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1 **_Williamsia faeni_ sp. nov., a novel actinomycete isolated from a hay meadow**

2

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6

7

8 **Keywords**: *Williamsia faeni,* polyphasic taxonomy, hay meadow

9 **Subject category**: New Taxa: Actinobacteria

10 **Running Title**: *Williamsia faeni* sp. nov.

11

12

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19

20 The GenBank accession number for the 16S rRNA gene sequence of strain N1350^{T} is

21 DQ157929.

22

23

24 The taxonomic status of an actinomycete isolated from soil collected from a hay
25 meadow was determined using a polyphasic approach. The strain, isolate N1350, had
26 morphological and chemotaxonomic properties consistent with its classification in the
27 genus *Williamsia* and formed a distinct phyletic line in the *Williamsia* 16S rRNA gene
28 tree. It shared its highest phylogenetic similarities with the type strains of *Williamsia*
29 *marianensis* (98.1%) and *Williamsia muralis* (98.3%) but was readily distinguished
30 from these and the other type strains of *Williamsia* species using a combination of
31 phenotypic properties. On the basis of these data the isolate is considered to represent
32 a new species of the genus *Williamsia.* The name proposed for this taxon is
33 *Williamsia faeni* sp. nov. with the type strain N1350^T (= DSM 45372^T = NCIB 14575^T
34 = NRRL B24794^T).
35

36 The genus *Williamsia* (Kämpfer *et al.*, 1999) has been classified in the family
37 *Nocardiaceae* together with the genera *Gordonia*, *Millisia*, *Nocardia*, *Rhodococcus*
38 and *Skermania* (Zhi *et al.*, 2009). At the time of writing the genus encompasses five
39 validly described species, *Williamsia muralis* (Kämpfer *et al.*, 1999), the type species,
40 was isolated from indoor building material of a children's day centre in Finland,
41 *Williamsia deligens* (Yassin & Hupfer, 2006) from human blood, *Williamsia*
42 *marianensis* (Pathom-aree *et al.*, 2006) from sediment taken from the Mariana Trench
43 in the Pacific Ocean, *Williamsia maris* (Stach *et al.*, 2004) from sediment collected
44 from the Sea of Japan, and *Williamsia serinedens* (Yassin *et al.*, 2007) from an oil-
45 contaminated soil. The type strains of these species form a clade within the
46 evolutionary radiation occupied by the suborder *Corynebacterineae* (Stackebrandt *et*
47 *al.*, 1997; Zhi *et al.*, 2009). The genus *Williamsia* can also be distinguished from the
48 other mycolic acid-containing genera using a combination of chemotaxonomic and

49 morphological properties (Soddell *et al.*, 2006; Adachi *et al.*, 2007). Similarly,
50 *Williamsia* species can be separated by using a range of phenotypic properties (Yassin
51 *et al.*, 2007).
52
53 The aim of the present study was to determine the taxonomic position of an
54 actinomycete, isolate N1350 that had been recovered from a hay meadow soil and
55 provisionally assigned to the genus *Williamsia*. The isolate was the subject of a
56 polyphasic taxonomic study which showed that it should be recognised as a new
57 species of *Williamsia*. The name proposed for this taxon is *Williamsia faeni* sp. nov.
58
59 Strain N1350 was isolated on Gauze's medium 2 supplemented with cycloheximide
60 (50µgml^{-1}), nalidixic acid (10µgml^{-1}), novobiocin (10µgml^{-1}) and nystatin (50µgml^{-1})
61 following inoculation with a soil suspension and incubation at 30°C for 21 days (Tan
62 *et al.*, 2006). The soil sample had been collected from underneath the surface of
63 Palace Leas meadow hay plot 6 (Atalan *et al.*, 2000) at Cockle Park Experimental
64 Farm, Northumberland, UK (National Grid Reference NZ 200913). The organism was
65 maintained on glucose-yeast extract agar (GYEA; Gordon & Mihm, 1962) at room
66 temperature and as glycerol suspensions (20%, v/v) at -20°C. Biomass for the
67 chemotaxonomic and 16S rRNA gene sequence analyses was grown in shake flasks of
68 GYE broth for 5 days at 28°C, checked for purity and harvested by centrifugation.
69 Cells for the chemosystematic studies were washed twice in distilled water and
70 freeze-dried; those for the 16S rRNA study in NaCl/EDTA buffer (0.1M EDTA, 0.1M
71 NaCl, pH 8.0) and stored at -20°C until required.
72

