*V. logei* was tested at 15°C.

[c]Each number is the % positive for the test after 48 h of incubation, except for oxidase and reduction of nitrate to nitrite, which were done only at 24 h. All species except *V. logei* were tested at 25°C. All testing was done in the CDC *Vibrio* Reference Laboratory using "enteric" media supplemented with marine cations (Na^+, K^+, and Mg^{++}). Results in this table may differ from those in the original descriptions of organisms due to variables in media, incubation temperature, reading time, and salt content.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

(*continued*)

TABLE BXII.γ.163. *(cont.)*

Characteristic	*V. nereis*	*V. nigripulchritudo*	*V. ordalii*	*V. orientalis*	*V. pectenicida*	*V. pelagius* biogroup 1	*V. pelagius* biogroup 2	*V. penaeicida*	*V. proteolyticus*	*V. scophthalmi*	*V. splendidus* biogroup 1	*V. splendidus* biogroup 2	*V. tapetis*	*V. trachuri*	*V. tubiashii*	Unnamed *Vibrio* group: *Vibrio* Group 509	*Vibrio* Group 510	*Vibrio* Group 511	*Vibrio* Group 512	Baumann Group E3	*Moritella*: *Moritella marina*	*Photobacterium*: *Photobacterium angustum*	*P. iliopiscarium*	*P. leiognathi*	*P. phosphoreum*	*Salinivibrio*: *Salinivibrio costicola*
myo-Inositol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0
Lactose	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	0	67	0	0	0	0	0	0	0	0	0
Maltose	100	67	100	100	100	100	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	60	100	0	100	80
D-Mannitol	0	0	0	100	0	100	100	0	100	0	100	100	0	100	100	100	100	100	100	100	0	20	0	0	0	60
D-Mannose	20	33	0	100	0	0	100	0	100	100	100	0	100	100	100	100	67	100	100	0	0	100	100	100	100	60
Melibiose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	50	0	0	0	0	0	0	0	0	0	0
α-Methyl-D-glucoside	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	50	100	0	0	0	0	0	0	0
Raffinose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L-Rhamnose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salicin	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0	100	67	0	0	0	0	0	0	0
L-Sorbitol	0	0	0	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0	100	0	0	0	0	0	0	0
Sucrose	100	0	100	100	0	100	67	0	0	100	75	0	0	100	100	100	100	100	100	100	0	60	0	0	0	60
Trehalose	100	100	0	100	100	100	100	0	100	100	75	100	100	100	100	100	100	100	100	100	0	40	50	0	0	80
D-Xylose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	100	0	0	0	0
Growth in:																										
0% NaCl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	0	0	0
0.1% NaCl	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	17	0	0	0	0	0	0	0
0.2% NaCl	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	100	100	100	0	0	0	0	0	0	0
0.3% NaCl	0	0	0	0	0	0	0	0	100	0	0	0	0	0	50	0	100	100	100	0	0	0	0	0	0	0
0.4% NaCl	0	0	0	0	0	0	0	0	100	0	0	0	0	50	50	50	100	100	100	0	0	0	0	0	0	0
0.5% NaCl	0	0	100	25	0	25	0	0	100	0	0	0	0	100	75	100	100	100	100	0	0	0	0	60	33	0
1.0% NaCl	100	100	100	100	0	100	100	100	100	25	75	0	0	100	100	100	100	100	100	100	0	40	50	100	67	40
2.0% NaCl	100	100	100	100		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	100	100	100	100	80
3.5% NaCl	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
6.0% NaCl	100	100	100	100	50	100	100	0	100	100	100	50	100	100	100	100	100	100	100	100	0	100	0	100	67	100
8.0% NaCl	100	0	0	100	0	100	0	0	100	0	50	50	0	100	50	100	100	100	100	100	0	0	0	0	0	100
10.0% NaCl	20	0	0	0	0	0	0	0	100	0	0	0	0	0	0	25	67	100	67	0	0	0	0	0	0	80
12.0% NaCl	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	60
Swarming	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0

[a]The strains for *V. fluvialis* and *V. furnissii* grew in 0% NaCl when incubated at 25°C but not at 36°C.

[b]*V. logei* was tested at 15°C.

[c]Each number is the % positive for the test after 48 h of incubation, except for oxidase and reduction of nitrate to nitrite, which were done only at 24 h. All species except *V. logei* were tested at 25°C. All testing was done in the CDC *Vibrio* Reference Laboratory using "enteric" media supplemented with marine cations (Na^+, K^+, and Mg^{++}). Results in this table may differ from those in the original descriptions of organisms due to variables in media, incubation temperature, reading time, and salt content.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

TABLE BXII.γ.164. Properties of the species and biogroups of *Vibrio*[a]*

Characteristics	*V. cholerae*	*V. aerogenes*	*V. aestuarianus*	*V. alginolyticus*	*V. anguillarum*	*V. campbellii*	*V. cincinnatiensis*	*V. cyclitrophicus*	*V. damsela*	*V. diabolicus*	*V. diazotrophicus*	*V. fischeri*	*V. fluvialis*	*V. furnissii*	*V. gazogenes*	*V. halioticoli*	*V. harveyi*	*V. hollisae*	*V. ichthyoenteri*	*V. logei*	*V. mediterranei*	*V. metschnikovii*	*V. mimicus*	*V. mytili*	*V. natriegens*
Number of strains tested	161	1	9	38	20	44	1	1	21	1	13	12	15	7	1	6	91	16	6	11	4	6	51	5	8
3–12 polar flagella	−	−		−	−	−		−				+	−	−	−	−	−		−	+		−			−
Lateral flagella/solid media	−			+	−	+	−	−			+	−	d	d	−	−	+	−		−		−			−
Swarming on complex media	−	−		+	−	−		−			−	−	−	−	−	−	−		−	−	−	−		−	−
Straight rods[b]	d			+	−	+		d				+	d	−	−		+			+		d			+
Curved rods	d			−	+	−		d				−	d	+	+		−			−		d			−
PHB accumulation	−	−		−	−	−						−	−	d	−	−	−		−	−		−		−	+
Pigment:																									
Yellow-orange	−	−		−	−	−		−				+	−	−	−	−	−			+	−	−		−	−
Blue-black	−			−	−	−		−				−	−	−	−	−	−			−	−	−		−	−
Red	−			−	−	−		−				−	−	−	+	−	−			−	−	−		−	−
Arginine dihydrolase[c]	−	+	+	−	+	−	−	w	+	−	+	−	+	+	−	−	−	−	−	−	−	−	−	+	−
Oxidase	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+
Reduction of NO_3 to NO_2	+	+		+	+	+	+	−	+	+	+	d	+	+	−	+	+	+	+	+	+	−	+	+	+
Luminescence	−[d]	−	−	−	−	−	−	−	−		−	+	−	−	−	−	d			+	d	−		−	−
Gas from D-glucose	−	+	−	−	−	−	−	−	+		−	−	−	+	+	−	−	−	−	−	−	−	−	+	−
Voges–Proskauer	+	−	−	+	+	−	+	−	+		−	−	−	−	−	−	−	−	−	−	−	+	−	−	−
Na^+ required for growth	−	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	d	−	+	+
Organic growth factors required	d	−		−	−	−		−				−	−	−	−	−	−			d		d			−
Growth at:																									
4°C	−	−	+	−	−	−	−	+			+	−	−	−	−	−	−		−	+	−	−		−	−
30°C	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+	−		+		+	+
35°C	+	+	+	+	+	+	+	+	+		+	d	+	+	+		+		−	−		+		+	+
40°C	+	−		+	−	−	−				d	−	+	+	+	−	d		−	−	−	+		−	+
Production of:																									
Alginase	−			−	−	−	−	−				−	−	−	−	+	d		−	−		−		−	−
Amylase	+	+		+	+	+	+				+	−	+	d	+	−	+		−	−	+	+		−	d
Chitinase	+			+	+	+	+	−			−	d	+	+	−	−	+		−	+	d	+		−	−
Gelatinase	+	+	+	+	+	+	−	+	−	+	−	−	+	d	+	−	+	−	−	−	−	+	+	−	d
Lipase	+	+		+	+	+	−	+	−	+	−	+	+	+	+	−	+	−	−	d	+	+	d	+	+
Utilization of:																									
β-Alanine	−			−	−	−						−	−	−	−		−			−		−			d
D-α-Alanine	d			+	+	+	+	+				−	+	+	−		+			−		d			
L-α-Alanine	−	+		+	d	d	+					−	+	+			d			−		+			
Aminobutyrate	−			−	−	−						−	+	+	−	−	−		−	−	−	−		−	+
Aminovalerate	−			−	−	−						−	−	d	−	−	−			−		−		−	+
L-Arabinose	−		−	−	+	−	+	−	−	−		−	+	+	+		d	+		−		−	−		d
Cellobiose	−	+	d	−	+	d	+	+	−	−		+	d	d	+	−	+	−		+		−	−	−	+

Citrate	d	+	+	+	+	d	+		−	+		d	+	+	+		+	−		−		d	+		
Ethanol	−			d	−	−	−					−	+	+	−		−			−		−			
D-Galactose	+	+		d	d	−		+		+		+	+	+	+		d	+	−	+		d	+		d
D-Galacturonate	−			−	−	−						−	+	+	−		−			−		−			
D-Gluconate	+			+	+	−	+			+		−	+	+	−	−	+		−	+	+	+		−	+
D-Glucuronate	−			−	−	−	−	+				−	+	−	−		+			−		−			+
L-Glutamate	+	+		+	+	−	+	+			+	d	+	+	+	−	+			+	+	+		+	+
Glutarate	−			−	−	−						−	−	+			−			−		−			
Heptanoate	−			+	−	−						−	+	+	−		+			−		d			
L-Histidine	d			+	+	−						−	+	d		−	d			−		d		−	+
p-Hydroxybenzoate	−			−	−	−	−	−				−	+	d	−		−			−		−			
Hydroxybutyrate	−	−		−	−	−	−					−	+	d	−		−			−		−			
myo-Inositol	−	+		−	d	−	+		−			−	−	−	−		−	−		−		d	−		
Ketoglutarate	+			+	+	+	+					−	+	+	+		+			−		−			
DL-Lactate	+			+	+	+		+				−	+	+	+		+			−		+			
Lactose	−	−	+	−	−	−	−		−		+	−	−	−	−		−[e]	−		−	+	d	d	+	+
L-Leucine	−			+	−	−	−					−	−	−	−		−		−	−		−			d
DL-Malate	+	−		+	+	d						−	+	+	+		d			−		+			
D-Mannitol	+	+	+	+	+	d	−		−	+		+	+	+	+		+	−		+		+	+		
D-Mannose	d	+	+	d	+	d	+	−	+	+	d	+	+	d	+		+	+		d	+	d	+	+	+
Melibiose	−	−	d	−	−	−			−			−	−	−		−	−	−		−		−	−	−	+
L-Proline	+			+	+	+	+				+	+	+	+	+		+			+	+	+		+	+
Propionate	+			+	+	+		−				−	+	+	+		+			−		−			
Putrescine	−			d	−	−	−					−	d	+	−	−	−		−	−	+	−		−	+
Pyruvate	+			+	+	+		+				−	+	+	+		+			−		+			
Salicin	−		−	−	−	−	+	−	−	−		d	+	−	+	−	d	−				−	−	+	d
L-Serine	d	−		+	+	d		+			d	−	+	+	+		+			d	+	+		+	+
D-Sorbitol	−	−	+	−	+	−	−		−			−	−	−	+		−	−		−		d	−		
Sucrose	+	+	+	+	+	−	+	+	−	+	+	−	+	+	+	−	d	−		−	+	+	−	+	+
Trehalose	+	−	+	+	+	+	+	+	−	+		d	+	+		−	+	−	−	−	−	+	+	−	+
L-Tyrosine	−			+	−	d	−	+				−	+	+	−	−	+	−		−		−	d	+	d
Valerate	−			+	−	−						−	−	−	−		−			−		−			
D-Xylose	−	+	−	−	−	−	+	+	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	+	−
Benzoate, betaine, hippurate, malonate, L-rhamnose, sarcosine, spermine	−			−	−	−						−	−	−	−		−			−		−			+

[a]Symbols: see standard definitions; w, weak; blank, data not determined or not applicable. Precautionary note: limitations of this table for identifying "unknown" cultures. This table is an attempt to revise and update Table 5.58 (pp. 532–533) of the *Vibrio* chapter in the first edition of this *Manual*, in which Baumann et al. studied each of the 20 *Vibrio* species. The data in their Table 5.58 were based on over 500 cultures tested under essentially identical conditions. Authors who described new *Vibrio* species since 1984 did not perform all of these tests and may have used different sets of media and methods. The following are probably the most important variables in the new data: media and methods used, incubation temperature, incubation time (final reading at 1–2 days vs 28 days or more), NaCl or "artificial seawater" added to media to speed growth and enhance metabolism, inoculation method and size, and "endpoint definition" (criteria for coding a test as positive or negative).

[b]Straight rods in exponential phase of growth becoming curved in stationary phase.

[c]Originally defined by Baumann et al. (1984b) as "the anaerobic production of ornithine from arginine." These authors have described the problems associated with the Thornley method, which measures alkali production from arginine.

[d]Luminous strains of this species have been found by Desmarchelier and Reichelt (1981).

[e]Wild-type strains are unable to utilize lactose, but many strains acquire this property by mutation. Most strains ferment lactose in "peptone type" media.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

TABLE BXII.γ.164. *(cont.)*

Characteristics	*V. navarrensis*	*V. nereis*	*V. nigripulchritudo*	*V. ordalii*	*V. orientalis*	*V. parahaemolyticus*	*V. pelagius* biogroup 1	*V. pelagius* biogroup 2	*V. pectenicida*	*V. penaeicida*	*V. proteolyticus*	*V. rumoiensis*	*V. scophthalmi*	*V. splendidus* biogroup 1	*V. splendidus* biogroup 2	*V. tapetis*	*V. trachuri*	*V. tubiashii*	*V. vulnificus* biogroup 1	*V. vulnificus* biogroup 2	*V. vulnificus* biogroup 3	*V. wodanis*	*Moritella marina*	*M. viscosa*	*P. iliopiscarium*	*Salinivibrio costicola*
Number of strains tested	11	6	14	11	5	134	7	4	5	6	1	1	6	4	15	24	3	6	15	3	19	35	1	20	37	2
3–12 polar flagella		−	−			−	−	−		−	−	−		−	−				−			+	−	−		−
Lateral flagella/solid media		−	−			+	−	−	+	−	+	−		−	d				−			−	−	−		−
Swarming on complex media	−	−	−		−	−	−	−	+	−	+		−	−	−	−		−	−			−	−			−
Straight rods		+	+[b]		−	+	+	−			+			−	d				−			d	+	d		−
Curved rods		−	−		+	−	−	+			−			+	d				+			d	−	d		+
PHB accumulation		+	d		+	−	−	−		−	−			−	−				−				−			−
Pigment:																										
Yellow-orange	−	−	−			−	−	−	−		−	−	−	−	−			−	−			+	−	−	−	−
Blue-black	−	−	+			−	−	−	−		−	−	−	−	−			−	−			−	−	−	−	−
Red	−	−	−			−	−	−	−		−	−	−	−	−			−	−			−	−	−	−	−
Arginine dihydrolase[c]	−	+	−	−	+	−	−	−	−	−	+	−	+	+	−	−	−	d	−	−	−		−	−	+	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+		+
Reduction of NO_3 to NO_2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+		+	d
Luminescence	−	−	−		+	−	−	−	−	−	−		−	+	−			−	−	−		−	−	−		−
Gas from D-glucose	−	−	−	−	−	−	−	−	−	−	−		−	−	−			−	−	−		−	−	−	+	−
Voges–Proskauer	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−		−	−	−	+	−
Na^+ required for growth	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Organic growth factors required		−	−		d	−	−	−			−			−	−				−				+			d
Growth at:																										
4°C		d	−		+	−	d	d		−	−	+	−	d	−	+		−	−	−		+	+	+	+	−
30°C	+	+	+		+	+	+	+		+	+	+	+	+	+	−			+			−	−	−	−	+
35°C	+	+	−	−	+	+	+	+		−	+	+		d	−	−		+	+	+		−	−	−	−	+
40°C	+	d	−	−	−	+	−	−		−	+	−	−	−	−	−			+			−	−	−	−	−
Production of:																										
Alginase		−	−		−	−	+	+	+	d	−	−		d	−	−		−	−			d	−	−		−
Amylase	+	−	+	−	+	+	−	+	+	d	+	+	−	+	+	+		+	+	+		+	−	+		−
Chitinase		d	+		+	+	d	+		+	+	+		+	+			+	+				+			−
Gelatinase	+	d	+	+	+	+	−	+	+	+	+	−	−	+	+	+		+	+	+		+	+	+	−	−
Lipase		−	+	−	+	+	+	+	+	+	+	+		+	+	+		+	+	+			+			−
Utilization of:																										
β-Alanine		−	d			−	−	d	−		+			−	−				−				−			−
D-α-Alanine						+													+							+
L-α-Alanine						d													+							+
Aminobutyrate	−	+	−			−	−	+		d	−			−	−	−		−	−				−			−
Aminovalerate	−	+	−			−	−	+			−		−	−	−				−				−			−
L-Arabinose		−	d	−		d	−	d	−		+			−	−				−	−			−			−
Cellobiose	−	+	−	−		−	−	+			−		−	−	−				+	+	−		−			−

Citrate				−		+												+	+	−				−
Ethanol						+												−						−
D-Galactose		+	−	d	−	+	−	−	−	−	−			−	−	−		+	+			−	+	−
D-Galacturonate						−												−						−
D-Gluconate	−	+	−		+	+	+	+		−	+			−		−	d	+				−		+
D-Glucuronate		−	−			d	−	−			−			−	−			+				−		−
L-Glutamate	+	+	+		+	+	+	+			+			+	d	−		+				+		−
Glutarate						−												−						−
Heptanoate						+												−						−
L-Histidine	−	d	+		+	+	d	d	+		+		−	d	d	−		d				−		+
p-Hydroxybenzoate						−												−						−
Hydroxybutyrate						−												−						−
myo-Inositol				−		−												−	−					−
Ketoglutarate						+												+						−
DL-Lactate						+												+						+
Lactose	+	+	+	−	+	−	+	+	−		+			+	+	−		d[c]	+	−		+		−
L-Leucine		+	−		−	+	−	−	−	−	−			−	−	−		−				−		−
DL-Malate						+												d						−
D-Mannitol				+		+												d	−	−				+
D-Mannose	+	d	+		d	+	+	+	−		+			+	d	−		+	+			+		−
Melibiose	−	d	+	−	+	−	d	d	+		+		−	d	d	−		−	+			−		−
L-Proline	+	+	+		+	+	+	+	−		+			+	+	−		+				+		−
Propionate						+												+						+
Putrescine	−	+	−		+	+	+	+		−	+			−		−	d	−				−		−
Pyruvate						+												+						+
Salicin	d	d	−	−	−	−	d	−			−			d	d		+	−	+	−		−		−
L-Serine	+	d	+		d	+	+	+	−		+			+	d	−						+		+
D-Sorbitol				−		−												−	−	−				−
Sucrose	+	+	+	+	+	−	+	+			+			+	d	−		−	−	−		+		+
Trehalose	−	+	−	−		+	−	+		d	−			−	−	−	−	+	+			−		+
L-Tyrosine	d	d	−		−	+	d	−			−			d	d		+	+				−		+
Valerate						−												−						−
D-Xylose	−	−	−	−		−	−	−	−	−	−	+		−	−		−	−	−			−	−	−
Benzoate, betaine, hippurate, malonate, L-rhamnose, sarcosine, spermine		−	−			−	−	−			−			−	−			−				−		−

[a]Symbols: see standard definitions; w, weak; blank, data not determined or not applicable. Precautionary note: limitations of this table for identifying "unknown" cultures. This table is an attempt to revise and update Table 5.58 (pp. 532–533) of the *Vibrio* chapter in the first edition of this *Manual*, in which Baumann et al. studied each of the 20 *Vibrio* species. The data in their Table 5.58 were based on over 500 cultures tested under essentially identical conditions. Authors who described new *Vibrio* species since 1984 did not perform all of these tests and may have used different sets of media and methods. The following are probably the most important variables in the new data: media and methods used, incubation temperature, incubation time (final reading at 1–2 days vs 28 days or more), NaCl or "artificial seawater" added to media to speed growth and enhance metabolism, inoculation method and size, and "endpoint definition" (criteria for coding a test as positive or negative).

[b]Straight rods in exponential phase of growth becoming curved in stationary phase.

[c]Originally defined by Baumann et al. (1984b) as "the anaerobic production of ornithine from arginine." These authors have described the problems associated with the Thornley method, which measures alkali production from arginine.

[d]Luminous strains of this species have been found by Desmarchelier and Reichelt (1981).

[e]Wild-type strains are unable to utilize lactose, but many strains acquire this property by mutation. Most strains ferment lactose in "peptone type" media.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

TABLE BXII.γ.165. Biochemical test results and other properties of the 12 *Vibrio* species that occur in human clinical specimens[a]*

Test	Percentage positive[b]											
	V. cholerae	*V. alginolyticus*	*V. cincinnatiensis*	*V. damsela*	*V. fluvialis*	*V. furnissii*	*V. harveyi*	*V. hollisae*	*V. metschnikovii*	*V. mimicus*	*V. parahaemolyticus*	*V. vulnificus*
Indole production (HIB, 1% NaCl)[c]	99	85	8	0	13	11	100	97	20	98	98	97
Methyl red (1% NaCl)	99	75	93	100	96	100	100	0	96	99	80	80
Voges–Proskauer (1% NaCl; Barritt)[c]	75	95	0	95	0	0	50	0	96	9	0	0
Citrate, Simmons	97	1	21	0	93	100	0	0	75	99	3	75
H_2S on TSI	0	0	0	0	0	0	0	0	0	0	0	0
Urea hydrolysis	0	0	0	0	0	0	0	0	0	1	15	1
Phenylalanine deaminase	0	1	0	0	0	0	NG	0	0	0	1	35
Arginine, Moellers, (1% NaCl)[c]	0	0	0	95	93	100	0	0	60	0	0	0
Lysine, Moellers, (1% NaCl)[c]	99	99	57	50	0	0	100	0	35	100	100	99
Ornithine, Moellers, (1% NaCl)[c]	99	50	0	0	0	0	0	0	0	99	95	55
Motility, (36°C)	99	99	86	25	70	89	0	0	74	98	99	99
Gelatin hydrolysis, (1% NaCl, 22°C)	90	90	0	6	85	86	0	0	65	65	95	75
KCN test (percentage that grow)	10	15	0	5	65	89	0	0	0	2	20	1
Malonate utilization	1	0	0	0	0	11	0	0	0	0	0	0
D-Glucose, acid production [c]	100	100	100	100	100	100	50	100	100	100	100	100
D-Glucose, gas production [c]	0	0	0	10	0	100	0	0	0	0	0	0
Acid production from:												
D-Adonitol	0	1	0	0	0	0	0	0	0	0	0	0
L-Arabinose [c]	0	1	100	0	93	100	0	97	0	1	80	0
D-Arabitol [c]	0	0	0	0	65	89	0	0	0	0	0	0
Cellobiose[c]	8	3	100	0	30	11	50	0	9	0	5	99
Dulcitol	0	0	0	0	0	0	0	0	0	0	3	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0
D-Galactose	90	20	100	90	96	100	0	100	45	82	92	96
Glycerol	30	80	100	0	7	55	0	0	100	13	50	1
myo-Inositol	0	0	100	0	0	0	0	0	40	0	0	0
Lactose[c]	7	0	0	0	3	0	0	0	50	21	1	85
Maltose[c]	99	100	100	100	100	100	100	0	100	99	99	100
D-Mannitol [c]	99	100	100	0	97	100	50	0	96	99	100	45
D-Mannose	78	99	100	100	100	100	50	100	100	99	100	98
Melibiose	1	1	7	0	3	11	0	0	0	0	1	40
α-Methyl-D-glucoside	0	1	57	5	0	0	0	0	25	0	0	0
Raffinose	0	0	0	0	0	11	0	0	0	0	0	0
L-Rhamnose	0	0	0	0	0	45	0	0	0	0	1	0
Salicin[c]	1	4	100	0	0	0	0	0	9	0	1	95
D-Sorbitol	1	1	0	0	3	0	0	0	45	0	1	0
Sucrose[c]	100	99	100	5	100	100	50	0	100	0	1	15
Trehalose	99	100	100	86	100	100	50	0	100	94	99	100
D-Xylose	0	0	43	0	0	0	0	0	0	0	0	0
Mucate-acid production	1	0	0	0	0	0	0	0	0	0	0	0
Tartrate-Jordan	75	95	0	0	35	22	50	65	35	12	93	84
Esculin hydrolysis	0	3	0	0	8	0	0	0	60	0	1	40
Acetate utilization	92	0	14	0	70	65	0	0	25	78	1	7
Nitrate reduced to nitrate[c]	99	100	100	100	100	100	100	100	0	100	100	100
Oxidase[c]	100	100	100	95	100	100	100	100	0	100	100	100
DNase (25°C)	93	95	79	75	100	100	100	0	50	55	92	50
Lipase[c]	92	85	36	0	90	89	0	0	100	17	90	92
ONPG test[c]	94	0	86	0	40	35	0	0	50	90	5	75
Yellow pigment at 25°C	0	0	0	0	0	0	0	0	0	0	0	0
Tyrosine clearing	13	70	0	0	65	45	0	3	5	30	77	75
Growth in nutrient broth with:												
0% NaCl[c]	100	0	0	0	0	0	0	0	0	100	0	0
1% NaCl[c]	100	99	100	100	99	99	100	99	100	100	100	99
6% NaCl[c]	53	100	100	95	96	100	100	83	78	49	99	65
8% NaCl[c]	1	94	62	0	71	78	0	0	44	0	80	0
10% NaCl[c]	0	69	0	0	4	0	0	0	4	0	2	0
12% NaCl[c]	0	17	0	0	0	0	0	0	0	0	1	0
Swarming (marine agar, 25°C)	−	+	+	−	−	−	100	−	−	−	+	−
String test	100	91	80	80	100	100	100	100	100	100	64	100
O/129, zone of inhibitionc	99	19	25	90	31	0	100	40	90	95	20	98
Polymyxin B, % with a zone of inhibition	22	63	92	85	100	89	100	100	100	88	54	3

[a]Symbols: +, most strains (generally about 90–100%) positive; −, most strains negative (generally about 0–10% positive); 1% NaCl in parentheses indicates 1% NaCl has been added to the standard media to enhance growth; HIB, heart infusion broth; TSI, triple sugar iron agar; ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

[b]The number gives the percentage positive after 48 h of incubation at 36°C (unless other conditions are indicated). Most of the positive reactions occur during the first 24 hours. NG (no growth) means that the organism does not grow, probably because the NaCl concentration is too low.

[c]Tests recommended as part of the routine set for *Vibrio* identification.

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

TABLE BXII.γ.166. Differential tests that divide the 12 *Vibrio* species found in clinical specimens into six groups[a,b]*

	Group 1		Group 2	Group 3	Group 4	Group 5			Group 6			
Test	*V. cholerae*	*V. mimicus*	*V. metschnikovii*	*V. cincinnatiensis*	*V. hollisae*	*V. damsela*	*V. fluvialis*	*V. furnissii*	*V. alginolyticus*	*V. harveyi*	*V. parahaemolyticus*	*V. vulnificus*
Growth in nutrient broth with no NaCl added	+	+	−	−	−	−	−	−	−	−	−	−
Oxidase			−	+	+	+	+	+	+	+	+	+
Nitrate reduced to nitrite			−	+	+	+	+	+	+	+	+	+
myo-Inositol fermentation	−	−	d	+	−	−	−	−	−	−	−	−
Arginine dihydrolase					−	+	+	+	−	−	−	−
Lysine decarboxylase					−				+	+	+	+

[a]All data are for reactions within 2 days at 35–37°C, unless otherwise specified.

[b]Symbols: +, most strains (generally about 90–100%) positive; d, strain-to-strain variation (generally about 25–75% positive); −, most strains negative (generally about 0–10% positive).

**Editorial Note: Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

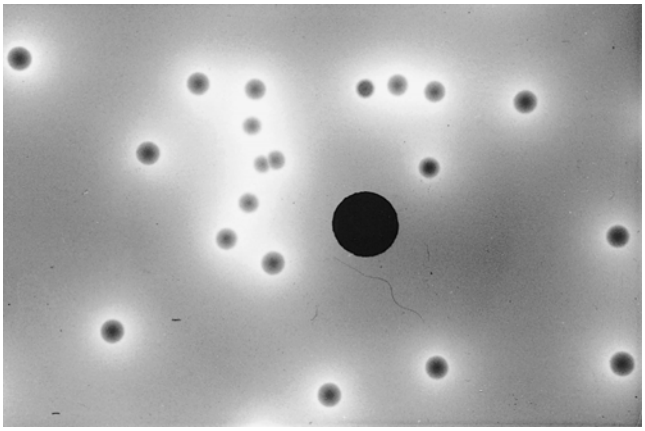

FIGURE BXII.γ.173. Colonies of *Vibrio cholerae* on TCBS agar. Yellow colonies, 2- to 3-mm in diameter are present after overnight incubation at 36°C. (Reproduced with permission from J.J. Farmer III and F.W. Hickman-Brenner, *In* Balows, Trüper, Dworkin, Harder and Schleifer (Editors), The Prokaryotes: A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications, 2nd Edition, Volume III, ©Springer-Verlag, New York, 1992, pp. 2952–3011.)

TABLE BXII.γ.168. Properties and differentiation of the classical and El Tor biogroups of *V. cholerae* serogroup O1[a]

	Biogroup	
Tests or property	Classical	El Tor
Frequency of isolation:		
On the Indian subcontinent	Occasional[b]	Common
In the rest of the world	Very rare	Common
Differential tests:		
Hemolysis of red blood cells	−	+
Voges–Proskauer test	−	+
Inhibition by polymyxin B (50-unit disks)	+	−
Agglutination of chicken red blood cells	−	+
Lysis by bacteriophage:		
Classical IV	+	−
FK	+	−
El Tor 5	−	+

[a]Symbols +, most strains (generally about 90–100%) positive; −, most strains negative (generally about 0–10 % positive).

[b]The classical biotype has reappeared on the Indian subcontinent, and has been isolated in some locations there.

TABLE BXII.γ.167. Subdivision of *Vibrio cholerae* below the level of species

Serogroup	Serotype	Biogroup
V. cholerae serogroup O1[a]	Inaba	Classical
	Inaba	El Tor
	Ogawa	Classical
	Ogawa	El Tor
V. cholerae serogroup O139		
V. cholerae "non-O1, non-O139" (≈150 serogroups)		

[a]A third serotype of serogroup O1 named Hikojima is recognized by a few authors.

TABLE BXII.γ.169. Key characteristics of *Vibrio cholerae* and *V. mimicus* and tests for their differentiation[a]

Tests or property	*V. cholerae*	*V. mimicus*
Frequency of isolation	Very common	Occasional
Oxidase	+	+
Growth in nutrient broth with:		
No added NaCl	+	+
1% NaCl	+	+
Lysine decarboxylase	+	+
Arginine dihydrolase	−	
Ornithine decarboxylase	+	+
Differentiation of the species:		
Sucrose fermentation	+[b]	−
Lipase (corn oil)	+	−
Voges–Proskauer	+[c]	−
Lactose fermentation (1–2 days)	−	d
Lactose fermentation (3–7 days)	d	d

[a]All data are for reactions within 2 days at 35–37°C unless otherwise specified. Symbols: +, 90–100% positive; d, 11–89% positive; −, 0–10% positive.

[b]Most of the positive reactions occur during the first 24 h of incubation

[c]Almost all strains of *V. cholerae* non-O1 and most strains of *V. cholerae* O1currently isolated are of the El Tor biogroup, which is almost always positive for the Voges–Proskauer test. The classical biogroup is negative for the Voges–Proskauer test.

3. **Vibrio aestuarianus** Tison and Seidler 1983, 699[VP]
aes.tu.a.ri.a′ nus. L. n. *aestuarium* estuary; N.L. masc. adj. *aestuarianus* pertaining to an estuary.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Straight or curved rods 0.5 × 1.5–2.0 μm; motile by a single polar flagellum. Colonies are β-hemolytic on sheep blood agar.

Isolated from estuary water, shellfish, and crabs off the coast of Oregon, USA. The type strain was isolated from an oyster.

Based on the results of DNA–DNA hybridization experiments, strains of *Vibrio aestuarianus* are highly related to the type strain (Tison and Seidler, 1983; Brenner et al., unpublished) and less than 25% related to other *Vibrio* species, including *V. anguillarum*, *V. nereis*, and *V. splendidus* which have similar phenotypic characteristics. The closest relative is *Vibrio anguillarum* to which it is 30–41% related (Brenner et al., personal communication). Fig. BXII.γ.171 illustrates the relationship of *Vibrio aestuarianus* to other *Vibrio* species, based on 16S rRNA gene sequence analysis (Brenner, personal communication).

The mol% G + C of the DNA is: 43–44 (T_m).

Type strain: OY-0-002, ATCC 35048.

GenBank accession number (16S rRNA): X74689.

4. **Vibrio alginolyticus** (Miyamoto, Nakamura and Takizawa 1961) Sakazaki 1968, 360[AL] (*"Oceanomonas alginolytica"* Miyamoto, Nakamura and Takizawa 1961, 481; *Beneckea alginolytica* (Miyamoto, Nakamura and Takizawa 1961, 481) Baumann, Baumann and Mandel 1971a, 289.)
al.gi.no.ly′ti.cus. L. fem. n. *alga* seaweed; M.L. adj. *alginolyticus* pertaining to alginic acid from seaweed; Gr. adj. *lyticus* dissolving; M.L. adj. *alginolyticus* alginic acid-dissolving.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. The morphology is illustrated in Figs. BXII.γ.159 and BXII.γ.162. Characters that differentiate *V. alginolyticus* from *V. harveyi*, *V. parahaemolyticus*, and *V. vulnificus* are given in Table BXII.γ.170. Characters that distinguish *V. alginolyticus* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165 and BXII.γ.166.

Occurs in human clinical specimens, particularly in soft tissue infections (Table BXII.γ.157) and in the marine environment.

Many reports describe the isolation of *V. alginolyticus* from soft tissue infections (Rubin and Tilton, 1975; Pien et al., 1977). Wound infections and ear infections are usually mentioned, with eye infections mentioned much less frequently. There is a clear association of *V. alginolyticus* with infection at these sites; however, the etiological role of *V. alginolyticus* has rarely been shown conclusively and little has been reported about the pathogenesis of these infections. Most authors list *V. alginolyticus* as a pathogen, particularly of wound and ear infections. Antibiotic treatment has been used in most cases; surgical debridement has been used in some.

The mol% G + C of the DNA is: 45–47 (T_m, Bd).

Type strain: 118, ATCC 17749, CCM 2578, DSM 2171, IMET 11295.

GenBank accession number (16S rRNA): X56576, X74690.

5. **Vibrio anguillarum** Bergman 1909, 28[AL] (*Listonella anguillarum* (Bergman 1909) MacDonell and Colwell 1986, 354; *Listonella anguillara* (sic) (Bergman 1909) MacDonell and Colwell 1985, 180.)*
an.guil.la′rum. L. n. *anguilla* eel; L. gen. pl. n. *anguillarum* of eels.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163, BXII.γ.164, and BXII.γ.171. In contrast to *V. ordalii*, whose strains belong only to O antigen group 2, *V. anguillarum* strains belong to O antigen groups 1–16.

Based on the results of DNA–DNA hybridization experiments, the type strain of *V. anguillarum* is closely related to other strains of this species. The closest relative is *V. ordalii* (Schiewe et al., 1981; also see Fig. BXII.γ.169, based on Brenner et al., 1983b) to which it is 58–76% related with T_m values of 2.5–4.0 (Brenner et al., unpublished). This close relationship has been confirmed by the structural similarity of superoxide dismutase (Fig. BXII.γ.170) and by 16S rRNA gene sequencing (Fig. BXII.γ.171). Although *V. an-*

**Editorial Note:* In the last edition of this *Manual*, Baumann et al. (1984b) used the names *V. anguillarum* biovar I and *V. anguillarum* biovar II. In this edition, these organisms are classified as *V. anguillarum* and *V. ordalii*, respectively. *V. anguillarum* has also been known as *Vibrio anguillarum* biotype 1 (Schiewe et al., 1977), *Beneckea anguillara* biotype I (Baumann et al., 1978), *Vibrio anguillarum* biotype I (Baumann et al., 1980b), and *Vibrio anguillarum* biovar I (Baumann et al., 1984b). In addition, the name *Listonella anguillarum* has standing in the nomenclature (MacDonell and Colwell, 1985); see description of *V. pelagius*.

TABLE BXII.γ.170. Differentiation of the arginine-negative, lysine-positive species *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus*, and *V. harveyi* [a]

Test or property	*V. alginolyticus*	*V. harveyi*	*V. parahaemolyticus*	*V. vulnificus*
Voges–Proskauer test	+	−	−	−
Growth in nutrient broth with:				
8% NaCl	+	−	(+)	−
10% NaCl	d	−	−	−
Fermentation of:				
Sucrose	+	+	−	(−)
Salicin	−	−	−	+
Cellobiose	−	−	−	+
Lactose	−	−	−	(+)
L-Arabinose	−	−	(+)	−
Swarming (marine agar, 25°C)	+	+	+	−
Size of zone of inhibition around:				
Colistin	Large	Small	Large	Small
Ampicillin	Small	Small	Small	Large
Carbenicillin	Small	Small	Small	Large

[a]All data for biochemical reactions are within 2 days at 35–37°C. Symbols: +, 90–100% positive, (+), 75–89.9% positive; d, 25.1–74.9% positive; (−), 10.1–25% positive; −, 0–10% positive.

guillarum and *V. ordalii* are closely related in a phylogenetic sense, they differ considerably in phenotypic properties (Table BXII.γ.171). *Vibrio anguillarum* is more versatile biochemically, has more diverse O antigens, and is less fastidious.

The mol% G + C of the DNA is: 44–46 (T_m, Bd).

Type strain: ATCC 19264, IFO 13266, LMG 4437, NCCB 72050.

GenBank accession number (16S rRNA): X16895.

6. **Vibrio campbellii** (Baumann, Baumann and Mandel 1971a) Baumann, Baumann, Bang and Woolkalis 1981, 217[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*Beneckea campbellii* Baumann, Baumann and Mandel 1971a, 288.)
camp.bel′li.i. M.L. gen. n. *campbelli* named to honor L.L. Campbell, an American bacteriologist.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. The cell morphology is illustrated in Fig. BXII.γ.174.

Occurs in ocean water some distance from the cost of Hawaii (Baumann et al., 1971a) and in open waters of the Atlantic ocean (Grimes et al., 1986).

Phylogenetically, *Vibrio campbellii* is one of the marine *Vibrio* species (Figs. BXII.γ.170 and BXII.γ.171). By DNA–DNA hybridization, it is most closely related to *V. harveyi* (61–74%); this result agrees with the close relationship indicated by 16S rRNA gene sequence analysis (Fig. BXII.γ.171). Based on the results of DNA–DNA hybridization experiments *V. campbellii* is 40–57% related to several of the marine *Vibrio* species (Reichelt et al., 1976; Brenner et al., 1983b).

TABLE BXII.γ.171. Properties and differentiation of *Vibrio anguillarum* and *V. ordalii*[a]

Test or property	*V. anguillarum*	*V. ordalii*
Number of strains studied	12	11
O antigens represented	O1–O16	O2 only
Colony size on TSA after 48 hours	3–5 mm	Barely visible
Growth at 37°C	92	0
Growth at 25°C	100	100
Growth at 22°C	100	100
Growth at 15°C	100	100
Growth in 0% NaCl	0	0
Growth in 0.5% NaCl	100	100
Growth in 3% NaCl	100	100
Growth in 7% NaCl	0	0
Indole production	58	0
Voges–Proskauer	100	0
Arginine dihydrolase	100	0
Nitrate reduction to nitrite	100	66
Citrate utilization (Simmons)	75	0
Citrate utilization (Christensen)	100	0
Starch hydrolysis	100	0
ONPG test	100	0
Lipase	100	0
L-Arabinose fermentation	58	0
Cellobiose fermentation	92	0
D-Galactose fermentation	100	55
Glycerol fermentation	100	0
D-Sorbitol fermentation	100	0
Trehalose fermentation	100	0

[a]Data are summarized from Table 2 of Schiewe et al. (1981). Conditions: incubation at 22°C; media were supplemented with NaCl to final concentration of 1.5%; final reading for negative tests was 14 days. Each number gives the percentage of strains that were positive for the test.

The mol% G + C of the DNA is: 46–48 (T_m, Bd).

Type strain: ATCC 25920, IFO 15631, LMG 11216, NCCB 73002.

GenBank accession number (16S rRNA): X56575.

7. **Vibrio cincinnatiensis** Brayton, Bode, Colwell, MacDonell, Hall, Grimes, West and Bryant 1986a, 355[VP] (Effective publication: Brayton, Bode, Colwell, MacDonell, Hall, Grimes, West and Bryant 1986b, 107.)
cin.cin.nat.i.en′sis. L. masc. adj. *cincinnatiensis* derived from Society of Cincinnati, from which the city of Cincinnati, Ohio, was named.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Characters that distinguish *V. cincinnatiensis* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.165 and BXII.γ.166. Small rods approximately 0.7–2.0 μm; motile; single polar flagellum. Colonies on nutrient agar are 1–2 mm in diameter, round, smooth, glossy, and cream colored. Grows poorly on TCBS medium (Table BXII.γ.160), but isolates from extraintestinal clinical specimens should grow on one of the non-selective plating media such as blood agar. Requires NaCl for growth (Tables BXII.γ.163 and BXII.γ.165). Ferments *myo*-inositol (Table BXII.γ.163).

Rarely isolated from human clinical specimens.

This organism was isolated by Brayton et al. (1986b) from blood and cerebral spinal fluid of a 70-year-old man with no known contact with seafood or saltwater. Biochemical testing and the 5S rRNA sequence led to the conclusion that this was a new species, which they named *V. cincinnatiensis*. Comparative 5S rRNA sequence analysis indicated that *V. cincinnatiensis* shared a recent ancestor with *V. gazogenes* (98% homology), but this postulated relationship has not been confirmed by other techniques including 16S rRNA gene sequencing (Fig. BXII.γ.171).

The mol% G + C of the DNA is: 45.0.

Type strain: ATCC 35912, CIP 104173, LMG 789.

GenBank accession number (16S rRNA): X74698.

FIGURE BXII.γ.174. Electron micrograph of *Vibrio campbellii* illustrating the appearance of the tubular appendages that are also observed in some other marine vibrios. Negatively stained preparation. × 36,000. (Reproduced with permission from R.D. Allen and P. Baumann, Journal of Bacteriology *107:* 295–302, 1971, ©American Society for Microbiology.)

8. **Vibrio cyclitrophicus** Hedlund and Staley 2001, 65[VP] (*Vibrio cyclotrophicus* (sic) Hedlund and Staley 2001, 65.)
cy′ cli.tro′ phi.cus. Gr. n. *kyklos* circle or ring; Gr. adj. *trophikos* pertaining to food.

Properties of *V. cyclitrophicus* are given in Table BXII.γ.164. Rods 0.6 × 1.5–2.5 μm. Motile by 1–2 polar or subpolar flagella. Grows in media containing ≥ 1.75% NaCl. Utilizes some polycyclic aromatic hydrocarbons as carbon and energy sources.

The type strain was isolated from creosote-contaminated marine sediment, Eagle Harbor, Puget Sound, Washington, USA, in 1993.

An analysis of the 16S rDNA sequences of 10 *Vibrio* species indicated that the closest relative of *V. cyclitrophicus* was *V. splendidus* (Hedlund and Staley, 2001). Fig. BXII.γ.171 illustrates the relationship of *V. cyclitrophicus* to other species of *Vibrio*.

The mol% G + C of the DNA is: 39 (T_m).
Type strain: P-2P44, ATCC 700982, CIP 106644.
GenBank accession number (16S rRNA): U57919.

9. **Vibrio damsela** Love, Teebken-Fisher, Hose, Farmer, Hickman, and Fanning 1982, 267[VP] (Effective publication: Love, Teebken-Fisher, Hose, Farmer, Hickman, and Fanning 1981, 1140.)*
dam.sel′ a. M.L. n. *damsela* from the modern zoological term damselfish.

Classified as *Listonella damsela* by MacDonell and Colwell (1985, 1986) and as *Photobacterium damselae* by Smith et al. (1991); Trüper and De' Clari (1997) corrected spelling of the species epithet of *Photobacterium damselae* to *damselae*.

Morphological, growth, physiological and biochemical characteristics are listed in Table BXII.γ.163. Biochemical and other phenotypic characteristics useful for identification are listed in Table BXII.γ.164. Additional characteristics are listed in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165, BXII.γ.166, and BXII.γ.173. Causes skin ulcers on the blacksmith *Chromis punctipinnis* (a damselfish) and causes human wound infections. Isolated from marine algae, which may be important in disease transmission to fish.

V. damsela was described by Love et al. (1981) who isolated the type strain from skin lesions on *Chromis punctipinnis* off the California coast. Koch's postulates were fulfilled when it was documented that this organism alone caused the fish lesions. Strains of *V. damsela* were then compared to a large collection of unidentified *Vibrio* strains from human clinical specimens, which revealed that *V. damsela* had occurred in human infections (Love et al., 1981). Morris et al. (1982) reviewed the case histories of six patients with wound infections positive for *V. damsela*. All had been acquired in coastal areas. *V. damsela* appears to cause human wound infections, but its causal role needs to be further documented. Most of the clinical isolates have been from wounds, and 9 of 10 were from leg or foot wounds (Vibrio Laboratory, CDC, unpublished). Strains were also from marine animals, particularly marine fish. Other sources include: fish from Senegal, sewage, oysters, and a wound culture from a raccoon (Vibrio Laboratory, CDC, unpublished). By DNA–DNA hybridization, strains of *V. damsela* are 76–97% related to the type strain and only 0–26% related to all other *Vibrio* species (Brenner et al., personal communication). Based on 16S rDNA sequencing *V. damsela* is more closely related to several *Photobacterium* species than to *Vibrio cholerae*.

The mol% G + C of the DNA is: 43 (Bd).
Type strain: CDC 2588-80, ATCC 33539, DSM 7482.
GenBank accession number (16S rRNA): X74700.

10. **Vibrio diabolicus** Raguénès, Christen, Guezennec, Pignet and Barbier 1997a, 994[VP]
di.a.bol′ i.cus. L. adj. *diabolicus* devilish, diabolic.

The morphological, growth, physiological and biochemical characteristics are listed in Tables BXII.γ.154 and BXII.γ.164. Small cells 0.8 × 2.2 μm, motile via a single polar flagellum in liquid media. Produce swarming colonies on solid media; the cells from these colonies have lateral flagella. The organisms synthesize a novel exopolysaccharide that contains high levels of uronic acids and hexosamines when grown in batch culture with glucose.

Only a single strain of this species has been described. This strain was isolated from the polychaete annelid *Alvinella pompejana* from a deep-sea (2600 m) hydrothermal vent in the East Pacific Rise and was found during a search for novel exopolysaccharides of marine origin.

Based on DNA–DNA hybridization experiments Raguénès et al., 1997a found that the closest relatives of *V. diabolicus* were *V. mytili* (27% relatedness) and *V. nereis* (15%). The closest relatives indicated by the results of 16S rRNA gene sequence analysis were *V. mytili* (98.9%), *V. alginolyticus* (98.9%), *V. pelagius* (98.9%), *V. parahaemolyticus* (98.8%), *V. natriegens* (98.7%), *V. nereis* (98.5%), *V. campbellii* (98.5%), and *V. tubiashii* (98.4%). The authors concluded that "*Vibrio diabolicus* belongs to a well-defined monophyletic unit that also includes *V. tubiashii*, *V. nereis*, and *V. mytili*." Fig. BXII.γ.171 illustrates the relationship of *V. diabolicus* to other *Vibrio* species based on 16S rRNA gene sequence analysis.

The mol% G + C of the DNA is: 49.6 (T_m).
Type strain: HE800, CNCM I-1629.
GenBank accession number (16S rRNA): X99762.

11. **Vibrio diazotrophicus** Guerinot, West, Lee and Colwell 1982, 356[VP]
di.a.zo.tro′ phi.cus. Gr. prefix *di* two, double; N.L. n. *azotum* nitrogen; Gr. n. *trophus* one that feeds; L. suffix *icus* relating to; M.L. adj. *diazotrophicus* one that feeds on dinitrogen, or less literally, nitrogen fixing.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Small cells 0.5 × 1.5–2.0 μm. Motile by means of a sheathed polar flagellum when grown in broth. Cells grown on solid media produce unsheathed lateral flagella with a short wavelength. The organisms fix nitrogen.

Distributed throughout estuarine and marine environments. Isolated from the intestinal tract of sea urchins, river water, Chesapeake Bay water, ditch water and sediment, and the surface of reeds growing in a drainage ditch.

This organism may occur in human clinical specimens.

*Editorial Note: *Photobacterium damselae* subsp. *damselae* is a junior objective synonym of *Vibrio damsela*.

Culture 2550-90 sent to the *Vibrio* Reference Laboratory was isolated from human blood and was reported as "*Vibrio diazotrophicus* group" (*Vibrio* Reference Laboratory, CDC, unpublished). If confirmed, it would be the first clinical isolate of this species.

Based on DNA–DNA hybridization experiments, Guerinot et al. (1982) found that four strains were 88–100% related to the type strain; however, one strain was only 62% related to the type strain. Also based on DNA–DNA hybridization experiments, Brenner et al. (unpublished) found that strains of *V. diazotrophicus* were highly related to the type strain (77–84% related), but distantly related to all other named *Vibrio* species. Unnamed "*Vibrio* group 510" was the closest relative (65–74% related).

The mol% G + C of the DNA is: 45.9–47.2 (T_m, Bd).
Type strain: NS1, ATCC 33466, DSM 2604, IAM 14402.
GenBank accession number (16S rRNA): X56577, X74701.

12. **Vibrio fischeri** (Beijerinck 1889) Lehmann and Neumann 1896, 342[AL] (*Photobacterium fischeri* Beijerinck 1889, 402.)
fisch′ er.i. M.L. gen. n. *fischeri* named to honor Bernhard Fischer, one of the earliest microbiologists to study bioluminescent bacteria.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. The cell morphology is as depicted in Figs. BXII.γ.161, BXII.γ.165, and BXII.γ.175. Produces a cell-associated yellow-orange pigment.

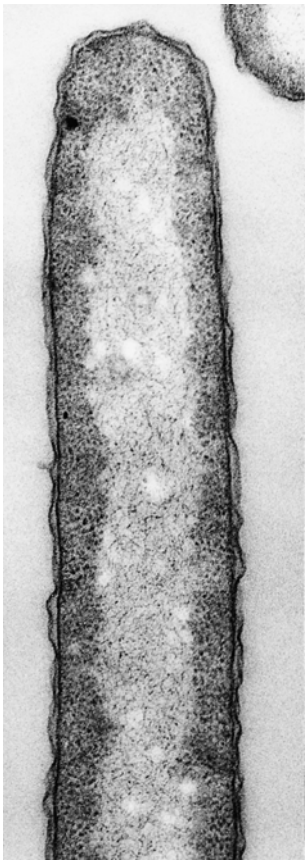

FIGURE BXII.γ.175. Ultrathin section of *Vibrio fischeri*. Exponential growth phase in yeast extract broth. × 40,000. (Courtesy of S.W. Watson.)

Isolated from seawater and marine animals.

Phenotypically and phylogenetically, *Vibrio fischeri* is closely related to *Vibrio logei* (Tables BXII.γ.163 and BXII.γ.164; Figs. BXII.γ.170 and BXII.γ.171). Based on DNA–DNA hybridization experiments, the two species are 36% related (Brenner et al., unpublished)

The mol% G + C of the DNA is: 39–41 (T_m, Bd).
Type strain: ATCC 7744 (strain 398, DSM 507.
GenBank accession number (16S rRNA): X70640.

13. **Vibrio fluvialis** Lee, Shread, Furniss and Bryant 1981c, 217[VP] (Effective publication: Lee, Shread, Furniss and Bryant 1981b, 92.)
flu.vi.a′ lis. L. adj. *fluvialis* of or belonging to a river.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Characters that distinguish *V. fluvialis* from *V. furnissii* are given in Tables BXII.γ.172 and BXII.γ.173. Characters that distinguish *V. fluvialis* from *Vibrio damsela* and *Aeromonas* spp. are given in Table BXII.γ.173. Characters that distinguish *V. fluvialis* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165 and BXII.γ.166.

Causes sporadic cases of human diarrhea and occasional outbreaks. Also isolated from natural waters (often from estuaries) and animals that live there.

Lee et al. (1981b) gave the name *Vibrio fluvialis* to a group of halophilic vibrios that had been previously been known as "marine aeromonads" and "Group F" in the United Kingdom (Furniss et al., 1977) and as "Group EF6" at the Special Bacteriology Laboratory, CDC (Huq et al., 1980). These organisms had been isolated from a number of sources throughout the world and from humans with diarrhea. Lee et al. (1981b) defined two biogroups in this species based on the source of isolation and the results of certain biochemical tests. *V. fluvialis* biogroup I strains did not produce gas during fermentation and were isolated from cases of human diarrhea as well as from the environment. *V. fluvialis* biogroup II strains produced gas during fermentation and were isolated from the environment, but not from humans with diarrhea. Brenner et al. (1983c) later used DNA–DNA hybridization to show that strains of *V.*

TABLE BXII.γ.172. Differentiation of *Vibrio fluvialis* and *V. furnissii*

Test or property	Percentage positive for: *V. fluvialis*	*V. furnissii*
Simple tests:[a]		
Gas production during fermentation	0	99
Esculin hydrolysis	72	0
Carbon source utilization:[b]		
Citrulline	97	4
D-Glucuronic acid	94	4
Putrescine	31	100
δ-Aminovalerate	0	63
Cellobiose	63	4
Glutaric acid[c]	−	+

[a]For the two simple tests, each number gives the percentage positive after 48h incubation at 35–37°C. Most of the positive reactions occur during the first 24 hours.

[b]Data from Lee et al. (1981b) except for glutaric acid, which is from Baumann et al. (1984b). These carbon-source utilization tests are usually done in research laboratories rather than in public health or clinical laboratories.

[c]Percentage data not available.

TABLE BXII.γ.173. Differentiation of the arginine-positive species *V. fluvialis*, *V. furnissii*, and *V. damsela* and comparison with *Aeromonas*[a]

Test or property	*V. damsela*	*V. fluvialis*	*V. furnissii*	*Aeromonas*
Growth in nutrient broth with:				
No added NaCl	−	−	−	+
1% NaCl	+	+	+	+
6% NaCl	+	+	+	−
Voges–Proskauer test	+	−	−	d
Citrate, Simmons	−	+	+	d
Fermentation of:				
D-Galacturonate	−	+	+	−
L-Arabinose	−	+	+	d
D-Mannitol	−	+	+	+
Sucrose	−	+	+	(+)
Gas production during fermentation	d	−	+	d

[a]All data are for reactions within 2 days at 35–37°C unless otherwise specified. Symbols: +, 90–100% positive, (+), 75–89.9% positive; d, 25.1–74.9 positive; −, 0–10% positive.

fluvialis biogroup II were related to strains of *V. fluvialis* biogroup I, but they were sufficiently different to be a new species, which was named *V. furnissii*.

The sources of early clinical isolates of *V. fluvialis* showed a marked association with diarrhea (Lee et al., 1981b), and further studies have strengthened its causative role. One difficulty in assessing the clinical symptoms and epidemiology of *V. fluvialis* has been the presence of other possible pathogens; most reports have come from geographical areas where several possible pathogens are often present in feces. Gastroenteritis associated with *V. fluvialis* is usually described as "cholera-like". Symptoms include watery diarrhea with vomiting (97% of patients), abdominal pain (75%), moderate to severe dehydration (67%), and fever (35%). Usually infants, children, and young adults are affected. Frank blood is found in a small percentage of stool samples, but red or white blood cells are found in most cases (75%). The pathogenesis of *V. fluvialis* diarrhea is beginning to be studied. Assays for heat-labile and heat-stable enterotoxin (LT and ST) and invasiveness have generally been negative. About 20% of isolates give a positive rabbit ileal loop test for enterotoxin. Recent studies have provided additional evidence for enterotoxin or for "enterotoxin-like" molecules (Lockwood et al., 1982; Nishibuchi et al., 1983).

The mol% G + C of the DNA is: 49.3–50.3 (T_m, Bd).

Type strain: VL 5125, ATCC 33809, IMET 11293, NCTC 11327.

