Amycolatopsis xylanica sp. nov., isolated from soil

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An actinomycete, designated CPCC 202699^T, was isolated from soil in Qinghai province, China, and its taxonomic status was established. Strain CPCC 202699^T contained *meso*-diaminopimelic acid in its peptidoglycan, arabinose and galactose as the diagnostic sugars in whole-cell hydrolysates, a phospholipid pattern consisting mainly of phosphatidylethanolamine, phosphatidylmethylethanolamine and phosphatidylcholine, MK-9(H₄) as the predominant menaquinone and iso-C_{16:0} (18.8%), iso-C_{15:0} (18.1%), iso-C_{14:0} (14.2%), C_{16:1} *cis*9 (10.9%) and C_{17:1} *cis*9 (10.3%) as the major fatty acids. In the phylogenetic tree based on 16S rRNA gene sequences, strain CPCC 202699^T formed a separate branch within the genus *Amycolatopsis*. However, strain CPCC 202699^T showed low 16S rRNA gene sequence similarities (<97.0%) with type strains of species with validly published names in the genus *Amycolatopsis*. On the basis of genotypic and phenotypic differences from the closest phylogenetic neighbours of strain CPCC 202699^T, a novel species, *Amycolatopsis xylanica* sp. nov., is proposed. The type strain is CPCC 202699^T (=DSM 45285^T =KCTC 19581^T =CCM 7627^T).

The genus *Amycolatopsis* was proposed by Lechevalier *et al.* (1986) to accommodate a number of nocardioform actinomycetes. It was later assigned to the family *Pseudonocardiaceae* on the basis of 16S rRNA gene sequence analysis (Embley *et al.*, 1988; Warwick *et al.*, 1994). Members of the genus *Amycolatopsis* form branching vegetative hyphae that undergo fragmentation into squarish subunits. Aerial mycelium, if present, may be sterile or may break down into chains of squarish oval fragments or spore-like structures (Lechevalier *et al.*, 1986). Species with validly published names in this genus share the chemotaxonomic characteristics of a type-IV cell wall, arabinose and galactose as the cell-wall sugars, MK-9(H₄) as the major menaquinone, complex mixtures of saturated and branched-chain fatty acids, a type-II or type-III phospholipid pattern and the absence of mycolic acids (Lechevalier *et al.*, 1986). At the time of writing, the genus *Amycolatopsis* comprised 39 recognized species; *Amycolatopsis fastidiosa* has since been reclassified as *Actinokineospora fastidiosa* (Tan *et al.*, 2006; Labeda *et al.*, 2010).

Strain CPCC 202699^T was isolated from a soil sample collected in Qinghai province, China, by selective isolation on medium MY10S, which contained xylan as the only

carbon and energy source, after incubation at 28 °C for 2 weeks. Strain CPCC 202699^T was maintained on International *Streptomyces* Project (ISP) yeast extract-malt extract agar slants (ISP 2; Shirling & Gottlieb, 1966) at 4 °C and as 20% (v/v) glycerol suspensions at –80 °C. Biomass for molecular systematic and chemotaxonomic studies was obtained by cultivation in shake flasks (about 150 r.p.m.) of tryptic soy broth (Difco) at 28 °C for 5–7 days.

Colours of substrate and aerial mycelia and soluble pigments were assessed using ISCC-NBS Color Charts (standard sample no. 2106; Kelly, 1964). Spore chains were observed using the coverslip technique (Zhou *et al.*, 1998). Morphological characteristics of spores and mycelia were recorded by examining dehydrated and gold-coated specimens of 1- to 4-week-old cultures grown on ISP 2 and ISP 4 agar by scanning electron microscopy (Quanta; FEI). Conditions for growth were tested on ISP 2 agar at 4, 10, 20, 28, 30, 32, 37, 40 and 45 °C and with 1, 3, 5 and 7–10% (w/v) NaCl (at intervals of 0.5% NaCl) and in agar-free ISP 2 medium at pH 4.0–11.0 (at intervals of 0.5 pH units). Freeze-dried cells were used for chemotaxonomic analysis. Utilization of sugars and other compounds listed for the API 50CH and API 20E systems (bioMérieux) and acid production from these sugars were reassessed in basal medium (Shirling & Gottlieb, 1966) supplemented with 1% (w/v) test substrate, with the basal medium as a control. Growth was monitored by measuring the OD₆₀₀ with an Ultrospec 2000 photometer (Pharmacia Biotech). A fingerprint of enzyme activities was obtained using the API ZYM system (bioMérieux), according to the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CPCC 202699^T is FJ529702.

