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FEMS Yeast Research 4 (2004) 579–586

FEMS Yeast Research

www.fems-microbiology.org

Four new yeast species of the genus *Sporobolomyces* from plant leaves

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Received 11 August 2003; received in revised form 12 November 2003; accepted 21 November 2003

First published online 15 January 2004

Abstract

Among the ballistoconidium-forming yeast strains isolated from various plant leaves collected in North and Northeast China, 12 strains forming orange to orange-red colored colonies were revealed to represent four novel species of the genus *Sporobolomyces* by conventional, chemotaxonomic and molecular phylogenetic studies, based on the 26S-rDNA D1/D2 domain and internal transcribed spacer (ITS) region sequences. *Sporobolomyces beijingensis* sp. nov., represented by eight strains (type strain CB 80^T = AS 2.2365^T = CBS 9730^T), and *Sporobolomyces jilinensis* sp. nov., represented by two strains (type strain CB 118^T = AS 2.2301^T = CBS 9728^T), clustered in the Johnsonii clade of the Sporidiobolus lineage. *Sporobolomyces clavatus* sp. nov., represented by strain CB 281^T (= AS 2.2318^T = CBS 9729^T), belonged to the Agaricostilbum lineage and showed a close relationship to *Sporobolomyces ruber* and *Sporobolomyces dracophylli*. *Sporobolomyces symmetricus* sp. nov., represented by strain CB 64^T (= AS 2.2299^T = CBS 9727^T), formed nearly symmetrical ballistoconidia and was closely related with *Sporobolomyces vermiculatus* and *Sporobolomyces gracilis* in the Gracilis clade of the Erythrobasidium lineage.

Keywords: *Sporobolomyces beijingensis* sp. nov.; *Sporobolomyces clavatus* sp. nov.; *Sporobolomyces jilinensis* sp. nov.; *Sporobolomyces symmetricus* sp. nov.

1. Introduction

In a survey of diversity in ballistoconidium-forming yeasts in the phyllosphere of North and Northeast China, more than one hundred strains forming ballistoconidia and orange or orange-red colored colonies were isolated from plant leaves collected from Baihua Mountain near Beijing and Changbai Mountain in Jilin Province, China, in October 1998. These strains were assigned to the genus *Sporobolomyces* by conventional and chemotaxonomical studies. On the basis of phenotypic characterization and grouping, representative strains were selected for molecular taxonomic studies. Twelve strains classified into four groups were revealed to represent four undescribed species by sequence analysis of the internal transcribed spacer (ITS) and 26S rDNA D1/D2 domains.

2. Materials and methods

2.1. Yeast strains

The strains studied are listed in Table 1. They were isolated from wilting leaves by using the improved ballistoconidia-fall method as described by Nakase and Takashima [1].

2.2. Conventional and chemotaxonomic characterization

Most of the morphological, physiological and biochemical characteristics were examined according to standard methods [2]. Assimilation of nitrogen compounds was investigated on solid media with starved inocula [3]. Extraction, purification and identification of

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doi:10.1016/j.femsyr.2003.11.002

Table 1
Yeast strains studied

Species	Strain	Source
Sporobolomyces beijingensis	CB 2	*Deutzia* sp., Baihua Mountain, Beijing
	CB 18	*Betula platyphylla*, Baihua Mountain, Beijing
	CB 22	*Lespedeza floridunda*, Baihua Mountain, Beijing
	CB 28	*Rabdosia japonica* var. *glaucocalyx*, Baihua Mountain, Beijing
	CB 39	*Aconitum kusnezoffii*, Baihua Mountain, Beijing
	CB 46	*Spodiopogon sibiricus*, Baihua Mountain, Beijing
	CB 69	*Aster ageratoides*, Baihua Mountain, Beijing
	CB 80^T	*Sorbus pohuashanensis*, Baihua Mountain, Beijing
Sporobolomyces clavatus	CB 281^T	*Sorbus pohuashanensis*, Changbai Mountain, Jilin
Sporobolomyces jilinensis	CB 118^T	*Pinus koraiensis*, Changbai Mountain, Jilin
	CB 137	*Tilia amurensis*, Changbai Mountain, Jilin
Sporobolomyces symmetricus	CB 64^T	*Betula platyphylla*, Baihua Mountain, Beijing

ubiquinones were carried out according to Yamada and Kondo [4].

