Amycolatopsis nigrescens sp. nov., an actinomycete isolated from a Roman catacomb

Ingrid Groth,¹ Geok Yuan Annie Tan,² Juan M. González,³ Leonila Laiz,³ Marc René Carlsohn,¹ Barbara Schütze,¹ Joachim Wink⁴ and Michael Goodfellow⁵

Correspondence

Ingrid Groth

Ingrid.Groth@hki-jena.de

¹Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e.V., Hans-Knöll-Institut, Beutenbergstrasse 11a, 07745 Jena, Germany

²Institute of Biological Sciences (Microbiology), Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

³Instituto de Recursos Naturales y Agrobiología, CSIC, Apartado 1052, 41080 Sevilla, Spain

⁴Sanofi-Aventis Deutschland GmbH, Industriepark Hoechst, 65926 Frankfurt (Main), Germany

⁵Division of Biology, University of Newcastle, Newcastle upon Tyne NE1 7RU, UK

The taxonomic status of two actinomycetes isolated from the wall of a hypogean Roman catacomb was established based on a polyphasic investigation. The organisms were found to have chemical and morphological markers typical of members of the genus *Amycolatopsis*. They also shared a range of chemical, molecular and phenotypic markers which served to separate them from representatives of recognized *Amycolatopsis* species. The new isolates formed a branch in the *Amycolatopsis* 16S rRNA gene sequence tree with *Amycolatopsis minnesotensis* NRRL B-24435^T, but this association was not supported by a particularly high bootstrap value or by the product of the maximum-parsimony tree-making algorithm. The organisms were distinguished readily from closely related *Amycolatopsis* species based on a combination of phenotypic properties and from all *Amycolatopsis* strains by their characteristic menaquinone profiles, in which tetra-hydrogenated menaquinones with 11 isoprene units predominated. The combined genotypic and phenotypic data indicate that the isolates merit recognition as representing a novel species of the genus *Amycolatopsis*. The name proposed for this novel species is *Amycolatopsis nigrescens* sp. nov., with type strain CSC17Ta-90^T (= HKI 0330^T = DSM 44992^T = NRRL B-24473^T).

The genus *Amycolatopsis* Lechevalier *et al.* 1986 is a member of the family *Pseudonocardiaceae* Embley *et al.* 1988 (as circumscribed by Warwick *et al.*, 1994). Members of this family are characterized by the presence of meso-diaminopimelic acid, arabinose and galactose in whole-cell hydrolysates (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), fatty acids that mainly consist of iso- and anteiso-branched components and *N*-acetylated muramic acid and by the lack of mycolic acids (Takahashi, 2001). *Amycolatopsis* strains can be separated from members of the other genera classified within the family *Pseudonocardiaceae* by using a range of chemotaxonomic and morphological

markers (Kim & Goodfellow, 1999) and by genus-specific oligonucleotide primers based on 16S rRNA gene sequences (Tan *et al.*, 2006).

Members of the genus *Amycolatopsis* are Gram-positive, non-acid-fast, non-motile actinomycetes that form branched vegetative hyphae that undergo fragmentation into rod-like and squarish elements. If present, aerial hyphae may be sterile or differentiate into squarish to ovoid spore-like structures (Chun *et al.*, 1999). The genus encompasses alkaliphilic, mesophilic, thermophilic and pathogenic species, which can be distinguished from one another by using a range of phenotypic properties (Saintpierre-Bonaccio *et al.*, 2005; Lee, 2006; Lee *et al.*, 2006). At the time of writing, the genus *Amycolatopsis* comprised 32 recognized species and subspecies, although there is compelling evidence that the genus is grossly underspeciated and that *Amycolatopsis fastidiosa* should be classified within a different taxon (Tan *et al.*, 2006). The present polyphasic study was designed to determine the taxonomic position of

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CSC17Ta-90^T and CSC17Ta-84 are respectively DQ486888 and DQ486887.

