Streptomyces panacagri sp. nov., isolated from soil of a ginseng field

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A Gram-positive, spore-forming, aerobic actinomycete, strain Gsoil 519^T, was isolated from soil of a ginseng field of Pocheon province in South Korea. The closest phylogenetic relatives were *Streptomyces marinus* Sp080513GE-26^T (97.94 % 16S rRNA gene sequence similarity), *Streptomyces albiacialis* NRRL B-24327^T (97.84 %), *Streptomyces albus* subsp. *albus* DSM 40313^T (97.84 %), *Streptomyces almquistii* NBRC 13015^T (97.81 %), *Streptomyces gibsonii* NBRC 15415^T (97.81 %), *Streptomyces rangoonensis* NBRC 13078^T (97.81 %), *Streptomyces sodiiphilus* YIM 80305^T (97.77 %) and *Streptomyces flocculus* NBRC 13041^T (97.67 %). The G + C content of the genomic DNA was 71.8 mol%. The chemotaxonomic data [MK-9(H₈) and MK-9(H₉) as the major menaquinones; LL-diaminopimelic acid as a component of the cell-wall peptidoglycan; ribose, xylose, mannose and glucose as the major cell-wall sugars; and anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{17:0}, anteiso-C_{17:0} and C_{16:0} as the major fatty acids] supported the affiliation of strain Gsoil 519^T to the genus *Streptomyces*. The physiological and biochemical characteristics and the low level of DNA–DNA relatedness differentiated the isolate genotypically and phenotypically from recognized members of the genus *Streptomyces*. The isolate, therefore, represents a novel species, for which the name *Streptomyces panacagri* sp. nov. is proposed, with Gsoil 519^T (=KCTC 19139^T=DSM 41871^T) as the type strain.

Waksman & Henrici (1943) first proposed the genus *Streptomyces*, the type genus of the family *Streptomycetaceae*. The general phenotypic properties of the genus are the production of a wide variety of pigments, which are responsible for the colour of the vegetative and aerial mycelia, and the utilization of a wide range of organic compounds as sole sources of carbon for energy and growth (Williams *et al.*, 1983; Kämpfer *et al.*, 1991; Korn-Wendisch & Kutzner, 1992). A lot of studies have been performed on this genus and it currently contains more than 500 species and subspecies with validly published names, making it the largest genus in the domain ‘*Bacteria*’ (Euzéby, 1997).

Strain Gsoil 519^T was isolated from soil of a ginseng field of Pocheon Province in South Korea. This soil sample was thoroughly suspended in 50 mM phosphate buffer (pH 7.0) and spread on 1/5 modified R2A agar (MR2A; Difco) as described by Im *et al.* (2010). Strain Gsoil 519^T was isolated after incubation at 30 °C for 1 month. The

strain was maintained on MR2A agar at 30 °C and stored in 20 % (w/v) glycerol at –70 °C.

The genomic DNA of strain Gsoil 519^T was extracted using a commercial genomic DNA extraction kit (Solgent). The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer pair 9F and 1512R and the purified PCR products were sequenced by Solgent, Daejeon, South Korea (Im *et al.*, 2010). The almost-complete sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using CLUSTAL_X (Thompson *et al.*, 1997) and gaps were edited in the program BioEdit (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in MEGA4 (Kumar *et al.*, 2008) and bootstrap values were calculated from 1000 replications (Felsenstein, 1985).

The 16S rRNA gene sequence of strain Gsoil 519^T determined in this study was a continuous stretch of

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 519^T is AB245388.

A supplementary figure is available with the online version of this paper.

