***Debaryomyces prosopidis* sp. nov., a yeast from exudates of mesquite trees**

Herman J. Phaff,¹ Ann Vaughan-Martini² and William T. Starmer³

Author for correspondence: Herman J. Phaff. Tel: +1 530 752 1465. Fax: +1 530 752 4759.
e-mail: hjphaff@ucdavis.edu

¹ Department of Food Science and Technology, University of California, Davis, CA 95616, USA

² Dipartimento di Biologia Vegetale, Università degli Studi, Perugia, Italy 06121

³ Department of Biology, Syracuse University, Syracuse, NY 13244, USA

Nine strains were studied of a new haploid species of the genus *Debaryomyces* Lodder & Kreger-van Rij that had been isolated from exudates of mesquite (*Prosopis juliflora*) trees in southern Arizona and from *Drosophila carbonaria* that breeds in these exudates. Their physiological characteristics, life cycle, and nuclear DNA base composition (approx. 37.5 mol% G+C) led to their original classification as *Debaryomyces hansenii* (Zopf) Kreger-van Rij. However, the two taxa are, at most, distantly related based on DNA reassociation values that indicated low base sequence complementarity. The two taxa also have distinctly different electrophoretic karyotypes. *Debaryomyces hansenii* has two varieties, *D. hansenii* var. *hansenii* and *D. hansenii* var. *fabryi*. *Debaryomyces prosopidis* can be differentiated phenotypically from both varieties by lack of growth on cellobiose after 2 weeks incubation and from the variety *hansenii* by a higher maximum temperature for growth. The type strain of *Debaryomyces prosopidis* sp. nov. is strain UCD-FST 84-100^T (= DBVPG 7010^T = CBS 8450^T = ATCC 201611^T).

Keywords: *Prosopis juliflora* (mesquite), *Debaryomyces prosopidis* sp. nov.

INTRODUCTION

Yeasts associated with decaying cactus tissue in the Americas and with *Drosophila* species breeding in these necroses have been studied extensively since the early seventies (Starmer *et al.*, 1990). These studies have shown that the great majority of yeast species occurring in this habitat are cactus specific and therefore not found in other plant tissues that support the growth of yeasts (Phaff & Starmer, 1987). Because some yeast species occur in cactus necroses as well as in other plant habitats, Ganter *et al.* (1986) investigated the yeast communities in a relatively narrow geographic area near Tucson, Arizona, USA. They compared the yeast community compositions in necrotic *Carnegiea gigantea* (saguaro) and in several *Opuntia* species with those found in exudates (slime fluxes) of several species of trees in the same area.

Among the yeast isolates that occurred only in tree exudates and not in cactus necroses were 30 strains identified as *Debaryomyces hansenii* that came from exudates of mesquite trees (*Prosopis juliflora*). An additional three strains were isolated from *Drosophila carbonaria* that breeds in exudates from mesquite trees.

The present study reports the results of a reinvestigation of nine representative strains of these yeast

isolates by molecular techniques. The results showed that these strains represent a new species of the genus *Debaryomyces* which is described here as *Debaryomyces prosopidis* sp. nov.

METHODS

Isolation and identification of organisms. Strains studied are listed in Table 1. Where possible, information is provided regarding the isolation source and location, other collection numbers, and original species designations if different from the present epithet. Cultures associated with *Prosopis juliflora* Amargo (mesquite) were collected in 1975 and 1984 from fluxes or from *Drosophila carbonaria* Patterson and Wheeler associated with the plant exudates.

All strains were compared phenotypically by standard methods as described by van der Walt & Yarrow (1984). Growth responses were determined in liquid medium on a roller drum at 25 °C. Maximum growth temperature was tested at one-degree intervals in tubes containing YEPG [1% (w/v) yeast extract, 1% (w/v) peptone, 2% (w/v) glucose] in a constant-temperature water bath with gentle shaking at 125 r.p.m. Ability to assimilate additional carbon compounds including hexadecane, methanol, D-glucosamine, N-acetyl-D-glucosamine, 2-propanol, acetone and ethyl acetate was also tested. The last three compounds, as well as hexadecane were tested as described by Phaff *et al.* (1997)

