Numerical Taxonomy of the Yeast Genus *Debaryomyces* Lodder & Kreger-van Rij

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A set of 142 strains of yeasts, originally described as *Debaryomyces* species, were examined for 64 morphological, physiological and serological characters and the results were subjected to numerical analysis. Three phenons and one individual strain emerged at the 77 % similarity level. The first two phenons were each divided into three subphenons at the 85 % similarity level. Assimilation of *n*-alkanes (C₆ to C₁₆) was most useful for the division of strains into the three phenons. The data agree with the published results of % GC content.

INTRODUCTION

The genus *Debaryomyces* Lodder & Kreger-van Rij comprises yeasts with globose or ellipsoidal cells, which reproduce by multilateral budding. Pseudomycelium may be formed. Heterogamous conjugation, between mother cell and bud, generally precedes ascus formation, but isogamous conjugation also occurs. The spores are spherical or oval with 'warty' walls. One or two spores are usually formed per ascus but in some species up to four may be present. Fermentation is slow, weak or absent; nitrate is not assimilated (Lodder, 1970).

Debaryomyces species are ubiquitous, occurring in soil, in water, on plants etc. They assimilate a large number of sugars and polyols, including pentoses, starch and inulin (Lodder, 1970) and phenol and catechol (Rao & Bhat, 1971). Strains are osmophilic and halophilic, tolerating more than 24 % NaCl in the medium (Norkrans, 1966, 1968; Norkrans & Kylin, 1969; Gezelius & Norkrans, 1970). Some strains accumulate a considerable amount of riboflavin (Shavlovsky, Ksheminskaya & Varivoda, 1970). Only little is known about hydrocarbon utilization by *Debaryomyces* species: Markowitz & Kallio (1964) showed that *D. kloecckerii* utilizes pure hydrocarbons and kerosene; Scheda & Bos (1966) showed the same for *D. hansenii* and *D. vanriji*; but Komagata, Nakase & Katsuya (1964) did not detect utilization of kerosene in any of 36 strains of five species of the genus *Debaryomyces*.

This paper presents the results of the numerical evaluation of 142 strains of *Debaryomyces* on the basis of 64 characters.

METHODS

Organisms. All strains (see Table 1) originated from the Czechoslovak Collection of Yeasts (CCY), Institute of Chemistry SAS, Bratislava.

Tests. The 64 morphological, physiological, genetical and immunological characters employed in the analysis were tested following the methods of Kocková-Kratochvílová *et al.* (1969).

Cell morphology. Lengths and widths of 100 to 200 cells from a 3-day-old culture in beer wort (7 %,

