THE MOLECULAR PHYLOGENY OF THE Q₉-EQUIPPED
ASCOMYCETOUS TELEOMORPHIC YEAST GENUS
DEBARYOMYCES LODDER ET KREGER-VAN RIJ
BASED ON THE PARTIAL SEQUENCES OF 18S
AND 26S RIBOSOMAL RIBONUCLEIC ACIDS¹

YUZO YAMADA,² TAKAHIKO NAGAHAMA, AND ISAO BANNO*

*Laboratory of Applied Microbiology, Department of Agricultural Chemistry,
Shizuoka University, Shizuoka 422, Japan*

**Institute for Fermentation, Osaka, Jusohon-machi,
Yodogawa-ku, Osaka 532, Japan*

(Received April 26, 1991)

The partial base sequences of 18S and 26S rRNAs were examined in eighteen strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species including two strains of *D. udonii*. All of the strains of *Debaryomyces* species constituted a single group (cluster) phylogenetically. In the partial base sequence (positions 493 through 622, 130 bases) of 26S rRNA, the maximum homologies were 79–99% among *Debaryomyces* species. *T. globosa* and *Y. philogaea* had 71–78% and 81–87% maximum homologies, respectively, with *Debaryomyces* species. In the partial base sequence (positions 1611 through 1835, 225 bases) of 26S rRNA, the base differences numbered 5–0 among *Debaryomyces* species. *T. globosa* and *Y. philogaea* had 15–13 and 10–8 base differences, respectively, with *Debaryomyces* species. In the partial base sequence (positions 1451 through 1618, 168 bases) of 18S rRNA, *Debaryomyces* species were divided into two subgroups (subclusters). The first subgroup was comprised of *D. hansenii*, *D. melissophilus*, *D. udonii*, and so on, and the second subgroup comprised of *D. castellii*, *D. polymorphus*, *D. yamadae*, and so on. The base difference numbered 1 between the two subgroups. *T. globosa* and *Y. philogaea* had 5–4 and 1–0 base differences, respectively, with *Debaryo-*

¹ This constitutes Part XLII of a series entitled "Significance of the coenzyme Q system in the classification of yeasts and yeast-like organisms." For Part XLI, see ref. 21.

² Address reprint requests to: Dr. Yuzo Yamada, Laboratory of Applied Microbiology, Department of Agricultural Chemistry, Shizuoka University, 836 Ohya, Shizuoka 422, Japan.

Abbreviations: Co-Q, coenzyme Q or ubiquinone; Q-9 or Q₉ and Q-6 or Q₆, coenzyme Q or ubiquinone homologues with 9 and 6 isoprene units, respectively, in a side chain; rRNA, ribosomal RNA; S, Svedberg value.

myces species. Between *T. globosa* and *S. cerevisiae*, there was 1 base difference. *D. tamarai* occupied a distant position (maximum homologies, 63–71%; base differences, 50–48 and 20–19, respectively).

The genus *Debaryomyces* Lodder et Kreger-van Rij was established with the type species, *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij (6–8, 11, 13–15). This genus is characterized morphologically by the formation of warty (or verrucate) ascospores (7, 8, 11), physiologically by the slow, weak but not vigorous fermentation (6, 7, 11, 14, 17), and chemotaxonomically by the Q-9 system (1, 13, 17, 20, 22, 23).

A new, soil-associated species was recently described in the genus *Debaryomyces* as *Debaryomyces udenii* van der Walt, Smith et Yamada (18). However, this species is recognized to be an intermediate between *P. abadiae*, *P. carsonii*, and *P. etchellsii* which form more or less glabrous ascospores and the presently accepted *Debaryomyces* species which form verrucate ascospores, since it produces colliculate to pusticulate rather than verrucate ascospores (18).

This paper is concerned with the partial base sequences of 18S and 26S rRNAs of *D. udenii* along with the presently accepted species of the genus *Debaryomyces* from the phylogenetic point of view.

MATERIALS AND METHODS

Yeast strains examined and cultivation. The yeast strains examined are listed in Table 1. The eighteen strains were grown as described previously (19).

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

Partial base sequencing of 18S and 26S rRNAs. The partial base sequences of 18S and 26S rRNAs of the yeast strains were determined by the method of Lane et al. (10) using reverse transcriptase. The oligonucleotide DNA primers used in this experiment were 5'-ACGGGCGGTGTGTAC-3', which is complementary to the sequence of positions 1641 through 1627 of 18S rRNA (in *Saccharomyces cerevisiae*) (12) and 5'-GGTCCGTGTTCAAGACGG-3' and 5'-TTGGAGACCTGCTGCGG-3', which are complementary to the sequences of positions 654 through 636 and 1857 through 1841, respectively, of 26S rRNA (in *S. cerevisiae*) (4).

Chemicals. The chemicals used in this experiment were the same as those described previously (19).

RESULTS

Partial base sequences in positions 493 through 622 of 26S rRNA

The primary partial base sequences of the strains examined in *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species were aligned (Fig. 1). The maximum

Table 1. The strains examined of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species.

