TAXONOMIC STUDIES ON *DEBARYOMYCES HANSENII*
(ZOPF) LODDER ET KREGER-VAN RIJ
AND RELATED SPECIES. II.
PRACTICAL DISCRIMINATION AND
NOMENCLATURE

TAKASHI NAKASE AND MOTOFUMI SUZUKI

*Japan Collection of Microorganisms, The Institute of Physical and
Chemical Research (RIKEN), Wako 351-01, Japan*

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Comparative taxonomic studies were carried out on strains of groups I, II and III, and subgroups Ia and Ib of *Debaryomyces hansenii* and related species which were found in the preceding paper on the basis of chemotaxonomic investigations, for the practical discrimination and clarification of nomenclature. These groups and subgroups could be discriminated from one another by maximum growth temperature, assimilability of propylene glycol, and electrophoretic patterns of glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and malate dehydrogenase (MDH, EC 1.1.1.37), in addition to the criteria reported in the preceding paper.

Strains of group III (*Candida saitoana* nov. nom.) could be easily discriminated from strains of other groups by their ability to assimilate propylene glycol and electrophoretic relative mobilities of G6PDH and MDH, in addition to their characteristic patterns of PMR spectra of mannans and cell surface antigens. Strains of subgroup Ia (*Debaryomyces hansenii* var. *hansenii*-*Candida famata* var. *famata*) could be discriminated by their low maximum growth temperature and the lack of G6PDH activity. Discrimination of strains of subgroup Ib (*Debaryomyces hansenii* var. *fabryi* nov. comb.-*Candida famata* var. *flareri* nov. comb.) from those of group II (*Debaryomyces nepalensis*-*Candida naganishii* nov. sp.) is not so easy in the present study. At present, electrophoretic comparison of MDH is the most convenient method for the separation of group II and subgroup Ib from each other because the differences found in the patterns of PMR spectra of mannans and cell surface antigens are not so clear-cut in several strains.

In the preceding paper (1), we reported that strains of *Debaryomyces hansenii*

and its anamorphs, and *Debaryomyces nepalensis* were classified into 3 groups, I, II and III, and that group I consisted of 2 subgroups, Ia and Ib. We considered that these 3 groups represented 3 distinct species and that these 2 subgroups represented 2 distinct varieties, respectively. The discrimination of these species and varieties, however, were difficult by taxonomic criteria commonly employed in the classification of yeasts. Practical discrimination and nomenclatural problems are discussed in the paper.

MATERIALS AND METHODS

Strains employed. Fifty strains of *Debaryomyces hansenii* and its anamorphs, and *Debaryomyces nepalensis*, which were employed in the preceding paper (1), were employed in the present study. *Debaryomyces coudertii* JCM 2387 (=CBS 5167, type strain) and *Debaryomyces maramba* JCM 1528 (=CBS 1958, type strain) were also employed in the electrophoretic comparison of enzymes.

Assimilation of carbon compounds. Assimilation of additional 33 carbon compounds listed in Table 1, which are not employed in ordinary tests in yeast classification, were investigated by the procedure described by VAN DER WALT (2).

Maximum growth temperature. The maximum growth temperature was determined by using liquid medium. Each tube (12 mm ϕ), containing 2 ml of YM broth (Difco Labs), was inoculated with a drop of light suspension of cultures to be tested and incubated at respective temperature at intervals of 1°C for 11 days in the metal block bath.

Assimilation of hydrocarbons. For the assimilation test of hydrocarbons, decane and hexadecane were employed as the carbon source. After inoculation of cultures to be tested, a 0.5 ml of each sterilized hydrocarbon was added onto the filter paper strip placed at the bottom of the agar slant which contained Yeast Nitrogen Base (Difco Labs) +2% agar as the basal medium. Agar slants were incubated at 25°C for 6 weeks. All of the strains of groups II and III, and subgroup Ib, and 10 strains of subgroup Ia were also examined for their assimilability of hexadecane by using the shaking culture. Loopful cultures to be tested were inoculated into the 500-ml Erlenmeyer flask containing 100 ml of Yeast Nitrogen Base (Difco Labs)+1 ml of hexadecane, then incubated at 25°C for 50 days on the rotary shaker (150 rpm).

Electrophoretic comparison of enzymes. Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and malate dehydrogenase (MDH, EC 1.1.1.37) were compared by slab-gel electrophoresis. The yeasts were cultivated in 500-ml flasks containing 200 ml of YM broth supplemented with 2% of glucose for 24 hr at 25°C with shaking. The cells were harvested by centrifugation at 5,000 rpm for 10 min at 4°C, and washed with 0.05 M Tris-HCl buffer, pH 7.8. The harvested cells were stored in a freezer at -20°C until they were used.

The cells were suspended in 1 to 2 volumes of 0.05 M Tris-HCl buffer, pH

7.8, and were transferred to a 50-ml glass vessel containing glass beads (0.45–0.50 mm ϕ). After cooling in ice water, the cells were disrupted for 2 min at 4,000 rpm with a mechanical cell homogenizer (Braun, Melsungen, West Germany). The homogenate was centrifuged at 11,000 rpm for 50 min at 4°C. The supernatant was used directly as an enzyme source for electrophoretic analysis. Slab-gel electrophoresis and staining procedures were performed as described by YAMAZAKI and KOMAGATA (3). After staining, the relative mobilities (*Rm*) of the enzyme bands were calculated as the ratio of the distance that the enzyme moved from the origin to the distance that the tracking dye (bromophenol blue) moved.

