Microbacterium insulae sp. nov., isolated from soil

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A Gram-positive, non-motile, rod- or coccoid-shaped *Microbacterium*-like bacterium, designated strain DS-66^T, was isolated from soil of Dokdo, Korea, and its exact taxonomic position was investigated by using a polyphasic approach. Strain DS-66^T grew optimally at 30 °C and pH 6.5–7.0 in the presence of 0.5–1.0 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain DS-66^T belonged to the genus *Microbacterium*. Strain DS-66^T had a peptidoglycan type based on B2β with partial substitution of glutamic acid by 3-hydroxy glutamic acid (Glu/Hyg–Gly–D–Orn), and galactose, rhamnose and ribose as whole-cell sugars. The acyl type was glycolyl. Strain DS-66^T contained MK-13, MK-12 and MK-14 as predominant menaquinones and anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{17:0} and iso-C_{16:0} as major fatty acids. Major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and an unidentified glycolipid. The DNA G + C content was 69.9 mol%. Phylogenetic distinctiveness, DNA–DNA relatedness data and differential phenotypic properties demonstrated that strain DS-66^T is distinguishable from recognized *Microbacterium* species. On the basis of the data presented, strain DS-66^T is considered to represent a novel species of the genus *Microbacterium*, for which the name *Microbacterium insulae* sp. nov. is proposed. The type strain is DS-66^T (=KCTC 19247^T=CCUG 54523^T).

The genus *Microbacterium* was proposed by Orla-Jensen (1919); at the time of writing, the genus comprised 50 species with validly published names, including several described since 2006: *Microbacterium koreense* (Lee *et al.*, 2006), *M. paludicola* (Park *et al.*, 2006), *M. aoyamense*, *M. deminutum* and *M. pumilum* (Kageyama *et al.*, 2006), *M. indicum* (Shivaji *et al.*, 2007), *M. sediminicola* and *M. marinilacus* (Kageyama *et al.*, 2007), *M. ginsengisoli* (Park *et al.*, 2008) and *M. hatanonis* (Bakir *et al.*, 2008). Here we report on the taxonomic characterization of a *Microbacterium*-like strain, designated DS-66^T, which was isolated from soil of Dokdo, Korea.

Strain DS-66^T was isolated by using the standard dilution plating technique at 25 °C by using 10 × diluted nutrient agar (Difco). The type strains of three recognized *Microbacterium* species were used as reference strains: *Microbacterium hominis* DSM 12509^T and *Microbacterium*

trichothecenolyticum DSM 8608^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany and *Microbacterium xylanilyticum* KCTC 19079^T was obtained from the Korean Collection for Type Cultures, Taejeon, Korea. The morphological, physiological and biochemical characteristics of strain DS-66^T were investigated by using routine cultivation on trypticase soy agar (TSA; Difco) at 30 °C. Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). Flagellation was determined by using transmission electron microscopy on cells from exponentially growing cultures negatively stained with 1 % (w/v) phosphotungstic acid. Grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth at various temperatures (4, 10, 15, 20, 25 and 28 °C, and from 30 to 40 °C in 1 °C increments) was measured on TSA. To investigate tolerance to NaCl, trypticase soy broth (TSB) was prepared according to the formula of the Difco medium and NaCl concentrations were varied [0.5 % (w/v) and 1.0–7.0 % (w/v) at increments of 1.0 %]. The pH range for growth was determined in nutrient broth (Difco) that was adjusted to various pH values (pH 4.5–10.5 at intervals of 0.5 pH

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DS-66^T is EU239498.

An extended neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain DS-66^T, the type strains of *Microbacterium* species and representatives of some other related taxa is available as supplementary material with the online version of this paper.

units) prior to sterilization by the addition of HCl or Na₂CO₃. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on TSA and on TSA supplemented with potassium nitrate (0.1 %, w/v). Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Hydrolysis of aesculin and reduction of nitrate were studied as described by Lányi (1987). Susceptibility to antibiotics was tested on TSA plates using antibiotic discs containing the following compounds: 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). Utilization of various substrates, enzyme activities, and other physiological and biochemical properties were tested by using the API 20E, API 20NE, API 50 CH and API ZYM systems (bioMérieux); utilization of various substrates was determined by inoculating API 20NE and API 50 CH strips with cells suspended in AUX medium (bioMérieux).

Cell biomass for DNA extraction and for analyses of cell-wall components, isoprenoid quinones and polar lipids was obtained from cultures grown by shaking at 150 r.p.m. in TSB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR by 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). The DNA G+C content was determined according to the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The presence or absence of diaminopimelic acid in the peptidoglycan was determined by using the method described by Komagata & Suzuki (1987). Preparation of cell walls and determination of peptidoglycan structure were carried out by using methods described by Schleifer & Kandler (1972), MacKenzie (1987) and Groth *et al.* (1996). Whole-cell sugars were determined as described by Komagata & Suzuki (1987). The cell-wall acyl type was determined as described by Uchida & Aida (1984). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and were analysed by using reversed-phase HPLC on a YMC ODS-A (250 × 4.6 mm) column. 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). For fatty acid methyl ester analysis, cell mass of strain DS-66^T was harvested from TSA plates after incubation for 7 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). DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989) by using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications 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 levels of DNA–DNA relatedness.

