Saccharothrix xinjiangensis sp. nov., a pyrene-degrading actinomycete isolated from Tianchi Lake, Xinjiang, China

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A pyrene-degrading isolate, strain PYX-6^T, was obtained from Tianchi Lake, Xinjiang Uygur Autonomous Region, China, and was identified by means of polyphasic taxonomy. On the basis of 16S rRNA gene sequence similarity, strain PYX-6^T was closely related to members of the genus *Saccharothrix*. Its 16S rRNA gene sequence similarity to each of *Saccharothrix syringae*, *Saccharothrix australiensis*, *Saccharothrix algeriensis* and *Saccharothrix coeruleofusca* was 98 %. Chemotaxonomic and physiological properties of strain PYX-6^T further supported its affiliation to the genus *Saccharothrix*. DNA–DNA hybridization results and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain PYX-6^T from recognized *Saccharothrix* species. Therefore, strain PYX-6^T represents a novel species, for which the name *Saccharothrix xinjiangensis* sp. nov. is proposed, with type strain PYX-6^T (=AS 4.1731^T=JCM 12329^T).

The genus *Saccharothrix* (Labeda *et al.*, 1984) has undergone several amendments based on accumulated chemotaxonomic and phylogenetic data (Labeda & Kroppenstedt, 2000; Labeda *et al.*, 2001). At the time of writing, the genus includes the following recognized species: *Saccharothrix australiensis*, *Saccharothrix coeruleofusca*, *Saccharothrix espanaensis*, *Saccharothrix longispora*, *Saccharothrix mutabilis*, *Saccharothrix mutabilis* subsp. *capreolus*, *Saccharothrix syringae*, *Saccharothrix texasensis* (Labeda *et al.*, 1984; Labeda, 1986, 1989; Labeda & Lechevalier, 1989; Grund & Kroppenstedt, 1989), *Saccharothrix albidocapillata*, *Saccharothrix violacea* (Lee *et al.*, 2000) and *Saccharothrix algeriensis* (Zitouni *et al.*, 2004). Involvement of *Saccharothrix* species in the degradation of polyaromatic compounds has not been described to date. During an ecological survey of the microbial diversity within Tianchi Lake, Xinjiang Uygur Autonomous Region, China, an isolate (strain PYX-6^T) capable of degrading pyrene and many other aromatic compounds was obtained.

Strain PYX-6^T was isolated from water samples from Tianchi Lake by plating 0.1 ml filtered (pore size = 0.22 µm) lake water onto agar plates with benzene, anthracene, phenanthrene or pyrene as the sole carbon source. Routine

cultivation of strain PYX-6^T was performed at 28 °C on inorganic salt agar with pyrene as the sole carbon source. Observation of the morphology of strain PYX-6^T was carried out with optical (Olympus BX51) and scanning electron (Hitachi S-570) microscopes.

Strain PYX-6^T grew well on mineral salt agar plates when pyrene was added as the sole carbon source. Culture characteristics were observed on ISP media (Shirling & Gottlieb, 1966), Santon's medium and ATCC 172 agar after 7–14 days of incubation at 28 °C. Colours were determined according to the standards of Ridgway (1912).

Tyrosine agar (ISP7) (Shirling & Gottlieb, 1966) was used to determine the production of melanoid pigment. ATCC 172 agar and malt agar (ISP3) were used to observe the production of soluble pigment. Physiological tests, including production of acid from carbohydrates, utilization of organic acids and the decomposition of adenine, hypoxanthine, tyrosine and casein, were determined by the methods of Gordon *et al.* (1974). Starch hydrolysis and nitrate reductase were evaluated following the method of Dong & Cai (2001). Sensitivity to lysozyme was studied according to the method of Gordon & Barnett (1977). Tolerance to salinity was investigated by supplementing ATCC 172 agar with various NaCl concentrations. Temperature range for growth was determined in liquid ATCC 172 medium.

