THE MOLECULAR PHYLOGENY OF THE ASCOMYCETOUS
ANAMORPHIC YEAST GENUS *ARXULA* VAN DER WALT,
SMITH ET YAMADA BASED ON THE PARTIAL
SEQUENCES OF 18S AND 26S RIBOSOMAL
RIBONUCLEIC ACIDS¹

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Nine strains of *Arxula*, *Stephanoascus*, *Endomyces*, *Yarrowia*, *Dipodascus*, and *Geotrichum* were examined regarding the partial sequences of 18S and 26S rRNAs. In the positions 471 through 627 of 26S rRNA, the two strains examined of the genera *Arxula* and *Stephanoascus* had 99% and 98% maximum homologies, respectively. The two species of the genus *Arxula*, *A. terrestris* and *A. adenivorans* had 80% maximum homology. The maximum homologies were 81–84% between the genera *Arxula* and *Stephanoascus*. *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species constituted their own clusters at 49–76% maximum homologies. In the positions 1685 through 1835 of 26S rRNA, the two strains examined of the genera *Arxula* and *Stephanoascus* had 2 and 0 base difference, respectively. The two species of the genus *Arxula* had 7–9 base differences. The base differences were 4–8 between the genera *Arxula* and *Stephanoascus*. *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species constituted their own clusters at 13–25 base differences. In the positions 1451 through 1618 of 18S rRNA, the two strains examined of the genera *Arxula* and *Stephanoascus* had no base difference. The two species of the genus *Arxula* had 2 base differences. The base differences were 2–4 between the genera *Arxula* and *Stephanoascus*. *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*,

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Abbreviations: S, Svedberg value; rRNA, ribosomal RNA; Co-Q, coenzyme Q or ubiquinone; Q-9 or Q-8, a coenzyme Q or ubiquinone homologue with 9 or 8 isoprene units in a side chain.

and *Saccharomyces* species constituted their own clusters at 5–9 base differences. The data obtained are discussed from the taxonomic and phylogenetic points of view.

The genus *Arxula* van der Walt, Smith et Yamada was introduced with two species, *Arxula terrestris* (van der Walt et Johannsen) van der Walt, Smith et Yamada (type species) and *Arxula adenivorans* (Middelhoven, Hoogkamer-te Niet et Kreger-van Rij) van der Walt, Smith et Yamada (14). The genus is characterized morphologically by producing arthroconidia, physiologically by xerotolerance and utilizing some purines such as uric acid and adenine as a sole source of both carbon and nitrogen, and chemotaxonomically by having the Q-9 system (10, 14).

This paper describes the molecular phylogeny of the genus *Arxula* based on the partial sequences of 18S and 26S rRNAs.

MATERIALS AND METHODS

Yeast strains examined. Nine strains of *Arxula*, *Stephanoascus*, *Endomyces*, *Yarrowia*, *Dipodascus*, and *Geotrichum* species were examined regarding partial sequencing of 18S and 26S rRNAs (Table 1). These yeast strains were cultured as described previously (17).

Preparation of rRNA. The rRNAs of the strains were prepared as described previously (17).

Partial sequencing of 18S and 26S rRNAs. The partial sequences of 18S and 26S rRNAs of the strains were determined by the method of Lane et al. (5) using reverse transcriptase. The oligonucleotide DNA primers used in the experiment were 5'-ACGGGCGGTGTGTAC-3' which is complementary to the sequence of the positions 1641 through 1627 (in *Saccharomyces cerevisiae*) (6) 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*) (1) of 26S rRNA.

Chemicals. The chemicals used in the experiment were the same as those described previously (17). The oligonucleotide DNA primer for the partial sequencing of 18S rRNA was supplied by H. Oyaizu, Toyama University, Toyama, Japan.

RESULTS

Partial sequencing in positions 471 through 627 of 26S rRNA

The primary partial sequences of the nine strains of *Arxula*, *Stephanoascus*, *Endomyces*, *Yarrowia*, *Dipodascus*, and *Geotrichum* species were aligned (Fig. 1). Because the base sequences were highly variable in the region, the maximum homologies (%) were calculated by a computer analysis using a Hitachi DNAsis (2).

