Sporidiobolus longiusculus sp. nov. and *Sporobolomyces patagonicus* sp. nov., novel yeasts of the Sporidiobolales isolated from aquatic environments in Patagonia, Argentina

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During a survey of carotenogenic yeasts carried out in north-western Patagonia (Argentina), several ballistoconidia-producing strains belonging to the order Sporidiobolales were isolated from aquatic environments. Five strains were found to represent two novel species, for which the names *Sporidiobolus longiusculus* and *Sporobolomyces patagonicus* are proposed, with CBS 9654^T (=PYCC 5818^T=CRUB 1044^T) and CBS 9657^T (=PYCC 5817^T=CRUB 1038^T) as the type strains, respectively. The elongated basidia, which are five to six times longer that those of the remaining species of the genus *Sporidiobolus*, are a particular micromorphological feature of *Sporidiobolus longiusculus*. On the basis of the sequences of the D1/D2 domains of the 26S rRNA gene, the species most closely related to *Sporidiobolus longiusculus* is *Sporobolomyces bannaensis*, whereas *Sporobolomyces marcillae* is the closest relative of *Sporobolomyces patagonicus*. Complete internal transcribed spacer sequence analysis confirmed the separate position of *Sporidiobolus longiusculus*, whereas for *Sporobolomyces patagonicus* no nucleotide differences were found with respect to *Sporidiobolus pararoseus* CBS 491^T. Negative mating experiments between strains of *Sporobolomyces patagonicus* and strains of *Sporidiobolus pararoseus* together with the low DNA–DNA reassociation values for the type strains of the two species validated the proposal of *Sporobolomyces patagonicus* as a distinct species. Information on additional Patagonian *Sporobolomyces* isolates is also included in this report.

Yeast species included in the polyphyletic genus *Sporobolomyces* are anamorphic basidiomycetes that produce bilaterally symmetrical ballistoconidia and have CoQ 10 or CoQ 10(H_2) as their major ubiquinone. The inability to ferment sugars, the absence of xylose in whole-cell hydrolysates and the positive diazonium blue B and urease reactions are also characteristics of this group (Boekhout & Nakase, 1998). On the basis of sequence analyses of the D1/D2 domains of the 26S rRNA gene (Fell *et al.*, 2000), the genus *Sporobolomyces* belongs to the Urediniomycetes and has representatives in the Microbotryomycetidae, where most species belong to the Sporidiobolales (Sampaio *et al.*, 2003), the Agaricostilbomycetidae and the *Naohidea–Rhodotorula minuta* group. The type species of *Sporobolomyces*, *Sporobolomyces salmonicolor*, belongs to the Sporidiobolales, and the sexual stages of this genus, classified within *Sporidiobolus* Nyland, also belong to this order. At present, the core group of *Sporobolomyces*, i.e. the species belonging to the Sporidiobolales, encompasses 15 taxa (Sampaio, 2004), whereas *Sporidiobolus* includes five species.

Sporidiobolus and *Sporobolomyces* appear to be associated mainly with the phylloplane of terrestrial plants (Nakase, 2000). Their presence in water reservoirs, including marine systems, is possibly caused by run-off (Hagler & Ahearn,

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Abbreviations: ITS, internal transcribed spacer; MSP-PCR, microsatellite-primed PCR.

The GenBank/EMBL/DDBJ accession numbers for the sequences determined in this study are AY552326, AY552327 and AY619557 (26S rRNA gene D1/D2 domain) and AY552328 and AY552329 (complete ITS region and 5·8S rRNA gene).

1987). During a survey on the occurrence of carotenogenic yeasts in aquatic environments of Argentinean north-western Patagonia, several ballistoconidia-forming yeasts of the genera *Sporobolomyces* and *Sporidiobolus* were found. Species of these genera prevailed in aquatic environments surrounded by dense forests and with a low anthropogenic influence (Libkind *et al.*, 2003). In that study, 64 carotenogenic yeast isolates were found to belong to six genera and 15 species. Four of these species were considered new to science, on the basis of their unique microsatellite-primed PCR (MSP-PCR) profiles and sequence analysis of the D1/D2 domains of the 26S rRNA gene. This report presents descriptions for two of those novel species, *Sporidiobolus longiusculus* and *Sporobolomyces patagonicus*, which belong to the order Sporidiobolales. It also represents the first description of novel yeast species from Patagonia, Argentina.

