Microbiology, Vol. 69, No. 2, 2000, pp. 225–228. Translated from Mikrobiologiya, Vol. 69, No. 2, 2000, pp. 276–279.

EXPERIMENTAL ARTICLES

A New Yeast Species, *Candida anutae* sp. nov., from the Fruiting Bodies of Agarics

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Received June 29, 1999; in final form, September 8, 1999

Abstract—Among the yeasts isolated from the fruiting bodies of different species of agarics picked in forests near Moscow and Turku (Finland) in 1995–1998, populations of an earlier unknown species, morphologically similar to *Metschnikowia lunata* but differing from it by physiological characteristics and the absence of asci with spores, were constantly found. Description of the new species is given within the genus *Candida* Berkhout.

Key words: yeast, agarics, taxonomy, mycetophilides

Strains whose morphology was similar to *Metschnikowia lunata* Golubev and *Candida peltata* Yarrow were found among yeasts isolated from the fruiting bodies of various species of agarics picked from May to October in forests near Moscow and Turku (Finland). However, the isolates differed from the above-mentioned species in physiological characteristics and DNA base composition. The attempts to obtain their asci with spores were not successful; therefore, we describe these isolates as members of the anamorphic genus *Candida* Berkhout.

MATERIALS AND METHODS

The regions and materials of the investigations were partially described earlier [1, 2]. On the whole, more than a hundred samples of fungi, both fresh and infected with the mycetophilide larvae, were picked

Table 1. List of the strains isolated

no.	Initial designation	no. in the KBP collection	Substrate, region	Isolation date
1	T-129-4	3476	*Russula* sp. fruiting body, Tver oblast, Kashinskii raion, spruce forest	May 1995
2	Sgr 1-2	3575 type	*Russula cyanoxanta* fruiting body infected by insect larvae, Moscow oblast, Istrinskii raion, mixed forest	September 1996
3	Sgr 1-9	3680	*Russula* sp. fruiting bodies at different stages of decomposition, mixed forest near Moscow	May–July 1996
4	Sgr 1-10	3681	"	"
5	Sgr 1-20	3682	"	"
6	Sgr 1-21	3683	"	"
7	Sgr 1-22	3884	"	"
8	Sgr 1-23	3685	"	"
9	Sgr 1-24	3686	"	"
10	Sgr 1-25	3687	"	"
11	21a-1	3688	*Amanita muscarina* fruiting body, Moscow oblast, Shakhovskoi raion, mixed forest	June 1997
12	Gf-2(c)-1	3671	Mycetophilide larvae from a *Pholiota flammans* fruiting body, Turku, Finland, forest	
13	Gf-2(b)-3	3670	"	"
14	Gf-24-12	3689	Mycetophilide larvae from a *Pholiota alnicola* fruiting body, Turku, Finland, forest	September 1998
15	Gf-24-13	3690	"	"
16	Gf-24-15	3691	"	"

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Table 2. Characteristics distinguishing the new species *C. anutae* from morphologically similar yeast species

Characteristic	*C. anutae*	*C. peltata*	*M. lunata*
Ascospore formation	–	–	+
Fermentation	–	+	+
Galactose	–	+	+
Sorbose	–	+	–
Sucrose	–	+	+
Maltose	–	+	+
Cellobiose	–	+	+
Melezitose	–	+	+
Starch	–	+/s	–
Xylose	–	+	+
L-Arabinose	–	+	–
D-Arabinose	–	+	–
Ribose	–	+	–
Rhamnose	–	+	–
Glucosamine	–	+/s	w
Ethanol	–	–	w
Erythritol	–	+/s	–
Ribitol	+/–	+	w
Dulcitol	–	+/s	–
Sorbitol	+/–	+	+
α-Methyl-D-glucoside	–	+	w/–
Salicin	–	+	+
Citrate	+	+	–
Growth at 30°C	–	+	+
Growth at 37°C	–	+	–
Growth on medium with 0.01% of cycloheximide	–	+	–
Formation of pseudomycelium	+	+	+

Note: The sign "+" denotes assimilation, "–" denotes lack of assimilation, "w" denotes weak assimilation, and "s" denotes slow assimilation.

and analyzed over four years. The pulp of the fruiting bodies and, separately, the larvae, which were ground with a soft pestle in a small volume of sterile water, were analyzed. The fruiting bodies of the fungi were preliminarily comminuted, and a weighted portion was used to prepare a 1 : 50 suspension in sterile water, which was treated in a tissue micropulverizer. Yeasts were isolated on wort agar (pH 3.5) at room temperature.

