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

Amanda L. Jones[1†], Gail, D. Payne[1‡] and Michael Goodfellow[1]

[1]School of Biology, University of Newcastle, Newcastle upon Tyne, NE1 7RU, UK

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Author for correspondence: †Amanda L. Jones, School of Applied Sciences, Ellison Building, Northumbria University, Newcastle upon Tyne, NE1 8ST, UK.

Tel: +44(0)191-2274895. Fax: +44(0)191-2273519.

E-mail: Amanda.L.Jones@northumbria.ac.uk

Present address: ‡Gail. D. Payne, Agencourt Bioscience, 500 Cummings Center, Beverly, MA 01915, USA.

The GenBank accession number for the 16S rRNA gene sequence of strain N1350^T 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^{o}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^{o}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 (A_2pm; 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*-A_2pm, 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 44343^T. 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 catalasepositive, 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 *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 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-aminobutyl, L-arginine, L- gelatin, D-gluconic acid, L-glycine, histidine, L-leucine, DL-methionine, mononethanolamine, DL-*nor*leucine, L-*nor*valine, 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-*iso*leucine, 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.

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References

Adachi, K., Katsuta, A., Matsuda, S., Peng, X., Misawa, N., Shizuri, Y., Kroppenstedt, R.M., Yokota, A. & Kasai, H. (2007). *Smaragdicoccus niigatensis* gen. nov., sp. nov., a novel member of the suborder *Corynebacterineae*. *Int J Syst Evol Microbiol* **57**, 297-301.

Atalan, E., Manfio, G.P., Ward, A.C., Kroppenstedt, R.M. & Goodfellow, M. (2000). Biosystematic studies on novel streptomycetes from soil. *Antonie van Leeuwenhoek* **77**, 337-353.

Collins, M.D. (1994). Isoprenoid quinones. In *Chemical Methods in Prokaryotic Systematics*, pp. 265-309. Edited by M. Goodfellow and A. G. O'Donnell. Chichester, John Wiley & Sons.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368-376.

Felsenstein, J. (1985). Confidence-limits on phylogenies - an approach using the bootstrap. *Evolution* **39**, 783-791.

Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package), version 3.5c. Department of Genetics, University of Washington, Seattle, USA.

Fitch, W.M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279-284.

Goodfellow, M. & Maldonado, L.A. (2005). The families *Dietziaceae*, *Gordoniaceae*, *Nocardiaceae* and *Tsukamurellaceae*. In *The Prokaryotes, Volume 3, Archaea, Bacteria, Firmicutes, Actinomycetes*, pp. 843-888. Edited by F. Dworkin, S. Falkow, K. H. Schleifer and E. Stackebrandt. New York, Berlin and Heidelberg, Springer.

Gordon, R.E. & Mihm, J.M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann NY Acad Sci* **98**, 628-636.

Hasegawa, T., Takizawa, M. & Tanida, S. (1983). A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* **29**, 319-322.

Jones, A.L., Koerner, R.J., Natarajan, S., Perry, J.D. & Goodfellow, M. (2008). *Dietzia papillomatosis* sp. nov., a novel actinomycete isolated from the skin of an immunocompetent patient with confluent and reticulated papillomatosis. *Int J Syst Evol Microbiol* **58**, 68-72.

Jukes, T.H. & Cantor, C.R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21-132. Edited by H. N. Munro. New York, Academic Press.

Kämpfer, P., Andersson, M.A., Rainey, F.A., Kroppenstedt, R.M. & Salkinoja-Salonen, M. (1999). *Williamsia muralis* gen. nov., sp. nov., isolated from the indoor environment of a children's day care centre. *Int J Syst Bacteriol* **49**, 681-687.

Kim, S.B., Falconer, C., Williams, E. & Goodfellow, M. (1998). *Streptomyces thermocarboxydovorans* sp. nov. and *Streptomyces thermocarboxydus* sp. nov., two moderately thermophilic carboxydotrophic species from soil. *Int J Syst Bacteriol* **48**, 59-68.

Kluge, A.G. & Farris, F.S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1-32.

Kroppenstedt, R.M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics*, pp. 173-199. Edited by M. Goodfellow and D. E. Minnikin. London., Academic Press.

Lechevalier, M.P. & Lechevalier, H. (1970). Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* **20**, 435-443.

Minnikin, D.E., Alshamaony, L. & Goodfellow, M. (1975). Differentiation of *Mycobacterium*, *Nocardia*, and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *J Gen Microbiol* **88**, 200-204.

Minnikin, D.E., O'Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J.H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Meth* **2**, 233-241.

Pathom-aree, W., Nogi, Y., Sutcliffe, I.C., Ward, A.C., Horikoshi, K., Bull, A.T. & Goodfellow, M. (2006). *Williamsia marianensis* sp. nov., a novel actinomycete isolated from the Mariana Trench. *Int J Syst Evol Microbiol* **56**, 1123-1126.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.

Soddell, J.A., Stainsby, F.M., Eales, K.L., Kroppenstedt, R.M., Seviour, R.J. & Goodfellow, M. (2006). *Millisia brevis* gen. nov., sp. nov., an actinomycete isolated from activated sludge foam. *Int J Syst Evol Microbiol* **56**, 739-744.

Stach, J.E., Maldonado, L.A., Ward, A.C., Bull, A.T. & Goodfellow, M. (2004). *Williamsia maris* sp. nov., a novel actinomycete isolated from the Sea of Japan. *Int J Syst Evol Microbiol* **54**, 191-194.

Stackebrandt, E., Rainey, F.A. & Ward-Rainey, N.L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479-491.

Staneck, J.L. & Roberts, G.D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226-231.

Sutcliffe, I.C. (2000). Characterisation of a lipomannan lipoglycan from the mycolic acid containing actinomycete *Dietzia maris*. *Antonie van Leeuwenhoek* **78**, 195-201.

Tan, G.Y., Ward, A.C. & Goodfellow, M. (2006). Exploration of *Amycolatopsis* diversity in soil using genus-specific primers and novel selective media. *Syst Appl Microbiol* **29**, 557-569.

Uchida, K., Kudo, T., Suzuki, K.I. & Nakase, T. (1999). A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49-56.

Wayne, L.G., Brenner, D.J., Colwell, R.R., Grimont, P.A.D., Kandler, O., Krichevsky, M.I., Moore, L.H., Moore, W.E.C., Murray, R.G.E., Stackebrandt, E., Starr, M.P. & Trüper, H.G. (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463-464.

Yassin, A.F. & Hupfer, H. (2006). *Williamsia deligens* sp. nov., isolated from human blood. *Int J Syst Evol Microbiol* **56**, 193-197.

Yassin, A.F., Young, C.C., Lai, W.A., Hupfer, H., Arun, A.B., Shen, F.T., Rekha, P.D. & Ho, M.J. (2007). *Williamsia serinedens* sp. nov., isolated from an oil-contaminated soil. *Int J Syst Evol Microbiol* **57**, 558-561.

Zhi, X.Y., Li, W.J. & Stackebrandt, E. (2009). An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* **59**, 589-608.

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

Reference strains: 1, Isolate N1350^T; 2, *W. deligens* DSM 44902^T; 3, *W. marianensis* DSM 44944^T (data from Pathom-aree *et al*., 2006); 4, *W. maris* DSM 44693^T; 5, *W. muralis* DSM 44343^T; 6, *W. serinedens* DSM 45037^T.
+, positive; -, negative; ND, not determined

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

Legend for Figure

Fig.1. Maximum likelihood tree (Felsenstein, 1981) based on a nearly complete 16S rRNA gene sequence of strain N1350^{T} 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.