The yeast strains of the genera *Sporobolomyces* and *Sporidiobolus* used in this report were isolated from aquatic environments in north-western Patagonia, by filtering subsurface water samples as described by Libkind *et al.* (2003). These aquatic environments are oligotrophic to ultra-oligotrophic temperate water bodies of glacial origin and have been classified as warm monomictic with a period of summer stratification (Díaz *et al.*, 2000). Water samples were collected between December and January in 2001–2002 and in 2002–2003. Water temperatures at the time of collection ranged between 14 and 18 °C.

For microscopy and assays of sexual compatibility, we used the procedures described by Valério *et al.* (2002). The colours of the cultures were assessed using the Mycological Colour Chart of Rayner (1970). The physiological characterization was done according to Yarrow (1998). Additional assimilation tests using aldaric acids and aromatic compounds were performed as described by Fonseca (1992) and Sampaio (1999), respectively.

For PCR fingerprinting, the MSP-PCR technique was employed. The DNA extraction protocol, primers, PCR and electrophoresis conditions and gel image-analysis procedures used were those described by Sampaio *et al.* (2001). DNA–DNA reassociation experiments were carried out in a Gilford Response UV-VIS spectrophotometer as described by Sampaio *et al.* (2001). DNA sequence analysis, DNA extraction, PCR amplification, purification and cycle sequencing were performed according to the protocol of Sampaio *et al.* (2001). DNA was amplified using primers ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and LR6 (5′-CGC CAG TTC TGC TTA CC-3′). Cycle sequencing of the 600–650 bp region at the 5′ end of the 26S rRNA gene D1/D2 domains employed forward primer NL1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′) and reverse primer NL4 (5′-GGT CCG TGT TTC AAG ACG G-3′). The internal transcribed spacer (ITS) region was sequenced using the forward primer ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and the reverse primer ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). Sequences were obtained with an Amersham Pharmacia ALF express II automated sequencer, using standard protocols. Alignments were made with MegAlign (DNAstar) and were corrected manually. To estimate phylogenetic relationships, we applied the Bayesian Markov chain Monte Carlo method of phylogenetic inference (Larget & Simon, 1999) as implemented in the computer program MRBAYES (Huelsenbeck & Ronquist, 2001). This method allows the *a posteriori* estimation of probability that groups of taxa are monophyletic given the DNA alignment. Four incrementally heated simultaneous Monte Carlo Markov chains were run over 1 000 000 generations using the general time-reversible model (six rate classes) of DNA substitution, additionally assuming a portion of invariable sites with gamma-distributed substitution rates of the remaining sites (GTR+I+G), random starting trees, and default starting parameters of the DNA substitution model. Trees were sampled every 100 generations, resulting in an overall sampling of 10 000 trees. From those trees that were sampled after the process had reached a stationary stage (burnin = 2000), a consensus tree was computed to obtain *a posteriori* estimates for the probabilities. This Bayesian approach to phylogenetic analysis was repeated at least three times, always using random starting trees and default starting values for the model parameters to test the reproducibility of the results.

Novel ballistoconidia-producing species from Patagonia

A preliminary characterization of the isolates was carried out using the MSP-PCR fingerprinting method. A figure with DNA banding profiles is available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm. The patterns of strains CRUB 1044^T, CRUB 1138 and CRUB 1139 were very similar and were distinct from the profiles of the remaining strains included in the analysis. A second group was formed by strains CRUB 1038^T and CRUB 1043. The fingerprints of these two strains showed some resemblance with the profile of *Sporidiobolus pararoseus* CBS 484 but were distinct from the pattern of the type strain of this species (CBS 491^T). Mating experiments using the first group of strains (ballistoconidia and yeast cells depicted in Fig. 1d and e, respectively) resulted in the formation of true mycelium with clamp connections and teliospores (Fig. 1c), whereas mating experiments employing strains of the second group (yeast cells and ballistoconidia depicted in Fig. 2), or strains of this group and different mating types of *Sporidiobolus pararoseus*, invariably gave negative results. For the sexual species, germination of teliospores was obtained by transferring agar blocks with 3-month-old teliospores grown in SGA medium (soytone, 2 %, w/v; glucose, 2 %, w/v; agar, 1·5 %, w/v) to water agar (2 %, w/v). The basidia differed from those of the other species of the genus *Sporidiobolus* because of their unusual length (compare Fig. 1a with Fig. 1b). Whereas the basidial length of known species in this genus does not exceed 50 μm (Statzell-Tallman & Fell, 1998), the basidia of the novel species

