Streptacidiphilus anmyonensis sp. nov., *Streptacidiphilus rugosus* sp. nov. and *Streptacidiphilus melanogenes* sp. nov., acidophilic actinobacteria isolated from *Pinus* soils

Sung-Heun Cho, Ji-Hye Han, Hye-Young Ko and Seung Bum Kim

Correspondence
Seung Bum Kim
sbk01@cnu.ac.kr

Department of Microbiology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yuseong, Daejeon 305-764, Republic of Korea

The taxonomic positions of 22 spore-forming, extensively branched actinobacteria isolated from *Pinus* soils were examined using a polyphasic approach. Analysis of the 16S rRNA gene sequences indicated that all of the isolates fell into three distinctive phylogenetic clusters within the genus *Streptacidiphilus* of the family *Streptomycetaceae*, and also that *Streptacidiphilus jiangxiensis* was the species closest to the three phylocusters, with 16S rRNA gene sequence similarities ranging from 98.0 to 99.2%. However, the low DNA–DNA relatedness values between representatives of the three clusters and *S. jiangxiensis* clearly differentiated them from one another. Representative isolates were also found to have chemotaxonomic features typical of the genus *Streptacidiphilus* and were distinguishable from all established species of *Streptacidiphilus* on the basis of a combination of phenotypic properties. It is evident from this study that each of the three phylocusters should be equated with three novel *Streptacidiphilus* species, for which the following names are proposed: *Streptacidiphilus anmyonensis* sp. nov. (type strain AM11^T = NBRC 103185^T = KCTC 19278^T), *Streptacidiphilus rugosus* sp. nov. (type strain AM16^T = NBRC 103186^T = KCTC 19279^T) and *Streptacidiphilus melanogenes* sp. nov. (type strain SB-B34^T = NBRC 103184^T = KCTC 19280^T).

Acidophilic actinobacteria with streptomycete-like features are common in terrestrial habitats such as acidic forest and mine-drainage soils, where they form a major constituent of the actinobacterial community (Williams *et al.*, 1971; Khan & Williams, 1975; Hagedorn, 1976; Goodfellow & Dawson, 1978; Lonsdale, 1985; Seong, 1992; Cho *et al.*, 2006). These actinobacteria are potential sources of commercially significant antifungal compounds and acid-stable enzymes (Williams & Khan, 1974; Williams & Flowers, 1978) and are likely to have a major role in the decomposition of fungal biomass in acid litters and soils (Williams & Robinson, 1981). It has been shown that acidophilic actinobacteria consistently form two distinct aggregate taxa (namely, the neutrotolerant acidophilic and strictly acidophilic cluster-groups) on the basis of numerical phenetic data (Khan & Williams, 1975; Lonsdale, 1985;

Seong *et al.*, 1993) and genotypic data for the 16S rRNA gene (Kim *et al.*, 2003, 2004; Xu *et al.*, 2006). Streptacidiphili are aerobic, Gram-positive, acidophilic (i.e. optimal growth around pH 4.5), chemo-organotrophic actinobacteria that form extensively branched substrate mycelium and aerial hyphae that differentiate into long chains of smooth-surfaced spores. The major chemotaxonomic features include the following: LL-diaminopimelic acid as the major diamino acid in the cell wall, galactose and rhamnose as the diagnostic sugars in whole-cell hydrolysates, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides as the main polar lipids, hexahydrogenated and octahydrogenated menaquinones [MK-9(H₈)] with nine isoprene units as the major respiratory quinones and a mixture of saturated, iso- and anteiso-branched fatty acids (Lonsdale, 1985; Seong, 1992). Strict acidophiles were found to constitute a novel genus within the family *Streptomycetaceae*, and three species, *Streptacidiphilus albus*, *Streptacidiphilus carbonis* and *Streptacidiphilus neutrinimicus* were initially described (Kim *et al.*, 2003). Two additional species, *Streptacidiphilus jiangxiensis* (Huang *et al.*, 2004) and *Streptacidiphilus oryzae* (Wang *et al.*, 2006), were described subsequently.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains AM-11^T, AM-16^T and SB-B34^T are DQ904546, DQ904547 and DQ994689; those of other strains described in this work are given in Supplementary Fig. S1.

An extended neighbour-joining phylogenetic tree and whole-cell fatty acid profiles for strains AM-11^T, AM-16^T and SB-B34^T and recognized *Streptacidiphilus* species are available as supplementary data in the online version of this paper.