Table 1. Strains studied

Species/strain designation*	Original designation/isolation information/source
<i>Debaryomyces castellii</i> Capriotti DBVPG 7068 ^T , UCD 69-35 ^T	Soil, Sweden
<i>Debaryomyces coudertii</i> DBVPG 7124 ^T , UCD 69-36 ^T	Faeces of Emperor penguin
<i>Debaryomyces hansenii</i> (Zopf) Lodder & Kreger-van Rij-like	
DBVPG 7010 ^T , UCD 84-100 ^T	<i>Prosopis juliflora</i> Amargo (mesquite) flux, Arizona, USA
DBVPG 7011, UCD 84-101	<i>Prosopis juliflora</i> Amargo (mesquite) flux, Arizona, USA
DBVPG 7012, UCD 84-102	<i>Drosophila carbonaria</i> Patterson and Wheeler near <i>Prosopis juliflora</i> Amargo (mesquite) flux, Arizona, USA
DBVPG 7109, UCD 76-11	<i>Prosopis juliflora</i> Amargo (mesquite) flux, Arizona, USA
DBVPG 7110, UCD 76-12	<i>Prosopis juliflora</i> Amargo (mesquite) flux, Arizona, USA
DBVPG 7111, UCD 76-13	<i>Prosopis juliflora</i> Amargo (mesquite) flux, Arizona, USA
DBVPG 7112, UCD 76-14	<i>Drosophila carbonaria</i> Patterson and Wheeler near <i>Prosopis juliflora</i> Amargo (mesquite) flux, Arizona, USA
DBVPG 7113, UCD 76-15	<i>Drosophila carbonaria</i> Patterson and Wheeler near <i>Prosopis juliflora</i> (mesquite) flux, Arizona, USA
DBVPG 7114, UCD 76-16	<i>Drosophila carbonaria</i> Patterson and Wheeler near <i>Prosopis juliflora</i> (mesquite) flux, Arizona, USA
<i>Debaryomyces hansenii</i> (Zopf) Lodder & Kreger-van Rij var. <i>fabryi</i> (Ota) Nakase & Suzuki	
DBVPG 6404, UCD 75-17	(<i>Debaryomyces subglobosus</i> Zach ^T) Diseased nail
DBVPG 7031 ^T , UCD 96-1 ^T	Interdigital mycotic lesion, Germany
DBVPG 7033, IFO 0037	(<i>Debaryomyces fukuyamaensis</i> Naganishi ^T) Rice-vinegar mash, Japan
DBVPG 7034, JCM 1441	(<i>Pichia adzetii</i> Jacob ^T) Tanning fluid, France
DBVPG 7035, JCM 2166	(<i>Blastodendron flaveri</i> Redaelli & Ciferri ^T) Erythematous-squamatic skin lesion, Italy
<i>Debaryomyces hansenii</i> (Zopf) Lodder & Kreger-van Rij var. <i>hansenii</i>	
DBVPG 6017, CBS 1795	Wound on hand
DBVPG 6050 ^T , UCD 74-86 ^T	Carlsberg laboratory
DBVPG 6405, UCD 72-47	(<i>Debaryomyces tyrocola</i> Giovannozzi ^T) Cheese
DBVPG 6406, CBS 796	(<i>Debaryomyces mucosus</i> Hufschmitt <i>et al.</i> ^T) Skin lesion
<i>Debaryomyces nepalensis</i> DBVPG 7123 ^T , UCD 72-46 ^T	Soil, Nepal

* CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; DBVPG, Collezione dei Lieviti Industriali (Industrial Yeast Collection), Sez. di Microbiologia, Dip. di Biologia Vegetale, Università di Perugia, Italy; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Japan; UCD, The H. J. Phaff Collection of Yeast and Yeast-like Organisms, University of California, Davis, CA, USA. T, Type strain of the species or variety.

Preparation and analyses of nuclear DNA. High-molecular-mass nuclear DNA for optical reassocination experiments was obtained from 24–48 h cultures grown in YEPG at 25 °C. Cells were suspended in a sucrose buffer [0.02 M Tris, 0.02 M EDTA, 15% (w/v) sucrose] together with an equal volume of 0.45–0.50 mm diameter glass beads and were disrupted mechanically using a Bead-Beater Cell Disrupter (Biospec Products). DNA was purified according to Bernardi *et al.* (1970) with modifications by Price *et al.* (1978). DNAs to be labelled with ¹²⁵I were further purified by banding in a caesium chloride gradient (Holzschu *et al.*, 1979).

DNA base compositions were determined by buoyant density using the techniques described by Price *et al.* (1978). Final results were calculated from duplicate or triplicate examinations.