Species and strain	Other designation	Co-Q system ^a
<i>Debaryomyces</i> Lodder et Kreger-van Rij		
<i>D. castellii</i> Capriotti		
IFO 1359*	CBS 2923, JCM 6177, A. Capriotti	Q-9
<i>D. coudertii</i> Saez		
JCM 2387*	CBS 5167, IFO 1817, H. Saez	Q-9
<i>D. hansenii</i> (Zopf) Lodder et Kreger-van Rij var. <i>hansenii</i>		
IFO 0083*	CBS 767, JCM 1990	Q-9
<i>D. hansenii</i> (Zopf) Lodder et Kreger-van Rij var. <i>fabryi</i> (Ota)		
Nakase et Suzuki		
IFO 0015*	CBS 789, JCM 2104, M. Ota	Q-9
<i>D. marama</i> di Menna		
IFO 0668*	CBS 1958, JCM 1528, M. E. di Menna	Q-9
IFO 1878	M. Mikata	
<i>D. melissophilus</i> (van der Walt et van der Klift) Kurtzman et Kreger-van Rij		
IFO 1900*	CBS 6344, JCM 1707, J. P. v.d. Walt	Q-9
<i>D. nepalensis</i> Goto et Sugiyama		
JCM 2095*	AJ 4967, CBS 5921, IFO 1428, J. Sugiyama	Q-9
<i>D. polymorphus</i> (Klöcker) Price et Phaff		
IFO 1166*	CBS 186, JCM 3647, O. Winge	Q-9
<i>D. pseudopolymorphus</i> (Ramirez et Boidin) Price et Phaff		
IFO 1026*	CBS 2008, JCM 3652, J. Boidin	Q-9
<i>D. tamaritii</i> Ohara et Nonomura		
IFO 0854*	CBS 4333, JCM 6351, Y. Ohara	Q-9
<i>D. udenii</i> van der Walt, Smith et Yamada		
JCM 7855*	CBS 7056, J. P. v.d. Walt	Q-9
JCM 7856	CBS 7057, J. P. v.d. Walt	
<i>D. vanrijiae</i> (van der Walt et Tscheuschner) Abadie, Pignal et Jacob var. <i>vanrijiae</i>		
IFO 0934*	CBS 3024, JCM 3657, J. P. v.d. Walt	Q-9
<i>D. vanrijiae</i> (van der Walt et Tscheuschner) Abadie, Pignal et Jacob var. <i>yarowii</i>		
(Santa Maria et Garcia-Aser) Kreger-van Rij		
IFO 1818*	CBS 6246, JCM 6190, J. Santa Maria	Q-9
<i>D. yamadai</i> (van der Walt et Johannsen) van der Walt, Smith et Yamada		
JCM 6191*	CBS 7036, J. P. v.d. Walt	Q-9
<i>Torulaspora</i> Lindner		
<i>T. globosa</i> (Klöcker) van der Walt et Johannsen		
IFO 1160*	CBS 764	Q-6
<i>Yamadazyma</i> Billon-Grand		
<i>Y. philogaea</i> (van der Walt et Johannsen) Billon-Grand		
CBS 6696*		Q-9

^a Data from Yamada and Kondo (20), Yamada et al. (22,23), Nakase and Suzuki (13), van der Walt and Johannsen (17), van der Walt et al. (18), and Billon-Grand (1,2).

* Type strain.

Abbreviations: IFO, Institute for Fermentation, Osaka, Osaka, Japan; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Wako, Saitama, Japan.

homology (%) was calculated in the respective pairs of the strains by computer analysis using a Hitachi DNAsis (Ver. 7, Hitachi Software Engineering Co., Ltd., Tokyo, Japan) instead of calculating the number of base differences, since the base

substitutions frequently occurred in this region.

As shown in Fig. 2, the maximum homologies were 79–99% among the strains of the species of the genus *Debaryomyces*, except for *D. tamarii* IFO 0854 which had abnormally low maximum homologies (63–71%) with the respective species of the genus *Debaryomyces*. The maximum homologies of *T. globosa* IFO 1160 were 71–78% and 87% with the strains of *Debaryomyces* species and *S. cerevisiae*, respectively. *Yamadazyma philogaea* CBS 6696 had 81–87% and 78% maximum homologies with the strains of *Debaryomyces* species and *T. globosa*, respectively.

Based on the calculated maximum homologies, a dendrogram was drawn by the simple linkage method (5). As shown in Fig. 3, the strains of *Debaryomyces* species constituted a single cluster with 86–99% maximum homologies. *Yamada-*

