Streptosporangium yunnanense sp. nov. and *Streptosporangium purpuratum* sp. nov., from soil in China

Li-Ping Zhang,¹ Cheng-Lin Jiang² and Wen-Xin Chen³

Correspondence

Li-Ping Zhang
zhlping@mail.hbu.edu.cn

¹College of Life Sciences, Hebei University, Baoding 071002, PR China

²The Key Laboratory for Microbial Resources of Ministry of Education, PR China, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

³Department of Microbiology, College of Life Sciences, China Agricultural University, Beijing 100094, PR China

Two strains of *Streptosporangium* were isolated from Yunnan Province, a region of China with specific geographical conditions that contribute to its great microbiological diversity. They were identified using a polyphasic approach employing phenotypic, genotypic and phylogenetic techniques, such as study of morphological and physiological properties, cell chemistry, G + C content of the genomic DNA, DNA–DNA hybridization and phylogenetic analysis. The strains belong to two novel species of *Streptosporangium* on the basis of 16S rRNA gene sequencing. The results of morphological, physiological and biochemical investigations and DNA–DNA hybridization indicated that the two strains are different from known members of the genus *Streptosporangium*. The names *Streptosporangium yunnanense* sp. nov. (type strain CY-11007^T = CCTCC AA 97009^T = CCRC 16307^T = DSM 44663^T) and *Streptosporangium purpuratum* sp. nov. (type strain CY-15110^T = CCTCC AA 97010^T = CCRC 16308^T = DSM 44688^T) are proposed. They have been deposited in CCTCC in Wuhan.

INTRODUCTION

On the basis of phylogenetic analysis of 16S rRNA gene sequences, Stackebrandt *et al.* (1997) proposed the family *Streptosporangiaceae*, containing the type genus *Streptosporangium* Couch 1955 as well as the genera *Herbidospira* Kudo *et al.* 1993, *Microbispora* Nonomura and Ohara 1957 emend. Zhang *et al.* 1998, *Microtetraspora* Thiemann *et al.* 1968 emend. Zhang *et al.* 1998, *Planobispora* Thiemann and Beretta 1968, *Planomonospora* Thiemann *et al.* 1967, *Planotetraspora* Hu *et al.* 1993 and *Nonomuraea* Zhang *et al.* 1998. In addition, the genus *Acrocarpospora* was proposed by Tamura *et al.* (2000) as another member of the *Streptosporangiaceae*. Description of taxa of each genus was based on phenotypic, genotypic and phylogenetic properties.

In the course of research on unknown microbial resources and microbiological biodiversity of actinomycetes, we isolated some strains of *Streptosporangium* from Yunnan Province, a region of south-western China that has specific geographical conditions that contribute to its great microbiological diversity. Strains CY-11007^T and CY-15110^T were

identified by a polyphasic approach. Comparative studies of morphology, physiology and biochemical composition of cells and phylogenetic analysis based on 16S rRNA gene sequences were carried out among isolated strains CY-11007^T and CY-15110^T and type strains of the genus *Streptosporangium* and of related genera. The results indicated that the two strains are different from known members of the genus *Streptosporangium*. Therefore, we consider that CY-11007^T and CY-15110^T represent two novel species, *Streptosporangium yunnanense* sp. nov. (CY-11007^T) and *Streptosporangium purpuratum* sp. nov. (CY-15110^T).

METHODS

Bacterial strains. Test strains CY-11007^T and CY-15110^T used in this study were isolated from soil samples collected from Yunnan Province, China. Type strains used for comparative studies were *Streptosporangium violaceochromogenes* JCM 3281^T (=DSM 43849^T), *Streptosporangium longisporum* JCM 3106^T (=DSM 43180^T), *Streptosporangium roseum* JCM 3005^T (=DSM 43021^T), *Streptosporangium vulgare* JCM 3028^T (=DSM 43802^T), *Streptosporangium nondiastaticum* JCM 3114^T (=DSM 43848^T), *Streptosporangium amethystogenes* JCM 3026^T (=DSM 43179^T), *Streptosporangium album* JCM 3025^T (=DSM 43023^T), *Streptosporangium fragile* JCM 6242^T (=DSM 43847^T), *Streptosporangium pseudovulgare* JCM 3115^T (=DSM 43181^T) and *Streptosporangium viridialbum* JCM 3027^T (=DSM 43801^T).

