Assignment of *Vibrio* sp. strain ABE-1 to *Colwellia maris* sp. nov., a new psychrophilic bacterium

Isao Yumoto,¹ Kosei Kawasaki,¹ Hideaki Iwata,^{1,2} Hidetoshi Matsuyama² and Hidetoshi Okuyama^{1,3}

Author for correspondence: Isao Yumoto. Tel: +81 11 8578925. Fax: +81 11 8578900.
e-mail: yumoto@hniri.go.jp

¹ Bioscience and Chemistry Division, Hokkaido National Industrial Research Institute, Tsukisamu-Higashi, Toyohira-ku, Sapporo 062-8517, Japan

² Department of Bioscience and Technology, School of Engineering, Hokkaido Tokai University, Minaminosawa, Minami-ku, Sapporo 005-8601, Japan

³ Laboratory of Environmental Molecular Biology, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan

A psychrophilic bacterium, previously described as *Vibrio* sp. strain ABE-1^T, has been reassigned by phenotypic characterization, chemotaxonomic analysis and 16S rRNA phylogenetic analysis. The organism was curved rods and it could reduce nitrate to nitrite and hydrolyse gelatin and DNA, but not chitin. NaCl was required for growth. This strain was susceptible to the vibriostatic compound O/129. The major isoprenoid quinone was ubiquinone-8 and the DNA G+C content was 39.4 mol%. The whole-cell fatty acids comprised saturated and monounsaturated fatty acids with 10–18 C atoms; saturated and monounsaturated C₁₆ fatty acids were predominant. Strain ABE-1^T contained the unique *trans*-unsaturated fatty acid, 9-*trans*-hexadecenoic acid. Although strain ABE-1^T has been identified as a *Vibrio* species, the strain did not ferment glucose. Phylogenetic analysis based on 16S rRNA sequencing indicated that strain ABE-1^T was more closely related to *Colwellia* species than to *Vibrio* species. However, strain ABE-1^T differed from other reported *Colwellia* species in terms of phylogenetic position, some phenotypic characteristics, chemotaxonomic analysis and relatedness by DNA–DNA hybridization. Accordingly, the name *Colwellia maris* is proposed. The type strain is ABE-1^T (= JCM 10085^T).

Keywords: *Colwellia maris* sp. nov., psychrophilic bacterium, *Vibrio*

INTRODUCTION

There have been many reports about micro-organisms that can grow in extreme environments such as low and high temperatures, high pressure, alkaline and acidic conditions, and high chemical concentrations (Horikoshi & Grant, 1991). Even though the temperature is below 10 °C in almost 80% of the Earth, little is known about the taxonomic diversity and physiology of micro-organisms which have adapted to the cold. Morita (1975) defined the psychrophile as having an optimum temperature for growth of about ≤ 15 °C and a maximum temperature for growth of ≤ 20 °C, and the psychrotroph as a cold-tolerant organism, with a maximum growth temperature above 20 °C. Compared with mesophiles, there are not many examples of isolation of psychrophiles. However,

several strains have been identified at the species level (Bowman *et al.*, 1997a, b; D'aoust & Kunshner, 1972; Deming *et al.*, 1988; Gounot, 1976; Irgens *et al.*, 1996; Morita & Haight, 1964; Pacha, 1968).

A psychrophilic bacterium, *Vibrio* sp. ABE-1^T, has been isolated previously (Takada *et al.*, 1979). The micro-organism exhibited optimum and maximum temperatures for growth of 15 °C and below 24 °C, respectively. The cellular fatty acids concerned with cold adaptation (Okuyama *et al.*, 1990, 1991), bioenergetic properties as a marine bacterium (Takada *et al.*, 1981, 1989a, 1991), and enzymology as a psychrophile (Ishii *et al.*, 1987, 1993) of the strain have been studied extensively. However, its taxonomic position at the species level remains to be determined.

In this study, phenotypic and chemotaxonomic characteristics and the phylogenetic position of strain ABE-1^T have been examined; results showed that the strain should be classified as a new species.

The GenBank/EMBL/DBJ accession number for the 16S rRNA sequence reported in this paper is AB002630.