	.493	.501	.511	.521	.531	.541
1 <u>D.castellii</u> IFO 1359*	GGCCAGCA	UCGGUUUGGA	UGGUGGGAUA	AUGACUUGGG	AAUGUG.CUNU	GCUCGGU.NA
2 <u>D.coudertii</u> JCM 2387*	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGAUUAAGG	AAUGUGGCUCU	GCUCGGUUGGA
3 <u>D.hansenii</u> var. <u>hansenii</u> IFO 0083*	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGACUAAGG	AAUGUGGCUCU	ACUUCGGUUGGA
4 <u>D.hansenii</u> var. <u>fabryi</u> IFO 0015*	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGACUAAGG	AAUGUGGCUCU	ACUUCGGUUGGA
5 <u>D.marana</u> IFO 0688*	GGCCAGCA	UCGGUUUGGA	CGGUAGGAUA	AUGAUUAAGG	AAUGUGGCUCU	GCUGCGGUUGGA
6 IFO 1878	GGCCAGCA	UCGGUUUGGA	CGGUAGGAUA	AUGAUUAAGG	AAUGUGGCUCU	ACUUCGGUUGGA
7 <u>D.melissophilus</u> IFO 1900*	GGCCAGCA	UCAAUUUGGA	UGAUUGGAUA	AUGAAUCAGG	AAUGUAGCUUU	ACUUCGGUGAA
8 <u>D.nepalensis</u> JCM 2095*	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGAUUAAGG	AAUGUGGCUCU	ACUUCGGUUGGA
9 <u>D.polymorphus</u> IFO 1186*	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGACUUGGG	AAUGUGACUUU	ACUUCGGUAAA
10 <u>D.pseudopolymorphus</u> IFO 1028*	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGACUUGGG	AAUGUGGCUUU	GCUCGGUAAA
11 <u>D.tamarii</u> IFO 0854*	GGCUAACA	UCAGUUUUGG	CGGAUGGAUA	A..A.U.GGU	AAAGUGGCAUU	..UUCGG.AU.
12 <u>D.udenii</u> JCM 7855*	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGAUUAAGG	A.UGUGAGCGC	..UUCGGUNU.
13 JCM 7856	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGAUUAAGG	AAUGUG.GCGC	..UUCGGUNU.
14 <u>D.vanriijiae</u> var. <u>vanriijiae</u> IFO 0934*	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGUCUUGGG	AAUGUGGCUUU	GCUCGGUAAA
15 <u>D.vanriijiae</u> var. <u>varrowii</u> IFO 1818*	GGCCAGCA	UCGGUUUGGA	UGGUAGGAUA	AUGACUUGGG	AAUGUGGCUUU	GCUCGGUAAA
16 <u>D.yamadae</u> JCM 8191*	GGCCAGCA	UCGGUUUGGA	UGGUGGGGAUA	AUGACAUIGG	AAUGUGGCUCU	GCUCGGUAGA
17 <u>T.globosa</u> IFO 1160*	GGCCAGCA	UCAGUUUUGG	CGGUAGGAUA	AAUCUGGAG.	AAUGUAGCUU.	GCUCGGC.AA
18 <u>Y.philogaea</u> CBS 6696*	GGCCAGCA	UCAGUUUUGGA	CGGUUGGGGAUA	AUAGCGCGAGG	AAUGUGGCUCG	GCUCGGUUNGA
19 <u>S.cerevisiae</u> IFO 2376	GGCCAGCA	UCAGUUUUGG	UGGCAGGAUA	AAUCCAUIAGG	AAUGUAGCUU.	GCUCGGU.AA

	.551	.561	.571	.581	.591	.601	.611	.621
1 GUGUUUAGC CCUN.GUUGAU ACCGCCUGUC UANCGCCGAG .CUGC.UC.. UUUGAC.UAGGA UGUGGGCAUA AU								
2 GUGUUUAGC CUUG.GUUGAU ACUGCCUGUC UAGAGACGAG .CUGCGUC.. UUUGAC.UAGGA UGUUGGCAUA AU								
3 GUGUUUAGC CUNN.GUUGAU ACUGCCNGUC UAGACCGAGG .CUGCGUC.U UUUGAC.UAGGA UGUUGGCAUA AU								
4 GUGUUUAGC CUUN.GUUGAU ACUGCCUGUC UAGACCGAGG .CAGCGUC.. UUUGAC.UAGGA UGCUGGCAUA AU								
5 GUGUUUAGC CUUN.GUUGAU GCUGCCUGUC UAGACCGAGG ACUGCGUV.U UUUGAC.UAGGA UCUUGGCAUA AU								
6 GUGUUUAGC CUUN.GUUGAU GCUGCCUGUC UAGACCGAGG ACUGCGUC.U UUUGAC.UAGGA UGCUGGCAUA AU								
7 GUGUUUAGC CUGU.GUUGAU ACCGUCUAGC UAGAUUGAGG ACUGCGUC.U UUUGAC.AAGGA UGUUGGCAUA AU								
8 GUGUUUAGC CUUG.GUUGAU ACUGCCUGUC UAGAN.GCGA GCUGCGUN.. UUUGAC.UAGGA UGCUGGCAUA AU								
9 GUGUUUAGC CCUN.GUUGAU ACUGCCUGUC UAGACCGAGG ACUGCGUC.. UUUGAC.UAGGA UGCUGGCAUA AU								
10 GUGUUUAGC CCUN.GUUGAU ACUACCUGUC UAGACCGAGG ACUGCGUC.. UUUGAC.UAGGA UGUUGGCAUA AU								
11 GUGUUUAGC UUUUUGCAAAU ACGUAACGGC GGAUUGAGG ACCGCG.C.U UUAUGC.AAGGA UGUUGGCAUA AU								
12 GUGUUUAGC CUUA.GUUGAU ACUGCCUGUC UAGACCGAGC .CUGC.UC.. UUUGAC.UAGGA UGUUGGCAUA AU								
13 NUGUUUAGC CUUA.GUUGAU ACUGCCUGUC UAGACCGA.G .CUGC.UC.. UUUGAC.UAGGA UGUUGGCAUA AU								
14 GUGUUUAGC CCUU.GUUGAU GCUACCUGUC UAGACCGAGG ACUGCGUC.. UUUGAC.UAGGA UGUUGGCAUA AU								
15 GUGUUUAGC GCUN.GUUGAU GCUACCUGUC UAGACCGAGG ACUGCGUC.. UUUGAC.UAGGA UGUUGGCAUA AU								
16 GUGUUUAGC GCUUUGUGUAG ACCACCUGUC UAGACCGUGG .CUGCGUC.. UUUGACUAGGA UGUUGGCAUA AU								
17 GUGUUUAGC CUGU.UGAAAU ACUGCCAGCC GGCACUGAGG ACUGCGACUU UUAGUC.AAGGA UGCUGGCAUA AU								
18 GUGUUUAGC CUNG.GUUGAU ACCGCCUGUC UAGAGCUGAG GCUGCGUCUU UGAGU...AGGA UGCUGGCAUA AU								
19 GUUUUAGC CUGU.GGGAUU ACUGCCAGCU GGCACUGAGG ACUGCGACGU A.AGUC.AAGGA UGCUGGCAUA AU								