A table giving the cellular fatty acid compositions of strains CSC17Ta-90^T and CSC17Ta-84 and *A. minnesotensis* NRRL B-24435^T is available as supplementary material in IJSEM Online.

two *Amycolatopsis*-like strains that had been isolated from a hypogean environment. The resultant data show that the isolates represent a novel species of the genus *Amycolatopsis*.

Strains CSC17Ta-84 (=HKI 0329) and CSC17Ta-90^T (=HKI 0330^T) were isolated from the wall of the last arcosolium near the exit of the Roman catacomb of St Callistus by touching the stone with a sterile cotton swab and suspending the adherent bacteria in 1:10-diluted organic medium 79 (Prauser & Falta, 1968). Aliquots of this suspension were spread over nutrient agar (Difco) plates, which were incubated at 28 °C for 14 days. The isolates were purified and maintained on yeast extract/malt extract agar (ISP medium 2; Shirling & Gottlieb, 1966) and preserved at -80 °C as a mixture of hyphae and fragmentation spores in organic medium 79 broth and glycerol medium that consisted of K₂HPO₄ (1.26 %), KH₂PO₄ (0.36 %), MgSO₄ (0.01 %), sodium citrate (0.09 %), (NH₄)₂SO₄ (0.18 %) and glycerol (8.8 %). Stock cultures of the two strains in liquid organic medium 79 supplemented with 5 % DMSO were additionally maintained in the vapour phase of liquid nitrogen.

Bacterial growth for chemotaxonomic investigations of the two isolates and the selected marker strains *Amycolatopsis minnesotensis* NRRL B-24435^T, *Amycolatopsis sacchari* DSM 44468^T and *Amycolatopsis sulphurea* IMET 7649^T (=ATCC 27624^T) was prepared by cultivating them for 24–48 h at 28 °C in liquid organic medium 79 or bacto-trypticase soy broth (Sigma-Aldrich). For MALDI-TOF mass spectrometry (MS) analysis the strains were cultivated on a sterile Sartorius filter (SM 11106) deposited on agar plates with a medium containing soluble starch (1 %), yeast extract (0.2 %), glucose (1 %), glycerol (1 %), cornsteep liquor (0.25 %), peptone (0.2 %), NaCl (0.1 %) and CaCO₃ (0.3 %) for 7 days at 28 °C. Morphological and cultural characteristics were studied by cultivating the isolates on ISP media 2, 3, 4 and 5 (Difco) (Shirling & Gottlieb, 1966) for up to 21 days at 28 °C. Growth parameters were determined by using organic medium 79. The pH range for growth was established in shake flasks of liquid medium adjusted to pH values between 4.5 and 10.0 with either 1 M HCl or 20 % (w/v) Na₂CO₃ solution. The cultures were incubated for up to 6 days at 28 °C. Physiological tests, including determination of enzyme activities and susceptibility to antibiotics, were carried out as described by Groth *et al.* (2003).

Standard HPLC and TLC procedures were used to determine the isomers of diaminopimelic acid in whole-organism hydrolysates (Rhuland *et al.*, 1955; Hasegawa *et al.*, 1983), predominant whole-organism sugars (Becker *et al.*, 1965; Saddler *et al.*, 1991), fatty acids (MIDI system; <http://www.midi-inc.com/>), predominant menaquinones (Collins *et al.*, 1977), muramic acid type (Uchida & Aida, 1984), polar lipids (Minnikin *et al.*, 1979; Collins & Jones, 1980) and the presence of mycolic acids (Minnikin *et al.*, 1975). For MALDI-TOF MS (Erhard *et al.*, 1997), the samples were collected from cellulose acetate filters, homogenized in a suspension of acetonitrile/methanol (1:1) and transferred

onto a stainless steel template. Each sample was mixed with the matrix (Kroppenstedt *et al.*, 2005) and positive-ion mass spectra were recorded using a MALDI-TOF mass spectrometer (Voyager). The MALDI-TOF MS spectra were analysed by using the Applied Biosystems Software Data Explorer and the peak lists were compared with the SARAMIS software (AnagnosTec).

