Short Communication

THE PHYLOGENETIC RELATIONSHIPS OF THE GENUS *SPOROPACHYDERMIA* RODRIGUES DE MIRANDA (SACCHAROMYCETACEAE) BASED ON THE PARTIAL SEQUENCES OF 18S AND 26S RIBOSOMAL RNAS[†]

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Since the genus *Sporopachydermia* Rodrigues de Miranda was proposed (9), the following three species, *Sp. lactativora* Rodrigues de Miranda (type species), *Sp. cereana* Rodrigues de Miranda, and *Sp. quercuum* Lachance (3) have been described (10). The anamorphs of the former two species, *Cryptococcus lactativorus* Fell et Phaff and *Cr. cereanus* Phaff et al. matched the definition of the genus *Cryptococcus* Kützing emend. Phaff et Spencer by their assimilation of inositol and their lack of pseudomycelium (1, 8, 11). However, they were distinct from other cryptococci by the absence of starch-like compounds or capsule. The two species were assumed not to be basidiomycetous in affinity (1, 8, 11), and the discovery of ascospores provided the final demonstration that the two anamorphs are related to ascomycetous yeasts (9, 10). When the third species, *Sp. quercuum*, was isolated, it was shown to give a positive reaction in a diazonium blue B test different from any other ascomycetous yeast species (4). The phylogenetic position of the genus *Sporopachydermia* is accordingly of interest.

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Abbreviations: Co-Q, coenzyme Q or ubiquinone; Q₉ and Q₈, Co-Q homologues with 9 and 8 isoprene units, respectively, in a side chain; rRNA, ribosomal RNA.

This paper describes the partial base sequences of 18S and 26S rRNAs of strains of the three ascomycetous yeast species mentioned above from the phylogenetic point of view.

Sporopachydermia lactativora IFO 1867 (=CBS 5771, mating type α , type strain of *Cr. lactativorus*) and IFO 10565 (=CBS 6192, mating type α , type strain), *Sp. cereana* IFO 10013 (=CBS 6644, type strain) and IFO 10188 (=CBS 6645), and *Sp. quercuum* IFO 10117 (=CBS 8070, type strain) were examined for the partial base sequences of 18S and 26S rRNAs (6, 12, 13) (Fig. 1).

In the partial base sequences of positions 493 through 622 (130 bases) in 26S rRNA, these five strains had very high maximum homologies (95–99%) to one another. There were 73–75% maximum homologies between the five strains and *Saccharomyces cerevisiae* IFO 2376.

In the partial base sequences of positions 1611 through 1835 (225 bases) in 26S rRNA, there were no base differences among the five strains examined. In contrast, the number of base differences was 21–20 between the five strains and *S. cerevisiae* IFO 2376.

In the partial base sequences of positions 1451 through 1618 (168 bases) in 18S rRNA, *Sp. lactativora* IFO 10565 showed base differences at 1482 (G to A), 1509 (U to G), 1552 (U to C), 1589 (C to U), 1592 (G to U), and 1600 (C to A) compared with *S. cerevisiae* IFO 2376. The two strains examined of *Sp. cereana* and *Sp. lactativora* showed no base differences within the respective species. *Sporopachydermia quercuum* IFO 10117 coincided in the base sequence with the two strains of *Sp. lactativora*. The 5-base sequences in the fingerprint segment (13) of the genus *Sporopachydermia* were the same as some of the Q₉-equipped yeast genera such as *Debaryomyces* Lodder et Kreger-van Rij (AUUAA) (18), *Schwanniomyces* Klöcker (AUUAG) (16), and *Holleya* Yamada (UUUUC) (14). The base sequences of the genus *Sporopachydermia* were divided into two subgroups: the first subgroup was comprised of UCGAC (*Sp. lactativora* and *Sp. quercuum*), and the second was of UCGUC (*Sp. cereana*). In addition, the two species classified in the first subgroup were characterized by A at position 1482, and the only species in the second subgroup was by G. Between the members of the two subgroups, there were 2 base differences. The number of base differences was 10 between the strains of *Sporopachydermia* species and *S. cerevisiae* IFO 2376.

