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EXPERIMENTAL ARTICLES

A Study of *Schizoblastosporion starkeyi-henricii* Isolates from Northern and Southern Hemispheres of the Earth[1]

W. I. Golubev[a, 2] and I. Pfeiffer[b]

[a] *Russian Collection of Microorganisms (VKM), Institute for Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, 142290 Pushchino, Russia*

[b] *Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary*

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Abstract—A species *Schizoblastosporion starkeyi-henricii* is common in peats and peaty soils. Fifteen strains of this species isolated from Europe and East Falkland Island were found homogenous in cultural, morphological and physiological characters, with the exception of one strain that was sensitive to elevated osmotic pressure. Analyses of the D1/D2 region of the 26S rDNA and internal transcribed spacers (ITS) confirm that these strains and also Asian isolates are conspecific. Our results show that ecological factors but not geographic distances determine the global distribution of yeast fungi.

Keywords: microbial distribution, global geographic range, yeast fungi

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The surveys of the yeast communities suggest that the species *Schizoblastosporion starkeyi-henricii* Ciferri (=*Nadsonia starkeyi-henricii* (Ciferri) Kurtzman et Robnett) has a global distribution. It has found in both hemispheres: in Europe (Denmark, Norway, Poland, Russia), Asia (Korea, Russia), North America (Canada, USA), New Zealand and some other islands of the Atlantic and the Pacific Oceans [1–8].

However, the results of molecular studies, as a rule, have demonstrated that the species with a widespread occurrence are usually taxonomically heterogeneous [9, 10]. But as for *S. starkeyi-henricii*, it should be emphasized that this species has rather specialized habitat: almost all strains of it have been isolated from peat and peaty soils. Unfortunately, most of strains ascribed to *S. starkeyi-henricii* have been identified by limited set of morphological and physiological characteristics, and accurate identification would be desirable to determine if the isolates of this imperfect yeast from widely separated geographical areas are truly representatives of the species.

In this paper, different criteria based on the morphological and physiological features, on sequencing data and RAPD analysis were used to characterize *S. starkeyi-henricii* strains isolated in opposite regions of the Earth.

MATERIALS AND METHODS

Strains. The strains studied are listed in Table 1.

Morphological and physiological characteristics. The morphological and physiological properties were tested by methods currently employed in yeast taxonomy [11]. The length and width of 100 cells were measured in strains.

DNA sequence analysis. Genomic DNA was extracted by using a previously described method [12]. The nucleotide sequence of the internal transcribed spacer (ITS) and the D1/D2 region of the ribosomal DNA were determined. The ITS region was amplified and sequenced with ITS1 and ITS4 primers while the D1/D2 region with NL-1 and NL-4 primers. The PCR reaction was performed in 50 μL volume mixture contained 200 μM of each dNTP, 20 pmol of each primer, 1.0 U DreamTaq DNA polymerase (Fermentas UAB, Vilnius, Lithuania) and 200 ng genomic DNA. The mixture was buffered with the recommended buffer of the DreamTaq polymerase. The amplification was carried out in a MJ Mini™ Personal Thermal Cycler (Bio-Rad, Hercules, CA, USA). The PCR reaction consisted of an initial denaturation for 5 min at 94°C followed by 30 steps of 1 min denaturation at 94°C, annealing for 30 s at 55°C and extension for 1 min at 72°C. The final extension step was carried out at 72°C for 5 min. The PCR products were purified with Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech Ltd., Taiwan). Sequencing of both strands was done with an ABI 3100 sequencer (Applied Biosystems Inc., Foster City, CA, USA). Sequence data have been deposited in the National Center for Biotechnology Information (NCBI) online service data bank. Based on the partial nuclear 26S D1/D2 and ITS1-5.8S-ITS2 sequences, the phy-

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[2] Corresponding author: wig@ibpm.pushchino.ru

Table 1. Origin of *Schizoblastosporion starkeyi-henricii* strains

Strain numbers	Source
Subantarctica	
VKM Y-1947–1949	Soddy-peaty soil, East Falkland Island
Northern Europe	
VKM Y-1998, 2534	Piny-sphagnum peat, high-land moor, Poland
VKM Y-1999, 2535–2542	Peaty podzolic gley soil, Novgorod region, Russia
VKM Y-276^{NT}	Peaty soil, Denmark

NT—Neotype strain.

