THE PHYLOGENETIC RELATIONSHIP OF THE GENERA
PHAFFIA MILLER, YONEYAMA ET SONEDA AND
CRYPTOCOCCUS KÜTZING EMEND. PHAFF ET
SPENCER (CRYPTOCOCCACEAE) BASED ON THE
PARTIAL SEQUENCES OF 18S AND 26S
RIBOSOMAL RIBONUCLEIC ACIDS¹

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The partial sequences of 18S and 26S rRNAs were studied in eighteen strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The positions determined were 1451 through 1618 (168 bases) of 18S rRNA, and 492 through 625 (134 bases), and 1686 through 1835 (150 bases) of 26S rRNA. These three determinations showed that the genus *Cryptococcus* is phylogenetically heterogeneous: the maximum homologies of 26S rRNA were 65–94% in the positions 492 through 625 and 69–93% in the positions 1686 through 1835, and the base differences of 18S rRNA were 0–13 in the positions 1451 through 1618. *Filobasidiella neoformans*, a teleomorph of the genus *Cryptococcus*, had partial sequences somewhat different from the species examined of the genus *Cryptococcus* (maximum homologies, 74–84% and 74–86%; base differences, 4–12, respectively). *Candida humicola* was included in the cluster of the genus *Cryptococcus*. However, the base differences of the species were not so small (maximum homologies, 69–77% and 72–84%; base differences, 5–13). The genus *Phaffia* constituted its own cluster separate from the species of *Cryptococcus*, *Candida*, and *Filobasidiella* (maximum homologies, 61–70% and 71–85%; base differences, 6–16, respectively). So the genus *Phaffia* is retained as an

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Abbreviations: S, Svedberg value; rRNA, ribosomal RNA; Co-Q, coenzyme Q or ubiquinone; Q-*n*, a Co-Q or ubiquinone homologue with a specified number of isoprene units in a side chain, e.g., Q-10.

independent genus.

The genus *Phaffia* Miller, Yoneyama et Soneda was introduced with a single species, *Phaffia rhodozyma* Miller, Yoneyama et Soneda (12). This genus is characterized by the ability to ferment sugars, the inability to assimilate inositol, the formation of a carotenoid pigment (astaxanthin), the production of amyloid compounds, and having the Q-10 system (11, 18). The characteristics distinguishing the genus *Phaffia* from the genus *Rhodotorula* Harrison are the production of amyloid compounds and the ability to ferment sugars, and from the genus *Cryptococcus* Kützing emend. Phaff et Spencer they are the ability to ferment sugars and the inability to assimilate inositol (2, 11, 14). The strains classified in the genus *Phaffia* were recently found to contain xylose in the cells, as in all of the strains classified in the genus *Cryptococcus* (15, 16).

This paper is concerned with the phylogenetic relationship of the genus *Phaffia* to the genus *Cryptococcus* on the basis of the partial sequences of 18S and 26S rRNAs. A preliminary report has been published (20).

MATERIALS AND METHODS

Yeast strains examined. The sixteen strains used in this experiment of *Phaffia*, *Cryptococcus*, and *Candida* species are listed in Table 1. In addition to the basidiomycetous, anamorphic yeast species mentioned above, we studied *Filobasidiella neoformans* Kwon-Chung, a teleomorph of *Cryptococcus neoformans* (Sanferice) Vuillemin, regarding the partial sequences of 18S and 26S rRNAs. These yeast strains were cultured as described previously (19).

Preparation of rRNA. The rRNAs of the strains were prepared as described previously (19).

Partial sequence determinations of 18S and 26S rRNAs. The partial sequences of 18S and 26S rRNAs were determined by the method of Lane et al. (9) using reverse transcriptase. The oligonucleotide DNA primers used in this experiment were 5'-ACGGGCGGTGTGTAC-3' which is complementary to the sequence of the positions 1641 through 1627 (in *Saccharomyces cerevisiae*) (10) of 18S rRNA, and 5'-GGTCCGTGTTTCAAGACGG-3' and 5'-TTGGAGACCTGCTGCGG-3', which are complementary to the sequences of the positions 654 through 636 and 1857 through 1841, respectively, (in *S. cerevisiae*) (3) of 26S rRNA.

Chemicals. The chemicals used in this experiment were the same as those described previously (19). The oligonucleotide DNA primer for the partial sequence determination of 18S rRNA was supplied by H. Oyaizu, Toyama University, Toyama, Japan.

Table 1. The strains examined of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species.

Species and strain	Other designation
<i>Cryptococcus</i> Kützing emend. Phaff et Spencer	
<i>Cr. albidus</i> (Saito) Skinner (1947) IFO 0378*	CBS 142
<i>Cr. ater</i> Castellani ex Cooke (1966) IFO 1862*	CBS 4685
<i>Cr. dimennae</i> Fell et Phaff (1967) IFO 1863*	CBS 5770
<i>Cr. elinovii</i> Golubev et Tauson (1979) IFO 10331*	CBS 7051
<i>Cr. flavus</i> (Saito) Phaff et Fell (1970) IFO 0407*	CBS 331, as <i>Rhodotorula flava</i>
<i>Cr. hungaricus</i> (Zolt) Phaff et Fell (1970) IFO 1052*	CBS 4214, as <i>Dioszegia hungarica</i>
<i>Cr. kuetzingii</i> Fell et Phaff (1967) IFO 1866*	CBS 1926
<i>Cr. laurentii</i> (Kufferath) Skinner (1947) var. <i>flavescens</i> (Saito) Lodder et Kreger-van Rij (1952) IFO 0384*	CBS 942
<i>Cr. laurentii</i> (Kufferath) Skinner (1947) var. <i>laurentii</i> IFO 0609*	CBS 139
<i>Cr. luteolus</i> (Saito) Skinner (1947) IFO 0411*	CBS 943
<i>Cr. magnus</i> (Lodder et Kreger-van Rij) Baptist et Kurtzman (1976) IFO 0698*	CBS 140
<i>Cr. skinneri</i> Phaff et do Carmo-Sousa (1962) IFO 1872*	CBS 5029
<i>Cr. terreus</i> di Menna (1954) IFO 0727*	IBL 537, CBS 1895
<i>Filobasidiella</i> Kwon-Chung	
<i>F. neoformans</i> Kwon-Chung (1975) YK-353* YK-354*	NIH 12, CBS 6956, mating type α NIH 430, CBS 6955, mating type α
<i>Candida</i> Berkhout	
<i>C. humicola</i> (Daszewska) Diddens et Lodder (1942) IFO 0760*	CBS 571, ATCC 14438, IBL 3387, as <i>Vanrija humicola</i>
<i>Phaffia</i> Miller, Yoneyama et Soneda	
<i>Ph. rhodozyma</i> Miller, Yoneyama et Soneda (1976) IFO 10129* IFO 10130	ATCC 24202, CBS 5905 ATCC 24228

