Williamsia faeni sp. nov., an actinomycete isolated from a hay meadow

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The taxonomic status of an actinomycete isolated from soil collected from a hay meadow was determined using a polyphasic approach. The strain, designated N1350T, had morphological and chemotaxonomic properties consistent with its classification in the genus Williamsia and formed a distinct phyletic line within the clade comprising the type strains of species of the genus Williamsia in the 16S rRNA gene tree. Strain N1350T shared highest 16S rRNA gene sequence similarities with Williamsia marianensis MTB1 (98.1 %) and Williamsia muralis MA140-96T (98.3 %). However, strain N1350T was readily distinguished from the type strains of Williamsia species using a combination of phenotypic properties. On the basis of these data, strain N1350T is considered to represent a novel species of the genus Williamsia. The name proposed for this taxon is Williamsia faeni sp. nov., with the type strain N1350T (=DSM 45372T =NCIMB 14575T =NRRL B-24794T).

The genus Williamsia (Kämpfer et al., 1999) has been classified in the family Nocardiaceae, together with the genera Gordonia, Millisia, Nocardia, Rhodococcus and Skermania (Zhi et al., 2009). At the time of writing, the genus encompasses five described species: Williamsia muralis (Kämpfer et al., 1999), the type species, isolated from indoor building material of a children’s day centre in Finland, Williamsia deligens (Yassin & Hupfer, 2006) from human blood, Williamsia marianensis (Pathom-Aree et al., 2006) from sediment taken from the Mariana Trench in the Pacific Ocean, Williamsia maris (Stach et al., 2004) from sediment collected from the Sea of Japan and Williamsia serinedens (Yassin et al., 2007) from an oil-contaminated soil. The type strains of these species form a clade within the evolutionary radiation occupied by the suborder Corynebacterineae (Stackebrandt et al., 1997; Zhi et al., 2009). The genus Williamsia can also be distinguished from the other mycolic-acid-containing genera using a combination of chemotaxonomic and morphological properties (Soddell et al., 2006; Adachi et al., 2007). Similarly, species of the genus Williamsia can be distinguished from each other by using a range of phenotypic properties (Yassin et al., 2007). The aim of the present study was to determine the taxonomic position of an actinomycete, strain N1350T, that had been recovered from a hay meadow soil and provisionally assigned to the genus Williamsia. Strain N1350T was isolated from a soil suspension inoculum on Gauze’s medium 2 supplemented with (μg ml⁻¹) cycloheximide (50), nalidixic acid (10), novobiocin (10) and nystatin (50) after incubation at 30 °C for 21 days (Tan et al., 2006). The soil sample had been collected from underneath the surface of Palace Leas meadow hay plot 6 (Atalan et al., 2000) at Cockle Park Experimental Farm, Northumberland, UK (national grid reference NZ 200913). Strain N1350T was maintained on glucose-yeast extract agar (GYEA; Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20 %, v/v) at −20 °C. Biomass for the chemotaxonomic and 16S rRNA gene sequence analyses was grown in shake flasks of GYE broth for 5 days at 28 °C, checked for purity and harvested by centrifugation. Cells for the chemosystematic studies were washed twice in distilled water and freeze-dried; cells for the 16S rRNA study were washed in NaCl/EDTA buffer (0.1 M NaCl, 0.1 M EDTA, pH 8.0) and stored at −20 °C until required.

The phylogenetic position of strain N1350T was determined in a 16S rRNA gene sequence analysis. Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified products were carried out as described by Kim et al. (1998). The almost-complete 16S rRNA gene sequence (1441 nt) was aligned manually with corresponding sequences of representatives of genera classified in the suborder Corynebacterineae retrieved from the DDBJ/EMBL/GenBank databases using the pairwise alignment option and 16S rRNA secondary structural information held in the PHYDIT program (available at http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Saitou & Nei, 1987) methods

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N1350T is DQ157929.

