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Myceligenerans crystallogenes sp. nov., isolated from Roman catacombs

Ingrid Groth,[1] Peter Schumann,[2] Barbara Schütze,[1] Juan M. Gonzalez,[3] Leonila Laiz,[3] Maija-Liisa Suihko[4] and Erko Stackebrandt[2]

Correspondence
Ingrid Groth
Ingrid.Groth@hki-jena.de

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

[2]DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, 38124 Braunschweig, Germany

[3]Instituto de Recursos Naturales y Agrobiologia, CSIC, Apartado 1052, 41080 Sevilla, Spain

[4]VTT Biotechnology, PO Box 1500, FI-02044 VTT, Finland

Three xylan-degrading actinobacterial strains were isolated from different sampling sites in the Roman catacombs of Domitilla and San Callisto. The organisms showed morphological and chemotaxonomic properties such as peptidoglycan type A4α, L-Lys–L-Thr–D-Glu; whole-cell sugars (glucose, mannose and galactose); octa-, hexa- and tetrahydrogenated menaquinones with nine isoprene units; phosphatidylglycerol and diphosphatidylglycerol as the major phospholipids; anteiso-$C_{15:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$ as the predominant fatty acids; and a DNA G+C content of 72 mol%. These features are consistent with affiliation of these isolates to the genus *Myceligenerans*. The three isolates shared a 16S rRNA gene similarity of 99·9 % and were most closely related to *Myceligenerans xiligouense* DSM 15700^T (97·9 % sequence similarity). The low level of DNA–DNA relatedness (about 14 %) and the differences in phenotypic characteristics between the novel strains and *M. xiligouense* DSM 15700^T justify the proposal of a novel species of the genus *Myceligenerans*, *Myceligenerans crystallogenes* sp. nov., with CD12E2-27^T (=HKI 0369^T=DSM 17134^T=NCIMB 14061^T=VTT E-032285^T) as the type strain.

The genus *Myceligenerans* was proposed recently by Cui *et al.* (2004) for a single strain that was isolated from an alkaline salt marsh soil in China. The type strain of the species *Myceligenerans xiligouense*, strain XLG9A10.2^T (=DSM 15700^T), forms a distinct phylogenetic line within the family *Promicromonosporaceae*, suborder *Micrococcineae*, order *Actinomycetales*, and shares 94·8–95·1 % 16S rRNA gene sequence similarity with representatives of the genus *Promicromonospora* Krasil'nikov *et al.* 1961 and 94·4–95·7 % similarity with strains of the genera *Xylanimonas* Rivas *et al.* 2003, *Xylanibacterium* Rivas *et al.* 2004 and *Isoptericola* Stackebrandt *et al.* 2004. *M. xiligouense* shares the ability to hydrolyse xylan with members of the last three genera and with *Xylanimicrobium pachnodae* (Stackebrandt & Schumann, 2004).

From different sampling sites in the Roman catacombs of Domitilla and San Callisto, three morphologically similar actinobacteria were isolated that were able to degrade xylan and to produce crystals of iodinin (1,6-phenazinediol 5,10-dioxide). Preliminary phylogenetic data obtained from 16S rRNA gene sequence comparison indicated a close relationship with *M. xiligouense* (DSM 15700^T). Therefore a polyphasic taxonomic study was carried out to establish the taxonomic position of these isolates.

Strain CD12E2-27^T (=HKI 0369^T) was isolated from a sample of tufa collected in the first arcosolium behind the entrance of the Roman catacomb of Domitilla using peptone/yeast extract/brain heart infusion agar (Yokota *et al.*, 1993) and a standard dilution plate procedure. Strain CD12Tz-28 (=HKI 0371=VTT E-032288) was obtained from the stone surface of the burial chamber at the same place 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 on peptone/yeast extract/brain heart infusion agar plates and incubated at 28 °C for 10 days. Strain CSC13Tb-79 (=HKI 0372=VTT E-032289) was isolated from a fresco in the cubiculum of Oceano, catacomb of San Callisto, Rome, using the same procedure and humic acid agar (Hayakawa & Nonomura, 1987) for growth.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CD12E2-27^T (=HKI 0369^T) is AY928181.

