ASPOROGENOUS YEASTS AND THEIR SUPPOSED ASCOSPOROGENOUS STATES: AN ELECTROPHORETIC COMPARISON OF ENZYMES

MASASHI YAMAZAKI¹ AND KAZUO KOMAGATA

*The Institute of Applied Microbiology, University of Tokyo,
Bunkyo-ku, Tokyo 113, Japan*

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The relationship of nine *Candida* species, five *Torulopsis* species, and five species and two varieties of *Kloeckera* to the ascosporogenous yeast species considered to be their teleomorphs was studied by electrophoretic comparison of enzymes. Close relationships were seen between fourteen teleomorphs and their anamorphic counterparts, *Candida guilliermondii* and *Pichia guilliermondii*, *Candida krusei* and *Issatchenkia orientalis* (*Pichia kudriavzevii*), *Candida lambica* and *Pichia fermentans*, *Candida melinii* and *Hansenula wingei*, *Candida utilis* and *Hansenula jadinii*, *Candida macedoniensis* and *Kluyveromyces marxianus*, *Candida pseudotropicalis* and *Kluyveromyces fragilis*, *Candida parapsilosis* (group 2) and *Lodderomyces elongisporus*, *Torulopsis holmii* and *Saccharomyces exiguus*, *Torulopsis colliculosa* and *Torulaspora delbrueckii* (*Saccharomyces fermentati*), *Torulopsis stellata* and *Torulaspora delbrueckii* (*Saccharomyces rosei*), *Kloeckera africana* and *Hanseniaspora vinea*, *Kloeckera apiculata* and *Hanseniaspora uvarum*, and *Kloeckera japonica* and *Hanseniaspora valbyensis*. There was no apparent relation between the four pairs: *Candida valida* and *Pichia membranaefaciens*, *Candida parapsilosis* (group 1) and *Lodderomyces elongisporus*, *Torulopsis molischiana* and *Hansenula capsulata*, and *Torulopsis candida* and *Debaryomyces hansenii* and *Debaryomyces marama*. From these results, this zymographic technique is considered to be a useful tool for the study of relationships between the different states of a yeast species.

In a previous paper (1), the present authors reported that an electrophoretic comparison of enzymes was a useful tool to clarify the taxonomical and physiological relationships among the strains of the genera *Rhodotorula* and *Rhodospiridium*.

¹ Present address: Research Institute of Fermentation, Yamanashi University, Kofu 400, Japan.

A number of *Torulopsis* and *Candida* species are considered to be anamorphs of ascosporogenous yeast species (2-18).

The present paper deals with the electrophoretic comparison of enzymes of some species of *Candida*, *Torulopsis*, and *Kloeckera* with their supposed teleomorphic states, and discusses the relationship between them.

YARROW and MEYER (19) proposed transferring the species classified in *Torulopsis* to the genus *Candida*, but we found it convenient to use the generic name of *Torulopsis* in this paper.

MATERIALS AND METHODS

Microorganisms. Thirteen strains of nine *Candida* species, thirteen strains of five *Torulopsis* species, seven strains of five *Kloeckera* species and two varieties, three strains of three *Pichia* species, one strain of *Issatchenkia orientalis*, two strains of two *Kluyveromyces* species, one strain of *Lodderomyces elongisporus*, three strains of three *Hansenula* species, three strains of *Torulaspora delbrueckii*, one strain of *Saccharomyces exiguus*, two strains of two *Debaryomyces* species, and four strains of three *Hanseniasopra* species were employed. Sources of cultures are shown in Tables 1-3.

Cultivation and harvesting of cells, preparation of cell-free extracts, and gel-slab electrophoresis. Methods for cultivation and harvesting of cells, preparation of cell-free extracts, and gel-slab electrophoresis were essentially the same as those reported previously (1).

Staining procedures. Fructose-1,6-bisphosphate aldolase (FA; EC 4.1.2.13.), 6-phosphogluconate dehydrogenase (6PGDH; EC 1.1.1.41), malate dehydrogenase (MDH; EC 1.1.1.37), hexokinase (HK; EC 2.7.1.1), phosphoglucomutase (PGm; EC 2.7.5.1), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49), glutamate dehydrogenase (GDH; EC 1.4.1.4), lactate dehydrogenase (LDH; EC 1.1.1.27), fumarase (EC; 4.2.1.2), and non-specific esterase (EC 3.1.1.1) were investigated. The staining procedures of the enzymes examined were those described by SICILIANO and SHAW (20). The relative mobilities (Rm) of the enzymes were calculated as the ratio of the distance that the enzyme moved from the origin to the distance that the tracking dye moved.

RESULTS

Figure 1 shows the electrophoretic patterns of seven enzymes of some pairs of asporogenous yeasts and their supposed ascosporogenous states.

Electrophoretic comparison of enzymes between nine Candida species and their supposed ascosporogenous states

The relative electrophoretic mobilities (Rm) of enzymes of nine *Candida* species and those of their supposed ascosporogenous states are shown in Table 1.

Fig. 1. Polyacrylamide gel stained for FA, HK, PGm, fumarase, LDH, MDH, and G6PDH in some pairs of anamorph and teleomorph counterparts.

The patterns for all ten enzymes of the strains of *Candida guilliermondii* YK 500 and *Pichia guilliermondii* YK 651, *Candida krusei* YK 501 and *Issatchenkia orientalis* (*Pichia kudriavzevii*) YK 652, *Candida lambica* YK 502 and *Pichia fermentans* YK 653, *Candida macedoniensis* YK 503 and *Kluyveromyces marxianus* YK 655, and *Candida pseudotropicalis* YK 505 and *Kluyveromyces fragilis* YK 656 were the same.