Table 2. Morphological characteristics of *Schizoblastosporion starkeyi-henricii* cells

Strain number	Size cells, μm (mean)	Length : width ratios (mean) A
VKMY-1947	3.4–5.1 (4.1) × 5.9–11.0 (8.2)	1.4–2.6 (2.0)
VKM Y-1948	2.5–4.3 (3.6) × 5.1–'9.4 (7.0)	1.5–2.5 (1.9)
VKM Y-1949	2.5–5.1 (4.2) × 4.2–9.4 (6.3)	1.2–2.3 (1.6)
VKM Y-1998	3.4–6.8 (4.4) × 5.1–9.4 (6.6)	1.1–2.0 (1.5)
VKM Y-1999	2.5–5.1 (3.8) × 5.1–9.4 (6.4)	1.2–2.3 (1.7)
VKM Y-2534	2.5–5.1(4.0) × 5.1–9.4(6.3)	1.2–2.2 (1.6)
VKM Y-2535	3.4–5.9 (4.6) × 6.9–9.4 (7.2)	1.2–2.0 (1.6)
VKMY-2536	3.4–5.9 (4.6) × 5.1–10.2 (7.3)	1.3–2.0 (1.6)
VKM Y-2537	3.4–5.9(4.2) × 5.1–10.2 (7.2)	1.2–2.4 (1.7)
VKM Y-2538	3.4–5.9 (4.4) × 5.1–9.4 (7.3)	1.2–2.2 (1.7)
VKM Y-2539	3.4–5.1 (4.1) × 5.1–10.2 (8.1)	1.2–2.8 (2.0)
VKM Y-2540	2.5–4.3 (3.5) × 5.9–10.2 (7.4)	1.6–2.8 (2.1)
VKMY-2541	3.4–5.9 (4.0) × 4.3–10.2 (7.5)	1.2–2.8 (1.9)
VKM Y-2542	2.5–4.3 (3.1) × 4.3– 8.5 (6.0)	1.2–3.0 (1.9)
VKM Y-2762^{NT}	2.5–5.1 (3.6) × 4.3–10.2 (6.4)	1.2–2.5 (1.8)

logenetic relationship of the strains with their selected relatives was estimated with Maximum Parsimony and Minimum Evolution methods using Mega v5.1 software [13] with the default settings. DNA sequences were aligned using ClustalX v1.83 [14] before the reconstructions. Conclusions based on the consensus of the two methods, presented on the Maximum Parsimony tree.

RAPD analysis. Random amplified polymorphic DNA (RAPD) analysis of the isolates was carried out using primers $(GACA)_4$, and OPC1, OPC2, OPC3, OPC4, OPC5, OPC6, OPC7, OPC11 of the Operon random primer kit (Operon Technologies, Inc., Alameda, CA, USA) according to the literature [15]. The amplification was carried out in the following conditions: initial denaturation for 3 min at 94°C followed by 30 steps of 1 min denaturation at 94°C, annealing for 30 s at 37°C and extension for 2 min at 72°C. The final extension step was carried out at 72°C for 5 min. Fragment presence/absence was coded in a binary data matrix. Then pair-wise strain similarity was estimated by Dice index.

Assay for mycocinogenic activity. Antagonistic interactions between *S. starkeyi-henricii* strains were tested on glucose-peptone agar with citrate-phosphate buffer (pH 4.5).

RESULTS

In glucose-yeast extract-peptone broth after 3 days at 24°C, the cells are subglobose to elongate, lemon-shaped, single, in pairs, or in short chains. Budding is bipolar on a broad base. The width and length of cells vary from 2.5 to 6.8 μm (mean—4.1) and from 4.2 to 11.0 μm (7.1), the width/length ratios do from 1.1 to 3.0 (1.8) (Table 2). After one month, the strains under study form a sediment and a ring, some strains (VKM Y-2536, 2539, 2540 and 2542) do a thin, creeping pellicle. Only the strains VKM Y-2537 and 2539 formed a rudimentary pseudomycelium.

Table 3. Physiological differences between *Schizoblastosporion starkeyi-henricii* strains

	Assimilation				
Strain number	L-sorbose	Ribitol	Xylitol	Mannitol	Cadaverine
VKM Y-1947, 1949, 2540–2542	d	–	–	d	+
VKM Y-1948, 1999	d	–	w	d	d
VKM Y-1998	–	–	w	d	–
VKM Y-2534–2536, 2539	–	–	w	d	d
VKM Y-2537	d	–	w	–	d
VKMY-2538	–	d	–	w	d
VKM Y-2762^{NT}	–	d	–	w	–

+ positive reaction, d—delayed, w—weak, —negative.