Chromosomal DNA of isolates CSC17Ta-90^T and CSC17Ta-84 was extracted following the method described by Marmur (1961). PCR amplification of the 16S rRNA genes was achieved using the conserved primers 27F (5'-AGAGTTTGATCCTGGCTCAG) and 1522R (5'-AAG-GAGGTGATCCAGCCGCA) (Edwards *et al.*, 1989) under the following conditions: initial denaturation at 95 °C for 1 min prior to the addition of BioTaq followed by 35 cycles of 95 °C for 15 s, 55 °C for 15 s and 72 °C for 2 min, and a final extension cycle at 72 °C for 10 min. Forward and reverse strands of the amplified DNA fragment were sequenced in an ABI 3700 sequencer (Applied Biosystems). The 16S rRNA gene sequences of the strains were aligned manually using the PHYDIT program (available at <http://plaza.snu.ac.kr/~jchun/phydit>) against corresponding sequences of representatives of the family *Pseudonocardiaceae* retrieved from the EMBL and GenBank databases. The algorithm of Jukes & Cantor (1969) was used to transform sequence dissimilarities into evolutionary distances, and phylogenetic trees were inferred by using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) tree-making methods. The topologies of the resultant trees were evaluated in bootstrap analyses based on 1000 replicates. All of the phylogenetic analyses were carried out by using the PHYLIP suite of programs (Felsenstein, 1993) and the resultant phylogenetic trees were viewed by using TREEVIEW (Page, 1996). The root position of the unrooted *Amycolatopsis* tree based on the neighbour-joining method was estimated by using *Prauserella rugosa* DSM 43194^T as the outgroup.

Almost-complete 16S rRNA gene sequences were generated for isolates CSC17Ta-84 (1459 nt) and CSC17Ta-90^T (1409 nt). Comparison of these sequences with corresponding nucleotide sequences of representatives of the genera classified in the family *Pseudonocardiaceae* showed that the hypogean organisms belonged to the genus *Amycolatopsis* (data not shown). In addition, isolates CSC17Ta-90^T and CSC17Ta-84 shared a range of chemotaxonomic and morphological markers consistent with their assignment to this genus (Lechevalier *et al.*, 1986; Yassin *et al.*, 1993; Takahashi, 2001) as they produced an extensively branched substrate mycelium that fragmented into rod-like elements, formed sparse to moderate aerial hyphae and contained *meso*-diaminopimelic acid, arabinose and galactose in whole-organism hydrolysates (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970) together with minor amounts of glucose, mannose, rhamnose and ribose. Furthermore, they were characterized by the presence of

N-acetylated muramic acid and a complex phospholipid pattern consisting of diphosphatidylglycerol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, two ninhydrin-positive lipids and several uncharacterized glycolipid and polar lipid components (phospholipid pattern 2 *sensu* Lechevalier *et al.*, 1977). The fatty acid profiles were rich in iso-branched, straight-chain saturated and unsaturated components. Mycolic acids were not detected. Isolates CSC17Ta-90^T and CSC17Ta-84 were also characterized by the presence of MK-11(H₄) as the major menaquinone component (52.4 and 54.3 % of the total, respectively) with smaller proportions of MK-12(H₄) (18.1 and 18.2 %), MK-9(H₄) (8.0 and 8.8 %) and MK-10(H₄) (9.0 and 9.1 %). By contrast, representatives of recognized *Amycolatopsis* species studied to date contain di-, tetra-, hexa- or octa-hydrogenated menaquinones with nine isoprene units as the predominant isoprenologue (Lechevalier *et al.*, 1986; Yassin *et al.*, 1991; Wink *et al.*, 2003; Lee, 2006). Furthermore, the type strain of *Amycolatopsis decaplanina* is reported to contain a mixture of tetra-hydrogenated menaquinones with eight and nine isoprene units (Wink *et al.*, 2004).