A thin-layer chromatogram of the phosphate-containing lipids of strain CPCC 202699^T and cellular fatty acid profiles of strain CPCC 202699^T and related type strains are available as supplementary material with the online version of this paper.

manufacturer's instructions. Sole nitrogen sources were determined in a basal liquid medium containing (1^{-1}) 1 g D-glucose, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.05 g NaCl, 0.001 g

$FeSO_4 \cdot 7H_2O$ and 0.01 g K_2HPO_4 . Other physiological and biochemical tests were performed according to the established methods of Williams *et al.* (1983) and Yuan *et al.* (2008).

Table 1. Phenotypic characteristics that differentiate strain CPCC 202699^T from related type strains of the genus *Amycolatopsis*

Strains: 1, *A. xylanica* sp. nov. CPCC 202699^T; 2, *A. alba* DSM 44262^T; 3, *A. azurea* DSM 43854^T; 4, *A. coloradensis* DSM 44225^T; 5, *A. decaplanina* DSM 44594^T; 6, *A. keratiniphila* subsp. *keratiniphila* DSM 44409^T; 7, *A. nigrescens* DSM 44992^T; 8, *A. saalfeldensis* DSM 44993^T. All data were obtained in this study. +, Positive; w, weakly positive; –, negative/none.

Characteristic	1	2	3	4	5	6	7	8
Colour of aerial mycelium	White	White	Usually white	White to olive-buff	Honey yellow	Light grey	White to orange	White
Colour of soluble pigment	Brown	–	Blue	Yellow	Red	–	Dark reddish-black	–
Growth at/in:								
10 °C	+	–	+	+	+	+	–	w
40 °C	+	w	–	–	w	+	+	–
45 °C	+	–	–	–	–	–	–	–
5 % NaCl	+	–	+	+	+	+	+	–
Use of sole carbon sources (1 %)								
Adonitol	–	+	+	–	–	+	–	+
(+)-L-Arabinose	+	+	+	–	+	+	+	+
D-Fructose	+	+	–	+	+	+	+	+
D-Galactose	+	+	–	+	–	+	+	–
Glycerol	+	+	+	+	w	+	w	w
Inositol	w	+	+	+	–	+	+	+
Lactose	–	+	+	–	–	+	–	+
D-Mannitol	+	+	–	+	+	+	+	+
Melibiose	–	+	+	–	+	+	–	+
Raffinose	–	+	+	–	–	+	+	–
(+)-L-Rhamnose	–	–	–	–	–	+	–	+
Sucrose	–	–	+	+	–	+	–	+
D-Xylose	+	–	–	+	–	+	+	+
Production of:								
Amylase	+	+	–	+	+	–	–	–
Arginine dihydrolase	+	w	+	–	–	+	–	–
Lysine decarboxylase	–	–	+	–	–	+	w	–
Nitrate reductase	–	–	+	+	+	+	+	–
Ornithine decarboxylase	–	+	+	–	–	+	–	+
Urease	+	+	+	–	+	+	+	+
API ZYM test results								
Alkaline phosphatase	+	+	–	–	+	+	+	+
Cystine arylamidase	–	+	–	+	–	+	+	–
Esterase (C4)	+	+	–	+	–	+	+	+
Esterase lipase (C8)	+	+	+	+	–	+	+	+
Leucine arylamidase	+	+	+	–	+	+	+	+
Lipase (C14)	w	–	–	–	–	+	+	–
Naphthol-AS-BI-phosphohydrolase	+	+	+	–	–	–	+	+
Trypsin	w	+	+	–	+	+	–	–
Valine arylamidase	w	+	–	–	+	+	w	+
α-Chymotrypsin	–	+	+	–	+	+	+	w
α-Fucosidase	–	+	–	+	–	–	–	–
α-Galactosidase	–	–	–	+	–	–	–	–
β-Galactosidase	–	+	+	–	+	+	+	–
α-Glucosidase	–	+	+	+	+	+	–	+
β-Glucosidase	–	+	+	+	+	+	w	w
α-Mannosidase	–	+	–	–	+	–	–	–

