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Recognition of the basidiomycetous yeast *Sporobolomyces ruberrimus* sp. nov. as a distinct species based on molecular and morphological analyses

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Abstract

Sporobolomyces ruberrimus Yamasaki and Fujii *nom. inval.* is established as a distinct species by ribosomal-DNA base composition in the D1, D2 and ITS regions and by morphology. A Latin description is given to validate the name with CBS 7500 as the type strain.

Keywords: Basidiomycete; Yeast; New species; rDNA; Ballistoconidium

1. Introduction

Yamasaki and Fujii [1] named the yeast *Sporobolomyces ruberrimus* and recognized two varieties, *S. ruberrimus* var. *ruberrimus* from air in Japan and *S. ruberrimus* var. *albus*, which was a spontaneous colorless mutant. The publication was in Japanese with tables and summary in English; however, the authors omitted the Latin description or diagnosis required by Article 36 of the International Code of Botanical Nomenclature [2]. Therefore the name was not validly published. The publication included drawings of reniform ballistoconidia and ovoid vegetative cells with apiculate and branched sterigmata, measurements of cells, color of the colonies and test results on the utilization of carbon and nitrogen sources. The species was not mentioned in the first three editions of The Yeasts [3–5]. Barnett et al. [6,7] listed the species with a question mark because they had not been able to examine cultures of the species. Boekhout and Nakase [8] included the species as a synonym of *Sporobolomyces roseus* Kluyver et van Niel. They designated both varieties as *nomina illegitima*, possibly because of the lack of a Latin diagnosis.

The application of molecular methods to yeast systematics has provided considerable insight into our knowledge of the relationships between taxa and has helped resolve some of the difficulties inherent with classical systematics. One particular problem has centered on the resolution of species definitions: many taxa have been reduced to synonymy on the basis of similarities in morphological and physiological properties. Subsequent molecular analyses have questioned some of these conclusions [9]. As a specific example, *S. ruberrimus* emerged as a separate species in the phylogenetic trees presented by Fell et al. [10,11], but the status of the species was not discussed in the text. The present contribution presents evidence to support the re-introduction of *S. ruberrimus* and the validation of the name.

2. Methods

Standard taxonomic methods followed the protocol of Yarrow [12]. Sequence analysis of the D1/D2 region of the large sub-unit rDNA (LrDNA) and the internal transcribed spacer (ITS) followed the methods of Fell et al. [11]. GenBank and CBS numbers for the strains are included in Figs. 1 and 2.

To prepare cells for scanning electron microscopy, cul-

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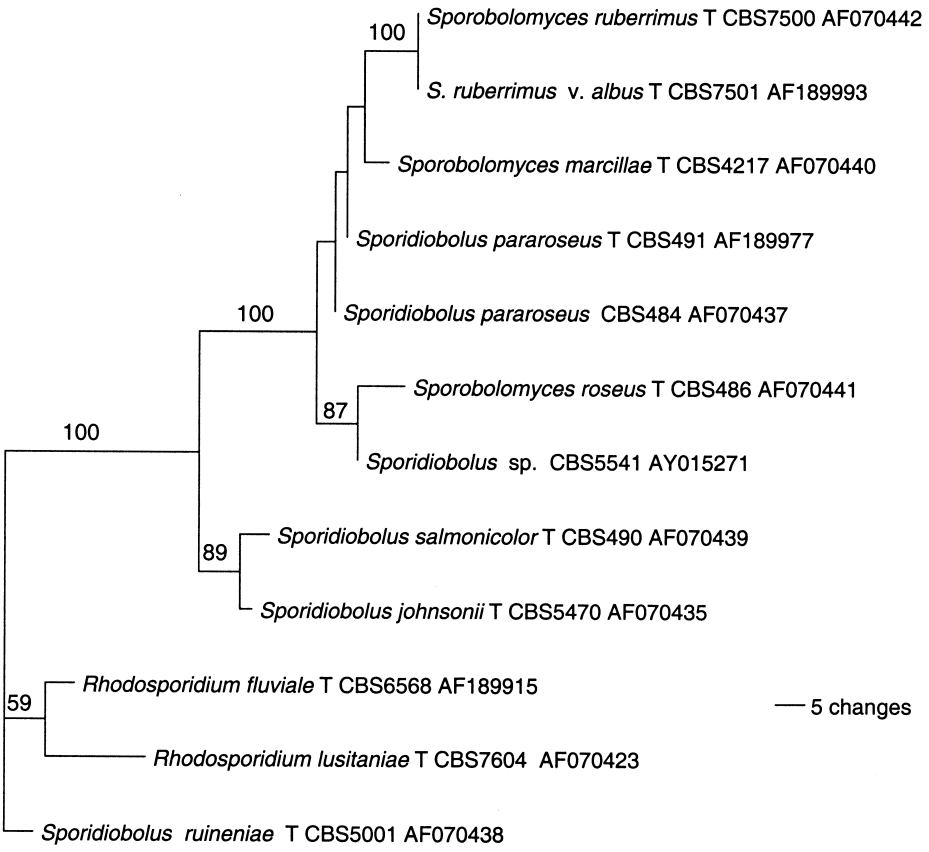