* Type strain.

Abbreviations: IFO, Institute for Fermentation, Osaka, Osaka, Japan; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; IBL, Laboratório de Microbiologia, Centro de Biologia, Instituto Gulbenkian de Ciencia, Oeiras, Portugal; YK, Yamazaki-Komagata, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan; NIH, National Institute of Health, Bethesda, Maryland, U. S. A.; ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.

RESULTS

Partial sequence determination of positions 492 through 625 of 26S rRNA

The primary partial sequences of the eighteen strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species were aligned (Fig. 1). Because the base sequences were highly variable in this region, the maximum homologies (%) were calculated by a computer analysis using a Hitachi DNAsis (7).

As shown in Fig. 2, the maximum homologies were 94% and 99% between the two strains of *Ph. rhodozyma* and *F. neoformans*, respectively. High maximum homologies were obtained between *Cr. albidus* and *Cr. kuetzingii* (90%), between *Cr. ater* and *Cr. magnus* (94%), and between *Cr. elinovii* and *Cr. terreus* (91%). The other maximum homologies were calculated as 64–85% among the species examined in the genus *Cryptococcus*.

Candida humicola had maximum homologies of 69–77% with *Cryptococcus*

				.501	.511	.521	.531	.541				
1	<i>Cr.albidus</i>	IFO 0378*		GGGUCAACA	UCAGGUUU.GAU	C.GAUGGAUA	AAGGCACGGGG	AAGGUAGCA.	CUCUCGGGUV	1		
2	<i>Cr.ater</i>	IFO 1862*		GGGUCAACA	UCCAGUUUUGAU	CCGC.GGAUA	A.GGCUGGAGG	AAGCUAG.UA	CCCUCCGGGUR	2		
3	<i>Cr.dimennae</i>	IFO 1863*		GGGUCAACA	U.CAGUUUCUGA.	CCGGCGGAAA	AGGGCUGGAG	AAGGUGGC..	CCCUVGGGUG	3		
4	<i>Cr.elinovii</i>	IFO 10331*		GGGUCAACA	U.CAGUUNUGAU	C.GCUGGAAA	AGGGCAGGAGG	AAUGUAGCA.	CUCUCGGGUV	4		
5	<i>Cr.flavus</i>	IFO 0407*		GGGUCAACA	U.CAGUUU.GAG	CCGGCGGAUA	AUGGCAGAGGG	AAUGUGGCA.	CCCCCGGGUG	5		
6	<i>Cr.hungaricus</i>	IFO 1052*		GGGUCAACA	U.CAGUUUUGGU	GGCGUGGAUA	AGAACCGAUUG	AAGGURGC..	CCCUVGGGUG	6		
7	<i>Cr.kuetzingii</i>	IFO 1866*		GGGU..AACA	U.CAGUUUUGAU	CGAUGGAUA	A.GGCACGGGG	AAGGUAGCA.	CUCUCGGGUG	7		
8	<i>Cr.laurentii</i> var. <i>flavescens</i>	IFO 0384*		GGGUCAACA	U.ACGUUUUG.U	UCGGUGGAUA	AGGGCAGUUGG	AAGGUGGCA.	CCCUVGGGUG	8		
9	<i>Cr.laurentii</i> var. <i>laurentii</i>	IFO 0609*		NGGUCAACA	U.CAGUUUUG.U	CCGGUGGAUA	AGGACGGAUUG	AAGGUGGC..	CCCUVGGGUG	9		
10	<i>Cr.luteolus</i>	IFO 0411*		GGGUCAACA	U.CAGUUUUG.A	GGCGUGGACC	AAGGUAGAGGG	AACGUAGCU.	CCCUCCGGGAG	10		
11	<i>Cr.magnus</i>	IFO 0698*		GGGUCAACA	U.CAGUUUUGAU	.CGCUGGAUA	AAGGCUGGAGG	AAGCUAG.UA	CCCUCCGGGUR	11		
12	<i>Cr.skinneri</i>	IFO 1872*		GGGUCAACA	U.CAGUUUGGAU	CGGCGGAUAA	AG..AUGAGGG	AAGGUGGCG.	CU..UVGGGUG	12		
13	<i>Cr.terreus</i>	IFO 0727*		GGGUCAACA	U.CAGUUUUGAU	.CGCUGGAAA	AGGGCAGGAGG	AAUGUAGCA.	CUCUCGGGUG	13		
14	<i>F.neoformans</i>	YK 353*		GGGUCAACA	U.CAGUUCUGAU	.CGGUGGAUA	AGGGCUVGAGG	AAUGUGGCAC	UCUUCGGGUG	14		
