Actinomadura miaoliensis sp. nov., a thermotolerant polyester-degrading actinomycete

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An actinomycete, strain BC 44T-5^T, with the ability to degrade poly(D-3-hydroxybutyrate) was isolated from a soil sample collected from Miaoli County, Taiwan. The isolate displayed substrate mycelia and short spore chains were borne on the aerial mycelia. Spores were non-motile, round, 1 µm in diameter and spiny. The aerial spore mass was blue. Strain BC 44T-5^T had *meso*-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan. Whole-cell sugars of the novel strain were identified as glucose, galactose and madurose. Diphosphatidylglycerol and phosphatidylinositol were detected. The predominant menaquinones were MK-9(H₄) and MK-9(H₂). Mycolic acids were not detected. Major cellular fatty acids were iso-C_{16:0} (14.82 %), C_{16:0} (14.63 %), C_{17:0} (13.79 %) and 10-methyl-C_{17:0} (23.77 %). The DNA G + C content of strain BC 44T-5^T was 70.6 mol%. On the basis of phenotypic and genotypic data, it is proposed that strain BC 44T-5^T (=FIRDI 002^T=BCRC 16873^T=LMG 24335^T) should be classified as the type strain of a novel species of the genus *Actinomadura*, *Actinomadura miaoliensis* sp. nov.

The genus *Actinomadura* was firstly described in 1970 (Lechevalier & Lechevalier, 1970) and currently comprises more than 30 species with validly published names (Lu *et al.*, 2003; Cook *et al.*, 2005; Lee & Jeong, 2006; Wang *et al.*, 2007; le Roes & Meyers, 2007). Members of the genus are aerobic, Gram-positive, non-acid-alcohol-fast, non-motile organisms. Non-fragmentary substrate mycelia and aerial hyphae that differentiate into spore chains are present. The spore chains are of various lengths and can be straight, hooked or spiral on the tips of the aerial mycelium. Spores are oval or short-rod-like with smooth, spiny or warty surfaces and non-motile. Cells contain *meso*-diaminopimelic acid (A₂pm) and the whole-cell sugars detected are madurose, glucose and galactose (type B; Lechevalier & Lechevalier, 1970). Major cellular fatty acids are iso-C_{16:0}, C_{16:0}, C_{17:0} and 10-methyl-C_{17:0}. Polar lipids belong to type I. The major menaquinones are MK-9(H₄) and MK-9(H₆). Mycolic acids are absent. Members of the genus have DNA G + C contents of 65–69 mol% (Meyer, 1989).

During our screening of polyester-degrading thermophilic/thermotolerant actinomycetes, some thermophilic/thermotolerant strains were isolated from soils in Taiwan (Tseng

et al., 2007). In this study, an isolate belonging to the genus *Actinomadura*, strain BC 44T-5^T, is described. Poly(D-3-hydroxybutyrate) degradation data for strain BC 44T-5^T will be published at a later date. Based on characteristics determined by a polyphasic taxonomic study, i.e. phenotypic properties and phylogenetic and genetic data, it is proposed that the strain should be classified as a representative of a novel species of the genus *Actinomadura*.

Strain BC 44T-5^T was isolated from a soil sample collected in Miaoli County, Taiwan, on HV agar (Hayakawa & Nonomura, 1987) incubated at 50 °C for 7 days. The strain was maintained on oatmeal agar and as a suspension of spores or mycelia fragments in glycerol (20 %, v/v) stored at –20 °C.

Morphological characteristics of strain BC 44T-5^T were observed by scanning electron microscopy (S-3000N; Hitachi) of cells that had been incubated on HV agar for 7 days at 50 °C, fixed with 4 % osmium tetroxide solution, dehydrated in an ethanol/acetone series and critical-point-dried. All physiological tests were performed at 50 °C. Growth temperature, hydrolysis of aesculin, casein, hypoxanthine, xanthine, adenine and L-tyrosine, and production of amylase, nitrate reductase, urease and melanin were assessed by the methods of Gordon *et al.* (1974). Sole carbon source utilization was tested by the method of Shirling & Gottlieb (1966).

Abbreviation: *meso*-A₂pm, *meso*-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BC 44T-5^T is EF116925.

Biomass for chemotaxonomic studies was prepared following growth in shake flasks (125 r.p.m.) of YG broth (10.0 g yeast extract, 10.0 g glucose, 1000 ml distilled water, pH 7.0) at 50 °C for 7 days. The diaminopimelic acid isomer and sugars in whole-cell hydrolysates were determined according to Hasegawa *et al.* (1983). The presence of mycolic acids was examined by TLC according to Minnikin *et al.* (1975) and phospholipids were extracted and identified following the method of Minnikin *et al.* (1984). Menaquinones were extracted and purified by the method of Collins *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 BC 44T-5^T was cultured on tryptic soy broth at 50 °C on a shaking incubator for 7 days at 125 r.p.m. Extracts of methylated fatty acids were prepared according to the protocol provided by the manufacturer.

DNA for 16S rRNA gene sequencing was prepared from cells of strain BC 44T-5^T grown in YG broth at 50 °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 DNA G+C content of strain BC 44T-5^T was determined by the HPLC method of Tamaoka & Komagata (1984). DNA was prepared by using the same method as above. The 16S rRNA gene was PCR-amplified according to Nakajima *et al.* (1999) and sequenced directly on an ABI model 3730 automatic DNA sequencer by 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 alignments 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 conducted using 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). DNA–DNA hybridization was carried out according to the method of Ezaki *et al.* (1989).

