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Myo-Inositol Assimilating New Species of *Rhodotorula* Harrison

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Abstract—A new species of anamorphic basidiomycetous yeasts, *Rhodotorula pinalis*, was isolated from needle litter of *Pinus sylvestris* L. collected in Moscow oblast (Russia). The cultures are nonpigmented; nitrate- and *myo*-inositol-positive, forming no ballistoconidia.

Keywords: yeast fungi, *myo*-inositol, *Rhodotorula*, ballistoconidia.

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Species identification of yeast fungi is based mainly on their different abilities to assimilate carbon and nitrogen compounds [1, 2]. Only in rare cases are these differences used for generic differentiation. In particular, utilization of *myo*-inositol (*i*-inositol, *meso*-inositol) as the sole carbon source is the key feature that differentiates the genus *Cryptococcus* Vuillemin from *Rhodotorula* Harrison. It should be noted that such differentiation is becoming less distinct: there are more and more reports on both inositol-positive *Rhodotorula* species and inositol-negative cryptococci [2, 3].

We have isolated a number of anamorphic basidiomycetous inositol-positive yeasts during microbiological investigation of dead conifer needles [4]. Among them, a few isolates with identical morphological and physiological–biochemical characteristics corresponded nearly in all features to the description of the species *Sporobolomyces inositophilus* Nakase et Suzuki but did not form ballistoconidia, although fresh isolates are usually exhibited very abundant sporulation. Direct comparison of these isolates (Ps-72, Ps-94, Ps-101, and Ps-116) with the strains of the above species, including the type strain VKM Y-2728, revealed that they also differed in the inability to utilize creatinine. The cultures mated neither with each other nor with the *S. inositophilus* strains. According to G. Scorzetti's data, they are related to Microbotryales (Microbotryomycetes) and are phylogenetically close to *S. inositophilus*; however, they differ in four nucleotides in the D1/D2 domains of 26S rDNA and more than 20 nucleotides in the ITS regions. The set of the above characteristics demonstrates that the strains isolated from the needle litter of *Pinus sylvestris* L. in Moscow oblast are a novel species, description of which was prepared in accordance with the recommended methods [1].

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Rhodotorula pinalis *Golubev sp. nov.*

In aqua glucosum et peptonum et extractum fermentati continente, post dies 3 ad 20°C, cellulae longi-ovoidae, fusiformes (1.7–4.3 × 6.8–21.3 μm) propagantes per gemmationem terminals. Post unum mensem, annulus, pellicula et sedimentum praesenta sunt. In agaro morphologico (Difco), post unum mensem ad 20°C, cultura griseo-cremea, mollis, glabra, nonnitida cum margine integre. Ballistoconidiae, teliosporae non format. In agaro farinae Zeae maydis pseudomycelium primitivum formatur.

Fermentatio nulla. D-Glucosum, sucrosum, trehalosum (lente), maltosum (lente), melezitosum, ethanolum, glycerolum, D-xylitolum (exigue), D-mannitolum, D-glucitolum (lente), myo-inositolum, DL-lactatum (exigue), succinatum (lente), citratum (lente), D-gluconatum, 5-keto-D-gluconatum, D-glucuronatum, D-glucaratum et acidum quinicum assimilantur at non inulinum, raffinosum, melibiosum, D-galactosum, lactosum, a-methyl-D-glucosidum, amylum solubile, cellobiosum, arbutinum, salicinum, L-sorbosum, L-rhamnosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, methanolum, erythritolum, L-arabitolum, ribitolum, galactitolum, allantoinum, D-glucosaminum, N-acetyl-D-glucosaminum nec hexadecanum. Kalium nitricum, kalium nitrosum, ethylaminum, L-lysinum (exigue), cadaverinum (lente), D-glucosaminum (lente) et L-tryptophanum assimilantur at non creatinum et creatininum. Ad crescentiam vitaminae externae neccessarium est. Materia amyloidea iodophila non formantur. Urea finditur. 25°C crescit neque 30°C.

Typus: VKM Y-2963 (originaliter ut Ps-101), isolatus ex foliis acicularibus Pinus sylvestris L. delapsis (Rossia), v. 2006, conservatur in collectione microorganismorum (Pushczino).

Three-day culture cells in glucose–peptone medium with yeast extract (20°C) are polar budding, elongate–oval, fusiform, 1.7–4.3 × 6.8–21.3 μm (average size, 2.8–13.5 μm); cell length to width ratio:

3.5 to 7.0 (average value, 4.9). A ring, a pellicle, and sediment are observed after a month. The cultures (1 month, 20°C) on morphological agar (Difco, United States) are grayish-creme, pastelike, smooth, semiopaque, with an entire margin. Do not form ballistoconidia and teliospores. Form primitive pseudomycelium on corn agar.

Do not ferment sugars. Assimilated compounds as the sole carbon or nitrogen sources in the medium are presented in Table 1. Do not grow in vitamin-free medium (Difco, United States). Do not synthesize starchlike substances. Are urease-positive. Grow at 25°C; do not grow at 30°C. Have no lipase and pectinase activities. Insensitive to the mycocins secreted by the *Rhodotorula* and *Sporidiobolus* Nyland species of the order *Sporidiobolales* (*Microbotryomycetes*).