Candida melinii YK 504 and *Hansenula wingei* YK 038 exhibited identical patterns except for esterase.

Candida utilis YK 506 was similar to *Hansenula jadinii* YK 014 for the respective enzymes except FA.

Of two *Candida valida* strains tested, the strain YK 508 was similar to *Pichia membranaefaciens* YK 654 with respect to 6PGDH, MDH, and LDH patterns but not for the other enzymes. The other strain, YK 507, produced similar patterns to *P. membranaefaciens* YK 654 for MDH, PGm, and G6PDH but not for the other enzymes.

Four *Candida parapsilosis* strains tested split into two groups on the basis of their enzymatic patterns. The first group comprised the two strains YK 509 and YK 510, and the second group the two strains YK 511 and YK 512. *Lodderomyces elongisporus* YK 690 produced the same enzymatic patterns as the second group.

Table 1. Electrophoretic comparison of enzymes between nine

Species	Strain	Source	FA	6PGDH	MDH	LDH
<i>Candida guilliermondii</i>	YK 500	IAM 4974	0.54	0.38	0.40	0.57
<i>Pichia guilliermondii</i>	YK 651	IFO 0961	0.54	0.38	0.40	0.57
<i>Candida krusei</i>	YK 501	IAM 12186	0.52	0.20 0.45	0.34	0.21 0.60
<i>Issatchenkia orientalis</i> (<i>Pichia kudriavzevii</i>)	YK 652	IFO 1279	0.52	0.20 0.45	0.34	0.21 0.60
<i>Candida valida</i>	YK 507	IAM 12190	0.52	0.20 0.45	0.35	0.21 0.60
	YK 508	IAM 12191	0.39	0.36	0.34	0.18 0.53
<i>Pichia membranaefaciens</i>	YK 654	IAM 4987	0.44	0.35	0.34	0.14 0.53
<i>Candida lambica</i>	YK 502	AJ 4461	0.51	0.46	0.36	0.19 0.62
<i>Pichia fermentans</i>	YK 653	IFO 1164	0.51	0.46	0.36	0.19 0.62
<i>Candida macedoniensis</i>	YK 503	IFO 0706	0.45	0.48	0.43	0.21 0.59
<i>Kluyveromyces marxianus</i>	YK 655	IFO 0690	0.45	0.48	0.43	0.21 0.59
<i>Candida pseudotropicalis</i>	YK 505	IAM 4829	0.45	—	0.43	0.19 0.55 0.57
<i>Kluyveromyces fragilis</i>	YK 656	IAM 12237	0.45	—	0.43	0.19 0.53 0.55 0.57
<i>Candida melinii</i>	YK 504	IFO 0747	0.49	0.30	0.39 0.49	0.22 0.55
<i>Hansenula wingei</i>	YK 038	NRRLY-2340	0.49	0.30	0.39 0.49	0.22 0.55

Candida species and their supposed ascosporeogenous states.

HK	PGm	G6PDH	Fumarase	GDH	Esterase	G+C contents of DNAs (mol%) ^a	Co Q ^b
0.51 0.68	0.50	0.28	0.24	0.13	0.54, 0.56	44.1	9
0.51 0.68	0.50	0.28	0.24	0.13	0.54, 0.56	44.6	9
0.39 0.58 0.60	0.40	0.40 0.42	0.27	0.17	0.61, 0.63 0.65, 0.68 0.75	39.3	7
0.39 0.58 0.60	0.40	0.38 0.40	0.27	0.17	0.61, 0.63 0.68, 0.84	38.5	7
0.39 0.55 0.60 0.63	0.40	0.38	0.27	0.17	0.79, 0.81 0.84	38.5	7
0.67	0.55	0.35	0.27	0.13	—	43.2	
0.28 0.63	0.40	0.38	0.30	0.11	0.67	42.0	7
0.58 0.65	0.53 0.55	0.44 0.46	0.35	0.15	0.56, 0.64 0.75, 0.78	42.9	7
0.58 0.61	0.55	0.40 0.42 0.44	0.35	0.15	0.56, 0.64 0.75	42.2	7
0.47 0.65	0.42 0.50	0.28 0.31	0.34	0.17	0.44, 0.48	39.8	6
0.47 0.65	0.50	0.28 0.31	0.34	0.17	0.41, 0.48	41.0	6
0.45 0.47 0.63 0.65	0.47	0.28 0.31	0.30 0.34	0.17	0.48	39.0	6
0.45 0.47 0.63 0.65	0.47	0.28 0.31	0.30 0.34	0.17	0.48	40.0	6
0.47 0.52	0.52	0.28 0.30 0.32	0.24	0.14	0.54, 0.61	39.5	7
0.47 0.52 0.56	0.52 0.56	0.28 0.30 0.32	0.24	0.14	0.59, 0.65	39.0	7

Table 1.

Species	Strain	Source	FA	6PGDH	MDH	LDH
<i>Candida utilis</i>	YK 506	IAM 4961	0.53	0.26	0.41	0.26 0.51
<i>Hansenula jadinii</i>	YK 014	NRRLY-1542	0.49	0.26	0.41	0.26 0.51
<i>Candida parapsilosis</i>	YK 509	IAM 12192	0.58	0.33	0.28 0.42	0.58
	YK 510	IAM 12270	0.58	0.33	0.28 0.42	0.58
	YK 511	AJ 4575	0.55	0.36	0.32 0.36	0.14 0.61
	YK 512	AJ 4571	0.55	0.36	0.32 0.36	0.14 0.61
<i>Lodderomyces elongisporus</i>	YK 690	AJ 5470	0.55	—	0.36	0.14
					0.38	0.61
					0.40	

^a NAKASE and KOMAGATA (3, 5, 7, 21)

^b YAMADA et al. (11, 40, 41)

Abbreviations: IAM, Institute of Applied Microbiology, University of Tokyo, Ajinomoto Co., Inc., Kawasaki, Japan; NRRL, ARS Culture Collection, Northern

Electrophoretic comparison of enzymes between five Torulopsis species and their supposed ascosporeogenous states

The relative electrophoretic mobilities (R_m) of the enzymes of five *Torulopsis* species and those of their supposed ascosporeogenous states are shown in Table 2.