The DNA base composition was determined by the thermal denaturation method [3] with slight modifications. Standard methods were used to determine the physiological characteristics [4]. Identification was performed using the determination manual [5].

RESULTS AND DISCUSSION

The collection consisting of 16 yeast strains with the characteristic crescent or horseshoe shape of cells was composed of isolates from the fruiting bodies of various species of agarics (Table 1). Such strains accounted for a very small share of the yeast species and in no case were the dominants. They were isolated from both fresh and decomposing fruiting bodies, as well as from the mycetophilide larvae infecting agarics. But never were they found in the surrounding substrates—in the soil and litter or on plants.

During the primary microscopic examination of these strains (immediately after their isolation), we observed, along with crescent, round, and oval budding cells, larger-sized round cells of the chlamydospore type. Some of them had appendages and resembled the "rackets" characteristic of *Metschnikowia pulcherrima* and *M. lunata* asci. However, no spores were observed when cultivating the isolates on any of the various media used: depleted agar, honey agar, Gorodkova's medium, media with high NaCl concentrations, glycerin agar, V8 medium, media containing the fruiting body extract of various agaric species, and all media of the assimilation spectrum.

Based on such traits as negative urease and DBB reactions, the DNA G+C content under 50 mol %, the holoblastic type of budding, and the absence of the sex stage, the isolates were assigned to the genus *Candida* Berkhout [5]. However, they did not correspond to any of the known species of this genus in the assimilation spectrum and exhibited a unique morphology. The species epithet *anutae* was chosen as a diminutive form of the name *Anna* in memory of Anna Kartintseva, an untimely deceased postgraduate student of the Department of Soil Biology, Moscow State University, who participated in the initial stages of this research [1].

DESCRIPTION OF *Candida anutae* sp. nov.

After 3 days of growth at 20°C in liquid wort or glucose–peptone medium with yeast extract, cells are predominantly round or oval, 1.6–2.4 × 3.2–5.6 μm, single or arranged in short chains; less frequently, the cells are curved (Fig. 1). A month later, a friable film is formed, settling down to the bottom when shaken gently.

After 7 days of growth at room temperature on wort agar, the streak is white, dull (shiny in some of the strains), smooth, or slightly rugose; the margin is even. The cells significantly vary in size and shape from short, 1.5–2.0 × 2.0–5.0 μm ovals, to elongated (7–9 μm) crescent cells. In some strains, the cells are horseshoe- or yoke-shaped. Curved cells usually form single buds on the convex surface or at the ends; however, sometimes up to three rod-shaped buds are formed at one of the ends (Fig. 2).

Some strains form highly branched pseudomycelium on plates with corn agar.

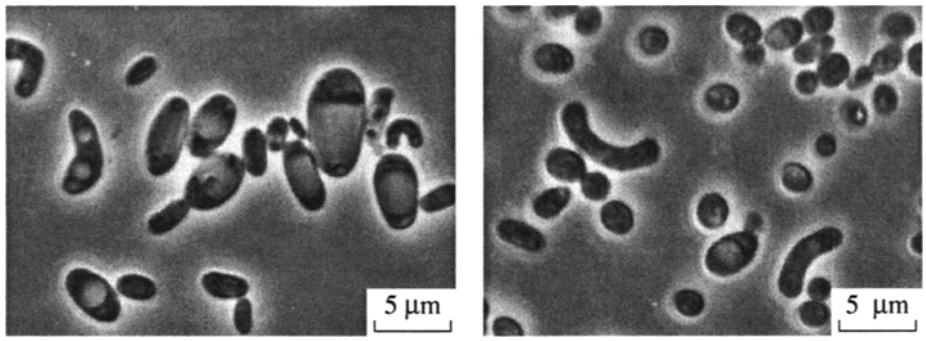

Fig. 1. *Candida anutae* cells grown on the glucose–peptone medium with yeast extract.

