Description of *Saccharomonospora xinjiangensis* sp. nov. based on chemical and molecular classification

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Comparative studies of morphology, physiology, biochemistry and chemical composition of cells, and phylogenetic analysis based on 16S rRNA gene sequences were carried out with strains XJ-54^T and XJ-58 of the genus *Saccharomonospora* and type strains of related genera. The results indicated that the two strains are different from known members of the genus *Saccharomonospora*. A new species with the name *Saccharomonospora xinjiangensis* sp. nov. is proposed. The new species is characterized by the presence of longitudinal pairs of spores on both the aerial and the vegetative hyphae and contains phosphatidyl ethanolamine, phosphatidyl choline and unknown glucosamine-containing phospholipids, and the major menaquinones MK-9(H₂), MK-9(H₄) and MK-7(H₄).

Keywords: *Saccharomonospora xinjiangensis* sp. nov., 16S rRNA

INTRODUCTION

The genus *Saccharomonospora* was first described by Nonomura & Ohara (21). The genus is characterized by the formation of single spores on the vegetative hyphae, and occasionally pairs and short chains on aerial hyphae, and contains *meso*-diaminopimelic acid, arabinose and galactose in the peptidoglycan (wall chemotype IV). Only one species, *Saccharomonospora viridis*, is described in *Bergey's Manual* (20). There are currently three validly described species of the genus, *Saccharomonospora azurea* Hu 1987 (8), *Saccharomonospora cyanea* Hu *et al.* 1988 (9) and *Saccharomonospora glauca* Greiner-Mai *et al.* 1988 (4). Recently, Yoon *et al.* (25, 26) investigated the rapid identification of the genus by using genomic DNA fragments and rRNA gene probes, and the RFLP analysis of PCR-amplified 16S rRNA genes. Kim *et al.* analysed the phylogenetic relationship among *Amycolatopsis*, *Pseudonocardia*, *Saccharomonospora* and *Saccharopolyspora* (11).

METHODS

Actinomycete strains. Strains XJ-54^T and XJ-58 were isolated with glycerol-asparagine agar and HV agar (7) from soil

samples collected from Xinjiang, People's Republic of China. Some of the type cultures for comparative studies were obtained from ATCC, NRRL and IFO.

Morphology. The media used for micromorphological studies were yeast extract-malt extract agar (ISP 2) and oatmeal agar (ISP 3) (23), and the incubation time was 14–21 d at 28 °C. Morphological observations of spores were made with a light microscope and a model EPMA-8705 electron microscope.

Cultural, physiological and biochemical tests. The media and procedures used for the study of the cultural and physiological characteristics of the strains were those described by Shirling & Gottlieb (23), Locci (17), Waksman (24), Gordon (5) and Gordon & Horan (6). Colour determinations were made by comparing the cultures with colour chips from the ISCC-NBS color charts standard samples no. 2106 (10).

Cell wall preparation. Cell walls were purified and analysed using the methods of Lechevalier & Lechevalier (15).

Whole-cell analysis of diagnostic cell wall amino acids. The procedures of Becker *et al.* (1) and Lechevalier & Lechevalier (14, 15) were used for whole-cell analysis.

Phospholipid analysis. The phospholipid analysis was carried out using the method of Lechevalier *et al.* (16).

Menaquinone analysis. Menaquinones were determined using the procedures of Collins (2).

16S rRNA gene sequencing. The chromosomal DNA was extracted using the procedures of Marmur (19), Jiang & Xu (12) and Zhou (27). The 16S rRNA gene was amplified by PCR (22) using *Taq* DNA polymerase (Sino-American

The GenBank accession numbers for the 16S rRNA gene sequences determined in this work are AF056710 (XJ-54^T) and AF056709 (XJ-58).

Biotechnology), primer A 7-26 (5' CCG TCG ACG AGC TCA GAG TTT GAT CCT GGC TCA G 3') and primer B 1523-1504r (5' CCC GGG TAC CAA GCT TAA GGA GGT GAT CCA GCC GCA 3'). The 1.5 kb amplified 16S rRNA gene fragment was purified by agarose gel electrophoresis. The purified fragments were directly sequenced using a FS-DNA sequencing kit (Applied Biosystems). The sequencing primers used were primer A, primer B, primer C 704-685r (5' TCT GCG CAT TTC ACC GCT AC 3') and primer D 1115-1100r (5' AGG GTT GCG CTC GTT G 3'). Sequencing was performed with a model 377 PRISM automatic sequencer (Applied Biosystems).