Nucleotide sequence homology was determined using two different techniques. Some DNA–DNA reassocination experiments were performed according to the optical method

of Kurtzman *et al.* (1980) using a Gilford 250 spectrophotometer equipped with a model 2527 Gilford thermo-programmer. Other studies were done using the technique of Holzschu *et al.* (1979) where DNA of strain DBVPG 7010 (UCD 84-100) was labelled *in vitro* with ¹²⁵I. Sequence complementarity was determined by renaturation in 0.28 M phosphate buffer (an equimolar mixture of monobasic and dibasic sodium phosphate, pH 6.8) at 25 °C below the *T_m* of native homologous DNA as described by Price *et al.* (1978). Reannealed DNA was separated from unreacted DNA by a hydroxylapatite (HA) procedure which separated single- from double-stranded molecules. Samples were counted in a Nuclear-Chicago model 1185 gamma counter where counting efficiency was 84%.

Intact chromosomal DNA for PFGE was prepared as previously reported (Vaughan-Martini *et al.*, 1993). All analyses were performed on a Chef Mapper (Bio-Rad) using gels composed of 1% agarose (Type II-A, medium EEO; Sigma) in 0.5 × TBE buffer. Temperatures of 12–15 °C were

maintained throughout the runs. The run time was 68 h with a ramp of 1 → 5 min at 4.5 V cm⁻¹ with an angle of 120 °.

RESULTS AND DISCUSSION

The 33 isolates from mesquite tree fluxes and *Drosophila carbonaria* in the Rincon and Catalina Mountains around Tucson, southern Arizona, USA (Ganter *et al.*, 1986) were originally identified as *Debaryomyces hansenii* based on their physiological characteristics and the determinative key of Kreger-van Rij (1984). In the present study nine representative strains from mesquite and associated *Drosophila* were investigated, using an expanded array of phenotypic criteria supplemented by DNA base composition determinations, DNA–DNA hybridization and electrophoretic karyotyping experiments. Currently two varieties are recognized, *Debaryomyces hansenii* var. *hansenii* and *D. hansenii* var. *fabryi* (Nakase & Suzuki, 1985a, b; Nakase *et al.*, 1998). Nakase & Suzuki (1985a) could not find any phenotypic criteria to distinguish the two varieties except by DNA hybridization, although in their accompanying paper (Nakase & Suzuki, 1985b) they stated that the maximum temperature for growth of the variety *hansenii* was 31–35 °C and that of the variety *fabryi* was 36–39 °C.

The G+C contents of four of the mesquite strains agreed fairly closely with those reported for the two varieties of *D. hansenii* (Price *et al.*, 1978; Nakase & Suzuki, 1985a). However, DNA reassociation experiments, using both optical reassociation (Table 2) and labelled DNA techniques (Table 3), clearly showed that, with only approximately 30 % DNA relatedness, *D. prosopidis* is a different species from *D. hansenii* var. *hansenii*. The relationship between *D. prosopidis* and *D. hansenii* var. *fabryi* appears to be closer as shown by a DNA relatedness value of 54 % between the type

Table 3. Percentage DNA–DNA relatedness between ¹²⁵I-labelled samples of *Debaryomyces prosopidis* DBVPG 7010^T and other strains of *Debaryomyces*

Sample*	Hybridization (%)
<i>D. prosopidis</i> 7010 ^T	(100)
7011	99.4
7012	97.6
<i>D. hansenii</i> var. <i>fabryi</i> 6404	48.4
<i>D. hansenii</i> var. <i>hansenii</i> 6050 ^T	32.5
6405	29.8
<i>D. coudertii</i> 7124 ^T	19.4
Calf thymus DNA	13.5
<i>D. nepalensis</i> 7123 ^T	13.3
<i>D. castellii</i> 7068 ^T	9.5

* DBVPG strain nos are given – see Table 1 for other designations.

strains of these two organisms (Table 2). Although this degree of kinship is significant, it is not high enough to consider the two taxa conspecific. The same could be said about the relatedness between the two varieties of *D. hansenii*, which is also in the range of 36–54 % [see Price *et al.* (1978) and Table 2]. The reason that *D. hansenii* var. *fabryi* has remained a variety is that so far there are no traditional phenotypic characteristics to distinguish the two varieties (Nakase & Suzuki, 1985a). In contrast, *D. prosopidis* can be differentiated from both varieties of *D. hansenii* by its inability to utilize cellobiose and salicin in Yeast Nitrogen Base after 2 weeks incubation. Nakase & Suzuki (1985a) tested