Morphological, cultural, physiological and biochemical characteristics of strain DS-66^T are given in the species description below or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain DS-66^T determined in the present study comprised 1447 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree based on the neighbour-joining algorithm, strain DS-66^T fell within the radiation of the cluster comprising *Microbacterium* species (Fig. 1; Supplementary Fig. S1 in IJSEM Online). Strain DS-66^T exhibited 16S rRNA gene sequence similarity values of 98.0, 98.0, 97.6 and 96.7 % with respect to the type strains of *M. hominis*, *M. trichothecenolyticum*, *M. xylanilyticum* and *Microbacterium halophilum*, respectively.

Under the condition of total hydrolysis (4 N HCl, 16 h at 100 °C), the total hydrolysate of the peptidoglycan of strain DS-66^T contained the amino acids ornithine, alanine, glycine, homoserine, glutamic acid and 3-hydroxy glutamic acid (Hyg). In addition, traces of threonine were detected. Quantitative analysis of the peptidoglycan amino acids by TLC, two-dimensional TLC and GC (Schleifer, 1985; MacKenzie, 1987; Groth *et al.*, 1996) showed that strain DS-66^T contained alanine, glycine, threonine, homoserine, ornithine and glutamic acid at a ratio of approximately 1.5:4.9:0.2:1.5:0.6:1.0. The amount of 3-hydroxy glutamic acid could not be quantified. Dinitrophenylation of the peptidoglycan revealed that D-ornithine represents the N terminus of the interpeptide bridge. From these data, it was concluded that strain DS-66^T has peptidoglycan type B2β with partial substitution of glutamic acid by 3-hydroxy glutamic acid (Glu/Hyg–Gly–D–Orn), as described by Schleifer & Kandler (1972). The amino acids in positions 1 and 3 were glycine and L-homoserine, respectively. The acyl type of the muramic acid was glycolyl. Cell-wall sugars were galactose, rhamnose and ribose. The predominant menaquinones detected in strain DS-66^T were MK-13, MK-12 and MK-14, at a peak area ratio of approximately 64, 17 and 12 %, respectively. Major polar lipids detected in strain DS-66^T were diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and an unidentified glycolipid. Fatty acids comprising more than 0.5 % of the total for strain DS-66^T were as follows: branched fatty acids anteiso-C_{15:0} (50.3 %), anteiso-C_{17:0} (15.9 %), iso-C_{17:0} (10.3 %), iso-C_{16:0} (10.2 %), iso-C_{15:0} (5.7 %), iso-C_{18:0} (3.7 %), anteiso-C_{19:0} (0.8 %) and iso-C_{14:0} (0.8 %); and straight-chain fatty acids C_{16:0} (1.0 %) and C_{18:0} (0.8 %). This fatty acid profile was similar to those of recognized

Table 1. Differential phenotypic characteristics between strain DS-66^T and phylogenetically related *Microbacterium* species

Strains: 1, DS-66^T (*M. insulae* sp. nov.); 2, *M. hominis* DSM 12509^T (data from Takeuchi & Hatano, 1998b; Kim *et al.*, 2005 and the present study); 3, *M. trichothecenolyticum* DSM 8608^T (Yokota *et al.*, 1993 and the present study); 4, *M. xylanilyticum* KCTC 19079^T (Kim *et al.*, 2005 and the present study). +, Positive reaction; –, negative reaction; w, weakly positive reaction. All strains are positive for Gram-staining, hydrolysis of aesculin, starch and Tweens 40, 60 and 80, utilization of glycerol, L-arabinose, D-xylose, galactose, glucose, fructose, mannose, mannitol, amygdalin, aesculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, melezitose, starch, gentiobiose, turanose and gluconate, activities of leucine arylamidase and α -glucosidase, and susceptibility to carbenicillin, cephalothin, chloramphenicol, oleandomycin, streptomycin and tetracycline. All strains are negative for production of H₂S and indole, hydrolysis of urea, utilization of erythritol, L-xylose, adonitol, sorbose, dulcitol, sorbitol, methyl α -D-mannoside, inulin, xylitol, D-tagatose, D-fucose, D-arabitol, L-arabitol, caprate and adipate, activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, β -glucuronidase and α -fucosidase, and susceptibility to polymyxin B.