15		YK 354*		GGGUCAACA	U.CAGUUCUGAU	.CGGUGGAUA	AGGGCUVGAGG	AAUGUGGCAC	UCUUCGGGUG	15		
16	<i>C.humicola</i>	IFO 0760*		GGGUCAACA	U.CAGUUUUG.U	CCGGUGGAUA	AAGGCAGGAAG	AAAGUGGCUC	.CCUCCGGGAG	16		
17	<i>Ph.rhodozyma</i>	IFO 10129*		AGGUCAGCA	U.CAGUUUCGG.	GGCGUGGAAA	ACGGGCGGGGG	AAGGUGGCA.	.UCUCCGGAU	17		
18		IFO 10130		AGGUCAGCA	U.CAGUUUCGG.	GGCGUGGAAA	ACGGGCGGGGG	AAGGUGGCA.	.UCUCCGGAU	18		
19	<i>S.cerevisiae</i>	IFO 2376		GGGCCAGCA	U.CAGUUUUGG.	UGGCAGGAUA	AAUCCAU..AGG	AAUGUAGCUU	GCCUCCGUAA	19		
				.551	.561	.571	.581	.591	.601	.611	.621	
1	AACUUUAGC	CACGCGUCAU	ACA.U.....UGAU	UGGGA.CUGAGG	AACGCAGCA.	UCCUN.AUGGC	CGGGAUUCGUCC	AVGGU				1
2	AACUUUAGC	CUNCGUGCAU	ACAGU.G.....G.U	UGGGA.CURAGG	AACGCAGCAA	CGCUUUNUGGC	CGGGAUUCGUCC	VA.GU				2
3	UGUUUAGC	UUCUGUGCAU	ACG.UCG.....G.U	UGGGA.CUGAGG	AAUGCACUUG	CGCA.A.GGC	NGGGGUUCSCCC	AS.GU				3
4	AACUUUAGC	CNNCYNUGU	ACAGUNG.....U	UGGGA.CUAAGG	AACGCAGCAU	.CCUUUAUAGGC	CGGGGUUCGCC	A.CG				4
5	UGUUUAGC	CYNNYUUGCAU	AC.GUCG.GC..CC.	..AGA.CUAAGG	AAUGCAGCUA	.CCUUUAUAGGC	CGGGGUUCGCC	CCN				5
6	UGUUUAGC	UUUCUGUGCAU	ACACU.GCCU..G..	..AGA.CUYAGG	ACUGCAGCU.	.CUAAC..GGR	CNGGCUUCGGC	A.CG				6
7	AACUUUAGC	CUUGGUGCAU	ACA.UAUACAUUGAU	UGGGACUUGAGG	AACGCAGCAU	.CCUUUU.GGC	CGGUUUNUGCC	AAGGU				7
8	UGUUUAGC	GCACGUGUGCA	GUACAGUCG...GAU	.GAGAGCUGAGG	AAUCGAGC.U	GCCUGUAUAGGC	CGGGGUUCGCC	GNNGU				8
9	UGUUUAGC	C.NNNUNGCAU	ACA.UCG.....GVU	.GAGA.CUNAGG	AACGCAGC.U	HCCUUUAUAGC	YGGGGUUNGCC	ACYGU				9
10	UGUUUAGC	CCNNUUUGCAU	ACACU.G.C.UUGA.	..GGAGCUGAGG	AAUGCAGCUG	CCCUUUAUAGGC	CGGGGUUCGCC	ACYGU				10
11	AACUUUAGC	CUNCGUGCAU	ACAGU.G.....G.U	UGGGA.CUNAGG	AACGCAGCAG	CCCUUUAUAGGC	CGGGGUUCGUCC	.CGGU				11
12	UGUUUAGC	CCRRUGUGU	AU.ACGCU.R..GUV..U	..RGACUNAGGV	SUGCGCU.N	S.VUUUNUGGC	CGGGGUUAGCCC	AGCGU				12
13	AACUUUAGC	CUNSGUGCAU	ACAGU.G.....G.U	UGGGA.CUNAGG	AACGCAGCA.	UCCUUUAUAGGC	CGGGCUUCGCC	AVNGU				13
14	GUGUUUAGC	CUCCUGUGCAU	ACACU.G.....G.U	UGGGA.CUGAGG	AAUGCAGCU.	CCCUUUAUAGGC	CGGGGUUCGCC	ACGGU				14
15	GUGUUUAGC	CUCCUGUGCAU	ACACU.G.....G.U	UGGGA.CUGAGG	AAUGCAGCU.	CCCUUUAUAGGC	CGGGGUUCGCC	AGGGU				15
16	UGUUUAGC	UUUCURUGCAU	ACACU.R..GAG...GAGA.CUYAGG	A.GC.UGCAG	CUCCUUUUGGC	CGGGGUUCGCC	AHAGU					16
17	GUGUUUAGC	CCCGUUUGAGG	..AGCAUCGCGUG..	.GGAGCUGAGG	AACCGCGCG	CCCUUUA..	CGGGGUUCGUCC	CGGA				17
18	GUGUUUAGC	CCCGUUUGGAU	..UGCAUCGCGUG..	.GGAGCUGAGG	AACCGCGCG	CCCUUUA..	AGGGGUUCGUCC	CGGA				18
19	GUUUUAGC	CUGUGGG.A.AU	AC..U.GCCA..GCU	.GGGAA.UGAGG	ACUGCAGCU	AAGU.CAAGGA	UG..CUGCAUA	AUGGU				19

Fig. 1. The primary partial sequences in the positions 492 through 625 of 26S rRNA in the strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The primary partial sequences were aligned. The numerals indicate the positions in *Saccharomyces cerevisiae* (3). N, A, G, C, or U; V, A, G, or C; H, A, C, or U; R, A, or G; S, G, or C; K, G, or U. *Type strain.

