DNA-DNA REASSOCIATION STUDIES OF STRAINS IN THE GENERA *RHODOSPORIDIUM* AND *RHODOTORULA*

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The genetic relatedness of 60 strains in the basidiomycetous yeast genera *Rhodosporidium* and *Rhodotorula* was studied by the DNA-DNA hybridization using the nitrocellulose filter technique. Degrees of the intra-specific reassociation among the DNAs from the strains of *Rhodosporidium infirmominiatum*, *Rhodosporidium diobovatum*, *Rhodosporidium sphaerocarpum*, and *Rhodosporidium dacryoidum* ranged from 64 to 99%. The mating-type strains of *Rhodosporidium toruloides* were divided into four homology clusters. The degrees of DNA-DNA reassociation between the mating-type strains and the self-sporulating strains in *Rhodosporidium toruloides* were very low (18%) indicating the absence of relatedness between them. The strains of *Rhodotorula glutinis* were divided into five homology clusters. Among these strains, some, including the type strain, showed close relatedness to *Rhodosporidium diobovatum*, some to *Rhodosporidium toruloides* (self-sporulating), and some to *Rhodotorula rubra*, and *Rhodotorula pilimanae*. The strains of *Rhodotorula minuta* were divided into four homology clusters. A close relationship was found between some strains of *Rhodotorula minuta* including the type strain and a strain of *Rhodotorula pallida*. There was a very close relationship between the *Rhodotorula sinensis* strain and the strains of *Rhodosporidium infirmominiatum*.

The DNA-DNA hybridization technique has proved valuable in determining genetic relatedness among various yeasts (1–23). Recently, the genera *Rhodosporidium* and *Rhodotorula* were found to consist of phylogenetically different strains based on the DNA base composition (24–26), the ubiquinone system (27, 28), the electrophoretic patterns of enzymes (29, 30), and the cellular carbohydrate composition (28, 31).

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Table 1. Strains used.

Species	Strain	Source ^a	DNA base composition (mol% G+C) ^b	Ubiquinone system ^c
<i>Rhodospiridium</i>				
<i>Rhodospiridium dacryoidum</i>	YK 231 ^T	CBS 6353 (mating type A1B1)	58.4	10
	YK 232	CBS 6354 (mating type A2B2)	58.1	10
	YK 233	CBS 6355 (mating type A2B1)	58.1	
	YK 234	CBS 6356 (mating type A1B2)	58.9	10
	YK 1037	CBS 6357 (AmutBx)	57.8	
<i>Rhodospiridium diobovatum</i>	YK 104	IFO 0688 (mating type α)	65.0	10
	YK 108	IFO 0667 (mating type α)	66.1	10
	YK 223	IAM 12259 (mating type a)	65.5	10
	YK 224	IFO 1828 (mating type a)	64.8	10
	YK 225 ^T	IAM 12260 (mating type α)	65.0	
	YK 226	IFO 1830 (mating type α)	65.2	
<i>Rhodospiridium infirmominium</i>	YK 240 ^T	IFO 1057 (mating type A1)	66.3	8
	YK 241	CBS 6350 (mating type A2)	67.1	8
	YK 242	CBS 6352 (mating type A3)	67.5	
	YK 243	IFO 1378	66.6	8
<i>Rhodospiridium sphaerocarpum</i>	YK 118	IFO 1438 (mating type a)	62.2	
	YK 220 ^T	IAM 12261 (mating type a)	62.7	10
	YK 221	IAM 12262 (mating type α)	62.5	10
	YK 222	CCY 62-1-1 (self-sporulating)	62.7	10
<i>Rhodospiridium toruloides</i>	YK 201 ^T	IFO 0559 (mating type A)	59.9	9
	YK 202	IFO 0413 (mating type A)	59.9	9
	YK 207	GSB G-5 (mating type A)	60.2	
	YK 208	GSB G-8 (mating type A)	60.5	
	YK 211	IAM 12256 (mating type a)	60.1	9
	YK 212	IFO 1236 (mating type a)		9
	YK 216	IFO 1638 (mating type a)	60.1	9
	YK 250	MMHW 8 (mating type a)	59.3	9
	YK 218	CCY 62-3-1 (self-sporulating)	64.7	10
	YK 219	CCY 62-3-2 (self-sporulating)	64.9	10
<i>Rhodotorula</i>				
<i>Rhodotorula glutinis</i>	YK 102	IFO 0414	64.8	
	YK 103	IAM 4988		10
	YK 106	IFO 0697	61.2	10
	YK 107	IFO 1099	60.1	10
	YK 111	EC 6-1	66.1	
	YK 112	H-3-9-1	51.5	10
	YK 113	H-3-9-2	50.9	
	YK 114	KC 4-4	61.0	
	YK 115	KC 9	60.4	10
	YK 116	IAM 12228	60.4	10
	YK 1017 ^T	IFO 1125	67.1	10
	YK 125 ^T	IFO 0928	52.4	10
	YK 126 ^T	IFO 0387	50.0	10
	YK 127	IAM 12233		
	YK 128	IFO 1434	48.2	10
<i>Rhodotorula marina</i>				
<i>Rhodotorula minuta</i>				