The 16S rRNA gene sequences of typical strains of related genera were obtained from GenBank.

Sequence alignment and phylogenetic analysis. The 16S rRNA gene sequences were manually aligned with the sequences of members of the order *Actinomycetales* by using the ae2 editor (18). Genetic distances were calculated by using PAUA 3.11. A phylogenetic tree was reconstructed by using treeing algorithms contained in the PHYLIP package.

RESULTS

Micromorphology

Strains XJ-54^T and XJ-58 were Gram-positive and non-acid fast. The vegetative hyphae were well developed, long, irregularly branched, fine (0.3–0.6 µm in diameter) and did not fragment. A large number of

paired spores were borne in longitudinal pairs on the vegetative hyphae. Most of spores were oval or ellipsoidal and 0.7–0.9 µm in diameter by 1.0–1.4 µm long; the spore surface was smooth. The aerial hyphae of strains XJ-54^T and XJ-58 were abundant on many media. Their aerial mycelium was irregularly branched, and fine (0.4–0.6 µm in diameter). Spores were borne in longitudinal pairs or singly on aerial mycelium. The spores were spherical or oval and 0.6–0.9 µm in diameter; the spore surface was smooth. The spores on both vegetative and aerial mycelium were non-motile. No sporangia were observed (Fig. 1).

Cultural characteristics

Table 1 shows the degree of growth and colour of both aerial and vegetative hyphae of strain XJ-54^T on various media tested. The aerial mycelium of strain XJ-54^T was abundant and yellow-white in colour on most of the media used but was light green-grey on Czapek agar. The reverse sides of colonies were light yellow on most media used. The sporulation of vegetative hyphae and aerial mycelium was abundant on most media tested. The strain produced light yellow-brown diffusible pigment on potato extract-glucose agar and did not produce melanin on tyrosine agar (ISP 7).

Physiological characteristics

The physiological reactions of strain XJ-54^T are shown in Table 2. Autolysis of strain XJ-54^T was observed when it was cultured on yeast extract-malt extract agar and nutrient agar.

Chemotaxonomy

The cell walls of strains XJ-54^T and XJ-58 contained *meso*-diaminopimelic acid. The whole-cell hydrolysates contained galactose and arabinose. No madurose was detected. Thus strains XJ-54^T and XJ-58 can be considered to have a type IV cell wall. The

Fig. 1. Scanning electron micrographs of spores of strain XJ-54^T.

Table 1. Cultural characteristics of strain XJ-54^T

Agar medium	Growth	Aerial mycelium	Vegetative mycelium	Diffusible pigment
Czapek agar	Good	Light grey green	Pale yellow	None
Glycerol-asparagine (ISP 5)	Moderate	None	Yellowish white	None
Inorganic salt-starch (ISP 4)	Poor	None	Yellowish brown	None
Oatmeal (ISP 3)	Good	Yellowish white	Pale yellow	None
Potato extract-glucose	Good	Pale yellowish pink	Light yellow	Light yellow-brown
Nutrient	Good	Yellowish white	Light yellow	None
Yeast extract-malt extract (ISP 2)	Good	Yellowish white	Light yellow	None

Table 2. Physiological and biochemical characters of strain XJ-54^T

Character	Reaction	Character	Reaction
Melanin	+	Enzyme production:	
Carbon source utilization:		Lecithinase	+
Adonitol	+	H ₂ S production	—
Cellobiose	+	NO ₃ reduction	+
D-Fructose	+	Autolysis	+
Inulin	+	Antibiosis:	
D-mannitol	+	<i>Aspergillus niger</i>	—
Raffinose	+	<i>Bacillus subtilis</i>	+
L-Rhamnose	+	<i>Escherichia coli</i>	—
D-xylose	+	Antibiotic resistance:	
Inositol	+	Neomycin (50 µg ml ⁻¹)	+
Nitrogen source utilization:		Rifampicin (50 µg ml ⁻¹)	+
Alanine	+	Growth at 45 °C	+
L-Histidine	+	50 °C	+
Proline	+		
Degradation of:			
Cellulose	+		
Urea	—		
Starch	+		

unusual type PIV phospholipid pattern was found (phosphatidyl ethanolamine, phosphatidyl choline and unknown glucosamine-containing phospholipids). The major menaquinones of XJ-54^T were MK-9(H₂), MK-9(H₄) and MK-7(H₄).

