

Full Paper

Transfer of '*Thermoactinomyces glaucus*' IFO 12530 and '*Thermoactinomyces monosporus*' IFO 14050 to the genus *Saccharomonospora* as members of *Saccharomonospora glauca*

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Two available strains of '*Thermoactinomyces glaucus*' and '*Thermoactinomyces monosporus*,' '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050, were considered not to be members of the genus *Thermoactinomyces* and that they belonged to the genus *Saccharomonospora* on the basis of the colors of colonies and 16S rDNA sequences. Some chemotaxonomic characteristics also showed that the two strains belong to the genus *Saccharomonospora*. The two strains contained *meso*-diaminopimelic acid, galactose, and arabinose in the cell wall and MK-9(H₄) as the predominant menaquinone. The genomic DNAs of the two strains had a G+C content of 69 mol%. The 16S rDNAs of '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 showed only 1 and 2 bp sequence differences, respectively, from that of the type strain of *Saccharomonospora glauca*. Furthermore, the two strains of '*T. glaucus*' and '*T. monosporus*' and the type strain of *S. glauca* shared identical 16S–23S rDNA ITS sequences. The levels of DNA-DNA relatedness confirm that the two strains of '*T. glaucus*' and '*T. monosporus*' are members of *Saccharomonospora glauca*. Therefore it is proposed that '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 should be considered as strains belonging to *Saccharomonospora glauca*.

Key Words—reclassification; *Saccharomonospora glauca*; '*Thermoactinomyces glaucus*'; '*Thermoactinomyces monosporus*'

Introduction

Some monosporic actinomycete genera, such as *Micromonospora* (Ørskov, 1923), *Saccharomonospora* (Nonomura and Ohara, 1971), *Thermoactinomyces* (Tsiklinsky, 1899), and *Thermomonospora* (Henssen,

1957), have been described in the past. Some species of these genera, however, had been taxonomically confused or misclassified before the appearance of reliable taxonomic tools such as chemotaxonomic and genetic analyses. For example, *Saccharomonospora viridis* was previously classified as *Thermoactinomyces viridis* (Schuurmans et al., 1956) and as *Thermomonospora viridis* (Küster and Locci, 1963) before proposal of the genus *Saccharomonospora* by Nonomura and Ohara (1971). Moreover, thermophilic actinomycetes that produced predominantly single spores were often classified as micropolysporas because oc-

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casional short chains of spores were observed on aerial and substrate hyphae, e.g., '*Saccharomonospora caesia*' (Kurup, 1981). This species, previously described to be *Micropolyspora caesia* (Kalakoutskii, 1964), is now considered to be a synonym of *Saccharomonospora azurea* (Yoon et al., 1997, 1999). Recently, some species of the genus *Thermomonospora* were reclassified as a new genus or transferred to other genera (Zhang et al., 1998).

The genus *Thermoactinomyces* was one of the earliest actinomycete genera to be named. However, the genus *Thermoactinomyces* produces endospores (Cross et al., 1968, 1971; Lacey and Vince, 1971) and has low G+C contents (Lacey and Cross, 1989) and unsaturated menaquinone profiles (Collins et al., 1982; Tseng et al., 1990), which are not usually found in actinomycetes. Therefore the genus *Thermoactinomyces* is no longer classified within the class *Actinobacteria* (Stackebrandt et al., 1997) and is now described as being in the family *Bacillaceae* (Lacey and Cross, 1989; Park et al., 1993; Stackebrandt and Woese, 1981). The 16S rRNA oligonucleotide sequencing also showed that the genus *Thermoactinomyces* is phylogenetically related to the genus *Bacillus* (Stackebrandt and Woese, 1981). There are currently eight validly described *Thermoactinomyces* species and some invalid ones, such as '*Thermoactinomyces glaucus*,' '*Thermoactinomyces monosporus*,' '*Thermoactinomyces thermophilus*,' '*Thermoactinomyces antibioticus*,' and '*Thermoactinomyces albus*,' which were also reported. '*T. antibioticus*' (Craveri et al., 1964) and '*T. albus*' (Lacey and Cross, 1989) were described as synonyms of *T. thalophilus* and *T. vulgaris*, respectively (Lacey and Cross, 1989). Strain(s) of '*T. thermophilus*' (Waksman, 1961) have been lost. '*T. glaucus*' and '*T. monosporus*' were described by Henssen (1957) and Waksman and Corke (1953), respectively, but the original strains of the two species have also been lost. Moreover, it appears that the authors did not designate the type strains for '*T. glaucus*' and '*T. monosporus*.' One strain of '*T. glaucus*' and one of '*T. monosporus*,' which are listed in catalogues of some culture collections, were those described by Fergus (1964) and Nonomura and Ohara (1969), respectively. The two available strains of '*T. glaucus*' and '*T. monosporus*' formed blue-green aerial mycelium in some agar media unlike other *Thermoactinomyces* species. From the phylogenetic analysis based on 16S rDNA sequences, the two strains of '*T. glaucus*' and '*T.*

monosporus' were found to be not members of the genus *Thermoactinomyces*, but of the genus *Saccharomonospora*. Therefore the aim of this study was to determine the exact taxonomic position of the available strains of '*T. glaucus*' and '*T. monosporus*,' '*T. glaucus*' IFO 12530, and '*T. monosporus*' IFO 14050 by using reliable taxonomic tools.