RESULTS AND DISCUSSION

Assimilation of additional 33 carbon compounds

Besides 34 carbon compounds commonly employed in yeast classification reported in the preceding paper (1), the assimilation of additional 33 compounds was investigated (Table 1). Fifty strains employed demonstrated similar patterns in the assimilation of these carbon compounds. The only exception was found in the assimilation of propylene glycol. All 6 strains of group III rapidly assimilated this compound, while strains of the other 2 groups did not assimilate this compound or assimilated it after 3 or 4 weeks of cultivation. Four strains of subgroup Ia, 3 strains of subgroup Ib, and a strain of group II weakly grew on this compound after 3 weeks of cultivation, and 2 of them (JCM 1441 and 2095) grew moderately after 4 weeks of cultivation. This characteristic is considered to be useful to distinguish the strains of group III from strains of the other 2 groups. The results of the assimilation test suggest that the expansion of carbon compounds employed for assimilation test is of a little value in differentiating these *Debaryomyces* species when such compounds belong to usual compounds such as sugars and sugar alcohols. Assimilation of unusual carbon compounds might be useful to differentiate these closely related species, as suggested by PRICE et al. (4).

Maximum growth temperature

Maximum growth temperatures of 50 strains employed distributed in rather wide range, 31–39°C (Table 1), however, those of each group or subgroup fell in narrow range; 31–35°C for subgroup Ia, 36–39°C for subgroup Ib, 36–37°C for group II, and 34–35°C for group III.

NAGANISHI and NAKAMURA (5) discussed the significance of maximum growth temperature in the classification of *Debaryomyces* and proposed to classify *Debaryomyces* species into 2 groups, semi-psychrophiles and mesophiles, based on this characteristic. According to them, species of semi-psychrophilic group can grow only below 34°C, while those of mesophilic group can grow also above 35°C. *Debaryomyces japonicus* and *Debaryomyces orientalis*, which belong to group II and subgroup Ib in the present study, were described by NAGANISHI (6) and were

Table 1. Assimilation of additional 33 carbon compounds and maximum growth

		Assimilation of																
Groups	Strains	D-Lyxose	L-Lyxose	D,L-Glyceraldehyde	Glucose	D-Fucose	L-Fucose	D-Turanose	D-Allose	D-Erythrose	D-Tagatose	D-Mannoheptulose	Stachyose	Dihydroxyacetone	β -Gentiobiose	Lactulose	D-Glucosamine-HCl	
Ia	JCM 1439	—	—	L	—	—	—	+	—	—	—	—	L	L	+	—	—	
	JCM 1440	—	L	L	—	—	—	+	—	L	—	+	—	/	+	—	—	
	JCM 1521	L	—	+	—	—	—	+	—	—	—	L	+	L	+	—	+	
	JCM 1526	—	—	+	—	—	—	+	—	L	—	+	L	+	+	—	w	
	JCM 1990	—	—	—	—	—	—	+	—	—	—	—	—	/	+	+	—	
	JCM 2086	—	—	+	—	—	—	+	—	—	—	+	L	L	+	+	—	
	JCM 2091	—	—	—	—	—	—	+	—	—	—	—	—	+	+	+	—	
	JCM 2094	—	—	—	—	—	—	+	—	—	—	/	—	L	+	+	—	
	JCM 2098	—	—	L	—	—	—	+	—	—	—	+	L	/	+	+	—	
	JCM 2105	—	—	+	—	—	—	+	—	—	—	—	L	/	+	—	—	
	JCM 2106	+	L	L	—	—	—	+	—	L	—	+	L	L	+	—	w	
	JCM 2107	+	L	L	—	—	—	+	—	w	—	+	+	L	+	—	—	
	JCM 2108	L	L	+	—	—	—	+	—	L	—	+	/	L	+	/	w	
	JCM 2109	L	—	+	—	—	—	+	—	/	—	+	+	L	+	/	+	
	JCM 2110	+	+	+	—	—	—	+	—	+	—	—	L	L	+	—	—	
	JCM 2111	+	—	+	—	—	—	+	—	/	—	—	L	L	+	—	/	
	JCM 2112	—	—	—	—	—	—	+	—	—	—	+	—	—	+	—	/	
	JCM 2113	/	—	L	—	—	—	+	—	—	—	+	+	L	—	—	w	
	JCM 2114	—	—	+	—	—	—	+	—	—	—	—	—	+	—	L	—	w
	JCM 2115	—	—	+	—	—	—	+	—	—	—	—	—	+	—	L	—	w
	JCM 2160	—	—	/	—	—	—	+	—	—	—	—	+	—	—	+	+	—
	JCM 2161	+	—	+	—	—	—	+	—	/	—	—	—	L	+	+	—	w
	JCM 2162	/	—	L	—	—	—	+	—	w	—	—	+	+	L	—	—	—
	JCM 2163	—	—	L	—	—	—	+	—	/	—	—	—	L	L	+	—	/
	JCM 2165	+	—	L	—	—	—	+	—	+	+	—	+	—	L	—	—	w

characterized in part by their high maximum growth temperatures. As stated by them, maximum growth temperature is an important criterion in the classification of *Debaryomyces*. Strains of group II and subgroup Ib can be distinguished by this criterion from strains of group III and subgroup Ia. PRICE et al. (4) stated that *Debaryomyces subglobosus* could be separated from *Debaryomyces hansenii* by its ability to grow at 37°C; however, growth test at 35 or 36°C is better from the result of the present study (Table 1).

