Psychrobacter cibarius sp. nov., isolated from jeotgal, a traditional Korean fermented seafood

Seo-Youn Jung,^{1,2} Mi-Hwa Lee,¹ Tae-Kwang Oh,¹ Yong-Ha Park^{1,2} and Jung-Hoon Yoon¹

Correspondence
Jung-Hoon Yoon
jhyoon@kribb.re.kr

¹Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejeon, Korea

²National Research Laboratory of Molecular Ecosystematics, Institute of Probiotics, Probionics Corporation, Bio-venture Center, Korea Research Institute of Bioscience and Biotechnology (KRIIBB), PO Box 115, Yusong, Taejeon, Korea

Two novel *Psychrobacter*-like bacterial strains, JG-219^T and JG-220, were isolated from jeotgal, a traditional Korean fermented seafood. Cells of strains JG-219^T and JG-220 were Gram-negative, non-motile coccobacilli. Growth of the two strains was observed at 4–32 °C. They grew optimally in the presence of 2–5 % (w/v) NaCl. Strains JG-219^T and JG-220 contained C_{18:1ω9c} and C_{17:1ω8c} as the major fatty acids and Q-8 as the predominant ubiquinone. The DNA G + C contents of strains JG-219^T and JG-220 were 43.5 and 43.0 mol%, respectively. The two strains showed no difference in their 16S rRNA gene sequences but exhibited minor differences in their phenotypic properties. Strains JG-219^T and JG-220 exhibited levels of 16S rRNA gene sequence similarity of 95.2–98.7 % to the type strains of recognized *Psychrobacter* species. The mean level of DNA–DNA relatedness between strains JG-219^T and JG-220 was 84.4 %. The two strains exhibited levels of DNA–DNA relatedness of 1.5–32.9 % to the type strains of eight phylogenetically related *Psychrobacter* species. On the basis of phenotypic data and phylogenetic and genetic distinctiveness, the two strains were classified as representing a novel species within the genus *Psychrobacter*, *Psychrobacter cibarius* sp. nov. The type strain is JG-219^T (=KCTC 12256^T=DSM 16327^T).

The genus *Psychrobacter* is classified in the family *Moraxellaceae* within the γ -*Proteobacteria* (Juni, 1991). The family also includes the genera *Moraxella* and *Acinetobacter* (Rossau *et al.*, 1991; Bowman *et al.*, 1996). At the time of writing, 17 species are recognized in the genus *Psychrobacter*, *Psychrobacter immobilis* (Juni & Heym, 1986), *Psychrobacter frigidicola*, *Psychrobacter urativorans*, *Psychrobacter phenylpyruvicus*, *Psychrobacter glacincola* (Bowman *et al.*, 1996, 1997), *Psychrobacter pacificensis* (Maruyama *et al.*, 2000), *Psychrobacter proteolyticus* (Denner *et al.*, 2001), *Psychrobacter faecalis* (Kämpfer *et al.*, 2002), *Psychrobacter submarinus*, *Psychrobacter marincola* (Romanenko *et al.*, 2002), *Psychrobacter jeotgali* (Yoon *et al.*, 2003), *Psychrobacter pulmonis* (Vela *et al.*, 2003), *Psychrobacter luti*, *Psychrobacter fozii* (Bozal *et al.*, 2003), *Psychrobacter*

okhotskensis (Yumoto *et al.*, 2003), *Psychrobacter maritimus* and *Psychrobacter arenosus* (Romanenko *et al.*, 2004). The genus *Psychrobacter* accommodates Gram-negative, coccoid or rod-shaped, oxidase-positive, non-motile, psychrophilic or psychrotolerant and halotolerant bacteria (Juni & Heym, 1986). *Psychrobacter* species have been isolated from various habitats or sources, including sea water, food, various human clinical samples, poultry, sea ice, krill and Antarctic ornithogenic soil (Juni & Heym, 1986; Shaw & Latty, 1988; Juni, 1991; Bowman *et al.*, 1996, 1997; Maruyama *et al.*, 2000; Denner *et al.*, 2001). In this study, we report on the taxonomic characterization of two strains, JG-219^T and JG-220, which were isolated from jeotgal, a traditional Korean fermented seafood. The two strains were compared with recognized *Psychrobacter* species using phenotypic characteristics and phylogenetic and genetic analyses.