Tables showing the physiological test results for the strains in this study are available as supplementary material in IJSEM Online.

(In the following text and in the tables and figures, only the HKI numbers are used for the isolates.)

General laboratory cultivation, morphological studies, determination of the optimal growth parameters (temperature, pH, oxygen requirements) and analysis of the susceptibility to antibiotics were performed using solid or liquid organic medium 79 and an incubation temperature of 28 °C. Cell morphology and cell dimensions were examined by phase-contrast microscopy using a Zeiss Axioscope 2 microscope equipped with image-analysing software (Axio Vision 2.05; Zeiss). The colony morphology of 2–10-day-old cultures was studied using an Olympus stereo microscope. Standard physiological tests were carried out according to the methods described by Cowan & Steel (1965), Gordon *et al.* (1974), Lanyi (1987) and Smibert & Krieg (1994). Acid production from carbon sources was studied using the API 50 CH system and API 50 CHB/E medium (bioMérieux) according to the manufacturer's instructions (incubation times of up to 7 days). API ZYM galleries (bioMérieux) were used to study enzymic activities. Additionally, the utilization of carbon sources was tested using Biolog GP2 MicroPlates and MicroLog computer software (Biolog Identification System). The cell density of the inoculum for the GP2 MicroPlates was adjusted to the range specified by the Biolog turbidity standard GP-COC/GP-ROD/GN-FAS (20 %T). Xylanolytic activity was determined on medium II, described by Cazemier *et al.* (2003), and using incubation times of up to 28 days. Susceptibility to antibiotics was examined by placing antibiotic discs (Difco) on agar plates that were seeded with suspensions of the test strains grown in a soft agar layer for 24 h at 28 °C. Oxygen requirements were studied with the GENbag microaer and GENbag anaer incubation systems (bioMérieux). The pH range for growth was established by using liquid medium adjusted to pH values between 4 and 11 with either 1 M HCl or 20 % (w/v) Na_2CO_3 solution and then incubating the samples at 28 °C for up to 10 days. The reference strains used for comparisons in physiological tests and DNA–DNA pairing studies were *Isoptericola variabilis* DSM 10177^T, *Xylanimicrobium pachnodae* DSM 12657^T, *Xylanimonas cellulosilytica* DSM 15894^T and *M. xiligouense* DSM 15700^T. Biomass for chemotaxonomic and molecular systematic studies was prepared by growing the strains in shake flasks containing liquid organic medium 79 or Bacto tryptic soy broth (Sigma-Aldrich) for 24–48 h. Stock cultures of the three isolates, HKI 0369^T, HKI 0371 and HKI 0372, in liquid organic medium 79 supplemented with 5 % DMSO were maintained either in the vapour phase of liquid nitrogen or at −80 °C as a 1 : 1 mixture of the liquid culture and a glycerol medium that consisted of K_2HPO_4 (1·26 %), KH_2PO_4 (0·36 %), $MgSO_4$ (0·01 %), sodium citrate (0·09 %), $(NH_4)_2SO_4$ (0·18 %) and glycerol (8·8 %).

The colonies of the three isolates under study were white to cream in colour, circular, convex and smooth (with diameters of about 1 mm). Larger wrinkled colonies with diameters of 2–4 mm were also observed. The colonies were distinguished from those of *M. xiligouense* DSM 15700^T by their white to cream colour, which never changed to yellow. Aerial mycelium was absent. The three organisms produced spore-like cells in the substrate mycelium after growth on solid organic medium 79: these cells were similar to those described for *M. xiligouense* by Cui *et al.* (2004). In liquid cultures, a well-developed primary mycelium was produced within 8–24 h (width 0·5–0·7 μm); this underwent fragmentation into irregular non-motile rods and cocci after about 48 h cultivation.