Fig. 1. The primary partial base sequences in positions 493 through 622 of 26S rRNA in strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species. The primary partial sequences were aligned. The arabic numerals indicate positions in *S. cerevisiae* (4). N, A, G, C, or U. *Type strain.

zyma philogaea CBS 6696 was linked to the cluster of *Debaryomyces* species at 87% maximum homology. *Torulaspora globosa* IFO 1160 and *S. cerevisiae* IFO 2376 constituted another cluster with 87% maximum homology. These two clusters were linked to each other at 78% maximum homology. The linkage of *D. tamaris* IFO 0854 was at 76% maximum homology.

D.castellii IFO 1359*
 88 D.coudertii JCM 2387*
 86 93 D.hansenii var.hansenii IFO 0083*
 87 93 96 D.hansenii var.fabryi IFO 0015*
 83 91 92 91 D.marama IFO 0668*
 84 92 94 95 96 IFO 1878
 81 84 86 84 82 84 D.melissophilus IFO 1900*
 85 96 92 94 90 93 82 D.nepalensis JCM 2095*
 89 88 90 93 87 91 85 89 D.polymorphus IFO 1166*
 89 89 90 92 88 90 83 88 97 D.pseudopolymorphus IFO 1026*
 63 68 69 68 69 70 71 66 66 67 D.tamaris IFO 0854*
 88 92 89 90 87 88 80 90 87 87 69 D.udenii JCM 7855*
 87 91 88 89 87 87 79 89 86 87 68 99 JCM 7856
 88 89 89 90 89 90 84 87 95 97 67 87 87 D.vanrijae var.vanrijae IFO 0934*
 88 88 89 90 89 90 83 86 95 97 67 87 86 98 D.vanrijae var.yarrowii IFO 1818*
 87 88 88 89 85 86 82 86 87 90 65 85 84 90 90 D.yamadae JCM 6191*
 71 76 76 76 76 78 77 74 77 78 76 75 74 77 76 72 T.globosa IFO 1160*
 82 87 86 87 83 85 82 85 83 84 67 81 81 82 82 85 78 Y.philogaea CBS 6696*
 70 76 75 75 73 75 76 74 76 76 72 73 72 75 75 73 87 74 S.cerevisiae IFO 2376

Fig. 2. A triangle matrix based on the calculated maximum homologies in the partial base sequences in positions 493 through 622 of 26S rRNA in strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species. The maximum homologies (%) were calculated by computer analysis using a Hitachi DNAsis in positions 493 through 622 (130 bases) of 26S rRNA. *Type strain.

Fig. 3. A dendrogram based on the calculated maximum homologies in the partial base sequences in positions 493 through 622 of 26S rRNA in strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species. The dendrogram was drawn by the simple linkage method (5). *Type strain.

Partial base sequences in positions 1611 through 1835 of 26S rRNA

The primary partial base sequences of the strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species were aligned (Fig. 4). The number of base differences was calculated among the strains examined.

As shown in Fig. 5, the base differences numbered 5–0 among the strains of *Debaryomyces* species except for *D. tamaritii* IFO 0854. There was no base difference among the strains of *D. hansenii* var. *hansenii*, *D. hansenii* var. *fabryi*, *D. marama*, and so on, and of *D. polymorphus*, *D. pseudopolymorphus*, *D. vanrijae* var. *vanrijae*, and *D. yamadae*, and between the strains of *D. udenii*, respectively. *Debaryomyces melissophilus* IFO 1900 had 5–2 base differences, compared with the strains of other *Debaryomyces* species. The number of base differences was very large in *D. tamaritii* IFO 0854 (50–48). *Torulaspora globosa* IFO 1160 and *Y. philogaea* CBS 6696 had 15–13 and 10–8 base differences, respectively, from the strains of *Debaryomyces* species.

