Kineococcus xinjiangensis sp. nov., isolated from desert sand

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A brown–orange-pigmented, non-spore-forming, coccus-shaped actinomycete, designated S2-20^T, was isolated from desert sand from Xinjiang Province in China. The isolate stains Gram-positive, is motile and produces a brownish diffusible pigment. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain S2-20^T was phylogenetically affiliated to the genus *Kineococcus*, and the sequence similarity to the type strains of *Kineococcus* species was less than 96 %, making it clear that strain S2-20^T represents a species that is separate from recognized *Kineococcus* species. Its major fatty acid was anteiso-C_{15:0}. The major menaquinone was MK-9(H₂). Whole-cell hydrolysates of strain S2-20^T contained *meso*-diaminopimelic acid, arabinose and galactose. The DNA G + C content was 77.8 mol%. On the basis of phylogenetic, phenotypic and chemotaxonomic characteristics, strain S2-20^T should be classified within a novel species of the genus *Kineococcus*, for which the name *Kineococcus xinjiangensis* sp. nov. is proposed. The type strain is S2-20^T (=CCTCC AB 207179^T =KCTC 19474^T).

Various bacterial species have the capacity to survive under conditions that are commonly considered extreme, such as cold, hot, saline and dry environments. Because of our interest in radiation-resistant bacteria and the correlation that has been made between desiccation and radiation resistance (Mattimore & Battista, 1996), we have studied bacteria that survive in the desert. Desiccation- and radiation-resistant bacteria have been observed in several genera, including *Deinococcus*, *Rubrobacter*, *Hymenobacter*, *Methylobacterium*, *Kocuria* and *Kineococcus* (Rainey *et al.*, 2005; Phillips *et al.*, 2002). The genus *Kineococcus* was first created by Yokota *et al.* (1993) with the species *Kineococcus aurantiacus*, based on a single strain isolated from soil. Two more species have been added to the genus in recent years; *Kineococcus radiotolerans* and *Kineococcus marinus* were reported in 2002 and 2006, respectively. In the present study, a strain with diffusible pigment production was characterized as a novel member of the genus *Kineococcus*.

Strain S2-20^T was isolated from a sand sample from a desert in Xinjiang Province using tenfold-diluted tryptic soy broth (TSB/10; Difco) agar. Colonies were brown–orange, circular, convex with entire edges and produced a brownish diffusible pigment but not clusters after 7 days of incubation on TSB/10 agar at 30 °C. Cell morphology was

examined by light microscopy (Olympus) and by electron microscopy (Hitachi) using methods published previously (Glauert, 1991; Bozzola & Russell, 1999). Motility was observed on motility agar (TSB/10 with 0.5 % agar). The isolate could also grow on tenfold-diluted marine agar 2216 (MA/10; Difco) but not on PTYG medium (Phillips *et al.*, 2002).

Genomic DNA for amplification of the 16S rRNA gene was extracted as described by Earl *et al.* (2002). The 16S rRNA gene was amplified by PCR with bacterial universal primers 27F and 1492R (Lane, 1991) and the PCR products were sequenced by Invitrogen Biotechnology. Similarity searches with the derived sequence were done in NCBI (<http://www.ncbi.nlm.nih.gov/>). Phylogenetic analysis was performed by using MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data via CLUSTAL_X (Thompson *et al.*, 1997). Distances were obtained using options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony method (data not shown). The topology of the phylogenetic tree reconstructed by the neighbour-joining method was evaluated by using bootstrap resampling (Felsenstein, 1985) with 1000 replications (Fig. 1).

The 16S rRNA gene sequence of strain S2-20^T (1385 bp) showed less than 96 % similarity to the type strains of recognized *Kineococcus* species. The highest 16S rRNA gene sequences similarities to members of the genus *Kineococcus*

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Abbreviation: A₂pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S2-20^T is EU543662.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing phylogenetic relationships between strain S2-20^T and other species of the genera *Kineococcus* and *Kineosporia*. Bootstrap values (expressed as percentages of 1000 replications) greater than 40% are given at nodes. Bar, 0.01 sequence variation. The sequence of *Agrococcus casei* LMG 22410^T was used as an outgroup.

were shown to *Kineococcus radiotolerans* SRS 30216^T (95.7 %) and *Kineococcus aurantiacus* IFO 15268^T (95.2 %). Although the highest sequence similarity was shown to *Kineosporia rhamnosa* JCM 9954^T (95.9 %), strain S2-20^T does not form spores (by light microscopy), and can therefore be differentiated easily from members of the genus *Kineosporia* (Kudo *et al.*, 1998).

