Candida milleri sp. nov.

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Three strains of yeast previously identified as *Torulopsis holmii* were found to have a considerably higher guanine-plus-cytosine content than the type and other strains of this species and are described as a new species, *Candida milleri*.

In the course of a revision of the genus *Saccharomyces*, 20 strains labeled *S. exiguus* Hansen and its presumed imperfect state *Torulopsis holmii* (Jørgensen) Lodder were examined. Three strains were found with a considerably higher percentage of guanine and cytosine in their deoxyribonucleic acid (DNA) than the others. These cannot be retained in *T. holmii* nor identified with any other known species; therefore, a new species will be described to accommodate them and named in honor of M. W. Miller of the University of California, Davis.

MATERIALS AND METHODS

The strains studied are listed in Table 1.

DNA was extracted from the cells by lysis with mercaptoethanol and sodium lauryl sulfate and then purified with a combination of the methods described by Marmur (4) and Kirby et al. (2). The T_m (midpoint of the thermal denaturation transition) was determined in standard saline citrate (0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0 ± 0.2) at 260 nm. DNA of *Candida parapsilosis* CBS 604 ($T_m = 85.9^\circ\text{C}$) was included as standard in all determinations. The moles percent guanine plus cytosine (G+C) was calculated from the formula $\text{mol\%} = (T_m - 69.3)/0.41$ (5).

The other tests were carried out as described by van der Walt in *The Yeasts—A Taxonomic Study* (3). The cultures used for the assimilation tests were incubated on a shaker at 30 cycles/min for 21 days at 25°C , and growth on ethylamine hydrochloride was tested on agar plates for 4 days. Growth on 50% glucose was tested by using the basal medium of Anand and Brown (1): 50 g of peptone, 2.5 g of yeast extract, 1.0 g of $\text{KH}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$, and 0.1 g of CaCl_2 per liter of demineralized water. Glucose was added to 50% (wt/wt), and the medium was solidified with agar. The cultures were sealed in plastic bags and incubated at 25°C for up to 6 weeks.

RESULTS AND DISCUSSION

The results of the T_m determinations and the calculated G+C contents, together with some characteristics, are given in Table 2.

Only one strain of *S. exiguus* and one of *T. holmii* grew well on glycerol and ethylamine hydrochloride and were sensitive to 0.1 g of

cycloheximide per liter, namely CBS 3019 and CBS 5530. Physiologically these two strains cannot be distinguished from an unidentified strain CBS 6750, an industrial strain deposited as *Saccharomyces bailii* Lindner, but which on examination was found to belong to the genus *Kluyveromyces*. The only difference between it and CBS 3019 is the persistent asci of the latter. The relationships between these strains and between them and the other strains of *S. exiguus* need to be clarified by DNA reassociation experiments.

Three strains labeled *T. holmii* had G+C contents ranging from 46.1 to 46.8 mol%, much higher than the range of 32.9 to 35.1 mol% found for the type and other strains. Two of these strains, CBS 6897 (= NRRL Y-7245) and NRRL Y-7244, were isolated from San Francisco sour dough (8). Physiologically these strains differ from *T. holmii* by failing to grow in the absence of niacin and by only weak delayed growth in the absence of calcium pantothenate. The other strain, CBS 2664, was described by Ramirez and Sierra (7) without a Latin description as *Torulopsis holmii* var. *acidi-lactici*. This strain also differs from *T. holmii* by being stimulated by calcium pantothenate, but unlike CBS 6897 and NRRL Y-7244 it does not require thiamin and niacin. Furthermore, it differs from these two strains by being resistant to 0.1 g of cycloheximide per liter. However, because of its general similarity in morphological and physiological properties it is here classified with the other two strains, although further investigation is necessary to confirm this. Nakase et al. (6) reported the isolation of six strains from the extraction process of water-soluble substances of defatted soybeans which are identifiable with CBS 2664. They suggested naming them *Torulopsis acidi-lactici*; however this epithet is not validly published in accordance with Article 36 of the International Code of Botanical Nomenclature.

Candida milleri Yarrow sp. nov. Cellulae globosae vel subovoidiae, $5-6 \times 4-6 \mu\text{m}$, singulae aut binae. Cultura butyrosa, glabra, nitida, eburna. Pseudohyphae nullae. Ascosporae nulla.