1493 bp. Sequence similarity calculations after alignment indicated that the closest relatives of strain Gsoil 519^T were *Streptomyces marinus* Sp080513GE-26^T (97.94 % 16S rRNA gene sequence similarity), *Streptomyces albiacialis* NRRL B-24327^T (97.84 %), *Streptomyces albus* subsp. *albus* DSM 40313^T (97.84 %), *Streptomyces almquistii* NBRC 13015^T (97.81 %), *Streptomyces gibsonii* NBRC 15415^T (97.81 %), *Streptomyces rangoonensis* NBRC 13078^T (97.81 %), *Streptomyces sodiophilus* YIM 80305^T (97.77 %) and *Streptomyces flocculus* NBRC 13041^T (97.67 %). The relationships between strain Gsoil 519^T and other members of the genus *Streptomyces* are shown in the neighbour-joining phylogenetic tree (Fig. 1). The isolate formed a distinct lineage in a cluster containing all the above-mentioned type strains except for *S. albiacialis* NRRL B-24327^T. The cluster was supported with a relatively high bootstrap value (64 %). Although *S. albiacialis* NRRL B-24327^T did not belong to the cluster containing the isolate, it should be considered as closely related because of the high sequence similarity. Results from the neighbour-joining analysis were supported by the maximum-parsimony analysis.

On the basis of the phylogenetic inference, *S. albiacialis* DSM 41799^T, *S. albus* subsp. *albus* KCTC 1082^T, *S.*

almquistii KCTC 9672^T, *S. flocculus* KCCM 40682^T, *S. gibsonii* DSM 43284^T, *S. rangoonensis* KCCM 40392^T and *S. sodiophilus* DSM 41839^T were obtained and used as reference strains in most phenotypic tests and in DNA–DNA hybridization experiments. Phenotypic data for *S. marinus* DSM 41968^T was obtained from Khan *et al.* (2010) as it grew extremely slowly and so comparable data could not be obtained in this study.

Gram-staining was performed by the non-staining method as described by Buck (1982). For strain Gsoil 519^T, the hyphal arrangement, spore chain morphology and spore surface ornamentation were observed by light microscopy (E600; Nikon) and scanning electron microscopy (JSM-5410LV; JEOL) after growth on International *Streptomyces* Project (ISP) 2 agar at 28 °C for 21–31 days. Catalase activity was determined by bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetra-methyl-*p*-phenylenediamine. Single-carbon-source assimilation studies were performed as described by Cui *et al.* (2009). Growth was examined visually on 96-well plates incubated at 30 °C for up to 7 days. Some physiological characteristics were determined with the API 20 E gallery (bioMérieux), according to the manufacturer's instructions. Anaerobic growth was tested in serum bottles of MR2A broth

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain Gsoil 519^T with members of the genus *Streptomyces*. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered using the maximum-parsimony algorithm. Bar, 0.005 substitutions per nucleotide position.

containing 1 g thioglycolate l⁻¹ with the upper air layer substituted with nitrogen gas and resazurin oxygen indicator. The tests for anaerobic and aerobic nitrate reduction were performed as described by Cui *et al.* (2009). Degradation of DNA (using DNase agar from Scharlau, supplemented with 1 M HCl), casein, chitin, starch (Atlas 1993), lipid (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were recorded after 10 days. Growth at 4, 15, 20, 25, 30, 37, 42 and 45 °C and at pH 5.0–10.0 (at intervals of 0.5 pH unit) was assessed on MR2A agar after 5 days. Growth with 1–10% (w/v) NaCl was tested on MR2A agar for 5 days. Growth on nutrient agar, trypticase soy agar and MacConkey agar was evaluated at 30 °C.

To determine resistance to antibiotics, MR2A agar was supplemented with (µg ml⁻¹) streptomycin sulphate (2, 4, 6, 12), gentamicin sulphate (2, 4, 6, 12), tetracycline hydrochloride (4.5, 7.2, 10, 15), chloramphenicol (5, 10, 15, 30), kanamycin (2, 4, 6, 12), penicillin (6, 12, 18, 24) and erythromycin (2, 4, 6, 9). Plates were incubated at 30 °C and examined after 5 days.

