Nesterenkonia jeotgali sp. nov., isolated from jeotgal, a traditional Korean fermented seafood

Jung-Hoon Yoon,¹ Seo-Youn Jung,¹ Wonyong Kim,² Sun-Woo Nam³ and Tae-Kwang Oh¹

Correspondence

Jung-Hoon Yoon
jhyoon@kribb.re.kr

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

²Department of Microbiology, College of Medicine, Chungang University, 221 Heukseok-dong, Seoul, Korea

³Health Technology Planning and Evaluation Board, 57-1 Noryangjin-dong, Seoul, Korea

A Gram-positive, non-motile, slightly halophilic actinomycete, strain JG-241^T, was isolated from jeotgal, a traditional Korean fermented seafood, and its taxonomic position was investigated by using a polyphasic approach. Strain JG-241^T grew optimally at 25–30 °C and in the presence of 2–5 % (w/v) NaCl. The physiological and biochemical properties of strain JG-241^T were distinguishable from those of recognized *Nesterenkonia* species. Strain JG-241^T had a peptidoglycan type based on L-Lys–Gly–D-Asp. It contained MK-7, MK-8 and MK-9 as the predominant menaquinones and anteiso-C_{15:0} and anteiso-C_{17:0} as the major fatty acids. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid. The DNA G+C content was 68.0 mol%. Comparative 16S rRNA gene sequence analysis showed that strain JG-241^T falls within the radiation of the cluster comprising *Nesterenkonia* species. Similarity values between the 16S rRNA gene sequence of strain JG-241^T and those of the type strains of *Nesterenkonia* species ranged from 96.7 to 99.7 %. DNA–DNA relatedness data and repetitive extragenic palindromic DNA-PCR genomic fingerprinting patterns showed that strain JG-241^T differs genetically from recognized *Nesterenkonia* species. On the basis of its phenotypic properties and phylogenetic and genetic distinctiveness, strain JG-241^T represents a novel species of the genus *Nesterenkonia*, for which the name *Nesterenkonia jeotgali* sp. nov. is proposed. The type strain is JG-241^T (=KCTC 19053^T=JCM 12610^T).

The genus *Nesterenkonia* was proposed through the reclassification of *Micrococcus halobius* (Onishi & Kamekura, 1972) as *Nesterenkonia halobia* (Stackebrandt *et al.*, 1995). Subsequently, five more *Nesterenkonia* species, *Nesterenkonia lacusekhoensis* (Collins *et al.*, 2002), *Nesterenkonia halotolerans* and *Nesterenkonia xinjiangensis* (Li *et al.*, 2004) and *Nesterenkonia sandarakina* and *Nesterenkonia lutea* (Li *et al.*, 2005) have been described. Phylogenetic analyses based on 16S rRNA gene sequences showed that the genus *Nesterenkonia* belongs to the family *Micrococcaceae* of the order *Actinomycetales* (Stackebrandt *et al.*, 1995, 1997). In the study presented here, we report on the taxonomic characterization of a slightly halophilic, vivid-yellow- or orange-yellow-pigmented *Nesterenkonia*-like

bacterial strain, JG-241^T, which was isolated from jeotgal, a traditional Korean fermented seafood.

Strain JG-241^T was isolated by the usual dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C. The type strains of six *Nesterenkonia* species were used as reference strains. *N. halobia* DSM 20541^T, *N. lacusekhoensis* DSM 12544^T, *N. halotolerans* DSM 15474^T and *N. xinjiangensis* DSM 15475^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. *N. sandarakina* KCTC 19011^T and *N. lutea* KCTC 19013^T were obtained from the Korean Collection for Type Cultures, Taejeon, Korea. The cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy. The presence of flagella was examined by transmission electron microscopy using cells from exponentially growing cultures. Colony morphology and colour were examined by using colonies grown on MA and on solid PYGV medium (Staley, 1968). The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various NaCl concentrations was

Abbreviation: rep-PCR, repetitive extragenic palindromic DNA-PCR.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JG-241^T is AY928901.