Cr.albidus IFO 0378*
 81 Cr.ater IFO 1862*
 72 72 Cr.dimennae IFO 1863*
 82 82 74 Cr.elinovii IFO 10331*
 70 75 76 75 Cr.flavus IFO 0407*
 69 66 73 67 71 Cr.hungaricus IFO 1052*
 90 79 69 78 68 66 Cr.kuetzingii IFO 1866*
 76 73 78 73 79 74 73 Cr.laurentii var.flavescens IFO 0384*
 71 72 75 74 76 72 70 80 Cr.laurentii var.laurentii IFO 0609*
 73 71 72 74 79 72 73 76 76 Cr.luteolus IFO 0411*
 84 94 74 84 74 70 80 76 74 75 Cr.magnus IFO 0698*
 65 67 69 67 73 69 64 71 70 68 72 Cr.skinneri IFO 1872*
 85 85 76 91 76 69 80 77 75 87 76 70 Cr.terreus IFO 0727*
 79 79 80 82 80 75 77 82 78 82 82 74 84 F.neoformans YK-353*
 79 79 80 81 79 74 77 82 78 82 81 75 84 99 YK-354*
 73 75 75 74 76 76 73 77 76 76 77 69 76 80 80 C.humicola IFO 0760*
 66 61 66 64 64 65 66 69 66 64 69 61 64 69 69 64 Ph.rhodozyma IFO 10129*
 66 61 67 64 66 65 66 70 67 65 70 60 65 70 70 65 94 IFO 10130

Fig. 2. A triangle matrix based on the calculated maximum homologies in the partial sequences of the positions 492 through 625 of 26S rRNA in the strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The maximum homologies (%) were calculated by a computer analysis using a Hitachi DNAsis(7) in the positions 492 through 652 (134 bases) of 26S rRNA. *Type strain.

Fig. 3. A dendrogram based on the calculated maximum homologies in the partial sequences of the positions 492 through 625 of 26S rRNA in the strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The dendrogram was drawn by the simple linkage method (8). *Type strain.

species. The maximum homologies of *F. neoformans* were 74–84% and 80% with respect to *Cryptococcus* species and *C. humicola*, respectively. *Phaffia rhodozyma* showed lower maximum homologies (60–70%) with any other species of the genera *Cryptococcus*, *Candida*, and *Filobasidiella*.

Based on the calculated maximum homologies, a dendrogram was drawn by the simple linkage method (8). As shown in Fig. 3, *Cr. albidus*, *Cr. kuetzingii*, *Cr. ater*, *Cr. magnus*, *Cr. terreus*, and *Cr. elinovii* constituted their own cluster at a maximum homology of 85%. *Filobasidiella neoformans* was linked at a maximum

homology of 84% to this cluster. The linkage between *Cr. luteolus* and *Cr. laurentii* var. *flavescens* was at a maximum homology of 82%, and that among *Cr. dimennae*, *Cr. flavus*, *Cr. laurentii* var. *laurentii*, and *C. humicola* was at a maximum homology of 80%. *Cryptococcus hungaricus* and *Cr. skinneri* were linked to the cluster with maximum homologies of 76% and 75%, respectively. *Phaffia rhodozyma* constituted its own separate cluster (maximum homology, 70%).

Partial sequence determination of positions 1686 through 1835 of 26S rRNA

The primary partial sequences of the eighteen strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species were aligned (Fig. 4). Because the base sequences were highly variable in this region, the maximum homologies (%) were calculated by a computer analysis using a Hitachi DNAsis (7).

As shown in Fig. 5, the maximum homologies were 98% and 99% between the two strains examined of *Ph. rhodozyma* and *F. neoformans*, respectively. High maximum homologies were found between *Cr. albidus* and *Cr. kuetzingii* (93%), between *Cr. ater* and *Cr. magnus* (89%), between *Cr. elinovii* and *Cr. terreus* (91%),