Strain Gsoil 519^T was Gram-positive, strictly aerobic, spore-forming and had morphological characteristics consistent with those of members of the genus *Streptomyces*. Colonies cultured on ISP 2 and ISP 4 agar for 7 days produced surface and aerial mycelia with extensively branched hyphae that penetrated the agar. Colonies were mustard on ISP 2 and white on ISP 4 agar. A scanning electron micrograph showing the spore chain morphology of strain Gsoil 519^T is given in Supplementary Fig. S1 (available in IJSEM Online). The phenotypic characteristics of strain Gsoil 519^T are summarized in the species description and compared with those of the reference strains in Table 1.

For the measurement of the G+C content of the chromosomal DNA, genomic DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995) and analysed as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Cellular fatty acid compositions were determined for strains grown in trypticase soy broth (TSB; BD) at 30 °C for 5 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids were analysed by GC (Hewlett Packard 6890) and identified using the Microbial Identification software (Sasser, 1990). For chemotaxonomic analysis (isoprenoid quinones, cell-wall peptidoglycan types and polar lipids), strain Gsoil 519^T was grown on TSB for 5 days at 30 °C. Harvested cells were lyophilized for 24 h. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated by vacuum and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane/quinone solution was purified using a Sep-Pak Vac silica cartridge (Waters) and subsequently analysed by HPLC as described elsewhere (Hiraishi *et al.*, 1996). The isomer type of the diamino acid of the cell-wall peptidoglycan was determined by TLC after hydrolysis with 6 M HCl at 100 °C for 18 h, as described by Komagata & Suzuki (1987).

Table 1. Physiological characteristics of strain Gsoil 519^T and related type strains of species of the genus *Streptomyces*

Strains: 1, *Streptomyces panacagri* sp. nov. Gsoil 519^T; 2, *S. albiacialis* DSM 41799^T; 3, *S. albus* subsp. *albus* KCTC 1082^T; 4, *S. almquistii* KCTC 9672^T; 5, *S. flocculus* KCCM 40682^T; 6, *S. gibsonii* DSM 43284^T; 7, *S. rangoonensis* KCCM 40392^T; 8, *S. sodiophilus* DSM 41839^T; 9, *S. marinus* DSM 41968^T. Data for columns 1–8 were taken from this study; data for column 9 were taken from Khan *et al.* (2010). All strains are positive for assimilation of D-glucose. +, Positive; w, weakly positive; –, negative; ND, not detected.

Characteristic	1	2	3	4	5	6	7	8	9
Utilization of:									
D-Galactose	+	+	–	+	+	+	+	–	ND
L-Rhamnose	–	+	+	–	–	–	+	+	–
D-Fructose	+	+	–	–	+	+	+	–	+
D-Arabinose	–	+	–	–	+	–	–	–	+
L-Arabinose	–	+	+	–	+	–	–	–	ND
D-Ribose	–	+	+	+	+	+	+	–	ND
D-Xylose	–	+	+	+	+	+	+	–	+
Citrate	w	+	+	+	+	+	+	–	ND
Cellobiose	+	+	+	+	+	+	+	–	ND
Lactose	+	+	+	+	+	+	+	–	ND
Maltose	+	+	+	–	+	–	+	–	ND
Sucrose	+	+	–	–	–	–	+	–	+
Raffinose	–	+	–	–	–	–	+	–	–
Inositol	+	+	–	–	+	+	+	–	–
D-Mannitol	–	+	+	+	+	+	+	–	+
D-Sorbitol	+	+	–	–	+	–	+	–	–
Glycerol	+	+	+	+	+	+	+	–	ND
Nitrate reduction to nitrite	–	+	+	+	–	–	+	+	–
Enzyme production									
Lysine decarboxylase	–	+	–	–	–	–	–	–	ND
Urease	–	+	+	+	+	+	+	+	–
Gelatinase	+	–	+	+	+	+	+	+	+

Polar lipids were extracted, examined by two-dimensional TLC and identified as described by Minnikin *et al.* (1977). Cell-wall sugars were analysed as described by Stanek & Roberts (1974). DNA–DNA hybridization experiments were performed between strain Gsoil 519^T and the reference strains by the method described by Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were converted to DNA–DNA relatedness.