The two new isolates shared a 16S rRNA gene sequence similarity of 99.9 %, a value that corresponds to a single

nucleotide difference at 1408 locations; isolate CSC17Ta-84 has a guanine but isolate CSC17Ta-90^T an adenine at *Escherichia coli* position 1463 (Brosius *et al.*, 1978). It is evident from Fig. 1 that the new isolates form a branch in the *Amycolatopsis* gene tree together with the type strain of *A. minnesotensis*, although this relationship is not supported by a high bootstrap value or by the results from the maximum-parsimony analysis. 16S rRNA gene sequence similarity between isolates CSC17Ta-84 and CSC17Ta-90^T and *A. minnesotensis* NRRL B-24435^T was 98.2 %, a value equivalent to 25 nt differences at 1458 and 1407 locations. In addition, the two isolates show relatively high 16S rRNA gene similarities with the type strains of *Amycolatopsis alba* (97.5 %), *Amycolatopsis azurea* (97.4 %), *Amycolatopsis keratiniphila* subsp. *keratiniphila* (97.4 %), *Amycolatopsis keratiniphila* subsp. *nogabecina* (97.2 %), *Amycolatopsis lurida* (97.5 %) and *A. sacchari* (97.3 %). DNA–DNA relatedness studies were not carried out between isolate CSC17Ta-90^T and its closest phylogenetic neighbours as it has been shown repeatedly that *Amycolatopsis* type strains which share much higher 16S rRNA gene similarities than those cited above have DNA–DNA relatedness values well below the 70 % cut-off point recommended by Wayne *et al.* (1987) for the delineation of strains which belong to the same genomic species (Chun *et al.*, 1999; Labeda *et al.*, 2003; Wink *et al.*, 2003).

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences showing the relationships between isolates CSC17Ta-84 and CSC17Ta-90^T and representatives of recognized *Amycolatopsis* species. F and P indicate branches that were also recovered using the least-squares (Fitch & Margoliash, 1967) and maximum-parsimony (Fitch, 1971) tree-making algorithms, respectively. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values ≥ 50 % are shown. Bar, 0.02 substitutions per nucleotide position.

Table 1. Differential physiological properties between strain CSC17Ta-90^T and the type strains of its nearest phylogenetic neighbours in the genus *Amycolatopsis*

Strains: 1, CSC17Ta-90^T (identical results for strain CSC17Ta-84 unless indicated); 2, *A. alba* DSM 44262^T; 3, *A. azurea* IMSNU 20053^T; 4, *A. keratiniphila* subsp. *keratiniphila* DSM 44409^T; 5, *A. keratiniphila* subsp. *nogabecina* DSM 44586^T; 6, *A. lurida* DSM 43134^T; 7, *A. minnesotensis* NRRL B-24435^T; 8, *A. sacchari* DSM 44468^T. Data are from Mertz & Yao (1993), Goodfellow *et al.* (2001), Kim *et al.* (2002), Al-Musallam *et al.* (2003), Wink *et al.* (2003) and this study. +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Colour of aerial mycelium	White to orange	White	Blue	Light grey	Not formed	White	White	White
Production of soluble pigment	+	–	+	–	–	–	–	–
Nitrate reductase activity	+	–	+	+	–	–	+	+
Decomposition of:								
Potato starch	–	+	–	–	–	–	–	–
Hypoxanthine	+	+	+	+	+	–	+	–
Xanthine	+*	+	–	+	+	+	–	+
Growth on sole carbon sources:								
L-Arabinose	+	–	+	+	+	+	–	+
D-Fructose	+	+	–	+	+	+	+	+
<i>myo</i> -Inositol	+	+	+	+	+	–	+	–
D-Raffinose	+	+	+	+	–	–	+	w
L-Rhamnose	w	–	–	–	+	–	–	+
Sucrose	–*	–	+	+	+	–	–	+
D-Xylose	+	–	–	+	+	–	w	+
Enzyme assays (API ZYM):								
Alkaline phosphatase	+	+	–	+	+	+	w	+
α -Chymotrypsin	+	+	+	+	+	+	–	+
Cystine arylamidase	+	+	–	+	+	–	–	+
Valine arylamidase	w	+	–	+	+	–	–	+
Esterase (C4)	+	+	–	+	–	–	+	+
α -Galactosidase	–	–	–	+	+	–	–	–
β -Galactosidase	+	+	+	+	+	+	–	–
α -Glucosidase	–	+	+	+	+	+	–	w
Lipase (C14)	+	–	–	+	+	+	–	+
α -Mannosidase	–	+	–	–	+	–	–	–
Naphthol-AS-BI-phosphohydrolase	+	+	+	–	+	+	w	+
Trypsin	–	+	+	+	+	+	–	–
Growth at:								
10 °C	–	–	+	+	ND	+	–	–
40 °C	+	w	–	+	+	+	–	+
45 °C	–	–	–	–	ND	–	–	+
Antibiotic susceptibility (μ g per disc):								
Kanamycin (30)	–	–	+	+	+	+	–	+
Nalidixic acid (30)	–	–	–	–	–	–	+	–
Streptomycin (10)	–	–	–	–	–	–	–	+
Sulfonamide (200)	–	–	w	–	w	w	+	–
Vancomycin (30)	–	–	–	–	–	–	–	+

