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 N1350^T, 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 N1350^T shared highest 16S rRNA gene sequence similarities with *Williamsia marianensis* MT8^T (98.1 %) and *Williamsia muralis* MA140-96^T (98.3 %). However, strain N1350^T was readily distinguished from the type strains of *Williamsia* species using a combination of phenotypic properties. On the basis of these data, strain N1350^T 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 N1350^T (=DSM 45372^T =NCIMB 14575^T =NRRL B-24794^T).

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 N1350^T, that had been recovered from a hay meadow soil and provisionally assigned to the genus *Williamsia*.

Strain N1350^T 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 N1350^T 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 N1350^T 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,

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1969) 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 N1350^T 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 N1350^T shared highest 16S rRNA gene sequence similarities with *W. muralis* MA140-96^T (98.3 %, corresponding to 24 nucleotide differences across 1416 locations) and *W. marianensis* MT8^T (98.1 %). DNA–DNA hybridization studies were not performed between these strains as *W. marianensis* MT8^T and *W. muralis* MA140-96^T, which formed a subclade with strain N1350^T, 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 N1350^T 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 N1350^T contained *meso*-diaminopimelic acid, arabinose and galactose in whole-organism hydrolysates (wall chemotype 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* Kroppenstedt, 1985; hexadecanoic ($C_{16:0}$, 21 % of total), monosaturated octadecanoic ($C_{18:1}$, 15 %), tridecanoic ($C_{13:0}$, 11 %), tuberculostearic (10-methyl $C_{18:0}$, 8 %) and octadecanoic ($C_{18:0}$, 7 %) acids] and minor proportions of tetradecanoic ($C_{14:0}$), pentadecanoic ($C_{15:0}$), iso-hexadecanoic (iso-$C_{16:0}$) and eicosanoic ($C_{20:0}$) acids. Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol were the major polar lipids. Strain N1350^T contained mycolic acids that co-migrated with those from *W. muralis* DSM 44343^T. This chemotaxonomic profile is consistent with the classification of strain N1350^T in the genus *Williamsia* (Goodfellow & Maldonado, 2006; Yassin & Hupfer, 2006).

Strain N1350^T 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 N1350^T 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 N1350^T in the genus *Williamsia* (Kämpfer *et al.*, 1999; Yassin *et al.*, 2007). Strain N1350^T could be readily distinguished from the type strains of *Williamsia* species using a combination of phenotypic properties (Table 1). Strain N1350^T could also be distinguished from *W. deligens*

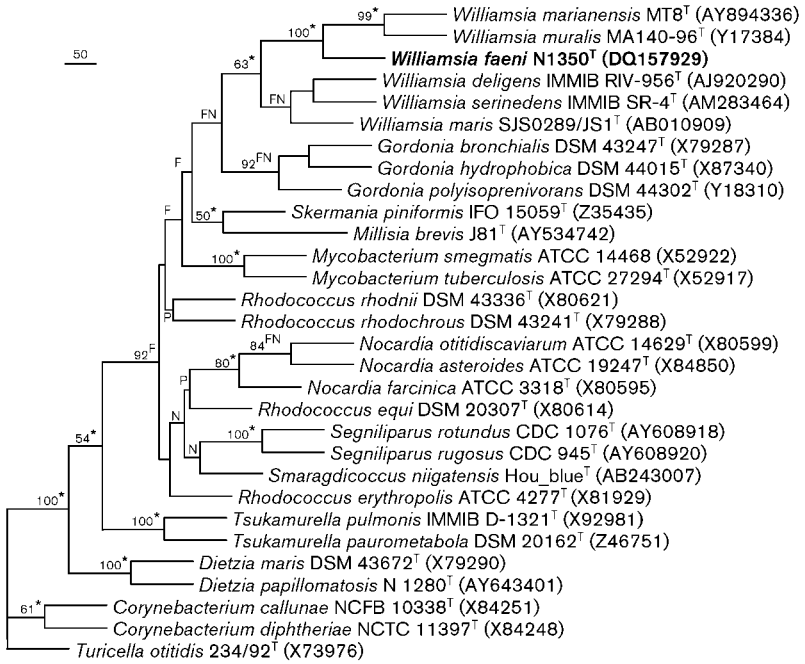

Fig. 1. Maximum-likelihood tree (Felsenstein, 1981) based on the nearly complete 16S rRNA gene sequence of strain N1350^T, showing the strain's relationships within the *Williamsia* clade. Asterisks indicate that the corresponding nodes were also recovered in trees generated with the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) algorithms. F, P and N indicate that the corresponding nodes were also recovered in trees generated with the least-squares, maximum-parsimony or neighbour-joining algorithms, respectively. Bootstrap values (>50 %) based on a neighbour-joining analysis of 1000 resampled datasets are shown at branch nodes. Bar, 50 changes.

