Millisia brevis gen. nov., sp. nov., an actinomycete isolated from activated sludge foam

Jacques A. Soddell,¹ Fiona M. Stainsby,² Kathryn L. Eales,¹
Reiner M. Kroppenstedt,³ Robert J. Seviour¹ and Michael Goodfellow²

Correspondence

Michael Goodfellow
m.goodfellow@ncl.ac.uk

¹Biotechnology Research Centre, La Trobe University, Bendigo, Victoria 3552, Australia

²Division of Biology, King George VIth Building, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

³DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1b, 38124 Braunschweig, Germany

The taxonomic position of two mycolic-acid-producing actinomycetes, isolates J81^T and J82, which were recovered from activated sludge foam, was clarified. Comparative 16S rRNA gene sequence studies indicated that the organisms formed a distinct lineage within the *Corynebacterineae* 16S rRNA gene tree. The taxonomic integrity of this group was underpinned by a wealth of phenotypic data, notably characteristic rudimentary right-angled branching. In addition, isolate J81^T contained the following: meso-diaminopimelic acid, arabinose and galactose; *N*-glycolated muramic acid residues; a dihydrogenated menaquinone with eight isoprene units as the predominant isoprenologue; a fatty acid profile rich in oleic and palmitoleic acids and with relatively small proportions of myristic, stearic and tuberculostearic acids; mycolic acids with 44–52 carbons; and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides as major polar lipids. Strain J81^T was found to have a chemotaxonomic profile that serves to distinguish it from representatives of all of the other taxa classified as belonging to the suborder *Corynebacterineae*. In the light of these data, it is proposed that the two isolates be classified in a novel monospecific genus. The name proposed for this taxon is *Millisia brevis* gen. nov., sp. nov.; strain J81^T (= DSM 44463^T = NRRL B-24424^T) is the type strain of *Millisia brevis*.

The taxonomy of mycolic-acid-containing actinomycetes has been clarified and extended by the application of genotypic and phenotypic procedures to representatives of established and novel taxa (Goodfellow *et al.*, 1998a, 1999; Gürtler *et al.*, 2004). Actinomycetes characterized by the presence of mycolic acids are classified in the suborder *Corynebacterineae* Stackebrandt *et al.* 1997, which encompasses the genera *Corynebacterium*, *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Segniliparus*, *Skermania*, *Tsukamurella* and *Williamsia* (Butler *et al.*, 2005; Goodfellow & Maldonado, 2006). Members of these taxa can be distinguished from one another using a combination of chemotaxonomic and morphological properties, and form distinct lineages in the *Corynebacterineae* 16S rRNA gene tree. Activated sludge wastewater-treatment plants with foaming problems (Soddell, 1999) are a rich source of mycolic-acid-containing actinomycetes (Lemmer & Kroppenstedt, 1984; Soddell & Seviour, 1990, 1995, 1998; Goodfellow *et al.*, 1998b; Stainsby *et al.*, 2002), as exemplified

by *Gordonia amarae* (Lechevalier & Lechevalier, 1974) Klatte *et al.* 1994, *Skermania piniformis* Chun *et al.* 1997, *Tsukamurella pseudospumae* Nam *et al.* 2004 and *Tsukamurella spumae* Nam *et al.* 2003.

Two actinomycetes that showed rudimentary right-angled branching and which produced salmon-pink filamentous colonies were isolated from activated sludge foam by using a micromanipulator (Soddell & Seviour, 1994). Subsequent studies based on numerical taxonomic and preliminary 16S rRNA gene sequence data indicated that the two strains, isolates J81^T and J82, might represent a novel genus in the suborder *Corynebacterineae* (Soddell & Seviour, 1998; Soddell *et al.*, 1998), a proposition underpinned by the results of the present investigation.

DNA from strains J81^T and J82 was extracted with a MoBio Laboratories UltraClean Soil DNA kit (Geneworks). 16S rRNA genes were PCR-amplified with universal primers 27f and 1525r, amplicons were purified with a QIA Quick PCR purification kit (Qiagen) and cloning was completed using pGEM-T Easy vector system II (Promega). The resultant preparations were fully sequenced with primers M13F,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of *Millisia brevis* strains J81^T and J82 are AY534742 and AY534743, respectively.