		.1691	.1701	.1711	.1721	.1731	.1741			
1	<i>Cr.albidus</i> IFO 0378*	UUCUC	CUUGACUAC	C U. AUAACCUUG	AAAUCCGGAU	AUCCGGAGCU	AAGGUACA	GGUAGGCCAA	1	
2	<i>Cr.ater</i> IFO 1862*	UUCUC	CUUGACCGU	C U. AUAACCUUG	AGAUCGGAU	GUCCGGAGU	AAGGUACA	GAUGGGCAAA	2	
3	<i>Cr.dimennae</i> IFO 1863*	UUCUC	CUNAACCUCU	UGAUGACCUUG	GAUUGCGGAU	AUCCGGAGCA	AAGGUUAC	GGAGRGUAGA	3	
4	<i>Cr.elinovii</i> IFO 10331*	UUCUC	CUVGACCGU	C U. AUAACCUUG	AAAUCCGGAU	AUACGGAGU	VAGGUAGA	GGCGGGVAGA	4	
5	<i>Cr.flavus</i> IFO 0407*	UUCUC	CNNAACC	UA. U. ACGACCUUG	GAUUGCGGAU	AUCCGGAGAU	AGGGUNAA	GGCGGGUAGA	5	
6	<i>Cr.hungaricus</i> IFO 1052*	UUCUC	CUGRAC	G. C. UUA. CGCCUUR	AAAUCCGGAU	AUCCGGAGU	AGGGUNAU	GGCGGGUAGA	6	
7	<i>Cr.kuetzingii</i> IFO 1866*	UUCUC	CUUGACUACU	C U. AUAACCUUG	AAAUCCGGAU	VUCCGGAGCU	AAGGUNVA	GGUAGGCCAA	7	
8	<i>Cr.laurentii</i> var. <i>flavescens</i> IFO 0384*	UUCUC	CUUGACCGU	CU. UACGAGCCUUG	AAAUCCGGAU	RUCCGGCGCU	GAGGURAU	GGCGGGCAGA	8	
9	<i>Cr.laurentii</i> var. <i>laurentii</i> IFO 0609*	UUCUC	CUUACCGG	CC. U. ACGACCUUG	AAAUCCGGAU	AUCCGGAGCU	AAGGUUAI	NGCNGNAUA	9	
10	<i>Cr.luteolus</i> IFO 0411*	UUCUC	CUUGACCUA	C U. ACGACCUUG	AAAUCCGGAU	AU. CGGAGU	AGGGUNAA	GUAGGGCAAA	10	
11	<i>Cr.magnus</i> IFO 0698*	UUCUC	CUKGCC	GU. C U. AUAACCUUG	AAAUCCGGAU	GUCCGGAGU	AAGGUAGA	GACGGGAAA	11	
12	<i>Cr.skinneri</i> IFO 1872*	UUCUC	CUUVACCG	CC. U. ACGACCUUG	AAAUCCGGAU	NUCCGGAGCU	NAGGUUAG	GGCGGGUAGA	12	
13	<i>Cr.terreus</i> IFO 0727*	UUCUC	CUUGACCGU	C U. AUAACCUUG	AAAUCCGGAU	VUACGGAGCU	GAGGUACA	GGCGGGGAG	13	
14	<i>F.neoformans</i> YK 353*	UUCUC	CUUACCGG	CC. U. ACGACCUUG	AAAUCCGGAU	AUCCGGAGCU	GAGGUUAI	GGUAGGUAAA	14	
15	YK 354*	UUCUC	CUUACCGG	CC. U. ACGACCUUG	AAAUCCGGAU	AUCCGGAGCU	GAGGUUAI	GGUAGGUAAA	15	
16	<i>C.humicola</i> IFO 0760*	UUCUC	CUUACCGG	CC. U. ACGACCUUG	AAAUCCGGAU	GUCCGGAGCU	GAGGUUAI	GGUAGGUAAA	16	
17	<i>Ph.rhodozyma</i> IFO 10129*	UUCUC	CUUACCGG	CC. U. ACCACCUUG	AAAUCCGGAU	AUCCGGAGCU	AGGGUACA	GGUAGGUAGA	17	
18	IFO 10130	UUCUC	CUUACCGG	CC. U. ACCACCUUG	AAAUCCGGAU	AUCCGGAGCU	AGGGUACA	GGUAGGUAGA	18	
19	<i>S.cerevisiae</i> IFO 2376	UUCUC	CUUACCGU	C U. AUCACCCCG	GAUUGCGGAU	AUCCGGAGU	GGGUUUAU	GGUAGGAAA	19	
		.1751	.1761	.1771	.1781	.1791	.1801	.1811	.1821	.1831
1	GUGCAACACAC	UUU. CGU. U	GC. GAGU. CCGGUGCGUC.	C. UAGACAGUC	CUUGAGAAAAC	GACGGAG. AGG	UAUUAUCUCAG	UGGCCUUGUC	GUUC. U	1
2	GUGCA. GCACU	GGUGUUG. .	C. AGU. CCUGUGCGUC.	C. UUAACAGUC	CU. UGAAAAAC	CGACGGA. AGG	UAUUGUGUUA.	UUGCCUUGU	GUUC. U	2
3	AGAC.	UGU. GUUGCGU.	CCGUGCGU.	AAUAGACAUCC	CU. UGAAAAAC	CGACGGA. AGG	UAUUA. UUCU.	AGCCUUNGU	GUUC. U	3
4	GCGCA. C. ACC	UUGUGUGUGU.	CCGUGCGU.	C. UUGRCAGUC	CUUGA. AAAAC	GACGGAACAUA	U. UA. CUUC.	UUGCCUUGU	GUUC. U	4
5	GCACGACA. UC	UGCGUUGUGU.	CCGUGCGU.	C. CUNACAGUC	CU. UGAAAAAC	CGACGGA. ACU	UAUUAUCUC.	AGCCUUNGU	GUUC. U	5
6	GCACGACA. CU	UGUGUUGUGU.	CCGUGCGU.	C. UUGACAGUC	CU. UGAAAAAC	CGACGGA. ACU	UAUUAUCUC.	AGCCUUNGU	GUUC. U	6
7	GUGCAAGAC. C	UNU. GGU. U	GC. GAGU. CCGGUGCGUC.	C. UAGACAGUC	CUUGAGAAAAC	GACGGAG. AGG	UAUUAUCUCAG	U. GCUUGUC	GUCC. U	7
8	GCGGAC. ACC	UCUGUGUGU.	CCGUGCGU.	C. UDRACAGUC	C. GUGAAAAAC	CGACGGA. AGG	UAUUAUCUC.	UUGCCUUGU	GUCC. U	8
9	GC. CGACACU	NUN. UCGUNU	CCGUGCGU.	C. UUAACAGUC	CUU. U. AAAAC	CGACGGA. AGG	UAUUA. UCUC.	CGCCUUGU	UAC. U	9
10	GCACAACGAGC	GUUUGUGU.	GGUGUGCGUGUGUGUG	CGUUGAUAGUC	C. GUGAAAAAC	CGGCGGA. AGG	UAUUAUCUC.	UUGCCUUGU	GUCC. U	10
11	GUGCAGGACU	GGUGUGU. .	C. AGU. CCGUGCGU.	C. UUAACAGUC	CU. UGAAAAAC	CGUCGGA. AGG	UAUUAUCUC.	UUGCCUUGU	GUCC. U	11
12	G. ACACAC. .	UU. CUGG. U	C. GU. C. CCGUGCGU.	C. UUAUAGUC	CU. UGAAAAAC	CGUCGGA. AGG	UAUUAUCUC.	CGCCUUGU	GUCC. U	12
13	AGGCGGACAC	UGUG. UC. .	G. U. G. U. CCGUGCGU.	C. UUGRCAGUC	CU. UGAAAAAC	CGACGGA. ACA	UAUUAUCUC.	UUGCCUUGU	GUCC. U	13
14	GCACAACAC. C	UCUGUGU. U	G. U. CCGUGG. GUC.	C. UUGACGAGC	CU. UGAAAAAC	CGACGGA. AGG	UAUUAUCUC.	AGCCUUNGU	GUCC. U	14
15	GCACAACAC. C	UCUGUGU. U	G. U. CCGUGG. GUC.	C. UUGACGAGC	CU. UGAAAAAC	CGACGGA. AGG	UAUUAUCUC.	AGCCUUNGU	GUCC. U	15
16	GCACAACACU	GUUGUGU. U	CCGUGCGU. G	C. UUAACAGU	CU. UGAAAAAC	CGACGGA. AGG	UAUUAUCUC.	CGCCUUGU	GUCC. U	16
17	G. CUU. AA. .	UUU. UGAC. .	GGGU. CCGUGCGU. U	C. CUAACAGUC	CU. UGAAAAAC	CAACGGAGAGC	UAUUAUCUCAG	CAGUACUAGUC	GUAC. U	17
18	G. CUU. AA. .	UUU. UGAC. .	GGGU. CCGUGCGU. U	C. CUAACAGUC	CU. UGAAAAAC	CAACGGAGAGC	UAUUAUCUCAG	CAGUACUAGUC	GUAC. U	18
19	GCGCAGC. ACC	UUUGUGGCU	CCGUGCGU. U	UG. UGACGGCC	C. GUGAAAAAC	CACGGA. AGG	AAUUAUUCU.	AGUACUAGUC	GUAC. U	19