Levels of DNA–DNA relatedness between strain JG-241^T and the type strains of six recognized *Nesterenkonia* species are shown in a supplementary table available in IJSEM Online.

investigated in marine broth 2216 (MB; Difco) and trypticase soy broth (Difco). Growth in the absence of NaCl was investigated in trypticase soy broth prepared according to the formula of the Difco medium except that no NaCl was used. Growth at various temperatures (4–40 °C) was measured on MA. The pH range for growth was determined in MB adjusted to various pH values (pH 4.5–9.5, with increments of 0.5 pH units) prior to sterilization by the addition of HCl and Na₂CO₃. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MA and on MA supplemented with nitrate, both of which had been prepared anaerobically using a nitrogen atmosphere. Catalase and oxidase activities and the hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). The hydrolysis of hypoxanthine, tyrosine and xanthine was investigated on MA at the substrate concentrations described by Cowan & Steel (1965). Nitrate reduction and the hydrolysis of aesculin, gelatin and urea were studied as described previously (Lanyi, 1987) with the modification that artificial seawater was used for preparation of media. The artificial seawater contained the following (l⁻¹ 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 (Bruns *et al.*, 2001). H₂S production was tested as described previously (Bruns *et al.*, 2001). Enzyme activity was determined by using the API ZYM system (bioMérieux). Acid production from carbohydrates was determined as described by Leifson (1963). The utilization of substrates as sole carbon and energy sources was tested according to the method of Baumann & Baumann (1981), using supplementation with 2 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957), 1 % (v/v) vitamin solution (Staley, 1968) and 0.01 % (w/v) yeast extract (Difco). Susceptibility to antibiotics was tested on MA plates, using antibiotic discs containing the following: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; and carbenicillin, 100 µg. Other physiological and biochemical tests were performed with the API 20E system (bioMérieux).

Cell mass for DNA extraction and for analyses of the cell wall, polar lipids and menaquinones was obtained by cultivation in MB at 30 °C. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used in combination with RNase A. The 16S rRNA gene sequence was amplified by means of a PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984), with the modification that the DNA was hydrolysed and the resultant nucleotides were analysed by reverse-phase HPLC. Preparation of cell-wall

peptidoglycan was carried out using the method described by Schleifer & Kandler (1972), and the peptidoglycan amino acids were determined using an automated amino acid analyser (model L-8500A; Hitachi). Preparation of cell-wall peptidoglycan and determination of the peptidoglycan structure was also performed at the DSMZ by using modified versions of the methods of Rhuland *et al.* (1955), Schleifer & Kandler (1972), Schleifer (1985) and MacKenzie (1987). Menaquinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid methyl ester analysis, cell mass of strain JG-241^T and reference strains was harvested from MA plates after cultivation for 5 days at 30 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and were identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells: five replications were performed for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values were quoted as the DNA–DNA relatedness values. Repetitive extragenic palindromic DNA-PCR (rep-PCR) genomic fingerprinting using REP, BOX and (GTG)₅ PCR primers was performed as described by Rademaker *et al.* (1998). Computer-assisted analysis of the genomic fingerprints was performed by using the software GelCompar II, version 1.5 (Applied Maths). Similarity among patterns was calculated on the basis of Pearson's similarity coefficient, and a dendrogram was constructed using the UPGMA algorithm (Sneath & Sokal, 1973).

Morphological, cultural, physiological and biochemical properties of strain JG-241^T are shown in Table 1 or are given in the species description (see below). The almost-complete 16S rRNA gene sequence of strain JG-241^T determined in this study comprised 1480 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. Comparative 16S rRNA gene sequence analyses revealed that strain JG-241^T is phylogenetically most closely related to *Nesterenkonia* species, showing highest sequence similarity (99.7 %) with *N. sandarakina* YIM 70009^T (Fig. 1). The 16S rRNA gene sequence similarity values between strain JG-241^T and the type strains of the other *Nesterenkonia* species were 96.7 % (*N. halobia* and *N. lacusekhoensis*), 97.0 % (*N. xinjiangensis*) and 99.3 % (*N. halotolerans* and *N. lutea*). Sequence similarities with respect to other species used in the phylogenetic analysis were below 95.6 % (Fig. 1).