M13R, 530F and 907R with Applied Biosystems PRISM BigDye 3.1 Terminator chemistry and then sequenced at the DNA Micromon Sequencing Facility of Monash University (Clayton, Australia). The 16S rRNA gene sequence results were compared with corresponding data retrieved from the Ribosomal Database Project (<http://rdp.cme.msu.edu/>) for representatives of the genera classified in the suborder *Corynebacterineae*. Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms. An evolutionary distance matrix was generated for the neighbour-joining algorithm using the distance model of Jukes & Cantor (1969). The unrooted tree topologies were subjected to bootstrap analysis (Felsenstein, 1985) of the neighbour-joining dataset, using the SEQBOOT and CONSENSE options from the PHYLIP package (Felsenstein, 1989).

Almost-complete 16S rRNA gene sequences (1519 nt) corresponding to *Escherichia coli* positions 27–1525 were obtained for the two strains. It is evident from Fig. 1 that the organisms have identical 16S rRNA gene sequences and form a deep-rooted lineage in the *Corynebacterineae* tree that can be equated with branches corresponding to recognized genera, a result consistent with their recognition as members of a novel genus. The strains tested are most closely related to members of *Gordonia* species with validly published names (93.7–95.7 % similarity) and to the type strain of *S. piniformis* (95.5 % similarity).

Chemotaxonomic studies were carried out to determine whether J81^T merited generic status within the suborder *Corynebacterineae*. Standard procedures were used for the extraction and analysis of fatty acids (MIDI system; <http://www.midi-inc.com/>), isoprenoid quinones (Minnikin *et al.*, 1984), muramic acid (Uchida *et al.*, 1999), mycolic acids (Minnikin *et al.*, 1980), polar lipids (Minnikin *et al.*, 1984) and sugars (Schaal, 1985). The chain lengths of the mycolic acids were determined according to the method described by Linos *et al.* (1999). The isolate contained the following: *meso*-diaminopimelic acid, arabinose and galactose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970); *N*-glycolated muramic acid; a dihydrogenated menaquinone with eight isoprene units [MK-8(H₂)] as the predominant menaquinone; mycolic acids within the range C₄₄–C₅₂; and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidyl-inositol mannosides as major polar lipids (phospholipid type 2; Lechevalier *et al.*, 1977). This chemical profile serves to distinguish the tested strain from members of all of the genera that encompass mycolic-acid-containing organisms (Table 1), apart from representatives of the genus *Rhodococcus* that have mycolic acids towards the upper carbon range (Goodfellow *et al.*, 1998a).

Strain J81^T was also characterized by the presence of large proportions of the fatty acids oleic acid (C_{18:1}; 46.5 % of the total), palmitic acid (C_{16:0}; 26.3 %) and palmitoleic acid (C_{16:1}; 16.8 %) and relatively small proportions of myristic

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987), based on almost-complete 16S rRNA gene sequences, showing relationships between strains J81^T and J82 and representatives of genera classified in the suborder *Corynebacterineae*. Asterisks indicate branches of the tree that were also found using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) tree-making algorithms. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 75 % are shown. Bar, 0.02 substitutions per nucleotide position.

Table 1. Chemical and morphological properties of strain J81^T and representatives of genera that contain mycolic-acid-containing organisms

Data for established genera are from Soddell & Seviour (1994), Butler *et al.* (2005) and Goodfellow & Maldonado (2006). PE indicates the presence (+) or absence (–) of phosphatidylethanolamine. ND, No data.