Assimilation of hydrocarbons

By citing the report of BOS and BRUYN (7), PRICE et al. (4) stated that *De-*

temperature of *Debaryomyces hansenii* and related species.

carbon compounds															Maximum growth temperature (°C)		
D-Galactosamine-HCl	D-Mannosamine-HCl	D-Glucuronic acid	D-Galacturonic acid	D-Arabitol	L-Arabitol	Xylitol	Esculin	Amygdalin	α -Me-D-mannoside	β -Me-D-glucoside	α -Me-D-xyloside	β -Me-D-xyloside	Ethylene glycol	Propylene glycol		Ethyl acetoacetate	Ethyl acetate
—	—	w	—	+	+	+	+	—	—	+	—	—	—	—	—	—	33–34
—	—	w	—	+	+	+	+	<i>l</i>	—	+	—	—	—	—	<i>l</i>	—	31–32
—	—	w	—	+	+	+	+	L	—	+	—	—	—	—	—	—	34–35
—	—	w	—	+	+	+	+	w	—	+	—	—	—	—	—	—	34–35
—	—	w	—	+	+	+	+	<i>l</i>	—	+	—	—	—	—	—	—	32–33
—	—	w	—	+	+	+	+	+	—	+	—	—	—	—	—	—	32–33
—	—	w	—	L	—	L	+	w	—	+	—	—	—	—	—	—	30–31
—	—	w	—	L	L	+	+	—	—	+	—	—	—	—	—	—	30–31
—	—	w	—	+	+	+	+	<i>l</i>	—	+	—	—	—	—	—	—	32–33
—	—	w	—	+	+	+	+	w	—	+	—	—	—	—	—	—	32–33
—	—	w	—	+	+	+	+	L	—	+	—	—	—	—	—	—	32–33
—	—	w	—	+	+	+	+	<i>l</i>	—	+	—	—	—	—	—	—	32–33
—	—	w	—	+	+	+	+	w	—	+	—	—	—	—	—	—	34–35
—	—	+	—	+	+	+	L	<i>l</i>	—	+	—	—	—	—	—	—	34–35
—	—	w	—	+	+	+	L	<i>l</i>	—	+	—	—	—	<i>l</i>	—	—	34–35
—	—	w	—	+	+	+	—	<i>l</i>	—	+	—	—	—	—	—	—	34–35
—	—	<i>l</i>	—	+	+	+	+	—	—	+	—	—	—	—	—	—	31–32
—	—	w	—	+	+	L	w	w	—	+	—	—	—	—	—	—	34–35
—	—	w	—	+	<i>l</i>	+	+	<i>l</i>	—	+	—	—	—	—	—	—	31–32
—	—	w	—	+	—	+	+	—	—	+	—	—	—	—	—	—	31–32
—	—	<i>l</i>	—	+	+	+	+	<i>l</i>	—	+	—	—	—	—	—	—	32–33
—	—	<i>l</i>	—	+	+	+	+	—	—	+	—	—	—	—	—	—	31–32
—	—	w	—	+	+	+	+	—	—	+	—	—	—	—	—	—	33–34
—	—	L	—	+	+	+	+	—	—	+	—	—	—	—	—	—	33–34
—	—	w	—	+	+	+	+	L	—	+	—	—	—	—	—	—	31–32

baryomyces nepalensis could be distinguished from *Debaryomyces hansenii* by its inability to assimilate of hydrocarbons. Therefore, we investigated the assimilation of hexadecane and decane for the purpose of discrimination of strains of groups I, II and III. As shown in Table 2, separation of these groups was found to be difficult by this criterion. All of the strains of group III grew well on hexadecane, however, assimilation of hexadecane by strains of other groups varied from strain to strain. Assimilation of decane was negative in strains of group II and variable in strains of other groups.

In group I, some strains grew well on hexadecane and also on decane, while other strains grew weakly or did not grow at all. In group II, type strain of

Table 1.

		Assimilation of															
Groups	Strains																
		D-Lyxose	L-Lyxose	DL-Glyceraldehyde	Glucose	D-Fucose	L-Fucose	D-Turanose	D-Allose	D-Erythrose	D-Tagatose	D-Mannoheptulose	Stachyose	Dihydroxyacetone	β -Gentiobiose	Lactulose	D-Glucosamine-HCl
Ia	JCM 2192	—	—	+	—	—	—	+	—	+	—	—	L	—	+	—	w
	JCM 2194	+	—	+	—	—	—	+	—	L	—	L	+	L	+	—	+
	JCM 2195	+	—	+	—	—	—	+	—	—	—	+	L	<i>l</i>	L	—	w
	JCM 2196	+	L	+	—	—	—	+	—	+	—	+	L	L	L	—	<i>l</i>
	JCM 2210	L	L	+	—	—	—	+	—	L	—	+	L	L	+	—	w
	JCM 2211	—	—	+	—	—	—	+	—	—	—	—	L	+	+	+	—
	JCM 2212	+	L	+	—	—	—	+	—	+	—	+	L	L	+	—	<i>l</i>
	JCM 2213	w	—	+	—	—	—	+	—	w	—	+	L	L	+	—	w
JCM 2224	—	—	L	—	—	—	L	—	—	—	+	L	L	+	—	—	
Ib	JCM 1441	L	—	L	—	—	—	+	+	—	—	—	L	+	+	—	w
	JCM 1527	—	—	L	—	—	—	+	—	—	—	—	—	—	+	—	w
	JCM 1989	w	L	+	—	—	—	+	—	—	—	+	L	+	+	+	w
	JCM 2104	+	<i>l</i>	+	—	—	—	+	+	<i>l</i>	—	—	L	+	+	—	w
	JCM 2166	L	L	+	—	—	—	+	—	—	—	+	+	L	+	+	w
	JCM 2207	L	L	L	—	—	—	+	L	—	—	—	L	+	+	—	w
II	JCM 2095	+	+	L	—	—	—	+	—	—	—	+	+	L	+	—	w
	JCM 2164	+	—	L	—	—	—	+	—	+	—	+	+	L	+	—	w
	JCM 2208	+	—	+	—	—	—	+	—	<i>l</i>	—	+	L	L	+	—	w
	JCM 2209	<i>l</i>	—	—	—	—	—	+	—	w	—	—	+	—	+	—	<i>l</i>
III	JCM 1438	—	—	+	—	—	—	+	—	<i>l</i>	—	—	+	+	+	+	w
	JCM 1522	—	—	+	—	—	—	+	—	—	—	—	+	+	+	+	+
	JCM 1523	—	—	+	—	—	—	+	—	w	—	—	+	+	+	+	w
	JCM 2118	—	—	+	—	—	—	+	—	—	—	—	+	+	+	+	L
	JCM 2197	L	—	+	—	—	—	+	—	—	—	—	+	+	+	+	w
	JCM 2198	—	—	L	—	—	—	+	—	—	—	—	+	+	+	+	w

