***DEBARYOMYCES POLYMORPHUS* VAR. *AFRICANUS*, A NEW VARIETY OF ASCOSPOROGENOUS YEAST FOUND IN SOUTH AFRICA**

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Two strains of the genus *Debaryomyces*, that are phenotypically and genetically close to *D. polymorphus*, were isolated from South African soils. Nuclear DNA comparison studies established that the two strains reassociate with the type strain of *D. polymorphus* for 49–59%. The two strains have consequently been assigned to the new variety *D. polymorphus* var. *africanus* which is practically distinguished from *D. polymorphus* var. *polymorphus* by the utilization of L-rhamnose.

Two strains of an ascosporeogenous yeast isolated from soils collected in South Africa were found to show characteristics similar to those of *Debaryomyces polymorphus*, but differentiated from this species by the utilization of L-rhamnose. DNA-DNA reassociation determinations revealed that these two isolates represent a new variety of *Debaryomyces polymorphus*. The new taxon is described in the present paper.

MATERIALS AND METHODS

Strains employed. Two strains, JCM 7442 (=CBS 6742) and JCM 7443 (=CBS 6741) were used in the present study. These strains were isolated from South African soil collected near Lydenburg and Graskop, respectively. *Debaryomyces polymorphus* (9) JCM 3647 (=CBS 186, type strain) and *Debaryomyces pseudopolymorphus* (9) JCM 3652 (=CBS 2008, type strain) were used for the

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comparative study. DNAs from *Candida parapsilosis* JCM 1785 (=CBS 604, type strain) and calf thymus were used as the reference in the DNA-DNA reassociation experiments.

Investigation of taxonomic criteria. Most of the methods used for the investigation of morphological, physiological and biochemical characteristics were those described by van der Walt and Yarrow (12). Assimilation of nitrogen compounds was investigated on solid media with starved inoculum. The vitamin requirement was investigated according to Komagata and Nakase (1). The maximum growth temperature was determined in YM broth using metal block baths.

Transmission electron microscopy. Observations by transmission electron microscope (TEM) were based on ascigerous material from a 10-day-old culture of JCM 7443 on YM agar, fixed in aqueous 1.5% potassium permanganate as described by van der Walt and von Arx (11).

Investigation of chemotaxonomic criteria. Extraction and purification of ubiquinones were carried out according to Nakase and Suzuki (8) using cells harvested in the stationary phase. Ubiquinone isoprenologues were identified by high performance liquid chromatography (HPLC).

DNAs were isolated and purified according to Nakase and Suzuki (6). The DNA base composition was analyzed by HPLC after the hydrolysis of DNA according to the procedure reported by Suzuki (10) as described in a previous paper (5). The DNA-DNA reassociation experiments were carried out by the membrane filter technique as previously reported (6).

Determination of the partial sequence of 18S rRNA. The rRNA was isolated and purified according to the procedure reported by Yamada and Kawasaki (13). The partial sequence of 18S rRNA was determined according to the method of Lane et al. (3) using reverse transcriptase. The oligonucleotide DNA primer used in the experiment was 5'-ACGGGCGGTGTGTAC-3' which is complementary to the sequence of the positions (in *Saccharomyces cerevisiae*) 1641 through 1627 of 18S rRNA (4, GenBank J01353D, M27607).

RESULTS AND DISCUSSION

The two yeast isolates obtained from South African soil have Q-9 as the major component of ubiquinone isoprenologues (Table 1), and produce ascospores with warty walls. These properties are diagnostic features of the genus *Debaryomyces*. In the taxonomic criteria commonly employed, these isolates resemble *Debaryomyces polymorphus* and *Debaryomyces pseudopolymorphus*. The G + C content of nuclear DNA of these two isolates showed a value of 32.5–33.0 mol% which was close to those of *D. polymorphus* and *D. pseudopolymorphus*, 31.8 and 31.7 mol%, respectively (Table 2). In the DNA-DNA reassociation experiment, these two isolates showed low relatedness to *D. pseudopolymorphus*, and intermediate relatedness of 50–59% (at 60°C) and 49–56% (at 70°C) to *D. polymorphus*. In comparisons of DNA relatedness and fertility, DNA relatedness of 65–70% or

greater suggests strains to be conspecific in most cases (2). The value of 49–56% or 50–59% indicates a close relationship of strains but is too low to regard them as conspecific. So, we decided to deal with these two isolates as a new variety of *D. polymorphus*, as done by Nakase and Suzuki (6, 7) in the case of *Debaryomyces hansenii*. It consequently proposed to subdivide *D. polymorphus* as follows:

1. *Debaryomyces polymorphus* (Klöcker) Price et Phaff 1979 var. *polymorphus*.
2. *Debaryomyces polymorphus* (Klöcker) Price et Phaff var. *africanus* van der Walt, Nakase et Suzuki, var. nov.

