Derxomyces amylogenes sp. nov., *Derxomyces bambusicola* sp. nov. and *Derxomyces corylopsis* sp. nov., three ballistoconidium-forming yeast species isolated from subtropical plant leaves

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Among ballistoconidium-forming yeast strains isolated from various plant leaves collected from subtropical forests in eastern and central China, four strains forming cream to yellowish coloured colonies were revealed to represent three novel *Derxomyces* species by conventional and molecular characterization. Phylogenetic analysis based on combined sequences of the internal transcribed spacer (ITS) and 26S rRNA gene D1/D2 domain showed that strains GT-753 and ZJJ-890^T were conspecific and closely related to *Derxomyces boninensis*, *Derxomyces mrakii* and *Derxomyces qinlingensis*. Strain ZJJ-394^T was basal to the branch formed by *Derxomyces komagatae*, *Derxomyces pseudoschimicola* and *Derxomyces schimicola* with strong bootstrap support. Strain GT-475^T was closely related to *Derxomyces linzhiensis*. The strains differed significantly from their close relatives in D1/D2 and ITS sequences and in physiological criteria. Three novel species are proposed: *Derxomyces amylogenes* sp. nov. (type strain ZJJ-890^T=CGMCC 2.4407^T=CBS 12233^T), *Derxomyces bambusicola* sp. nov. (type strain GT-475^T=CGMCC 2.4411^T=CBS 12234^T) and *Derxomyces corylopsis* sp. nov. (type strain ZJJ-394^T=CGMCC 2.4409^T=CBS 12259^T).

The genus *Bullera* is highly polyphyletic and its species belong to the orders Tremellales, Filobasidiales and Trichosporonales (Fell *et al.*, 2000; Fungsin *et al.*, 2006; Nakase *et al.*, 2002; Wang *et al.*, 2004; Scorzetti *et al.*, 2002). Reclassification of *Bullera* and related genera of basidiomycetous yeasts has been under way and three new genera (*Derxomyces*, *Hannaella* and *Mingxiaea*) have been proposed for the *Bullera* species that belong to three clades of the Tremellales (Wang & Bai, 2008; Wang *et al.*, 2011). The number of *Derxomyces* species that formerly belonged to the *Bullera mrakii* clade has increased rapidly in recent years (Bai *et al.*, 2001; Wang & Bai, 2008; Wang *et al.*, 2004), suggesting that members of the genus are commonly distributed in nature. Of the currently recognized 20 *Derxomyces* species, 12 were described from China: seven species from tropical regions, two species from subtropical regions and three species from temperate regions in China (Wang & Bai, 2008). Compared with tropical and

temperate regions, subtropical regions of China have rarely been sampled in our study on phylloplane yeast diversity. Therefore, we have increased the number of samples collected from subtropical forests in recent years. Approximately 1700 strains were isolated from 80 plant leaf samples collected in Zhejiang and Hunan provinces located in eastern and central China, respectively. A total of 82 species (including representatives of 27 proposed novel species) belonging to 31 genera of basidiomycetous yeasts were identified based on 26S rRNA gene D1/D2 domain sequence analysis. Four of the strains representing three novel *Derxomyces* species were subjected to further characterization; it is proposed that they are representatives of three novel species, *Derxomyces amylogenes* sp. nov., *Derxomyces bambusicola* sp. nov. and *Derxomyces corylopsis* sp. nov.

The yeast strains studied are listed in Table 1. They were isolated from wilting leaves of different plants by using the improved ballistoconidia-fall method (Nakase & Takashima, 1993) in July and August, 2010. Morphological, physiological and biochemical characteristics were examined according to standard methods (Yarrow, 1998).

Nuclear DNA was extracted by the method of Makimura *et al.* (1994). PCR amplification and sequencing of the

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the ITS region and 26S rRNA gene D1/D2 domain sequences of strains ZJJ-890^T, ZJJ-394^T and GT-475^T are HQ890372, HQ890374 and HQ890376, respectively.