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CY-11007^T and CY-15110^T are AF191733 and AF191735.

Morphology. Strains CY-11007^T, CY-15110^T and other type strains were cultured for 3, 5, 7 and 14 days at 28 °C on International *Streptomyces* project medium 3 (ISP3; oatmeal agar) (Shirling & Gottlieb, 1966) and HV agar (Hayakawa & Nonomura, 1987) and observed by light microscopy (Olympus) and scanning electron microscopy (model KYKY-AMRAY-100B).

Cultural, physiological and biochemical tests. Cultural characteristics were studied by using 14-day-old cultures grown at 28 °C on various agar media. Colours were determined by comparing the cultures with colour chips from the ISCC-NBS colour charts standard samples no. 2106 (Kelly, 1964). The physiological characteristics of the strains were tested according to the standards for *Streptosporangium* (Nonomura, 1989).

Analysis of chemotaxonomic characteristics. Cultures of the test strains were grown in shake flasks containing Bennett's broth to prepare biomass. These cultures were incubated for 5–7 days at 28 °C. Cell walls were purified from 10 g wet biomass. Amino acids of purified cell walls were analysed by the methods of Lechevalier & Lechevalier (1980). Amino acids and sugars of whole-cell hydrolysates were determined by the methods of Becker *et al.* (1964). Phospholipids were obtained from freeze-dried biomass (approx. 100 mg) and analysed by the methods of Lechevalier *et al.* (1981). Menaquinone analysis (from 100 mg freeze-dried biomass) was performed as described previously by Collins (1985). Methyl esters of cellular fatty acids (from 10 mg freeze-dried biomass) were determined by the methods of Miller (1982) and Kuykendall *et al.* (1989).

DNA base composition. The G+C content of the chromosomal DNA was determined from the melting point value of the thermal denaturation profile using a spectrophotometer (Ultrospec 2000) equipped with a programmable temperature-control unit by the equation of Marmur & Doty (1962), as modified by De Ley (1970).

DNA–DNA hybridization. Chromosomal DNA of strains CY-11007^T and CY-15110^T was prepared as described by Marmur (1961). The initial reassociation rate method (De Ley *et al.*, 1970) was used for determining percentage DNA–DNA hybridization.

16S rRNA gene sequencing. Chromosomal DNA was extracted as described by Marmur (1961) and Jiang & Xu (1990). 16S rRNA genes were amplified by PCR (Saiki *et al.*, 1988) using a PCR kit (Sino-American Biotechnology Co.) with primers A 8–38f (5'-CG-GGATCCAGAGTTTGATCCTGGCTCAGAACGAACGCT-3') and B 1479–1506r (5'-CGGGATCCTACGGCTACCTTGTACGACTTCA-CCCC-3') and the 1.5 kb amplified fragment was purified by 0.8 % low-melting-point agarose gel electrophoresis, by the methods of Wieslander (1979). Purified PCR products and plasmid pUC18 vector were cut with *Bam*HI and ligated at 18 °C for 20 h. Ligated plasmids were transformed into *Escherichia coli* DH5 α (Wieslander, 1979) and transformants were selected by blue-white selection (Sambrook *et al.*, 1989). Plasmids were extracted and purified according to the methods of Tiesman & Rizzino (1991). Purified plasmids containing PCR products were sequenced with a model 377 Prism automatic sequencer by American Cybersyn Company. The sequencing primer was 5'-TTCAGCAGGGACGAAGTTGA-3'. The 16S rRNA gene sequences of type strains of species of related genera were obtained from GenBank.

Phylogenetic analysis. The 16S rRNA gene sequences were aligned by the CLUSTAL X program (Thompson *et al.*, 1997) with corresponding nucleotide sequences of representatives of the genus *Streptosporangium* retrieved from GenBank (Benson *et al.*, 1997). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-parsimony (Fitch, 1971) algorithms from the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices were

generated according to the model of Kimura (1980). Tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset using SEQBOOT and CONSENSE options from the PHYLIP suite of programs.