Fig. 1. Phylogenetic analysis of the D1/D2 region large sub-unit rDNA of species in the *Sporidiobolus salmonicolor* cluster of the *Sporidiobolus* clade of the urediniomycetous yeasts. (Paup*4.0; heuristic search, random stepwise addition, tree bisection–reconnection). Tree represents a single, most parsimonious tree, 620 characters, 524 constant characters, 17 parsimony uninformative characters, 79 parsimony informative characters. Tree length 136, consistency index 0.799, retention index 0.853. Numbers on branches represent bootstrap percentages from 1000 full heuristic bootstrap replications. T = type strain, numbers after species names represent CBS and GenBank accession numbers. *Rhodosporidium fluviale*, *Rhodosporidium lusitaniae* and *Sporidiobolus ruineniae* were employed as outgroup species.

tures were grown on potato dextrose agar (PDA) at 17°C. After 3 days of growth, cells were fixed in 2.5% glutaraldehyde/0.2 M sodium cacodylate buffer at 4°C for 2 h, and post-fixed in 1% OsO_4/0.2 M sodium cacodylate buffer for 1.5 h at 4°C. Samples were dehydrated through an ethanol series (30, 50, 60, 70, 80, 90, 95, 100%), and critical-point-dried in liquid CO_2. Dry samples were sputter-coated with palladium (20-nm-thick coat on a Hummer VII Sputtering System) and examined in a Philips XL 30 ESEM-FEG under 20 kV.

3. Results and discussion

S. ruberrimus is a member of the *Sporidiobolus* clade of the urediniomycetous yeasts [11]. Analysis of data from the D1/D2 and ITS regions (Figs. 1 and 2) confirms that the two varieties represent a single species, which differ in the color of their colonies and the utilization of the test compounds salicin, arbutin, starch and D-gluconate (Table 1). *S. ruberrimus* is related to a group of ballistoconidia-forming species comprising *Sporidiobolus pararoseus*, *Sporobolomyces marcillae*, *S. roseus* and strain CBS5541 (Figs. 1 and 2). *S. pararoseus* is a heterothallic, teliospore-forming species, which has been isolated from the atmosphere, soils and seawater [13]. The two strains of *S. pararoseus* depicted in Figs. 1 and 2 are opposite mating types and represent the species most closely related to *S. ruberrimus*. CBS 491, the type strain, differs from *S. ruberrimus* by 11 bp in the D1/D2 region and 9 bp in the ITS region. The *S. pararoseus* strains differ from each other by 2 bp in the D1/D2 and ITS regions.

S. marcillae Santa María, an anamorphic species, was found as an air-borne contaminant on an agar plate in Spain [14] and was subsequently considered to be a synonym of *S. pararoseus* Fell and Tallman [4]. Sequence analysis [11] demonstrated that *S. marcillae* and *S. pararoseus* were phylogenetically distinct. This taxonomic distinction was indicated by differences in DNA base composition: 55.0 mol% for *S. marcillae* strain CBS 4217, and 51.5 mol% for *S. pararoseus* strain CBS 484 [15]. Strains of *S. roseus* were isolated from plants, soils and human diseases [16]. Strain CBS 5541 was isolated from a *Fumaria* sp. flower in France and subsequently identified as a self-

Fig. 2. Phylogenetic analysis of the internal transcribed spacer region of species in the *S. salmonicolor* cluster of the *Sporidiobolus* clade of the urediniomycetous yeasts. (Paup*4.0; heuristic search, random stepwise addition, tree bisection–reconnection). Tree represents one of two equally most parsimonious trees, 626 characters, 463 constant characters, 31 parsimony uninformative characters, 132 parsimony informative characters. Tree length 243, consistency index 0.844, retention index 0.867. Numbers on branches represent bootstrap percentages from 1000 full heuristic bootstrap replications. T = type strain, numbers after species names represent CBS and GenBank accession numbers. *R. fluviale*, *R. lusitaniae* and *S. ruineniae* were employed as outgroup species.

sporulating strain of *S. pararoseus* [13]. However, the analyses presented in Figs. 1 and 2 illustrate that CBS 5541 is genetically distinct from *S. pararoseus*. The complete life cycle of CBS 5541 has not been studied, consequently a formal taxonomic description is not presented here.