GenBank accession number (16S rRNA): X74703, X76335.

14. **Vibrio furnissii** Brenner, Hickman-Brenner, Lee, Steigerwalt, Fanning, Hollis, Farmer, Weaver, Joseph and Seidler 1984b, 91[VP] (Effective publication: Brenner, Hickman-Brenner, Lee, Steigerwalt, Fanning, Hollis, Farmer, Weaver, Joseph and Seidler 1983c, 823.)
fur.niss′i.i. L. gen. n. *furnissii* of Furniss; named to honor A.L. Furniss, Maidstone Public Health Laboratories, Maidstone, England who was involved in the original discovery and naming of the "group F" vibrios.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Characters that distinguish *V. furnissii* from *V. fluvialis* are given in Tables BXII.γ.172 and BXII.γ.173. Characters that distinguish *V. furnissii* from *Vibrio damsela* and *Aeromonas* spp. are given in Table BXII.γ.173. Characters that distinguish *V. furnissii* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165 and BXII.γ.166.

Widely distributed in natural waters (often in estuaries) and animals that live there; occasionally isolated from animal feces. Although *V. furnissii* strains were isolated from two outbreaks of acute gastroenteritis, other pathogens or possible pathogens were also isolated (*V. parahaemolyticus*, *V. cholerae* non-O1, *V. fluvialis*, *Salmonella*, and *Plesiomonas*). Therefore, the role of *V. furnissii* as the actual cause of the diarrhea was only speculative.

V. furnissii was established for strains that resembled *V. fluvialis* but produced gas during fermentation. Based on DNA–DNA hybridization experiments, three strains of *V. furnissii* were shown to be 79% or more related to the type strain of *V. furnissii* but only 50% related to the type strain of *V. fluvialis* (Brenner et al., 1984b).

The mol% G + C of the DNA is: 50.4 (T_m).

Type strain: ATCC 35016, CDC B3215, CIP 102972, LMG 7910.

GenBank accession number (16S rRNA): X74704, X76336.

15. **Vibrio gazogenes** (Harwood, Bang, Baumann and Nealson 1980) Baumann, Baumann, Bang and Woolkalis 1981, 217[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*Beneckea gazogenes* (ex Harwood 1978) Harwood, Bang, Baumann and Nealson 1980, 655.)
gaz.og′e.nes. Fr. n. *gaz* gas; L. n. *genesis* birth; M.L. adj. *gazogenes* gas-producing.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Relatedness of strains based on DNA–DNA hybridization experiments is given in Figs. BXII.γ.172 and BXII.γ.176. Three characters distinguish *V. gazogenes* from all other *Vibrio* species: production of a non-diffusible red ("prodigiosin-like") pigment, a negative reaction for oxidase, and an inability to reduce NO_3^- to NO_2^-. The determination of the oxidase reaction is complicated by the red pigmentation of colonies; this difficulty can be overcome by testing spontaneous white colonial mutants, which are produced by most cultures held for long periods in agar slants or deeps.

Isolated from estuary water and salt marsh mud off the eastern coast of the United States (Farmer et al., 1988),

and from a hypersaline lake in Baja California (Giovannoni and Margulis, 1981).

DNA–DNA hybridization experiments (Figs. BXII.γ.172 and BXII.γ.176) showed that strains of *V. gazogenes* fall into three highly related groups (Farmer et al., 1988). However, the three groups cannot be completely differentiated by phenotype. The type strain is less than 15% related to other *Vibrio* species tested (Brenner et al., unpublished). Analysis of 16S rRNA gene sequences showed that *V. gazogenes* is not closely related to other *Vibrio* species (Fig. BXII.γ.171).

The mol% G + C of the DNA is: 47.1 (T_m).
Type strain: PB1, ATCC 29988.
GenBank accession number (16S rRNA): X74705.

16. **Vibrio halioticoli** Sawabe, Sugimura, Ohtsuka, Nakano, Tajima, Ezura and Christen 1998b, 578[VP]
hal.i.o.ti′ co.li. M.L. n. *Haliotis* genus name for abalone, a rock-climbing gastropod mollusk; Gr. n. *colon* gut; M.L. gen. n. *halioticoli* pertaining to the gut of *Haliotis*.

The morphological, growth, physiological and biochemical characteristics are listed in Tables BXII.γ.154 and BXII.γ.164. Cells are rod shaped with rounded ends when grown in Zobell's medium 2216A but are spherical when grown in this medium with added alginate. Nonmotile. Cells bind to and utilize alginic acid.

Isolated from gut of the abalone *Haliotis discus hannai*, where this bacterium may aid in the digestion of algae that contain alginic acid.

Sawabe et al. (1998b) concluded that *Vibrio halioticoli* is a separate *Vibrio* species with no close relatives based on DNA–DNA hybridization experiments and 16S rRNA gene sequence analysis (also see Fig. BXII.γ.171).

The mol% G + C of the DNA is: 41.6–43.1 (HPLC).
Type strain: A431, ATCC 700680, IAM 14596.
GenBank accession number (16S rRNA): AB000390.

17. **Vibrio harveyi** (Johnson and Shunk 1936) Baumann, Baumann, Bang and Woolkalis 1981, 217[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*"Achromobacter harveyi"* Johnson and Shunk 1936, 587; *Lucibacterium harveyi* (Johnson and Shunk 1936) Hendrie, Hodgkiss and Shewan 1970, 166; *Beneckea harveyi* (Johnson and Shunk 1936) Reichelt and Baumann 1973b, 320; *Vibrio carchariae* Grimes, Stemmler, Hada, May, Maneval, Hetrick, Jones, Stoskopf and Colwell 1985, 224.)
har′ vey.i. M.L. gen. n. *harveyi* of Harvey; named to honor E.N. Harvey, a biologist who was a pioneer in the systematic study of bioluminescence.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Characters that differentiate *V. harveyi* from *V. alginolyticus*, *V. parahaemolyticus*, and *V. vulnificus* are given in Table BXII.γ.170. Characters that distinguish *V. harveyi* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.165 and BXII.γ.166. The morphology is as illustrated in Figs. BXII.γ.163 and BXII.γ.177.

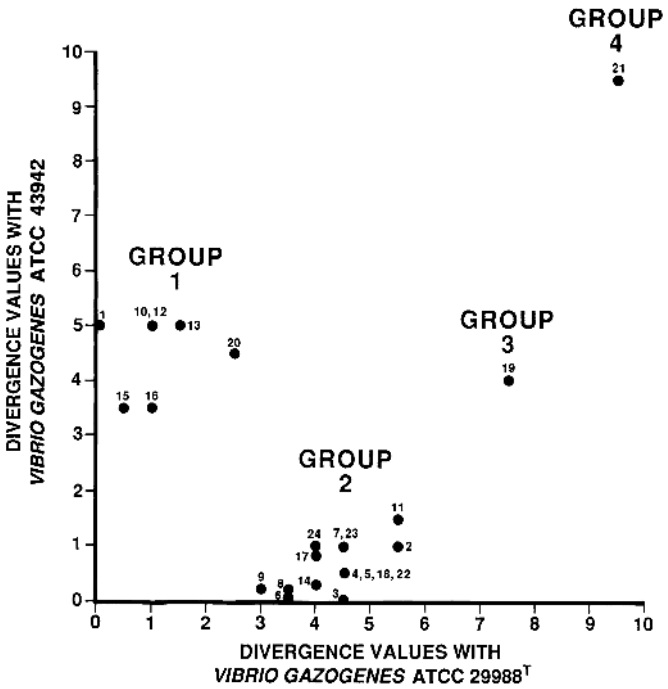

FIGURE BXII.γ.176. Subgroups in the species *Vibrio gazogenes* based on divergence values in DNA–DNA hybridization experiments. Divergence values reflect non-hybridizing DNA sequences. The numbers above or beside the dots are the strain numbers given in Farmer et al. (1988). (Reproduced with permission from J.J. Farmer III et al., Journal of Clinical Microbiology *21:* 46–76, 1988, ©American Society for Microbiology.)

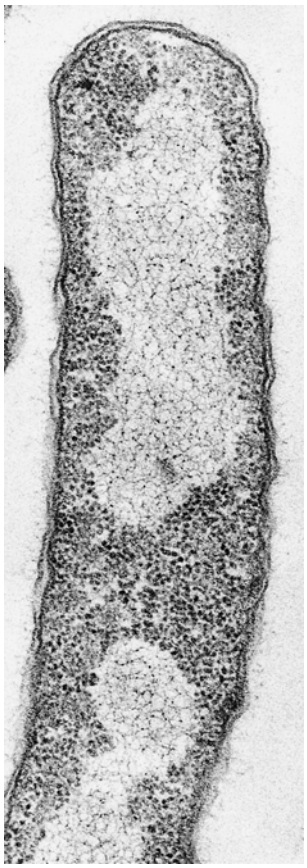

FIGURE BXII.γ.177. Ultrathin section of *Vibrio harveyi*. Exponential growth phase in yeast extract broth. × 43,000. (Courtesy of S.W. Watson.)

Occurs in marine animals, the mouth of sharks (reported as *Vibrio carchariae*), and the marine environment. Isolated from a human wound infection (reported as *Vibrio carchariae*) associated with a shark bite. The type strain was isolated from an amphipod (*Talorchestia* sp.).

Grimes et al. (1984), studied two urease-positive halophilic vibrios isolated from a brown shark (*Carcharhinus plumbeus*) that had died in captivity in a large aquarium. One of the organisms (strain 1116a) was identified as *V. damsela*, but the other (strain 1116b) could not be identified. After further phenotypic testing and DNA–DNA hybridization, Grimes et al. (1984) concluded that this was a new species and named it *V. carchariae*. *V. carchariae* has been isolated from other sharks since the original report, but it was recently shown to occur in human clinical specimens. Pavia et al. (1989) described a case in which the organism was isolated from a wound following a shark bite.

The name *Vibrio carchariae* was used for this organism until the identity of *V. carchariae* with *V. harveyi* was shown conclusively (Farmer and Hickman-Brenner, 1992; Pedersen et al., 1998; Brenner et al., unpublished; see Fig. BXII.γ.171). *Vibrio carchariae* and *Vibrio harveyi* are objective synonyms.

The mol% G + C of the DNA is: 46–48 (T_m).

Type strain: 384, ATCC 14126, CCUG 28584, CIP 103192, IFO 15634, LMG 4044, NCMB 1280.

GenBank accession number (16S rRNA): X56578, X74706.

18. **Vibrio hollisae** Hickman, Farmer, Hollis, Fanning, Steigerwalt Weaver, and Brenner 1982b, 384[VP] (Effective publication: Hickman, Farmer, Hollis, Fanning, Steigerwalt, Weaver and Brenner 1982a, 398.)
hol′ lis.ae. M.L. gen. n. *hollisae* of Hollis; named to honor Dannie G. Hollis, who first recognized this organism as a new and distinct vibrio.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Characters that distinguish *V. hollisae* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165 and BXII.γ.166. Small cells, 0.5 × 1.5–2 μm with a single polar flagellum when grown in liquid media. On sheep blood agar colonies are 1–2 mm in diameter and are weakly hemolytic. Does not grow on TCBS or MacConkey agar.

Appears to cause sporadic cases of diarrhea in humans and has been isolated from blood. Rarely isolated from other human or environmental sources. The type strain was isolated from human feces.

V. hollisae is strongly associated with human diarrhea, but additional evidence is needed to further document the causal role and pathogenic mechanisms of this organism. Fifteen of the original sixteen strains were from feces; many of those patients had diarrhea (Hickman et al., 1982a). Morris et al. (1982) described the clinical and epidemiological features of 11 cases of diarrhea from which *V. hollisae* was isolated.

Based on DNA–DNA hybridization experiments, strains of *V. hollisae* are 76–97% related to the type strain and only 0–4% related to all other *Vibrio* species (Brenner et al. unpublished). 16S rRNA gene sequence analysis indicates that *V. hollisae* is not closely related to other *Vibrio* species (Fig. BXII.γ.171).

V. hollisae has also been known as "Group EF-13" of the Special Bacteriology Laboratory, CDC, unpublished (see Hickman et al., 1982a).

The mol% G + C of the DNA is: 49.3–51.0.

Type strain: ATCC 33564, CDC 0075-80, IMET 12291.

GenBank accession number (16S rRNA): X56583, X74707.

19. **Vibrio ichthyoenteri** Ishimaru, Akagawa-Matsushita and Muroga 1996, 159[VP]
ich.thy.o.en′ te.ri. Gr. n. *ichthys* fish; Gr. n. *enteron* gut; Gr. gen. n. *ichthyoenteri* of fish gut.

The characteristics are as described for the genus and as listed in Tables BXII.γ.154, BXII.γ.163 and BXII.γ.164. Short rods, straight or slightly curved, 1.6–2.5 × 0.6–0.8 μm. Motile by a single polar flagellum. No lateral flagella are produced when grown on solid media. Most strains produce yellow colonies on TCBS agar, but the type strain is a weak sucrose fermenter and produces green colonies.

Causes opaque intestines, intestinal necrosis, and enteritis with high mortality rates in Japanese flounder larvae (*Paralichthys olivaceus*) reared in marine hatcheries.

V. ichthyoenteri was previously known as "*Vibrio* species INFL" (Masumura et al., 1989). Ishimaru et al. (1996) found that five strains were 89–100% related to the type strain based on DNA–DNA hybridization experiments. There was only one close relative; strain P-8706 of "*Vibrio* species INFL-2" from a diseased flounder larva, which was 37% related. Other *Vibrio* species were only 3–18% related.

The mol% G + C of the DNA is: 43.4–44.3 (HPLC).

Type strain: : F-2, ATCC 700023, IFO 15847.

20. **Vibrio logei** (Harwood, Bang, Baumann and Nealson 1980) Baumann, Baumann, Bang and Woolkalis 1981, 217[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*Photobacterium logei* (ex Bang, Baumann and Nealson 1978) Harwood, Bang, Baumann and Nealson 1980, 655; *Vibrio salmonicida* Egidius, Wiik, Anderson, Hoff and Hjeltnes 1986, 519.)
log′ e.i. M.L. gen. n. *logei* of Loge; from the German Loge, Norse god of fire and mischief.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Produces a characteristic yellow-orange pigment that is cell-associated.

Causes Hitra disease ("hemorrhagic syndrome")—a disease originally thought to be caused by *Vibrio salmonicida*—in salmonid fish raised in ponds in Norway. Isolated from scallops, intestinal contents of fish, marine sediments, and lesions of the exoskeleton of tanner crabs (Baumann and Baumann, 1981a). The type strain was isolated from the intestine of arctic mussel.

The mol% G + C of the DNA is: 40–42 (T_m, Bd).

Type strain: 584, ATCC 29985, CIP 104991.

21. **Vibrio mediterranei** Pujalte and Garay 1986, 279[VP]
me.di.ter.ra′ ne.i. L. gen. n. *mediterranei* of the Mediterranean Sea.

The characteristics are as described for the genus and as listed in Tables BXII.γ.154, BXII.γ.163, and BXII.γ.164. Cells are 0.5 × 1.0–2.0 μm and possess polar flagella when grown in liquid medium. Colonies are circular, translucent,

nonswarming, and nonpigmented on marine agar and yellow on TCBS agar.

Isolated from plankton, sediments, and seawater off the coast of Valencia, Spain.

Based on DNA–DNA hybridization experiments, the type strain of *V. mediterranei* is highly related (75–100%) to other strains included in the species but is not closely related to other *Vibrio* species. *V. harveyi* was the closest relative (11%) in the original study and *V. alginolyticus* was the closest relative (25%) in the DNA–DNA hybridization study of Brenner et al. (unpublished). Fig. BXII.γ.171 illustrates its relationships to other *Vibrio* species based on 16S rRNA gene sequence analysis.

The mol% G + C of the DNA is: 42–43 (T_m).

Type strain: ATCC 43341, CECT 621, CIP 103203, NCTC 11946.

GenBank accession number (16S rRNA): X74710.

22. **Vibrio metschnikovii** Gamaléia 1888, 485[AL] (*Vibrio cholerae* biovar proteus Shewan and Véron 1974, 344.)
metsch.ni.kov′ i.i. M.L. masc. gen. n. *metschnikovii* of Metschnikoff; named to honor E. Metschnikoff, a Russian biologist.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Characters that distinguish *V. metschnikovii* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165 and BXII.γ.166. Oxidase negative. Does not reduce nitrate to nitrite.

Frequently isolated from fresh, brackish, and marine waters, but rarely from human clinical specimens (Lee et al., 1978). The type strain was isolated from fowl.

In 1888, Gamaléia reported the isolation of a new organism, *Vibrio metschnikovii*, from a fowl that had died of a "cholera-like" disease (Lee et al., 1978). Little was written about the organism until Lee et al. (1978) began isolating similar organisms from marine and fresh water environments in the United Kingdom. These strains were oxidase negative and did not reduce nitrate to nitrite but otherwise were typical halophilic vibrios. Lee et al. (1978) proposed that the strains be classified as *V. metschnikovii*, and emended the description of this organism. Lee et al. (1978) described 40 strains of *V. metschnikovii*, but none were from human clinical specimens. Jean-Jacques et al. (1981) reported the first clinically significant isolate of this newly redefined species. A blood culture from an 82-year-old woman with peritonitis and an inflamed gallbladder yielded *V. metschnikovii*. Nonhuman sources include rivers, sewage, cockles, shrimp, lobster, crab, and fowl (Lee et al., 1978; *Vibrio* Laboratory, CDC, unpublished). These data suggest that *V. metschnikovii* is widely distributed in the environment and that humans may acquire the organism from these sources.

The mol% G + C of the DNA is: 44–46 (T_m, Bd).

Type strain: ATCC 700040, LMG 11664, NCTC 8443.

23. **Vibrio mimicus** Davis, Fanning, Madden, Steigerwalt, Bradford, Smith and Brenner 1982, 267[VP] (Effective publication: Davis, Fanning, Madden, Steigerwalt, Bradford, Smith and Brenner 1981a, 636.)
mim′ i.cus. M.L. adj. *mimicus* pertaining to a mimic; named to indicate its close phenotypic similarity to *V. cholerae*.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Characters that distinguish *V. mimicus* from *V. cholerae* are given in Table BXII.γ.169. Characters that distinguish *V. mimicus* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165 and BXII.γ.166. *V. mimicus* is similar to *Vibrio cholerae* non-O1 in its ecology, distribution, and pathogenicity. Does not require Na^+.

Occurs in aquatic environments. Some strains apparently cause diarrhea and human intestinal infections, and some virulence factors have been postulated. Occasionally causes extraintestinal human infections. The type strain was isolated from a human ear culture.

V. mimicus was discovered during a DNA–DNA hybridization study of "biochemically atypical" strains of *V. cholerae* (Davis et al., 1982). Strains that were originally characterized as "*Vibrio cholerae* sucrose$^+$" were highly related to, but distinct from, *V. cholerae* by DNA–DNA hybridization; the new species *V. mimicus* was based on these results (Davis et al., 1982).

The mol% G + C of the DNA is: unknown.

Type strain: ATCC 33653, CDC 1721-77.

GenBank accession number (16S rRNA): X74713.

24. **Vibrio mytili** Pujalte, Ortigosa, Urdaci, Garay and Grimont 1993, 360[VP]
my.ti′ li. L. gen. n. *mytili* of mussels, from the genus name *Mytilus*.

The characteristics are as described for the genus and as listed in Tables BXII.γ.154, BXII.γ.163, and BXII.γ.164. Coccobacilli. Motile by a single polar flagellum. Colonies are nonpigmented on marine agar and yellow on TCBS agar. No growth occurs on MacConkey agar.

Isolated from mussels (*Mytilus edulis*) off the Atlantic coast of Spain.

Based on DNA–DNA hybridization experiments Pujalte et al. (1993) concluded that strains of *V. mytili* were highly related (94–100%) to the type strain but not closely related to 23 other species of *Vibrio* and *Photobacterium*. *Vibrio parahaemolyticus* was the closest relative (25%). Brenner et al. (unpublished) found 25–40% relatedness of *V. mytili* to *V. alginolyticus* and other marine *Vibrio* species. Fig. BXII.γ.171 illustrates the relationships of *V. mytili* to other *Vibrio* species based on 16S rRNA gene sequence analysis.

The mol% G + C of the DNA is: 45–46 (T_m).

Type strain: 165, ATCC 51288, CECT 632.

25. **Vibrio natriegens** (Payne, Eagon and Williams 1961) Baumann, Baumann, Bang and Woolkalis 1981, 217[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*"Pseudomonas natriegens"* Payne, Eagon and Williams 1961, 125; *"Vibrio natriegens"* (Payne, Eagon and Williams 1961) Webb and Payne 1971, 1080; *Beneckea natriegens* (Payne, Eagon and Williams 1961) Baumann, Baumann and Mandel 1971a, 291.)
na.tri.e′ gens. M.L. *natrium* sodium; L. *egens* pres. part. *egere* to be in need; M.L. adj. *natriegens* sodium-requiring.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Cells usually have a single, long, polar flagellum and occasionally 2–3

flagella. Cells often contain poly-β-hydroxybutyrate granules (Fig. BXII.γ.178). Doubling time (generation time) is 9.8 minutes (Eagon, 1962). *V. natriegens* is differentiated from other species of *Vibrio* by its short generation time and its ability to utilize a much wider variety of carbon sources than most other species (Table BXII.γ.163).

Isolated from salt marsh mud and coastal seawater (Payne et al., 1961; Furniss et al., 1978; Baumann and Baumann, 1981a). The type strain was isolated from a salt marsh mud, Sapelo Island, Georgia, USA.

Based on DNA–DNA hybridization experiments, strains of *V. natriegens* are highly related (83% or greater) to the type strain and 33–53% related (Fig. BXII.γ.169) to other marine *Vibrio* species (Reichelt and Baumann, 1973b). These results were confirmed by Brenner et al. (unpublished) who found the closest relatives of *V. natriegens* according to DNA–DNA hybridization data to be *V. alginolyticus*, *V. campbellii*, *V. harveyi*, and *V. parahaemolyticus* (31–45% related). Based on 16S rRNA gene sequence analysis (Fig. BXII.γ.171 and analysis of the immunological relationships of superoxide dismutase (Fig. BXII.γ.170), *Vibrio natriegens* falls into the marine *Vibrio* group.

The mol% G + C of the DNA is: 45.1–47 (T_m, Bd).
Type strain: ATCC 14048, DSM 759, NCMB 857.
GenBank accession number (16S rRNA): X74714.

26. **Vibrio navarrensis** Urdaci, Marchand, Ageron, Arcos, Sesma and Grimont 1991, 293[VP]
na.var.ren′ sis. M. L. masc. adj. *navarrensis* pertaining to Navarra, Spain, the Spanish province where the organism was isolated.

Morphological, growth, physiological, and biochemical characteristics are listed in Tables BXII.γ.154 and BXII.γ.164. Cells are 0.8–1.0 × 1.0–2.0 μm and are motile by a single polar flagellum when grown on solid and liquid media. Colonies are 2–3 mm in diameter on nutrient agar with 2% NaCl after overnight incubation at 30°C and are round, opaque, and nonpigmented. Colonies on TCBS agar are yellow. Slight requirement for Na^+. Seven of ten strains studied grew weakly in peptone water without added NaCl. Synthesizes a unique fatty acid of undetermined structure not found in 22 other *Vibrio* species (Urdaci et al., 1991) that accounts for 1–3 % of the total fatty acids of *V. navarrensis*.

Isolated from sewage, irrigation water and river water in Spain.

Based on DNA–DNA hybridization experiments, Urdaci et al. (1991) found that eight strains of *V. navarrensis* were highly related (82–98% with T_m values of 0–2.0) to the type strain; another strain (1381-1) was 74% related.. The closest relative was *Vibrio natriegens*, which was 39% related. The other *Vibrio* species tested were only 3–15% related. Based on DNA–DNA hybridization experiments, Brenner et al. (unpublished) found 14–22% relatedness of *V. navarrensis* to *V. natriegens* and 28–36% relatedness of *V. navarrensis* to *V. harveyi*, *V. parahaemolyticus*, *V. pelagius*, and *V. vulnificus*. Fig. BXII.γ.171 illustrates the relationship of *V. navarrensis* to other species of *Vibrio*.

The mol% G + C of the DNA is: 45–47 (T_m).
Type strain: ATCC 51183, CIP 103381.
GenBank accession number (16S rRNA): X74715.

27. **Vibrio nereis** (Harwood, Bang, Baumann and Nealson 1980) Baumann, Baumann, Bang and Woolkalis 1981, 217[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*Beneckea nereida* (ex Baumann, Baumann and Mandel 1971a) Harwood, Bang, Baumann and Nealson 1980, 655.)
ne′ re.is. L. n. *nereis* a sea nymph.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. The morphology is illustrated in Figs. BXII.γ.179 and BXII.γ.180.

Isolated from seawater off the coast of Hawaii. Kusuda et al. (1986) isolated *V. nereis* from an outbreak of fatal infections of sea bream (*Acanthopagrus schlegeli*) at hatcheries in western Japan, but did not think this organism was causing disease in the fish. The type strain was isolated from seawater off the coast of Hawaii.

Based on DNA–DNA hybridization experiments, Reichelt et al. (1976) found that strains of *Vibrio nereis* were

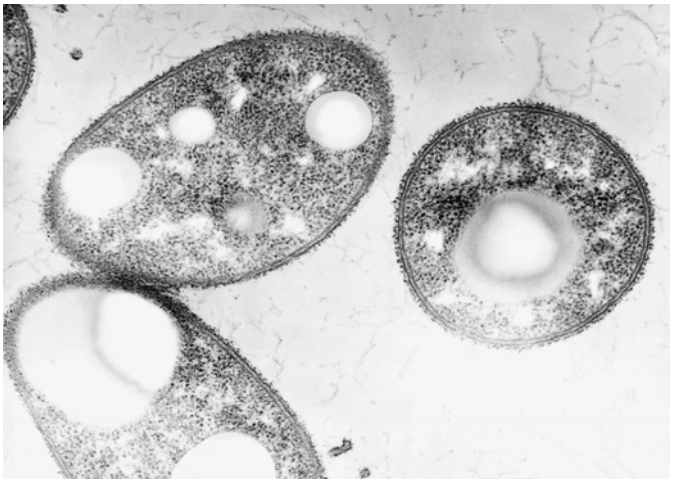

FIGURE BXII.γ.178. Ultrathin section of *Vibrio natriegens* containing granules of poly-β-hydroxybutyrate that accumulate during stationary phase of growth. Grown in basal medium containing limiting nitrogen and excess β-hydroxybutyrate. × 32,000. (Courtesy of R.D. Allen.)

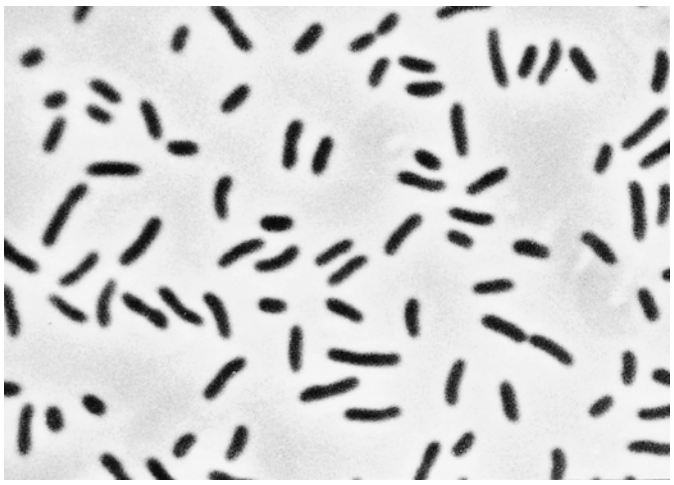

FIGURE BXII.γ.179. Phase contrast micrograph of *Vibrio nereis*. Exponential growth phase in yeast extract broth. × 3000.

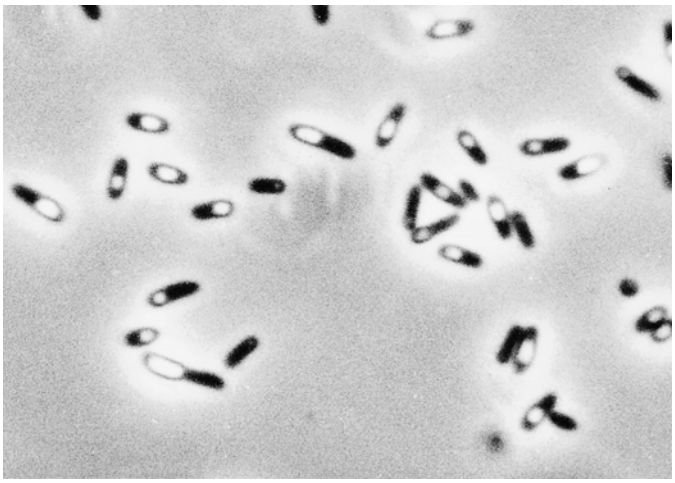

FIGURE BXII.γ.180. Phase contrast micrograph of *Vibrio nereis* containing refractile granules of poly-β-hydroxybutyrate that accumulate during stationary phase. The organisms were grown in basal medium containing limiting nitrogen and excess β-hydroxybutyrate. × 3000.

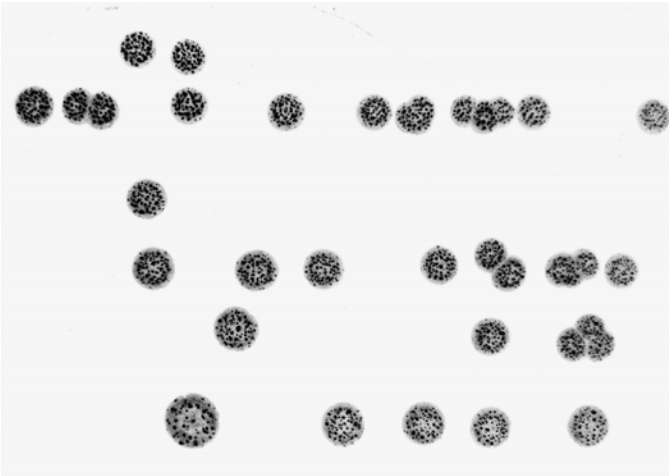

FIGURE BXII.γ.181. Colonies of *Vibrio nigripulchritudo* containing crystals of a blue-black pigment. Incubation for 2 days on basal medium agar with 0.2% [v/v] glycerol. × 4.5. (Reproduced with permission from P. Baumann et al., Journal of Bacteriology *108:* 1380–1383, 1971, ©American Society for Microbiology.)

highly related (87–94%) to the type strain and less than 30% related to 13 other *Vibrio* species. These results were confirmed by Brenner et al. (unpublished), who found the closest relatives by DNA–DNA hybridization to be *V. alginolyticus*, *V. parahaemolyticus*, *V. harveyi*, *V. proteolyticus*, and two unnamed *Vibrio* groups (all 22–40% related). By DNA–DNA hybridization (Fig. BXII.γ.169), 16S rRNA sequencing (Fig. BXII.γ.171), and the immunological relationship of superoxide dismutase (Fig. BXII.γ.170), *Vibrio nereis* falls into the marine *Vibrio* group.

The mol% G + C of the DNA is: 46–47 (T_m, Bd).

Type strain: ATCC 25917, CIP 103194, IFO 15637, LMG 3895, NCCB 73020.

GenBank accession number (16S rRNA): X74716.

28. **Vibrio nigripulchritudo** (Baumann, Baumann, Mandel and Allen 1971a) Baumann, Baumann, Bang and Woolkalis 1981, 217[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*Beneckea nigripulchritudo* Baumann, Baumann, Mandel and Allen 1971b, 1383.)
ni.gri.pul.chri.tu' do. L. *niger* black; L. *pulchritudo* beauty; M.L. adj. *nigripulchritudo* black beauty, which refers to its striking blue-black colonies.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Instantly recognizable and differentiated from other *Vibrio* species by its formation of striking blue-black colonies (Fig. BXII.γ.181).

Isolated from sea water off the coast of Hawaii.

Based on DNA–DNA hybridization experiments, Reichelt et al. (1976) found that strains of *Vibrio nigripulchritudo* were highly related (91–96%) to the type strain and less than 20% related to 15 other *Vibrio* species. These results were essentially confirmed by Brenner et al. (unpublished) who found the closest relative by DNA–DNA hybridization to be *V. penaeicida* (36% related). By 16S rRNA gene sequence analysis (Fig. BXII.γ.171 and the immunological relationship of superoxide dismutase (Fig. BXII.γ.170), *Vibrio nigripulchritudo* falls into the marine *Vibrio* group, but may have diverged slightly from that group.

The mol% G + C of the DNA is: 46–47 (T_m, Bd).

Type strain: ATCC 27043, CCUG 28586, CIP 103195, LMG 3896.

GenBank accession number (16S rRNA): X74717.

29. **Vibrio ordalii** Schiewe, Trust and Crosa 1982, 384[VP] (Effective publication: Schiewe, Trust and Crosa 1981, 347.)
or.dal' i.i. M.L. gen. n. *ordalii* of Ordal; named to honor Erling J. Ordal for his many contributions to the current understanding of the biology of bacterial fish pathogens

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. The cells are 2.5–3.0 × 1.0 μm in size and are motile by a single polar flagellum. Grows very slowly, forming visible colonies on trypticase soy agar only after 4–6 days (Table BXII.γ.171). Growth is enhanced by the addition of NaCl. All isolates of *V. ordalii* belong to O antigen group 2, in contrast to *V. anguillarum* (O antigen groups 1–16). Closely related phylogenetically to *V. anguillarum* (Figs. BXII.γ.169, BXII.γ.170, and BXII.γ.171), but less biochemically versatile and more likely to have additional nutritional requirements (Table BXII.γ.171).

Important fish pathogen isolated from diseased salmonid fish in Washington, Oregon, British Columbia, and Japan (Schiewe et al., 1981), and from ayu (*Plecoglossus altivelis*) and rockfish fingerlings (*Sebastes schlegeli*) in Japan (Muroga et al., 1986). The type strain was isolated from the kidney of a moribund Coho salmon (*Oncorhynchus kisutch*) at a commercial salmon farm in Puget Sound, Washington, U.S.A.

V. ordalii is a halophilic *Vibrio* species that is very closely related to *Vibrio anguillarum*. Previously, other investigators had studied vibriosis of marine fish and isolated a halophilic vibrio similar to, but less biochemically versatile than *V. anguillarum*. This organism was referred to as "*Vibrio* sp. 1669 group", "*Vibrio* sp. RT group", *"Beneckea anguillara"* biotype I or *Vibrio anguillarum* biotype 2. However, DNA–DNA hybridization studies by Schiewe et al. (1981) indi-

cated that, although closely related to *V. anguillarum*, *V. ordalii* was a separate species.

The mol% G + C of the DNA is: 43–44 (T_m).

Type strain: DF3K, ATCC 33509.

GenBank accession number (16S rRNA): X70641, X74718.

30. **Vibrio orientalis** Yang, Yeh, Cao, Baumann, Baumann, Tang and Beaman 1983b, 673[VP] (Effective publication: Yang, Yeh, Cao, Baumann, Baumann, Tang and Beaman 1983a, 98.)
or.i.en.ta′ lis. L. adj. *orientalis* of or belonging to the Orient.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Motile by a single, sheathed, polar flagellum. All the original strains were reported to be bioluminescent, but this property is apparently lost after storage and subculture. Differentiated from other bioluminescent *Vibrio* species because it accumulates poly-β-hydroxybutyrate and grows at 4°C.

Isolated from seawater and shrimp from the Yellow Sea, China.

V. orientalis was originally defined and named because it was distinct from other species of *Vibrio* and *Photobacterium* in phenotype and in the relationship of its superoxide dismutase (Yang et al., 1983a). Based on DNA–DNA hybridization experiments, the type strain of *Vibrio orientalis* was highly related (75% or greater with 0.5% divergence) to three other strains (Brenner et al., unpublished). The closest relatives were *V. parahaemolyticus*, *V. pelagius*, *V. tubiashii*, *Vibrio* group 515 and *Vibrio* group 9084-82 (19–37% related) (Brenner et al., unpublished). Fig. BXII.γ.171 illustrates the relationship of *V. orientalis* to other *Vibrio* species based on 16S rRNA gene sequence analysis. Based on the immunological relationship of its superoxide dismutase *V. orientalis* falls into the marine *Vibrio* group (see Fig. 3 of Yang et al., 1983a).

The mol% G + C of the DNA is: 45.3–45.8 (Bd).

Type strain: ATCC 33934, CCUG 16389, CIP 102891, IFO 15638, LMG 7897.

GenBank accession number (16S rRNA): X74719.

31. **Vibrio parahaemolyticus** (Fujino, Okuno, Nakada, Aoyama, Fukai, Mukai and Ueho 1951) Sakazaki, lwanami and Fukumi 1963, 181[AL] (*"Pasteurella parahaemolytica"* Fujino, Okuno, Nakada, Aoyama, Fukai, Mukai and Ueho 1951, 11; *"Oceanomonas parahaemolytica"* (Fujino, Okuno, Nakada, Aoyama, Fukai, Mukai and Ueho 1951) Miyamoto, Nakamura and Takizawa 1961, 477; *Beneckea parahaemolytica* (Fujino, Okuno, Nakada, Aoyama, Fukai, Mukai and Ueho 1951) Baumann, Baumann, and Mandel 1971a, 291.)
para.hae.mo.ly′ ti.cus. Gr. prep. *para* by the side of, beside; Gr. n. *haema* blood; Gr. adj. *lyticus* dissolving; M.L. adj. *parahaemolyticus* dissolving blood.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Characters that differentiate *V. parahaemolyticus* from *V. alginolyticus*, *V. harveyi*, and *V. vulnificus* are listed in Table BXII.γ.170. Characters that distinguish *V. parahaemolyticus* from other *Vibrio* spp. found in human clinical specimens are listed in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165 and BXII.γ.166.

Occurs in human clinical specimens (Table BXII.γ.157) and is an important cause of diarrhea. Also occurs in marine environments.

Outbreaks of gastroenteritis caused by *V. parahaemolyticus* occur worldwide. In Japan, *V. parahaemolyticus* causes 50–70% of foodborne enteritis cases (Sakazaki and Balows, 1981), which are invariably associated with seafood. In the early 1970s, several articles described *V. parahaemolyticus* as the cause of severe wound infections and bacteremia (Roland, 1970, 1971; Zide et al., 1974; Weaver and Ehrenkranz, 1975). It is now known that the infectious agent in the case was really *Vibrio vulnificus* (Weaver and Ehrenkranz, 1975), not *V. parahaemolyticus*. Similarly, many ecological and environmental reports about *V. parahaemolyticus* may be incorrect because of our limited understanding of the large number of *Vibrio* species that inhabit these environments.

The mol% G + C of the DNA is: 46–47 (T_m, Bd).

Type strain: 113, ATCC 17802, DSM 30189, NCMB 1326.

GenBank accession number (16S rRNA): M59161, X56580, X74720.

32. **Vibrio pectenicida** Lambert, Nicolas, Cilia and Corre 1998, 486[VP]
pec.ten.i.ci′ da. M.L. n. *Pecten* genus name of scallops; M.L. v. *caedo* to kill; *pectenicida* scallop-killer.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Cells are curved rods with a large single polar flagellum when grown in liquid medium. Smaller, unsheathed, lateral flagella are produced during growth on solid medium. Colonies after 48 h of growth are circular, smooth, and unpigmented and exhibit waves of swarming. Isolated from moribund scallop (*Pecten maximus*) larvae during outbreaks of disease in a hatchery in France.

Lambert et al. (1998) compared the type strain of *Vibrio penaeicida* to the type strains of 30 other *Vibrio* and *Photobacterium* species by 16S rRNA gene sequence analysis. The two closest relatives were *Vibrio tapetis* (97.2% sequence similarity) and *V. splendidus* (95.5% sequence similarity). DNA–DNA hybridization was used to show that the type strain of *V. pectenicida* was 86–100% related to four other strains and less than 5% related to the type strains of *V. tapetis* and *V. splendidus*. Brenner et al. (unpublished) showed that the type strain of *V. pectenicida* was 98% related to one other strain, and 1–25% related to all other *Vibrio* species tested based on the results of DNA–DNA hybridization experiments. Fig. BXII.γ.171 shows the relationship of *Vibrio pectenicida* to other *Vibrio* species based on 16S rRNA gene sequence analysis. *V. logei* is the closest relative of *V. pectenicida* based on phenotypic similarities.

The mol% G + C of the DNA is: 39–41 (T_m).

Type strain: A365, ATCC 700783, CIP 105190.

GenBank accession number (16S rRNA): Y13830.

33. **Vibrio pelagius** (Baumann, Baumann and Mandel 1971a) Baumann, Baumann, Bang and Woolkalis 1981, 217[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*Beneckea pelagia* Baumann, Baumann and Mandel 1971a, 291.)*
pe.la′ gi.us. L. adj. *pelagius* of the sea.

**Editorial Note:* The name *Listonella pelagia* also has standing in the nomenclature (MacDonell and Colwell, 1985).

TABLE BXII.γ.174. Nomenclature, classification and differentiation of the two groups of *Vibrio pelagius* that are distinct by DNA hybridization and phenotype

Test or property	*Vibrio pelagius* group 1	*Vibrio pelagius* group 2
Type or reference strain	ATCC 25916[T] (strain 99)	ATCC 33786 (strain 106)[a]
Other strains in ATCC[b]	33781 (96)	33504 (105)
	33782 (100)	33784 (103)
	33783 (101)	33785 (104)
	33808 (97)	33786 (106)
Nomenclature and classification according to:		
Baumann et al. (1971a)	Group F, cluster 1	Group F, cluster 2
Baumann et al. (1971a)	*Vibrio pelagius*	*Vibrio pelagius*
Reichelt et al. (1976)	*Vibrio pelagius* biotype I	*Vibrio pelagius* biotype II
Baumann et al. (1984b)	*Vibrio pelagius* biotype I	*Vibrio pelagius* biotype II
MacDonell and Colwell (1985)	*Listonella pelagia*	*Listonella pelagia*
Differential tests:		
Cellular morphology	Straight rods	Curved rods
Amylase production	0	100
Gelatinase production	0	100
γ-Aminobutyrate utilization	0	100
δ-Aminovalerate utilization	0	100

[a]This strain was the labeled strain in the DNA hybridization study of Reichelt et al. (1976).

[b]The ATCC number is listed first. The strain number from Baumann et al. (1971a) is listed in parentheses.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Nomenclature, classification, and differential phenotypic traits of the two groups of *Vibrio pelagius*, which are also distinct based on the results of DNA hybridization experiments (see Taxonomic Comments), are given in Table BXII.γ.174.

Isolated from seawater of the coast of Hawaii. Furniss et al. (1978) found that *V. pelagius* is one of the most common vibrios isolated along the British coasts.

Beneckea pelagia was first described and named by Baumann et al. (1971a). It was defined as a single species based on phenotypic properties of 11 strains isolated from waters off the coast of Hawaii; however, the authors noted that the strains "clustered in two groups." Reichelt et al. (1976) confirmed the existence of two groups by DNA–DNA hybridization experiments. The two groups were classified as biotypes and referred to as *Beneckea pelagia* I and II. Baumann et al. (1980b) "abolished" the genus *Beneckea*, and proposed that *Beneckea pelagia* be classified in the genus *Vibrio* as *V. pelagius*.

Brenner et al. (1983b) showed by DNA–DNA hybridization experiments that the type strain of *V. pelagius* was 30–45% related to type strains of *V. campbellii*, *V. harveyi*, *V. parahaemolyticus*, and *V. splendidus*. Recently Brenner et al. (unpublished) used DNA–DNA hybridization experiments to compare strains of the two groups of *V. pelagius* and other strains that had been identified as belonging to this species. They confirmed the results of Reichelt et al. (1976) that showed that *V. pelagius* II was distinct from *V. pelagius* I and suggested that the two groups be classified as subspecies of *V. pelagius*, rather than biogroups. They also showed that the most closely related species (20–44%) are *"V. algosus"*, *V. harveyi* biogroup 2, *V. mytili*, *V. orientalis*, *V. parahaemolyticus*, *V. splendidus* biogroup 2, and *Vibrio* groups 514, 515, and 516. Fig. BXII.γ.171 shows the relationship of *Vibrio pelagius* to other *Vibrio* species based on 16S rRNA gene sequence analysis. Based on the immunological relationship of their superoxide dismutases (Fig. BXII.γ.170) the two groups of *Vibrio pelagius* are distinct, and both cluster in the marine *Vibrio* group (Baumann et al., 1984b).

Based on similarity of 5S rRNA sequences, MacDonell and Colwell (1985) proposed that *V. pelagius* should be united with *Vibrio anguillarum* and *Moritella marina* species in a new genus *Listonella*, and they proposed the name *Listonella pelagia*. Thus, *V. pelagius* currently has standing in nomenclature in three different genera as *Beneckea pelagia*, *Vibrio pelagius*, and *Listonella pelagia* (Table BXII.γ.162). All three names are based on the same type strain.

The mol% G + C of the DNA is: 45–47 (T_m, Bd).

Type strain: ATCC 25916, CECT 4202.

GenBank accession number (16S rRNA): X74722.

34. **Vibrio penaeicida** Ishimaru, Akagawa-Matsushita and Muroga 1995, 138[VP]

pe.nae.i.ci'da. L. n. *Penaeus* genus of kuruma prawns; L. v. *caedo* to kill; L. adj. *penaeicida Penaeus* killer.

The characteristics are as described for the genus and as listed in Tables BXII.γ.154, BXII.γ.163, and BXII.γ.164. Small rods, straight or slightly curved, 0.5–0.8 × 1.5–2.0 μm. Motile by means of a single polar flagellum. No lateral flagella are produced during growth on solid media. Colonies are low, cream colored, and translucent with entire margins. All reported isolates have the same O antigen.

Causes vibriosis of kuruma prawns (*Penaeus japonicus*) in Japan. Isolated from diseased and apparently healthy kuruma prawns and from water samples from ponds where prawns are grown.

V. penaeicida was formerly known as "*Vibrio* species PJ" (de la Peña et al., 1992). Ishimaru et al. (1995) showed by DNA–DNA hybridization experiments that the type strain of *V. penaeicida* was highly related (87–99%) to five other strains but was only 4–18% related to 29 other *Vibrio* and *Photobacterium* species tested. On the basis of DNA–DNA hybridization experiments, Brenner et al. (unpublished) also confirmed that *V. penaeicida* is a distinct species and found that its closest relative is *V. nigripulchritudo* (36% related).

The mol% G + C of the DNA is: 46.2–47 (HPLC).
Type strain: KH-1, ATCC 51841, IFO 15640, JCM 9123.

35. **Vibrio proteolyticus** (Merkel, Traganza, Mukherjee, Griffin and Prescott 1964) Baumann, Baumann, Bang and Woolkalis 1982, 267[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*"Aeromonas proteolytica"* Merkel, Traganza, Mukheriee, Griffin and Prescott 1964, 1230; *Aeromonas hydrophila* subsp. *proteolytica* (Merkel, Traganza, Mukheriee, Griffin and Prescott 1964) Schubert 1969, 412.)
pro.te.o.ly′ti.cus. Ger. *protein* from Gr. *protos* first; Gr. adj. *lyticus* dissolving; M.L. adj. *proteolyticus* protein-dissolving.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Grows on solid media as swarming waves. Isolated from *Limnoria tripunctata* taken from wood pilings Charleston, South Carolina, U.S.A.

The type strain was isolated from the alimentary canal of the marine isopod crustacean.

Reichelt et al. (1976) showed by DNA–DNA hybridization that the type strain of *V. proteolyticus* was only 0–25% related to 11 other *Vibrio* species. These results were confirmed by Brenner et al. (1983b) who showed that the type strain was less than 5% related to type strains of 16 other *Vibrio* species. Brenner et al. (unpublished) studied almost all newly described *Vibrio* species and concluded that *V. proteolyticus* is a distinct species whose closest relatives are CDC *Vibrio* groups 515 and 516 (33% relatedness), which have not yet been described in the literature. Fig. BXII.γ.171 shows the relationship of *V. proteolyticus* to other *Vibrio* species based on 16S rRNA gene sequence analysis.

The mol% G + C of the DNA is: 50.5 (Bd).
Type strain: ATCC 15338, DSM 30189, IFO 13287, NCMB 1326.
GenBank accession number (16S rRNA): X56579, X74723.

36. **Vibrio rumoiensis** Yumoto, Iwata, Sawabe, Ueno, Ichise, Matsuyama, Okuyama and Kawasaki 1999b, 935[VP] (Effective publication: Yumoto, Iwata, Sawabe, Ueno, Ichise, Matsuyama, Okuyama and Kawasaki 1999a, 71.)
ru.moi.en′sis. L. adj. *rumoiensis* from Rumoi, location of isolation.

Morphological, growth, physiological, and biochemical characteristics are listed in Table BXII.γ.164. Cells 0.5–0.9 × 0.7–2.1 μm. No flagella. Numerous blebs occur on the cell surface (Fig. BXII.γ.182). Colonies are white and circular. Produces high level of catalase.

Isolated from a drainage pool at a fish product processing plant where hydrogen peroxide was used as a bleaching agent.

Based on DNA–DNA hybridization experiments carried out using the fluorometric method, Yumoto et al. (1999a) found that the type strain of *V. rumoiensis* was only 3.2–9.0% related to 11 other *Vibrio* species. Based on the results of 16S rRNA gene sequence analysis, the type strain of *V. rumoiensis* was 92.4–95.5% similar to *Vibrio* and *Photobacterium* species. Fig. BXII.γ.171 illustrates the relationship of *V. rumoiensis* to other species of *Vibrio*.

The mol% G + C of the DNA is: 43.2 (HPLC).
Type strain: S-1, FERM-P 14531.
GenBank accession number (16S rRNA): AB013297.

37. **Vibrio salmonicida** Egidius, Wiik, Andersen, Hoff and Hjeltnes 1986, 519[VP]
sal.mon.i.ci′da N. L. n. *salmon* salmon; L. v. *caedo* to kill; N. L. adj. *salmonicida* salmon killer.

See description of *V. logei*.

By DNA–DNA hybridization experiments, Brenner et al. (unpublished) showed that the type strain of *Vibrio salmonicida* was highly related to the type strain of *Vibrio logei* (see also Fig. BXII.γ.171) and concluded that these two organisms should be classified in the single species, *Vibrio logei*.

The mol% G + C of the DNA is: 42 (T_m).
Type strain: HI 7751, ATCC 43839, CIP 103166, LMG 14010, NCIMB 2262.

38. **Vibrio scophthalmi** Cerdà-Cuéllar, Rosselló-Mora, Lalucat, Jorfe and Blanch 1997, 60[VP]
scoph.thal′mi. L. gen. n. *scophthalmi* derived from the genus name *Scophthalmus* because all the original isolates were from the turbot (fish) species *Scophthalmus maximus*.

The characteristics are as described for the genus and as listed in Tables BXII.γ.154, BXII.γ.163, and BXII.γ.164. Halophilic; grows poorly in media containing ≤ 0.5% NaCl. Colonies are unpigmented on TSA and yellow on TCBS agar.

Isolated from the intestines of juvenile turbot (*Scophthalmus maximus*) in a hatchery in Spain.

Based on DNA–DNA hybridization experiments (dot hybridization method) Cerdà-Cuéllar et al. (1997) found that the type strain was highly related to five other strains. Brenner et al. (unpublished) confirmed these results and concluded that *V. scophthalmi* is a distinct species. They also found that the type strain of *Vibrio scophthalmi* was 99% related to the reference strain of *Vibrio* INFL-2. The closest other relative was *V. ichthyoenteri*, which was 32% related. Based on the results of 16S rRNA gene sequence analysis, Cerdà-Cuéllar et al. (1997) found that the closest relative was *V. aestuarianus*, which had a sequence similarity of 97.8%. Fig. BXII.γ.171 shows the relationship of *Vibrio scophthalmi* to other *Vibrio* species based on 16S rRNA gene sequence analysis.

The mol% G + C of the DNA is: 44–44.5 (HPLC).
Type strain: A089, CECT 4638.
GenBank accession number (16S rRNA): U46579.

39. **Vibrio splendidus** (Beijerinck 1900a) Baumann, Baumann, Bang and Woolkalis 1981, 217[VP] (Effective publication: Baumann, Baumann, Bang and Woolkalis 1980b, 128) (*"Photobacter splendidum"* Beijerinck 1900a, 362; *Beneckea splendida* (Beijerinck 1900a) Reichelt, Baumann and Baumann 1979, 80.)
splen′di.dus. L. adj. *splendidus* brilliant.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. The cell morphology is shown in Fig. BXII.γ.183. Properties and differentiation of the three biogroups described by Reichelt et al. (1976) are given in Table BXII.γ.175.

Isolated from marine fish and seawater.

Reichelt et al. (1976) determined that *V. splendidus* consisted of three distinct groups that could be defined on the basis of both phenotypic characteristics and DNA–DNA hybridization experiments. These groups were referred to as

FIGURE BXII.γ.182. Electron micrograph of *Vibrio rumoiensis* illustrating the appearance of "blebs" on the cell surface. Negatively stained preparation. Bar = 1 μm. (Reproduced with permission from Yumoto et al., Applied and Environmental Microbiology, *65:* 67–72, 1999a ©American Society for Microbiology.)

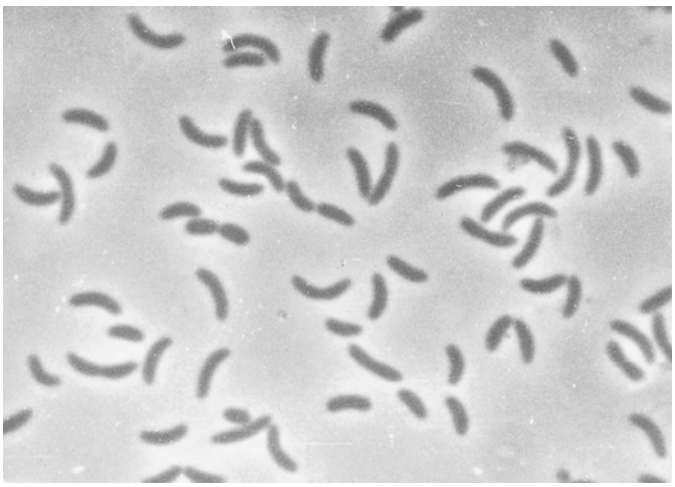

FIGURE BXII.γ.183. Phase contrast micrograph of *Vibrio splendidus* biogroup II. Exponential growth phase in yeast extract broth. × 3000.

biotypes I, II, and a third unnamed biotype (Table BXII.γ.175). Brenner et al. (unpublished) also confirmed that *Vibrio splendidus* is a distinct *Vibrio* species based on DNA–DNA hybridization experiments and showed that the type strain of *V. splendidus* was 30–45% related to the type strains of *V. campbellii*, *V. harveyi*, *V. parahaemolyticus*, and *V. pelagius*. Based on the immunological relationship of superoxide dismutases (Fig. BXII.γ.170). Baumann et al. (1984b) concluded that *V. splendidus* biogroups I and II are closely related, and (along with *V. fischeri* and *V. logei*) are more distantly related to the marine *Vibrio* group. Fig. BXII.γ.171 shows the relationship of the type strain of *V. splendidus*, which belongs to biogroup 1, to other *Vibrio* species based on 16S rRNA gene sequence analysis.

The mol% G + C of the DNA is: 45–46 (T_m, Bd).
Type strain: ATCC 33125, NCMB 1.
GenBank accession number (16S rRNA): X74724.

40. **Vibrio tapetis** Borrego, Castro, Luque, Paillard, Maes, Garcia and Ventosa 1996, 483[VP]
ta.pe′ tis. L. gen. n. *tapetis* of clams (the genus *Tapes*).

The characteristics are as described for the genus and as listed in Tables BXII.γ.154, BXII.γ.163, and BXII.γ.164. Coccobacilli, 0.5 × 1.0–1.5 μm. Motile by a single polar flagellum. Grows at 4–22°C; optimum growth at 22°C. Colonies on marine agar circular, translucent, and nonpigmented. Colonies on TCBS agar are green.

Causes brown ring disease in several species of wild and cultured clams. The type strain was isolated from cultured manila clam (*Tapes philippinarum*), Landeda, France.

Based on DNA–DNA hybridization experiments, Borrego et al. (1996) found that the type strain of *Vibrio tapetis* was highly related (100%) to eight other strains and less related to seven other *Vibrio* species. *Vibrio vulnificus* (58%), *V. alginolyticus* (53%), *V. fischeri* (48%), and *V. splendidus* (46%) were the closest relatives. Based on DNA–DNA hybridization experiments, Brenner et al., (unpublished) confirmed that *Vibrio tapetis* is a distinct species, and found that *V. wodanis* was the closest relative (19%). Results of this study indicated that *Vibrio tapetis* was 15% or less related to other *Vibrio* species, including the species listed above. Fig. BXII.γ.171 shows the relationship of *V. tapetis* to other *Vibrio* species based on 16S rRNA gene sequence analysis.