Table 1. Yeast strains studied

Species	Strain	Other designation	Source
<i>Derxomyces amylogenes</i> sp. nov.	GT-753	CGMCC 2.4406	Leaf of <i>Pinus</i> sp., Gutian Mountain, Zhejiang, China
	ZJJ-890 ^T	CGMCC 2.4407 ^T , CBS 12233 ^T	Leaf of <i>Acer</i> sp., Tianmen Mountain, Zhangjiajie, Hunan, China
<i>Derxomyces bambusicola</i> sp. nov.	GT-475 ^T	CGMCC 2.4411 ^T , CBS 12234 ^T	Leaf of bamboo (<i>Phyllostachys heterocycla</i>), Gutian Mountain, Zhejiang, China
<i>Derxomyces corylopsis</i> sp. nov.	ZJJ-394 ^T	CGMCC 2.4409 ^T , CBS 12259 ^T	Leaf of <i>Corylopsis</i> sp., Tianzi Mountain, Zhangjiajie, Hunan, China

DNA fragment covering the internal transcribed spacer (ITS) region (including the 5.8S rRNA gene) and 26S rRNA gene D1/D2 domain were performed using methods described previously (Bai *et al.*, 2002). Sequences were aligned using the program CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic trees were constructed from evolutionary distance data calculated from Kimura's two-parameter model (Kimura, 1980) by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analyses (Felsenstein, 1985) were performed from 1000 random resamplings.

Phylogenetic analysis

Phylogenetic analysis based on the combined sequences of the ITS region (including 5.8S rRNA gene) and the 26S rRNA gene D1/D2 domain placed strains GT-475^T, GT-753, ZJJ-394^T and ZJJ-890^T in the *Derxomyces* clade with strong bootstrap support (Fig. 1). Strain GT-475^T was most closely related to *Derxomyces linzhiensis*, but differed from the latter by 14 (2.3 %) mismatches (12 substitutions and 2 indels) in the D1/D2 domain and by 52 (18.2 %) mismatches (29 substitutions and 23 indels) in the ITS region. Strain ZJJ-394^T was located in a branch together with *Derxomyces komagatae*, *Derxomyces pseudoschimicola* and *Derxomyces schimicola* with 99 % bootstrap support. Strain ZJJ-394^T differed from these three species by 11–13 (1.6–1.9 %) mismatches (8–9 substitutions and 3–5 indels) in the D1/D2 domain and 50–74 (15.5–23.0 %) mismatches (25–42 substitutions and 25–32 indels) in the ITS region.

Strains GT-753 and ZJJ-890^T had identical D1/D2 sequences and differed by only one substitution in the ITS region, thus indicating their conspecificity. They were closely related to *Derxomyces boninensis*, *Derxomyces mrakii* and *Derxomyces qinlingensis* (Fig. 1). They differed from the type strain of *D. boninensis*, the closest relative, by 7 (1.0 %) substitutions in the D1/D2 domain and 38–39 (11.4–11.7 %) mismatches (26 substitutions and 14 indels) in the ITS region.

Compared with the result of Wang & Bai (2008), addition of the novel strains to the tree drawn from combined D1/D2 and ITS sequences (Fig. 1) slightly changed the positions of a few *Derxomyces* species, for example, the relative relationships between *D. linzhiensis*, *Derxomyces hubeiensis* and *Derxomyces simaoensis*. However, these species still clustered in the same subclade with strong

bootstrap support. Likewise, the other subclades of *Derxomyces* remained stable. The clear separation of the entire clade of *Derxomyces* from closely related clades was also maintained, supporting the distinction of the genus.

Morphology and physiology

The four strains studied formed yellowish colonies with butyrous or wrinkled textures, which were similar to the colonies formed by other *Derxomyces* species (Wang & Bai, 2008). They reproduced asexually by forming polar or multilateral budding cells and rotationally symmetrical ballistoconidia (Fig. 2). Physiological differences between the novel species and closely related taxa are shown in Table 2. Specifically, strain GT-475^T could be differentiated from its close relative *D. linzhiensis* by assimilation reactions of D-arabinose, D-glucitol, inositol, soluble starch and ethylamine. Strain ZJJ-394^T could be distinguished from its close relatives by its negative assimilation reactions for D-ribose, L-rhamnose, D-glucitol and inositol and positive assimilation reaction of inulin. Strains GT-753 and ZJJ-890^T differed from closely related species by their ability to form starch-like substances and to assimilate inulin.