RESULTS AND DISCUSSION

Morphology

Strain CY-11007^T produced aerial mycelium with single or clustered spherical sporangia. The sporangia were generally 4–20 μ m in diameter. Spores within the sporangium were non-motile and formed by the septation of unbranched hyphae within the spore vesicle. Sporangiospores were spherical or oblate in shape (Fig. 1a, b). Strain CY-15110^T produced globose, single or clustered sporangia, usually 2–5 μ m in diameter on the aerial mycelium. Sporangiospores were formed by septation of a coiled, unbranched mycelium. Sporangiospores within the sporangium were spherical and non-motile (Fig. 1c, d).

Cultural characteristics

Strains CY-11007^T and CY-15110^T grew well on various inorganic or organic media and produced spherical sporangia; the vegetative and aerial mycelium grew well on the media tested. Table 1 shows the degree of growth and colour of both aerial and vegetative hyphae of strains CY11007^T and CY-15110^T on various media. The aerial mycelium of CY-11007^T was abundant and pale-pink (7. p. Pink; Research Group of Actinomycetes, 1970) to yellowish-pink (31. p.y. Pink) on most of the media used. The substrate mycelium was brownish and produced pigment on some media tested. Colonies of strain CY-15110^T were deep red (13. deep Red) to deep purplish-red (257. v.deep p. R), lacking visible aerial mycelium by eye

Fig. 1. Scanning electron micrographs (a, c) and light micrographs (b, d) of cells of strains CY-11007^T (a, b) and CY-15110^T (c, d) grown on oatmeal agar for 14 days at 28 °C, showing mycelia and sporangia. Bars, 10 μ m.

Table 1. Cultural characteristics of strains CY-11007^T and CY-15110^T

Medium	Growth		Aerial mycelium		Vegetative mycelium		Diffusible pigment	
	CY-11007 ^T	CY-15110 ^T	CY-11007 ^T	CY-15110 ^T	CY-11007 ^T	CY-15110 ^T	CY-11007 ^T	CY-15110 ^T
Glycerol-asparagine agar (ISP5)	Good	Good	Pale-pink	Pinkish white	Brown	Deep red	Pale brown	None
Oatmeal agar (ISP3)	Abundant	Good	Yellowish pink	Pinkish white	Brownish yellow	Deep red to deep purplish red	Pale brown	None
Oatmeal-yeast extract agar	Abundant	Good	Yellowish pink	Pink	Brownish yellow	Deep purplish red	Pale brown	None
Bennett's agar	Abundant	Abundant	Pale pink	None	Brown	Deep purplish red	None	None
Inorganic salts-starch agar (ISP4)	Abundant	Good	Pinkish	Pinkish white	Brown	Deep purplish red	Pale brown-yellow	None
Yeast extract-malt extract agar (ISP2)	Abundant	Abundant	Pinkish	Poor	Brown	Deep purplish red	None	None
Czapek agar	Good	Good	Pinkish	Pinkish	Brownish yellow	Deep red	None	None

Table 2. Comparison of physiological characteristics of strains CY-11007^T and CY-15110^T and related strains

Strains: 1, CY-11007^T; 2, *S. nondiastaticum* JCM 3114^T; 3, *S. pseudovulgare* JCM 3115^T; 4, CY-15110^T; 5, *S. longisporum* JCM 3106^T. All strains are positive for utilization of D-glucose and D-sucrose and hydrolysis of succinate and uric acid and negative for utilization of inositol, L-rhamnose, lactose and methyl α -D-glucoside and iodinin production.

Property	1	2	3	4	5
Carbon source utilization:					
L-Arabinose	–	+	+	–	+
D-Galactose	–	+	–	–	–
D-Fructose	–	+	+	+	–
D-Mannose	–	+	+	+	–
D-Mannitol	–	+	+	–	–
D-Xylose	–	+	+	+	+
D-Raffinose	–	+	+	–	–
D-Cellobiose	+	+	+	+	–
Sorbitol	–	+	+	–	–
Sorbose	–	+	+	–	–
Erythrose	–	+	+	–	–
Melibiose	–	+	+	–	–
Dextrin	–	+	+	–	–
Degradation of:					
Cellulose	+	–	+	+	–
Starch	+	–	+	+	+
Gelatin liquefaction	+	+	+	–	–
Requirement for B vitamins	–	+	+	–	–
Nitrite from nitrate	+	+	+	–	+
Melanin produced	+	+	+	+	–
Uricase	–	–	–	–	+
Hydrolysis of:					
Malate	+	+	+	–	–
Citrate	+	–	+	–	–
Hippurate	+	+	+	–	–

on organic media and poor aerial mycelium on inorganic media used. The strain did not produce soluble pigment.

