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

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Two Gram-negative, non-motile, non-spore-forming, moderately halophilic strains, JG-100^T and JG-102, were isolated from squid jeotgal, a traditional Korean fermented seafood. The two strains grew optimally at 30 °C and in the presence of 2–3 % (v/w) NaCl. Strains JG-100^T and JG-102 were characterized chemotaxonomically; they both had ubiquinone-8 as the predominant respiratory lipoquinone and C_{18:1}ω₉c as the major fatty acid. Their DNA G+C content was 44 mol%. Strains JG-100^T and JG-102 showed 1 bp difference in their 16S rRNA gene sequences and a mean DNA–DNA relatedness level of 88 %. Phylogenetic analysis based on 16S rRNA gene sequences showed that strains JG-100^T and JG-102 form a distinct phylogenetic lineage within the cluster comprising *Psychrobacter* species. The 16S rRNA gene sequences of strains JG-100^T and JG-102 had similarity levels of 95.2–98.4 % to sequences of the type strains of recognized *Psychrobacter* species. Levels of DNA–DNA relatedness between strains JG-100^T and JG-102 and the type strains of some phylogenetically related *Psychrobacter* species were 6–24 %. On the basis of phenotypic and phylogenetic data and genomic distinctiveness, strains JG-100^T and JG-102 should be placed in the genus *Psychrobacter* as a novel species, for which the name *Psychrobacter alimentarius* sp. nov. (type strain, JG-100^T=KCTC 12186^T=DSM 16065^T) is proposed.

Members of the genus *Psychrobacter* are widely distributed in various habitats, including fish, poultry, food, clinical specimens, Antarctic ornithogenic soils and sea water (Juni, 1991; Juni & Heym, 1986; Shaw & Latty, 1988; Bowman *et al.*, 1996; Vela *et al.*, 2003). Recently, many *Psychrobacter* species have been isolated from marine locations and related environments/materials and innovations in the study of bacterial systematics have led to a considerable increase in the number of isolates identified as *Psychrobacter* species (Bowman *et al.*, 1997; Maruyama *et al.*, 2000; Denner *et al.*, 2001; Romanenko *et al.*, 2002; Bozal *et al.*, 2003; Yoon *et al.*, 2003; Yumoto *et al.*, 2003). Currently, the genus *Psychrobacter* comprises 17 valid species: *Psychrobacter immobilis* (Juni & Heym, 1986); *Psychrobacter frigidicola*, *Psychrobacter urativorans* and *Psychrobacter phenylpyruvicus* (Bowman

et al., 1996); *Psychrobacter glacincola* (Bowman *et al.*, 1997); *Psychrobacter pacificensis* (Maruyama *et al.*, 2000); *Psychrobacter proteolyticus* (Denner *et al.*, 2001); *Psychrobacter submarinus* and *Psychrobacter marincola* (Romanenko *et al.*, 2002); *Psychrobacter faecalis* (Kämpfer *et al.*, 2002); *Psychrobacter pulmonis* (Vela *et al.*, 2003); *Psychrobacter jeotgali* (Yoon *et al.*, 2003); *Psychrobacter luti* and *Psychrobacter fozii* (Bozal *et al.*, 2003); *Psychrobacter okhotskensis* (Yumoto *et al.*, 2003); and *Psychrobacter maritimus* and *Psychrobacter arenosus* (Romanenko *et al.*, 2004). Recently, two Gram-negative, moderately halophilic bacterial strains, JG-100^T and JG-102, were isolated from squid jeotgal, a traditional Korean fermented seafood. 16S rRNA gene sequence analysis has shown that these organisms are phylogenetically related to members of the genus *Psychrobacter*. *P. jeotgali* has been recently isolated from shrimp jeotgal (Yoon *et al.*, 2003). Accordingly, the aim of the present study was to determine the exact taxonomic positions of strains JG-100^T and JG-102 by polyphasic taxonomic characterization.

Strains JG-100^T and JG-102 were isolated by the dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C from jeotgal that had been prepared with squid. The type

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JG-100^T and JG-102 are AY513645 and AY513646, respectively.

