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EXPERIMENTAL ARTICLES

A New Yeast Species, *Candida aurita* sp. nov., from Oligotrophic Bogs of Western Siberia

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Abstract—Five anamorphous yeast strains of ascomycetous affinity with a specific mode of budding were isolated from high bog soils of the Bakcharskoe Bog (Tomsk oblast). According to their morphological and physiological properties, these strains belong to the genus *Candida* but differ from all species described previously. The level of DNA–DNA homology with species similar in the assimilation spectrum was as low as 7%. Based on these data, the new species *Candida aurita* sp. nov. is described.

Key words: yeasts, peat, new species.

Yeast fungi are permanent but poorly studied components of the bog soil microbiota [1, 2]. In recent years, we have analyzed the specific taxonomic composition of yeast fungi in the peat of high bogs [3]. It was shown that, along with yeast species of basidiomycetous affinity, which frequently occur in various soils, ascomycetous yeasts, primarily of the genus *Candida*, are widespread in bog soils. Among the latter yeasts, we found five strains of specific morphology that differed in their morphological and physiological features from all hitherto known species of the genus *Candida*.

MATERIALS AND METHODS

Peat samples were taken in August 1998 and July 1999 from Bakcharskoe Bog near the Plotnikovo station (lat. 57° N, long. 82° E, Tomsk oblast, western Siberia). Yeasts were isolated by plating on wort agar acidified with lactic acid at room temperature. The strains isolated were identified from their morphological and physiological traits by conventional methods [4, 5].

Ascospore formation was induced by cultivation on nutrient-free agar, acetate agar, Gorodkova medium, Starky medium, and V8 medium [6]. Ascospores were also detected by microscopic examination of the cultures grown on various media used to study the assimilation spectrum.

DNA was isolated and purified by the Marmur method [7], the G+C content was determined by the method of thermal denaturation [8], and DNA–DNA hybridization was performed by the method of optical reassociation [9].

RESULTS

The five yeast strains of the ascomycetous affinity with a specific bud shape (the buds were significantly longer than the maternal cells) were isolated from peat from a depth of 10 cm under *Carex rostrata* and from leaves of *Menyanthes trifoliata*.

Microscopic examination of various-age cultures revealed no asci with ascospores. Neither were they revealed upon yeast cultivation on special media promoting spore formation or after mixing different strains or using a temperature shock.

The strains were assigned to the genus *Candida* based on the negative urease reaction, low G+C content of DNA (36.6%), genuine budding on a holoblastic-type narrow base, the absence of arthrospores, and sexual reproduction. In its assimilation spectrum, the species described was most related to *C. austromarina* Meyer et Yarrow, the only strain of which was isolated from Antarctic seawater [4]. *C. austromarina* differs from our strains in morphology (its description contains no indications of the formation of elongated buds); it is unable to grow at temperatures higher than 20°C, and the G+C content of its DNA is higher. Sequence analysis of the D1/D2 region (5'-end of 26S rRNA) of *C. austromarina* showed a less than 1% similarity with the conspecific *Candida sake* van Uden & Buckley ex Meyer & Ahearn 1983 [10]. Comparison of our strains with the type strain of *C. sake* revealed significant morphological and physiological distinctions (table). The DNA–DNA homology among the isolates there was at least 98%, whereas there was only a 7% homology between these strains and the type strain of *C. sake.*

Thus, our isolates are conspecific and have a low level of DNA–DNA homology with phenotypically similar species; this allows us to describe them as a new

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Distinctive traits of *C. aurita* sp. nov.

Trait	*Candida aurita* sp. nov.			*C. austromarina*	*C. sake*
	VKM 2910 (type strain)	C14-4	B9		
Glucose fermentation	+	+	s	–/s	+
Assimilation of					
Glucose	+	+	+	+	+
Galactose	+	+	+	+	+
L-Sorbose	–	–	–	–	+
Sucrose	–	–	–	–	+
Maltose	–	–	–	–	+
Trehalose	+	+/s	+	+	+
Lactose	–	–	–	–	–
Melizitose	–	–	–	–	+
D-Xylose	–	–	–	–	+
Ethanol	–	–	–	V	+
Glycerol	–	–	–	–	+
Mannitol	–	–	–	–	+
Dulcitol	–	–	–	–	+
Succinate	+	+	+	+	+
Gluconate	–	–	–	–	V
2-Ketogluconate	–	–	–	–	+
Maximum growth temperature, °C	30	30	30	<25	30
Pseudomycelium	–	–	–	–	V
G+C Content, mol %	36.1	?	36.7	39.0	37.8–41.0

Note: "+" denotes assimilation; "–," lack of assimilation; "s," poor assimilation; "V" means that the character varies.

species of the genus *Candida*. The species epithet *aurita* (literally, big-eared) is based on the most pronounced morphological trait of the species—the capacity to form on a maternal cell two elongated buds, resembling long hare ears.

Description of *Candida aurita* sp. nov. Growth at 20°C in liquid wort and glucose–peptone medium containing yeast extract exhibits the following features: on the third day, cells are oval, elongated, (1–2) × (4–6) μm, and occur singly (Fig. 1); over a week, they measure (1.5–2) × (6–7) μm and sometimes form chains (Fig. 2). Precipitate and ring are formed in liquid wort.

On wort agar, white, dense colonies are formed, which have a smooth surface, even edges, and a slight luster. After a week of growth at 20°C, the colony diameter reaches 15 mm.

Pseudomycelium is not formed.

The capacity for glucose fermentation varies among strains. Other sugars are not fermented.

Glucose, galactose, trehalose, and succinic acid are assimilated.