Physiological characteristics

The physiological and biochemical reactions of strains CY-11007^T and CY-15110^T are shown in Table 2. The optimal growth temperatures of strains CY-11007^T and CY-15110^T were respectively 30 and 28 °C and optimal pH for both strains was 7.2.

Chemotaxonomy

Pure cell walls of strains CY-11007^T and CY-15110^T contained *meso*-diaminopimelic acid (*meso*-DAP). Whole-cell hydrolysates contained madurose as the diagnostic sugar. The two strains can be considered to have a type III/B cell wall. Phospholipids consisted of phosphatidylethanolamine (PE), phosphatidylinositol (PI), diphosphatidylglycerol (DPG), phosphatidylmethylethanolamine (PME, only found

Table 3. DNA–DNA reassociation between isolates CY-11007^T and CY-15110^T and related strains

Strain	Hybridization (%) with:	
	CY-11007 ^T	CY-15110 ^T
CY-11007 ^T	100	–
CY-15110 ^T	30.1	100
<i>S. roseum</i> JCM 3005 ^T	23.6	43.3
<i>S. album</i> JCM 3025 ^T	21.4	11.2
<i>S. amethystogenes</i> JCM 3026 ^T	26.7	15.7
<i>S. viridialbum</i> JCM 3027 ^T	25.6	13.8
<i>S. vulgare</i> JCM 3028 ^T	30.4	16.7
<i>S. longisporum</i> JCM 3106 ^T	9.0	55.3
<i>S. nondiasticum</i> JCM 3114 ^T	56.8	43.6
<i>S. pseudovulgare</i> JCM 3115 ^T	53.1	45.2

in strain CY-11007^T) and phospholipids of unknown structure containing glucosamine (GluNus), and belong to the type PIV phospholipid pattern *sensu* Lechevalier *et al.* (1981). The major menaquinones of strain CY-11007^T were MK-9(H₀), MK-9(H₂) and MK-9(H₄) (in descending order of abundance) and for strain CY-15110^T were MK-9(H₂), MK-9(H₀) and MK-9(H₄). The predominant fatty acids in whole-cell methyl esters were C_{16:0} (14.73 %), C_{17:0} (4.95 %), C_{18:0} (10.24 %), C_{19:0} (29.64 %) and C_{16:3} (2.54 %) (strain CY-11007^T) and C_{15:0} (23.74 %), C_{16:0} (42.41 %), C_{17:0} (24.84 %), C_{18:0} (0.88 %) and C_{17:3} (0.66 %) (strain CY-15110^T).

DNA base composition and DNA hybridization

The G + C contents of the DNA of strains CY-11007^T and CY-15110^T were respectively 71.06 and 69.10 mol%. The levels of DNA–DNA relatedness with type strains of *Streptosporangium* ranged from 9 to 56.8 % (Table 3).

16S rRNA gene sequence comparisons and phylogenetic analysis

The nearly complete 16S rRNA gene sequences of strains CY-11007^T (1436 nucleotides) and CY-15110^T (1422 nucleotides) were determined. The sequences were compared with the corresponding sequences of representatives of the *Streptosporangiaceae*. The results showed that strains CY-11007^T and CY-15110^T were clustered into a group. The evolutionary distance between the two strains was 2.956 %. Fig. 2 shows a neighbour-joining phylogenetic tree constructed on the basis of evolutionary distances calculated by using the method of Kimura (1980).