2.3. Sequencing and molecular phylogenetic analysis

Nuclear DNA was extracted by using the method of Makimura et al. [5]. The DNA fragment covering the ITS region (including 5.8S rDNA) and 26S-rDNA D1/D2 domain was amplified with a pair of primers ITS1 (5′-GTC GTA ACA AGG TTT CCG TAG GTG-3′) and NL4 (5′-GGT CCG TGT TTC AAG ACG G-3′). The polymerase chain reaction (PCR) was performed using 36 cycles with denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min and extension at 72 °C for 1.5 min. After purification, the PCR products were directly sequenced with the forward primers ITS1 and NL1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′) and the reverse primers ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) and NL4 using the ABI BigDye terminator cycle sequencing kit. Electrophoresis was done on an ABI PRISM 377 DNA sequencer.

The sequences were aligned with the Clustal X program [6]. The phylogenetic trees were constructed from the evolutionary distance data calculated from Kimura's two-parameter model [7] by using the neighbor-joining method [8]. Bootstrap analysis [9] was performed from 1000 random resamplings. Reference sequences retrieved from GenBank are indicated in the trees, as are the accession numbers generated in this study.

3. Results and discussion

3.1. Morphology and physiology

The 12 ballistoconidium-forming yeast strains studied formed orange to orange-red colored colonies. All but CB 64 produced asymmetrical ballistoconidia. CB 64 formed nearly symmetrical ballistoconidia, which were relatively longer than those formed by species of the genus *Bullera* and other hymenomycetous, ballistoconidium-forming yeast species. The genus *Sporobolomyces* has been defined as forming typically asymmetrical ballistoconidia [10]. However, the original descriptions or pictures of the ballistoconidia of *Sporobolomyces coprosmae*, *Sporobolomyces gracilis*, *Sporobolomyces oryzicola* and *Sporobolomyces vermiculatus* showed that they formed nearly symmetrical ballistoconidia [11–14]. Strain CB 281 differed morphologically from the other strains studied by forming semi-dried and wrinkled cultures and pyriform to clavate vegetative cells.

The remaining 10 strains with similar morphological characters were classified into two groups by physiological tests. The first group included CB 80 and seven other strains, and the second group included CB 118 and CB 137. They differed in the assimilation reactions of nitrate, nitrite and cadaverine. In physiological characters, the former group was similar to the *Sporobolomyces roseus* complex, while the latter were similar to the *Sporidiobolus pararoseus* complex [10,15,16].

For each of the two groups including multiple strains, mating tests were performed on 5% malt extract agar and corn meal agar at 20 or 25 °C. Clamped hyphae or other sexual structures were not observed in the mating cultures within two months. Sexual structures were also looked for in the cultures of CB 64 and CB 118, using the same conditions but were not observed either.

3.2. Sequence analysis

In agreement with the grouping of the strains studied based on morphological and physiological characteristics, four groups represented by CB 64, CB 80, CB 118 and CB 281 were also recognized from the 26S-rDNA D1/D2 domain and ITS sequence comparisons. The strains in the CB 80 group were identical in the D1/D2 sequences. Except for CB 46, the strains in this group also had identical ITS sequences. CB 46 differed from the other strains in this group by only one nucleotide in the ITS region. CB 118 and CB 137 had identical D1/D2 sequences and differed by only one base in the ITS region.

The phylogenetic relationships of the representative strains CB 64, CB 80, CB 118 and CB 281 with closely related species were depicted in the trees drawn from the D1/D2 (Fig. 1(a)) and ITS (Fig. 1(b)) sequences. The positions of the four strains in these two trees were very similar. CB 80 and CB 118 clustered in the Johnsonii