+: good growth, L: latent or slow growth, w: weak growth, *l*: latent and weak growth,

Debaryomyces nepalensis (JCM 2095) formed several colonies on agar slant containing hexadecane after 35 days of cultivation and grew weakly in liquid medium containing hexadecane after 50 days of shaking culture. This finding on the type strain of this species clearly indicates that assimilation of hydrocarbons could not differentiate *Debaryomyces nepalensis* (strains of group II) from *Debaryomyces hansenii* (strains of group I).

In results which differ from those of Bos and BRUYN (7), 18 strains out of 40 of *Debaryomyces hansenii* (group I) did not assimilate hydrocarbons. This suggests

Continued.

carbon compounds																	
D-Galactosamine-HCl	D-Mannosamine-HCl	D-Glucuronic acid	D-Galacturonic acid	D-Arabitol	L-Arabitol	Xylitol	Esculin	Amygdalin	α -Me-D-mannoside	β -Me-D-glucoside	α -Me-D-xyloside	β -Me-D-xyloside	Ethylene glycol	Propylene glycol	Ethyl acetate	Ethyl acetate	Maximum growth temperature (°C)
—	—	w	—	+	+	+	+	—	—	+	—	—	—	—	—	—	31–32
—	—	w	—	+	+	+	+	<i>l</i>	—	+	—	—	—	—	—	—	32–33
—	—	w	—	+	+	+	+	w	—	+	—	—	—	—	—	—	34–35
—	—	—	—	+	+	+	+	<i>l</i>	—	+	—	—	—	<i>l</i>	—	—	33–34
—	—	w	—	+	+	+	+	<i>l</i>	—	—	—	—	—	<i>l</i>	—	—	32–33
—	—	—	—	+	+	+	+	—	—	+	—	—	—	<i>l</i>	—	—	34–35
—	—	w	—	+	+	+	+	w	—	—	—	—	—	—	—	—	34–35
—	—	<i>l</i>	—	+	+	+	+	<i>l</i>	—	+	—	—	—	—	—	—	34–35
—	—	w	—	+	+	+	+	<i>l</i>	—	+	—	—	—	—	—	—	32–33
—	—	w	—	+	+	+	+	w	—	+	—	—	—	L	—	—	38–39
—	—	—	—	+	+	+	+	—	—	+	—	—	—	—	—	—	36–37
—	—	—	—	+	+	+	+	—	—	+	—	L	—	<i>l</i>	—	—	37–38
—	—	<i>l</i>	—	+	+	+	+	—	—	+	—	—	—	<i>l</i>	—	—	36–37
—	—	—	—	+	+	+	+	—	—	+	—	—	—	—	—	—	37–38
—	—	w	—	+	+	+	+	—	—	+	—	—	—	—	—	—	36–37
—	—	—	—	+	+	+	+	w	—	+	—	+	—	L	—	—	36–37
—	—	w	—	+	+	+	+	<i>l</i>	—	+	—	—	—	—	—	—	36–37
—	—	—	—	+	+	+	+	—	—	+	—	—	—	—	—	—	36–37
—	—	<i>l</i>	—	+	+	+	+	—	—	+	—	—	—	—	—	—	36–37
—	—	—	—	+	+	+	+	w	—	+	—	<i>l</i>	—	+	—	—	34–35
—	—	—	—	+	L	+	+	—	—	+	—	+	—	+	—	—	34–35
—	—	—	—	+	+	+	+	—	—	+	—	L	—	+	—	—	34–35
—	—	—	—	+	+	+	+	—	—	+	—	L	—	+	—	—	34–35
—	—	—	—	+	+	+	+	—	—	+	—	L	—	+	—	—	34–35
—	—	—	—	+	+	+	+	—	—	+	—	L	—	+	—	—	34–35

—: no growth.

that conditions employed in the present experiment might not be proper as compared with those by BOS and BRUYN (7), who observed assimilation of hydrocarbons by all 45 strains employed. However, we did not carry out further investigation in this respect because this criterion was found not to be useful to differentiate *Debaryomyces nepalensis* from *Debaryomyces hansenii*.