Strain CPCC 202699^T displayed the typical morphological properties of the genus *Amycolatopsis*. The aerial mycelium was white and the substrate mycelium was white–brown to brown on most media tested. Spore surfaces were smooth. Growth was observed at 10–45 °C, at pH 6.0–9.5 and with 0–5 % (w/v) NaCl. Strain CPCC 202699^T was catalase-positive and oxidase-negative. Urea, starch and aesculin were hydrolysed, but gelatin was not. Nitrate was reduced. H₂S was not produced. Milk was coagulated and peptonized. The detailed physiological and biochemical characteristics of strain CPCC 202699^T are given in the species description and Table 1.

The isomer of diaminopimelic acid and the whole-cell sugars were determined following the procedures developed by Hasegawa *et al.* (1983) and Lechevalier & Lechevalier (1980). Polar lipids were extracted and identified according to the method of Minnikin *et al.* (1984). Menaquinones were isolated using the method of Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1997). The acid methanolysis procedure was used for the extraction and analysis of

mycolic acids (Minnikin *et al.*, 1975). Fatty acids were extracted, methylated and estimated by GC using the standard Sherlock Microbial Identification system (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996).

Genomic DNA preparation and PCR amplification of the 16S rRNA gene were performed according to Chun & Goodfellow (1995) and sequencing was performed using an automatic sequencer (ABI PRISM 3730XL; Applied Biosystems). The G+C content of the genomic DNA was determined with the thermal denaturation method (Marmur & Doty, 1962) and *Escherichia coli* K-12 as a control. Multiple alignments of sequences of members of the genus *Amycolatopsis* and calculation of sequence similarities were carried out using the EzTaxon server (Chun *et al.*, 2007). A distance matrix and phylogenetic tree were constructed from K_{nuc} values (Kimura, 1980, 1983) with the neighbour-joining method of Saitou & Nei (1987) using MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1981) with 1000 resamplings.

Fig. 1. Neighbour-joining tree, based on almost-complete 16S rRNA gene sequences, showing the relationships between strain CPCC 202699^T and type strains of the genus *Amycolatopsis*. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. Bar, 0.005 substitutions per nucleotide position.

The cell-wall diamino acid in the peptidoglycan layer of strain CPCC 202699^T was *meso*-diaminopimelic acid and the major sugars in whole-cell hydrolysates were arabinose and galactose (cell-wall chemotype IV according to Lechevalier & Lechevalier, 1980). The predominant menaquinone was MK-9(H₄) (83.9%), with small amounts of MK-9(H₆) (11.3%) and MK-8(H₄) (4.7%). Phosphatidylethanolamine, phosphatidylmethylethanolamine and phosphatidylcholine were predominant in the phospholipid profile (Supplementary Fig. S1, available in IJSEM Online). The major cellular fatty acids were iso-C_{16:0} (18.8%), iso-C_{15:0} (18.1%), iso-C_{14:0} (14.2%), C_{16:1} *cis*9 (10.9%) and C_{17:1} *cis*9 (10.3%). The detailed cellular fatty acid profiles of strain CPCC 202699^T and related type strains of the genus *Amycolatopsis* are given in Supplementary Table S1. No mycolates were detected. The genomic DNA G + C content was 65.9 mol%.

