Desmospora activa gen. nov., sp. nov., a thermoactinomycete isolated from sputum of a patient with suspected pulmonary tuberculosis, and emended description of the family Thermoactinomycetaceae Matsuo et al. 2006

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A novel Gram-positive, aerobic, catalase-positive, filamentous micro-organism, designated strain IMMIB L-1269T, originating from sputum was characterized using phenotypic and molecular taxonomic methods. It showed cell-wall chemotype III, phospholipid type PII (with phosphatidylethanolamine as the diagnostic phospholipid) and contained an unsaturated menaquinone with seven isoprene units (MK-7) as the predominant isoprenoid quinone. It synthesized long-chain cellular fatty acids of the straight-chain saturated, monounsaturated and iso- and anteiso-branched types (with iso-C15:0, C16:0 and iso-C17:0 predominating) and possessed a DNA G+C content of 49.3 mol%. On the basis of its morphological, biochemical and chemical characteristics, strain IMMIB L-1269T did not conform to any presently recognized taxon. Comparative analyses based on 16S rRNA gene sequences confirmed the distinctiveness of the isolate, as it displayed sequence-divergence values greater than 7.7 % with respect to recognized Gram-positive taxa. Phylogenetic treeing analysis served to reinforce the view that strain IMMIB L-1269T was distinct from recognized taxa, as it formed a relatively long subline branching within a 16S rRNA gene sequence cluster that encompassed the genera Thermoactinomyces, Laceyella, Mechercharimyces, Thermoflavimicrobium, Planifilum, Seinonella and Shimazuella of the family Thermoactinomycetaceae. On the basis of phenotypic and molecular phylogenetic evidence, strain IMMIB L-1269T represents a novel genus and species, for which the name Desmospora activa gen. nov., sp. nov. is proposed. The type strain of Desmospora activa is strain IMMIB L-1269T (=DSM 45169T =CCUG 55916T). An emended description of the family Thermoactinomycetaceae is also given.