The morphological and physiological similarities of the three strains under study underline the genomic coherence of these isolates (see Supplementary Tables S1 and S2 available in IJSEM Online). Although the novel strains share numerous physiological characteristics with *M. xiligouense* DSM 15700^T, they could be readily distinguished from the latter by means of the physiological characteristics listed in Table 1. Full details of the results of the physiological tests are available as Supplementary Tables S1 and S2 in IJSEM Online.

Table 1. Physiological characteristics that differentiate the strains of *M. crystallogenes* from *M. xiligouense* DSM 15700^T

−, Negative; +, positive; (+), weakly positive; V, variable.

Test	*M. xiligouense* DSM 15700^T	*M. crystallogenes*
Growth in the presence of 8·0 % NaCl	(+)	−
Decomposition of:		
Tyrosine	−	+
Urea	−	+
Utilization of:		
Aconitate	−	+
Citrate	−	V*
Enzyme assay (API ZYM)		
Trypsin	(+)	V†
Antibiotic susceptibility		
Ampicillin (10 μg)	−	+
Kanamycin (30 μg)	−	V†
Penicillin G (10 IU)	−	+
Polymyxin B (300 IU)	−	+
Biolog GP2 MicroPlate (24 h incubation)		
Glycogen	+	−
Arbutin	+	−
Methyl α-D-galactoside	+	−
D-Sorbitol	+	−
L-Lactic acid	+	V†
L-Alaninamide	+	−
Uridine	+	−
Nitrate reduction	−	+

*The type strain reacts positively.
†The type strain reacts negatively.

The optimal growth parameters of the three catacomb isolates corresponded well with the data given by Cui *et al.* (2004) for *M. xiligouense* DSM 15700^T. However, in this study growth at 6 and 42 °C, at pH 5 and 10 and at a NaCl concentration of 10 % (w/v) was not observed for strain DSM 15700^T or for the three isolates, as shown in Supplementary Table S1. Furthermore, it should be mentioned that differentiation between the isolates and *M. xiligouense* DSM 15700^T using the API 50 CHB/E kit was not possible because of the generally weakly expressed metabolic activity in all strains. The results (data not shown) obtained at different incubation temperatures (28 and 35 °C) did not allow an unambiguous interpretation (exceptions were the utilization of aesculin and arbutin). Weak activities were also observed in the utilization of carbon sources provided in the Biolog GP2 MicroPlates. The low colour intensities in the wells and the mycelial growth of the strains under study contributed to the fact that numerous reactions had to be considered as variable.

For sequence analysis of the 16S rRNA gene, the genomic DNA was extracted by following the method described by Marmur (1961). PCR-mediated amplification of the 16S rRNA gene, sequencing of the amplified DNA fragment and phylogenetic analysis were carried out as described previously (Groth *et al.*, 2005).

Phylogenetic analysis of the almost-complete 16S rRNA gene (1437 bases) revealed that strain HKI 0369^T is a member of the family *Promicromonosporaceae*, showing between 93·0 and 97·9 % similarity with its members. The highest level of relatedness (97·9 %) was obtained with *M. xiligouense* DSM 15700^T. A bootstrap value of 100 confirmed the isolated position of the two strains within the radiation of the *Promicromonosporaceae* (Fig. 1).

For DNA–DNA relatedness studies, DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970), incorporating the modifications described by Huß *et al.* (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with an *in situ* temperature probe (Varian).

Ribotyping was performed using the standard method of the automated ribotyping device RiboPrinter System (DuPont Qualicon) according to the manufacturer's instructions, as described by Bruce (1996). The restriction enzyme used was *Pvu*II (DuPont Qualicon).

The three catacomb isolates shared a 16S rRNA gene similarity of 99·9 %. This close phylogenetic relationship was underlined by the results of the riboprint analysis (Fig. 2). Strains HKI 0369^T and HKI 0372 showed identical riboprint patterns and were affiliated to the same ribogroup. In strain HKI 0371, an additional band occurred that was unique to this strain and which did not occur in the pattern of *M. xiligouense* DSM 15700^T.

