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EXPERIMENTAL ARTICLES

A New Basidiomycetous Yeast Species, *Cryptococcus mycelialis*, Related to *Holtermannia* Saccardo et Traverso

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Abstract—A new species of the genus *Cryptococcus*, *Cr. mycelialis* (the type strain VKM Y-2863), is described based on the taxonomic study of four strains isolated from soil and plant samples collected on the South Georgia and East Falkland islands. This species differs from the known *Cryptococcus* species in its ability to form a true monokaryotic mycelium with pseudoclamp connections and haustoria. The species can be distinguished from the phylogenetically related and phenotypically similar species *Holtermannia corniformis* and *Cr. nyarrowii* by some assimilatory reactions, maximum growth temperature, and sensitivity to mycocins.

Key words: dimorphic heterobasidiomycetes, taxonomy, *Holtermannia*, *Cryptococcus.*

The investigations of yeast mycobiota in the subpolar regions of the Northern and Southern Hemispheres, carried out in the early 1970s, allowed us to isolate several strains of anamorphic basidiomycetes from the soil and plant samples collected on the South Georgia and East Falkland islands located in the southern part of the Atlantic Ocean [1]. In their physiological and biochemical properties, the isolated strains corresponded to the genus *Cryptococcus* Kützing emend. Phaff et Spencer [2, 3], but differed from the known representatives of this genus in its ability to form an abundant true mycelia with clamp connections and haustoria in old cultures [4]. According to the modified description of the genus *Cryptococcus* [5], this genus may include mycelial organisms. However, the strains that we isolated differ from the known representatives of the genus *Cryptococcus*, including the closest species *Cr. laurentii* (Kufferath) Skinner, in a number of phenotypic properties. Moreover, the partial sequencing of the rDNA genes of the isolates showed that they are phylogenetically distant from the type strain of *Cr. laurentii* but are close to the tremelloid fungus *Holtermannia corniformis* Kobayasi [6] and to the yeast *Cr. nyarrowii* Thomas-Hall et Watson from Antarctica [7]. Our isolates differ from the latter organism in a number of morphological and physiological traits, the nucleotide sequence of rDNA, the composition of cellular proteins and fatty acids, and sensitivity to mycocins. All this allows the isolates to be considered as representatives of a new species.

This paper deals with the description of this species.

MATERIALS AND METHODS

The strains under study were isolated by the method of serial dilutions from the soil and plant samples collected in 1971 (Table 1). The inoculated glucose–peptone agar plates containing 80 mg/l streptomycin were incubated at 5°C. The isolates were studied by conventional methods [8] using *Cr. laurentii* VKM Y-1595, *Cr. laurentii* VKM Y-1665, *Cr. nyarrowii* VKM Y-2901, *H. corniformis* VKM Y-2803, and *H. corniformis* VKM

Table 1. The origin of the isolates under study

Isolates	Strain designations in different collections	Years, locations, and substrates from which the strains were isolated
C-66-II	CBS 7743, NRRL Y-11957	South Georgia, herbaceous plants, 1972
C-89-II	CBS 7713, NRRL Y-11958	East Falkland, soil, 1972
C-104-II	VKM Y-2863, CBS 7712, NRRL Y-11959	South Georgia, soil, 1972
C-138-II	CBS 7714, NRRL Y-11960	South Georgia, soil, 1975

Note: VKM, All-Russia Collection of Microorganisms, Pushchino, Russia; CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; NRRL, ARS Culture Collection, Northern Regional Research Center, Peoria, Illinois, USA.