The chemotaxonomic properties of strain JG-241^T support the results of monothetic phylogenetic classification,

Table 1. Differential phenotypic characteristics of *Nesterenkonia* species

Taxa: 1, strain JG-241^T; 2, *N. sandarakina* (data from Li *et al.*, 2005 unless indicated); 3, *N. halotolerans* (Li *et al.*, 2004, 2005); 4, *N. lutea* (Li *et al.*, 2005); 5, *N. halobia* (Onishi & Kamekura, 1972; Stackebrandt *et al.*, 1995; Collins *et al.*, 2002); 6, *N. lacusekhoensis* (Collins *et al.*, 2002); 7, *N. xinjiangensis* (Li *et al.*, 2004). Symbols: +, positive; –, negative; w, weakly positive; ND, no data available. Data in parentheses are results for the type strain. All taxa are Gram-positive, non-spore-forming, positive for catalase and negative for indole production.

Characteristic	1	2	3	4	5	6	7
Morphology	Cocci	Cocci	Cocci	Cocci	Cocci	Short rods, occasionally branching and cocci	Short rods
Colony pigmentation (PYGV medium)	Light yellow	Orange–yellow	Deep orange–yellow	Light yellow to primrose yellow	Colourless	Bright yellow	Light yellow
Motility	–	–	+	+	–	–	–
Oxidase	–	–	–	–	+	–	–
Urease	–	–	+	–	–	ND	+
Temperature range for growth (°C)	4–36	4–36*	4–40	4–36*	10–40*	8.5 to > 42	4–40*
NaCl requirement	–	+	–	–	+	–	–
Maximum NaCl conc. for growth (%)	16	15	25	20	> 23	> 15	25
H ₂ S production	–	–	–	–	–	w	–
Nitrate reduction	–	–	–	+	–	–	–
Voges–Proskauer reaction	+	–	+	–	+	–	ND
Indole production	–	–	–	–	–	–	ND
Hydrolysis of:							
Starch	–	–	–	–	+	–	–
Gelatin	–	+	+	–	–	–	+
Tween 80	–	–	–	–	–	–	ND
Utilization of:							
D-Glucose	+	+	+	w	ND	+	+
D-Xylose	+	+	–	+	ND	ND	+
L-Arabinose	+	w	–	w	ND	ND	+
D-Cellobiose	+	+	–	+	ND	ND	+
D-Trehalose	+	+	–	–	(–)	+	–
D-Fructose	+	+	+	+	ND	+	+
D-Mannose	w	+	+	+	(–)	+	+
Sucrose	+	+	+	+	ND	+	+
Maltose	+	+	+	+	ND	+	+
Acid production from:							
D-Galactose	+	+	–	+	w	–	–
Lactose	–	–	–	+	+	–	–
D-Trehalose	w	–	–	+	–	+	–
D-Xylose	+	+	–	+	+	–	–
Mannitol	+	+	–	+	+	–	–
Enzyme activity							
α -Galactosidase	–	+	–	+	ND	ND	ND
β -Galactosidase	–	+	+	+	ND	ND	ND
α -Glucosidase	+	–	+	+	ND	ND	ND
β -Glucosidase	–	+	+	+	ND	ND	ND
β -Glucuronidase	–	+	–	–	ND	ND	ND
Lysine decarboxylase	–	+	+	+	ND	ND	ND
Susceptibility to:							
Streptomycin	+	–	+	–	ND	ND	ND
Tetracycline	–	+	–	–	ND	ND	ND

