Actinomadura xylanilytica sp. nov., an actinomycete isolated from soil

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The taxonomic position of a soil isolate, strain BK147T, was established using data from a polyphasic study. The organism showed a combination of chemotaxonomic and morphological characteristics consistent with its classification in the genus Actinomadura. It formed a distinct phyletic line in the phylogenetic tree based on 16S rRNA gene sequences of members of the genus Actinomadura and was most closely, albeit loosely, related to Actinomadura bangladeshensis DSM 45347T, Actinomadura meyerae DSM 44715T and Actinomadura napierensis NRRL B-24319T but was readily distinguished from these strains using a range of phenotypic properties. Based on the combined genotypic and phenotypic data it is proposed that isolate BK147T (=KACC 20919T=NCIMB 14771T=NRRL B-24852T) be classified as the type strain of a novel species of the genus Actinomadura, for which the name Actinomadura xylanilytica sp. nov. is proposed.

The genus Actinomadura Lechevalier & Lechevalier (1970a) belongs to the family Thermomonosporaceae and can be distinguished from other members of the taxon, namely the genera Actinolomurus, Actinocorallia, Spirillospora and Thermomonospora, by using a combination of genotypic and phenotypic properties (Tamura et al., 2009; Goodfellow & Trujillo, 2012). Members of the genus Actinomadura produce a stable, extensively branched substrate mycelium and aerial hyphae, which when formed, differentiate into chains of spores. They also tend to be characterized by the presence of meso-diaminopimelic acid (meso-A_{2pm}) and madurose in whole-organism hydrolysates (wall chemotype IIIB; Lechevalier & Lechevalier, 1970b), acetylated muramic acid in the peptidoglycan, hexahydrogenated menaquinones with nine isoprene units [MK-9 (H_{6})] as the predominant isoprenologue, diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol mannosides as major phospholipids (Lechevalier et al., 1977), and complex mixtures of fatty acids with major amounts of hexadecanoic (C_{16:0}), 14-methylpentadecanoic (iso-C_{16:1}) and 10-methyloctadecanoic acid (tuberculostearic acid) (fatty type 3a; Kroppenstedt et al., 1985). At the time of writing, the genus encompasses 50 species with validly published names (Euzéby, 2012) which can be distinguished from one another using chemotaxonomic, morphological and phenotypic properties (Lee & Lee, 2010; Promnuan et al., 2011; Trujillo & Goodfellow, 2012).

Most species of the genus Actinomadura have been isolated from soil although a few are causal agents of the debilitating human disease actinomycetoma (Trujillo & Goodfellow, 2012). As part of a screening programme for antibiotic-producing actinomycetes, isolate BK147T was recovered from a hay meadow soil and provisionally assigned to the genus Actinomadura. In the present taxonomic study using a polyphasic approach, the isolate was shown to form a new centre of taxonomic variation in the genus Actinomadura. It is therefore proposed that the isolate be recognized as representing a novel species of the genus Actinomadura.

Isolate BK147T was recovered from a plate of starch-casein agar (Küster & Williams, 1964), supplemented with cycloheximide and nystatin (each at 25 µg ml^{-1}), which had been inoculated with a pre-heated (55 °C for 20 min) soil suspension and incubated at 28 °C for 21 days. The soil sample was collected from Palace Leas hay meadow plot 6 (Atalan et al., 2000) at Cockle Park Experimental Farm, Northumberland, UK (National Grid Reference NZ 200913). The organism was maintained on oatmeal agar slopes [International Streptomycyes Project (ISP) medium 3; Shirling & Gottlieb (1966)] at 4 °C and as mycelial fragments in 20% (v/v) glycerol at −20 °C. Biomass for the chemotaxonomic and molecular systematic studies was grown in shake flasks of tryptone-yeast extract broth (ISP medium 1; Shirling & Gottlieb, 1966) for 7 days at 28 °C, harvested by centrifugation and washed twice in distilled water; cells for chemical studies were freeze-dried.