73 The phylogenetic position of isolate N1350 was determined in a 16S rRNA gene
74 sequence analysis. Isolation of chromosomal DNA, PCR amplification and direct
75 sequencing of the purified products were carried out after Kim et al. (1998) The
76 almost complete 16S rRNA gene sequence (1441 nucleotides [nt]) was aligned
77 manually with corresponding sequences of representatives of the genera classified in
78 the suborder *Corynebacterineae*, retrieved from the DDBJ/EMBL/GenBank
79 databases, using the pairwise alignment option and 16S rRNA secondary structural
80 information held in the PHYDIT program (available at
81 http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic trees were inferred using the
82 least-squares (Fitch & Margoliash, 1967), neighbour-joining (Saitou & Nei, 1987),
83 maximum-parsimony (Kluge & Farris 1969) and maximum-likelihood (Felsenstein,
84 1981), tree-making algorithms from the PHYLIP suite of programs (Felsenstein,
85 1993), and evolution distance matrices prepared after Jukes and Cantor (1969). The
86 resultant unrooted tree topologies were evaluated in a bootstrap analysis (Felsenstein,
87 1985) based on 1,000 resamplings of the neighbour-joining dataset using the
88 CONSENSE and SEQBOOT options from the PHYLIP package.
89
90 It can be seen in Figure 1 that strain N1350 was recovered within the *Williamsia* 16S
91 rRNA gene clade, an association supported by all of the tree-making algorithms and
92 by a 100% bootstrap value in the neighbour-joining analysis. The organism showed its
93 highest similarity with the type strain of *W. muralis*, the two strains shared a 16S
94 rRNA gene similarity of 98.3%, a value that corresponds to 24 nt differences at 1416
95 locations. DNA:DNA relatedness studies were not carried out between these strains as
96 the type strain of *W. marianensis* and *W. muralis*, which form a subclade with isolate
97 N1350, shared a much higher 16S rRNA similarity value (99.5%) but have a DNA-

98 DNA homology value of only 11% (Pathom-aree *et al.*, 2006), a figure well below the
99 70% cut-off point recommended for the delineation of bacterial species (Wayne *et al.*,
100 1987).
101
102 Strain N1350 was examined for key chemotaxonomic markers to establish if it had
103 chemical properties characteristic of *Williamsia* strains. Standard procedures were
104 used to determine the diagnostic isomers of diaminopimelic acid (A_2pm; Staneck &
105 Roberts, 1974), fatty acids (Sutcliffe, 2000), isoprenoid quinones (Collins, 1994),
106 muramic acid type (Uchida *et al.*, 1999), mycolic acids (Minnikin *et al.*, 1975), polar
107 lipids (Minnikin *et al.*, 1984) and whole-organism sugars (Hasegawa *et al.*, 1983).
108 The organism contained *meso*-A_2pm, arabinose and galactose in whole-organism
109 hydrolysates (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970), N-glycolyl
110 muramic acid, dihydrogenated menaquinones with nine isoprene units as the sole
111 isoprenologue, major proportions of straight chain saturated, unsaturated and
112 tuberculostearic acids (fatty acid type 1b *sensu* Kroppenstedt, 1985),
113 phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and
114 phosphatidylinositol as major polar lipids, and mycolic acids that co-migrated with
115 those from *W. muralis* DSM 44343^T. This chemotaxonomic profile is consistent with
116 the classification of the strain in the genus *Williamsia* (Goodfellow & Maldonado,
117 2005; Yassin & Hupfer, 2006).
118
119 Strain N1350 was examined for a range of phenotypic properties using a range of
120 media and methods known to yield data of value for the classification and
121 identification of mycolic-acid-containing actinomycetes (Jones *et al.*, 2008). The
122 organism is aerobic, Gram-positive, non-acid-alcohol fast, asporogenous and catalase-

