Jaminaea angkorensis gen. nov., sp. nov., a novel anamorphic fungus containing an S943 nuclear small-subunit rRNA group IB intron represents a basal branch of Microstromatales

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In the course of molecular characterization of yeasts isolated from decaying leaves collected in Cambodia, anamorphic yeast strains were detected that clustered with the Microstromatales in molecular phylogenetic analyses of the chromosomal regions coding for the D1/D2 domain of the large-subunit 26S rRNA gene, the small-subunit 18S rRNA gene and the internal transcribed spacer ITS1-5.8S rRNA gene-ITS2. In the trees obtained, the isolates formed a distinct, basal branch of Microstromatales supported with high bootstrap values. As the isolates could not be identified with any known genus, we have described them as *Jaminaea angkorensis* gen. *et* sp. nov. (type strain C5b^T=CBS 10918^T=CCY 88-1-1^T). In the chromosomal region coding for the 18S rRNA gene, the type strain of the novel species contained a group IB intron that was similar in location and sequence to introns found in certain species of Exobasidiales and Entylomatales. As no similar introns have been detected in Microstromatales, the new genus might represent a phylogenetic link connecting these three orders.

Microstromatales, Exobasidiales and Entylomatales are related orders of the class Exobasidiomycetes that comprise species lacking teliospores and mostly occur on woody bushes and trees (Bauer *et al.*, 1997; Begerow *et al.*, 2006). The class Exobasidiomycetes belong to the basidiomycetous subphylum Ustilaginomycotina, containing species that are usually dimorphic, having both yeast and hyphal phases (Begerow *et al.*, 2006). Ustilaginomycota also contains anamorphic species such as members of *Acaromyces*, *Malassezia*, *Meira*, *Rhodotorula*, *Tilletiopsis* or *Sympodiomyces* (Boekhout *et al.*, 2003, 2006; Xu *et al.*, 2007; Weijman *et al.*, 1988). The latter genus is classified within the Microstromatales and accommodates a species, *Sympodiomyces paphiopedili* (Sugiyama *et al.*, 1991; Suh & Sugiyama, 1994), which secretes an antifungal glycolipid into the culture medium (Kulakovskaya *et al.*, 2004). Antifungal antagonism is a property of numerous yeast genera and can be exploited in bioprotection of crops and

horticultural produce (for a review see Spadaro & Gullino, 2004). Certain antagonistic yeasts are pigmented and their antimicrobial activity can be a consequence of the pigment synthesis (Sipiczki, 2006). In a yeast isolation project intended to identify novel pigmented antagonistic yeasts, we isolated two strains from fallen leaves that produced pink colonies. Although they failed to show antimicrobial activities under laboratory conditions, their preliminary examination indicated that they might be interesting from a taxonomic point of view. In this paper we describe their morphology, taxonomy and phylogenesis and propose a species name for them.

Isolation and phenotypic characterization of yeast strains

Fallen leaves were collected from two different localities of the world heritage site Angkor, Cambodia in 2006. The collected plant material was macerated in sterile water and samples were streaked on YPGA (1% yeast extract, 1% peptone, 2% glucose, 2% agar, w/v) plates. After incubation for 14 days at 25 °C, individual yeast colonies were isolated. Two isolates (C5b^T and C6b) were selected for further examination. Both strains produced smooth, slightly pink colonies on YPGA medium, which upon prolonged incubation gradually became darker with venose and occasionally warty surfaces, and slightly eroded margins with occasional farinose sectors (Fig. 1a). The

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DDBJ accession numbers for the sequences of isolate C5b^T (CBS 10918^T) are EU587489 (D1/D2 domain of the large-subunit rRNA gene), EU604148 (18S rRNA gene), EU604147 (ITS1-5.8S rRNA gene-ITS2) and EU604149 (group IB intron).

Figures showing a CLUSTAL alignment of the rRNA group I introns of *Ustilago maydis* and strain C5b^T and a maximum-likelihood analysis of the S943 nuclear small-subunit rRNA group IB intron sequences are available as supplementary material with the online version of this paper.