Glucosum, galactosum, sucrosum, et raffinose fermentantur. Glucosum, galactosum, sucrosum, trehalosum, raffinose, inulinum, (variabile), glycerolum (variabile), acidum lacticum (variabile), acidum succinicum (variabile), et glucono- δ -lactonum (variabile) assimilantur. Crescere

potest in 37°C. Ad crescentiam vitaminarum necessariae sunt. Proportio molaris guanini + cytosini in acido deoxyribonucleico 46.1–46.8 mol%.

Typus isolatus ex fermento San Franciscoensis, depositus in collectione Centraalbureau voor Schimmelcultures, Delphi (CBS 6897) et North-

TABLE 1. *Strains examined*

Strain no.	Name	Source
CBS ^a 134	<i>Torulopsis holmii</i>	W. B. Bierberg
CBS 135	<i>T. holmii</i>	Buttermilk, A. Oelmeyer; type
CBS 1514	<i>T. holmii</i>	Cucumber brine, J. L. Etchells
CBS 2664	<i>T. holmii</i>	Alpechin, C. Ramirez
CBS 2671	<i>T. holmii</i>	Sputum
CBS 2680	<i>T. holmii</i>	Sputum, E. Dietrichson
CBS 5530	<i>T. holmii</i>	Intestine of <i>Pygidiae</i> larva, J. P. van der Walt
CBS 6897	<i>T. holmii</i>	San Francisco sour dough, NRRL
NRRL ^b Y-7244	<i>T. holmii</i>	San Francisco sour dough, NRRL
CBS 379	<i>Saccharomyces exiguus</i>	CLMR; type
CBS 1515	<i>S. exiguus</i>	Cucumber brine, J. L. Etchells
CBS 2141	<i>S. exiguus</i>	Soil, J. P. van der Walt
CBS 3019	<i>S. exiguus</i>	Soil, J. P. van der Walt
CBS 4660	<i>S. exiguus</i>	Grape must, E. Minárik
CBS 5647	<i>S. exiguus</i>	Sauerkraut, S. Steinbuch
CBS 6388	<i>S. exiguus</i>	Strawberry, T. Nakase
CBS 6440	<i>S. exiguus</i>	Sewage slick, I. Campbell
CBS 6712	<i>S. exiguus</i>	Sauerkraut, C. T. Verrips
CBS 6946	<i>S. exiguus</i>	Soft drink, F. E. M. J. Sand
B-17	<i>S. exiguus</i>	Mayonnaise
CBS 6750	<i>Kluyveromyces species</i>	R. T. S. van Gorp

^a CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands.

^b NRRL, Northern Regional Research Laboratory, Peoria, Ill.

TABLE 2. *DNA base composition and some properties of strains labeled S. exiguus and T. holmii*

Strain no.	$T_m \pm SD^a$ (°C)	G+C (mol%)	Growth on:		Resistance to 0.1 g of cycloheximide per liter
			Glycerol	Ethylamine	
CBS 134	82.8 $\pm$ 0.10	32.9	—	—	+
CBS 135	83.4 $\pm$ 0.18	34.4	—	—	+
CBS 1514	83.6 $\pm$ 0.08	34.9	—	—	+
CBS 2671	83.5 $\pm$ 0.00	34.6	—	—	+
CBS 2680	83.3 $\pm$ 0.05	34.1	—	—	+
CBS 5530	83.7 $\pm$ 0.05	35.1	+	+	—
CBS 2664	88.2 $\pm$ 0.00	46.1	+	—	+
CBS 6897	88.5 $\pm$ 0.06	46.8	—	—	—
NRRL Y-7244	88.3 $\pm$ 0.06	46.3	+ slow	—	—
CBS 379	83.1 $\pm$ 0.09	33.7	—	—	+
CBS 1515	83.2 $\pm$ 0.11	33.9	—	—	+
CBS 2141	83.1 $\pm$ 0.05	33.7	—	—	+
CBS 3019	83.4 $\pm$ 0.08	34.4	+	+	—
CBS 4660	83.4 $\pm$ 0.15	34.4	—	—	+
CBS 5647	82.8 $\pm$ 0.08	32.9	—	—	+
CBS 6388	82.8 $\pm$ 0.15	32.9	—	—	+
CBS 6440	83.2 $\pm$ 0.11	33.9	—	—	+
CBS 6712	83.6 $\pm$ 0.21	34.9	—	—	+
CBS 6946	83.0 $\pm$ 0.05	33.4	—	—	+
B-17	83.6 $\pm$ 0.11	34.9	—	—	+
CBS 6750	83.5 $\pm$ 0.17	34.6	+	+	—