Table 1. (continued).

Species	Strain	Source ^a	DNA base composition (mol% G + C) ^b	Ubiquinone system ^c
	YK 129	CBS 5695	49.2	10
	YK 130	IFO 1540	49.3	
	YK 131	IFO 0932	50.1	10
	YK 132	IFO 0879	50.1	10
	YK 135	IFO 0920	49.2	10
	YK 136	IAM 12231		10
	YK 137	IFO 1432	49.5	10
<i>Rhodotorula pallida</i>	YK 138 ^T	IFO 0715	50.6	10
	YK 160	CBS 5475	50.1	10
<i>Rhodotorula pilimanae</i>	YK 139	AJ 5217	59.6	10
	YK 140	CCY 20-15-1	59.5	
<i>Rhodotorula rubra</i>	YK 141	IAM 4989	59.7	10
	YK 155	IFO 0898	59.2	10
	YK 156	I 6-1	59.0	
	YK 1036 ^T	ATCC 9449	59.0	10
<i>Rhodotorula sinensis</i>	YK 165 ^T	CBS 6962	66.9	8

^a Abbreviations: AJ, Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan; ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; CCY, Czechoslovak Collection of Yeasts, Bratislava, Czechoslovakia; GSB, Greifswald, Sektion Biologie, West Germany; IAM, Institute of Applied Microbiology, The University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan.

^b From HAMAMOTO et al. (26).

^c From YAMADA and KONDO (27) and SUGIYAMA et al. (28).

^T Type strain.

Here, we report a study of the DNA-DNA reassociation of the strains in the genera *Rhodospiridium* and *Rhodotorula* and discuss the relationships among them with regard to the other biochemical characteristics.

MATERIALS AND METHODS

Yeast strains. We used 29 strains representing five species in the genus *Rhodospiridium* and 31 strains representing seven species in the genus *Rhodotorula*. The sources of these strains are shown in Table 1.

Cultivation and harvesting of cells, and isolation and purification of DNA. These procedures were as previously reported (26).

In vitro labeling of DNA probes. Purified native DNA was labeled in vitro with [1', 2', 5-³H]cytidine 5'-triphosphate by the nick translation technique described by RIGBY et al. (32), using the ³H-Nick Translation kit (Code TRK. 625 and Code N. 5000, Amersham International plc, Buckinghamshire, England). The DNA preparations were assigned to specific activities of 2.8×10^4 to 4.6×10^4 cpm/g.

DNA-DNA hybridization. The DNA-DNA hybridization was carried out by

Table 2. Intraspecific DNA relatedness of *Rhodospiridium infirmominiatum*, *Rhodospiridium diobovatum*, *Rhodospiridium sphaerocarpum*, *Rhodospiridium dacryoidum*, and *Rhodospiridium toruloides*.

Source of unlabeled DNA	% relative binding of DNA from					
	<i>Rhodospiridium infirmominimatum</i> YK 240 ^T	<i>Rhodospiridium diobovatum</i> YK 225 ^T	<i>Rhodospiridium sphaerocarpum</i> YK 220 ^T	<i>Rhodospiridium dacryoidum</i> YK 231 ^T	<i>Rhodospiridium toruloides</i> YK 201 ^T YK 207 YK 216 YK 218	
<i>Rhodospiridium infirmominimatum</i>						
YK 240 ^T	100					
YK 241	80					
YK 242	99					
YK 243	97					
<i>Rhodospiridium diobovatum</i>						
YK 104		65				
YK 108		64				
YK 223		95				
YK 224		75				
YK 225 ^T		100				
YK 226		91				

the procedure described by KANEKO (33). The labeled probe DNA was sheared by sonication in distilled water and denatured by heating, after which it was poured into 10% SDS and $20\times$ SSC to give a final concentration of $2\times$ SSC containing 0.1% SDS and a specific activity of approximately 6.0×10^3 cpm/ml. The unlabeled DNA in $2\times$ SSC (30 g/ml) was denatured by heating, trapped on a 25 mm filter (TOYO, TM-3, Toyo Roshi Co., Ltd., Tokyo, Japan) by suction, and fixed by heating for 3 hr in an oven at 90°C . The DNA-filters in vials were incubated at 60°C for 45 hr with 1 ml of the labeled DNA solution.