Table 2. Optical DNA–DNA reassociation within *Debaryomyces hansenii* and related taxa

Species*	6017	6050 ^T	6405	6406	6404	7031 ^T	7010 ^T
<i>D. hansenii</i> var. <i>hansenii</i>							
6017	(100)	–	–	–	–	–	–
6050 ^T	82	(100)	–	–	–	–	–
6405	–	75	(100)	–	–	–	–
6406	57	80	58	(100)	–	–	–
<i>D. hansenii</i> var. <i>fabryi</i>							
6404	57	54	31	61	(100)	–	–
7031 ^T	–	36	–	–	79	(100)	–
7033	–	–	–	–	–	96	67
7034	–	–	–	–	–	96	62
7035	–	–	–	–	98	75	–
<i>D. prosopidis</i>							
7010 ^T	26	–	30	–	47	54	(100)
7011	–	–	–	46	–	–	95
7012	–	45	–	–	60	–	99

* DBVPG strain nos are given – see Table 1 for other designations.

Fig. 1. Electrophoretic karyotypes of *D. prosopidis* and related strains (DBVPG strain nos given – see Table 1 for other designations). *D. hansenii* var. *fabryi* 7033 (lane 1) and 7031^T (lane 2); *D. prosopidis* 7012 (lane 3), 7011 (lane 4), 7010^T (lane 5), 7114 (lane 6), 7113 (lane 7), 7112 (lane 8), 7111 (lane 9), 7110 (lane 10) and 7109 (lane 11); *D. hansenii* var. *hansenii* 6406 (lane 12) and 6050^T (lane 13). STD1, standard DNA of *Pichia canadensis* (syn. *Hansenula wingei*) and STD2, standard DNA of *Saccharomyces cerevisiae* (both from Bio-Rad).

34 strains of *D. hansenii* var. *hansenii* and six strains of *D. hansenii* var. *fabryi* which all grew on cellobiose and β -methyl-D-glucoside. Most strains of *D. prosopidis* including the type grew at 37 °C, except strains UCD 76-13, 76-14 and 76-15, which grew at 35–36 °C, thus enabling further discrimination between *D. prosopidis* and *D. hansenii* var. *hansenii*.

Also supporting the establishment of the new species, *D. prosopidis*, is the fact that the electrophoretic karyotypes of all strains of the new species are identical (Fig. 1) and significantly different from the type strain and other strains of the two varieties of *D. hansenii*. Selected diagnostic characteristics of *Debaryomyces* species are given in Table 4.

Latin diagnosis of *Debaryomyces prosopidis* sp. nov.

In medio liquido cum glucoso, peptono et extracto levedinis (post dies 3 ad 25 °C) cellulae ovoideae, 3.5–5.5 × 1.5–2.5 µm, singulae aut binae; sedimentum; pellicula nulla. Cultura in agarso malto (post dies 25 ad 25 °C) eburnea, cana-cremea, butyrosa, glabra, nitida. In agarso farinae Zea mays post dies 10 pseudomycelium nullum. Asci formantur ex transformatione cellularum vegetarum (post dies 3–5, 20–25 °C). Ascospores

Table 4. Selected characteristics of *Debaryomyces prosopidis* and related taxa

–, No growth; +, strong growth in 3 d; +s, growth in 6 d; +w, growth in 14 d; +vw, growth in 21 d; $T_{\max}$, maximum growth temperature.

Species*	Assimilation of:		$T_{\max}$ (°C)	G + C content (mol %) [†]
	CEL	MEL		
<i>D. prosopidis</i>				
7010 ^T	–	–	37	37.54 ± 0.25 ^a
7011	–	–	37	37.42 ± 0.3 ^a
7012	–	–	37	37.51 ± 0.3 ^a
7109	–	–	37	
7110	+vw	–	37	37.7 ± 0.4 ^a
7111	–	–	36	
7112	–	–	35	
7113	+vw	+w	35	
7114	+w	–	37	
<i>D. hansenii</i> var. <i>fabryi</i>				
6404	+	+w	37	37.3 ± 0.2 ^b
7031 ^T	+	+w	35	36.5 ^c
7033	+	+s	35	36.8 ^c
7034	+	+w	36	36.6 ^c
7035	+	+s	37	36.8 ^c
<i>D. hansenii</i> var. <i>hansenii</i>				
6017	+	+	< 35	37.5 ^c
6050 ^T	+	+w	< 35	36.8 ^c
6405	+	+w	< 35	37.2 ^c
6406	+	+w	< 35	

* DBVPG strain nos are given – see Table 1 for other designations.