Table 4. Maximum temperatures, concentrations of NaCl and glucose for growth of *Schizoblastosporion starkeyi-henricii* strains

Strain number	T_{max}, °C	NaCl, %	Glucose, %
VKMY-1947, 1948, 2539	30	4.5	27
VKMY-1949	29	4.5	26
VKMY-1998	28	3.5	27
VKMY-1999	28	4.5	27
VKMY-2534	28	3.0	27
VKMY-2535	29	1.0	10
VKM Y-2536, 2541	30	3.5	26
VKM Y-2537	29	3.5	26
VKMY-2538	29	4.5	27
VKM Y-2540	31	3.5	26
VKM Y-2542	30	2.5	26
VKM Y-2762^{NT}	28	2.5	26

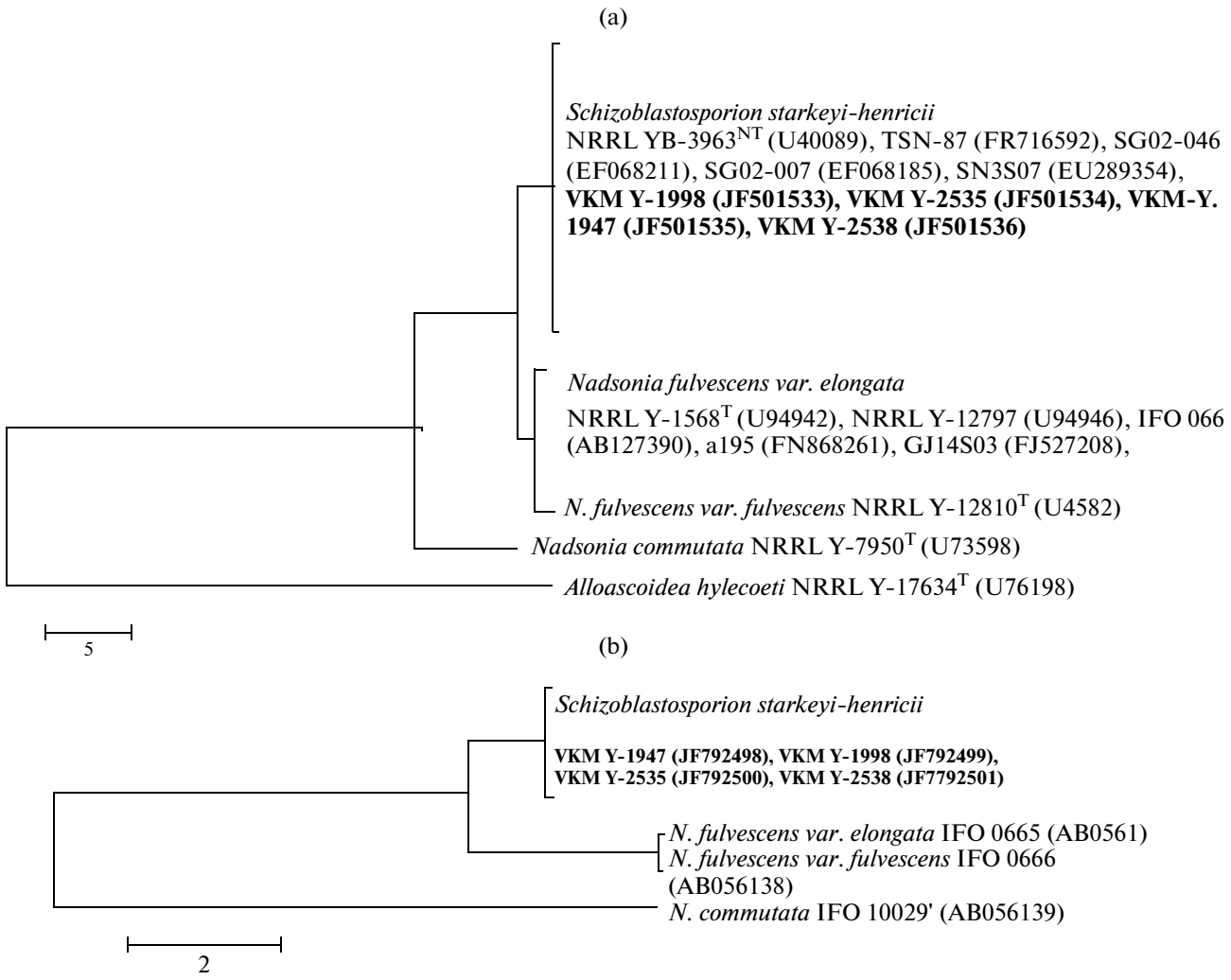
The growth of all the strains on malt agar is similar: cream-white, smooth, dull, pasty; the margin is entire, sinuated.

The strains alone or mixed in pairs did not produce ascospores on all sporulation media recommended.