Strain BC 44T-5^T produced branched, non-fragmented, substrate mycelia; short spore chains were borne on the aerial mycelia. Spores were non-motile, round, 1 µm in diameter and spiny (Fig. 1). The aerial spore mass was blue. No soluble pigment was produced in any medium tested. The results of physiological and biochemical tests are shown in Table 1.

Whole-cell hydrolysates of strain BC 44T-5^T contained *meso*-A₂pm, madurose, glucose and galactose. The predominant menaquinones found were MK-9(H₄) and MK-9(H₂); mycolic acids were not detected. Diphosphatidylglycerol and phosphatidylinositol were detected. The major fatty acid methyl esters were iso-C_{16:0} (14.82%), C_{16:0} (14.63%), C_{17:0} (13.79%) and 10-methyl-C_{17:0} (23.77%). The DNA G+C content was 70.6 mol%.

Fig. 1. Scanning electron micrograph of cells of strain BC 44T-5^T grown on oatmeal agar for 7 days at 50 °C. Bar, 5 µm.

Table 1. Physiological characteristics that differentiate strain BC 44T-5^T from representatives of phylogenetically related species of the genus *Actinomadura*

Strains: 1, BC 44T-5^T; 2, *A. viridilutea* BCRC 13638^T; 3, *A. rubrobrunea* BCRC 16817^T.

Characteristic	1	2	3
Growth temperature (°C)	22–55	40–60	40–55
Aerial mass colour	Blue	Grey	Grey
Hydrolysis of L-tyrosine	+	–	–
Growth on sole carbon source:			
Fructose	+	–	+
Glucose	+	–	+
Inositol	–	+	+
Inulin	–	+	–
Sorbitol	+	–	+
Trehalose	+	+	–
Xylose	+	+	–

The almost-complete 16S rRNA gene sequence (1514 nt) of strain BC 44T-5^T was determined. Preliminary comparison of the sequence against those in GenBank revealed high sequence similarity values with strains of the genus *Actinomadura*. The phylogenetic tree based on 16S rRNA gene sequences of strain BC 44T-5^T and strains of recognized species of the genus *Actinomadura* and other related species is shown in Fig. 2. Phylogenetically, strain BC 44T-5^T was most closely related to *Actinomadura viridilutea* BCRC 13638^T and *Actinomadura rubrobrunea* BCRC 16817^T, two thermophilic species of the genus *Actinomadura*. DNA–DNA hybridization values of strain BC 44T-5^T with these two type strains were 50.1 and 53.2%, respectively (Table 2). DNA–DNA hybridization values with recognized members of the genus *Actinomadura* clearly indicated that strain BC 44T-5^T represents a novel species. The distinctiveness of the isolate was also evident from phenotypic data (Table 1). On the basis of phenotypic and genotypic characteristics, it is proposed that the isolate should be classified as a representative of a novel species of the genus *Actinomadura*, *Actinomadura miaoliensis* sp. nov.

Fig. 2. Neighbour-joining tree based on almost complete 16S rRNA gene sequences showing the phylogenetic position of strain BC 44T-5^T among strains of the genus *Actinomadura* and related species. Numbers at nodes indicate percentages from 1000 bootstrap resamplings; only values over 50% are given. GenBank accession numbers are given in parentheses. Bar, 0.01 substitutions per nucleotide position.

Description of *Actinomadura miaoliensis* sp. nov.

Actinomadura miaoliensis (mi.ao.li.en'sis. N.L. fem. adj. *miaoliensis* of Miaoli county, where the type strain was isolated).

Cells are Gram-positive, aerobic, non-acid-alcohol-fast and thermotolerant. Short spore chains are borne on the tips of aerial mycelium. Spores are round, 1 µm in diameter, spiny and non-motile. Aerial spore mass is blue. No soluble pigment is produced. Growth occurs between 25 and 55 °C. Hydrolyses casein, aesculin and L-tyrosine. Positive for nitrate reductase production. Glucose, xylose,

rhamnose, sorbitol, fructose, trehalose and lactose can be utilized, but inositol, arabinose, galactose and inulin are not utilized. The cell-wall peptidoglycan contains *meso*-A₂pm. Madurose, glucose and galactose are detected in whole-cell hydrolysates. Predominant menaquinones are MK-9(H₄) and MK-9(H₂). Mycolic acids are not detected. The diagnostic phospholipids are diphosphatidylglycerol and phosphatidylinositol. Major cellular fatty acids are iso-C_{16:0}, C_{16:0}, C_{17:0} and 10-methyl-C_{17:0}.

The type strain is BC 44T-5^T (=FIRDI 002^T=BCRC 16873^T=LMG 24335^T), isolated from soil collected from Miaoli, Taiwan. The DNA G + C content of the type strain is 70.6 mol%.

Table 2. DNA–DNA hybridization rates of strain BC 44T-5^T and closely related *Actinomadura* species

Probe DNA	Hybridization (%) with DNA from:		
	BC 44T-5 ^T	<i>A. viridilutea</i> BCRC 13638 ^T	<i>A. rubrobrunea</i> BCRC 16817 ^T
BC 44T-5 ^T	100.0	47.6	27.8
<i>A. viridilutea</i> BCRC 13638 ^T	50.1	100.0	69.6
<i>A. rubrobrunea</i> BCRC 16817 ^T	53.2	64.7	100.0

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