Amycolatopsis taiwanensis sp. nov., from soil

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An actinomycete strain (0345M-7^T) was isolated from a soil sample from Yilan county, Taiwan. The isolate displayed substrate mycelia, upon which were borne short spore chains. The spore chains were composed of non-motile, smooth-surfaced, oval spores. Strain 0345M-7^T had meso-diaminopimelic acid in its peptidoglycan. Whole-cell sugars were galactose, glucose, arabinose and ribose. The only phospholipid found was phosphatidylethanolamine. The predominant menaquinone was MK-9(H₄). Mycolic acids were not detected. Major cellular fatty acids were iso-C_{16:0} (38.1 %) and C_{17:1} (25.4 %). The DNA G+C content of strain 0345M-7^T was 68.9 mol%. On the basis of phenotypic and genotypic data, it is proposed that strain 0345M-7^T (= BCRC 16802^T = KCTC 19116^T) should be classified as the type strain of a novel species of the genus *Amycolatopsis*, *Amycolatopsis taiwanensis* sp. nov.

The genus *Amycolatopsis* was first described by Lechevalier *et al.* (1986) and at the time of writing comprises 27 recognized species. Members of the genus are aerobic, Gram-positive, non-acid-fast, non-motile organisms that form a branched substrate mycelium that fragments into square-shaped elements. Aerial hyphae may be sterile or differentiate into long chains of spore-like structures. *Amycolatopsis* species contain meso-diaminopimelic acid (meso-A₂pm), arabinose and galactose in the cell wall (wall chemotype IV of Lechevalier & Lechevalier, 1970), are rich in iso- and anteiso-branched fatty acids and contain di-, tetra- and hexahydrogenated menaquinones with nine isoprene units as the predominant isoprenologue. Phosphatidylethanolamine (PE) and phosphatidylglycerol are major polar lipids with diphosphatidylinositol mannosides variably present. Members of the genus have DNA G+C contents in the range 66–73 mol%.

During our investigation of the ecology of novel actinomycetes, we isolated several new strains from soils in Taiwan. In this study, we describe one isolate, 0345M-7^T, as belonging to the genus *Amycolatopsis*. On the basis of the polyphasic data presented, we propose that strain 0345M-7^T should be classified within a novel species of the genus *Amycolatopsis*.

Strain 0345M-7^T was isolated from a soil sample collected from Yilan county, Taiwan, by using HV agar (Hayakawa & Nonomura, 1987), and incubated at 28 °C for 4 weeks. The

strain was maintained on oatmeal agar and stored at –20 °C as a suspension of spores or mycelial fragments in 20 % (v/v) glycerol.

Morphological characteristics of cells of strain 0345M-7^T were observed via scanning electron microscopy (S-420; Hitachi), following incubation on HV agar for 14 days at 28 °C and fixation in 4 % osmium tetroxide solution, dehydration through a graded ethanol series and acetone, followed by critical-point drying. Cultural characteristics were tested by using 14-day-old cultures grown at 28 °C on various agar media (Table 1). Colour designations of the substrate mycelium were according to Kelly (1964). All physiological tests were performed at 28 °C. Growth temperature, NaCl tolerance, hydrolysis of aesculin, casein, gelatin, hypoxanthine and xanthine and production of amylase, nitrate reductase and urease were tested following the methods and procedures of Gordon *et al.* (1974). Carbon source utilization was examined on ISP 9 as the basal medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1 % of the tested carbon source.

Biomass for chemotaxonomic studies was prepared following growth in shaking flasks (125 r.p.m.) of YG broth (10.0 g yeast extract and 10.0 g glucose per litre distilled water, pH 7.0) at 28 °C for 7 days. The isomer of A₂pm and sugars in whole-cell hydrolysates were determined by the method of Hasegawa *et al.* (1983). The N-acyl types of muramic acid were determined by the method of Kawamoto *et al.* (1981). Presence of mycolic acids was examined via TLC following Minnikin *et al.* (1975) and phospholipids were extracted and identified following the method of Minnikin *et al.* (1984). Menaquinones were extracted and purified according to the method of Collins

Abbreviations: A₂pm, diaminopimelic acid; PE, phosphatidylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 0345M-7^T is DQ160215.