Strains JG-219^T and JG-220 were isolated by the dilution plate technique on marine agar 2216 (MA; Difco) at 30 °C. Growth at various temperatures from 4 to 37 °C was measured on MA, and growth at various pH values and tolerance to NaCl were measured in marine broth 2216 (MB; Difco). Growth in the absence of NaCl was investigated in trypticase soy broth (Difco) lacking NaCl. Growth

Published online ahead of print on 5 November 2004 as DOI 10.1099/ijs.0.63398-0.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains JG-219^T and JG-220 are AY639871 and AY639872, respectively.

DNA–DNA relatedness data and a neighbour-joining tree are available as supplementary material in IJSEM Online.

under anaerobic conditions was determined after incubation in an anaerobic chamber on anaerobically prepared MA. Cell morphology and presence of flagella were examined by light microscopy (Nikon E600) and transmission electron microscopy of cells grown on MA. Gram reaction was determined using the bioMérieux Gram staining kit according to the manufacturer's instructions. Catalase activity was determined by bubble production in a 3% (v/v) hydrogen peroxide solution on MA. Oxidase activity was determined by oxidation of 1% (w/v) *p*-aminodimethylaniline oxalate. Nitrate reduction was investigated as described by Lanyi (1987). Hydrolysis of hypoxanthine, tyrosine and xanthine was performed on MA using the substrate concentrations recommended by Cowan & Steel (1965). Urease activity and hydrolysis of Tween 80, casein, gelatin, aesculin and starch were determined as described by Cowan & Steel (1965). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of various substrates for growth was determined as described by Yurkov *et al.* (1994). Requirements of yeast extract (0.005 g), biotin (10 µg), *p*-aminobenzoic acid (1 mg), thiamin hydrochloride (1 mg) and vitamin B₁₂ (1 mg) were investigated in the liquid medium as used for the substrate utilization test supplemented with 0.1% (w/v) acetate as the sole carbon and energy source. The API ZYM system (bioMérieux) was used to determine enzyme activity. Antibiotic sensitivity was tested by spreading bacterial suspension on MA and applying discs impregnated with the following antibiotics (content per disc): ampicillin (10 µg), penicillin G (10 U), lincomycin (15 µg), polymyxin B (300 U), streptomycin (30 µg), tetracycline (30 µg) and neomycin (15 µg).

Cell biomass for isoprenoid quinone analysis and for DNA extraction was obtained from cultivation for 3 days in MB at 30 °C. Respiratory lipoquinones were analysed as described by Komagata & Suzuki (1987) using reversed-phased HPLC. For fatty acid methyl ester (FAME) analysis, cell mass of strains JG-219^T and JG-220 was harvested from agar plates after cultivation for 6 days on MA at 30 °C. FAMES were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990).

Chromosomal DNA was extracted and purified by the procedure described by Yoon *et al.* (1996). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA reassociation was determined by the microplate hybridization method (Ezaki *et al.*, 1989) using photobiotin-labelled DNA probes. Type strains of *P. fozii*, *P. okhotskensis*, *P. pulmonis*, *P. luti*, *P. faecalis*, *P. glacincola*, *P. proteolyticus* and *P. urativorans* were used as reference strains for DNA–DNA hybridization. *P. faecalis* DSM 14664^T, *P. glacincola* DSM 12194^T, *P. proteolyticus* DSM 13887^T and *P. urativorans* DSM 14009^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig,

Germany. *P. fozii* CECT 5889^T, *P. luti* CECT 5885^T and *P. pulmonis* CECT 5989^T were obtained from the Colección Española de Cultivos Tipo (CECT), Burjassot, Spain. *P. okhotskensis* JCM 11840^T was obtained from the Japan Collection of Microorganisms (JCM), Wako-shi, Japan.

16S rRNA gene sequence amplification was performed according to the method described by Yoon *et al.* (1998) using two universal primers. Sequencing of the amplified 16S rRNA gene was performed as described by Yoon *et al.* (2003). Alignment of sequences was carried out with the CLUSTAL W program (Thompson *et al.*, 1994) and gaps at the 5' and 3' ends of the alignment were omitted from further analysis. Evolutionary distances were calculated using the Kimura two-parameter correction with the CLUSTAL W package (Thompson *et al.*, 1994). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) on the basis of distance matrix data. The reliability of grouping was assessed by 1000 bootstrap resamplings of the neighbour-joining dataset using the CLUSTAL W package.