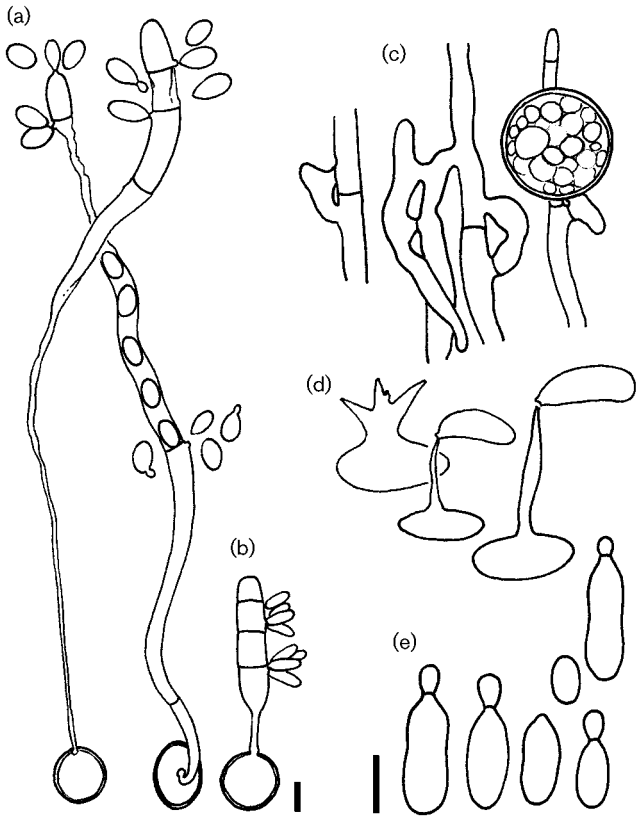

Fig. 1. Line drawings of *Sporidiobolus longiusculus* and comparison with *Sporidiobolus microsporus*. (a) Germinated teliospores, basidia and basidiospores of CBS 9654^T × CBS 9655. Note that the basidia are very long [compare with (b)] and one of them has endospores in one of its compartments. (b) Teliospore, basidium and basidiospores of *Sporidiobolus microsporus* CBS 7041^T. (c) Details of hyphae with clamp connections and teliospore of CBS 9654^T × CBS 9656. (d) Ballistoconidia-producing cells and ballistoconidia of CBS 9654^T. (e) Budding yeast cells of CBS 9654^T. Bars, 5 μm; bar beside (b) applies to parts (a) and (b); bar beside (e) applies to parts (c), (d) and (e).

reached 275 μm. The phylogenetic analysis of the D1/D2 domains and complete ITS sequences confirmed that the two groups of strains represent two undescribed species (Fig. 3).

Latin diagnosis of *Sporidiobolus longiusculus* Libkind, van Broock *et* Sampaio sp. nov.

Fungus dimorphus. Urediniomycetum, Microbotryomycetidarum, Sporidiobolalium. In statu unicellulari cellulae ovoideae ad cylindraceae, (2) 3–4 × 6–9 (10) μm. *Ballistoconidia ovoidea ad reniformia*, 2–3 × 5–7 μm. *Mycelium* 1·5–2 μm *diametro, conjugatione culturarum compatibilium procreatur, fibulatum. Teliosporae globosae*, 9–10 (12) μm, *ad ellipsoideae*, 9–10 × 9–12 μm. *Basidia transversalitre septata, longiuscula* (4–5 × 120–275 μm), *plerumque* 4 *cellulata. Basidiosporae ovoideae* (3–4 × 6–7 μm). *Characteres biochemici physiologicique in Tabula* 1 *et* http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm *describuntur. Positio phylogenetica in* Fig. 3 *illustratur.*

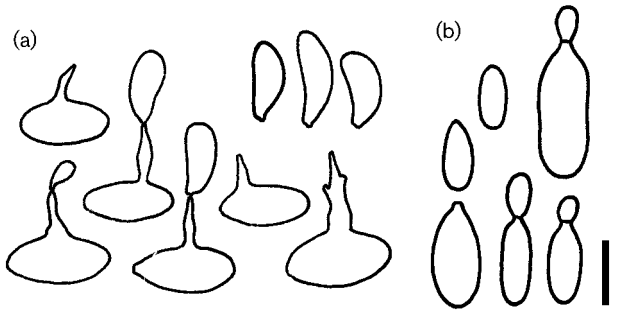

Fig. 2. Line drawings of *Sporobolomyces patagonicus* CBS 9657^T. (a) Ballistoconidia-producing cells and ballistoconidia. (b) Budding yeast cells. Bar, 5 μm.