A table showing the fatty acid composition of strains JG-100^T and JG-102 is available as supplementary material in IJSEM Online.

strains of 13 *Psychrobacter* species were used as reference strains for DNA–DNA hybridization: *P. faecalis* DSM 14664^T, *P. submarinus* DSM 14161^T, *P. marincola* DSM 14160^T, *P. glacincola* DSM 12194^T, *P. frigidicola* DSM 12411^T, *P. urativorans* DSM 14009^T and *P. proteolyticus* DSM 13887^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. *P. okhotskensis* JCM 11840^T was obtained from the Japan Collection of Microorganisms (JCM), Saitama, Japan. *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), Valencia, Spain. *P. jeotgali* KCCM 41559^T was obtained from the study of Yoon *et al.* (2003). *P. maritimus* KMM 3646^T was obtained from Dr Olga I. Nedashkovskaya, Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Science, Russia. Cell biomass for respiratory lipoquinone analysis and DNA extraction was obtained from marine broth 2216 (MB; Difco) cultures at 30 °C. All strains were cultivated on a gyratory shaker at 150 r.p.m. For fatty acid methyl ester analysis, cell mass of strains JG-100^T and JG-102 was obtained from agar plates after incubation for 6 days at 30 °C on MA. Cell morphology was examined using a Nikon E600 light microscopy and a Philips CM-20 transmission electron microscope. The presence or absence of flagella was determined using transmission electron microscopy with cells from exponentially growing cultures. Gram reaction was determined using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Growth at various NaCl concentrations was investigated in MB or trypticase soy broth. Growth in the absence of NaCl was investigated in trypticase soy broth. Growth at various temperatures (4–45 °C) was measured on MA. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber with anaerobically prepared MA. All physiological and biochemical tests were performed aerobically at 30 °C, except for temperature range determinations. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and nitrate reduction were determined as described previously (Lanyi, 1987). Hydrolysis of casein, starch, and Tween 20, 40, 60 and 80 was determined as described by Cowan & Steel (1965). Hydrolysis of gelatin and urea was determined as described by Lanyi (1987), with the modification that artificial sea water was used. The artificial sea water contained (per litre of distilled water): 23.6 g NaCl; 0.64 g KCl; 4.53 g MgCl₂·6H₂O; 5.94 g MgSO₄·7H₂O; and 1.3 g CaCl₂·2H₂O (Levring, 1946). Hydrolysis of hypoxanthine, tyrosine and xanthine was examined on MA using the substrate concentrations described previously (Cowan & Steel, 1965). H₂S production was tested as described previously (Bruns *et al.*, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). Enzyme activity was determined using the API ZYM system (bioMérieux). Other physiological tests were performed with the API 20E system (bioMérieux). Utilization of various substrates for growth was determined as described by Yurkov *et al.* (1994). Growth requirements for yeast

extract and vitamins were determined in the liquid medium used for substrate utilization tests, with yeast extract and vitamin B₁₂ omitted and supplemented with 0.1 % (w/v) acetate as the sole carbon and energy sources. Yeast extract and vitamins were added to the medium in the following amounts (l⁻¹): yeast extract, 0.005 g; *p*-aminobenzoic acid, 1 mg; biotin, 10 µg; thiamine hydrochloride, 1 mg; and vitamin B₁₂, 1 mg. Susceptibility to antibiotics was detected after 2–3 days on MA plates using discs containing the following antibiotics: polymyxin B, 100 U; streptomycin, 50 µg; penicillin, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; erythromycin, 15 µg; and tetracycline, 30 µg.

Chromosomal DNA was isolated and purified according to a method described previously (Yoon *et al.*, 1996), with the exception that ribonuclease T1 was used together with ribonuclease A. Respiratory lipoquinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. For quantitative analysis of cellular fatty acid composition, a loopful of cell mass was harvested and fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G + C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). DNA–DNA hybridization was performed fluorometrically using the method of Ezaki *et al.* (1989) with photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. Of the values obtained, the highest and lowest values for each sample were excluded; DNA relatedness values were calculated as the means of the remaining three values.

Strains JG-100^T and JG-102 were Gram-negative, non-spore-forming, non-motile cocci (1.4–2.0 µm in diameter) on MA. Neither strain required yeast extract or vitamins for their growth in minimal salt medium. However, both strains grew better in the presence of yeast extract than in the presence of vitamins. Ethanol and benzoate were utilized only by strain JG-102. Other morphological, cultural, physiological and biochemical characteristics of strains JG-100^T and JG-102 are given in the species description or are shown in Table 1 together with those of some *Psychrobacter* species.