The following substrates are not assimilated: glycerol, mannitol, sorbose, glucosamine, ribose, xylose, L-arabinose, D-arabinose, rhamnose, sucrose, maltose, α-methyl-D-glucoside, cellobiose, salicin, arbutin, melibiose, lactose, raffinose, melezitose, inulin, starch, erythritol, dulcitol, inositol, 2- and 5-ketogluconate, glucuronic acid, gluconic, citric, and lactic acids, methanol, ethanol, ribitol, and sorbitol.

Nitrate and nitrite are not used as the sources of nitrogen.

Starch is not formed.

Urease reaction is negative.

Thiamine–HCl is required for growth.

Cycloheximide at a concentration of 0.01% inhibits the strain growth.

The maximum growth temperature is 30°C.

Gelatin is not liquified.

Growth on medium containing 50% glucose is poor.

Growth is possible on media containing up to 7% NaCl.

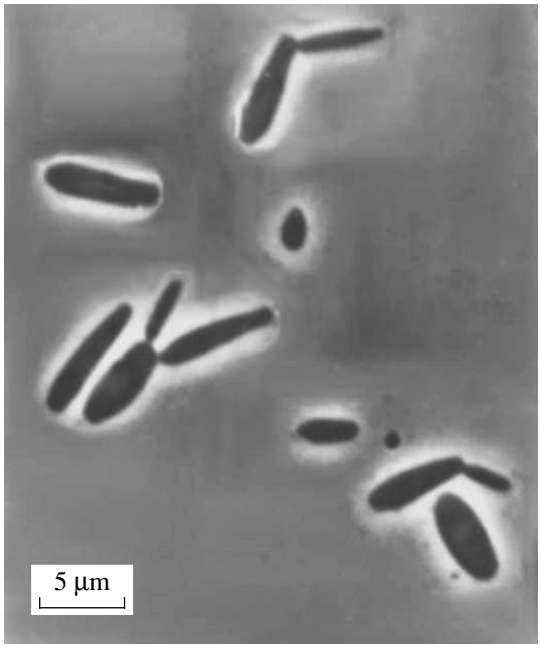

Fig. 1. Cells of *Candida aurita* in a 3-day culture grown on glucose–peptone medium containing yeast extract (phase-contrast micrograph).

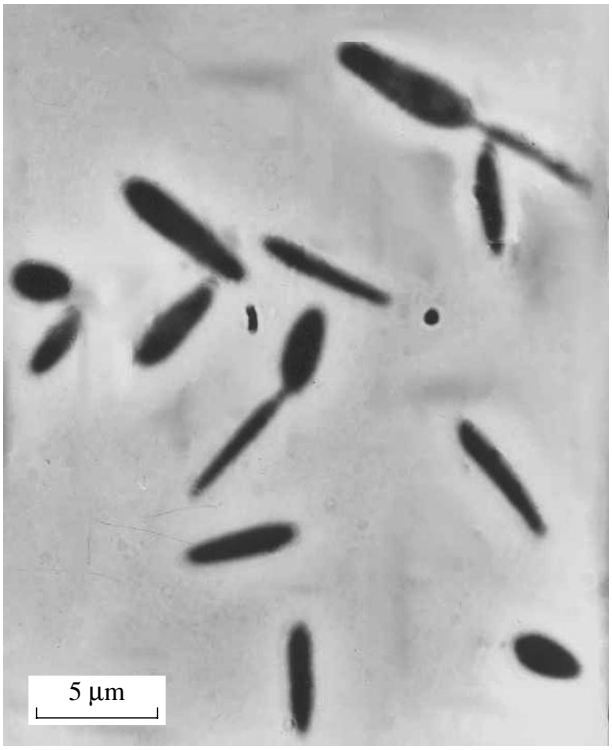

Fig. 2. Cells of *Candida aurita* in a 7-day culture grown on wort-agar (phase contrast).

In the type strain, the G+C content of DNA is 36.6 mol %.

The type strain KBP3738, VKM 2910 is stored in the collection of the Department of Soil Biology (Soil Science Faculty, Moscow State University) and in VKM at the Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences. It was isolated from high bog soil from a depth of 10 cm (Bakcharskii raion, Tomsk oblast).

Latin diagnosis. In medio liquido post dies 3 ad 20°C cellulae ovoidal vel elongatae(1–2) × (4–6) μm, singulae. Post dies 7 ad 20°C – singulae aut in catenis brevis (1.5–2) × (6–7) μm.

Cultura in agaro malti post unum mensem ad 20°C 15 mm in diametro,alba, glabra, semi-nitida; margine glabra.

Fermentatio glucosi variabilis.

Assimilatio carbo-compositorum: glucosum, trehalosum, galactosum, succinatum et non: glicerolum, D-mannitolum, L-sorbosum, glucosaminum, ribosum, xylosum, L- et D-arabinosum, rhamnosum, α-methylglucosidum, cellobiosum, salicinim, arbutinum, melibiosum, lactosum, raffinosum, melezitosum, inulinum, amylum, erythritolum, galactitolum, inositolum, ethanolum, methanolum, 2- et 5-ketogluconatum, glucuronatum, lactatum, citratum, ribitolum et glucitolum.

Non assimilantur kalium nitricum, kalium nitrosum.

Urea non finditur.

Ad crescentiam thiaminum necessariaea sunt.

Maxima temperatura crescentiae: 30°C.

Gelatinum non liquescit.

Proportio molaris guanini + cytosini in acido deoxyribonucleico: 36.6 mol % (typus).

Nominacio "aurita" – proponitur formae et posisionis causa oculeurum quae longis leporum auribus sunt similia.

Typus: Stirps in collectione zymotica, Moskva, Rossia; ex turfa isolata est.

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