The mol% G + C of the DNA is: 42.9–45.5 (T_m).
Type strain: B1090, CECT 4600.
GenBank accession number (16S rRNA): Y08430.

41. **Vibrio trachuri** Iwamoto, Suzuki, Kurita, Watanabe, Shimizu, Ohgami and Yanagihara 1996, 625[VP] (Effective publication: Iwamoto, Suzuki, Kurita, Watanabe, Shimizu, Ohgami and Yanagihara 1995, 836.)

TABLE BXII.γ.175. The three biotypes (subgroups) of *Vibrio splendidus*

Test or property	Biotype I	Biotype II	Unnamed biotype
Type[a] or reference strain	ATCC 33125^T = NCMB 1	ATCC 33789 (strain 2)[b]	Strain 16 (not in ATCC)
Other strains	ATCC 33869 (strain 378); ATCC 33870 (strain 379); ATCC 33871 (strain 380)	ATCC 25914 (strain 8)	
Differential tests:[c]			
Bioluminescence	+	−	−
Arginine dihydrolase	+	−	
D-Mannose utilization	+	−	
D-Galactose utilization	+	−	
D-Glucuronate utilization	+	−	
L-Arginine utilization	+	−	
L-Citrulline utilization	+	−	
Heptonate utilization	+	−	

[a]The type strain for the species *Vibrio splendidus* is ATCC 33125.

[b]The strain numbers of Reichelt et al. (1976) are given in parentheses. Reichelt et al. (1976) labeled strain ATCC 33789 for DNA–DNA hybridization experiments and showed it was 82–91% related to the other four strains included in biotype II.

[c]Symbols: +, positive; −, negative.

tra′ chu.ri. L. gen. masc. n. *trachuri* derived from *Trachurus japonicus,* Japanese horse mackerel (fish).

The characteristics are as described for the genus and as listed in Tables BXII.γ.154, BXII.γ.163, and BXII.γ.164.

Causes vibriosis of "ma-aji", Japanese horse mackerel (*Trachurus japonicus*). The type strain was isolated from Japanese horse mackerel grown at Uchiura Bay, Numazu, Japan.

Based on DNA–DNA hybridization experiments (fluorometric assay in microdilution plates), Iwamoto et al. (1995) found that the type strain of *Vibrio trachuri* was highly related (95–98%) to two other strains, but less related (1–40%) to 15 other *Vibrio* species tested. The closest relative was *V. harveyi*, to which *Vibrio trachuri* was 40% related. *Vibrio trachuri* was 24–25% related to *V. alginolyticus* and *V. campbellii*, and 10% or less related to 12 other *Vibrio* species. Based on DNA–DNA hybridization experiments, Brenner et al. (unpublished) also concluded that *Vibrio harveyi* is the closest relative of *V. trachuri*. However, they found "species level relatedness" between the type strain of *V. trachuri* and the type strain of *V. harveyi*. Since these two species have different type strains, they concluded that *V. trachuri* would probably become a junior objective synonym of *V. harveyi*. Different methods were used in the two DNA–DNA hybridization studies; this fact may account for the differences in the results.

The mol% G + C of the DNA is: 45.4–47.8 (HPLC after nuclease P1 digestion).

Type strain: T9210, JCM 9677.

42. **Vibrio tubiashii** Hada, West, Lee, Stemmler and Colwell 1984, 2^{VP}

tu.bi.ash′ i.i. L. gen. n. *tubiashii* of Tubiash; named to honor H.S. Tubiash who first isolated the organism.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Short rods, 0.5 × 1.5 μm. Motile by means of a single polar flagellum when grown in liquid medium. Lateral short-wavelength flagella are synthesized when cultures are grown on solid medium. Colonies on marine agar smooth, circular, off-white and sometimes mucoid; yellow on TCBS agar.

Causes disease, with resulting economic losses, in larvae and juveniles of bivalve mollusks. Isolated from larvae and juveniles of the hard clam (*Mercenria mercenaria*), oyster spat (*Crassostrea virginica*), and adult oyster.

Based on DNA–DNA hybridization experiments (nitrocellulose method), Hada et al. (1984) found that the type strain of *V. tubiashii* was highly related (68–96%) to five other strains, but was only 28% related to *Vibrio anguillarum*. They also found that *V. tubiashii* strain NCMB 2166 was only 13–26% related to 11 other *Vibrio* species. Brenner et al. (unpublished) agreed that *V. tubiashii* is a distinct species, and found the closest relatives of *V. tubiashii* to be *V. orientalis* and *Vibrio* groups 515 and 516 (29–33% related). Fig. BXII.γ.171 illustrates the relationship of *V. tubiashii* to other *Vibrio* species based on 16S rRNA gene sequence analysis.

The mol% G + C of the DNA is: 43–45% (T_m).

Type strain: ATCC 19109, CCUG 38428, CIP 102760, IFO 15644, LMG 10936.

GenBank accession number (16S rRNA): X74725.

43. **Vibrio vulnificus** (Reichelt, Baumann and Baumann 1979) Farmer 1980, 656^{VP} (*Beneckea vulnifica* Reichelt, Baumann and Baumann 1979, 80.)

vul.ni′ fi.cus. L. adj. *vulnificus* inflicting wounds.

The characteristics are as described for the genus and as listed in Tables BXII.γ.163 and BXII.γ.164. Characters that differentiate *V. vulnificus* from *V. alginolyticus*, *V. harveyi*, and *V. parahaemolyticus* are given in Table BXII.γ.170. Characters that distinguish *V. vulnificus* from other *Vibrio* spp. found in human clinical specimens are given in Tables BXII.γ.156, BXII.γ.157, BXII.γ.160, BXII.γ.161, BXII.γ.165 and BXII.γ.166. Differential characteristics of the three biogroups of *V. vulnificus* are given in Table BXII.γ.176.

Occurs in human clinical specimens (Table BXII.γ.157) and the marine environment. Cause of wound infections, bacteremia, and septicemia. The type strain was isolated from human blood.

The type stain and most other strains belong to biogroup 1. All information given below on mol% G + C of the DNA, type strain accession numbers, and GenBank accession numbers pertains to biogroup 1. Biogroups 2 and 3 differ in phenotype, host range, ecology, and several other ways.

V. vulnificus biogroup 2 was described by Tison et al. (1982). Deposited strains include ATCC 33147, 33148, and

TABLE BXII.γ.176. Differentiation of three *Vibrio vulnificus* biogroups[a]

Test	Biogroup: 1	2	3
Citrate (Simmons)	+	+	−
Indole production	+	−	+
ONPG test[b]	+	+	−
Ornithine decarboxylase	+	−	+
Fermentation of:			
Cellobiose	+	+	−
Lactose	+	+	−
D-Mannitol	+	−	−
Salicin	+	+	−
D-Sorbitol	−	+	−

[a]Symbols: +, 75–100% of strains positive; −, 75–100% of strains negative. Data from Bisharat et al., 1999.

[b]ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

33149. Strains of biogroup 2 were originally shown to be pathogenic for eels (Tison et al., 1982). Biogroup 2 appears to be a cause of human wound infections as well (Amaro and Biosca, 1996).

Biogroup 3 strains were described as a separate biogroup by Bisharat et al. (1999). Biogroup 3 strains were originally identified as a variety of different *Vibrio* species (Bisharat and Raz, 1996); all isolates were associated with human wound infections and exposure to cultured tilapia. There are no deposited strains.

The mol% G + C of the DNA is: 46–48 (T_m, Bd) (biogroup 1).

Type strain: B9629, ATCC 27562, DSM 10143, IMET 11292 (biogroup 1).

GenBank accession number (16S rRNA): X56582, X74726, X76333 (biogroup 1).

44. **Vibrio wodanis** Lunder, Sørum, Holstad, Steigerwalt, Mowinckel and Brenner 2000, 446[VP]

wo.da′ nis. M. L. gen. n. *wodanis* from Wodan, the Norse god of art, culture, war, and the dead.

Morphological, growth, physiological, and biochemical characteristics are listed in Table BXII.γ.164. This is the second of the two new *Vibrio* species from Atlantic salmon with "winter ulcer" that were described by Lunder et al. (2000). Fig. BXII.γ.171 illustrates the relationship of *V. wodanis* to other species of *Vibrio*.

The mol% G + C of the DNA is: 40 (T_m).

Type strain: NVI 88/441, ATCC BAA-104, NCIMB 13582.

GenBank accession number (16S rRNA): AJ132227.

Other Organisms

1. Baumann Group E-3[1]

Properties are given in Table BXII.γ.163. This group was originally described and given the vernacular name "E-3" by Baumann et al. (1971a). The group included Baumann strains 94 and 95. Based on the immunological relationships of the enzyme superoxide dismutase from different *Vibrio* and *Photobacterium* species, Bang et al. (1981) showed that Group E-3 was more closely related to three *Photobacterium* species than to *V. cholerae*, *V. alginolyticus*, and other *Vibrio* species (see Fig. 4 of Bang et al., 1981). Group E-3 has been referred to as *Vibrio* group 513 by Farmer (unpublished).

Deposited strain: ATCC 33523 from seawater, Oahu, Hawaii (strain 95 of Baumann et al. [1971]; isolated with acetate enrichment).

2. *Vibrio* Group 509[2]

Properties are given in Table BXII.γ.163. The name "*Vibrio* Group 509" includes a group of four Na^+-requiring *Vibrio* strains isolated from blood cultures of Kemp's Ridley sea turtles during an outbreak at the National Marine Fisheries Services facilities, Galveston, Texas. The organisms were seen in stained sections of lung tissue and the animals were observed to vomit blood. This condition was thought to resemble "hemorrhagic septicemia". Based on DNA–DNA hybridization experiments, Brenner et al. (unpublished) found that the strains were 85% related under stringent hybridization conditions.. The strains of *Vibrio* Group 509 strains were later found to be closely related to the type strain of *V. harveyi* (Brenner et al., personal communication).

3. *Vibrio* group 510

Properties are given in Table BXII.γ.163. This group includes the reference strain, which was isolated from an infected human leg wound, and five additional strains. Based on DNA–DNA hybridization experiments (Brenner et al., personal communication), these strains had an average relatedness to the reference strain of 78% under stringent hybridization conditions. *Vibrio* group 510 is closely related to *V. diazotrophicus* and may eventually become a named biogroup (Table BXII.γ.164) or subspecies of *V. diazotrophicus*. *Vibrio* group 510 can be distinguished phenotypically from *V. diazotrophicus* by being arginine negative at 7 days, fermenting D-mannose, and failing to ferment salicin. *V. diazotrophicus* strains usually have the opposite reactions.

4. *Vibrio* group 511

Properties are given in Table BXII.γ.163. *Vibrio* group 511 appears closely related to or perhaps a synonym of *V. cincinnatiensis* (see Table BXII.γ.164, and *V. cincinnatiensis*).

5. *Vibrio* group 512

Properties are given in Table BXII.γ.163. Strains of *Vibrio* group 512 were isolated from seawater and mud in the

1. "Baumann Groups"—In the 1970s, many different vibrios were studied in the laboratories of Paul and Linda Baumann. Scientific names were assigned to many of their distinct groups. However, some groups were never given scientific names. Also, in the Other Organisms Section of the *Vibrio* chapter in the last edition of this *Manual*, Baumann et al. (1984b) discussed four strains or groups of strains that they considered were distinct from all the species and named groups known at that time. These four vibrio groups should be studied with the techniques now available.

2. For many years vernacular names such as "*Vibrio* group 509" were assigned by one of us (JJF) to a new or unusual strain or group of strains. This nomenclature is similar to the one that assigned a vernacular name "Enteric Group" to an organism thought to belong in the family *Enterobacteriaceae* (Farmer et al., 1985a). The "*Vibrio* group names" have been included for many years in the databases (see Table BXII.γ.163) and computer identification programs distributed by the *Vibrio* Reference Laboratory. Many have been included in various DNA–DNA hybridization studies (Brenner et al., 1983b; Brenner et al., unpublished). Four of the groups are described below. Additional groups have been defined but require further study and will be described in the future. The relationships of these groups to other *Vibrio* species as shown by DNA–DNA hybridization experiments are given in Fig. BXII.γ.172.

United Kingdom. Based on DNA–DNA hybridization experiments, five strains were found to be 89% related to the reference strain under stringent hybridization conditions (Brenner et al., unpublished). The closest relative of group 512 is *V. cincinnatiensis* (including *Vibrio* group 511), which is 15% related under stringent hybridization conditions.

Genus II. **Photobacterium** *Beijerinck 1889, 401*[AL]

AN THYSSEN AND FRANS OLLEVIER

Pho.to.bac.te′ ri.um. Gr. n. *phos* light; Gr. neut. dim. n. *bakterion* a small rod; M.L. neut. n. *Photobacterium* light (-producing) bacterium.

Plump, straight rods, 0.8–1.3 × 1.8–2.4 μm; some coccobacillary and bipolar. Involution forms are usually seen in old cultures or under adverse conditions of cultivation. Gram negative. **Motile by 1–3 unsheathed polar flagella**; some species nonmotile. **Facultative anaerobic.** Chemoorganotrophic, having **both a respiratory and a fermentative type of metabolism**. Optimal growth temperature range appears to be 18–25°C, except for the psychrophilic species *P. profundum*. **Sodium ions are required for growth.** D-Glucose and D-mannose are catabolized with the production of acid and, in the case of *P. damselae* subsp. *damselae*, *P. iliopiscarium*, and *P. phosphoreum*, with gas. Oxidase reaction is variable. Most strains are lysine decarboxylase and arginine dihydrolase positive. Ornithine decarboxylase negative. **Accumulate poly-β-hydroxybutyrate** (PBH) under certain conditions of cultivation; **do not use the exogenous monomer β-hydroxybutyrate**. Most strains **grow in a minimal medium containing a seawater base, D-glucose, and NH_4Cl**; other strains also require L-methionine. Utilize D-glucose, D-fructose, glycerol, and D-mannose. Some species are bioluminescent.

The mol% G + C of the DNA is: 39–44 (Baumann and Baumann, 1977).

Type species: **Photobacterium phosphoreum** (Cohn 1878) Beijerinck 1889, 401 (*Micrococcus phosphoreus* Cohn 1878, 126.)

FURTHER DESCRIPTIVE INFORMATION

The genus *Photobacterium* is situated in the family *Vibrionaceae* and belongs to the class *Gammaproteobacteria*. Within the genus *Photobacterium*, the group of *P. damselae* subsp. *piscicida*, *P. damselae* subsp. *damselae*, and *P. leiognathi* forms a distinct subcluster (Nogi et al., 1998c). *P. phosphoreum*, *P. angustum*, and *P. profundum* are also closely related. High DNA–DNA hybridization values (which vary with the hybridization techniques used) have been reported between *P. damselae* subsp. *piscicida* and *P. damselae* subsp. *damselae* (Gauthier et al., 1995b; Thyssen et al., 2000). Low DNA–DNA hybridization values have been reported between (i) *P. damselae* subsp. *piscicida* and *P. leiognathi* (Thyssen et al., 2000), (ii) *P. damselae* subsp. *damselae* and *P. leiognathi* (Nogi et al., 1998c; Thyssen et al., 2000), (iii) *P. angustum* and *P. phosphoreum* (Baumann and Baumann, 1981b; Nogi et al., 1998c), (iv) *P. angustum* and *P. profundum* (Nogi et al., 1998c), (v) *P. phosphoreum* and *P. profundum* (Nogi et al., 1998c), (vi) *P. phosphoreum* and *P. leiognathi* (Baumann and Baumann, 1981b).

Photobacterium spp. display morphologic characteristics typical of members of the *Vibrionaceae*, appearing as straight rods; *P. damselae* subsp. *damselae*, and *P. leiognathi* appear as coccobacilli. In the early stationary phase of growth, PHB, which is stored by the cells as a reserve product, can be detected as discrete, refractile, intracellular granules (Reichelt and Baumann, 1973b). When a substantial amount of PHB is accumulated, the entire cell appears refractile. PHB is only formed when cells are grown in a basal medium with glucose as the sole carbon source; PHB is not formed when cells are grown in rich media containing peptone. Except for *P. damselae* subsp. *piscicida*, all *Photobacterium* species are motile. One to three polar flagella have been described (Reichelt and Baumann, 1973b). These flagella, unlike those of *Vibrio*, are not enclosed in a sheath.

The fatty acid profile of *Photobacterium* species has been recently determined by Nogi et al. (1998c), who found high concentrations of $C_{16:1}$ and $C_{16:0}$ fatty acids. Moreover, $C_{12:0}$, $C_{12:0\ 3OH}$, $C_{18:1}$, and to a lesser extent $C_{14:0}$, $C_{14:0\ 3OH}$, and $C_{14:1}$ fatty acids are detected. Q-8 and Q-7 have been reported as major isoprenoid quinones in *Photobacterium* species (Nogi et al., 1998c).

Colonies of *Photobacterium* are entire, smooth, semitranslucent, convex, and ivory. The color of the colonies is somewhat whiter than that of many other Gram-negative marine bacteria, probably due to a relatively low content of cytochromes.

Photobacterium species are unable to grow in the absence of Na^+. For optimal growth, 160–280 mM Na^+ is required. Most *Photobacterium* strains have no organic growth factor requirement; some *P. phosphoreum* strains, however, require L-methionine, either alone or in combination with other amino acids (Reichelt and Baumann, 1973b; Ruby et al., 1980). All species except *P. profundum* are able to grow at 20°C; *P. iliopiscarium*, *P. phosphoreum*, *P. profundum*, and some *P. angustum* strains are able to grow at 4°C.

The nutritional versatility of *Photobacterium* species is relatively limited; only 7–22 carbon compounds can be utilized as sole or principal sources of carbon and energy. These compounds include hexoses and a few pentoses, disaccharides, sugar acids, tricarboxylic acid cycle intermediates, and amino acids. Almost none of the *Photobacterium* species can utilize D-mannitol as the sole source of carbon energy; most species, however, except *P. damselae* subsp. *piscicida* and *P. leiognathi*, can utilize maltose. Most species are able to use *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, D-fructose, glycerol, glycogen, DL-lactate, D-glucose, D-galactose, maltose, D-mannose, and turanose as sole carbon sources. Adonitol, L-arabinose, cellobiose, *myo*-inositol, α-D-lactose, raffinose, L-rhamnose, D-sorbitol, and sucrose are not used. Acid with gas from D-glucose fermentation is detected with some of the *Photobacterium* species. None of the species have an extra-

TABLE BXII.γ.177. Differential characteristics of species of *Photobacterium*[a]

Characteristic	1. *P. phosphoreum*	2. *P. angustum*	3a. *P. damselae* subsp. *damselae*	3b. *P. damselae* subsp. *piscicida*	4. *P. iliopiscarium*	5. *P. leiognathi*	6. *P. profundum*
Luminescence	+	−	−	−	−	+	−
Motility	+	+	+	−	+	+	+
Growth, 4°C	+	d	−	−	+	−	+
Growth, 37°C	−	+	d	d	−	+	−
Gas production from D-glucose	+	−	+	−	+	−	−
Indole production	−	−	−	−	−	−	+
Nitrate reduction	+	−	+	−	+	+	+
Gelatinase	−	d	d	−	−	−	nd
Lipase	−	d	d	+	nd	+	nd
Acetate utilization	−	+	−	nd	nd	+	nd
DL-Glycerate utilization	+	−	−	nd	nd	d	nd
Maltose utilization	+	d	+	−	nd	−	+
L-Proline utilization	−	−	nd	nd	nd	+	nd
Pyruvate utilization	−	+	nd	−	nd	+	nd
D-Xylose utilization	−	+	−	−	nd	−	nd

[a]For symbols, see standard definitions; nd, not determined.

cellular gelatinase or alginase; some strains have an extracellular chitinase, lipase, or amylase. The methyl red and Voges–Proskauer tests are positive. Oxidase reaction is variable; catalase reaction is positive. Indole, hemolysin, and H_2S are not produced; except in the case of *P. profundum*, which is able to produce indole. Most species reduce nitrate, except *P. angustum* and *P. damselae* subsp. *piscicida* (Table BXII.γ.177).

Phylogenetic analyses based on the 5S rRNA, 16S rRNA, and 16S rDNA sequences have been carried out to clarify the taxonomic and phylogenetic position of members of the *Vibrionaceae* and species belonging to the genus *Photobacterium* (MacDonell and Colwell, 1985; Kita-Tsukamoto et al., 1993; Gauthier et al., 1995b; Nogi et al., 1998c). Moreover, the levels of homology of the chromosomal DNA of the different *Photobacterium* species have been intensively studied by Nogi et al. (1998c) and Thyssen et al. (2000).

Several serological typing systems have been developed, but these focus primarily on *P. damselae* subsp. *piscicida* and *P. damselae* subsp. *damselae* (Fouz et al., 1992; Magariños et al., 1992; Bakopoulos et al., 1997; Kawahara et al., 1998). In addition, plasmid profiling and a variety of DNA-based typing methods, such as amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), and ribotyping, have been used in epidemiological studies (Magariños et al., 1992, 1997; Pedersen et al., 1997; Thyssen et al., 2000). Plasmids have been described in a variety of species, including *P. damselae* subsp. *piscicida* and *P. damselae* subsp. *damselae* (Fouz et al., 1992; Kim and Aoki, 1993; Pedersen et al., 1997). Chloramphenicol, tetracycline, sulfamonomethoxine, and kanamycin resistances have been shown to be plasmid mediated and transferable (Kim and Aoki, 1993).

Photobacterium species are susceptible to vibriostatic agent O/129 (10 μg and 150 μg). Drug resistance has been reported for many species, especially for *P. damselae* subsp. *piscicida*, which is resistant to a variety of antimicrobial agents (Aoki, 1992).

P. damselae subsp. *piscicida* and *P. damselae* subsp. *damselae* are pathogenic for several marine fish species. The polysaccharide capsular layer, iron uptake system, phopholipase activity of *P. damselae* subsp. *piscicida*, and extracellular products have been recognized as virulence factors (Romalde and Magariños, 1997). A phospholipase toxin with hemolytic and cytotoxic activities has been documented in *P. damselae* subsp. *damselae* (Toranzo and Barja, 1993).

Photobacterium strains are widespread in the marine environment and have been isolated from seawater, sediment, the surface and intestines of diseased marine animals, and the specialized light organs from marine fish. Some strains are found as symbionts in specialized luminous organs of marine fish.

Strains of *P. leiognathi* and *P. phosphoreum* are able to emit light due to the presence of luciferase. The reaction catalyzed by luciferase involves the luminescent oxidation of reduced flavin mononucleotide ($FMNH_2$) and a long chain aliphatic aldehyde by molecular oxygen. The reaction can be expressed as $FMNH_2 + O_2 + RCHO \rightarrow h\nu + FMN + RCOOH$.

Enrichment and Isolation Procedures

Photobacterium species may be recovered from the marine environment and on the surfaces and the intestinal contents of marine animals using blood agar, brain–heart infusion agar, tryptic soy agar (supplemented with 1% (w/v) NaCl), thiosulfate citrate bile sucrose agar, and marine agar. Details of the isolation of *Photobacterium* in luminous organs of fish are given by Baumann and Baumann (1981b) and Hastings and Nealson (1979). Nutrient agents affecting the growth, luminescence intensity, and luciferase synthesis are described by Rodicheva et al. (1993).

Maintenance Procedures

Photobacterium strains may be maintained at room temperature on long-term preservation medium (West and Colwell, 1984) for several months and reactivated on brain–heart infusion agar (Difco) to which 1% (w/v) NaCl is added. For long-term storage, cultures should be frozen and maintained at −70°C. A method for long-term preservation of luminous bacteria, such as *P. leiognathi* and *P. phosphoreum*, is described by Janda and Opekarova (1989).

Procedures for Testing Special Characters

A somewhat unusual combination of properties of *Photobacterium* is the ability to accumulate PHB as an intracellular reserve product, coupled with the inability to utilize the exogenous monomer, β-hydroxybutyrate, as a sole or principal source of carbon and energy. Accumulation of refractile PHB granules can be readily detected by phase microscopy in early stationary-phase cultures grown in basal medium containing 0.2% (w/v) D-glucose. In the case of organisms requiring growth factors, the medium is supplemented with 0.05–0.1% (w/v) yeast extract.

Differentiation of the genus *Photobacterium* from other genera

Classical biochemical differentiation of the genus *Photobacterium* from related genera, such as *Vibrio*, *Aeromonas*, and *Plesiomonas*, is primarily achieved via the identification of the individual species. *Photobacterium* can be differentiated from *Vibrio*, *Aeromonas*, and *Plesiomonas* by its production of arginine dihydrolase, Voges–Proskauer reaction, inability to produce alginase, gelatinase, and indole, fermentation of sucrose, utilization of D-mannitol, and requirement of over 100 mM Na^+ for optimal growth. Moreover, *Photobacterium* can be distinguished from *Vibrio* by the presence of unsheathed polar flagella. Analysis of poly-β-hydroxybutyrate enables clear differentiation of *Photobacterium* strains from *Vibrio* strains, but is of limited value in a routine diagnostic laboratory.

The decay kinetics of light emission by luciferase in the presence of dodecanal subdivides luminous bacteria into two major groups having either "slow" or "fast" kinetics (Jensen et al., 1980; Hastings and Nealson, 1981). "Slow" decay kinetics are characteristic of the enzyme from *Vibrio harveyi*, *V. splendidus* biovar I, *V. cholerae*, and *Xenorhabdus luminescens*, while "fast" decay kinetics occur with the luciferases of *Photobacterium*, *V. fischeri*, *V. logei*, and *Alteromonas hanedai*. Although *Photobacterium* is not unique in having "fast" decay kinetics, this property still has diagnostic value, since the luminous species of *Vibrio* and *Alteromonas* are readily distinguished from *Photobacterium* by a few easily determined properties; *V. fischeri* and *V. logei* have a yellow-orange, cell-associated pigment, while *Photobacterium* does not, and *A. hanedai* is a strict aerobe, whereas *Photobacterium* is facultative anaerobe.

Taxonomic Comments

Since its creation in 1889, a variety of Gram-negative, facultatively anaerobic, PHB-producing bacteria have been included in the genus *Photobacterium*. Originally, the genus was composed of 3 species: *P. angustum*, *P. leiognathi*, and *P. phosphoreum*. Later phylogenetic work based on ribosomal RNA and DNA sequence analysis and/or hybridization experiments has revealed the presence of other species in this genus, such as *P. fischeri* (formerly *V. fischeri*) (Reichelt and Baumann, 1973b), *P. logei* (Harwood et al., 1980), *P. histaminum* (Okuzumi et al., 1994), *P. damselae* subsp. *piscicida* (formerly *Pasteurella piscicida*) (Gauthier et al., 1995b), *P. damselae* subsp. *damselae* (formerly *Vibrio damsela* or *Listonella damsela*) (Gauthier et al., 1995b), *P. profundum* (Nogi et al., 1998c), and *P. iliopiscarium* (formerly *V. iliopiscarius*) (Urakawa et al., 1999a). *P. logei*, however, has been removed from *Photobacterium* and classified in the genus *Vibrio*. The taxonomic position and classification of *P. fischeri* (*Vibrio fischeri*) remains uncertain. More details are given in the chapter on *Vibrionaceae*.

There are some taxonomic problems at the species level. Although the names *P. damselae* subsp. *piscicida* and *P. damselae* subsp. *damselae* have been validly published, some doubt exists whether or not these organisms should be classified as two separate species. They are phenotypically and genotypically dissimilar, develop different diseases and have different ecological niches (Thyssen et al., 1998; Thyssen et al., 2000). DNA–DNA hybridization and 16S rRNA sequence analysis, however, indicate that they are closely related (Gauthier et al., 1995b).

Another problem concerns the taxonomic position of *P. iliopiscarium* and *P. histaminum*. *P. iliopiscarium* was originally placed in the genus *Vibrio* (Onarheim et al., 1994), but has recently been reclassified as *P. iliopiscarium* based on 16S rRNA gene sequences (Urakawa et al., 1999a). *P. histaminum* (Okuzumi et al., 1994) is considered to be a later heterotypic synonym of *P. damselae* subsp. *damselae*, based on 16S rDNA sequencing and DNA–DNA hybridization (Kimura et al., 2000).

List of species of the genus Photobacterium

1. **Photobacterium phosphoreum** (Cohn 1878) Beijerinck 1889, 401[AL] (*Micrococcus phosphoreus* Cohn 1878, 126.)
phos.pho′re.um. Gr. v. *phosphoreo* bring light; M.L. neut. adj. *phosphoreum* light-bearing.

Straight rod. Motile by means of unsheathed polar flagella. Luminescent. Contains high concentrations of $C_{16:1}$, $C_{16:0}$, and $C_{14:0}$ fatty acids, as well as $C_{12:0}$ and $C_{12:0\ 3OH}$, and to a lesser extent $C_{14:1}$, $C_{14:0\ 3OH}$, $C_{18:0}$, and $C_{18:1}$ (Nogi et al., 1998c). Isoprenoid quinone Q-8 accounts for 100% of the total isoprenoid quinones (Nogi et al., 1998c). May require L-methionine, either alone or in combination with other amino acids. Able to grow at 4°C, but not at 35°C or 40°C. Optimum growth at 160–280 mM Na^+ and 20–25°C. The description is the same as that for the genus. Gas production. Oxidase, caseinase, and lipase are not produced. L-arabinose, cellobiose, *i*-inositol, D-mannitol, salicin, D-sorbitol, sucrose, trehalose, and D-xylose are not fermented. Acetate, alanine, arginine, citrate, ethanol, glycine, leucine, putrescine, proline, pyruvate, and threonine are not used as sole carbon source. See Table BXII.γ.178 for other characteristics.

DNA–DNA hybridization experiments have revealed a DNA relatedness of 20.5% between *P. phosphoreum* and *P. profundum* (Nogi et al., 1998c). Moreover, there was a DNA–rRNA relatedness of 98.7% to *P. leiognathi* (Baumann and Baumann, 1981b). Bacteriophages active against *P. phosphoreum* have been isolated from the marine environment (Spencer, 1963). Isolated from the light organs of five fish families (*Macrouridae*, *Merluccidae*, *Opisthoproctidae*, *Trachichthydae*, and *Moridae*) that are usually found in deep, cold waters. *P. phosphoreum* is a psychrophilic, deepwater species found at depths of 50–500 m.

The mol% G + C of the DNA is: 40.8 (HPLC).

Type strain: ATCC 11040, LMG 4233.

GenBank accession number (16S rRNA): D25310, X74687, Z19107.

2. **Photobacterium angustum** Reichelt, Baumann and Baumann 1979, 79[AL]

TABLE BXII.γ.178. Other biochemical characteristics of *Photobacterium* species[a]

Characteristic	1. *P. phosphoreum*	2. *P. angustum*	3a. *P. damselae* subsp. *damselae*	3b. *P. damselae* subsp. *piscicida*	4. *P. iliopiscarium*	5. *P. leiognathi*	6. *P. profundum*
Growth at 20°C	+	+	+	+	+	+	−
Growth at 30°C	d	+	+	+	−	+	−
Growth at 40°C	−	−	−	−	−	−	−
Oxidase	−	d	d	+	nd	d	+
Catalase	+	+	+	+	+	d	+
Arginine dihydrolase	+	+	+	+	+	+	+
Lysine decarboxylase	+	−	d	−	+	−	−
Ornithine decarboxylase	−	−	−	−	−	−	−
Hemolysin production	nd	−	d	−	−	−	nd
Amylase	−	−	d	d	−	−	nd
Alginase	−	−	−	−	nd	−	nd
Production H_2S	−	−	−	−	−	−	−
Utilization of:							
N-Acetyl-D-galactosamine	−	+	+	nd	nd	+	−
N-Acetyl-D-glucosamine	+	+	+	+	+	+	−
Adonitol	−	−	−	−	−	−	−
L-Arabinose	−	−	−	−	−	−	−
Cellobiose	−	−	+	−	−	−	−
D-Fructose	+	+	+	+	+	+	−
D-Galactose	+	+	+	+	+	+	+
Glycogen	+	+	+	−	nd	+	+
D-Gluconate	d	+	−	−	d	+	nd
D-Glucose	+	+	+	+	+	+	+
L-Glutamate	d	−	nd	+	nd	d	nd
Glycerol	+	+	+	−	d	+	+
myo-Inositol	−	−	−	−	−	−	−
DL-Lactate	d	+	+	d	nd	+	nd
α-D-Lactose	−	−	−	−	nd	−	−
Maltose	+	+	+	+	+	+	+
D-Mannitol	−	−	−	−	−	−	+
D-Mannose	+	+	+	+	+	+	+
Raffinose	−	−	−	−	−	−	−
L-Rhamnose	−	−	−	−	−	−	−
D-Ribose	d	+	+	d	+	+	nd
D-Sorbitol	−	−	−	−	−	−	−
Sucrose	−	d	−	−	−	−	−
Trehalose	−	d	d	−	d	−	+
Turanose	−	+	+	nd	−	+	+
Tween 40	+	−	+	nd	nd	−	+
Tween 80	−	−	−	+	nd	−	+

[a]For symbols, see standard definitions; nd, not determined.

an.gus′tum. L. neut. adj. *angustum* limited, with respect to nutritional versatility.

Rod. Motile by means of polar flagella. Not luminescent. The fatty acid profile has recently been determined by Nogi et al. (1998c), who have found that the bacterium contains high concentrations of $C_{16:1}$, $C_{16:0}$, and $C_{18:1}$ fatty acids. Moreover, $C_{12:0}$ and $C_{12:0\ 3OH}$, and to a lesser extent $C_{14:0}$, $C_{14:0\ 3OH}$, $C_{14:1}$, $C_{15:0}$, $C_{17:0}$, $C_{17:1}$, and $C_{18:0}$ fatty acid have been detected. The major isoprenoid quinone is Q-8 (Nogi et al., 1998c). Optimal growth at 20–25°C and 160–280 mM Na^+. No growth in absence of NaCl, or at NaCl concentration higher than 8%. Not able to grow at 42°C. No gas production from D-glucose. Oxidase and DNase positive. Urease, lysine decarboxylase, and phenylalanine deaminase are not produced. D-adonitol, L-arabinose, cellobiose, esculin, *i*-inositol, D-mannitol, L-rhamnose, salicin, and D-sorbitol are not fermented. Arginine, citrate, citrulline, ethanol, glycine, leucine, malate, methionine, proline, and putrescine are not used as sole carbon sources. Some strains are gelatinase positive. See Table BXII.γ.178 for other characteristics.

DNA–DNA hybridization experiments have indicated relatedness between *P. angustum* and *P. damselae* subsp. *piscicida*, *P. damselae* subsp. *damselae*, *P. leiognathi*, *P. phosphoreum*, and *P. profundum* of, respectively, 21%, 18.5%, 43.5%, 33.5%, and 24.5% (Nogi et al., 1998c; Thyssen et al., 2000). Susceptible to vibriostatic agent O/129 (2,4-diamino-6,7-diisopropyl pteridine) (10 μg and 150 μg). Resistant to penicillin G, oxacillin, streptomycin, and amoxicillin. Isolated from the open ocean and the light organs of several fish species.

The mol% G + C of the DNA is: 41.0 (HPLC).

Type strain: ATCC 25915.

GenBank accession number (16S rRNA): D25307, X74685.

3. **Photobacterium damselae** (Love, Teebken-Fisher, Hose, Farmer, Hickman and Fanning 1981) Smith, Sutton, Fuerst

and Reichelt 1991, 533^{VP} (*Vibrio damsela* Love, Teebken-Fisher, Hose, Farmer, Hickman and Fanning 1981, 1140.) *dam.se' la.e.* L. *damselae* named after damselfish.

Not luminescent. Na^+ required for growth. Does not produce phenylalanine deaminase. D-Glucose and D-mannose are fermented. No fermentation of L-arabinose, lactose, D-mannitol, melibiose, or trehalose. Several molecular techniques, such as AFLP, ribotyping, and RFLP, have been used to characterize and differentiate between the two subspecies of *Photobacterium damselae*, *P. damselae* subsp. *piscicida* and *P. damselae* subsp. *damselae* (Magariños et al., 1992, 1997; Pedersen et al., 1997; Thyssen et al., 2000). Plasmids have been reported. Isolated from fish.

The mol% G + C of the DNA is: 40.6–41.4 (enzymatic hydrolysis + HPLC).

Type strain: ATCC 33539.

a. **Photobacterium damselae** *subsp.* **damselae** (Love, Teebken-Fisher, Hose, Farmer, Hickman and Fanning 1981) Gauthier, Lafay, Ruimy, Breittmayer, Nicolas, Gauthier and Christen 1995b, 142^{VP} emend. Kimura, Hokimoto, Takahashi and Fujii 2000, 1341 (*Photobacterium damselae* Smith, Sutton, Fuerst and Reichelt 1991, 533; *Vibrio damsela* Love, Teebken-Fisher, Hose, Farmer, Hickman and Fanning 1981, 1140.)

Analyses of lipopolysaccharides and outer membranes have shown that different patterns can be seen, although some bands were common (Fouz et al., 1992). The fatty acid profile of the bacterium contains high concentrations of $C_{16:1}$, $C_{16:0}$, and $C_{18:1}$ fatty acids; $C_{12:0}$ and $C_{12:0\ 3OH}$, and, to a lesser extent, $C_{14:0}$, $C_{14:0\ 3OH}$, $C_{14:1}$, $C_{15:0}$, $C_{17:0}$, $C_{17:1}$, and $C_{18:0}$ fatty acids are also present (Nogi et al., 1998c) . The major isoprenoid quinone is Q-8, and a minor quinone is Q-7; these account for 90% and 10% of the total isoprenoid quinones, respectively (Nogi et al., 1998c). Round to slightly irregular, off-white, opaque, flat, shiny colonies. Able to grow at 1–6% NaCl, with an optimum of 3% NaCl. Produces urease, phosphatase, DNase, and arginine dihydrolase, but not gelatinase or ornithine decarboxylase. Production of amylase and lysine decarboxylase is variable. Some strains produce β-hemolysis. Able to grow on TCBS and MacConkey agar. Gas is produced during fermentation of carbohydrates. Citrate is not used as a sole source of carbon and energy. Maltose is fermented. No fermentation of D-adonitol, D-arabitol, dulcitol, erythritol, *i*-inositol, raffinose, L-rhamnose, salicin, D-sorbitol, or sucrose. See Table BXII.γ.178 for other characteristics.

DNA–DNA hybridization experiments show a DNA relatedness of 21%, 21%, and 19.5% between *P. damselae* subsp. *damselae* and *P. leiognathi*, *P. phosphoreum*, and *P. profundum*, respectively (Nogi et al., 1998c). Plasmids have been reported to be common in *P. damselae* subsp. *damselae* (Fouz et al., 1992). Plasmids can be heterogeneous in size, varying from very small (3.0 kb) to very large (~190 kb) (Pedersen et al., 1997). A high-molecular-weight plasmid band can be detected in most strains. Antigenic differences exist among different *P. damselae* subsp. *damselae* strains, and at least 4 distinct groups are recognized. The serological results are supported by lipopolysaccharide profiles and the outer membrane protein patterns (Fouz et al., 1992).

Resistant to penicillin G, ampicillin, erythromycin, streptomycin, and kanamycin. Susceptible to vibriostatic agent O/129 (150 μg). Virulence tests have shown that *P. damselae* subsp. *damselae* strains are pathogenic for turbot and rainbow trout (Fouz et al., 1992). Pathogenicity is due to production of toxins. *P. damselae* subsp. *damselae* strains have been shown to possess similar virulence determinants for poikilothermic and homeothermic hosts, secreting a potent, lethal phospholipase toxin with hemolytic and cytotoxic activities (Toranzo and Barja, 1993). Isolated from several marine fish (damselfish, yellowtail, seabream, brown shark, lemon shark, etc.), mammals (dolphin), reptiles (turtle), mollusks (octopus), and uninfected fish. Also associated with wound infections in humans.

The mol% G + C of the DNA is: 40.6–41.4 (enzymatic hydrolysis, HPLC).

Type strain: ATCC 33539, DSM 7482.

GenBank accession number (16S rRNA): X74700.

b. **Photobacterium damselae** *subsp.* **piscicida** (ex Janssen and Surgalla 1968) Gauthier, Lafay, Ruimy, Breittmayer, Nicolas, Gauthier and Christen 1995b, 142^{VP} *pis.ci.ci' da.* L. n. *piscis* a fish; L. suff. *-cida* from L. v. *caedo* to cut or to kill; M.L. n. *piscicida* fish killer.

Nonflagellated, straight rods with bipolar staining, measuring 1.5 ×1.0 μm. Pleomorphic, changing from coccoidal to long rods under different culture conditions. The toxicity and chemistry of the lipopolysaccharides (LPS) has been studied by Salati et al. (1989). LPS structures are similar for different isolates (Nomura and Aoki, 1985), possessing O-side chains consisting of high-molecular-mass bands in a ladder-like pattern (Magariños et al., 1992). Outer membrane protein patterns (OMP) are similar for different strains (Magariños et al., 1992); when grown under iron-restricted conditions, strains are able to produce iron-regulated outer membrane proteins (IROMPs) (Bakopoulos et al., 1997). Capsular proteins (CP) are not produced by all strains, but production can be induced by growth under special conditions (Magariños et al., 1996). The chemical composition of the capsular polysaccharides has been studied by Bonet et al. (1994). Fatty acid analysis has revealed that saturated and unsaturated fatty acids of 16 carbon atoms are the predominant fatty acids (Romalde et al., 1995). Colonies are regular, uniformly round, 1–2 mm in diameter, glistening, convex, opaque, and viscid. Capsules may be produced. Cells are not able to grow at NaCl concentrations lower than 0.02 M. Optimal growth at 0.3 M NaCl; osmotic requirement of 0.5–3% NaCl. Cells are able to grow at 25°C, but not at 4°C. Growth at 37°C is variable. Cells are able to grow at pH 6–9; optimal growth is at pH 6.8. Oxidase and catalase variable. Lecithinase and lipase positive. Does not produce urease, elastinase, caseinase, phospholipase, and lysine decarboxylase. The following carbon compounds are utilized as sole carbon and energy sources: glutamate, adipate, and L-proline. Fructose, D-galactose, sucrose, and raffinose are fermented anaerobically. L-rhamnose, D-glucitol, D-xylose, maltose, glycerol, galactitol, and cellobiose are not fermented. Gas is not produced. See Table BXII.γ.178 for other characteristics.

By DNA–DNA hybridization, DNA relatedness between *P. damselae* subsp. *piscicida* and *P. damselae* subsp. *damselae* is 77%. DNA relatedness between *P. damselae* subsp. *piscicida* and both *P. leiognathi* and *P. angustum* is much lower, at 23% and 21%, respectively (Thyssen et al., 2000). Direct analysis of DNA sequences has revealed that the rRNA sequences of *P. damselae* subsp. *piscicida* and *P. damselae* subsp. *damselae* differ only by one nucleotide (Gauthier et al., 1995b). Considerable variation in the prevalence, number, and size of plasmids found in *P. damselae* subsp. *piscicida* has been described. R-plasmids encode resistance for chloramphenicol, tetracycline, sulfamonomethoxine, and kanamycin (Kim and Aoki, 1993). *P. damselae* subsp. *piscicida* was formerly thought to be serologically homogeneous (Magariños et al., 1992), but serological differences have been recently detected (Bakopoulos et al., 1997; Kawahara et al., 1998). The median values for minimum inhibitory concentrations of antibiotics are (μg/ml): oxytetracycline, 6.4; penicillin, 0.1; tetracycline, 0.8; amoxicillin, 0.1; ampicillin, 0.05; chloramphenicol, 1.6; flumequin, 0.2; gentamycin, 12.8; neomycin, 12.8; nitrofurantoin, 25.6; sulfonamide, 51.2; streptomycin, 25.6; and trimethoprim, 6.4. Can be extremely virulent for fish. The presence of capsules, the iron uptake system, and the phospholipase are thought to play an important role in the virulence (Romalde and Magariños, 1997). Isolated from diseased fish (mainly *Seriola quinqueradiata*, *Sparus aurata*, and *Dicentrarchus labrax*). Restricted to the East Coast of North America, Japan, and the Mediterranean area.

The mol% G + C of the DNA is: 41.1–41.3 (enzymatic hydrolysis and HPLC).

Type strain: NCMB 2058.

GenBank accession number (16S rRNA): X78105.

4. **Photobacterium iliopiscarium** (Onarheim, Wiik, Burghardt and Stackebrandt 1994) Urakawa, Kita-Tsukamoto and Ohwada 1999a, 260^{VP} (*Vibrio iliopiscarius* Onarheim, Wiik, Burghardt and Stackebrandt 1994, 377.)

i.li.o.pis.ca′ ri.um. L. n. pl. *ilia* intestines, guts; *ilio* pertaining to intestines; L. adj. *piscarius* belonging to fish *iliopiscarium*, belonging to intestines of fish.

Cells are highly pleomorphic. Morphology of both the colonies and the cells varies, even under apparently identical growth conditions. Bacteria growing in broth cultures at salt levels higher than 2% tend to aggregate. Other factors that influence the morphology are the presence of glucose, temperature, and age of the culture. In broth medium with 2% NaCl and lacking glucose, the dominant cell morphology is rods and curved rods. Motile. Not luminescent. The dominant colony morphology of young cultures comprises small, colorless to grayish, opaque colonies with entire edges. At least 0.5% NaCl is required for growth; this varies with the temperature. No growth occurs at NaCl concentrations of ≥5%. Optimum growth in media with 2% NaCl. The ability to grow at different temperatures varies with the medium composition. No growth is observed at 30°C; 20°C is the optimal temperature when grown in peptone water containing 2% salt. Bacteria are able to grow at 4°C. Growth is better under aerobic conditions rather than anaerobic conditions.

Produce gas from glucose. Cells possess lysine decarboxylase. Negative with respect to urease, and tryptophan deaminase. Citrate, *i*-inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdaline, erythritol, D-xylose, L-xylose, adonitol, β-methylxyloside, L-sorbose, dulcitol, α-methyl-D-mannoside, α-methyl-D-glucoside, arbutin, salicin, melibiose, inulin, melezitose, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol, 2-cetogluconate and 5-cetogluconate are not utilized. See Table BXII.γ.178 for other characteristics. Maximum-likelihood, maximum-parsimony, and neighbor-joining methods of nearly complete 16S rRNA gene sequences have shown that the nearest phylogenetic neighbor is *P. phosphoreum* (Urakawa et al., 1999a). Susceptible to vibriostatic agent O/129 at 150 μg/disc, but only slightly sensitive at 10 μg/disc. Resistant to penicillin-G, but sensitive to novobiocin, chloramphenicol, sulfamethoxazole, tetracycline, nitrofurantoin, oxytetracycline, and trimethoprim. Slightly sensitive to ampicillin and streptomycin. Isolated from the intestines of fish (herring, coal fish, cod, and salmon) living in cold seawater.

The mol% G + C of the DNA is: 39 ± 1 (T_m).

Type strain: PS1, ATCC 51760, DSM 9896.

GenBank accession number (16S rRNA): AB000278.

5. **Photobacterium leiognathi** (Hendrie, Hodgkiss and Shewan 1970) Boisvert, Chatelain and Bassot 1967, 521^{AL} (*Photobacterium mandapamensis* Hendrie, Hodgkiss and Shewan 1970, 165.)

lei.o.gna′ thi. M.L. gen. n. *leiognathi* named after fish of the family *Leiognathidae*.

Coccobacillus, approximately 1.6 × 3.2 μm. Motile. Luminescent. The fatty acid profile of the bacterium contains high concentrations of $C_{16:1}$, $C_{16:0}$, and $C_{18:1}$ fatty acids; as well as $C_{12:0}$, $C_{12:0\ 3OH}$, $C_{14:0}$, and, to a lesser extent, $C_{14:1}$, $C_{14:0\ 3OH}$, $C_{15:0}$, $C_{17:0}$, $C_{17:1}$, and $C_{18:0}$ fatty acids (Makemson et al., 1997; Nogi et al., 1998c). The major isoprenoid quinone is Q-8, and a minor quinone is Q-7; these account for 90% and 10% of the total isoprenoid quinones, respectively (Nogi et al., 1998c). Colonies are 1 mm in diameter, regular, convex, smooth, transparent, and yellow. The optimal temperature is 30°C. Growth can be observed at 37°C, but not at 4°C. Cells can grow at a salt concentration of 0.5–6%. Optimal NaCl concentration is 1.5%. Negative for the production of oxidase and for tryptophan deaminase. No production of lysine decarboxylase. Esculin is not hydrolysed. Fermentation occurs without gas production from fructose, D-glucose, D-galactose, glycerin, D-mannose, *N*-acetylglucosamine, and ribose. Cells are not able to ferment L-arabinose, cellobiose, dulcitol, *i*-inositol, lactose, maltose, D-sorbitol, saccharose, trehalose, or D-xylose. Citrate, citruline, glucuronate, leucine, malonate, methionine, putrescine, and tartrate are not utilized. See Table BXII.γ.178 for other characteristics.

DNA relatedness of *P. leiognathi* to *P. phosphoreum* and *P. profundum* is 26% and 19%, respectively (Nogi et al., 1998c). DNA–rRNA hybridization experiments have shown a relatedness of >98% between *P. leiognathi* and *P. phosphoreum* (Baumann and Baumann, 1981b). Susceptible to vibriostatic agent O/129, chloramphenicol, kanamycin, thiam-

phenicol, tetracycline, erythromycin, novobiocin, nitrofurantoin, polymyxin, trimethoprim, ampicillin, flumequin, gentamycin, and neomycin. Resistant to penicillin G, oxacillin, and amoxicillin. Isolated from the light organs of Leiognathidae and Apogonidae, which are usually found in warm, shallow, tropical water.

The mol% G + C of the DNA is: 41.6 (enzymatic hydrolysis, HPLC).

Type strain: ATCC 25521.

GenBank accession number (16S rRNA): D25309, X74686.

6. **Photobacterium profundum** Nogi, Masui and Kato 1998d, 631[VP] (Effective publication: Nogi, Masui and Kato 1998c, 6.)

pro.fun′dum. L. adj. n. *profundum* deep, living within the depth of the oceans.

Cells are rod shaped; 0.8–1.0 × 2–4 μm. Motile by means of single, unsheathed polar flagella. Nonluminescent. The fatty acid profile is very distinct from those of other *Photobacterium* species and is particularly characterized by substantial amounts of $C_{16:0\ iso}$ and $C_{20:5}$. The dominant cellular fatty acids are $C_{16:1}$, $C_{16:0\ iso}$, $C_{16:0}$, $C_{18:1}$, and $C_{20:5\ \omega 3c}$ (IPA). The major isoprenoid quinone is Q-8, and a minor quinone is Q-7; these account for 95% and 5% of the total isoprenoid quinones, respectively (Nogi et al., 1998c). Colonies are entire, smooth, semitranslucent, and ivory. They are 0.7–1.0 mm in diameter after 48 h of incubation at 10°C. Growth occurs between 4°C and 18°C at atmospheric pressure, with optimum growth displayed between 8°C and 12°C. No growth occurs at 0°C or 20°C. Growth occurs at pressures between 0.1 MPa and 70 MPa at 10°C, with optimum growth displayed at 10 MPa. Best growth occurs at a NaCl concentration of ~3%. No growth occurs in the absence of NaCl. Moderately barophilic and psychrophilic.

Acid is produced from D-glucose, but gas is not produced. Production of indole. D-Mannitol, but not *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, or D-fructose, can be utilized as a sole carbon and energy source. See Table BXII.γ.178 for other characteristics. The level of DNA relatedness of *P. profundum* to *P. angustum*, *P. damselae* subsp. *damselae*, *P. leiognathi*, and *P. phosphoreum* is 24.5%, 19.5%, 19%, and 20.5% respectively (Nogi et al., 1998c). Resistant to vibriostatic agent O/129 (10 μg and 150 μg). Isolated from sediment at a depth of 5110 m.

The mol% G + C of the DNA is: 42 (reverse-phase HPLC).

Type strain: DSJ4, JCM 10084.

GenBank accession number (16S rRNA): D21226 for Strain DSJ4.

Genus III. **Salinivibrio** *Mellado, Moore, Nieto and Ventosa 1996, 820[VP]*

ANTONIO VENTOSA

Sa.li.ni.vib′ri.o. L. adj. *salinus* saline; L. v. *vibrio* move rapidly to and from, vibrate; M.L. masc. n. *Salinivibrio* saline organism which vibrates.

Gram-negative curved rods (0.5–06 × 1.0–3.2 μm). Cells occur singly, in pairs, or occasionally united by S shapes or spirals. **Motile by one polar flagellum**. Spores are not formed.

Moderately halophilic. The optimum NaCl concentration for growth is between 2.5 and 10% at 37°C; grows between 0 and 20% NaCl. Temperature range for growth: 5–50°C; optimum temperature: 37°C. pH range for growth: 5–10; optimum pH: 7.3–7.5. No growth factors are required for growth.

Facultatively anaerobic. Chemoorganotrophic. Catalase and oxidase are produced. Acid is produced from D-glucose. Gelatin is hydrolyzed. Voges–Proskauer and arginine decarboxylase tests are positive. Indole, β-galactosidase, lysine, and ornithine decarboxylase tests are negative. Colonies are circular, convex, opaque, smooth, and cream colored. Broth cultures are uniformly turbid.

The mol% G + C of the DNA is: 49.4–50.5.

Type species: **Salinivibrio costicola** (Smith 1938) Mellado, Moore, Nieto and Ventosa 1996, 820 emend. Huang, Garcia, Patel, Cayol, Baresi and Mah 2000, 620 (*Vibrio costicola* Smith 1938, 29.)

FURTHER DESCRIPTIVE INFORMATION

The genus is currently represented by a single species, *S. costicola*, with two subspecies: *S. costicola* subsp. *costicola* and *S. costicola* subsp. *vallismortis*. The genus was established to separate *Vibrio costicola* from the other species of the genus *Vibrio*, based on their phenotypic features and the lack of a close phylogenetic relationship between *S. costicola* and other *Vibrio* species (Mellado et al., 1996). The species *S. costicola* was isolated from hypersaline environments (salterns, saline soils) and from salted food (Garcia et al., 1987b; Mellado et al., 1996). Recently, Huang et al. (2000) isolated a single strain from a hypersaline pond located in Death Valley, California, USA, with phenotypic and genotypic features typical of *S. costicola*. Based on 16S rRNA gene sequence comparison, it was closely related to *S. costicola* (mean sequence similarity 97.7%) and DNA–DNA hybridization studies showed a high degree of relatedness (93%). However, there were some phenotypic differences and it was proposed to place the new strain in a subspecies of *S. costicola*, designated *S. costicola* subsp. *vallismortis*, which automatically created *S. costicola* subsp. *costicola* (Huang et al., 2000).

The most peculiar feature of the species *S. costicola* is its ability to grow optimally at salt concentrations ranging from 2.5 to 10%, with an upper limit for growth at 20–25% salts. For that reason it is classified as a moderately halophilic bacterium (Ventosa et al., 1998) and especially with respect to its physiology and biochemistry, has been considered a model organism representative of that physiological group (Ventosa et al., 1998). Several studies have determined that *S. costicola* has a specific requirement for Na^+. Addition of high concentrations of compounds such as glucose or glycerol lowered the NaCl requirement from 0.5 M to 0.3 M NaCl (Adams et al., 1987). Temperature and medium composition affect the salt requirement (Kushner, 1978; Adams and Russell, 1992).

S. costicola strains have simple growth requirements. The minimum growth requirements have been determined, and two synthetic media have been described. One contained D-glucose, L-

cysteine or cystine, glutamate, L-arginine, L-valine, L-isoleucine, and salts (Flannery and Kennedy, 1962). Kamekura et al. (1985) reported a minimal medium based on D-glucose, glutamate, biotin, thiamine, choline, and salts. Complex media stimulate growth at high salt concentrations. The effect may be due to the presence in the medium of compatible solutes or their precursors, or to the fact that other growth factors may be synthesized more slowly under the high salt conditions (Ventosa et al., 1998).

Strains of *S. costicola* show a heterogeneous response to antibiotics; they are very sensitive to chloramphenicol and rifampin (Nieto et al., 1993). The salt concentration may influence the susceptibility of *S. costicola* to antimicrobial compounds. However, *S. costicola* shows a high sensitivity to rifampin and trimethoprim, regardless of the salt concentration (Coronado et al., 1995). The susceptibility of *S. costicola* to several heavy metals has also been studied (Garcia et al., 1987a). All 58 strains tested were susceptible to cadmium, copper, silver, zinc, and mercury, but showed tolerance to lead (Garcia et al., 1987a).