Following a previous report of the isolation of acidophilic actinobacteria from soils associated with *Pinus thunbergii* (Cho *et al.*, 2006), the taxonomic status of representative isolates belonging to the genus *Streptacidiphilus* was examined in this study by using a polyphasic approach.

All of the strains were maintained and examined on agar media adjusted to pH 4.5–5.0. They were kept on acidified modified Bennett's agar and starch-casein agar plates (Seong, 1992) at room temperature and as suspensions of mycelial fragments and spores in glycerol (20%, v/v). Biomass for the diaminopimelic acid, fatty acid, menaquinone and molecular systematic analyses was obtained from shake-flask cultures and test tubes containing modified Bennett's broth (pH 5.0) grown at 30 °C for 7 days. Biomass for chemical and molecular systematic studies was washed twice in distilled water and then freeze-dried; biomass for preservation was stored at –20 and –70 °C.

The strains were inoculated onto acidified modified Bennett's and acidified inorganic salts-starch (ISP 4 medium; Shirling & Gottlieb, 1966) agar plates and incubated at 30 °C for 14 days. The plates were examined visually to determine the colour of the aerial spore mass, substrate mycelial pigments and soluble pigments. Yeast extract-malt extract (ISP 2 medium; Shirling & Gottlieb, 1966) agar was used to determine the production of soluble pigments. Spore-chain morphology and spore-surface ornamentation were examined using scanning electron microscopy, as described previously (O'Donnell *et al.*, 1993). Phenotypic properties of the strains were examined using established procedures (Seong *et al.*, 1993; Williams *et al.*, 1983), but with acidified media.

Isoprenoid quinones and diaminopimelic acid were investigated using reversed-phase HPLC procedures as described by Komagata & Suzuki (1987) and El-Waziry *et al.* (1996), respectively. Fatty acids were extracted, methylated and analysed by GC using the standard Microbial Identification System (MIDI) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Isolation of chromosomal DNA, PCR amplification and direct sequencing of the PCR products were carried out as described previously (Park *et al.*, 2005). The resultant 16S rRNA gene sequences were aligned together with corresponding sequences from representative species of the genera *Kitasatospora*, *Streptacidiphilus* and *Streptomyces* by using the PHYDIT program (version 3.1; available at <http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using the Fitch–Margoliash, maximum-likelihood, maximum-parsimony and neighbour-joining methods according to previously described procedures (Kim *et al.*, 2003; Park *et al.*, 2006).

Levels of DNA–DNA relatedness were determined using the procedure described by Cho & Giovannoni (2004). The hybridization temperature was 55 °C. Densitometric analyses were carried out using a Personal Densitometer with ImageQuant imaging software (Bio-Rad).

All of the novel isolates were Gram-positive, aerobic, acidophilic actinobacteria. All formed extensively branched substrate mycelia and aerial hyphae that differentiated into long, straight to flexuous chains of smooth-surfaced spores. Brown or cream-coloured colonies carrying moderate to abundant white or greyish-white aerial hyphae were formed on acidified oatmeal agar, ISP 4 agar, ISP 2 agar and modified Bennett's agar.

The major diamino acid of the peptidoglycan was LL-diaminopimelic acid, although minor amounts of the *meso* isomer were detected. Representative isolates AM-11^T, AM-16^T and SB-B34^T contained 13-methyltetradecanoic acid (iso-C_{15:0}; 15–20% of the total fatty acid composition), 12-methyltetradecanoic acid (anteiso-C_{15:0}; 7–15%), 14-methylpentadecanoic acid (iso-C_{16:0}; 15–25%), n-hexadecanoic acid (C_{16:0}; 15–16%), 15-methylhexadecanoic acid (iso-C_{17:0}; 3–7%) and 14-methylhexadecanoic acid (anteiso-C_{17:0}; 4–7%) (see Supplementary Table S1, available in IJSEM Online). All of the three strains contained hexahydrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H₆) and MK-9(H₈), comprising 18–24 and 59–69% of the total, respectively] as the predominant isoprenologues.