[†] *a*, This study (calculated from the mean of 2–3 buoyant density determinations); *b*, Price *et al.* (1978) (by buoyant density); *c*, Nakase & Suzuki (1985a) (by T_m).

rotundae; 1–(2) in asco. Fermentatio glucosi nullum. Glucosum, galactosum, sorbosum, maltosum, saccharosum, trichalosum (lente), raffinsum, melezitum, amyllum solubile, D-xylosum, L-arabiosum, D-ribosum (exigue), ethanolum, glycerolum, erythritolum, ribitololum, D-mannitololum, D-glucitololum, methylum α -D-glucosidum, glucono- δ -lactonum, 2-ketogluconatum, acidum succinicum, acidum citricum, acidum gluconatum, N-acetylglucosaminum, ethyl acetat (exigue), hexadecanum assimilantur at non cellobiosum, lactosum, melibiosum, inulinum, D-arabiosum, L-rhamnosum, galactitololum, salicinum, acidum lacticum, inositololum, methanololum, glucosaminum, acetolum, 2-propanolum. Lysinum, cadaverinum et ethylaminum assimilantur. Natrium nitricum et natrium nitosum non assimilantur. Ad crescentiam additae vitaminiae necessariae sunt. Temperatura maxima crescentiae 37 °C +, 38 °C –. Crescere potest in 12.5% NaCl/5% glucoso, 50% glucoso, 0.1 g cycloheximido l⁻¹. Typus depositus in collectione H. J. Phaff Collection of Yeast and Yeast-like Organisms, UCD 84-100^T) et Collezione dei Lieviti Industriali, Perugia (DBVPG 7010^T), CBS 8450^T, ATCC 201611^T.

Description of the type strain of *Debaryomyces prosopidis* sp. nov.

Debaryomyces prosopidis (pro.so'pi.dis. L. masc. gen. n. *prosopidis* of *Prosopis*, referring to the host tree of the new species).

In YM (Difco) liquid medium after 5 d at 25 °C, the cells are ovoid or spherical, (3.5–5.5 × 1.5–2.5 µm) and occur singly or in pairs; budding is multilateral. A sediment develops after about 10 d; a pellicle or ring is not present. After 1 month at 20 °C, on malt agar the streak is butyrous, smooth, glistening and cream-coloured. In Dalmau plate cultures on cornmeal agar after 10 d, no pseudohyphae or true mycelium is produced. Asci resulting from mother–daughter conjugation containing one, occasionally two, globose ascospores (diameter 3 µm) were observed after 6 d at 20–25 °C, on Gorodkova agar. Fermentation is absent. Assimilates the following carbon compounds: glucose, galactose, L-sorbose, maltose, sucrose, trehalose (weakly), raffinose, melezitose, soluble starch, D-xylose, L-arabinose, D-ribose (weakly), ethanol, glycerol, erythritol, ribitol, D-mannitol, D-glucitol, methyl α -D-glucoside, glucono- δ -lactone, 2-ketogluconate, succinate, citrate, gluconate, N-acetyl-D-glucosamine, ethylacetate (weakly), hexadecane. Does not assimilate cellobiose, lactose, melibiose, inulin, D-arabinose, L-rhamnose, galactitol, salicin, DL-lactate, inositol, methanol, glucosamine, acetone, 2-propanol. KNO₃ and KNO₂ are not utilized as sole sources of nitrogen; ethylamine, cadaverine and lysine are utilized. Does not grow in vitamin-free medium. Grows weakly in presence of 100 p.p.m. cycloheximide. Grows in YM agar containing 12.5% (w/w) NaCl. Grows in the presence of 50% glucose. Does not show casein or gelatin hydrolysis; lipolytic and urease activity are lacking. Growth at 37 °C +; 38 °C –. The habitat is

exudate of mesquite trees (*Prosopis juliflora*). Cultures of the type strain have been deposited in The H. J. Phaff Collection of Yeast and Yeast-like Organisms, Department of Food Science and Technology, University of California, Davis, CA, USA, as UCD 84-100, in the Collezione dei Lieviti Industriali, Dipartimento di Biologia Vegetale, Università di Perugia, Italy (DBVPG 7010^T), in the Centraalbureau voor Schimmelcultures, Delft, The Netherlands, as CBS 8450^T, and the American Type Culture Collection, Rockville, MD, USA, as ATCC 201611^T.