*Strain CSC17Ta-84 grew weakly on sucrose and did not degrade xanthine.

The isolates gave almost identical phenotypic profiles that served to distinguish them from representatives of their phylogenetic neighbours, including *A. minnesotensis* (Table 1). In addition, they grew well on ISP media 2–7, between 20 and 40 °C and from pH 5 to 9. Strains CSC17Ta-84 and CSC17Ta-90^T showed highly similar fatty acid patterns in which 14-methylpentadecanoic acid (iso-C_{16:0})

was the major component (27.7 and 27.1 % of the total fatty acid composition, respectively); fatty acids found in much smaller proportions included C_{17:0} (7.3 and 9.3 %), C_{17:1 cis9} (8.4 and 9.8 %), C_{16:0} (7.4 and 7.2 %), iso-C_{16:0} 2-OH (7.9 and 6.4 %), iso-C_{15:0} (5.2 and 5.7 %), C_{18:1 cis9} (4.9 and 4.8 %), iso-C_{16:1} (4.2 and 4.4 %), anteiso-C_{17:0} (3.8 and 3.7 %), C_{16:1 cis9} (3.8 and 3.1 %), C_{18:0} (3.3 %) and an

Fig. 2. Dendrogram showing the relationships between strains CSC17Ta-84 and CSC17Ta-90^T and representatives of the genus *Amycolatopsis* based on MALDI-TOF MS data.

unidentified component (3.7 and 3.0 %). Similar complex fatty acid patterns have been recorded for recognized members of the genus *Amycolatopsis* (Yassin *et al.*, 1993; Wink *et al.*, 2004). However, differences in the profiles obtained from concurrent fatty acid analyses of the two new isolates and their closest phylogenetic neighbour, *A. minnesotensis* NRRL B-24435^T, clearly distinguished strains CSC17Ta-84 and CSC17Ta-90^T from this species (see Supplementary Table S1 available in IJSEM Online). The same was true for the MALDI-TOF MS-based fingerprinting data (Fig. 2), which underlined the distinctiveness of strains CSC17Ta-84 and CSC17Ta-90^T from *A. minnesotensis* and all other studied less closely related *Amycolatopsis* species. Furthermore, these data indicated again the close relationship between the two novel organisms as they shared mass similarity values above 70 % (Kroppenstedt *et al.*, 2005).

It is apparent from the genotypic and phenotypic data presented that isolates CSC17Ta-84 and CSC17Ta-90^T represent a single species that can be distinguished from representatives of all recognized *Amycolatopsis* species. It is proposed that the isolates be classified in the genus *Amycolatopsis* as *Amycolatopsis nigrescens* sp. nov.

Description of *Amycolatopsis nigrescens* sp. nov.