From the phylogenetic analysis of 1358 nucleotide positions from the 16S rRNA gene sequences, the isolates formed three distinctive phylogenetic lines within the taxonomic variation encompassed by the genus *Streptacidiphilus* (Fig. 1). The expanded phylogenetic tree including all 22 isolates tested also showed three independent phylocusters (Supplementary Fig. S1). It was notable that the isolates from the Anmyeon area (AM prefixes) were split into two separate phylocusters, whereas isolates from the Sambong area (SB prefixes) formed an independent phylocuster. The strains belonging to each phylocuster shared the same morphological and cultural characteristics.

The DNA–DNA relatedness data for the representative strains and the type strain of *S. jiangxiensis* confirmed that all of the taxa could be clearly separated from one another (Table 1). Strains AM-16^T and *S. jiangxiensis* 33214^T shared 34% DNA–DNA relatedness. Strain SB-B34^T exhibited 30% DNA–DNA relatedness with respect to *S. jiangxiensis* 33214^T, 42% with respect to strain AM-11^T and 23% with respect to strain AM-16^T. No obvious correlation was observed between the 16S rRNA gene sequence similarity and DNA–DNA relatedness data (Table 1).

The representative isolates could be separated from other *Streptacidiphilus* species with validly published names and also from one another by using a combination of phenotypic tests (Table 2). It is evident from the results of the polyphasic study, including the phenotypic data, the diamino acid present in the peptidoglycan, the fatty acid profiles, the DNA–DNA hybridization data and the phylogenetic results for the 16S rRNA gene, that each of the three novel phylocusters of *Streptacidiphilus* should be recognized as an independent species. The names proposed

Fig. 1. Neighbour-joining phylogenetic tree (Saitou & Nei, 1987), based on almost-complete 16S rRNA gene sequences, showing the relationships between the isolates and representatives of the genus *Streptacidiphilus*. Asterisks indicate branches that were also recovered using the Fitch–Margoliash, maximum-likelihood and maximum-parsimony methods. Numbers at nodes indicate bootstrap percentages (based on neighbour-joining analysis of 1000 resampled datasets using Jukes–Cantor distances); only values over 50% are given. Bar, 0.01 substitutions per nucleotide position. An extended version of this tree, including all 22 novel isolates, is available as Supplementary Fig. S1.

for the three novel species are *Streptacidiphilus anmyonensis* sp. nov. (type strain AM-11^T), *Streptacidiphilus rugosus* sp. nov. (type strain AM-16^T) and *Streptacidiphilus melanogenes* sp. nov. (type strain SB-34^T).

Description of *Streptacidiphilus anmyonensis* sp. nov.

Streptacidiphilus anmyonensis (an.myon.en'sis. N.L. masc. adj. *anmyonensis* of Anmyon, where the first strains were isolated).

Forms cream-coloured colonies that carry moderate to abundant, white to greyish-white aerial hyphae on acidified oatmeal, ISP 4, ISP 2 and modified Bennett's agar plates. Substrate mycelium is brownish grey or brown on acidified oatmeal agar and ISP 2 agar, but cream on ISP 4 agar and modified Bennett's agar. Aerial hyphae differentiate into long flexuous chains of spores ($0.6 \times 0.9 \mu\text{m}$); the spore surface is smooth. Soluble pigments are not produced on any of the above-mentioned media. Growth occurs at pH 3.0–8.0 and 28–35 °C. Starch and Tween 80 are

degraded, but xanthine and Tweens 20 and 40 are not. Glycerol, D-gluconic acid, D(+)-glucosamine hydrochloride, myo-inositol, melibiose, D-sorbitol, sucrose and D(+)-xylose (each at 1%, w/v) and L-arginine (at 0.1%, w/v) are used as sole carbon sources for energy and growth, but L-aspartic acid and sodium oxalate (each at 0.1%, w/v) are not. L-Isoleucine is used as a sole nitrogen source. Chemotaxonomic properties are typical of those for the genus *Streptacidiphilus*. The major fatty acids are iso-C_{15:0} (18.4% of the total fatty acid composition), anteiso-C_{15:0} (11.4%), iso-C_{16:0} (19.1%), C_{16:0} (14.7%), iso-C_{17:0} (7.4%) and anteiso-C_{17:0} (7.5%). Contains hexahydrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H₆) and MK-9(H₈); 24.1 and 55.8% of the total, respectively] as the predominant isoprenologues. The diamino acid of the peptidoglycan is LL-diaminopimelic acid (89% of the total diaminopimelic acid composition), although a minor amount of the *meso* isomer (11%) is also detected.