Strain/genus	Major menaquinone	Muramic acid acyl type	Mycolic acids (no. of carbons)	PE	DNA G+C content (mol%)	Cellular micromorphology
Strain J81 ^T	MK-8(H ₂)	Glycolated	44–52	+	64.7	Characteristic rudimentary right-angled branching
<i>Dietzia</i>	MK-8(H ₂)	Acetylated	34–39	+	66–73	Short rods and cocci
<i>Corynebacterium</i>	MK-8(H ₂)	Acetylated	22–38	–	51–67	Pleomorphic rods
<i>Gordonia</i>	MK-9(H ₂)	Glycolated	46–66	+	63–69	Rods and cocci/moderately branched hyphae
<i>Mycobacterium</i>	MK-9(H ₂)	Glycolated	60–90	+	62–70	Rods, occasionally branched filaments
<i>Nocardia</i>	MK-8(H ₄ , co-cycl.)	Glycolated	40–64	+	64–72	Mycelium that fragments into rods and cocci
<i>Rhodococcus</i>	MK-8(H ₂)	Glycolated	30–54	+	67–73	Rods to extensively branched elements that fragment
<i>Segniliparus</i>	ND	ND	ND	ND	68–72	Rods
<i>Skermania</i>	MK-8(H ₄ , co-cycl.)	Glycolated	58–64	+	67.5	Mycelium resembling a pine tree
<i>Tsukamurella</i>	MK-9	Glycolated	64–78	+	67–74	Rods and coccobacilli
<i>Williamsia</i>	MK-9(H ₂)	Glycolated	50–56	+	64–65	Irregular rods or cocci

acid (1.8%), stearic acid (2.3%) and tuberculostearic acid (3.0%). The large proportion of oleic acid and the low proportion of tuberculostearic acid distinguish the organism from representatives of all of the genera composed of mycolic-acid-containing actinomycetes (Kämpfer *et al.*, 1999; Yoon *et al.*, 2000; Gürtler *et al.*, 2001; Kattar *et al.*, 2001; Kim *et al.*, 2002; Linos *et al.*, 2002; Nam *et al.*, 2003; Butler *et al.*, 2005). The highest identification score in the MIDI system (0.041) for the tested strain was with representatives of the genera *Dietzia* and *Rhodococcus*, but this score is too low to allow assignment of the strain to either of these genera; consequently, strain J81^T cannot be assigned to any of the taxa present in the MIDI database. One-dimensional TLC showed that the organism contained mycolic acids with an *R_f* value similar to those found for the *Gordonia* and *Rhodococcus* marker strains (*R_f* 0.37–0.38). Strain J81^T was also compared with representatives of mycolic-acid-containing genera by using a Curie-point pyrolysis mass spectrometric procedure described by Goodfellow *et al.* (1998b). It is apparent from Fig. 2 that the organism is well separated from the other strains, as it occupies a distinct area in the resultant taxospace.

The DNA G+C content was determined for strain J81^T. Isolation and purification of the DNA was achieved by following the procedure described by Pitcher *et al.* (1989). The G+C content of the DNA preparation was determined using the reverse-phase HPLC method described by Tamaoka (1994). The molar G+C content, calculated using the method described by Mesbah *et al.* (1989), was 64.7 mol%.

It is apparent from the excellent congruence found between the present chemotaxonomic and earlier numerical phenetic

data (Soddell *et al.*, 1998) that strain J81^T should be classified in a novel genus within the suborder *Corynebacterineae*. This conclusion is strongly supported by the 16S rRNA sequence data, which show that strains J81^T and J82 form a distinct

Fig. 2. Three-dimensional ordination diagram based on Curie-point pyrolysis mass spectral data showing relationships between strain J81^T and strains representing genera classified in the suborder *Corynebacterineae*. The plot is based on the first three canonical variates of a principal component-canonical variate analysis. The first three axes account for 77.3, 16.1 and 3.5% of the total variation between the strains. N1015^T, duplicated cultures of *Dietzia maris*; N654^T, *Gordonia bronchialis*; M6^T, *Mycobacterium peregrinum*; N318^T, *Nocardia brasiliensis*; N54^T, *Rhodococcus rhodochrous*; N663^T, *Tsukamurella paurometabola*.

lineage in the *Corynebacterineae* gene tree, and by the distinctive cellular morphology of these organisms (Soddell & Seviour, 1994). It is proposed that these strains be classified in a novel monospecific genus. The name proposed for this taxon is *Millisia brevis* gen. nov., sp. nov.

Members of mycolic-acid-containing genera have been assigned to six suprageneric taxa, the families *Corynebacteriaceae*, *Dietziaceae*, *Gordoniaceae*, *Mycobacteriaceae*, *Nocardiaceae* and *Tsukamurellaceae*, mainly on the basis of 16S rRNA signature nucleotides (Stackebrandt *et al.*, 1997; Goodfellow & Maldonado, 2006). The *Millisia* strains have signature nucleotides that are characteristic of the subclass *Actinobacteridae* and of the suborder *Corynebacterineae*, but not of those of their nearest phylogenetic neighbours in the family *Gordoniaceae*. Further comparative taxonomic studies on additional *Millisia* strains are needed to determine whether this taxon should be assigned to a novel family. An organism associated with a deep-water marine invertebrate has been found to share an identical partial 16S rRNA gene sequence with *M. brevis* J81^T (Sfanos *et al.*, 2005).