Torulopsis holmii YK 559 produced the same enzymatic patterns as *Saccharomyces exiguus* YK 610.

The patterns of *Torulopsis colliculosa* YK 561 and two strains YK 611 and YK 659 of *Torulaspora delbrueckii* were identical.

The patterns of *Torulopsis molischiana* YK 560 differed from those of *Hansenula capsulata* YK 013.

Of two *Torulopsis stellata* strains tested, the strain YK 563 was similar to *Torulaspora delbrueckii* YK 612 with respect to LDH, HK, fumarase, GDH, and G6PDH patterns but not to FA, PGm, and esterase patterns. The other strain YK 562 produced similar enzymatic patterns to *T. delbrueckii* YK 612 for FA and fumarase but not for MDH, LDH, HK, PGm, G6PDH, and GDH.

The eight *Torulopsis candida* strains fell into three groups on the basis of their enzymatic patterns. The first group consisted of five strains: YK 551, YK 552, YK 553, YK 554, and YK 555. These strains produced identical en-

Continued

HK	PGm	G6PDH	Fumarase	GDH	Esterase	G+C contents of DNAs (mol %) ^a	Co Q ^b
0.55	0.68	0.40	0.29	0.13	0.82	44.0	7
0.61		0.42					
0.55	0.68	0.40	0.29	0.13	0.82	43.2	7
0.58		0.42					
0.61							
0.56	0.56	0.28	0.22	0.06	0.58	40.0	
0.61		0.31	0.29	0.12			
0.56	0.56	0.28	0.22	0.06	0.58		9
0.61		0.31	0.29	0.12			
0.45	0.59	0.31	0.24	0.13	0.62	39.3	
0.61		0.34	0.27				
0.66							
0.45	0.59	0.31	0.24	0.13	0.62	39.3	
0.61		0.34	0.27				
0.66							
0.45	0.59	0.31	0.24	0.13	0.62		9
0.61		0.32	0.27				
0.66		0.34					

Japan; IFO, Institute for Fermentation, Osaka, Japan; AJ, Central Research Laboratories, Regional Research Center, U.S. Department of Agriculture, Peoria, U.S.A.

zymatic patterns for FA, 6PGDH, LDH, PGm, G6PDH, fumarase, and GDH and showed a slight variation with respect to MDH (Rm 0.54–0.58), HK (Rm 0.41–0.45), and esterase (Rm 0.53–0.56). The second group consisted of two strains, YK 556 and YK 557, which produced identical patterns for all ten enzymes. This group did not show the same enzymatic patterns as did the first group except for G6PDH and GDH. The third group comprised one strain, YK 558, which was similar to the first group with respect to fumarase and the second group with respect to LDH and esterase but not to the other enzymes. The first group was similar to *Debaryomyces hansenii* YK 657 with respect to 6PGDH, PGm, and G6PDH, and to *Debaryomyces marama* YK 658 with respect to MDH (Rm 0.58) and HK (Rm 0.45), but not to either *Debaryomyces* species for the other enzymes. The second group produced the same enzymatic patterns as *D. hansenii* YK 657 for G6PDH and *D. marama* YK 658 for 6PGDH but not for the other enzymes. The third group was not similar to *D. hansenii* YK 657 and *D. marama* YK 658 for any of the enzymes.

Electrophoretic comparison of enzymes between the genera Klöeckera and Hanseniaspora

The relative electrophoretic mobilities (Rm) of enzymes of the genera

Table 2. Electrophoretic comparison of enzymes between five

Species	Strain	Source	FA	6PGDH	MDH	LDH
<i>Torulopsis holmii</i>	YK 559	IFO 0660	0.58	0.39	0.30	—
<i>Saccharomyces exiguus</i>	YK 610	AJ 4075	0.58	0.39	0.30	—
<i>Torulopsis colliculosa</i>	YK 561	IAM 4426	0.46	0.28	0.33	0.51
<i>Torulasporea delbrueckii</i> (<i>Saccharomyces fermentati</i>)	YK 611	IFO 0422	0.46	0.28	0.33	0.51
<i>Torulasporea delbrueckii</i>	YK 659	IAM 12236	0.46	0.28	0.33	0.51
<i>Torulopsis molischiana</i>	YK 560	KL 3 ^c	0.49	0.42 0.55	0.32 0.53	0.23 0.50
<i>Hansenula capsulata</i>	YK 013	NRRLY-1842	—	0.51	0.43	0.21 0.60
<i>Torulopsis stellata</i>	YK 562	IFO 0703	0.46	0.39	0.42	0.43
	YK 563	AJ 4394	0.48	0.27	—	0.50
<i>Torulasporea delbrueckii</i> (<i>Saccharomyces rosei</i>)	YK 612	IAM 4991	0.46	—	0.47 0.48	0.50
<i>Torulopsis candida</i>	YK 551	IFO 0644	0.31	0.38	0.58	0.67
	YK 552	IFO 0623	0.31	0.38	0.56	0.67
	YK 553	IFO 1084	0.31	0.38	0.54	0.67
	YK 554	IFO 0856	0.31	0.38	0.54	0.67
	YK 555	IFO 0728	0.31	0.38	0.54	0.67
	YK 556	IFO 0380	0.23	0.43	0.35 0.43 0.45 0.47	0.63
	YK 557	IFO 0768	0.23	0.43	0.35 0.43 0.45 0.47	0.63
	YK 558	AJ 4385	0.60	0.36	0.37	0.64
<i>Debaryomyces hansenii</i>	YK 657	IAM 4978	0.44	0.38	0.31	0.14 0.54
<i>Debaryomyces marama</i>	YK 658	IFO 0668	—	0.43	0.58	0.61

^a NAKASE and KOMAGATA (4, 6, 21).^b YAMADA et al. (11, 34, 40).^c LEE and KOMAGATA (26).