The species currently includes the type strain AM-11^T (=NBRC 103185^T =KCTC 19278^T) and strains AM-02, AM-06, AM-08, AM-10, AM-17, AM-18, AM-23, AM-24, AM-25 and AM-30, isolated from *Pinus*-associated soils from Anmyeon, near the coastal areas of Tae-An, Chungnam, Republic of Korea (Cho *et al.*, 2006).

Description of *Streptacidiphilus rugosus* sp. nov.

Streptacidiphilus rugosus (ru.go'sus. L. masc. adj. *rugosus* wrinkled).

Forms cream-coloured, rugose colonies that carry moderate to abundant white aerial hyphae on acidified oatmeal, ISP 4, ISP 2 and modified Bennett's agar plates. Substrate mycelium is cream on acidified ISP 4, ISP 2 and modified Bennett's agars, but yellowish brown or brown on oatmeal

Table 1. DNA–DNA relatedness and 16S rRNA gene sequence similarity among strains AM-11^T, AM-16^T, SB-B34^T and *S. jiangxiensis* 33214^T

Values above the diagonal are 16S rRNA gene sequence similarity values (%). Values below the diagonal are mean percentages from three reciprocal DNA–DNA hybridization reactions.

Strain	33214 ^T	AM-11 ^T	AM-16 ^T	SB-B34 ^T
33214 ^T	–	99.3	98.1	98.8
AM-11 ^T	35	–	98.5	99.3
AM-16 ^T	34	29	–	99.2
SB-B34 ^T	30	42	28	–

Table 2. Phenotypic properties that serve to distinguish strains AM-11^T, AM-16^T and SB-B34^T from type strains of *Streptacidiphilus* species with validly published names

Strains: 1, AM-11^T; 2, AM-16^T; 3, SB-B34^T; 4, *S. jiangxiensis* 33214^T; 5, *S. albus* JL83^T; 6, *S. carbonis* JL415^T; 7, *S. neutrinimicus* JL206^T; 8, *S. oryzae* TH49^T. All strains were positive for the utilization of glycerol and sucrose and also for growth at pH 4, 5 and 6.

Characteristic	1	2	3	4	5	6	7	8
Degradation of:								
Starch	+	+	+	+	+	+	+	–
Tween 20	–	–	–	–	–	–	–	+
Tween 40	–	–	+	–	+	+	–	+
Tween 80	+	+	+	–	–	–	–	+
Xanthine	–	–	+	+	+	+	–	–
Growth on sole carbon sources at 1% (w/v)								
D-Gluconic acid	+	+	+	+	–	+	–	+
D-Glucosamine hydrochloride	+	–	+	+	+	+	+	+
myo-Inositol	+	+	+	+	–	+	+	+
Melibiose	+	–	+	+	+	+	+	+
D-Sorbitol	+	+	+	+	–	+	+	+
D-Xylose	+	–	+	–	–	+	+	+
Growth on sole carbon sources at 0.1% (w/v)								
L-Arginine	+	+	+	+	+	–	–	+
L-Aspartic acid	–	–	–	+	+	+	+	+
Sodium oxalate	–	+	+	+	–	+	+	+
Growth on L-isoleucine (0.1%, w/v) as sole nitrogen source	+	+	+	+	–	+	+	+
Growth at pH 3	+	–	+	–	+	+	–	+

agar. Aerial hyphae differentiate into long flexuous chains of spores ($0.5 \times 1.2 \mu\text{m}$); the spore surface is smooth. Soluble pigments are not produced on any of the above-mentioned media. Growth occurs at pH 3.0–8.0 and 28–35 °C. Starch and Tween 80 are degraded, but xanthine and Tweens 20 and 40 are not. Glycerol, D-gluconic acid, myo-inositol, D-sorbitol and sucrose (each at 1%, w/v), L-arginine and sodium oxalate (each at 0.1%, w/v) are used as sole carbon sources for energy and growth, but D(+)-glucosamine hydrochloride, melibiose and D(+)-xylose (each at 1%, w/v) and L-aspartic acid (at 0.1%, w/v) are not. L-Isoleucine is used as a sole nitrogen source. Chemotaxonomic properties are typical of those for the genus *Streptacidiphilus*. The major fatty acids are iso-C_{15:0} (15% of the total fatty acid composition), anteiso-C_{15:0} (14.9%), iso-C_{16:0} (25.4%), n-C_{16:0} (15.6%), iso-C_{17:0} (3.0%) and anteiso-C_{17:0} (4.7%). Contains hexahydrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H₆) and MK-9(H₈); 19.8 and 59.6% of the total, respectively] as the predominant isoprenologues. The diamino acid of the peptidoglycan is LL-diaminopimelic acid (94% of the total diaminopimelic acid composition), although a minor amount of the *meso* isomer (6%) is also detected.