Fig. 4. The primary partial sequences in the positions 1686 through 1835 of 26S rRNA in the strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The primary partial sequences were aligned. The numerals indicate the positions in *Saccharomyces cerevisiae* (3). N, A, G, C, or U; V, A, G, or C; R, A, or G. *Type strain.

Cr.albidus IFO 0378*
 83 Cr.ater IFO 1862*
 73 71 Cr.dimennae IFO 1863*
 81 78 74 Cr.elinovii IFO 10331*
 73 74 77 77 Cr.flavus IFO 0407*
 76 75 76 80 87 Cr.hungaricus IFO 1052*
 93 81 72 79 73 74 Cr.kuetzingii IFO 1866*
 76 75 74 81 79 82 75 Cr.laurentii var.flavescens IFO 0384*
 76 76 75 78 78 80 74 81 Cr.laurentii var.laurentii IFO 0609*
 76 72 69 73 77 76 75 77 72 Cr.luteolus IFO 0411*
 79 89 69 77 74 76 79 74 72 71 Cr.magnus IFO 0698*
 74 76 75 78 79 79 73 81 84 73 75 Cr.skinneri IFO 1872*
 79 80 72 91 75 76 79 80 77 80 71 77 Cr.terreus IFO 0727*
 79 77 78 79 82 86 76 86 83 74 78 82 77 F.neoformans YK-353*
 78 76 78 79 82 86 76 86 83 74 78 82 77 99 YK-354*
 74 73 74 75 77 83 76 79 79 73 74 78 72 84 84 C.humicola IFO 0760*
 77 71 72 74 78 80 73 76 74 69 72 74 72 76 76 74 Ph.rhodozyma IFO 10129*
 76 71 71 74 77 79 72 85 84 69 72 74 72 76 76 74 98 IFO 10130

Fig. 5. A triangle matrix based on the calculated maximum homologies in the partial sequences of the positions 1686 through 1835 of 26S rRNA in the strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The maximum homologies (%) were calculated by a computer analysis using a Hitachi DNAsis (7) in the positions 1686 through 1835 (150 bases) of 26S rRNA. *Type strain.

Fig. 6. A dendrogram based on the calculated maximum homologies in the partial sequences of the positions 1686 through 1835 of 26S rRNA in the strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The dendrogram was drawn by the simple linkage method (8). *Type strain.

and between *Cr. flavus* and *Cr. hungaricus* (87%). The other maximum homologies were calculated as 71–84% among the species examined of the genus *Cryptococcus*.

Candida humicola had maximum homologies of 72–83% with *Cryptococcus* species. The maximum homologies of *F. neoformans* were 74–86% and 84% with respect to *Cryptococcus* species and *C. humicola*, respectively. *Phaffia rhodozyma* showed maximum homologies (71–85%) with *Cryptococcus*, *Candida*, and *Filobasidiella* species.

Based on the calculated maximum homologies, a dendrogram was drawn by

the simple linkage method (8). As shown in Fig. 6, *Cr. albidus* and *Cr. kuetzingii* constituted a cluster with a maximum homology of 93%, and *Cr. ater* and *Cr. magnus* were in a separate cluster with a maximum homology of 89%. A third small cluster was composed of *Cr. elinovii* and *Cr. terreus* (maximum homology, 91%). *Filobasidiella neoformans* was linked to *Cr. flavus*, *Cr. hungaricus*, and *Cr. laurentii* var. *flavescens* at a maximum homology of 86%. All the species examined of the genera *Cryptococcus*, *Candida*, and *Filobasidiella* were linked to one another with maximum homologies of 81–84% except for two *Cryptococcus* species. *Phaffia rhodozyma* constituted its own cluster (maximum homology, 80%). The exceptional two species were *Cr. luteolus* and *Cr. dimennae*, the linkages of which were at maximum homologies of 80% and 78%, respectively.