Torulopsis species and their supposed ascosporeogenous states.

HK	PGm	G6PDH	Fumarase	GDH	Esterase	G+C contents of DNAs (mol%) ^a	Co Q ^b
0.34 0.52 0.34 0.37 0.45 0.52	0.32 0.32	0.35 0.37 0.33 0.35 0.37	0.27 0.27	0.19 0.19	0.51, 0.58 0.66 0.51, 0.58 0.66	34.1 33.7	6 6
0.35 0.35 0.35	0.25 0.36 0.36	0.38 0.40 0.36 0.38 0.36 0.38	0.24 0.24 0.24	0.14 0.14 0.14	— —	42.2 43.2 42.9	6 6 6
0.42 0.69 0.39 0.56	0.43 0.59	0.46 —	0.36 0.36	0.15 0.19	0.72 0.42, 0.65	47.2 ^c 47.1 ^c	8 ^c 8 ^c
0.66 0.67 0.35 0.35	0.48 0.38 0.26	0.24 0.36 0.38 0.38 0.41	0.24 0.24 0.24	0.08 0.14 0.14	0.58 0.41 0.33	 42.9 42.9	8 6 6
0.44 0.43 0.45 0.43 0.41 0.40 0.59	— 0.43 0.43 0.43 0.43 0.58	— 0.38 0.38 0.38 0.38 0.38 0.38	0.35 0.38 0.35 0.38 0.35 0.38 0.34 0.35	0.16 0.16 0.16 0.16 0.16 0.16	0.56 0.53 0.53, 0.78 0.53, 0.78 0.54 0.65, 0.69	 36.8	 9
0.40 0.59	0.60	0.38	0.34 0.35	0.16	0.65, 0.69		
0.55 0.28 0.45	0.56 0.43 —	0.32 0.34 0.36 0.38 0.36	0.35 0.36 0.38 0.30 0.31	0.21 0.12 0.12	0.65 0.51 0.60	42.9 34.5 36.6	 9 9

Table 3. Grouping of the genera *Kloeckera* and

Group	Species	Strain	Source	FA	6PGDH	MDH
1	<i>K. africana</i>	YK 803	IAM 4984	0.49	0.34	0.42
	<i>H. vinea</i>	YK 660	AJ 5186	0.49	0.34	0.42
2	<i>K. javanica</i> var. <i>javanica</i>	YK 805	AJ 5195	0.60	—	0.38
	<i>K. javanica</i> var. <i>lafarii</i>	YK 806	AJ 5197	0.60	—	—
3	<i>K. apiculata</i>	YK 800	IAM 12219	—	—	0.36
	<i>H. uvarum</i>	YK 662	AJ 5184	—	—	0.36
4	<i>K. apiculata</i>	YK 801	AJ 4806	0.55	—	0.36
	<i>K. japonica</i>	YK 802	IAM 12220	—	—	0.36
	<i>H. valbyensis</i>	YK 663	IFO 1414	0.55	—	0.36
5	<i>H. valbyensis</i>	YK 661	IAM 4972	0.58	—	0.35
6	<i>K. corticis</i>	YK 804	IFO 0631	0.49	—	0.38

^a NAKASE and KOMAGATA (32).

Kloeckera and *Hanseniaspora* are shown in Table 3. These yeasts fell into six groups on the basis of their enzymatic patterns. *Kloeckera africana* YK 803 and *Hanseniaspora vinea* YK 660 were included in group 1, and they exhibited identical patterns for all enzymes. *Kloeckera javanica* var. *javanica* YK 805 and *K. javanica* var. *lafarii* YK 806 were included in group 2, and the patterns for FA,

Hanseniaspora on the basis of the enzymatic patterns.

LDH	HK	PGm	G6PDH	Fumarase	GDH	Esterase	G+C contents of DNAs (mol%) ^a
0.15	0.43	0.49	0.28	0.24	0.14	0.60	38.0
0.53	0.60	0.53	0.30				
0.15	0.43	0.53	0.30	0.24	0.14	0.60	37.8
0.53	0.60	0.55					
0.46	0.45	0.58	0.29 0.31 0.33	0.27	0.16	0.52 0.59	34.6
0.44	0.45 0.58 0.60	0.52	0.31 0.33	0.27	0.16	0.52	33.9
0.46	0.58 0.61	0.56	—	0.29 0.35	—	—	31.7
0.46	0.58 0.61	0.44 0.54	—	0.29 0.35	—	—	31.5
0.47	0.51 0.55	0.50	0.32 0.34 0.36 0.40 0.41	0.20 0.25	—	0.62	27.1
0.46	0.51 0.55	0.50	0.33 0.35 0.38 0.40 0.41	0.20 0.25	—	0.62	27.1
0.46	0.51 0.55	0.51 0.53	0.33 0.35 0.38 0.40 0.41	0.20 0.25	—	0.70	27.6
0.50	0.49 0.55 0.61 0.63	0.48 0.53	0.45 0.47	0.24 0.29	—	0.36 0.52	31.2
0.19 0.43	0.40 0.58	—	0.32 0.33 0.34	0.27	0.12	—	37.8