Amycolatopsis nigrescens (ni.gres'cens. L. v. *nigresco* to become black; L. part. adj. *nigrescens* becoming black).

Aerobic, Gram-positive, non-acid-alcohol-fast, non-motile, catalase-positive actinomycete which forms an extensively branched vegetative mycelium (diameter of hyphae 0.7–0.9 µm) that fragments into squarish rod-like elements. The colour of the substrate mycelium changes from orange

to black with the production of a dark reddish-black soluble pigment. The substrate mycelium carries sparse to moderate white or pale-orange aerial hyphae. Optimal growth occurs between 28 and 35 °C, but growth is not evident at either 10 or 42 °C. Grows well between pH 5 and 9 and in the presence of 6.0 % (w/v) NaCl, but does not grow at either pH 4.5 or 10 or in the presence of more than 10.0 % (w/v) NaCl. Aesculin and urea are hydrolysed and H₂S is produced. Casein, hippurate, Tween 80 and tyrosine are degraded but adenine is not. Utilizes acetate, aconitate, citrate, malate, succinate, D-fructose, D-glucose and D-mannitol as sole carbon sources, but not benzoate, DL-tartrate or cellulose. Produces leucine arylamidase, esterase lipase (C8), *N*-acetyl-β-glucosamidase and acid phosphatase, but not α-fucosidase or β-glucuronidase (API ZYM tests). Susceptible to (per disc) chloramphenicol (30 µg), imipenem (10 µg), ofloxacin (10 µg), oxytetracycline hydrochloride (30 µg) and rifampicin (30 µg), but resistant to (per disc) lincomycin hydrochloride (2 µg), meticillin (5 µg) and norfloxacin (10 µg). Additional phenotypic properties are given in Table 1. Chemotaxonomic characters are typical for *Amycolatopsis* species, apart from the menaquinone profile, which is characterized by the presence of a large proportion of tetra-hydrogenated menaquinones with 11 isoprene units.

The type strain, CSC17Ta-90^T (= HKI 0330^T = DSM 44992^T = NRRL B-24473^T), was isolated from the wall of the hypogean Roman catacomb of St Callistus. Strain CSC17Ta-84 (= HKI 0329) is a reference strain.

Acknowledgements

This work was supported by the European Commission Energy, Environment and Sustainable Development Programme (contract

EVK4-CT-2000-00028). We are grateful to Renate Schön, Carmen Schult and Christiane Weigel for excellent technical assistance and to Peter Schumann (DSMZ, Braunschweig) for analysing the menaquinones.