The molecular and phenotypic comparisons described above indicate that the four strains studied represent three novel *Derxomyces* species, for which the names *Derxomyces amylogenes* sp. nov. (strains GT-753 and ZJJ-890^T), *Derxomyces bambusicola* sp. nov. (strain GT-475^T) and *Derxomyces corylopsis* sp. nov. (strain ZJJ-394^T) are proposed. Although the latter two species are represented by only one strain each, their distinction from close relatives is quite clear (Fig. 1). The formal descriptions of these species will be helpful for understanding the phylogenetic diversity and geographical distribution of species in the recently established genus *Derxomyces* (Wang & Bai, 2008).

Latin diagnosis of *Derxomyces amylogenes* F.-Y. Bai, X.-Z. Liu & T. Boekhout sp. nov.

In YM (Difco) liquido post dies 7 ad 20 °C, cellulae vegetativae ovoideae aut ellipsoideae, (2.5–6.5 × 4.7–10.0 µm), singulae aut binae. Annulus et sedimentum formantur. In agar YM post unum mensem ad 20 °C, cultura flavae, non nitida, glabra

Fig. 1. Phylogenetic tree drawn from neighbour-joining analysis of the combined sequences of the ITS region (including 5.8S rRNA gene) and the 26S rRNA gene D1/D2 domain, depicting the relationship of the three novel *Derxomyces* species with closely related taxa. Bootstrap percentages below 50% are not shown. Sequences are from type strains of the species compared, either determined in this study (in bold) or retrieved from GenBank under the accession numbers indicated. Bar, 0.005 substitutions per nucleotide position.

vel rugosa, *butyracea*, *marginata*, *glabra*. Mycelium et pseudo-mycelium non formantur. Ballistoconidia subglobosa, ellipsoideae vel napiformia (5.0–8.7 × 5.0–8.4 µm). Fermentatio nulla. Glucosum, galactosum, L-sorbosum (lente et exigue vel infirme), cellobiosum, maltosum, melibiosum, sucrosum, trehalosum, melezitosum, raffinose, D-arabiosum (lente), L-arabiosum, D-ribosum (vel lente), L-rhamnosum (infirmum), D-xylosum, erythritolum (varium), galactitolum (lente et exigue), D-glucitolum (lente et exigue), inositolum (lente vel infirmum), D-mannitolum (lente vel infirmum), ribitolum (lente et exigue), acidum citricum (vel lente et exigue), acidum succinicum (vel lente), methyl α-D-glucoside (vel lente), salicinum (infirmum) et inulinum (lente) assimilantur, at non lactosum, ethanolum, glycerolum, methanolum, acidum DL-lacticum, D-glucosaminum nec hexadecanum. Ammonium sulfatum, kalium nitricum (lente et exigue) et L-lysinum

assimilantur, at non natrum nitrosum, ethylaminum nec cadaverinum. Vitaminarum externarum ad crescentiam necessaria sunt. Maxima temperatura crescentiae: 25–29 °C. Materia amyloidea iodophila formantur. Urea finditur. Diazonium caeruleum B positivum. Typus: ZJJ-890^T, isolatus ex folio *Acer* sp., depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (=CGMCC 2.4407^T=CBS 12233^T).

Description of *Derxomyces amylogenes* F.-Y. Bai, X.-Z. Liu & T. Boekhout sp. nov.

Derxomyces amylogenes [a.my.lo.ge'nes. Gr. n. *amulon* starch; N.L. suff. -*genes* (from Gr. v. *gennaō* to produce) producing; N.L. masc. adj. *amylogenes* starch-producing, referring to the ability of the species to form starch-like compounds].