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
Lincomycin	+	+	+	—	ND	ND	ND
Oleandomycin	+	+	+	—	ND	ND	ND
Peptidoglycan type	L-Lys–Gly– D-Asp	L-Lys–Gly– D-Asp	L-Lys–Gly– D-Asp	L-Lys–Gly– D-Asp	L-Lys–Gly– L-Glu	L-Lys–L-Glu	L-Lys–Gly– L-Glu
Major menaquinones	MK-7, MK-8, MK-9	MK-7, MK-8	MK-7, MK-8	MK-8, MK-7	MK-8, MK-9	MK-7, MK-8	MK-7, MK-8, MK-9
Polar lipids†	DPG, PG, PI, GL	DPG, PG, PI, GL	DPG, PG, PI, GL	DPG, PG, PI, GL	DPG, PG, PI, GL	DPG, PG, PC, GL	DPG, PG, PC
DNA G + C content (mol%)	68.0	68.3‡	66.9‡	67.4‡	70–72	66.1	66.7

*Data obtained in this study.

†DPG, Diphosphatidylglycerol; GL, unidentified glycolipid; PC, phosphatidylcholine; PG, phosphatidylglycerol; PI, phosphatidylinositol.

‡Data obtained in this study by the HPLC method.

indicating that it represents a member of the genus *Nesterenkonia*. Cell-wall analysis showed that strain JG-241^T had the A4 α peptidoglycan type based on L-Lys–Gly–D-Asp (described by Schleifer & Kandler, 1972). The predominant menaquinones found in strain JG-241^T were MK-7 (59.8%), MK-8 (20.3%), MK-9 (11.0%) and MK-6 (8.8%). The cellular fatty acid profile showed the presence of large amounts of straight and branched fatty acids; the major fatty acids were anteiso-C_{15:0} and anteiso-C_{17:0} (Table 2). This fatty acid profile was generally similar to those of *Nesterenkonia* species analysed in this study (Table 2). For some fatty acids, the proportions present in *Nesterenkonia* species differed between this study and the studies of Li *et al.* (2004, 2005), perhaps because of differences in cultivation conditions. The major polar lipids detected in strain JG-241^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid. This polar lipid profile was similar to those

of *Nesterenkonia* species, although it differed from those of *N. lacusekhoensis* and *N. xinjiangensis* in that phosphatidylcholine was absent (Table 1). The DNA G + C content of strain JG-241^T was 68.0 mol%.

Strain JG-241^T could be distinguished from *Nesterenkonia* species by notable differences in rep-PCR genomic

Table 2. Cellular fatty acid compositions (%) of *Nesterenkonia* species

Strains: 1, strain JG-241^T; 2, *N. sandarakina* KCTC 19011^T; 3, *N. halotolerans* DSM 15474^T; 4, *N. lutea* KCTC 19013^T; 5, *N. halobia* DSM 20541^T; 6, *N. lacusekhoensis* DSM 12544^T; 7, *N. xinjiangensis* DSM 15475^T. Fatty acids that represented <0.5% in all strains are omitted. —, Not detected.

Fatty acid	1	2	3	4	5	6	7
Straight-chain fatty acids							
C _{14:0}	0.4	2.8	—	0.4	—	2.5	—
C _{15:0}	0.9	2.1	—	—	—	—	0.2
C _{16:0}	2.6	14.9	1.1	2.6	1.6	6.1	0.7
C _{18:0}	0.7	5.1	—	—	—	—	—
Branched fatty acids							
iso-C _{14:0}	0.2	0.9	0.6	1.8	—	0.5	0.2
iso-C _{15:0}	1.4	0.8	2.2	5.5	1.7	1.3	6.2
iso-C _{15:1} G	—	—	—	1.0	—	—	1.2
anteiso-C _{15:1} A	0.4	—	—	7.8	—	0.6	0.4
anteiso-C _{15:0}	43.7	59.7	72.8	53.6	33.8	62.1	21.9
iso-C _{16:0}	13.0	4.4	9.8	17.8	8.3	6.7	9.6
iso-C _{16:1} G	—	—	—	0.4	—	—	4.8
iso-C _{17:0}	1.7	—	0.4	0.5	2.3	—	6.2
anteiso-C _{17:0}	33.8	7.1	12.2	8.0	52.4	19.8	39.7
anteiso-C _{17:1} A	—	—	—	—	—	—	8.5
Unsaturated fatty acids							
C _{14:1} ω 5c	—	0.9	—	—	—	—	—
10-Methyl C _{16:0}	—	1.4	—	—	—	—	—