Taxonomic conclusions

The typical characteristics of members of the genus *Streptosporangium* are as follows. They produce globose/spherical sporangia on aerial mycelium. Sporangiospores are formed by septation of a coiled, unbranched hypha within the sporangium; they are spherical, oval or rod-shaped and non-motile. Cell walls contain *N*-acetylated muramic acid

Fig. 2. Neighbour-joining tree (Saitou & Nei, 1987), based on nearly complete 16S rRNA gene sequences, showing relationships between strains CY-11007^T and CY-15110^T and members of the genus *Streptosporangium*. Numbers at nodes indicate levels of bootstrap support (%) based on neighbour-joining analyses of 1000 resampled datasets; only values over 50 % are given. Accession numbers are given in parentheses. *Actinomadura madurae* JCM 7436^T was used as the outgroup. The scale bar indicates 0.01 substitutions per nucleotide position.

and *meso*-DAP but no characteristic sugars. Whole-cell hydrolysates contain madurose. Major phospholipids include unknown glucosamine-containing compounds, but no phosphatidylcholine or phosphatidylglycerol (PG). They are Gram-positive, aerobic and mesophilic, and a few species are thermotolerant. The G + C content of the DNA is 69.5–71 mol% (*T_m*) (Nonomura, 1989).

The two strains CY-11007^T and CY-15110^T should be placed in the genus *Streptosporangium* based on their morphological characteristics, menaquinones, predominant fatty acids in whole-cell methyl esters and DNA base composition and the similarity of their 16S rRNA gene sequences. The results of phylogenetic analysis based on 16S rRNA gene sequences show that strains CY-11007^T and CY-15110^T were clustered into a group, and the evolutionary distance between them was below 5 %. The novel isolates and other representatives of *Streptosporangium* appear to be relatively distantly related. DNA–DNA hybridization is the standard criterion for designation of species, and the criterion for a species is ≥70 % DNA–DNA relatedness (Wayne *et al.*, 1987). The DNA–DNA relatedness of strains CY-11007^T and CY-15110^T with related type strains was <70 %. Cultural characteristics, physiological properties and biochemical reactions indicated that strain CY-11007^T is different from strain CY-15110^T. We therefore propose that the two isolates represent novel species, *Streptosporangium yunnanense* sp. nov.

Table 4. Differences between isolates CY-11007^T and CY-15110^T and related strains of *Streptosporangium*

All species, including the novel isolates, contain MK-9(H₀), MK-9(H₂), MK-9(H₄) as predominant menaquinones and produce non-motile sporangiospores.

Characteristic	<i>Streptosporangium</i> species	CY-11007 ^T	CY-15110 ^T
Sporangia	Globose/spherical	Spherical	Spherical
Sporangiospore shape	Spherical, oval or rod	Spherical	Spherical or oblate
Cell wall	N-acetylated muramic acid, <i>meso</i> -DAP	<i>meso</i> -DAP	<i>meso</i> -DAP
Whole-cell hydrolysates	Madurose	Madurose, glucose, rhamnose	Madurose, glucose, rhamnose
Phospholipids	GluNus	PE, PME, DPG, PI, GluNus	PE, DPG, PI, GluNus
DNA G+C content (mol%)	69.5–71	71.06	69.10
Fatty acids		C _{16:0} (14.73 %), C _{17:0} (4.95 %), C _{18:0} (10.24 %), C _{19:0} (29.64 %), C _{16:3} (2.54 %)	C _{15:0} (23.74 %), C _{16:0} (42.41 %), C _{17:0} (24.84 %), C _{18:0} (0.88 %), C _{17:3} (0.66 %)

(CY-11007^T) and *Streptosporangium purpuratum* sp. nov. (CY-15110^T). Differences between isolates CY-11007^T and CY-15110^T and related strains of *Streptosporangium* are shown in Table 4.

Description of *Streptosporangium yunnanense* sp. nov.

Streptosporangium yunnanense (yun.nan.en'se. N.L. neut. adj. *yunnanense* pertaining to Yunnan, a province of south-west China).