An almost-complete (1451 nt) 16S rRNA gene sequence of strain CPCC 202699^T was determined. Comparison of the sequence with those of representatives of the family *Pseudonocardiaceae* and other related taxa showed clearly that strain CPCC 202699^T was most closely related to members of the genus *Amycolatopsis* (16S rRNA gene sequence similarities 95.0–96.9%). In the phylogenetic tree (Fig. 1), strain CPCC 202699^T formed a separate branch within the *Amycolatopsis* clade, which permitted classification of this strain within a novel species of the genus *Amycolatopsis* (Stackebrandt & Goebel, 1994; Rosselló-Mora & Amann, 2001).

Based on the phenotypic differentiation and genotypic data presented above, we propose strain CPCC 202699^T to be a representative of a novel species of the genus *Amycolatopsis*, with the name *Amycolatopsis xylanica* sp. nov.

Description of *Amycolatopsis xylanica* sp. nov.

Amycolatopsis xylanica (xy.la.ni'ca. N.L. n. *xylanum* xylan; L. fem. suff. *-ica* suffix used with the sense of pertaining to; N.L. fem. adj. *xylanica* pertaining to xylan, referring to the energy source used for the isolation of the type strain).

Cells are Gram-stain-positive, aerobic and non-motile and form well-developed aerial and vegetative mycelium. Catalase-positive and oxidase-negative. Colony colour is dependent on the test medium. Aerial mycelium is white on ISP 2, 4 and 5 and Bennett agar. Vegetative mycelium is brown on ISP 2 and Bennett agar, white–brown on ISP 4 and white on ISP 5. Diffusible pigments are light brown on ISP 2, yellow–brown on ISP 4 and ISP 5 and brown on Bennett agar; no discernible diffusible pigments on ISP 6. The temperature range for growth is 10–45 °C and good growth is observed at 28 °C. Cells can grow with 0–5% NaCl, but not with more than 7% NaCl. Growth occurs at pH 6.0–9.5 (optimum pH 7.0–8.0). Utilizes L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glycine, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, L-tryptophan, L-valine and potassium nitrate as sole nitrogen sources. Utilizes L-arabinose, D-fructose, D-galactose, D-glucose, D-xylose, D-mannitol, D-mannose, trehalose, D-xylose, aesculin ferric

citrate, N-acetylglucosamine and glycerol as sole carbon sources for energy and growth, but not D-arabinose, cellobiose, D-fucose, lactose (bovine origin), melezitose, melibiose, raffinose, L-rhamnose, D-ribose, D-sorbose, sucrose, D-tagatose, L-xylose, arbutin, amygdalin, D-adonitol, D- or L-arabitol, dulcitol, erythritol, D-sorbitol, xylitol, methyl α -D-glucopyranoside, methyl α -D-mannopyranoside, methyl β -D-xylopyranoside, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate, D-glycogen, D-inulin or salicin. With API ZYM, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl- β -glucosaminidase, weakly positive for lipase (C14), valine arylamidase and trypsin, but negative for cystine arylamidase, α -chymotrypsin, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase and α -mannosidase. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, arabinose and galactose. The predominant menaquinone is MK-9(H₄); small amounts of MK-9(H₆) and MK-8(H₄) are detected. In the phospholipid profile, phosphatidylethanolamine, phosphatidylmethylethanolamine and phosphatidylcholine are predominant and glucosamine-containing phospholipids, phosphatidylinositol mannosides and phosphatidylinositol dimannosides can also be detected. The major cellular fatty acids (>10%) are iso-C_{16:0}, iso-C_{15:0}, iso-C_{14:0}, C_{16:1} *cis*9 and C_{17:1} *cis*9. The genomic DNA G + C content of the type strain is 65.9 mol%.

The type strain is CPCC 202699^T (=DSM 45285^T =KCTC 19581^T =CCM 7627^T), isolated from soil in Qinghai, China.

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