After the incubation, the vials were cooled at room temperature, and the filters were rinsed by two consecutive immersions in beakers containing about 200 ml of $2\times$ SSC, and washed twice by suction with 2 ml of $2\times$ SSC. Then the non-hybridized radioactive DNA was removed by washing the filters by suction with 1 ml and 5 ml of 5 mM Tris buffer (pH not adjusted) (34). The filters were dried at room temperature and counted in a PACKARD TRI-CARB 300 model scintillation spectrometer (Packard Instrument Co., Downers Grove, Illinois, U.S.A.). The relative binding was calculated as follows: Relative binding (%) = [(cpm of homologous filters)/(cpm of homologous filters)] $\times 100$.

All hybridizations were done in triplicate. The values of relative binding reported are the averages for three filters.

RESULTS

Intraspecific hybridization of the strains in Rhodosporidium infirmominiatum, Rhodosporidium diobovatum, Rhodosporidium sphaerocarpum, Rhodosporidium dacryoidum, and Rhodosporidium toruloides

Table 2 shows the results of DNA-DNA hybridization with the strains of the five species in the genus *Rhodosporidium*, i.e., *Rhodosporidium infirmominiatum*, *Rhodosporidium diobovatum*, *Rhodosporidium sphaerocarpum*, *Rhodosporidium dacryoidum*, and *Rhodosporidium toruloides*.

With DNA probes prepared from the type strains of the respective species, the degrees of intraspecific relative binding among the strains of four of the species, i.e., *Rhodosporidium infirmominiatum*, *Rhodosporidium diobovatum*, *Rhodosporidium sphaerocarpum*, and *Rhodosporidium dacryoidum*, ranged from 64 to 99%.

In *Rhodosporidium toruloides* with DNA probes from the three mating-type strains YK 201 (type strain), YK 207, and YK 216, the degrees of intraspecific relative binding among the mating-type strains ranged from 30 to 82%. With the DNA probe from the type strain, the self-sporulating strains gave low degrees of relative binding (18%) in comparison with the mating-type strains. The degrees of relative binding between the two self-sporulating strains was 79%.

Intraspecific hybridization of the strains in Rhodotorula glutinis and Rhodotorula rubra

Table 3 shows the results of DNA-DNA hybridization obtained with the

Table 3. Intraspecific DNA relatedness of *Rhodotorula glutinis* and *Rhodotorula rubra*.

Source of unlabeled DNA	% relative binding of DNA from		
	<i>Rhodotorula glutinis</i>		<i>Rhodotorula rubra</i>
	YK 1017 ^T	YK 115	YK 1036 ^T
<i>Rhodotorula glutinis</i>			
YK 1017 ^T	100		
YK 111	98		
YK 102	32		
YK 103	43		
YK 114		99	
YK 115	33	100	
YK 116	34	81	
YK 106	32	38	
YK 107	12	26	
YK 112	23		
YK 113	25		
<i>Rhodotorula rubra</i>			
YK 1036 ^T			100
YK 141			96
YK 155			99
YK 156			98

^T Type strain.

strains of *Rhodotorula glutinis* and *Rhodotorula rubra*.

Rhodotorula glutinis exhibited large intraspecific variation in the degrees of relative binding (12 to 99%).

With the DNA probe from strain YK 1017, strain YK 111 showed a high degree of relative binding (98%) but the other eight strains, YK 102, YK 103, YK 115, YK 116, YK 106, YK 107, YK 112, and YK 113, had low degrees of relative binding (12 to 43%).

Although the DNA base compositions of the five strains, YK 114, YK 115, YK 116, YK 106, and YK 107, were very close (range 60.1 to 61.2 mol% G+C) (26), these strains were divided into two groups with respect to the DNA-DNA hybridization, with the DNA probe from strain YK 115; strains YK 114 and YK 116 showed 81 to 99% relative binding; strains YK 106 and YK 107 showed 26 to 38% relative binding.