Based on the calculated number of base differences, a dendrogram was drawn by the simple linkage method (5). As shown in Fig. 6, all the strains of *Debaryomyces* species constituted a single cluster within 2 base differences. *Yamadazyma philogaea* CBS 6696 was linked to the cluster of *Debaryomyces* species at 8 base differences. *Torulaspora globosa* IFO 1160 and *S. cerevisiae* IFO 2376 constituted a

	.1611	.1621	.1631	.1641	.1651	.1661	.1671	.1681	.1691	.1701
1 <i>D. castellii</i> IFO 1358*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
2 <i>D. coudertii</i> JCM 2387*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
3 <i>D. hansenii</i> var. <i>hansenii</i> IFO 0083*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
4 <i>D. hansenii</i> var. <i>fabryi</i> IFO 0015*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
5 <i>D. marama</i> IFO 0668*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
6 IFO 1878	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
7 <i>D. melissophilus</i> IFO 1900*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
8 <i>D. nepalensis</i> JCM 2095*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
9 <i>D. polymorphus</i> IFO 1186*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
10 <i>D. pseudopolymorphus</i> IFO 1026*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
11 <i>D. tamaritii</i> IFO 0854*	ACUUGAUU	GGAGUUUCCA	CGGUAACG	AUUGAUGG	CGAGCGCG	CACAGACC	GGGAGGAG	UUCUUCUU	UUUACGGA	AGCGACCCG
12 <i>D. udenii</i> JCM 7855*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
13 JCM 7856	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
14 <i>D. vanrijae</i> var. <i>vanrijae</i> IFO 0934*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
15 <i>D. vanrijae</i> var. <i>varrowii</i> IFO 1818*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
16 <i>D. yamadae</i> JCM 6191*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
17 <i>T. globosa</i> IFO 1160*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
18 <i>Y. philogaea</i> CBS 6696*	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	CUNNUUCU	CUUACAGC	U-AUACCCG
19 <i>S. cerevisiae</i> IFO 2376	ACUUGAUU	GGA-UUCU	CGGUAACG	ACUGAUGG	GAGACGUG	CGGAGGCC	GGGAGGAG	AUCUUCUU	CUUACAGC	U-AUACCCG

	.1711	.1721	.1731	.1741	.1751	.1761	.1771	.1781	.1791	.1801	.1811	.1821	.1831
1 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
2 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
3 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
4 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
5 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
6 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
7 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
8 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
9 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
10 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
11 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
12 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
13 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
14 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
15 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
16 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
17 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
18 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU
19 GAUUGGUU	AUCCGAGU	AGGGUNUU	GGGUGAAG	GGGCAUUA	CU	UUUGUGCU	CGGUGGCU	UACGCGGC	CUUGAAAU	CACAGGAG	AUUGUUUU	AUCCGAGU	GUACU

Fig. 4. The primary partial base sequences in positions 1611 through 1835 of 26S rRNA in strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species. The primary partial sequences were aligned. The arabic numerals indicate positions in *S. cerevisiae* (4). N, A, G, C, or U. *Type strain.

<i>D. castellii</i> IFO 1359*									
3	0	0	0	0	0	0	0	0	<i>D. coudertii</i> JCM 2387*
3	0	0	0	0	0	0	0	0	<i>D. hansenii</i> var. <i>hansenii</i> IFO 0083*
3	0	0	0	0	0	0	0	0	<i>D. hansenii</i> var. <i>fabryi</i> IFO 0015*
3	0	0	0	0	0	0	0	0	<i>D. marama</i> IFO 0668*
3	0	0	0	0	0	0	0	0	IFO 1878
3	4	4	4	4	4	4	4	4	<i>D. melissophilus</i> IFO 1900*
3	0	0	0	0	0	0	4	4	<i>D. nepalensis</i> JCM 2095*
1	2	2	2	2	2	2	2	2	<i>D. polymorphus</i> IFO 1166*
1	2	2	2	2	2	2	2	2	<i>D. pseudopolymorphus</i> IFO 1026*
50	50	50	50	50	50	50	48	48	<i>D. tamarai</i> IFO 0854*
4	1	1	1	1	1	5	1	3	<i>D. udenii</i> JCM 7855*
4	1	1	1	1	1	5	1	3	JCM 7856
1	2	2	2	2	2	2	0	0	<i>D. vanrijae</i> var. <i>vanrijae</i> IFO 0934*
3	4	4	4	4	4	4	2	2	<i>D. vanrijae</i> var. <i>yarrowii</i> IFO 1818*
1	2	2	2	2	2	2	0	0	<i>D. yamadae</i> JCM 6191*
15	15	15	15	15	15	14	15	14	<i>T. globosa</i> IFO 1160*
9	10	10	10	10	10	10	8	8	<i>Y. philogaea</i> CBS 6696*
19	18	18	18	18	18	18	18	54	<i>S. cerevisiae</i> IFO 2376

Fig. 5. A triangle matrix based on the calculated number of base differences in the partial base sequences in positions 1611 through 1835 of 26S rRNA in strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species. The number of base differences was calculated in positions 1611 through 1835 (225 bases) of 26S rRNA. *Type strain.

Fig. 6. A dendrogram based on the calculated number of base differences in the partial base sequences of positions 1611 through 1835 of 26S rRNA in strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species. The dendrogram was drawn by the simple linkage method (5). *Type strain.

separate cluster with 10 base differences. These two clusters were linked to each other at 13 base differences. The linkage of *D. tamarai* IFO 0854 was very distant (48 base differences).