^a SD, Standard deviation.

ern Regional Research Laboratories, Peoria (NRRL Y-7245).

Growth on morphology agar: after 48 h at 25°C the cells are globose or slightly ovoid, 5 to 6 by 4 to 6 μ m, single or in pairs.

After 1 month at 20°C on glucose-peptone-yeast extract agar the streak is butyrous, smooth, glistening, and cream colored.

No pseudohyphae were observed in slide cultures on cornmeal agar, potato agar, and morphology agar.

Ascospores were not observed in the cultures either individually or when mixed.

Fermentation: D-glucose, +; D-galactose, +; sucrose, +; maltose, +; lactose, -; raffinose, + $\frac{1}{2}$.

Assimilation: D-glucose, +; D-galactose, +; L-sorbose, -; sucrose, +; maltose, -; cellobiose, -; trehalose, +; lactose, -; melibiose, -; raffinose, +; melezitose, -; inulin, - or + latent; soluble starch, -; D-xylose, -; L-arabinose, -; D-arabinose, -; D-ribose, -; L-rhamnose, -; methanol, -; glycerol, + or -; *D*-erythritol, -; ribitol, -; galactitol, -; D-mannitol, -; D-glucitol, -; α -methylglucoside, -; salicin, -; arbutin, -; lactic acid, + or -; succinic acid, + weak or -; citric acid, -; *D*-inositol, -; glucono- δ -lactone, + latent or -; keto-2-gluconate, -; keto-5-gluconate, -.

Assimilation of KNO₃: -.

Assimilation of ethylamine hydrochloride: - or +.

Resistance to 0.1 g of cycloheximide per liter: - or +.

Growth on 50% (wt/wt) glucose agar: -.

Growth at 37°C, -; 35°C, + or -; 30°C, +.

Growth in vitamin-free medium: -, two strains require biotin, thiamin, and niacin, the other requires biotin; all three are stimulated by calcium pantothenate.

Production of acid: + weak.

Cultures of the type strain have been deposited in the collections of the Centraalbureau voor Schimmelcultures, Delft (CBS 6897), and the Northern Regional Research Laboratory, Peoria, Ill. (NRRL Y-7245).

Strains of this species can be distinguished from those of *T. holmii* by their requirement for calcium pantothenate.

REPRINT REQUESTS

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LITERATURE CITED

1. Anand, J. C., and A. D. Brown. 1968. Growth rate patterns of the so-called osmophilic and non-osmophilic yeasts in solutions of polyethylene glycol. *J. Gen. Microbiol.* 52:205-212.
2. Kirby, K. S., E. Fox-Carter, and M. Guest. 1967. Isolation of deoxyribonucleic acid and ribosomal nucleic acid from bacteria. *Biochem. J.* 104:258-262.
3. Lodder, J. (ed.). 1970. The yeasts—a taxonomic study. North Holland Publishing Co., Amsterdam.
4. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* 3: 208-218.
5. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* 5:109-118.
6. Nakase, T., K. Komagata, and S. Konishi. 1977. Yeasts found in the extraction process of water-soluble substances of defatted soybean flakes. *Trans. Mycol. Soc. Jpn.* 18:186-196.
7. Ramirez, C., and G. Sierra. 1956. Una nueva variedad de *Torulopsis holmii* (Jørgensen) y estudio de sus productos metabólicos. *Microbiol. Espan.* 9:147-162.
8. Sugihara, T. F., L. Kline, and M. W. Miller. 1971. Microorganisms of the San Francisco sour dough bread process. I. Yeasts responsible for the leavening action. *Appl. Microbiol.* 21:456-458.