Williamsia serinedens sp. nov., isolated from an oil-contaminated soil

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The taxonomic status of a bacterium designated strain IMMIB SR-4^T isolated from an oil-contaminated soil sample was characterized by using a polyphasic approach. Chemotaxonomic investigations revealed the presence of cell-wall chemotype IV, short-chain mycolic acids that co-migrated with those extracted from members of the genus *Williamsia* and that on pyrolysis GC produce C_{16:0} and C_{18:0} fatty acids, and dihydrogenated menaquinone with nine isoprene units as the predominant menaquinone. The generic assignment was confirmed by 16S rRNA gene sequence analysis. Comparative analysis of the 16S rRNA gene sequence showed that strain IMMIB SR-4^T formed a distinct phyletic line within the genus *Williamsia*, displaying sequence similarities of 95.5–98.1 % with the type strains of recognized *Williamsia* species. Strain IMMIB SR-4^T was distinguished from the type strains of recognized species of the genus *Williamsia* based on a set of phenotypic features. The genotypic and phenotypic data indicated that strain IMMIB SR-4^T represents a novel species of the genus *Williamsia*, for which the name *Williamsia serinedens* sp. nov. is proposed. The type strain is IMMIB SR-4^T (= DSM 45037^T = CCUG 53151^T).

The genus *Williamsia* (Kämpfer *et al.*, 1999) as currently defined is a member of the family *Gordoniaceae* (Stackebrandt *et al.*, 1997) and accommodates actinomycetes with atypical cell morphology that are unable to grow at temperatures above 30 °C, and possesses mycolic acids with carbon chain length of C_{50:0}–C_{56:0}. At the time of writing, the genus *Williamsia* comprised four recognized species, *Williamsia deligens* (Yassin & Hupfer, 2006) isolated from human blood, *Williamsia muralis* (Kämpfer *et al.*, 1999) isolated from indoor building material of a children's day-care centre in Finland, *Williamsia maris* (Stach *et al.*, 2004) isolated from deep sediments of the Sea of Japan and *Williamsia marianensis* (Pathom-Aree *et al.*, 2006) isolated from a deep-sea sediment. These species form a distinct clade within the evolutionary radiation occupied by mycolic-acid-containing actinomycetes, i.e. by organisms classified in the suborder *Corynebacterineae* (Stackebrandt *et al.*, 1997). In the present paper we describe an actinobacterial strain, designated IMMIB SR-4^T, which was isolated from an oil-contaminated soil sample. Based on

phylogenetic and phenotypic evidence, it is proposed that this isolate should be classified as representing a novel species of the genus *Williamsia*.

Isolate IMMIB SR-4^T was isolated from a soil sample collected from an oil-contaminated site located in Chyai County, Taiwan. The organism was cultured on Columbia agar supplemented with 5 % sheep blood and brain heart infusion (BHI) agar to determine its morphological characteristics. Air-dried smears were stained via Gram's method in order to determine the Gram reaction and cell morphology. Ziehl-Neelsen's method was used to determine acid-fastness. Growth temperatures were determined by incubating the organism at 22, 28, 30, 37 and 42 °C. The physiological properties of the strain were determined by using tests for hydrolysis of complex substrates as described by Gordon (1966, 1967) and Gordon & Mihm (1957) as well as tests for carbon source utilization according to Yassin *et al.* (1995). The isomeric form of diaminopimelic acid was determined according to the method of Becker *et al.* (1964) and whole-cell sugars were identified by using the method of Lechevalier (1968). Lipids were extracted by using acid methanolysis and mycolic acids were detected with TLC as described by Minnikin *et al.* (1980); pyrolysis GC of the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMMIB SR-4^T is AM283464.

mycolate was performed according to Yassin *et al.* (1993a). Fatty acids were purified, identified and quantified by GC as described by Yassin (1988). Phospholipids were extracted, purified and identified as described by Yassin *et al.* (1993b). Menaquinones were extracted and purified according to Collins *et al.* (1977). Mass spectra of the menaquinones were recorded in positive-ion mode on a Q-TOF 2 mass spectrometer (Micromass) equipped with a nanospray source as described by Yassin & Hupfer (2006).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were carried out according to the procedures of Rainey *et al.* (1996). Purified PCR products were sequenced by using a Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) as described in the manufacturer's protocol. An Applied Biosystems 310 DNA Genetic Analyzer was used for electrophoresis of the sequence reaction products. The 16S rRNA gene sequences of recognized species of the genus *Williamsia* retrieved from GenBank were added to the ARB database (Ludwig *et al.*, 2004) and were aligned by using the respective tool within the ARB package. The resulting alignment was corrected manually and evolutionary trees were inferred using maximum-parsimony (Kluge & Farris, 1969), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. The evolutionary distance matrix was calculated using the correction of Jukes & Cantor (1969). The topology of the resultant tree was evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining data based on 1000 resamplings by using the ARB package.