16S rRNA gene sequence comparisons and phylogenetic analysis

The nearly complete 16S rRNA sequence (1468 nucleotides) of strain XJ-54^T was determined. The sequence was compared with the corresponding sequences of the representative reference strains of cell wall types I–IV of the other genera of the order *Actinomycetales*. The results show that strain XJ-54^T, *Saccharomonospora viridis* (21), *Saccharomonospora caesia* (3) and *Saccharopolyspora hirsuta* are clustered into a group. The evolutionary distances among the four strains were 0.0096–0.0448. Fig. 2 shows a neighbour-joining phylogenetic tree constructed on the basis of the evolutionary distances calculated by using the 1516 positions that could be aligned.

DISCUSSION

Morphological characteristics and phospholipid pattern of XJ-54^T are the same as those of the genus *Actinobispora* (13). However, on the basis of the similarity of 16S rRNA gene sequences, strain XJ-54^T should be placed in the genus *Saccharomonospora*. The two strains, XJ-54^T and XJ-58 are clearly different from *Saccharomonospora azurea* and *Saccharomonospora cyanea* in morphological and cultural characteristics and growth temperatures. No aerial

hyphae of strains XJ-54^T and XJ-58 were observed on ISP 4 and ISP 5, spores were borne in pairs on vegetative hyphae, and growth temperature was between 45 and 50 °C while *Saccharomonospora azurea* and *Saccharomonospora cyanea* have white and dark blue aerial hyphae, no spores on vegetative hyphae and growth temperature of 28–40 °C. 16S rRNA gene sequences of strain XJ-54^T, *Saccharomonospora viridis* and *Saccharomonospora glauca* are different from each other, and the evolutionary distances are greater than 0.04. Therefore we propose strains XJ-54^T and XJ-58 as a new species named *Saccharomonospora xinjiangensis* sp. nov.

Description of *Saccharomonospora xinjiangensis* sp. nov.

Saccharomonospora xinjiangensis (xin.jiang.en'sis. M.L. neut. adj. *xinjiangensis* pertaining to Xinjiang, a province of north-west China).

Light yellowish vegetative hyphae. Yellow-white aerial mycelium on most media tested, light green-grey on Czapek agar. The sporulation of both vegetative hyphae and aerial mycelium are abundant on most media tested. The spores are borne in longitudinal pairs on vegetative hyphae, and in longitudinal pairs (or in a few cases singly) on aerial hyphae. Light yellow-brown diffusible pigment is produced on potato extract-glucose agar and no melanin is produced on tyrosine agar. Adonitol, inulin, cellobiose, fructose, rhamnose, mannitol, raffinose, xylose and inositol are utilized but no acid is produced from these carbon sources. Alanine, histidine and proline are utilized.

