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

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The GenBank accession number for the 16S rRNA gene sequence of strain N1350T is DQ157929.
The taxonomic status of an actinomycete isolated from soil collected from a hay meadow was determined using a polyphasic approach. The strain, isolate N1350, had morphological and chemotaxonomic properties consistent with its classification in the genus *Williamsia* and formed a distinct phyletic line in the *Williamsia* 16S rRNA gene tree. It shared its highest phylogenetic similarities with the type strains of *Williamsia marianensis* (98.1%) and *Williamsia muralis* (98.3%) but was readily distinguished from these and the other type strains of *Williamsia* species using a combination of phenotypic properties. On the basis of these data the isolate is considered to represent a new 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 = NCIB 14575^T = NRRL B24794^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 validly described species, *Williamsia muralis* (Kämpfer et al., 1999), the type species, was 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, *Williamsia* species can be separated 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, isolate N1350 that had been recovered from a hay meadow soil and provisionally assigned to the genus *Williamsia*. The isolate was the subject of a polyphasic taxonomic study which showed that it should be recognised as a new species of *Williamsia*. The name proposed for this taxon is *Williamsia faeni* sp. nov.

Strain N1350 was isolated on Gauze’s medium 2 supplemented with cycloheximide (50µgml⁻¹), nalidixic acid (10µgml⁻¹), novobiocin (10µgml⁻¹) and nystatin (50µgml⁻¹) following inoculation with a soil suspension and 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). The organism 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; those for the 16S rRNA study in NaCl/EDTA buffer (0.1M EDTA, 0.1M NaCl, pH 8.0) and stored at -20°C until required.
The phylogenetic position of isolate N1350 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 after Kim et al. (1998) The almost complete 16S rRNA gene sequence (1441 nucleotides [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 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 (Felsenstein, 1981), tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993), and evolution distance matrices prepared after Jukes and Cantor (1969). The resultant unrooted tree topologies were evaluated in a bootstrap analysis (Felsenstein, 1985) based on 1,000 resamplings of the neighbour-joining dataset using the CONSENSE and SEQBOOT options from the PHYLIP package.

It can be seen in Figure 1 that strain N1350 was recovered within the *Williamsia* 16S rRNA gene clade, an association supported by all of the tree-making algorithms and by a 100% bootstrap value in the neighbour-joining analysis. The organism showed its highest similarity with the type strain of *W. muralis*, the two strains shared a 16S rRNA gene similarity of 98.3%, a value that corresponds to 24 nt differences at 1416 locations. DNA:DNA relatedness studies were not carried out between these strains as the type strain of *W. marianensis* and *W. muralis*, which form a subclade with isolate N1350, shared a much higher 16S rRNA similarity value (99.5%) but have a DNA-
DNA homology value of only 11% (Pathom-aree et al., 2006), a figure well below the 70% cut-off point recommended for the delineation of bacterial species (Wayne et al., 1987).

Strain N1350 was examined for key chemotaxonomic markers to establish if it had chemical properties characteristic of *Williamsia* strains. Standard procedures were used to determine the diagnostic isomers of diaminopimelic acid (A₂pm; 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). The organism contained *meso*-A₂pm, arabinose and galactose in whole-organism hydrolysates (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), N-glycolyl muramic acid, dihydrogenated menaquinones with nine isoprene units as the sole isoprenologue, major proportions of straight chain saturated, unsaturated and tuberculostearic acids (fatty acid type 1b *sensu* Kroppenstedt, 1985), phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol as major polar lipids, and mycolic acids that co-migrated with those from *W. muralis* DSM 44343ᵀ. This chemotaxonomic profile is consistent with the classification of the strain in the genus *Williamsia* (Goodfellow & Maldonado, 2005; Yassin & Hupfer, 2006).