LDH, HK, G6PDH, fumarase, GDH, and esterase were similar, but not for PGm. The activity of 6PGDH was not detected in either variety. *Kloeckera apiculata* YK 800 and *Hanseniaspora uvarum* YK 662 were included in group 3, and they produced identical patterns for MDH, LDH, HK, and fumarase, but there was a slight variation for PGm. However, the activities of FA, 6PGDH, GDH,

G6PDH, and esterase were not detected. *Kloeckera apiculata* YK 801, *Kloeckera japonica* YK 802, and *Hanseniaspora valbyensis* YK 663 were included in group 4, and they had similar patterns for FA, MDH, LDH, HK, G6PDH, and fumarase. However, the activities of 6PGDH and GDH were not detected in any of them. *Hanseniaspora valbyensis* YK 661 was accommodated in group 5 and *Kloeckera corticis* YK 804 in group 6.

DISCUSSION

In the previous paper (1), we reported that possible haploid mating partners can be detected by comparing the enzymatic patterns of various strains in the genera *Rhodotorula* and *Rhodospiridium* and that such strains did indeed cross with the known haploid mating-type strains. From these results, it was considered that the technique of polyacrylamide slab gel electrophoresis with specific staining for various enzymes is a useful tool for clarifying the relationship between anamorphic and teleomorphic strains.

Relationship between nine Candida species and their supposed ascosporeogenous states on the basis of their enzymatic patterns

The genus *Candida* is considered to be a heterogeneous collection of ascosporeogenous yeast species, and consists of anamorphic strains of basidiomycetous and ascomycetous yeasts (2). NAKASE and KOMAGATA (7) reported that *Candida* species without urease activity possessed deoxyribonucleic acids (DNAs) with guanine-plus-cytosine (G+C) contents from 30.0 to 56.6 mol%, and could be regarded as anamorphs of ascomycetous yeasts. However, the teleomorphs of most of the *Candida* species have not yet been described.

Mating strains of *Candida guilliermondii* are now classified in the heterothallic species *Pichia guilliermondii*. At present, *C. guilliermondii* contains the strains which for some reason are incapable mating (2). *C. guilliermondii* produced the same enzymatic patterns as *P. guilliermondii*, and this result agreed with those of studies of other biochemical properties (3, 9, 11). Therefore, *C. guilliermondii* is considered to be the anamorph of *P. guilliermondii*.

NAKASE (8) reported that 21 strains of *Candida krusei* could not be distinguished from *Pichia kudriavzevii* on the basis of their physiological properties and the G+C contents of their DNAs. Recently, *P. kudriavzevii* was assigned to the genus *Issatchenkia* as *I. orientalis*, and *C. krusei* was shown to be the anamorph of *I. orientalis* by DNA-DNA hybridization experiments (17). Three strains in this study, *C. krusei* YK 501, *C. valida* YK 507, and *I. orientalis* (*P. kudriavzevii*) YK 652, produced identical patterns for their enzymes. Two of the *C. valida* strains tested were once named *Candida mycoderma*, their DNAs having different G+C contents (38.5 and 43.2 mol%) (7). *C. valida* has been regarded as the anamorph of *Pichia membranaefaciens* because both species show the same morphological and physiological properties (2). However, of

two *C. valida* strains, YK 507 is similar to *I. orientalis* YK 652 and has an identical G+C content (38.5 mol %), both strains differing from *P. membranaefaciens* in their G+C contents (3, 7). Therefore, *C. valida* YK 507 should be regarded as an anamorph of *I. orientalis*. On the other hand, *C. valida* YK 508 has the same G+C content (43.2 mol %) as *P. membranaefaciens*, but it was not similar to *P. membranaefaciens* as regards the enzymatic patterns. From these results, further studies are required for clarification of the anamorph of *P. membranaefaciens*.

Close relationships were seen between two pairs of anamorph and teleomorph counterparts, *Candida macedoniensis* and *Kluyveromyces marxianus*, and *Candida pseudotropicalis* and *Kluyveromyces fragilis*, on the basis of their enzymatic patterns. These four species exhibited identical G+C contents (7, 21) and antigenic structures (9). Physiologically, *K. marxianus* occupies an intermediate position between *Kluyveromyces wickerhamii* and *K. fragilis*, and differs from *K. fragilis* chiefly in the fact that lactose is not or only seldom weakly and latently fermented (2). BAK and STENDERUP (13) indicated the existence of a close relationship between *C. pseudotropicalis* and *K. fragilis*, relative DNA-DNA homology 92%, and MARTINI and PHAFF (22) showed 92% of DNA-DNA homology between *K. fragilis* and *K. marxianus*. Moreover, VAN DER WALT and JOHANNSEN (23) proposed to join ten species, including *K. marxianus* and *K. fragilis*, as a single species after mating studies with auxotrophic mutants. LACHANCE and PHAFF (24) pointed out that the molecular weights of the *exo*- β -glucanase and immunological distance of *K. fragilis* are the same as those of *K. marxianus*. From these studies and their enzymatic patterns, *K. fragilis* and *K. marxianus* should be combined, and their anamorph should be called *C. macedoniensis* because the description of *C. macedoniensis* antedates that of *C. pseudotropicalis*.

BAK and STENDERUP (13) reported that *Candida melinii* and *Hansenula wingei* show 97% of relative DNA-DNA homology. The similarity in the enzymatic patterns of these species agreed with this.