The genome organization of *S. costicola* has been investigated by pulsed field gel electrophoresis. The genome sizes of six strains ranged from 2100 to 2600 Kb. (Mellado et al., 1997). Several plasmids and one megaplasmid have also been reported (Fernandez-Castillo et al., 1992; Mellado et al., 1997). Conjugation is the only genetic transfer mechanism that has been described for this organism. A bacteriophage, phage UTAK, that infected and lysed *S. costicola* was isolated from salterns in Alicante (Spain), and propagated optimally at 1–2 M NaCl (Goel et al., 1996). However, genetic transfer by transduction has not been studied.

Besides its presence in salted foods, the natural environment of *S. costicola* is hypersaline habitats such as salt lakes and salterns. This organism is present in ponds of salterns with 10–25% salts, and is one of the predominant bacteria in ponds with less that 15% salts, as determined by isolation of *S. costicola* from these habitats in culture media (Rodriguez-Valera et al., 1985; Márquez et al., 1987). However, its presence in hypersaline soils is less evident (Quesada et al., 1982).

Since *S. costicola* has served as a model organism in different studies of moderately halophilic bacteria, there is much information concerning its ecology, physiology, and biochemistry (internal ions concentration and ion pumps, compatible solutes, cytoplasmic and membrane-bound enzymes, polar lipid and fatty acid composition, etc.). Further information can be found in a review on moderately halophilic bacteria (Ventosa et al., 1998).

Enrichment and Isolation Procedures

Selective procedures for the enrichment and isolation of *S. costicola* have not been reported. It can be isolated in complex media with the addition of a salt mixture and incubated at 37°C under aerobic or preferentially anaerobic (to avoid the growth of strictly aerobic species) conditions. The isolation medium described by Ventosa et al. (1982) can be used. Another isolation procedure has been described by Huang et al. (2000). Enrichments are prepared by inoculating samples into serum bottles containing 25 ml anaerobic enrichment medium (12% NaCl and 0.5% D-glucose) and incubation at 37°C without shaking.

Maintenance Procedures

Salinivibrio costicola may be maintained on complex medium containing 10% salts. An appropriate medium is described by Garcia et al. (1987b). Long-term storage of members of this genus can be carried out by freeze-drying, storage at −80°C, or by storing in liquid nitrogen.

Differentiation of the genus *Salinivibrio* from other genera

The genus *Salinivibrio* can be differentiated from other related genera, except *Vibrio*, based on morphology, since it contains curved rods. Typical features of *Salinivibrio costicola* are its optimal growth in media containing 10% salts, and its ability to produce arginine decarboxylase. The genus *Salinivibrio* can also be differentiated from *Vibrio* and other related genera of the family *Vibrionaceae* by comparative analysis of 16S rRNA gene sequences. Besides, in comparison with species of the genus *Vibrio*, *Salinivibrio costicola* has two unique helical sequences and secondary structures at positions 178–197 and 197–219 of the 16S rRNA (*E. coli* 16S rRNA gene sequence numbering) (Huang et al., 2000).

Taxonomic Comments

The single species of the genus *Salinivibrio* was isolated from rib bones in Australian bacon and described originally by Smith (1938), as *"Vibrio costicolus"*. In *Bergey's Manual of Determinative Bacteriology* (7th ed.), this species was included as a member of the genus *Vibrio* (Breed et al., 1957) and in the 8th edition it was named correctly as *Vibrio costicola* (Shewan and Véron, 1974). Very few taxonomic studies were performed on this species and they were carried out on isolates from cured meats (Gardner, 1980). Garcia et al. (1987b) amended the description of this species, including strains isolated from cured meats as well as 54 isolates from several salterns located in different areas of Spain. Further DNA–DNA hybridization studies determined that isolates from cured meats and salterns have a high level of DNA relatedness and should be considered members of the same species (Gutiérrez et al., 1989). The analysis of complete 16S rRNA gene sequences of six strains showed that phylogenetically they form a monophyletic branch that is distinct from other *Vibrio* species and related genera (Fig. BXII.γ.184). This evidence, together with several phenotypic differences, permitted the establishment of a different genus for this species, *Salinivibrio costicola* (Mellado et al., 1996).

Acknowledgments

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Further Reading

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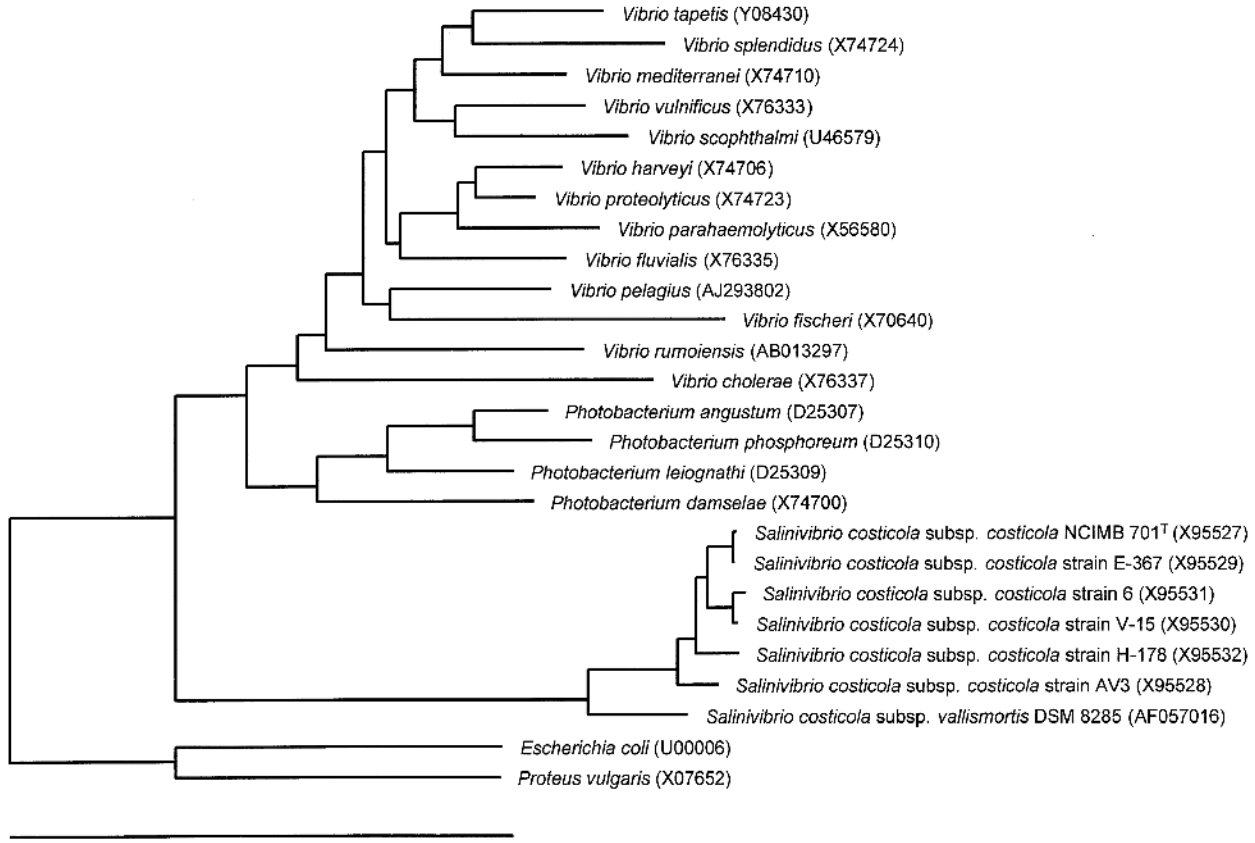

FIGURE BXII.γ.184. Phylogenetic tree derived from the analysis of 16S rRNA gene sequences of strains of *Salinivibrio costicola* and related species of the family *Vibrionaceae*, as well as two outgroup representatives. Figures in parenthesis indicate the accession numbers of 16S rRNA gene sequence data. Bar = 5% sequence difference.

List of species of the genus Salinivibrio

1. **Salinivibrio costicola** (Smith 1938) Mellado, Moore, Nieto and Ventosa 1996, 820^{VP} emend. Huang, Garcia, Patel, Cayol, Baresi and Mah 2000, 620 (*Vibrio costicola* Smith 1938, 29.)

cos.ti′ co.la. L. n. *costa* rib; L. subst. *cola* dweller; M.L. n. *costicola* rib dweller.

Cells are Gram-negative, curved rods, 0.5–0.6 × 1.0–3.2 μm and occur singly, in pairs, or rarely in chains. Motile by one, or rarely two, polar or subpolar flagella. Spores are not formed. Colonies are circular, convex, opaque, smooth, and cream colored. Broth cultures are uniformly turbid. Moderately halophilic. Grows at NaCl concentrations between 0 and 20%; the optimum NaCl concentration for growth is between 2.5 and 10% at 37°C. Growth occurs at 5–50°C (optimal growth at 37°C) and at pH 5–10 (optimal growth at pH 7.5). No growth factors are required, but yeast extract enhances growth. Facultatively anaerobic. Chemoorganotrophic. Catalase and oxidase are produced. Acid is produced from D-glucose, maltose, sucrose, and D-trehalose. Gelatin is hydrolyzed. Voges–Proskauer and arginine decarboxylase tests are positive. Indole, β-galactosidase, lysine, and ornithine decarboxylase tests are negative. Nitrates usually not reduced to nitrites. Nitrites are not reduced. Isolated from hypersaline environments and from salted food.

The mol% G + C of the DNA is: 49.4–50.5 (T_m).

Type strain: ATCC 33508, CCM 3575, DSM 11403, NCIMB 701.

GenBank accession number (16S rRNA): X95527, X74699.

a. **Salinivibrio costicola** *subsp.* **costicola** (Smith 1938) Mellado, Moore, Nieto and Ventosa 1996, 820^{VP} Huang, Garcia, Patel, Cayol, Baresi and Mah 2000, 620 (*Vibrio costicola* Smith 1938, 29.)

Cells are Gram-negative, curved rods, 0.5 × 1.5–3.2 μm, motile by one polar flagellum (Fig. BXII.γ.185). The optimum salts concentration for growth is 10% at 37°C. Grows in the presence of 0.5–20% salts. No growth in the absence of NaCl. Other features are reported in the species description. Some characteristics differentiating this subspecies from *S. costicola* subsp. *vallismortis* are shown in Table BXII.γ.179. The following compounds are utilized as sole carbon and energy sources: D-trehalose, glycerol, acetate, *N*-acetylglucosamine, fumarate, glutamate, lactate, DL-malate, pyruvate, propionate, and succinate. The following compounds are not utilized as sole carbon and energy sources: amygdalin, D-arabinose, D-cellobiose, esculin, D-galactosamine, D-gluconolactone, lactose, D-melibiose, L-rhamnose, D-xylose, erythritol, *cis*-aconitate, benzoate, *p*-hydroxybenzoate, hip-

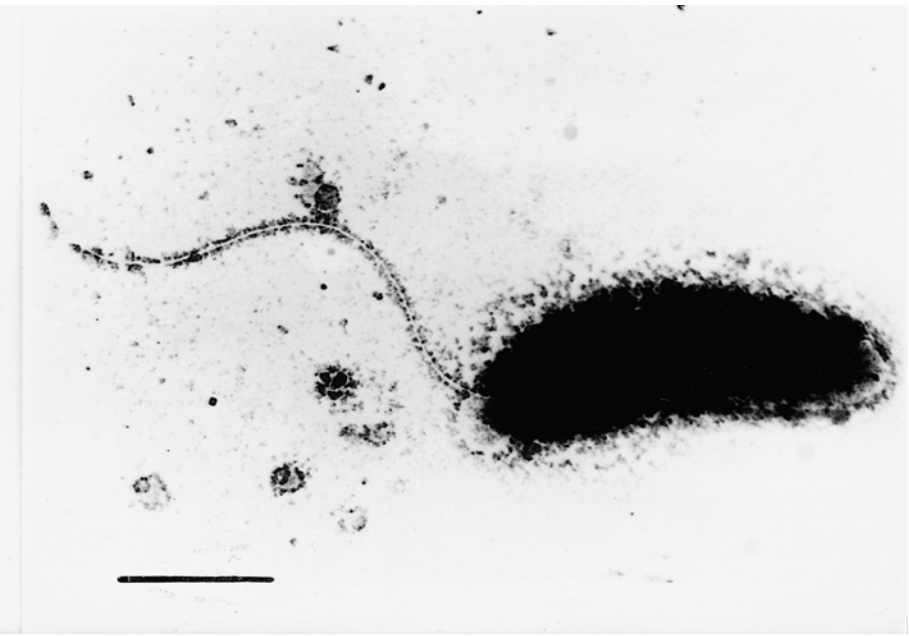

FIGURE BXII.γ.185. Electron micrograph of polar monotrichously flagellated cell of *Salinivibrio costicola*. Bar = 1 μm. (Reprinted with permission from M.T. Garcia et al., International Journal of Systematic Bacteriology *37:* 251–256, 1987, ©American Society for Microbiology.)

purate, malonate, quinate, salicylate, suberate, and D-tartrate. The following compounds are utilized as sole carbon, nitrogen, and energy sources: L-alanine, L-asparagine, L-glutamine, L-ornithine, L-proline, and L-serine. The following compounds are not utilized as sole carbon, nitrogen, and energy sources: L-allantoin, L-aspartic acid, betaine, creatine, ethionine, L-isoleucine, L-methionine, putrescine, sarcosine, L-tryptophan, and L-valine. Isolated from hypersaline habitats and from salted food.

The mol% G + C of the DNA is: 49.4–50.5 (T_m).

TABLE BXII.γ.179. Differential characteristics of the subspecies of *Salinivibrio costicola*[a]

Characteristic	*S. costicola* subsp. *costicola*	*S. costicola* subsp. *vallismortis*
Growth without NaCl	−	+
Growth in the presence of 20% NaCl	+	−
Anaerobic gas production	−	+
Hydrolysis of:		
Starch	−	+
Esculin	+	−
Utilization of:		
L-Alanine	+	−
Lactate	+	−
D-Mannose	−	+
D-Mannitol	+	−
L-Serine	+	−

[a]For symbols see standard definitions.

Type strain: ATCC 33508, CCM 3575, DSM 11403, NCIMB 701.

GenBank accession number (16S rRNA): X95527.

b. **Salinivibrio costicola** *subsp.* **vallismortis** Huang, Garcia, Patel, Cayol, Baresi and Mah 2000, 620[VP]

val.lis.mor′tis. L. gen. n. *vallis* of the valley; L. gen. n. *mortis* of death; M.L. fem. n. *vallismortis* of the valley of death, named after Death Valley, California, USA.

Cells are Gram-negative, short, curved rods, 0.5–0.6 × 1.0–1.8 μm, highly motile by means of one, or rarely two, subpolar flagella. Optimum growth at 2.5% NaCl. Growth occurs at NaCl concentrations between 0 and 12.5%. Other features are reported in the species description. Some characteristics differentiating this subspecies from *S. costicola* subsp. *costicola* are shown in Table BXII.γ.179. The following compounds are used aerobically: D-glucose, D-mannose, sucrose, trehalose, starch, adipate, caproate, D-gluconate, pyruvate, L-glutamic acid, and yeast extract. The following compounds are not used: L-arabinose, cellobiose, D-galactose, D-galacturonate, lactose, maltose, L-rhamnose, salicin, citrate, 2-oxoglutarate, DL-lactate, L-malate, malonate, phenylacetate, propionate, valerate, ethanol, D-mannitol, *m*-inositol, D-sorbitol, DL-α-alanine, β-alanine, L-leucine, L-serine, L-tyrosine, benzoate, *p*-hydroxybenzoate, betaine, sarcosine and spermine. Isolated from Death Valley, California, USA.

The mol% G + C of the DNA is: 50 (HPLC).

Type strain: DV, DSM 8285.

GenBank accession number (16S rRNA): AF057016.

Order XII. **Aeromonadales** *ord. nov.**

AMY MARTIN-CARNAHAN AND SAMUEL W. JOSEPH

Ae.ro.mo.na.da′ les. M.L. fem. n. *Aeromonas* type genus of the family and order; suff. *-ales* to denote an order; M.L. pl. n. *Aeromonadales* the *Aeromonas* order.

The order *Aeromonadales*, which contains a single family, *Aeromonadaceae*, with the *Aeromonas*, is herein proposed. Gram-negative, straight, rigid rods. Facultatively anaerobic; oxidase positive with rare exceptions and catalase positive. Includes motile (single, polar flagellum) and nonmotile species, as well as mesophilic and psychrophilic species. Primarily aquatic inhabitants of freshwater, marine, and especially estuarine environments, often associated with aquatic animals. Some species are either primary or opportunistic pathogens in humans, as well as a variety of other warm-blooded and cold-blooded animals and invertebrates.

The mol% G + C of the DNA is: 57–63.

Type genus: **Aeromonas** Stanier 1943, 213.

Editorial Note: The family *Succinivibrionaceae* has been placed in this chapter by the editors as *familia incertae sedis* based on a phylogenetic analysis that indicated distant relationships between the 16S rDNA sequences of the type species of the four genera included in the *Succinivibrionaceae* and the 16S rDNA sequences of some members of the genus *Aeromonas*. This placement should not be taken to imply that members of the *Succinivibrionaceae* are or should be included in the definition of the order *Aeromonadales* as proposed here by Martin-Carnahan and Joseph.

Family I. **Aeromonadaceae** Colwell, MacDonell and De Ley 1986, 474[VP]

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Ae.ro.mo.na.da′ ce.ae. M.L. fem. n. *Aeromonas* type genus of the family; suff. *-aceae* to denote a family; M.L. fem. pl. n. *Aeromonadaceae* the *Aeromonas* family.

Gram-negative, straight, rigid, nonsporeforming rods. Motility by means of polar flagella with some evidence of lateral flagella; some species are considered nonmotile. Occur singly, occasionally in pairs, or in short chains.

Facultatively anaerobic and chemoorganotrophic. Fermentative or respiratory with oxygen as a universal electron acceptor. Reduce nitrates, but do not denitrify. Ammonium salts utilized by most isolates as a sole source of nitrogen. Oxidase positive, except for *Tolumonas*. Catalase positive. Most species are mesophilic, but psychrophilic species do exist. *Oceanimonas* requires NaCl.

Primarily aquatic, most frequently isolated from fresh and estuarine waters and in association with aquatic animals; also found in sewage, surface waters, sediments, and biofilms; several species are pathogenic for humans, other warm-blooded animals, fish, eels, frogs, as well as other vertebrates and invertebrates, such as leeches.

The mol% G + C of the DNA is: 57–63.

Type genus: **Aeromonas** Stanier 1943, 213.

TAXONOMIC COMMENTS

The analysis of rRNA–DNA hybridization studies of the members of the family *Vibrionaceae*, in which *Aeromonas* was then included, was summarized in the first edition of *Bergey's Manual of Systematic Bacteriology* (Baumann and Schubert, 1984). The evidence from those studies suggested that the genus *Aeromonas* was sufficiently different from other genera in *Vibrionaceae* to merit its placement in a new family.

Subsequent analyses by Colwell et al. (1986) using 16S rRNA cataloging, 5S rRNA gene sequence comparisons, and rRNA–DNA hybridization data revealed that aeromonads demonstrated an evolutionary divergence that was approximately equidistant from the *Enterobacteriaceae* and the *Vibrionaceae*, thereby justifying classification of the genus *Aeromonas* in its own family. These authors formally proposed the creation of the family *Aeromonadaceae* to incorporate the single genus, *Aeromonas*, which was later supported in 1992 by analyses using 16S rDNA sequencing and dendrograms showing that members of the genus *Aeromonas* form a distinct line within the *Gammaproteobacteria* (Martinez-Murcia et al., 1992a).

Additional evidence for the separation of the family *Aeromonadaceae* from the family *Vibrionaceae* soon followed with the analysis of 50 reference strains from 10 genera on the basis of 600 16S rRNA nucleotides by using reverse transcriptase sequencing (Kita-Tsukamoto et al., 1993). Shortly thereafter, the sequencing of nearly complete 16S rRNAs of 54 reference strains from the genera *Vibrio*, *Photobacterium*, *Aeromonas*, and *Plesiomonas* was added to all other known sequences for bacteria belonging to the *Gammaproteobacteria* to generate a database of 70 sequences for subsequent comparison (Ruimy et al., 1994). Their results showed that the family *Vibrionaceae* should include only *Vibrio* and *Photobacterium*, and that the genus *Aeromonas* should be moved to a separate family, *Aeromonadaceae*. The genus *Oceanimonas* has provisionally been placed in the family *Aeromonadaceae*.

Genus *Incertae Sedis* Although the genus *Tolumonas* is included as a member of the family *Aeromonadaceae* in this edition of *Bergey's Manual*, there is evidence to question the validity of this placement. In the genus description of *Tolumonas* in this edition, Fischer-Romero and Tindall have described the genus and single species, *Tolumonas auensis*, as either an anaerobic or aerobic Gram-negative rod that is oxidase negative, with a mol% G + C content of 49 (T_m). This description also differs from that given herein for the family *Aeromonadaceae*.

Genus I. **Aeromonas** *Stanier 1943, 213*[AL]

AMY MARTIN-CARNAHAN AND SAMUEL W. JOSEPH

Ae.ro.mo'nas. Gr. n. *aer* air, gas; Gr. n. *monas* unit, monad; M.L. fem. n. *Aeromonas* gas(-producing) monad.

Cells straight, coccobacillary to bacillary with rounded ends, 0.3–1.0 × 1.0–3.5 µm. Occur singly, in pairs, or rarely in short chains. Gram negative. **Most species are motile by a single, polar flagellum** of 1.7 µm wavelength; peritrichous flagella may be formed on solid media in young cultures and lateral flagella occur in some species. Facultatively anaerobic. Chemoorganotrophic, displaying oxidative and fermentative metabolism of D-glucose. Acid and often acid with gas produced from many carbohydrates, especially D-glucose. Nitrate is reduced to nitrite. A variety of exoenzymes such as arylamidases, amylase, DNase, esterases, peptidases, and other hydrolytic enzymes are produced. Main cellular fatty acids are hexadecanoic acid ($C_{16:0}$), hexadecenoic acid ($C_{16:1}$), and octadecenoic acid ($C_{18:1}$). **Usually oxidase positive and catalase positive.** Optimum growth temperature varies between 22°C and 37°C; growth temperature can range from 0 to 45°C, and some species do not grow at 35°C. **Generally resistant to 150 µg of the vibriostatic agent 2,4 diamino-6, 7-diisopropylpteridine (0/129).** Occur in fresh, brackish, tap, well, and chlorinated water, as well as biosolids and sewage. Some of the species have been associated with disease in a wide variety of warm-blooded and cold-blooded animals, including humans, domestic animals, frogs, fresh and salt water fish, and invertebrates. The phylogenetic position of *Aeromonas*, as determined by 16S rRNA gene sequence analysis, is in the *Gammaproteobacteria*, with its closest relatives in the families *Vibrionaceae* and *Enterobacteriaceae*. 16S rDNA sequences (signature sequences) have been determined for nearly all validly named species and are deposited in GenBank, EMBL, or RDP databases (Table BXII.γ.180).

The mol% G + C of the DNA is: 57–63.

Type species: **Aeromonas hydrophila** (Chester 1901) Stanier 1943, 213 (*Bacillus hydrophilus* Chester 1901, 235.)

FURTHER DESCRIPTIVE INFORMATION

The genus *Aeromonas* has traditionally been divided into two major groups of species, i.e., motile versus nonmotile species and mesophilic versus psychrophilic species. This general approach will be used here with the caveat that there are isolated instances where species considered to be nonmotile (*Aeromonas salmonicida*) do harbor flagellin genes (Umelo and Trust, 1997) and although *A. salmonicida* was generally considered not to grow above 30°C, it may actually be able to grow at 37°C (Austin, 1993).

The larger of these main categories includes the mesophilic, motile aeromonads which include 14 phenospecies that correspond to at least 17 genomospecies (DNA hybridization groups or HGs) (Table BXII.γ.181). The majority of human clinical isolates to date have been isolated from only six genomospecies, namely 1, 4, 8/10, 9, 12, and 14 (Holmberg et al., 1986a; Kuijper et al., 1989a; Altwegg, 1990; Altwegg et al., 1990; Abbott et al., 1992; Carnahan and Joseph, 1993). The category of psychrophilic, nonmotile aeromonads contains *A. salmonicida* (genomospecies 3), which includes four subspecies and is a major cause of fish disease worldwide (Austin et al., 1989a; Hänninen and Siitonen, 1995; Austin and Adams, 1996).

Phylogenetic treatment In the early 1980s, DNA relatedness studies of the motile aeromonads resulted in the establishment of three phenotypic species, namely *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae*, from among the eight reported genomospecies or DNA–DNA hybridization groups (Popoff et al., 1981). The number of aeromonad hybridization groups was subsequently expanded to 12 (Fanning et al., 1985; Farmer et al., 1986), and there are at present at least 17 hybridization groups, of which only two are unnamed: DNA hybridization Group 11 and *Aeromonas* Group 501 (HG 13) (Table BXII.γ.181).

Current recommendations define a genetic species as strains with approximately 70% or greater DNA–DNA relatedness and with 5°C or less ΔT_m to enable clear-cut differentiation of genomospecies (Wayne et al., 1987). 16S rDNA sequence analysis, which is routinely used to distinguish and establish relationships between genera, is often not as clear cut at the species level and has a lower discriminatory value when sequence analysis reveals a similarity of 97% or higher (Stackebrandt and Goebel, 1994).

TABLE BXII.γ.180. Nucleotide composition at variable positions of the 16S rDNA sequences determined from all known species of *Aeromonas*[a]

Species	77	129	131–132	154–156	165–167	199	218	230–232	250	258	264	457–464	469–476
A. hydrophila	A	A	A U	A G U	A C U	C	G	A U G	A	A	U	U G A U G C C U	C G U A U C A A
A. allosaccharophila	–	G	U C	– – –	– – –	–	–	G A A	–	–	–	– – G – A G – G	A C – G C – – G
A. bestiarum	–	G	U C	– – –	– – –	G	U	G A A	–	G	–	– – G C – – – –	– – – G – – – –
A. caviae	–	–	– –	– – –	– – –	–	–	– – –	–	–	–	C A G – A G – –	U C – G C U G G
A. encheleia	–	G	U C	– – –	– – –	G	U	G A A	–	G	–	– – – – – – – –	– – – – – – – –
A. eucrenophila	–	G	U C	– – –	G – –	–	–	G A A	–	G	–	– – – – – – – –	– – C – – – – G
A. jandaei	–	G	U C	U A C	G U A	–	–	G A A	–	–	C	C A G – A G – –	U C – G C U G G
A. media	–	–	– –	– – –	– – –	–	–	– – –	U	–	–	– – – – – – – –	– C – – – – G
A. popoffii	–	G	U C	– – –	– – –	G	U	G A A	–	G	–	– – – U – – – –	– C C – G – – –
A. salmonicida	–	G	U C	– – –	– – –	G	U	G A A	–	G	–	– – G C – – – –	– – – G – – – –
A. schubertii	G	G	U C	U A C	G U A	–	–	G A A	U	–	C	– – G – – G U –	– C – G C – – G
A. sobria	–	G	U C	– – –	– – –	G	U	G A A	–	G	–	– – G C A G – –	U C – G – – – G
A. trota	–	–	– –	– – –	– – –	–	–	– – –	–	–	–	C A G – A G – –	U C – G C U G G
A. veronii	–	G	U C	U A C	G U A	–	–	G A A	–	–	–	– – G – A G – –	A C – G C– – – G
Aeromonas sp. DHG 11	–	G	U C	– – –	– – –	G	U	G A A	–	G	–	– – G – C G – –	A C G G C – – –
Aeromonas sp. G501	–	G	U C	U A C	G U A	–	–	G A A	U	–	C	– – G – A G – –	A C – G C – – G

[a]Numbers are homologous positions following the numbering system in *E. coli* (Brosius et al., 1978). Dashes indicate the same nucleotide as that of *A. hydrophila*. The two triplets characteristic of species from the "Schubertii" branch are underlined. Martínez-Murcia, unpublished.

TABLE BXII.γ.180. *(cont.)*

Species	647	649–650	650	707	834	839	847	1009–1011	1018–1019	1153	1285	1308	1329	1355	1367
A. hydrophila	C	A G	G	C	C	U	A	U G C	G C	G	A	C	G	A	U
A. allosaccharophila	–	– –	–	–	–	–	–	C U A	U G	–	–	–	–	–	–
A. bestiarum	–	– –	–	–	–	–	–	– – –	– –	–	–	–	–	G	C
A. caviae	–	– –	–	–	–	–	–	– – –	– –	–	–	U	A	–	–
A. encheleia	–	– –	–	–	–	–	–	– – U	A –	–	–	–	–	–	–
A. eucrenophila	–	– –	–	G	–	–	–	– – U	A –	–	–	–	–	–	–
A. jandaei	–	– –	–	–	–	–	–	– – –	– –	–	–	–	–	–	–
A. media	–	– –	–	–	–	–	–	– – –	– –	–	–	–	–	–	–
A. popoffii	–	– –	–	–	–	–	–	– – –	– –	–	–	–	–	–	–
A. salmonicida	–	– –	–	–	–	–	–	– – U	A –	–	–	–	–	G	C
A. schubertii	–	G A	A	–	U	C	G	– – U	A –	A	U	U	A	–	–
A. sobria	U	– –	–	–	–	–	–	– – –	A –	–	–	–	–	–	–
A. trota	–	G –	–	–	–	–	–	– – –	– –	–	–	U	A	–	–
A. veronii	–	– –	–	–	–	–	–	– – U	A –	–	–	–	–	–	–
Aeromonas sp. DHG 11	–	– –	–	–	–	–	–	– – U	A –	–	–	–	–	–	—
Aeromonas sp. G501	–	G A	A	–	U	C	G	– – –	– –	A	U	U	A	–	–

[a]Numbers are homologous positions following the numbering system in *E. coli* (Brosius et al., 1978). Dashes indicate the same nucleotide as that of *A. hydrophila*. The two triplets characteristic of species from the "Schubertii" branch are underlined. Martínez-Murcia, unpublished.

The comparative analysis of the 16S rRNA gene sequences for *Aeromonas* species generally correlates with species designations derived from DNA–DNA hybridization studies (Martinez-Murcia et al., 1992a). While there is some lack of congruence between DNA–DNA hybridization studies and 16S rDNA sequencing results, the overall differentiation between groups is very similar. Table BXII.γ.180 provides the nucleotide composition at variable positions of the 16S rDNA sequences (signature sequences) determined from all currently recognized *Aeromonas* species. Fig. BXII.γ.186 is a phylogenetic tree showing the relationships between described *Aeromonas* species (type strains and reference strains) as determined by a continuous 1502-nucleotide 16S rDNA sequence comparison using the neighbor-joining method (Martinez-Murcia, 1999). Within the genus *Aeromonas*, the species are closely related and high levels of sequence similarity (~97.8–100%, which corresponds to 0–33 base differences) are observed. For example, the 16S rDNA of *A. hydrophila* ATCC 7966^T (HG 1) exhibits only three nucleotide differences with the sequence of *A. media* ATCC 33907^T (HG 5B). *A. jandaei* and *A. schubertii* represent quite separate lines, and together with *A. veronii*, seem to form a separate subbranch or affiliation within the genus (Fig. BXII.γ.187). Support for this loose association comes from the presence of paired signature triplets at the V2 region (positions 154–156 and 165–167) seen in Table BXII.γ.180 (Martinez-Murcia, unpublished), which distinguishes this "Schubertii" cluster from all other aeromonads. *A. schubertii* reveals the highest values of nucleotide differences (up to 33) within the genus and shows the highest number of sites with "unique" nucleotide composition.

Quite presciently, Sneath suggested that there was evidence for genetic crossing-over or recombination based on his analysis of the ribosomal sequences of Martinez-Murcia et al. (1992a) (Sneath, 1993). Although crossing-over may obliterate true phylogenetic relationships, Sneath reconstructed a speculative evolutionary phenogram from the right and left parts of the ribosomal sequence that seemed to suggest that *A. schubertii* might be the basal lineage species of the genus.

Further problems in using 16S rDNA for species identification of aeromonads were revealed when multiple strains from each species were sequenced. This subsequent research used Multilocus Sequence Typing (MLST) to examine a large collection of 94 well-characterized aeromonads, including type and reference strains, for four gene loci: 16S rDNA, *recA*, *chiA*, and *gyrB* (Carnahan, 2001). 16S rDNA exhibited less than 1% variable nucleotides within a species, which means that 16S rDNA sequencing can clearly define an isolate at the genus level only. In contrast, the other three loci were all more variable than 16S and could be more helpful in species identification. The phylogenetic analyses from these three loci arranged the aeromonads into their expected species clusters based on previous phenotypic and genotypic data. However, there were several instances where a single strain would cluster with its proper species designation for 2 of 3 loci, but for the other locus that strain would fall into an entirely different species cluster, again suggesting that recombination or lateral gene transfer is occurring within *Aeromonas*. For each locus examined, *A. schubertii* appeared to be the basal lineage species, consistent with Sneath's earlier assertion.

These observations of recombination across species boundaries and the recent touting of MLST as a "method of great promise" for the re-evaluation of the species definition in bacteriology (Stackebrandt et al., 2002) strongly suggest that the analysis of multiple gene loci should undoubtedly be pursued in future taxonomic studies of the genus *Aeromonas*.

Cell Morphology The members of the genus *Aeromonas* are Gram-negative, straight rods with rounded ends, but sometimes can appear as coccobacilli or with filamentous forms. Cells are 0.3–1.0 × 1.0–3.5 μm and can occur singly, in pairs, or even as short chains (Altwegg, 1999).

Cell Wall Composition Early studies of the composition of core oligosaccharides from motile aeromonads, with respect to combinations of hexose and heptose monosaccharide residues present in the core region of the cell wall lipopolysaccharides, revealed three general groups (Shaw and Hodder, 1978). These three groups generally matched later species designations of *A. hydrophila*, *A. caviae*, and *A. sobria* (Popoff et al., 1981). Analysis of the electrophoretic mobilities of purified or partially purified (proteinase K-treated) lipopolysaccharide (LPS) components by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or either autoradiography or silver staining techniques indicated at least three major resolvable fractions (Chart et al., 1984; Dooley et al., 1985). Three major patterns among LPS O polysaccharide side chain profiles include a common heterogeneous profile that appears as a series of ladder-like bands ex-

TABLE BXII.γ.181. Current genomospecies and phenospecies within the genus *Aeromonas*

DNA hybridization group (HG)	Type strain[T] and/or HG definition strains or reference strains	Genomospecies	Phenospecies	Remarks
1	ATCC 7966[T]	*A. hydrophila*	*A. hydrophila*	Isolated from clinical specimens
2	ATCC 14715[T]	*A. bestiarum*	*A. hydrophila*-like	Isolated from clinical specimens
3	ATCC 33658[T]	*A. salmonicida*	*A. salmonicida* subsp. *salmonicida*a	
3	ATCC 33659[T]	*A. salmonicida*	*A. salmonicida* subsp. *achromogenes*[a]	
3	ATCC 27013[T]	*A. salmonicida*	*A. salmonicida* subsp. *masoucida*[a]	
3	ATCC 49393[T]	*A. salmonicida*	*A. salmonicida* subsp. *smithia*[a]	
3	CDC 0434-84 (DS), Popoff C316	unnamed	*A. hydrophila*-like[b]	Isolated from clinical specimens
4	ATCC 15468[T]	*A. caviae*	*A. caviae*	Isolated from clinical specimens
5A	CDC 0862-83(DS)	*A. media*	*A. caviae*-like	Isolated from clinical specimens
5B	CDC 0435-84 (DS)	*A. media*	*A. media*	
5B	ATCC 33907[T]	*A. media*	*A. media*	
6	ATCC 23309[T], NCMB 74[T]	*A. eucrenophila*	*A. eucrenophila*[c]	
7	CIP 7433[T], NCMB 12065[T]	*A. sobria*	*A. sobria*	
8X	CDC 0437-84 (DS)	*A. veronii*	*A. sobria*	
8Y	ATCC 9071 (RS)	*A. veronii*	*A. veronii* biovar sobria[d]	Isolated from clinical specimens
9	ATCC 49568[T]	*A. jandaei*	*A. jandaei*	Isolated from clinical specimens;
10	ATCC 35624[T]	*A. veronii* biovar veronii	*A. veronii* biovar veronii	Isolated from clinical specimens; ornithine decarboxylase positive
11	ATCC 35941 (RS)	unnamed	*Aeromonas* sp. (ornithine positive)[e]	
12	ATCC 43700[T]	*A. schubertii*	*A. schubertii*	Isolated from clinical specimens
13	ATCC 43946 (RS)	*Aeromonas* Group 501	*A. schubertii*-like	Isolated from clinical specimens
14	ATCC 49657[T]	*A. trota*	*A. trota*[f]	Isolated from clinical specimens; ampicillin susceptible
15	ATCC 51208[T], CECT 4199[T]	*A. allosaccharophila*	*A. allosaccharophila*	
16	ATCC 51929[T],CECT 4342[T]	*A. encheleia*	*A. encheleia*[g]	
17	LMG 17541[T]	*A. popoffii*	*A. popoffii*	

[a]This includes nonmotile, psychrophilic strains.

[b]This includes motile, mesophilic strains.

[c]It has been proposed that *A. eucrenophila* Subgroup II may reside within *A. encheleia* (Huys et al., 1996a, 1997a).

[d]*A. ichthiosmia* appears to be synonymous with *A. veronii* biovar sobria (Martinez-Murcia et al., 1992a; Collins et al., 1993; Huys et al., 2001).

[e]It has been proposed that members of HG Group 11 may reside within *A. encheleia* (Huys et al., 1996a, 1997a).

[f]*A. enteropelogenes* appears to be synonymous with *A. trota* (Martinez-Murcia et al., 1992a; Collins et al., 1993).

[g]It has been proposed to emend the description of *A. encheleia* to include members of HG 11 and Subgroup II of *A. eucrenophila* (Huys et al., 1997a).

tending throughout the gel lane; a second homogeneous pattern that appears as a small number of prominent O side chain bands, sometimes arranged as a doublet; and a third pattern often associated with serologically rough strains and characterized by a lack of detectable side chains (Dooley et al., 1985; Kokka et al., 1990, 1991a).

The major fatty cellular fatty acids produced are hexadecanoic acid ($C_{16:0}$), hexadecenoic acid ($C_{16:1}$), and octadecenoic acid ($C_{18:1}$) (Lambert et al., 1983; Huys et al., 1994). Gas–liquid chromatographic analysis of cellular fatty acid methyl esters (FAMEs) can be used to separate the basic phenotypic clusters of *A. hydrophila*, *A. caviae*, and *A. sobria* (Canonica and Pisano, 1988; Huys et al., 1995). However, the growth medium used can affect the cellular fatty acid composition, and hence the chemotaxonomic differentiation ability of FAME analysis (Huys et al., 1997c). There is limited general information on *Aeromonas* outer membrane profiles (OMPs) other than studies of particular subsets of virulent aeromonads (Aoki and Holland, 1985; Kuijper et al., 1989b).

Fine structure *Aeromonas* species exhibit many fine structures outside the cell membrane and cell wall such as S-layer, flagella, pili, and capsule. A review of these features can be found in the chapter on "Pathogenic mechanisms" in *The Genus Aeromonas* (Gosling, 1996b). Further discussion of these individual features can be found in the pathogenicity section of this chapter *vide infra*.

Colonial or cultural characteristics On standard laboratory media, colonies of motile mesophilic aeromonads are 1–3 mm in diameter, smooth, circumscribed, circular, convex, translucent,

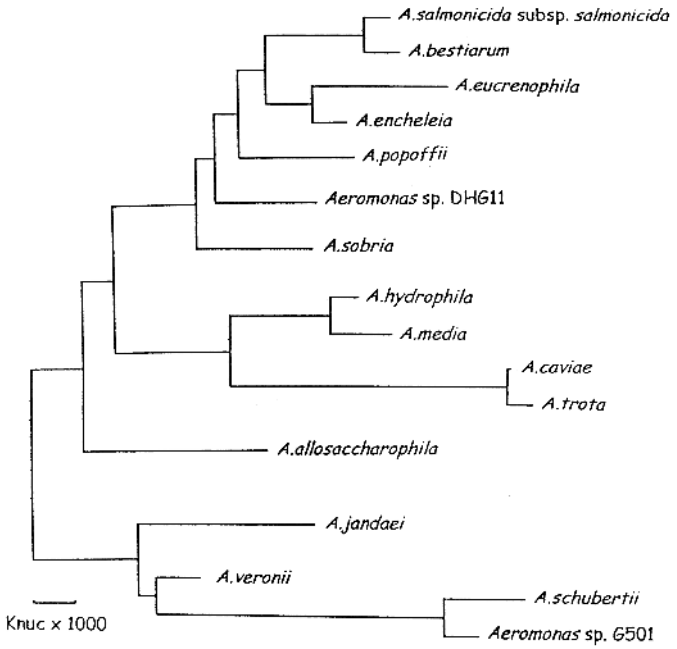

FIGURE BXII.γ.186. Phylogenetic relationships of described *Aeromonas* genomospecies as determined by a continuous 1502-nucleotide 16S rDNA sequence comparison using the neighbor-joining method (Saitou and Nei, 1987) and the MEGA Program version 1.01. Strain designations from different culture collections (in parentheses) and 16S rDNA sequence accession numbers [in brackets] are: *Aeromonas hydrophila* (HG 1) ATCC 7966^T [X60404]; *A. bestiarum* (HG 2) ATCC 51108^T (CIP 7430^T) [X60406]; *A. salmonicida* (HG 3) ATCC 33658^T (NCIMB 1102^T) [X60405]; *A. caviae* (HG 4) ATCC 15468^T (NCIMB 13016^T) [X60408]; *A. media* (HG 5) ATCC 33907^T [X60410]; *A. eucrenophila* (HG 6) NCIMB 74^T [X60411]; *A. sobria* (HG 7) CIP 7433^T (NCIMB 12065^T) [X60412]; *A. veronii* (HGs 8/10) ATCC 35624^T [X60414]; *A. jandaei* (HG 9) ATCC 49368^T [X60413]; *Aeromonas* sp. (HG 11) ATCC 35941 [X60417], *A. schubertii* (HG 12) ATCC 43700^T [X60416]; *Aeromonas* Group 501 (HG 13) ATCC 43946 (CECT 4254) [U88663]; *Aeromonas trota* (HG 14) ATCC 49657^T [X60415], *Aeromonas allosaccharophila* (HG 15) CECT 4199^T (S39232); *Aeromonas encheleia* (HG 16) CECT 4342^T [AJ224309]; and *Aeromonas popoffii* (HG 17) LMG 17541^T [AJ224308]. The sequences that start with the letter "X" are assigned with EMBL accession numbers and the other prefix letters, such as AJ, S, and U, can be found on the NCBI Website at http://www.ncbi.nlm.nih.gov (Reprinted with permission from A.J. Martinez-Murcia, International Journal of Systematic Bacteriology *49:* 1403–1408, 1999, ©International Union of Microbiological Societies.)

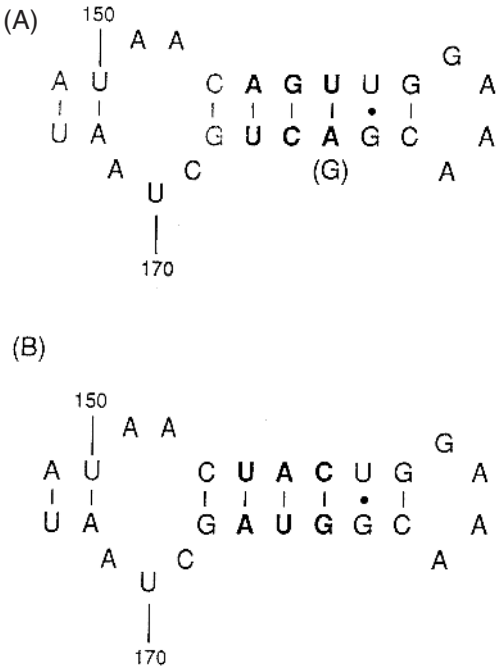

FIGURE BXII.γ.187. Primary structures of the diagnostic sequences at the V2 regions of the 16S rDNA (position ~160) which distinguish the *Aeromonas* "type" groups (*A*) from the "Schubertii" cluster (*B*). The nucleotide in parentheses occurs only in *Aeromonas eucrenophila.* (Reprinted with permission from A.J. Martinez-Murcia et al., International Journal of Systematic Bacteriology *42:* 412–421, 1992a, ©International Union of Microbiological Societies.)

and grayish-white to buff with a sometimes buttery consistency after 24–48 h of incubation at 35°C. Older colonies can develop a greenish hue, similar to that seen with certain *Vibrio* species, and a somewhat strong odor. There can be variation in colony size in some instances, either on original isolation or after subculture. *A. media* strains can produce a brown diffusible pigment on trypticase soy agar (TSA) (Allen et al., 1983). Colonies of the psychrophilic, nonmotile *A. salmonicida* subspecies are pinpoint in size after 18–24 h at 20–22°C, but after 4 d of incubation are circular, convex, entire, friable, and 1–2 mm in diameter (Griffin et al., 1953). Several of the *A. salmonicida* subspecies produce a brown, diffusible pigment after 5 d, especially on media containing tyrosine (Bernoth and Artzt, 1989). The presence of conspicuous encapsulation among several of the species results in the appearance of glistening to mucoid colonies.

There is variability in hemolysin production both within and between the species, as well as differences in the presence and/or type of hemolysis displayed depending on the type of red cells used. In one study, out of erythrocytes from nine animal species tested, the most sensitive blood agar medium contained mouse red cells, and sheep red cells were among the least sensitive for detection of β-hemolysis (Brenden and Janda, 1987). *A. hydrophila* and *A. veronii* are two of the more commonly isolated species that are strongly β-hemolytic on sheep blood agar, with less commonly isolated species often exhibiting β-hemolysis as well (Table BXII.γ.182). This β-hemolysis can be a broad zone, a double zone of partial hemolysis, or a narrow zone of β-hemolysis just under the colony edge. Many *A. caviae* isolates are partially hemolytic, displaying α-hemolysis, but can display narrow zones of β-hemolysis as well (Carnahan and Joseph, 1993).

On MacConkey agar, the majority of the mesophilic species grow as nonlactose fermenters, but a fair number of *A. caviae* isolates can ferment lactose. This can lead to confusion when testing water sources for coliforms, which are also lactose fermenters.

Nutrition and growth conditions The aeromonads grow over a wide temperature range (0–45°C), with the mesophilic strains growing between 10 and 42°C (Hänninen, 1994). The optimum temperature range is 22–37°C depending on the strain. While earlier studies have reported 28°C as the optimum temperature for motile aeromonads (Popoff, 1984a), at least one study on the effect of incubation temperature on growth and soluble protein profiles suggests that in some cases 37°C may be the optimum growth temperature (Statner et al., 1988). The psychrophilic

TABLE BXII.γ.182. Descriptive characteristics among all motile *Aeromonas* species[a]

Characteristic	*A. hydrophila*	*A. allosaccharophila* (1)	*A. bestiarum*	*A. caviae*	*A. encheleia* (4)	*A. eucrenophila*	*A. jandaei*	*A. media*	*A. popoffii*	*A. salmonicida*[b]	*A. schubertii*	*A. sobria*	*A. trota*	*A. veronii* biovar veronii	*A. veronii* biovar sobria
Motility	+	+	+	d	+	+	+	d	+	d	+	−	+	+	+
Indole	+	+	+	+	+	+	+	+	d	+	−	+	+	+	+
Urea	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ONPG	+	+	+	+	+	+	+	+	d	+	d	+	+	+	+
Citrate	d	d	d	+	−	−	+	d	+	d	d	+	d	+	d
Acetate	+	+	+	+	+	d	+	d	d	+	d	+	+	+	+
Malonate	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
KCN	+	−	d	+	+	+	d	d	d	d	−	−	d	−	d
Voges–Proskauer	+	−	d	−	−	−	d	−	d	d	d	−	−	d	d
Gelatin	+	+	d	+	d	d	+	d	+	+	d	−	+	+	+
Lysine decarboxylase	+	+	d	−	−	−	+	−	−	d	d	+	+	+	+
Arginine dihydrolase	+	d	d	+	d	d	+	d	+	d	+	−	+	−	+
Ornithine decarboxylase	−	d	−	−	−	−	−	−	−	−	−	−	−	+	−
Phenylpyruvic acid production	d	+	d	+	d	d	+	d	d	d	d	+	+	+	+
DNase	+	−	d	+	+	d	+	d	d	d	d	−	+	d	d
Corn oil lipase	+	+	d	d	+	d	+	d	+	+	+	+	−	+	+
D-Glucose (gas)	+	+	d	−	d	d	+	−	+	d	−	d	d	d	d
L-Arabinose	d	d	+	+	−	d	−	+	d	+	−	−	−	−	d
L-Rhamnose	d	d	d	−	d	d	−	−	−	−	−	−	−	−	−
D-Xylose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Cellobiose	−	+	d	+	−	d	d	+	−	d	−	+	+	d	d
Lactose	d	−	d	d	−	d	d	d	−	+	−	−	−	d	d
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	d	d	−	+	−	+	−	+	d	+	+
D-Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Raffinose	−	d	−	−	−	−	−	−	−	−	−	−	−	−	−
Adonitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Dulcitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Erythritol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glycerol	+	+	+	d	+	d	+	d	+	+	−	+	d	+	+
Inositol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-Mannitol	+	+	+	+	+	+	+	+	+	+	−	+	d	+	+
D-Sorbitol	−	−	−	−	−	−	−	−	−	d	−	−	−	−	−
α-Methyl-D-glucoside	d	−	d	−	−	−	d	−	+	d	−	d	−	+	d
Salicin	d	−	d	+	d	d	−	d	−	d	−	−	−	+	−
Melibiose	−	−	−	−	−	−	d	−	−	−	−	−	−	−	−
D-Amygdalin	−	−	−	d	−	−	−	−	−	−	−	−	−	−	−
D-Arabitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
H_2S from gelatin cysteine throsulfate	+	d	d	−	d	d	d	−	+	d	−	+	−	d	d
Gluconate oxidation[c]	d	−	d	−	−	−	d	−	−	−	−	−	−	d	d
Elastase	d	−	d	−	−	−	−	−	−	d	−	−	−	−	−
Ascorbate	nd	nd	d	nd	nd	−	d	−	nd	−	−	nd	−	−	nd
Esculin hydrolysis	+	d	+	+	+	d	−	d	−	d	−	−	−	+	−
Growth in 0% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in 3% NaCl	+	+	+	+	+	d	+	+	+	+	+	−	+	+	+
Hemolysis[d]	+	d	+	−	d	d	+	d	−	d	d	−	d	+	+
Ampicillin (S)[e]	−	−	−	−	nd	−	−	d	−	d	−	d	+	−	−
Cephalothin (S)[f]	d	−	d	−	d	d	d	d	−	d	d	+	−	d	+
O/129 (R)	+	+	+	+	+	d	+	+	+	+	+	+	+	+	+
Pyrazinamidase[g]	−	nd	−	d	nd	+	−	d	−	d	−	−	d	−	d
Mannose	+	nd	+	d	nd	+	+	+	+	+	+	+	+	+	+
Stapholysin	d	−	d	−	nd	−	−	−	−	d	−	−	−	−	−
Oxidase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polypectate (25°C)	−	−	−	−	−	−	−	−	−	d	−	−	−	−	−
Mucate[h]	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a]Symbols: +, >90%; −, <10%; d, 11 89% positive with incubation at 35°C for 7 d except for *A. popoffii* and *A. sobria*, which were incubated at 25°C (data from Abbott et al., 2003); nd, not determined.

[b]The *A. salmonicida* results are for the motile, mesophilic strains residing in HG 3 that are a currently unnamed subspecies.

[c]For determination of gluconate oxidation, the culture was incubated for only 48 h.

[d]Hemolysis detected on TSA on 5% sheep blood agar.

[e]Ampicillin susceptibility performed with 10 μg/disk and the culture incubated for only 24 h.

[f]Cephalothin susceptibility performed with 30 μg/disk and the culture incubated for only 24 h.

[g]Pyrazinamidase activity slants were incubated for only 48 h.

[h]Utilization as a sole carbon source.

strains in the *A. salmonicida* subspecies grow at temperatures generally ranging from 2 to 30°C.

Clinical isolates are generally incubated at 37°C, and environmental isolates are quite often incubated at 22–30°C, so that interlaboratory comparisons of biochemical reactions are difficult to make. Some mesophilic strains appear to be biochemically more active at 22°C than at 37°C. There are even cases of some biochemical tests having quite different results at the two different temperatures for the same strain (Ali et al., 1996a). In brain heart infusion medium at 28°C, the growth range for *A. hydrophila* is between pH 4.5 and 9.0 and 0–4% NaCl (Palumbo et al., 1985b).

Metabolism Aeromonads are facultative anaerobes that ferment D-glucose to acid or acid with gas and are oxidase and catalase positive, reduce nitrates to nitrites, and are enzymatically very active. They have been reported to produce amylase, DNase, chitinase, elastase, esterases, peptidases, arylamidases, and other hydrolytic enzymes (Hsu et al., 1981; Waltman et al., 1982; Janda, 1985; Carnahan et al., 1988; Hänninen, 1994). Recently, an adenylyl cyclase 2 was isolated from *A. hydrophila* with thermophilic properties and sequence similarities to proteins from hyperthermophilic *Archaea* (Sismeiro et al., 1998). There is new evidence that *Aeromonas* may exhibit anaerobic respiration and dissimilatory metal reduction (Knight and Blakemore, 1998).

Numerous large-scale phenotypic biochemical studies on *Aeromonas* have been published (Eddy, 1960, 1962; Ewing and Johnson, 1960; Ewing et al., 1961; Eddy and Carpenter, 1964; Popoff, 1969; McCarthy, 1975; Popoff and Veron, 1976; Lee, 1987). Both motile and nonmotile aeromonads utilize a number of other carbohydrates in addition to glucose (Popoff, 1984a; Arduino et al., 1988; Renaud et al., 1988; Carnahan et al., 1989a; Kuijper et al., 1989a; Altwegg et al., 1990; Abbott et al., 1992; Kämpfer and Altwegg, 1992; Carnahan and Joseph, 1993; Hänninen and Siitonen, 1995; Noterdaeme et al., 1996; Oakey et al., 1996a). In two recent major biochemical studies examining representatives of all known hybridization groups, excluding *A. encheleia* (HG 16), *A. popoffii* (HG 17), and nonmotile psychrophilic *A. salmonicida* subspecies (HG 3), it was determined that members of HGs 1–15 (including motile, mesophilic HG 3 *A. hydrophila*-like strains) shared a number of common features other than the aforementioned production of acid from glucose, oxidase and catalase activity, and reduction of nitrate (Abbott et al., 1992, 2003). These included the production of acid from D-trehalose; failure to utilize malonate or mucate as the sole carbon source; inability to ferment adonitol, dulcitol, erythritol, inositol, and D-xylose; and growth in nutrient broth containing 0 and 3% NaCl. Test results for several other phenotypic properties were nearly always positive (98–99%) and included motility, β-galactosidase activity, and resistance to O/129; fewer than 2% of the strains tested hydrolyzed urea, degraded pectin, or produced acid from arabitol, D-raffinose, or D-amygdalin. Variable results were obtained for 36 tests which included production of acetyl methyl carbinol (Voges–Proskauer test), indole, phenylpyruvic acid, acetate, citrate, and ascorbate utilization; elaboration of elastase, hemolysin, stapholysin, deoxyribonuclease, and corn oil lipase; esculin and gelatin hydrolysis; growth in KCN (potassium cyanide) broth; ornithine and lysine decarboxylase, arginine dihydrolase, and pyrazinamidase activities; H_2S production in GCF (gelatin–cysteine–thiosulfate) medium, pigment production at 25°C, gluconate oxidation; susceptibility to cephalothin and ampicillin; and acid production from L-arabinose, cellobiose, glycerol, lactose, α-methylglucoside, maltose, D-mannitol, D-mannose, melibiose, L-rhamnose, salicin, and sorbitol fermentation (Abbott et al., 1992, 2003) (Table BXII.γ.182). Nine tests were determined by these authors as well as others to be discriminatory enough to use as an initial primary battery of tests to separate aeromonads from the more commonly isolated species in the clinical laboratory (Carnahan et al., 1991a; Abbott et al., 1992, 2003; Furuwatari et al., 1994). However, these test results may not be reliable for the identification of HG 3 nonmotile, psychrophilic *A. salmonicida* subspecies strains, since the most recent studies noted above were conducted at 35–37°C and generally evaluated only motile aeromonad isolates. Therefore, the HG 3 strains in the Abbott et al. (1992) study were members of the unnamed, motile subspecies within *A. salmonicida* (Hänninen, 1994), which are genetically identical with nonmotile, psychrophilic fish pathogens (HG 3), but phenotypically resemble HG 1 *A. hydrophila* strains (Table BXII.γ.183). It should be noted that rapid identification systems to date are not very accurate at identifying motile *Aeromonas* species other than *A. hydrophila*, *A. caviae*, and *A. veronii* biovar sobria (HG 8; formerly termed *A. sobria* HG 7) from clinical specimens. Often the rarer clinical species are misidentified by these systems (Hickman-Brenner et al., 1987; Carnahan et al., 1991a; Janda, 1991; Abbott et al., 1992, 1998; Janda and Abbott, 1998b; Altwegg, 1999).