Abbreviations: meso-A_{2pm}, meso-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BK147T is FR692101.

A supplementary figure is available with the online version of this paper.


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The phylogenetic position of strain BK147<sup>T</sup> was determined by 16S rRNA gene sequence analysis. Genomic DNA was extracted from the isolate and PCR amplification and 16S rRNA gene sequencing were achieved using procedures described previously (Kim et al., 1996). The resultant, almost-complete 16S rRNA gene sequence (1454 nt) was aligned manually against corresponding sequences of representatives of the genus *Actinomadura* retrieved from the EzTaxon database (Chun et al., 2007) using MEGA5 software (Tamura et al., 2011). Phylogenetic trees were inferred by using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms drawn from the MEGA5 (Tamura et al., 2011) and PHYML (Guindon & Gascuel, 2003) software packages. The Jukes & Cantor (1969) model was used to generate an evolutionary distance matrix for the neighbour-joining algorithm. Topologies of the resultant trees were evaluated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based upon 1000 replicates using the MEGA5 program. *Actinocorallia herbida* NBRC 15485<sup>T</sup> (GenBank accession number D85473) and *Streptosporangium album* DSM 43023<sup>T</sup> (GenBank accession number X89934) were used as outgroups.

The classification of strain BK147<sup>T</sup> in the genus *Actinomadura* was confirmed by its assignment to the *Actinomadura madurae* subclade, the taxonomic integrity of which was underpinned by all of the tree-making algorithms and by a 93 % bootstrap value (Fig. 1). Strain BK147<sup>T</sup> and its nearest neighbour, *Actinomadura napierensis* NRRL B-24319<sup>T</sup>, formed a group towards the periphery of the *Actinomadura madurae* subclade that was supported by all three treeing algorithms and a bootstrap value of 64%; the two strains shared a 16S rRNA gene sequence similarity of 98.4 %, a value that corresponded to 22 nt differences at 1347 locations. Strain BK147<sup>T</sup> also shared relatively high 16S rRNA gene similarities with *Actinomadura bangladeshensis* DSM 45347<sup>T</sup> (98.3 %, 24 nt differences) and *Actinomadura meyerae* DSM 44715<sup>T</sup> (98.2 %, 25 nt differences). Its relationships with the remaining type strains recovered in the *Actinomadura madurae* subclade fell within the range 96.5–98.4 % 16S rRNA gene sequence similarity.

DNA–DNA relatedness studies between the isolate and its closest phylogenetic neighbours were not carried out as type strains of species assigned to the *Actinomadura madurae* subclade have been shown to have gene sequence similarities over 99 % and DNA–DNA relatedness values well below the 70 % cut-off point recommended for the delineation of genomic species (Wayne et al., 1987). *A. bangladeshensis* DSM 45347<sup>T</sup> and *Actinomadura chokoriensis* JCM 13932<sup>T</sup>, for instance, share high 16S rRNA gene sequence similarity (99.5 %, 7 nt differences) but have a DNA–DNA relatedness of only 43.6 ± 2.3 % (mean ± SD of four determinations; Ara et al., 2008). Similarly, the type strain of *Actinomadura geliboluensis* A8036<sup>T</sup> shares 99.2 % 16S rRNA gene sequence similarity with the type strain of *A. meyerae* DSM 44715<sup>T</sup> but a DNA–DNA relatedness value of only 19.5 % (Sazak et al., 2012).