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and maximum-likelihood (Felsenstein, 1981) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993) and evolution distance matrices were prepared according to Jukes & Cantor (1969). The resulting unrooted tree topologies were evaluated in a bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset using the CONSENSE and SEQBOOT options from the PHYLIP package.

The 16S rRNA gene phylogenetic tree is shown in Fig. 1. Strain N1350T fell within the 16S rRNA gene clade for the genus Williamsia. This association was supported by all of the tree-making algorithms and by a 100 % bootstrap value in the neighbour-joining analysis. Strain N1350T shared highest 16S rRNA gene sequence similarities with Williamsia muralis MA140-96T (98.3 %, corresponding to 24 nucleotide differences across 1416 locations) and Williamsia marianensis MT8T (98.1 %). DNA–DNA hybridization studies were not performed between these strains as Williamsia marianensis MT8T and Williamsia muralis MA140-96T, which formed a subclade with strain N1350T, share a higher 16S rRNA sequence similarity (99.5 %) with each other but a DNA–DNA relatedness value of only 11 % (Pathom-Aree et al., 2006), which is well below the 70 % cut-off point recommended for the delineation of bacterial species (Wayne et al., 1987).

Strain N1350T was examined for key chemotaxonomic markers that are characteristic for the genus Williamsia. Standard procedures were used to determine the diagnostic isomers of diaminopimelic acid (Staneck & Roberts, 1974), fatty acids (Sutcliffe, 2000), isoprenoid quinones (Collins, 1994), muramic acid type (Uchida et al., 1999), mycolic acids (Minnikin et al., 1975), polar lipids (Minnikin et al., 1984) and whole-organism sugars (Hasegawa et al., 1983). Strain N1350T contained meso-diaminopimelic acid, arabinose and galactose in whole-organism hydrolysates (Wall chomotype IV sensu Lechevalier & Lechevalier, 1970), N-glycolyl muramic acid and dihydrogenated menaquinones with nine isoprene units as the sole isoprenologue. The fatty acid profile included major proportions of straight-chain saturated, unsaturated and tuberculostearic acids [fatty acid type 1b sensu Kropinnenstedt, 1985; hexadecanoo (C16 : 0, 21 % of total), monosaturated octadecanoic (C18 : 1, 15 %), tridecanoic (C13 : 0, 11 %), tuberculostearic (10-methyl C18 : 0, 8 %) and octadecanoic (C18 : 0, 7 %) acids] and minor proportions of tetradecanoic (C14 : 0), pentadecanoic (C15 : 0), iso-hexadecanoic (iso-C16 : 0) and eicosanoic (C20 : 0) acids. Phosphatidylethanolamine, phosphatidyleglycerol, diphosphatidyleglycerol and phosphatidylinositol were the major polar lipids. Strain N1350T contained mycolic acids that co-migrated with those from Williamsia muralis DSM 44343T. This chemotaxonomic profile is consistent with the classification of strain N1350T in the genus Williamsia (Goodfellow & Maldonado, 2006; Yassin & Hupfer, 2006).

Strain N1350T was examined for a range of phenotypic properties using a range of media and methods known to yield data of value for the classification and identification of mycolic-acid-containing actinomycetes (Jones et al., 2008). Strain N1350T was aerobic, Gram-staining-positive, non-acid–alcohol-fast, asporogenous and catalase-positive and used a diverse range of compounds as sole carbon sources. These properties were in line with the classification of strain N1350T in the genus Williamsia (Kämpfer et al., 1999; Yassin et al., 2007). Strain N1350T could be readily distinguished from the type strains of Williamsia species using a combination of phenotypic properties (Table 1). Strain N1350T could also be distinguished from Williamsia faeni sp. nov.
Table 1. Phenotypic properties that distinguish strain N1350T from the type strains of Williamsia species