In extracto malti post triduum ad 25°C cellulae vegetativae ellispoideae, ovoideae vel botuliformes, 2.0–9.0 × 2.0–6.5 µm, singulae, binae raro aggregatae. Pellicula hebes repens annulus et sedimentum formantur.

In agaro malti post unum mensem ad 17°C, cultura butyrosa, cremea, patens, glabra, striata transverse tenuiter, margine integro vel undulato aliquando lobato. In agaro farina *Zae maydis* post dies 10 ad 25°C hyphae et pseudophyphae nullae. Asci per automixi, ovoidei, ellipsoidei vel elongati, 3.0–14.0 × 2.0–5.0 µm, sparse per fusionem cellularum duarum discretarum, 1–2 spori, persistentes. Ascosporae

Table 1. Ubiquinone systems of strains of *Debaryomyces polymorphus* var. *africanus* and related yeasts.

Strain		Molar ratio of ubiquinones (%)			
		Q-7	Q-8	Q-9	Q-10
<i>D. polymorphus</i> var. <i>africanus</i>	JCM 7442	0.8	10.4	88.6	0.2
	JCM 7443 ^T	0.8	7.2	91.5	0.5
<i>D. polymorphus</i> var. <i>polymorphus</i>	JCM 3647 ^T	Trace	2.6	96.9	0.5
<i>D. pseudopolymorphus</i>	JCM 3652 ^T	Trace	6.4	93.4	0.2

^T, type strain.

Table 2. DNA relatedness among strains of *Debaryomyces polymorphus* var. *africanus*, *Debaryomyces polymorphus* var. *polymorphus* and *Debaryomyces pseudopolymorphus*.

Source of unlabeled DNA			% Relative binding of DNA				
			Mol% G + C of DNA	<i>D. polymorphus</i> var. <i>africanus</i> JCM 7443 ^T		<i>D. polymorphus</i> var. <i>polymorphus</i> JCM 3647 ^T	
				60°C	70°C	60°C	70°C
<i>D. polymorphus</i> var. <i>africanus</i>	JCM 7442	33.0	100	100	59	56	
	JCM 7443 ^T	32.5	93	92	55	53	
<i>D. polymorphus</i> var. <i>polymorphus</i>	JCM 3647 ^T	31.8	50	49	100	100	
<i>D. pseudopolymorphus</i>	JCM 3652 ^T	31.7	24	23	26	25	
<i>Candida parapsilosis</i>	JCM 1785 ^T	38.0	6	6	9	9	
Calf thymus			1	0	0	1	

^T, type strain.

Fig. 1. *Debaryomyces polymorphus* var. *africanus*.

TEM micrograph showing the electron-dense warty surface structure of the ascospore. The bar represents 0.5 μm .

maturae globosae, diam. 1.5–3.0 μm , verruculosae, brunnea, olei continentes.

Glucosum, galactosum (lente et exiguum), sucrosum, maltosum (lente, vel lente et exiguum) et raffinsum fermentantur at non trehalosum nec lactosum.

D-Glucoso, D-galactoso, L-sorbose, sucroso, maltoso, cellobioso (lente), trehaloso, lactoso (lente vel nullo), melibioso, raffinoso, melezitoso, inulino, amylo solubile, D-xyloso, L-arabinoso (lente), D-arabinoso (lente), D-riboso, L-rhamnoso, ethanololo, ribitololo, galactitololo, D-mannitololo, D-glucitololo, α -methyl-D-glucosido, salicino (fortasse lente), acido 2-ketogluconico, acido 5-ketogluconico, D-glucosamino (lente), N-acetyl-D-glucosamino, hexadecano, acido DL-lactico (lente et exiguo, vel nullo), acido succinico et acido citrico utitur, at non methanololo, acido D-glucuronicolo, acido D-galacturonicolo, acido saccharico nec inositololo.

Maxima temperatura crescentiae: 39–42°C. Ad crescentiam vitaminarum non necessariae sunt. Materia amyloidea iodophila non formatur. Non color ruber Sale Diazonii Cerulei B formans. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 32.5–33.0 (per HPLC). Ubiquinonum majus: Q-9.

Holotypus: Cultura JCM 7443, a J. P. van der Walt e terra prope Graskop (Africa australis) isolata et in collectione culturarum "Japan Collection of Microorganisms (Wako, Saitama, Japonia)" conservatur.