Candida utilis has the same morphological and physiological properties as *Hansenula jadinii*, but they apparently differ in sexuality and pathogenicity, *H. jadinii* having the slight ability to produce both ascospores and disease in mammals (2). However, 93–98% of DNA sequence similarities between *C. utilis* and *H. jadinii* has been shown by MANACHINI (14). Furthermore, KURTZMAN et al. (15) reported that these two species share $85 \pm 6.1\%$ DNA sequence similarity. Thus, the similarity of the enzymatic patterns agreed with the results of DNA-DNA homology.

Lodderomyces elongisporus was indicated by VAN DER WALT (12) as the teleomorph of *Candida parapsilosis* because *L. elongisporus* agreed closely with *C. parapsilosis* both morphologically and physiologically. However, MEYER and PHAFF (25) indicated that these yeasts were not closely related by DNA-DNA hybridization. Following this, NAKASE et al. (16) performed a comparative

taxonomic study on 22 strains of *C. parapsilosis* and one strain of *L. elongisporus* from the viewpoints of physiological properties, serological characteristics, proton-magnetic-resonance spectra of cell-wall mannans, and G+C contents of DNAs, and found that *C. parapsilosis* strains could be assigned to one of two forms. Form II differed from form I in the assimilation of L-arabinose, requirement of thiamine, lack of antigen 24, proton-magnetic-resonance spectra of cell-wall mannans, and lack of excretion of excess riboflavin into synthetic media; these properties are shared with *L. elongisporus*. From these results, NAKASE et al. (16) regarded the strains of *C. parapsilosis* form II as the anamorph of *L. elongisporus*. On electrophoretic comparison of enzymes, *C. parapsilosis* strains were split into two groups. Two strains, YK 509 and YK 510 (group 1), corresponded to form I, and two strains, YK 511 and YK 512 (group 2), to form II. Thus, the opinion of NAKASE et al. (16) was confirmed by the electrophoretic comparison of enzymes. MEYER and PHAFF (25) employed strains of *C. parapsilosis* belonging to form I and the type strain of *L. elongisporus* (YK 690 was derived from the type strain) in DNA-DNA hybridization experiments. Therefore, it is natural that the DNAs of these two species are not homologous. *C. parapsilosis* group 2 (YK 511 and YK 512) should be regarded as the anamorph of *L. elongisporus* on the basis of enzymatic patterns and the study of NAKASE et al. (16). Furthermore, the teleomorph corresponding to *C. parapsilosis* group 1 (YK 509 and YK 510) might be expected in other ascosporeogenous yeasts.

Relationship between five Torulopsis species and their supposed ascosporeogenous states on the basis of their enzymatic patterns

The heterogeneity of the genus *Torulopsis* was clearly shown by the serological investigation made by TSUCHIYA et al. (9), G+C contents of DNAs by NAKASE and KOMAGATA (6), and coenzyme Q system by YAMADA and KONDO (11). A number of *Torulopsis* species are considered to be haploid mating types of ascosporeogenous yeast species. However, the clarification of the relatedness between *Torulopsis* species and ascosporeogenous yeasts has hardly been started yet.

Torulopsis holmii is morphologically and physiologically similar to *Saccharomyces exiguus*, and is regarded as its anamorph (2). The above two species produced identical enzymatic patterns, and result of their enzymatic comparison agreed with those of investigations of antigenic structures (9), G+C contents of DNAs (6), and coenzyme Q system (11). Therefore, *T. holmii* is regarded as the anamorph of *S. exiguus*.

Saccharomyces fermentati, *S. rosei*, and *Torulopsis colliculosa* are currently considered synonyms of *Torulaspora delbrueckii* (18). *Torulaspora delbrueckii* YK 611 and YK 612 were once named *Saccharomyces fermentati* and *S. rosei*, respectively. Three strains of *Torulaspora delbrueckii*, *Torulopsis colliculosa* YK 561, and *Torulopsis stellata* YK 563 possessing Q-6² show similar enzymatic

² The abbreviation used for coenzyme Q or ubiquinone is Q-*n*, with *n* denoting the number of isoprene units in the side-chain.

patterns. Moreover, the results of the enzymatic comparison made by the above workers agreed with those of investigations of G+C contents of DNAs (4, 6) and the coenzyme Q system (11). From these results, *S. fermentati* and *S. rosei* are synonyms of *Torulaspora delbrueckii*, and *Torulopsis colliculosa* and *T. stellata* possessing Q-6 are regarded as anamorphs of *T. delbrueckii*. Furthermore, the teleomorph corresponding to *T. stellata* possessing Q-8 might be expected in other ascosporogenous yeasts.

Torulopsis molischiana is regarded as the anamorph of the homothallic species *Hansenula capsulata* (2). *T. molischiana* YK 560 has been isolated and described as a methanol-assimilating yeast by LEE and KOMAGATA (26). *H. capsulata* also assimilates methanol as a sole carbon source. Moreover, *T. molischiana* and *H. capsulata* had identical G+C contents of DNAs and coenzyme Q system (26). LEE and KOMAGATA (26) examined the electrophoretic pattern of methanol oxidase in one strain each of these two species and reported that the strains exhibited identical patterns. However, in this study on the patterns of the enzymes of glucose metabolism, the two strains were not identical. Therefore, it is not clear whether *T. molischiana* is the anamorph of *H. capsulata*. Further investigations are required for clarification on the relationship between these two species.