Description of *Sporidiobolus longiusculus* Libkind, van Broock & Sampaio sp. nov.

Sporidiobolus longiusculus (long.i.us′cu.lus. L. masc. adj. *longiusculus* rather long, referring to the long basidia).

Dimorphic. Belonging to the class Urediniomycetes, subclass Microbotryomycetidae, order Sporidiobolales. Yeast cells after 1 week on MYP agar (malt extract, 0·7 %, w/v; yeast extract, 0·05 %, w/v; soytone peptone, 0·25 %, w/v; agar, 1·5 %, w/v) are ovoid to cylindrical, measuring (2) 3–4 × 6–9 (10) μm (Fig. 1e; additional images available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm). Ballistoconidia abundantly produced, ovoid to reniform, measuring 2–3 × 5–7 μm and originating at the end of sterigmata measuring 1–3 × 5–11 μm (Fig. 1d). After 1 month at room temperature, the streak culture is scarlet (Rayner, 1970), the surface is smooth and glistening, the texture is mucous and the margin is entire. Mycelium (1·5–2 μm in diameter) is formed after mating of sexually compatible strains. Clamp connections are present. Teliospores are spherical [9–10 (12) μm] to ellipsoidal (9–10 × 9–12 μm), terminal or intercalary. Basidia are transversally septate, not stalked, long (120–275 × 4–5 μm) and normally four-celled (Fig. 1a). Basidiospores are ovoid (3–4 × 6–7 μm) (Fig. 1a). The species is heterothallic and two mating types are known. The physiological and biochemical properties of *Sporidiobolus longiusculus* are depicted in Table 1 (available in tabular format at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm). The salient physiological features of *Sporidiobolus longiusculus* are depicted in Table 2 and its phylogenetic placement is shown in Fig. 3. The strains of *Sporidiobolus longiusculus* have been deposited at the Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, at the Portuguese Yeast Culture Collection (PYCC), Caparica, Portugal and at the Centro Regional

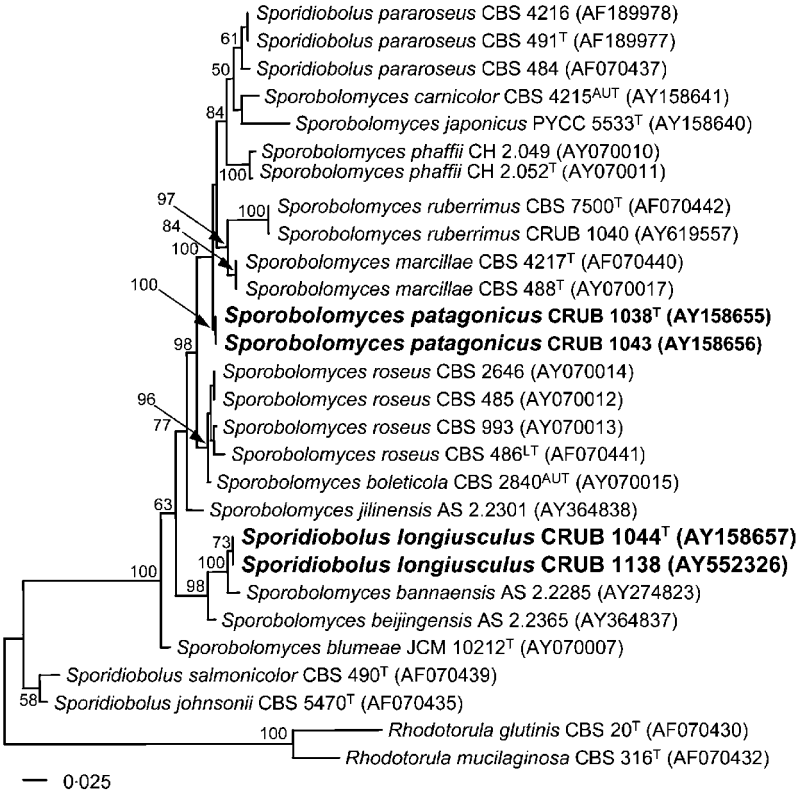

Fig. 3. Phylogenetic placement of *Sporidiobolus longiusculus* and *Sporobolomyces patagonicus* within the Sporidiobolales, on the basis of Bayesian Markov chain Monte Carlo analysis of an alignment of 26S rRNA gene (D1/D2 domain) sequences. Numbers on branches are *a posteriori* estimates for probabilities, i.e. probabilities that the respective groups are monophyletic, given the alignment (values lower than 50 % are not shown). The topology was rooted with *Rhodotorula glutinis* and *Rhodotorula mucilaginosa*. Names shown in bold type correspond to the organisms described in this report. GenBank/EMBL/DDBJ accession numbers of the sequences are given in parentheses.