<table>
<thead>
<tr>
<th>Strains: 1, Williamsia faeni sp. nov. N1350T; 2, W. marianensis DSM 44944T (data from Pathom-Aree et al., 2006); 3, W. muralis DSM 44343T; 4, W. deligens DSM 44902T; 5, W. maris DSM 44693T; 6, W. serinedens DSM 45037T. Data were obtained in this study unless indicated. +, Positive; −, negative; ND, not determined.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>+</td>
<td>+</td>
<td>−</td>
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<td>−</td>
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<tr>
<td>10</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>−</td>
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<td>+</td>
<td>+</td>
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<td>45</td>
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<td>−</td>
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<td>Adonitol</td>
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<td>(−)-L-Arabinose</td>
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<td>−</td>
<td>−</td>
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<td>(+)-Cellobiosi</td>
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<tr>
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<td>+</td>
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<tr>
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<tr>
<td>α-L-Rhamnose</td>
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<td>+</td>
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<tr>
<td>Growth with (0.1 %, w/v):</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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</tr>
<tr>
<td>Growth with (1 %, v/v):</td>
<td>1,2-Propanediol</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

Williamsia faeni sp. nov.

Williamsia faeni (fae'ni. L. n. faenum hay; L. gen. n. faeni of hay, referring to the isolation of the type strain from a hay meadow).

Forms coccoid elements. Produces irregular, convex, matt yellow–pink-pigmented colonies on GYEA after incubation for 5 days at 28 °C. Grows at 10–30 °C, but not at 37 °C. Exhibits chemotaxonomic markers characteristic of the genus Williamsia. Hydrolyses allantoin and urea, but not arbutin. Degrades DNA, RNA, starch and uric acid, but not adenine, chitin, elastin, xanthine or xylan. Uses (−)-D-amylglucoside, (−)-D-arabinose, (−)-D-arabitol, arbutin, (−)-D-fructose, (−)-D-fucose, (−)-D-glucose, inulin, (−)-lactose, (−)-D-mannose, (−)-melibiose, methyl 2-D-glucoside, (−)-D-ribose, (−)-turhanose (all at 1 %, w/v), butan-1,3-diol, butan-1,4-diol, butan-1-ol, butan-2,3-diol, ethanol, propan-1-ol, propan-2-ol (all at 1 %, w/v), 3-methyl-1-butanol (isooamyl alcohol), benzoic acid, fumaric acid, glyceral, glycogen, (−)-L-lactic acid, L-malic acid, oleic acid, propanoic acid, pyruvic acid, sodium acetate, sodium n-butyrate, (−)-L-tartaric acid, valeric acid and xylitol (all at 0.1 %, w/v) as sole carbon sources for energy and growth, but not dulcitol, salicin (all at 1 %, w/v), adipic acid, citric acid, glutaric acid, malonic acid, D-mandelic acid, oxalic acid, sebacic acid, suberic acid or succinic acid (all at 0.1 %, w/v). Uses acetamide, L-alanine, L-α-aminobutyric acid, L-arginine, L-gelatin, D-glucic acid, L-glycine, histidine, L-leucine, DL-methionine, mono-ethanolamine, DL-norleucine, L-norvaline, DL-phenylalanine, L-proline, serine, uric acid, urea and L-valine as sole carbon and nitrogen sources, but not L-cysteine, L-glutamic acid, L-isoleucine or L-ornithine (all at 0.1 %, w/v). Additional phenotypic properties are indicated in the text and in Table 1. The fatty acid profile includes major amounts of hexadecanoic (C16 : 0), octadecenoic (C18 : 1), tridecanoic (C13 : 0), tetradecanoic (C14 : 0) and octadecanoic (C18 : 0) acids and minor amounts of tetradecanoic (C14 : 0), pentadecanoic (C15 : 0), iso-hexadecanoic (iso-C16 : 0) and eicosanoic (C20 : 0) acids.

The type strain, N1350T (=DSM 45372T =NCIB 14575T =NRRL B24794T), was isolated from a hay meadow plot at Cockle Park Experimental Farm, Northumberland, UK.

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References


Williamsia faeni sp. nov.