Table 1. *Strains studied*

CCY no.†	Original name	Origin† and date
41-1-1	<i>Debaryomyces nicotianae</i> Giovannozzi var. <i>minor</i> Giovannozzi	BI CZAS 365 (1957)
41-1-2	<i>D. nicotinae</i> Giovannozzi	Isolated from mushroom <i>Pluteus nannus</i> in Kuřim (1962)
41-2-1	<i>D. globosus</i> Klöcker	Carlsberg Lab., Copenhagen, Denmark (1947)
41-2-2	<i>D. globosus</i> Klöcker	CBS (1947)
41-2-3	<i>Saccharomyces kloeckerianus</i> van der Walt	LKB-D-1 (1973)
41-2-4 to 41-2-6	<i>D. globosus</i> Klöcker	VKM-Y-92, VKM-Y-1035, VKM-Y-93 (1973)
41-3-1	<i>D. kloeckeri</i> Guilliermond & Péju	Carlsberg Lab., Copenhagen, Denmark (1947)
41-3-2	<i>D. kloeckeri</i> Guilliermond & Péju	BI CZAS 364/2 (1957)
41-3-3	<i>D. kloeckeri</i> Guilliermond & Péju	MGU 1923 (1973)
41-3-4 to 41-3-8	<i>D. kloeckeri</i> Guilliermond & Péju	VKM-Y-96, VKM-Y-1042 to VKM-Y-1045 (1973)
41-3-9	<i>D. kloeckeri</i> Guilliermond & Péju race S	VKM-Y-1046 (1973)
41-3-10	<i>D. kloeckeri</i> Guilliermond & Péju (syn. <i>D. matruchoti</i> Grigoraki & Péju)	VKM-Y-101 (1973)
41-3-11	<i>D. kloeckeri</i> Guilliermond & Péju (syn. <i>D. matruchoti</i> Grigoraki & Péju race <i>fabryi</i>)	VKM-Y-102 (1973)
41-3-12	<i>D. kloeckeri</i> Guilliermond & Péju (syn. <i>D. matruchoti</i> Grigoraki & Péju race <i>gruetzii</i>)	VKM-Y-103 (1973)
41-3-13	<i>D. kloeckeri</i> Guilliermond & Péju (syn. <i>D. matruchoti</i> Grigoraki & Péju race <i>hildegardii</i>)	VKM-Y-104 (1973)
41-3-14	<i>D. kloeckeri</i> Guilliermond & Péju (syn. <i>D. membranaefaciens</i> Naganishi var. <i>hollandicus</i> Lodder)	VKM-Y-108 (1973)
41-4-1	<i>D. subglobosus</i> (Zach) Lodder & Kreger-van Rij	BI CZAS 363 (1957)
41-5-1	<i>D. vini</i> Zimmermann (syn. <i>Pichia vini</i>)	NRRL Y-1459 (1970)
41-6-1	<i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij	BI CZAS 361/1 (1951)
41-6-2	<i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij	BI CZAS 361/2 (1951)
41-6-3	<i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij	BI CZAS 361/3 (1951)
41-6-4	<i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij	Plant sample (Mecklenburg, GDR) (1971)
41-6-5	<i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij	LKB-D-774 (1973)
41-6-6 to 41-6-8	<i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij	MGU 2367 to MGU 2369 (1973)
41-6-9	<i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij (syn. <i>D. tyrocola</i> var. <i>hansenii</i>)	VKM-Y-116 (1973)
41-6-10	<i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij	VKM-Y-1040 (1973)
41-6-11	<i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij	LKB-D-1 (1973)
41-7-1	<i>D. phaffii</i> Capriotti	IFO 1362 (1969)
41-7-2*	<i>D. phaffii</i> Capriotti	CBS 4346 (1973)
41-8-1*	<i>D. cantarellii</i> Capriotti	NRRL YB-5160 (1970)
41-8-2	<i>D. cantarellii</i> Capriotti	LKB-D-2 (1973)
41-8-3 to 41-8-12	<i>D. cantarellii</i> Capriotti	MGU 2397 to MGU 2406 (1973)
41-8-13 to 41-8-26	<i>D. cantarellii</i> Capriotti	MGU 2407 to MGU 2420 (1973)
41-8-27	<i>D. cantarellii</i> Capriotti	MGU 2433 (1973)
41-8-28, 41-8-29	<i>D. cantarellii</i> Capriotti	VKM-Y-1562, VKM-Y-1563 (1973)
41-9-1*	<i>D. castellii</i> Capriotti	NRRL Y-5983 (1970)
41-9-2, 41-9-3	<i>D. castellii</i> Capriotti	MGU 2431, MGU 2432 (1973)
41-9-4 to 41-9-6	<i>D. castellii</i> Capriotti	MGU 2449 to MGU 2451 (1973)
41-9-7	<i>D. castellii</i> Capriotti	MGU A-128 (1973)
41-10-1	<i>D. marama</i> di Menna	NRRL Y-2171 (1970)
41-10-2	<i>D. marama</i> di Menna	VKM-Y-100 (1973)
41-10-3*	<i>D. marama</i> di Menna	CBS 1958 (1973)
41-12-1	<i>D. coudertii</i> Saëz	Plant sample (Mecklenburg, GDR) (1971)
41-12-2*	<i>D. coudertii</i> Saëz	CBS 5167 (1973)
41-13-1	<i>D. franciscae</i> (Capriotti) Kodama, Kyono, Naganishi & Takahara (<i>Saccharomyces pretoriensis</i> van der Walt & Tschuschner)	LKB-D-3 (1973)
41-14-1	<i>D. nilssonii</i> (Capriotti) Kodama, Kyono, Naganishi & Takahara (<i>Saccharomyces microellipsodes</i> Osterwalder)	LKB-D-637 (1973)
41-15-1 to 41-15-3	<i>D. formicarius</i> Golubev & Babyeva	MGU 2342 to MGU 2344 (1973)
41-15-4 to 41-15-5	<i>D. formicarius</i> Golubev & Babyeva	MGU 2346, MGU 2347 (1973)
41-15-6 to 41-15-8	<i>D. formicarius</i> Golubev & Babyeva	MGU 2349 to MGU 2351 (1973)
41-15-9, 41-15-10	<i>D. formicarius</i> Golubev & Babyeva	MGU 2354, MGU 2355 (1973)
41-15-11 to 41-15-20	<i>D. formicarius</i> Golubev & Babyeva	MGU 2357 to MGU 2366 (1973)
41-15-21	<i>D. formicarius</i> Golubev & Babyeva	IBPM 650 (1973)
41-15-22*	<i>D. formicarius</i> Golubev & Babyeva	VKM-Y-1555 (1973)
41-16-1	<i>D. mancharicus</i> Naganishi (<i>Saccharomyces montanus</i> Phaff, Miller & Shifrine)	IBPM 240 (1973)
41-16-2	<i>D. mancharicus</i> Naganishi (<i>Saccharomyces montanus</i> Phaff, Miller & Shifrine)	VKM-Y-99 (1973)
41-17-1	<i>D. dekkeri</i> Mrak et al. ex Kudriavzev [<i>Saccharomyces fermentati</i> (Saito) Lodder & Kreger-van Rij]	IBPM 235 (1973)
41-17-2, 41-17-3	<i>D. dekkeri</i> Mrak et al. ex Kudriavzev	VKM-Y-91, VKM-Y-707 (1973)
41-18-1	<i>D. delbrueckii</i> (Lindner) Kudriavzev (syn. <i>Torulaspora delbrueckii</i> Lindner, <i>Saccharomyces delbrueckii</i> Lindner)	VKM-Y-706 (1973)
41-19-1	<i>D. disporus</i> Dekker [<i>Saccharomyces disporus</i> (Dekker) Kreger-van Rij]	VKM-Y-1034 (1973)
41-20-1	<i>D. guilliermondii</i> Dekker	VKM-Y-94 (1973)