Electrophoretic comparison of enzymes

The relative mobilities (*R_m*) of G6PDH and MDH are shown in Table 3.

Table 2. Assimilation of hydrocarbons by *Debaryomyces hansenii* and related species.^a

Group	Strain	Growth in agar medium										Growth in liquid medium with shaking ^b Hexadecane			
		Decane					Hexadecane								
		Days										7	14	28	50
		11	18	26	35	42	11	18	26	35	42				
Ia	JCM 1439	—	—	—	—	—	—	—	—	—	+				
	JCM 1440	—	+	+	+	+	+	++	++	++	++				
	JCM 1521	—	—	—	—	—	—	—	—	—	—	+			
	JCM 1526	—	—	+	+	+	—	—	—	+	+				
	JCM 1990	—	—	—	—	—	—	—	—	—	—				
	JCM 2086	—	—	—	—	—	—	—	—	+	+				
	JCM 2091	—	—	—	—	—	—	—	—	—	—				
	JCM 2094	—	—	—	—	—	—	—	—	—	—				
	JCM 2098	—	—	—	—	—	—	—	+	+	+				
	JCM 2105	—	—	—	—	—	—	—	—	—	—				
	JCM 2106	—	—	—	—	—	—	—	—	—	—				
	JCM 2107	—	—	—	—	—	—	—	—	—	—				
	JCM 2108	—	—	—	—	+	—	—	—	+	+				
	JCM 2109	—	—	—	+	+	—	—	—	—	—				
	JCM 2110	—	—	—	—	+	++	+++	+++	+++	+++				
	JCM 2111	—	+	+	+	+	+	++	+++	+++	+++				
	JCM 2112	—	—	—	—	—	—	—	—	—	+	++			
	JCM 2113	—	++	++	++	++	—	—	—	—	—				
	JCM 2114	—	—	—	—	—	—	—	—	—	—				
	JCM 2115	—	—	—	—	—	—	—	—	—	—				
	JCM 2160	—	—	—	—	—	—	—	—	—	—	+			
	JCM 2161	—	—	—	—	—	—	—	—	—	—	—			
	JCM 2162	—	+	++	++	++	—	—	—	—	—	—			
	JCM 2163	—	—	—	—	—	—	—	—	—	—	—			
	JCM 2165	—	—	+	+	+	+	+	+	++	++	++	—	—	—

^a +++: good growth, ++: moderate growth, +: weak growth (sometimes several colonies), —: no growth.

Strains of subgroup Ia did not exhibit the activity of G6PDH. G6PDH of strains of subgroup Ib and group II exhibited similar *Rm* values, while those of group III exhibited different values. MDH of strains of subgroup Ia fell into 2 groups. Namely, MDH from 20 strains possessed *Rm* values of 0.55–0.56, while those of the remaining 13 strains showed *Rm* values of 0.58–0.60 (Table 3). These 2 kinds of *Rm* values of MDH were similar to those of subgroup Ib and group II, respectively (Table 3).

The patterns of G6PDH and MDH of strains of group III were found to be quite different from those of the other 2 groups.

YAMAZAKI and KOMAGATA (8) investigated the electrophoretic patterns of 10 enzymes of *Torulopsis candida* and found that strains of this species fell into 3 groups. The first and the second groups of YAMAZAKI and KOMAGATA (8) are

Table 2. Continued.

Group	Strain	Growth in agar medium										Growth in liquid medium with shaking ^b Hexadecane			
		Decane					Hexadecane								
		Days													
		11	18	26	35	42	11	18	26	35	42	7	14	28	50
Ia	JCM 2192	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	JCM 2194	—	—	+	+	+	—	—	—	—	+	—	—	—	—
	JCM 2195	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	JCM 2196	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	JCM 2210	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	JCM 2211	+	++	++	++	++	+	++	++	++	++	—	++	+++	+++
	JCM 2212	—	—	—	+	+	—	++	+++	+++	+++	—	—	++	+++
	JCM 2213	+	+	++	++	++	—	—	+	++	++	—	—	—	—
	JCM 2224	+	+	+	+	++	+	++	++	++	+++	—	—	+	++
Ib	JCM 1441	—	—	—	—	—	—	—	—	—	—	—	—	—	++
	JCM 1527	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	JCM 1989	—	—	+	+	+	—	++	++	+++	+++	—	++	+++	+++
	JCM 2104	++	++	++	++	+++	++	++	++	++	++	—	—	—	—
	JCM 2166	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	JCM 2207	—	—	—	—	—	—	—	+	++	++	—	—	—	—
II	JCM 2095	—	—	—	—	—	—	—	—	+	+	—	—	—	+
	JCM 2164	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	JCM 2208	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	JCM 2209	—	—	—	—	—	—	—	—	—	—	—	—	—	—
III	JCM 1438	++	++	++	++	++	++	+++	+++	+++	+++	—	+++	+++	+++
	JCM 1522	+	+	++	++	++	++	++	+++	+++	+++	—	+++	+++	+++
	JCM 1523	++	++	+++	+++	+++	++	+++	+++	+++	+++	++	+++	+++	+++
	JCM 2118	—	—	—	—	—	++	++	+++	+++	+++	++	+++	+++	+++
	JCM 2197	—	—	+	+	+	++	+++	+++	+++	+++	—	++	+++	+++
	JCM 2198	—	—	—	+	+	++	+++	+++	+++	+++	—	+++	+++	+++

^b Twenty-four strains of subgroup Ia (from JCM 1439 to 2163) were not tested by this method.

considered to correspond to subgroup Ia and group III, respectively, in the present study, and the third group is identical to *Candida glabrosa* (unpublished data). However, *R_m* values and the number of isozymes reported by them differed slightly from the results of the present experiment. Further, they detected G6PDH activity on 4 strains out of 5 of their first group. These difference might be ascribed to the difference of cultivation media and also to the different persons who prepared slab-gels.