Description of *Millisia* gen. nov.

Millisia (Mil.li'si.a. N.L. fem. n. *Millisia* named after Professor Emeritus Nancy F. Millis AC, MBE, a celebrated Australian microbiologist who promoted wastewater microbiology in Australia).

The description is based on data taken from this and previous studies (Soddell & Seviour, 1998; Soddell *et al.*, 1998). Aerobic, Gram-positive to Gram-variable, acid-alcohol-fast, non-motile, catalase-positive actinomycetes that form non-spore-forming rods showing rudimentary right-angled branching and which contain polyphosphate storage granules. Form salmon-pink, irregular colonies with filamentous margins and sparse, unbranched aerial hyphae on glucose/yeast extract agar. Colonies are matt and dry in appearance, soft in texture and easy to emulsify. Diffusile pigments are not formed. Whole-organism hydrolysates are rich in *meso*-diaminopimelic acid, arabinose and galactose. Contain *N*-glycolated muramic acid residues. The predominant menaquinone has eight isoprene units, one of which is hydrogenated. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides are the major polar lipids. The mycolic acids present have 44–52 carbons (principal components C₄₈, C₅₀ and C₅₂). Oleic, palmitic and palmitoleic acids are the predominant fatty acids, and there are relatively small proportions of myristic, stearic and tuberculostearic acids. The G+C content of the genomic DNA of the type strain of the type species is 64.7 mol%. The genus forms a distinct lineage in the *Corynebacterineae* 16S rRNA gene tree. The type species is *Millisia brevis*.

Description of *Millisia brevis* sp. nov.

Millisia brevis (bre'vis. L. fem. adj. *brevis* short, denoting the formation of short, branched rods).

The description is based upon data taken from Soddell *et al.* (1998). In addition to having the properties given in the genus description, the organism degrades Tweens 20, 40 and 60, but not adenine, casein, elastin, hypoxanthine, tyrosine or xanthine. Hydrolyses allantoin (weakly) and urea (strongly) but does not reduce nitrate or produce *m*- or *p*-nitrophenol oxidases. Grows between 15 and 35 °C, but not at 10 or 37 °C, and at pH 5.5–9.5. Cellobiose, ethanol, fructose, glucose, glycerol, *myo*-inositol, mannitol, mannose, sucrose and trehalose are used as sole sources of carbon for energy and growth, but arabinose, galactose, inulin, lactose, maltose, melezitose, raffinose, rhamnose, salicin, sorbitol and xylose (all at 0.1 %, w/v) are not. Similarly, *m*-hydroxybenzoic acid, sebacic acid, sodium butyrate, sodium gluconate, sodium lactate, sodium octanoate and sodium pyruvate are used as sole carbon sources, but acetamide, benzamide, *p*-hydroxybenzoic acid, pimelic acid, sodium benzoate, sodium succinate, sodium tartrate, testosterone and tyrosine (0.01 %, w/v) are not; variable results are obtained with *p*-cresol, sodium acetate, sodium adipate, sodium citrate, sodium fumarate, sodium malate and sodium propionate (all at 0.01 %, w/v). Coconut oil, glycerol trioleate, kerosene, hexadecane, olive oil, paraffin oil, safflower oil and xylene (weakly) are used as sole carbon sources in shake-flask liquid culture (all at 1 %, v/v). Variable results are obtained with acetamide and serine as sole carbon and nitrogen sources, but trimethyldiamine does not support growth. Grows in the presence of crystal violet (0.0001 %, w/v), phenol (0.01 %, w/v) and phenyl ethanol (0.02 and 0.03 %, v/v), but is sensitive to crystal violet (0.001 %, w/v), phenol (0.1 %, w/v), sodium azide (0.01 and 0.02 %, w/v), sodium chloride (5 and 7 %, w/v) and penicillin G (10 IU). Produces acid phosphatase, alkaline phosphatase (weakly), esterase (C₄), esterase lipase (C₈), α -glucosidase, β -glucosidase, leucine arylamidase, α -mannosidase (weakly), naphthol-AS-BI-phosphohydrolase and valine arylamidase, but not chymotrypsin, lipase (C₁₄), α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, *N*-acetyl- β -glucosaminidase or trypsin; variable results are obtained for cystine arylamidase.