Partial sequence determination of positions 1451 through 1618 of 18S rRNA

The primary partial sequences of the eighteen strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species were aligned (Fig. 7). The type strain of *Cr. laurentii* var. *laurentii* (IFO 0609) showed the following base differences when compared with *S. cerevisiae* IFO 2376: U (position 1475), no base (1496), G (1509),

	.1451	.1461	.1471	.1481	.1488	.1501	.1511	
1 <i>Cr. albidus</i> IFO 0378*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	1
2 <i>Cr. ater</i> IFO 1862*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	2
3 <i>Cr. dimennae</i> IFO 1863*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UCAUCA. CCUU. GCDC	AGAGGUCUGG	GUAAUCUUGU	3
4 <i>Cr. elinovii</i> IFO 10331*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	4
5 <i>Cr. flavus</i> IFO 0407*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUU. GCDC	AGAGGUCUGG	GUAAUCUUGU	5
6 <i>Cr. hungaricus</i> IFO 1052*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGAGCC	AGAGGUCUGG	GUAAUCUUGU	6
7 <i>Cr. kuetzingii</i> IFO 1865*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	7
8 <i>Cr. laurentii</i> var. <i>flavescens</i> IFO 0384*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGAGCC	AGAGGUCUGG	GUAAUCUUGU	8
9 <i>Cr. laurentii</i> var. <i>laurentii</i> IFO 0609*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	9
10 <i>Cr. luteolus</i> IFO 0411*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	10
11 <i>Cr. magnus</i> IFO 0698*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	11
12 <i>Cr. skinneri</i> IFO 1872*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	12
13 <i>Cr. terreus</i> IFO 0727*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	13
14 <i>F. neoformans</i> YK 353*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	CUUACGSCCUUGGCCG	AGAGGUCUGG	GUAAUCUUGU	14
15 <i>C. humicola</i> YK 354*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	CUUACGSCCUUGGCCG	AGAGGUCUGG	GUAAUCUUGU	15
16 <i>C. humicola</i> IFO 0760*	GGGCCGCAGC	CGCGCUACAC	UGACUGAGCC	AGCGAGU	UUUAUA. CCUUGAGCC	AGAGGUCUGG	GUAAUCUUGU	16
17 <i>Ph. rhodozyma</i> IFO 10129*	GGGCCGCAGC	CGCGCUACAC	UGACAGAGCC	AGCGAGU	UUUUUU. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	17
18 <i>S. cerevisiae</i> IFO 10130	GGGCCGCAGC	CGCGCUACAC	UGACAGAGCC	AGCGAGU	UUUUUU. CCUUGGCCG	AAAGGUCUGG	GUAAUCUUGU	18
19 <i>S. cerevisiae</i> IFO 2376	GGGCCGCAGC	CGCGCUACAC	UGACGAGGCC	AGCGAGU	CUAA. . . CCUUGGCCG	AGAGGUCUGG	GUAAUCUUGU	19

	.1521	.1531	.1541	.1551	.1561	.1571	.1581	.1591	.1601	.1611	
1 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		1
2 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		2
3 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		3
4 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		4
5 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		5
6 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		6
7 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		7
8 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		8
9 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		9
10 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		10
11 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		11
12 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		12
13 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		13
14 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		14
15 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		15
16 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		16
17 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		17
18 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		18
19 GAAACUCAGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUAUUG	CUCUUAACG	AGGAAUCCU	AGUAAAGCGU	AGUCAUAGC	UUGCGUUGAU	UAGCUCCC		19

Fig. 7. The primary partial sequences in the positions 1451 through 1618 of 18S rRNA in the strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The primary partial sequences were aligned. The numerals indicate the positions in *Saccharomyces cerevisiae* (10). *Type strain.

A (1528), A (1577), U (1589), no base (1596), and C (1599). The base differences of the type strain of *Ph. rhodozyma* (IFO 10129) were A (position 1475), A (1502), G (1509), U (1528), and C (1552). The finger print segment (19) was comprised of UUAUAA (6 bases) in *Cr. albidus*, *Cr. ater*, *Cr. elinovii*, *Cr. hungaricus*, *Cr. kuetzingii*, *Cr. magnus*, *Cr. terreus*, and *C. humicola*, UCAUCA (6 bases) in *Cr. dimennae*, UCAUAA (6 bases) in *Cr. flavus*, UUAUCA (6 bases) in *Cr. laurentii* var. *flavescens*, *Cr. laurentii* var. *laurentii*, *Cr. luteolus*, and *Cr. skinneri*. *Filobasidiella neoformans* had a quite different base sequence comprised of 7 bases (CUUACCG). The base sequence of *Ph. rhodozyma* was different (UUCUUU, 6 bases) from any

Cr.albidus	IFO 0378*																
4	Cr.ater	IFO 1862*															
10	8	Cr.dimennae	IFO 1863*														
3	1	7	Cr.elinovii	IFO 10331*													
8	6	2	5	Cr.flavus	IFO 0407*												
6	4	5	3	3	Cr.hungaricus	IFO 1052*											
0	4	10	3	8	6	Cr.kuetzingii	IFO 1866*										
7	9	6	7	6	7	7	Cr.laurentii	var.flavescens	IFO 0384*								
7	9	5	10	8	9	10	6	Cr.laurentii	var.laurentii	IFO 0609*							
11	9	6	8	6	7	11	4	10	Cr.luteolus	IFO 0411*							
5	1	9	2	7	5	5	10	13	10	Cr.magnus	IFO 0698*						
5	4	5	2	5	3	5	6	9	4	6	Cr.skinneri	IFO 1872*					
3	1	7	0	5	3	3	7	11	2	8	2	Cr.terreus	IFO 0727*				
12	10	4	9	6	7	12	8	9	11	7	7	9	F.neoformans	YK-353*			
12	10	4	9	6	7	12	8	9	11	7	7	9	0	YK-354*			
11	9	8	9	8	5	11	10	13	10	6	8	9	10	10	C.humicola	IFO 0760*	
6	10	14	9	12	11	6	11	15	11	15	9	9	15	15	16	Ph.rhodozyma	IFO 10129*
6	10	14	9	12	11	6	11	15	11	15	9	9	15	15	16	0	IFO 10130

Fig. 8. A triangle matrix based on the calculated number of base differences in the partial sequences of the positions 1451 through 1618 of 18S rRNA in the strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The number of base differences was calculated in the positions 1451 through 1618 (168 bases) of 18S rRNA. *Type strain.