Based on the results of the DNA-DNA hybridization in this study and the DNA base compositions (26), the strains of *Rhodotorula glutinis* were divided into five groups (Table 4), presented as "homology clusters" in this study. Homology cluster I included strains YK 102 and YK 103 with 64.8 mol% G+C; homology cluster II included strains YK 114, YK 115, and YK 116 with 60.4–61.0 mol% G+C; homology cluster III included strains YK 112 and YK 113 with 50.9–

Table 4. Grouping of *Rhodotorula glutinis* based on the DNA-DNA hybridization and DNA base composition.

Homology cluster	Strain	DNA base composition (mol% G+C) ^a	Group based on the enzyme pattern ^{b,c}
I	YK 102	64.8	1
	YK 103		1
II	YK 114	61.0	2
	YK 115	60.4	2
	YK 116	60.4	2
III	YK 112	51.5	3
	YK 113	50.9	3
IV	YK 106	61.2	4
	YK 107	60.1	4
V	YK 111	66.1	4
	YK 1017 ^T	67.1	4

^a From HAMAMOTO et al. (26).^b From YAMAZAKI and KOMAGATA (29).^c From HAMAMOTO et al. (30).^T Type strain.

51.5 mol% G+C; homology cluster IV included strains YK 106 and YK 107 with 60.1–61.2 mol% G+C; and homology cluster V included strains YK 111 and YK 1017 (type strain) with 66.1–67.1 mol% G+C. These five groups were almost the same as the four groups based on the electrophoretic patterns of fructose-1, 6-bisphosphate aldolase (FA) and 6-phosphogluconate dehydrogenase (6PGDH) reported by YAMAZAKI and KOMAGATA (29), and almost the same as the overall similarity of the electrophoretic mobility of enzymes reported by HAMAMOTO et al. (30). The four strains, YK 106, YK 107, YK 111, and YK 1017, included in the same group by those authors were separated into two homology clusters based on their DNA base compositions; strains YK 106 and YK 107 were included in homology cluster IV; strains YK 111 and YK 1017 were in homology cluster V.

The degrees of intraspecific relative binding among the strains of *Rhodotorula rubra* ranged from 96 to 99%, with the DNA probe prepared from the type strain.

Interspecific hybridizations among the strains Rhodotorula glutinis, Rhodotorula rubra, Rhodotorula pilimanae, Rhodosporidium toruloides (self-sporulating), Rhodosporidium diobovatum, and Rhodosporidium sphaerocarpum

Table 5 shows the results of the DNA-DNA hybridizations among the strains of *Rhodotorula glutinis*, *Rhodotorula rubra*, *Rhodotorula pilimanae*, *Rhodosporidium toruloides* (self-sporulating), *Rhodosporidium diobovatum*, and *Rhodosporidium sphaerocarpum*, with the DNA probes from *Rhodotorula rubra* YK 1036 (type

Table 5. Interspecific DNA relatedness of *Rhodotorula glutinis*, *Rhodotorula rubra*, *Rhodotorula pilimanae*, *Rhodosporidium toruloides* (self-sporulating), *Rhodosporidium diobovatum*, and *Rhodosporidium sphaerocarpum*.

Source of unlabeled DNA	% relative binding of DNA from				
	<i>Rhodotorula glutinis</i>		<i>Rhodotorula rubra</i>	<i>Rhodosporidium toruloides</i>	<i>Rhodosporidium diobovatum</i>
	YK 1017 ^T	YK 115	YK 1036 ^T	YK 218 (ss) ^a	YK 225 ^T
<i>Rhodotorula glutinis</i>					
YK 102				71	
YK 103				81	
YK 114			82		
YK 115		100	67		
YK 116			66		
YK 111					52
YK 1017 ^T	100				69
<i>Rhodotorula pilimanae</i>					
YK 139		71	93		
YK 140		77	99		
<i>Rhodosporidium diobovatum</i>					
YK 108	78				
YK 223	60				
YK 224	75			36	
YK 225 ^T	58			37	100
YK 226	55				
<i>Rhodotorula rubra</i>					
YK 1036 ^T		72	100		
YK 141		75			
YK 155		73			
YK 156		74			
<i>Rhodosporidium sphaerocarpum</i>					
YK 220 ^T				25	
<i>Rhodosporidium toruloides</i>					
YK 218 (ss)				100	47

^a ss: self-sporulating strain.