By comparing the electrophoretic patterns of these 2 enzymes, groups I, II and III, and subgroups Ia and Ib could be discriminated from each other. In practical identification, comparison of enzyme patterns with authentic strain of each group or subgroup is necessary until a standard method for this criterion is established.

Table 3. Electrophoretic comparison of glucose-6-phosphate dehydrogenase and malate dehydrogenase from strains of *Debaryomyces hansenii* and related species.

Group or species	Strain	Relative mobility (<i>R_m</i>)	
		G6PDH	MDH
Ia	JCM 1439	— ^a	0.55
	JCM 1440	NT ^b	NT
	JCM 1521	—	0.55
	JCM 1526	—	0.60
	JCM 1990	—	0.55
	JCM 2086	—	0.55
	JCM 2091	—	0.55
	JCM 2094	—	0.55
	JCM 2098	—	0.55
	JCM 2105	—	0.55
	JCM 2106	—	0.60
	JCM 2107	—	0.60
	JCM 2108	—	0.60
	JCM 2109	—	0.55
	JCM 2110	—	0.60
	JCM 2111	—	0.59
	JCM 2112	—	0.55
	JCM 2113	—	0.59
	JCM 2114	—	0.55
	JCM 2115	—	0.55
	JCM 2160	—	0.56
	JCM 2161	—	0.55
	JCM 2162	—	0.59
	JCM 2163	—	0.55
	JCM 2165	—	0.55
	JCM 2192	—	0.60

^a — : Enzyme activity was not detected.^b NT: Not tested.

Debaryomyces coudertii (9) and *Debaryomyces marama* (10) also resemble *Debaryomyces hansenii* and related species investigated in the present study though they are easily discriminated by their psychrophilic nature and formation of 1–4 ascospores in the ascus, respectively. These 2 species were easily distinguished from each other and from strains of groups I, II and III by electrophoretic patterns of G6PDH and MDH.

Differential characteristics

Differential characteristics of groups I, II, and III, and subgroups Ia and Ib are summarized in Table 4. Strains of group III can easily be discriminated from strains of the other 2 groups by their characteristic PMR spectra of mannans, serological characteristics, ability to assimilate propylene glycol, and electrophoretic patterns of G6PDH and MDH. Strains of subgroup Ia can be discriminated from

Table 3. Continued.

Group or species	Strain	Relative mobility (<i>R_m</i>)		
		G6PDH	MDH	
Ia	JCM 2194	—	0.55	
	JCM 2195	—	0.55	
	JCM 2196	—		0.58
	JCM 2210	—		0.60
	JCM 2211	—	0.56	
	JCM 2212	—		0.59
	JCM 2213	—		0.60
	JCM 2224	—	0.56	
Ib	JCM 1441	0.30	0.56	
	JCM 1527	0.30	0.56	
	JCM 1989	0.29	0.56	
	JCM 2104	0.30	0.56	
	JCM 2166	0.29	0.56	
	JCM 2207	0.30	0.56	
II	JCM 2095	0.31		0.60
	JCM 2164	0.31		0.60
	JCM 2208	0.31		0.60
	JCM 2209	0.31		0.60
III	JCM 1438	0.35	0.30	0.45
	JCM 1522	0.35	0.30	0.45
	JCM 1523	0.35	0.30	0.45
	JCM 2118	0.35	0.30	0.45
	JCM 2197	0.35	0.30	0.45
	JCM 2198	0.35	0.30	0.45
<i>D. coudertii</i>	JCM 2387	0.29	0.10	0.43
<i>D. marama</i>	JCM 1528	0.34		0.48

G6PDH: Glucose-6-phosphate dehydrogenase, MDH: Malate dehydrogenase.

strains of other groups and subgroup Ib by their low maximum growth temperature and the lack of G6PDH activity.

Discrimination of strains of subgroup Ib from those of group II is not so easy. Types of PMR spectra of mannans and serological characteristics can be employed to aid discrimination, however, the differences found in these criteria are not so clear-cut as discussed in the preceding paper (1). At present, the most convenient characteristic to discriminate these 2 groups is electrophoretic *R_m* values of MDH as shown in Table 4. We have to find more convenient criteria for the differentiation of subgroup Ib and group II.

Nomenclature and description

After precise nomenclatural investigations, the names of taxa recognized in

Table 4. Differential characteristics of *Debaryomyces hansenii* and related species.

Group	Type of PMR spectra of mannans (I)	Agglutination with absorbed antisera		Maximum growth temperature (°C)	Assimilation of propylene glycol	Electrophoretic <i>R_m</i> values of ^c	
		A ^a	B ^b			G6PDH	MDH
Ia	1	++	—	31–35	— ^d	ND ^e	0.55–0.56 or 0.58–0.60
Ib	1	++	—	36–39	— ^d	0.29–0.30	0.56
II	2	++	+	36–37	— ^d	0.31	0.60
III	3	—	++	34–35	+	0.35	0.30, 0.45

^a A: Antiserum for *D. hansenii* JCM 1990 absorbed with *T. candida* JCM 1438 (I).

^b B: Antiserum for *C. glabrosa* JCM 1616 absorbed with *T. famata* JCM 1521 (I).