Partial base sequences in positions 1451 through 1618 of 18S rRNA

The primary partial base sequences of the strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species were aligned (Fig. 7). The base differences of *D.*

hansenii var. *hansenii* IFO 0083 were at positions 1482 (G to A), 1509 (U to G), and 1512 (U to A), compared with *S. cerevisiae* IFO 2376. The finger print segment (19) (positions 1488 through 1491 in *S. cerevisiae*) was characterized by AUUAA (3 base differences with *S. cerevisiae* IFO 2376) in all the strains of *Debaryomyces* species. The base sequences of *T. globosa* IFO 1160 (CUAA) and *Y. phillogaea* CBS 6696 (AUUAA) coincided in the finger print segment with those of *S. cerevisiae* IFO 2376 and *D. hansenii* var. *hansenii* IFO 0083, respectively. The number of base differences was calculated among the strains examined.

As shown in Fig. 8, the base difference numbered 1 to 0: the strains of *Debaryomyces* species constituted a single group, which was divided into two subgroups. The first subgroup including the type species of the genus *Debaryomyces* was comprised of the strains of *D. hansenii* var. *hansenii*, *D. hansenii* var. *fabryi*, *D. marama*, *D. coudertii*, *D. melissophilus*, *D. nepalensis*, and *D. udenii*, and the second subgroup was comprised of the strains of *D. castellii*, *D. polymorphus*, *D. pseudo-*

	.1451	.1461	.1471	.1481	.1488	.1492	.1501	.1511
1 <i>D. castellii</i> IFO 1358*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
2 <i>D. coudertii</i> JCM 2387*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
3 <i>D. hansenii</i> var. <i>hansenii</i> IFO 0083*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
4 <i>D. hansenii</i> var. <i>fabryi</i> IFO 0015*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
5 <i>D. marama</i> IFO 0688*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
6 IFO 1878	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
7 <i>D. melissophilus</i> IFO 1900*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
8 <i>D. nepalensis</i> JCM 2095*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
9 <i>D. polymorphus</i> IFO 1166*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
10 <i>D. pseudopolymorphus</i> IFO 1026*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
11 <i>D. tamarii</i> IFO 0854*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	ACAUAAACCUUGGCCG	AAAGGUCUGG	GUAAUUCUUG	
12 <i>D. udenii</i> JCM 7855*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
13 JCM 7856	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AACGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
14 <i>D. vanriijiae</i> var. <i>vanriijiae</i> IFO 0934*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
15 <i>D. vanriijiae</i> var. <i>yarowii</i> IFO 1818*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
16 <i>D. yamadae</i> JCM 6191*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
17 <i>T. globosa</i> IFO 1160*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	CUAA.CCUUGGCCG	AGAGGUCUGG	GUAAUUCUUG	
18 <i>Y. phillogaea</i> CBS 6696*	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	AUUAA.CCUUGGCCG	AGAGGUCUGG	GAAAUUCUUG	
19 <i>S. cerevisiae</i> IFO 2376	GGGCCGACG	CGCGCUACAC	UGACGGAGCC	AGCGAGU	CUAA.CCUUGGCCG	AGAGGUCUGG	GUAAUUCUUG	

	.1521	.1531	.1541	.1551	.1561	.1571	.1581	.1591	.1601	.1611
1 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
2 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
3 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
4 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
5 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
6 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
7 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
8 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
9 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
10 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
11 UAAACUCCGU	CGUGCUGGGG	ACAGAGCUUU	GCAAUUUUUG	CUCUCGAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCA.C	UUGCGUUGAU	UACGUCCC	
12 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
13 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
14 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
15 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
16 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
17 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
18 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	
19 GAAACUCCGU	CGUGCUGGGG	AUAGAGCAUU	GUAAUUUUG	CUCUUAACG	AGGAUUUCCU	AGUAAGCGCA	AGUCAUCAGC	UUGCGUUGAU	UACGUCCC	

Fig. 7. The primary partial base sequences in positions 1451 through 1618 of 18S rRNA in strains of *Debaryomyces*, *Torulaspora*, and *Yamadazyma* species. The primary partial sequences were aligned. The arabic numerals indicate positions in *S. cerevisiae* (12). *Type strain.

Based on the calculated number of base differences, a dendrogram was drawn by the simple linkage method (5). As shown in Fig. 9, all the strains of *Debaryomyces* species were linked to each other at 1–0 base difference. The type strain of the type species of the genus *Debaryomyces*, *D. hansenii* var. *hansenii* IFO 0083 was included in the first subcluster (subgroup) of the genus *Debaryomyces*. *Yamadazyma philogaea* CBS 6696 was included in the second subcluster. *Torulaspora globosa* IFO 1160 was linked to *S. cerevisiae* IFO 2376 at 1 base difference, and then to the strains of *Debaryomyces* and *Yamadazyma* species at 4 base differences. *Debaryomyces tamarii* IFO 0854 was quite different, the linkage of which was at 19 base differences.