Fig. 2. Vegetative cells (a, c, e) grown in YM broth for 7 days at 20 °C and ballistoconidia (b, d, f) produced on cornmeal agar after 10 days at 20 °C. (a, b) *Derxomyces amylogenes* ZJJ-890^T; (c, d) *Derxomyces bambusicola* GT-475^T; (e, f) *Derxomyces corylopsis* ZJJ-394^T. Bars, 10 µm.

In YM broth, after 7 days at 20 °C, cells are ovoid and ellipsoidal ($2.5\text{--}6.5 \times 4.7\text{--}10.0$ µm) (Fig. 2a). Budding is singly or in pairs. Sediment and a ring are formed. On YM agar, after 1 month at 20 °C, the streak culture is yellow, butyrous, dull, smooth or somewhat wrinkled, and has an entire margin. In Dalmau plate culture on cornmeal agar,

hyphae and pseudohyphae are not formed. Ballistoconidia are produced on cornmeal agar and are subglobose, ellipsoidal or napiform ($5.0\text{--}8.7 \times 5.0\text{--}8.4$ µm) (Fig. 2b). Fermentation of glucose is negative. Glucose, galactose, L-sorbose (delayed and weak or slow positive), cellobiose, maltose, melibiose, sucrose, trehalose, melezitose, raffinose, D-arabinose (delayed), L-arabinose, D-ribose (positive or delayed), L-rhamnose (slow positive), D-xylose, erythritol (variable), galactitol (delayed and weak), D-glucitol (delayed and weak), inositol (delayed or slow positive), D-mannitol (delayed or slow positive), ribitol (delayed and weak), citric acid (positive or delayed and weak), succinic acid (positive or delayed), methyl α -D-glucoside (positive or delayed), salicin (slow positive), inulin (delayed) are assimilated. Lactose, ethanol, glycerol, methanol, DL-lactic acid, D-glucosamine and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate (delayed and weak) and L-lysine are assimilated. Sodium nitrite, ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 25–29 °C. Growth in vitamin-free medium is negative. Starch-like compounds are produced. Urease activity is positive. Diazonium blue B reaction is positive.

The type strain is ZJJ-890^T (=CGMCC 2.4407^T=CBS 12233^T), isolated from a wilting leaf of *Acer* sp. collected in Tianmen Mountain, Zhangjiajie, Hunan Province in August 2010.

Latin diagnosis of *Derxomyces bambusicola* F.-Y. Bai, X.-Z. Liu & T. Boekhout sp. nov.

In YM (Difco) liquido post dies 7 ad 20 °C, cellulae vegetativae ovoideae, ellipsoideae aut cylindrarum ($3.7\text{--}5.5 \times 7.5\text{--}12.0$ µm), singulae. Annulus et sedimentum formantur. In agar YM post unum mensem ad 20 °C, cultura flavae, non nitida, glabra, butyracea, margine glabra. Mycelium et pseudomycelium non formantur. Ballistoconidia napiformia ($4.7\text{--}7.5 \times 5.0\text{--}8.5$ µm). Fermentatio nulla. Glucosum, galactosum, cellobiosum, maltosum, melibiosum, sucrosus, trehalosum, melezitosum, raffinolum, L-arabinosum, L-rhamnosum (lente et exigue), D-xylosum (lente), galactitolum (lente), D-mannitolum, acidum succinicum, methyl α -D-glucoside (lente), salicinum (lente) et inulinum assimilantur, at non L-sorbosum, lactosum, D-arabinosum, D-ribosum, ethanolum, erythritolum, D-glucitolum, glycerolum, inositolum, methanolum, ribitolum, acidum citricum, acidum DL-lacticum, D-glucosaminum nec hexadecanum. Ammonium sulfatum, kalium nitricum (lente et exigue), L-lysinum (lente) et ethylaminum assimilantur, at non natrum nitrosus nec cadaverinum. Vitaminae externae ad crescentiam necessariae sunt. Maxima temperatura crescentiae: 29 °C. Materia amyloidea iodophila non formantur. Urea finditur. Diazonium caeruleum B positivum. Typus: GT-475^T, isolatus ex folio *Phyllostachys heterocycla*, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (=CGMCC 2.4411^T=CBS 12234^T).