ACKNOWLEDGEMENTS

We are indebted to Stefania Barcaccia for determining the electrophoretic karyotypes and for performing the optical DNA reassociation experiments; to James Haudenschild for his help with the labelled DNA reassociation experiments and to Don Vacek for his assistance in collecting the samples. This work was supported by a grant from the Italian Council for Research: Progetto coordinato CNR: Biodiversità microbica in ambienti agro-industriali. 'GERMIC' Codice 6623 to A. V. M.

REFERENCES

- Bernardi, G., Faures, M., Piperno, G. & Slonimski, P. P. (1970). Mitochondrial DNAs from respiratory-sufficient and cytoplasmic respiratory-deficient mutants of yeasts. *J Mol Biol* **48**, 23–43.
- Ganter, P. F., Starmer, W. T., Lachance, M. A. & Phaff, H. J. (1986). Yeast communities from host plants and associated *Drosophila* in southern Arizona: new isolations and analysis of the relative importance of hosts and vectors on community composition. *Oecologia (Berlin)* **70**, 386–392.
- Holzschu, D. L., Presley, H. L., Miranda, M. & Phaff, H. J. (1979). Identification of *Candida lusitanae* as an opportunistic yeast in humans. *J Clin Microbiol* **10**, 202–205.
- Kreger-van Rij, N. J. W. (1984). Genus *Debaryomyces* Lodder et Kreger-van Rij. In *The Yeasts: a Taxonomic Study*, 3rd edn, pp. 130–145. Edited by N. J. W. Kreger-van Rij. Amsterdam: Elsevier.
- Kurtzman, C. P., Smiley, M. J., Johnson, C. J., Wickerham, L. J. & Fuson, G. B. (1980). Two new and closely related heterothallic species, *Pichia amylophila* and *Pichia mississippiensis*: characterization by hybridization and deoxyribonucleic acid reassociation. *Int J Syst Bacteriol* **30**, 208–216.
- Nakase, T. & Suzuki, M. (1985a). Taxonomic studies on *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and related species. I. Chemotaxonomic investigations. *J Gen Appl Microbiol* **31**, 49–69.
- Nakase, T. & Suzuki, M. (1985b). Taxonomic studies on *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and related species. II. Practical discrimination and nomenclature. *J Gen Appl Microbiol* **31**, 71–86.
- Nakase, T., Suzuki, M. & Phaff, H. J. (1998). Genus *Debaryomyces* Lodder et Kreger-van Rij. In *The Yeasts: a Taxonomic Study*, 4th edn, pp. 157–173. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.
- Phaff, H. J. & Starmer, W. T. (1987). Yeasts associated with plants, insects, and soil. In *The Yeasts*, 2nd edn, vol. 1, pp. 253–296. Edited by A. H. Rose & J. S. Harrison. London: Academic Press.

Phaff, H. J., Blue, J., Hagler, A. N. & Kurtzman, C. P. (1997). *Dipodascus starmeri* sp. nov., a new species of yeast occurring in cactus necroses. *Int J Syst Bacteriol* **47**, 307–312.

Price, C. W., Fuson, G. B. & Phaff, H. J. (1978). Genome comparison in yeast systematics: delimitation of species within the genera *Schwanniomyces*, *Saccharomyces*, *Debaryomyces* and *Pichia*. *Microbiol Rev* **42**, 161–193.

Starmer, W. T., Lachance, M. A., Phaff, H. J. & Heed, W. B. (1990). The biogeography of yeasts associated with decaying cactus

tissue in North America, the Caribbean, and Northern Venezuela. *Evol Biol* **24**, 253–296.

Vaughan-Martini, A., Martini, A. & Cardinali, G. (1993). Electrophoretic karyotyping as a taxonomic tool in the genus *Saccharomyces*. *Antonie Leeuwenhoek* **63**, 145–156.

van der Walt, J. P. & Yarrow, D. (1984). Methods for the isolation, maintenance classification and identification of yeasts. In *The Yeasts: a Taxonomic Study*, 3rd edn, pp. 45–104. Edited by N. J. W. Kreger-van Rij. Amsterdam: Elsevier.