Table 1. Cultural characteristics of strain 0345M-7^T

Mycelium colours were taken from Kelly (1964).

Medium	Growth	Substrate mycelium	Sporulation
Tryptone-yeast extract agar (ISP 1)	Good	Purple-yellow	None
Yeast extract-malt extract agar (ISP 2)	Good	Light yellow	None
Oatmeal agar (ISP 3)	Good	Yellowish white	Poor
Inorganic salts-starch agar (ISP 4)	Good	Yellowish white	Poor
Glycerol-asparagine agar (ISP 5)	Good	Purple-yellow	Poor
Glucose-asparagine agar	Poor	Yellowish white	Poor
Peptone-yeast-iron agar (ISP 6)	Good	Light yellow	None
Tyrosine agar (ISP 7)	Good	Light yellow	Poor
Glucose-asparagine agar	Poor	Yellowish white	Poor
Bennett's agar	Poor	Light yellow	None
Nutrient agar	Poor	Light yellow	None

et al. (1977) and then analysed by HPLC (model 600; Waters) with a Nova-Pak C18 column. For quantitative analysis of the cellular fatty acid content, strain 0345M-7^T was cultured using TSB medium at 28 °C on a shaking incubator at 125 r.p.m. for 7 days. Extracts of the methylated fatty acids were prepared according to the protocol provided by MIDI Inc.

For the extraction of DNA to be used for sequencing of the 16S rRNA gene, strain 0345M-7^T was grown in YG broth at 28 °C for 7 days. Cells were removed from the broth using a pipette tip and total DNA was extracted by using the QIAGEN Genomic DNA kit. The G + C content of the DNA was determined by HPLC according to the method of Tamaoka & Komagata (1984). DNA was prepared by using the same method as above. The 16S rRNA gene was PCR-amplified according to the methods of Nakajima *et al.* (1999) and directly sequenced on an ABI model 3730 automatic DNA sequencer using the BigDye Terminator v3.1 kit (Applied Biosystems). Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by using CLUSTAL X (Thompson *et al.*, 1997). Calculation of evolutionary distances (distance options according to the Kimura two-parameter model; Kimura, 1980, 1983) and clustering were with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985).

Strain 0345M-7^T produced branched and non-fragmented substrate mycelia, borne on which were short spore chains. No aerial mycelia were found after growth on any of the test media. Spores were non-motile, oval and smooth-surfaced (Fig. 1). The cultural characteristics of strain 0345M-7^T are detailed in Table 1. Good growth and pale-yellow substrate mycelia were observed but sporulation was poor on most of the media tested. No soluble pigment was produced in all media tested. The physiological and biochemical characteristics of strain 0345M-7^T are given in Table 1 and in the species description below.

Strain 0345M-7^T contained *meso*-A₂pm, arabinose, galactose, glucose and ribose in whole-cell hydrolysates. The predominant menaquinone was MK-9(H₄); mycolic acids were not detected. PE was detected. The major fatty acid methyl esters were iso-C_{16:0} (38.1%), C_{17:1} (25.4%), anteiso-C_{17:0} (5.2%), C_{16:0} (5.1%) and C_{17:0} (4.0%), but tuberculostearic acid was not detected. The G + C content of the DNA was 68.9 mol%.

The almost-complete 16S rRNA gene sequence (1507 nt) of strain 0345M-7^T was determined. Preliminary comparison of the sequence against the GenBank database revealed high sequence similarity values with members of the genus *Amycolatopsis*. The phylogenetic tree based on the 16S rRNA gene sequences of strain 0345M-7^T and recognized members of the genus *Amycolatopsis* is shown in Fig. 2. Strain 0345M-7^T had 16S rRNA gene similarity values ranging between 92.7% (*Amycolatopsis fastidiosa* IMSNU 20054^T) and

Fig. 1. Scanning electron micrograph of cells of strain 0345M-7^T grown on HV agar for 14 days at 28 °C. Bar, 1.5 μm.

Fig. 2. Neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing the phylogenetic position of strain 0345M-7^T within the radiation of *Amycolatopsis* species. Numbers at nodes indicate percentages of 1000 bootstrap resamplings; only values >50 % are given. Bar, 0.02 substitutions per nucleotide position.