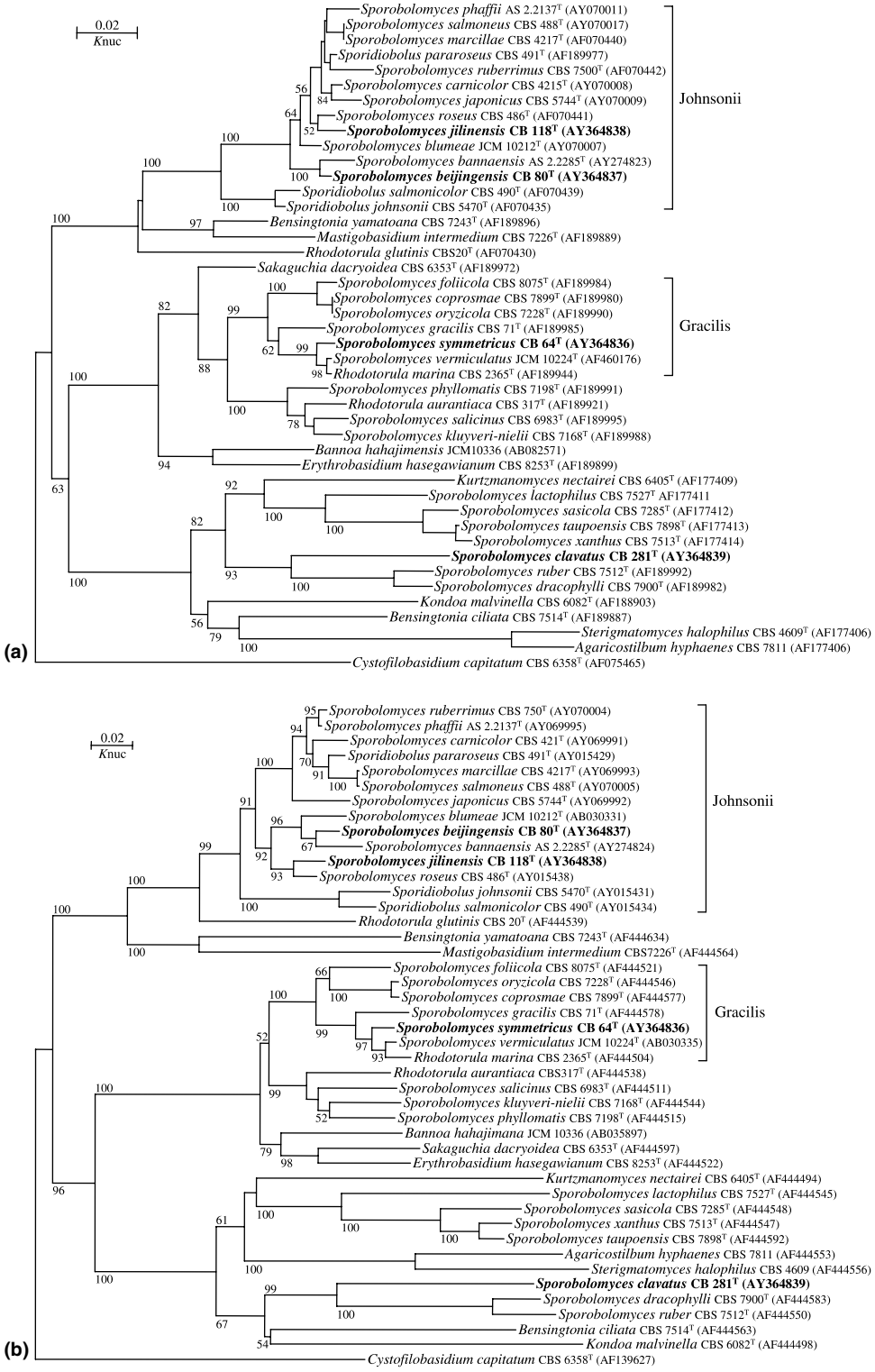

Fig. 1. Phylogenetic trees based on neighbor-joining analysis of (a) the 26S-rDNA D1/D2 domain and (b) the ITS region (including 5.8S rDNA) sequences, depicting the relationships of the four novel *Sporobolomyces* species with closely related taxa. Bootstrap percentages over 50% from 1000 bootstrap replicates are shown. Reference sequences retrieved from GenBank are indicated, as are the accession numbers of sequences of the new species.

clade of the Sporidiobolus lineage [17]. CB 80 was found to be closely related with *Sporobolomyces bannaensis*, a newly described species isolated from Yunnan Province, Southwest China [18]. This strain differed from *S. bannaensis* by 9 and 22 bases in the D1/D2 and ITS regions, respectively. CB 118 was most closely related with *S. roseus*. It differed from the type strain of *S. roseus* by 9 and 21 bases in the D1/D2 and ITS regions, respectively.

Strain CB 64 belonged to the Gracilis clade of the Erythrobasidium lineage and showed close relationships to *S. vermiculatus* and *Rhodotorula marina*. It differed from these two species by 7 and 18–21 bases in the D1/D2 and ITS regions, respectively. It is interesting to note that the other three *Sporobolomyces* species forming symmetric ballistoconidia (see above) were also clustered in this clade (Fig. 1). It is not clear if the remaining ballistoconidium-forming species in this clade, *S. foliicola*, formed symmetric ballistoconidia or not, as this was not indicated in the original description of the species [19] or other literature [10,16].