METHODS

Bacterial strains and cultivation. The strain examined was *Vibrio* sp. ABE-1^T (Takada *et al.*, 1979). It was isolated from seawater which was obtained from the Abashiri coast (44°3' N, 144°16' E), off the Okhotsoku Sea, in Hokkaido, Japan, in the season when drifting ice from Siberia flowed into the region. The organism was cultivated aerobically until the late exponential phase of growth at 15 °C in PYSE medium (pH 7.5), unless otherwise stated. The PYSE medium (pH 7.5) contained (per litre 50% Herbst's artificial seawater) 8.0 g peptone (Kyokuto), 3.0 g yeast extract (Merck), and 15 g agar (if needed). Herbst's artificial seawater contained the following (per litre distilled water): 30 g NaCl; 0.7 g KCl; 5.3 g MgSO₄·7H₂O; 1.3 g CaSO₄·2H₂O; and 10.8 g MgCl₂·6H₂O. *Colwellia psychrerythraea* ATCC 27364^T (type strain) was used as a comparison for the test of metabolism of D-glucose and DNA-DNA hybridization. The strain was grown at 15 °C on marine agar or broth 2216 (Difco).

Phenotypic characterization. For phenotypic characterization, PYSE medium was used as the basal medium, the culture was incubated at 15 °C for 2 weeks, and the experiment was performed more than twice unless otherwise stated. Morphological, physiological and biochemical tests were performed as described in Cowan & Steel's manual (Barrow & Feltham, 1993). The metabolism of carbohydrates was tested by the method of Leifson (1963). The experiment was repeated five times. The result was checked daily for 1 month after inoculation. Alginase activity was determined after a preparation was overlaid with ethanol following 10 d incubation. Sensitivity to the vibriostatic agent O/129 (2,4-diamino-6,7-diisopropylpteridine) was determined after 1 week cultivation on PYSE agar plates using diagnostic disks (10 and 150 µg; Oxoid). The utilization of the substrate as the sole carbon and energy source was performed with USTM medium (pH 7.5) containing 0.2% substrate, 50 mM Tris, 190 mM NH₄Cl, 0.33 mM K₂HPO₄, 0.1 mM FeSO₄·7H₂O in 1 l 50% Herbst's artificial seawater (described above).

Electron microscopy. Cells which were grown on PYSE agar medium were suspended in physiological saline. A small drop of the suspension was placed on a carbon-coated copper grid and negatively stained with 1% phosphotungstic acid for observation with a transmission electron microscope (Hitachi H-800).

Analysis of isoprenoid quinones. Isoprenoid quinones were extracted by treating 500 mg freeze-dried cells with 150 ml chloroform/methanol (2:1, v/v) for 2 h in a reciprocal shaker (120 strokes per min) at room temperature. The extracted solution was concentrated and transferred by acetone. The resulting solution was concentrated, separated by TLC using n-hexane/dimethyl ether (85:15, v/v) as the solvent, and recovered from the TLC plate using acetone. The obtained isoprenoid quinones were analysed by HPLC equipped with a Novapak C18 column (Waters) at room temperature. The HPLC system consisted of a solvent delivery pump (model CCPM-II; Tosoh) and a spectrophotometer detector (model UV-8020; Tosoh) set at 275 nm.

Analysis of cellular fatty acids. Whole cell fatty acids were extracted from 100 mg freeze-dried cells, which were cultivated on PYSE medium, esterified by acid methanolysis and analysed by GLC equipped with a flame-ionization detector (model GC-7A; Shimadzu) and a 25 m BPX70 column (SGE) with an oven temperature of 170 °C. Fatty acids were identified by comparing them with fatty acid

methyl ester standards (Supelco) and by GC/MS (model INCOS 50; Finnigan mat) connected to GLC (model 3400; Varian).

DNA base composition and DNA-DNA hybridization. DNA was prepared from bacterial cells by the method of Marmur (1961). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984). The obtained DNA was digested with nuclease P1 (Yamasa Shoyu). The resulting nucleotides were separated by HPLC. The HPLC system was as described above. An equimolar mixture of four deoxyribonucleotides (Yamasa Shoyu) was used as the standard.