Universitario Bariloche (CRUB), Bariloche, Argentina. Strain CBS 9654^T (=PYCC 5818^T=CRUB 1044^T) is designated as the type strain, and mating type A1 and strains CBS 9655 (=CRUB 1138) and CBS 9656 (=CRUB 1139) are assigned to mating type A2. All strains were isolated from subsurface water by D. Libkind. Strain CBS 9654^T was isolated in 2002 from Lake Fonck (41° 20′ S, 71° 37′ W) whereas CBS 9655 and CBS 9656 were isolated in 2003 from Lake Ilon (41° 11′ S, 71° 56′ W). The two lakes are located in the Nahuel Huapi National Park, Patagonia, Argentina.

Latin diagnosis of *Sporobolomyces patagonicus* Libkind, van Broock *et* Sampaio sp. nov.

Fungus Urediniomycetum, Microbotryomycetidarum, Sporidiobolalium. Cellulae zymosae ovoideae ad cylindraceae, 2–4 × 5–9 (11) μm. *Ballistoconidia ovoidea ad reniformia*, 2–4 × 3–7 μm. *Mycelium verum non formatur. Characteres biochemici physiologicique in Tabula* 1 *et* http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm *describuntur. Positio phylogenetica in* Fig. 3 *illustratur.*

Description of *Sporobolomyces patagonicus* Libkind, van Broock & Sampaio sp. nov.

Sporobolomyces patagonicus (pa.ta.go′ni.cus. N.L. masc. adj. *patagonicus* pertaining to Patagonia, referring to the name of the region from where the species was isolated).

Anamorphic yeast belonging to the class Urediniomycetes, subclass Microbotryomycetidae, order Sporidiobolales. Yeast cells after 1 week on MYP agar are ovoid to cylindrical, measuring 2–4 × 5–9 (11) μm (Fig. 2; additional images available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm). Ballistoconidia abundantly produced, ovoid to reniform, measuring 2–4 × 3–7 μm and originating at the end of sterigmata measuring 1–3 × 3–5 (8) μm (Fig. 2). True mycelium is not formed. After 1 month at room temperature, the streak culture is salmon-pink to scarlet (Rayner, 1970), the surface is smooth and glistening, the texture is mucous and the margin is entire. The physiological and biochemical properties of *Sporobolomyces patagonicus* are depicted in Table 1 (available in tabular format at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm). The salient physiological features of *Sporobolomyces patagonicus* are depicted in Table 2 and its phylogenetic placement is shown in Fig. 3. The strains have been deposited at the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands (CBS), at the Portuguese Yeast Culture Collection (PYCC) and at the Centro Regional Universitario Bariloche, Bariloche, Argentina (CRUB). Strain CBS 9657^T (=PYCC 5817^T=CRUB 1038^T) is designated the type strain. The two strains were isolated in 2002 from subsurface water by D. Libkind. Strain CBS 9657^T was isolated from Lake Fonck (41° 20′ S, 71° 37′ W), whereas CBS 9658 (=CRUB 1043) was isolated from Lake Hess (41° 36′ S, 71° 73′ W). The two lakes are located in the Nahuel Huapi National Park, Patagonia, Argentina.