Table 1. The strains examined of *Arxula* and related species.

Species and strain	Other designation	Co-Q system ^a
<i>Arxula</i> van der Walt, Smith et Yamada		
<i>A. terrestris</i> (van der Walt et Johannsen) van der Walt, Smith et Yamada		
CBS 6697*	CSIR 803, from soil (South Africa)	Q-9
CBS 7376	CSIR 914, from soil (South Africa)	Q-9
<i>A. adenivorans</i> (Middelhoven, Hoogkamer-te Niet et Kreger-van Rij) van der Walt, Smith et Yamada		
CBS 8244*	CSIR 577, from soil (The Netherlands)	Q-9
<i>Stephanoascus</i> Smith, van der Walt et Johannsen		
<i>St. ciferrii</i> Smith, van der Walt et Johannsen		
IFO 1854*	CBS 5295, mating type α , from pig, D. Swierstra	Q-9
IFO 1855*	CBS 6699, mating type α , from soil, J. P. van der Walt	Q-9
<i>Endomyces</i> Reess		
<i>E. fibuliger</i> Lindner		
IFO 1744*	CBS 2521, NRRL Y-2388	Q-8
<i>Yarrowia</i> van der Walt et von Arx		
<i>Y. lipolytica</i> (Wickerham, Kurtzman et Herman) van der Walt et von Arx		
IFO 1548*	CBS 6124, NRRL YB-423, from material of corn-processing plant, L. J. Wickerham	Q-9
<i>Dipodascus</i> de Lagerheim		
<i>D. albidus</i> de Lagerheim		
IFO 1984	CBS 766.85, AJ 4896, from exudate of tree, T. Nakase	Q-9
<i>Geotrichum</i> Link		
<i>G. candidum</i> Link ex Persoon		
IFO 4597	FAT, Kral Museum, as <i>Oidium lactis</i>	Q-9

^a Data from van der Walt et al. (14), Yamada and Kondo (18), Yamada and Smith (19), Yamada et al. (20, 22), and the present work.

* Type strain.

Abbreviations: CBS, Centraalbureau voor Schimmelcultures, Delft & Baarn, The Netherlands; CSIR, Council for Scientific and Industrial Research, Pretoria, South Africa; IFO, Institute for Fermentation, Osaka, Osaka, Japan; NRRL, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Illinois, U.S.A.; AJ, Central Research Laboratories, Ajinomoto Co., Ltd., Kawasaki, Japan; FAT, Faculty of Agriculture, University of Tokyo, Tokyo, Japan.

As shown in Fig. 2, the maximum homologies were calculated to be 99% and 98%, respectively, between the two strains examined of *A. terrestris* and *St. ciferrii*. Within the genus *Arxula*, the maximum homologies were 80–99%. It is of interest that there were higher maximum homologies (81–84%) between the strains classified in the genera *Arxula* and *Stephanoascus*. The strains of *Arxula* species had 65–73% maximum homologies with the strains examined of *Endomyces*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species. The similar maximum homologies were calculated (63–73%) in the strains of *St. ciferrii*. *Yarrowia lipolytica* IFO 1548 (type strain) occupied a phylogenetically unique situation: the maximum homologies were very low (45–49%).

Based on the calculated maximum homologies, a dendrogram was drawn by the simple linkage method (3). As shown in Fig. 3, the strains of *Arxula* and *Stephanoascus* species were linked to one another at 81–84% maximum homologies.

Fig. 1. The primary partial sequences in the positions 471 through 627 of 26S rRNA in the strains of *Arxula*, *Stephanoascus*, *Endomyces*, *Yarrowia*, *Dipodascus*, and *Geotrichum* species. The primary partial sequences were aligned. The data of *S. cerevisiae* IFO 2376 are cited from ref. 17. The numerals indicate the positions in *S. cerevisiae* (1). N, A, G, C, or U; V, A, G, or C; R, A, or G; S, G, or C. *Type strain.