Table 1. (cont.)

CCY no. †	Original name	Origin† and date
41-20-2	<i>D. guilliermondii</i> Dekker	VKM-Y-1036 (1973)
41-20-3	<i>D. guilliermondii</i> Dekker (syn. <i>D. kloeckeri</i> var. <i>major</i>)	VKM-Y-97 (1973)
41-20-4	<i>D. guilliermondii</i> Dekker (syn. <i>D. matruchoti</i> var. <i>subglobosus</i> Zach)	VKM-Y-106 (1973)
41-20-5, 41-20-6	<i>D. guilliermondii</i> Dekker (syn. <i>D. tyrocola</i>)	VKM-Y-1052, VKM-Y-1053 (1973)
41-20-7	<i>D. guilliermondii</i> Dekker (syn. <i>D. tyrocola</i> strain 'Sb')	VKM-Y-115 (1973)
41-20-8	<i>D. guilliermondii</i> Dekker	VKM-Y-1037 (1973)
41-20-9, 41-20-10	<i>D. guilliermondii</i> Dekker var. <i>nova-zeelandicus</i> Lodder	VKM-Y-1039, VKM-Y-1038 (1973)
41-21-1	<i>D. hudeloi</i> da Fonseca (syn. <i>D. kloeckeri</i> Guilliermond & Péju)	VKM-Y-95 (1973)
41-22-1	<i>D. konokotinae</i> Kudriavzev	VKM-Y-98 (1973)
41-23-1 to 41-23-3	<i>D. mucosus</i> [Torulopsis <i>candida</i> (Sito) Lodder]	VKM-Y-1047, VKM-Y-1048, VKM-Y-109 (1973)
41-24-1 to 41-24-3	<i>D. rosei</i> (Guilliermond) Kudriavzev (syn. <i>Torulasporea rosei</i> Guilliermond)	VKM-Y-1049, VKM-Y-708, VKM-Y-710 (1973)
41-24-4	<i>D. rosei</i> (Guilliermond) Kudriavzev (syn. <i>Zygosaccharomyces globiformis</i> forma <i>typica</i> Kroemer)	VKM-Y-864 (1973)
41-25-1	<i>D. sake</i> Saito & Ota	VKM-Y-1050 (1973)
41-26-1, 41-26-2	<i>D. tyrocola</i> Konokotina	VKM-Y-113, VKM-Y-112 (1973)
41-26-3	<i>D. tyrocola</i> Konokotina race 'OP'	VKM-Y-1051 (1973)
41-26-4	<i>D. tyrocola</i> Konokotina	VKM-Y-1054 (1973)
41-26-5	<i>D. tyrocola</i> Konokotina (syn. <i>D. matruchoti</i> var. <i>cesarii</i> Dekker)	VKM-Y-105 (1973)
41-26-6	<i>D. tyrocola</i> Konokotina (syn. <i>D. membranaefaciens</i> Naganishi)	VKM-Y-107 (1973)
41-26-7	<i>D. tyrocola</i> Konokotina (syn. <i>D. nicotianae</i> Giovannozzi)	VKM-Y-110 (1973)
41-26-8	<i>D. tyrocola</i> Konokotina (syn. <i>D. nicotianae</i> var. <i>minor</i> Giovannozzi)	VKM-Y-111 (1973)
41-27-1	<i>D. cavensis</i> Giovannozzi [syn. <i>D. hansenii</i> (Zopf) Lodder & Kreger-van Rij]	VKM-Y-90 (1973)
41-28-1*	<i>D. tamarii</i> Ohara & Nonomura	CBS 4333 (1973)
41-29-1*	<i>D. nepalensis</i> Goto	CBS 5921 (1973)
41-29-2*	<i>D. nepalensis</i> Goto	NP-2-17-5, S. Goto, Japan (1973)
41-30-1*	<i>D. yarrowii</i> Santa Maria & Aser	CBS 6246 (1973)