A basic morphological characteristic of the species in this phylogenetic cluster (Figs. 1 and 2) is the presence of ballistoconidia. Yamasaki and Fujii [1] depicted structures that they considered to be characteristic ballistoconidia. However, our examination failed to observe either the formation of apiculate sterigmata or the forceful ejection of conidia. In contrast, we observed conidiophores with blunt tips rather than sterigmata (Figs. 3 and 4), which suggested an inability to forcefully eject conidia. The conidia produced on these conidiophores were reniform in shape (Figs. 3–5), which is typical of the ballistoconidia of *Sporobolomyces*. The loss of the ability to form ballistoconidia during prolonged laboratory culture is not an uncommon occurrence. There is considerable information regarding the mechanism of ballistospore discharge [17], although the genetics of the process are unknown. The potential that the inability of *S. ruberrimus* to produce ballistoconidia is due to a loss of genetic function or to our failure to provide adequate environmental conditions is unresolved, in spite of our repeated attempts with standard media (YM agar, potato dextrose agar, Difco yeast morphology agar and cornmeal agar). As a consequence, we retained *S. ruberrimus* in *Sporobolomyces*, rather than including the species in *Rhodotorula*, a genus that does not produce ballistoconidia.

4. Standard description

Sporobolomyces ruberrimus Yamasaki et Fujii ex Fell, Pinel, Scorzetti, Statzell-Tallman et Yarrow.

Basionym: *Sporobolomyces ruberrimus* Yamasaki et Fujii (in Yamasaki and Fujii 1950. Studies on *Sporobolomyces* Red Yeast. Part 7. Classification of the Genera *Sporobolomyces* and *Bullera*. Agric. Chem. Soc. Jpn. Bull. 24, p. 13, Fig. 4, sine descr. Latin).

Synonym: *Sporobolomyces ruberrimus* var. *albus* Yamasaki et Fujii.

In extracto malti post dies tres ad 25°C, cellulae ovoideae vel elongatae (3–5)×(2–3) μm, singulae vel binae. Annulus non formatur, sedimentum exiguum atque insulae parvae formantur. Post unum mensem ad 25°C annulus aest et sedimentum moderatum est.

Table 1
Physiological properties of *S. ruberrimus* and related species and strains

	S. ruberrimus CBS7500	*S. r.* v. *albus* 7501	*S. pararoseus* 491	*S. pararoseus* 484	*S. marcillae* 4217	*S. roseus* 486	*Sporidiobolus* sp. 5541	*S. salmonicolor* 490	*Sporidiobolus johnsonii* 5470
Carbon compounds									
D-Glucose	+	+	+	+	+	+	+	+	+
D-Galactose	D	−	+	+	D	−	D	−	−
L-Sorbose	−	−	+	D	+	−	+	D	D
D-Glucosamine	−	−	−	−	−	−	−	−	−
D-Ribose	−	D	D	+	D	D	+	D	+
D-Xylose	−	−	−	+	D	−	D	D	D
L-Arabinose	−	−	−	+	−	−	−	−	−
D-Arabinose	−	D	D	+	+	−	+	+	+
L-Rhamnose	−	−	−	−	−	−	−	−	−
Sucrose	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	−	+
α,α-Trehalose	+	+	+	+	+	−	+	+	+
Methyl-α-D-glucoside	−	−	+	+	D	−	D	−	+
Cellobiose	+	D	+	+	+	+	+	−	+
Salicin	+	−	+	W	+	W	+	−	+
Arbutin	+	−	+	+	+	W	+	−	+
Melibiose	−	−	−	−	−	−	−	−	−
Lactose	−	−	−	−	−	−	−	−	−
Raffinose	+	+	+	WD	+	+	D	+	−
Melezitose	+	+	+	+	+	+	+	−	+
Inulin	−	−	−	−	−	−	−	−	−
Starch	−	+	+	+	D	+	+	−	+
Glycerol	D	+	+	−	D	D	+	D	+
Erythritol	−	−	−	−	−	−	−	−	−
Ribitol	−	−	−	D	−	D	+	+	D
Xylitol	−	−	D	D	D	−	+	D	D
L-Arabinitol	−	−	−	−	−	−	−	−	−
D-Glucitol	−	−	+	+	D	−	+	+	+
D-Mannitol	D	D	+	+	D	−	+	+	+
Galactitol	−	−	−	−	−	−	−	−	−
myo-Inositol	−	−	−	−	−	−	−	−	−
D-Glucono-1,5-lactone	−	−	+	+	D	−	+	+	+
2-Keto-D-gluconate	−	−	−	−	−	−	−	−	−
D-Gluconate	−	+	+	D	+	−	+	D	+
D-Glucuronate	−	−	−	−	−	−	−	−	−
D-Galacturonate	−	−	−	−	−	−	−	−	−
DL-Lactate	−	−	D	−	−	−	D	−	−
Succinate	+	W	+	−	+	W	D	+	+
Citrate	D	D	+	−	+	W	−	+	+
Methanol	−	−	−	−	−	−	−	−	−
Ethanol	+	D	+	WD	+	WD	+	+	+
Propane-1,2-diol	D	−	+	+	D	−	+	D	WD
Butane-1,2-diol	−	−	−	−	−	−	−	−	−
Quinic acid	+	D	+	+	+	+	+	−	+
D-Glucarate	−	−	−	−	−	−	−	−	−
D-Galactonate	−	−	−	−	−	−	−	−	−
Nitrogen compounds									
Nitrate	+	+	−	−	−	−	+	+	+
Nitrite	+	+	−	−	−	−	+	+	+
Ethylamine	−	−	+	−	−	−	−	W	+
L-Lysine	WD	W	+	+	−	−	−	−	−
Cadaverine	+	+	+	+	−	−	−	−	−
Creatine	−	−	−	−	−	−	−	−	−
Creatinine	−	−	−	−	−	−	−	−	−
Glucosamine	−	−	−	−	−	−	−	−	−
Imidazole	−	−	−	−	−	−	−	−	−
D-Tryptophan	W	+	−	−	−	−	−	−	−
Other tests									
W/0 vitamins	+	+	+	+	WD	−	+	+	+