CB 281 clustered in the Agaricostilbum lineage. This strain clustered in a branch together with *Sporobolomyces ruber* and *S. dracophylli* with strong bootstrap support (Fig. 1). It differed from the latter two species by 65 and 71 nucleotides in the D1/D2 region, respectively. The divergences in the ITS sequences were even much higher.

The sequence comparisons demonstrated that the four groups represented by strains CB 64, CB 80, CB 118 and CB 281 represent four novel species in the genus *Sporobolomyces*, for which the names *Sporobolomyces symmetricus* sp. nov., *Sporobolomyces beijingensis* sp. nov., *Sporobolomyces jilinensis* sp. nov. and *Sporobolomyces clavatus* sp. nov. are proposed, respectively.

3.3. Descriptions of the new species

3.3.1. *Latin diagnosis of* Sporobolomyces beijingensis *F.Y. Bai et Q.M. Wang sp. nov.*

In liquido malti post dies 7 ad 17 °C, cellulae vegetativae, ellipsoideae, (3.0–6.2) × (5.0–10.0) μm, singulae. Annulus, pelliculum et sedimentum formantur. In agaro malti post unum mensem ad 17 °C, cultura rosea, glabra vel rugosa, butyracea vel viscida, nitida, margine glabra. In agaro farinae zeae pseudomycelium non formantur. Ballistosporae reniformes vel ellipsoidales, (2.5–5.0) × (6.2–8.0) μm.

Fermentatio nulla. Glucosum, L-sorbosum (lente et exigue), saccharosum, maltosum, cellobiosum, trehalosum, raffinosum, melezitosum, amylum solubile, D-arabinosum (lente), ethanolum, glycerolum (variabile), D-mannitolum, D-glucitolum (lente), salicinum (variabile) et acidum succinicum assimilantur at non galactosum, lactosum, melibiosum, inulin, D-xylosum, L-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, methanolum, erythritolum, ribitolum, galactitolum, methyl α-D-glucosidum, acidum DL-lacticum, acidum citricum, inositolum nec hexadecanum. Ammonium sulfatum et L-lysinum assimilantur at non kalium nitricum, natrum nitrosum, ethylaminum nec cadaverinum. Maxima temperatura crescentiae 32–34 °C. Materia amyloidea iodophila non formantur. Ad crescentiam vitaminum non necessarium est. Urea finditur. Diazonium caeruleum B positivum. *Ubiquinonum majus*: Q-10. Typus: Isolatus ex folio *Sorbus pohuashanensis* (Hance) Hedl., CB 80^T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.2365^T).

3.3.2. *Description of* Sporobolomyces beijingensis *F.Y. Bai et Q.M. Wang sp. nov.*

In malt extract, after 7 days at 17 °C, the cells are ellipsoidal and occur singly, (3.0–6.2) × (5.0–10.0) μm (Fig. 2(a)). A ring, pellicle and sediment are formed. On malt extract agar, after 1 month at 17 °C, the streak culture is butyrous, reddish orange, smooth or slightly wrinkled, glistening. The margin is entire or erose. Pseudohyphae are not formed in Dalmau plate culture on corn meal agar. On corn meal agar, ballistoconidia are produced abundantly, and asymmetric, reniform or ellipsoidal, (2.5–5.0) × (6.2–8.0) μm (Fig. 2(b)).

Fermentation is negative. Glucose, L-sorbose (delayed and weak), sucrose, maltose, cellobiose, trehalose, raffinose, melezitose, soluble starch, D-arabinose (delayed), ethanol, glycerol (variable), D-mannitol, D-glucitol (delayed), salicin (variable) and succinic acid are assimilated. Galactose, lactose, melibiose, inulin, D-xylose, L-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, erythritol, ribitol, galactitol, methyl α-D-glucoside, DL-lactic acid, citric acid, inositol

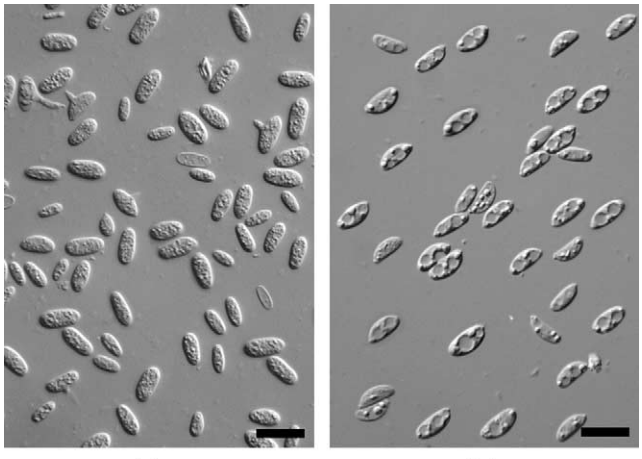

Fig. 2. *Sporobolomyces beijingensis* sp. nov. CB 80^T (a) vegetative cells grown in YM broth for 5 days at 17 °C and (b) ballistoconidia produced on corn meal agar after 5 days at 20 °C. Bars indicate 10 μm.