* Type culture.

† BI CZAS, Biological Institute of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia (now Institute of Microbiology CZAS); CBS, Centraalbureau voor Schimmelmicrocultures, Delft, The Netherlands; CCY, Czechoslovak Collection of Yeasts, Institute of Chemistry SAS, Bratislava, Czechoslovakia; IBPM, Institute of Biochemistry and Physiology of Microorganisms, Academy of Sciences USSR, Pushchino on Oka, U.S.S.R.; IFO, Institute of Fermentation, Osaka, Japan; LKB, Laboratorium of Kodama Brewing, Iitaga, Japan; MGU, State University of Moscow, U.S.S.R.; NRRL, Northern Regional Research Laboratory, Peoria, Illinois, U.S.A.; VKM, All Union Collection of Microorganisms, Institute of Microbiology AS USSR, Moscow, U.S.S.R.

w/w, extract) were measured. The ratio of length:width indicates the form of the average cell, and the correlation coefficients between lengths and widths indicate the degree of the ploidy (Sarachek, 1958; Scheda, 1963; Kocková-Kratochvílová & Pokorná, 1964; Pokorná & Kocková-Kratochvílová, 1965).

Culture morphology. Pseudomycelium was demonstrated on slides with onion agar (250 g peeled onion was boiled with 1 l water, filtered and 1 g glucose l⁻¹ and 15 g agar l⁻¹ were added) by a method described by Langeron (1945). For the description of growth in liquid media, beer wort was used. Giant colonies on wort agar were studied during 3 weeks cultivation at room temperature; the diameter of the colony was measured after 1, 7, 14 and 21 days and the average radial growth rate was calculated as the increase in diameter (mm) in 100 h.

Physiological characters. Growth at 5 and 42 °C on wort agar was compared visually with cultures grown at 28 °C. Sporulation activity was estimated on Fowell agar containing 5 g sodium acetate l⁻¹ by counting the number of sporulation elements (spores, asci, zygotes) within a set of 500 cells. Tolerance to ethanol was studied in beer wort containing 1, 4, 8, 12, 16 or 18 % (v/v) ethanol and the growth limiting concentration was noted. Osmophily was determined in a solution of 4 g powdered yeast extract l⁻¹ containing either 600 g sucrose l⁻¹ or 100 g glucose l⁻¹ and 50 g NaCl l⁻¹; growth was compared with that in yeast extract containing 20 g glucose l⁻¹ by measuring turbidity in a nephelometer. Halophily was tested in medium containing 4 g yeast extract l⁻¹ supplemented with 20 g glucose l⁻¹ and 100, 150 or 200 g NaCl l⁻¹. Vitamin requirement was tested in Difco Bacto-vitamin-free yeast base; the culture was transferred twice into fresh medium with a 1 week interval and the turbidity of the culture was then compared with that of a culture grown in medium supplemented with vitamins (Lodder, 1970, p. 82). The autolysis rate was measured on 3-day-old yeast cultures from wort agar; yeast cells corresponding to about 1 mg dry wt were transferred into 15 ml distilled water, incubated for 17 h at 50 °C, centrifuged, and then the content of amino-nitrogen in the supernatant was determined by a method described by Herbert, Phipps & Strange (1971). Catalase activity was measured according to Lang & Böhm (1961), the remaining H₂O₂ being determined by titration with KMnO₄; the catalase in control samples was inactivated by simultaneous incubation at 100 °C. The phosphomolybdic acid test was carried out according to Holland & Kunz (1961) and Kocková-Kratochvílová & Vojtková-Lepšíková (1963) on agar medium containing 10 g peptone l⁻¹, 40 g sucrose l⁻¹, and 15 g agar l⁻¹, supplemented with 125 g phosphomolybdic acid l⁻¹; the colour of the smear was recorded after 3 days incubation at 28 °C.