Based on the calculated number of base differences in the partial base sequences of 18S rRNA, a dendrogram was drawn by the simple linkage method among the strains of the three species of the genus *Sporopachydermia* along with those of the Q₉-equipped species of the genera *Debaryomyces*, *Schwanniomyces*, *Holleya*, *Dipodascus* de Lagerheim, *Endomyces* Reess, *Metschnikowia* Kamienski, *Stephanoascus* Smith et al., *Yarrowia* van der Walt et von Arx, *Arxula* van der Walt et al., and *Geotrichum* Link (Fig. 2). The members of the two subgroups of the genus *Sporopachydermia* constituted their own cluster with 2 base differences. To this cluster, *H. sinecauda* was linked at 7 base differences (number of base differences was 7 between *Sp. cereana* and *H. sinecauda*). *Dipodascus albidus*, *E.*

(A)		.493	.501	.511	.521	.531	.541				
1	<i>Sp.cereana</i> IFO 10013*	GGCCAAC	AUCGAUUUGAG	UGGUAGGAUA	AUGCCGUUGG	AAUGUAGCUU	CAUUCGUGAA				
2	<i>Sp.cereana</i> IFO 10188	-----	-----	-----	-----	-----	-----				
3	<i>Sp.lactativora</i> IFO 1867	-----	-----	-----	-A-	-----	-----				
4	<i>Sp.lactativora</i> IFO 10565*	-----	-----	-----	-A-	-----	-A-				
5	<i>Sp.quercuum</i> IFO 10117*	-----	-----	A	-----	-----	-----				
6	<i>S.cerevisiae</i> IFO 2376	---G-	--AG--UG-	--C-	---AU--A-	-----	GCC---GU--				
		.551	.561	.571	.581	.591	.601	.611	.621		
1		GUGUUUAGG	CUUCGGUGAU	ACUGCCNACU	UGGAUCGAGG	ACUGCGUCUU	UUGACUAGGA	UNGUUGGCUUA	AU		
2		-----	-----	-----	N-	-----	-----	-G-	--		
3		-----	-U-	-----	-U-	C-	-----	-G-	--		
4		-----	-U-	-----	-U-	C-	-----	-G-	--		
5		-----	-----	-A-	-U-	-----	-----	-G-	--		
6		--A-----C	--GU--GA--	-----AG-	G--CU-	-----A-G-	AA-U-A-	-C-----A-	--		
(B)		.1611	.1621	.1631	.1641	.1651	.1661				
1	<i>Sp.cereana</i> IFO 10013*	ACCUGGAUUAU	GGAUUCUUA	CGUAACGUA	ACUGAAUGUG	GAGACGCCG	CGUAAGCCCU				
2	<i>Sp.cereana</i> IFO 10188	-----	-----	-----	-----	-----	-----				
3	<i>Sp.lactativora</i> IFO 1867	-----	-----	-----	-----	-----	--N-				
4	<i>Sp.lactativora</i> IFO 10565*	-----	-----	-----	-----	-----	-----				
5	<i>Sp.quercuum</i> IFO 10117*	-----	-----	-----	-----	-----	-----				
6	<i>S.cerevisiae</i> IFO 2376	--U-	-----	-----	-----	-----	-U--G	--CG-			
		.1671	.1681	.1691	.1701	.1711	.1721	.1731	.1741		
1		AGGAGGAGUC	AUNUNUUCUU	CUUACACGU	UAUCACCCUG	GAAUUGGUU	AUCNGGAGAU	AGGGUNUUU	GGCUNGAAGA		
2		-----	-----	-----	-----	-----	-----	-----	-----		
3		-----	-----	-----	-----	-----	--C-	-----	-----		
4		-----	--N-	-----	-----	-----	--C-	-----	-----		
5		-----	-----	-----	-----	-----	-----	-----	-----		
6		G-----U	--C-U-	-----	-----	-----	--C-	G--C-	---G-		
		.1751	.1761	.1771	.1781	.1791	.1801	.1811	.1821	.1831	
1		GCUCAGACAU	UCACGUAGGU	CUNGUGCGCU	UACNCGGCC	CUUGAAAAUC	CACAGGAAGG	AAUAGUUUUC	AUGCCAGGUN	GUACU	
2		-----	-----	-----	-----	-----	--N-	-----	--N-	-----	
3		-----	-----	-----	-----	-----	--N-	-----	--N-	-----	
4		-----	-----	-----	-----	-----	--N-	-----	--N-	-----	
5		-----	-----	-----	--GA-	-----	--N-	-----	-----	-----	
6		-G-C-	---C	-UU--G-C-	-CG-	-GUGA-	-G-	-----	-----	-U--C	
(C)		.1451	.1461	.1471	.1481	.1491	.1501	.1511			
1	<i>Sp.cereana</i> IFO 10013*	GGGCGCGACG	CGCGCUACAC	UGACGGAGCC	AGCGAGUUCGU	CCCUUGGCCG	AGAGGUCUGG	GUAAUCUUGU			
2	<i>Sp.cereana</i> IFO 10188	-----	-----	-----	-UCGU	C-	-----	-----			
3	<i>Sp.lactativora</i> IFO 1867	-----	-----	-----	-A-	-UCGA	C-	-----			
4	<i>Sp.lactativora</i> IFO 10565*	-----	-----	-----	-A-	-UCGA	C-	-----			
5	<i>Sp.quercuum</i> IFO 10117*	-----	-----	-----	-A-	-UCGA	C-	-----			
6	<i>S.cerevisiae</i> IFO 2376	-----	-----	-----	-----	-CUA	A-	-----			
		.1521	.1531	.1541	.1551	.1561	.1571	.1581	.1591	.1601	.1611
1		GAACACUCCGU	CGUCGUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGUA	AUUCAUACAGA	UUGCGUUGAU	UACGUCCC
2		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
3		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
4		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
5		-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
6		-----	-----	-----	-U-	-----	-----	-C-	-G-	-----	-C