Characteristic	1	2	3	4
Catalase*	–	+	+	+
Oxidase*	+	–	–	–
Growth at 37 °C	+	+	–	+
Growth in 6% (w/v) NaCl	–	w	–	+
Nitrate reduction	–	–	+	+
Hydrolysis of:*				
Casein	–	+	+	–
Gelatin	+	+	+	–
Hypoxanthine	–	–	–	+
Tyrosine	–	–	–	+
Tween 20	+	+	+	–
Xanthine	–	–	–	+
Utilization of:*				
D-Arabinose	–	–	–	+
D-Ribose	–	–	–	+
Methyl β -D-xyloside	–	–	+	–
Rhamnose	+	+	+	–
Inositol	–	–	+	–
Methyl α -D-glucoside	+	+	–	+
N-Acetylglucosamine	–	+	+	+
Arbutin	–	+	+	–
Melibiose	–	+	+	+
Raffinose	–	+	+	+
Glycogen	w	+	+	+
D-Lyxose	–	+	–	–
L-Fucose	–	–	+	+
2-Ketogluconate	–	+	–	+
5-Ketogluconate	–	+	+	w
Citrate	–	+	+	–
Malate	–	+	+	+
Phenylacetate	–	–	–	+

Table 1. cont.

Characteristic	1	2	3	4
API ZYM*				
Esterase (C4)	w	+	+	–
Esterase lipase (C8)	w	+	+	–
Acid phosphatase	–	–	–	+
α -Galactosidase	–	+	–	+
β -Galactosidase	–	+	w	w
β -Glucosidase	+	+	+	–
N-Acetyl- β -glucosaminidase	+	+	+	–
α -Mannosidase	–	–	–	+
Susceptibility to antibiotics*				
Penicillin G	+	+	–	+
Ampicillin	+	+	–	–
Gentamicin	–	–	–	+
Novobiocin	+	+	+	–
Kanamycin	–	–	–	+
Lincomycin	+	–	–	–
Neomycin	–	–	+	+
Cell-wall sugar†	Gal, Rha, Rib	Rha, 6dT, Gal, Man	Gal, Glc	Gal, Glc
Amino acid at position 3	Homo-serine	Lysine	Homo-serine	Homo-serine
Predominant menaquinones	12, 13, 14	11, 12	12, 13	11, 12, 13
DNA G + C content (mol%)	69.9	71.2‡	69.0	69.7

*Data from the present study.

†Gal, Galactose; Glc, glucose; Man, mannose; Rha, rhamnose; Rib, ribose; 6dT, 6-deoxytalose.

‡Value of 71.2 mol% in Takeuchi & Hatano (1998b), but 70.9 mol% in Kim *et al.* (2005).

Microbacterium species (Takeuchi & Hatano, 1998a, b; Kim *et al.*, 2005; Kageyama *et al.*, 2006; Lee *et al.*, 2006). The DNA G + C content of strain DS-66^T was 69.9 mol%. Based on the results of phylogenetic and chemotaxonomic investigations, it is clear that strain DS-66^T is a member of the genus *Microbacterium* (Takeuchi & Hatano, 1998a, b; Kim *et al.*, 2005; Kageyama *et al.*, 2006; Lee *et al.*, 2006).

Strain DS-66^T exhibited levels of DNA–DNA relatedness of 11–17% to the type strains of its three closest phylogenetic neighbours, namely *M. xylanilyticum* KCTC 19079^T (13%), *M. hominis* DSM 12509^T (17%) and *M. trichothecenolyticum* DSM 8608^T (11%). These values indicate that strain DS-66^T represents a genomic species that is distinct from *M. xylanilyticum*, *M. hominis* and *M. trichothecenolyticum* (Wayne *et al.*, 1987). Strain DS-66^T could also be distinguished from these three recognized *Microbacterium* species based on differences in several phenotypic properties, as shown in Table 1. The phylogenetic distinctiveness of strain DS-66^T, together with genetic distinctiveness and differential phenotypic properties, is sufficient to

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain DS-66^T among the type strains of selected *Microbacterium* species. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points; only values >50 % are shown. *Arthrobacter globiformis* DSM 20124^T (GenBank accession number M23411; not shown) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position. An extended phylogenetic tree from which Fig. 1 was taken is available as Supplementary Fig. S1 in IJSEM Online.

permit the allocation of this strain to a species that is separate from recognized species of the genus *Microbacterium* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain DS-66^T is considered to represent a novel species of the genus *Microbacterium*, for which the name *Microbacterium insulae* sp. nov. is proposed.

Description of *Microbacterium insulae* sp. nov.

Microbacterium insulae (in.su'lae. L. fem. gen. n. *insulae* of an island, where the type strain was isolated).