The predominant respiratory lipoquinone found in strains JG-100^T and JG-102 was ubiquinone-8 (Q-8) at a peak ratio of approximately 88–90 %. Strains JG-100^T and JG-102 had cellular fatty acid profiles that contained large amounts of the unsaturated fatty acids C_{18:1}ω9c (46.5 and 54.8 %, respectively) and C_{17:1}ω8c (16.6 and 13.4 %, respectively) (see the supplementary table available in IJSEM Online). These fatty acid profiles have been observed in other

Table 1. Differential phenotypic characteristics of *Psychrobacter alimentarius* and some other *Psychrobacter* species

1, *P. alimentarius* ($n=2$); 2, *P. submarinus* ($n=1$; Romanenko *et al.*, 2002); 3, *P. marincola* ($n=1$; Romanenko *et al.*, 2002); 4, *P. jeotgali* ($n=2$; Yoon *et al.*, 2003); 5, *P. faecalis* ($n=1$; Kämpfer *et al.*, 2002); 6, *P. pulmonis* ($n=2$; Vela *et al.*, 2003); 7, *P. proteolyticus* ($n=1$; Denner *et al.*, 2001; Bozal *et al.*, 2003); 8, *P. glacincola* ($n=4$; Bowman *et al.*, 1997; Romanenko *et al.*, 2002); 9, *P. okhotskensis* ($n=1$; Yumoto *et al.*, 2003); 10, *P. luti* ($n=1$; Bozal *et al.*, 2003); 11, *P. fozii* ($n=2$; Bozal *et al.*, 2003); 12, *P. immobilis* ($n=20$; Juni & Heym, 1986; Bowman *et al.*, 1996; Romanenko *et al.*, 2002); 13, *P. frigidicola* ($n=4$; Bowman *et al.*, 1996; Romanenko *et al.*, 2002). +, Positive reaction; –, negative reaction; ND, not determined; w, weakly positive reaction; v, variable reaction. Data given in parentheses are for the type strain. All species were positive for catalase and oxidase. All species were negative for Gram-staining, spore formation, motility, anaerobic growth, acid production from D-mannitol, and utilization of D-glucose, arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Cell morphology	Cocci	Ovoid	Cocci	Cocci	Rods	Cocci	Coccobacilli	Cocci	Rods	Coccobacilli	Coccobacilli	Coccobacilli	Cocci
Urease	–	–	–	+	–	–	+	v(–)	–	–	+	v(+)	–
Tryptophan deaminase	–	–	–	–	ND	–	–	–	ND	–	–	v(–)	+
Nitrate reduction	–	–	–	+	ND	+	–	v(+)	+	+	–	v(–)	–
Hydrolysis of:													
Casein	–	–	–	–	ND	ND	+	–	–	+	–	–	–
Gelatin	–	–	–	–	ND	–	+	–	–	–	–	–	–
Tween 40	+	+	–	ND	ND	ND	ND	+	+	ND	ND	+	–
Tween 80	+	+	+	+	ND	ND	w	+	+	+	–	+	–
Growth in:													
0% NaCl	+	–	–	+	ND	ND	ND	+	+	+	v(+)	+	+
10% NaCl	+	+	+	+	+	ND	+	+	+	–	+	v(+)	–
15% NaCl	–	+	+	–	ND	ND	ND	–	–	–	–	–	–
Growth temperature (maximum; °C)	35	35	35	36	36	<42	<35	19–22	35	30	30	(<35–37)	19–22
Optimum growth temperature (°C)	30	25–28	25–28	25–30	15–30	ND	19–25	13–15	25	15	15	(20)	14–16
Acid production from:													
D-Glucose	+	–	–	–	–	–	+	–	–	–	–	+	–
D-Xylose	+	–	–	–	–	ND	ND	–	–	–	–	+	–
L-Arabinose	+	+	–	–	–	–	+	–	–	–	–	+	–
D-Fructose	–	+	–	–	ND	ND	ND	–	–	–	–	–	–
L-Rhamnose	+	–	–	–	–	–	+	–	ND	–	–	+	–
D-Galactose	+	–	–	–	ND	ND	ND	–	–	–	–	+	–
Maltose	–	–	–	–	–	ND	ND	–	–	–	–	–	–
Lactose	+	–	–	–	–	ND	ND	–	–	–	–	v(–)	–
Utilization of:													
Acetate	+	–	–	+	+	ND	+	+	ND	+	v(+)	+	+
Pyruvate	+	ND	ND	+	+	ND	ND	+	+	ND	+	+	–
Citrate	+	–	–	–	+	–	+	v	ND	+	–	v(–)	–
Malate	+	–	–	v(–)	+	–	–	–	+	+	+	+	+
Succinate	+	ND	ND	+	ND	ND	ND	–	ND	+	+	+	–
Lactate	–	–	–	–	+	ND	ND	+	ND	ND	ND	+	–
Enzymic activities (API ZYM):													
Acid phosphatase	w	ND	ND	–	ND	–	+	–	ND	–	v(–)	–	–
Alkaline phosphatase	+	ND	ND	+	ND	–	+	–	ND	+	+	v(+)	–
Esterase (C4)	+	ND	ND	+	ND	+	+	–	ND	+	+	v(+)	–
Lipase (C14)	–	ND	ND	–	ND	–	–	+	ND	+	–	v(+)	–
Cystine arylamidase	–	ND	ND	+	ND	+	–	–	ND	–	v(+)	–	–
Valine arylamidase	–	ND	ND	–	ND	+	w	–	ND	–	–	–	–
Naphthol-AS-BI-phosphohydrolase	w	ND	ND	v(w)	ND	–	+	+	ND	–	–	ND	–
Sensitivity to antibiotics (per disc):													
Ampicillin (10 µg)	v(w)	+	+	ND	ND	ND	ND	–	ND	ND	ND	(–)	(+)
Gentamicin (10 µg)	+	+	+	ND	ND	ND	ND	+	ND	ND	ND	(+)	(w)
Polymyxin B (300 U)	+	+	+	ND	ND	ND	ND	+	ND	ND	ND	(+)	(+)
Streptomycin (30 µg)	+	+	+	ND	ND	ND	ND	+	ND	ND	ND	(+)	(+)
Tetracycline (30 µg)	–	–	+	ND	ND	ND	ND	–	ND	ND	ND	(–)	(w)
DNA G+C content (mol%)	44	47	51	43–44	ND	ND	44	43–44	47	45	44–46	44–47	41–42