Levels of DNA relatedness were determined fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microplates.

Amplification of 16S rRNA gene and sequencing. The 16S rRNA gene was amplified by PCR. The sequences of the primers used for amplification were 5' AGAGTTTGAT-CCTGGCT 3' and 5' AAGGAGGTGATCCAGCCGCA 3', corresponding to positions 8–24 and 1528–1544, respectively, in the 16S rRNA sequence of *Escherichia coli* (Brosius *et al.*, 1978). The 1.5 kb PCR product was directly sequenced by the dideoxynucleotide chain-termination method with a DNA sequencer (model 377; Applied Biosystems). Multiple alignments of the sequence were performed, nucleotide substitution rates (K_{mut} value) were calculated, and a neighbour-joining phylogenetic tree (Kimura, 1980; Saitou & Nei, 1987) was constructed using the CLUSTAL W program (Thompson *et al.*, 1994). The similarity values of the sequences were calculated using the GENETYX computer program (Software Development).

Nucleotide sequence accession numbers. The DDBJ/EMBL/GenBank accession numbers for the sequences used as reference sequences are as follows: *Colwellia*-like bacterium S51-W(gv)1 (Gosink & Staley, 1995), U14581; *C. psychrerythraea* (DeLong *et al.*, 1993), L10939; small subunit rRNA gene cloned from macroaggregate of the sea (AGG53) (DeLong *et al.*, 1993), L10950; *Pseudoalteromonas haloplanktis* subsp. *haloplanktis* ATCC 14393^T, X67024; *Shewanella hanedai* CIP 103207^T, X82132; *Photobacterium angustum* ATCC 25915^T, X74685; *Vibrio cholerae* ATCC 14035^T, X74695; *Alteromonas macleodii* IAM 12920^T, X82145; *Aeromonas hydrophila* ATCC 35654, X74676; *Escherichia coli* (Carbon *et al.*, 1979), J01859; *Moritella marinus* NCIMB 1144^T, X82142; *Marinomonas vaga* ATCC 27119, X67025.

RESULTS

Morphology

When the bacterium was grown at 15 °C in PYSE medium, the cells appeared as single motile curved rods of 0.6–0.8 by 2.0–4.0 µm in size. Flagella-staining light microscopic observation and transmission electron micrograph (Fig. 1) revealed a single polar flagellum. Spore formation was absent and Gram-staining was negative.

Phenotypic characteristics

Strain ABE-1^T exhibited the following physiological and biochemical characteristics. Oxidase and catalase activities were positive. Acids were produced from D-glucose under aerobic conditions; no acids, however,

Fig. 1. Electron micrograph of negatively stained cells of strain ABE-1^T showing curved rod and polar flagellum. Bar, 1 µm.

were produced under anaerobic conditions. No acids were produced from L-arabinose, D-fructose, maltose, D-mannose, melibiose and sucrose under either aerobic or anaerobic conditions. The strain did not grow in the absence of NaCl in the culture medium. It grew in media supplemented with 3 and 4% NaCl, but not in media with salinity higher than 6.5% NaCl. Susceptibility to the vibriostatic compound O/129 (10 and 150 µg) was also detected. Growth occurred at 0–22 °C, but not at temperatures higher than 25 °C. The strain was negative for methyl red, Voges–Proskauer and indole production, but did reduce nitrate to nitrite. It hydrolysed gelatin, DNA and Tweens 20, 40, 60 and 80, but not casein, chitin, starch or alginic acid. The strain utilized D-glucose as a sole carbon and energy source for growth, but not D-mannose, raffinose, D-xylose, L-arabinose, D-fructose, glycerol, lactose, maltose, melibiose or sucrose.

Chemotaxonomic characteristics

The isoprenoid quinones isolated from strain ABE-1^T using TLC were analysed by HPLC. Analysis revealed that ubiquinone-8 (Q-8) was the predominant isoprenoid quinone in the strain.