and hexadecane are not assimilated. Ammonium sulfate and L-lysine are assimilated. Potassium nitrate, sodium nitrite, ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Growth in vitamin-free medium is positive. Maximum growth temperature is 32–34 °C. Starch-like compounds are not produced. Urease activity is positive. Diazonium Blue B reaction is positive. Growth on 50% (w/w) glucose-yeast extract agar is positive. The major ubiquinone is Q-10. The type strain, CB 80^T, was isolated from a wilting leaf of *Sorbus pohuashanensis* (Hance) Hedl. collected in Baihua Mountain, Beijing in October 1998. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.2365^T, and in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as CBS 9730^T.

Etymology: The specific epithet *beijingensis* refers to the geographic origin of the species.

Physiologically, *Sporobolomyces beijingensis* sp. nov. differs from the taxa in the *Sporidiobolus pararoseus* complex in methyl α-D-glucoside assimilation reaction. The new species differs from its closest relative *S. bannaensis* by its negative assimilation reactions of inulin, methyl α-D-glucoside, potassium nitrate and sodium nitrite and positive assimilation reaction of L-lysine.

3.3.3. Latin diagnosis of Sporobolomyces clavatus *F.Y. Bai et Q.M. Wang sp. nov.*

In YM (Difco) liquido post dies 7 ad 17 °C, cellulae vegetativae clavatae vel pyriformes, (2.0–7.5) × (7.7–17.4) μm, singulae. Sedimentum formantur. In agaro YM post unum mensem ad 17 °C, cultura rubro-aurantiaca, rugosa, non-nitida, margine erosa. In agaro farinae zeae pseudomycelium et mycelium formantur. Ballistosporae reniformes vel falcatas, (2.5–4.9) × (5.0–12.4) μm. Fermentatio nulla. Glucosum, saccharosum, maltosum, cellobiosum, trehalosum, melibiosum, raffinosum, melezitosum, amylum solubile, D-xylosum (lente), D-ribosum (lente et exigue), ribitolum, D-mannitolum et D-glucitolum assimilantur at non galactosum, L-sorbosum, lactosum, inulin, L-arabinosum, D-arabinosum, L-rhamnosum, D-glucosaminum, methanolum, ethanolum, glycerolum, erythritolum, galactitolum, methyl α-D-glucosidum, salicinum, acidum DL-lacticum, acidum succinicum, acidum citricum, inositolum nec hexadecanum. Ammonium sulfatum, ethylaminum et cadaverinum assimilantur at non kalium nitricum, natrum nitrosum nec L-lysinum. Vitaminae externae ad crescentiam necessaria sunt. Maxima temperatura crescentiae 25 °C. Materia amyloidea iodophila non formantur. Diazonium caeruleum B positivum. *Ubiquinonum majus*: Q-10. Typus: Isolatus ex folio *Sorbus pohuashanensis* (Hance) Hedl., CB 281^T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.2318^T).

3.3.4. Description of Sporobolomyces clavatus *F.Y. Bai et Q.M. Wang sp. nov.*

In YM broth, after 7 days at 17 °C, cells are clavate, pyriform or elongate, and occur singly, (2.0–7.5) × (7.7–17.4) μm (Fig. 3(a)). Budding is polar. Sediment is formed. On YM agar, after 1 month at 17 °C, the streak culture is orange-red, ridged and dull. The margin is eroded. In Dalmau plate culture on corn meal agar, pseudohyphae and true hyphae are formed. Ballistoconidia are produced on corn meal agar, and are reniform to falcate, (2.5–4.9) × (5.0–12.4) μm (Fig. 3(b)).