Fig. 1. Dendrogram of strains of the genus *Debaryomyces*. Phenons and subphenons: AI *cantarellii*, AII *castellii*, AIII *formicarius*, BI *hansenii*, BII *tyrocola*, BIII *coudertii*, C *torulaspora*, D *tamaritii*.

Fermentation and assimilation tests. Fermentation of sugars was tested in Durham tubes containing 4 g powdered yeast extract l⁻¹ and 20 g sugar l⁻¹. Assimilation of sugars and other carbon and nitrogen sources was tested using Difco Bacto-yeast carbon base or Bacto-yeast nitrogen base agar on the surface of which were placed filter paper discs, which had been soaked with 50 g test substance l⁻¹ and sterilized by ultra-violet light.

The utilization of raffinose is an important taxonomic character and depends on the presence of the enzymes β -fructofuranosidase, α -glucosidase and α -galactosidase. Metabolic products of raffinose (D-galactose, melibiose and sucrose) were determined chromatographically (Kocková-Kratochvílová, Vojtková-Lepšíková & Fischerová, 1959) using diphenylamine for detection.

The assimilation of ethanol, ethanediol and glycerol was evaluated in liquid medium with the alcohol as sole source of carbon using Bacto-yeast nitrogen base. The assimilation of *n*-alkanes (C₆ to C₁₆) was tested as described by Markowitz & Kallio (1964). The growth on Bacto-yeast nitrogen base agar was compared visually with control samples on the same medium containing glucose as a carbon source.

Serological tests. Rabbit sera against *Debaryomyces hansenii* CCY41-6-2 and *Pichia membranaefaciens* CCY39-1-4 were kindly supplied by Dr J. Šandula; the simple agglutination test was done in tubes.

Effect of inhibitory substances. Inhibition by actidione was estimated by the disc method (1, 50 or 100 µg actidione per disc), measuring the diameter of the clear zone around the disc after incubation on beer wort agar at 28 °C. The inhibitory effect of 20 g DL-lactic acid l⁻¹ or 20 g DL-tartaric acid l⁻¹ was measured nephelometrically in a medium containing 4 g powdered yeast extract l⁻¹ and 20 g glucose l⁻¹ by comparing the absorbance of the test broth with that of a control.

Coding of features for computation. Features existing in two mutually exclusive states were scored as 1 or 0. Other qualitative features were divided into three or more mutually exclusive states, e.g. character of giant colonies, growth in liquid media, type of pseudomycelium. Multistate quantitative characters were divided into states according to the frequency distribution curves, e.g. cell sizes, radial growth rate, inhibition tests. The non-additive code in the 0 and 1 scale was used for more than two-state characters.

Computation. The similarity between strains was computed by the 'matching' coefficient method of Sokal & Michener (1958); both positive and negative matches were included. This coefficient (S_{SM}) was calculated from the equations:

$$S_{SM} = 1 - d_{jk}^2$$

$$\text{and } d_{jk}^2 = \frac{\sum_{i=1}^n (x_j - x_k)^2}{n}$$

(which are valid for the 0-1 scale used) where x_j and x_k are matches for strains *j* and *k*, *n* is the number of matches included in the calculation and d_{jk} is the taxonomic distance between strains *j* and *k*. The dendrogram was prepared by the method of unweighted average linkage (Sokal & Sneath, 1963). The homogeneity of clusters was checked using the χ^2 test. Single phenons at the 77% similarity level were characterized according to the percentage frequency of positive characters (Goodfellow, 1971). The individual phenons were named according to the centroid strain or group of strains.

The calculations were programmed in Algol and carried out on the Gier III computer at the Institute of Technical Cybernetics of the Slovak Academy of Sciences, Bratislava.