GC analysis of the methyl ester derivatives of the fatty acids of the strain revealed that the major components were C_{16:0} (18%), C_{16:1}9t (20%) and C_{16:1}9c (20%). Smaller, but substantial amounts of C_{16:1}7c (6%), C_{17:0} (5%) and C_{18:1}11c (6%) were also detected.

16S rRNA sequence analysis

The almost complete 16S rRNA sequence of strain ABE-1^T, consisting of 1514 nucleotides, showed 92.9% similarity to the 16S rRNA sequence of *C. psychrerythraea*, whereas its similarity to the sequences of *Pseudoalteromonas*, *Shewanella*, *Photobacterium*, *Vibrio*, *Moritella*, *Alteromonas*, *Escherichia*, *Pasteurella* and *Aeromonas* species was less than 90%. The strain showed high similarity (97.8%) with a gas vacuolate

bacterium, strain S51-W(gv)1 (Gosink & Staley, 1995), isolated from Antarctica. However, this strain has not yet been identified at the species level. Strain ABE-1^T also clustered with amplified 16S rRNAs of AGG53 (DeLong *et al.*, 1993) and PVB OTU12 (Moyer *et al.*, 1995) (data not shown) which were isolated from sea macroaggregate and a hydrothermal vent system, respectively. The 16S rRNA sequence of strain ABE-1^T exhibited high similarity to that of AGG53 (95.3%) and PVB OTU12 (92.9%).

DNA base composition and DNA–DNA hybridization

The DNA G+C content of strain ABE-1^T was 39.4 mol%, a value that falls within the definition range of the genera *Colwellia* and *Vibrio*.

According to 16S rRNA sequence analysis, strain ABE-1^T was closely related to the genus *Colwellia*. Only two species belonging to genus *Colwellia*, *C. psychrerythraea* and *Colwellia hadaliensis*, have previously been recognized. However, the type strain of *C. hadaliensis* is difficult to obtain. Therefore, the level of DNA relatedness between strain ABE-1^T and only obtainable species, *C. psychrerythraea* ATCC 27364^T, was determined; this value was 22%.

DISCUSSION

Phenotypic characterization and chemotaxonomic analysis of strain ABE-1^T in this study were comparable to previous results (Okuyama *et al.*, 1990; Takada *et al.*, 1979, 1989b), except for the casein hydrolysis and DNA G+C content results. Strain ABE-1^T was tentatively identified as belonging to the genus *Vibrio*, due to the characteristic phenotypic similarities to *Vibrio marinus*, although it has been reported as being negative for fermentation of D-glucose (Takada *et al.*, 1979). In the present study, in addition to the phenotypic characteristics, the almost complete sequence of the 16S rRNA was determined. Phylogenetic analysis based on the 16S rRNA sequence revealed that strain ABE-1^T was more closely related to *Colwellia* species than to *Vibrio* species (Fig. 2). These results suggest that strain ABE-1^T should be classified as *Colwellia* rather than *Vibrio*.

Although phylogenetic analysis has revealed that the genera *Colwellia* and *Vibrio* are very different (Fig. 2) and strain ABE-1^T and *C. psychrerythraea* do not ferment D-glucose, *Colwellia* species have been described in the genus *Vibrio* in terms of phenotypic characterization (Holt *et al.*, 1994). Actually, the *Colwellia* strains, *C. psychrerythraea* and *C. hadaliensis*, have been reported as facultatively anaerobic bacteria (D'aoust & Kunshner, 1972; Deming *et al.*, 1988), but in this study, generally (four-fifths of trial), *C. psychrerythraea* did not produce acids from D-glucose under either aerobic or anaerobic conditions during 1 month. However, acids were occasionally produced under aerobic conditions (one-fifth of trial). These results suggested that phenotypic characteristics of the genus *Colwellia* may also be totally different from

Fig. 2. Phylogenetic tree derived from 16S rRNA sequence data of strain ABE-1^T and other species within the gamma 3 subclass of the class *Proteobacteria*. *Marinomonas vaga* was used as outgroup for the gamma 3 subclass of *Proteobacteria*. Bar, 0.01 K_{nuc} .