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1. **Becker, B., Gordon, R. E., Lechevalier, H. A. & Lechevalier, M. P. (1964).** Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole cell hydrolysates. *Appl Microbiol* **12**, 421–423.
2. **Collins, M. D. (1985).** Isoprenoid quinone analysis in bacterial classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
3. **Greiner-Mai, E., Kroppenstedt, R. M., Korn-Wendisch, F. & Kutzner, H. J. (1987).** Morphological and biochemical characterization and emended descriptions of thermophilic actinomycetes species. *Syst Appl Microbiol* **9**, 97–109.
4. **Greiner-Mai, E., Korn-Wendisch, F. & Kutzner, H. J. (1988).** Taxonomic revision of the genus *Saccharomonospora* and description of *Saccharomonospora glauca* sp. nov. *Int J Syst Bacteriol* **38**, 398–405.
5. **Gordon, R. E. (1967).** *The Taxonomy of Soil Bacteria*, pp. 293–321. Liverpool: Liverpool University Press.
6. **Gordon, R. E. & Horan, A. C. (1968).** A piecemeal description of *Streptomyces griseus* (Krausky) Waksman and Henrici. *J Gen Microbiol* **50**, 223–233.
7. **Hayakawa, M. (1990).** Selective isolation methods and distribution of soil actinomycetes. *Actinomycetologica* **4**, 103–112.
8. **Hu, R. (1987).** *Saccharomonospora azurea* sp. nov., a new species from soil. *Int J Syst Bacteriol* **37**, 60–61.
9. **Hu, R., Lin, C. & Wei, G. (1988).** *Saccharomonospora cyanea* sp. nov. *Int J Syst Bacteriol* **38**, 444–446.
10. **Kelly, K. L. (1964).** Inter-Society Color Council-National Bureau of Standards color-name charts illustrated with centroid colors published in US.
11. **Kim, S.-B., Hoon, J.-H., Kim, H., Lee, S. T., Park, Y.-H. & Goodfellow, M. (1995).** A phylogenetic analysis of the genus *Saccharomonospora* conducted with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 351–356.
12. **Jiang, C. L. & Xu, L. H. (1990).** Improvement on cell disruption methods of actinomycetes in DNA preparation. *Microbiology* **17**, 178–182.
13. **Jiang, C. L., Xu, L. H., Yang, Y. R., Guo, G. Y., Ma, J. & Liu, Y. (1991).** *Actinobispora*, a new genus of the order *Actinomycetales*. *Int J Syst Bacteriol* **41**, 526–528.
14. **Lechevalier, M. P. (1968).** Identification of aerobic actinomycetes of clinical importance. *J Lab Clin Med* **71**, 934–944.
15. **Lechevalier, M. P. & Lechevalier, H. A. (1980).** The chemotaxonomy of actinomycetes. In *Actinomycete Taxonomy* (Special Publication 6), pp. 227–291. Edited by A. Dietz & J. Thayer. Arlington, VA: Society for Industrial Microbiology.
16. **Lechevalier, M. P., De Bievre, C. & Lechevalier, H. A. (1977).** Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* **5**, 249–260.
17. **Locci, R. (1989).** Streptomycetes and related genera. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2451–2508. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
18. **Maidak, B. L., Larsen, N., McCaughey, M. J., Overbeek, R., Olsen, G. J., Fogel, K., Blandy, J. & Woese, C. R. (1994).** The

- Ribosomal Database Project. *Nucleic Acids Res* **22**, 3485–3487.
19. **Marmur, J. (1961).** A procedure for the isolation of DNA from microorganisms. *J Mol Biol* **3**, 208–218.
20. **McCarthy, A. J. (1989).** Genus *Saccharomonospora* Nonomura and Ohara 1971c 899^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, pp. 2402–2404. Edited by S. T. Williams, M. E. Sharpe & J. G. Holt. Baltimore: Williams and Wilkins.
21. **Nonomura, H. & Ohara, Y. (1971).** Distribution of actinomycetes in soil. X. New genus and species of monosporic actinomycetes in soil. *J Ferment Technol* **49**, 895–903.
22. **Saiki, R. K., Gelfand, D. H., Stoffe, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988).** Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491.
23. **Shirling, E. B. & Gottlieb, D. (1966).** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
24. **Waksman, S. A. (1967).** *The Actinomycetes*. New York: Ronald Press.
25. **Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996).** Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
26. **Yoon, J.-H., Lee, S. T., Kim, S.-B., Kim, W. Y., Goodfellow, M. & Park, Y.-H. (1997).** Restriction fragment length polymorphism analysis of PCR-amplified 16S ribosomal DNA for rapid identification of *Saccharomonospora* strains. *Int J Syst Bacteriol* **47**, 111–114.
27. **Zhou, H. (1978).** Determination of the G-C content of *Corynebacterium pekinense* and other bacterial species. *Acta Microbiol Sin* **18**, 134–139.