Growth in malt extract: After 3 days at 25°C, cells are ellipsoid, ovoid to botuliform, 2.0–9.0 $\times$ 2.0–6.5 μm , and occur singly, in pairs, rarely in aggregates. A dull, creeping pellicle, ring and sediment are formed.

Growth on malt agar: After 4 weeks at 17°C, the culture is butyrous, cream-colored, spreading, smooth with faint transverse striations, and with an entire, undulating, occasionally lobate margin.

Formation of ascospores: Asci arising from the conjugation of a mother-cell and bud, are ellipsoid, ovoid to elongate, 3.0–14.0 $\times$ 2.0–5.0 μm , one- to two-spored (usually one-spored), and persistent (Fig. 1). Conjugation of independent cells is

less common. Mature ascospores are globose, 1.5–3.0 μm in diameter, verruculose, and contain lipid globules. Actively sporulating cultures are light brown.

Dalmau plate culture on corn meal agar: After 10 days at 25°C, neither hyphae nor pseudohyphae are formed. Chains of cells may, however, be produced under the coverslip.

Fermentation:

Glucose	+	Trehalose	—
Galactose	+ (latent and weak)	Lactose	—
Sucrose	+	Raffinose	+
Maltose	+ (latent, or latent and weak)		

Utilization of carbon compounds:

D-Glucose	+	Ribitol	+
D-Galactose	+	Galactitol	+
L-Sorbose	+	D-Mannitol	+
Sucrose	+	D-Glucitol	+
Maltose	+	α -Methyl-D-glucoside	+
Cellobiose	+ (latent)	Salicin	+ (may be latent)
Trehalose	+		
Lactose	+ (latent) or —	D-Gluconate	+
Melibiose	+	Glucono- δ -lactone	+
Raffinose	+	2-Ketogluconic acid	+
Melezitose	+	5-Ketogluconic acid	+
Inulin	+	D-Glucuronic acid	—
Soluble starch	+	D-Galacturonic acid	—
D-Xylose	+	D-Glucosamine	+ (latent)
L-Arabinose	+ (latent)	N-Acetyl-D-glucosamine	+
D-Arabinose	+ (latent)	Hexadecane	+
D-Ribose	+	DL-Lactic acid	+ (latent and weak) or —
L-Rhamnose	+		
Ethanol	+	Succinic acid	+
Methanol	—	Citric acid	+
Glycerol	+	Saccharic acid	—
Erythritol	+	Inositol	—

Assimilation of nitrogen compounds:

Ammonium sulfate	+	Ethylamine hydrochloride	+
Potassium nitrate	—	L-Lysine hydrochloride	+
Sodium nitrite	—	Cadaverine dihydrochloride	—

Maximum growth temperature: 39–42°C.

Vitamins required: None.

Production of starch-like substances: Absent.

Urease: Absent.

Liquefaction of gelatin: Absent.

Hydrolysis of fat: Absent.

Growth on 50% glucose-yeast extract agar: Weak.

Growth on 60% glucose-yeast extract agar: Absent.

Growth in 10% NaCl + 5% glucose medium: Positive.

Acid production on chalk agar: Absent.

Cycloheximide resistance (1,000 ppm): Positive.

Diazonium Blue B color reaction: Absent.

G + C content of nuclear DNA: 32.5–33.0 mol% (by HPLC).

Major ubiquinone system: Q-9.

Holotype strain: JCM 7443 (=CBS 6741) isolated from soil collected near Graskop (South Africa).

Etymology: *africanus* L. adj. african, which refers to the habitat of the new variety.

Practically, *D. polymorphus* var. *africanus* is distinguished from *D. polymorphus* var. *polymorphus* by the utilization of L-rhamnose, and from *D. pseudopolymorphus* by the utilization of inulin and D-arabinose, and the absence of any vitamin requirement.

In positions 1451 through 1618 (in *Saccharomyces cerevisiae*) of 18S rRNA, *Debaryomyces polymorphus* var. *africanus* has the identical nucleotide sequences to a group of *Debaryomyces* including *D. castellii*, *D. polymorphus* var. *polymorphus*, *D. pseudopolymorphus*, *D. vanrijae* var. *vanrijae*, *D. vanrijae* var. *yarrowii*, *D. yamadae*, and *Yamadaea philogaea* (14). The sequence data of *D. polymorphus* var. *africanus* JCM 7443 and JCM 7442 will appear in the DDBJ, EMBL and GenBank Nucleotide Sequence Database with the numbers D31790 and D31791, respectively.

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