those of the genus *Vibrio*. It is considered that there are several difficulties in judging Leifson's modified oxidation/fermentation test (Leifson, 1963) due to the long period of incubation at low temperature when studying psychrophiles. Interestingly, the *Colwellia* species are more closely related to *Alteromonas macleodii* (Fig. 2), a strictly aerobic marine bacterium, than to facultative anaerobes, such as the genera *Vibrio*, *Photobacterium* and *Aeromonas*, on the basis of phylogenetic analysis. From the results described above, it is concluded that strain ABE-1^T belongs to the genus *Colwellia*, and that the definition of *Colwellia* should be amended in terms of D-glucose metabolism.

The phenotypic characterization of halophilic genera that commonly occur in marine habitats is shown in Table 1. The genus *Colwellia* can be differentiated

from four other genera of halophilic marine microorganisms on the basis of phenotypic and chemotaxonomic characteristics as follows: genera *Vibrio*, *Photobacterium* and *Moritella* are facultatively anaerobic; and the genus *Halomonas* exhibits a G+C content of DNA of 52–68 mol% and the major isoprenoid quinone detected is Q-9. However, it is very difficult to differentiate between the genus *Colwellia* and the genera *Alteromonas*, *Pseudoalteromonas*, *Shewanella* and *Marinomonas* using these characteristics. In some cases, it is possible to differentiate between strain ABE-1^T, which belongs to the genus *Colwellia*, and the cold-adapted strains that belong to these other genera on the basis of phenotypic and chemotaxonomic characteristics. For example, recently, *Pseudoalteromonas antarctica* was isolated from an Antarctic coastal environment (Bozal *et al.*, 1997). Strain ABE-1^T was differentiated from *P. antarctica* in terms of cell shape, growth temperature range (4–30 °C), utilization and metabolism of substrates, nitrate reduction and starch hydrolysis.

The available data of phenotypic characteristics of the already known *Colwellia* species, *C. psychrerythraea* and *C. hadaliensis*, are limited (D'aoust *et al.*, 1972; Deming *et al.*, 1988). Strain ABE-1^T can be differentiated from the other two reported *Colwellia* species on the basis of phenotypic characteristics and DNA G+C content as follows: *C. psychrerythraea* produces red pigment, hydrolyses starch and has a growth temperature range of 0–19 °C; *C. hadaliensis* hydrolyses chitin, is obligately barophilic and has a DNA G+C content of 45.7 mol%. The similarity value between strain ABE-1^T and *C. psychrerythraea* was 92.9% on the basis of 16S rRNA sequencing, and the DNA–DNA relatedness value between strain ABE-1^T and *C. psychrerythraea* was 22%. These values also indicate that these two strains do not belong to the same species.

Colwellia-like gas vacuolate bacterium strain S51-W(gv)1 (Gosink & Staley, 1995), from the Antarctic sea, exhibits a high 16S rRNA sequence similarity value with strain ABE-1^T (97.8%). Strain ABE-1^T can

Table 1. Characteristics of genus *Colwellia* and genera that commonly occur in marine habitats

Data were obtained from the following references: Akagawa-Matsushita *et al.* (1992); Baumann *et al.* (1984); Collins & Jones (1981); D'aoust & Kunshner (1972); Deming *et al.* (1988); Dobson & Franzmann (1996); Gauthier *et al.* (1995); MacDonell & Colwell (1985); and Van Landschoot & De Ley (1983). 1, *Colwellia*; 2, *Vibrio*; 3, *Photobacterium*; 4, *Moritella*; 5, *Alteromonas*; 6, *Pseudoalteromonas*; 7, *Shewanella*; 8, *Halomonas*; and 9, *Marinomonas*. +, Positive; –, negative; ND, not determined.

