Amycolatopsis keratiniphila sp. nov., a novel keratinolytic soil actinomycete from Kuwait

Azza A. Al-Musallam,¹ Sheikha S. Al-Zarban,¹ Yunis A. Fasasi,¹ Reiner M. Kroppenstedt² and Erko Stackebrandt²

¹Kuwait University, Department of Biological Sciences, Microbiology Division, P.O. Box 5969, 13060 Safat, Kuwait
²DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen, Mascheroder Weg 1b, 38124 Braunschweig, Germany

A keratinolytic, nocardioform actinomycete, strain D2T, was isolated from Kuwait marsh soil by the bait technique using animal wool. Strain D2T was an aerobic, Gram-positive organism that produced light-grey aerial mycelium but no specific spore chains. 16S rDNA sequence analyses and chemotaxonomic markers were consistent with the classification of strain D2T in the genus Amycolatopsis, i.e. it had meso-diaminopimelic acid in its peptidoglycan, arabinose and galactose as its diagnostic sugars, the polar lipids phosphatidylinositol, phosphatidylethanolamine, hydroxypenicillolethanolamine and diphosphatidyglycerol, menaquinone MK-9(H₄) and an iso-/anteiso-branched fatty acid pattern combined with 10-methyl-branched and 2-hydroxybranched fatty acids. Amycolatopsis japonica was the closest phylogenetic neighbour of strain D2T, showing 99.4% 16S rDNA sequence similarity with the novel strain. A. japonica and strain D2T could be clearly separated from each other on the basis of their low DNA–DNA reassociation value (55–9%). These data, together with its distinct physiological traits, led to the conclusion that strain D2T represented a novel species within the genus Amycolatopsis, for which the name Amycolatopsis keratiniphila (type strain D2T = DSM 44409T = NRRL B24117T) is proposed.

Strain D2T was isolated from marsh soil in Kuwait that had been baited with sterilized and defatted wool; the strain was deposited with the DSMZ as Amycolatopsis keratiniphila DSM 44409T. Determinations of morphological traits, colour of the aerial and substrate mycelia, and of soluble pigments were performed as described previously (Shirling & Gottlieb, 1966). Biochemical tests were done as described by Lechevalier et al. (1986). Strain D2T showed the typical macroscopic and microscopic appearance of a member of the genus Amycolatopsis (Henssen et al., 1987; Kothe et al., 1989; Embley, 1992; Holt et al., 1994), having dirty-white aerial mycelium which became light-grey in ageing cultures grown on GYM medium (4 g glucose L⁻¹, 4 g yeast extract L⁻¹, 10 g malt extract L⁻¹). No specific pigments were produced by the strain. In older cultures, aerial hyphae disintegrated into spore-like structures.

Cell material used for chemotaxonomic analyses was obtained in trypticase soy broth (BBL) after 4 days incubation of strain D2T at 28°C on a rotary shaker. The cell material was harvested by centrifugation and washed twice with distilled water. Analyses of amino acids and sugars were carried out using the methods of Staneck & Roberts (1974). Menaquinones and polar lipids were extracted following the procedure of Minnikin et al. (1984). Polar lipids and menaquinones were analysed by
hydrolysates of strain D2\textsuperscript{T} contained meso-diaminopimelic acid as the diamino acid of the peptidoglycan and galactose and arabinose as the major cell-wall sugars. 

Menaquinone with a tetrahydrogenated isoprenoid chain of nine isoprene units [MK-9(H\textsubscript{4})] was found as the dominant isoprenoid quinone. The polar lipid pattern was composed of phosphatidylglycerol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine and diphosphatidylglycerol. This pattern matched quite well with the patterns for other Amycolatopsis spp. reported by Lechevalier et al. (1986). The fatty acid pattern was composed mainly of iso-anteiso-branched fatty acids. Of diagnostic value is the combination of 10-methyl-branched iso-branched fatty acids together with 2-hydroxy-branched fatty acids (fatty acid pattern 3f).

Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA and purification of PCR products were carried out as described previously (Rainey et al., 1996). The AE2 editor (Maidak et al., 1999) was used to align the almost-complete 16S rDNA sequence of strain D2\textsuperscript{T} (1439 nt) against the 16S rDNA gene sequences of representatives of the main actinobacterial lineages and then against members of the genus Amycolatopsis. Phylogenetic analyses (De Soete, 1983; Felsenstein, 1993) followed described methods. The almost-complete 16S rDNA sequence of strain D2\textsuperscript{T}, consisting of 1439 nt, was compared to 16S rDNA sequences of members of the class Actinobacteria. With intrageneric relationships ranging between 99-4 and 94-3\% sequence similarity, strain D2\textsuperscript{T} was most closely related to members of the genus Amycolatopsis, especially to Amycolatopsis japonica (99-4\% sequence similarity). A phylogenetic dendrogram (Fig. 1) was constructed from a distance matrix using the treeing algorithm of De Soete (1983). Distance-matrix and maximum-likelihood analyses gave consistent results, in that strain D2\textsuperscript{T} clustered with the type strains of A. japonica, Amycolatopsis azurea and Amycolatopsis orientalis (Fig. 1).

Strain D2\textsuperscript{T} was able to utilize all of the tested carbon sources (Table 1) and could grow in the presence of 5\% NaCl. The strain grew at 10°C but not at 45°C; the optimal growth temperature was between 25 and 28°C. In addition, strain D2\textsuperscript{T} could grow on feathers as sole C and N sources, and tolerated 25\% NaCl for growth. Based on these properties, strain D2\textsuperscript{T} could be distinguished from its closest phylogenetic relatives (Table 1).

DNA was isolated by chromatography on hydroxyapatite by the method of Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970), with the modifications described by Huss et al. (1983) and Escara & Hutton (1980), using a Gilford System model 2600 spectrometer equipped with a Gilford model 2527-R thermoprogrammer and plotter. Renaturation rates were computed with the TRANSFER.BAS program (Jahnke, 1992). The DNA–DNA relatedness of strain D2\textsuperscript{T} to A. japonica was 59-5\%, indicating that they were distinct species based on the criteria set forth by Wayne et al. (1987) for strains of the same species.

Based on phenotypic and genotypic data, we conclude that strain D2\textsuperscript{T} merits separate species status within the genus Amycolatopsis. We, therefore, propose the name Amycolatopsis keratiniphila for strain D2\textsuperscript{T}, the sole strain of this species.

**Description of Amycolatopsis keratiniphila sp. nov.**

Amycolatopsis keratiniphila (ke.rat.i.ni’phi.la. N.L. n. keratinum keratin; Gr. adj. philos loving; N.L. adj. keratiniphila keratin-loving, referring to the ability of the species to degrade keratin).

Aerobic, Gram-positive, non-motile actinomycete, which forms light-grey aerial mycelium. No specific endo- or exopigments are produced. In ageing cultures, aerial and...
Table 1. Characteristics useful for distinguishing between species of the Amycolatopsis orientalis–Amycolatopsis mediterranei group

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<td>White to olive-buff</td>
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<td>Production of soluble pigment</td>
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<td>D–Galactose</td>
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<td>Glycerol</td>
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<td>Growth in the presence of 5 % (w/v) NaCl</td>
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The type strain of Amycolatopsis keratiniphila is D2ᵀ (= DSM 44409ᵀ = NRRL B24117ᵀ). Isolated from agricultural soil in Kuwait using animal wool as bait.

Acknowledgements

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References


substrate mycelia disintegrate into spore-like structures. Optimal growth is obtained on GYM medium (4 g glucose 1⁻¹, 4 g yeast extract 1⁻¹, 10 g malt extract 1⁻¹) at 28 °C. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinositol, arabinose and galactose. The predominant menaquinone is MK-9(H₄). The polar lipids are phosphatidylinosito...


