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EXPERIMENTAL ARTICLES

A New Species of Psychrophilic Basidiomycetous Yeasts *Leucosporidium fasciculatum* sp. nov.

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Abstract—A psychrophilic yeast with a basidiomycetous developmental cycle and properties corresponding to the genus *Leucosporidium* Fell et al. was isolated from the fruiting body of the edible spring mushroom *Gyromitra esculenta* Pers. picked near Moscow. However, the isolate differed from all *Leucosporidium* species described to date in a number of characteristics. The results of the study of the developmental cycle and of the cultural, morphological, physiological, and biochemical properties of the new isolate, strain KBP Y-3696, allow it to be assigned to a new species of the genus *Leucosporidium*.

MATERIALS AND METHODS

The yeast from the fruiting body of the edible spring mushroom *Gyromitra esculenta* Pers. picked in spring 1992 in a mixed forest near Moscow (Istra region) was isolated by shaking the disintegrated fruiting body in sterile water for 10 min with subsequent inoculation with a drop of wort agar acidified to pH 4.5.

The inoculated dishes were incubated at 5 and 20°C.

Along with other yeast isolates, the strain with the initial index Hb-106 (=KBP Y-3696) isolated into a pure culture was investigated according to the standard scheme using conventional methods for the identification of yeast cultures [1, 2]. All tests were carried out at temperatures below 21°C.

Isolation of DNA and determination of its G+C content were carried out using the thermal denaturation procedure [3] with slight modifications [4].

To observe the germination of teliospores, a cornmeal agar culture was incubated for 2 weeks at 19°C. Agar blocks with mycelium and teliospores were then soaked in distilled water at 7°C for 6 weeks. Germination of the teliospores was induced by alternating freezing and thawing of the agar blocks.

DESCRIPTION OF *LEUCOSPORIDIUM FASCICULATUM* SP. NOV.

Latin diagnosis

In extracto malti post 3 dies cellulae sunt ovoideae ad cylindratae, (1.2–3.2) × (4.8–16.0) μm, singulare vel binae. Post unum mensem sedimentum et pellicula formantur.

Coloniae in agaro malti post 1 mensem glabra albidum, 10 mm in diametro.

Pseudomycelium et mycelium formantur.

Teliosporae intercalares vel terminales, spheroidales sunt. Metabasidia ex germinatione teliosporarum oriontur et quattuor cellulis habent, numerosi basidiosporae producuntur.

Species homothallica.

Fermentatio nulla. Assimilatio carbo-compositorum: D-glucosum, L-sorbosum, D-xylosum, L-arabinosum, cellobiosum, sucrosum (lente), maltosum (lente), α-methyl-D-glucosidum (lente); melezitosum (exiguum), acidum lacticum et citricum (exiguum) assimilantur at non galactosum, D-glucosaminum, D-ribosum, L-rhamnosum, trehalosum, arbutinum, melibiosum, lactosum, raffinosum, inulinum, amylum, glycerolum, erythritolum, D-glucitolum, D-sorbitolum, dulcitolum, inositolum, 2-keto-gluconatum, 5-keto-gluconatum, acidum D-glucuronatum, acidum succinicum, methanolum et ethanolum.

Creatinum assimilat.

Kalii nitratum non assimilat.

Vitamina externa non necessaria sunt.

Maxima temperatura crescentiae 21°C.

Fig. 1. *Leucosporidium fasciculatum* sp. nov. Vegetative cells grown on wort for three days at 13°C. The bar represents 10 μm.

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Materiae amyloideae non formatur.

Ureum hydrolysatur.

Diazonium caeruleum B: positivum.

Proportio molaris guanini + cytosini in acido deoxyribonucleico: 54.2%.

Holotypus: cultura KBP Y-3696 (= isolatus Hb-106 ex Gyromitra esculenta Pers.) in collectione zymotica Moskva, Rossia; VKM Y-2869.

After three days of growth in liquid wort (13°C), single cells or cells with oval or cylindrical polar buds (1.2–3.2) × (4.8–16) μm in size are observed; the average size is 1.8 × 8 μm (Fig. 1). Two weeks later, a film and a sediment are formed. After one month of growth in glucose–peptone medium with yeast extract, the culture forms a ring and a sediment, but not a film.

The colonies on wort agar after one month of growth at 7°C are 10 mm in diameter, light cream in color, with a dull velvety surface. The margin is entire, but it is not sharply outlined due to the formation of a zone of substrate mycelium. The center is elevated over the peripheral part and has a crater-like depression. The colony is clearly differentiated into a central convex and peripheral flat parts.