The family Thermoactinomycetaceae Matsuo et al. 2006 was suggested to accommodate the genera Thermoactinomyces, Laceyella, Seinonella, Thermoflavimicrobium, Planifilum and Mechercharimyces. Members of these genera are characterized by the formation of single, sessile spores on the aerial and substrate hyphae, or chains of spores on simple or branched sporophores. The spores are like bacterial endospores in terms of their structure and properties (Tsilinsky, 1899; Lacey & Cross, 1989; Hatayama et al., 2005; Yoon et al., 2005). Members of the family Thermoactinomycetaceae are aerobic, Gram-positive, non-acid-fast and chemo-organotrophic. Most of the species are thermophilic, growing at 30–60 °C. Only four of the species, Seinonella peptonophila, Mechercharimyces mesophilus, Mechercharimyces asporophorgenus and Shimazuella kribbensis, are mesophilic, growing only below 45 °C (Nonomura & Ohara, 1971; Matsuo et al., 2006). Members of the Thermoactinomycetaceae are found widely in nature. They have been isolated from mouldy hay, cereal...
Strain IMMIB L-1269T was isolated from sputa from a patient with suspected pulmonary tuberculosis, using Columbia agar supplemented with 5% sheep blood. The organism was grown on yeast extract-malt extract agar (ISP medium 2), oatmeal agar (ISP medium 3) and inorganic salts-starch agar (ISP medium 4), as described by Shirling & Gottlieb (1966), and was examined for pigmentation, aerial mycelium and other morphological characteristics. Air-dried smears from Columbia agar were stained using the Gram and Ziehl–Neelsen methods to determine the Gram staining reaction and acid-fastness, respectively. The micromorphology of the organism was determined by using a culture grown on ISP medium 2 at 42 °C for 1, 2 and 4 days. Electron micrographs were taken with a Zeiss digital scanning electron microscope (model DSM 950). Growth temperatures were determined by incubating the organism at 30, 37, 47 and 55 °C. Physiological properties of strain IMMIB L-1269T were determined by using tests to assess the hydrolysis of complex substrates, as described previously (Gordon, 1966, 1967; Gordon & Mihm, 1957), as well as tests for carbon-source utilization (performed according to Yassin et al., 1995). Peptide-extract-iron agar (ISP medium 6) and tyrosine agar (ISP medium 7) (Shirling & Gottlieb, 1966) were used to investigate melanoid pigment production. Resistance to antibiotics was investigated by using the method of Bauer et al. (1966). The isomeric form of the diaminopimelic acid was determined by using the methods of Becker et al. (1964) and whole-cell sugars were determined by using the method of Lechevalier (1968). The acyl type of the muramic acid was determined by using the colorimetric method (Uchida & Aida, 1977). Lipids were extracted using acid methanolysis and mycolic acids were detected with TLC as described by Minnikin et al. (1980). Non-glycerol hydroxylated fatty acids were analysed as described recently by Yassin et al. (2007). Polar lipids were extracted, purified and identified using two dimensional TLC as described previously by Yassin et al. (1993). Menaquinones were extracted and purified according to Collins et al. (1977). Mass spectral analyses of the menaquinones were recorded as described by Yassin & Hupfer (2006) in positive-ion mode on a Q-TOF 2 mass spectrometer (Micromass) equipped with a nanospray source. The DNA G+C content (mol%) was determined by using HPLC according to Mesbah et al. (1989).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were carried out using procedures described previously (Rainey et al., 1996). The purified PCR products were sequenced using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) as described in the manufacturer’s protocol. A genetic analyser (310; Applied Biosystems) was used for electrophoresis of the sequencing reaction products. The 16S rRNA gene sequences of isolate IMMIB L-1269T and recognized members of the genera of the family Thermoactinomycetaceae (obtained from GenBank) were added to the ARB database (Ludwig et al., 2004) and aligned using the appropriate tool from the ARB package. The resulting alignment was corrected manually and evolutionary trees were inferred using maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. The evolutionary distance matrix for the neighbour-joining method was calculated using the correction of Jukes & Cantor (1969). The topology of the neighbour-joining tree was evaluated using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Cultures of strain IMMIB L-1269T produced abundant growth on Columbia agar and ISP media 2, 3 and 4. On agar media, a branched, non-fragmenting, vegetative mycelium formed leathery colonies usually covered with non-fragmenting aerial mycelium. The production of aerial mycelium was abundant on Columbia agar and ISP medium 2, but was sparse on ISP media 3 and 4. Aerial mycelia were long, moderately branched, straight or flexuous (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online) and were yellow in colour. Upon microscopic examination, single endospores on unbranched sporophores were seen on both substrate and aerial mycelia (Fig. 1). In electron micrographs, chains of arthrospores were seen on the tips of aerial mycelia (Fig. 2 and Supplementary Fig. S2). A zigzag arrangement of developing arthrospores was present on the aerial mycelium. No diffusible pigments were produced on ISP media 2, 3 or 4. Melanoid pigments were not produced on ISP medium 6 or 7. The mycelia of strain IMMIB L-1269T stained Gram-positive and were non-acid-fast. The organism grew at temperatures between 37 and 50 °C and was aerobic, catalase-positive and chemo-organotrophic. A battery of phenotypic and biochemical tests were performed on strain IMMIB L-1269T, the results of which are shown in the genus and species descriptions and in Table 1.

Cell-wall analysis of strain IMMIB L-1269T revealed the presence of meso-diaminopimelic acid and the absence of any characteristic sugars in whole-cell wall hydrolysates, indicating that the cells possessed wall chemotype III sensu Lechevalier & Lechevalier (1970). The muramic acid residues of the peptidoglycan were N-glycolylated. Mycolic
Acids were not detected in whole-cell acid methanolysates. Analysis of the non-hydroxylated long-chain cellular fatty acids of strain IMMIB L-1269<sup>T</sup> revealed the presence of straight-chain saturated fatty acids C<sub>14:0</sub> (7.17% of total fatty acids), C<sub>15:0</sub> (0.76%), C<sub>16:0</sub> (14.48%) and C<sub>18:0</sub> (0.20%), branched-chain fatty acids iso-C<sub>13:0</sub> (1.14%), anteiso-C<sub>13:0</sub> (0.20%), iso-C<sub>14:0</sub> (2.94%), iso-C<sub>15:0</sub> (41.35%), iso-C<sub>16:0</sub> (4.67%), iso-C<sub>17:0</sub> (12.48%) and anteiso-C<sub>17:0</sub> (1.41%) and monounsaturated fatty acids C<sub>16:1ω7c</sub> (8.51%), iso-C<sub>17:1ω11c</sub> (2.48%) and C<sub>18:1ω9c</sub> (0.22%). The phospholipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylmonomethylethanolamine (phospholipid type PII; Lechevalier et al., 1977) and two additional unknown phospholipids (Supplementary Fig. S3). Mass spectral analysis showed that the predominant respiratory quinone was MK-7. The DNA G+C content was 49.3 mol%.