Strain HKI 0369^T and the type strain of *M. xiligouense* DSM 15700^T shared a 16S rRNA gene similarity of 98 %, which corresponded to a low level of DNA–DNA similarity [14·4 and 13·3 %, measurement in duplicate; hybridization buffer, 2 × SSC + 10 % (v/v) formamide; hybridization temperature, 68 °C]. These low values together with the pronounced differences in the riboprint patterns between the three strains under study and *M. xiligouense* clearly indicate that the catacomb isolates and *M. xiligouense* DSM 15700^T represent different genospecies.

The following chemotaxonomic characteristics were determined as described previously: the structure of the peptidoglycan (Schleifer & Kandler, 1972; Schleifer, 1985; MacKenzie, 1987; Groth *et al.*, 1996); the muramic acid type (Uchida & Aida, 1984); the whole-cell sugars (Becker *et al.*, 1965; Saddler *et al.*, 1991); the menaquinones (Groth *et al.*, 1996); the polar lipids (Minnikin *et al.*, 1979; Collins & Jones, 1980); and the mycolic acids (Minnikin *et al.*, 1975).

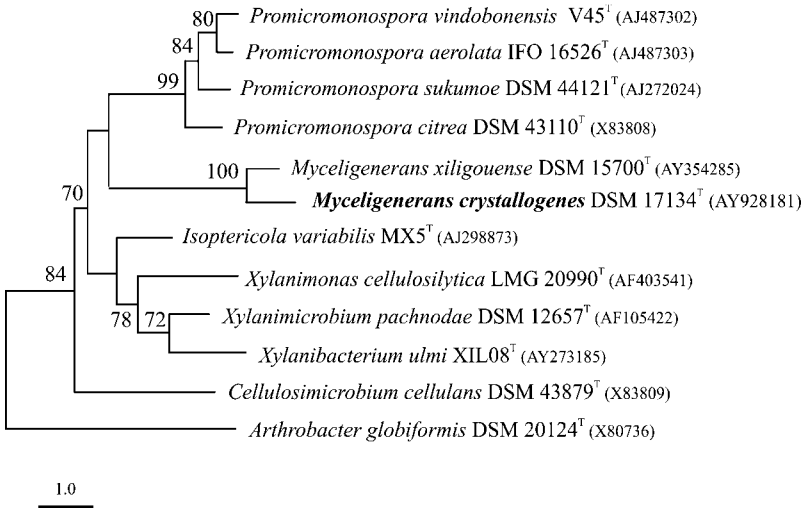

Fig. 1. Phylogenetic relatedness among members of the genera *Myceligenerans*, *Isoptericola*, *Xylanimonas*, *Xylanimicrobium*, *Xylanibacterium*, *Cellulosimicrobium* and *Promicromonospora*, based upon 16S rRNA gene sequence comparison. *Arthrobacter globiformis* DSM 20124^T (X80736) was used as the outgroup. The dendrogram was generated by neighbour-joining analysis (Felsenstein, 1993). Numbers within the dendrogram indicate the percentages of occurrence of the branching order in 500 bootstrapped trees (only values of 50 % and above are shown). Bar, 1 nucleotide substitution per 100 nucleotides.

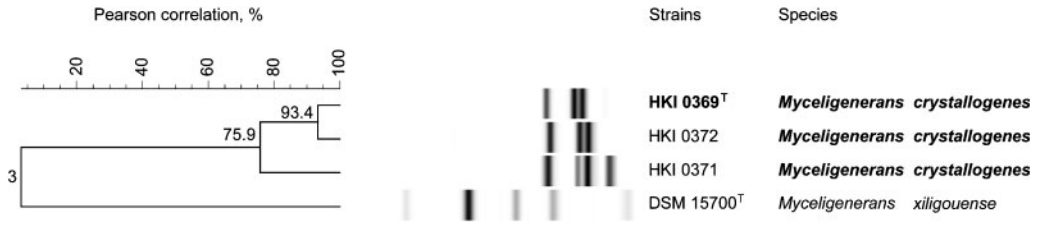

Fig. 2. Riboprint patterns of the three catacomb isolates and *M. xiligouense* DSM 15700^T. The dendrogram was constructed using the Pearson correlation coefficient and UPGMA with the software package BioNumerics (Applied Maths).