To investigate chemotaxonomic characteristics, biomass was obtained from cultures grown in TSB/10 at 30 °C for 7 days on a rotary shaker. Cells were harvested by centrifugation and washed twice with distilled water. Analyses of amino acids and sugars in whole-cell hydrolysates were performed according to procedures described by Stanek & Roberts (1974). The diagnostic diamino acid of the peptidoglycan was *meso*-diaminopimelic acid (*meso*-A₂pm). Whole-cell hydrolysates contained arabinose and galactose as characteristic sugars. Fatty acid methyl esters were analysed utilizing the Sherlock Microbial Identification System (MIDI, 2005) according to the manufacturer's instructions after 48 h cultivation on TSB agar at 30 °C. Strain S2-20^T had anteiso-C_{15:0} as the major fatty acid. Menaquinones were extracted and then identified by HPLC as described by Xie & Yokota (2003). Strain S2-20^T contained MK-9(H₂) as the major component. The DNA G + C content of strain S2-20^T, determined using HPLC (Mesbah *et al.*, 1989), was 77.8 mol%. These characteristics also suggest that strain S2-20^T is not a member of the genus *Kineosporia*. Members of the genus *Kineosporia* contain almost equal amounts of LL- and *meso*-A₂pm or mainly LL- or *meso*-A₂pm as the diaminopimelic acid isomer and galactose, glucose, mannose and ribose as cell-wall sugars and lack anteiso-branched fatty acids (Kudo *et al.*, 1998); members of the genus *Kineococcus* contain *meso*-A₂pm, arabinose and galactose in the cell wall and anteiso-C_{15:0} as the major fatty acid (Yokota *et al.*, 1993; Phillips *et al.*, 2002; Lee, 2006).

To investigate further physiological and biochemical characteristics, we used the methods described by Smibert & Krieg (1994) for the following tests: oxidase and catalase reaction, H₂S production and hydrolysis of starch, casein, aesculin, gelatin and urea. The KOH test (Gregersen, 1978) was used to test the Gram reaction. Anaerobic growth was assessed on TSB/10 agar (both with and without KNO₃) incubation in airtight jars containing an AnaeroPack (Oxoid). Temperature tolerance was tested

by checking growth at 4, 14, 21, 30, 37, 40 and 42 °C and tolerance of salinity was tested with growth in TSB/10 with 0, 1, 2 and 3 % (w/v) NaCl. The pH range (pH 5–10 at intervals of 1 pH unit) for growth was determined in tenfold-diluted marine broth (Difco). In addition, carbohydrate utilization was tested using a defined medium solidified with deionized water-washed agar, as described previously (Rainey *et al.*, 2005). Growth was examined visually on plates incubated at 30 °C for up to 7 days. Negative-control plates did not include carbon sources. The physiological and biochemical characteristics of strain S2-20^T are listed in Table 1 and in the species description. In addition, the UV-C resistance of strain S2-20^T and *Kineococcus marinus* NRRL B-24439^T was compared, showing that the survival rate of strain S2-20^T was higher than that of *Kineococcus marinus* NRRL B-24439^T (data not shown).

It has been recognized that organisms with more than 3 % 16S rRNA gene sequence dissimilarity are likely to belong to different genomic species (Stackebrandt & Goebel, 1994). Thus, from the phylogenetic analysis, physiological tests and chemotaxonomic features, it is evident that strain S2-20^T represents a distinct, previously undescribed species within the genus *Kineococcus*, for which the name *Kineococcus xinjiangensis* sp. nov. is proposed.

Description of *Kineococcus xinjiangensis* sp. nov.

Kineococcus xinjiangensis (xin.jiang.en'sis. N.L. masc. adj. *xinjiangensis* pertaining to Xinjiang, a province in north-west China, where the type strain was isolated).

Cells stain Gram-positive and are strictly aerobic, motile, non-spore-forming cocci, 0.8–1.2 µm in diameter. Colonies are brown–orange-coloured and produce a brownish diffusible pigment. Catalase-positive and oxidase-negative. Growth occurs at 14–40 °C (optimum 37 °C), pH 6–10 (optimum pH 7–9) and at up to 2 % NaCl (optimum 0–1 %). Does not hydrolyse starch or urea. Hydrolysis of aesculin, casein and gelatin is positive. H₂S is not produced. A variety of carbon sources are used including rhamnose, sucrose, maltose, D-glucose, L-arabinose and inositol, but not mannitol, D-ribose or sorbitol. The major fatty acid is anteiso-C_{15:0}. The diagnostic diamino acid of the peptidoglycan is *meso*-A₂pm. Whole-cell hydrolysates contain arabinose and galactose as

Table 1. Differential phenotypic characteristics of strain S2-20^T and other *Kineococcus* species

Taxa: 1, strain S2-20^T; 2, *Kineococcus radiotolerans*; 3, *Kineococcus aurantiacus*; 4, *Kineococcus marinus*. +, Positive; –, negative; ND, no data available. Data from Phillips *et al.* (2002), Yokota *et al.* (1993), Lee (2006) and this study. All strains are positive for utilization of D-glucose, sucrose and L-arabinose.

Characteristic	1	2	3	4
Production of brown diffusible pigment	+	–	–	–
Utilization of:				
Mannitol	–	+	–	+
Rhamnose	+	–	–	+
D-Ribose	–	–	–	+
Inositol	+	+	–	–
Sorbitol	–	+	ND	+
Maltose	+	–	–	+
Hydrolysis of:				
Aesculin	+	ND	–	+
Starch	–	ND	–	+
Temperature range for growth (°C)	14–40	11–41	9–36	4–37
pH range for growth	6.0–10.0	5.0–9.0	6.0–9.0	5.1–10.1
Growth on 7% NaCl	–	–	–	+
DNA G+C content (mol%)	77.8	ND	73.9	76.6

characteristic sugars. The major menaquinone is MK-9(H₂). The DNA G+C content of the type strain is 77.8 mol%.

The type strain is S2-20^T (=CCTCC AB 207179^T =KCTC 19474^T), isolated from desert sand in Xinjiang Province, China.

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