Aerobic, Gram-positive. Abundant aerial mycelium is pale-pink (7.p. Pink) to yellowish-pink (31. p.y. Pink) on most of the media tested such as glycerol-asparagine agar (ISP5), oatmeal agar (ISP3), oatmeal-yeast extract agar and Bennett's agar. Substrate mycelium is brownish and pigment is produced on various media tested. Spherical sporangia are produced on aerial mycelium on HV agar and oatmeal agar. Sporangiospores are formed by septation of coiled, unbranched hyphae and are spherical and non-motile. Cell walls contain *meso*-DAP; whole-cell hydrolysates contain madurose, glucose and rhamnose. Phospholipids consist of PE, PME, DPG, PI and GluNus; PG is not detected. Major menaquinones are MK-9(H₀), MK-9(H₂) and MK-9(H₄). Predominant fatty acids in whole-cell methyl esters are 16:0 (14.73 %), 17:0 (4.95 %), 18:0 (10.24 %), 19:0 (29.64 %) and 16:3 (2.54 %). G+C content of the DNA of the type strain is 71.06 mol%. Optimal growth temperature is 30 °C; growth occurs at 10 and 42 °C but not at 50 °C. Optimal pH for growth is 7.2. D-Glucose, D-sucrose and D-cellobiose are utilized; L-arabinose, D-galactose, D-fructose, D-mannose, D-mannitol, L-rhamnose, inositol, D-raffinose, D-xylose, sorbitol, sorbose, erythrose, lactose, melibiose, methyl α-D-glucoside and dextrin are not utilized. Degradation of cellulose and starch is positive. Reduction of nitrate and liquefaction of gelatin are positive. Vitamin B is not required for growth and iodinin production is negative. Melanin is produced. Uricase is negative. Succinate, malate, citrate, uric acid and hippurate are hydrolysed.

The type strain is CY-11007^T (=CCTCC AA 97009^T = CCRC 16307^T = DSM 44663^T).

Description of *Streptosporangium purpuratum* sp. nov.

Streptosporangium purpuratum (pur.pur.at'um. L. neut. adj. *purpuratum* clad in purple-violet, referring to the colony colour).

Aerobic, Gram-positive. Colonies are deep red (13. deep Red) to deep purplish-red (257. v. deep p. R) on most media tested such as glycerol-asparagine agar (ISP5), oatmeal agar (ISP3), oatmeal-yeast extract agar and Bennett's agar. Aerial mycelium is very poor so that it is invisible by eye. Substrate mycelium is deep red and soluble pigment is not produced on various media tested. Spherical sporangia are produced on aerial mycelium on HV agar and oatmeal agar, usually 2–5 μm in diameter. Sporangiospores are formed by septation of coiled, unbranched hyphae; they are spherical or oblate in shape and non-motile. Cell walls contain *meso*-DAP; whole-cell hydrolysates contain madurose, glucose and rhamnose. Phospholipids consist of PE, DPG, PI and GluNus. Major menaquinones are MK-9(H₂), MK-9(H₀) and MK-9(H₄). Predominant fatty acids in whole-cell methyl esters are 15:0 (23.74 %), 16:0 (42.41 %), 17:0 (24.84 %), 18:0 (0.88 %) and 17:3 (0.66 %). Optimal growth temperature is 28 °C; grows at 42 and 50 °C. Optimal pH for growth is 7.2. D-Glucose, D-sucrose, D-cellobiose, D-fructose and D-xylose are utilized; L-arabinose, D-galactose, D-mannose, D-mannitol, inositol, D-raffinose, L-rhamnose, sorbitol, sorbose, erythrose, lactose, melibiose, methyl α-D-glucoside and dextrin are not utilized. Degradation of cellulose and starch is positive. Reduction of nitrate and liquefaction of gelatin are negative. Vitamin B is not required for growth and iodinin and melanin are produced. Uricase is negative. Succinate, uric acid, malate, citrate and hippurate are hydrolysed.

The type strain is strain CY-15110^T (=CCTCC AA 97010^T = CCRC 16308^T = DSM 44688^T).

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (no. 30270002) and the Key Laboratory for Microbial Resources of Ministry of Education, PR China.