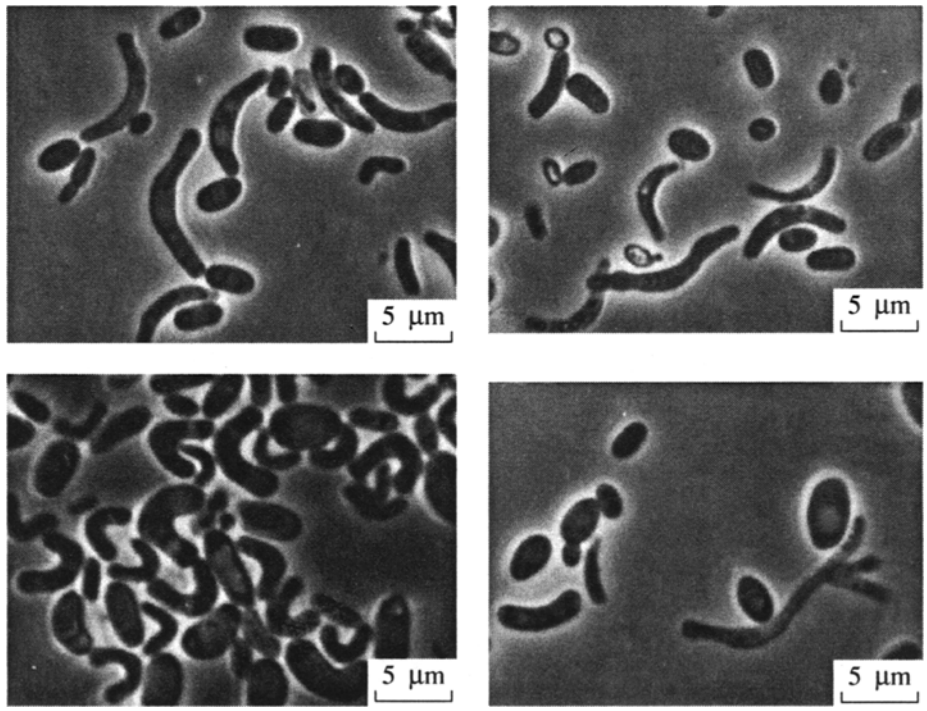

Fig. 2. *Candida anutae* cells of different shape in 7-day cultures on wort agar.

Fermentation does not occur.

Glucose, trehalose, glycerin, mannitol, succinic and citric acids are utilized; growth on ribitol and sorbitol varies in different strains.

Galactose, 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-ketogluconates, glucuronic and lactic acids, methanol, and ethanol are not utilized.

No growth occurs with nitrate, nitrite, creatine, or creatinine as the sources of nitrogen.

Starch is not formed.

Urease reaction is negative.

No growth occurs on vitamin-free medium.

Cycloheximide at a concentration of 0.01% inhibits growth.

The maximum growth temperature is 27°C.

Gelatin is weakly liquefied.

Abundant growth occurs on medium with 50% glucose.

Growth is possible on medium with NaCl at a concentration of up to 7%.

The DNA G+C content is 47.5 mol % for the type strain.

The type strain KBP-3575 (=VKM Y-2868) is kept in the collection of yeast cultures of the Department of Soil Biology, Faculty of Soil Science, Moscow State University, and in the All-Russian Collection of Micro-

organisms (Pushchino, Moscow oblast). It was isolated from a decomposing fruiting body of *Russula cyanoxanta* Schaeff. (mixed forest, Istra raion, Moscow oblast).

Latin diagnosis

Cultura in agaro malti post unum mensem ad 20°C 15–20 mm in diametro, cremeum.

In extracto malti cellulae lunatae curvatae aut ellipsoidales 1.6–2.4 × 3.2–5.6 mkm. Post unum mensem sedimentum et pellicula (exique) formantur.

Fermentatio nulla.

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

Non assimilantur kalium nitricum, kalium nitrosum, creatinum, creatininum.

Materia amyloidea iodophila non formantur.

Maxima temperatura crescentiae: 27°C.

Proportio molaris guanini + cytosini in acido deoxyribonucleico: 47.5 mol % (typus).

Etymology "anutae"—in memoriam A.A. Kartintseva denominata est.

Typus: KBP-3575 in collectione zymotica, Moskva, Rossia.

The species described is, above all, characterized by the peculiar cellular morphology, known for two yeast species, namely, *Candida peltata* and *Metschnikowia lunata*. However, it differs greatly from these species in its physiological characteristics; in addition, *M. lunata* forms ascospores, and *C. peltata* does not form pseudomycelium. The fact that all strains of the new species were isolated from one type of habitats—fungal fruiting bodies or insect larvae infecting them—allows us to purposefully search for new isolates to reveal the teleomorphic stage of this yeast.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research (grant no. 98-04-48965).

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