	1	2	3	4	5	6	7	8	9
Na ⁺ requirement for growth	+	+	+	+	+	+	+	+	+
Fermentation of glucose	–	+	+	+	–	–	–	–	–
Respiration	+	+	+	+	+	+	+	+	+
Major isoprenoid quinone(s)	Q-8	MK-8, Q-8, Q-9	ND	ND	Q-8	Q-8	MK-7, Q-7, Q-8	Q-9	Q-8
Marine inhabitant	+	+	+	+	+	+	+	+	+
DNA G+C (mol %)	39–46	38–51	40–44	42.2	44–47	37–50	44–47	52–68	44–48

be differentiated from strain S51-W(gv)1 in that strain S51-W(gv)1 does not contain a unique *trans*-unsaturated fatty acid in the membrane lipid (Gosink & Staley, 1995). Furthermore, strain ABE-1^T is not a gas vacuolate bacterium.

DeLong *et al.* (1997) studied the evolutionary relationship of cultivated psychrophilic and barophilic deep-sea bacteria. All the strains used in their experiments were affiliated with one of five genera: *Colwellia*, *Shewanella*, *Photobacterium*, *Moritella* and a new group. Bowman *et al.* (1997c) estimated the diversity of psychrophilic bacteria from Antarctic sea ice. Their results revealed that these psychrophilic strains contained both *Colwellia* species and a closely related group. These results indicated that the genus *Colwellia* is well-distributed in cold sea environments.

DeLong *et al.* (1993) and Moyer *et al.* (1995) cloned *Colwellia*-like rRNA from isolates from Santa Barbara, California, USA, in the spring season (AGG53) and the hydrothermal vent system (PVB OTU12), respectively. These results suggested that the genus *Colwellia* consists not only of psychrophiles, but also of mesophiles or thermophiles. However, further studies are necessary to establish this. Interestingly, considering only isolated strains, there have been no reports of the isolation of *Colwellia* species from any environments other than cold ones.

On the basis of the above results, the name *Colwellia maris* sp. nov. is proposed and the type strain is designated ABE-1^T. A description of the new species is given below.

Description of *Colwellia maris* sp. nov.

Colwellia maris sp. nov. (mar'is. L. gen. n. *maris* of the sea).

Cells are curved rods (0.6–1.0 by 2–4 µm), Gram-negative and motile by means of a single polar flagellum. Catalase and oxidase reactions are positive. Acids are produced from D-glucose under aerobic conditions; however, no acids are produced in anaerobic conditions. No acids are produced from L-arabinose, D-fructose, maltose, D-mannose, melibiose and sucrose either aerobically or anaerobically. No growth is observed in the absence of NaCl in the culture medium, but growth occurs in media supplemented with 3 and 4% NaCl and not in media with salinity higher than 6.5% NaCl. Susceptibility to vibriostatic compound O/129 (10 and 150 µg) is observed. Growth occurs at 0–22 °C, but not at temperatures higher than 25 °C. Voges–Proskauer test, methyl red test and indole production are negative, but reduction of nitrate is positive. Hydrolyses gelatin, DNA and Tweens 20, 40, 60 and 80, but does not hydrolyse casein, chitin, starch and alginic acid. Utilizes D-glucose as a sole carbon and energy source for growth, but not D-mannose, raffinose, D-xylose, L-arabinose, D-fructose, glycerol, lactose, maltose, melibiose and sucrose. The major isoprenoid quinone is

Q-8. The whole-cell fatty acids contain saturated and monounsaturated fatty acids with 10–18 C atoms; saturated and mono-unsaturated C₁₆ fatty acids are predominant in cells grown at 15 °C. The strain contains a unique *trans*-unsaturated fatty acid (9-*trans*-hexadecenoic acid, C_{16:1}9t). The DNA G+C content is 39.4 mol% (determined by HPLC). The type strain of *Colwellia maris* is ABE-1^T (= JCM 10085^T).

ACKNOWLEDGEMENTS

The authors would like to thank Dr Y. Nodasaka (Hokkaido University) for his help with transmission electron microscopic observation and Dr K.-I. Suzuki (Japan Collection of Microorganisms, Institute of Physical and Chemical Research) for critically reading the manuscript.

