Williamsia marianensis sp. nov., a novel actinomycete isolated from the Mariana Trench

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The taxonomic status of an actinomycete isolated from sediment collected from the Mariana Trench was established using a combination of genotypic and phenotypic data. Isolate MT8^T had chemotaxonomic and morphological properties consistent with its classification in the genus *Williamsia*, and formed a distinct phyletic line in the 16S rRNA gene tree together with the type strain of *Williamsia muralis*. The isolate was readily distinguished from the latter, and from representatives of other *Williamsia* species, using DNA–DNA relatedness and phenotypic criteria. Predominant cellular fatty acids were oleic, palmitic and tuberculostearic acids and a hexadecenoic acid. The DNA G + C content was 65.2 mol%. It is apparent that the isolate belongs to a novel species of *Williamsia*. Strain MT8^T (= DSM 44944^T = NCIMB 14085^T) was thus considered to be the type strain of a novel species in the genus *Williamsia*, for which the name *Williamsia marianensis* sp. nov. is proposed.

The monospecific genus *Williamsia* was proposed by Kämpfer *et al.* (1999) to accommodate an unusual mycolic-acid-containing actinomycete. The taxon currently contains three recognized species, *Williamsia muralis* Kämpfer *et al.* 1999, *Williamsia maris* Stach *et al.* 2004 and *Williamsia deligens* Yassin and Hupfer 2006, species that encompass strains isolated from indoor building materials, deep-sea sediment and human blood, respectively. These species form a distinct 16S rRNA clade within the evolutionary radiation occupied by mycolic-acid-containing actinomycetes, i.e. by organisms classified in the suborder *Corynebacterineae* Stackebrandt *et al.* 1997 (Butler *et al.*, 2005; Soddell *et al.*, 2006).

The present investigation was designed to determine the taxonomic status of an actinobacterial strain, isolate MT8^T, that had been isolated from a deep-sea sediment and was considered to represent a member of the genus *Williamsia* (Pathom-aree *et al.*, 2006). The isolate was the subject of a polyphasic taxonomic study, which showed that it merited recognition within a novel species of the genus *Williamsia*.

Strain MT8^T was isolated from sediment collected from the Mariana Trench in the north-west Pacific Ocean [Challenger Deep (10 898 m): 11° 19' 911" N 142° 12' 372" E] using the remotely operated unmanned submersible *Kaiko* (Kato *et al.*, 1997). The sample (2 ml), which was collected during dive number 74 on 21 May 1998, was transported to the UK in an insulated container at 4 °C and then stored at –20 °C. The isolate was recovered from a suspension of the sediment sample used to inoculate a raffinose–histidine agar plate (Vickers *et al.*, 1984) supplemented with cycloheximide and nystatin. The organism was maintained on glucose–yeast extract agar (Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20 %, v/v) at –20 °C.

The phylogenetic position of isolate MT8^T was determined by 16S rRNA gene sequence analysis. The organism was grown at 28 °C for 7 days in a glucose–yeast extract shake culture (Gordon & Mihm, 1962), and the resultant biomass was harvested by centrifugation and washed twice in distilled water. Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified products of the strain were carried out according to the methods of Kim *et al.* (2000). The almost complete 16S rRNA gene sequence (1443 nt) was aligned manually with corresponding sequences of representatives of the genera classified in the suborder *Corynebacterineae*, retrieved from the DDBJ/EMBL/GenBank databases, using the pairwise alignment option and

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MT8^T is AY894336.

16S rRNA secondary structure information held within the PHYDIT program (available at <http://plaza.snu.ac.kr/~jchun/phydit/>).

Phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices were generated for the least-squares and neighbour-joining methods after Jukes & Cantor (1969) and the resultant unrooted tree topologies were evaluated in a bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings from the neighbour-joining dataset using the SEQBOOT and CONSENSE options from the PHYLIP package. It is evident from Fig. 1 that isolate MT8^T not only falls within the zone of evolutionary radiation occupied by the genus *Williamsia* but forms a distinct phylogenetic line together with the type strain of *W. muralis*, an association underpinned by all of the tree-making algorithms and by a 100% bootstrap value in the neighbour-joining analysis. The two organisms share 99.5% 16S rRNA gene similarity, a value that corresponds to 7 nt differences over the 1450 locations available for alignment.

Isolate MT8^T was the subject of chemotaxonomic analyses designed to determine whether it has chemical properties

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rRNA gene sequences showing relationships between isolate MT8^T and representatives of the sub-order *Corynebacterineae*. Asterisks indicate branches that were also recovered using the least-squares (Fitch & Margoliash, 1967), 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 50% are given. Bar, 0.02 substitutions per nucleotide position.

characteristic of *Williamsia* strains. The required biomass was prepared from glucose-yeast extract broth cultures (Gordon & Mihm, 1962) as described above. Standard procedures were used to extract and to analyse the isomeric forms of diaminopimelic acid (Staneck & Roberts, 1974), fatty acids (Sutcliffe, 2000), mycolic acids (Hamid *et al.*, 1993), isoprenoid quinones (Collins, 1994), muramic acid type (Uchida *et al.*, 1999), polar lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Hasegawa *et al.*, 1983). The organism was characterized by the presence of *meso*-diaminopimelic acid, arabinose and galactose in whole-organism hydrolysates (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), *N*-glycolyl muramic acid residues, dihydrogenated menaquinones with nine isoprene units as the sole isoprenologue, major proportions of straight-chain, unsaturated and tuberculostearic acids (fatty acid type 1b; Kroppenstedt, 1985), phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol as major polar lipids (phospholipid type II *sensu* Lechevalier *et al.*, 1977) and by mycolic acids that comigrated with those of *W. muralis* DSM 44343^T. This chemical profile is consistent with the classification of the isolate in the genus *Williamsia* (Goodfellow & Maldonado, 2006; Yassin & Hupfer, 2006).