DISCUSSION

The type species, *Debaryomyces globosus* Klöcker, of the genus *Debaryomyces* Klöcker, was classed in the genus *Torulaspora* Lindner as *T. globosa* (Klöcker) van der Walt et Johannsen (16). In the present study, the accommodation of the species as *T. globosa* (16) and the proposal of the genus *Debaryomyces* Lodder et Kreger-van Rij with the type species, *D. hansenii* (Zopf) Lodder et Kreger-van Rij (11) proved to be adequate phylogenetically. Phylogenetically, the Q₆-equipped species, *T. globosa* was close to *S. cerevisiae* (Q-6) (1 base difference in partial base sequence of 18S rRNA), rather than to *D. hansenii* (Q-9) (13,20,22).

The members of the genus *Debaryomyces* Lodder et Kreger-van Rij constituted a single group (cluster) phylogenetically. All the species of the genus *Debaryomyces* had partial base sequences similar to each other except for *D. tamarii*. The maximum homologies were 79–99% in the partial base sequence (positions 493 through 622, 130 bases) of 26S rRNA, and the number of base differences was 1–0 in the partial base sequence (positions 1451 through 1618, 168 bases) of 18S rRNA. Similar results were obtained in the partial base sequence in positions 1611 through 1835 (225 bases) of 26S rRNA (base differences, 5–0).

Kurtzman and Robnett (9) reported the partial base sequences of 18S and 25S (26S) rRNAs of the members of the genus *Debaryomyces*. They recognized three subclusters in the genus *Debaryomyces* Lodder et Kreger-van Rij except for *D. tamarii*. Our results coincided with theirs, however, they did not examine the strains of *D. udonii*. According to their sequence data of 18S rRNA (9), the same base sequences, AUUAA and CUAA (their positions 1467–1489), as ours for *Debaryomyces* species and *S. cerevisiae*, respectively, are read in the finger print segment which represents one of the genus-specific base sequences (19). Based on the partial base sequences in positions 1451 through 1618 (168 bases) of 18S rRNA, we divided the genus *Debaryomyces* into two subgroups (subclusters). *Debaryomyces melissophilus* constituted their third subcluster (9). In contrast, this species was placed in our first subgroup as *D. hansenii*, *D. marama*, and *D. nepalensis* were. However, our partial base sequencing of 26S rRNA gave a somewhat different situation to this species; the maximum homologies were slightly lower (79–86%),

and the base differences were slightly higher (5–2).

Kreger-van Rij (7) observed that the strain of *D. tamarii* does not produce ascospores. The present study has demonstrated that the species is not classified in the genus *Debaryomyces*.

Debaryomyces udenii was recently described as a new species of the genus *Debaryomyces* (18). This species is somewhat different morphologically from the presently accepted species of the genus *Debaryomyces*. It produces colliculate to pusticulate rather than verrucate ascospores. The results obtained have indicated that *D. udenii* was not included in the second subgroup (subcluster) as the physiologically related *Debaryomyces* species, *D. castellii*, *D. pseudopolymorphus*, and *D. yamadae*, but in the first subgroup as *D. hansenii*, the type species of the genus *Debaryomyces*, in the partial base sequence determinations of 18S rRNA (positions 1451 through 1618, 168 bases).

It is notable that *Y. philogaea*, the type species of the Q₉-equipped genus *Yamadazyma* Billon-Grand (3), had the same partial base sequence in 18S rRNA as the members in the second subgroup (or subcluster) of the genus *Debaryomyces* (Q-9) such as *D. castellii*, *D. pseudopolymorphus*, and *D. yamadae*. However, the number of base differences was not so small (10–8), compared with *Debaryomyces* species, in the partial base sequence (positions 1611 through 1835, 225 bases) of 26S rRNA. The species of the genus *Yamadazyma* are characterized by producing hat-shaped ascospores. The partial base sequences of 18S and 26S rRNAs of the remaining species of the genus *Yamadazyma* will be presented elsewhere.

We express our thanks to Dr. J. P. van der Walt, Pretoria, South Africa for his guidance and encouragement of this experiment. Thanks are also due to Dr. M. Suzuki and Dr. T. Nakase, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Wako, Saitama, Japan and to Dr. D. Yarrow, Centraalbureau voor Schimmelcultures, Delft, The Netherlands, for sending us yeast cultures. This work was supported by a Grant-in-Aid (No. 2806022) for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

- 1) Billon-Grand, G., Coenzyme Q de quelques espèces du genre *Pichia*. Détermination qualitative et quantitative. *Mycopathologia*, **90**, 101–106 (1985).
- 2) Billon-Grand, G., Minor ubiquinones of the yeast coenzyme Q system: Importance in the taxonomy of the yeasts. *J. Gen. Appl. Microbiol.*, **33**, 381–390 (1987).
- 3) Billon-Grand, G., A new ascosporogenous yeast genus: *Yamadazyma* gen. nov. *Mycotaxon*, **35**, 201–204 (1989).
- 4) Georgiev, O. I., Nikolaev, N., Hadjiolov, A. A., Skryabin, K. G., Zakharyev, V. M., and Bayev, A. A., The structure of the yeast ribosomal RNA genes. 4. Complete sequence of 25S rRNA gene from *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **9**, 6953–6958 (1981).
- 5) Kaneko, T., 7. Data kaiseki (7. Data analysis). In *Biseibutsu no Kagaku Bunrui Jikkenho* (Methods in Chemotaxonomy of Microorganisms) (in Japanese), ed. by Komagata, K., Japan Scientific Societies Press, Tokyo (1982), p. 309–346.
- 6) Kocková-Kratochvilová, A., Sláviková, E., and Jensen, V., Numerical taxonomy of the yeast genus *Debaryomyces* Lodder et Kreger-van Rij. *J. Gen. Microbiol.*, **104**, 257–268 (1978).