Morphological, cultural, physiological and biochemical characteristics of strains JG-219^T and JG-220 are shown in Table 1 or are given under the species description below. The major respiratory lipoquinone of strains JG-219^T and JG-220 was ubiquinone-8 (Q-8), at peak area ratios of approximately 83 and 86%, respectively. The result of cellular fatty acid analysis of strains JG-219^T and JG-220 is summarized in Table 2. The two strains had cellular fatty acid profiles that contained large amounts of straight-chain and unsaturated fatty acids. These fatty acid profiles were similar to those of *Psychrobacter* species in containing C_{18:1ω9c} and C_{17:1ω8c} as the predominant components (Bozal *et al.*, 2003; Romanenko *et al.*, 2002, 2004; Yoon *et al.*, 2003; Yumoto *et al.*, 2003). The DNA G+C contents of strains JG-219^T and JG-220 were 43.5 and 43.0 mol%, respectively.

The almost-complete 16S rRNA gene sequences of strains JG-219^T and JG-220 determined in this study comprised 1494 nt. The two strains showed no difference in their 16S rRNA gene sequences. Comparative 16S rRNA gene sequence analysis and estimation of phylogenetic relationships showed that strains JG-219^T and JG-220 are most closely related to members of the genus *Psychrobacter* (Fig. 1). The two strains exhibited 16S rRNA gene sequence similarity levels of 98.7, 98.7, 98.2, 98.1, 97.9, 97.7, 97.6 and 97.0% to the type strains of *P. fozii*, *P. okhotskensis*, *P. pulmonis*, *P. luti*, *P. faecalis*, *P. glacincola*, *P. proteolyticus* and *P. urativorans*, respectively. The two strains exhibited 16S rRNA gene sequence similarity values of less than 95.2% to the type strains of the other *Psychrobacter* species. Strains JG-219^T and JG-220 exhibited a mean DNA–DNA relatedness value of 84.4% when their DNAs were used individually as a labelled DNA probe for cross-hybridization. This value indicated that strains JG-219^T and JG-220 were members of the same genomic species (Wayne *et al.*, 1987). Levels of DNA–DNA relatedness between strains JG-219^T