References

- Al-Musallam, A. A., Al-Zarban, S. S., Fasasi, Y. A., Kroppenstedt, R. M. & Stackebrandt, E. (2003). *Amycolatopsis keratiniphila* sp. nov., a novel keratinolytic soil actinomycete from Kuwait. *Int J Syst Evol Microbiol* 53, 871–874.
- Becker, B., Lechevalier, M. P. & Lechevalier, H. A. (1965). Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Appl Microbiol* 13, 236–243.
- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* 75, 4801–4805.
- Chun, J., Kim, S. B., Oh, Y. K., Seong, C.-N., Lee, D.-H., Bae, K. S., Lee, K.-J., Kang, S.-O., Hah, Y. C. & other authors (1999). *Amycolatopsis thermoflava* sp. nov., a novel soil actinomycete from Hainan Island, China. *Int J Syst Bacteriol* 49, 1369–1373.
- Collins, M. D. & Jones, D. (1980). Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2,4-diaminobutyric acid. *J Appl Bacteriol* 48, 459–470.
- Collins, M. D., Goodfellow, M. & Minnikin, D. E. (1977). Isoprenoid quinones in the classification of coryneform and related bacteria. *J Gen Microbiol* 110, 127–136.
- Edwards, U., Rogall, T., Blöcker, H., Emde, M. & Böttger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* 17, 7843–7853.
- Embley, T. M., Smida, J. & Stackebrandt, E. (1988). The phylogeny of mycolate-less wall chemotype IV actinomycetes and description of *Pseudonocardiaceae* fam. nov. *Syst Appl Microbiol* 11, 44–52.
- Erhard, M., von Dören, H. & Jungblut, P. (1997). Rapid typing and elucidation of new metabolites of intact cyanobacteria using MALDI-TOF mass spectrometry. *Nat Biotechnol* 15, 906–909.
- Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5.1. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Fitch, W. M. (1971). Towards 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.
- Goodfellow, M., Kim, S. B., Minnikin, D. E., Whitehead, D., Zhou, Z.-H. & Mattinson-Rose, A. D. (2001). *Amycolatopsis sacchari* sp. nov., a moderately thermophilic actinomycete isolated from vegetable matter. *Int J Syst Evol Microbiol* 51, 187–193.
- Groth, I., Schütze, B., Boettcher, T., Pullen, C. B., Rodriguez, C., Leistner, E. & Goodfellow, M. (2003). *Kitasatospora putterlickiae* sp. nov., isolated from rhizosphere soil, transfer of *Streptomyces kifunensis* to the genus *Kitasatospora* as *Kitasatospora kifunensis* comb. nov., and emended description of *Streptomyces aureofaciens* Duggar 1948. *Int J Syst Evol Microbiol* 53, 2033–2040.
- Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* 29, 319–322.
- 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.
- Kim, S. B. & Goodfellow, M. (1999). Reclassification of *Amycolatopsis rugosa* Lechevalier *et al.* 1986 as *Prauserella rugosa* gen. nov., comb. nov. *Int J Syst Bacteriol* 49, 507–512.
- Kim, B., Sahin, N., Tan, G. Y. A., Zakrewska-Czerwinska, J. & Goodfellow, M. (2002). *Amycolatopsis eurytherma* sp. nov., a thermophilic actinomycete isolated from soil. *Int J Syst Evol Microbiol* 52, 889–894.
- Kroppenstedt, R. M., Mayilraj, S., Wink, J. M., Kallow, W., Schumann, P., Secondini, C. & Stackebrandt, E. (2005). Eight new species of the genus *Micromonospora*, *Micromonospora citrea* sp. nov., *Micromonospora echinaurantiaca* sp. nov., *Micromonospora echinofusca* sp. nov., *Micromonospora fulviviridis* sp. nov., *Micromonospora inyonensis* sp. nov., *Micromonospora peucetia* sp. nov., *Micromonospora sagamiensis* sp. nov., and *Micromonospora viridifaciens* sp. nov. *Syst Appl Microbiol* 28, 328–339.
- Labeda, D. P., Donahue, J. M., Williams, N. M., Sells, S. F. & Henton, M. M. (2003). *Amycolatopsis kentuckyensis* sp. nov., *Amycolatopsis lexingtonensis* sp. nov. and *Amycolatopsis pretoriensis* sp. nov., isolated from equine placentas. *Int J Syst Evol Microbiol* 53, 1601–1605.
- Lechevalier, M. P. & Lechevalier, H. A. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* 20, 435–443.
- Lechevalier, M. P., De Bièvre, C. & Lechevalier, H. A. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* 5, 249–260.
- Lechevalier, M. P., Prauser, H., Labeda, D. P. & Ruan, J.-S. (1986). Two new genera of nocardioform actinomycetes: *Amycolata* gen. nov. and *Amycolatopsis* gen. nov. *Int J Syst Bacteriol* 36, 29–37.
- Lee, S. D. (2006). *Amycolatopsis jejuensis* sp. nov. and *Amycolatopsis halotolerans* sp. nov., novel actinomycetes isolated from a natural cave. *Int J Syst Evol Microbiol* 56, 549–553.
- Lee, S. D., Kinkel, L. L. & Samac, D. A. (2006). *Amycolatopsis minnesotensis* sp. nov., isolated from a prairie soil. *Int J Syst Evol Microbiol* 56, 265–269.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* 3, 208–218.
- Mertz, F. P. & Yao, R. C. (1993). *Amycolatopsis alba* sp. nov., isolated from soil. *Int J Syst Bacteriol* 43, 715–720.
- Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-organism methanolytates. *J Gen Microbiol* 88, 200–204.
- Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1979). Fatty acid and polar lipid composition in the classification of *Cellulomonas*, *Oerskovia* and related taxa. *J Appl Bacteriol* 47, 87–95.
- Page, R. D. M. (1996). TREEVIEW: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 12, 357–358.
- Prauser, H. & Falta, R. (1968). Phagensensibilität, Zellwand-Zusammensetzung und Taxonomie von Actinomyceten. *Z Allg Mikrobiol* 8, 39–46 (in German).
- Rhuland, L. E., Work, E., Denman, R. F. & Hoare, D. S. (1955). The behavior of the isomers of α,ϵ -diaminopimelic acid on paper chromatograms. *J Am Chem Soc* 77, 4844–4846.
- Saddler, G. S., Tavecchia, P., Lociuero, S., Zanol, M., Colombo, L. & Selva, E. (1991). Analysis of madurose and other actinomycete whole cell sugars by gas chromatography. *J Microbiol Methods* 14, 185–191.
- Saintpierre-Bonaccio, D., Amir, H., Pineau, R., Tan, G. Y. A. & Goodfellow, M. (2005). *Amycolatopsis plumensis* sp. nov., a novel bioactive actinomycete isolated from a New-Caledonian brown hypermagnesian ultramafic soil. *Int J Syst Evol Microbiol* 55, 2057–2061.

- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for constructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Takahashi, Y. (2001).** Family *Pseudonocardiaceae*. In *Identification Manual of Actinomycetes*, pp. 227–239. Edited by The Society for Actinomycetes Japan. Tokyo: The Business Centre for Academic Societies.
- Tan, G. Y. A., Ward, A. C. & Goodfellow, M. (2006).** Exploration of *Amycolatopsis* diversity in soil using genus-specific primers and novel selective media. *Syst Appl Microbiol* **29**, 557–569.
- Uchida, K. & Aida, K. (1984).** An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.
- Warwick, S., Bowen, T., McVeigh, H. & Embley, T. M. (1994).** A phylogenetic analysis of the family *Pseudonocardiaceae* and the genera *Actinokineospora* and *Saccharothrix* with 16S rRNA sequences and a proposal to combine the genera *Amycolata* and *Pseudoa-mycolata* in an emended genus *Pseudonocardia*. *Int J Syst Bacteriol* **44**, 293–299.
- 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.
- Wink, J. M., Kroppenstedt, R. M., Ganguli, B. N., Nadkarni, S. R., Schumann, P., Seibert, G. & Stackebrandt, E. (2003).** Three new antibiotic producing species of the genus *Amycolatopsis*, *Amycolatopsis balhimycina* sp. nov., *A. tolypomycina* sp. nov., *A. vancor-esmycina* sp. nov., and description of *Amycolatopsis keratiniphila* subsp. *keratiniphila* subsp. nov. and *A. keratiniphila* subsp. *noga-becina* subsp. nov. *Syst Appl Microbiol* **26**, 38–46.
- Wink, J., Gandhi, J., Kroppenstedt, R. M., Seibert, G., Sträubler, B., Schumann, P. & Stackebrandt, E. (2004).** *Amycolatopsis decaplanina* sp. nov., a novel member of the genus with unusual morphology. *Int J Syst Evol Microbiol* **54**, 235–239.
- Yassin, A. F., Schaal, K. P., Brzezinka, H., Goodfellow, M. & Pulverer, G. (1991).** Menaquinone patterns of *Amycolatopsis* species. *Zentralbl Bakteriol* **274**, 465–470.
- Yassin, A. F., Haggenei, B., Budzikiewicz, H. & Schaal, K. P. (1993).** Fatty-acid and polar lipid-composition of the genus *Amycolatopsis* – application of fast-atom-bombardment mass-spectrometry to structure-analysis of underivatized phospholipids. *Int J Syst Bacteriol* **43**, 414–420.