Fig. 1. Growth of strain C5b^T (*Jaminaea angkorensis* gen. nov., sp. nov.) on solid media. (a) Yeast colony on YPGA after 14 days. (b) Dimorphic colony on CMA after 1 month. (c) Invasive mycelium. (d and e) Formation of satellite yeast colonies (hypha-to-yeast transition) in the substrate colonized by the invasive mycelium. y, Yeast colony; m, mycelium; sc, satellite yeast colony. Bar, 100 µm.

colonies contained yeast cells of variable shape and size, propagating by apical budding (Fig. 2a). Cell morphology was examined by using phase-contrast microscopy. Photographs were taken using an Olympus BH-2 microscope. Around and below the yeast colonies, slowly growing invasive mycelium was observable, particularly when CMA (Corn Meal Agar; van der Walt & Yarrow, 1984) medium was used (Fig. 1b–e). The mycelium grew towards uncolonized parts of the medium (Fig. 1b). A similar phenomenon was described in *Schizosaccharomyces japonicus*, where the hyphal extension was directed by a gradient of nutrients between the colonized (shortage of nutrients) and uncolonized (rich in nutrients) parts of the substrate (Sipiczki *et al.*, 1998). The mycelial phase mainly consisted of long hyphae producing apical conidia-like cells on sterigma-like protuberances (Fig. 2c) that either developed into new hyphae or produced small ‘satellite’ colonies (Fig. 1d, e) consisting of individual yeast cells or pseudo-hypha-like chains of unseparated yeast cells

(Fig. 2d, e). Occasionally, septa were visible in longer hyphae (true mycelium) (Fig. 2b). The septate hyphae branched at certain septa. The isolates were further subjected to tests standard to yeast taxonomy using methods described by van der Walt & Yarrow (1984). The results of the physiological and growth tests are shown in Table 1. No sporulation was detected in the cultures even after prolonged incubation.

Sequence analysis of the D1/D2 domain of the 26S rRNA gene and the ITS1-5.8S-ITS2 region

To reveal the taxonomic affiliation of the isolates, we isolated nuclear DNA from overnight cultures grown in YPG broth and amplified the chromosomal regions coding for the D1/D2 domain of the LSU 26S rRNA gene with primers NL-1 and NL-4 (O'Donnell, 1993), as described by Sipiczki (2003). The same primers were used for sequencing of the fragments obtained. The sequences of the

Fig. 2. Microscopic morphology of strain C5b^T (*Jaminaea angkorensis* gen. nov., sp. nov.). (a) Budding yeast cells propagating in the liquid medium YPG. (b) Invasive septate hypha branching at a septum. (c) Hyphae producing apical conidia-like cells (marked by arrows). (d and e) Pseudo-hypha-like chains of budding yeast cells establishing satellite yeast colonies at the tips of invasive hyphae. Bar, 10 µm.

isolates proved to be identical. A megablast similarity search in the NCBI database (<http://www.ncbi.nlm.nih.gov/blast>) revealed 97% similarity between them and the sequence of the unspecified organism *Sympodiomyces* sp. LM418 (12 nucleotide differences). However, they differed from the corresponding sequence of the type strain of *Sympodiomyces paphiopedili*, the only characterized species of the genus, by 6% (at 35 nucleotides). Somewhat higher similarities (95%; differences: 27–30 nucleotides; gaps: 1–7 nucleotides) were detected with the D1/D2 domain sequences of various strains of three *Rhodotorula* (*R. phylloplana*, *R. hinnulea*, *R. baccarum*),

one *Microstroma* (*M. juglandis*) and three *Quambalaria* (*Q. pitereka*, *Q. cyanescens*, *Q. eucalypti*) species, all belonging to Microstromatales. As none of these species differ in the D1/D2 sequence from the rest of the group by more than 5%, the difference between them and the Cambodian isolates strongly suggests that the latter represent a novel species. A previous comparative sequence analysis of large groups of basidiomycetous yeasts demonstrated that the D1/D2 and internal transcribed spacer (ITS) regions are suitable for species identification (Fell *et al.*, 2000; Scorzetti *et al.*, 2002). In ascomycetous yeasts, a minimum of 1% divergence in the D1/D2 domain of the 26S rRNA gene is usually sufficient to resolve individual ascomycetous yeast species (Kurtzman & Robnett, 1998).