Table 1. Differential phenotypic characteristics of *Psychrobacter* species

Species: 1, *P. cibarius* sp. nov.; 2, *P. immobilis* (Juni & Heym, 1986; Bowman *et al.*, 1996); 3, *P. frigidicola* (Bowman *et al.*, 1996); 4, *P. glacincola* (Bowman *et al.*, 1997; Romanenko *et al.*, 2002); 5, *P. phenylpyruvicus* (Bowman *et al.*, 1996); 6, *P. urativorans* (Bowman *et al.*, 1996); 7, *P. marincola* (Romanenko *et al.*, 2002); 8, *P. submarinus* (Romanenko *et al.*, 2002); 9, *P. proteolyticus* (Denner *et al.*, 2001; Kämpfer *et al.*, 2002; Bozal *et al.*, 2003); 10, *P. luti* (Bozal *et al.*, 2003); 11, *P. fozii* (Bozal *et al.*, 2003); 12, *P. jeotgali* (Yoon *et al.*, 2003); 13, *P. pulmonis* (Vela *et al.*, 2003); 14, *P. pacificensis* (Maruyama *et al.*, 2000); 15, *P. okhotskensis* (Yumoto *et al.*, 2003). +, Positive reaction; –, negative reaction; ND, not determined; w, weakly positive reaction; v (+), variable reaction but type strain positive; v (–), variable reaction but type strain negative. All species were positive for catalase and oxidase.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Urease activity	–	v (+)	–	v (–)	+	v (+)	–	–	+	–	+	+	–	+	–
Nitrate reduction	+	v (–)	–	v (+)	+	v (–)	–	–	–	+	–	+	+	–	+
Tween 80 hydrolysis	+	+	–	+	–	–	+	+	w	+	–	+	ND	ND	+
Acid production from carbohydrates	–	+	–	–	–	–	–	+	+	–	–	–	–	+	–
Maximum growth temperature	32	35–39	19–22	19–22	39	25–27	35	35	<35	30	30	36	<42	38	35
Optimum growth temperature (°C)	25–30	27–31	14–16	13–15	32	17–19	25–28	25–28	19–25	15	15	25–30	ND	25	25
Utilization of:															
Acetate	+	+	+	+	+	v (+)	–	–	+	+	v (+)	+	ND	v (–)	ND
Pyruvate	+ or w	+	–	+	+	+	ND	ND	ND	ND	+	+	ND	ND	+
Succinate	v (+)	+	–	–	–	–	ND	ND	ND	+	+	+	ND	ND	ND
Citrate	–	v (–)	–	+	+	–	–	–	+	+	–	–	–	–	ND
Enzymic activity															
Alkaline phosphatase	v (+)	v (–)	–	–	+	–	ND	ND	+	+	+	+	–	ND	ND
Esterase lipase (C8)	+	+	+	+	+	+	ND	ND	+	+	+	+	+	ND	ND
Leucine arylamidase	+	+	+	+	+	+	ND	ND	+	+	+	+	+	ND	ND
Valine arylamidase	v (+)	–	–	–	–	–	ND	ND	w	–	–	–	+	ND	ND
Naphthol-AS-BI-phosphohydrolase	+	+	ND	–	–	–	ND	ND	+	–	–	v (–)	–	ND	ND
Sensitivity to antibiotics															
Ampicillin (10 µg per disc)	+	–	+	–	+	w	+	+	ND	ND	ND	ND	ND	ND	ND
Penicillin G (10 U per disc)	+	–	–	–	+	–	–	–	ND	ND	ND	ND	ND	ND	ND
Lincomycin (15 µg per disc)	–	–	–	–	–	–	+	–	ND	ND	ND	ND	ND	ND	ND
Polymyxin B (300 U per disc)	+	+	+	+	+	+	+	+	ND	ND	ND	ND	ND	ND	ND
Streptomycin (30 µg per disc)	+	+	+	+	+	+	+	+	ND	ND	ND	ND	ND	ND	ND
Tetracycline (30 µg per disc)	–	–	w	–	+	+	+	–	ND	ND	ND	ND	ND	ND	ND
Neomycin (15 µg per disc)	+	+	+	+	w	+	+	+	ND	ND	ND	ND	ND	ND	ND
Major fatty acid(s)	C _{18:1} ω9c C _{17:1} ω8c	C _{18:1} ω9c C _{16:1} ω7c	C _{18:1} ω9c C _{16:1} ω7c	C _{18:1} ω9c C _{16:1} ω7c	C _{18:1} ω9c C _{16:1} ω7c	C _{16:1} ω7c C _{18:1} ω9c	C _{18:1} ω9c	C _{18:1} ω9c	C _{18:1} ω9c C _{16:1} ω7c	C _{18:1} ω9c C _{16:1} ω7c	C _{18:1} ω9c C _{16:1} ω7c	C _{18:1} ω9c	C _{18:1} ω9c	ND	C _{18:1} ω9c C _{17:1}
DNA G+C content (mol%)	43.0–43.5	44–47	41–42	43–44	43	44–46	50.7	46.8	43.6	45	44–46	43–44	ND	43–44	46.7

Table 2. Cellular fatty acid compositions (%) of strains JG-219^T and JG-220

Fatty acid*	Strain JG-219 ^T	Strain JG-220
Straight-chain fatty acid		
C _{9:0}	2.3	2.4
C _{10:0}	2.0	2.7
C _{16:0}	0.9	1.5
C _{17:0}	1.7	1.8
C _{18:0}	2.9	4.6
Unsaturated fatty acid		
C _{17:1ω8c}	20.7	19.4
C _{18:1ω9c}	53.6	57.0
Hydroxy fatty acid		
C _{12:0} 3-OH	2.4	3.0
Summed feature†		
1	1.8	1.8
3	2.6	3.0
6	4.1	1.3

*Fatty acids representing less than 1.0% in the two strains were omitted.

†Summed feature represents groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contained one or more of C_{13:0} 3-OH, iso-C_{15:1} I and/or iso-C_{15:1} H. Summed feature 3 contained one or more of C_{16:1ω7c} and/or iso-C_{15:0} 2-OH. Summed feature 6 contained one or more of C_{19:1ω11c} and/or C_{19:1ω9c}.

and JG-220 and the type strains of *P. fozii*, *P. okhotskensis*, *P. pulmonis*, *P. luti*, *P. faecalis*, *P. glacincola*, *P. proteolyticus* and *P. urativorans* were in the range 1.5–32.9% (DNA–DNA relatedness data are available as supplementary material in IJSEM Online).