Over the years, there have likewise been extensive biochemical studies of large numbers of nonmotile *A. salmonicida* strains (both typical and atypical) (Griffin et al., 1953; Smith, 1963; Schubert, 1974; Popoff, 1984a; Austin et al., 1989a, 1998a; Dalsgaard et al., 1994, 1998; Hänninen et al., 1995, 1997). A recent review and examination of the results from these studies revealed several discrepancies for key reactions previously published as useful for separating the various subspecies (Millership, 1996). This is most likely due to a combination of reasons, namely that only a limited number of strains were available in older studies, and that test methods and reagents varied, particularly incubation temperature and length of incubation. Recent extensive biochemical studies of the *A. salmonicida* subspecies, including the increasing numbers of "atypical" strains, are those by Hänninen et al. (1995) and Hänninen and Hirvelä-Koski (1997), who studied Scandinavian fish isolates (Table BXII.γ.183); Austin et al. (1998a); and a review article by Wiklund and Dalsgaard (1998). There is an urgent need for standardization of methods both within and between laboratories (Dalsgaard et al., 1998) (*vide infra* Taxonomic Issues).

Genetic classification of aeromonads has been accomplished by mol% G + C composition (Popoff, 1984a), DNA–DNA relatedness studies (Popoff et al., 1981; Fanning et al., 1985; Farmer et al., 1986), 16S rDNA sequence analysis (Martinez-Murcia et al., 1992a; Martinez-Murcia, 1999), and multilocus sequence typing (MLST) analysis (Carnahan, 2001). Popoff et al. (1981) originally designated eight DNA–DNA hybridization groups using the DNA S1 nuclease method. The number of HGs was expanded to 12 (Fanning et al., 1985; Farmer et al., 1986) using the hydroxyapatite method and the recommended specified values of >70% relatedness at the optimum temperature of 60°C, >55% relatedness at the stringent temperature of 75°C, and divergence of 5% or less.

A variety of other molecular methods has been employed for both taxonomic purposes and/or epidemiological typing of aeromonads. An excellent review of these can be found in the articles by Carnahan and Altwegg (1996) and Altwegg (1996), and include plasmid analysis, restriction enzyme analysis (REA), ribo-

TABLE BXII.γ.183. Differential characteristics for psychrophilic nonmotile *Aeromonas* species and subspecies (HG 3)

Characteristic	*A. salmonicida* subsp. *salmonicida*	*A. salmonicida* subsp. *salmonicida*	*A. salmonicida* subsp. *achromogenes*	*A. salmonicida* subsp. *masoucida*	*A. salmonicida* subsp. *smithia*	Atypical *A. salmonicida*
Strain	ATCC 33658[T]	47 strains[a]	ATCC 33659[T]	ATCC 27013[T]	ATCC 49393[T]	34 strains[a]
Motility	−	− (100%)	−	−	−	− (100%)
β-Hemolysis[b]	+	+ (100%)	−	+	−	− (100%)
Oxidase	+	+ (100%)[c]	+	+	+	+ (100%)
Brown diffusible pigment[d]	+	+ (100%)	+	−	−	+ (68%)
Esculin hydrolysis	+	+ (100%)	−	+	−	− (97%)
Indole	−	− (100%)	+	+	−	+ (18%)
Arginine dihydrolase	−	− (100%)	+	+	−	nd
Voges–Proskauer	−	nd	−	+	−	nd
Fermentation of D-glucose	+	+ (100%)	+	+	+	+ (100%)
Gas from D-glucose	+	+ (100%)	−	+	−	− (97%)
Fermentation of:						
Sucrose	−	− (100%)	+	+	+	+ (100%)
Maltose	+	+ (100%)	+	+	+	+ (100%)
Salicin	+	+ (100%)	−	−	−	− (100%)
Arbutin[e]	+	+ (91%)	−	−	−	− (100%)
D-Galactose[e]	+	+ (100%)	+	+	−	+ (76%)
D-Mannitol	+	+ (100%)	−	+	−	+ (94%)
L-Arabinose[e]	+	+ (100%)	−	+	−	− (100%)
i-Inositol[e]	−	− (100%)	−	−	−	− (100%)
N-Acetyl glucosamine assimilation[e]	+	+ (100%)	−	−	+	+ (37%)

[a]Data from Hirvelä-Koski et al. (1994) (deals with both typical *A. salmonicida* subsp. *salmonicida* strains (n 47), as well as atypical strains (n = 34), Hänninen and Hirvelä-Koski (1997) (atypical strains).

[b]β-Hemolysis detected on ox blood agar.

[c]Oxidase-negative strains have been isolated from turbots.

[d]Pigment production for all strains in this table were evaluated using the medium containing tyrosine (Bernoth and Artzt, 1989) (see text for medium formulation).

[e]Tested with API 50 CH.

typing, restriction fragment length polymorphism (RFLP), and amplified fragment length polymorphism (AFLP). In addition, the use of pulsed-field gel electrophoresis (PFGE) (Hänninen and Hirvelä-Koski, 1997), species-specific probes, polymerase chain reaction (PCR) tests, and RFLP-PCR of 16S rDNA has been proposed (Husslein et al., 1992; Ash et al., 1993a, 1993b; Dorsch et al., 1994; Cascon et al., 1996; Oakey et al., 1996b, 1998; Borrell et al., 1997; Hoie et al., 1997; Khan and Cerniglia, 1997; Graf, 1999a; Figueras et al., 2000). These findings reaffirm the opinion of some investigators that the separation of species and accurate identification requires a polyphasic approach (Altwegg, 1993; Carnahan and Joseph, 1993).

In addition, a variety of phenotypic methods outside of conventional or rapid identification kits have been used for subtyping of *Aeromonas* isolates. These include phage typing (DeMarta and Peduzzi, 1984); resistotyping (determining antimicrobial susceptibility patterns) (Motyl et al., 1985; Overman and Janda, 1999); protein analysis using SDS-PAGE protein profiles (Stephenson et al., 1987); SDS-PAGE of outer membrane proteins (OMPs) (Kuijper et al., 1989b); immunoblots of SDS-PAGE gels (Mulla and Millership, 1993); analysis of quinone, polyamine, and fatty acid patterns (Kämpfer et al., 1994); FAME (gas liquid chromatographic analysis of cellular fatty acid methyl esters) (Hansen et al., 1991; Huys et al., 1994, 1995); and enzyme analyses (Altwegg, 1996). Methods to detect the presence or absence of a particular enzyme using chromogenic substrates have already been discussed, but a second method of enzyme analysis involves the analysis of electrophoretic mobilities of enzymes using nondenaturing gel electrophoresis followed by staining for particular enzyme activities. Electrophoretic typing of esterases in an *Aeromonas* hospital outbreak (Picard and Goullet, 1985) was followed by the development of multilocus enzyme electrophoresis (MEE) based on the relative frequencies of four alleles at only four loci to separate HGs 1–12 (Altwegg et al., 1991).

Antigenic structure There are several serotyping schema for aeromonads that are based on the presence of unique heat-stable somatic (O) determinants (Sakazaki and Shimada, 1984; Fricker, 1987; Guinee and Jansen, 1987; Cheasty et al., 1989; Thomas et al., 1990). Only one comparison study of two of these schema has been published (Shimada and Kosako, 1991). The original Sakazaki and Shimada scheme recognizes 44 serogroups with an additional 52 provisional serogroups (Albert et al., 1995b). Using the Sakazaki and Shimada scheme on 268 strains, *Aeromonas* species were found to be serologically heterogeneous, with individual serogroups present in more than one species (Janda et al., 1996a). Most type and reference strains were not serologically representative of the genomospecies at large. Further, three particular serogroups—O:11 (24%), O:16 (14%), and O:34 (10%)—were predominant. They were not necessarily associated with any particular genomospecies, but did appear to be associated with clinical infections (48%). Following the report of Albert et al. (1995b) that some strains of *Aeromonas trota* cross-reacted with *Vibrio cholerae* O:139 antisera, it was determined that most of the *A. trota* strains examined by another investigator did not express the O139 antigen of *Vibrio cholerae* (Janda et al., 1996a).

Antimicrobial susceptibility Most motile *Aeromonas* species are generally resistant to penicillin, ampicillin, carbenicillin, and ticarcillin, but continue to be susceptible to second- and third-generation cephalosporins, aminoglycosides, carbapenems, chloramphenicol, tetracyclines, trimethoprim-sulfamethoxazole, and the quinolones (Koehler and Ashdown, 1993; Janda and Abbott, 1998b; Altwegg, 1999). In the three earliest studies on antimicrobial susceptibility, all clinical isolates were considered

to be *A. hydrophila*, so that species-related differences in susceptibility could not be inferred (Overman, 1980; Fass and Barnishan, 1981; Fainstein et al., 1982b). A study comparing the "*in vitro*" susceptibilities among *A. hydrophila*, *A. veronii* biovar sobria (formerly considered to be *A. sobria*), and *A. caviae* found *A. hydrophila* generally more resistant than the other species (Motyl et al., 1985). Most aeromonad species produce "inducible" chromosomal β-lactamases, and at least three distinct types have been noted (Bakken et al., 1988; Iaconis and Sanders, 1990; Morita et al., 1994; Walsh et al., 1997b). There have been reports of rapid commercial susceptibility systems that were unable to accurately detect this inducible resistance factor (Schadow et al., 1993). It appears that cephalothin can be a potential marker for clinical strains of *A. veronii* biovar sobria from clinical sources (Janda and Motyl, 1985) and that a certain minimum inhibitory concentration (MIC) of colistin (4 μg/ml) could be a marker for *A. jandaei* (Carnahan et al., 1991c). *Aeromonas trota* has a unique susceptibility to ampicillin, with up to 30% of some *A. caviae* isolates being susceptible as well (Kilpatrick et al., 1987; Carnahan et al., 1991b). Therefore, the use of ampicillin in selective media may lead to underestimation of these species. Examination of the more rarely isolated clinical species for antimicrobial resistance patterns included 17 *A. jandaei*, 12 *A. schubertii*, 15 *A. trota*, and 12 *A. veronii* biovar veronii strains (Overman and Janda, 1999). All of the *A. trota* isolates were susceptible to all of the agents tested except for 20% resistance to cefazolin and 13% to cefoxitin. All of the *A. schubertii*, *A. veronii* biovar veronii, and 88% of the *A. jandaei* isolates were resistant to ampicillin. Overall, *A. veronii* biovar veronii and *A. schubertii* had markedly increased resistance to tobramycin; *A. veronii* biovar veronii and *A. jandaei* were generally resistant to imipenem; and *A. schubertii* and *A. trota* were less susceptible to cefoxitin, an expanded-spectrum cephalosporin, than to broad-spectrum cephalosporins. These species-related antibiograms may be useful adjuncts to species identification of clinical isolates.

Antimicrobial resistance to tetracycline, trimethoprim-sulfamethoxazole, some extended-spectrum cephalosporins, and aminoglycosides seems to be increasing among clinical aeromonad isolates in Taiwan, as compared to isolates from Australia and the United States (Ko et al., 1996). A study of the spectrum of extraintestinal disease due to *Aeromonas* species in Queensland, Australia found that in nine cases, the empirical antibiotic regimen prescribed did not adequately cover infection due to *Aeromonas* (Kelly et al., 1993). This suggests that identification to the species level may play a role in the selection of appropriate antimicrobial therapy for infection with motile *Aeromonas* species.

Reports from several countries indicate increasing resistance of *A. salmonicida* isolates from fish. As with the motile species, there is a report of at least three β-lactamases among isolates of *A. salmonicida* (Hayes et al., 1994). Farmed Atlantic salmon in Scotland were assessed for antimicrobial disk diffusion values and compared with MIC results for amoxycillin, oxolinic acid, oxytetracycline, and potentiated sulfonamide (Grant and Laidler, 1993). Of the 65 isolates tested, 23 were susceptible to all four antimicrobials, and 12, 13, and 16 isolates showed resistance to 1, 2, or 3 antimicrobials, respectively. Among 35 Finnish isolates, resistance was found only to oxytetracycline in strains from 9 of the 35 farms (Hirvelä-Koski et al., 1994). A total of 130 strains originating from farmed salmonid fish isolated in Denmark, Norway, Scotland, Canada, and the United States were tested for susceptibility to 22 antimicrobial agents (Dalsgaard et al., 1994). Antibiograms revealed increasing resistance to oxytetracycline and the quinolones, with multiresistant strains found in several countries.

Plasmids carrying resistance to streptomycin, chloramphenicol, tetracycline, and sulfathiazole as R factors have been isolated from *A. salmonicida* (Aoki et al., 1971; Toranzo et al., 1983). They have likewise been detected in motile aeromonads (Chang and Bolton, 1987). There is widespread plasmid-mediated resistance by means of 20–30 MDa plasmids in fish farms and eel ponds, and this most likely affects the potential benefit of antimicrobial compounds (Toranzo et al., 1983; Aoki, 1988). Broth conjugation experiments showed the transfer of the oxytetracycline (OT)-resistant phenotype from *A. salmonicida* to *Escherichia coli* and found this resistant phenotype to be encoded by high-molecular-weight R-plasmids that could be characterized by restriction digest profiles (Adams et al., 1998). One study of *Aeromonas* from clinical and environmental sources, representing seven species, found single or multiple resistance in 107/108 strains examined, with the highest incidence shown for β-lactam antibiotics other than cefotaxime. Thirty-five strains were found to contain transferable resistance plasmids, encoding resistance to ampicillin, cephalexin, cefoxitin, erythromycin, and furazolidone, either alone or in combination (Chaudhury et al., 1996).

Pathogenicity The early interest of von Graevenitz in *Aeromonas* stimulated others to begin to study this aquatic-borne organism, which seemed to be more common in clinical samples than previously realized (von Graevenitz and Mensch, 1968). While initially believed to be an opportunistic organism capable of infecting only immunocompromised individuals, a body of evidence now indicates that *Aeromonas* is a primary cause of extraintestinal illness and is strongly associated with gastrointestinal disease (Kelly et al., 1993; Janda and Abbott, 1996, 1998b; Joseph, 1996). Isolation rates have ranged from <1% to more than 60% in diarrheic populations in various geographic locations. The first well-documented extraintestinal infection was a case of septicemia in a menstruating woman who had been swimming in Jamaican waters. The organism was identified as *"Vibrio jamaicensis"* at the time but was later shown to be an *Aeromonas* sp. (Hill et al., 1954; Caselitz, 1955). The lack of final proof that *Aeromonas* is an etiologic agent of gastrointestinal disease lies in the absence of significantly large outbreaks and in one unsuccessful human volunteer study (Morgan et al., 1985). However, there is significant circumstantial evidence in the literature of sporadic cases and small outbreaks (Joseph, 1996; Janda and Abbott, 1998b).

Before the aeromonads were recognized as human pathogens, they were commonly isolated from poikilothermic animals, particularly amphibians, reptiles, and fish (Marcus, 1971, 1981; Shotts et al., 1972; Brackee et al., 1992; Burns et al., 1996; Gosling, 1996a; Schillinger, 1997). One of the first diseases attributed to *Aeromonas* was "red-leg" disease in frogs (Sanarelli, 1891). Aeromonads are also found with some frequency to cause various types of diseases in birds and domestic animals including pneumonia, peritonitis, and various localized infections (Gray, 1984; Shane et al., 1984; Shane and Gifford, 1985; Gosling, 1996a). Various species of fish develop hemorrhagic disease, ulcerative disease, furunculosis, red sore disease, and septicemia, resulting from infections with both motile and nonmotile *Aeromonas* species (Joseph and Carnahan, 1994; Austin and Adams, 1996). Humans can suffer from a variety of extraintestinal infections including wound infections, septicemia, meningitis, ophthalmitis, and infections associated with leech therapy, as well as gastroenteritis, especially in children <5 years (Altwegg, 1985; Janda

and Duffey, 1988; Altwegg and Geiss, 1989; Janda, 1991; Fedorko, 1993; Janda and Abbott, 1996, 1998b; Graf, 1999b). The species most frequently isolated from clinical specimens are *A. hydrophila* (HG 1), *A. veronii* biovar sobria (HG 8), *A. caviae* (HG 4), *A. jandaei* (HG 9), *A. schubertii* (HG 12), and *A. trota* (HG 14) (Altwegg, 1990; Altwegg et al., 1990; Carnahan et al., 1991a; Janda, 1991; Carnahan and Joseph, 1993; Janda and Abbott, 1998b). There is some indication from experimental mouse lethality studies that some species are more virulent than others (Janda and Kokka, 1991).

Current theory suggests that the virulence of *Aeromonas* species may be multifactorial. Possible virulence-associated substances and components described for *Aeromonas* spp. include toxins (cytotoxic and cytotonic), proteases, hemolysins, lipases, adhesins, agglutinins, pili, enterotoxins, various enzymes, and outer membrane arrays (Cahill, 1990; Janda and Abbott, 1996; Gosling, 1996a, b; Howard et al., 1996). Unfortunately, with some of the early work, the species identification of the organisms was not consistent with present day criteria, so it is difficult to determine to which and to how many aeromonads these results apply.

Filamentous structures on aeromonads are diverse. There are two broad morphological types of pili (short, rigid and long, wavy), as well as polar and lateral flagella. Short, rigid pili are the main pilus type expressed on heavily piliated aeromonads. Amino acid sequencing of their pilin showed they are homologous to *E. coli* type 1 and Pap pili (Ho et al., 1992). Long, wavy pili on fecal isolates belong predominantly to a family of a type IV pili which has been designated Bfp for "bundle-forming pilus" (Kirov and Sanderson, 1996). Their pilins have a molecular mass of 19–23 kDa. Despite the tendency to form bundles, the pilins of Bfp pili have closer N-terminal homology to the type IVA pilins, such as MSHA of *Vibrio cholerae*, than they do to the type IVB pilins of enteropathogenic *E. coli* and *V. cholerae*. There is compelling evidence that this pilus type mediates adherence to enterocytes as revealed by observations made with EM and immunoelectron microscopy, reduction of adhesion when pili are removed from the organism, adhesions to Henle 407 cells, and blocking experiments with purified pili or antibody to Bfp (Honma and Nakasone, 1990; Iwanaga and Hokama, 1992; Kirov et al., 1999). Bfp pili have not yet been genetically characterized. However, a type IV pilus gene cluster (*tapABCD*) has been identified in *Aeromonas* spp. It was originally cloned from a strain of *A. bestiarum* AH65 (previously published as *A. hydrophila*), and the product was designated Tap for type IV *Aeromonas* pilus (Pepe et al., 1996). *TapD* encodes a type IV leader peptidase/methyltransferase, which in addition to processing Tap pilin, is responsible for the extracellular secretion of aerolysin and other enzymes via the type II secretion pathway (Strom and Lory, 1993; Strom and Pepe, 1999). The *tap* cluster was subsequently cloned from a strain of *A. veronii* biovar sobria from which Bfp had been purified and it was clearly established that Tap pili was a second and distinct family of pili from the Bfp family (Barnett et al., 1997). Tap pili have a predicted molecular mass of approximately 17 kDa.

To date, there is little known regarding the function(s) of Tap pili. If assembled on the cell surface, they represent a minor pilus species on fecal isolates grown under standard laboratory conditions. Studies with Tap pilin mutants in *in vitro* adhesion assays and in animal models have found no evidence that Tap pili are important enterocyte adhesins or colonization factors. The widespread conservation of the *tap* gene cluster among all *Aeromonas* spp., however, suggests that the cluster does play an important function (Barnett and Kirov, 1999a, b).

Genetic characterization of the polar (Pof) and lateral (Laf) flagella of *Aeromonas* spp. has been described very recently (Shaw and Kirov, 1999). Evidence was presented that Pof are adhesins for *Aeromonas* spp. The Laf, when purified from a strain of *A. caviae*, showed considerable homology to the Laf of *Vibrio parahaemolyticus*. Laf genes are present in approximately 50% of *Aeromonas* isolates and lateral flagella are optimally expressed when bacteria are grown on solid media for <8 h at 37°C or 22°C. Preliminary evidence suggests that Laf serve as accessory colonization factors at the cell surface (Shaw and Kirov, 1999).

Enterotoxic (aerolysin), cytotoxic (Act), and two cytotonic toxins (Alt and Ast) have been detected in *A. hydrophila* (Chopra et al., 1996; Xu et al., 1998; Chopra and Houston, 1999). It appears that there may be a family of related hemolytic, cytotoxic enterotoxins. Because presumed "aerolysin" has been described in different species and/or strains, it is difficult to determine if these are all the same protein owing to some variance in the description of this toxin. Comparisons of Act of *A. hydrophila* SSU with the aerolysin of *A. trota* AH-2 (Chakraborty et al., 1987) and that of *A. bestiarum* (formerly *A. hydrophila*) AH-65 (Buckley et al., 1981) show differences in restriction maps, divergent flanking sequences, possibly different host cell receptors, and variability in the role of selected amino acid residues in biological function, e.g., in post-cleavage activity of the pro-toxin and eventual folding. Act appears to increase levels of tumor necrosis factor α (TNF-α) and interleukin (IL)-1 β in macrophage cell line RAW 264.7, as well as other pro-inflammatory cytokines, which could mediate inflammation and tissue damage during *Aeromonas* infections. There was also an increase in prostaglandin activity (PG_1 or PG_2). Use of PG inhibitors such as NS 398 and Celebrex eliminated activity or reduced the time of production of PG including INOS and $SsPLA_2$ (GrpV) levels in Chinese hamster ovary cells (Chopra and Houston, 1999).

Aerolysin from *A. trota* AH2 (formerly *A. hydrophila/A. sobria*) was first shown to be an important virulence feature by marker exchange mutagenesis, where the mutants were found to be much less toxic to mice (Chakraborty et al., 1987). It is first released as a proaerolysin, which is inactive until it is cleaved proteolytically and a C-terminal fragment of approximately 40 amino acids is removed.

A type II secretion pathway was described for a different "aerolysin" from *A. bestiarum* AH 65 that contained two operons, *exeAB* and *exeC–N*, forming the central components of this pathway in *Aeromonas*. The products of these genes form an apparatus that appears to culminate in an outer membrane secretion port. Phosphate bond hydrolysis and proton motive force are required for secretion through this port. The two operons appear to form an inner membrane complex, and *in vitro* mutagenesis of *exeA* suggests that this complex functions in the energy-dependent gating of the port (Howard et al., 1993, 1996; Howard, 1999). Their proaerolysin is a dimer in crystalline form and in solution (van der Goot et al., 1993). Murine erythrocytes, which have a glycoprotein receptor, are particularly susceptible to aerolysin activity and are easily lysed. The mode of action of this "aerolysin" involves channel formation in the target cell, and it functions in similar fashion to the action of membrane porins. The heptameric toxin inserts into the membrane of the cell and forms a 1–2 nm channel, causing the cell to lose its stability because of increased permeability and it is eventually destroyed (Howard, 1999).

Aeromonas also produces a lipase, named glycerophospholipid:cholesterol acyltransferase (GCAT), which can produce cholesteryl esters and can act as a phospholipase by digesting plasma membranes (Buckley, 1983). Two distinct types of proteases have been isolated from aeromonads including metallo- and serine proteases (Rodriguez et al., 1992). While considered accessory toxins in most instances, some studies with *A. salmonicida* suggest that they are primary virulence features. These enzymes probably function to protect the organism in its various environments, including humans, by inducing tissue destruction and assisting in the degradation of substrates for catabolic metabolism. Other miscellaneous toxins include amylase, chitinase, elastase, lecithinase, and nucleases (Gosling, 1996a).

The presence of a polysaccharide capsule has been reported for *A. salmonicida* and *A. hydrophila* serogroups O:11 and O:34 (Garrote et al., 1992; Martinez et al., 1995). Functionally, it is presumed that the capsule is a virulence factor that aids in complement resistance and/or in adherence to and invasion of fish cell lines (Merino et al., 1996, 1997).

S-layers of *Aeromonas* spp. are paracrystalline structures composed of identical protein subunits that are translocated across the cytoplasmic membrane, periplasm, and outer membrane to the cell surface, where they are assembled and tethered to the cell via an interaction with the O-polysaccharide side chains of the lipopolysaccharide. The S-Layer was originally referred to as an "A-Layer" first described in *A. salmonicida* (Udey and Fryer, 1978). It was considered a virulence feature because strains with this feature were more virulent than those without it. Specifically, strains with the S-layer could cause furunculosis in fish, while those without S-layers were avirulent. S-layer mutants have an altered ability to produce disease (Noonan and Trust, 1997). The significance of the S-layer in mesophilic aeromonads such as *A. hydrophila* and *A. veronii* biovar sobria appears to be less significant. Studies to correlate the presence of an S-layer on human isolates of *Aeromonas* through an autoagglutination phenomenon yielded inconclusive evidence for a direct involvement in mouse pathogenicity (Janda et al., 1987; Paula et al., 1988; Kokka et al., 1990, 1991a, b).

Aeromonads also possess more than one type of siderophore. In *A. salmonicida* there are at least two different mechanisms for iron acquisition (Chart and Trust, 1983). Mesophilic aeromonads usually produce either a unique siderophore, amonabactin (a four-peptide-based *bis*-catecholate siderophore), or an enterobactin-like siderophore (Telford and Raymond, 1997). Interestingly, *A. sobria* (HG 7), a species not isolated from humans thus far, does not produce siderophores (Zywno et al., 1992). *Aeromonas* spp. display significantly increased siderophore production under iron limiting conditions, and clinical strains are generally more productive than environmental strains (Naidu and Yadav, 1997).

Ecology Mesophilic aeromonads are indigenous to and have been isolated from various aquatic environments worldwide including fresh, estuarine (brackish), surface water (especially recreational), drinking water (including treated, well water, and bottled water), and polluted waters (Zimmermann, 1890; Hazen et al., 1978; Rippey and Cabelli, 1980, 1985; Seidler et al., 1980; Kaper et al., 1981; LeChevallier et al., 1982; Van der Kooj, 1988; Araujo et al., 1989; Alonso et al., 1994; Holmes and Nicolls, 1995; Holmes et al., 1996), as well as waste water effluent sludge (Schubert, 1975; Montfort and Baleux, 1991). They are not generally considered marine organisms, but can be found naturally in marine systems that interface with fresh waters, and found at all salinities, except the most extreme (Hazen et al., 1978). However, they are usually not a part of the autochthonous bacterial population of groundwater, which is normally poor in nutrients (Havelaar et al., 1990). However, in nutrient-rich waters, *Aeromonas* species can grow to large numbers, generally reaching a peak in the warmer temperatures of the summer months in both temperate freshwater lakes and chlorinated drinking water, showing a seasonal distribution (Burke et al., 1984a, b). While not considered to be of fecal origin, they seem to have a capacity to tolerate polluted environments including those of chemical origin (Seidler et al., 1980).

There is increasing interest in the incidence of *Aeromonas*, both during water treatment and distribution, particularly with regard to public health interest and to their role as indicators of chlorine resistance, disinfection efficacy, regrowth potential and biofilm development, even at 4°C (Holmes et al., 1996; Kuhn et al., 1997a, b; Sisti et al., 1998; Chamorey et al., 1999; Massa et al., 1999). Following the detection of increased numbers of aeromonads in the Netherlands in 1985, the public health authorities defined "maximum values" for *Aeromonas* densities in drinking water. These values are currently 20 CFU/100 ml of water for water leaving the production plant and 200 CFU/100 ml for drinking water in distribution (Van der Kooj, 1988). Examination of biofilms from exhumed pipe lengths revealed that 30% of the pipes examined contained an average population of aeromonads of 118 CFU/g dry weight (LeChevallier et al., 1987). After disinfection with 1 mg/l of chlorine, they could still isolate *Aeromonas* from 10% of the pipe lengths, with an average population of 51 CFU/g wet weight, suggesting that even though free cells of *Aeromonas* may be relatively susceptible to disinfection, populations may survive high chlorine dosing when associated with biofilms (LeChevallier et al., 1987; Holmes and Nicolls, 1995). Recent research revealed the apparent role of "quorum sensing" in biofilm formation on stainless steel by *A. hydrophila* (Lynch et al., 1999).

Aeromonas spp. have a capacity to infect fishes, reptiles, amphibians, humans, and other animals after exposure to such aquatic environments (Austin and Adams, 1996; Gosling, 1996a; Janda and Abbott, 1996, 1998b). There is good evidence that the species associated with clinical infections in humans are different hybridization groups than those isolated from drinking water and environmental sources, although putative virulence factors can be found in both categories (Holmberg et al., 1986a; Millership et al., 1986; Cahill, 1990; Havelaar et al., 1992; Hänninen, 1994; Kirov et al., 1994; Hänninen and Siitonen, 1995; Holmes and Nicolls, 1995; Kuhn et al., 1997a, b). Given the fact that the presence of aeromonads in water supplies may be connected with the increased incidence of *Aeromonas*-related gastroenteritis, the significance of which HGs are isolated from water as opposed to clinical isolates needs to be studied in greater detail (Holmes et al., 1996; Joseph, 1996).

Aeromonas species have been isolated from various food sources, including raw meats and fresh grocery produce, thereby establishing a possible source for *Aeromonas* human consumption (Kirov, 1993; Palumbo, 1996). These include raw chicken (Kirov et al., 1990; Akan et al., 1998); milk (Santos et al., 1994, 1996; Eneroth et al., 1998); cheese (Santos et al., 1994, 1996); ground meats (Okrend et al., 1987; Singh, 1997); seafood (Tsai and Chen, 1996; Lipp and Rose, 1997; Pianetti et al., 1997); poultry eggs (Yadav and Verma, 1998); fish, fish eggs, and shrimp (Hänninen et al., 1997); and a swine slaughter plant (Palumbo et al.,

1999). Produce sources include vegetables (Callister and Agger, 1987; Pedroso et al., 1997).

The presence of *Aeromonas* species in foods most likely reflects contact of these foods with water, as reflected in the name of the type species, *A. hydrophila*, which means "water-loving". The factors controlling the numbers of these organisms in these products include temperature, the trophic state of water, pH, NaCl, nitrite, atmosphere, and miscellaneous inhibitors such as essential oils of clove, coriander, nutmeg, and pepper (Palumbo et al., 1985b; Stecchini et al., 1993; Palumbo, 1996). However, a conclusive link between the consumption of *Aeromonas*-containing food and diarrheal disease has not yet been identified. As with the water isolates discussed above, putative virulence factors are found in both clinical and food isolates (Palumbo, 1996; Pedroso et al., 1997; Pin et al., 1997). It may be that temperature-related expression of virulence features, as yet undiscovered virulence features, and/or the existence of unique virulence "subsets" within several *Aeromonas* species can help to explain the absence of disease when *Aeromonas* is so ubiquitous (Daily et al., 1981; Palumbo, 1993; Joseph, 1996).

A. salmonicida is an inhabitant of natural waters and especially aquaculture ponds. It has been long considered a chief cause of furunculosis in various species of fishes (Shotts et al., 1972; Austin and Adams, 1996; Wiklund and Dalsgaard, 1998). Surprisingly, the organism has been difficult to isolate from the environment but is isolated from infected fish, particularly in the kidney. However, this organism was recently isolated in association with purple-pigmented bacteria in sediment from a Scottish loch (Austin et al., 1998b). Another possible reason for its cryptic character may be found in the recovery of cell wall deficient forms or L forms (McIntosh and Austin, 1990).

Enrichment and Isolation Procedures

The use of enrichments for the increased recovery of *Aeromonas* isolates from specimens is somewhat controversial. Ecological studies attempting to collect environmental samples should not use enrichments since the predominant strain(s) will quickly overgrow other strains that may be present. Enrichment broth can be useful as a presence–absence test for aeromonads in drinking water and food, or for monitoring marine populations in shellfish harvesting areas (Moyer, 1996). Among fish samples, both typical and atypical *A. salmonicida* have been recovered using an enrichment procedure with tryptone soy broth (TSB) (Austin and Adams, 1996). Food samples may be placed in enrichment broths when small numbers of aeromonads are expected and if there is the probability of cell injury from environmental pressures. The most frequently used broths are tryptic soy ampicillin (30 mg/l) broth (TSAB) and alkaline peptone water (APW). There are various formulations for these broths, as APW has been used with or without ampicillin for enrichment and enumeration of aeromonads from foods by the most probable number (MPN) technique.

The use of enrichment broths in clinical laboratories for the detection of low numbers of *Aeromonas* spp. has been exclusively used for the recovery from stool (fecal) samples. Early comparative studies found that nutrient broth gave the best recovery; but the authors contended that enrichment led to isolation of aeromonads in low concentrations that might be expected in convalescent patients, carriers, and those with subclinical infections. They further suggested that enrichment not be routinely used since it would interfere with interpretation of epidemiological studies trying to interpret the relationship between *Aeromonas* spp. and acute diarrhea (Robinson et al., 1984, 1986). The currently recommended broth for enrichment from fecal samples is APW adjusted to pH 8.6–9.8 (Shread et al., 1981; Millership et al., 1983; Moulsdale, 1983; von Graevenitz and Bucher, 1983; Moyer et al., 1991). The basic formulation contains 1% peptone in water at pH 8.6, but some investigators have increased the pH to 9.8 and added ampicillin (10–40 mg/l) and/or 0.5% desoxycholate to increase the selectivity of the medium (Khardori and Fainstein, 1988). The matter of the proper incubation temperature for APW enrichments is controversial as well, with some researchers finding better growth at 25°C than at 37°C (Millership and Chattopadhyay, 1984), but others finding no difference at all between these two temperatures (Price and Hunt, 1986). The use of GN (Gram-negative) broth for enrichment of aeromonads is only half as efficient as using APW (Millership et al., 1983), and Moyer et al. (1991) found that enrichment with APW increased the number of *A. caviae* isolates, suggesting that APW may be detecting not bona fide pathogens, but merely transient colonizers. The question of clinical relevance of aeromonads detected from enrichment broths only is clearly open to interpretation at this date and enrichment broths are generally not recommended for routine cultures.

Several studies have been conducted on the use of Cary–Blair transport medium for the transport of *Aeromonas* isolates from a variety of simulated clinical specimens (Siitonen and Mattila, 1990; Altwegg and Lüthy-Hottenstein, 1992; Koehler and Ashdown, 1993). Comparisons were made with other transport media, such as Amies medium, modified Stuart's medium, and glycerol-buffered saline. The consensus was that Cary–Blair transport medium was the best overall choice for transport of viable *Aeromonas*. Altwegg and Lüthy-Hottenstein (1992) also compared the behavior of *Aeromonas* species in Cary–Blair transport medium tubes incubated at 4°C, 25°C, and 37°C and found that the strains behaved similarly at both 25°C and 37°C, but very differently at 4°C with an initial drop in the colony count of all six strains examined during the first 24 h followed by a rapid increase for only the two *A. hydrophila* strains. The colony count of both *A. caviae* strains steadily dropped over the next few weeks, producing results consistent with those reported by Siitonen and Mattila (1990) in that storage at 4°C resulted in higher numbers of organisms only after several days.

Aeromonas species can be isolated from fish, food, water, and clinical specimens (most notably fecal) on a variety of primary plating media. They grow routinely on eugonic media such as blood and chocolate as well as on most enteric differential agar, although somewhat inhibited (Desmond and Janda, 1986). The following references include tables listing differential and selective media, their abbreviations, the application for which they are used, and the literature citation containing their formulation (von Graevenitz and Bucher, 1983; Joseph et al., 1988; Farmer et al., 1992; Moyer, 1996).

Aside from the enrichment above for *A. salmonicida* from fish, nearly all *A. salmonicida* strains can be isolated on trypticase soy agar (TSA). They require incubation at 22–25°C for 48 h and one should try to detect the presence of brown pigmentation, although furunculosis agar (recipe given below) seems superior (Bernoth and Artzt, 1989). There is only sparse growth of *A. salmonicida* on enteric agars and thiosulfate citrate bile salts sucrose (TCBS) medium, which is generally used for the isolation of *Vibrio* species. Rimler–Schotts (RM) medium (Shotts and Rimler, 1973) and a starch–glutamate–ampicillin–penicillin-based medium (SGAP-10C) (Jenkins and Taylor, 1995), both solid me-

dia, have been recommended for isolating *Aeromonas* species in general from piscine sources.

Methods for isolation of aeromonads from foods have been somewhat standardized (Palumbo et al., 1992). Some of the current recommended media used for the detection of motile aeromonads from food are starch–ampicillin agar (SA), blood ampicillin agar (BA, 30 mg/l), bile salts irgasan brilliant green agar (BIBG), Ryan Aeromonas agar (Oxoid, Basingstoke, England), and APW (Palumbo et al., 1985a; Moyer, 1996). Several comparison studies of media for food isolation resulted in slightly different recommendations for the preferred medium (Fricker and Tompsett, 1989; Ciufecu et al., 1990; Gobat and Jemmi, 1995). Trypticase soy ampicillin broth (TSBA, 30 mg/l) and MacConkey agar are currently considered the best combination for the isolation of *Aeromonas* from oysters (Abeyta et al., 1986).

An early recommendation for the detection of aeromonads from environmental samples was the use of dextrin fuchsin sulfite (DFS) in conjunction with membrane filtration for enumeration of aeromonads from clear waters (Schubert 1967a, 1987). This was followed by a selective and differential Rimler Shotts (RS) agar, which was subsequently modified into MRSM (Shotts and Rimler, 1973; Seidler et al., 1980). Rippey and Cabelli (1979) formulated mAeromonas medium or mA (a medium with trehalose, ampicillin, and ethanol) for use with a membrane filter. A comparison study of 11 media for recovery of aeromonads from polluted waters found mA to be the preferred medium followed by DNase–toluidine blue–ampicillin agar (DNTA), MacConkey Tween-80 (MACT), and starch bile (SB) agar (Arcos et al., 1988). A later study also found mA agar to be useful (Poffe and Op de Beeck, 1991). Analysis of surface waters using three media (mA, RS, and Ryan Aeromonas agar) found mA to be the most suitable (Bernagozzi et al., 1994). Ampicillin dextrin agar (ADA), mA, starch ampicillin (SA), Pril xylose ampicillin agar (PXA), and SGAP-10 appear to be the most widely used plating media (Moyer, 1996). However, there is no published comparison of these media nor does there appear to be a single optimal recovery medium.

Isolation of aeromonads from drinking water sources (chlorinated, unchlorinated, and well water) has been accomplished using a variety of media and methods, including anaerobic incubation (Burke et al., 1984a, b; Millership and Chattopadhyay, 1985; Cunliffe and Adcock, 1989; Havelaar et al., 1990; Moyer et al., 1992). In a study comparing ADA, xylose ampicillin agar (XAA), Ryan Aeromonas agar, and Aeromonas agar (Difco), Ryan's medium was found superior (Holmes and Sartory, 1993). The presence of lactose-fermenting aeromonad species, usually *A. caviae* phenotypes, has interfered with coliform determination by MF, MPN, and single tube coliform detection methods (Moyer, 1996; Landre et al., 1998). Aeromonads have been isolated from bottled waters by investigators in Canada, Saudi Arabia, Spain, and elsewhere (Gonzalez et al., 1987; Warburton et al., 1992; Hunter, 1993).

The recovery of *Aeromonas* from clinical specimens can be achieved with a number of media, many of which have already been discussed. Isolates from normally sterile sites should be easily detected on either blood or MacConkey agar. Blood agar has the advantage of allowing the simultaneous detection of hemolysis, oxidase, and indole production from a single primary medium, as well as the presence of more than one *Aeromonas* species (Janda et al., 1984). However, detecting fecal isolates of *Aeromonas*, particularly lactose-fermenting *A. caviae*-like strains on MacConkey, could be problematic as they would generally not be selected for further identification. Clinically significant *Aeromonas* species are also generally inhibited on TCBS, the selective medium for vibrios. Nearly all *Aeromonas* species can be detected on a modification of CIN (cefsulodin–irgasan–novobiocin) agar, which is normally used for the selection of *Yersinia enterocolitica* (Altorfer et al., 1985). This modified CIN, known as Yersinia selective agar (YSA, Difco), has a lower concentration of cefsulodin (4 mg/l) than the original CIN I formulation (15 mg/l) which tended to inhibit some species (Alonzo et al., 1996). This allows for simultaneous detection of both *Aeromonas* and *Yersinia* by streaking the plates with fecal matter and incubating at 25°C for 28 h. The incorporation of varying concentrations of ampicillin (10, 15, 20, and 30 mg/l) into blood agar for the purpose of inhibiting normal enteric flora has presented a few problems. Since several species of clinically significant *Aeromonas* are susceptible to ampicillin, most notably *A. trota* and a fair percentage of *A. caviae* (the most commonly isolated species from pediatric fecal samples), the incorporation of ampicillin in the media is controversial (Kilpatrick et al., 1987; Carnahan et al., 1991a; Singh and Sanyal, 1994). Most investigators use the lowest ampicillin concentration of 10 mg/l, if at all. A comparison of five selective media for detection of *Aeromonas* species from human and animal feces found that sheep blood agar with 30 mg/l of ampicillin (ASBA30) yielded a higher percentage of positive specimens, and that the addition of DNase–toluidine blue agar allowed them to detect 98% of all isolates (Mishra et al., 1987). Another comparison of five media included blood agar, blood agar with 20 mg/l of ampicillin, MacConkey, ampicillin Tween-80 agar (MAT), and YSA (modified CIN) for isolation of aeromonads from stools transported in Cary–Blair medium. Optimal recovery required the use of both BA20 and YSA (Kelly et al., 1988). An international multilaboratory study to establish the optimal culture media, incubation time, and incubation temperature found that APW enrichment streaked to blood agar with 10 mg/l of ampicillin gave the highest recovery of any single plate medium tested (Moyer et al., 1991). Ryan Aeromonas Medium Base with an ampicillin-selective supplement can also be used for clinical species isolation.

It should be noted that a modified MIO semisolid agar known as AH medium (Kaper's multitest medium) was proposed for screening presumptive *Aeromonas* colonies taken from selective medium (Kaper et al., 1979). It allowed for the determination of D-mannitol and *i*-inositol fermentation, ornithine decarboxylation, indole production, motility, H_2S, and gas production. It was evaluated by other investigators using an incubation temperature of 25°C for 24 h, rather than the originally described 30°C for 24 h (Toranzo et al., 1986). They detected false-negative indole reactions and the production of gas was difficult to determine. Furthermore, with species that are D-mannitol and indole negative (*A. schubertii*) and ornithine decarboxylase positive (*A. veronii* biovar veronii), the investigator would be better served to use one of several well-developed schema employing conventional biochemical tests (Carnahan et al., 1991a; Abbott et al., 1992; Altwegg, 1999).

Maintenance Procedures

The maintenance of stock cultures of *Aeromonas* can be accomplished by lyophilization, freezing in trypticase soy broth (TSB) with 15% glycerol at −20°C for short periods (months), TSB with 20% glycerol at −70°C for long periods (several years), or freezing in serum inositol broth (containing 25 g of *i*-inositol dissolved in 50 ml of distilled water, filter sterilized, and asepti-

cally added to 450 ml of sterile calf serum) at −70°C for long-term storage. For short-term storage, working cultures of *Aeromonas* spp. grown on nutrient media that do not contain fermentable carbohydrates can be placed at 5°C and subcultured every 2–3 weeks (Moyer, 1996). There is a medium for maintenance of marine strains that contains 0.5% peptone, 0.1% yeast extract, 2.4% NaCl, 0.7% Mg_2SO_4, 0.075% KCl, and 1.5% agar (Effendi and Austin, 1991). There is also a long-term room temperature maintenance medium (up to 15 years) from H.M. Atkinson that was originally developed for salmonellae, but has been adapted for use in storing aeromonads (Crowder, 1974).

Procedures for Testing Special Characters

H_2S from GCF (gelatin–cysteine–thiosulfate) (Véron and Gasser, 1963) Aeromonads were assayed for production of H_2S from cysteine using a medium containing Lab Lemco powder (Oxoid, 3 g), tryptone (Oxoid, 10 g), KCl (4 g), L-cysteine hydrochloride (0.1 g), sodium thiosulfate (0.1 g), ferric ammonium citrate (0.4 g), and agar (Difco Bacto, 12 g), w/v in 1000 ml of distilled water and steamed to dissolve all ingredients. The pH was adjusted to 7.4 and the media dispensed in 3-ml volumes (as broths). Special autoclave instructions included 10-lb pressure for 10 min and then the small agar butts were cooled and refrigerated until used. Inoculation involved stabbing the semisolid agar tubes with growth from an 18–24-h TSA culture and then incubating at 35–37°C. A positive reaction is indicated by a diffuse blackening of the medium radiating from the stab line after 72 h of incubation.

Pyrazinamidase Activity (Carnahan et al., 1990) Aeromonads were assayed using a medium obtained from Remel (Lenexa, KS) that contained tryptic soy agar (15 g), yeast extract (1.5 g), Tris-maleate (0.2 M, pH 6, 500 ml) buffer, and pyrazinamide (0.5 g), and was dispensed in 5-ml aliquots into screw-capped tubes (16 × 150 mm), autoclaved at 121°C for 15 min, and slanted. Slants were inoculated with 18–24 h bacterial growth taken from tryptic soy agar and incubated for 48 h at 36°C. One millimeter of 1% (w/v) freshly prepared ferrous ammonium sulfate (aqueous) solution was flooded over each slant, and a positive (pinkish rust color) or negative reaction (colorless) was recorded after 15 min. Positive pyrazinamidase activity indicated the presence of pyrazinoic acid resulting from the action of the enzyme pyrazinamidase.

DL-Lactate utilization (Altwegg et al., 1990; Janda et al., 1996a) Aeromonads were tested for utilization of DL-lactate as a carbon source using slants prepared according to the following formula, per liter: DL-lactic acid (60% w/v; Sigma, St. Louis, MO.), 2.5 ml; NaCl, 5 g; $NH_4H_2PO_4$, 1 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; agar, 15 g; and 0.2% bromothymol blue, 40 ml, adjusted to a pH of 6.8. The medium was then autoclaved, dispensed in 5-ml slants, and inoculated with growth from an 18–24-h culture. Incubation was carried out at 25°C, 30°C, or 35°C for 48 h, 72 h, or 5 d with a positive reaction indicated by growth and blue color that begins to develop from the top of the slant.

Urocanic acid utilization (Hänninen, 1994) Aeromonads were tested for utilization of urocanic acid with a medium containing NaCl (5 g), $NH_4H_2PO_4$ (1 g), K_2HPO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), agar (9 g), distilled water (1000 ml), and bromothymol blue (0.2% solution) 40 ml, adjusted to a pH of 6.8. The urocanic acid carbon source was added after the autoclaving of the basic medium as a filter-sterilized solution (2 g/l). The medium was dispensed in 5-ml amounts and slanted. Slants were inoculated and incubated as described above for DL-lactate testing and a positive reaction was recorded as growth and a bright blue color on the slant.

Furunculosis agar (Bernoth and Artzt, 1989 Hänninen and Hirvelä-Koski, 1997) The most sensitive method for brown pigment production among *A. salmonicida* isolates is furunculosis agar (tryptone, 10 g; yeast extract, 5 g; L-tyrosine, 1 g; NaCl, 2.5 g; agar, 15 g; 1000 ml water, pH 7.3). This medium was incubated at 22°C for 3, 7, and 18 d.

Differentiation of the Genus *Aeromonas* from Other Genera

Aeromonas is most closely related to *Vibrio* and *Plesiomonas*. Key tests to differentiate these genera are given in Table BXII.γ.184. It should be noted that the classic test of resistance to 150 mg of O/129 bears the caveat that there are reports of increasing resistance to O/129 among isolates of *Vibrio cholerae* from India and Bangladesh (Ramamurthy et al., 1992).

Taxonomic Comments

Great strides have been made over the past 20 years in clarifying the taxonomic order of the aeromonads. Prior to 1985, only three species were recognized. Now there are at least 14 species and a new family, *Aeromonadaceae*, has been proposed. Though much of the earlier confusion has been dispelled, some concerns remain including the taxa HG 3 (motile, mesophilic subspecies of *A. salmonicida* only), HG 5A, and HG 5B; the biovars in *A. veronii*; the status of *A. ichthiosmia* and *A. enteropelogenes*; and whether to consider *A. trota* as HG 13 or HG 14. Several discrepancies can occur when trying to group isolates of *A. salmonicida* into one of four subspecies, subsp. *salmonicida*, subsp. *achromogenes*, subsp. *masoucida*, and subsp. *smithia*, thus creating a number of atypical groups caused by lack of congruence of phenotypic and molecular data (Table BXII.γ.183). These atypical groups require attention because members of these groups are increasingly being isolated, especially from marine fish (Hänninen et al., 1995; Hänninen and Hirvelä-Koski, 1997; Austin et al., 1998a; Wiklund and Dalsgaard, 1998).

In HG 3 (*A. salmonicida*) there is an orphan group that is motile, grows well at 37°C, and has been isolated from humans.

TABLE BXII.γ.184. Differential characteristics for the genera *Aeromonas*, *Plesiomonas*, and *Vibrio*[a]

Characteristic	*Aeromonas*	*Plesiomonas*	*Vibrio*
Resistance to O/129[b] (150 μg)	R	S	S[c]
String Test[d]	−	−	+
Growth in 6.5% NaCl	−	−	(+)
Ornithine decarboxylase	(−)	+	+
Fermentation of i-inositol	−	+	(−)
Fermentation of D-mannitol	(+)	−	+
Fermentation of sucrose	(+)	−	(+)
Gelatin liquefaction	+	−	+
Growth on TCBS[e]	(−)	−	(+)

[a]Symbols: +, positive for the majority of species within the genus; −, negative for the majority of species within the genus; (+), positive for most speices, but a few species may be negative; (−), negative for most species, but a few may be positive; R, resistant; S, susceptible.

[b]Vibriostatic agent (2,4-diamino-6,7-diisopropylpteridine) (150 μg/disk).

[c]Vibrios are generally susceptible to O/129 with increased reports of resistance in *V. cholerae* strains from India and Bangladesh (Ramamurthy et al., 1992).

[d]Performed using a 0.5% solution of sodium desoxycholate.

[e]Thiosulfate citrate bile salts sucrose agar.

TABLE BXII.γ.185. Identification of genomospecies within the phenospecies for *Aeromonas hydrophila*[a,b]

	HG 1		HG 2		HG 3[c]	
	A. hydrophila		*A. bestiarum*		Motile, mesophilic *A. salmonicida* subspecies	
Characteristic	25°C	35°C	25°C	35°C	25°C	35°C
Acid from:						
D-Rhamnose	−	−	+	+	−	−
D-Sorbitol	−	−	−	−	+	+
Lactose	−	d	−	d	d	+
Utilization of:						
DL-Lactate	d	+	−	−	−	−
Urocanic acid	−	−	+	+	+	+
Elastase	+	d	+	−	+	d
Gluconate oxidation	nd	d	−	−	nd	−
Lysine decarboxylation	+	+	+	−	+	d
Maximum growth Temperature	41°C		38–39°C		38–39°C	

[a]Symbols: +, >75% of strains positive; −, <25% of strains positive; d, 26–74% of strains positive; nd, not determined.

[b]Data from Abbott et al. (1992); Hänninen (1994); Ali et al. (1996a); and Altwegg (1999); note that results for some tests vary in relation to the temperature of incubation.

[c]These are motile, nonpigmented, indole-positive strains that grow at 35°C.

It is unlike the other members of the group but exhibits subspecies level DNA relatedness to HG 3. See Tables BXII.γ.182 and BXII.γ.185 for differentiation of this motile *A. hydrophila*-like group from HG 1 (*A. hydrophila*) and HG 2 (*A. bestiarum*). It remains undesignated at present, but it is possible that a subspecies for this group could be proposed in the future.

For many years, the remaining subspecies members of the nonmotile *A. salmonicida* group were thought to be phenotypically distinct, but after additional taxonomic studies of the group by several researchers, it became evident that there are many more atypical strains in the environment than previously realized. An *Aeromonas* Taxonomy working group within the Subcommittee on the Taxonomy of *Vibrionaceae* has been established and is presently studying *A. salmonicida* in an attempt to clarify the position of atypical organisms and to reevaluate the presently accepted taxonomic designations for this group. It is questionable whether the currently recognized subspecies, as well as the possible incorporation of *Haemophilus piscium*, should be retained in the future (Austin et al., 1989a, 1998a; Thornton et al., 1999).

HG 5 is currently subdivided into HG 5A and HG 5B. HG 5A genetically resembles *A. media* but phenotypically resembles *A. caviae*. HG 5B genetically and phenotypically is *A. media*. It has been suggested that the two groups should be considered as either two subspecies of *A. media*, since HG 5B includes the type strain for *A. media* (Altwegg, 1990), or as biovars of the genomospecies *A. media* (Huys et al., 1996b).

HG 7, *A. sobria*, has not been isolated from human clinical specimens. However, there is a continuing use of this species name for clinical isolates, which resemble *A. sobria* but are actually *A. veronii* biovar sobria (HG 8) as opposed to *A. veronii* biovar veronii (HG 10). The terminology *A. veronii* biovar sobria for HG 8 and *A. veronii* biovar veronii HG 10 was suggested (Joseph et al., 1991) to clarify the situation and has been generally accepted based on usage in current literature and inclusion in the databases of most rapid identification systems. Although this suggestion to use the term " biovar sobria" for the biovar designation was to clarify that these HG 8 clinical isolates were genomically *A. veronii*, but phenotypically most similar to what we had heretofore called *A. sobria*, some concern has been expressed over the possibility that the term *sobria*, being used as both a species designation for HG 7 and the biovar designation for HG 8, might lead to confusion.

The proposed species *Aeromonas ichthiosmia* (Schubert et al., 1990b) has now been shown to actually be *A. veronii* biovar sobria (HG 8) by 16S ribosomal DNA sequencing (Collins et al., 1993), AFLP (Huys et al., 1996b, and DNA–DNA hybridization studies (Huys et al., 2001). Therefore, it is a junior synonym of *A. veronii* biovar sobria.

There is a proposal to divide *A. eucrenophila* into two groups, *A. eucrenophila* I and *A. eucrenophila* II based on ribotyping, AFLP fingerprinting, electrophoretic profiling of soluble cellular proteins, and cellular fatty acid methyl ester profiles (Huys et al., 1996a), with *A. encheleia* and HG 11 strains also residing in Group II. DNA hybridization studies (optical renaturation) (De Ley et al., 1970) of Group I and II *A. eucrenophila* and strains of *A. encheleia* and HG 11 grouped them into two distinct hybridization groups, with the first group composed only of *A. eucrenophila* I. The other group comprised *A. eucrenophila* subgroup II, *A. encheleia*, and HG 11 strains. DNA relatedness between strains in the *A. eucrenophila* I group versus strains in the second group, which included *A. encheleia*, *A. eucrenophila*, subgroup II, and HG 11, ranged from 42 to 52%. These findings suggest that members of this second group, which includes *A. encheleia*, *A. eucrenophila* subgroup II, and HG 11 strains, are genetically (DNA relatedness of values of 74–105%) and phenotypically very similar and should therefore all reside in *A. encheleia* (Huys et al., 1997a). However, a recent rebuttal to this suggestion was published with equally compelling arguments for their continued separation based on 16S rDNA sequence data and conflicting DNA relatedness values below the species level using the competitive nitrocellulose filter method (Martinez-Murcia, 1999). It appears that additional investigation is required to resolve this matter.

Aeromonas Group 501 was initially designated by Hickman-Brenner et al. (1988a) to identify three strains, that were phenotypically close to the newly proposed *Aeromonas schubertii*, and genotypically close to each other, but not related at the species level to any of the other HG groups, including *A. schubertii*. The group of unnamed organisms currently resides in HG 13 (Carnahan et al., 1991a) (Table BXII.γ.181). 16S rDNA sequencing of one Group 501 strain shows that there is a four-nucleotide difference between this strain and *A. schubertii* (Table BXII.γ.180).

Aeromonas trota (HG 14) and *A. enteropelogenes* are synonymous (Collins et al., 1993). While the frequency of terminology usage in the literature shows a marked preference for *A. trota*, the name

A. enteropelogenes was validated earlier (Schubert et al., 1990a). A request for a Judicial Opinion will eventually be made to decide which validated name will be accepted. Although they do not share the same type strain, phenotypic, typing, and molecular studies suggest they are the same organism (Carnahan, 1992; Collins et al., 1993; Huys et al., 2002a).