^T Type strain.

strain), *Rhodotorula glutinis* YK 1017 (type strain) and YK 115, *Rhodosporidium toruloides* YK 218 (self-sporulating), and *Rhodosporidium diobovatum* YK 225 (type strain).

The degrees of interspecific relative binding among the strains of homology cluster I of *Rhodotorula glutinis* (YK 102 and YK 103) and the self-sporulating strains of *Rhodosporidium toruloides* ranged from 71 to 78%; the degrees among the strains of homology cluster II of *Rhodotorula glutinis* (YK 114, YK 115, and YK 116), *Rhodotorula rubra*, and *Rhodotorula pilimanae* ranged from 66 to 99%; the degrees among the strains of homology cluster V of *Rhodotorula glutinis* (YK 111 and YK 1017 (type strain)) and *Rhodosporidium diobovatum* ranged from 52 to 78%.

Table 6. Intra- and interspecific DNA relatedness of *Rhodotorula minuta*, *Rhodotorula pallida*, *Rhodotorula marina*, and strains YK 112 and YK 113 of *Rhodotorula glutinis*.

Source of unlabeled DNA	% relative binding of DNA from				
	<i>Rhodotorula minuta</i>				<i>Rhodotorula glutinis</i>
	YK 126 ^T	YK 129	YK 135	YK 128	YK 112
<i>Rhodotorula minuta</i>					
YK 126 ^T	100	22	17	14	10
YK 127	98	25	25	19	
YK 131	65	25	23	19	
YK 132	98	23	26	19	
YK 129	31	100	66	23	9
YK 130	25	78	68	26	
YK 135	25	63	100	26	7
YK 137	28	84	63	19	
YK 136	23	20	20	38	
YK 128	22	20	17	100	9
<i>Rhodotorula pallida</i>					
YK 138 ^T	24	15	15	37	8
YK 160	97	26	25	32	9
<i>Rhodotorula marina</i>					
YK 125 ^T	18	12	11	20	9
<i>Rhodotorula glutinis</i>					
YK 112	13	9	8	11	100
YK 113	13	8	7	17	87

^T Type strain.

Intra- and interspecific hybridizations among the strains of Rhodotorula minuta, Rhodotorula pallida, Rhodotorula marina, and the two strains of Rhodotorula glutinis

Table 6 shows the results of DNA-DNA hybridization among the strains of *Rhodotorula minuta*, *Rhodotorula pallida*, *Rhodotorula marina*, and strains YK 112 and YK 113 of *Rhodotorula glutinis*, with the DNA probes from the four strains of *Rhodotorula minuta*, YK 126 (type strain), YK 128, YK 129, and YK 135 and *Rhodotorula glutinis* YK 112.

Rhodotorula minuta exhibited large intraspecific variations in the degrees of relative binding (14 to 98%).

Rhodotorula pallida YK 160 showed 97% relative binding with the DNA probe from *Rhodotorula minuta* YK 126 (type strain). On the other hand, *Rhodotorula pallida* YK 138 (type strain) did not show particularly high degrees of relative binding with any of the DNA probes studied (8 to 37%).

Rhodotorula marina YK 125 (type strain) did not show high degrees of relative binding with any of the DNA probes studied (9 to 20%).

Rhodotorula glutinis YK 112 and YK 113 showed very low degrees of relative

Table 7. Interspecific DNA relatedness of *Rhodotorula sinensis* and *Rhodosporidium infirmominiatum*.

Source of unlabeled DNA	% relative binding of DNA from	
	<i>Rhodosporidium infirmominiatum</i> YK 240 ^T	<i>Rhodotorula sinensis</i> YK 165 ^T
<i>Rhodosporidium infirmominiatum</i>		
YK 240 ^T	100	75
YK 241	80	78
YK 242	99	89
YK 243	97	87
<i>Rhodotorula sinensis</i>		
YK 165 ^T	96	100

^T Type strain.

binding with the DNA probes from the four strains of *Rhodotorula minuta* (7 to 17%).

Interspecific hybridization between the strains of Rhodotorula sinensis and Rhodosporidium infirmominiatum

Table 7 shows the results of the DNA-DNA hybridizations among the strains of *Rhodotorula sinensis* and *Rhodosporidium infirmominiatum*, with the DNA probes from *Rhodotorula sinensis* YK 165 (type strain) and *Rhodosporidium infirmominiatum* YK 240 (type strain). The degrees of interspecific relative binding among the strains of *Rhodotorula sinensis* and *Rhodosporidium infirmominiatum* ranged from 75 to 99%.