The DNA G+C content of strain Gsoil 519^T was 71.8 mol%. The respiratory quinone system of strain Gsoil 519^T contained MK-9(H₆) and MK-9(H₈) as the predominant quinones, which is consistent with the quinone system of the genus *Streptomyces*. The cell-wall peptidoglycan of strain Gsoil 519^T contained LL-diaminopimelic acid and the cell-wall sugars were glucose, ribose, xylose and mannose. The fatty acid compositions of the isolate and the reference strains are shown in Table 2. The fatty acid composition of

Table 2. Cellular fatty acid compositions (%) of strain Gsoil 519^T and related type strains of species of the genus *Streptomyces*

Strains: 1, *Streptomyces panacagri* sp. nov. Gsoil 519^T; 2, *S. albiacialis* DSM 41799^T; 3, *S. albus* subsp. *albus* KCTC 1082^T; 4, *S. almquistii* KCTC 9672^T; 5, *S. flocculus* KCCM 40682^T; 6, *S. gibsonii* DSM 43284^T; 7, *S. rangoonensis* KCCM 40392^T; 8, *S. sodiophilus* DSM 41839^T; 9, *S. marinus* DSM 41968^T. Data for columns 1–8 were taken from this study after culture on TSB for 5 days at 30 °C; data for column 9 were taken from Khan *et al.* (2010). Major fatty acids (>10 %) are shown in bold type. Fatty acids that accounted for <1.0 % of the total in each strain are not shown.

Fatty acid	1	2	3	4	5	6	7	8	9
Saturated									
C _{10:0}	—	—	—	1.02	—	—	—	—	—
C _{14:0}	1.17	—	3.63	—	—	—	—	—	—
C _{15:0}	—	1.60	—	1.55	2.96	—	—	—	—
C _{16:0}	11.90	3.94	6.85	8.04	8.92	2.93	3.22	4.15	4.00
C _{18:0}	2.57	—	11.83	—	—	11.22	13.30	1.39	—
C _{19:0}	—	—	3.77	—	—	1.64	2.13	—	—
C _{20:0}	—	—	18.95	—	—	6.60	8.76	—	—
Unsaturated									
C _{16:1} ω ₉ c	—	—	—	—	—	—	—	—	2.00
iso-C _{17:1} ω ₉ c	—	—	—	—	—	—	—	3.41	—
anteiso-C _{17:1} ω ₉ c	—	—	—	—	—	—	—	3.81	—
C _{18:3} ω ₆ c (6,9,12)	—	—	—	—	—	—	—	1.31	—
Branched-chain									
iso-C _{15:0}	19.09	10.55	9.51	14.41	13.92	12.83	12.03	8.31	11.00
iso-C _{16:0}	5.52	24.40	2.39	20.57	20.28	1.70	2.74	30.67	30.00
iso-C _{16:1} H	—	—	—	—	—	—	—	—	4.00
iso-C _{16:1} G	—	1.66	—	—	—	—	—	2.45	—
iso-C _{17:0}	14.78	5.82	2.65	6.61	10.08	10.88	9.46	4.32	2.00
C _{17:0} cyclo	—	1.66	—	—	—	—	—	—	—
iso-C _{19:0}	—	—	—	—	—	7.29	6.67	—	—
anteiso-C _{15:0}	31.35	25.46	27.26	30.41	24.06	31.07	29.87	18.82	24.00
anteiso-C _{17:0}	11.38	21.50	3.44	14.31	16.63	10.15	7.93	18.07	8.00
anteiso-C _{19:0}	—	—	—	—	—	3.68	2.95	—	—
Unknown ECL 13.566*	—	—	—	—	—	—	—	2.34	—

*Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified; ECL, equivalent chain-length.