Strain N1350 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). The organism is aerobic, Gram-positive, non-acid-alcohol fast, asporogenous and catalase-
positive, and uses a diverse range of compounds as sole carbon sources, properties in line with its classification in the genus *Williamsia* (Kämpfer *et al.*, 1999; Yassin *et al.*, 2007). It can be seen from Table 1 that the isolate can be readily distinguished from the type strains of *Williamsia* species using a combination of phenotypic properties. It can also be distinguished from *W. deligens DSM 449002* 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 capacity to degrade tributyrin, but not hypoxanthine (Pathom-aree *et al.*, 2006).

It can be concluded from the genotypic and phenotypic data that isolate N1350 can be readily distinguished from the validly 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 *Williamsia 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 its isolation from a hay meadow).

Forms coccoid elements. Irregular, convex, matt yellow pink pigmented colonies are produced on glucose-yeast extract agar after incubation for 5 days at 28°C. Grows between 10 and 30°C, but not at 37°C. Hydrolyses allantoin and urea, but not arbutin. DNA, RNA, starch and uric acid are degraded, but not adenine, chitin, elastin, xanthine or xylan. D(-) amygdalin, D(-) arabinose, D(+) arabitol, arbutin, D(-) fructose, D(-) fucose, D(-) glucose, inulin, D(+) lactose, D(+) mannose, D(+) melibiose, α-methyl-D-glucoside, D(-) ribose, and D(+) turanose are used as sole
carbon sources for energy and growth, but not dulcitol or salicin (all at 1%, w/v); butan-1,3-diol, butan-1,4-diol, butan-1-ol, butan-2,3-diol, ethanol, propan-1-ol and propan-2-ol are also used as sole carbon sources (all at 1% v/v) with iso-amyl 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 used as sole carbon sources (all at 0.1%, w/v), but not adipic acid, citric acid, glutaric acid, malonic acid, D-mandelic acid, oxalic acid, sebacic acid, suberic acid or succinic acid. Acetamide, L-alanine, L-aminobuty1, L-arginine, L-gelatin, D-gluconic acid, L-glycine, histidine, L-leucine, DL-methionine, mononethanolamine, DL-norleucine, L-norvaline, DL-phenylalanine, L-proline, serine, uric acid, urea and L-valine are used as sole carbon and nitrogen sources (all at 0.1%, w/v), but not L-cysteine, L-glutamic acid, L-isoleucine, L-ornithine, Additional phenotypic properties are cited either in the text or in Table 1. Exhibits chemotaxonomic markers characteristic of the genus Williamsia. The fatty acid profile includes major amounts of hexadecanoic (C_{16:0}, 21% of total), monosaturated octadecanoic (C_{18:1}, 15%), tridecanoic (C_{13:0}, 11%), tuberculostearic (10-methyl octadecanoate, 8%) and octadecanoic (C_{18:0}, 7%) acids; minor components include; tetradecanoic (C_{14:0}); pentadecanoic (C_{15:0}); iso-hexadecanoic (iC_{16:0}) and eicosanoic acids (C_{20:0}).

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.

Acknowledgements
The authors are indebted to Dr Iain Sutcliffe (University of Northumbria) for help with the fatty acid analysis of the *Williamsia faeni* strain.

References


Table 1. Phenotypic properties that distinguish strain N1350\textsuperscript{T} from the type strains of Williamsia species

Reference strains: 1, Isolate N1350\textsuperscript{T}; 2, W. deligens DSM 44902\textsuperscript{T}; 3, W. marianensis DSM 44944\textsuperscript{T} (data from Pathom-aree et al., 2006); 4, W. maris DSM 44693\textsuperscript{T}; 5, W. muralis DSM 44343\textsuperscript{T}; 6, W. serinedens DSM 45037\textsuperscript{T}. 

+-, positive; -, negative; ND, not determined

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**Legend for Figure**

Fig.1. Maximum likelihood tree (Felsenstein, 1981) based on a nearly complete 16S rRNA gene sequence of strain N1350ᵀ showing its position in the *Williamsia* clade. Asterisks indicate branches of the tree that were also found using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms; the symbols F, P and N indicate branches recovered using the least-squares, maximum-parsimony and neighbour-joining methods, respectively. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 re-sampled datasets; only values above 50% are given. The scale bar indicates 10 substitutions per nucleotide position. ᵀ, type strain.