Isolate BK147<sup>T</sup> was examined for chemical markers known to be characteristic of the genus *Actinomadura* (Trujillo & Goodfellow, 2012). Standard chromatographic procedures were used to determine the isomers of diaminopimelic acid (A<sub>Dpa</sub> Hasegawa et al., 1983), menaquinones (Collins, 1985), muramic acid type (Uchida et al., 1999), menaquinones (Collins, 1985), polar lipids (Minnikin et al., 1984) and whole-cell sugars (Hasegawa et al., 1983), using appropriate controls. Fatty acids were extracted, methylated and analysed by GC using the standard Sherlock Microbial Identification (MIDI) system and the

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**Fig. 1.** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences (approx. 1400 bp) showing relationships between isolate BK147<sup>T</sup> and the type strains of phylogenetically closely related members of the genus *Actinomadura*. Stars indicate branches of the tree that were also recovered with the maximum-likelihood and maximum-parsimony tree-making algorithms; the open diamond indicates a branch which was also recovered by the maximum-parsimony tree-making algorithm. Numbers at nodes are percentage bootstrap values based on a neighbour-joining analysis of 1000 resampled datasets; only values >50 % are shown. The arrow indicates the inferred root position using *Actinocorallia herbida* NBRC 15485<sup>T</sup> (GenBank accession no. D85473) and *Streptosporangium album* DSM 43023<sup>T</sup> (X89934) as outgroup. Bar, 0.005 substitutions per nucleotide position.
ACTINO version 5 database (Sasser, 1990). The isolate contained meso-A2pm, galactose, glucose, madurose and xylose in whole-organism hydrolysates (wall chemotype IIIIB sensu Lechevalier & Lechevalier, 1970b), N-acetylated muramic acid in the peptidoglycan, saturated and branched chain fatty acids with hexadecanoic acid (C16:0) as the major component, major amounts of diphostatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides (Fig. S1, available in IJSEM Online; phospholipid pattern 1; Lechevalier et al., 1977), and hexahydrogenated and octahydrogenated menaquinones with nine isoprene units [MK-9(H6,H8)] as predominant isoprenologues in a ratio of 4:3; minor amounts of MK-9(H4) were also detected. The detailed fatty acid profile was C 14 : 0 (2.9 %), iso-C 14 : 0 (0.7 %), iso-C15 : 0 (1.3 %), C16 : 0 (26.8 %), 10-methyl C16 : 0 (1.1 %), anteiso-C16 : 0 (0.9 %), iso-C16 : 0 (11.7 %), C16 : 1v7c/iso-C15 : 0 2-OH (8.4 %), C17 : 0 (0.7 %), 10-methyl C17 : 0 (1.8 %), anteiso-C17 : 0 (0.4 %), C18 : 0 (3.2 %), iso-C18 : 0 (1.5 %), C18 : 0 9c (16.0 %) and 10-methyl C18 : 0 (22.8 %). Taken together all these data provide further evidence that the isolate belongs to the genus Actinomadura (Lechevalier & Lechevalier, 1970a; Trujillo & Goodfellow, 2012).

Strain BK147T was also examined for cultural and morphological features after growth on several standard nutrient media at 28 °C for 3 weeks. Cultural properties were investigated using glucose-yeast extract-malt extract (GYM, DSMZ medium 65) and modified Bennett’s (Jones, 1949) agars and on tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic salts-starch, glycerol-asparagine and tyrosine agars (ISP media 1–5 and 7; Shirling & Gottlieb, 1966). Spore arrangement and spore surface ornamentation were investigated by examining a gold-coated, dehydrated preparation taken from the ISP 3 plate, using a scanning electron microscope (Stereoscan 240; Cambridge) and the procedure described by O’Donnell et al. (1993). The isolate formed a pale yellow substrate mycelium on GYM, ISP media 1–3 and modified Bennett’s agar, but did not grow on the remaining media. Sparse white aerial hyphae were produced on GYM, and on ISP media 1 and 3; there was no evidence of spore formation on ISP medium 3 after incubation at 28 °C for 4 weeks.