Fermentation is negative. Glucose, sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch, D-xylose (delayed), D-ribose (delayed and weak), ribitol, D-mannitol and D-glucitol are assimilated. Galactose, L-sorbose, lactose, inulin, L-arabinose, D-arabinose, L-rhamnose, D-glucosamine, methanol, ethanol, glycerol, erythritol, galactitol, methyl α-D-glucoside, salicin, DL-lactic acid, succinic acid, critic acid, inositol and hexadecane are not assimilated. Ammonium sulfate, cadaverine dihydrochloride and ethylamine hydrochloride are assimilated. Potassium nitrate, sodium nitrite and L-lysine are not assimilated. Maximum growth temperature is 25 °C. Growth in vitamin-free medium is negative. Starch-like substances are not produced. Growth on 50% (w/w) glucose-yeast extract agar is negative. Diazonium Blue B reaction is positive. The major ubiquinone is Q-10. The type strain of, CB 281^T, was isolated from a wilting leaf of *Sorbus pohuashanensis* (Hance) Hedl. collected in Changbai Mountain, Jilin Province, China in October 1998. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.2318^T, and in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as CBS 9729^T.

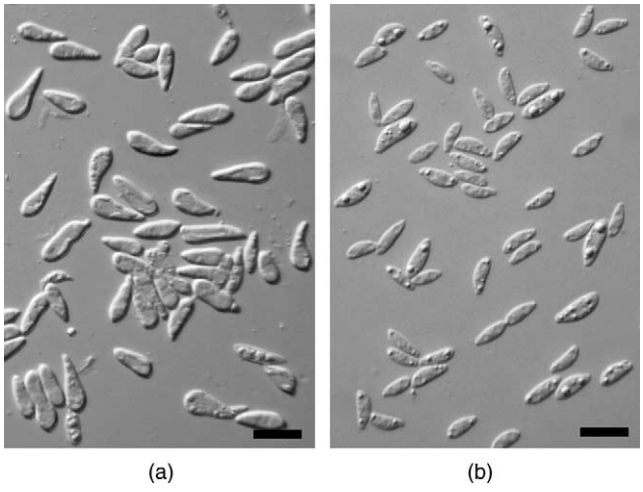

Fig. 3. *Sporobolomyces clavatus* sp. nov. CB 281^T (A) vegetative cells grown in YM broth for 5 days at 17 °C and (B) ballistoconidia produced on corn meal agar after 5 days at 20 °C. Bars indicate 10 μm.

Etymology: The specific epithet *clavatus* refers to the shape of vegetative cells of the species.

The morphology of *S. clavatus* sp. nov. is unique because it forms pyriform to clavate vegetative cells (Fig. 3(a)). The new species is also unusual physiologically. It did not grow on standard Christensen's urea agar, nor in the standard method using Difco Bacto Urea R Broth [2]. Thus, the urea hydrolysis test was negative. In other physiological properties, *S. clavatus* differs from its closely related species *S. ruber* and *S. dracophylli* in the assimilation reactions of trehalose, soluble starch, D-xylose, succinic acid, ethylamine and cadaverine.

3.3.5. Latin diagnosis of Sporobolomyces jilinensis *F.Y. Bai et Q.M. Wang sp. nov.*

In liquido malti post dies 7 ad 17 °C, cellulae ellipsoideae vel cylindraceae, (2.5–5.0) × (5.0–10.0) μm, singulae aut binae. Annulus, pelliculum et sedimentum formantur. In agaro malti post unum mensem ad 17 °C, cultura aurantiaca, glabra, butyracea vel viscida, nitida, margine glabra. In agaro farinae zeae pseudomycelium non formantur. Ballistosporae reniformes, (2.5–5.0) × (5.0–8.0) μm. Fermentatio nulla. Glucosum, galactosum, L-sorbosum (lente et exigue), saccharosum, maltosum, trehalosum, raffinosum, melezitosum, amylum solubile, D-xylosum (lente et exigue), ethanolum, glycerolum (lente), D-mannitolum, D-glucitolum, methyl α-D-glucosidum, acidum succinicum et hexadecanum (variabile) assimilantur at non cellobiosum, lactosum, melibiosum, inulin, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, methanolum, erythritolum, ribitolum, galactitolum, salicinum, acidum DL-lacticum, acidum citricum nec inositolum. Ammonium sulfatum, natrum nitrosum, kalium nitricum, cadaverinum et L-lysinum assimilantur at non ethylaminum. Maxima temperatura crescentiae 33 °C. Materia amyloidea iodophila non formantur. Ad crescentiam vitaminum non necessarium est. Urea finditur. Diazonium caeruleum B positivum. *Ubiquinonum majus*: Q-10. Typus: Isolatus ex folio *Pinus koraiensis* Sieb. & Zucc., CB 118^T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.2301^T).