Fig. 1. The primary partial base sequences of 18S and 26S rRNAs of strains of species in the genus *Sporopachydermia*. The primary partial base sequences of 26S rRNA (A and B) and 18S rRNA (C) were aligned. The Arabic numerals indicate positions in *Saccharomyces cerevisiae* (2,7,13). IFO, Institute for Fermentation, Osaka, Japan. N represents A, G, C, or U. *Type strain.

fibuliger, *G. candidum*, *S. cerevisiae*, *D. hansenii*, *Schw. occidentalis*, *St. ciferrii*, and *A. terrestris* were also linked at 7 base differences (number of base differences was 7 between *H. sinicauda* and *Schw. occidentalis*). *Metschnikowia bicuspidata* and *Y. lipolytica* were at 8 and 9 base differences, respectively.

Lachance (4) described the third species of the genus *Sporopachydermia*, *Sp. quercuum*, to have a unique physiological character: it gives a positive reaction in

Fig. 2. A dendrogram based on the calculated number of base differences in the partial base sequences of 18S rRNA in *Sporopachydermia* species and their related, Q_8 -equipped species. The dendrogram was drawn by the simple linkage method. The number of base differences was calculated from the sequence data obtained presently and previously (positions 1451 through 1618, 168 bases) (14-16, 18). The only species, *E. fibuliger* is Q_8 -equipped (15). IFO, Institute for Fermentation, Osaka, Japan; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Wako, Saitama, Japan. * Type strain.

the diazonium blue B (DBB) test. This character is generally restricted to basidiomycetous yeasts. According to his data (5), *Sp. lactativora* (type species) also has this character. Based on the DBB reaction, the first subgroup of the genus *Sporopachydermia* in the partial base sequences of 18S rRNA is comprised of DBB-positive species. In contrast, the second subgroup is of a DBB-negative species. The members of the genus *Sporopachydermia* are assumed not to be so homogeneous phylogenetically as those of the genus *Debaryomyces*; the number of base differences was 2 in the former and 1 in the latter.

In the present experiment, the three species of the genus *Sporopachydermia* were found to be phylogenetically classified in ascomycetous yeasts, although two of the three react positively with the DBB reagent (5).

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