The almost-complete 16S rRNA gene sequence of strain IMMIB SR-4^T was determined [1479 nt; 95.9 % of the *Escherichia coli* sequence (Brosius *et al.*, 1978)]. 16S rRNA gene sequence comparison clearly indicated that strain IMMIB SR-4^T was a member of the suborder *Corynebacterineae* (Stackebrandt *et al.*, 1997), and the sequence contained all signature nucleotides expected for this suborder as well as those described recently (Yassin & Hupfer, 2006) for the family *Gordoniaceae*. Furthermore, the new isolate contained the signature nucleotides at positions 293–304 (G–C) and 307 (C) shared with other members of

the genus *Williamsia*. However, strain IMMIB SR-4^T differed from recognized *Williamsia* species in that it possessed C–G rather than G–C at positions 1007:1022. Therefore, the current pattern of signature nucleotides for members of the genus *Williamsia* may need to be updated as novel species are described.

The phylogenetic tree constructed (Fig. 1) shows the position of strain IMMIB SR-4^T within the radiation of representative phylogenetic groups of the suborder *Corynebacterineae*. It is evident from the tree that strain IMMIB SR-4^T not only falls within the phylogenetic radiation occupied by the genus *Williamsia* but forms a distinct phyletic line that is loosely associated (bootstrap value of 55 %) with the type strains of *W. maris* and *W. deligens*. Strain IMMIB SR-4^T showed 16S rRNA gene sequence similarities of 97.0 and 98.1 % to *W. maris* SJS0289/JS1^T and *W. deligens* IMMIB RIV-956^T, respectively, and 95.5 and 95.6 % to *W. marianensis* MT8^T and *W. muralis* MA140/96^T, respectively. Therefore, the distinctiveness of strain IMMIB SR-4^T from *W. maris*, *W. marianensis* and *W. muralis* is underpinned by exhibiting ≥3 % sequence divergence (Stackebrandt & Goebel, 1994) from these three species. It is known that the type strains of *W. marianensis* and *W. muralis* share high 16S rRNA gene sequence similarity (99.5 % or 7 nt differences out of 1443 positions) but belong to genomic species with a mean DNA–DNA relatedness value of 11 % (Pathom-Aree *et al.*, 2006). It is therefore evident that the high 16S rRNA gene sequence similarity found between strain IMMIB SR-4^T and the type strain of *W. deligens* (98.1 %, corresponding to 30 nt differences) indicates that the test strain represents a distinct taxonomic entity within the genus *Williamsia*. It is also clear from Table 1 that strain IMMIB SR-4^T can be distinguished readily from the type strain of *W. deligens* based on a set of biochemical properties.

Chemotaxonomically, strain IMMIB SR-4^T possessed chemical markers that supported its assignment to the genus *Williamsia*. The cell wall contained *meso*-diaminopimelic acid as well as arabinose and galactose (i.e. wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970). One-dimensional TLC of the whole-cell acid methanolsate of the

Fig. 1. Neighbour-joining tree showing the position of strain IMMIB SR-4^T within the radiation of mycolic-acid-containing taxa. The tree was based on a comparison of 16S rRNA gene sequences that were at least 90 % complete (with regard to the *E. coli* sequence). Bootstrap values of >50 % (based on 1000 resamplings) are indicated at nodes. Bar, 5.0 % sequence divergence.

organism revealed the presence of two lipid spots on the chromatogram. The lower spot corresponded to mycolic acids as identified by its R_f value (0.55) and the higher spot corresponded to non-hydroxylated fatty acids. Pyrolysis GC of the purified mycolic acid methyl esters from strain IMMIB SR-4^T released fatty acid methyl esters of C_{16:0} (55.0 % of the total cleavage products) and C_{18:0} (45.0 %) as pyrolysis cleavage products. GC analyses of the non-hydroxylated fatty acid methyl esters revealed the presence of tetradecanoate (1.7 % of the total fatty acids), pentadecanoate (0.15 %), *cis*-hexadecenoate (0.35 %), hexadecanoate (15.6 %), 10-methyl hexadecanoate (0.52 %), heptadecanoate (0.16 %), octadecenoate (4.9 %), octadecanoate (7.4 %), tuberculostearic acid (10-methyl octadecanoate; 17.1 %), eicosenoate (1.2 %), eicosanoate (0.57 %), docosanoate (15.8 %), tetracosenoate (1.3 %) and tetracosanoate (34.3 %) as the major cellular fatty acid methyl esters. Polar lipid analysis showed that the organism contained phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol and diphosphatidylglycerol as characteristic phospholipids (i.e. phospholipid type PII *sensu* Lechevalier *et al.*, 1977). Mass spectral analysis of the main respiratory quinone components from strain IMMIB SR-4^T revealed a strong peak at m/z 809.5 attributable to $[M+Na]^+$ in the high-mass region. This corresponds to a dihydrogenated menaquinone with nine isoprene units, MK-9(H₂). The second band revealed a strong peak at m/z 741.58 attributable to $[M+Na]^+$ in the high-mass region. This corresponds to a dihydrogenated menaquinone with eight isoprene units, MK-8(H₂).