The fatty acid profile was determined using the MIDI system (Agilent).

The chemotaxonomic characteristics of the catacomb isolates were consistent with their affiliation to the genus *Myceligenerans*. The peptidoglycan of strain HKI 0369^T contained *N*-acetylated muramic acid and corresponded to type A4α, L-Lys–L-Thr–D-Glu (A11·57 according to http://www.dsmz.de/species/murein.htm). This type has been found in members of the family *Cellulomonadaceae* and from members of the family *Promicromonosporaceae* only in the genus *Myceligenerans*. The organisms under study shared with *M. xiligouense* DSM 15700^T the characteristic sugars analysed in whole-cell hydrolysates, the major phospholipids and the predominant fatty acids [anteiso-$C_{15:0}$ (54·4 mol%), iso-$C_{15:0}$ (18·5 mol%) and iso-$C_{16:0}$ (16·5 mol%)]. The minor fatty acids were iso-$C_{14:0}$ (4·8 mol%) and anteiso-$C_{17:0}$ (3·7 mol%). The polar lipids were identified as diphosphatidylglycerol, phosphatidylglycerol, two unknown phospholipids and three unknown glycolipids. In contrast to the data given by Cui *et al.* (2004), phosphatidylinositol was not detected in preparations of *M. xiligouense* DSM 15700^T or in those of the three isolates. The three strains under study could be differentiated from *M. xiligouense* by the patterns of their menaquinones. Strain HKI 0369^T was characterized by the major menaquinones MK-9(H_8), MK-9(H_4), MK-9(H_6) and MK-9(H_2), with peak areas of 36 : 19 : 16 : 13, and the minor amounts of MK-8(H_4), MK-9 and MK-8(H_2) (peak areas 6 : 3 : 1), while for *M. xiligouense* DSM 15700^T, the major menaquinones were MK-9(H_4) and MK-9(H_6).

The DNA G+C value for strain HKI 0369^T, determined by HPLC according to Groth *et al.* (1996), was 72·3 mol%.

It is evident from the genotypic and phenotypic data that the three strains from the Roman catacombs merit recognition as a novel species in the genus *Myceligenerans*. The name proposed for this new taxon is *Myceligenerans crystallogenes* sp. nov.

Description of *Myceligenerans crystallogenes* sp. nov.

Myceligenerans crystallogenes [crys.tall.o.ge′nes. Gr. n. *krustallos* crystal; Gr. v. *gennao* produce; N.L. neut. adj. *crystallogenes* producing crystals of iodinin (1,6-phenazinediol 5,10-dioxide)].

Gram-positive, aerobic to microaerophilic actinomycete with a well-developed primary mycelium (diameter of hyphae 0·5–0·7 μm) that undergoes fragmentation into short, irregular, non-motile rods and cocci in the stationary growth phase. Aerial mycelium is absent. Spore-like cells occur in the substrate mycelium. Colonies on organic medium 79 are wrinkled, circular, smooth and white to cream (diameter about 1–4 mm). Grows between 10 and 40 °C (optimal growth is at 28 °C) and at pH values in the range pH 6–9. NaCl in the culture medium is well tolerated up to 5 %. Physiological characteristics (utilization of carbohydrates, enzymic activities and susceptibility to antibiotics) are listed in Supplementary Tables S1 and S2 in IJSEM Online. The peptidoglycan type is A4α, L-Lys–L-Thr–D-Glu. The whole-cell sugars are glucose, mannose and galactose. The acyl type is acetyl. The menaquinones are MK-9(H_8), MK-9(H_4), MK-9(H_6), MK-9(H_2) and minor amounts of MK-8(H_4), MK-9 and MK-8(H_2). The predominant fatty acids are anteiso-$C_{15:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$. The phospholipids are diphosphatidylglycerol, phosphatidylglycerol, two unknown phospholipids and three unknown glycolipids. Mycolic acids are absent. The DNA G+C content is 72·0–72·3 mol% (type strain, 72·3 mol%).