There is a discrepancy between Opinion 48 of the Judicial Commission in response to a request from Schubert (1971) and the Approved List of Bacterial Names (Amended Edition, 1989). Opinion 48 states that Kluyver and van Niel (1936) are no longer to be associated with the genus designation of *Aeromonas* (Editorial Board, 1973). In the future, Stanier (1943) should be associated with both the genus *Aeromonas* and the type species, *A. hydrophila*. A recent request for an Opinion from the Judicial Commission to correct the Kluyver and Van Niel citation in the 1980 Approved Lists for the genus *Aeromonas* has been submitted.

A. punctata and *A. caviae* have been considered objective synonyms and share the same type strain, ATCC 15468. This is because the type strain of *A. punctata* is incorrectly listed on the 1980 Approved List of Bacterial Names as ATCC 15468, when it should have been NCMB 74 (ATCC 23309). As part of the same request for an Opinion from the Judicial Commission on the Kluyver and van Niel issue mentioned above, it has also been registered to correct this *A. punctata/A. caviae* mistake and make *A. caviae* the single legitimate name for the type strain ATCC 15468. This would, however, cause *A. punctata* and *A. eucrenophila* to then become objective synonyms since both species would then share the same type strain of NCMB 74 (ATCC 23309). Although *A. punctata* is older and would have priority (it has a confusing history), it has been reported to have differing phenotypes and is not being used by the scientific community. Therefore, a second request for an Opinion from the Judicial Commission has been made to conserve the name *A. eucrenophila* Schubert and Hegazi, 1988a as the correct name for HG 6 with the type strain of NCMB 74 (ATCC 23309).

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Differentiation of the species of the genus *Aeromonas*

Each validly named species *vide infra* has a particular HG (hybridization group) designation and can be found in either Tables BXII.γ.182 and BXII.γ.186 (motile aeromonad HGs) or in Table BXII.γ.183 (nonmotile aeromonad HGs).

List of species of the genus Aeromonas*

1. **Aeromonas hydrophila** (Chester 1901) Stanier 1943, 213[AL] (*Bacillus hydrophilus* Chester 1901, 235.)
hy.dro′phi.la. Gr. n. *hydro* water; Gr. adj. *philos* loving; M.L. adj. *hydrophila* water-loving.

Hybridization group 1 of 17 (Popoff et al., 1981; Farmer et al., 1986).

At 37°C hydrolyzes esculin; produces acetoin from D-glucose (positive Voges-Proskauer test); displays pyrazinamidase activity; produces acid from D-mannitol and sucrose and variably from arabinose; resistant to ampicillin and cephalothin. Decarboxylates lysine but not ornithine; produces indole, H_2S (from GCF medium), and gas from D-glucose; β-hemolysis on TSA with 5% sheep blood agar. Differential tests to aid in the identification of *A. hydrophila* from all validly named motile species are listed in Tables BXII.γ.185 and BXII.γ.186. Descriptive tests for all the validly named motile species are listed in Table BXII.γ.182. Isolated from fresh and marine waters, diseased fish and poikilothermic aquatic animals (e.g., frogs with red leg disease), and warm-blooded animals; associated with both extraintestinal and diarrheal disease in humans (Austin and Adams, 1996; Gosling, 1996a ; Holmes et al., 1996; Janda and Abbott, 1996; Joseph, 1996).

The mol% G + C of the DNA is: 58–62 (Bd, T_m).
Type strain: ATCC 7966, DSM 30187.
GenBank accession number (16S rRNA): X60404 (16S rDNA).

2. **Aeromonas allosaccharophila** Martinez-Murcia, Esteve, Garay and Collins 1992c, 511[VP] (Effective publication: Martinez-Murcia, Esteve, Garay and Collins 1992b, 203.)
al.lo.sa.ca.ro′phi.la. Gr. adj. *allos* different; Gr. n. *saccharo* sugar; Gr. adj. *philos* loving; M.L. adj. *allosaccharophila* different sugar loving.

Hybridization Group 15 of 17 (Martinez-Murcia et al., 1992b; Esteve et al., 1995b).

Motile. Diffusible brown pigment is not produced. Growth occurs in 0–3% (w/v) NaCl. Resistant to 2,4-diamino-6,7-diisopropylpteridine (vibriostatic agent 0/129). Acid and gas are produced from D-glucose. Lysine is decarboxylated (Moeller's medium); indole, and β-galactosidase are positive. H_2S production from thiosulfate and Voges–Proskauer test are negative. Growth occurs at 4–42°C and at pH 9.0, but not at pH 4.5. Acid is produced from sucrose, D-cellobiose, maltose, D-trehalose, D-galactose, D-mannose, glycerol, and D-mannitol, but not from adonitol, arbutin, D-xylose, lactose, *myo*-erythritol, dulcitol, *myo*-inositol, or D-sorbitol. Sole carbon and energy sources include L-arabinose, sucrose, D-cellobiose, maltose, D-trehalose, D-galactose, D-mannose, glycerol, D-mannitol, L-histidine, L-arginine, L-proline, L-glutamate, D-gluconate, fumarate, and

Editorial Note: Since the submission and review of this chapter, two new subspecies and one new species have been proposed and validly published. These are *Aeromonas hydrophila* subsp. *dhakensis* (Huys et al., 2002b), *Aeromonas hydrophila* subsp. *ranae* (Huys et al., 2003) and *Aeromonas culicicola* (Pidiyar et al., 2002).

TABLE BXII.γ.186. Differential characteristics among motile *Aeromonas* species[a]

Characteristic	*A. hydrophila*[b] HG 1	*A. allosaccharophila*[c] HG 15	*A. bestiarum*[d] HG 2	*A. caviae*[b] HG 4	*A. encheleia*[e] HG 16	*A. eucrenophila*[f] HG 6	*A. jandaei*[b] HG 9	*A. media*[g] HG 5	*A. popoffii*[h] HG 17	*A. schubertii*[b] HG 12	*A. sobria*[i] HG 7	*A. trota*[b] HG 14	*A. veronii* biovar veronii[b] HG 10	*A. veronii* biovar sobria[b] HG 8
Esculin hydrolysis	+	d	+	+	+	+	−	+	−	−	−	−	+	−
Gas from D-glucose	+	+	+	−	+	+	+	−	+	−	+	+	+	+
Voges–Proskauer	+	−	+	−	−	−	+	−	+	d	weak +	−	+	+
Indole production	+	+	+	+	+	+	+	d	d	−	+	+	+	+
Pyrazinamidase[j]	+	nd	−	+	nd	+	−	d	nd	−	nd	−	−	−
Acid from:														
L-Arabinose	d	d	+	+	−	+	−	+	nd	−	−	−	−	−
D-Mannitol	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Sucrose	+	+	+	+	+	d	−	+	−	−	+	−	+	+
Decarboxylase:														
Lysine	+	+	−	−	−	−	+	−	−	+	weak +	+	+	+
Ornithine	−	d	−	−	−	−	−	−	−	−	−	−	+	−
Arginine dihydrolase	+	d	+	+	d	+	+	+	+	+	−	+	−	+
Arbutin hydrolysis	+	nd	+	+	−	+	−	+	nd	−	nd	d	+	−
H_2S from GCF	+	nd	+	−	nd	+	+	−	nd	−	nd	+	+	+
Hemolysis on SBA	+	nd	+	d		−	+	−	−	+	−	+	+	+
Susceptibility:[k]														
Ampicillin (10 μg)	R	R	R	R	R	S	R	S	R	R	R	S	R	R
Carbenicillin (30 μg)	R	nd	R	R	R	nd	R	nd	nd	R	S	S	R	R
Cephalothin (30 μg)	R	nd	R	R	nd	S	S	d	nd	S	S	R	d	S
Colistin (4 μg/ml)	d	nd	d	S	nd	S	R	S	nd	S	nd	S	S	S

[a]Symbols: +, ≥75% of the strains are positive; −, ≤25% of the strains are positive; d, 26–74% of the strains are positive; nd, not determined.

[b]Data from Carnahan et al. (1991a); all analyses were performed at 36°C ± 1°C for 3 d.

[c]Data from Martinez-Murcia et al. (1992b); analyses were performed at 35°C.

[d]Data from Ali et al. (1996a) and Carnahan and Joseph (1993); analyses were performed at 35°C for 7 d (see Table BXII.γ.185 for results at 25°C).

[e]Data from Esteve et al. (1995a) and Huys et al. (1996a, 1997a); analyses were performed at 28°C unless otherwise indicated.

[f]Data from Schubert and Hegazi (1988a); analyses were performed at 30°C and 20°C; Carnahan et al. (1991a) (36°C ± 1°C); and Abbott et al. (1992) (35°C for 7 d).

[g]Data from Allen et al. (1983); analyses were performed at 22°C for 7 d; Carnahan et al. (1991a) (36°C ± 1°C); and Abbott et al. (1992) (35°C for 7 d). Although originally described as nonmotile, later work by Austin and Austin (1990) reported on the motility of this species at 37°C; original description by Allen et al. (1983) also included production of a brown diffusible pigment on TSA (trypticase soy agar).

[h]Data from Huys et al. (1997b); analyses were performed at 28°C and 37°C for 7 d unless otherwise indicated.

[i]Data from Carnahan(unpublished data); analyses were performed at 25°C for 7 d incubation, unless otherwise indicated.

[j]Data from Carnahan et al. (1990); analysis performed at 35°C for 48 h.

[k]Antimicrobial susceptibility results were incubated at 35°C for 24 h; R, resistance; S, susceptibility; d, 26–74% susceptibility.

succinate. There is no growth on lactose, *myo*-erythritol, dulcitol, *myo*-inositol, ethanol, L-citrulline, L-leucine, L-alanine, glycine, L-serine, L-glutamine, DL-3-hydroxybutyrate, propionate, γ-aminobutyrate, D-glucuronate, α-ketoglutarate, or putrescine. Gelatin, casein, egg yolk, Tween 80, starch, and DNA are degraded but not urea, sodium dodecyl sulfate, or elastin (Martinez-Murcia et al., 1992b). Differential tests to aid in the identification of *A. allosaccharophila* from all motile species are listed in Table BXII.γ.186. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Found in diseased elvers and diarrheic stools.

The mol% G + C of the DNA is: 59.5 (method unknown).

Type strain: ATCC 51208, CECT 4199, DSM 11576.

GenBank accession number (16S rRNA): S39232.

3. **Aeromonas bestiarum** Ali, Carnahan, Altwegg, Lüthy-Hottenstein, and Joseph 1996b, 1189[VP] (Effective publication: Ali, Carnahan, Altwegg, Lüthy-Hottenstein, and Joseph 1996a, 163.)

bes.ti.a′rum. L. n. *bestiarum* of beasts (whether wild or domestic).

Hybridization Group 2 of 17 (Popoff et al., 1981; Fanning et al., 1985; Farmer et al., 1986; Ali et al., 1996a).

At 25°C and 35°C it produces acid from L-rhamnose; does not usually produce acid from lactose or D-sorbitol; utilizes urocanic acid, but does not utilize DL-lactate. At 25°C it hydrolyzes elastin; utilizes phenylpyruvate and decarboxylates lysine. Differential tests to aid in the identification of *A. bestiarum* from all motile species are listed in Table BXII.γ.186 and tests for separation from closely related species are listed in Table BXII.γ.185. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Primarily found in the environment in both wild and domestic animals and birds and fish. Isolated from human feces (Figueras et al., 1999).

The mol% G + C of the DNA is: not reported.

Type strain: ATCC 51108, CDC 9533-76.

GenBank accession number (16S rRNA): X60406 (16S rDNA).

Additional Remarks: X60406 is identical to *A. salmonicida* subsp. *achromogenes* X60407.

4. **Aeromonas caviae** (ex Eddy 1962) Popoff 1984b, 355[VP] (Effective publication: Popoff 1984a, 548.)
ca′ vi.ae. M.L. fem. n. *cavia* generic name of a guinea pig; M.L. gen. n. *caviae* of a guinea pig

Hybridization Group 4 of 17 (Popoff et al., 1981; Farmer et al., 1986).

Does not produce gas during D-glucose fermentation, Voges–Proskauer (acetoin) negative, lysine decarboxylase negative, H_2S not produced from GCF media. Utilizes DL-lactate and citrate as sole source of carbon. Differential tests to aid in the identification of *A. caviae* from all motile species are listed in Table BXII.γ.186 with supplementary tests to separate *A. caviae* given in Table BXII.γ.187. Descriptive tests for all the motile species are in Table BXII.γ.182. Found in fresh water, sewage, and on domestic and wild animals, birds, and fish. May be associated with gastrointestinal disease in adults (Holmberg et al., 1986a; Kuijper et al., 1989a; Altwegg et al., 1990; Joseph, 1996). Etiological agent of gastroenteritis in young children (Altwegg, 1985; Namdari and Bottone, 1990). Causes extraintestinal disease primarily in immunocompromised humans (Janda and Duffey, 1988; Janda, 1991; Janda and Abbott, 1998b).

The mol% G + C of the DNA is: 61–63 (Bd, T_m).

Type strain: ATCC 15468, DSM 7323, NCIMB 13016.

GenBank accession number (16S rRNA): X74674.

5. **Aeromonas encheleia** Esteve, Gutiérrez and Ventosa 1995a, 464[VP] emend. Huys, Kämpfer, Altwegg, Coopman, Janssen, Gillis and Kersters 1997a, 1162.
en.che′ le.ia. Gr. n. *encheyls* eel; M.L. adj. *encheleia* from eels.

Hybridization Group 16 of 17 (Martinez-Murcia et al., 1992b).

Motile. Colonies develop within 24 h at 28°C on TSA and are not pigmented. Old cultures (10–15 d) may have colonies with light brown pigmented colonies. No brown water-soluble pigment is produced. Growth occurs on MacConkey agar but not on thiosulfate–citrate–bile salts–sucrose agar. Acid is produced from D-glucose; gas production is variable. Resistant to vibriostatic agent 0/129. Growth in the presence of 0–3% NaCl at 4 and 37°C and under alkaline conditions (pH 9.0). Arginine dihydrolase variable; indole positive. H_2S variable, and lysine and ornithine decarboxylase negative. Voges–Proskauer reactions are usually negative. Acid is produced from D-mannose, D-trehalose, D-galactose, and D-mannitol, but not from L-arabinose, D-cellobiose, lactose, D-xylose, D-melibiose, D-raffinose, and *myo*-erythritol, dulcitol, *myo*-inositol, or D-sorbitol. Acid is sometimes produced from sucrose, salicin, maltose, L-rhamnose, and glycerol. Hydrolyzes esculin, arbutin, casein, collagen, chitin, Tween 80, egg yolk, and DNA, but not chondroitin sulfate, elastin, keratin, or urea. SDS-alkyl sulfatase negative. Fibrinogen and starch, but not mucin, are sometimes hydrolyzed. Human erythrocytes are hemolyzed. Sole carbon and energy sources include sucrose, salicin, maltose, D-mannose, D-trehalose, L-proline, L-serine, L-malate, fumarate, and D-mannitol. L-Histidine is sometimes utilized. The following are not used as sole carbon or energy sources: L-arabinose, D-cellobiose, L-rhamnose, lactose, D-raffinose, L-arginine, L-citrulline, L-leucine, L-alanine, glycine, L-glutamine, L-tyrosine, citrate, L-aspartate, DL-3-hydroxybutyrate, propionate, D-gluconate, D-glucuronate, α-ketoglutarate, *myo*-inositol, *myo*-erythritol, dulcitol, ethanol, and putrescine. L-Glutamate and D-galactose are sometimes utilized (Esteve et al., 1995a) See Table BXII.γ.182 for tests to differentiate *A. encheleia* from other *Aeromonas* spp. Differential tests to aid in the identification of *A. encheleia* from all motile species are listed in Table BXII.γ.186. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Found in healthy European eels reared in a freshwater farm; not pathogenic for mice.

The mol% G + C of the DNA is: 59.4–60.8 (T_m).

Type strain: ATCC 51929, CECT 4342, DSM 11577.

6. **Aeromonas eucrenophila** Schubert and Hegazi 1988b, 449[VP] (Effective publication: Schubert and Hegazi 1988a, 34) emend. Huys, Kämpfer, Altwegg, Coopman, Janssen, Gillis and Kersters 1997a, 1162.
eu.cre.no.phi′ la. Gr. fem. n. *eu* good; *krene* well; Gr. adj. *philos* loving; *eucrenophila* good well water.

Hybridization Group 6 of 17 (Popoff et al., 1981; Farmer et al., 1986).

Rod-shaped with rounded ends to coccoid 1.0–4.4 μm in diameter, occasionally forming filaments up to 8 μm long; occurs singly, in pairs, or chains. Motile by polar flagella, generally monotrichous. Voges–Proskauer negative. Facultatively anaerobic. Grows on a mineral medium with ammonia as the sole source of nitrogen and one of the following as the sole source of carbon: D-glucose, arginine, asparagine, or histidine. No growth at 40°C; maximum growth temperature is 37°C; minimum growth temperature is 0–5°C; optimal growth temperature 30°C, although incubation at 20°C is best for some tests; growth at pH range 5.5–9.0. Resistant to vibriostatic agent 0/129. Produces gas from D-glucose, but not from glycerol. Fructose, maltose, trehalose, and esculin are usually utilized; D-adonitol, dulcitol, i-inositol, inulin, melezitose, D-sorbose, and D-xylose are not fermented. Starch and dextrin are hydrolyzed; gelatin is liquefied; deoxyribonuclease, arginine dihydrolase, and phosphatase are usually produced; glutamic acid is not decarboxylated; urea is not hydrolyzed. Differential tests to aid in the identification of *A. eucrenophila* from all motile species are listed in Table BXII.γ.186. Descriptive tests for

TABLE BXII.γ.187. Identification of the genomospecies within the phenospecies *Aeromonas caviae*[a,b]

Characteristic	HG 4 *A. caviae*	HG 5A (unnamed)	HG 5B *A. media*[c]
Acid from:			
Lactose	d	+	+
Cellobiose	d	+	+
Utilization of:			
DL-Lactate	+	−	+
Citrate	+	−	−

[a]Symbols: +, >75% strains positive; −, <25% strains positive; d, 25–75% strains positive.

[b]Results derived from API50 and API 32GN strips at an incubation temperature of 30°C for 48 hr incubation. (Reprinted with permission from M. Altwegg. *In* Murry, Baron, Pfaller, Tenover and Yolken (Editors), *Manual of Clinical Microbiology*, 7th. Ed., American Society for Microbiology, Washington D.C., pp. 507 516, 1999.)

[c]HGs 5A and 5B may constitute two subspecies with HG 5B consisting of two biovars.

all the motile species are listed in Table BXII.γ.182. Found in unpolluted surface and groundwater such as oligosaprobic (living in an environment rich in organic matter and relatively free of oxygen) streams and clean well waters.

The mol% G + C of the DNA is: 59.8–62.6 (Bd, T_m).

Type strain: ATCC 23309, NCMB 74.

GenBank accession number (16S rRNA): X74675.

7. **Aeromonas jandaei** Carnahan, Fanning and Joseph 1992b, 191[VP] (Effective publication: Carnahan, Fanning and Joseph 1991c, 562.)

jan.dae′ i. M.L. gen. n. *jandaei* named after J. Michael Janda.

Hybridization Group 9 of 17 (Farmer et al., 1986).

Its unique biochemical profile includes negative reactions for esculin hydrolysis, fermentation of sucrose and cellobiose, and resistance to colistin (4 μg/ml). It differs from *A. schubertii* (HG 12), because *A. jandaei* is indole positive, ferments D-mannitol, and produces gas from D-glucose fermentation. A sucrose-negative reaction usually differentiates *A. jandaei* from other *Aeromonas* spp., although sucrose-negative strains that are not *A. jandaei* can occur. Differential tests to aid in the identification of *A. jandaei* from all motile species are listed in Table BXII.γ.186. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Isolation has been from specimens of diverse geographic origin including wounds, blood, and feces of humans, aquatic sources, fish, and prawns (Carnahan et al., 1991c; Joseph et al., 1991; Janda and Abbott, 1996).

The mol% G + C of the DNA is: not determined.

Type strain: ATCC 49568, DSM 7311.

GenBank accession number (16S rRNA): X74678.

8. **Aeromonas media** Allen, Austin and Colwell 1983, 603[VP]

me′ di.a. M.L. fem. gen. n. *media* in the middle.

Hybridization Group 5 of 17 (Popoff et al., 1981; Farmer et al., 1986).

Colonies on TSA are creamy, shiny, smooth, round, raised, entire, and 2 mm in diameter after incubation for 2 d at 22°C. Originally described as nonmotile, but motility can be expressed under certain laboratory conditions using appropriate media, temperature, and incubation periods (Austin and Austin, 1990). A diffusible, brown, nonfluorescent pigment is produced. Cultures in peptone broth are uniformly turbid. Chemoorganotrophic; metabolism is fermentative. Temperature range of 4–37°C; no growth at 42°C. Growth occurs in 0–3% NaCl. β-Galactosidase and arginine dihydrolase are usually produced. Phosphatase is not produced. Lysine and ornithine are not decarboxylated. Blood, gelatin, starch, ribonucleic acid, Tween 20, Tween 40, Tween 60, and Tween 80 are degraded, but cellulose, elastin, lecithin, xanthine, and urea are not. Methyl red test positive. Voges–Proskauer, gluconate oxidation, and H_2S production are negative. Utilizes DL-lactate, L-arabinose, glycerol, D-mannitol, L-proline, L-serine, sodium acetate, sodium glutamate, sodium pyruvate, and sodium succinate as sole carbon sources for energy and growth. Does not utilize either adonitol, D-cellobiose, *meso*-erythritol, ethanol, D-fructose, *meso*-inositol, inulin, L-leucine, D-melezitose, *p*-hydroxybenzoic acid, D-raffinose, sodium benzoate, sodium formate, sodium malonate, D-sorbitol, sucrose, D-trehalose, L-valine, or D-xylose (Allen et al., 1983). Differential tests to aid in the identification of *A. media* from all motile species are listed in Tables BXII.γ.186 and BXII.γ.187. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Strains isolated from fishponds and other aquatic sources (Allen et al., 1983). One rare report of association with human diarrheal disease (Rautelin et al., 1995a).

The mol% G + C of the DNA is: 62.3 (Bd, T_m).

Type strain: ATCC 33907, DSM 4881.

GenBank accession number (16S rRNA): X74679.

9. **Aeromonas popoffii** Huys, Kämpfer, Altwegg, Kersters, Lamb, Coopman, Lüthy-Hottenstein, Vancanneyt, Janssen and Kersters 1997b, 1170[VP]

po.pof′ fi.i. M.L. gen. n. *popoffii* of Popoff, named after Michel Popoff.

Hybridization Group 17 of 17 (Huys et al., 1997b).

Motile. Reduces nitrate to nitrite; resistant to vibriostatic agent 0/129. Optimal growth after 24 h at 28°C on TSA medium; acid and gas produced from D-glucose and glycerol. Growth in KCN broth but not in 3, 6, 8, and 10% NaCl; positive for arginine dihydrolase, DNase, and Voges–Proskauer test; variable production for indole; no production of urease, tryptophan deaminase, ornithine and lysine decarboxylase; H_2S is sometimes produced; citrate and malonate utilization is variable. Acid is uniformly produced from D-galactose, D-mannitol, D-mannose, methyl-D-glucoside, and D-trehalose, but not from adonitol, D-arabitol, D-cellobiose, dulcitol, erythritol, *myo*-inositol, lactose, α-D-melibiose, D-raffinose, L-rhamnose, salicin, D-sorbitol, D-sucrose, and D-xylose. The following substrates are used as carbon and energy sources: *N*-acetyl-D-glucosamine, L-aspartate, fumarate, D-galactose, D-gluconate, v-glucose, L-glutamate, L-glutamine, glycerol, L-histidine, L-malate, D-mannitol, D-mannose, D-maltose, putrescine, pyruvate, D-ribose, succinate, L-serine, D-trehalose, and L-tyrosine. The following are not utilized: acetate, *cis*-aconitate, *trans*-aconitate, adipate, adonitol, β-alanine, 4-aminobutyrate, arbutin, azelate, D-cellobiose, citrullin, dulcitol, ethanol, erythritol, D-glucuronate, DL-3-hydroxybutyrate, *myo*-inositol, itaconate, L-leucine, maltitol, α-D-melibiose, mesaconate, L-ornithine, oxoglutarate, phenylacetate, L-phenylalanine, L-proline, propionate, D-raffinose, L-rhamnose, salicin, D-sorbitol, suberate, D-sucrose, L-tryptophan, and D-xylose.

It hydrolyzes the following substrates: L-alanine-*p*NA, casein, 2-deoxythymidine-5′-*p*NP-phosphate, gelatin, lecithin, *bis*-*p*NP-phenylphosphonate, *p*NP-phosphoryl choline, L-proline-*p*NA, starch, and Tween 80. It does not hydrolyze chitin, esculin, L-glutamate-γ-3-carboxy-*p*NA, *p*NP-α-D-glucopyranoside, *p*NP-β-D-glucuronide, and *p*NP-β-D-xyloside (Huys et al., 1997b). Differential tests to aid in the identification of *A. popoffii* from all motile species are listed in Table BXII.γ.186. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Found in drinking water production plants and reservoirs.

The mol% G + C of the DNA is: 57.7–59.6 (T_m).

Type strain: LMG 17541.

GenBank accession number (16S rRNA): AJ224308.

10. **Aeromonas salmonicida** (Lehmann and Neumann 1896) Griffin, Snieszko and Friddle 1953, 138[AL] (*Bacterium salmonicida* Lehmann and Neumann 1896, 240.)

sal.mon.ic′ i.da. L. n. *salmo, salmonis* salmon; L. suff. *-cida* from L. v. *caedo* cut or kill; M.L. n. *salmonicida* salmon-killer.

Hybridization group 3 of 17 (Popoff et al., 1981; Fanning et al., 1985; Farmer et al., 1986).

Short Gram-negative rods, some pleomorphism and coccobacillary forms. In nutrient broth, pairs, chains, and clumps are usually observed in phase-contrast preparations. Nonencapsulated (Popoff, 1984a). Rarely motile. Usually possesses an S-layer (Noonan and Trust, 1997). Optimum growth temperature is 22–25°C. Maximum growth temperature in nutrient broth is 34.5°C (Griffin et al., 1953). Colonies on agar after 24 h are pinpoint. After 48–72 h colonies are circular, raised, convex, translucent, entire, and friable. On blood agar there is similar morphology and some subspecies produce β-hemolysis after 2–4 d (Millership, 1996). Colony pigmentation, when present, is usually yellowish; absence is indicated by grayish white colony. A brown, diffusible pigment appears after 24 h and reaches maximum color within 48–72 h. Differential and descriptive tests to aid in the identification of all nonmotile *A. salmonicida* subspecies, as well as atypical strains, are listed in Table BXII.γ.183. Differential and descriptive tests for the motile *A. salmonicida* strains within HG 3, which may be either a new subspecies or a biovar, are listed in Tables BXII.γ.182 and BXII.γ.185. Various diseases, including swelling at the vent and in the kidney, external and internal hemorrhages, ascitic fluid, and especially furunculosis are caused in a variety of fish. Interestingly, though not usually isolated from related aquatic locations, *A. salmonicida* was recently recovered in co-culture with purple-pigmented bacteria from fresh water lake sediment (Austin et al., 1998b).

The mol% G + C of the DNA is: 57–59 (Bd, T_m).
Type strain: ATCC 33658, NCMB 1102.
Additional Remarks: Neotype proposed Schubert, 1967b.

a. **Aeromonas salmonicida** *subsp.* **salmonicida** (Lehmann and Neumann 1896) Griffin, Snieszko and Friddle 1953, 138[AL] (*Bacterium salmonicida* Lehmann and Neumann 1896, 240.)

RBrown water-soluble pigment production on agar containing 0.1% tyrosine or phenylalanine. β-Hemolytic on ox blood agar, hydrolyzes esculin, indole negative, gas produced during D-glucose fermentation, ferments salicin and L-arabinose. Differential and descriptive tests to aid in the identification of this nonmotile *A. salmonicida* subspecies, as well as atypical strains, are listed in Table BXII.γ.183.

The mol% G + C of the DNA is: 57–59 (Bd, T_m).
Type strain: ATCC 33658, NCMB 1102.
GenBank accession number (16S rRNA): X60405.
Additional Remarks: Neotype proposed Schubert, 1967b.

b. **Aeromonas salmonicida** *subsp.* **achromogenes** (Smith 1963) Schubert 1967c, 278[AL] (*Necromonas achromogenes* Smith 1963, 273.)
a.chro.mo.ge′ nes. Gr. adj. *achromos* colorless; Gr. v. *gennaio* produce; M.L. adj. *achromogenes* not producing color.

Generally do not produce brown, water-soluble pigment unless grown on specific media such as furunculosis agar. May produce indole, arginine dihydrolase, ferment D-mannitol, and assimilate *N*-acetyl glucosamine. Gas is not produced during glucose fermentation; esculin is not hydrolyzed; no β-hemolysis on ox blood agar. Differential and descriptive tests to aid in the identification of all nonmotile *A. salmonicida* subspecies, as well as atypical strains, are listed in Table BXII.γ.183.

The mol% G + C of the DNA is: 57–59 (Bd, T_m).
Type strain: ATCC 33659, NCMB 1110.
GenBank accession number (16S rRNA): X60407.

c. **Aeromonas salmonicida** *subsp.* **masoucida** Kimura 1969, 52[AL]
ma.sou.ci′ da. Japan. n. *masou* specific epithet of *Onchorhynchus masou*, (fish); L. v. suff. *-cida* from L. v. *caedo* cut or kill; M.L. fem. n. *masoucida Onchorhyncus masou*-killer.

Does not produce brown, water-soluble pigment on agar; β-hemolytic on ox blood agar; may hydrolyze esculin; arginine dihydrolase positive; gas produced during glucose fermentation; L-arabinose fermented; *N*-acetyl glucosamine not assimilated. Differential and descriptive tests to aid in the identification of this nonmotile *A. salmonicida* subspecies, as well as atypical strains, are listed in Table BXII.γ.183.

The mol% G + C of the DNA is: not available.
Type strain: ATCC 27013.
GenBank accession number (16S rRNA): X74680.

d. **Aeromonas salmonicida** *subsp.* **pectinolytica** Pavan, Abbott, Zorzópulos and Janda 2000, 1123[VP]
Isolated from heavily polluted river.
The mol% G + C of the DNA is: not known.
Type strain: 34mel, DSM 12609.
GenBank accession number (16S rRNA): AF134065.

e. **Aeromonas salmonicida** *subsp.* **smithia** Austin, McIntosh and Austin 1989b, 495[VP] (Effective publication: Austin, McIntosh and Austin 1989a, 288.)
smi′ thi.a. M.L. fem. gen. n. *smithia* of Smith, named after Isabel W. Smith.

Nonmotile, approximately 1–2 μm in size, with rounded ends. Cultures on trypticase soy agar (TSA) dissociate into "rough", "smooth", and "G-phase" colonies (Duff, 1937). Does not readily produce brown diffusible pigment. In peptone broth, cultures are uniformly turbid. Metabolism is fermentative. Growth occurs at 4–25°C but not at 30°C and in 0–2%, but not 3% NaCl. No growth on MacConkey agar. Catalase, β-galactosidase, H_2S, oxidase, phosphatase, and phosphoamidase are produced, but not indole, arginine dihydrolase, lysine or ornithine decarboxylase, or phenylalanine deaminase. DNA, gelatin, RNA, and starch are degraded, but not aesculin, blood, chitin, elastins, guanine, hypoxanthine, lecithin, Tween 20, 40, 60, 80, or xanthine. Carbon-containing compounds are not readily utilized (Austin et al., 1989a). Differential and descriptive tests to aid in the identification of this nonmotile *A. salmonicida* subspecies, as well as atypical strains, are listed in Table BXII.γ.183. Pathogenic for fish, with mortality within 24–72 h. External hemorrhages are evident in the vent and on the fins, as is tail rot. Accumulation of ascitic fluid occurs in the peritoneal cavity, along with extensive muscle hemorrhaging, liquefaction, and gastroenteritis.

The mol% G + C of the DNA is: 55.9 (T_m).
Type strain: 138, ATCC 49393, CCM 4103.
GenBank accession number (16S rRNA): AJ009859.

11. **Aeromonas schubertii** Hickman-Brenner, Fanning, Arduino, Brenner and Farmer 1989, 205[VP] (Effective publication: Hickman-Brenner, Fanning, Arduino, Brenner and Farmer 1988a, 1563.)
schu.ber′ti.i. M.L. masc. gen. n.*schubertii,* named after Ralph H.W. Schubert.

Hybridization Group 12 of 17 (Farmer et al., 1986).

At 36°C, positive reactions for methyl red, lysine decarboxylase, arginine dihydrolase, motility, lipase, DNase, reduction of nitrate to nitrite, oxidase, and growth in nutrient broth with 0 and 1% NaCl. No growth in 6% NaCl. Fermentation of D-glucose, D-galactose, maltose, D-mannose, and trehalose. No fermentation of adonitol. L-arabinose, D-arabitol, cellobiose, dulcitol, erythritol, *myo*-inositol, lactose, D-mannitol, melibiose, α-methyl-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, and D-xylose. Esculin is not hydrolyzed. String test negative. Differential reactions include failure to ferment D-mannitol, sucrose, L-arabinose, and salicin; failure to produce indole, or hydrolyze esculin, and lack of gas production from glucose (Hickman-Brenner et al., 1988a; Carnahan et al., 1991a). A D-mannitol negative reaction usually differentiates *A. schubertii* from other *Aeromonas* spp. although mannitol-negative strains that are not *A. schubertii* can occur. Extracellular amylase, protease, and DNases produced. High cell surface charge but low hydrophobicity. Major cell wall fatty acids are hexadecenoic ($C_{16:1}$), hexadecanoic ($C_{16:0}$), and octadecanoic acid ($C_{18:1}$) (Kokka et al., 1992). Differential tests to aid in the identification of *A. schubertii* from all motile species are listed in Table BXII.γ.186. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Strains isolated from wounds, abscesses, pleural fluid, and blood. There have been no isolates from human stools (Hickman-Brenner et al., 1988a; Carnahan et al., 1989b).

The mol% G + C of the DNA is: undetermined.
Type strain: ATCC 43700, DSM 4882.
GenBank accession number (16S rRNA): X74682.

12. **Aeromonas sobria** Popoff and Véron 1981, 215[VP] (Effective publication: Popoff and Véron 1976, 20.)
so.bri′a. M.L. fem. adj. *sobria* moderate.

Hybridization Group 7 of 17 (Popoff et al., 1981; Farmer et al., 1986).

Biochemical results obtained at 25°C after 7 d of incubation. Produces acid and gas from glucose, acid from mannitol and sucrose, and is weakly positive Voges–Proskauer reaction. Lysine decarboxylase is weakly positive after 7 d; arginine dihydrolase and ornithine decarboxylase are not produced. Does not produce acid from L-arabinose. Does not hydrolyze esculin. Does not produce β-hemolysis on sheep blood agar (SBA) (5%). There is no growth at 42°C. Produces gas from glucose at 25°C and no gas at 37°C after 48 h. This species closely resembles *A. veronii* biovar sobria (HG 8) biochemically, although they differ in their arginine dihydrolase and hemolytic reaction (SBA) (Table BXII.γ.186) and their ability to grow at 42°C. Other differences include the ability of *A. sobria* to produce a positive string test (with 0.5% sodium desoxycholate) and susceptibility to O129 (150 μg). Differential tests to aid in the identification of *A. sobria* from all motile species are listed in Table BXII.γ.186. Descriptive tests for all the motile species are listed in Table BXII.γ.182.

The few known strains were isolated from fish, sewage, and water. These strains generally grow optimally at 25–30°C with few exceptions. Thus far, there are no reported human or animal clinical isolates. (There is frequent incorrect reference to this species as a human clinical isolate; see Taxonomic Comments.)

The mol% G + C of the DNA is: 58–60 (T_m).
Type strain: ATCC 43979, CIP 7433, NCIMB 12065.
GenBank accession number (16S rRNA): X74683.

13. **Aeromonas trota** Carnahan, Chakraborty, Fanning, Verma, Ali, Janda and Joseph 1992a, 191[VP] (Effective publication: Carnahan, Chakraborty, Fanning, Verma, Ali, Janda and Joseph 1991b, 1207.)
tro′ta. Gr. adj. *trotos* vulnerable; M.L. fem. adj. *trota* vulnerable.

Hybridization Group 14 of 17 (Carnahan et al., 1991b).

Its unique biochemical profile includes negative reactions for esculin hydrolysis, L-arabinose fermentation, Voges–Proskauer test, and gluconate oxidation. Positive for cellobiose fermentation, lysine decarboxylase, and citrate utilization. Susceptible to ampicillin (in contrast to almost all other aeromonads) and carbenicillin (Tables BXII.γ.182 and BXII.γ.183). Differential tests to aid in the identification of *A. trota* from all motile species are listed in Table BXII.γ.186. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Strains isolated from human stools, aquatic sources, and extraintestinal human infections. First thought to be found primarily in Southern Asia. Now considered to have worldwide distribution (Carnahan and Joseph, 1993). Documented case of pediatric diarrhea (Reina and Lopez, 1996b).

The mol% G + C of the DNA is: undetermined.
Type strain: AH2, ATCC 49657, DSM 7312.

The species name *Aeromonas enteropelogenes* has been validly published (Schubert et al., 1990a). It appears to be identical to *A. trota*, a more frequently used name (Carnahan et al., 1991b; Collins et al., 1993; Huys et al., 2002a) (see Taxonomic Comments).

14. **Aeromonas veronii** Hickman-Brenner, MacDonald, Steigerwalt, Fanning, Brenner and Farmer 1988b, 220[VP] (Effective publication: Hickman-Brenner, MacDonald, Steigerwalt, Fanning, Brenner and Farmer 1987, 901.)
ve.ro′ni.i. M.L. gen. n. *veronii* of Véron, named after M.M. Véron.

Hybridization Groups 8/10 of 17 (Popoff et al., 1981; Farmer et al., 1986).

Subsequent to the proposal for this species (formerly termed Enteric Group 77 and HG 10), it was learned that although they had different biochemical reactions, HG 8 was genetically identical to HG 10. Thus, *A. veronii* contained two biogroups (Kuijper et al., 1989a; Altwegg et al., 1990). The first was the originally proposed ornithine decarboxylase positive species, *A. veronii* (HG 10) (Hickman-Brenner et al., 1987). The second group was lysine decarboxylase positive, arginine dihydrolase positive, and ornithine decarboxylase negative, and contained the so-called "clinical" *A. sobria* (HG 8). While the HG 7 environmental strains of *A. sobria* and the former HG 8 *A. sobria* are phenotypically very similar, they are genetically and clinically quite different. For this reason, it was decided to place the

two biogroups of *A. veronii* into two separate biovars, namely biovar veronii and biovar sobria (Joseph et al., 1991).

The mol% G + C of the DNA is: undetermined.

Type strain: ATCC 35624, DSM 7386.

GenBank accession number (16S rRNA): X74684.

Note that the species name *Aeromonas ichthiosmia* (Schubert et al., 1990b) has been validly published, but has been unarguably shown to be identical to *A. veronii* (Carnahan, 1992; Collins et al., 1993, Huys et al., 2001) (see Taxonomic Comments).

a. **Aeromonas veronii** *biovar* **veronii**

String test negative, produces gas from D-glucose, is resistant to O/129, and grows in the absence of added NaCl. This biovar is resistant to ampicillin (10 μg) and carbenicillin (30 μg), and sensitive to cephalothin (30 μg) and colistin (4 μg/ml). Indole positive and Voges–Proskauer positive, produces acid from D-mannitol and sucrose, does not produce acid from L-arabinose, and is pyrazinamidase negative. β-Hemolytic on sheep blood agar (5%).

Aeromonas veronii biovar veronii is characteristically ornithine decarboxylase positive, arginine dihydrolase negative, and esculin and arbutin hydrolysis positive. This biovar can be misidentified as *Vibrio cholerae* in some rapid identification systems (Hickman-Brenner et al., 1987). Differential tests to aid in the identification of *Aeromonas veronii* biovar veronii from all motile species are listed in Table BXII.γ.186. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Isolated from aquatic environments, including leeches (Graf, 1999b) and specimens from human clinical disease including diarrhea, wounds, pulmonary complications, sinusitis (Hickman-Brenner et al., 1987), wounds (Joseph et al., 1991), and bacteremia (Abbott et al., 1994; Janda and Abbott, 1996).

The mol% G + C of the DNA is: 57.6–58.2 (Bd, T_m).

Type strain: ATCC 35624, DSM 7386.

GenBank accession number (16S rRNA): X74684.

b. **Aeromonas veronii** *biovar* **sobria**

String test negative, produces gas from D-glucose, is resistant to O/129, and grows in the absence of added NaCl. This biovar is resistant to ampicillin (10 μg) and carbenicillin (30 μg), and sensitive to cephalothin (30 μg) and colistin (4 μg/ml). Indole positive and Voges–Proskauer positive, produces acid from D-mannitol and sucrose, does not produce acid from L-arabinose, and is pyrazinamidase negative. β-Hemolytic on sheep blood agar (5%).

Aeromonas veronii biovar sobria is characteristically ornithine decarboxylase negative; lysine decarboxylase and arginine dihydrolase positive, and esculin and arbutin hydrolysis negative; β-hemolytic on 5% sheep blood agar and able to grow at 42°C. Differential tests to aid in the identification of *Aeromonas veronii* biovar sobria from all motile species are listed in Table BXII.γ.186. Descriptive tests for all the motile species are listed in Table BXII.γ.182. Associated with gastrointestinal disease and a wide array of extraintestinal and systemic disease including septicemia, wound infections, meningitis, peritonitis, septic arthritis, and hepatobiliary disease (Steinfeld et al., 1998; Janda and Abbott, 1996, 1998b). The reference strain for these clinical isolates, which were formerly considered to be HG 7 *A. sobria*, but in actuality are HG 8 *Aeromonas veronii* biovar sobria, is ATCC 9071.

The mol% G + C of the DNA is: undetermined.

Type strain: ATCC 9071.

Other Organisms

1. *"Aeromonas arequipensis"*

Isolated from a lake in the Arequipa region of Peru; may have diarrheagenic potential for humans (Matte et al., 1999).

2. *"Aeromonas dechromatica"*

This organism was proposed by Kvasnikov et al. (1985). Isolated from chromium-containing industrial wastewater; has basic characteristics of *Aeromonas* spp. Exceptions are requirement for sodium chloride (optimal 3%), mixed flagellation, Gram variable, absence of catalase activity, presence of a crystalline microcapsule; so named because it reduces highly toxic hexavalent chromium ions.

3. *"Aeromonas guangheii"*

This organism was isolated from eels. Unpublished; only one strain.

Deposited strain: 95-173.

GenBank accession number (16S rRNA): AB028881.

4. *"Aeromonas pastoria"*

This organism was proposed (Torres, 1990; Torres et al., 1991) and later included in a large phenotypic study of *Aeromonas* isolates (Noterdaeme et al., 1996). Two strains of *"Aeromonas pastoria"* were examined, one of which fell into their Cluster 2A, which was characterized by nonfermentation of L-arabinose and α-methyl-D-glucoside and by assimilation of citrate. Another strain of *"A. pastoria"*, which was received separately, fell into Cluster 7, which was characterized by the following reactions: negative for production of ornithine decarboxylase; hydrolysis of esculin; assimilation and fermentation of L-arabinose; fermentation of α-methyl-D-glucoside, arbutin, salicin, and lactose; and positive for the assimilation of citrate.

5. "*Aeromonas* Group 501" (formerly Enteric Group 501).

A designation given to two strains in the CDC collection by Hickman-Brenner et al. (1988a), which closely resembled *A. schubertii* but were genotypically and phenotypically different and currently reside in HG 13; see Taxonomic Comments above for further discussion.

Deposited strain: ATCC 43946.

GenBank accession number (16S rRNA): U88663.

6. "*Aeromonas* species ornithine positive" (formerly Enteric Group 77)

Currently resides in HG 11 (Fanning et al., 1985; Farmer et al., 1986; Hickman-Brenner et al., 1987); see Taxonomic Comments above for further discussion about these strains.

Deposited strain: ATCC 35941.

GenBank accession number (16S rRNA): X60417.

7. "Halophilic *Aeromonas* species"

There is a report of a strictly halophilic *Aeromonas* sp. from Atlantic salmon in association skin lesions leading to severe widespread lesions elsewhere, a condition that sug-

gested production of a potent toxin(s). On primary culture, the organism was slow growing (best in the presence of horse blood, 10% w/v) and had a strict requirement for at least 1.0% NaCl. Optimum growth temperature is between 15 and 20°C. No growth at 37°C. Growth at 30°C after 72 h yielded small (1–2 mm), round, low, convex, mucoid, translucent, β-hemolytic colonies. Individual cells were motile, straight, Gram-negative rods. Resistant to vibriostatic agent 0/129 (10 and 150 μg) and susceptible to oxytetracycline, oxalinic acid, furazolidone, and penicillin. Oxidase positive, reduced nitrates, and used D-glucose fermentatively. Majority of biochemical tests were negative, except for gelatinase and D-glucose. Voges–Proskauer test was variable. The organism (NCIMB 2263) was at and was deposited in the National Collection of Industrial and Marine Bacteria Ltd., Aberdeen (Cox et al., 1986).

Species Incertae Sedis

1. **Haemophilus piscium** Snieszko, Griffin and Friddle 1950, 699[AL]
pis.ci′um. L. n. *piscis* a fish; L. gen. pl. *piscium* of fishes.

This microorganism was removed from the genus *Haemophilus* in the first edition of *Bergey's Manual of Systematic Bacteriology* (Kilian and Biberstein, 1984). This exclusion was made on the basis of its DNA base composition, growth factor requirements, cell wall structure, respiratory requirements, respiratory quinones, antigenic composition, and phage susceptibility. It was later concluded that it represents an atypical form of *Aeromonas salmonicida* (Paterson et al., 1980; Austin et al., 1989a). 16S rRNA encoding genes for the reference strains of *A. salmonicida* subsp. *smithia* and *H. piscium*, when amplified by PCR, revealed that these organisms showed 99.4 and 99.6% 16S rRNA gene sequence identity, respectively, with *A. salmonicida* subsp. *salmonicida* (Thornton et al., 1999).

It should be noted that an authentic type strain of *Haemophilus piscium* was not available in any culture collection, so the *H. piscium* strain (NCIMB 1952) was obtained for use from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (Thornton et al., 1999).

The mol% G + C of the DNA is: 55.1 (T_m).
Type strain: ATCC 10801.
GenBank accession number (16S rRNA): AJ009860.

Genus II. **Oceanimonas** *Brown, Sutcliffe and Cummings 2001, 71*[VP]

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O.ce.a.ni.mo′ nas. Gr. n. *okeanus* ocean; Gr. fem. n. *monas* monad unit; M.L. fem. n. *Oceanimonas* ocean monad.

Gram-negative motile rods (0.7–1.2 × 2.0–2.5 mm). Up to four polar flagella. **Respiratory metabolism** using O_2. Phosphatidylethanolamine, phosphatidylglyerol, and diphosphatidylglycerol are the main phospholipids. **Require Na^+ for growth**

The mol% G + C of the DNA is: 54 (HPLC).

Type species: **Oceanimonas doudoroffii** (Baumann, Bowditch, Baumann and Beamann 1983b) Brown, Sutcliffe and Cummings 2001, 71 (*Pseudomonas doudoroffii* Baumann, Bowditch, Baumann and Beamann 1983b, 863.)

FURTHER DESCRIPTIVE INFORMATION

These organisms are chemoorganotrophs found in marine environments.

ENRICHMENT AND ISOLATION PROCEDURES

O. doudoroffii strains were first isolated from Pacific Ocean surface water by enrichment in liquid medium containing allantoin, benzoate, creatine, caprylate, or D-aminovalerate followed by streaking on the same medium (Baumann et al., 1972). *O. baumannii* was isolated from estuary mud by enrichment in liquid minimal salts medium containing phenol as a carbon source and 5% NaCl, followed by streaking onto the same medium solidified with agar (Brown et al., 2001).

MAINTENANCE PROCEDURES

O. doudoroffii strains were maintained on Marine Agar (Difco) slopes with monthly transfer (Baumann et al., 1972).

DIFFERENTIATION OF THE GENUS *OCEANIMONAS* FROM OTHER GENERA

O. doudoroffii DSM 7028[T] and *O. baumannii* GB6[T] can be distinguished from the type strains of both *Aeromonas hydrophila* and *Tolumonas auensis* by the inability of the former two strains to utilize arabinose, gluconate, glucose, maltose, mannitol, mannose, and sucrose, and by the inability of the latter two strains to utilize malate. *O. doudoroffii* DSM 7028[T] and *O. baumannii* GB6[T] can be further distinguished from *Tolumonas auensis* by the inability of the latter to grow above 25°C and from *A. hydrophila* by the inability of the latter to utilize citrate and by its ability to produce indole, to produce acid from glucose, to produce arginine hydrolase, to hydrolyze esculin, and to utilize *N*-acetylglucosamine.

TAXONOMIC COMMENTS

The major reevaluation of the taxonomy of the genus *Pseudomonas* and the reassignment of species in RNA groups II–V to other genera are discussed elsewhere in this volume. In the first edition of the *Manual*, Palleroni (1984) placed *Pseudomonas doudoroffii* in a separate section devoted to organisms whose relationships to those in the *Pseudomonas* RNA groups I–V were not known. Subsequent studies (De Vos et al., 1989; Kersters et al., 1996; Anzai et al., 2000) did not clarify the taxonomic relationships of *Pseudomonas doudoroffii*. Brown et al. (2001) place the genus *Oceanimonas* in the *Gammaproteobacteria* but do not assign it to a family.

List of species of the genus Oceanimonas

1. **Oceanimonas doudoroffii** (Baumann, Bowditch, Baumann and Beamann 1983b) Brown, Sutcliffe and Cummings 2001, 71[VP] (*Pseudomonas doudoroffii* Baumann, Bowditch, Baumann and Beamann 1983b, 863.)
dou.do.rof′ fi.i. M.L. gen. n. *doudoroffii* of Doudoroff; named after M. Doudoroff.

Gram-negative rods (0.7–1.2 × 2.0–2.5 μm). One to three bipolar flagella. Utilizes acetate, aconitate, D-α-alanine, L-α-alanine, β-alanine, allantoin, γ-aminobutyrate, δ-aminovalerate, L-arginine, L-aspartate, benzoate, betaine, butylamine, caprate, citrate, creatine, ethanol, D-fructose, fumarate, L-glutamate, glutarate, DL-glycerate, glycine, glycolate, histamine, L-histidine, DL-β-hydroxybutyrate, α-ketoglutarate, DL-lactate, DL-malate, L-ornithine, phenol, L-proline, putrescine, pyruvate, L-serine, sarcosine, spermine, and succinate. Does not utilize galactose or glycerol. Oxidase positive. Accumulates poly-β-hydroxybutyrate. Fatty acids include dodecanoic, hexdecanoic, *cis*-hexadecenoic, and *cis*-octadecenoic.

The mol% G + C of the DNA is: 54 (HPLC).

Type strain: ATCC 27123, DSM 7028.

GenBank accession number (16S rRNA): AB019390.

2. **Oceanimonas baumannii** Brown, Sutcliffe and Cummings 2001, 71[VP]

bau.man′ ni.i. M.L. gen. n. *baumannii* of Baumann; named after Paul and Linda Baumann, who first studied these organisms.

Gram-negative rods (1 × 2 μm). Aerobic growth on L-alanine, betaine, caprate, citrate, ethanol, L-glutamate, malate, L-proline, phenol, sarcosine, and succinate. Grows in NaCl up to 7% (w/v). Growth temperatures 10–30°C. Utilizes galactose and glycerol. Fatty acids include dodecanoic, hexdecanoic, *cis*-hexadecenoic, and *cis*-octadecenoic.

The mol% G + C of the DNA is: 54 (HPLC).

Type strain: GB6, ATCC 700832, NICMB 13685.

GenBank accession number (16S rRNA): AF168367.

Genus incertae sedis III. **Tolumonas** *Fischer-Romero, Tindall and Jüttner 1996, 187*[VP]

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To.lu.mo′ nas. L. n. *toluolum* toluol (German for toluene); Gr. n. *monas* unit; M.L. fem. n. *Tolumonas* toluene-producing unit.

Cells are **nonmotile**, Gram-negative rods (2.5–3.2 × 0.9–1.2 μm) that occur singly or in pairs. **Growth** occurs under **aerobic and anaerobic** conditions. Anaerobic cultures are catalase and oxidase negative; aerobic cultures are **catalase positive** and **oxidase negative**. **Toluene is produced from phenylalanine, phenylacetate, phenyllactate, and phenylpyruvate** only in the presence of an additional carbon source. Phenol is produced when phenylalanine is replaced by tyrosine. The optimum conditions for anaerobic growth are **22°C** and pH 7.2. The major fermentation products in peptone–yeast extract–glucose cultures are ethanol, acetic acid, and formic acid. Under anaerobic conditions D-arabinose, D-cellobiose, D-fructose, D-glucose, glycogen, inulin, maltose, D-mannose, D-melecitose, melibiose, D-raffinose, L-rhamnose, D-ribose, salicin, sucrose, D-trehalose, D-mannitol, and D-sorbitol are used as carbon sources. In the presence of air the following organic substances are used: Tween 40, Tween 80, L-arabinose, D-fructose, D-galactose, D-glucose, methyl-pyruvate, monomethyl succinate, acetic acid, *cis*-aconitic acid, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, β-hydroxybutyric acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, quinic acid, sebacic acid, succinic acid, glucuronamide, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, L-threonine, urocanic acid, and inosine. The enzymes lipase, lecithinase, urease, and exoprotease are not produced. Indole, lipase, and H_2S are not formed. According to the results of 16S rDNA sequence analysis this genus belongs to the *Gammaproteobacteria*.

The mol% G + C of the DNA is: 49.

Type species: **Tolumonas auensis** Fischer-Romero, Tindall and Jüttner 1996, 187.

FURTHER DESCRIPTIVE INFORMATION

Ecological studies (Fischer-Romero, unpublished results) on the distribution of *Tolumonas auensis* in different lakes and streams of central Europe suggest that this bacterium is not ubiquitously distributed. Cells were not found in surface samples, whereas in the water column the number of *Tolumonas auensis* increased with the depth and reached a maximum in the sediment and the overlying water. In the course of one year the maximum number of *Tolumonas auensis* cells was counted in samples taken in autumn, before the autumnal circulation, concurring with the highest seasonal toluene concentration in the hypolimnion.

ENRICHMENT AND ISOLATION PROCEDURES

Members of the genus *Tolumonas* can be enriched in TP medium inoculated with anoxic freshwater sediment and isolated by agar deep dilutions (Fischer-Romero et al., 1996).

MAINTENANCE PROCEDURES

Strains of the genus *Tolumonas* can be maintained in TP medium at 4°C, after incubation to allow good growth, with monthly transfers or preserved by lyophilization or by storage in liquid nitrogen.

PROCEDURES FOR TESTING SPECIAL CHARACTERS

To determine the concentration of toluene in the culture media, the method described by Jüttner (1988) may be used. The medium (0.1–75 ml) was supplied with perdeutero-toluene (1 μl perdeutero-toluene/ethanol; 1:1000 v/v) as internal standard and stripped for 30 min in a closed-loop stripping apparatus. Toluene was adsorbed on a Tenax-filled cartridge (150 g Tenax TA) and transferred from these by thermodesorption into a combined GC–MS. Toluene was separated by gas–liquid chromatography on a DB 1301 fused silica capillary column (30 m × 0.32 mm, J & W Scientific) and detected by single ion monitoring (*m/z* 91 and *m/z* 98) on a Hewlett Packard 5970A mass spectrometer.

DIFFERENTIATION OF THE GENUS *TOLUMONAS* FROM OTHER GENERA

Tolumonas transforms phenylalanine and other phenyl precursors into toluene. A few bacteria that are able to transform amino acids into aromatic hydrocarbons have been described. *"Clostridium aerofoetidum"* transforms phenylalanine in a Stickland reaction with methionine into toluene (Pons et al., 1984). *Lacto-*

TABLE BXII.γ.188. Differentiation of the genus *Tolumonas* from the closest phylogenetic relatives[a]

Characteristics	*Tolumonas*	*Aeromonas*	*Citrobacter*	*Escherichia*	*Hafnia*	*Plesiomonas*	*Serratia*	*Vibrio*
Mol% G + C	49	57–63	50–52	48–52	48–49	51	52–60	38–51
Motility	−	+	+	D	D	+	+	+
Optimal temperature, °C	22	22–28	37	37	30–37	37	30–37	20
Glycerol utilization	−	d	+	D	+	+	+	+
Fucose utilization	−	nd	nd	nd	nd	nd	+	nd
Catalase	+	+	+	+	+	+	+	nd
Oxidase	−	+	−	−	−	+	nd	+

[a]For symbols see standard definitions; nd, not determined.

bacillus sp. (Yokoyama and Carlson, 1981) and *Clostridium* sp. strains (Elsden et al., 1976) liberate *p*-cresol and phenol from tyrosine. These organisms differ from *Tolumonas* in their cytophysiological features and in the biochemistry of toluene production.