DISCUSSION

DNA-DNA reassociation studies have been found useful for conspecificity in a few basidiomycetous yeasts (20–22). We discuss the usefulness of DNA-DNA reassociation as a criterion for conspecificity in the genera *Rhodosporidium* and *Rhodotorula* and the relationships among the strains of these genera with regard to the other biochemical characteristics.

Rhodosporidium

The intraspecific DNA-DNA reassociations of *Rhodosporidium infirmominiatum*, *Rhodosporidium diobovatum*, *Rhodosporidium sphaerocarpum*, and *Rhodosporidium dacryoidum* showed high values, respectively (64 to 99%). These data support the previous conclusion that these four species are well-defined species on the basis of interfertility (35), the DNA base composition (25, 26), the ubiquinone system (27, 28), and the overall similarity of the electrophoretic mobility of the enzymes studied (30). Therefore, the strains of the respective species were

considered to be a homogeneous taxon.

YAMAZAKI and KOMAGATA (29) reported that the self-sporulating strain YK 235 (originating from CBS 6357) of *Rhodospiridium dacryoidum* showed a different pattern of enzymes from the four mating-type strains, and that more information is needed to determine the correct taxonomic position of strain YK 235. We obtained a culture of the self-sporulating strain (CBS 6357) of *Rhodospiridium dacryoidum* from Centraalbureau voor Schimmelcultures (Delft, Netherlands) and studied the DNA base composition (26) and the DNA-DNA reassociation with the culture. In this study we found that the self-sporulating strain YK 1037 (originating from CBS 6357) has a DNA base composition similar to the mating-type strains and shows high degrees of relative binding with the type strain of *Rhodospiridium dacryoidum*. Thus, strain YK 1037 was regarded as a strain of *Rhodospiridium dacryoidum*. Possibly that the YK 235 strain used by YAMAZAKI and KOMAGATA (29) was mislabeled.

The two self-sporulating strains of *Rhodospiridium toruloides*, YK 218 and YK 219, showed very low values of reassociation with the type strain of *Rhodospiridium toruloides*. The data support the previous conclusion that the two self-sporulating strains were not related to the mating-type strains of *Rhodospiridium toruloides*, based on the electrophoretic patterns of enzymes (29), the ubiquinone system (28), and the DNA base compositions (26). Moreover, the DNA-DNA reassociation values between strain YK 218 and the strains of *Rhodospiridium diobovatum* and *Rhodospiridium sphaerocarpum* which have identical ubiquinone systems and similar DNA base compositions, were low in comparison to the values among the strains of the respective species (Table 5). Therefore, the two self-sporulating strains of *Rhodospiridium toruloides* were not very closely related to *Rhodospiridium diobovatum* and *Rhodospiridium sphaerocarpum*. The self-sporulating strains YK 218 and YK 219 should be reclassified as a new taxon in the genus *Rhodospiridium*.

The eight mating-type strains of *Rhodospiridium toruloides* examined in this study showed widely ranging reassociation values within the taxon. The intraspecific reassociation values among some strains in this species were rather low (30 to 54%) in comparison with the other four species of the genus *Rhodospiridium* (Table 2).

PRICE et al. (11) suggested that strains with 80 to 100% DNA base sequence relatedness almost certainly belong to the same species. Strains showing reassociation values of 20 to 25% or less are generally thought to belong to different species (1, 6-8, 10, 11). Values in the 20 to 65% range have been found only infrequently between yeasts, and their significance has not yet been assessed.

Recently, VAN DER WALT and JOHANNSEN (14) confirmed that the strains of the ten species in the genus *Kluyveromyces* showing 20% or less DNA-DNA reassociation were interfertile, and that the low reassociation values could not confidently be used to differentiate between species. KURTZMAN et al. (17) reported that the DNA-DNA reassociation values between *Issatchenkia scutulata* var. *scutulata* and *Issatchenkia scutulata* var. *exigua* averaged only 24%; yet intervarietal

mating gave 3 to 6% viable ascospores. Based on that observation, they (17) suggested that the DNA-DNA reassociation values indicative of conspecificity need to be extended considerably.

We believe that there can be no absolute level of DNA-DNA reassociation that separates specific taxonomic levels, and that the morphological, physiological, biochemical, and sometimes ecological characteristics should be used to analyze speciation.