Fig. 2. A triangle matrix based on the calculated maximum homologies in the partial sequences of the positions 471 through 627 of 26S rRNA in the strains of *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species. The maximum homologies (%) were calculated by a computer analysis using a Hitachi DNAsis (2) in the positions 471 through 627 (157 bases) of 26S rRNA. *Type strain.

Fig. 3. A dendrogram based on the calculated maximum homologies in the partial sequences of the positions 471 through 627 of 26S rRNA in the strains of *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species. The dendrogram was drawn by the simple linkage method (3). *Type strain.

Arxula, *Endomyces*, *Saccharomyces*, *Dipodascus*, *Geotrichum* and *Yarrowia* species constituted their own clusters separating from each other at 49–73% maximum homologies. The linkage of *Y. lipolytica* was extremely low (maximum homology, 49%).

Partial sequencing in positions 1685 through 1835 of 26S rRNA

The primary partial sequences of the nine strains of *Arxula*, *Stephanoascus*, *Endomyces*, *Yarrowia*, *Dipodascus*, and *Geotrichum* species were aligned (Fig. 4). The number of base differences was calculated among the strains examined.

As shown in Fig. 5, the two strains examined of *Arxula terrestris* and *St. ciferrii*

		' 1691	' 1701	' 1711	' 1721	' 1731	' 1741
1	<i>A. terrestris</i> CBS 6697*	UUUCUU CUUACAG. UU	UAUCACCCUG	AAAUUGGUUU	NUCCGGAGCU	AGGGUNUUAG	AACUGGAAGA
2	CBS 7376	UUUCUU CUUACAG. UU	UAUCACCCUG	AAAUUGGUUU	AUCCGGAGCU	AGGGUNUUAG	AACUGGAAGA
3	<i>A. adeninivorans</i> CBS 8244*	UUUCUU CUUACAG. UC	UAUCACCCUG	AAAUUGGUUU	NUCCGGAGCU	AGGGUNUUAG	GGCUGGAAGA
4	<i>St. ciferrii</i> IFO 1854*	UUUCUU CUUACAG. CU	UAUCACCCUG	AAAUUGGUUU	NUCCGGAGCU	AGGGUNUUAG	GGCUGGAAGA
5	IFO 1855*	UUUCUU CUUACAG. CU	UAUCACCCUG	AAAUUGGUUU	NUCCGGAGCU	AGGGUNUUAG	GGCUGGAAGA
6	<i>E. fibuliger</i> IFO 1744*	UUUCUU CUUACAG. CU	UAUCACCCUG	AAAUUGGUUU	AUCCGGAGAU	AGGGUNUUAG	GGCUGGAAGA
7	<i>Y. lipolytica</i> IFO 1548*	UUUCUU UUUAAACGAGU	CAACACCCUG	AAAUUGGUUU	NUCUAGAGAU	AGGGUNUUAG	UGCNGGAAGA
8	<i>D. albidus</i> IFO 1984	UUUCUU UUUAAACAA. UU	UAUCACCCUG	AAAUUGGUUU	GUCUAGAGAU	AAGGUCUUAG	AAUUGGAAGA
9	<i>G. candidum</i> IFO 4597	UUUCUU UUUAAACAG. UG	UAUCACCCUG	AAAUUGGUUU	GUCUAGAGAU	AGGGUNUUAG	CACUGGAAGA
10	<i>S. cerevisiae</i> IFO 2376	UUUCUU CUUACAG. CU	UAUCACCCUG	AAAUUGGUUU	AUCCGGAGAU	GGGGUCUUAG	GGCUGGAAGA