Rudimentary pseudomycelium and true mycelium without buckles but with terminal and intercalary spherical or pear-shaped teliospores 4.8–11.2 μm in diameter (8 μm on average) are formed within a week (15°C) on cornmeal agar plates.

The life cycle is of the homothallic type. Teliospores germinate in the form of two- to four-celled club-shaped metabasidia on which numerous sporidia are formed terminally and on the lateral parts in the septal regions. Each intercalary metabasidial cell is surrounded by a halo of sporidia; the apical cell carries a bundle of six basidiospores (Figs. 2a, 2b).

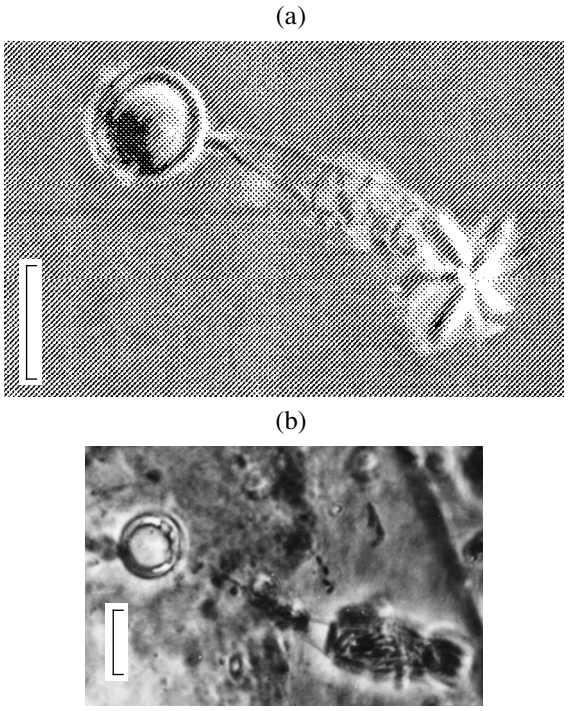

Fig. 2. Germinated teliospores: (a) a 4-celled basidium with a bundle of sporidia on the apical cell and (b) massive formation of sporidia. The bar represents 10 μm.

Obligate psychrophile does not grow above 21°C.

Does not ferment sugars.

Table 1. Differentiating characteristics of close taxa of teliosporous yeasts

Taxa	Type of basidial structures	Physiological and biochemical characteristics					
		pigmentation	fermentation	nitrate assimilation;	starch formation	inositol/glucuronate assimilation	G+C, mol %
Rhodosporidium	Transversely septate basidium	+	–	v	–	–/v	57.8–67.3
Cystofilobasidium	Tubular holometabasidium	+	–	+	+	+/+	56.6–66.3
C. lari-marini		–	+	+	+	+	n
Mrakia	Holometabasidium	–	+	+	+	v/s	52.9–56.1
Leucosporidium (3 species)	Phragmometabasidium with bundles of terminal and lateral basidiospores	–	–	+	–	–/v	50.5–61.1
L. fasciculatum sp. nov.		–	–	–	–	–/–	54.2

Note: The table does not include the teliosporous yeast genera *Sporidiobolus* and *Sterigmatosporidium* because they are characterized by a peculiar mode of asexual reproduction involving the formation of ballistospores or buds on sterigmas. Neither does the table include the pigmented yeast of the genera *Kondoa* and *Sakaguchia*, whose taxonomic position is still unclear. Designations here and in Table 2 are as follows: "+" means that the character is positive; "–" means that the character is negative; "w" means that the character is weakly pronounced; "s" means that the mainfestation of the character occurs slowly; "v" means that the character varies; "n" signifies that no information is available.

Table 2. Differences between the species *L. fasciculatum*, *L. fellii*, *L. scottii*, and *L. antarcticum*