3.3.6. Description of Sporobolomyces jilinensis *F.Y. Bai et Q.M. Wang sp. nov.*

In malt extract, after 7 days at 17 °C, the cells are ellipsoidal to cylindrical, occur singly or in pairs, (2.5–5.0) × (5.0–10.0) μm (Fig. 4(a)). A ring, pellicle and sediment are formed. On malt extract agar, after 1 month at 17 °C, the streak culture is butyrous to mucoid, orange, smooth and glistening with an entire margin. Pseudohyphae are not formed in Dalmau plate culture on corn meal agar. On corn meal agar, ballistoconidia are formed abundantly, reniform, (2.5–5.0) × (5.0–8.0) μm (Fig. 4(b)).

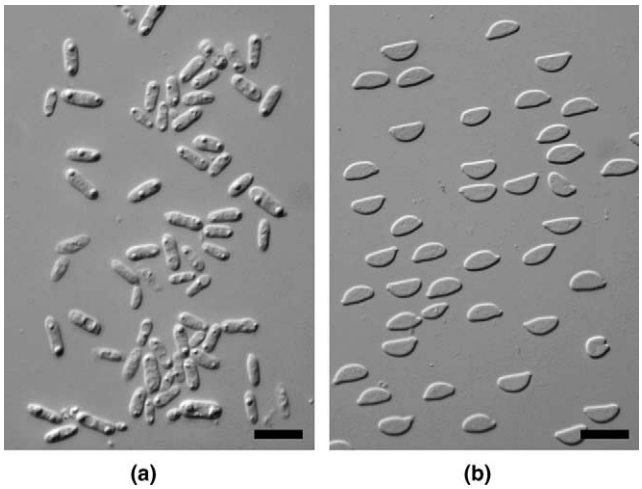

Fig. 4. *Sporobolomyces jilingensis* sp. nov. CB 118^T (a) vegetative cells grown in YM broth for 5 days at 17 °C and (b) ballistoconidia produced on corn meal agar after 5 days at 20 °C. Bars indicate 10 μm.

Fermentation is negative. Glucose, galactose, L-sorbose (delayed and weak), sucrose, maltose, trehalose, raffinose, melezitose, soluble starch, D-xylose (delayed and weak), ethanol, glycerol (delayed), D-mannitol, D-glucitol, methyl α-D-glucoside, succinic acid and hexadecane (variable) are assimilated. Cellobiose, lactose, melibiose, inulin, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, erithritol, ribitol, galactitol, salicin, DL-lactic acid, citric acid and inositol are not assimilated. Ammonium sulfate, sodium nitrite, potassium nitrate, L-lysine and cadaverine dihydrochloride are assimilated. Ethylamine hydrochloride is not assimilated. Growth in vitamin-free medium is positive. Maximum growth temperature is 33 °C. Starch-like compounds are not produced. Urease activity is positive. Diazonium Blue B reaction is positive. Growth on 50% (w/w) glucose-yeast extract agar is negative. The major ubiquinone is Q-10. The type strain, CB 118^T, was isolated from a wilting leaf of *Pinus koraiensis* Sieb. & Zucc. collected in Changbai Mountain, Jilin Province, China in October 1998. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.2301^T, and in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as CBS 9728^T.

Etymology: The specific epithet *jilinensis* refers to the geographic origin of the species.

Sporobolomyces jilinensis sp. nov. cannot be distinguished from taxa in the *Sporobolomyces roseus* complex by phenotypic characters.

3.3.7. Latin diagnosis of Sporobolomyces symmetricus *F.Y. Bai et Q.M. Wang sp. nov.*

In YM (Difco) liquido post dies 7 ad 17 °C, cellulae vegetativae ellipsoideae vel ovoideae, (2.5–5.2) × (3.7–

7.7) μm, singulae, sedimentum formantur. In agaro YM post unum mensem ad 17 °C, cultura aurantiaca aut rugosa, glabra, butyracea, nitida, margine glabra. Pseudomycelium non formantur. Ballistosporae ellipsoideae vel amygdaloideae, (2.5–5.0) × (5.0–7.5) μm. Fermentatio nulla. Glucosum, saccharosum, cellobiosum, trehalosum, melezitosum, glycerolum, ribitolum et acidum succinicum assimilantur at non galactosum, L-sorbosum, maltosum, lactosum, melibiosum, raffinosum, inulin, amylum solubile, D-xylosum, Larabinosum, D-arabinosum, D-ribosum, L-rhamnosum, D-glucosaminum, methanolum, ethanolum, erythritolum, galactitolum, D-mannitolum, D-glucitolum, methyl α-D-glucosidum, salicinum, acidum DL-lacticum, acidum citricum, inositolum nec hexadecanum. Ammonium sulfatum et ethylaminum assimilantur at non kalium nitricum, natrum nitrosum, L-lysinum nec cadaverinum. Maxima temperatura crescentiae 30 °C. Materia amyloidea iodophila non formantur. Ad crescentiam vitaminum non necessarium est. Urea finditur. Diazonium caeruleum B positivum. *Ubiquinonum majus*: Q-10. Typus: Isolatus ex folio *Betula platyphylla* Suk, CB 64^T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.2299^T).