	' 1751	' 1761	' 1771	' 1781	' 1791	' 1801	' 1811	' 1821	' 1831
1	GCCUGACACC	UUUGUCAGGU	SCGGUGCGSU	UAUGASGUCC	CUUGAAAAUC	CNCAGGAAGG	AAUUGUUUUC	AUNCCAAGUY	GUNCU
2	GCCUGACACC	UUUGUCAGGU	SCGGUGCGSU	UAUGASGUCC	CUUGAAAAUC	CACAGGAAGG	AAUUGUUUUC	AUNCCAAGUY	GUNCU
3	GCCUGACACC	UNUGUCAGGU	SCGGUGCGCU	UAUGASGUCC	CUUGAAAAUC	CACUGGAAGG	UUUUGUUUUC	AUGCCAAGUY	GUNCU
4	GCCUGACAU	UUUGUCAGGU	SCGGUGCGSU	UAUGACGUCC	CUUNAAAAUC	CACAGGAAGG	AAUUGUUUUC	AUGCCAAGUY	GUNCU
5	GCCUGACAU	UUUGUCAGGU	SCGGUGCGSU	UAUGACGUCC	CUUNAAAAUC	CACAGGAAGG	AAUUGUUUUC	AUGCCAAGUY	GUNCU
6	GCCCAGUACC	UUUACUGGCU	CCGGUGCGCU	UAUACGGGCC	CUUUAAAAUC	CNCAGGAAGG	AAUUGUUUUC	AUNCCAAGUY	GUNCU
7	GGGGGCGAGC	UUUGUCCNCU	CCGAUGCGCU	UGUUACGGCC	CUUUAAAAUC	CACAGGAAGG	AAUUGUUUUC	AUGCCAAGUY	GUNCU
8	GCCUGGCAAU	UCAGUCAGGU	CUGGUGCGCU	UAUGACGUCC	CUUGAAAAUC	CACAGGAAGG	AAUUGUUUUC	AUGUCAAAGC	GUNCU
9	GCGUGGCAAU	UCAGUCAGGU	CUGGUGCGCU	UAUACGGGCC	CUUGAAAAUC	CACAGGAAGG	AAUUGUUUUC	AUGUCAAAGC	GUNCU
10	GGCCAGCACC	UUUGUCGCU	CCGGUGCGCU	UGUACGGGCC	CGUGAAAAUC	CACAGGAAGG	AAUUGUUUUC	AUGUCAAAGC	GUACU

Fig. 4. The primary partial sequences in the positions 1685 through 1835 of 26S rRNA in the strains of *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species. The primary partial sequences were aligned. The data of *S. cerevisiae* IFO 2376 are cited from ref. 17. The numerals indicate the positions in *S. cerevisiae* (1). N, A, G, C, or U; S, G, or C; Y, C, or U. *Type strain.

<i>A. terrestris</i> CBS 6697*									
2									
	CBS 7376								
7	9	<i>A. adeninivorans</i> CBS 8244*							
4	7	8	<i>St. ciferrii</i> IFO 1854*						
4	7	8	0	IFO 1855*					
21	22	24	17	17	<i>E. fibuliger</i> IFO 1744*				
27	30	29	25	25	25	<i>Y. lipolytica</i> IFO 1548*			
21	22	25	25	25	36	35	<i>D. albidus</i> IFO 1984		
22	22	25	26	26	35	31	13	<i>G. candidum</i> IFO 4597	
22	22	25	19	19	16	29	34	36	<i>S. cerevisiae</i> IFO 2376

Fig. 5. A triangle matrix based on the calculated number of base differences in the partial sequences of the positions 1685 through 1835 of 26S rRNA in the strains of *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species. The number of base differences were calculated in the positions 1685 through 1835 (151 bases) of 26S rRNA. *Type strain.

had 2 and 0 base difference, respectively. Within the genus *Arxula*, the base differences were calculated to be 2–9. The genera *Arxula* and *Stephanoascus* are phylogenetically related to each other: the base differences were 4–8. In contrast, the strains classified in the genera *Arxula* and *Stephanoascus* had 21–30 and 17–26 base differences, respectively, with the strains examined of *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species. *Yarrowia lipolytica* IFO 1548 (type strain) had 25–35 base differences.