Strain BK147T, A. bangladeshensis DSM 45347T, A. meyerae DSM 44715T and A. napierensis NRRL-B-24319T were examined for a range of phenotypic properties using media and methods described by Williams et al. (1983). Strain BK147T was distinguished from all of these organisms, including A. napierensis NRRL B-24319T, using a combination of phenotypic properties, notably by its ability to

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**Table 1.** Characteristics that differentiate isolate BK147T from the type strains of phylogenetically closely related species of the genus *Actinomadura*

<table>
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<tr>
<th>Characteristic</th>
<th>1</th>
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<td>Colony characteristics on yeast extract-malt extract agar (ISP medium 2)</td>
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<tr>
<td>Aerial mycelium</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
<td>Grey</td>
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<td>Reverse colour</td>
<td>Pale yellow</td>
<td>Bamboo</td>
<td>Light yellow</td>
<td>Browny grey</td>
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<td>Production of diffusible pigments on tyrosine agar (ISP medium 7)</td>
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<td>Degradation of:</td>
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<td>Gelatin</td>
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<td>Hypoxanthine</td>
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<td>Tween 80</td>
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<td>Xanthine</td>
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<td>Xylan</td>
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<td>Growth on sole carbon sources (1 %, w/v)</td>
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<td>Adonitol</td>
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<td>L-Arabinose</td>
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<td>D-Mannitol</td>
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<td>Melibiose</td>
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<td>D-Rhamnose</td>
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<td>Sucrose</td>
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<tr>
<td>D-Xylose</td>
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<td>+</td>
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<tr>
<td>Growth in the presence of 5 % (w/v) NaCl</td>
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<td>+</td>
<td>+</td>
<td>-</td>
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</table>

*Strains: 1, BK147T; 2, A. bangladeshensis DSM 45347T; 3, A. meyerae DSM 44715T; 4, A. napierensis NRRL B-24319T. All data were acquired in this study.*
degrade xanthine and xylan, and its inability to grow on a range of sugars as sole carbon sources. Additional phenotypic properties are cited in the species description.

It is evident from the genotypic and phenotypic data that isolate BK147T can be distinguished readily from its closest phylogenetic neighbours and hence should be recognized as representing a novel species of the genus Actinomadura, for which the name Actinomadura xylanilytica sp. nov. is proposed.

**Description of Actinomadura xylanilytica sp. nov.**

*Actinomadura xylanilytica* sp. nov. [xy.la.ni.ly’tic.a. N.L. n. xylanum xylan; N.L. fem. adj. lytica (from Gr. fem. adj. lutikê able to loosen, dissolving); N.L. fem. adj. xylanilytica xylan-dissolving].

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, non-motile actinomyceTe which forms an extensively branched, pale yellow mycelium on oatmeal agar; aerial hyphae are rare but when present do not differentiate into spores. Grows at 10–37 °C and pH 5.0–9.0, but not in the presence of 3.0 % (w/v) NaCl. Hydrolyses aesculin and arbutin, but not allantoin. Degrades adenine, but not chitin, elastin or L-tyrosine. Maltose is used as a sole carbon source for energy and growth, but not glycerol, glycogen, myo-inositol, lactose, D-mannose, D-ribose, D-sorbitol or trehalose (at 1.0 %, w/v). Susceptible to (μg ml⁻¹) gentamicin sulphate (5) but not to ampicillin (4), cephaloridine hydrochloride (2), ciprofloxacin (2), kanamycin sulphate (8), novobiocin (10), penicillin G (2 IU ml⁻¹), streptomycin sulphate (4), rifampicin (15), tetracycline hydrochloride (8) or vancomycin hydrochloride (2). Additional properties are cited in the text or in Table 1. The fatty acid profile contains C₁₄:₀, iso-C₁₅:₀, C₁₆:₀, 10-methyl C₁₆:₀, anteiso-C₁₅:₀, iso-C₁₇:₀, C₁₇:₁, C₁₇:₀, 10-methyl C₁₇:₀, anti-C₁₇:₀, C₁₈:₃, C₁₈:₀ and 10-methyl C₁₈:₀. The remaining chemotaxonomic properties are also typical of the genus.

The type strain, BK147T (=KACC 20919T=NCIMB 14771T=NRRL B-24852T), was isolated from hay meadow plot 6 soil at Cockle Park Experimental Farm, Northumberland, UK. The species description is based on a single strain and hence serves as the description of the type strain.

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**References**