The type strain Ps-101 isolated in 2006 from the dead needles of *Pinus sylvestris* L. (Moscow oblast) is maintained in the All-Russian Collection of Microorganisms (Pushchino) as VKM Y-2963.

In spite of the phenotypic and phylogenetic similarity of the newly described yeast to *S. inositophilus*, the absence of ballistoconidia prevents its inclusion in the genus *Sporobolomyces* Kluyver et van Niel. At the same time, classification of the new species within the genus *Rhodotorula* should be considered as preliminary, because this genus is currently interpreted as an extremely heterogenic one: it is phylogenetically related to different orders, classes *Pucciniomycotina* and *Ustilagomycotina* [5]. One of the reasons for heterogeneity is the transfer of assimilating D-glucuronic acid basidiomycetous of *Candida* Berkhout species to the genus *Rhodotorula* [6]. D-glucuronic acid is the first intermediate of the glucuronate–gulonate pathway of *myo*-inositol catabolism in eukaryotes [7]. Due to its functioning in fungi, all inositol-positive organisms, with rare exceptions, are also glucuronate-positive. The opposite is untrue; quite a number of yeast species are known to assimilate glucuronate but not inositol [2]. They probably lack inositol oxygenase (EC 1.13.99.1), the synthesis of which in cryptococci is encoded by three genes localized on different chromosomes [8]. In the presence of oxygen, this monooxygenase cleaves the ring between the first and the sixth carbon atoms of the given cyclitol (hexahydroxy cyclohexane) with the formation of D-glucuronic acid. The latter is further catabolyzed via the pentose–phosphate cycle.

The ability to utilize glucuronate seems to be a more resistant and conservative characteristic than inositol utilization. The correlation between the ability to assimilate D-glucuronate together with *myo*-inositol naturally suggests joint application of the inositol and glucuronate tests for identification. Such a combination may be an additional control of the testing results and may provide more reliable diagnostics of yeasts [7].

At present, nearly all inositol-positive yeasts are concentrated in three orders of *Tremellomycetes* (*Agaricomycotina*), whereas most of the glucuronate-positive but inositol-negative *Rhodotorula* species, together with the so far few in number inositol- and glucuronate-positive species, are included in *Microbotryales* (*Microbotryomycetes, Pucciniomycotina*).

Table 1. Assimilation characteristics of *Rhodotorula pinalis*

Carbon sources			
Glucose	+	Glycerol	+
Inulin	–	Erythritol	–
Sucrose	+	Arabitol	–
Raffinose	–	D-Xylitol	w
Melibiose	–	Ribitol	–
Galactose	–	Dulcitol	–
Lactose	–	D-Mannitol	+
Trehalose	s	D-Sorbitol	s
Maltose	s	*myo*-Inositol	+
Melezitose	+	DL-Lactate	w
α-Methyl-D-glucoside		Succinate	s
Soluble starch	–	Citrate	s
Xylan	–	Glucarate	+
Cellobiose	–	Quinic acid	+
Arbutin	–	*p*-Oxybenzoic acid	+
Salicin	–	*m*-Oxybenzoic acid	–
L-Sorbose	–	Homoveratric acid	–
L-Rhamnose	–	Veratric alcohol	–
D-Xylose	–	Allantoin	–
L-Arabinose	–	D-Gluconate	+
D-Arabinose	–	5-keto-D-gluconate	+
D-Ribose	–	D-Glucuronate	+
Methanol	–	D-Glucosamine	–
Ethanol	+	N-Acetyl-D-glucosamine	–
Phenol	–	Hexadecane	–
Nitrogen sources			
Nitrates	+	L-Lysine	w
Nitrites	+	Cadaverine	s
Creatine	–	D-Glucosamine	s
Creatinine	–	L-Tryptophan	s
Ethylamine	+		

Note: +, assimilates; s, slowly; w, weakly; –, does not assimilate.

R. pinalis is characterized by the narrowest spectrum of utilized carbon sources and may be differentiated from the currently known inositol-positive species of *Rhodotorula* by quite a number of tests (Table 2).

Table 2. Differentiating characters of inositol-positive *Rhodotorula* species (*Microbotryales*)

Carbon sources	*R. pinalis*	*R. rosulata*	*R. silvestris*	*R. straminea*	*R. yarrowii*
Raffinose	–	–	+	+	+
Melibiose	–	–	s	–	+
Cellobiose	–	s	s	–	–/w
Arbutin	–	s	s	–	–
Salicin	–	s	s	–	–
L-Sorbose	–	–	s	w	w
L-Rhamnose	–	+	–	–	–
Ribitol	–	w	s	s	s
N-Acetyl-D-glucosamine	–	+	+	+	+
Nitrogen sources					
Creatine	–	+	–	–	–
Creatinine	–	+	–	–	–

Note: Symbols are as in Table 1.

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