REFERENCES

- Akagawa-Matsushita, M., Itoh, T., Katayama, Y., Kuraishi, H. & Yamasato, K. (1992). Isoprenoid quinone composition of some marine *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* species. *J Gen Microbiol* **138**, 2275–2281.
- Barrow, G. L. & Feltham, R. K. A. (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edn. Cambridge: Cambridge University Press.
- Baumann, P., Ralph, H. & Schubert, W. (1984). The *Vibrionaceae*. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 516–550. Edited by N. R. Krieg & J. H. Holt. Baltimore: Williams & Wilkins.
- Bowman, J. P., McCammon, S. A. & Skerratt, J. H. (1997a). *Methylosphaera hansonii* gen. nov., sp. nov., a psychrophilic, group I methanotroph from Antarctic marine-salinity, meromictic lakes. *Microbiology* **143**, 1451–1459.
- Bowman, J. P., McCammon, S. A., Brown, J. L., Nichols, P. D. & McMeekin, T. A. (1997b). *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria isolated from Antarctic lacustrine and sea ice habitats. *Int J Syst Bacteriol* **47**, 670–677.
- Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. (1997c). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**, 3068–3078.
- Bozal, N., Tudela, E., Rosselló-Mora, R., Lalucat, J. & Guinea, J. (1997). *Pseudoalteromonas antarctica* sp. nov., isolation from an Antarctic coastal environment. *Int J Syst Bacteriol* **47**, 345–351.
- Brosius, J., Palmer, J. L., Kennedy, J. P. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci USA* **75**, 4801–4805.
- Carbon, P., Ehresmann, C., Ehresmann, B. & Ebel, J. P. (1979). The complete nucleotide sequence of the ribosomal 16-S RNA from *Escherichia coli*. *Eur J Biochem* **100**, 399–410.
- Collins, M. D. & Jones, D. (1981). Distribution of isoprenoid quinone structure types in bacterial and their taxonomic implications. *Microbiol Rev* **45**, 316–354.
- D'aoust, J. Y. & Kunshner, D. J. (1972). *Vibrio psychroerythrus* sp. n.: classification of the psychrophilic marine bacterium, NRC 1004. *J Bacteriol* **111**, 340–342.
- DeLong, E. F., Franks, D. G. & Alldredge, A. L. (1993). Phylogenetic diversity of aggregate vs. free-living marine bacterial assemblages. *Limnol Oceanogr* **38**, 924–934.