Ability to ferment sugars is lacking. All strains assimilate glucose, galactose (slowly or weakly), glucitol, ethanol, glycerol, gluconate, succinate, lactate (weakly), fumarate and acetate (slowly) but not D-glucosamine, inulin, sucrose, raffinose, melibiose, lactose, trehalose, maltose, melezitose, α-methylglucoside, cellobiose, soluble starch, salicin, arbutin, L-rhamnose, xylose, L-and D-arabinose, D-ribose, erythritol, L-arabitol, galactitol, i-inositol, glucuronate, citrate, 2- and 5-ketogluconate, α-ketoglutarate, tartrate, oxalate, mucic acid and ethylene glycol. They utilize ethylamine and L-lysine but not nitrate, nitrite, creatine and creatinine. Do not grow in vitamin-free medium. Biotin required for growth of all strains and some strains (VKM Y-2535–2542) need pyridoxine. The strain VKM Y-1998 requires also thiamin and nicotinic acid stimulates growth of the strain VKM Y-2534. Urease activity is absent. Gelatin is not liquefied. Starch-like substances are not produced. Acid production on glucose-chalk agar is negative. The physiological differences between strains are few in number (Table 3). In most cases sharp distinctions in utilization carbon compounds are not observed, growth of positive strains is usually delayed or weak. Maximum temperatures for growth (malt agar) of the strains studied are about 30°C (±1–2°C), maximum concentrations of glucose in medium are 26–27%, with the exception of the strain VKM Y-2535 that does not grow at glucose concentration above 10% (Table 4). Also, this strain is sensitive to increased NaCl concentrations, other strains can grow at 2.5–4.5% of NaCl.

All strains under study were examined for mycocinogenic activity by cross-testing within *S. starkeyi-henricii*. No activity has been detected in any strains.

Sequence analyses showed that *S. starkeyi-henricii* composes a distinct unit but it is very close to *Nadsonia*



(a) Phylogenetic tree of *Schizoblastosporion starkeyi-henricii* strains and closely related species based on the alignment of D1/D2 LSU rDNA sequences. Tree was rooted to *Alloascoidea hylecoeti*. (b) Phylogenetic tree of *Schizoblastosporion starkeyi-henricii* strains and *Nadsonia* spp. based on the alignment of ITS sequences. Tree was rooted to *Nadsonia commutata*. The GenBank accession numbers of the sequences are indicated in brackets after the strain numbers (^{T}, type strain; ^{NT}, neotype strain). Sequences determined in the present study are typed in boldface.

species. It differs from these latter only in three positions in the D1/D2 and the ITS regions (figure (a), (b)). Eight strains of *S. starkeyi-henricii*, including the neotype one, showed identical sequences in the D1/D2 region. Also, any variability in the ITS region is not revealed between our four strains and four strains of this species isolated from soil of Europe (Denmark, Germany) and Asia (Korea, Taiwan).

RAPD analysis of the *S. starkeyi-henricii* strains was carried out by using 8 random decamer and $(GACA)_4$ primers. Only those thirty-seven bands were taken into account and coded in the binary matrix, which were amplified in all three separate RAPD reactions. The RAPD patterns were variable (data not shown) and isolates were separated from each other. Based on Dice dissimilarity index (d) Falkland isolate, VKM Y-1947, was similar to Russian one, VKM Y-2538, ($d = 0.55$) while more distant from Polish isolate, VKM Y-1998 ($d = 0.82$). The distance of strain VKM Y-2535, sensitive to elevated osmotic pressure, was similar from the three other strains ($d = 0.65$–0.68).

DISCUSSION

The strains of *S. starkeyi-henricii* studied are rather homogenous in their cultural, morphological and physiological properties. No clearly differentiating characteristics can be found between them. There are only some variations in cell sizes (Table 2) and differences in the assimilation of L-sorbose, polyols and cadaverine which are usually weakly or slowly utilized by some strains (Table 3). Among 15 strains studied, the strain VKM Y-2535 is distinguished for its sensitiv-

ity to increased osmotic pressure (Table 4). All these differences are obviously strain-related as well as the RAPD patterns that have been widely used in microbial strain characterization below the species level [16]. Phenotypic and RAPD variations are not related to geographic origin of the strains compared.

Comparison of the D1/D2 domain sequence as well as the ITS sequence of the subantarctic and European isolates (including the strain VKM Y-2535) indicated that they are conspecific.

Global distribution of *S. starkeyi-henricii* is not unique among both ascomycetous and basidiomycetous yeast fungi. Specifically, during our microbiological studies several cultures of *Sporobolomyces* species were isolated from polar regions: South Georgia island and arctic East-Siberian tundra [4, 17]. It was found later these isolates mate with each other and yield clamped mycelium with teliospores, i.e. they are haplophase of heterothallic species *Sporidiobolus salmonicolor* Fell et Tallman. It is interesting to note that the closest relative of *S. starkeyi-henricii*, ascogenous yeast *Nadsonia commutata* Golubev, was isolated from East Falkland Island (South Atlantic ocean) and Carpathian Mts. (Eastern Europe) [18].

Owing to their sizes and many forces in the natural environment that must drive their dispersal (e.g., atmospheric circulations, ocean currents, bird migrations and so on) any geographic distances on the Earth are not obstacles for distribution of free-living microorganisms. The possibilities for their development in some habitat are determined mainly by ecological factors (physicochemical characteristics of environment and substrates).

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