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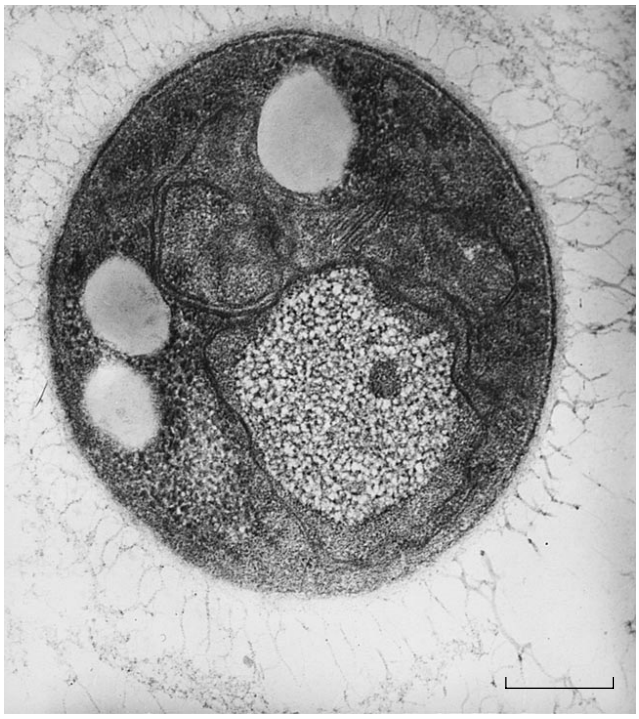

Fig. 1. Electron microscopy of a 3-day-old yeast cell of *Cr. mycelialis* VKM Y-2863 cultivated on malt extract agar at 20°C. The cell was fixed with glutaraldehyde and permanganate. The bar represents 1 μm.

Y-2804 (http://www.vkm.ru) as the reference strains. The cell nuclei were stained by the Giemsa method. Mycocinotyping, the electron microscopy of ultrathin sections, the isolation and analysis of nuclear DNA and extracellular polysaccharides were performed as described elsewhere [9–12].

RESULTS

Description of Cryptococcus mycelialis W. et N. Golubev nov. sp.

Growth in glucose–peptone–yeast extract medium. Three-day-old cultures are composed of budding, encapsulated, monokaryotic, oval cells, from 3.4–6.0 × 5.9–11.1 μm (on the average, 4.7 × 7.8 μm) in size (Fig. 1). The length-to-width ratio varies from 1.2 to 2.5, averaging 1.7. The lamellar cell wall is of basidiomycetous type. Growth over a period of one month is accompanied by the formation of a precipitate, ring, and film.

Growth on malt extract agar. After incubation on malt extract agar for one month, the species produces smooth, dull, pasty, cream-colored colonies, which are rhizoid due to the development of a substrate mycelium with clamp connections and opposite haustoria and blastospores (Fig. 2). Ballisto-, arthro-, telio-, and basidiospores (as well as basidia) are not observed. Sugars are not fermented.

The assimilation of carbon sources, scored as + (good growth), w (weak growth), s (slow, latent growth), and – (no growth), is as follows:

Glucose	+	Inulin	–
Galactose	+	Starch	s
Sorbose	–	Glycerol	s
N-Acetylglucosamine	+	Erythritol	–
Glucosamine	–	Ribitol	–
Ribose	+	Xylitol	w
Xylose	+	Arabitol	w/–
L-Arabinose	+	Sorbitol	s
D-Arabinose	w	Mannitol	+
Rhamnose	s	Dulcitol	s/–
Sucrose	+	Inositol	+
Maltose	+	Gluconate	+
Trehalose	+	2-Ketogluconate	+
α-Methylglucoside	+	5-Ketogluconate	+
Cellobiose	+	Glucuronate	+
Salicin	+	Glucarate	w
Arbutin	s	Lactate	s
Melibiose	w	Succinate	s
Lactose	+	Citrate	w/–
Raffinose	s	Quinic acid	–
Melezitose	+	Methanol	–
Ethanol	w		

The assimilation of nitrogen sources is as follows:

Nitrates	–	Glucosamine	+
Nitrites	–	Cadaverine	w
Creatine	–	Ethylamine	s
Creatinine	–	Lysine	w

The species requires biotin and thiamine for growth.

The maximum growth temperature on malt extract agar is 26–27°C.

The species is urease positive.

Acids on chalk agar are not produced.

In media with low pH values, the species produces starch-like substances.

Extracellular polysaccharides are composed of glucose, mannose, xylose, galactose, and glucuronic acid.