Table 1 (*continued*)

	S. ruberrimus CBS7500	*S. r.* v. *albus* 7501	*S. pararoseus* 491	*S. pararoseus* 484	*S. marcillae* 4217	*S. roseus* 486	*Sporidiobolus* sp. 5541	*S. salmonicolor* 490	*Sporidiobolus johnsonii* 5470
Cycloheximide 0.01%	−	−	D (0.1%−)	−	−	−	−	+(0.1%+)	+(0.1%+)
Acetic acid 1%	−	−	−	−	−	−	−	−	−
Temperature max °C for growth	29	29	30 (35−)	30 (35−)	25 (30−)	25 (30−)	30 (35−)	30 (35−)	37 (40−)
Salinity max % for growth	10-W	10-W	10-W	10-W	10 (16-W)	10-W	10 (16-W)	10-W	10-W

In agaro malti post unum mensem cultura lactea vel aurantiaca, leviter rugosa, plana, opaca, margine expresso.

In agaro farina Zea maydi confecto, post unum mensem ad 25°C pseudohyphae rudimentales formantur.

Ballistoconidia non manifesta.

Fermentatio nulla.

Crescit in medio addito D-glucoso, D-galactoso (tarde vel crescit non), D-riboso (tarde vel crescit non), D-arabinoso (tarde vel crescit non), sucroso, maltoso, α,α-trehaloso, cellobioso (nonnumquam tarde), salicino (varibiliter), arbutino (varibiliter), raffinoso, melezitoso, amylosolubile (varibiliter), glycerolo (nonnumquam tarde), D-mannitolo (tarde), D-gluconato (varibiliter), succinato (nonnumquam exigue), citrato (tarde), ethanolo (nonnumquam tarde), propano-1,2-diolo (tarde vel crescit non), acido quinico (nonnumquam tarde), kalii nitrato, natrii nitrato, cadaverino, L-lysino (exigue), D-tryptophano (nonnumquam exigue; non in medio addito L-sorboso, D-glucosamino, D-xyloso, L-arabinoso, L-rhamnoso, α-methyl-D-glucosido, melibioso, lactoso, inulino, erythritolo, ribitolo, xylitolo, L-arabinitolo, D-glucitolo, galactitolo, *myo*-inositolo, D-glucono-1,5-lactono, 2-keto-D-gluconato, D-glucuronato, D-galacturonato, DL-lactato, methanolo, butano-2,3-diolo, D-glucarato, D-galactonato, ethylamino, creatino, creatinino, D-glucosamino, imidazolo.

Ad crescentiam vitamina haud necessaria.