Therefore, the eight mating-type strains of *Rhodosporidium toruloides* examined in this study, showing 30–82% reassociation values, are considered to belong to the same species, because the strains mate with the opposite mating type (29, 36, 37) and they have identical ubiquinone systems (27), similar DNA base compositions (26), and similar enzyme patterns (29, 30). However, it is not clear why the mating-type strains of only *Rhodosporidium toruloides* in the genus *Rhodosporidium* studied showed rather low intraspecific DNA-DNA reassociation values. Further molecular biology investigations will be required to determine the correlation between DNA homology and interfertility.

Rhodotorula

The strains of *Rhodotorula glutinis* studied were confirmed to be heterogeneous and were considered to be divided into five taxonomic groups based on the DNA base compositions (26) and the DNA-DNA homology (Table 3).

The strains of homology cluster I in *Rhodotorula glutinis* (YK 102 and YK 103) and strain YK 218 (presently classified in *Rhodosporidium toruloides*) show high DNA-DNA reassociation values (Table 5). They have identical ubiquinone systems (27, 28) and similar DNA base compositions (26), and they form a cluster in the numerical taxonomy based on the overall similarity of the electrophoretic mobility of the enzymes studied (30). Therefore, strains YK 102 and YK 103 are considered to be the anamorphic strains of *Rhodosporidium toruloides* self-sporulating strains YK 218 and YK 219.

The strains of homology cluster II in *Rhodotorula glutinis* (YK 114, YK 115, and YK 116) and the strains of *Rhodotorula rubra* showed high reassociation values (Table 5). They have identical ubiquinone systems (27, 28) and similar DNA composition (26), and they form a cluster in the numerical taxonomy (30). Therefore, strains YK 114, YK 115, and YK 116 are considered to be included in *Rhodotorula rubra*. We confirm the current inadequacy of discrimination between *Rhodotorula glutinis* and *Rhodotorula rubra* based on the ability to assimilate nitrate.

The strains of homology cluster V in *Rhodotorula glutinis* (YK 111 and YK 1017 (type strain)) and the strains of *Rhodosporidium diobovatum* showed high reassociation values (Table 5). They have identical ubiquinone systems (27, 28) and similar DNA composition (26), and they form a cluster in the numerical taxonomy (30). Therefore, strains YK 111 and YK 1017 are considered to be the anamorphic strains of *Rhodosporidium diobovatum*.

In this study none of the strains examined in the genera *Rhodosporidium* and *Rhodotorula* appeared to be closely related to the strains of homology cluster III (YK 112 and YK 113) or homology cluster IV (YK 106 and YK 107) in *Rhodotorula glutinis*, on the basis of the DNA-DNA homology or the other biochemical characteristics (26–28, 30).

Strains YK 112 and YK 113 of homology cluster III in *Rhodotorula glutinis*, isolated from the soil of Laya in Bhutan, were identified as *Rhodotorula glutinis* based on their close agreement with the standard description of *Rhodotorula glutinis* (38) by GOTO and SUGIYAMA (39). The two strains have the same ubiquinone system (27) and a DNA base composition (26) similar to *Rhodotorula minuta*, *Rhodotorula pallida*, and *Rhodotorula marina*, but the degrees of the DNA-DNA relative binding between the two strains and with the strains of the three species above are very low (Table 6). Moreover, the two strains differ from the other strains of the genera *Rhodosporidium* and *Rhodotorula* in enzymatic comparison (29, 30) and the Diazonium Blue B (DBB) color test (28). Therefore, we conclude that strains YK 112 and YK 113 should be assigned to another genus.

The strains of *Rhodotorula pilimanae* and *Rhodotorula rubra* showed high DNA-DNA reassociation values (Table 5) and similar biochemical characteristics (26–28, 30). This supports the decision to place *Rhodotorula pilimanae* in synonymy with *Rhodotorula rubra*, based on their nearly identical patterns of sugar assimilation and similar DNA base composition (40).

Rhodotorula minuta, *Rhodotorula pallida*, and *Rhodotorula marina*, which had previously been separated based on the differences in the assimilation patterns of lactose and maltose (41), have identical ubiquinone systems (27, 28) and similar DNA base compositions (26). However, the strains of these three species were not certainly determined to be one homogeneous taxonomic group, considering their DNA-DNA reassociation (Table 6) and the overall similarity of the electrophoretic mobility of their enzymes (30). Recently, FELL et al. (40) stated that *Rhodotorula pallida* and *Rhodotorula marina* were placed in synonymy with *Rhodotorula minuta* because of their similar DNA base composition and essential requirement for *p*-aminobenzoic acid. Our results do not fully support their conclusion. Further genetic and biochemical investigations will be required to define the three species and to reveal the taxonomic relationships among them.