Table 1. Physiological and biochemical characteristics of *Sporidiobolus longiusculus* and *Sporobolomyces patagonicus*

Taxa: 1, *Sporidiobolus longiusculus*; 2, *Sporobolomyces patagonicus*. Characteristics are scored as follows: +, growth; −, no growth; d, delayed growth; W, weak growth; V, variable growth. Both taxa were positive for assimilation of the carbon compounds D-glucose, sucrose, raffinose, melezitose, soluble starch, glucono-δ-lactone, D-gluconic acid, succinic acid and nitrogen compound L-lysine. Both taxa were positive for growth with vitamin-free medium, growth at 25 °C, splitting of arbutin, hydrolysis of urea and the diazonium blue B reaction. Both taxa were negative for fermentation of D-glucose, assimilation of the carbon compounds D-glucosamine, L-arabinose, L-rhamnose, melibiose, lactose, inulin, erythritol, xylitol, galactitol, inositol, D-glucuronic acid, DL-lactic acid, citric acid, L-tartaric acid, D-tartaric acid, *m*-tartaric acid, saccharic acid, mucic acid, methanol, vanillic acid, ferulic acid, veratric acid, gallic acid, salicylic acid, gentisic acid, catechol, phenol and the nitrogen compounds sodium nitrite, creatine and creatinine. Both taxa were negative for growth with 0·1 % cycloheximide, growth at 35 °C and formation of starch-like compounds.

Characteristic	1	2
Carbon compounds		
D-Galactose	+	d
L-Sorbose	+	d
D-Ribose	+	dW
D-Xylose	+	−
D-Arabinose	V	−
Maltose	+	V
α,α-Trehalose	+	V
Methyl α-D-glucoside	V	V
Cellobiose	V	V
Salicin	V	−
Glycerol	+	d
Ribitol	V	−
D-Glucitol	V	V
D-Mannitol	+	d
L-Malic acid	+	V
Ethanol	+	−
Protocatechuic acid	+	−
p-Hydroxybenzoic acid	+	−
m-Hydroxybenzoic acid	+	−
Nitrogen compounds		
Potassium nitrate	−	+
Ethylamine	−	+
Cadaverine	−	V
D-Glucosamine	+	−
Other tests		
Cycloheximide (0·01 %)	+	V
Growth at 30 °C	−	+

Table 2. Salient physiological differences between *Sporidiobolus longiusculus*, *Sporobolomyces patagonicus* and related ballistoconidia-producing taxa

Taxon	NO_3	Xylitol	D-Ribose	30 °C
Sporidiobolus longiusculus	−	−	+	−
Sporidiobolus pararoseus	−	−	−	+
Sporobolomyces marcillae	−	+	+	−
Sporobolomyces carnicolor	−	−	+	+
Sporobolomyces beijingensis	−	ND	−	+
Sporobolomyces blumeae	−	−	−	+
Sporobolomyces patagonicus	+	−	+	+
Sporobolomyces roseus	+	−	+	−
Sporobolomyces ruberrimus	+	−	−	−
Sporobolomyces bannaensis	+	ND	−	+
Sporobolomyces phaffii	+	ND	−	+
Sporobolomyces jilinensis	+	ND	−	+
Sporobolomyces japonicus	+	−	−	+

ND, Not determined.

Phylogenetic placement and salient physiological markers

Recent publications dealing with the core group of the genus *Sporobolomyces*, i.e. taxa assigned to the Sporidiobolales, are the description of *Sporobolomyces phaffii* and the re-evaluation of *Sporobolomyces roseus* and *Sporidiobolus pararoseus* (Bai *et al.*, 2002), the validation of *Sporobolomyces ruberrimus* (Fell *et al.*, 2002) and the descriptions of *Sporobolomyces bannaensis* (Zhao *et al.*, 2003), *Sporobolomyces beijingensis* and *Sporobolomyces jilinensis* (Wang & Bai, 2004). The phylogenetic analysis shown in Fig. 3 is based on sequence data (D1/D2 domains of the 26S rRNA gene) of representative species of the Sporidiobolales, including the recently described taxa mentioned above. The closest relative of *Sporidiobolus longiusculus* is *Sporobolomyces bannaensis* (four mismatches). The closest teleomorphic species is *Sporidiobolus pararoseus*. *Sporobolomyces patagonicus* occupied a somewhat isolated position and differed from *Sporobolomyces marcillae* by four base substitutions. Recent observations made by Bai *et al.* (2002) that D1/D2 and ITS sequence analyses may not be concordant for this group of yeasts encouraged us to study the ITS regions of the two novel species (phylogenetic tree available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm). As observed with the D1/D2 region, *Sporidiobolus longiusculus* maintained its close relationship with *Sporobolomyces bannaensis* but a higher number of nucleotide substitutions (17 mismatches) was observed. With respect to *Sporobolomyces patagonicus* no nucleotide differences were observed in the comparison with the type strain of *Sporidiobolus pararoseus*, whereas five mismatches were observed in the D1/D2 region for these two species. To elucidate the relationship between *Sporobolomyces patagonicus* and *Sporidiobolus pararoseus*, DNA–DNA reassociation experiments were performed between *Sporobolomyces patagonicus* CBS 9657^T and strains CBS 491^T and CBS 484 of *Sporidiobolus pararoseus*. Low DNA homology values (0–12 %) were recorded in the assays involving *Sporobolomyces patagonicus* and both strains of *Sporidiobolus pararoseus*, whereas DNA reassociation values