Table 2. Physiological characteristics that differentiate the three novel *Derxomyces* species from closely related taxa

Group I species: 1, *D. amylogenes* sp. nov.; 2, *D. boninensis*; 3, *D. mrakii*; 4, *D. qinlingensis*. Group II species: 1, *D. bambusicola* sp. nov.; 2, *D. linzhiensis*. Group III species: 1, *D. corylopsis* sp. nov.; 2, *D. komagatae*; 3, *D. pseudoschimicola*; 4, *D. schimicola*. +, Positive; –, negative; l, delayed; s, slow positive; w, weak; lw, delayed and weak; where more than one symbol appears in a cell, all characteristics may be observed.

Characteristic	Group I				Group II		Group III			
	1	2	3	4	1	2	1	2	3	4
Assimilation of:										
D-Arabinose	l	+	+	+	–	+	+, w	l	+, l	+
D-Ribose	l, +	+	+	+	–	–	–	l	l	+
L-Rhamnose	s	+	+	+	lw	–	–	l	+, l	+
D-Glucitol	lw	+, l	w, –	–	–	+	–	+	+, –	+
Inositol	l, s	+, l	+	+	–	w	–	l	+, l, w	+
D-Mannitol	l, s	+, l	l	–	+	+	s, l	l	+, l	+
Salicin	s	+	+	+	l	–	lw	+	+, l, w	+
Inulin	l	–	–	–	+	+	l	–	–	–
Soluble starch	s, l	+	+	–	+	–	l	+	+	+
L-Lysine	+	+	+	+	l	+	l	+	+	+
Ethylamine	–	–	–	–	+	–	–	–	–	–
Starch formation	+	–	–	–	–	–	–	–	–	–

Description of *Derxomyces bambusicola* F.-Y. Bai, X.-Z. Liu & T. Boekhout sp. nov.

Derxomyces bambusicola [bam.bu.si'co.la. N.L. n. *Bambusa* scientific name of bamboo; L. suff. *-cola* (from L. n. *incola*) inhabitant, dweller; N.L. n. *bambusicola* bamboo dweller, referring to the source of the type strain of the species].

In YM broth, after 7 days at 20 °C, cells are ovoid, ellipsoidal and cylindrical (3.7–5.5 × 7.5–12.0 µm) (Fig. 2c). Budding is singly. Sediment and a ring are formed. On YM agar, after 1 month at 20 °C, the streak culture is yellow, butyrous, dull and smooth, with an entire margin. In Dalmau plate culture on cornmeal agar, hyphae and pseudohyphae are not formed. Ballistoconidia are produced on cornmeal agar and are napiform, (4.7–7.5 × 5.0–8.5 µm) (Fig. 2d). Fermentation of glucose is negative. Glucose, galactose, cellobiose, maltose, melibiose, sucrose, trehalose, melezitose, raffinose, L-arabinose, L-rhamnose (delayed and weak), D-xylose (delayed), galactitol (delayed), D-mannitol, succinic acid, methyl α-D-glucoside (delayed), salicin (delayed) and inulin are assimilated. L-Sorbose, lactose, D-arabinose, D-ribose, ethanol, erythritol, D-glucitol, glycerol, inositol, methanol, ribitol, citric acid, DL-lactic acid, D-glucosamine and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate (delayed and weak), L-lysine (delayed) and ethylamine hydrochloride are assimilated. Sodium nitrite and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 29 °C. Growth in vitamin-free medium is negative. Starch-like compounds are not produced. Urease activity is positive. Diazonium blue B reaction is positive.

The type strain is GT-475^T (=CGMCC 2.4411^T=CBS 12234^T), isolated from a wilting leaf of *Phyllostachys heterocycla* collected in Gutian Mountain, Zhejiang Province, China in July 2010.