^c Should be compared with the authentic strain of each group.

^d Some strains assimilate this compound after 3 to 4 weeks of cultivation.

^e ND: Not detected.

the present paper and their synonymy are enumerated below:

Ia. *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij var. *hansenii*, The Yeasts, ed. 1, 280, 1952.

Basionym: *Saccharomyces hansenii* Zopf, *Ber. Deut. Bot. Ges.*, 7: 94, 1889.

Synonyms: *Debaryomyces tyrocola* Konokotina (1913)
Debaryomyces membranaefaciens Naganishi (1917)
Debaryomyces kloeckeri Guilliermond et Péju (1919)
Debaryomyces matruchoti Grigoraki et Péju (1921)
?Debaryomyces hudeloi da Fonseca (1922)
Debaryomyces hildegaardi Ota (1923)
Debaryomyces laedegaardi Ota (1923)
Debaryomyces gruetzii Ota (1924)
Debaryomyces tyrocola Konokotina var. *hansenii* (Zopf) Dekker (1931)
Debaryomyces matruchoti Grigoraki et Péju var. *cesarii* Dekker (1931)
Debaryomyces guilliermondii Dekker (1931)
Debaryomyces sake Saito et Oda (1932)
Debaryomyces membranaefaciens Naganishi var. *hollandicus* Lodder (1932)
Debaryomyces kloeckeri Guilliermond et Péju var. *major* Lodder (1932)
?Debaryomyces guilliermondii Dekker var. *nova-zelandicus* Lodder (1932)
Debaryomyces miso Mogi (1938)
Debaryomyces nicotianae Giovannozzi (1939)

Debaryomyces nicotianae Giovannozzi var. *minor*
Giovannozzi (1939)

?*Debaryomyces marylandii* Giovannozzi (1941)

Torulopsis westerdijkii Novák et Zsolt (1964)

Anamorph: *Candida famata* (Harrison) Meyer et Yarrow var. *famata*, *Int. J. Syst. Bacteriol.* **28**: 612, 1978.

Basionym: *Mycotorula famata* Harrison, *Trans. Roy. Soc. Can. V.* **22**: 187, 1928.

Synonyms: *Geotrichum famatum* (Harrison) C. W. Dodge (1935)
Torulopsis famata (Harrison) Lodder et Kreger-van Rij (1952)

Candida famata (Harrison) Novák et Zsolt (1961)

Cryptococcus minor Pollacci et Nannizzi (1934)

Torulopsis minor (Pollacci et Nannizzi) Lodder (1934)

Parentomyces minor (Pollacci et Nannizzi) C. W. Dodge (1935)

Debaryomyces membranaefaciens was not employed in the present study; however, PRICE et al. (4) reported the high DNA relatedness between the authentic strain of this species and the type strain of *Debaryomyces hansenii* so that it is listed as the synonym of this variety. Synonyms with question marks are those whose type or authentic strains were not investigated in the present study. Of the synonyms listed, *Debaryomyces sake* Saito et Oda is often written as *Debaryomyces sake* Saito et Ota, however, Dr. M. Ota and Dr. M. Oda are different persons. Dr. M. Ota is a medical mycologist and Dr. M. Oda is a fermentation technologist. In this case, "Saito et Oda" is correct.

Ib. *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij var. *fabryi* (Ota) Nakase et Suzuki nov. comb.

Basionym: *Debaryomyces fabryi* Ota, *Dermatol. Wochenschrift* **78**: 287, 1924.

Synonyms: *Eutorulopsis subglobosus* Zach (1934)
Debaryomyces fukuyamaensis Naganishi (1941)
?*Debaryomyces orientalis* Naganishi (1941)
Debaryomyces subglobosus (Zach) Lodder et Kreger-van Rij (1952)
Pichia adzetii Jacob (1969)

Anamorph: *Candida famata* (Harrison) Meyer et Yarrow var. *flareri* (Ciferri et Redaelli) Nakase et Suzuki nov. comb.

Basionym: *Blastodendron flareri* Ciferri et Redaelli, *Arch. Mikrobiol.* **6**: 9, 1935.

Synonym: *Candida flareri* (Ciferri et Redaelli) Langeron et Guerra (1939)

II. *Debaryomyces nepalensis* Goto et Sugiyama, *J. Jpn. Bot.* **43**: 7, 1968.

Synonyms: *Debaryomyces cavensis* Giovannozzi (1941), syn. nov.

[nom. illegit. public. sine diagn. Latine]

Debaryomyces japonicus Naganishi (1941), syn. nov.

[nom. illegit. public. sine diagn. Latine]

Anamorph: *Candida naganishii* Nakase et Suzuki nov. sp.

Species a *Debaryomycete nepalensi* differt: Asci et ascosporae non formantur. Typus: IFO 1428 (=JCM 2095=CBS 5921).

Growth in YM broth: After 3 days at 25°C, the cells are spherical, almost spherical, or short oval, (2–6) × (2.5–6.5) μm ; single, in pairs, or in short chains. A sediment, a ring, and occasionally a thin, smooth or wrinkled pellicle or islets are formed.

Growth on YM agar: After 1 month at 17°C, the streak culture is greyish to yellowish, soft, shiny or dull, smooth or wrinkled.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudomycelium is absent or very primitive.