REFERENCES

- Becker, B., Lechevalier, M. P., Gordon, R. E. & Lechevalier, H. A. (1964). Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell hydrolysates. *Appl Microbiol* **12**, 421–423.
- Benson, D. A., Boguski, M. S., Lipman, D. J. & Ostell, J. (1997). GenBank. *Nucleic Acids Res* **25**, 1–6.
- Collins, M. D. (1985). Isoprenoid quinone analysis in bacterial classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Couch, J. N. (1955). A new genus and family of the *Actinomycetales*, with a revision of the genus *Actinoplanes*. *J Elisha Mitchell Sci Soc* **71**, 148–155.
- De Ley, J. (1970). Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J Bacteriol* **101**, 738–754.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Felsenstein, J. (1985). Confidence limits on phylogenies: a approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1993). PHYLIP – Phylogenetic Inference Package version 3.5c. Distributed by the author. University of Washington, Seattle, USA.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees: a method based on mutation distances as estimated from cytochrome *c* sequences is of general applicability. *Science* **155**, 279–284.
- Hayakawa, M. & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J Ferment Technol* **65**, 501–509.
- Hu, R., Wei, G. & Li, J. (1993). A new genus of actinomycetes, *Planotetraspora* gen. nov. *Int J Syst Bacteriol* **43**, 468–470.
- Jiang, C. L. & Xu, L. H. (1990). Improvement on cell disruption method of actinomycetes in DNA preparation. *Microbiology (Beijing)* **17**, 178–182.
- Kelly, K. L. (1964). *Inter-Society Color Council – National Bureau of Standards Color-Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Kudo, T., Itoh, T., Miyadoh, S., Shomura, T. & Seino, A. (1993). *Herbidospora* gen. nov., a new genus of the family *Streptosporangiaceae* Goodfellow et al. 1990. *Int J Syst Bacteriol* **43**, 319–328.
- Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1989). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.
- Lechevalier, M. P. & Lechevalier, H. A. (1980). The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy*, Special Publication no. 6, pp. 227–291. Edited by A. Dietz & J. Thayer. Arlington, VA: Society for Industrial Microbiology.
- Lechevalier, M. P., Stern, A. E. & Lechevalier, H. A. (1981). Phospholipids in the taxonomy of actinomycetes. In *The Actinomycetes*, pp. 111–116. Edited by K. P. Schaal & G. Pulverer. New York: Gustav Fischer Verlag.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.
- Nonomura, H. (1989). Genus *Streptosporangium* Couch 1955, 148^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2545–2551. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Nonomura, H. & Ohara, Y. (1957). Distribution of actinomycetes in soil. II. *Microbispora*, a new genus of *Streptomycetaceae*. *J Ferment Technol* **35**, 307–311.
- Research Group of Actinomycetes (1970). *Manual of Determinative Actinomycetes*. Edited by X. C. Yan. Beijing: Science Press.
- Saiki, R. K., Gelfand, D. H., Stoffe, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). In *Molecular Cloning: a Laboratory Manual*, 2nd edn, pp. 4.21–4.43. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- 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.
- Tamura, T., Suzuki, S. & Hatano, K. (2000). *Acrocarpospora* gen. nov., a new genus of the order *Actinomycetales*. *Int J Syst Evol Microbiol* **50**, 1163–1171.
- Thiemann, J. E. & Beretta, G. (1968). A new genus of the *Actinoplanaceae*: *Planobispora* gen. nov. *Arch Mikrobiol* **62**, 157–166.
- Thiemann, J. E., Pagani, H. & Beretta, G. (1967). A new genus of the *Actinoplanaceae*: *Planomonospora*, gen. nov. *G Microbiol* **15**, 27–38.
- Thiemann, J. E., Pagani, H. & Beretta, G. (1968). A new genus of the *Actinomycetales*: *Microtetraspora* gen. nov. *J Gen Microbiol* **50**, 295–303.
- 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.
- Tiesman, J. & Rizzino, A. (1991). A rapid and reliable method for the purification of high-quality plasmid DNA for double-stranded sequencing. *Biotechniques* **10**, 319, 326–328.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Wieslander, L. (1979). A simple method to recover intact high molecular weight RNA and DNA after electrophoretic separation in low gelling temperature agarose gels. *Anal Biochem* **98**, 305–309.
- Zhang, Z., Wang, Y. & Ruan, J. (1998). Reclassification of *Thermomonospora* and *Microtetraspora*. *Int J Syst Bacteriol* **48**, 411–422.