The species currently includes the type strain AM-16^T (NBRC 103186^T = KCTC 19279^T) and strains AM-20, AM-21, AM-22, AM-26, AM-27, AM-28 and AM-29, isolated from *Pinus*-associated soils from Anmyeon, near the coastal areas of Tae-An, Chungnam, Republic of Korea.

Description of *Streptacidiphilus melanogenes* sp. nov.

Streptacidiphilus melanogenes [me.la.no'gen.es. Gr. n. *melas* -anos black; Gr. v. suff. -genes producing; N.L. part. adj. *melanogenes* producing black (pigment)].

Forms cream-coloured colonies that carry moderate to abundant white aerial hyphae on acidified oatmeal, ISP 4, ISP 2 and modified Bennett's agar plates. Substrate mycelium is brownish grey or brown on acidified ISP 4 agar and ISP 2 agar, but cream on oatmeal agar and modified Bennett's agar. Aerial hyphae differentiate into long flexuous chains of spores ($0.6 \times 1.0 \mu\text{m}$); the spore surface is smooth. Brownish-grey diffusible pigments are formed on acidified oatmeal agar and ISP 2 agar. Soluble pigments are not produced on ISP 4 agar or modified Bennett's agar. Growth occurs at pH 3.0–8.0 and 28–35 °C. Starch, Tweens 40 and 80 and xanthine are degraded, but Tween 20 is not. Glycerol, D-gluconic acid, D(+)-glucosamine hydrochloride, myo-inositol, melibiose, D-sorbitol, sucrose and D(+)-xylose (each at 1%, w/v), L-arginine and sodium oxalate (each at 0.1%, w/v) are used as sole carbon sources for energy and growth, but L-aspartic acid (at 0.1%, w/v) is not. L-Isoleucine is used as a sole nitrogen source. Chemotaxonomic properties are typical of those for the genus *Streptacidiphilus*. The major fatty acids are iso-C_{15:0} (20.4% of the total fatty acid composition), anteiso-C_{15:0} (6.7%), iso-C_{16:0} (14.7%), n-C_{16:0} (15.7%), iso-C_{17:0} (7.3%) and anteiso-C_{17:0} (3.8%). Contains hexahydrogenated and octahydrogenated

menaquinones with nine isoprene units [MK-9(H₆) and MK-9(H₈); 18.1 and 68.6% of the total, respectively] as the predominant isoprenologues. The diamino acid of the peptidoglycan is LL-diaminopimelic acid (100% of the total diaminopimelic acid composition).

The species currently includes the type strain SB-B34^T (=NBRC 103184^T =KCTC 19280^T) and strains SB-B33 and SB-B35, isolated from *Pinus*-associated soils from Sambong, near the coastal areas of Tae-An, Chungnam, Republic of Korea.