The species is sensitive to the mycocins of *Cr. laurentii* VKM Y-1627 and Y-1665, *Filobasidium capsuligenum* VKM Y-1439, is slightly sensitive to the mycocins of *Bullera alba* VKM Y-2829 and *Cr. perniciosus* VKM Y-2905 and 2907, and is insensitive to the mycocins of *B. hannae* VKM Y-2832, *B. sinensis* var. *lactis* VKM Y-2826, *B. unica* VKM Y-2830, *Cr. laurentii* VKM Y-1628, *Cr. nemorosus* VKM Y-2906, *Cr. podzolicus* VKM Y-2247 and Y-2249, *Cystofilobasidium bisporidii* VKM Y-2700, and *Cyst. infirmominiatum* VKM Y-2897.

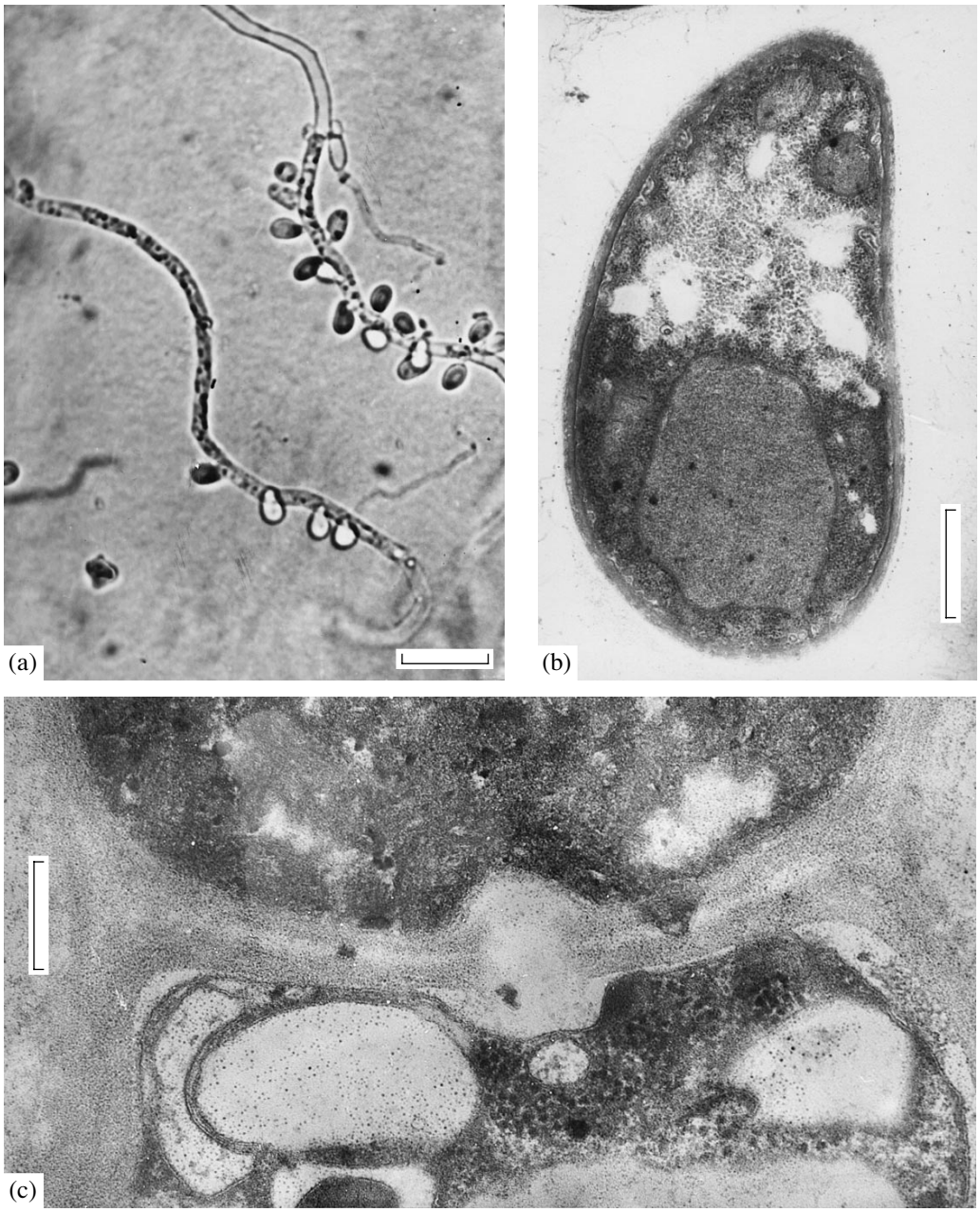

Fig. 2. The mycelial structures of *Cr. mycelialis* VKM Y-2863 grown on malt extract agar at 20°C for 2 weeks: (a) a substrate mycelium with clamp connections, haustoria, and blastospores (the bar represents 10 μm); (b) an ultrathin section of a blastospore (fixation with glutaraldehyde and permanganate; the bar represents 1 μm); (c) an ultrathin section of a doliporous septum (fixation with glutaraldehyde and permanganate; the bar represents 0.2 μm).

The G+C content of DNA is 55.6 mol %.

The type strain is VKM Y-2863 (= CBS 7712 and NRRL Y-11959). For the abbreviations of relevant culture collections, see note to Table 1.

The Latin Description of Cryptococcus mycelialis W. et N. Golubev nov. sp.

In aqua glucosum et extractum fermentati et peptonum continente, post dies 3 cellulae subglobosae et ovoidae (3.3–6.0 × 5.9–11.4 μm), incapsulatae. Post unum mensem sedimentum, annulus et pellicula formantur. In agaro morphologico et agaro malti post unum mensem cultura in striis glabra, opaca, mollis, cremea et filamentosa ad margineum. Mycelium fibulatum cum rami haustorialis formantur. Arthrosporae, ballistosporae et teliosporae non format. Fermentatio nulla. Glucosum, galactosum, *N*-acethylglucosaminum, ribosum, xylosum. Larabinosum, D-arabinosum (exigue), rhamnosum (lente), sucrosum, maltosum, trehalosum, α-methylglucosidum, cellobiosum, salici-