RESULTS AND DISCUSSION

Formation of phenons

The 142 strains of the genus *Debaryomyces* were classified into three phenons (A, B and C) and one individual strain (phenon D) at the 77.7% similarity level (Fig. 1). Phenon A contained 62 strains and was divided at the level of 82.2% of average similarity into three subphenons: I, *cantarellii*; II, *castellii*; and III, *formicarius*. Phenon B contained 61 strains and was divided at the level of 84.6% of average similarity into three subphenons: I, *hansenii*; II, *tyrocola*; and III, *coudertii*. Phenon C contained 18 strains at the level of 81.8% of average similarity and was named *torulaspora*. (It was given this name because most of the strains originally described as of the genus *Torulaspora* were included in this phenon.) The individual strain, the type culture of *D. tamarii*, will be the subject of a future communication.

Table 2. *Percentage frequency of positive characters found in individual phenons at the 80 % similarity level*

Character	Phenon A			Phenon B			Phenon C (18)
	I *(30)	II (8)	III (24)	I (30)	II (22)	III (9)	
Cell morphology:							
Average cell length							
< 5.5 μm	6	0	0	100	72	88	28
5.6–7.5 μm	80	87	75	0	28	12	72
> 7.5 μm	14	13	25	0	0	0	0
Average cell width							
< 4.0 μm	6	0	0	50	19	55	0
4.1–5.0 μm	80	0	13	50	59	33	66
> 5.0 μm	14	100	87	0	22	12	34
Length:width ratio							
1.0–1.10	0	75	0	50	45	77	39
1.11–1.50	67	25	93	47	55	22	61
> 1.50	33	0	7	3	0	0	0
Surface:volume ratio							
< 1.0	3	0	25	0	13	0	0
1.01–1.30	83	100	75	0	28	12	72
> 1.30	14	0	0	100	59	88	28
Correlation coefficient between lengths and widths of cells							
0–0.30	3	13	0	0	0	0	0
0.31–0.60	27	37	33	10	10	0	0
0.61–1.00	70	50	67	90	90	100	100
Culture morphology:							
Pseudomycelium							
None	0	13	8	56	86	100	88
Rudimentary	53	13	70	23	10	0	0
Dendroidal	47	74	22	21	4	0	12
Growth in 7 % (w/v) beer wort							
Sediment only	0	0	0	20	9	22	28
Ring	36	75	17	43	73	78	72
Islets on surface	33	25	71	6	0	0	0
Pellicle	31	0	12	31	18	0	0
Giant colonies							
Smooth	40	87	30	60	90	100	66
Semi-rough	60	13	70	33	5	0	28
Rough	0	0	0	7	5	0	6
Radial growth rate [mm (100 h) ⁻¹]							
< 3.0	0	0	7	7	0	0	28
3.1–5.0	33	25	43	66	63	66	72
5.1–7.0	67	62	50	25	37	22	0
> 7.0	0	13	0	2	0	12	0
Growth at 5 °C:							
None	0	0	100	0	0	0	28
Moderate	96	100	0	83	77	100	72
Good	4	0	0	17	23	0	0
Growth at 42 °C:							
None	70	100	4	70	59	23	11
Weak	30	0	4	30	36	77	56
Good	0	0	91	0	5	0	33
Tolerance to ethanol (% v/v, in medium):							
4	96	100	79	93	36	66	56
8	4	0	21	7	59	23	44
12	0	0	0	0	5	11	0
Growth in 60 % (w/v) sucrose:							
< 30 %	10	0	0	50	0	0	6
30–60 %	80	100	91	46	23	56	0
60–80 %	10	0	9	4	54	22	6
80–100 %	0	0	0	0	23	22	88

Table 2 (cont.)