Torulopsis candida is a versatile, variable, widely distributed species, and may be regarded as the anamorph of both *Debaryomyces hansenii* and *D. marama* (2). *T. candida* and *D. hansenii* are exclusive species of the floras in cheese (27). NAKASE and KOMAGATA (4) stated that *T. candida* strains form two groups according to the G+C contents of their DNAs (about 36 and 42 mol%), and that the DNAs of *D. hansenii* and *D. marama* had G+C contents of 36.6 to 37.3 mol%. Therefore, the strains of *T. candida* with a G+C content of about 36 mol% might be anamorphs of *D. hansenii* and *D. marama*. In this study, the eight *T. candida* strains tested formed three groups. All the strains belonging to the first group (YK 551 to YK 555) were once named *T. famata*, and those of the second group (YK 556 and YK 557) were previously named *T. candida*. LODDER and KREGER-VAN RIJ (28) distinguished *T. candida* from *T. famata* mainly on the basis of lactose assimilation. However, lactose assimilation ranged from nearly negative, over weakly positive to clearly positive, and these differences in lactose assimilation did not correlate with other differences in morphological or physiological properties (2). For this reason, *T. candida* and *T. famata* were combined in one species (2). The enzymatic comparison supports the opinion of LODDER and KREGER-VAN RIJ (28). Further studies from other viewpoints are required to determine whether *T. famata* should be included in *T. candida*. The strain YK 558 belonging to the third group was isolated from frozen foods by KOMAGATA and NAKASE (29). This strain differed from the members of the first and the second groups of *T. candida* in the enzymatic comparison and G+C content of its DNA (4). Therefore, the strain YK 558 should be separated from *T. candida*. The enzymatic patterns of *D. hansenii* YK 657 and *D. marama* YK

658 do not resemble those of these groups of *T. candida*. From these results, the teleomorph of *T. candida* should be sought in strains of *D. hansenii* and *D. marama* other than YK 657 and YK 658 or in other species.

Relation between the genera Kloeckera and Hanseniaspora on the basis of their enzymatic patterns

The genus *Hanseniaspora* and its anamorph counterpart *Kloeckera* are the most frequently encountered genera of apiculate yeasts in various natural products, and have been studied systematically by several workers (30–36).

MILLER and PHAFF (30) stated that the species of the genus *Hanseniaspora* and its anamorph counterpart *Kloeckera* made a natural group, and that these yeasts comprised four groups. TSUCHIYA et al. (31) recognized three groups in *Hanseniaspora-Kloeckera* on the basis of antigenic structures; and NAKASE and KOMAGATA (32) reported that the G+C content of DNAs from *Hanseniaspora-Kloeckera* were distributed between 26.8 and 38.0 mol%, and classified these species into four groups by the G+C content of their DNAs and physiological properties. Furthermore, only the coenzyme Q-6 system was observed in the two genera by YAMADA et al. (34). Since then MEYER et al. (36) have revealed the existence of six distinct species in the genus *Hanseniaspora*, namely, *Hanseniaspora valbyensis*, *H. uvarum*, *H. guilliermondii*, *H. occidentalis*, *H. osmophila*, and *H. vinea* with their anamorphic states *Kloeckera japonica*, *K. apiculata*, *K. apis*, *K. javanica*, *K. corticis*, and *K. africana*, respectively, on the basis of DNA base composition and DNA-DNA reassociation. The relationships of *Hanseniaspora* and *Kloeckera* species proposed by the various authors (30–33, 35, 36) are presented in Table 4. The grouping of *Hanseniaspora* and *Kloeckera* by their en-

Table 4. The relationships of *Hanseniaspora* to

Group	Anamorph	MILLER and PHAFF (30)	TSUCHIYA et al. (31)	NAKASE and KOMAGATA (32)
1	<i>K. africana</i>	?	<i>H. osmophila</i> <i>H. vinea</i>	<i>H. osmophila</i> <i>H. vinea</i>
2	<i>K. javanica</i>	?	?	?
3	<i>K. apiculata</i>	<i>H. uvarum</i>	<i>H. guilliermondii</i>	<i>H. guilliermondii</i>
		<i>H. valbyensis</i>	<i>H. valbyensis</i> <i>H. uvarum</i>	<i>H. valbyensis</i> <i>H. uvarum</i>
4	<i>K. japonica</i>	<i>H. uvarum</i>	<i>H. guilliermondii</i>	<i>H. valbyensis</i>
		<i>H. valbyensis</i>	<i>H. valbyensis</i> <i>H. uvarum</i>	
5	<i>K. apis</i>	?	?	?
6	<i>K. corticis</i>	<i>H. osmophila</i>	<i>H. osmophila</i>	<i>H. osmophila</i>
			<i>H. vinea</i>	<i>H. vinea</i>

?, unknown; —, not tested.

zymatic patterns coincided with the grouping made by MEYER et al. (36) by DNA-DNA reassociation.

The members of group 1 consisted of *K. africana* and *H. vinea* from their enzymatic patterns. NAKASE and KOMAGATA (32) included *K. corticis* in this group because of the G+C content of its DNA. However, *K. corticis* was not similar to *H. vinea* and *K. africana* for the respective enzymes and was, therefore, accommodated in group 6. MILLER and PHAFF (30) pointed out that *K. corticis* differed from *K. africana* because the former could not assimilate sucrose. TSUCHIYA et al. (31) included *K. africana* in the group of *K. corticis* on the basis of antigenic structures. Moreover, NAKASE and KOMAGATA (32) stated that the separation of *K. africana* from *K. corticis* by assimilation of sucrose was not a reasonable proposition because sucrose assimilation was variable. In the DNA reassociation study made by MEYER et al. (36), *K. africana* and *K. corticis* exhibited a relative homology of 45–56%. Therefore, *K. corticis* was regarded as the anamorph of *H. osmophila* (36). In this study, the results of MILLER and PHAFF (30) and MEYER et al. (36) were supported.