Psychrobacter species, although there are differences in the proportions of some fatty acids, which may be caused by different cultivation conditions, e.g. cultivation media (Maruyama *et al.*, 2000; Romanenko *et al.*, 2002; Bozal *et al.*, 2003). The DNA G+C contents of strains JG-100^T and JG-102 were the same (44 mol%).

The almost-complete 16S rRNA gene sequences of strains JG-100^T and JG-102 determined in this study comprised 1494 nt, corresponding to the region between positions 28 and 1524 by comparison with the *Escherichia coli* 16S rRNA gene sequence. The sequences of strains JG-100^T and JG-102 had a 1 bp difference in the region compared. 16S rRNA gene sequence comparison revealed that the two strains had highest similarity to members of the γ -*Proteobacteria*, particularly to species of the genus *Psychrobacter*. Phylogenetic trees based on 16S rRNA gene sequences showed that strains JG-100^T and JG-102 fell within the radiation of the cluster comprising *Psychrobacter* species (Fig. 1). Strains JG-100^T and JG-102 exhibited 16S rRNA gene sequence similarity levels of 95.2–98.5 % and 95.3–98.5 %, respectively, to the type strains of all validly published *Psychrobacter* species. The sequence similarities between strains JG-100^T and JG-102 and all other taxa included in the

phylogenetic analysis were less than 93.2 %. DNA–DNA hybridization was performed to determine the genomic relatedness between strains JG-100^T and JG-102 and between the two strains and the type strains of some phylogenetically related *Psychrobacter* species. Strains JG-100^T and JG-102 exhibited a mean level of DNA–DNA relatedness of approximately 88 % when their DNAs were used individually as labelled DNA probes for cross-hybridization. Levels of DNA–DNA relatedness between these two strains and the type strains of 13 *Psychrobacter* species, which had 16S rRNA gene sequence similarity values of more than 97 % to strains JG-100^T and JG-102, were in the range 6–24 %.