between CBS 491^T and CBS 484 were high and ranged between 95 and 100 %. Therefore, D1/D2 sequence data, DNA reassociation values and the absence of mating between *Sporobolomyces patagonicus* and *Sporidiobolus pararoseus* validate the proposal of *Sporobolomyces patagonicus* as a distinct species. Our results are similar to those of Bai *et al.* (2002) for the pair *Sporobolomyces ruberrimus* and *Sporobolomyces phaffii*. These two species belong also to the *Sporidiobolus pararoseus* complex and have more nucleotide differences in the D1/D2 domains (15 mismatches) than in the ITS region (three mismatches). In both situations, species with similar ITS sequences show more divergent D1/D2 sequences. Our data and also the results of Bai *et al.* (2002) suggest that, for the *Sporidiobolus pararoseus* species complex, D1/D2 data are more useful than ITS data for resolving species relationships.

The salient physiological differences between *Sporidiobolus longiusculus*, *Sporobolomyces patagonicus* and closely related ballistoconidia-producing taxa are presented in Table 2. *Sporidiobolus longiusculus* and *Sporidiobolus pararoseus* are the two nitrate-negative species of the genus *Sporidiobolus*. Other physiological features of *Sporidiobolus longiusculus* include limited utilization of aromatic compounds, growth with D-ribose and an inability to grow at 30 °C. *Sporobolomyces patagonicus* belongs to the group of nitrate-positive species. In contrast to its closest relatives, *Sporobolomyces patagonicus* is unable to utilize aromatic compounds, a rare characteristic among the Sporidiobolales.

Additional ballistoconidia-producing species from Patagonia

Other pink-coloured, ballistoconidial yeasts of the genera *Sporobolomyces* and *Sporidiobolus* were isolated from aquatic environments in north-western Patagonia. The MSP-PCR fingerprints of the novel isolates were compared with those of the various type strains (representative fingerprints and strain information are available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm). Four strains (CRUB 1039, CRUB 1051, CRUB 1052 and CRUB 1053) were identified as *Sporidiobolus salmonicolor*, six strains (CRUB 1040, CRUB 1041, CRUB 1137, CRUB 1182, CRUB 1184 and CRUB 1141) as *Sporobolomyces ruberrimus* and two strains (CRUB 1042 and CRUB 1140) as *Sporobolomyces roseus*. The type strain of *Sporidiobolus salmonicolor* (PYCC 4111^T) and the four Patagonian isolates showed similar MSP-PCR banding patterns using the $(GTG)_5$ primer. CRUB 1039 belongs to mating type A1 since true mycelium and teliospores were produced after mating with PYCC 4112 (mating type A2) but not when crossed with PYCC 4111^T (mating type A1). The other strains of the group were considered as anamorphs of *Sporidiobolus salmonicolor* since they failed to react with the different sexually compatible strains of this species.

Recently, *Sporobolomyces ruberrimus* was recognized as a distinct species, on the basis of morphological and molecular analyses (Fell *et al.*, 2002). However, the authors failed to observe the production of ballistoconidia in the strains under study. The absence of ballistoconidia was attributed to a loss of the genetic function or to a failure to provide adequate environmental conditions. The Patagonian isolates of *Sporobolomyces ruberrimus* were able to produce forcefully ejected conidia (images available at http://www.crem.fct.unl.pt/dimorphic_basidiomycetes/Databases/databases.htm). To confirm the identification of the Patagonian isolates, selected strains were studied by sequence analysis of the D1/D2 domains: no discrepancies were found with respect to the type strain of the species (Fig. 3). Our findings suggest that the inability to produce ballistoconidia reported by Fell *et al.* (2002) for two strains may be due to loss of the characteristic because of prolonged maintenance in the laboratory. Negative results were obtained in crossings involving the novel isolates and also strains PYCC 5678^T (=CBS 7500^T) and PYCC 5679 (=CBS 7501) of *Sporobolomyces ruberrimus*.

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