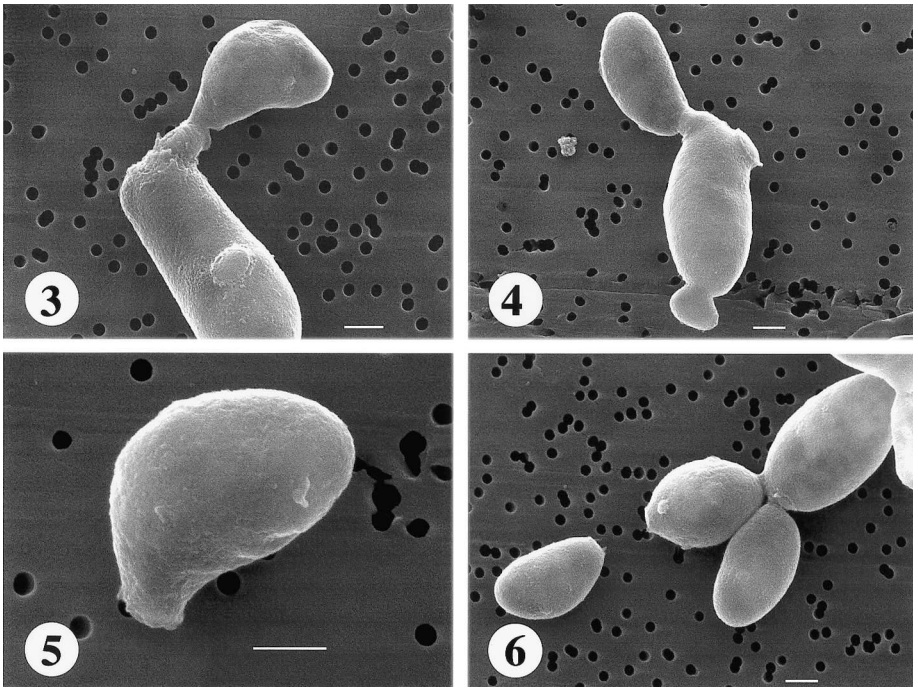
Typus: CBS 7500 isolatus ex atmospherica.

Maxima temperatura crescentiae: 29°C.

Crescit in 10% natrii cloridii (exigue).

Crescere non potest in medio 0.01% cicloheximido neque in medio 1% acido acetico continente.

Growth in malt extract: After 3 days at 25°C the cells were ovoid or elongated (3–5)×(2–3) μm (Figs. 3–6), single or in small groups. Cells reproduced by blastoconidia that formed directly on the parental cell (Fig. 6) or on



Figs. 3–6. *S. ruberrimus*. Scanning electron microphotomicrographs taken with a Philips XL 30 ESEM-FEG. Scale bars = 1 μm. Fig. 3, cell with a sterigma-like conidiophore and reniform bud. Figs. 4 and 6, blastoconidia formation. Fig. 5, reniform blastoconidia.

short conidiophores that resembled blunt tipped sterigmata (Figs. 3 and 4). Buds were ovoid or reniform in shape (Figs. 3–6). A ring was absent; small islands and a sparse sediment were present. After 1 month a ring was absent and the sediment moderate.

Growth on malt agar: After 1 month at 25°C the streak culture was either whitish or orange, slightly wrinkled, flat and dull with a lobate margin.

Dalmau slide culture on cornmeal agar at 25°C: Rudimentary pseudohyphae were formed.

Formation of ballistoconidia: Not observed at 25°C on Difco yeast morphology agar, potato-dextrose agar, yeast extract–malt extract agar and cornmeal agar.

Heterothallic sexual reproduction: Not observed when mixed on cornmeal agar at 25°C with CBS strains 481, 484, 486, 4216. 4217, 5541 and 7253. (CBS 5541 is a self-sporulating strain.)

Fermentation: Absent.

Growth on carbon sources, nitrogen sources and other tests: See Table 1.

Origin of the strains: CBS 7500 was isolated from the atmosphere in Japan in June 1941; CBS 7501 was a spontaneous white mutant of CBS 7500 isolated in January 1950 [1]. Both strains were deposited at CBS by A. Shiraishi in January 1990. CBS 7292 and CBS 7293 were subcultures of the same strains deposited by J.A. Barnett in January 1987.

Type strain: CBS 7500, isolated from the atmosphere at Fukuoka in Japan by I. Yamasaki and S. Morisita in June 1941, freeze-dried and also frozen in the collection of the Centraalbureau voor Schimmelcultures, Utrecht.

GenBank Nos.: CBS 7500, D1/D2: AF070442; ITS: AY015439; CBS 7501, D1/D2: AF189993; ITS: AY015436.

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