To ascertain the phylogenetic relationships of strain IMMIB L-1269<sup>T</sup>, its 16S rRNA gene was sequenced and subjected to a comparative analysis. The almost-complete gene sequence (1495 nt) was determined. Comparative 16S rRNA gene sequence analysis revealed that strain IMMIB L-1269<sup>T</sup> represented an unknown taxon. The results of a phylogenetic analysis using neighbour-joining, maximum-likelihood and maximum-parsimony methods showed that strain IMMIB L-1269<sup>T</sup> formed a distinct subline within the *Thermoactinomyces–Laceyella–Mechercharimyces–Thermo flavimicrobium–Planifilum–Seinonella–Shimazuella* cluster of the family *Thermoactinomycetaceae* (Fig. 3). Of the aforementioned taxa, strain IMMIB L-1269<sup>T</sup> displayed the highest sequence similarity with respect to the type strains of *Thermoflavimicrobium dichotomicum* (92.3%), *M. asporophagenens* (92.3%), *M. mesophilus* (92.1%), *Laceyella sacchari* (92.2%), *Laceyella putida* (91.9%), *Thermoactinomyces vulgaris* (91.9%), *Thermoactinomyces intermedius* (91.7%), *Planifilum* species (91.9% or less), *Shimazuella kribbensis* (90.4%) and *Seinonella peptonophila* (89.0%). The association of strain IMMIB L-1269<sup>T</sup> with members of the genus *Planifilum* was supported by a bootstrap resampling value of 73% (Fig. 3). The relatively high divergence values (>7.7%), together with the deep branching position of strain IMMIB L-1269<sup>T</sup>, showed that the organism was only distantly related to these taxa and merited classification at a similar taxonomic rank (i.e. genus).

It should also be noted that examination of the nucleotide signatures present in the 16S rRNA gene sequence revealed
Table 1. Differential phenotypic and molecular genetic characteristics of isolate IMMIB L-1269\textsuperscript{T} and the genera of the family Thermoactinomycetaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<th>4</th>
<th>5</th>
<th>6</th>
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<th>8</th>
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<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>Yellow</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Yellow</td>
<td>ND</td>
<td>White</td>
<td>White</td>
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<td>Degradation of:</td>
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<td>Casein</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Aesculin</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>ND</td>
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<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
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<td>–</td>
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<td>Hypoxanthine</td>
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<td>Xanthine</td>
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<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>Optimal temperature for growth ((^\circ\text{C}))</td>
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<tr>
<td>Major menaquinone</td>
<td>MK-7</td>
<td>MK-9</td>
<td>MK-7</td>
<td>MK-9</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-9</td>
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<tr>
<td>Major fatty acids</td>
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<tr>
<td>DNA G+C content ((\text{mol}%))</td>
<td>49.3</td>
<td>45.0</td>
<td>48.0</td>
<td>48.0–49.0</td>
<td>43.0</td>
<td>58.7–60.3</td>
<td>40.0</td>
<td>39.4</td>
</tr>
</tbody>
</table>

16S rRNA gene signature nucleotides at positions:

370 : 391 G–C 371 : 390 U–A

Fig. 3. Neighbour-joining phylogenetic tree showing the position of strain IMMIB L-1269\textsuperscript{T} within the radiation of species of the family Thermoactinomycetaceae. The tree was based on a comparison of 16S rRNA gene sequences that were at least 90 % complete with regard to the \textit{Escherichia coli} sequence (GenBank accession no. J01695). Numbers at nodes indicate bootstrap percentages (based on neighbour-joining analyses of 1000 resampled datasets); filled circles indicate that the corresponding nodes (groupings) were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 2 % sequence divergence.
Support for the distinctiveness of strain IMMIB L-1269T is also evident from phenotypic considerations, as the strain could be readily distinguished from all currently described members of the family *Thermoactinomycetaceae* (Table 1). In particular, it differed from the genera *Mechechariomycetes*, *Laceyella* and *Shimazuela* in terms of growth temperature, mycelium colour and by the fact that it possesses MK-7 as the major menaquinone. Similarly, strain IMMIB L-1269T differed from members of *Thermoactinomyces*, *Thermoflavimicrobium* and *Planifilum* by its inability to grow above 50°C. Members of *Thermoflavimicrobium* differed further from strain IMMIB L-1269T by the ability to hydrolyse hypoxanthine and xanthine. In addition, unlike strain IMMIB L-1269T, members of the genus *Seinonella* are not able to degrade casein or hydrolyse gelatin. Therefore, on the basis of the sequence divergence values (>7.7%) for IMMIB L-1269T with respect to its closest named phylogenetic relatives, its distinct and deep subline and its distinctive phenotypic characteristics, the isolate merits assignment to a novel genus and species, for which the name *Desmospora activa* gen. nov., sp. nov. is proposed.