Cells are Gram-positive, non-motile, non-spore-forming rods or cocci (0.2–0.5 × 0.5–1.8 µm). Colonies on TSA are circular, convex, smooth, glistening, yellow and 1.5–2.0 mm in diameter after 7 days incubation at 30 °C. Optimal temperature for growth is 30 °C. Growth occurs at 20 and 38 °C, but not at 15 or 39 °C. Optimal pH for growth is between 6.5 and 7.0; growth occurs at pH 5.5 and 8.0, but not at pH 5.0 or 8.5. Growth occurs in the presence of 0–5.0 % (w/v) NaCl with an optimum of 0.5–1.0 % (w/v). Anaerobic growth does not occur on TSA or on TSA supplemented with nitrate. Cell-wall peptidoglycan type is B2β with partial substitution of glutamic acid by 3-hydroxy glutamic acid (Glu/Hyg–Gly–D–Orn). The amino acid in position 3 of the peptidoglycan is L-homoserine. The acyl type of the muramic acid is glycolyl. Cell-wall sugars are galactose, rhamnose and ribose. Predominant menaquinones are MK-13, MK-12 and MK-14. Major fatty acids (>10 % of the total) are anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{17:0} and iso-C_{16:0}. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, an unidentified phospholipid and an unidentified glycolipid. The DNA G + C content of the type strain is 69.9 mol% (determined

by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, DS-66^T (=KCTC 19247^T=CCUG 54523^T), was isolated from soil of Dokdo, Korea.

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References

- Bakir, M. A., Kudo, T. & Benno, Y. (2008). *Microbacterium hatanonis* sp. nov., isolated as a contaminant of hairspray. *Int J Syst Evol Microbiol* **58**, 654–658.
- 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.
- Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.
- Kageyama, A., Takahashi, Y. & Ōmura, S. (2006). *Microbacterium deminutum* sp. nov., *Microbacterium pumilum* sp. nov. and *Microbacterium aoyamense* sp. nov. *Int J Syst Evol Microbiol* **56**, 2113–2117.
- Kageyama, A., Takahashi, Y., Matsuo, Y., Kasai, H., Shizuri, Y. & Ōmura, S. (2007). *Microbacterium sediminicola* sp. nov. and *Microbacterium marinilacus* sp. nov., isolated from marine environments. *Int J Syst Evol Microbiol* **57**, 2355–2359.
- Kim, K. K., Park, H. Y., Park, W., Kim, I. S. & Lee, S.-T. (2005). *Microbacterium xylanilyticum* sp. nov., a xylan-degrading bacterium isolated from a biofilm. *Int J Syst Evol Microbiol* **55**, 2075–2079.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Lee, J.-S., Lee, K. C. & Park, Y.-H. (2006). *Microbacterium koreense* sp. nov., from sea water in the South Sea of Korea. *Int J Syst Evol Microbiol* **56**, 423–427.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyryl 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.
- Orla-Jensen, S. (1919). *The Lactic Acid Bacteria*. Copenhagen: Høst and Son.
- Park, H. Y., Kim, K. K., Jin, L. & Lee, S.-T. (2006). *Microbacterium paludicola* sp. nov., a novel xylanolytic bacterium isolated from swamp forest. *Int J Syst Evol Microbiol* **56**, 535–539.

- Park, M.-J., Kim, M. K., Kim, H.-B., Im, W.-T., Yi, T.-H., Soung, N.-K. & Yang, D.-C. (2008). *Microbacterium ginsengisoli* sp. nov., a β -glucosidase-producing bacterium isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **58**, 429–433.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- 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.
- Shivaji, S., Bhadra, B., Rao, R. S., Chaturvedi, P., Pindi, P. K. & Raghukumar, C. (2007). *Microbacterium indicum* sp. nov., isolated from a deep-sea sediment sample from the Chagos Trench, Indian Ocean. *Int J Syst Evol Microbiol* **57**, 1819–1822.
- 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.
- Takeuchi, M. & Hatano, K. (1998a). Union of the genera *Microbacterium* Orla-Jensen and *Aureobacterium* Collins *et al.* in a redefined genus *Microbacterium*. *Int J Syst Bacteriol* **48**, 739–747.
- Takeuchi, M. & Hatano, K. (1998b). Proposal of six new species in the genus *Microbacterium* and transfer of *Flavobacterium marinotypicum* ZoBell and Upham to the genus *Microbacterium* as *Microbacterium maritypicum* comb. nov. *Int J Syst Bacteriol* **48**, 973–982.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & 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.
- Yokota, A., Takeuchi, M., Sakane, T. & Weiss, N. (1993). Proposal of six new species in the genus *Aureobacterium* and transfer of *Flavobacterium esteraromaticum* Omelianski to the genus *Aureobacterium* as *Aureobacterium esteraromaticum* comb. nov. *Int J Syst Bacteriol* **43**, 555–564.
- 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 *Nocardioidea* and related taxa based on 16S rRNA gene 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.