Other characteristics that differentiate members of the genus *Tolumonas* from their closest phylogenetic relatives, *Escherichia*, *Citrobacter*, *Hafnia*, *Plesiomonas*, *Serratia*, *Vibrio*, and *Aeromonas* (Holt et al., 1994) are shown in Table BXII.γ.188.

Based on the 16S rDNA sequence analysis *Tolumonas auensis* shows about 10% sequence divergence from its nearest neighbors. These include members of the genera *Escherichia*, *Serratia*, *Enterobacter* (i.e., members of the family *Enterobacteriaceae*), *Vibrio*, *Aeromonas*, *Shewanella*, *Alteromonas*, and *Pseudoalteromonas*. A greater degree of dissimilarity is observed between *Tolumonas* and members of the genera *Haemophilus*, *Actinobacillus*, *Pasteurella* (i.e., members of the family *Pasteurellaceae*) *Succinivibrio*, *Succinimonas*, *Anaerobiospirillum*, and *Ruminobacter* (Hippe et al., 1999).

T. auensis produces both ubiquinone 8 and menaquinone 8 (Fischer-Romero et al., 1996). Ubiquinone 8 is found in all members of the genera *Escherichia*, *Serratia*, *Enterobacter* (i.e., members of the family *Enterobacteriaceae*), *Vibrio*, *Aeromonas*, *Shewanella*, *Alteromonas*, and *Pseudoalteromonas* (Wilkinson, 1988; Akagawa-Matsushita et al., 1992a). In some cases, menaquinones with the same isoprenoid chain length are also found, and may comprise both menaquinones and demethyl menaquinones. Members of the genera *Alteromonas* and *Pseudoalteromonas* do not produce menaquinones, while those members of the genus *Shewanella* examined to date produce ubiquinone 7 and 8, but menaquinone 7, and usually, but not always, methylated menaquinone 7 (Akagawa-Matsushita et al., 1992a). Menaquinones are absent from *Ruminobacter amylophilus* (Stackebrandt and Hippe, 1986), while members of the family *Pasteurellaceae* produce either menaquinones and/or demethylnaphthoquinones with seven or eight isoprene units; ubiquinones with the corresponding isoprenoid side chain length may be found, although they apparently are not always present (Kroppenstedt and Mannheim, 1989; Engelhard et al., 1991).

The fatty acid composition of *T. auensis* is dominated by $C_{16:0}$ and $C_{16:1}$ fatty acids (Fischer-Romero et al., 1996), as are the fatty acids of members of the majority of genera listed above. The most notable exceptions are members of the genus *Shewanella* where *iso*- and *anteiso*-branched fatty acids are present in significant quantities. A $C_{14:0\ 3OH}$ fatty acid is also present in *Tolumonas auensis*, approximately half of which appears to be amide linked, probably to the lipopolysaccharide. In members of the family *Enterobacteriaceae* it is well documented that the lipopolysaccharides contain 3-hydroxy fatty acids, in which $C_{14:0\ 3OH}$ predominates, and is present in both an ester- and an amide-linked form (Wilkinson, 1988). It is interesting to note that, in addition to members of the genera *Vibrio*, *Aeromonas*, *Haemophilus*, *Actinobacillus*, *Pasteurella*, *Succinivibrio*, *Succinimonas*, *Anaerobiospirillum*, and *Ruminobacter* also contain a $C_{14:0\ 3OH}$ fatty acid (Miyagawa et al., 1979; Moore et al., 1994a; Urdaci et al., 1990; Engelhard et al., 1991; Huys et al., 1994; Weyant et al., 1996). Depending upon the genus, additional hydroxylated and nonhydroxylated fatty acids may be present, allowing some degree of differentiation between the genera; 2-hydroxy fatty acids are absent in *T. auensis*, and all of the genera listed above.

Phosphatidylethanolamine and phosphatidylglycerol are the major phospholipids present in *T. auensis* (Fischer-Romero et al., 1996). Based on the scant data currently available it appears the same phospholipids predominate in the closest evolutionary relatives, although differentiation between the genera may also be possible based on the presence of additional polar lipids (Wilkinson, 1988; Engelhard et al., 1991).

List of species of the genus Tolumonas

1. **Tolumonas auensis** Fischer-Romero, Tindall and Jüttner 1996, 187[VP]

au.en′ sis. M.L. gen. n. *auensis* of Lake Au, the location of the first isolation of this organism.

The characteristics are those of the genus, as described in the text and in Table BXII.γ.189. The major lipoquinones present are ubiquinone 8 and menaquinone 8 under both oxic and anoxic growth conditions. The fatty acid profile of the cells is: $C_{12:0}$, $C_{14:0}$, $C_{14:0\ 3OH}$, $C_{16:1\ \omega7c}$, $C_{16:0}$, and $C_{18:1\ \omega7c}$. Isolated from the anoxic sediment of a freshwater lake.

The mol% G + C of the DNA is: 49 (T_m).

Type strain: TA 4, DSM 9187.

GenBank accession number (16S rRNA): X92889.

TABLE BXII.γ.189. Biochemical characteristics of the genus *Tolumonas*

Characteristics	Reaction/Result
Gram stain	−
Motility	−
Toluene production	+
Catalase	+
Oxidase	−
D-Glucose acid production	+
Hydrogen sulfide production	−
Indole production	−
Lipase	−

Family *incertae sedis* II. **Succinivibrionaceae** Hippe, Hagelstein, Kramer, Swiderski and Stackebrandt 1999, 782[VP]*

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Suc.ci.ni.vib.ri.o.na' ce.ae M. L. masc. n. *Succinivibrio* type genus of the family; *-aceae* ending to denote a family; M. L. fem. pl. n. *Succinivibrionaceae* the *Succinivibrio* family.

Gram-negative, short to long, **straight, curved, or helical rods.** Nonsporeforming. **Strict anaerobes.** Catalase negative. **Ferment carbohydrates to succinate and acetate.** No production of gas or reduction of nitrate. Take up CO_2.

The mol% G + C of the DNA is: 39–44.

Type genus: **Succinivibrio** Bryant and Small 1956, 22.

**Editorial Note:* This family was proposed based on 16S rDNA sequence analysis (Hippe et al., 1999).

Genus I. **Succinivibrio** *Bryant and Small 1956, 22*[AL]*

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Suc.ci.ni.vib' ri.o. M.L. n. *acidum succinicum* succinic acid; M.L. masc. n. *vibrio* that which vibrates, a generic name; M.L. masc. n. *Succinivibrio* the succinic acid vibrio.

Curved rods, 0.4–0.6 × 1.0–7.0 μm, with pointed ends. The cells are helically twisted with less than one coil to three or more coils per cell. The cells may become straight or only slightly curved after maintenance on artificial media. Gram negative. **Possess a progressive vibrating type of motility by means of a single polar flagellum.** Strictly anaerobic. Chemoorganotrophic, having a fermentative type of metabolism with carbohydrates being the main fermentable substrates. **The major products of glucose fermentation are succinate, acetate, formate, and sometimes lactate.** Butyrate and H_2 are not formed. A large net uptake of CO_2 may occur. Occur in the rumen of cattle and sheep.

The mol% G + C of the DNA is: unknown.

Type species: **Succinivibrio dextrinosolvens** Bryant and Small 1956, 22.

FURTHER DESCRIPTIVE INFORMATION

Cells from young cultures on RGCA slants[1] are short with one or less than one complete coil, but longer cells containing two or three coils are commonly present also. Cells are mainly single but a few short chains may occur. In aging cultures swollen, giant spirillar forms and round bodies may be present.

Surface colonies on RGCA agar in roll tubes are 1–2 mm in diameter, entire, translucent, slightly convex, and light tan in color after 3 d incubation at 37°C. Deep colonies are lenticular. In liquid media with glucose as the energy source growth occurs as a heavy, flocculent sediment that is easily dispersed; light turbidity also occurs.

Good growth of *S. dextrinosolvens* occurs in a chemically defined medium containing glucose, minerals, *p*-aminobenzoic acid, 1,4-naphthoquinone, ammonium ions, cysteine, methionine, leucine, and serine. The use of a CO_2/HCO_3^- buffer system (pH 6.7) is necessary for good growth (Gomez-Alarcon et al., 1982). Growth occurs at 30–39°C but not at 22° or 45°C.

The final pH in lightly buffered glucose medium is 4.8–5.2. *S. dextrinosolvens* requires a fermentable carbohydrate for growth and does not ferment amino acids. Succinic and acetic acids, and often formic and lactic acids, are produced from fermentation of glucose. Gas is not produced. Xylose, galactose, glucose, maltose, and dextrin can serve as fermentable substrates. Arabinose, fructose, cellobiose, sucrose, esculin, salicin, and mannitol are fermented by some strains. No fermentation of lactose, trehalose, cellulose, xylan, inulin, glycerol, or inositol occurs. Starch is partially fermented but not completely hydrolyzed. Nitrate is not reduced. Gelatin is not hydrolyzed. Acetoin, indole, and H_2S are not produced. The catalase test is negative.

Urease is produced by many strains but is strongly repressed in media containing large amounts of ammonia or other utilizable nitrogen sources (Wozny et al., 1977).

S. dextrinosolvens is not pathogenic for animals as far as is known. Isolates corresponding to the description of the organism have been isolated from a few cases of human bacteremia (Southern, 1975; Porschen and Chan, 1977).

S. dextrinosolvens is found in the rumen of cattle and sheep, especially when high-grain diets containing large amounts of starch are fed (Bryant and Small, 1956; Bryant et al., 1961; Wozny et al., 1977). The organism appears to be a major fermenter of dextrins under these conditions.

ENRICHMENT AND ISOLATION PROCEDURES

S. dextrinosolvens is sometimes a predominant organism from the rumen of cattle fed high-grain diets, and can be isolated nonselectively in anaerobic roll tubes of RGCA medium incubated for 3 d or longer at 37°C.

MAINTENANCE PROCEDURES

S. dextrinosolvens can be maintained on stab-inoculated RGCA slants at −70°C for 1 year or longer. The organisms can also be preserved indefinitely by lyophilization.

DIFFERENTIATION OF THE GENUS *SUCCINIVIBRIO* FROM OTHER GENERA

Succinivibrio differs from *Anaerobiospirillum* by having monotrichous rather than lophotrichous flagella. It differs from the genus *Anaerovibrio* by not producing a large amount of propionate, by not fermenting glycerol, and by its ability to ferment dextrin.

**Editorial Note:* The chapter on *Succinivibrio* is reprinted from the 1st edition of *Bergey's Manual of Systematic Bacteriology*.

1. RGCA slants (g/l): K_2HPO_4, 0.23 g; KH_2PO_4, 0.45; $(NH_4)_2SO_4$, 0.45; NaCl, 0.45; $MgSO_4$, 0.023; $CaCl_2$, 0.023; resazurin, 0.001; glucose, 0.5, cellobiose, 0.5; soluble starch, 0.5; rumen fluid (centrifuged), 400; cysteine·HCl, 0.25; $Na_2S{\cdot}9H_2O$, 0.25; Na_2CO_3, 4.0, agar (Difco), 10.0; pH 6.7; gas phase, 100% CO_2. Prepared and used with the Hungate technique (Bryant, 1972).

Further Reading

Bryant, M.P. and N. Small. 1956. Characteristics of two new genera of anaerobic curved rods isolated from the rumen of cattle. J. Bacteriol. *72*: 22–26.

Porschen, R.K. and P. Chan. 1977. Anaerobic vibrio-like organisms cultured from blood: *Desulfovibrio desulfuricans* and *Succinivibrio* species. J. Clin. Microbiol. *5*: 444–447.

List of species of the genus Succinivibrio

1. **Succinivibrio dextrinosolvens** Bryant and Small 1956, 22.[AL] *dex.tri.no.sol' vens.* M.L. n. *dextrinosum* dextrin; L. part. adj. *solvens* dissolving; M.L. part. adj. *dextrinosolvens* dextrin-dissolving.

The characteristics are as described for the genus.

Found in the bovine and ovine rumen.

The mol% G + C of the DNA is: unknown.

Type strain: 554, 24 of Bryant and Small (1956), ATCC 19716, DSM 3072.

GenBank accession number (16S rRNA): Y17600.

Genus II. **Anaerobiospirillum** *Davis, Cleven, Brown and Balish 1976, 503*[AL] *emend. Malnick 1997, 383*

Henry Malnick

An.ae.ro.bi.o.spi.ril' lum. Gr. prefix *an* not; Gr. n. *aer* air; Gr. n. *bios* life; M.L. dim. neut. n. *spirillum* a small spiral; M.L. neut. n. *Anaerobiospirillum* anaerobic small spiral.

Helical rods 0.6–0.8 × 3–15 µm, usually occurring singly. Some cells are up to 32 µm in length. **Motile by bipolar tufts of flagella**. Do not form endospores. Gram negative. **Anaerobic, having a strictly fermentative type of metabolism**. Catalase negative or weakly positive. Oxidase negative. Do not hydrolyze esculin, gelatin, hippurate, or urea. Do not reduce nitrate. Lipase activity does not occur. Indole is not produced and meat is not digested. **Ferment carbohydrates; produce succinic and acetic acids from glucose**. May also produce traces of lactic and formic acids. Optimal temperature 37–44°C. **Isolated from feces of dogs and cats. Pathogenic for humans, causing septicemia and/or diarrhea**. Belongs to the class *Gammaproteobacteria*, order *Aeromonadales*, and family *Succinivibrionaceae*.

The mol% G + C of the DNA is: 39–44.

Type species: **Anaerobiospirillum succiniciproducens** Davis, Cleven, Brown and Balish 1976, 503.

Further descriptive information

The diameter of the cell helix is 0.9–1.1 µm. Straight rods and spherical forms may occur, especially in liquid cultures. Some strains of *Anaerobiospirillum succiniciproducens*, including the type strain, can be pleomorphic, often producing bizarre forms. This feature seems to be rare in *A. thomasii*. The cells have a corkscrew-like motility. The flagella are 14 nm in diameter and are arranged as bipolar tufts of ~16 flagella.

Ultrastructural examination of *A. succiniciproducens* has shown that the cells possess fibrils arranged along the longitudinal axis within the cell. So far, this has not been described in any other organism. The flagellar base within the cell is disc shaped (Wecke and Horbach, 1999).

Surface colonies on most types of blood agar are 0.5–1.0 mm in diameter, circular, convex, and translucent after 2–3 days anaerobic incubation at 37°C. Colonies may have raised centers. Spreading or feathery edges from heavily inoculated areas is not uncommon on some media. Growth is most rapid at 37–40°C and does not occur below 33°C or above 44°C. Cells do not survive 80°C for 10 min.

Although requiring an anaerobic atmosphere for growth, *Anaerobiospirillum* spp. will survive in an atmosphere of reduced oxygen within a jar until other bacteria present have reduced the atmosphere. It is in this way that fecal isolates of *A. thomasii* were isolated on Skirrow's *Campylobacter* isolation medium.

At usual therapeutic levels, isolates of *Anaerobiospirillum* spp. are susceptible to amoxicillin-clavulanate, azithromycin, cefoxitin, ciprofloxacin, chloramphenicol, and trovafloxacin, and are resistant to cephalexin, clarithromycin, clindamycin, lincomycin, nalidixic acid, polymyxin B, sulfamethoxazole, and vancomycin. Susceptibility to ampicillin, erythromycin, penicillin G, and metronidazole is variable. *A. succiniciproducens* is mezlocillin resistant and gemifloxacin variable, and has an MIC for trimethoprim of 4 µg/ml, whereas *A. thomasii* is mezlocillin and gemifloxacin sensitive and has an MIC for trimethoprim of >256 µg/ml (McNeil et al., 1987b; Goldstein et al., 1999, Malnick, unpublished results).

Under favorable conditions of pH 6.2 and high CO_2 concentrations, *A. succiniciproducens* can produce industrially significant levels of succinate (up to 35 g/l) (Datta, 1989). The phosphoenolpyruvate-carboxykinase-encoding gene (*pckA*) involved has been sequenced, and the amino acid sequence—deduced from the data—encodes a 532-residue polypeptide. Phosphoenolpyruvate carboxykinase is a CO_2-fixing enzyme (Laivenieks et al., 1997).

Anaerobiospirillum species have been reported as causing septicemia and diarrhea in man. There appears to be a difference in pathogenicity between the two species. *A. succiniciproducens* has been isolated from blood cultures of patients suffering from septicemia, and in a number of cases it was reported either as the cause of death or as a contributory factor. Most of the patients involved had underlying disorders. So far, the isolation of *A. thomasii* has only been reported from cases of diarrhea (McNeil et al., 1987b; Malnick et al., 1990).

Of three isolates of *A. succiniciproducens* described by Davis et al. (1976), one came from the throat and two from cecal homogenate of dogs. Further studies have shown both species are found as part of the fecal flora in cats and dogs. Although the organisms seem to be part of the normal intestinal flora of these animals, no definite ecological function is known (Malnick et al., 1990).

Enrichment and Isolation Procedures

An enrichment and serial dilution method for obtaining isolates from throat and cecal contents of dogs was used without the aid of a selective medium by Davis et al. (1976). A selective medium for the isolation of *Anaerobiospirillum* spp. has been described (Malnick et al., 1990). It consists of a suitable anaerobic agar medium supplemented with (per liter): polymyxin, 2500 IU; vancomycin, 20 mg; sulfamethoxazole, 100 mg; Victoria blue B (Cl 44045 Sigma), 250 mg; and horse blood lysed with saponin, 50 ml. The Victoria blue was initially dissolved in 4 ml of ethanol. After 48–72 h of anaerobic incubation, *Anaerobiospirillum* species produce dark blue colonies, depending on the basal medium. Other organisms produce white or pale blue colonies.

Maintenance Procedures

Anaerobiospirillum spp. can be maintained at −70°C or in liquid nitrogen on beads, with any of a variety of cryoprotectants. The organisms can also be preserved by lyophilization.

Differentiation of the genus *Anaerobiospirillum* from other genera

The genus is distinguished from *Succinivibrio* and *Campylobacter* by its lophotrichous flagellar arrangement. In clinical situations, *Anaerobiospirillum* species have most often been confused with *Campylobacter*; however, *Campylobacter* species are oxidase positive and do not ferment carbohydrates. Production of succinic and acetic acids from glucose may help differentiate *Anaerobiospirillum* from other curved and spiral anaerobic bacteria.

Taxonomic Comments

The phylogenetic relationship between *Anaerobiospirillum* and other Gram-negative, strictly anaerobic bacteria has been determined by 16S rDNA sequence analysis, placing these organisms in the family *Succinivibrionaceae* (Hippe et al., 1999).

The DNA–DNA hybridization values between the type strain of *A. succiniciproducens* and the type strain of *A. thomasii* are less than 6% (Malnick, 1997).

Differentiation of the species of the genus *Anaerobiospirillum*

Characteristics useful in differentiating the two species of the genus are listed in Table BXII.γ.190.

List of species of the genus Anaerobiospirillum

1. **Anaerobiospirillum succiniciproducens** Davis, Cleven, Brown and Balish 1976, 503[AL]
suc.ci.ni.ci.pro.du′cens. M.L. n. *acidum succinicum* succinic acid; L. pres. part. *producens* producing; M.L. part. adj. *succiniciproducens* producing succinic acid.

The characteristics are as described for the genus and in Table BXII.γ.190, with the following additional information. Helical rods 0.6–0.8 × 3–15 μm. Produces weak β-hemolysis. Fixes CO_2.
The mol% G + C of the DNA is: 42–44 (T_m).
Type strain: S411, ATCC 29305, NCTC 11536.
GenBank accession number (16S rRNA): U96412.

2. **Anaerobiospirillum thomasii** Malnick 1997, 383[VP]
thom′as.i.i. N.L. gen. n. *thomasii* of Thomas, named after M.E.M. Thomas (Public Health Laboratory Service, 1947–1984), whose interest in and enthusiasm for uncommon medical bacterial infections led to the initiation of this study.

The characteristics are as described for the genus and in Table BXII.γ.190, with the following additional information. Helical rods, 0.5–0.7× 5–32 μm. Optimum temperature 37°C; range, 33–43°C. Colonies are flat with even, spreading edges, 1–2 mm in diameter after 24–48 h on horse blood agar. Hemolysis does not occur. Isolated from cat and dog feces and as a pathogen in human diarrheal feces.
The mol% G + C of the DNA is: 39–41 (T_m).
Type strain: NCTC 12467.
GenBank accession number (16S rRNA): AJ420985.

TABLE BXII.γ.190. Differential characteristics of *A. succiniciproducens* and *A. thomasii*[a,b]

Characteristics	1. *A. succiniciproducens*	2. *A. thomasii*
Acid production from:		
Adonitol	−	+
Fructose	+	−
Inulin	+	−
Lactose	+	−
Raffinose	+	−
Sucrose	+	−
Enzyme production:[c]		
β-D-Galactosidase	+	−
α-Glucosidase	+	−
α-Maltosidase	d	−
L-Lysine arylamidase	−	+
Glycine arylamidase	d	+
Methionine arylamidase	−	+

[a]Symbols: see standard definitions.
[b]Data from Davis et al. (1976), Malnick (1997), and Malnick (unpublished results).
[c]API-ZYM enzyme test kits (API Biomerieux, Basingstoke, Hants, UK).

Genus III. **Ruminobacter** *Stackebrandt and Hippe 1987, 179*[VP] *(Effective publication: Stackebrandt and Hippe 1986, 205)*

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Ru.mi.no.bac' ter. L. adj. *ruminalis* of the rumen; M.L. masc. n. *bacter* equivalent of bacterium, a small rod; M.L. masc. n. *Ruminobacter* small rod of the rumen.

Oval to long rods. Gram negative. Nonsporeforming. Nonmotile. Chemoorganotroph. **Metabolize carbohydrates; fermentation products include succinate, acetate, and formate; trace amounts of lactate and ethanol may be formed. No rumen fluid requirement for growth. Obligately anaerobic.** CO_2 required, which is incorporated into succinic acid. NH_3 is essential as a nitrogen source. No cytochromes. Peptidoglycan contains *meso*-diaminopimelic acid. Sphingophospholipids absent. Straight chain saturated $C_{16:0}$ fatty acid predominates, while $C_{14:0}$ acid and the monounsaturated $C_{16:1}$ and $C_{18:1}$ acids occur in smaller amount. Low levels of iso- and anteiso-methyl branched fatty acids. Analysis of 16S rDNA places the genus into the family *Succinivibrionaceae* of the *Gammaproteobacteria*.

The mol% G + C of the DNA is: 40–42.

Type species: **Ruminobacter amylophilus** (Hamlin and Hungate 1956) Stackebrandt and Hippe 1987, 179 (Effective publication: Stackebrandt and Hippe 1986, 205) (*Bacteroides amylophilus* Hamlin and Hungate 1956, 552.)

FURTHER DESCRIPTIVE INFORMATION

As the genus currently contains a single species, the description of the genus is that of the species. The presence of an A–U base pair at positions 19 and 916 indicates that *R. amylophilus* belongs to a broad phylogenetic cluster of organisms within the *Gammaproteobacteria*, all of which are defined by the presence of this signature, such as members of the *Succinivibrionaceae*, *Aeromonadaceae*, *Enterobacteriaceae*, *Vibrionaceae*, *Pasteurellaceae*, *Methylococcaceae*, *Halomonadaceae*, and *Oceanospirillaceae*.

The colonial and cultural properties are based on the descriptions of Hamlin and Hungate (1956), Holdeman and Moore (1974), Cato et al. (1978), and Holdeman et al. (1984b). Cells of the type strain of *R. amylophilus* are pleomorphic after incubation for 2 d in peptone–yeast extract-maltose broth. Gram-negative, oval to long rods with tapered or round ends, and include some swollen forms and irregularly curved cells, 0.9–1.2 × 1.1–8.0 μm (Cato et al., 1978). Surface colonies on rumen fluid-glucose-cellobiose agar roll tubes are 1 mm in diameter, circular, entire, slightly convex, translucent, smooth, glistening, white to tan. Colonies on blood roll streak tubes are pinpoint to 2 mm in diameter, circular, entire to slightly erose, convex to slightly erose, convex to umbonate, translucent, colorless, shiny, and smooth after incubation for 4 d. Growth is sparse on freshly prepared blood agar plates incubated anaerobically. Surface colonies on rumen fluid–glucose–cellobiose agar roll tubes are 1 mm in diameter, circular, entire, slightly convex, translucent, smooth, glistening, white to tan. Colonies in deep agar are 0.8–1 mm in diameter, lenticular, entire or irregular, white, and soft butyrous. Growth is not stimulated by amino acids, volatile fatty acids (Miura et al., 1980), vitamins, or hemin (Macy and Probst, 1979). No colonies appear on egg yolk agar plates and on the surface of plates incubated in an aerobic jar.

The organism is strictly anaerobic. Only bicarbonate and/or CO_2, a fermentable carbohydrate, and minerals are required for growth. Rumen fluid is not required (Bryant and Robinson, 1962). Ammonia is essential as a nitrogen source. CO_2 is fixed and ammonia is assimilated. In the presence of CO_2 maltose is fermented to succinate: 2 maltose + $2CO_2 \rightarrow$ 2 succinate + 2 acetate + 2 formate (Kühn, 1979). Production of succinic acid has been patented (U.S. Pat. 5,143,833). The organism is able to grow well in basal medium supplemented with starch. Starch broth cultures are turbid and have a final pH of 5.3–5.5. Amylase production is stimulated by Tween 80 (McWethy and Hartman, 1977). Autolysis occurs after growth ceases and the degradation of bacterial protein is accompanied by the production of branched-chain amino acids (Miura et al., 1980). The organism is strongly proteolytic (Abou-Akkada and Blackburn, 1963), and protease is produced in the exponential growth phase (Blackburn and Hallah, 1974).

Optimum temperature for growth is near 37°C. Hydrogen is not produced; no growth in 20% bile; esculin not digested; milk and meat reaction negative. Cellulose is not digested. Casein is not utilized but is digested when cells are grown in ammonia-containing media (Caldwell et al., 1973). Protease is produced in the exponential growth phase (Blackburn, 1968; Blackburn and Hallah, 1974). Acid is produced from dextrin, glycogen, maltose, and starch. No acid is produced from amygdalin, arabinose, cellobiose, esculin, fructose, D-galactose, D-glucose, glycerol, inositol, inulin, lactose, D-mannitol, raffinose, L-rhamnose, ribose, salicin, sucrose, trehalose, and D-xylose. Strains are very sensitive to inhibition by heavy metals and trace elements (Forsberg, 1978).

An almost complete *rrn* operon, consisting of the 5′ upstream promoter region, the 16S rDNA gene of 1539 nucleotides (accession number Y15992), the intergenic spacer, including a tRNA (*gln*) gene (Martens et al., 1987), and the 23S rDNA (Spiegl et al., 1988) (accession number X06765) has been sequenced from a BgII-Kpn1 fragment of strain DSM 1361[T] (plasmid number DSM 4825). A partial 16S rDNA sequence, consisting of 1429 nucleotides, of a *R. amylophilus* strain with no assigned strain number has recently been released under the accession number AB004908. The similarity of the common stretch of the two 16S rDNA sequences is 99.7%.

Cytochromes (Reddy and Bryant, 1977) and sphingolipids have not been detected (Kunsman and Caldwell, 1974; Miyagawa et al., 1978). Menaquinones (R. Kroppenstedt, personal communication) are absent. Peptidoglycan contains *meso*-diaminopimelic acid (Miyagawa et al., 1981).

Ruminobacter amylophilus is one of the predominant anaerobic bacteria obtained in pure culture from ruminal contents of a Holstein cow. The bacterium thrives on basal medium supplemented with maltose and ammonia. A casein hydrolysate plus volatile fatty acids is only slightly stimulatory and glucose and cellobiose cannot substitute for maltose (Bryant and Robinson, 1962). *R. amylophilus* occurs sporadically in the rumen contents of cattle but, when present, may be the predominant starch digester and may constitute as much as 10% of the bacterial population of the rumen. It also occurs also in the ovine rumen (Blackburn and Hobson, 1962; Bryant and Robinson, 1962).

Enrichment and Isolation Procedures

None of the media described in the literature on the isolation of *Ruminobacter (Bacteroides) amylophilus* was designed to specifically enrich this organism. The media used are those to survey proteolytic bacteria present in the rumen of sheep (Blackburn and Hobson, 1962) and cows (Hamlin and Hungate, 1956; Bryant and Robinson, 1962). In some of these studies, the animals were fed different diets. As *R. amylophilus* does not require rumen fluid, a nonrumen fluid medium is described that has been used to isolate *R. amylophilus* (Bryant and Robinson, 1962). The basal medium contains 0.3% maltose, 0.0001% resazurin, 0.4% Na_2CO_3, 0.025% (each) cysteine-HCl·H_2O and Na_2S·$9H_2O$, 0.09% (each) KH_2PO_4 and NaCl, 0.002% (each) $CaCl_2$ and $MgCl_2$·$6H_2O$, 0.001% $MnCl_2$·H_2O, 0.001% $CoCl_2$·$6H_2O$, 0.2 mg per 100 ml (each) of thiamine-HCl, Ca-D-pantothenate, nicotinamide, riboflavine, and pyridoxal, 0.01 mg per 100 ml of *p*-aminobenzoic acid, 0.05 mg per 100 ml (each) of biotin, folic acid, and DL-thioctic acid, 0.02 mg per 100 ml of cobalamin, and is equilibrated with oxygen-free CO_2. All ingredients except cysteine, Na_2S, and Na_2CO_3 were mixed, adjusted to pH 6.5 with H_2SO_4, and autoclaved at 15 psi for 15 min. Sterile, CO_2-equilibrated Na_2CO_3 solution is added and the medium is added to tubes (13 × 100 mm) in 2.8 ml amounts, using anaerobic methods and CO_2 (Bryant and Robinson, 1961). Two milliliters each of 0.09% $(NH_4)_2SO_4$, and 2 mg/100 ml hemin plus 0.2 ml of a solution of cysteine, and Na_2S brings the medium constituents to final concentration.

According to Blackburn and Hobson (1962), samples are taken via a rumen cannula and rapidly strained to remove coarse debris. Tenfold dilutions of rumen fluid are made under CO_2 in a solution prepared exactly like the medium. Bacteria are cultured in 50-ml flasks each containing 25 ml of medium. For dilutions, the role tube method can be used as described by Bryant and Burkey (1953).

Maintenance Procedures

R. amylophilus is routinely maintained in the DSM-German Collection of Microorganisms and Cell Cultures in medium DSM 147 (DSM catalog of strains, 2001), containing (per 1000 ml distilled water): KH_2PO_4, 0.45 g; K_2HPO_4, 0.45 g; $(NH_4)_2SO_4$, 0.9g; NaCl, 0.9g; $MgSO_4$·$7H_2O$, 0.18g; soluble starch, 5.0 g; casitone (Difco), 10.0 g; resazurin, 1.0 mg; cysteine hydrochloride, 0.5 g; $NaHCO_3$, 6.0 g; pH 7.0, at a gas atmosphere of 100 CO_2. For short-term storage, stab cultures in a modified medium DSM 147 is recommended, in which the starch content is reduced to 0.1%. For long-term conservation, freezing in liquid nitrogen is recommended.

Differentiation of the genus *Ruminobacter* from other genera

Recent phylogenetic analyses on some Gram-negative strictly anaerobic, nonsporeforming bacteria indicated *Ruminobacter amylophilus* to be a member of a separate line of descent that includes *Succinimonas amylolytica*, *Succinivibrio dextrinosolvens*, and members of the genus *Anaerobiospirillum*. This phylogenetic group has been described as *Succinivibrionaceae* (Hippe et al., 1999) (Fig. BXII.γ.188).

Taxonomic Comments

The first culture of *Bacteroides amylophilus*, isolated and described by Hamlin and Hungate (1956), was lost. Since then, organisms conforming to the original description of *B. amylophilus* have been isolated frequently from the rumen (Blackburn and Hobson, 1962; Bryant and Robinson, 1962; Caldwell et al., 1969; Holdeman et al., 1977). Cato et al. (1978) designated strain H 18 isolated by Blackburn and Hobson (1962), as the neotype strain of *B. amylophilus*. This species differs from the majority of *Bacteroides* species in a number of chemotaxonomic properties, e.g., fatty acid composition (Miyagawa et al., 1979) and the lack of sphingophospholipids (Kunsman and Caldwell, 1974; Miyagawa et al., 1978). These differences led Miyagawa et al. (1979) and Shah and Collins (1983) to the conclusion that *B. amylophilus* should be removed from *Bacteroides sensu stricto*. These conclusions were supported by the finding that *B. amylophilus* and authentic members of *Bacteroides* belong to different main phylogenetic lines of descent. The results of 16S ribosomal RNA cataloging (Woese et al., 1985) and later by 16S rDNA analysis (Martens et al., 1987) revealed that *B. amylophilus* groups loosely with the "core" organisms (e.g., enterobacteria, vibrios, oceanospirilla, alteromonads) of the *Gammaproteobacteria*, while authentic *Bacteroides* species (e.g., *B. fragilis*, *B. asaccharolyticus*, *B. distasonis*, *B. melaninogenicus*, *B. ovatus*, *B. ruminicola*, *B. thetaiotaomicron*, *B. uniformis*, and *B. vulgatus*) form a coherent cluster within the *Bacteroides-Cytophaga-Flavobacterium* line of descent (Paster et al., 1985). Consequently, *Bacteroides amylophilus* was reclassified as *Ruminobacter amylophilus* (Stackebrandt and Hippe, 1986). The generic name "*Ruminobacter*" was originally used to describe Gram-negative, nonmotile, anaerobic chemoheterotrophic cellulose fermenting bacteria (Kaars Sijpesteijn, 1949). Prévot (1966) considered this genus as *incertae sedis*, but at the same time transferred the *Bacteroides* species *"Bacteroides amylogenes"*, *B. ruminicola*, *B. succinogenes*, and the noncellulolytic species *B. amylophilus* into this genus. The taxon *Ruminobacter* was not included in the Approved Lists of Bacterial Names (Skerman et al., 1980); hence, it had no standing in nomenclature and could be proposed to accommodate the former *B. amylophilus*.

Analysis of 16S rDNA sequence is to date the most reliable method by which isolates can be affiliated to *Ruminobacter amylophilus*. Without sequence information and placement, this species can be misclassified as *Prevotella ruminicola* (Shah and Collins, 1990) or *Fibrobacter succinogenes* (Montgomery et al., 1988), organisms that were previously classified as nonmotile members of *Bacteroides*. *Ruminobacter amylophilus* differs considerably from these organisms in a combination of properties, e.g., the pattern of carbohydrates utilized, fermentation products, mol% G + C of their DNA, and fatty acid composition. *Ruminobacter* differs phenotypically from other genera previously grouped in the family *Bacteroidaceae* as defined by Holdeman et al. (1984a). *Ruminobacter* can be distinguished from *Fusobacterium* and *Leptotrichia* by the end-products of carbohydrate fermentation (Holdeman et al., 1984a). On the basis of metabolic properties *Ruminobacter* can also be distinguished unambiguously from several other genera containing Gram-negative obligate-anaerobes that have been described recently, viz. *Acidaminobacter* (Stams and Hansen, 1984), *Ilyobacter* (Stieb and Schink, 1985), *Pelobacter* (Schink and Pfennig, 1982a), *Propionigenium* (Schink and Pfennig, 1982b), and *Propionispira* (Schink et al., 1982), all of which are phylogenetically related to members of the *Clostridium-Bacillus* line of descent.

Further Reading

Blackburn, T.H. and P.N. Hobson. 1962. Further studies on the isolation of proteolytic bacteria from sheep rumen. J. Gen. Microbiol. *29*: 69–81.

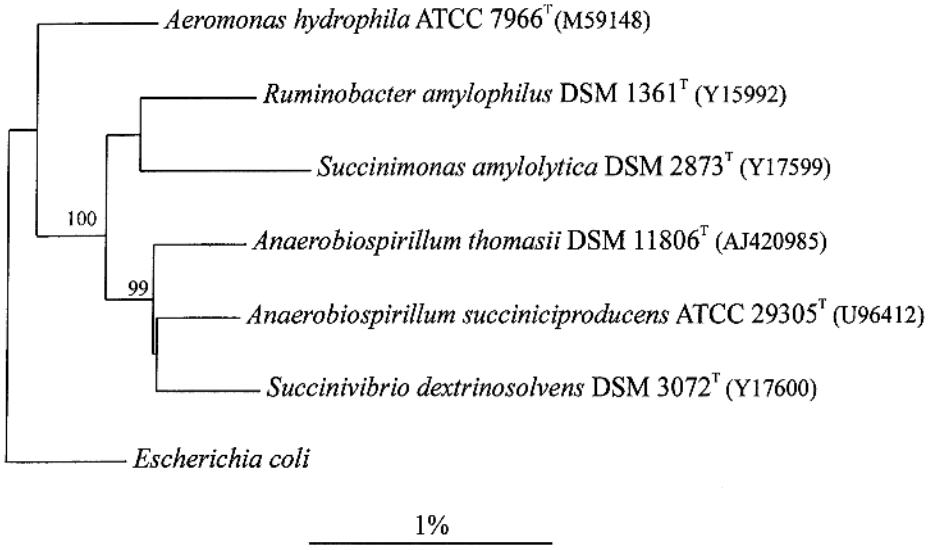

FIGURE BXII.γ.188. Dendrogram showing the phylogenetic position of *Ruminobacter amylophilus* next to its phylogenetic neighbors within the *Gammaproteobacteria. R. amylophilus* represents the family *Succinivibrionaceae,* which includes the genera *Ruminococcus, Anaerobiospirillum, Succinivibrio,* and *Succinimonas* (Hippe et al., 1999). The tree was constructed by the neighbor-joining method (Saitou and Nei, 1987), using corrected distance values (Jukes and Cantor, 1969). Bootstrap values (expressed as percentages of 500 replications) of 70% or more are indicated at the branch points. Bar = 1% sequence divergence.

Cato, E.P., W.E.C. Moore and M.P. Bryant. 1978. Designation of neotype strains for *Bacteroides amylophilus* Hamlina and Hungate 1956 and *Bacteroides succinogenes* Hungate 1950. Int. J.Syst. Bacteriol. *28*: 491–495.

Miura, H., M. Horiguchi and T. Matsumoto. 1980. Nutritional interdependence among rumen bacteria, *Bacteroides amylophilus, Megasphaera elsdenii* and *Ruminococcus albus.* Appl. Environ. Microbiol. *40*: 294–300.

Stackebrandt, E. and H. Hippe. 1986. Transfer of *Bacteroides amylophilus* to a new genus *Ruminobacter* gen. nov., nom. rev. as *Ruminobacter amylophilus* comb. nov. Syst. Appl. Microbiol. *8*: 204–207.

List of species of the genus Ruminobacter

1. **Ruminobacter amylophilus** (Hamlin and Hungate 1956) Stackebrandt and Hippe 1987, 179[VP] (Effective publication: Stackebrandt and Hippe 1986, 205) (*Bacteroides amylophilus* Hamlin and Hungate 1956, 552.)
am.y.lo'phi.lus. Gr. n. *amylo* starch; Gr. part. *philo* loving; M.L. adj. *amylophilus* starch loving.

The characteristics are described for the genus.

Occurs sporadically in the rumen of the bovine and sheep.

The mol% G + C of the DNA is: 40-42 (Bd).

Type strain: H 18, ATCC 29744, DSM 1361, VIP 2502B.

GenBank accession number (16S rRNA): Y15992.

Additional Remarks: GenBank accession number for 23S rRNA gene is X06765.

Genus IV. **Succinimonas** *Bryant, Small, Bouma and Chu 1958, 21*[AL]*

MARVIN P. BRYANT

Suc.ci.ni.mo'nas. M.L. n. *acidum succinicum* succinic acid; Gr. n. *monas* a unit, monad; M.L. fem. n. *Succinimonas* succinic acid monad.

Short, straight rods to coccobacilli, 1.0–1.5 µm × 1.0–3.0 µm, with rounded ends. No spores or resting stages are produced. Gram negative. **Motile by a single polar flagellum.**

Strictly anaerobic. Chemoorganotrophic, having a fermentative type of metabolism. **Glucose, maltose, dextrin, or starch can serve as the energy source. Succinate and acetate are the main products of glucose fermentation.** No butyrate or gas is formed. Catalase negative. Occur in the bovine rumen.

The mol% G + C of the DNA is: unknown.

Type species: **Succinimonas amylolytica** Bryant, Small, Bouma and Chu 1958, 21.

Editorial Note: The chapter on *Succinimonas* is reprinted from the 1st edition of *Bergey's Manual of Systematic Bacteriology.*

FURTHER DESCRIPTIVE INFORMATION

In the water of syneresis of RGCA slants[1] incubated overnight at 37°C, the cells are arranged as singles, pairs, and clumps. No capsules are evident. Motility is relatively slow and progressive and is rapidly lost upon exposure to air.

Surface colonies on RGCA agar in roll tubes are smooth, convex, translucent, light tan, and 0.7–1.5 mm in diameter after 3 d incubation at 37°C. Deep colonies are lenticular and 0.7–1.0

1. RGCA slants (g/l): K_2HPO_4, 0.23 g; KH_2PO_4, 0.45; $(NH_4)_2SO_4$, 0.45; NaCl, 0.45; $MgSO_4$, 0.023; $CaCl_2$, 0.023; resazurin, 0.001; glucose, 0.5; cellobiose, 0.5; soluble starch, 0.5; rumen fluid (centrifuged), 400; cysteine·HCl, 0.25; $Na_2S{\cdot}9H_2O$, 0.25; Na_2CO_3, 4.0; agar (Difco), 10.0; pH 6.7; gas phase, 100% CO_2. Prepared and used with the Hungate technique (Bryant, 1972).

mm in diameter. In liquid media with glucose as the energy source growth occurs as light, uniform turbidity.

The type strain of *S. amylolytica* grows well in a glucose medium with rumen fluid replaced by trypticase and yeast extract. It also grows in a chemically defined medium containing glucose, CO_2/HCO_3 buffer, minerals, B vitamins, and acetate. Ammonium ions serve as the nitrogen source and cannot be replaced by amino acids or peptides. Sulfide serves as both the reducing agent and the sulfur source. Acetate (30 mM) is a highly stimulatory supplement even though the organisms produce acetate from glucose (Bryant and Robinson, 1962; Roberton and Bryant, unpublished data). During fermentation of glucose to succinate and acetate, a net uptake of CO_2 occurs. Of the substrates tested, only glucose, maltose, dextrin, and starch are fermented. Arabinose, xylose, fructose, cellobiose, lactose, sucrose, cellulose, inulin, xylan, glycerol, esculin, mannitol, lactate, amino acids, and peptides are not fermented. H_2S and indole are not produced. Gelatin is not liquefied. The Voges–Proskauer test is variable. Nitrate is not reduced.

Growth occurs at 30° and 37°C but not at 22° or 45°C. The final pH in poorly buffered, liquid glucose medium is 5.2–5.8. Good growth occurs at pH 6.5–7.0. The upper pH limit has not been determined.

S. amylolytica appears to be nonpathogenic for humans or animals. It occurs in the rumen of cattle fed diets containing roughage and some grain, where it is involved in fermentation of starch and its hydrolytic products. It is usually present as only a small proportion of the total viable bacteria in the bovine rumen (less than 6% of total). Whether *S. amylolytica* occurs in the rumen of ruminants other than cattle or in nonruminal ecosystems is not known.

Enrichment and Isolation Procedures

Succinimonas is isolated nonselectively in anaerobic roll tubes of RGCA medium from the rumen of cattle fed hay-grain diets. It constitutes only a small proportion of the colonies that develop after 3 d or longer of incubation at 37°C.

Maintenance Procedures

S. amylolytica strains can be maintained on stab-inoculated RGCA slants at −70°C for a year or more. They can also be preserved indefinitely by lyophilization.

Further Reading

Bryant, M.P., N. Small, C. Bouma and H. Chu. 1958. *Bacteroides ruminicola*, sp. nov. and *Succinimonas amylolytica*, gen. nov., species of succinic acid-producing anaerobic bacteria of the bovine rume. J. Bacteriol. *76*: 15–23.

Bryant, M.P. and I.M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. *84*: 605–614.

List of species of the genus Succinimonas

1. **Succinimonas amylolytica** Bryant, Small, Bouma and Chu 1958, 21.[AL]
   am.y.lo.1y′ti.ca. Gr. n. *amylum* fine meal, starch; Gr. adj. *lyticus* loosening, dissolving; M.L. fem. adj. *amylolytica* starch dissolving.
   The characteristics are as described for the genus.
   Occur in the bovine rumen.
   The mol% G + C of the DNA is: unknown.
   Type strain: ATCC 19206, DSM 2873, VPI 13846.
   GenBank accession number (16S rRNA): Y17599.

Order XIII. **"Enterobacteriales"**

En.te.ro.bac.te.ri.a′les. M.L. n. *enterobacterium* an intestinal bacterium; *-ales* ending to denote order; M.L. fem. n. *Enterobacteriales* the *enterobacterium* order.

Description is the same as for the family *Enterobacteriaceae*.

Type genus: **Escherichia** Castellani and Chalmers 1919, 941.

Family I. **Enterobacteriaceae** Rahn 1937, Nom. Fam. Cons. Opin. 15, Jud. Comm. 1958a, 73; Ewing, Farmer, and Brenner 1980, 674; Judicial Commission 1981, 104

Don J. Brenner and J.J. Farmer III

En.te.ro.bac.te.ri.a′ce.ae. M.L. n. *enterobacterium* an intestinal bacterium; *-aceae* ending to denote a family; M.L. fem. pl. n. *Enterobacteriaceae* the family of the enterobacteria. Rahn's original derivation of the name *Enterobacteriaceae* is not certain. It may have come from his genus *Enterobacter*, or may have come from the root enterobacterium.

Gram-negative straight rods, 0.3–1.0 × 1.0–6.0 μm, except for *Arsenophonus*, which is 7–10 μm in length. Motile by peritrichous flagella, except for *Tatumella*, or nonmotile. Do not form endospores or microcysts; not acid-fast. Grow in the presence and absence of oxygen. Grow well on peptone, meat extract, and usually MacConkey's medium, except for *Calymmatobacterium* and insect symbionts, which have not been cultivated. Most grow well at 22–35°C; optimal growth and maximal biochemical capacity of a number of genera (*Yersinia*, *Hafnia*, *Xenorhabdus*, *Photorhabdus*, and many erwiniae) occurs at 25–28°C. Some grow on D-glucose as the sole source of carbon; some require vitamins and/or amino acids. **Chemoorganotrophic; having both a respira-**

mm in diameter. In liquid media with glucose as the energy source growth occurs as light, uniform turbidity.

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S. amylolytica appears to be nonpathogenic for humans or animals. It occurs in the rumen of cattle fed diets containing roughage and some grain, where it is involved in fermentation of starch and its hydrolytic products. It is usually present as only a small proportion of the total viable bacteria in the bovine rumen (less than 6% of total). Whether *S. amylolytica* occurs in the rumen of ruminants other than cattle or in nonruminal ecosystems is not known.

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Maintenance Procedures

S. amylolytica strains can be maintained on stab-inoculated RGCA slants at −70°C for a year or more. They can also be preserved indefinitely by lyophilization.

Further Reading

Bryant, M.P., N. Small, C. Bouma and H. Chu. 1958. *Bacteroides ruminicola*, sp. nov. and *Succinimonas amylolytica*, gen. nov., species of succinic acid-producing anaerobic bacteria of the bovine rume. J. Bacteriol. *76*: 15–23.

Bryant, M.P. and I.M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. *84*: 605–614.

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1. **Succinimonas amylolytica** Bryant, Small, Bouma and Chu 1958, 21.[AL]
   am.y.lo.1y′ti.ca. Gr. n. *amylum* fine meal, starch; Gr. adj. *lyticus* loosening, dissolving; M.L. fem. adj. *amylolytica* starch dissolving.
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Order XIII. **"Enterobacteriales"**

En.te.ro.bac.te.ri.a′les. M.L. n. *enterobacterium* an intestinal bacterium; *-ales* ending to denote order; M.L. fem. n. *Enterobacteriales* the *enterobacterium* order.

Description is the same as for the family *Enterobacteriaceae*.

Type genus: **Escherichia** Castellani and Chalmers 1919, 941.

Family I. **Enterobacteriaceae** Rahn 1937, Nom. Fam. Cons. Opin. 15, Jud. Comm. 1958a, 73; Ewing, Farmer, and Brenner 1980, 674; Judicial Commission 1981, 104

Don J. Brenner and J.J. Farmer III

En.te.ro.bac.te.ri.a′ce.ae. M.L. n. *enterobacterium* an intestinal bacterium; *-aceae* ending to denote a family; M.L. fem. pl. n. *Enterobacteriaceae* the family of the enterobacteria. Rahn's original derivation of the name *Enterobacteriaceae* is not certain. It may have come from his genus *Enterobacter*, or may have come from the root enterobacterium.

Gram-negative straight rods, 0.3–1.0 × 1.0–6.0 μm, except for *Arsenophonus*, which is 7–10 μm in length. Motile by peritrichous flagella, except for *Tatumella*, or nonmotile. Do not form endospores or microcysts; not acid-fast. Grow in the presence and absence of oxygen. Grow well on peptone, meat extract, and usually MacConkey's medium, except for *Calymmatobacterium* and insect symbionts, which have not been cultivated. Most grow well at 22–35°C; optimal growth and maximal biochemical capacity of a number of genera (*Yersinia*, *Hafnia*, *Xenorhabdus*, *Photorhabdus*, and many erwiniae) occurs at 25–28°C. Some grow on D-glucose as the sole source of carbon; some require vitamins and/or amino acids. **Chemoorganotrophic; having both a respira-**

tory and a fermentative metabolism. Acid and visible gas are often produced during fermentation of D-glucose, other carbohydrates and polyhydroxyl alcohols. Not halophilic. **Most are catalase-positive**, except for *Shigella dysenteriae* O group 1 and *Xenorhabdus*. **Most are oxidase negative**, except for *Plesiomonas*. **Most reduce nitrate to nitrite**, except *Saccharobacter fermentatus* (Yaping et al., 1990) and some strains of *Erwinia* and *Yersinia*.

The mol% G + C of the DNA is: 38–60 (63.5 for *S. fermentatus* (Yaping et al., 1990)).

Type genus: **Escherichia** Castellani and Chalmers 1919, 941. Designated type genus Nom. Fam. Cons. Opin. 15, Jud. Comm. 1958a, 73.

Further descriptive information

The definition of the family circumscribes a large biochemically and genetically related group that shows substantial heterogeneity in its ecology, host range, and pathogenic potential for man, animals, insects and plants. The phylogenetic position of the family *Enterobacteriaceae* lies within the *Gammaproteobacteria*. Its nearest neighbors are the families *Alteromonadaceae*, *Vibrionaceae*, *Aeromonadaceae*, and *Pasteurellaceae*. Phylogenetic relationships of genera within the *Enterobacteriaceae* based on analysis of the 16S rRNA sequences of the type strains of the type species are shown in Fig. BXII.γ.189. Phenotypic characteristics useful in differentiating *Enterobacteriaceae* from its nearest phenotypic neighbors are shown in Table BXII.γ.191. The genera *Vibrio*, *Photobacterium*, and *Aeromonas* are oxidase positive, have polar flagella when grown in liquid media, and do not contain the enterobacterial common antigen—characteristics which distinguish them from *Enterobacteriaceae*. However, at least two *Vibrio* species (*V. metschnikovii* and *V. gazogenes*) are oxidase negative; strains of other species are oxidase negative or weakly positive; and, under certain conditions (often on solid media), members of these genera produce peritrichous flagella. Some *Aeromonas* species show higher DNA relatedness to *E. coli*, the type species of the type genus of *Enterobacteriaceae*, than that seen between several genera within the family. 16S rRNA gene sequence comparisons do not reveal any overlap between these families.

Biochemical variability and fastidiousness of a growing number of new species added to *Enterobacteriaceae*, the proposed inclusion in the family of the genus *Plesiomonas*, and the inclusion of *Calymmatobacterium* and a number of genera that are endosymbionts of insects has made a literal description of this family difficult. *Calymmatobacterium* is very fastidious, and cultivation is rarely successful—no strains are available in culture collections. The insect symbionts in the genera *Arsenophonus* (Gherna et al., 1991; Hypsa and Dale, 1997), *Buchnera* (Munson et al., 1991b; Clark et al., 1992), and *Wigglesworthia* (Aksoy, 1995b) have not been cultivated on bacteriological media, and therefore no biochemical profiles are available. These species can only be identified by their 16S rRNA gene sequences and perhaps by their host specificity. The description of the family therefore includes many traits for which there are exceptions. For example, *Plesiomonas*, a biochemically well-defined genus with a single species, is oxidase positive genus in an extremely large, oxidase negative family. Similarly, all cultivated species except *Erwinia chrysanthemi* contain enterobacterial common antigen, a trait that is specific for the family *Enterobacteriaceae*; two members of the family *Pasteurellaceae*, *Actinobacillus equuli* and *Actinobacillus suis*, also possess this antigen (Le Minor et al., 1972; Ramia et al., 1982; Böttger et al., 1987).

The relationships of many members of *Enterobacteriaceae* have been defined based on DNA relatedness (Wayne et al., 1987; Fox et al., 1992; Stackebrandt and Goebel, 1994). DNAs from species within most genera are at least 20% related to one another and to *Escherichia coli*, the type species of the type genus of the family. Notable exceptions are species of *Plesiomonas*, *Yersinia*, *Proteus*, *Providencia*, *Hafnia*, *Edwardsiella*, *Xenorhabdus*, and *Photorhabdus*, whose DNAs are usually 5–20% related to those of species from other genera. Most species have been analyzed by their 16S rDNA sequence. 16S rDNA sequence analysis is the method of choice for determining genera and higher taxa (Stackebrandt and Goebel, 1994); however, DNA relatedness can also be used to approximate evolutionary divergence within genera (Fig. BXII.γ.190).

The numbers of genera and species in the family have markedly increased during the past 25 years. There were 12 genera and 36 species in 1974 when the 8th edition of *Bergey's Manual of Determinative Bacteriology* was published (Buchanan and Gibbons, 1974). By 1984, when the 1st edition of *Bergey's Manual of Systematic Bacteriology* was published, the family contained 20 genera and 76 species (Krieg and Holt, 1984). The total rose to 30 genera and 107 species by the 9th edition of *Bergey's Manual of Determinative Bacteriology* in 1994 (Holt et al., 1994). In the classification used in this chapter, the family contains 44 genera and 176 named species. Unnamed published and unpublished genomospecies and presently undescribed groups undoubtedly include a number of new genera and species. A prediction of 225 or more species contained in 60 or more genera by the next edition of this *Manual* may be conservative.

Compared with the 1st edition of *Bergey's Manual of Systematic Bacteriology* and the 9th edition of *Bergey's Manual of Determinative Bacteriology*, the present volume contains proposed changes in classification, new genera, and new species. Taxonomic proposals since the last edition of the *Manual* are shown in Table BXII.γ.192 and discussed below and in the chapters describing individual genera. If there is any certainty with respect to the *Enterobacteriaceae*, it is that the family will continue to change and to pose a challenge to microbiologists in all specialties.

Plant, animal, and human disease *Enterobacteriaceae* are distributed worldwide. They are found in soil, water, fruits, meats, eggs, vegetables, grains, flowering plants and trees, and in animals from insects to man. Their pathogenicity for man and animals and economic importance, as well as their rapid generation time, ability to grow on defined media, and ease of genetic manipulation have made them the objects of intense laboratory study.

Many species are of considerable economic importance. Erwiniae and pectobacteria cause blight, wilt and soft-rot disease in corn, potatoes, apples, sugar cane, pineapples, and many other crops, often destroying substantial amounts of these crops (Starr and Chatterjee, 1972). For example, it has been estimated that plant pathogenic species causing soft rot diseases have caused at least $50 million of damage annually (Pérombelon and Kelman, 1980). The commercial and tropical fish industries are severely affected by the diseases caused by *Yersinia ruckeri* and species of *Edwardsiella* (Ewing et al., 1978; Shotts and Snieskzo, 1976).