strain Gsoil 519^T was mainly composed of anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{17:0}, anteiso-C_{17:0} and C_{16:0}, which is typical for members of the genus *Streptomyces*. However, some qualitative and quantitative differences in fatty acid content could be observed between strain Gsoil 519^T and the reference strains. DNA–DNA relatedness between strain Gsoil 519^T and the reference strains was <40 %, i.e. 19 % (*S. marinus* DSM 41968^T), 8 % (*S. albiacialis* DSM 41799^T), 10 % (*S. albus* subsp. *albus* KCTC 1082^T), 2 % (*S. almquistii* KCTC 9672^T), 5 % (*S. gibsonii* DSM 43284^T), 9 % (*S. rangoonensis* KCCM 40392^T), 6 % (*S. sodiophilus* DSM 41839^T) and 1 % (*S. flocculus* KCCM 40682^T). These values are low enough to assign strain Gsoil 519^T to a novel species of the genus *Streptomyces* (Wayne *et al.*, 1987).

In summary, the characteristics of strain Gsoil 519^T were consistent with the description of the genus *Streptomyces* with regard to morphological, biochemical and chemotaxonomic properties. However, phylogenetic distances and the low level of DNA–DNA relatedness (Wayne *et al.*,

1987) between strain Gsoil 519^T and recognized members of the genus, and phenotypic characteristics of the isolate (Table 1) support the assignment of the isolate to a novel species of the genus *Streptomyces*, for which the name *Streptomyces panacagri* sp. nov. is proposed.

Description of *Streptomyces panacagri* sp. nov.

Streptomyces panacagri (pa.na.ca'gri. N.L. n. *Panax -acis* scientific name of ginseng; L. n. *ager agri* a field; N.L. gen. n. *panacagri* of a ginseng field).

Cells are Gram-positive, strictly aerobic, spore-forming and non-motile. Hyphae are abundant and well developed. Produces mustard-coloured surface and aerial mycelia after 7 days culture on ISP 2 agar. Spore chains are *rectiflexibiles* and the spore surface is smooth. Grows well at 20–30 °C, but not at 15 or 37 °C, and at pH 5.5–8.5. Grows with 0–5.0 % (w/v) NaCl, but not with 6.0 % NaCl. Grows on nutrient agar, but not on MacConkey agar. Catalase- and oxidase-positive. Does not reduce nitrate to nitrite. H₂S is

not produced. Gelatinase is positive, but urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, β -galactosidase and tryptophan deaminase are negative. Does not produce any acid or gas from glucose. Degrades starch, but not xylan, chitin, cellulose, casein or DNA. The following compounds are utilized as sole carbon sources: D-galactose, D-glucose, D-mannose, D-fructose, D-lyxose, L-xylose, *N*-acetylglucosamine, pyruvic acid, acetate, propionate, 3-hydroxybutyrate, valerate, phenylacetate, 3-hydroxybenzoate, salicin, citrate, lactate, malate, malonate, tartaric acid, D-cellobiose, lactose, maltose, sucrose, trehalose, gluconate, D-adonitol, dulcitol, inositol, D-sorbitol, xylitol, glycerol, glycogen, alanine, glutamic acid, glutamine, histidine, phenylalanine and threonine. The following compounds are not utilized as sole carbon sources: D-fucose, ethanol, L-rhamnose, L-sorbose, D- and L-arabinose, D-ribose, D-xylose, formic acid, caprate, maleic acid, fumaric acid, benzoic acid, 4-hydroxybenzoate, succinic acid, glutaric acid, itaconate, adipate, suberate, oxalic acid, melibiose, D-raffinose, D-mannitol, amygdalin, methanol, inulin, dextran, arginine, asparagine, aspartic acid, cysteine, glycine, isoleucine, leucine, lysine, methionine, proline, serine, tryptophan, tyrosine and valine. MK-9(H_6) and MK-9(H_8) are the predominant menaquinones and anteiso- $C_{15:0}$, iso- $C_{15:0}$, iso- $C_{17:0}$, anteiso- $C_{17:0}$ and $C_{16:0}$ are the major fatty acids. The cell-wall peptidoglycan contains LL-diaminopimelic acid. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol mannosides. The cell-wall sugars are ribose, xylose, mannose and glucose.