Based on the calculated number of base differences, a dendrogram was drawn by the simple linkage method (3). As shown in Fig. 6, the strains of *Arxula* and *Stephanoascus* species were linked to one another at 4–7 base differences. *Arxula*,

Fig. 6. A dendrogram based on the calculated number of base differences in the partial sequences of the positions 1685 through 1835 of 26S rRNA in the strains of *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species. The dendrogram was drawn by the simple linkage method (3). *Type strain.

		'1451	'1461	'1471	'1481	'1488	'1501	'1511			
1	<u>A.terrestris</u> CBS 6697*	GGGCGGCACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AAUAACCUUGGCCG	AGAGGUUUGG	GUAAUCUUGU			
2	CBS 7376	GGGCGGCACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AAUAACCUUGGCCG	AGAGGUUUGG	GUAAUCUUGU			
3	<u>A.adeninivorans</u> CBS 8244*	GGGCGGCACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	AAUAACCUUGGCCG	AGAGGUUUGG	GUAAUCUUGU			
4	<u>St.ciferrii</u> IFO 1854*	GGGCGGCACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	ACUAACCUUGGCCG	AGAGGUUUGG	GUAAUCUUGU			
5	IFO 1855*	GGGCGGCACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	ACUAACCUUGGCCG	AGAGGUUUGG	GUAAUCUUGU			
6	<u>E.fibuliger</u> IFO 1744*	GGGCGGCACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUAU...CCUUGGCCG	AGAGGUUUGG	GUAAUCUUGU			
7	<u>Y.lipolytica</u> IFO 1548*	GGGCGGCACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	CGA...CCAAC.CCG	AGAGGUUUGG	GUAAUCUUGU			
8	<u>D.albidus</u> IFO 1984	GGGCGGCACG	CGCGCUACAA	UGACGGAGCC	AGCGAGU	AUAU...CCUUGGCCG	AGAGGUUUGG	GUAAUCUUGU			
9	<u>G.candidum</u> IFO 4597	GGGCGGCACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	CUAA...CCUUGGCCG	AGAGGUUUGG	GUAAUCUUGU			
10	<u>S.cerevisiae</u> IFO 2376	GGGCGGCACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	CUAA...CCUUGGCCG	AGAGGUUUGG	GUAAUCUUGU			
		'1521	'1531	'1541	'1551	'1561	'1571	'1581	'1591	'1601	'1611
1	UAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
2	UAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
3	UAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
4	UAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
5	UAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
6	GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGUA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
7	GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
8	GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
9	GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
10	GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GCAAUUUAUUG	CUCUUAACG	AGGAAUUCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	

Fig. 7. The primary partial sequences in the positions 1451 through 1618 of 18S rRNA in the strains of *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, and *Saccharomyces* species. The primary partial sequences were aligned. The data of *S. cerevisiae* IFO 2376 are cited from ref. 17. The numerals indicate the positions in *S. cerevisiae* (6). *Type strain.

Endomyces, *Saccharomyces*, *Dipodascus*, *Geotrichum*, and *Yarrowia* species constituted their own clusters separating from each other at 13–25 base differences. *Yarrowia lipolytica* is phylogenetically distant from any other species examined: the linkage was at 25 base differences.

Partial sequencing in positions 1451 through 1618 of 18S rRNA

The primary partial sequences of the nine strains of *Arxula*, *Stephanoascus*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum* species were aligned (Fig. 7). The finger print segment (positions 1488 through 1491 in *S. cerevisiae*) (17) was characterized by AAAUAA in the genus *Arxula*, by ACAUAA in the genus *Stephanoascus*, by AUAU in the genus *Endomyces*, by CGA in the genus *Yarrowia*, by AUAU in the genus *Dipodascus*, and by CUAA in the genus *Geotrichum*. The number of base differences was calculated among the strains examined.