In group 2, each strain of two varieties of *K. javanica* exhibited identical enzymatic patterns, and they could not be separated from each other. MILLER and PHAFF (30) separated *K. lafarii* as *K. javanica* var. *lafarii* from *K. javanica* var. *javanica* on the basis of the lack of assimilation and fermentation of raffinose. However, NAKASE and KOMAGATA (32) intimated that assimilation and fermentation of raffinose by the *K. javanica* group were always latent, or latent and weak, and that these properties have minor taxonomic value in the classification of *Hanseniaspora* and *Kloeckera*, and therefore, there is no need to retain *K. javanica* var. *lafarii*. The results of our enzymatic comparison support the opinion of

Kloeckera species as proposed by several workers.

SMITH (33)	SMITH et al. (35)	MEYER et al. (36)	The enzymatic patterns
—	—	<i>H. vinea</i>	<i>H. vinea</i>
<i>H. occidentalis</i>	—	<i>H. occidentalis</i>	—
—	<i>H. uvarum</i>	<i>H. uvarum</i>	<i>H. uvarum</i>
—	—	<i>H. valbyensis</i>	<i>H. valbyensis</i>
—	<i>H. guilliermondii</i>	<i>H. guilliermondii</i>	—
—	—	<i>H. osmophila</i>	—

NAKASE and KOMAGATA (32). Therefore, *K. javanica* var. *lafarii* should be regarded as the synonym of *K. javanica*. TSUCHIYA et al. (31) deduced from the antigenic structures that the *K. javanica* group was a hybrid of the *K. africana* and *K. apiculata* groups. This assumption was supported by SPENCER and GORIN (37) on the basis of proton-magnetic-resonance spectra of mannans isolated from these yeasts. On the other hand, NAKASE and KOMAGATA (32) stated that two microorganisms whose DNA differed in G+C content by about 7 mol% seem unlikely to be able to hybridize. This assumption of hybridization of the *K. africana* group with the *K. apiculata* group could not be supposed by the electrophoretic comparison of enzymes. Much more evidence is required for the deduction of TSUCHIYA et al. (31). *H. occidentalis* was regarded as the teleomorph of *K. javanica* by SMITH (33). Moreover, a high degree of DNA-DNA reassociation (75–100%) was demonstrated between the two species by MEYER et al. (36). Since the teleomorphs with enzymatic patterns similar to those with their anamorphic *Candida* states also had homologous DNAs it may be supposed that the above two species will be found to have similar enzymatic patterns.

The members of group 3 consisted of the species *K. apiculata* and *H. uvarum* from their enzymatic patterns. NAKASE and KOMAGATA (32) included *H. valbyensis* YK 661 (IAM 4972=AJ 4277) in this group from the G+C content of the DNAs. However, *H. valbyensis* YK 661 was not similar to the starins of group 3 for the respective enzymes, therefore, *H. valbyensis* YK 661 was accommodated in group 5. There is a high degree of DNA-DNA reassociation (95–100%) between *K. apiculata* and *H. uvarum* but not between *K. apiculata* and *H. valbyensis* (11–16%) (36). From these results, *K. apiculata* belonging to group 3 should be regarded as the anamorph of *H. uvarum*.

Three species *K. apiculata*, *K. japonica*, and *H. valbyensis* produced identical enzymatic patterns and formed group 4. In the monograph of LODDER and KREGER-VAN RIJ (28), *K. japonica* described by SAITO and OHTANI (38) was treated as a synonym of *K. apiculata*. The strains of *K. apiculata* were split between two groups, group 3 and group 4, on the basis of their enzymatic patterns and G+C contents of DNAs. The type strain of *K. apiculata* is in group 3. A high degree of DNA-DNA reassociation (94–100%) between *K. japonica* and *H. valbyensis* was demonstrated by MEYER et al. (36). Therefore, *K. apiculata* YK 801 belonging to group 4 should be identified as *K. japonica*, and *K. japonica* should be regarded as the anamorph of *H. valbyensis*.

The strains of *H. valbyensis* divided into two groups, group 4 and group 5. LODDER and KREGER-VAN RIJ (28) indicated *H. guilliermondii* as being a synonym of *H. valbyensis*, while KREGER-VAN RIJ and AHEARN (39) suggested a separation of *H. guilliermondii* from *H. valbyensis* by the number of ascospores per ascus and growth at 37°. NAKASE and KOMAGATA (32) divided *H. valbyensis* strains between group 3 and group 4 of their system and included *H. guilliermondii* in group 3 on the basis of the G+C content of their DNAs. The *H. valbyensis*

strain in group 4 is regarded as the teleomorph of *K. japonica* from the enzymatic patterns and DNA-DNA reassociation experiments done (36). The strain YK 661 of *H. valbyensis* should be identified as *H. guilliermondii*. SMITH et al. (35) described *K. apis* as the anamorph of *H. guilliermondii* on the basis of DNA-DNA reassociation studies. *K. apis* was not investigated in this study. However, a high degree of DNA-DNA reassociation (91–100%) was demonstrated between *K. apis* and *H. guilliermondii* by MEYER et al. (36). Therefore, these species may be expected to have similar patterns when their enzymes are compared electrophoretically.

As shown above, the enzymatic patterns of *Candida*, *Torulopsis*, and *Kloeckera* species coincided with those of their supposed ascosporogenous states. Furthermore, a close relationship between the degree of DNA-DNA homology and electrophoretic similarity of the enzymes can be discerned. We conclude from this that the electrophoretic comparison of enzymes is useful for studying the relationship of an asporogenous yeast species to a sporogenous one and allows the relationship between a teleomorph species and its anamorphic state to be more clearly established.

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