In this study *Rhodotorula sinensis* was considered to be the anamorph of *Rhodosporidium infirmominiatum* based on the DNA-DNA reassociation (Table 7) and other biochemical characteristics (26–28, 30). *Rhodotorula sinensis* has been placed in synonymy with *Rhodosporidium infirmominiatum* by FELL and TALLMAN (42), with no explanation. Our result supports their treatment.

This study is the first to report the DNA-DNA homology of the strains in the basidiomycetous yeast genera *Rhodosporidium* and *Rhodotorula*. As shown above, the DNA-DNA reassociation was considered to be one of the most useful criteria for conspecificity in the genera *Rhodosporidium* and *Rhodotorula*.

The results of the DNA-DNA reassociation in this study and the other biochemical characteristics, the ubiquinone system, the G+C contents of the DNAs, etc., indicate that the genus *Rhodospordium* should be divided into several taxa and that the species of the genus *Rhodotorula* will have to be reclassified in the future.

It is difficult to select the DNA-DNA relatedness values which can be used for circumscription of species. Generally, it has been accepted that DNA-DNA relatedness values of 80 to 100% indicate strains belonging to the same species, whereas values of 25% or less characterize different species (11). DNA-DNA relatedness values of 65 to 80% are rather commonly observed and are generally regarded to represent the better differentiated strains within a species (19, 21).

Relatedness values of ca. 30 to 65% were found in some strains of the genera *Rhodospordium* and *Rhodotorula* in this study. Intermediate levels of relatedness such as these occur infrequently. However, such levels have been reported by MEYER et al. (12) and AULAKH et al. (20). MEYER et al. (12) reported that the DNA-DNA relatedness values among the strains of *Hanseniaspora osmophilia* and *Kloeckera cortis* with *Hanseniaspora vineae* and *Kloeckera africana* ranged between 38 and 60% and that these yeasts were more closely related to one another than some other species of *Hanseniaspora* and *Kloeckera* species were. AULAKH et al. (20) reported that the DNA-DNA relatedness values between *Filobasidiella neoformans* and *Filobasidiella bacillispora* ranged from 55 to 63% and concluded that the two species were closely related but were distinct species because of their ecological, epidemiological, genetic, and biochemical differences.

The interpretation of the intermediate levels of the DNA relatedness has been discussed by KURTZMAN et al. (17). They argue cogently that the DNA-DNA relatedness is an important determinant of species boundaries but this must be viewed in the context of the entire set of the characteristics of the organisms in question, particularly those pertaining to mating. In fact, they (17) found that mating between *Issatchenkia scutulata* var. *scutulata* and *Issatchenkia scutulata* var. *exigua*, showing ca. 24% DNA-DNA relatedness values, produced viable ascospores. Similarly, VAN DER WALT and JOHANNSEN (14) confirmed interfertility between some strains of the genus *Kluyveromyces* with DNA-DNA reassociation values of 20% or less.

Recently, BAHARAEEN et al. (22) stated that the correlation of the DNA homology and the production of fertile offspring (as an indicator of conspecificity) was further complicated by the probability that very minor changes in total genome might interdict mating, while far more extensive changes could leave the mating process intact.

We believe there can be no particular level of DNA-DNA reassociation that absolutely separates the specific taxonomic level and that interfertility, the DNA-DNA reassociation, and the other characteristics should serve to differentiate species as follows: (1) Interfertility is the most important criterion for conspecificity. (2) In the asporogenous and self-sporulating yeasts: (2-a) If the morphological,

physiological, biochemical, and sometimes ecological characteristics are similar, degrees of DNA-DNA reassociation greater than ca. 65% can be applied as a criterion for conspecificity between strains; (2-b) Very low degrees of DNA-DNA reassociation are indicative of the absence of relatedness between strains; (2-c) The mean degree of the DNA-DNA reassociation between strains which have similar morphological, physiological, and biochemical characteristics is indicative of close relatedness, but the strains may be classified into distinct species if there is an absence of interfertility. Consequently, we should consider how our data influence the use of the DNA-DNA reassociation for yeast taxonomic studies. In the future, DNA-DNA reassociation experiments between the progenies will be required to show the correlation of DNA homology and interfertility.

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