Table 1. Phenotypic properties that distinguish strain N1350^T from the type strains of *Williamsia* species

Strains: 1, *Williamsia faeni* sp. nov. N1350^T; 2, *W. marianensis* DSM 44944^T (data from Pathom-Aree *et al.*, 2006); 3, *W. muralis* DSM 44343^T; 4, *W. deligens* DSM 44902^T; 5, *W. maris* DSM 44693^T; 6, *W. serinedens* DSM 45037^T. Data were obtained in this study unless indicated. +, Positive; −, negative; ND, not determined.

Characteristic	1	2	3	4	5	6
Growth at (°C):						
4	+	+	−	−	−	−
10	+	+	+	−	+	+
37	−	−	+	+	+	−
45	−	−	+	−	−	−
Aesculin hydrolysis	+	−	−	−	ND	−
Growth with (1 % (w/v):						
Adonitol	+	−	+	−	−	+
(−)-L-Arabinose	+	+	−	−	−	+
(+)-Cellobiose	+	−	−	−	−	−
(−)-D-Galactose	+	−	−	−	−	+
myo-Inositol	+	−	−	−	−	−
(+)-Maltose	+	−	−	+	−	+
(−)-D-Mannitol	+	+	+	+	−	+
(+)-Melibiose	+	−	+	−	−	+
(+)-D-Raffinose	+	−	−	−	−	−
α-L-Rhamnose	+	+	+	−	+	−
(−)-D-Sorbitol	+	+	+	+	−	+
(+)-Sucrose	+	+	+	+	−	+
(+)-Trehalose	+	+	−	+	+	+
(+)-D-Xylose	+	−	−	+	+	+
Growth with (0.1 %, w/v):						
m-Hydroxybenzoic acid	−	−	+	−	−	+
p-Hydroxybenzoic acid	+	−	−	−	−	−
Growth with (1 %, v/v):						
1,2-Propanediol	+	−	−	−	−	+

DSM 44902^T and *W. serinedens* DSM 45037^T by its ability to degrade L-tyrosine (Yassin & Hupfer, 2006; Yassin *et al.*, 2007) and from *W. marianensis* DSM 44944^T by its ability to degrade tributyrin but not hypoxanthine (Pathom-Aree *et al.*, 2006).

It can be concluded from the genotypic and phenotypic data that strain N1350^T can be readily distinguished from described *Williamsia* species and, hence, should be classified as a representative of a novel species in the genus *Williamsia*. The name proposed for this taxon is *Willliamsia faeni* sp. nov.

Description of *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-amygdalin, (−)-D-arabinose, (+)-D-arabitol, arbutin, (−)-D-fructose, (−)-D-fucose, (−)-D-glucose, inulin, (+)-lactose, (+)-D-mannose, (+)-melibiose, methyl α-D-glucoside, (−)-D-ribose, (+)-turanose (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 %, v/v), 3-methyl-1-butanol (isoamyl alcohol), benzoic acid, fumaric acid, glycerol, 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-gluconic acid, L-glycine, histidine, L-leucine, DL-methionine, monoethanolamine, 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 ($C_{16:0}$), octadecenoic ($C_{18:1}$), tridecanoic ($C_{13:0}$), tuberculostearic (10-methyl $C_{18:0}$) and octadecanoic ($C_{18:0}$) acids and minor amounts of tetradecanoic ($C_{14:0}$), pentadecanoic ($C_{15:0}$), iso-hexadecanoic (iso-$C_{16:0}$) and eicosanoic ($C_{20:0}$) acids.

The type strain, N1350^T (=DSM 45372^T =NCIB 14575^T =NRRL B24794^T), was isolated from a hay meadow plot at Cockle Park Experimental Farm, Northumberland, UK.

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