The type strain, strain J81^T (=DSM 44463^T=NRRL B-24424^T), was isolated from activated sludge foam from Tamworth Sewage Treatment Plant, New South Wales, Australia.

References

- Butler, W. R., Floyd, M. M., Brown, J., Toney, S. R., Daneshvar, M., Cooksey, R. S., Carr, J., Steigerwalt, A. & Charles, N. (2005). Novel mycolic acid-containing bacteria in the family *Segniliparaceae* fam. nov., including the genus *Segniliparus* gen. nov., with descriptions of *Segniliparus rotundus* sp. nov. and *Segniliparus rugosus* sp. nov. *Int J Syst Evol Microbiol* 55, 1615–1624.
- Chun, J., Blackall, L. L., Kang, S.-O., Hah, Y.-C. & Goodfellow, M. (1997). A proposal to reclassify *Nocardia pinensis* Blackall *et al.* as *Skermania piniformis* gen. nov., comb. nov. *Int J Syst Bacteriol* 47, 127–131.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 17, 368–376.

- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Goodfellow, M. & Maldonado, L. A. (2006). The families Dietziaceae, Gordoniaceae, Nocardiaceae and Tsukamurellaceae. In *The Prokaryotes. A Handbook on the Biology of Bacteria*, 3rd edn, vol. 3, Archaea and Bacteria: Firmicutes, Actinomycetes. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer (in press).
- Goodfellow, M., Alderson, M. & Chun, J. (1998a). Rhodococcal systematics: problems and developments. *Antonie van Leeuwenhoek* **74**, 3–20.
- Goodfellow, M., Stainsby, F. M., Davenport, R., Chun, J. & Curtis, T. (1998b). Activated sludge foaming: the true extent of actinomycete diversity. *Water Sci Technol* **37** (4–5), 511–519.
- Goodfellow, M., Isik, K. & Yates, E. (1999). Actinomycete systematics: an unfinished synthesis. *Nova Acta Leopold NF80* (312), 47–82.
- Gürtler, V., Smith, R., Mayall, B., Pötter-Reinemann, G., Stackebrandt, E. & Kroppenstedt, R. M. (2001). *Nocardia veterana* sp. nov., isolated from human bronchial lavage. *Int J Syst Evol Microbiol* **51**, 933–936.
- Gürtler, V., Mayall, B. C. & Seviour, R. (2004). Can whole organism genome analysis refine the taxonomy of the genus *Rhodococcus*? *FEMS Microbiol Rev* **28**, 377–403.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kämpfer, P., Andersson, M. A., Rainey, F. A., Kroppenstedt, R. M. & Salkinoja-Salonen, M. (1999). *Williamsia muralis* gen. nov., sp. nov., isolated from the indoor environment of a children's day care centre. *Int J Syst Bacteriol* **49**, 681–687.
- Kattar, M. M., Cookson, B. T., Carlson, L. C. & 7 other authors (2001). *Tsukamurella strandjordae* sp. nov., a proposed new species causing sepsis. *J Clin Microbiol* **39**, 1467–1476.
- Kim, K. K., Roth, A., Andrees, S., Lee, S. T. & Kroppenstedt, R. M. (2002). *Nocardia pseudovaccinii* sp. nov. *Int J Syst Evol Microbiol* **52**, 1825–1829.
- Klatte, S., Rainey, F. A. & Kroppenstedt, R. M. (1994). Transfer of *Rhodococcus aichiensis* Tsukamura 1982 and *Nocardia amarae* Lechevalier and Lechevalier 1994 to the genus *Gordonia* as *Gordonia aichiensis* comb. nov. and *Gordonia amarae* comb. nov. *Int J Syst Bacteriol* **44**, 769–773.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 434–443.
- Lechevalier, M. P. & Lechevalier, H. A. (1974). *Nocardia amarae* sp. nov., an actinomycete common in foaming activated sludge. *Int J Syst Bacteriol* **24**, 278–288.
- Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Evol* **5**, 249–260.
- Lemmer, H. & Kroppenstedt, R. M. (1984). Chemotaxonomy and physiology of some actinomycetes isolated from scumming activated sludge. *Syst Appl Microbiol* **5**, 124–135.