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequence data, showing the phylogenetic position of strain JG-241^T and representatives of some related taxa. Bootstrap values (1000 replications) are shown as percentages at each node only if they are 50% or greater. *Cellulomonas flavigena* DSM 20109^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Fig. 2. Consensus dendrogram showing relationships among *Nesterenkonia* species based on similarity values derived by using the Jaccard correlation coefficient and the UPGMA algorithm in an analysis of the combined rep-PCR fingerprints generated by REP, BOX and (GTG)₅ PCR primers. The cut-off point for the recognition of clades was set at 30 % similarity.

fingerprinting patterns (Fig. 2). The DNA–DNA relatedness data were sufficient to identify strain JG-241^T as representing a genomic species that is distinct from recognized *Nesterenkonia* species (Wayne *et al.*, 1987). Mean levels of DNA–DNA relatedness between strain JG-241^T and the type strains of six recognized *Nesterenkonia* species were in the range 11–53 % (see Supplementary Table S1 in IJSEM Online). There were differences between strain JG-241^T and recognized *Nesterenkonia* species in several phenotypic, particularly chemical, properties, including the presence of MK-9, differences in the content of some fatty acids (particularly anteiso-C_{17:0}), NaCl requirements and some physiological and biochemical properties (Table 1). Therefore, on the basis of the data presented above, strain JG-241^T should be placed within the genus *Nesterenkonia* as a member of a novel species, for which the name *Nesterenkonia jeotgali* sp. nov. is proposed.

Description of *Nesterenkonia jeotgali* sp. nov.

Nesterenkonia jeotgali (je.ot.ga'li. N.L. gen. n. *jeotgali* of jeotgal, from which the type strain was isolated).

Cells are cocci (diameter 0.5–0.8 µm) on MA at 30 °C; some cells are oval in the very early growth phase. Colonies are smooth, circular, slightly convex or raised, glistening and 1.0–2.0 mm in diameter after 5 days cultivation at 30 °C on MA. Colonies on MA are vivid yellow in colour at 30 °C, but orange–yellow in colour at 25 °C. Colonies on solid PYGV medium are light yellow in colour at 25 and 30 °C. Optimal growth temperature is 25–30 °C; growth occurs at 4 and 36 °C, but not at 37 °C. Optimal pH for growth is 7.5–8.5; growth occurs at pH 6.0, but not at pH 5.5. Optimal growth occurs in the presence of 2–5 % (w/v) NaCl; growth occurs in the presence of 0–16 % NaCl (w/v). Growth does not occur under anaerobic conditions on MA or on MA supplemented with nitrate. Tyrosine is hydrolysed, but aesculin, casein, hypoxanthine, xanthine and Tweens 20, 40 and 60 are not hydrolysed. Methyl red reaction is positive. In assays with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8) and leucine arylamidase are present, but lipase (C14), valine arylamidase, cystine

arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, ornithine decarboxylase and tryptophan deaminase are absent. D-Galactose, acetate, citrate, succinate, L-malate and pyruvate are utilized. Benzoate, salicin, formate and L-glutamate are not utilized. Acid is produced from L-arabinose, D-cellobiose, D-glucose, maltose, D-mannose, D-melezitose, D-ribose and sucrose, but not from D-fructose, melibiose, D-raffinose, L-rhamnose, myo-inositol or D-sorbitol. Susceptible to penicillin G, chloramphenicol, ampicillin, cephalothin, novobiocin and carbenicillin, but not to polymyxin B, gentamicin, kanamycin or neomycin. The cell-wall peptidoglycan type is L-Lys-Gly-D-Asp. The predominant menaquinones are MK-7, MK-8 and MK-9. The major fatty acids are anteiso-C_{15:0} and anteiso-C_{17:0}. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid. The DNA G + C content is 68.0 mol% (determined by HPLC). Other phenotypic properties are given in Table 1.