Table 2. Characteristics that differentiate strain 0345M-7^T from its nearest phylogenetic neighbours

Strains: 1, strain 0345M-7^T; 2, *Amycolatopsis eurytherma* DSM 44348^T; 3, *Amycolatopsis methanolica* IMSNU 20055^T; 4, *Amycolatopsis thermoflava* IFO 14333^T. Data for reference strains were taken from Chun *et al.* (1999) and Kim *et al.* (2002). +, Positive; –, negative; w, weak growth or reaction; ND, no data available. For all four strains, the cell-wall peptidoglycan contains *meso*-A₂pm and mycolic acids are absent.

Characteristic	1	2	3	4
Presence of aerial mycelium	–	+	+	+
Spores on substrate hyphae	+	–	–	–
Production of soluble pigment	–	–	–	+
Growth at 5 % NaCl (w/v)	–	+	+	+
Growth at 45 °C	–	+	+	+
Decomposition of:				
Aesculin	+	–	w	+
Casein	+	+	–	+
Gelatin	+	+	+	–
Hypoxanthine	w	w	+	+
Xanthine	–	–	–	+
Production of:				
Amylase	–	ND	–	–
Nitrate reductase	+	+	+	–
Urease	–	+	–	+
Polar lipids*	PE	DPG, PE, PI, PIM, PME, PL	DPG, PE	ND

*DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unidentified polar lipid; PME, phosphatidylmonomethylethanolamine.

95.4% (*Amycolatopsis methanolica* IMSNU 20055^T). It is generally recognized that organisms displaying similarity values of <97% do not belong to the same species (Stackebrandt & Goebel, 1994). The sequence divergence values of >4.0% with recognized members of the genus *Amycolatopsis* clearly indicate that strain 0345M-7^T represents a novel species.

Morphologically, species of the genus *Amycolatopsis* have not previously been described to form short spore chains on mature substrate mycelia and without the formation of aerial mycelia. These morphological features have been described for members of the genus *Catellatospora* (Asano & Kawamoto, 1986). However, chemotaxonomic data, including the predominant menaquinone, polar lipid and major fatty acids, indicate that strain 0345M-7^T should be classified within the genus *Amycolatopsis*.

The distinctiveness of the isolate is also shown by phenotypic comparison with its nearest phylogenetic neighbours (Table 2). On the basis of the phenotypic and genotypic data presented, 0345M-7^T should be classified as the type strain of a novel species of the genus *Amycolatopsis*, for which the name *Amycolatopsis taiwanensis* sp. nov. is proposed.

Description of *Amycolatopsis taiwanensis* sp. nov.

Amycolatopsis taiwanensis (tai.wan.en'sis. N.L. fem. adj. *taiwanensis* of Taiwan, where the type strain was isolated).

Gram-positive, aerobic, non-acid-fast and mesophilic. No aerial mycelia are present. Short spore chains are formed on substrate mycelia; spores are oval with a smooth surface and are non-motile. The substrate mycelium is yellowish white to purple yellow. No soluble pigment is produced. Growth is good on most test media, but sporulation is very poor. Good growth occurs between 20 and 40 °C; no growth occurs above 1% NaCl. Hydrolyses casein and aesculin and is positive for gelatin liquefaction. Able to reduce nitrate. Glucose, arabinose, cellulose and sucrose can be used as sole carbon sources for growth, but not fructose or salicin; use of rhamnose, inositol, raffinose, mannitol and xylose is questionable. The cell-wall peptidoglycan contains meso-A₂pm; arabinose, galactose, glucose and ribose are detected in whole-cell hydrolysates. The predominant menaquinone is MK-9(H₄). Mycolic acids are not detected. The diagnostic phospholipid is PE. Major cellular fatty acids are iso-C_{16:0} (38.1%) and C_{17:1} (25.4%). The G + C content of the DNA of the type strain is 68.9 mol%.

The type strain, 0345M-7^T (=BCRC 16802^T=KCTC 19116^T), was isolated from a soil collected from Yilan county, Taiwan.

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