Strain IMMIB SR-4^T was examined for a range of phenotypic markers. The organism showed morphological properties consistent with its assignment to the genus *Williamsia*. It was aerobic, and built smooth, orange- to orange-red-pigmented colonies on BHI agar and Columbia agar supplemented with 5 % sheep blood. Cells were Gram-positive rods and were non-acid-alcohol-fast. The organism was able to grow at 22 and 30 °C but not at higher temperatures. It is evident from Table 1 that strain IMMIB SR-4^T can be readily differentiated from the type strains of the four recognized *Williamsia* species. It is of particular note that only isolate IMMIB SR-4^T was able to hydrolyse gelatin and to utilize acetamide and serine as simultaneous nitrogen and carbon sources for energy. The biochemical properties of strain IMMIB SR-4^T are given in the species description below. It is apparent from the genotypic and phenotypic data presented that strain IMMIB SR-4^T represents a novel species of the genus *Williamsia*, for which the name *Williamsia serinedens* sp. nov. is proposed.

Description of *Williamsia serinedens* sp. nov.

Williamsia serinedens (se.ri.ne'dens. N.L. n. *serinum* serine; L. part. pres. *edens* eating; N.L. part. adj. *serinedens* eating serine).

Forms smooth, orange- to orange-red-pigmented colonies on agar media. Cells are Gram-positive rods and are

Table 1. Differential physiological characteristics between strain IMMIB SR-4^T and type strains of *Williamsia* species

Strains: strain IMMIB SR-4^T; 2, *W. deligens* DSM 44902^T; 3, *W. marianensis* DSM 44944^T; 4, *W. maris* DSM 44693^T; 5, *W. muralis* DSM 44343^T. Data were obtained in this study. All strains were positive for utilization of acetate, citrate, glucose, mannitol, paraffin and urea and negative for utilization of adipic acid, cellobiose, isoamyl alcohol, lactose, melezitose, ornithine, proline and raffinose and hydrolysis of adenine, casein, elastin, aesculin, guanine, hypoxanthine, tyrosine and xanthine. –, Negative; +, positive; W, utilized weakly after 3 weeks incubation.

Characteristic	1	2	3	4	5
Hydrolysis of:					
Gelatin	+	–	–	–	–
Testosterone	–	–	+	–	+
Utilization as sole sources of carbon and energy:					
Adonitol	+	–	+	–	+
L-Arabinose	+	–	+	–	+
2,3-Butandiol	+	+	+	–	+
<i>meso</i> -Erythritol	+	–	+	–	+
Galactose	+	–	–	+	–
Gluconate	–	–	+	–	+
Lactate	+	–	–	+	+
<i>myo</i> -Inositol	–	–	–	+	–
Maltose	+	+	–	+	–
Rhamnose	–	–	+	–	+
D-Sorbitol	+	+	+	–	+
Sucrose	+	+	+	–	+
Trehalose	+	+	+	–	+
1,2-Propandiol	+	–	–	+	+
<i>m</i> -Hydroxybenzoate	–	–	–	–	+
<i>p</i> -Hydroxybenzoate	+	–	–	+	+
D-Xylose	+	+	+	–	+
Utilization as sole sources of carbon and nitrogen:					
Acetamide	+	W	–	–	–
L-Alanine	+	+	+	–	+
Serine	+	–	–	–	–

non-acid-alcohol-fast. Grows at 22–30 °C, but not at higher temperatures. Contains the salient chemotaxonomic characteristics of the genus *Williamsia*. Its mycolic acids were cleaved on pyrolysis to release C_{16:0} and C_{18:0} fatty acids as the major products. The fatty acid profile consists mainly of straight-chain saturated, unsaturated and 10-methyl-branched fatty acids. Hydrolyses gelatin and urea but not adenine, casein, elastin, aesculin, guanine, hypoxanthine, testosterone, tyrosine or xanthine. Assimilates acetate, adonitol, L-arabinose, 2,3-butandiol, citrate, *meso*-erythritol, galactose, glucose, *p*-hydroxybenzoate, lactate, maltose, mannitol, paraffin, 1,2-propandiol, sucrose, sorbitol, trehalose and xylose as carbon sources, but not adipate, isoamyl alcohol, cellobiose, gluconate, *m*-hydroxybenzoate, *myo*-inositol, lactose, melezitose, raffinose or rhamnose.

Utilizes acetamide, L-alanine and serine as simultaneous carbon and nitrogen sources, but not arginine, gelatin, ornithine or proline.

The type strain, IMMIB SR-4^T (=DSM 45037^T=CCUG 53151^T), was isolated from an oil-contaminated soil sample in Chyai County, Taiwan.

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