Compounds	*L. fasciculatum* KBP Y-3696	*L. fellii**	*L. scottii**	*L. antarcticum**
Galactose	–	–	v	v
Sorbose	+	s	+	–
Sucrose	s	w/s	+	v
Maltose	s	–	+	v
Cellobiose	+	+	w	–
Trehalose	–	+	+	v
Raffinose	–	–	+	v
Melezitose	w	–	+	–
Xylose	+	s	+	v
L-Arabinose	+	–	v	–
Rhamnose	–	+	+	–
Glucosamine	–	+	+	–
Ethanol	–	+	+	v
Glycerol	–	+	+	+/w
Ribitol	–	+	v	–
Sorbitol;	–	+	+	–
α-Methyl-D-glucoside	w/s	–	+	–
Salicin	w	+	+	–
Nitrates	–	+	+	+
2-Ketogluconate	–	n	+	+
5-Ketogluconate;	–	n	+	–
Glucuronic acid	–	+	+	–
Arbutin	–	+	n	n
Creatine	+	–	n	n
50% glucose	+	–	–	–
Gelatin liquefaction	+	–	+	+
0.01% cycloheximide	–	n	n	n
Maximal growth temperature, °C	20	28	30	17
DNA G+C, mol %	54.2	57.7	61.1	50.5

* The data for these species are given according to [5]. The traits by which *Leucosporidium* species do not differ from each other are not tabulated.

Assimilates glucose, sorbose, xylose, L-arabinose, cellobiose, and mannitol as the only sources of carbon. Slowly assimilates sucrose and maltose. The assimilation of α-methyl-D-glucoside salicin, melezitose, lactic acid, and citric acids is weak.

Does not assimilate galactose, glucosamine, ribose, D-arabinose, rhamnose, trehalose, arbutin, melibiose, lactose, raffinose, inulin, starch, glycerol, erythritol, ribitol, sorbitol, dulcitol, inositol, 2-ketogluconate, 5-ketogluconate, glucuronic and succinic acids, methanol, or ethanol.

Does not assimilate nitrates; able to utilize creatine as the only source of nitrogen.

Grows in vitamin-free medium.

Maximal concentration of NaCl is 3%.

Does not grow in medium with 0.01% of cycloheximide.

Does not form starchy substances.

Good growth occurs on a medium with 50% glucose.

Liquefies gelatin and hydrolyzes urea.

The DNA G+C content is 54.2 mol %.

The type strain KBP Y-3696 is stored in the collection of yeast cultures at the Department of Soil Biology, Faculty of Soil Science, Moscow State University, and in the All-Russian Collection of Microorganisms (VKM Y-2869). It was isolated from the fruiting body of *Gyromitra esculenta*, Moscow region, Russia.

DISCUSSION

The new species described in this paper belongs to the group of teliosporous yeasts, among which there are colorless and pigmented taxa. The latter are included in the genera *Rhodosporidium*, *Sporidiobolus*, and *Cystofilobasidium*. The genera *Leucosporidium*, *Sterigmatosporidium*, *Mrakia*, as well as one species of the genus *Cystofilobasidium*, *C. lari-marini*, are colorless taxa. In addition to pigmentation, the teliosporous genera differ in the morphology of basidia and in the type of asexual structures (buds, ballistospores, buds on sterigmas) (Table 1).

By its psychrophilic properties and DNA G+C content, the new yeast species is similar to the members of the genus *Mrakia*. However, it substantially differs from them in in the type of sexual structures, represented by holometabasidia in the genus *Mrakia* and by transversely septate phragmometabasidia in *L. fasciculatum*. The latter characteristic permitted the new isolate to be assigned to the genus *Leucosporidium*.

As shown in Table 1, the new species shares all of the properties of the *Leucosporidium* members, except for their capacity for nitrate assimilation, a characteristic that is not currently considered to be taxonomically important at the generic level.

At present [2], the genus *Leucosporidium* Fell *et al.* includes three species which differ in their type of life cycle, mode of formation of basidiosphores (sporidium), and in the spectra of the carbon sources utilized (Table 2). Only in one species, *L. scottii* Fell, Statzell, Hunter, and Phaff, was some of the strain able to grow at 30°C; other species are psychrophilic, and one of them, *L. antarcticum* Fell, Statzell, Hunter, and Phaff, is a an obligate psychrophile incapable of growth at temperatures above 17°C. The species of this genus can

be isolated from polar regions and other low-temperature habitats.

The new species *L. fasciculatum* shares psychrophilic properties common to the *Leucosporidium* species, in addition to features such as the inability to hydrolyze polysaccharides (starch, inulin), the inability to synthesize starchy compounds, or to utilize inositol as a carbon source.

At the same time, the new species differs from other species of the genus *Leucosporidium* in the morphology of sexual structures, the spectrum of utilized substrates, and the DNA base composition.

This provides the basis for describing the new species *L. fasciculatum* as a constituent of the genus *Leucosporidium*, family *Sporidiobolaceae*, order *Sporidiales*.

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