**Description of Desmospora gen. nov.**

*Desmospora* (Des.mo.spo’ra. Gr. n. desmos chain; Gr. fem. n. spora seed; N.L. fem. n. Desmospora spore chain).

Cells are Gram-positive, non-acid-fast, aerobic, catalase-positive and chemo-organotrophic. Non-fragmentary vegetative mycelium forms leathery colonies that are covered with aerial mycelium. Aerial mycelia are long, moderately branched, straight or flexuous. On agar media, aerial mycelium is yellow in colour. Aerial mycelium bears both long chains of arthrospores and sessile endospores that are formed singly on simple unbranched sporophores. Motile elements are not produced. Growth at 37–50°C. The peptidoglycan contains *meso*-diaminopimelic acid; no characteristic sugars are detected in whole cell hydrolysates (i.e. the cell wall is of chemotype III sensu Lechevalier & Lechevalier, 1970). The predominant respiratory menaquinone is MK-7. The phospholipid pattern consists predominantly of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositolphosphatidylethanolamine (phospholipid pattern type PII sensu Lechevalier et al., 1977) and two additional unknown phospholipids. The major cellular fatty acids are iso-C₁₅ : 0, iso-C₁₇ : 0 and C₁₆ : 0. The muramic acid residues of the peptidoglycan are N-glycated. The DNA G + C content of the type strain of the type species is 49.3 mol%. The type species is *Desmospora activa*.

**Description of Desmospora activa sp. nov.**

*Desmospora activa* (acti’va. L. fem. adj. activa active, referring to the metabolic activity of the type strain).

Displays the following properties in addition to those given in the genus description. Thermotolerant. Colonies are yellow with radial wrinkles. No diffusable pigments are produced on ISP medium 2, 3 or 4. Melanoid pigments are not formed in ISP medium 6 or 7. Casein, elastin, aesculin, gelatin and urea are hydrolysed; adenine, guanine, hypoxanthine, keratin, testosterone, tyrosine and xanthine are not hydrolysed. Assimilates acetate, isomyl alcohol, 2,3-butanediol, citrate, D-galactose, D-glucose, D-glucuronate, *myo*-inositol, L-lactate, D-lactose, 1,2-propanediol, D-sorbitol, sucrose, trehalose and D-xylose as carbon sources, but not adipate, adonitol, L-arabinose, cellobiose, meso-erythritol, *m*-hydroxybenzoate, *p*-hydroxybenzoate, maltose, *d*-mannitol, melezitose, raffinose or L-rhamnose. Acetamide, L-alanine, arginine, gelatin, L-proline and L-serine are utilized as simultaneous sources of carbon and nitrogen, but ornithine is not. Susceptible to the following antibiotics (μg): amikacin (30), ciprofloxacin (5), erythromycin (15), fusidic acid (10), gentamicin (10), imipenem (10), levofloxacin (5), linezolid (30), meropenem (10), teicoplanin (30) and vancomycin (30). Resistant to the following antibiotics (μg): ampicillin (10), ampicillin–sulbactam (30), aztreonam (30), cefazolin (30), cefotaxime (30), cefoxitin (30), cefpodoxime (10), ceftazidime (30), ceftriaxone (30), clindamycin (10), fosfomycin (30), mezlocillin (30), netilmicin (10), oxacillin (5), penicillin (10), piperacillin–tazobactam (110) and trimethoprim–sulfamethoxazole (25).

The type strain, IMMIB L-1269T (=DSM 45169T =CCUG 55916T), was isolated from sputa from a patient with suspected pulmonary tuberculosis.

**Emended description of Thermoactinomycetaceae Matsuo et al. 2006**

*Thermoactinomycetaceae* (Ther.mo.ac.ti.no.my.cae.ta ’ce.ae. N.L. masc. n. *Thermoactinomyces* type genus of the family; -aece ending to denote a family; N.L. fem. pl. n. *Thermoactinomycetaceae* the *Thermoactinomyces* family). The description of the family *Thermoactinomycetaceae* is as given by Matsuo et al. (2006), with the following amendment. The pattern of 16S rRNA gene signatures consists of C–G at positions 415:428, C–G at 441:493, C–G at 681:709, G–C at 682:708 and G at 694.

**Acknowledgements**

We thank Professor Dr Hans-Georg Trüper for nomenclatural advice.

**References**