Fermentation:

Glucose	— or + (weak)	Lactose	—
Galactose	—	Raffinose	— or + (weak)
Sucrose	— or + (weak)	Melibiose	—
Maltose	—		

Assimilation of carbon compounds:

Glucose	+	Ethanol	+
Galactose	+	Glycerol	+
L-Sorbose	+	Erythritol	+
Sucrose	+	Ribitol	+
Maltose	+	Galactitol	— or + (latent)
Cellobiose	+	D-Mannitol	+
Trehalose	+	D-Glucitol	+
Lactose	— or + (latent)	α -Methyl-D-glucoside	+
Melibiose	+	Salicin	+
Raffinose	+	Glucono- δ -lactone	+ (latent)
Melezitose	+	2-Ketogluconic acid	+
Inulin	—	5-Ketogluconic acid	—
Soluble starch	+	DL-Lactic acid	variable
D-Xylose	+	Succinic acid	+
L-Arabinose	+	Citric acid	+
D-Arabinose	variable	Inositol	—
D-Ribose	+ (may be latent)	Propylene glycol	— or + (extremely latent)
L-Rhamnose	—		

Assimilation of nitrogen compounds:

Nitrate	—	Ethylamine-HCl	+
Nitrite	+		

Maximum growth temperature: 36–37°C.

Vitamin required: Biotin.

G+C content of the nuclear DNA: 37.6–38.0 mol%.

We propose to name the anamorph of *Debaryomyces nepalensis* as *Candida naganishii* in honor of Dr. H. Naganishi who first found the teleomorph of this species and described it as *Debaryomyces japonicus* without Latin diagnosis (6).

III. *Candida saitoana* Nakase et Suzuki nov. nom.

Basionym: *Torula candida* Saito, *Jpn. J. Bot.* 1: 41, 1922.

Synonyms: *Torulopsis candida* (Saito) Lodder (1934)

Cryptococcus candidum (Saito) Skinner (1947)

Growth in YM broth: After 3 days at 25°C, the cells are spherical, almost spherical, or short oval, $(3.5\text{--}9.5) \times (3.5\text{--}10) \mu\text{m}$; single, in pairs, or in short chains. A sediment, a ring, and occasionally a thin, smooth pellicle are formed.

Growth on YM agar: After 1 month at 17°C, the streak culture is greyish to yellowish, soft, shiny or dull, smooth or delicately wrinkled.

Dalmau plate culture on corn meal agar: After 7 days at 25°C, pseudomycelium is usually absent or very primitive. A strain (JCM 1523) forms well-developed, tree-like pseudomycelium, especially in the aerobic condition.

Fermentation: Absent.

Assimilation of carbon compounds:

Glucose	+	Ethanol	+	(may be latent and weak)
Galactose	+	Glycerol	+	
L-Sorbose	+	Erythritol	—	
Sucrose	+	Ribitol	+	
Maltose	+	Galactitol	—	
Cellobiose	+	D-Mannitol	+	
Trehalose	+	D-Glucitol	+	
Lactose	+	α -Methyl-D-glucoside	+	
Melibiose	+	Salicin	+	
Raffinose	+	Glucono- δ -lactone	variable	
Melezitose	— or + (latent and weak)	2-Ketogluconic acid	+	
Inulin	variable	5-Ketogluconic acid	—	
Soluble starch	— or + (weak, latent and weak)	DL-Lactic acid	+	(may be latent)
D-Xylose	+	Succinic acid	+	
L-Arabinose	+	Citric acid	+	
D-Arabinose	— or + (latent and weak)	Inositol	—	
D-Ribose	variable	Propylene glycol	+	
L-Rhamnose	—			

Assimilation of nitrogen compounds:

Nitrate	—	Ethylamine-HCl	+
Nitrite	variable		

Maximum growth temperature: 34–35°C.

Vitamin required: Biotin.

G+C content of nuclear DNA: 36.3–37.5 mol%.

The name *Torula candida* Saito 1922 (11) has priority, however, it cannot be adopted because the combination *Candida candida* is tautonymous. Therefore, a new name *Candida saitoana* Nakase et Suzuki is proposed. The specific epithet was chosen in honor of Dr. K. Saito who described *Torula candida*. The teleomorph of this species is not yet known. CBS 940 (=IFO 0380=JCM 1438) is the type of this species.

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REFERENCES

- 1) T. NAKASE and M. SUZUKI, *J. Gen. Appl. Microbiol.*, **31**, 49 (1985).
- 2) J. P. VAN DER WALT, *In The Yeasts: A Taxonomic Study*, 2nd ed., ed. by J. LODDER, North-Holland Publ. Co., Amsterdam (1970), p. 34.
- 3) M. YAMAZAKI and K. KOMAGATA, *Int. J. Syst. Bacteriol.*, **31**, 361 (1981).
- 4) C. W. PRICE, G. B. FUSON and H. J. PHAFF, *Microbiol. Rev.*, **42**, 161 (1978).
- 5) H. NAGANISHI and J. NAKAMURA, *Jozogaku Zasshi*, **15**, 40 (1937).
- 6) H. NAGANISHI, *Jozogaku Zasshi*, **19**, 14 (1941).
- 7) P. BOS and J. C. DE BRUYN, *Antonie van Leeuwenhoek; J. Serol. Microbiol.*, **39**, 99 (1973).
- 8) M. YAMAZAKI and K. KOMAGATA, *J. Gen. Appl. Microbiol.*, **28**, 119 (1982).
- 9) H. SAËZ, *Bull. Mens. Soc. Linn. Lyon*, **29**, 288 (1960).
- 10) M. E. DI MENNA, *J. Gen. Microbiol.*, **10**, 65 (1954).
- 11) K. SAITO, *Jpn. J. Bot.*, **1**, 1 (1922).