Table 2. Phenotypic distinctions between various representatives of the cluster *Holtermannia*

Characteristic	*Cr mycelialis*	*Cr. nyarrowii*	*H. corniformis*
Mycelium	+	–	–*
Assimilation of:			
N-acetylglucosamine	+	+	–
melibiose	w	–	–
lactose	+	–	–
raffinose	s	–	–
ribitol	–	w	s
Growth at 25°C	+	–	+
Sensitivity to the mycocins of:			
Bullera alba VKM Y-2829	w	–	+
Cryptococcus laurentii VKM Y-1627, 1665	+	–	+
Cr. perniciosus VKM Y-2905, 2907	w	–	+
Filobasidium capsuligenum VKM Y-1439	+	–	w

Note: s—slow reaction; w—weak reaction.

* When cultivated in monocultures, strains VKM Y-2803 and Y-2804 with the A_1B_1 and A_2B_2 types of hybridization, respectively, grow as yeasts. But when cultivated on corn agar in a mixed culture, they produce a true mycelium with clamp connections and haustoria.

num, arbutinum (lente), melibiosum (exigue), lactosum, raffinosum (lente), melezitosum, amylum (lente), glycerolum (lente), xylitolum (exigue), glucitolum (lente), mannitolum, inositolum, gluconatum, 2- et 5-ketogluconatum, glucuronatum, lactatum (lente), succinatum (lente), glucaratum (exigue) et ethanolum (exigue) assimilantur neque sorbosum, glucosaminum, inulinum, erythritolum, ribitolum, acidum quinicum et methanolum. Assimilatio galactitolum, arabinitolum et citratum variabilis. Ammonium, glucosaminum, cadaverinum (exigue), ethylaminum (lente) et lysinum (exigue) assimilantur, at non kalium nitricum, kalium nitrosum, creatinum et creatininum. Ad crescentiam biotinm et thiaminum necessarium est. Maxima temperatura crescentiae: 26–27°C. Urea finditur. Materia iodophila formantur. Glucosum, mannosum, xylosum, galactosum et acidum glucuronicum in polysaccharides externis demonstrable est. Proportio molaris guanini + cytosini in acido deoxyribonucleico: 55.6%. Typus: cultura VKM Y-2863 conservatur in collectione microorganismorum Acad. Sci. Rossicum, Pushczino, Rossia.