The phenotypic characteristics of strains JG-219^T and JG-220 were similar to those of members of the genus *Psychrobacter* (Table 1). Strains JG-219^T and JG-220 are similar in most phenotypic characteristics except for properties such as utilization of some carbon sources and some enzyme activities (Table 1). The predominant respiratory lipoquinone and fatty acid profiles of strains JG-219^T and JG-220 were similar to those of the type strains of the other *Psychrobacter* species (Bozal *et al.*, 2003; Romanenko *et al.*, 2002, 2004; Yoon *et al.*, 2003; Yumoto *et al.*, 2003). The phylogenetic distinctiveness and DNA–DNA relatedness data provide evidence that strains JG-219^T and JG-220 differ from *Psychrobacter* species with validly published names. On the basis of the data presented, strains JG-219^T and JG-220 should be placed as members of a novel species of the genus *Psychrobacter*, for which the name *Psychrobacter cibarius* sp. nov. is proposed.

Description of *Psychrobacter cibarius* sp. nov.

Psychrobacter cibarius (ci.ba.ri'us. L. masc. adj. *cibarius* relating to food).

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strains JG-219^T and JG-220 and related taxa, based on 16S rRNA gene sequence analysis. Only bootstrap values (expressed as percentages of 1000 replications) of greater than 50% are shown at the branch points. Bar, 0.01 substitution per nucleotide position. The tree from which Fig. 1 was taken is available as supplementary material in IJSEM Online.

Cells are Gram-negative, non-motile coccobacilli. Growth occurs at 4–32 °C (optimum 25–30 °C). Optimum pH for growth is 7.0–8.0; growth occurs weakly at pH 5.0, but not at 4.5. Growth occurs in the presence of 10 % (w/v) NaCl, but not in the presence of >11 % NaCl; optimum growth occurs in the presence of 2–5 % (w/v) NaCl. Growth does not occur without NaCl. Anaerobic growth does not occur on MA. Yeast extract, biotin, *p*-aminobenzoic acid, thiamin hydrochloride and vitamin B₁₂ are not required for growth. Tyrosine is hydrolysed, but casein, gelatin, aesculin, hypoxanthine, xanthine and starch are not. Nitrate is reduced to nitrite. Using the API ZYM system (bioMérieux), esterase (C4), lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. DL-Alanine and DL-malate are utilized as sole carbon and energy source. D-Glucose, D-fructose, D-mannose, sucrose, D-cellobiose, D-mannitol, glycerol, D-gluconate, L-glutamate, aspartate, L-arginine, hexadecane, lactate, formate, methanol, ethanol and benzoate are not utilized. Utilization of butyrate and L-serine is variable (positive for type strain). Acid is not produced from D-sorbitol, *myo*-inositol, D-xylose, D-ribose, D-fructose, D-mannitol, melibiose, L-arabinose, D-melezitose, D-glucose, D-galactose, adonitol, L-rhamnose, D-mannose, D-cellobiose, lactose, sucrose, maltose, D-trehalose or D-raffinose. The major fatty acids are C_{18:1} ω 9c and C_{17:1} ω 8c. The predominant ubiquinone is Q-8. The DNA G+C content is 43.0–43.5 mol% (HPLC). Other characteristics are given in Table 1.

The type strain, JG-219^T (= KCTC 12256^T = DSM 16327^T), was isolated from jeotgal, a traditional Korean fermented seafood.

Acknowledgements

This work was supported by the 21C Frontier Program of Microbial Genomics and Applications (grant MG02-0401-001-1-0-0) and the NRL research programme (grant M10104000294-01J000012800) from the Ministry of Science and Technology (MOST) of the Republic of Korea.