Biomass for chemotaxonomic analysis was harvested from

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ATCC 172 broth cultures on a rotary shaker (100 r.p.m., 28 °C). The isomeric form of diaminopimelic acid of cell walls, the predominant sugars and the phospholipid pattern were analysed with the methods of Becker *et al.* (1964), Lechevalier & Lechevalier (1970) and Hasegawa *et al.* (1983), respectively. Menaquinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989), with *S. australiensis* as the reference. The fatty acid profile of whole cells was analysed by GC with a model HP6890 equipped with an hydrogen ionization detector (FID). Peaks were identified with pre-installed software, HPCHEM-STATION (version A5.01).

Genomic DNA of strain PYX-6^T was extracted according to the method of Dong & Cai (2001), and its G + C content was determined by thermal denaturation, with *Escherichia coli* as the reference strain. The 16S rRNA gene of strain PYX-6^T was amplified and sequenced as described by Zhang *et al.* (2003). Alignments of 16S rRNA gene sequences of strain PYX-6^T and other type strains of the genus *Saccharothrix* were carried out with CLUSTAL X version 1.8 (Thompson *et al.*, 1997). Phylogenetic trees (Fig. 1) were constructed according to tree-making algorithms of the neighbour-joining method (Saitou & Nei, 1987). Calculation of evolutionary distances was performed according to the method of Kimura (1980). DNA–DNA relatedness was determined by the method of De Ley *et al.* (1970) at 63 °C.

Strain PYX-6^T exhibited good growth on ISP2, ISP3, ATCC 172 and Sauton's media, as well as in mineral salt medium with pyrene as the sole carbon and energy source. Only weak growth was observed on ISP4. The well-developed, branched substrate mycelium occasionally fragmented into non-motile elements, and the weak aerial mycelium exhibited a characteristic 'zigzag' morphology during sporulation, with spores generally having a smooth surface. The colour of the substrate mycelium was pinkish buff

on ISP2 and ochraceous-salmon on ISP3. Aerial mycelium was greyish white on ISP2 and pale fresh colour on ISP3. On ATCC 172 medium, substrate mycelium was pale orange in colour and turned pale brown with extended incubation times. Pale brown soluble pigment was produced on ATCC 172 and tyrosine agar media and bright rose-red soluble pigment was produced on ISP3 malt splices.

Cell walls of strain PYX-6^T were type III and contained *meso*-diaminopimelic acid. The whole cell hydrolysates contained galactose and mannose. Cells contained MK-9(H₄) (85 %) as the major and MK-10(H₄) (15 %) as the minor menaquinone. Cell membranes contained phosphatidylethanolamine (PE) and diphosphatidylglycerol (DPG) and the phospholipid pattern was type II. The predominant fatty acids were iso-branched molecules (iso-C_{16:0}, 35.3 %; anteiso-C_{15:0}, 12.7 %; Table 1). The DNA G + C content of strain PYX-6^T was 70.4 mol%. These characteristics support the identification of strain PYX-6^T as a member of the genus *Saccharothrix*. Similarity based on 16S rRNA gene sequences indicated that strain PYX-6^T exhibited high relatedness to *S. australiensis*, *S. syringae*, *S. algeriensis* and *S. coeruleofusca* (all 98 %).

DNA–DNA hybridization and physiological properties distinguished strain PYX-6^T from recognized species of the genus *Saccharothrix*. DNA–DNA hybridization values of strain PYX-6^T to *S. syringae*, *S. australiensis*, *S. algeriensis* and *S. coeruleofusca* were 50, 29.9, 21.0 and 14.5 %, respectively. Significant physiological differences among strain PYX-6^T and the type strains of *S. syringae*, *S. australiensis*, *S. algeriensis* and *S. coeruleofusca* are listed in Table 2.

Based on the above characterization, strain PYX-6^T was identified as representing a novel species of *Saccharothrix*, for which the name *Saccharothrix xinjiangensis* sp. nov. is proposed.

Fig. 1. Distance matrix tree based on 16S rRNA gene sequences showing phylogenetic relationships between *Saccharothrix xinjiangensis* sp. nov. and other species of the genus *Saccharothrix*. Bootstrap confidence percentages obtained from 1000 replications are given at branch points. Bar, 0.02 substitutions per nucleotide position.