DISCUSSION

Like other anamorphic genera, the genus *Cryptococcus* is polyphyletic and, according to rDNA sequencing data, includes hymenomycetous yeasts of the orders *Cr. nyarrowii*, *Tremellales*, *Trichosporonales*, and *Cystofilobasidiales* [13]. The phylogenetic position of *Cr. mycelialis*, *Cr. nyarrowii*, and *H. corniformis* is not entirely known. According to the data of partial 26S rDNA sequencing, these three species form a distinct cluster ranking as an order. However, according to the sequence data of internal transcribed spacer rDNA regions, the species *Cr. mycelialis* and *Cr. nyarrowii* and the genus *Holtermannia* belong to the order *Tremellales* [6]. This discrepancy could be resolved by investigating a large number of species (especially teleomorphic ones) of this group. Unfortunately, even the most extensive known collections of live microbial cultures have only one (*H. corniformis*) of the six known species of the genus *Holtermannia.*

Many characteristics of *Cr. mycelialis* (the presence of urease; the inability to ferment sugars; the absence of arthro- and ballistospores; the synthesis of xylose-containing polysaccharides and, under acidic conditions, starch-like substances; and the ability to assimilate inositol and glucuronic acid) correspond closely to the modern description of the genus *Cryptococcus* [5]. The most distinguishing feature of *Cr. mycelialis* is the formation of an abundant substrate mycelium with clamp connections (Fig. 2a), which, however, are most likely false, since we were unable to observe true clamp connections fused with the adjacent hyphal cell during the electron microscopic examination of the thin sections of the mycelium. Like yeast cells (Fig. 1) and blastospores (Fig. 2b), *Cr. mycelialis* hyphae are monokaryotic and have, with very rare exceptions, nonporous septa (Fig. 2c). These observations, together with the failure to induce the formation of basidia, suggest that the dimorphic species *Cr. mycelialis* is haploid and heterothallic. Furthermore, the presence of haustoria suggests that this species is parasitic at the mycelial stage of its life cycle. This is in agreement with the fact that some representatives of the order *Tremellales* are mycoparasitic [15]. The cross hybridization of four *Cr. mycelialis* isolates on corn agar was not successful, but the teleo-

morphic state of this species can likely be achieved by cultivating it together with its host.

Phenotypically, the four isolates under study are similar to the species *Cr. laurentii* and, hence, according to the modern taxonomic conceptions [5, 15], can formally be ascribed to this very heterogeneous taxon [17]. However, the type strain *Cr. laurentii* VKM Y-1665 differs greatly from *Cr. mycelialis* not only phylogenetically [6, 13] but also phenotypically (in the absence of mycelial structures; its ability to utilize erythritol, ribitol, creatine, and creatinine; and ability to grow at 30°C). It should, however, be noted in this regard that some strains preliminarily identified as *Cr. laurentii* var. *laurentii* turned out to produce, when hybridized, hyphae with chlamydospores and dolipore-containing septa but without clamp connections, although some hyphae were found to be bikaryotic [18, 19]. Our observations showed that these strains grown in monocultures on corn agar are able to produce a true substrate mycelium with clamp connections and haustoria. The sequence analysis of these strains carried out in Fell's laboratory showed that they are identical to the type strain *Cr. laurentii* (Kufferath) Skinner var. *flavescens* (Saito) Lodder et Kreger-van Rij in the cluster *Cr. laurentii* [6] and, hence, are phylogenetically distant from *Cr. mycelialis.*

The anamorphic species *Cr. mycelialis* is close to the yeast fungus *H. corniformis* both phylogenetically and phenotypically. These two organisms are virtually identical in their sensitivity to mycocins but can be differentiated by their ability to assimilate *N*-acetylglucosamine, lactose, raffinose, and ribitol (Table 2). The species *Cr. nyarrowii* differs from *Cr. mycelialis* in the maximum growth temperature (22°C) and resistance to the mycocins of the genera *Bullera*, *Cryptococcus*, *Cystofilobasidium*, and *Filobasidium.*

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