Character	Phenon A			Phenon B			Phenon C
	I	II	III	I	II	III	
Growth in 10 % (w/v) glucose and 5% (w/v) NaCl:							
< 30 %	0	0	0	4	0	0	0
30-60 %	0	0	0	8	5	11	6
60-80 %	50	13	0	46	23	56	0
80-100 %	50	87	100	42	72	33	94
Halophily:							
No growth in 10 % NaCl	76	13	91	43	9	44	100
Growth in 10 % NaCl	24	87	0	43	36	12	0
15 % NaCl	0	0	4	14	41	44	0
20 % NaCl	0	0	4	0	14	0	0
Growth in vitamin-free medium (% of growth in supplemented medium):							
15-50	0	100	0	93	100	100	17
51-90	0	0	9	7	0	0	83
91-100	100	0	91	0	0	0	0
Autolysis rate [mg amino-N (100 mg dry wt) ⁻¹]:							
< 1.0	30	87	70	93	95	100	77
> 1.0	70	13	30	7	5	0	23
Fermentation of:							
Glucose	100	100	100	13	13	44	100
Galactose	0	0	0	0	0	0	27
Sucrose	93	100	0	10	4	44	94
Maltose	0	0	0	0	0	0	0
Lactose	0	0	0	0	0	0	0
Assimilation of:							
Galactose	100	75	100	100	100	88	38
Sucrose	100	100	100	100	100	100	94
Maltose	100	100	100	100	100	100	44
Lactose	83	87	0	23	40	55	6
Melibiose	97	100	100	16	93	74	54
Trehalose	100	100	100	93	100	100	66
Cellobiose	100	100	0	100	100	100	11
Melezitose	100	87	100	100	100	100	50
Maltotriose†	76	100	100	30	45	77	16
Starch	100	100	100	86	55	0	0
Sorbose	100	100	100	16	93	100	100
Xylose	100	100	100	93	86	100	22
Arabinose	0	12	0	76	100	66	22
Rhamnose	0	12	100	46	18	66	27
Ribose	26	12	50	73	13	22	0
Inulin	0	0	0	4	0	0	16
Mannitol	100	100	100	96	100	100	88
Galactitol	56	0	29	0	13	0	0
Ribitol	100	100	100	93	72	100	11
Arabitol	100	100	100	96	100	100	50
Erythritol	100	50	100	96	50	55	5
Glucitol	100	100	100	96	90	100	44
Ethanol	100	87	100	36	40	55	27
Ethanediol	46	0	4	0	0	0	0
Glycerol	100	100	100	10	95	45	33
Lysine	100	100	100	93	100	88	83
Utilization of <i>n</i> -alkanes:							
Slight or none	5	0	0	77	77	100	100
Moderate	5	0	0	23	18	0	0
Strong	90	100	100	0	5	0	0
Metabolic end products of raffinose:							
Meiibiose	3	0	4	51	7	30	83
D-Galactose	0	0	0	23	7	11	0
No detectable sugar	97	100	96	26	86	44	11
No assimilation	0	0	0	0	0	15	6

Table 2 (cont.)

Character	Pheno ^u A			Phenon B			Phenon C
	I	II	III	I	II	III	
Inhibition by:							
Actidione (50 µg/disc) (diam. of clear zone, mm)							
No inhibition	100	100	100	33	5	0	23
1-15	0	0	0	26	5	22	11
16-20	0	0	0	15	5	22	5
> 20	0	0	0	26	86	56	61
DL-Lactic acid (2 %, w/v) (% of growth of control)							
< 10	0	0	0	4	40	12	5
11-50	0	75	0	83	50	88	18
51-100	83	25	34	10	10	0	72
> 100	17	0	66	4	0	0	5
DL-Tartaric acid (2 %, w/v) (% of growth of control)							
< 10	0	25	0	17	14	0	17
11-50	0	62	4	83	86	100	50
51-100	13	13	50	0	0	0	33
> 100	87	0	46	0	0	0	0
Sporulation activity (% sporulation elements):							
None	4	87	0	90	96	70	60
< 10	80	13	0	10	4	11	33
10-50	16	0	30	0	0	19	9
> 50	0	0	70	0	0	0	0
Catalase activity [% H ₂ O ₂ metabolized (mg dry wt) ⁻¹ min ⁻¹]:							
< 4.7	0	0	87	0	8	0	0
4.75-5.8	0	38	13	30	30	0	10
5.85-9.0	90	62	0	70	58	100	90
> 9.0	10	0	0	0	4	0	0
Colour of streak on phosphomolybdic acid agar:							
White	0	0	0	83	60	11	5
Olive	70	0	8	17	4	11	5
Brown	13	0	0	0	0	0	0
Blue	10	0	4	0	30	0	72
Dark blue	7	100	88	0	6	78	18
Agglutination with rabbit sera against:							
<i>D. hansenii</i> CCY41-6-2							
No agglutination	40	13	70	54	54	70	82
Dilution titre 20-80	60	87	30	23	40	30	6
Dilution titre 160-320	0	0	0	23	6	0	12
<i>P. membranaefaciens</i> CCY39-1-4							
No agglutination	30	0	0	17	54	100	82
Dilution titre 20-80	43	62	75	60	34	0	12
Dilution titre 160-320	27	38	25	23	12	0	6

* Numbers of strains analysed are given in parentheses.

† Maltotriose was only weakly assimilated by the percentages of strains indicated.