Table 1. Fatty acid profile of *Saccharothrix xinjiangensis* sp. nov. PYX-6^T

Fatty acid	Percentage of total
iso-C _{14:0}	6.9
iso-C _{15:0}	9.0
anteiso-C _{15:0}	12.7
C _{15:0}	1.0
iso-C _{16:1}	1.9
iso-C _{16:0}	35.3
C _{16:0}	5.2
iso-C _{17:0}	1.5
anteiso-C _{17:0}	6.8
C _{17:1ω8c}	4.5
C _{17:0}	2.0
C _{18:1ω9c}	1.1
C _{18:0}	1.4

Table 2. Differential properties of *S. xinjiangensis* sp. nov. and other members of the genus *Saccharothrix*

Strains: 1, *S. xinjiangensis* sp. nov. PYX-6^T; 2, *S. australiensis* NRRL B-11239^T; 3, *S. syringae* NRRL B-16468^T; 4, *S. algeriensis* NRRL B-16468^T; 5, *S. coeruleofusca* NRRL B-16115^T. +, Positive; w, weakly positive; –, negative; NR, not reported.

Characteristic	1	2	3	4	5
Decomposition of:					
Starch	+	–	+	–	–
Tyrosine	–	+	+	+	–
Production of nitrate reductase	–	+	–	+	–
Assimilation of lactic acid	–	–	+	NR	–
Acid from:					
Arabinose	+	–	+	–	+
Lactose	+	–	+	–	+
Melibiose	+	–	+	–	+
Raffinose	+	–	+	–	–
Rhamnose	+	–	+	–	+
Sorbitol	–	+	–	–	–
Sucrose	+	–	+	–	–
Xylose	+	–	+	–	+
Methyl α -D-glucoside	+	–	–	–	w
Growth in the presence of:					
4 % NaCl	–	+	+	+	–
5 % NaCl	–	–	+	–	–

Description of *Saccharothrix xinjiangensis* sp. nov.

Saccharothrix xinjiangensis (xin.ji.ang.en'sis. N.L. fem. adj. *xinjiangensis* pertaining to Xinjiang, where the type strain was isolated).

Assimilates anthracene, phenathrene, pyrene and other aromatics as sole carbon and energy sources. Aerobic, Gram-positive. The colour of the substrate mycelium is pinkish buff on ISP2 and ochraceous-salmon on ISP3. Aerial mycelium is greyish white on ISP2 and pale fresh colour on ISP3. On medium ATCC 172, substrate mycelium is pale orange in colour and turns pale brown after prolonged cultivation. Pale brown soluble pigment is produced on ATCC 172 and tyrosine agar and bright rose-red soluble pigment is produced on ISP3 malt splices. Growth occurs between 13 and 50 °C, but not at or above 52 °C. Utilizes starch, sucrose, raffinose, glucose, lactose, arabinose, cellobiose, xylose, rhamnose and melibiose. Citrate, sorbitol, tartrate and malonate do not support growth. Growth occurs in the presence of 0.005 % lysozyme. Nitrate is not reduced. Cell wall is type III (*meso*-diaminopimelic acid, galactose, mannose). The major menaquinone is MK-9(H₄) and the minor menaquinone is MK-10(H₄). Cell membranes contain PE and DPG and the phospholipid pattern is type II. The predominant fatty acid is iso-C_{16:0} (35.3 %), with lesser proportions of anteiso-C_{15:0} (12.7 %), iso-C_{15:0} (9.0 %) and anteiso-C_{17:0} (6.8 %). Growth occurs

in the presence of 1.5 % NaCl, weak growth at 3.0 % NaCl but none at 4 or 5 %. The DNA G + C content is 70.4 mol%.

The type strain, strain PYX-6^T (=AS 4.1731^T=JCM 12329^T), was isolated from a water sample of Tianchi Lake, Xinjiang Uygur Autonomous Region, China.

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