Latin diagnosis of *Derxomyces corylopsis* F.-Y. Bai, X.-Z. Liu & T. Boekhout sp. nov.

In YM (Difco) liquido post dies 7 ad 20 °C, cellulae vegetativae ovoideae aut ellipsoideae (2.5–6.2 × 5.0–10.0 µm), singulae. Annulus et sedimentum formantur. In agar YM post unum mensem ad 20 °C, cultura flavae, non nitida, rugosa, butyracea, margine glabra. Pseudomycelium formantur. Ballistoconidia subglobosa, ellipsoideae vel napiformia (5.0–7.5 × 5.0–9.4 µm). Fermentatio nulla. Glucosum, galactosum, cellobiosum, maltosum, melibiosum, sucrosum (vel infirme), trehalosum, melezitosum, raffinolum, D-arabiosum (vel exigue), L-arabiosum, D-xylosum, galactitolum (varium), D-mannitolum (infirmum vel lente), ribitolum (lente et exigue), acidum succinicum, methyl α-D-glucoside (infirmum), salicinum (lente et exigue) et inulinum (lente) assimilantur, at non L-sorbosum, lactosum, D-ribosum, L-rhamnosum, ethanolum, erythritolum, D-glucitolum, glycerolum, inositolum, methanolum, acidum citricum, acidum DL-lacticum, D-glucosaminum nec hexadecanum. Ammonium sulfatum, kalium nitricum (lente et exigue) et L-lysinum (lente) assimilantur, at non natrum nitrosum, ethylaminum nec cadaverinum. Vitaminae externae ad crescentiam necessaria sunt. Maxima temperatura crescentiae: 29 °C. Materia amyloidea iodophila non formantur. Urea finditur. Diazonium caeruleum B positivum. Typus: ZJJ-394^T, isolatus ex folio *Corylopsis* sp., depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (=CGMCC 2.4409^T=CBS 12259^T).

Description of *Derxomyces corylopsis* F.-Y. Bai, X.-Z. Liu & T. Boekhout sp. nov.

Derxomyces corylopsis (co.ryl'op.sis. N.L. gen. n. *corylopsis* of *Corylopsis*, isolated from a wilting leaf of *Corylopsis* sp.).

In YM broth, after 7 days at 20 °C, cells are ovoid and ellipsoidal ($2.5\text{--}6.2 \times 5.0\text{--}10.0 \mu\text{m}$) (Fig. 2e). Budding is singly. Sediment and a ring are formed. On YM agar, after 1 month at 20 °C, the streak culture is yellow, butyrous, dull and wrinkled. The margin is entire. In Dalmau plate culture on cornmeal agar, pseudohyphae are formed. Ballistoconidia are produced on cornmeal agar, which are subglobose, ellipsoidal or napiform ($5.0\text{--}7.5 \times 5.0\text{--}9.4 \mu\text{m}$) (Fig. 2f). Fermentation of glucose is negative. Glucose, galactose, cellobiose, maltose, melibiose, sucrose (positive or slow positive), trehalose, melezitose, raffinose, D-arabinose (positive or weak), L-arabinose, D-xylose, galactitol (variable), D-mannitol (slow positive or delayed), ribitol (delayed and weak), succinic acid, methyl α -D-glucoside (slow positive), salicin (delayed and weak) and inulin (delayed) are assimilated. L-Sorbose, lactose, D-ribose, L-rhamnose, ethanol, erythritol, D-glucitol, glycerol, inositol, methanol, citric acid, DL-lactic acid, D-glucosamine and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate (delayed and weak) and L-lysine (delayed) are assimilated. Sodium nitrite, ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 29 °C. Growth in vitamin-free medium is negative. Starch-like compounds are not produced. Urease activity is positive. Diazonium blue B reaction is positive.

The type strain is ZJJ-394^T (=CGMCC 2.4409^T=CBS 12259^T), isolated from a wilting leaf of *Corylopsis* sp. collected in Tianzi Mountain, Zhangjiajie, Hunan Province in August 2010.

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