Materials and Methods

Bacterial strains and culture conditions. '*Thermoactinomyces glaucus*' IFO 12530 and '*Thermoactinomyces monosporus*' IFO 14050 were obtained from IFO (Institute for Fermentation, Osaka, Japan). For an investigation of morphological characteristics, '*Thermoactinomyces glaucus*' IFO 12530 and '*Thermoactinomyces monosporus*' IFO 14050 were cultivated at 45°C on GC agar (Greiner-Mai et al., 1988), JCM (Japan Collection of Microorganisms, Wako, Japan) medium No. 104 agar, and IFO medium No. 229 agar. JCM medium No. 104 agar contains (per liter) yeast extract, 1 g; beef extract, 1 g; N-Z amine (type A), 2 g; sucrose, 10 g; and agar, 15 g (pH 7.3). IFO medium No. 229 agar contains (per liter) yeast extract, 5 g; glycerol, 50 g; CaCO₃, 1 g; and agar, 20 g (pH 7.3). The cell mass for the analyses of cell walls and menaquinones was obtained from trypticase soy broth (BBL Cockeysville, U.S.A.) culture. For DNA extraction, it was produced on trypticase soy broth (BBL) supplemented with glucose (0.75%, w/v). The two strains were cultivated at 45°C on a horizontal shaker at 150 rpm. The broth cultures were microscopically checked for purity before they were harvested by centrifugation.

Isolation of DNA. Chromosomal DNA was isolated and purified by the method described previously (Yoon et al., 1996).

Chemotaxonomic characterization. The isomer of diaminopimelic acid in the peptidoglycan was analyzed by using TLC according to the method described previously (Komagata and Suzuki, 1987). The sugar composition of the cell wall was determined by the method described by Saddler et al. (1991). Menaquinones were analyzed as described by Komagata and Suzuki (1987), using reversed-phase HPLC.

Determination of G+C content. The guanine plus cytosine (G+C) content was determined by the method of Tamaoka and Komagata (1984). DNA was hydrolyzed and the resultant nucleotides were ana-

lyzed by reversed-phase HPLC.

Sequence analysis of 16S rDNA and 16S–23S rDNA ITS. The sequencing of 16S rDNA and 16S–23S rDNA internally transcribed spacer (16S–23S ITS) were performed as described previously (Yoon et al., 1997, 1998). The alignments of sequences were performed by using CLUSTAL W (Thompson et al., 1994).

DNA-DNA hybridization. DNA-DNA hybridization was performed fluorometrically by the method of Ezaki et al. (1989), using photobiotin-labeled DNA probes and microdilution wells.

Nucleotide sequence accession numbers. The 16S rDNA sequences of '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 have been deposited in the GenBank under accession numbers AF139879 and AF139880, respectively. The 16S–23S ITS sequences of '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 have been deposited in the GenBank under accession numbers AF139881 and AF139882, respectively.

Results and Discussion

Macroscopic appearance

Colonies of '*Thermoactinomyces glaucus*' IFO 12530 and '*Thermoactinomyces monosporus*' IFO 14050 produced light green to bluish green aerial mycelia and dark green substrate mycelia on JCM medium No. 104 agar and IFO medium No. 229 agar and on GC agar as shown with the type strains of *Saccharomonospora glauca* (Greiner-Mai et al., 1988).

Chemotaxonomy

'*Thermoactinomyces glaucus*' IFO 12530 and '*Thermoactinomyces monosporus*' IFO 14050 contained meso-diaminopimelic acid, galactose, and arabinose in the cell wall, indicating that the wall chemotype is type IV (Lechevalier and Lechevalier, 1970). However, the genus *Thermoactinomyces* contains meso-diaminopimelic acid but no diagnostic sugars in the cell wall (Lacey and Cross, 1989), indicating the wall chemotype III (Lechevalier and Lechevalier, 1970). The two strains of '*T. glaucus*' and '*T. monosporus*' were found to have MK-9(H₄) as major isoprenoid quinone, whereas the genus *Thermoactinomyces* has been known to have MK-7 or MK-9 by Collins et al. (1982) and MK-7 or MK-8 and MK-9 by Tseng et al. (1990). Both '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 had a G+C content of 69 mol%, which is a high G+C content characteristic of actinomycetes, whereas *Thermoactinomyces* species have a G+C content ranging from 52 to 54.8 mol% (Tm) (Lacey and Cross, 1989). Therefore these characteristics support that '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 are members of the genus *Saccharomonospora*, not the genus *Thermoactinomyces* (Table 1).