The type strain is CD12E2-27^T (=HKI 0369^T=DSM 17134^T=NCIMB 14061^T=VTT E-032285^T), which was isolated from the catacomb of Domitilla in Rome, Italy.

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References

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.

Bruce, J. (1996). Automated system rapidly identifies and characterizes microorganisms in food. *Food Technol* **50**, 77–81.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Cazemier, A. E., Verdoes, J. C., Reubsaet, F. A. G., Hackstein, J. H. P., van der Drift, C. & Op den Camp, H. J. M. (2003). *Promicromonospora pachnodae* sp. nov., a member of the (hemi)-cellulolytic hindgut flora of larvae of the scarab beetle *Pachnoda marginata*. *Antonie Van Leeuwenhoek* **83**, 135–148.

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.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. Cambridge: Cambridge University Press.

Cui, X., Schumann, P., Stackebrandt, E., Kroppenstedt, R. M., Pukall, R., Xu, L., Rohde, M. & Jiang, C. (2004). *Myceligenerans xiligouense* gen. nov., sp. nov., a novel hyphae-forming member of the family *Promicromonosporaceae*. *Int J Syst Evol Microbiol* **54**, 1287–1293.

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. (1993). PHYLIP (phylogeny inference package), version 3.5.1. Distributed by the author, Department of Genome Sciences, University of Washington, Seattle, USA.

Gordon, R. E., Barnett, D. A., Handerhan, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca, Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* **24**, 54–63.

Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.

Groth, I., Schumann, P., Schütze, B., Gonzalez, J. M., Laiz, L., Saiz-Jimenez, C. & Stackebrandt, E. (2005). *Isoptericola hypogeus* sp. nov., isolated from the Roman catacomb of Domitilla. *Int J Syst Evol Microbiol* **55**, 1715–1719.

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.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Krasil'nikov, N. A., Kalakoutskii, L. V. & Kirillova, N. F. (1961). New genus of the *Actinomycetales*: *Promicromonospora* gen. nov. *Izv Akad Nauk SSSR Ser Biol* **1**, 107–112 (in Russian).

Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyryl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Minnikin, D. E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *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.

Prauser, H. & Falta, R. (1968). Phage sensitivity, cell wall composition and taxonomy of actinomycetes. *Z Allg Mikrobiol* **8**, 39–46 (in German).

Rivas, R., Sánchez, M., Trujillo, M. E., Zurdo-Piñeiro, J. L., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2003). *Xylanimonas cellulosilytica* gen. nov., sp. nov., a xylanolytic bacterium isolated from a decayed tree (*Ulmus nigra*). *Int J Syst Evol Microbiol* **53**, 99–103.

Rivas, R., Trujillo, M. E., Schumann, P., Kroppenstedt, R. M., Sánchez, M., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2004). *Xylanibacterium ulmi* gen. nov., sp. nov., a novel xylanolytic member of the family *Promicromonosporaceae*. *Int J Syst Evol Microbiol* **54**, 557–561.

Saddler, G. S., Tavecchia, P., Lociuro, 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.

Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. *Methods Gen Mol Bacteriol* **25**, 607–654.

Stackebrandt, E. & Schumann, P. (2004). Reclassification of *Promicromonospora pachnodae* Cazemier *et al.* 2004 as *Xylanimicrobium pachnodae* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **54**, 1383–1386.

Stackebrandt, E., Schumann, P. & Cui, X.-L. (2004). Reclassification of *Cellulosimicrobium variabile* Bakalidou *et al.* 2002 as *Isoptericola variabilis* gen. nov. comb. nov. *Int J Syst Evol Microbiol* **54**, 685–688.

Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacteria cell walls. *J Gen Appl Microbiol* **30**, 131–134.

Yokota, A., Takeuchi, M., Sakane, T. & Weiss, N. (1993). Proposal of six new species of the genus *Aureobacterium* and transfer of *Flavobacterium esteraromaticum* Omelianski to the genus *Aureobacterium* as *Aureobacterium esteraromaticum* comb. nov. *Int J Syst Bacteriol* **43**, 555–564.