- DeLong, E. F., Franks, D. G. & Yayanos, A. A. (1997). Evolutionary relationship of cultivated psychrophilic and barophilic deep-sea bacteria. *Appl Environ Microbiol* **63**, 2105–2108.
- Deming, J. W., Somers, L. K., Straube, W. L., Swartz, D. G. & Macdonell, M. T. (1988). Isolation of an obligately barophilic bacterium and description of a new genus, *Colwellia* gen. nov. *Syst Appl Microbiol* **10**, 152–160.
- Dobson, S. J. & Franzmann, P. D. (1996). Unification of genera *Deleya* (Baumann et al. 1983), *Halomonas* (Vreeland et al. 1980), and *Halovibrio* (Fendrich 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a single genus, *Halomonas*, and placement of the genus *Zymobacter* in the family *Halomonadaceae*. *Int J Syst Bacteriol* **46**, 550–558.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Gauthier, G., Gauthier, M. & Christen, R. (1995). Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and proposal of twelve new species combinations. *Int J Syst Bacteriol* **45**, 755–761.
- Gosink, J. J. & Staley, J. T. (1995). Biodiversity of gas vacuolate bacteria from Antarctic sea ice and water. *Appl Environ Microbiol* **61**, 3486–3489.
- Gounot, A.-M. (1976). Effects of temperature on the growth of psychrophilic bacteria from glaciers. *Can J Microbiol* **22**, 839–846.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (1994). *Bergey's Manual of Determinative Bacteriology*, 9th edn. Baltimore: Williams & Wilkins.
- Horikoshi, K. & Grant, W. D. (1991). *Superbugs*. Tokyo: Japan Scientific Societies Press.
- Irgens, R. L., Gosink, J. J. & Staley, J. T. (1996). *Polaromonas vacuolata* gen. nov., sp. nov., a psychrophilic, marine, gas vacuolate bacterium from Antarctica. *Int J Syst Bacteriol* **46**, 822–826.
- Ishii, A., Imagawa, S., Fukunaga, N., Sasaki, S., Minowa, O., Mizuno, Y. & Shiokawa, H. (1987). Isozymes of isocitrate dehydrogenase from an obligate psychrophilic bacterium, *Vibrio* sp. strain ABE-1: purification, and modulation of activities by growth conditions. *J Biochem* **102**, 1489–1498.
- Ishii, A., Suzuki, M., Sahara, T., Takada, Y., Sasaki, S. & Fukunaga, N. (1993). Genes encoding two isocitrate dehydrogenase isozymes of a psychrophilic bacterium, *Vibrio* sp. strain ABE-1. *J Bacteriol* **175**, 6873–6880.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- MacDonell, M. T. & Colwell, R. R. (1985). Phylogeny of *Vibrionaceae* and recommendation for two genera, *Listonella* and *Shewanella*. *Syst Appl Microbiol* **6**, 171–182.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J Mol Biol* **3**, 208–218.
- Morita, R. Y. (1975). Psychrophilic bacteria. *Bacteriol Rev* **39**, 144–167.
- Morita, R. Y. & Haight, R. D. (1964). Temperature effects on the growth of an obligate psychrophilic marine bacterium. *Limnol Oceanogr* **9**, 102–106.
- Moyer, C. L., Dobbs, F. C. & Karl, D. M. (1995). Phylogenetic diversity of the bacterial community from a microbial mat at an active, hydrothermal vent system, Loihi seamount, Hawaii. *Appl Environ Microbiol* **61**, 1555–1562.
- Okuyama, H., Sasaki, S., Higashi, S. & Murata, N. (1990). A trans-unsaturated fatty acid in a psychrophilic bacterium, *Vibrio* sp. strain ABE-1. *J Bacteriol* **172**, 3515–3518.
- Okuyama, H., Okajima, N., Sasaki, S., Higashi, S. & Murata, N. (1991). The *cis/trans* isomerization of the double bond of fatty acid as a strategy for adaptation to changes in ambient temperature in the psychrophilic bacterium, *Vibrio* sp. strain ABE-1. *Biochim Biophys Acta* **1084**, 13–20.
- Pacha, R. E. (1968). Characteristics of *Cytophaga psychrophila* (Borg) isolated during outbreaks of bacterial cold-water disease. *Appl Microbiol* **21**, 97–101.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Takada, Y., Ochiai, T., Okuyama, H., Nishi, K. & Sasaki, S. (1979). An obligately psychrophilic bacterium isolated on the Hokkaido coast. *J Gen Appl Microbiol* **25**, 11–19.
- Takada, Y., Fukunaga, N. & Sasaki, S. (1981). Temperature-dependence and distribution of NADH dehydrogenase in a psychrophilic bacterium, *Vibrio* sp. strain ABE-1. *J Gen Appl Microbiol* **27**, 327–337.
- Takada, Y., Fukunaga, N. & Sasaki, S. (1989a). Coupling site of the respiration-dependent sodium pump in a psychrophilic bacterium, *Vibrio* sp. strain ABE-1. *J Gen Appl Microbiol* **35**, 33–42.
- Takada, Y., Fukunaga, N. & Sasaki, S. (1989b). Identification of respiratory quinones from a psychrophilic bacterium, *Vibrio* sp. strain ABE-1. *Agric Biol Chem* **53**, 3047–3049.
- Takada, Y., Fukunaga, N. & Sasaki, S. (1991). Na⁺-driven ATP synthesis of a psychrophilic marine bacterium, *Vibrio* sp., strain ABE-1. *FEMS Microbiol Lett* **82**, 225–228.
- Tamaoka, J. & Komagata, K. (1984). Determination of base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL w: improving the sensitivity of progressive multiple sequence weighing, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Van Landschoot, A. & De Ley, J. (1983). Intra- and intergeneric similarities of the rRNA cistron of *Alteromonas*, *Marinomonas* (gen. nov.) and some other Gram-negative bacteria. *J Gen Microbiol* **129**, 3057–3074.