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References

- Cho, J. C. & Giovannoni, S. J. (2004). *Oceanicola granulosus* gen. nov., sp. nov. and *Oceanicola batsensis* sp. nov., poly- β -hydroxybutyrate-producing marine bacteria in the order 'Rhodobacterales'. *Int J Syst Evol Microbiol* **54**, 1129–1136.
- Cho, S. H., Han, J. H., Seong, C. N. & Kim, S. B. (2006). Phylogenetic diversity of acidophilic spiroactinobacteria isolated from various soils. *J Microbiol* **44**, 600–606.
- El-Waziry, A. M., Tomita, Y., Ling, J. R. & Onodera, R. (1996). Measurement of total and separate stereoisomers of diaminopimelic acid in rumen bacteria by high-performance liquid chromatography. *J Chromatogr B Biomed Appl* **677**, 53–59.
- Goodfellow, M. & Dawson, D. (1978). Qualitative and quantitative studies of bacteria colonizing *Picea sitchensis* litter. *Soil Biol Biochem* **10**, 303–307.
- Hagedorn, C. (1976). Influences of soil acidity on *Streptomyces* populations inhabiting forest soils. *Appl Environ Microbiol* **32**, 368–375.
- Huang, Y., Cui, Q., Wang, L., Rodriguez, C., Quintana, E., Goodfellow, M. & Liu, Z. (2004). *Streptacidiphilus jiangxiensis* sp. nov., a novel actinomycete isolated from acidic rhizosphere soil in China. *Antonie van Leeuwenhoek* **86**, 159–165.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Khan, M. R. & Williams, S. T. (1975). Studies on the ecology of actinomycetes in soil. VIII. Distribution and characteristics of acidophilic actinomycetes. *Soil Biol Biochem* **7**, 345–348.
- Kim, S. B., Lonsdale, J., Seong, C. N. & Goodfellow, M. (2003). *Streptacidiphilus* gen. nov., acidophilic actinomycetes with wall chemotype I and emendation of the family Streptomycetaceae (Waksman and Henrici 1943^{AL}) emend. Rainey (1977). *Antonie van Leeuwenhoek* **83**, 107–116.
- Kim, S. B., Seong, C. N., Jeon, S. J., Bae, K. S. & Goodfellow, M. (2004). Taxonomic study of neutrotolerant acidophilic actinomycetes isolated from soil and description of *Streptomyces yeochonensis* sp. nov. *Int J Syst Evol Microbiol* **54**, 211–214.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lonsdale, J. T. (1985). *Aspects of the biology of acidophilic actinomycetes*. PhD thesis, University of Newcastle, Newcastle, UK.
- O'Donnell, A. G., Falconer, C., Goodfellow, M., Ward, A. C. & Williams, E. (1993). Biosystematics and diversity amongst novel carboxydrotrophic actinomycetes. *Antonie van Leeuwenhoek* **64**, 325–340.
- Park, M. S., Jung, S. R., Lee, M. S., Kim, K. O., Do, J. O., Lee, K. H., Kim, S. B. & Bae, K. S. (2005). Isolation and characterization of bacteria associated with two sand dune plant species, *Calystegia soldanella* and *Elymus mollis*. *J Microbiol* **43**, 219–227.
- Park, M. S., Jung, S. R., Lee, K. H., Lee, M. S., Do, J. O., Kim, S. B. & Bae, K. S. (2006). *Chryseobacterium soldanellicola* sp. nov. and *Chryseobacterium taeanense* sp. nov., isolated from roots of sand-dune plants. *Int J Syst Evol Microbiol* **56**, 433–438.
- 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.
- Seong, C. N. (1992). *Numerical taxonomy of acidophilic and neutrotolerant actinomycetes isolated from acid soil in Korea*. PhD thesis, Seoul National University, Seoul, Republic of Korea.
- Seong, C. N., Goodfellow, M., Ward, A. C. & Hah, Y. C. (1993). Numerical classification of acidophilic actinomycetes isolated from acid soil in Korea. *J Microbiol* **31**, 355–363.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Wang, L., Huang, Y., Liu, Z., Goodfellow, M. & Rodriguez, C. (2006). *Streptacidiphilus oryzae* sp. nov., an actinomycete isolated from rice-field soil in Thailand. *Int J Syst Evol Microbiol* **56**, 1257–1261.
- Williams, S. T. & Flowers, T. H. (1978). The influence of pH on starch hydrolysis by neutrophilic and acidophilic actinomycetes. *Microbios* **20**, 99–106.
- Williams, S. T. & Khan, M. R. (1974). Antibiotics – a soil microbiologist's viewpoint. *Postepy Hig Med Dosw* **28**, 395–408.
- Williams, S. T. & Robinson, C. S. (1981). The role of streptomycetes in decomposition of chitin in acidic soils. *J Gen Microbiol* **127**, 55–63.
- Williams, S. T., Davies, F. L., Mayfield, C. I. & Khan, M. R. (1971). Studies on the ecology of actinomycetes in soil. II. The pH requirements of streptomycetes from two acid soils. *Soil Biol Biochem* **3**, 187–195.
- 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.
- Xu, C., Wang, L., Cui, Q., Huang, Y., Liu, Z., Zheng, G. & Goodfellow, M. (2006). Neutrotolerant acidophilic *Streptomyces* species isolated from acidic soils in China: *Streptomyces guanduensis* sp. nov., *Streptomyces paucisporeus* sp. nov., *Streptomyces rubidus* sp. nov. and *Streptomyces yanglinensis* sp. nov. *Int J Syst Evol Microbiol* **56**, 1109–1115.