123 positive, and uses a diverse range of compounds as sole carbon sources, properties in
124 line with its classification in the genus *Williamsia* (Kämpfer *et al.*, 1999; Yassin *et al.*,
125 2007). It can be seen from Table 1 that the isolate can be readily distinguished from
126 the type strains of *Williamsia* species using a combination of phenotypic properties. It
127 can also be distinguished from *W. deligens* DSM 449002^T and *W. serinedens* DSM
128 45037^T by its ability to degrade L-tyrosine (Yassin & Hupfer, 2006; Yassin *et al.*,
129 2007) and from *W. marianensis* DSM 44944^T by its capacity to degrade tributyrin, but
130 not hypoxanthine (Pathom-aree *et al.*, 2006).
131
132 It can be concluded from the genotypic and phenotypic data that isolate N1350 can be
133 readily distinguished from the validly described *Williamsia* species and hence should
134 be classified as a representative of a novel species in the genus *Williamsia*. The name
135 proposed for this taxon is *Willliamsia faeni* sp. nov.
136
137 **Description of *Williamsia faeni* sp. nov.**
138 *Williamsia faeni* (fae'ni. L.n. *faenum*, hay; L. gen.n. *faeni* of hay, referring to its
139 isolation from a hay meadow).
140
141 Forms coccoid elements. Irregular, convex, matt yellow pink pigmented colonies are
142 produced on glucose-yeast extract agar after incubation for 5 days at 28°C. Grows
143 between 10 and 30°C, but not at 37°C. Hydrolyses allantoin and urea, but not arbutin.
144 DNA, RNA, starch and uric acid are degraded, but not adenine, chitin, elastin,
145 xanthine or xylan. D(-)-amygdalin, D(-)-arabinose, D(+)-arabitol, arbutin, D(-)-
146 fructose, D(-)-fucose, D(-)-glucose, inulin, D(+)-lactose, D(+)-mannose, D(+)-
147 melibiose, α-methyl-D-glucoside, D(-)-ribose, and D(+)-turanose are used as sole

148 carbon sources for energy and growth, but not dulcitol or salicin (all at 1%, w/v);
149 butan-1,3-diol, butan-1,4-diol, butan-1-ol, butan-2,3-diol, ethanol, propan-1-ol and
150 propan-2-ol are also used as sole carbon sources (all at 1% v/v); with *iso*-amyl
151 alcohol, benzoic acid, fumaric acid, glycerol, glycogen, L+lactic acid, L-malic acid,
152 oleic acid, propanoic acid, pyruvic acid, sodium acetate, sodium *n*-butyrate, L+tartaric
153 acid, valeric acid and xylitol used as sole carbon sources (all at 0.1%, w/v), but not
154 adipic acid, citric acid, glutaric acid, malonic acid, D-mandelic acid, oxalic acid,
155 sebacic acid, suberic acid or succinic acid. Acetamide, L-alanine, L-aminobutyl, L-
156 arginine, L- gelatin, D-gluconic acid, L-glycine, histidine, L-leucine, DL-methionine,
157 mononethanolamine, DL-*nor*leucine, L-*nor*valine, DL-phenylalanine, L-proline,
158 serine, uric acid, urea and L-valine are used as sole carbon and nitrogen sources (all at
159 0.1%, w/v), but not L-cysteine, L-glutamic acid, L-*iso*leucine, L-ornithine, Additional
160 phenotypic properties are cited either in the text or in Table 1. Exhibits
161 chemotaxonomic markers characteristic of the genus *Williamsia*. The fatty acid profile
162 includes major amounts of hexadecanoic ($C_{16:0}$, 21% of total), monosaturated
163 octadecanoic ($C_{18:1}$, 15%), tridecanoic ($C_{13:0}$, 11%), tuberculostearic (10-methyl
164 octadecanoate, 8%) and octadecanoic ($C_{18:0}$, 7%) acids; minor components include;,
165 tetradecanoic ($C_{14:0}$); pentadecanoic ($C_{15:0}$); *iso*-hexadecanoic ($iC_{16:0}$) and eicosanoic
166 acids ($C_{20:0}$).
167
168 The type strain, N1350^T (= DSM 45372^T = NCIB 14575^T = NRRL B24794^T), was
169 isolated from a hay meadow plot at Cockle Park Experimental Farm, Northumberland,
170 UK.
171
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173

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175 with the fatty acid analysis of the *Williamsia faeni* strain.

176

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299

01

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.