- Linos, A., Steinbüchel, A., Spröer, C. & Kroppenstedt, R. M. (1999). *Gordonia polyisoprenovorans* sp. nov., a rubber-degrading actinomycete isolated from an automobile tyre. *Int J Syst Bacteriol* **49**, 1785–1791.
- Linos, A., Berekaa, M. M., Steinbüchel, A., Kim, K. K., Spröer, C. & Kroppenstedt, R. M. (2002). *Gordonia westfalica* sp. nov., a novel rubber-degrading actinomycete. *Int J Syst Evol Microbiol* **52**, 1133–1139.
- 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., Hutchinson, I. G., Goodfellow, M. & Caldicott, A. B. (1980). Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr* **188**, 221–233.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. K. (1984). An integrated procedure for the extraction of isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Nam, S.-W., Chun, J., Kim, S., Kim, W., Zakrzewska-Czerwinska, J. & Goodfellow, M. (2003). *Tsukamurella spumae* sp. nov., a novel actinomycete associated with foaming in activated sludge plants. *Syst Appl Microbiol* **26**, 367–375.
- Nam, S.-W., Kim, W., Chun, J. & Goodfellow, M. (2004). *Tsukamurella pseudospumae* sp. nov., a novel actinomycete isolated from activated sludge foam. *Int J Syst Evol Microbiol* **54**, 1209–1212.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schaal, K. P. (1985). Laboratory diagnosis of actinomycete diseases. In *Chemical Methods in Bacterial Systematics*, pp. 359–381. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Sfanos, K., Harmody, D., Dang, P., Ledger, A., Pomponi, S., McCarthy, P. & Lopez, J. (2005). A molecular systematic survey of cultured microbial associates of deep-water marine invertebrates. *Syst Appl Microbiol* **28**, 242–264.
- Soddell, J. A. (1999). Foaming. In *Microbiology of Activated Sludge*, pp. 161–202. Edited by S. J. Seviour & L. L. Blackall. Dordrecht: Kluwer.
- Soddell, J. A. & Seviour, R. J. (1990). Microbiology of foaming in activated sludge plants – a review. *J Appl Bacteriol* **69**, 145–176.
- Soddell, J. A. & Seviour, R. J. (1994). Incidence and morphological variability of *Nocardia pinensis* in Australian activated sludge plants. *Water Res* **28**, 2343–2351.
- Soddell, J. A. & Seviour, R. J. (1995). Relationship between temperature and growth of organisms causing *Nocardia* foams in activated sludge plants. *Water Res* **29**, 1555–1558.
- Soddell, J. A. & Seviour, R. J. (1998). Numerical taxonomy of *Skermania piniformis* and related isolates from activated sludge. *J Appl Microbiol* **84**, 272–284.
- Soddell, J. A., Seviour, R. J., Blackall, L. L. & Hugenholtz, P. (1998). New foam-forming nocardioforms found in activated sludge. *Water Sci Technol* **37** (4–5), 495–502.
- 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.
- Stainsby, F. M., Soddell, J., Seviour, R., Upton, J. & Goodfellow, M. (2002). Dispelling the “*Nocardia amarae*” myth: a phylogenetic and phenotypic study of mycolic acid-containing actinomycetes isolated from activated sludge foam. *Water Sci Technol* **46** (1–2), 81–90.
- Tamaoka, J. (1994). Determination of DNA base composition. In *Chemical Methods in Prokaryotic Systematics*, pp. 463–470. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.

Uchida, K., Kudo, T., Suzuki, K. & Nakase, T. (1999). A new rapid method of glycolate test by diethyl ether extraction which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49–56.

Yoon, J.-H., Cho, Y.-G., Kang, S.-S., Kim, S. B., Lee, S. T. & Park, Y.-H. (2000). *Rhodococcus koreensis* sp. nov., a 2,4-dinitrophenol-degrading bacterium. *Int J Syst Evol Microbiol* **50**, 1193–1201.