The type strain, JG-241^T (= KCTC 19053^T = JCM 12610^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 MG05-0401-2-0) and the NRL research program from the Ministry of Science and Technology (MOST) of the Republic of Korea.

References

- Baumann, P. & Baumann, L. (1981). The marine Gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes*, pp. 1302–1331. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- 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.
- Cohen-Bazire, G., Sistrom, W. R. & Stanier, R. Y. (1957).** Kinetic studies of pigment synthesis by nonsulfur purple bacteria. *J Cell Comp Physiol* **49**, 25–68.
- Collins, M. D., Lawson, P. A., Labrenz, M., Tindall, B. J., Weiss, N. & Hirsch, P. (2002).** *Nesterenkonia lacusekhoensis* sp. nov., isolated from hypersaline Ekho Lake, East Antarctica, and emended description of the genus *Nesterenkonia*. *Int J Syst Evol Microbiol* **52**, 1145–1150.
- Cowan, S. T. & Steel, K. J. (1965).** *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- 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.
- Komagata, K. & Suzuki, K. (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.
- Li, W.-J., Chen, H.-H., Zhang, Y.-Q., Schumann, P., Stackebrandt, E., Xu, L.-H. & Jiang, C.-L. (2004).** *Nesterenkonia halotolerans* sp. nov. and *Nesterenkonia xinjiangensis* sp. nov., actinobacteria from saline soils in the west of China. *Int J Syst Evol Microbiol* **54**, 837–841.
- Li, W.-J., Chen, H.-H., Kim, C.-J., Zhang, Y.-Q., Park, D.-J., Lee, J.-C., Xu, L.-H. & Jiang, C.-L. (2005).** *Nesterenkonia sandarakina* sp. nov. and *Nesterenkonia lutea* sp. nov., novel actinobacteria, and emended description of the genus *Nesterenkonia*. *Int J Syst Evol Microbiol* **55**, 463–466.
- MacKenzie, S. L. (1987).** Gas chromatographic analysis of amino acids as the N-heptafluorobutyl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984).** An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Onishi, H. & Kamekura, M. (1972).** *Micrococcus halobius* sp. n. *Int J Syst Bacteriol* **22**, 233–236.
- Rademaker, J. L. W., Louws, F. J. & de Bruijn, F. J. (1998).** Characterization of the diversity of ecologically important microbes by rep-PCR genomic fingerprinting. In *Molecular Microbial Ecology Manual*, supplement 3, pp. 3.4.3.1–3.4.3.26. Edited by A. D. L. Akkermans, J. D. van Elsas & F. J. de Bruijn. Dordrecht: Kluwer.
- Rhuland, L. E., Work, E., Denman, R. F. & Hoare, D. S. (1955).** The behaviour of the isomers of α,ϵ -diaminopimelic acid on paper chromatograms. *J Am Chem Soc* **77**, 4844–4846.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*. Technical Note 101. Newark, DE: MIDI.
- Schleifer, K. H. (1985).** Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972).** Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Sneath, P. H. A. & Sokal, R. R. (1973).** *Numerical Taxonomy*. San Francisco: Freeman.
- Stackebrandt, E., Koch, C., Gvozdiak, O. & Schumann, P. (1995).** Taxonomic dissection of the genus *Micrococcus*: *Kocuria* gen. nov., *Nesterenkonia* gen. nov., *Kytococcus* gen. nov., *Dermacoccus* gen. nov., and *Micrococcus* Cohn 1872 gen. emend. *Int J Syst Bacteriol* **45**, 682–692.
- Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997).** Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.
- Staley, J. T. (1968).** *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* **95**, 1921–1942.
- Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- 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 rRNA gene sequences. *Int J Syst Bacteriol* **48**, 187–194.
- Yoon, J.-H., Kim, H., Kim, I.-G., Kang, K. H. & Park, Y.-H. (2003).** *Erythrobacter flavus* sp. nov., a slight halophile from the East Sea in Korea. *Int J Syst Evol Microbiol* **53**, 1169–1174.