Fig. 9. A dendrogram based on the calculated number of base differences in the partial sequences of the positions 1451 through 1618 of 18S rRNA in the strains of *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* species. The dendrogram was drawn by the simple linkage method (8). *Type strain.

other species of the genera *Cryptococcus* and *Filobasidiella*.

The number of base differences was calculated. As shown in Fig. 8, the two strains of *Ph. rhodozyma* and *F. neoformans* showed no base differences within the respective species. The no-base differences were found between *Cr. albidus* and *Cr. kuetzingii* and between *Cr. elinovii* and *Cr. terreus*. Among the *Cryptococcus* species examined, there were 0–13 base differences. *Cryptococcus hungaricus* had 3–9 base differences with respect to other *Cryptococcus* species, *C. humicola* had 5–13 base differences, and *F. neoformans* had 4–12 base differences. *Phaffia rhodozyma* had 6–16 base differences with *Cryptococcus*, *Candida*, and *Filobasidiella* species.

Based on the calculated number of base differences, a dendrogram was drawn by the simple linkage method (8). As shown in Fig. 9, *Cr. albidus*, *Cr. kuetzingii*, *Cr. ater*, *Cr. magnus*, *Cr. terreus*, *Cr. elinovii*, *Cr. skinneri*, *Cr. hungaricus*, *Cr. flavus*, and *Cr. dimennae* constituted their own cluster (base differences, 0–3). These species were characterized mostly by the base sequence of UUAUAA in the finger print segment, and sometimes by the base sequence of UCAUCA, UCAUAA, or UUAUCA. *Filobasidiella neoformans* was linked to the cluster at 4 base differences, and the other *Cryptococcus* and *Candida* species were linked at 5–6 base differences. The linkage of *Ph. rhodozyma* to *Cryptococcus*, *Candida*, and *Filobasidiella* species was at 6 base differences.

DISCUSSION

All the species examined of the genera *Phaffia*, *Cryptococcus*, *Candida*, and *Filobasidiella* gave complete radioautograms as to the partial sequence determinations in the positions 1686 through 1835 of 26S rRNA. These species are characterized by the presence of xylose in the cells (15, 16).

Guého et al. (4, 5) reported the partial sequences of 18S and 25S rRNAs of *F. neoformans* and *Ph. rhodozyma*. They determined the base sequences in the positions 277 through 626 (350 bases) of 25S rRNA and in the positions 1288 through 1626 (339 bases) of 18S rRNA and placed the above-mentioned teleomorphic and anamorphic yeast species in phylogenetic clusters separate from each other. Our results coincided with theirs, although our sequenced bases (134 and 168 bases, respectively) were only half as many as theirs in both regions.

The genus *Cryptococcus* was confirmed to be heterogeneous from the phylogenetic point of view: the base differences in the positions 1451 through 1618 (168 bases) of 18S rRNA were calculated as 0–13. And there were four kinds of base sequences in the finger print segment (UUAUAA, UCAUCA, UCAUAA, and UUAUCA). *Filobasidiella neoformans*, a teleomorph of the genus *Cryptococcus*, was quite different in the base sequence of the finger print segment from other *Cryptococcus* species: the base sequence was comprised of 7 bases (CUUACCG). The physiological and biochemical discriminations between *Cr. neoformans* and other *Cryptococcus* species are in the vigorous growth at 37°C (14) and the formation of carotenoid pigments within the cells (6): the former grows at 37°C and produces

no carotenoid pigments. Fell et al.(1) proposed that the genus *Cryptococcus* Vuillemin is conserved with the type species, *Cr. neoformans* (Sanfelice) Vuillemin instead of *Cryptococcus* Kützing. As described above, the genus *Cryptococcus* is phylogenetically heterogeneous. The genus will, therefore, be divided into two or more genera.

Moore(13) introduced the genus *Vanrija* Moore with the type species, *Vanrija humicola* (Daszewska) Moore. In this experiment, *C. humicola* ($\equiv V. humicola$) was found to be not so close phylogenetically to *F. neoformans* (teleomorph of *Cr. neoformans*, type species of genus *Cryptococcus* Vuillemin) and to any other species examined of the genus *Cryptococcus*.

The genus *Phaffia* is characterized by the ability to ferment sugars and by the inability to assimilate inositol(11, 12). These physiological properties distinguish the genus *Phaffia* from the genus *Cryptococcus*(11, 14). As has been described above, the genus *Phaffia* is not so close phylogenetically to the species examined of the genera *Cryptococcus* and *Filobasidiella*. Guého et al.(4) supported such a phylogenetic relationship between *F. neoformans* and *Ph. rhodozyma*. Therefore, the name of *Phaffia* is qualified to be retained contrary to the opinion of Weijman et al.(17) that *Phaffia* is synonymous with *Cryptococcus*, because the genus *Phaffia* has a cluster separate from the genus *Cryptococcus* which had a heterogeneous nature from the phylogenetic point of view.

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