The type strain, Gsoil 519^T (=KCTC 19139^T=DSM 41871^T), was isolated from soil of a ginseng field of Pocheon province, South Korea. The DNA G+C content of the type strain is 71.8 mol% (HPLC).

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References

- Atlas, R. M. (1993). *Handbook of Microbiological Media*. Edited by L. C. Parks. Boca Raton, FL: CRC Press.
- Buck, J. D. (1982). Nonstaining (KOH) method for determination of gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Cui, Y.-S., Lee, S.-T. & Im, W.-T. (2009). *Nocardioideus ginsengisoli* sp. nov., isolated from soil of a ginseng field. *Int J Syst Evol Microbiol* **59**, 3045–3050.
- Euzéby, J. P. (1997). List of bacterial names with standing in nomenclature: a folder available on the Internet. *Int J Syst Bacteriol* **47**, 590–592. <http://www.bacterio.cict.fr/>
- 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.
- Felsenstein, J. (1985). Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hiraishi, A., Ueda, Y., Ishihara, J. & Mori, T. (1996). Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J Gen Appl Microbiol* **42**, 457–469.
- Im, W.-T., Kim, S.-Y., Liu, Q.-M., Yang, J.-E., Lee, S.-T. & Yi, T.-H. (2010). *Nocardioideus ginsengisegetis* sp. nov., isolated from soil of a ginseng field. *J Microbiol* **48**, 623–628.
- Khan, S. T., Tamura, T., Takagi, M. & Shin-Ya, K. (2010). *Streptomyces tateyamensis* sp. nov., *Streptomyces marinus* sp. nov. and *Streptomyces haliclona* sp. nov., isolated from the marine sponge *Haliclona* sp. *Int J Syst Evol Microbiol* **60**, 2775–2779.
- Kämpfer, P., Kroppenstedt, R. M. & Dott, W. (1991). A numerical classification of the genera *Streptomyces* and *Streptoverticillium* using miniaturized physiological tests. *J Gen Microbiol* **137**, 1831–1891.
- Kimura, M. (1983). *The Neutral Theory of Molecular Evolution*. Cambridge: Cambridge University Press.
- Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Korn-Wendisch, F. & Kutzner, H. J. (1992). The family *Streptomycetaceae*. In *The Prokaryotes*, 2nd edn, vol. 1, pp. 921–995. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.
- Kouker, G. & Jaeger, K.-E. (1987). Specific and sensitive plate assay for bacterial lipases. *Appl Environ Microbiol* **53**, 211–213.
- Kumar, S., Nei, M., Dudley, J. & Tamura, K. (2008). MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief Bioinform* **9**, 299–306.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Minnikin, D. E., Patel, P. V., Alshamaony, L. & Goodfellow, M. (1977). Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- Moore, D. D. & Dowhan, D. (1995). Preparation and analysis of DNA. In *Current Protocols in Molecular Biology*, pp. 2–11. Edited by F. W. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- 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*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- Ten, L. N., Im, W.-T., Kim, M.-K., Kang, M.-S. & Lee, S.-T. (2004). Development of a plate technique for screening of polysaccharide-degrading

microorganisms by using a mixture of insoluble chromogenic substrates. *J Microbiol Methods* **56**, 375–382.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Waksman, S. A. & Henrici, A. T. (1943). The nomenclature and classification of the actinomycetes. *J Bacteriol* **46**, 337–341.

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.

Williams, S. T., Goodfellow, M., Alderson, G., Wellington, E. M. H., Sneath, P. H. A. & Sackin, M. J. (1983). Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* **129**, 1743–1813.