References

- Bowman, J. P., Cavanagh, J., Austin, J. J. & Sanderson, K. (1996). Novel *Psychrobacter* species from Antarctic ornithogenic soils. *Int J Syst Bacteriol* **46**, 841–848.
- Bowman, J. P., Nichols, D. S. & McMeekin, T. A. (1997). *Psychrobacter glacincola* sp. nov., a halotolerant, psychrophilic bacterium isolated from Antarctic sea ice. *Syst Appl Microbiol* **20**, 209–215.
- Bozal, N., Montes, M. J., Tudela, E. & Guinea, J. (2003). Characterization of several *Psychrobacter* strains isolated from Antarctic environments and description of *Psychrobacter luti* sp. nov. and *Psychrobacter fozii* sp. nov. *Int J Syst Evol Microbiol* **53**, 1093–1100.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Denner, E. B. M., Mark, B., Busse, H.-J., Turkiewicz, M. & Lubitz, W. (2001). *Psychrobacter proteolyticus* sp. nov., a psychrotrophic, halotolerant bacterium isolated from the Antarctic krill *Euphausia superba* Dana, excreting a cold-adapted metalloprotease. *Syst Appl Microbiol* **24**, 44–53.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution 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.
- Juni, E. (1991). The genus *Psychrobacter*. In *The Prokaryotes*, pp. 3241–3246. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Juni, E. & Heym, G. A. (1986). *Psychrobacter immobilis* gen. nov., sp. nov.: genospecies composed of gram-negative, aerobic, oxidase-positive coccobacilli. *Int J Syst Bacteriol* **36**, 388–391.
- Kämpfer, P., Albrecht, A., Buczolits, S. & Busse, H.-J. (2002). *Psychrobacter faecalis* sp. nov., a new species from a bioaerosol originating from pigeon faeces. *Syst Appl Microbiol* **25**, 31–36.
- Komagata, K. & Suzuki, K.-I. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Maruyama, A., Honda, D., Yamamoto, H., Kitamura, K. & Higashihara, T. (2000). Phylogenetic analysis of psychrophilic bacteria isolated from the Japan Trench, including a description of the deep-sea species *Psychrobacter pacificensis* sp. nov. *Int J Syst Evol Microbiol* **50**, 835–846.
- Romanenko, L. A., Schumann, P., Rohde, M., Lysenko, A. M., Mikhailov, V. V. & Stackebrandt, E. (2002). *Psychrobacter submarinus* sp. nov. and *Psychrobacter marincola* sp. nov., psychrophilic halophiles from marine environments. *Int J Syst Evol Microbiol* **52**, 1291–1297.
- Romanenko, L. A., Lysenko, A. M., Rohde, M., Mikhailov, V. V. & Stackebrandt, E. (2004). *Psychrobacter maritimus* sp. nov. and *Psychrobacter arenosus* sp. nov., isolated from coastal sea ice and sediments of the Sea of Japan. *Int J Syst Evol Microbiol* **54**, 1741–1745.
- Rossau, R., Van Landschoot, A., Gillis, M. & De Ley, J. (1991). Taxonomy of *Moraxellaceae* fam. nov., a new bacterial family to accommodate the genera *Moraxella*, *Acinetobacter*, and *Psychrobacter* and related organisms. *Int J Syst Bacteriol* **41**, 310–319.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*. Newark, DE: MIDI Inc.
- Shaw, B. G. & Latty, J. B. (1988). A numerical taxonomic study of non-motile non-fermentative gram-negative bacteria from foods. *J Appl Bacteriol* **65**, 7–21.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA 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 alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Vela, A. I., Collins, M. D., Latre, M. V., Mateos, A., Moreno, M. A., Hutson, R., Domínguez, L. & Fernández-Garayzábal, J. F. (2003).

Psychrobacter pulmonis sp. nov., isolated from the lungs of lambs. *Int J Syst Evol Microbiol* **53**, 415–419.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.

Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.

Yoon, J.-H., Kang, K. H. & Park, Y.-H. (2003). *Psychrobacter jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **53**, 449–454.

Yumoto, I., Hirota, K., Sogabe, Y., Nodasaka, Y., Yokota, Y. & Hoshino, T. (2003). *Psychrobacter okhotskensis* sp. nov., a lipase-producing facultative psychrophile isolated from the coast of the Okhotsk Sea. *Int J Syst Evol Microbiol* **53**, 1985–1989.

Yurkov, V., Stackebrandt, E., Holmes, A. & 7 other authors (1994). Phylogenetic positions of novel aerobic, bacteriochlorophyll *a*-containing bacteria and description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov. *Int J Syst Bacteriol* **44**, 427–434.