Sequence analyses of the 16S rDNA and 16S–23S ITS

'*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 exhibited the highest levels of 16S rDNA similarity to the genus *Saccharomonospora*, but not to the genus *Thermoactinomyces*. In particular, 16S rDNAs

Table 1. Some phenotypic characteristics of '*Thermoactinomyces glaucus*' IFO 12530, '*Thermoactinomyces monosporus*' IFO 14050, and the genera *Thermoactinomyces* and *Saccharomonospora*.

Taxon	Blue-green mycelium	Cell wall chemotype ^a	Major menaquinone(s)	G+C content (mol%)
' <i>Thermoactinomyces glaucus</i> ' IFO 12530	+	IV	MK-9(H ₄)	69
' <i>Thermoactinomyces monosporus</i> ' IFO 14050	+	IV	MK-9(H ₄)	69
Genus <i>Thermoactinomyces</i> ^b	–	III	MK-7 or MK-9, ^c MK-7 or MK-8 and MK-9 ^d	52–54.8
Genus <i>Saccharomonospora</i> ^e	+	IV	MK-9(H ₄)	66–70

^aBased on Lechevalier and Lechevalier (1970).

^bData from Lacey and Cross (1989).

^cData from Collins et al. (1982).

^dData from Tseng et al. (1990).

^eData from McCarthy (1989) and Embley (1991).

Table 2. Levels of DNA-DNA relatedness between '*Thermoactinomyces glaucus*' IFO 12530 and '*Thermoactinomyces monosporus*' IFO 14050 and between the two strains and *Saccharomonospora* species.

Species	Percentage reassociation with	
	' <i>T. glaucus</i> ' IFO 12530	' <i>T. monosporus</i> ' IFO 14050
' <i>Thermoactinomyces glaucus</i> ' IFO 12530	100	95.1
' <i>Thermoactinomyces monosporus</i> ' IFO 14050	102	100
<i>Saccharomonospora glauca</i> KCTC 9479 ^T	97.5	90.8
<i>Saccharomonospora azurea</i> KCTC 9475 ^T	16.3	15.5
<i>Saccharomonospora cyanea</i> KCTC 9478 ^T	18.8	17.9
<i>Saccharomonospora viridis</i> KCTC 9115 ^T	9.6	8.7

of '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 showed only 1 and 2 bp sequence differences, respectively, to 16S rDNA of the type strain of *Saccharomonospora glauca* (DSM 43769^T). The 16S–23S rDNA internally transcribed spacer (16S–23S ITS) sequences, together with 16S rDNA sequences, also provide supporting evidence that the two strains of '*T. glaucus*' and '*T. monosporus*' are closely related to *Saccharomonospora glauca*. '*T. glaucus*' IFO 12530, '*T. monosporus*' IFO 14050, and the type strain of *Saccharomonospora glauca* (DSM 43769^T) were found to have identical 16S–23S ITS sequences. The 16S–23S ITS sequences have already been proven to be useful for elucidating the interspecific and intraspecific relationships of the genus *Saccharomonospora* (Yoon et al., 1997). Six strains of *Saccharomonospora glauca* had identical 16S–23S ITS sequences and exhibited levels of 16S–23S ITS similarity of 83.7–91.5% with other validly described *Saccharomonospora* species (Yoon et al., 1997).

DNA relatedness

DNA-DNA hybridization was performed between '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 and between the two strains and the type strains of some *Saccharomonospora* species. The result of DNA-DNA relatedness confirms that the two strains of '*T. glaucus*' and '*T. monosporus*' are members of *Saccharomonospora glauca* (Wayne et al., 1987). Levels of DNA-DNA relatedness between '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 were 95.1 or 102% (Table 2). '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 exhibited levels of DNA-DNA relatedness of 97.5 and 90.8% with the type strain of *Saccharomonospora glauca*, respectively, but levels of

DNA-DNA relatedness below 20% with type strains of the other three validly described *Saccharomonospora* species, *S. azurea*, *S. cyanea*, and *S. viridis* (Table 2).

The results of morphological and chemotaxonomic properties and genetic similarities showed that '*T. glaucus*' IFO 12530 and '*T. monosporus*' IFO 14050 are more taxonomically related to the genus *Saccharomonospora*, especially to *S. glauca*, than to the genus *Thermoactinomyces* (Tables 1 and 2). Therefore, it is proposed that these two strains formerly described as '*T. glaucus*' and '*T. monosporus*' should be transferred to the genus *Saccharomonospora* as members of *Saccharomonospora glauca*.

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