Salmonellosis in poultry and in eggs is a worldwide problem, both for poultry farmers and as a vehicle for human disease (Williams, 1965; Von Rockel, 1965; Hall, 1965; Mishu et al., 1994). Salmonellosis is also common in pigs, cows, horses, dogs, and cats (Barnes and Sorensen, 1975; Ewing, 1969). Stillbirths and wool damage in sheep are usually caused by salmonellae (Jensen,

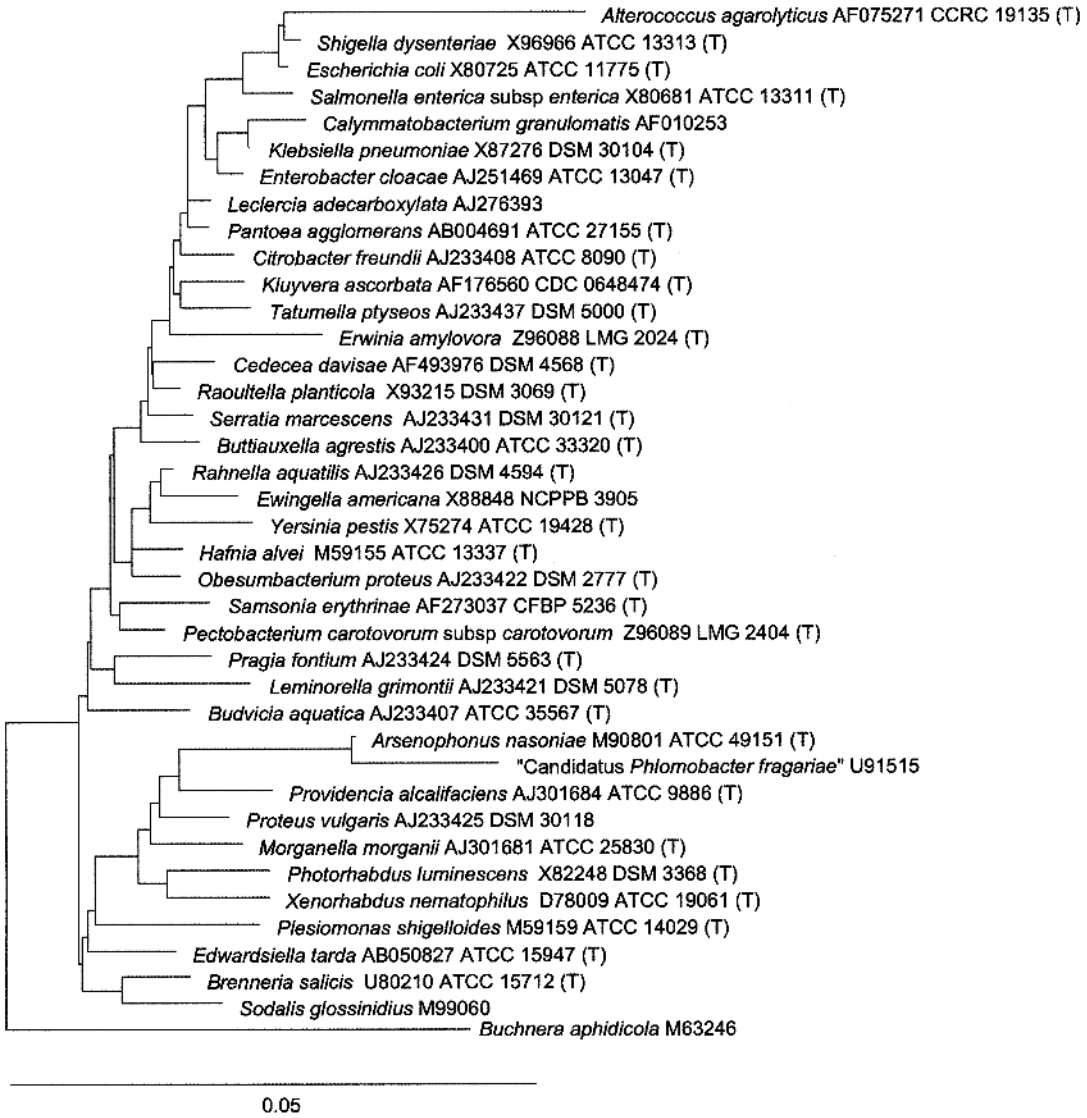

FIGURE BXII.γ.189. Phylogenetic relationships of the genera of the family *Enterobacteriaceae*. The distances in the tree were calculated using 1101 positions (the least-squares method, Jukes-Cantor model). (Courtesy T. Lilburn of the Ribosomal Database Project.)

1974). Enterotoxigenic *E. coli* strains are primarily responsible for diarrhea in lambs, piglets, and calves (Bruner and Gillespie, 1973). Klebsiellae and *Citrobacter freundii* are causes of bovine mastitis. Species of *Enterobacteriaceae* are responsible for numerous other animal infections. Examples include sexually transmitted uterine infections in horses caused by *Klebsiella pneumoniae*; infections in snakes, turtles and lizards caused by salmonellae; diarrheal and septicemic infection in rabbits, mink, and other rodents caused by yersiniae; and shigellosis in monkeys.

Salmonella serotype Typhi (*Salmonella typhi*) is the cause of typhoid fever, and *Yersinia pestis* causes bubonic and pneumonic plague. Many other *Enterobacteriaceae* are pathogenic for humans, causing a wide variety of diseases. These include diarrheal disease, usually transmitted by contaminated food or water; septicemia; respiratory disease; wound and burn infections; urinary tract infections; and meningitis. The causative agents of these diseases can be loosely divided into species that are normally pathogenic (*Salmonella*, *Shigella*, *K. pneumoniae*, *Y. pestis*, various serotypes of *E. coli*, and *Y. enterocolitica*) and species that cause disease under certain circumstances. Members of this second group are often referred to as opportunistic pathogens. The compromised host (for example, the malnourished, diabetic, immunosuppressed, catheterized, burn, cancer, respiratory, AIDS, or elderly patient) is vulnerable to nosocomial infections caused by opportunistic pathogens. *Enterobacteriaceae* have long been responsible for a substantial percentage of nosocomial infections in the United States (Jarvis et al., 1984), including urinary tract infections, surgical wound infections, lower respiratory infections, and bacteremias.

Salmonellae, *E. coli*, and shigellae are frequent causes of foodborne disease outbreaks (Beane et al., 1990), and *Yersinia enterocolitica* also causes foodborne disease. More recently, *E. coli* O157:H7, *Salmonella* serotype Typhimurium phage type 104 (definitive type 104, resistant to at least 5 antimicrobials), and *Salmonella* serotype Enteritidis phage type 4, have emerged as major foodborne pathogens (Boyce et al., 1995; Altekruse et al., 1997). *Salmonella* serotypes Typhimurium and Enteritidis are the two most prevalent causes of human salmonellosis in the United

TABLE BXII.γ.191. Some differential characteristics of the family *Enterobacteriaceae* and their nearest relatives[a]

Characteristic	*Enterobacteriaceae*	*Aeromonadaceae*	*Pasteurellaceae*	*Vibrionaceae*
Cell diameter, microns	0.3–1.5	0.3–1.0	0.2–0.4	0.3–1.3
Straight rods	+	D	+	D
Curved rods	−	D	−	D
Motility	D	+[b]	−	+[c]
Flagellar arrangement (liquid medium):				
Polar	−[d]	+		+
Lateral	+[d]	−		−
Oxidase test	−[e]	+[f]	+	+[f]
Sodium required or stimulatory for growth	−	−	−	D
Contain enterobacterial common antigen	+[g]	−	−[g]	−
Cells contain menaquinones[h]	D	D	−	D
Parasitic on mammals and birds	D	−[c]	+	−[c]
Heme and/or nicotinamide adenine dinucleotide required for growth	−	−	D	−
Plant pathogenicity	D	−	−	−
Organic nitrogen sources required	−[c]	−	+	−[c]

[a]Symbols: see standard definitions.

[b]Except *Ruminobacter*, *Tolumonas*, and certain biogroups of *Aeromonas salmonicida*.

[c]A few exceptions may occur.

[d]Except *Plesiomonas*, which has lateral polar flagella, and *Tatumella*, which may have polar, subpolar or lateral flagella.

[e]Except *Plesiomonas*.

[f]Except *Vibrio metschnikovii* and *Vibrio gazogenes* in *Vibrionaceae* and the genus *Tolumonas* in *Aeromonadaceae*.

[g]*Erwinia chrysanthemi* does not contain the antigen; *Actinobacillus equuli* and *Actinobacillus suis* contain the antigen.

[h]*Pasteurellaceae* do contain demethylmenaquinones but not menaquinones; ubiquinones may or may not be produced. *Enterobacteriaceae*, *Vibrionaceae*, and *Aeromonadaceae* may contain menaquinones, demethylmenaquinones, and ubiquinones.

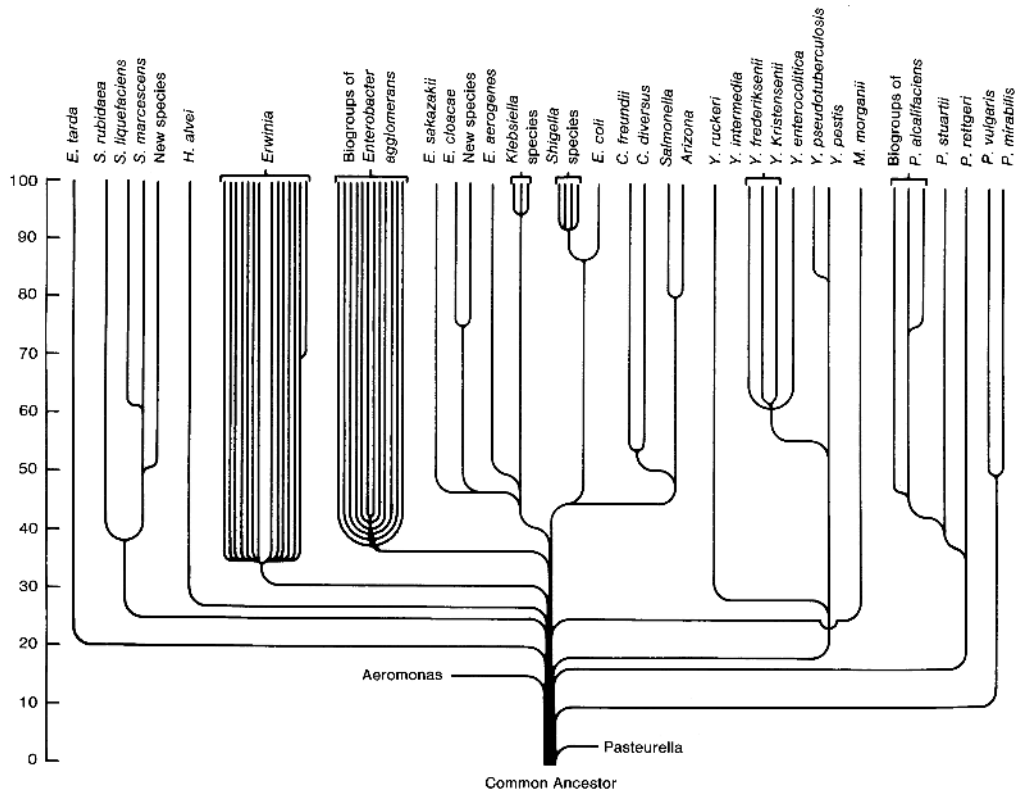

FIGURE BXII.γ.190. Divergence of *Enterobacteriaceae*. The ordinate is percentage of relatedness. This figure is a simplified attempt to depict relatedness of species of enterobacteria to other species. It assumes a common ancestor from which all of the organisms have diverged. The horizontal branches depict the degree of relatedness of the group of organisms to all organisms that have not yet branched. For example, *E. tarda* is 20% related to all organisms except *Aeromonas*, *Proteus*, *Providencia* and *Pasteurella*; *Citrobacter* species are 45% related to all species that branch above them and *C. diversus* and *C. freundii* were speciated at a point in time such that they are now 50% related.

TABLE BXII.γ.192. Comparison of the classification with that in the *Bergey's Manual of Systematic Bacteriology*, First Edition, and *Bergey's Manual of Determinative Bacteriology*, Ninth Edition[a]

Classification	Synonyms	Classification in *Bergey's Manual of Systematic Bacteriology*, First Edition 1, and *Bergey's Manual of Determinative Bacteriology*, Ninth Edition
Alterococcus agarolyticus		NL
Arsenophonus nasoniae		*A. nasoniae*
Candidatus Arsenophonus triatominarum		NL
Brenneria alni	*Erwinia alni*	NL
Brenneria nigrifluens	*Erwinia nigrifluens*	*E. nigrifluens*
Brenneria paradisiaca	*Erwinia paradisiaca*	NL
Brenneria quercina	*Erwinia quercina*	*E. quercina*
Brenneria rubrifaciens	*Erwinia rubrifaciens*	*E. rubrifaciens*
Brenneria salicis	*Erwinia salicis*	*E. salicis*
Buchnera aphidicola		NL
Budvicia aquatica		*B. aquatica*
Buttiauxella agrestis		*B. agrestis*
Buttiauxella brennerae		NL
Buttiauxella ferragutiae	Enteric group 63	Enteric group 63
Buttiauxella gaviniae	Enteric group 64	Enteric group 64
Buttiauxella izardii		NL
Buttiauxella noackiae		NL
Buttiauxella warmboldiae		NL
Calymmatobacterium granulomatis	*Klebsiella granulomatis*	*C. granulomatis*
Cedecea davisae		*C. davisae*
Cedecea lapagei		*C. lapagei*
Cedecea neteri		*C. neteri*
Cedecea species 3		Cedecea species 3
Cedecea species 5		Cedecea species 5
Citrobacter amalonaticus	*Levinea amalonatica*	*C. amalonaticus*
Citrobacter braakii		NL
Citrobacter farmeri	*C. amalonaticus* biogroup 1	*C. amalonaticus* biogroup 1
Citrobacter freundii		*C. freundii*
Citrobacter gillenii	*Citrobacter* unnamed species 10	NL
Citrobacter koseri	*L. malonatica, C. diversus*	*C. diversus*
Citrobacter murliniae	*Citrobacter* unnamed species 11	NL
Citrobacter rodentium	*Citrobacter* unnamed species 9	NL
Citrobacter sedlakii		NL
Citrobacter werkmanii		NL
Citrobacter youngae		NL
Edwardsiella hoshinae		*E. hoshinae*
Edwardsiella ictaluri		*E. ictaluri*
Edwardsiella tarda	*E. anguillimortifera*	*E. tarda*
Enterobacter amnigenus		*E. amnigenus*
Enterobacter asburiae		*E. asburiae*
Enterobacter cancerogenus	*Enterobacter taylorae, Erwinia cancerogena*	*E. taylorae*
Enterobacter cloacae		*E. cloacae*
Enterobacter dissolvens	*Erwinia dissolvens*	*Enterobacter dissolvens*
Enterobacter gergoviae		*E. gergoviae*
Enterobacter hormaechei		*E. hormaechei*
Enterobacter kobei		NL
Enterobacter nimipressuralis	*Erwinia nimipressuralis*	*Enterobacter nimipressuralis*
Enterobacter pyrinus		NL
Enterobacter sakazakii	yellow-pigmented *Enterobacter cloacae*	*E. sakazakii*
Erwinia amylovora		*E. amylovora*
Erwinia aphidicola		NL
Erwinia billingiae		NL
Erwinia mallotivora		*E. mallotivora*
Erwinia persicina	*"E. nulandii"*	*E. persicinus*
Erwinia psidii		*E. psidii*
Erwinia pyrifoliae		NL
Erwinia rhapontici	*Pectobacterium rhapontici*	*E. rhapontici*
Erwinia tracheiphila		*E. tracheiphila*
Escherichia blattae		*E. blattae*
Escherichia coli		*E. coli*
Escherichia fergusonii		*E. fergusonii*
Escherichia hermannii		*E. hermannii*
Escherichia vulneris		*E. vulneris*
Ewingella americana		*E. americana*
Hafnia alvei		*H. alvei*
Klebsiella mobilis	*Enterobacter aerogenes*	*Enterobacter aerogenes*
Klebsiella oxytoca		*K. oxytoca*
Klebsiella ornithinolytica	*Raoultella ornithinolytica*	NL
Klebsiella planticola	*Raoultella planticola*	*K. planticola*
Klebsiella pneumoniae subsp. *ozaenae*	*K. ozaenae*	*Klebsiella pneumoniae* subsp. *ozaenae*
Klebsiella pneumoniae subsp. *pneumoniae*		*Klebsiella pneumoniae* subsp. *pneumoniae*

(*continued*)

TABLE BXII.γ.192. *(cont.)*

Current classification	Synonyms	Classification in *Bergey's Manual of Systematic Bacteriology*, First Edition 1, and *Bergey's Manual of Determinative Bacteriology*, Ninth Edition
Klebsiella pneumoniae subsp. *rhinoscleromatis*	*K. rhinoscleromatis*	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*
Klebsiella terrigena	*Raoultella terrigena*	*K. terrigena*
Kluyvera ascorbata		*K. ascorbata*
Kluyvera cochleae	*Enterobacter intermedius*[b]	*Enterobacter intermedium*
Kluyvera cryocrescens		*K. cryocrescens*
Kluyvera georgiana	*Kluyvera* species 3	*Kluyvera* species 3
Leclercia adecarboxylata	*Escherichia adecarboxylata*, *Enterobacter agglomerans* DNA group XI	*L. adecarboxylata*
Leminorella grimontii		*L. grimontii*
Leminorella richardii		*L. richardii*
Leminorella species 3		
Moellerella wisconsensis		*M. wisconsensis*
Morganella morganii subsp. *morganii*	*Proteus morganii*	*M. morganii*
Morganella morganii subsp. *sibonii*	*Proteus morganii*	*M. morganii*
Obesumbacterium proteus	*Hafnia protea*	*O. proteus*
Pantoea agglomerans	*Enterobacter agglomerans* DNA group XIII, *Erwinia herbicola*, *Erwinia milletiae*	*Enterobacter agglomerans*
Pantoea ananatis	*Pantoea ananas*, *Erwinia ananas*, *Enterobacter agglomerans* DNA group VI, *Erwinia uredovora*	*Erwinia ananas*
Pantoea citrea		NL
Pantoea dispersa	*E. agglomerans* DNA group III, phenon 8	*P. dispersa*
Pantoea punctata		NL
Pantoea stewartii subsp. *indologenes*		NL
Pantoea stewartii subsp. *stewartii*		*Erwinia stewartii*
Pantoea terrea		NL
Pectobacterium cacticida	*Erwinia cacticida*	*Erwinia cacticida*
Pectobacterium carotovorum subsp. *atrosepticum*	*Erwinia atroseptica*, *E. carotovora* subsp. *atroseptica*	*Erwinia carotovora* subsp. *atroseptica*
Pectobacterium carotovorum subsp. *betavasculorum*	*Erwinia carotovora* subsp. *betavasculorum*	*Erwinia carotovora* subsp. *betavasculorum*
Pectobacterium carotovorum subsp. *carotovorum*	*Erwinia carotovora* subsp. *carotovora*	*Erwinia carotovora* subsp. *carotovora*
Pectobacterium carotovorum subsp. *odoriferum*	*Erwinia carotovora* subsp. *odorifera*	NL
Pectobacterium carotovorum subsp. *wasabiae*	*Erwinia carotovora* subsp. *wasabiae*	*Erwinia carotovora* subsp. *wasabiae*
Pectobacterium chrysanthemi	*Erwinia chrysanthemi*	*Erwinia chrysanthemi*
Pectobacterium cypripedii	*Erwinia cypripedii*	*Erwinia cypripedii*
Candidatus Phlomobacter fragariae		NL
Photorhabdus luminescens subsp. *luminescens*	*Xenorhabdus luminescens*	*X. luminescens*
Photorhabdus luminescens subsp. *akhurstii*		NL
Photorhabdus asymbiotica		NL
Photorhabdus temperata		NL
Plesiomonas shigelloides[b]		*P. shigelloides*[b]
Pragia fontium		*P. fontium*
Proteus mirabilis		*P. mirabilis*
Proteus myxofaciens		*P. myxofaciens*
Proteus penneri		*P. penneri*
Proteus vulgaris		*P. vulgaris*
Providencia alcalifaciens		*P. alcalifaciens*
Providencia heimbachae		*P. heimbachae*
Providencia rettgeri		*P. rettgeri*
Providencia rustigianii		*P. rustigianii*
Providencia stuartii		*P. stuartii*
Rahnella aquatilis		*R. aquatilis*
Rahnella genomospecies 2		NL
Rahnella genomospecies 3		NL
Saccharobacter fermentatus		NL
Salmonella bongori	*Salmonella* subsp. *bongori*, *Salmonella* subsp. V	*"S. bongori"*[c], *Salmonella bongori*[d]
Salmonella enterica subsp. *arizonae*	*S. arizonae* (monophasic), *Salmonella* subsp. IIIa	*Salmonella arizonae*[c], *S. choleraesuis* subsp. *arizonae*[d]
Salmonella enterica subsp. *diarizonae*	*S. arizonae* (diphasic), *Salmonella* subsp. IIIb	*S. choleraesuis* subsp. *diarizonae*[d]
Salmonella enterica subsp. *enterica* Choleraesuis	*Salmonella* subsp. I	*Salmonella choleraesuis*[c], *Salmonella choleraesuis* subsp. *choleraesuis* Choleraesuis[d]
Salmonella enterica subsp. *enterica* Enteritidis		*Salmonella enteritidis*[c]
Salmonella enterica subsp. *enterica* Gallinarum		*"Salmonella gallinarum"*[c], *Salmonella choleraesuis* subsp. *choleraesuis* Gallinarum[d]
Salmonella enterica subsp. *enterica* Paratyphi A		*"Salmonella paratyphi-A"*[c], *Salmonella choleraesuis* subsp. *choleraesuis* Paratyphi A[d]
Salmonella enterica subsp. *enterica* Paratyphi B		NL

(*continued*)

TABLE BXII.γ.192. *(cont.)*

Current classification	Synonyms	Classification in *Bergey's Manual of Systematic Bacteriology*, First Edition 1, and *Bergey's Manual of Determinative Bacteriology*, Ninth Edition
Salmonella enterica subsp. *enterica* Paratyphi C		NL
Salmonella enterica subsp. *enterica* Typhi		*Salmonella typhi*[c], *Salmonella choleraesuis* subsp. *choleraesuis* Typhi[d]
Salmonella enterica subsp. *enterica* Typhimurium		*Salmonella typhimurium*[c]
Salmonella enterica subsp. *houtenae*	*Salmonella* subsp. IV	*"Salmonella houtenae"*[c] *S. choleraesuis* subsp. *houtenae*[d]
Salmonella enterica subsp. *indica*	*Salmonella* subsp. V	*S. choleraesuis* subsp. *indica*[d]
Salmonella enterica subsp. *salamae*	*Salmonella* subsp. II	*"Salmonella salamae"*[c], *S. choleraesuis* subsp. *salamae*[d]
Serratia entomophila		*S. entomophila*
Serratia ficaria		*Serratia ficaria*
Serratia fonticola		*S. fonticola*
Serratia grimesii		*S. grimesii*
Serratia liquefaciens		*S. liquefaciens*
Serratia marcescens		*S. marcescens*
Serratia odorifera		*S. odorifera*
Serratia plymuthica		*S. plymuthica*
Serratia proteamaculans subsp. *proteamaculans*		*S. proteamaculans* subsp. *proteamaculans*
Serratia proteamaculans subsp. *quinovora*		*S. proteamaculans* subsp. *quinovora*
Serratia rubidaea		*S. rubidaea*
Shigella boydii		*S. boydii*
Shigella dysenteriae		*S. dysenteriae*
Shigella flexneri		*S. flexneri*
Shigella sonnei		*S. sonnei*
Sodalis glossinidius		NL
Tatumella ptyseos		*T. ptyseos*
Trabulsiella guamensis		NL
Wigglesworthia glossinidia		NL
Xenorhabdus beddingii	*X. nematophilus* subsp. *beddingii*	*X. beddingii*
Xenorhabdus bovienii	*X. nematophilus* subsp. *bovienii*	*X. bovienii*
Xenorhabdus japonica		NL
Xenorhabdus nematophila		*X. nematophilus*
Xenorhabdus poinarii	*X. nematophilus* subsp. *poinarii*	*X. poinarii*
Yersinia aldovae		*Y. aldovae*
Yersinia bercovieri		*Y. bercovieri*
Yersinia enterocolitica		*Y. enterocolitica*
Yersinia frederiksenii		*Y. frederiksenii*
Yersinia intermedia		*Yersinia intermedia*
Yersinia kristensenii		*Y. kristensenii*
Yersinia mollaretii		*Y. mollaretii*
Yersinia pestis		*Y. pestis*
Yersinia pseudotuberculosis		*Y. pseudotuberculosis*
Yersinia rohdei		*Y. rohdei*
Yersinia ruckeri		*Y. ruckeri*
Yokenella regensburgei	*Koserella trabulsii*, *Hafnia* hybridization group 3, Enteric group 45, Enteric group 45, NIH (Japan) biogoup 9	*Y. regensburgei*

[a]NL, not listed.

[b]*Enterobacter intermedius* and *Kluyvera cochleae* have identical 16S rRNA sequences.

[c]Name used in *Bergey's Manual of Systematic Bacteriology*, First Edition, 1984.

[d]Name used in *Bergey's Manual of Determinative Bacteriology*, Ninth Edition, 1994.

States and in the United Kingdom. Outbreaks due to *E. coli* O157:H7 have occurred in the United States, Europe, Asia, and Africa. In the United States, this organism is the leading cause of hemolytic uremic syndrome, the main cause of acute kidney failure in children (Boyce et al., 1995). *Enterobacteriaceae* are also significant causes of waterborne disease outbreaks.

Maintenance Procedures The following procedures have been used for many years in the Enteric Reference Laboratory, CDC, and seem to work well for all the genera and species of *Enterobacteriaceae*. Many other procedures have been described in the literature, but there have been few, if any, good comparative studies.

Long term preservation of cultures. Compared to many other organisms, strains of *Enterobacteriaceae* are simple to grow and maintain. However, strains will die if they are allowed to dry on plating media, agar slants or in "deeps." This die off is mainly due to drying. Freezing and storage at low temperature is the method of choice for long term preservation and maintenance. An alternative method is lyophilization and storage at 4°C, which is used routinely, with good results, by the American Type Culture Collection.

Preparation of a permanent frozen stock of the "whole culture." All important cultures of *Enterobacteriaceae* should be frozen and stored in liquid nitrogen. If liquid nitrogen storage is not available, they can be stored in a laboratory freezer at −70°C, or even in a "home freezer" at −10 to −20°C. The lowest tem-

perature available should be used for long term storage. The culture to be maintained is first inoculated heavily and grown on a blood agar plate for 24 h (or longer if it grows slowly). Growth from the heavy area of growth (designated as the "whole culture," as opposed to a single colony) is removed with a cotton swab, and a very heavy suspension is made in 10% skim milk (a variety of other suspending media are also in general use). One ml of this suspension is then removed to a sterile plastic freezer vial. A permanent marking pen is used to write the genus and species names and the strain number. The vial is then frozen in an alcohol bath kept in the freezer. Vials are then transferred to a 100-compartment storage box and stored at the lowest temperature available.

Preparation of a "working stock" culture of the single colony. This procedure is recommended for cultures that are used frequently, since it avoids the inconvenience of repeatedly going to a freezer. Growth from the top of a single, isolated colony is touched and stabbed to the bottom of a 13 × 100 mm screw-cap tube of working stock medium. The culture is grown overnight and checked to insure that the tube has good growth. The tube is sealed by closing the cap tightly. These working stocks are convenient for daily use. A small amount of growth is removed with a loop and transferred to a plate or tube of a good growth medium. After incubation, the culture is ready to use. The sealing method described above does not give an air-tight fit, thus water from the semi-solid medium will begin to evaporate at a rate dependent on the tightness of the fit. Drying is not a problem for many months, but eventually the viable count of the culture may begin to drop. To avoid this problem, two alternative sealing methods are used. A small volume (about 0.3 ml) of sterile mineral oil can be added to cover the surface (about 3 mm above the interface), which prevents evaporation of the medium. This works extremely well in preventing evaporation, but the mineral oil is messy and must be considered when the culture is transferred. A second method to prevent drying is to insert a number "000" white rubber stopper into the neck of the screw-cap tube to seal it. Cultures are conveniently stored at room temperature in the dark in 40-compartment divided boxes. Many species of *Enterobacteriaceae* survive for decades with this method, but some species die more quickly than others. To avoid the death of cultures, they should be frozen in addition to being stored at room temperature.

Differentiation of genera and species of *Enterobacteriaceae*
Genera and species of the family *Enterobacteriaceae* have traditionally been differentiated based on biochemical tests. Unfortunately, the media and tests are not completely standardized, and few laboratories use exactly the same formulations or procedures. Even with these variables, this approach usually results in correct identifications of the common species of *Enterobacteriaceae*. Biochemical reactions for *Enterobacteriaceae* are presented in Table BXII.γ.193. All species were studied in a single laboratory (the Enteric Reference Laboratories at the Centers for Disease Control and Prevention, Atlanta, GA, USA) by a single set of methods (Edwards and Ewing, 1972; Farmer et al., 1980a; Hickman and Farmer, 1978). Because different methods and tests are often used in different laboratories, the percentage of positive reactions obtained may differ somewhat from those presented in the chapters on specific genera. The purpose of Table BXII.γ.193 is not to advocate any given set of tests or to put undue emphasis on the percentages obtained, but to present a comprehensive comparison derived from a single set of data obtained by tests commonly done in a diagnostic laboratory. Commercial identification systems and computer-aided identification are also widely used. These methods can be supplemented by identification based on genus- and/or species-specific tests, which can be both sensitive and specific. (An example is bacteriophage O1, which is both sensitive and specific for the most common serotypes of *Salmonella*.) Several of these tests are listed in Table BXII.γ.194. Many genera and species of *Enterobacteriaceae* also have typical patterns of resistance and susceptibility to antibiotics; thus, the antibiogram of an isolate can also be used as an aid to identification (Table BXII.γ.195). Detailed discussions of practices in clinical microbiology laboratories for isolation, maintenance, storage, computer-aided identification, use of commercial test "kits", antibiotic susceptibility testing, and difficulties presented by "problem strains" of *Enterobacteriaceae* can be found in Brenner (1992b) and Farmer (1999).

From 50 to more than 200 biochemical tests have been used in phenetic or numerical taxonomic studies of *Enterobacteriaceae* (Bascomb et al., 1971; Johnson et al., 1975; Véron, 1975; Véron and Le Minor, 1975a, b). These include tests for the fermentation of a large number of carbohydrates and polyhydroxyl alcohols, tests for the ability to use a wide variety of organic substrates as the sole source of carbon and energy, and tests for the presence of specific enzymes. A number of these tests are useful for the differentiation of species, subspecies, or biogroups within *Enterobacteriaceae* (Véron and Le Minor, 1975a, b). Some tests of particular diagnostic value are for nitrate reductase type A or type B (Pichinoty and Piéchaud, 1968; Pichinoty et al., 1969), tetrathionate reductase (Richard, 1977), fermentation or growth on D-galacturonate (Le Minor et al., 1979), presence of α-glutamyl transferase (Giammanco et al., 1980), and fermentation or growth on 2-ketogluconate (Buissière et al., 1981). A summary of data obtained at the Institut Pasteur for these tests is given in Table BXII.γ.196. Other nonroutine tests of value in differentiating between phenotypically similar organisms are ascorbate and D-arabitol for *Buttiauxella* and *Kluyvera*; L-fucose, 5-ketogluconate, D-xylose, and D-sorbose to differentiate *Klebsiella* from *Enterobacter*; susceptibility to *Hafnia*-specific bacteriophage to differentiate *Hafnia* from other genera; pectinase to differentiate erwiniae from other genera; ascorbate, D-glucose fermentation at 5°C within 21 d, and irgasan susceptibility to differentiate *Kluyvera* species; tyrosine clearing to differentiate *Leminorella* species from biochemically similar species; growth in *trans*-aconitate, adonitol, benzoate, *m*-erythritol, gentisate, D-malate, L-rhamnose, and *m*-tartrate to differentiate *S. liquefaciens*, *S. proteamaculans*, and *S. grimesii*; growth in histamine, D-melizitose, and D-tartrate to differentiate *S. rubidaea* subspecies; and bioluminescence, pigment on Loeffler's blood serum, colony color on MacConkey agar, and adsorption of bromthymol blue to differentiate *Photorhabdus* and *Xenorhabdus* species. Reactions for species in these specialized tests are given in the following genus chapters.

Molecular methods can be used to identify bacteria at taxonomic levels from the family down to the strain; furthermore, molecular tests based on virulence and pathogenicity genes can be used to distinguish pathogenic and nonpathogenic isolates. Many of these methods have been described for *Enterobacteriaceae*. For example, detection of the *phoE* gene by PCR amplification provides a sensitive and specific test for *Escherichia* and *Shigella*. A growing number of genus or species-specific probes have been reported in the literature. A number of systems for identification by either complete or partial 16S rRNA gene sequencing are now under development.

TABLE BXII.γ.193. Biochemical reactions of the named species and unnamed Enteric Groups of the family *Enterobacteriaceae*[a, b]

Characteristic	*Escherichia coli*	*Escherichia coli*, inactive	*Escherichia blattae*	*Escherichia fergusonii*	*Escherichia hermannii*	*Escherichia vulneris*	*Budvicia aquatica*	*Buttiauxella agrestis*	*Buttiauxella brennerae*	*Buttiauxella ferragutiae*	*Buttiauxella gaviniae*	*Buttiauxella izardii*	*Buttiauxella noackiae*	*Buttiauxella warmboldiae*	*Cedecea davisae*	*Cedecea lapagei*
Indole production	98	80	0	98	99	0	0	0	0	0	0	0	33	0	0	0
Methyl red	99	95	100	100	100	100	93	100	100	100	100	100	100	100	100	40
Voges–Proskauer	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	80
Citrate (Simmons)	1	1	50	17	1	0	0	100	0	0	20	0	33	33	95	99
Hydrogen sulfide (TSI)	1	1	0	0	0	0	80	0	0	0	0	0	0	0	0	0
Urea hydrolysis	1	1	0	0	0	0	33	0	0	0	0	0	0	0	0	0
Phenylalanine deaminase	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0	0
Lysine decarboxylase	90	40	100	95	6	85	0	0	0	100	0	0	0	0	0	0
Arginine dihydrolase	17	3	0	5	0	30	0	0	0	0	20	0	67	0	50	80
Ornithine decarboxylase	65	20	100	100	100	0	0	100	33	80	0	100	0	0	95	0
Motility	95	5	0	93	99	100	27	100	100	60	80	100	100	100	95	80
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth in KCN	3	1	0	0	94	15	0	80	100	40	60	67	100	33	85	100
Malonate utilization	0	0	100	35	0	85	0	60	100	0	100	100	100	100	91	99
Esculin hydrolysis	35	5	0	46	40	20	0	100	100	100	100	100	100	100	45	100
Tartrate, Jordan's	95	85	50	96	35	2	27	60	0	0	40	67	100	0	0	0
Acetate utilization	90	40	0	96	78	30	0	0	0	0	0	0	0	0	0	60
Lipase (corn oil)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	91	100
DNase (25°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrate oxidized to nitrite	100	98	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	95	45	0	83	98	100	93	100	100	100	100	100	100	100	90	99
Yellow pigment	0	0	0	0	98	50	0	0	0	0	0	0	0	0	0	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	95	5	100	95	97	97	53	100	100	100	40	100	100	100	70	100
Fermentation of:																
Adonitol	5	3	0	98	0	0	0	0	67	0	100	0	0	0	0	0
L-Arabinose	99	85	100	98	100	100	80	100	100	100	100	100	100	100	0	0
D-Arabitol	5	5	0	100	8	0	27	0	67	0	80	0	0	0	100	100
Cellobiose	2	2	0	96	97	100	0	100	100	100	100	100	100	100	100	100
Dulcitol	60	40	0	60	19	0	0	0	0	0	0	0	0	0	0	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	75	65	100	20	3	25	0	60	67	0	0	33	0	0	0	0
myo-Inositol	1	1	0	0	0	0	0	0	0	0	0	0	0	67	0	0
Lactose	95	25	0	0	45	15	87	100	0	0	60	100	0	0	19	60
Maltose	95	80	100	96	100	100	0	100	100	100	60	100	100	100	100	100
D-Mannitol	98	93	0	98	100	100	60	100	100	100	100	100	100	100	100	100
D-Mannose	98	97	100	100	100	100	0	100	100	100	100	100	100	100	100	100
Melibiose	75	40	0	0	0	100	0	100	100	0	0	67	0	0	0	0
α-Methyl-D-glucoside	0	0	0	0	0	25	0	0	0	40	0	0	33	0	5	0
Mucate	95	30	50	0	97	78	20	100	67	60	80	100	100	0	0	0
Raffinose	50	15	0	0	40	99	0	100	100	0	0	33	0	0	10	0
L-Rhamnose	80	65	100	92	97	93	100	100	33	100	100	100	100	100	0	0
Salicin	40	10	0	65	40	30	0	100	100	100	100	100	100	100	99	100
D-Sorbitol	94	75	0	0	0	1	0	0	0	100	0	0	0	0	0	0
Sucrose	50	15	0	0	45	8	0	0	0	0	0	0	0	0	100	0
Trehalose	98	90	75	96	100	100	0	100	100	100	100	100	100	100	100	100
D-Xylose	95	70	100	96	100	100	93	100	100	100	100	100	100	100	100	0

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Cedecea neteri*	*Cedecea* species 3	*Cedecea* species 5	*Citrobacter amalonaticus*	*Citrobacter freundii*	*Citrobacter braakii*	*Citrobacter farmeri*	*Citrobacter gillenii*	*Citrobacter koseri* (*C. diversus*)	*Citrobacter murliniae*	*Citrobacter rodentium*	*Citrobacter sedlakii*	*Citrobacter werkmanii*	*Citrobacter youngae*	*Edwardsiella tarda*	*Edwardsiella tarda* biogroup 1
Indole production	0	0	0	100	33	33	100	0	99	100	0	83	0	15	99	100
Methyl red	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Voges–Proskauer	50	50	50	0	0	0	0	0	0	0	0	0	0	0	0	0
Citrate (Simmons)	100	100	100	95	78	87	10	33	99	100	0	83	100	75	1	0
Hydrogen sulfide (TSI)	0	0	0	5	78	60	0	67	0	67	0	0	100	65	100	0
Urea hydrolysis	0	0	0	85	44	47	59	0	75	67	100	100	100	80	0	0
Phenylalanine deaminase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lysine decarboxylase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100
Arginine dihydrolase	100	100	50	85	67	67	85	33	80	67	0	100	100	50	0	0
Ornithine decarboxylase	0	0	50	95	0	93	100	0	99	0	100	100	0	5	100	100
Motility	100	100	100	95	89	87	97	67	95	100	0	100	100	95	98	100
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth in KCN	65	100	100	99	89	100	93	100	0	100	0	100	100	95	0	0
Malonate utilization	100	0	0	1	11	0	0	100	95	0	100	100	100	5	0	0
Esculin hydrolysis	100	100	100	5	0	0	0	0	1	0	0	17	0	5	0	0
Tartrate, Jordan's	0	0	0	96	100	93	93	100	90	100	100	100	100	100	25	0
Acetate utilization	0	50	50	86	44	53	80	0	75	33	0	83	100	65	0	0
Lipase (corn oil)	100	100	50	0	0	0	0	0	0	0	0	0	0	0	0	0
DNase (25°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrate oxidized to nitrite	100	100	100	99	100	100	100	100	100	100	100	100	100	85	100	100
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	100	100	100	97	89	80	100	67	99	100	100	100	100	90	0	0
Yellow pigment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	100	100	100	97	89	93	96	100	98	100	100	100	100	75	100	50
Fermentation of:																
Adonitol	0	0	0	0	0	0	0	0	99	0	0	0	0	0	0	0
L-Arabinose	0	0	0	99	100	100	100	100	99	100	100	100	100	100	9	100
D-Arabitol	100	100	100	0	0	0	0	0	98	0	0	0	0	5	0	0
Cellobiose	100	100	100	100	44	73	100	67	99	100	100	100	0	45	0	0
Dulcitol	0	0	0	1	11	33	2	0	40	100	0	100	0	85	0	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	0	0	0	60	100	87	65	67	99	100	0	83	100	90	30	0
myo-Inositol	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0
Lactose	35	0	0	35	78	80	15	67	50	67	100	100	17	25	0	0
Maltose	100	100	100	99	100	100	100	100	100	100	100	100	100	95	100	100
D-Mannitol	100	100	100	100	100	100	100	100	99	100	100	100	100	100	0	100
D-Mannose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Melibiose	0	100	100	0	100	80	100	67	0	33	0	100	0	10	0	0
α-Methyl-D-glucoside	0	50	0	2	11	33	75	0	40	0	0	0	0	0	0	0
Mucate	0	0	0	96	100	100	100	67	95	100	100	100	100	100	0	0
Raffinose	0	100	100	5	44	7	100	0	0	33	0	0	0	10	0	0
L-Rhamnose	0	0	0	100	100	100	100	100	99	100	100	100	100	100	0	0
Salicin	100	100	100	30	0	0	9	0	15	33	0	17	0	10	0	0
D-Sorbitol	100	0	100	99	100	100	98	100	99	100	100	100	100	100	0	0
Sucrose	100	50	100	9	89	7	100	33	40	33	0	0	0	20	0	100
Trehalose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	0	0
D-Xylose	100	100	100	99	89	100	100	100	100	100	100	100	100	100	0	0

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Edwardsiella hoshinae*	*Edwardsiella ictaluri*	*Enterobacter cloacae*	*Enterobacter aerogenes*	*Enterobacter amnigenus* biogroup 1	*Enterobacter amnigenus* biogroup 2	*Enterobacter asburiae*	*Enterobacter cancerogenus* (*E. taylorae*)	*Enterobacter dissolvens*	*Enterobacter gergoviae*	*Enterobacter hormaechei*	*Enterobacter intermedius*	*Enterobacter nimipressuralis*	*Enterobacter pyrinus*	*Enterobacter sakazakii*	*Ewingella americana*
Indole production	50	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0
Methyl red	100	0	5	5	7	65	100	5	0	5	57	100	100	29	5	84
Voges–Proskauer	0	0	100	98	100	100	2	100	100	100	100	100	100	86	100	95
Citrate (Simmons)	0	0	100	95	70	100	100	100	100	99	96	65	0	0	99	95
Hydrogen sulfide (TSI)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urea hydrolysis	0	0	65	2	0	0	60	1	100	93	87	0	0	86	1	0
Phenylalanine deaminase	0	0	0	0	0	0	0	0	0	0	4	0	0	0	50	0
Lysine decarboxylase	100	100	0	98	0	0	0	0	0	90	0	0	0	100	0	0
Arginine dihydrolase	0	0	97	0	9	35	21	94	100	0	78	0	0	0	99	0
Ornithine decarboxylase	95	65	96	98	55	100	95	99	100	100	91	89	100	100	91	0
Motility	100	0	95	97	92	100	0	99	0	90	52	89	0	43	96	60
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth in KCN	0	0	98	98	100	100	97	98	100	0	100	65	100	0	99	5
Malonate utilization	100	0	75	95	91	100	3	100	100	96	100	100	100	86	18	0
Esculin hydrolysis	0	0	30	98	91	100	95	90	100	97	0	100	100	100	100	50
Tartrate, Jordan's	0	0	30	95	9	0	30	0	0	97	13	100	0	0	1	35
Acetate utilization	0	0	75	50	0	0	87	35	100	93	74	0	0	0	96	10
Lipase (corn oil)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DNase (25°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrate oxidized to nitrite	100	100	99	100	100	100	100	100	100	99	100	100	100	100	99	97
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	0	0	99	100	91	100	100	100	100	97	95	100	100	100	100	85
Yellow pigment	0	0	0	0	0	0	0	0	0	0	0	0	0	0	98	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	35	50	100	100	100	100	95	100	100	98	83	100	100	100	98	0
Fermentation of:																
Adonitol	0	0	25	98	100	0	0	0	0	0	0	0	0	0	0	0
L-Arabinose	13	0	100	100	100	100	100	100	100	99	100	100	100	100	100	0
D-Arabitol	0	0	15	100	0	0	0	0	0	97	0	0	0	0	0	99
Cellobiose	0	0	99	100	100	100	100	100	100	99	100	100	100	100	100	10
Dulcitol	0	0	15	5	0	0	0	0	0	0	87	100	0	0	5	0
Erythritol	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glycerol	65	0	40	98	0	0	11	1	0	100	4	100	0	0	15	24
myo-Inositol	0	0	15	95	100	0	0	0	0	0	0	0	0	100	75	0
Lactose	0	0	93	95	70	35	75	10	0	55	9	100	0	14	99	70
Maltose	100	100	100	99	100	100	100	99	100	100	100	100	100	100	100	16
D-Mannitol	100	0	100	100	100	100	100	100	100	99	100	100	100	100	100	100
D-Mannose	100	100	100	95	100	100	100	100	100	100	100	100	100	100	100	99
Melibiose	0	0	90	99	100	100	0	0	100	97	0	100	100	0	100	0
α-Methyl-D-glucoside	0	0	85	95	55	100	95	1	100	2	83	100	100	0	96	0
Mucate	0	0	75	90	35	100	21	75	100	2	96	100	100	0	1	0
Raffinose	0	0	97	96	100	0	70	0	100	97	0	100	0	0	99	0
L-Rhamnose	0	0	92	99	100	100	5	100	100	99	100	100	100	100	100	23
Salicin	50	0	75	100	91	100	100	92	100	99	44	100	100	100	99	80
D-Sorbitol	0	0	95	100	9	100	100	1	100	0	0	100	100	0	0	0
Sucrose	100	0	97	100	100	0	100	0	100	98	100	65	0	100	100	0
Trehalose	100	0	100	100	100	100	100	100	100	100	100	100	100	100	100	99
D-Xylose	0	0	99	100	100	100	97	100	100	99	96	100	100	0	100	13

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Hafnia alvei*	*Hafnia alvei* biogroup 1	*Klebsiella pneumoniae* subsp. *ozaenae*	*Klebsiella pneumoniae* subsp. *pneumoniae*	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*	*Klebsiella oxytoca*	*Klebsiella oxytoca*, ornithine positive	*Klebsiella planticola*	*Klebsiella terrigena*	*Kluyvera ascorbata*	*Kluyvera cryocrescens*	*Kluyvera georgiana*	*Leclercia adecarboxylata*	*Leminorella grimontii*	*Leminorella richardii*	*Moellerella wisconsensis*
Indole production	0	0	0	0	0	99	100	20	0	92	90	100	100	0	0	0
Methyl red	40	85	98	10	100	20	96	100	60	100	100	100	100	100	0	100
Voges–Proskauer	85	70	0	98	0	95	70	98	100	0	0	0	0	0	0	0
Citrate (Simmons)	10	0	30	98	0	95	100	100	40	96	80	100	0	100	0	80
Hydrogen sulfide (TSI)	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100	0
Urea hydrolysis	4	0	10	95	0	90	100	98	0	0	0	0	48	0	0	0
Phenylalanine deaminase	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Lysine decarboxylase	100	100	40	98	0	99	100	100	100	97	23	100	0	0	0	0
Arginine dihydrolase	6	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithine decarboxylase	98	45	3	0	0	0	100	0	20	100	100	100	0	0	0	0
Motility	85	0	0	0	0	0	0	0	0	98	90	100	79	0	0	0
Gelatin hydrolysis (22°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Growth in KCN	95	0	88	98	80	97	100	100	100	92	86	83	97	0	0	70
Malonate utilization	50	45	3	93	95	98	100	100	100	96	86	50	93	0	0	0
Esculin hydrolysis	7	0	80	99	30	100	100	100	100	99	100	100	100	0	0	0
Tartrate, Jordan's	70	30	50	95	50	98	100	100	100	35	19	50	83	100	100	30
Acetate utilization	15	0	2	75	0	90	95	62	20	50	86	83	28	0	0	10
Lipase (corn oil)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
DNase (25°C)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nitrate oxidized to nitrite	100	100	80	99	100	100	100	100	100	100	100	100	100	100	100	90
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	90	30	80	99	0	100	100	100	100	100	100	100	100	0	0	90
Yellow pigment	0	0	0	0	0	1	0	1	0	0	0	0	37	0	0	0
D-Glucose, acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Glucose, gas	98	0	50	97	0	97	100	100	80	93	95	17	97	33	0	0
Fermentation of:																
Adonitol	0	0	97	90	100	99	100	100	100	0	0	0	93	0	0	100
L-Arabinose	95	0	98	99	100	98	100	100	100	100	100	100	100	100	100	0
D-Arabitol	0	0	95	98	100	98	100	100	100	0	0	0	96	0	0	75
Cellobiose	15	0	92	98	100	100	100	100	100	100	100	100	100	0	0	0
Dulcitol	0	0	2	30	0	55	10	15	20	25	0	33	86	83	0	0
Erythritol	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Glycerol	95	0	65	97	50	99	100	100	100	40	5	33	3	17	0	10
myo-Inositol	0	0	55	95	95	98	95	100	80	0	0	0	0	0	0	0
Lactose	5	0	30	98	0	100	100	100	100	98	95	83	93	0	0	100
Maltose	100	0	95	98	100	100	100	100	100	100	100	100	100	0	0	30
D-Mannitol	99	55	100	99	100	99	100	100	100	100	95	100	100	0	0	60
D-Mannose	100	100	100	99	100	100	100	100	100	100	100	100	100	0	0	100
Melibiose	0	0	97	99	100	99	100	100	100	99	100	100	100	0	0	100
α-Methyl-D-glucoside	0	0	70	90	0	98	100	100	100	98	95	100	0	0	0	0
Mucate	0	0	25	90	0	93	96	100	100	90	81	83	93	100	50	0
Raffinose	2	0	90	99	90	100	100	100	100	98	100	100	66	0	0	100
L-Rhamnose	97	0	55	99	96	100	100	100	100	100	100	83	100	0	0	0
Salicin	13	55	97	99	98	100	100	100	100	100	100	100	100	0	0	0
D-Sorbitol	0	0	65	99	100	99	100	92	100	40	45	0	0	0	0	0
Sucrose	10	0	20	99	75	100	100	100	100	98	81	100	66	0	0	100
Trehalose	95	70	98	99	100	100	100	100	100	100	100	100	100	0	0	0
D-Xylose	98	0	95	99	100	100	100	100	100	99	91	100	100	83	100	0

[a]Each number is the percentage of positive reactions after 2 d at 36°C unless otherwise indicated (gelatin liquefaction and deoxyribonuclease; *Photorhabdus luminescens*).

[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

(*continued*)

TABLE BXII.γ.193. *(cont.)*

Characteristic	*Morganella morganii* subsp. *morganii*	*Morganella morganii* subsp. *sibonii*	*Morganella morganii* biogroup 1	*Obesumbacterium proteus* biogroup 2	*Pantoea agglomerans*	*Pantoea dispersa*	*Photorhabdus luminescens* (all tests at 25°C)	*Photorhabdus* DNA hybridization group 5	*Pragia fontium*	*Proteus vulgaris*	*Proteus mirabilis*	*Proteus myxofaciens*	*Proteus penneri*	*Providencia alcalifaciens*	*Providencia heimbachae*	*Providencia rettgeri*
Indole production	95	50	100	0	20	0	50	0	0	98	2	0	0	99	0	99
Methyl red	95	86	95	15	50	82	0	0	100	95	97	100	100	99	85	93
Voges–Proskauer	0	0	0	0	70	64	0	0	0	0	50	100	0	0	0	0
Citrate (Simmons)	0	0	0	0	50	100	50	20	89	15	65	50	0	98	0	95
Hydrogen sulfide (TSI)	20	7	15	0	0	0	0	0	89	95	98	0	30	0	0	0
Urea hydrolysis	95	100	100	0	20	0	25	60	0	95	98	100	100	0	0	98
Phenylalanine deaminase	95	93	100	0	20	9	0	0	22	99	98	100	99	98	100	98
Lysine decarboxylase	1	29	100	100	0	0	0	0	0	0	0	0	0	0	0	0
Arginine dihydrolase	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ornithine decarboxylase	95	64	80	100	0	0	0	0	0	0	99	0	0	1	0	0
Motility	95	79	0	0	85	100	100	100	100	95	95	100	85	96	46	94
Gelatin hydrolysis (22°C)	0	0	0	0	2	0	50	80	0	91	90	100	50	0	0	0
Growth in KCN	98	79	90	0	35	82	0	20	0	99	98	100	99	100	8	97
Malonate utilization	1	0	5	0	65	9	0	0	0	0	2	0	0	0	0	0
Esculin hydrolysis	0	0	0	0	60	0	0	0	78	50	0	0	0	0	0	35
Tartrate, Jordan's	95	100	100	15	25	9	50	60	0	80	87	100	85	90	69	95
Acetate utilization	0	0	0	0	30	100	0	20	0	25	20	0	5	40	0	60
Lipase (corn oil)	0	0	0	0	0	0	0	0	0	80	92	100	45	0	0	0
DNase (25°C)	0	0	0	0	0	0	0	0	0	80	50	50	40	0	0	0
Nitrate oxidized to nitrite	90	100	90	100	85	91	0	0	100	98	95	100	90	100	100	100
Oxidase, Kovacs	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ONPG test	10	0	20	0	90	91	0	0	0	1	0	0	1	1	0	5
Yellow pigment	0	0	0	0	75	27	50	60	0	0	0	0	0	0	0	0
D-Glucose, acid	99	100	100	100	100	100	75	100	100	100	100	100	100	100	100	100
D-Glucose, gas	90	86	93	0	20	0	0	0	0	85	96	100	45	85	0	10
Fermentation of:																
Adonitol	0	0	0	0	7	0	0	0	0	0	0	0	0	98	92	100
L-Arabinose	0	0	0	0	95	100	0	0	0	0	0	0	0	1	0	0
D-Arabitol	0	0	0	0	50	100	0	0	0	0	0	0	0	0	92	100
Cellobiose	0	0	0	0	55	55	0	0	0	0	1	0	0	0	0	3
Dulcitol	0	0	0	0	15	0	0	0	0	0	0	0	0	0	0	0
Erythritol	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	75
Glycerol	5	7	100	0	30	27	0	0	0	60	70	100	55	15	0	60
myo-Inositol	0	0	0	0	15	0	0	0	0	0	0	0	0	1	46	90
Lactose	1	0	0	0	40	0	0	0	0	2	2	0	1	0	0	5
Maltose	0	0	0	50	89	82	25	0	0	97	0	100	100	1	54	2
D-Mannitol	0	0	0	0	100	100	0	0	0	0	0	0	0	2	0	100
D-Mannose	98	100	100	85	98	100	100	100	0	0	0	0	0	100	100	100
Melibiose	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	5
α-Methyl-D-glucoside	0	0	0	0	7	0	0	0	0	60	0	100	80	0	0	2
Mucate	0	7	0	0	40	0	0	0	0	0	0	0	0	0	0	0
Raffinose	0	0	0	0	30	0	0	0	0	1	1	0	1	1	0	5
L-Rhamnose	0	0	0	15	85	91	0	0	0	5	1	0	0	0	100	70
Salicin	0	0	0	0	65	0	0	0	78	50	0	0	0	1	0	50
D-Sorbitol	0	0	0	0	30	0	0	0	0	0	0	0	0	1	0	1
Sucrose	0	7	0	0	75	1	0	0	0	97	15	100	100	15	0	15
Trehalose	0	100	0	85	97	100	0	0	0	30	98	100	55	2	0	0
D-Xylose	0	0	0	15	93	100	0	0	0	95	98	0	100	1	8	10

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[b]Table taken from Farmer, 1999.

[c]In a Request for an Opinion published in 1987, Le Minor and Popoff proposed replacement of the type species of *Salmonella* (*Salmonella choleraesuis* subsp. *choleraesuis*) with *Salmonella enterica* as the former was considered to be a source of confusion. Although the Request was denied by the Judicial Commission, their proposal resulted in an alternative naming convention which has found widespread endorsement in the public health community. This matter was revisited in July 2002 by the Judicial Commission during the IUMS Congress in response to several new Requests for an Opinion and will likely result in a decision to replace the type strain *Salmonella choleraesuis* subsp. *choleraesuis* with *Salmonella enterica* subsp. *enterica* LT2, while preserving the former rather than placing it on the list of rejected names. We view the six subspecies of *Salmonella choleraesuis* as deprecated. Readers are also advised that the names *Salmonella enteritidis*, *Salmonella paratyphi*, *Salmonella typhi*, and *Salmonella typhimurium* are synonyms of *Salmonella enterica* subsp. *enterica* and refer to specific serovars. These names have not been deprecated at this time as they remain in use by some public health reporting agencies.

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