- 7) Kreger-van Rij, N. J. W., Genus 7. *Debaryomyces* Lodder et Kreger-van Rij nom. cons. In *The Yeasts: A Taxonomic Study*, 3rd ed., ed. by Kreger-van Rij, N. J. W., Elsevier Science Publishers, Amsterdam (1984), p. 130-145.
- 8) Kreger-van Rij, N. J. W. and Veenhuis, M., Ultrastructure of the ascospores of some species of the *Torulaspora* group. *Antonie van Leeuwenhoek: J. Microbiol. Serol.*, **42**, 445-455 (1976).
- 9) Kurtzman, C. P. and Robnett, C. J., Phylogenetic relationships among species of *Saccharomyces*, *Schizosaccharomyces*, *Debaryomyces*, and *Schwanniomyces* determined from partial ribosomal RNA sequences. *Yeast*, **7**, 61-72 (1991).
- 10) Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L., and Pace, N. R., Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 6955-6959 (1985).
- 11) Lodder, J. and Kreger-van Rij, N. J. W., Proposal (446) for the conservation of the generic name *Debaryomyces* Lodder et Kreger-van Rij against *Debaryomyces* Klöcker. *Taxon*, **27**, 306-307 (1978).
- 12) Mankin, A. S., Skryabin, K. G., and Rubtsov, P. M., Identification of ten additional nucleotides in the primary structure of yeast 18S rRNA. *Gene*, **44**, 143-145 (1986).
- 13) Nakase, T. and Suzuki, M., Taxonomic studies on *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and related species. I. Chemotaxonomic investigations. *J. Gen. Appl. Microbiol.*, **31**, 49-69 (1985).
- 14) Nakase, T. and Suzuki, M., Taxonomic studies on *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and related species. II. Practical discrimination and nomenclature. *J. Gen. Appl. Microbiol.*, **31**, 71-86 (1985).
- 15) Price, C. W., Fuson, G. B., and Phaff, H. J., Genome comparison in yeast systematics: Delimitation of species within the genera *Schwanniomyces*, *Saccharomyces*, *Debaryomyces*, and *Pichia*. *Microbiol. Rev.*, **42**, 161-193 (1978).
- 16) van der Walt, J. P. and Johannsen, E., The genus *Torulaspora* Lindner. *C.S.I.R. Res. Rep.*, 325, 1-23 (1975).
- 17) van der Walt, J. P. and Johannsen, E., *Debaryozyma yamadae* spec. nov. (Saccharomycetaceae). *J. Gen. Appl. Microbiol.*, **26**, 217-222 (1980).
- 18) van der Walt, J. P., Smith, M. Th., and Yamada, Y., *Debaryomyces udenii* sp. nov. (Saccharomycetaceae), a new species from soil. *Antonie van Leeuwenhoek: J. Gen. Mol. Microbiol.*, **56**, 233-239 (1989).
- 19) Yamada, Y. and Kawasaki, H., The molecular phylogeny of the Q₈-equipped basidiomycetous yeast genera *Mrakia* Yamada et Komagata and *Cystofilobasidium* Oberwinkler et Bandoni based on the partial sequences of 18S and 26S ribosomal ribonucleic acids. *J. Gen. Appl. Microbiol.*, **35**, 173-183 (1989).
- 20) Yamada, Y. and Kondo, K., Taxonomic significance of the coenzyme Q system in yeasts and yeast-like fungi (2). In *Fermentation Technology Today: Proc. 4th Int. Ferment. Symp.*, Kyoto 1972, ed. by Terui, G., Society of Fermentation Technology, Japan, Osaka (1972), p. 781-784.
- 21) Yamada, Y., Nagahama, T., Banno, I., Giménez-Jurado, G., and van Uden, N., The phylogenetic relationship of *Kurtzmanomyces tardus* Giménez-Jurado et van Uden (Cryptococcaceae) based on the partial sequences of 18S and 26S ribosomal RNAs. *J. Gen. Appl. Microbiol.*, **37**, 321-324 (1991).
- 22) Yamada, Y., Nojiri, M., Matsuyama, M., and Kondo, K., Coenzyme Q system in the classification of the ascosporogenous yeast genera *Debaryomyces*, *Saccharomyces*, *Kluyveromyces*, and *Endomycopsis*. *J. Gen. Appl. Microbiol.*, **22**, 325-337 (1976).
- 23) Yamada, Y., Okada, T., Ueshima, O., and Kondo, K., Coenzyme Q system in the classification of the ascosporogenous yeast genera *Hansenula* and *Pichia*. *J. Gen. Appl. Microbiol.*, **19**, 189-208 (1973).