3.3.8. Description of Sporobolomyces symmetricus *F. Y. Bai et Q. M. Wang sp. nov.*

In YM broth, after 7 days at 17 °C, the cells are ellipsoidal or ovoid, and occur singly, (2.5–5.2) × (3.7–7.7) μm (Fig. 5(a)). Budding is polar. Sediment is formed. On YM agar, after 1 month at 17 °C, the streak culture is orange-red, butyrous, smooth, and shining. The margin is entire. In Dalmau plate culture on corn meal agar, pseudohyphae are not formed. Ballistoconidia are produced abundantly on corn meal agar, and are symmetrical, ellispsoidal or amygdaloid, (2.5–5.0) × (5.0–7.5) μm (Fig. 5(b)).

Fermentation is negative. Glucose, sucrose, cellobiose, trehalose, melezitose, glycerol, ribitol and succinic acid are assimilated. Galactose, L-sorbose, maltose, lactose, melibiose, raffinose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, ethanol, erythritol, galactitol, D-mannitol, D-glucitol, methyl α-D-glucoside, salicin, DL-lactic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate and ethylamine hydrochloride are assimilated. Potassium nitrate, sodium nitrite, L-lysine and cadaverine dihydrochloride are not assimilated. Growth in vitamin-free medium is positive. Maximum growth temperature is 30 °C. Starch-like compounds are not produced. Urease activity is positive. Diazonium Blue B reaction is positive. Growth on 50% (w/w) glucose-yeast extract agar is negative. The major ubiquinone is Q-10. The type strain, CB 64^T, was isolated from a wilting leaf of *Betula platyphylla* Suk. collected in Baihua Mountain, Beijing, China in October 1998. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.2299^T, and in the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as CBS 9727^T.

Etymology: The specific epithet *symmetricus* refers to the shape of ballistoconidia produced by the species.

Phylogenetically, *S. symmetricus* sp. nov. is more closely related to *S. vermiculatus* than to *S. gracilis* (Fig. 1). Phenotypically, however, the new species is similar to *S. gracilis*. S.*symmetricus* sp. nov. differs from *S. gracilis* in positive assimilation reactions of sucrose, melezitose, and ethylamine and negative assimilation reactions of D-xylose, D-ribose and D-mannitol.

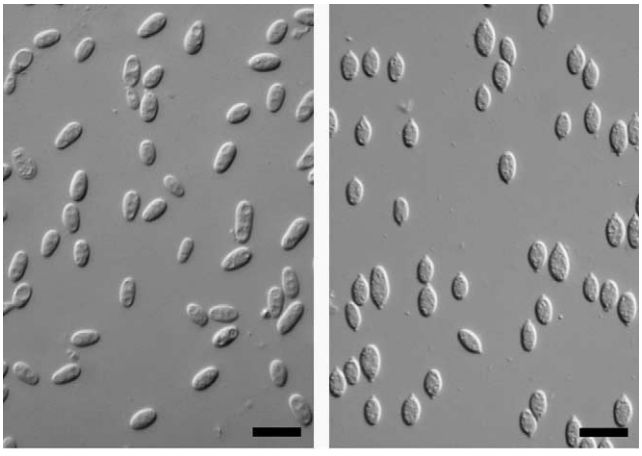

Fig. 5. *Sporobolomyces symmetricus* sp. nov. CB 64^T (a) vegetative cells grown in YM broth for 5 days at 17 °C and (b) ballistoconidia produced on corn meal agar after 5 days at 20 °C. Bars indicate 10 μm.

Acknowledgements

This study was supported by Grant No. 30170002 from the National Natural Science Foundation of China (NSFC), No. KSCX2-SW-101C from the Chinese Academy of Sciences and No. 2001AA227131 of the '863 program' from the Ministry of Science and Technology, China.

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