The fatty acid profile was dominated by palmitic acid (C_{16:0}, 30% of total), a hexadecenoic acid (C_{16:1}, 22%), oleic acid (C_{18:1}, 15%) and tuberculostearic acid (10-methyl octadecanoate, 32%). This profile is generally consistent with that reported for the type strain of *W. muralis* (Kämpfer *et al.*, 1999), except that the latter organism was reported to contain significant quantities of palmitoleic acid (C_{16:1}:cis9) whereas we detected a fatty acid provisionally identified as a hexadecenoic acid but clearly resolved from a palmitoleic acid standard. In this respect it is notable that *W. muralis* DSM 44343^T was reported to contain a minor amount of C_{16:1}:cis11 (1.4%; Kämpfer *et al.*, 1999). The fatty acid profiles of MT8^T and *W. muralis* DSM 44343^T are thus both notably distinct from that reported for the type strain of *W. deligens* as the latter contained only minor amounts of C_{16:1} and significant quantities (40%) of longer chain (≤C₂₀) saturated and unsaturated fatty acids (Yassin & Hupfer, 2006).

DNA–DNA hybridization experiments were carried out between isolate MT8^T and the type strains of *W. maris* and *W. muralis* using DNA prepared following the methods of Kim *et al.* (1998). The analyses were performed using the microplate method, as described by Ezaki *et al.* (1989). Mean DNA–DNA relatedness values were calculated from triplicate hybridization experiments. The G + C content of the DNA of isolate MT8^T was determined by reversed-phase HPLC (Tamaoka & Komagata, 1984). The mean levels of DNA–DNA relatedness found between isolate MT8^T and *W. maris* DSM 44693^T and *W. muralis* DSM 44343^T were 10 ± 2.8 and 11 ± 0.0%, respectively, values well below the cut-off point recommended for the assignment of bacterial strains to the same genomic species (Wayne *et al.*,

1987). The DNA G+C content of strain MT8^T was 65.2 mol%.

Isolate MT8^T was examined for a range of phenotypic markers using established procedures (Stach *et al.*, 2004). It is evident from Table 1 that it can be differentiated readily from the type strains of the three recognized *Williamsia* species. It is particularly interesting that only isolate MT8^T grows at 4 °C and in the presence of high concentrations of sodium chloride.

It is evident from the genotypic and phenotypic data presented here that isolate MT8^T represents a novel species within the genus *Williamsia*. The name proposed for this taxon is *Williamsia marianensis* sp. nov.

Description of *Williamsia marianensis* sp. nov.

Williamsia marianensis (ma.ri.an.en'sis. N.L. fem. adj. *marianensis* pertaining to the Mariana Trench, the source of the type strain).

Non-acid-alcohol-fast actinomycete that forms short rods and coccoid-like elements, and produces round, entire, convex, orange colonies with smooth matt surfaces on glucose-yeast extract agar after 5 days incubation at 28 °C. Grows well on trypticase soy agar, and between 4 and 30 °C, with an optimum temperature for growth around 28 °C.

Table 1. Phenotypic properties that differentiate strain MT8^T from the type strains of *Williamsia* species

Reference strains: 1, *W. deligens* DSM 44902^T (data from Yassin & Hupfer, 2006); 2, *W. maris* DSM 44693^T (Stach *et al.*, 2004); 3, *W. muralis* DSM 44343^T (Kämpfer *et al.*, 1999).

Characteristic	MT8 ^T	1	2	3
Growth on sole carbon sources at				
1 % (w/v):				
Adonitol	—	—	—	+
L(+)-Arabinose	+	—	—	+
meso-Erythritol	+	—	—	+
D(+)-Galactose	—	—	+	—
m-Hydroxybenzoate	—	—	—	+
p-Hydroxybenzoate	—	—	+	—
myo-Inositol	—	—	+	—
D(+)-Maltose	—	+	+	—
D(+)-Rhamnose	+	—	—	+
D(+)-Trehalose	+	+	+	—
D(+)-Xylose	—	+	+	—
Growth on:				
5 % (w/v) NaCl	+	—	—	—
7 % (w/v) NaCl	+	—	—	—
1 % (v/v) 1,2-Propandiol	—	—	+	—
Growth at:				
4 °C	+	—	—	—
37 °C	—	+	+	+

Neither aesculin nor arbutin are hydrolysed. Casein, cellulose, hypoxanthine, starch and uric acid are degraded, but not gelatin, guanine, Tween 80, L-tyrosine or xanthine. D(+)-Fructose, D(+)-mannitol, D(+)-mannose, sucrose, D(+)-sorbitol and xylitol are used as sole carbon sources for energy and growth, but not L(–)-arabitol, D(+)-cellobiose, dextran, dextrin, D(+)-glycerol, glycogen, D(+)-melezitose, D(+)-raffinose, D(+)-salicin or L(+)-sorbitose. Additional phenotypic properties are given in Table 1. Exhibits chemical markers characteristic of the genus *Williamsia*. The predominant fatty acid components are palmitic and tuberculostearic acids. The G+C content of the DNA is 65.4 mol%.

The type and only strain, MT8^T (=DSM 44944^T=NCIMB 14085^T), was isolated from sediment collected from the Challenger Deep of the Mariana Trench in the north-west Pacific Ocean.

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