As shown in Fig. 8, the base difference was 0 between the two strains examined of *A. terrestris* and *St. ciferrii*. Within the genus *Arxula*, the base differences were 2. The genera *Arxula* and *Stephanoascus* are phylogenetically related to each other: the base differences were 2–4. The strains classified in the genera *Arxula* and

<i>A. terrestris</i>	CBS 6697*
0	CBS 7376
2	2 <i>A. adeninivorans</i> CBS 8244*
2	2 4 <i>St. ciferrii</i> IFO 1854*
2	2 4 0 IFO 1855*
7	7 8 8 8 <i>E. fibuliger</i> IFO 1744*
14	14 12 14 14 13 <i>Y. lipolytica</i> IFO 1548*
10	10 10 11 11 8 14 <i>D. albidus</i> IFO 1984
8	8 7 9 9 5 9 7 <i>G. candidum</i> IFO 4597
8	8 6 9 9 8 9 10 5 <i>S. cerevisiae</i> IFO 2376

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 *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* 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 *Arxula*, *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species. The dendrogram was drawn by the simple linkage method (3). *Type strain.

Stephanoascus had 6–14 and 7–14 base differences, respectively, with the strains examined of *Endomyces*, *Yarrowia*, *Dipodascus*, *Geotrichum*, and *Saccharomyces* species. *Yarrowia lipolytica* IFO 1548 (type strain) had 9–14 base differences.

Based on the calculated number of base differences, a dendrogram was drawn by the simple linkage method (3). As shown in Fig. 9, the strains of *Arxula* and *Stephanoascus* species were linked to one another at 2 base differences. *Arxula*, *Endomyces*, *Saccharomyces*, *Dipodascus*, *Geotrichum*, and *Yarrowia* species constituted their own clusters separating from each other at 5–9 base differences. *Yarrowia lipolytica* is phylogenetically distant from any other species examined: the species was linked at 9 base differences.

DISCUSSION

Arxula terrestris was first described as *Trichosporon terrestre* van der Walt et Johannsen (1975) (12), and *A. adeninivorans* as *Trichosporon adeninivorans* Middelhoven, Hoogkamer-te Niet et Kreger-van Rij (1984) (8).

van der Walt and Johannsen (12) found that *T. terrestre* is characterized by an essentially ascomycetous, double-layered cell wall and has a ploidy of greater than unity. van der Walt et al. (15) reexamined the species by transmission electron microscopy and reported it as having multiperforate septa, as observed in the genus *Geotrichum* Link and its teleomorphic genus *Dipodascus* de Lagerheim. *Trichosporon terrestre* was once classified in the genus *Geotrichum* (16). The present study has demonstrated that *A. terrestris* ($\equiv T. terrestre$) is phylogenetically separate from the species classified in the genera *Geotrichum* and *Dipodascus*.

Middelhoven et al. (10) reported that the two species of the genus *Trichosporon* (*T. terrestre* and *T. adeninivorans*) are common in assimilating purines such as uric acid and adenine as a sole source of carbon and nitrogen. The assimilation characters are in agreement with those of *St. ciferrii* (9, 10). The extracellular polysaccharide enzyme-linked immunosorbent assay pointed out that *St. ciferrii* is related to the two species, *T. terrestre* (and *T. adeninivorans*) (7, 9). The present study has demonstrated that the anamorphic and teleomorphic species are phylogenetically related to each other.

There were 7–9 and 2 base differences between the two species of the genus *Arxula*, respectively, in the positions 1685 through 1835 (151 bases) of 26S rRNA and in the positions 1451 through 1618 (168 bases) of 18S rRNA. The discrimination between the two species is in the ability to ferment sugars: *A. adeninivorans* has it but *A. terrestris* does not (8, 12). Considering the calculated number of base differences, the genus *Arxula* is not so small phylogenetically.

It is of interest that *Y. lipolytica* ($= Candida lipolytica$, $\equiv Saccharomycopsis lipolytica$) occupies a position phylogenetically distant from any other species examined. *Yarrowia lipolytica* has been characterized in the finger print segment by three bases composed of CGA. The species has a urease activity differing from any other ascomycetous, teleomorphic and anamorphic yeast species (4, 13), and

different antigenic structures (in the *Schizosaccharomyces* group)(11). The results obtained are considered to reflect such unique physiological and serological properties of *Y. lipolytica*.

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