Characteristics of the phenons

The characteristics of the taxa are given in Table 2. The characters which differentiate phenons A, B and C are given in Table 3. Whilst phenon C is clearly separated from the other two, phenons A and B are only reliably separated on the basis of their different GC contents. The difference, however, is slight and further evaluation of the position of these phenons is probably required.

Table 3. *Characters for differentiating between individual phenons*

Phenon	Subphenon	GC content (mol %)	Utilization of	
			Starch	n-Alkanes
A	I, II, III	32.4-34.4	+	+
B	I	36.6-37.3	+	+
	II		+/-	+
	III		-	-
C		42.9-45.6	-	-

Table 4. *Characters for differentiating between individual subphenons within phenon A*

Character	Subphenon I	Subphenon II	Subphenon III
Cell form	Elongate	Slightly ovoid	Ellipsoidal
Pseudomycelium	Rudimentary	Dendroidal	Rudimentary
Growth at 5 °C	+	+	-
Growth at 42 °C	-	-	+
Growth in vitamin-free medium	+	-	+
Fermentation of sucrose	+	+	-
Assimilation of lactose	+/-	+	-
Assimilation of cellobiose	+	+	-
Assimilation of rhamnose	-	-	+
Tolerance* to lactic acid	+	-	+
Tolerance* to tartaric acid	+	-	+
Sporulation activity	Weak	Weak	> 50 %
Colour of streak on phosphomolybdic acid agar	Olive	Dark blue	Dark blue

+, Present; -, absent

* Growth not markedly inhibited, i.e. > 50 % of control.

The distinctive features of each phenon are as follows:

Phenon A. Cells are ellipsoidal to elongated, tend to form dendroidal pseudomycelium, have a fast radial growth rate, low tolerance to ethanol, 60 % sucrose and 10 % NaCl, are resistant to actidione, lactic acid and tartaric acid, and are agglutinated by *P. membranaefaciens* antiserum.

Phenon B. Cells are spherical to slightly ellipsoidal with a high surface:volume ratio and a high correlation coefficient between cell length and width. They have no pseudomycelium, are able to grow at 5 °C, but grow only slightly or not at all at 42 °C. They require vitamins, slowly autolyse and show very weak or no fermentation of glucose and sucrose, but assimilate arabinose. They do not sporulate, and are sensitive to actidione, lactic acid and tartaric acid.

Phenon C. Cells are spherical or ellipsoidal, their surface:volume ratio is between 1.0 and 1.3, in keeping with diploid cells, and they have a high correlation coefficient between cell length and width. They have no pseudomycelium, have a slow radial growth rate, high osmophily and low halophily, rapidly ferment glucose, galactose and sucrose, assimilate sorbose but not soluble starch or galactitol, are sensitive to actidione, turn phosphomolybdic acid medium blue, and do not agglutinate with *D. hansenii* or *P. membranaefaciens* antiserum.

The characters for differentiating the three subphenons of phenon A are given in Table 4. The subphenons of phenon B could be differentiated on the basis of their different catalase activities and colour produced on phosphomolybdic acid medium (see Table 2).

Phenon C resembles those groups of *Saccharomyces sensu lato* in which two proliferating cells unite before ascus formation, i.e. *S. bailii*, *S. fermentati*, *S. delbrueckii* (Kocková-Kratochvílová & Nakase, 1971; Kocková-Kratochvílová, 1976). The GC content of these *Saccharomyces* groups varies from 40.0 to 44.1 mol % (Yarrow & Nakase, 1975). A number of other morphological and physiological features of these *Saccharomyces* groups and

phenon C are also similar. We therefore propose to combine the above mentioned groups into one group named *torulaspora*, which corresponds to the genus *Torulaspora* Lindner in classical taxonomy.

Recently, the heterogeneity of *Saccharomyces*, *Debaryomyces* and related genera has been discussed. Yarrow & Nakase (1975) reported the GC content of 48 species of *Saccharomyces sensu lato* and classified these strains into four groups, only the first of which could be considered as *Saccharomyces sensu stricto*. Similarly, van der Walt & Johannsen (1975) proposed the name *Torulaspora* Lindner for a number of different species, including the whole genus *Debaryomyces*. Kurtzman & Baker (1975) published scanning electron micrographs of spores of various strains previously classified in the genus *Saccharomyces* (*S. pretoriensis*, *S. microellipsodes*) and demonstrated the rough surface of their spore walls, a characteristic which is sufficient for separating these species from *Saccharomyces sensu stricto*.

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