In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, the cluster comprising strains JG-100^T and JG-102 and *Psychrobacter* species was distinguished from the genus *Moraxella* by a bootstrap confidence level of 100 % (Fig. 1). Chemotaxonomic data for the isolates, i.e. predominant respiratory lipoquinone, cellular fatty acid profile and DNA G+C content, are similar to those of the genus *Psychrobacter* (Yoon *et al.*, 2003; Yumoto *et al.*, 2003). Strains JG-100^T and JG-102 show almost identical phenotypic characteristics (Table 1). The genomic

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strains JG-100^T and JG-102 and representatives of some other related taxa based on 16S rRNA gene sequences. Bar, 0.01 substitution per nucleotide position. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at the branch points.

DNA relatedness indicated that strains JG-100^T and JG-102 are members of the same species (Wayne *et al.*, 1987). Strains JG-100^T and JG-102 can be differentiated from phylogenetically related *Psychrobacter* species in some physiological and biochemical characteristics, such as growth temperature, acid production from carbohydrates and the ability to utilize certain substrates (Table 1). There are widely recognized criteria for species definition in current bacteriology; strains with DNA–DNA relatedness levels of less than 70 % or with more than 3 % difference in 16S rRNA gene sequences are considered to represent different species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). The DNA–DNA relatedness values between the two strains and the type strains of 13 *Psychrobacter* species were lower than the threshold value (70 %) suggested for species delineation in current bacterial systematics (Wayne *et al.*, 1987); the 16S rRNA gene sequence similarities between the two strains and the type strains of 13 *Psychrobacter* species were more than 97 %. The level of DNA relatedness, together with differential phenotypic properties and 16S rRNA gene sequence similarity data, justify taxonomic discrimination of strains JG-100^T and JG-102 from all recognized *Psychrobacter* species. Therefore, on the basis of the data presented, strains JG-100^T and JG-102 should be placed in the genus *Psychrobacter* as representatives of a novel species, for which the name *Psychrobacter alimentarius* sp. nov. is proposed.

Description of *Psychrobacter alimentarius* sp. nov.

Psychrobacter alimentarius (a.li.men.ta'ri.us. L. adj. *alimentarius* relating to food).

Cells are cocci, 1.4–2.0 µm in diameter. Colonies on MA are smooth, low convex, circular to slightly irregular and cream in colour. Aerobic. Growth occurs at 4 and 35 °C, but not above 36 °C. Optimal pH for growth is 6.5–7.5; growth occurs at pH 5.0, but not at pH 4.5. Growth occurs in the presence of 0–12 % (w/v) NaCl, with optimum growth at 2–3 %. Yeast extract, biotin, *p*-aminobenzoic acid, thiamine hydrochloride and vitamin B₁₂ are not required for growth. Tyrosine is hydrolysed, but aesculin, starch, hypoxanthine and xanthine are not. Indole and H₂S are not produced. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. When assayed with the API ZYM system, lipase (C8) and leucine arylamidase are present, but trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. Butyrate is utilized. D-Glucose, D-fructose, glutamate, formate and methanol are not utilized. Utilization of benzoate and ethanol is variable (negative for type strain). Acid is produced from D-cellobiose, D-mannose, melibiose and D-ribose. Acid is not produced from adonitol, maltose, D-melezitose, *myo*-inositol, D-raffinose, D-sorbitol, sucrose or D-trehalose. Sensitive to cephalothin (30 µg), chloramphenicol (100 µg) and erythromycin (15 µg) and weakly

sensitive to novobiocin (5 µg) and penicillin (20 U). The predominant respiratory lipoquinone is Q-8. The DNA G + C content of the type and reference strains is 44 mol% (HPLC). Other characteristics are shown in Table 1.

The type strain is JG-100^T (=KCTC 12186^T=DSM 16065^T), isolated from squid jeotgal, a traditional Korean fermented seafood. Reference strain is JG-102 (=KCTC 12187=DSM 16066).

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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.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.
- 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). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–206.

- 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.
- Levring, T. (1946).** Some culture experiments with *Ulva* and artificial seawater. *K Fysiogr Sallsk Lund Forh* **16**, 45–56.
- 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 isolated 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.
- Sasser, M. (1990).** Identification of bacteria by gas chromatography of cellular fatty acids. *Technical Note 101*. Newark, DE: MIDI.
- 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.
- Stackebrandt, E. & Goebel, B. M. (1994).** Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- 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.