To determine the phylogenetic position of the Cambodian isolates, we derived phylogenetic trees from the D1/D2 domain sequences of isolate C5b^T, the corresponding Microstromatales sequences available in the NCBI database and certain additional species representing related basidiomycetous orders. For multiple alignment of sequences, the CLUSTAL W 1.7 (Thompson *et al.*, 1994) and the MAFFT version 6 (Katoh & Toh, 2008) algorithms were used. The phylogenetic analysis was carried out using the Fitch–Margoliash, maximum-likelihood, neighbour-joining and DNA parsimony methods of the PHYLIP software package, version 3.67 (Felsenstein, 2007). Confidence limits for phylogenetic trees were estimated from bootstrap analysis (1000 replications; SEQBOOT and CONSENSE of the PHYLIP package). Phylogenetic trees were visualized with the TreeView program (Page, 1996). In the trees obtained (example in Fig. 3), strain C5b^T and the uncharacterized *Sympodiomyces* sp. LM418 formed a clade with the Microstromatales species and appeared as a basal branch with high bootstrap support (ranging from 89 to 98%, depending on the method used).

Sequence analysis of the 18S rRNA gene and the ITS1–5.8S–ITS2 region

The chromosomal segment coding for the small-subunit 18S rRNA gene, ITS1, 5.8S rRNA gene and ITS2 was amplified with the primers Fungi-18S-up (TTGTCTCAAAGATT-AAGCCATGCATGTCTAA) (Zimmer *et al.*, 2006) and ITS4 (TCCTCCGCTTATTGATATGC) (White *et al.*, 1990). Using these primers we could only sequence the ends of the fragment. For sequencing of the entire fragment we also used the internal primers Sin1 (GACAGTTGGGGGCATT-AGTAT) and Sin2 (AAAGGGCAGGGACGTAATCAA).

The phylogenetic analysis of the ITS1–5.8S–ITS2 region confirmed the basal position of strain C5b^T with even higher bootstrap values than in the D1/D2 analysis. The D1/D2 and ITS trees were also congruent in showing no close phylogenetic affiliation for this branch with any known genera of Microstromatales. We therefore propose a new genus, *Jaminaea* Sipiczki & Kajdacs gen. nov., to accommodate these organisms and a novel species, *Jaminaea angkorensis* Sipiczki & Kajdacs sp. nov., for the Cambodian isolates.

Table 1. Comparison of physiological characteristics of strain C5b^T (*Jaminaea angkorensis* gen. nov., sp. nov.) and *Sympodiomyopsis paphiopedili*

+, Growth; –, no growth; d, delayed growth; w, weak growth. Both species were positive for assimilation of D-glucose, L-sorbose, D-xylose, D-arabinose, sucrose, maltose, cellobiose, raffinose, melezitose, glycerol, L-arabinitol, D-glucitol, D-mannitol, meso-inositol, succinate, citrate, ethanol and nitrate. Both species grew on vitamin-free medium, did not grow at 37 °C and were negative for assimilation of arbutin and production of acetic acid.

Characteristic	Strain C5b ^T	<i>Sympodiomyopsis paphiopedili</i> *
Assimilation of carbon compounds		
D-Galactose	+	d
D-Ribose	+	d
D-Arabinose	+	d
L-Rhamnose	d	–
Trehalose	d	+
Salicin	d	–
Melibiose	+	d
Lactose	–	d
Inulin	+	–
Starch	+	–
Erythritol	–	+
Ribitol	+	d
Xylitol	–	d
D-Glucuronate	d	w
D-Galacturonate	w	–
DL-Lactate	+	–
1,2-Propanediol	+	d
Assimilation of nitrogen compounds		
Nitrite	–	+
L-Lysine	+	–
Other tests		
Methanol	+	–
Growth in 60 % glucose	w	+

*Data from CBS (<http://www.cbs.knaw.nl/yeast/BioloMICS.aspx>).

A megablast search in the NCBI database found 95–97 % sequence similarity with all species identified in the D1/D2 and ITS searches. However, it also revealed a 392-nucleotide long intron starting at nucleotide 1101, which corresponds to position 943 in the *Escherichia coli* 16S numbering. In the 18S phylogenetic trees inferred after the exclusion of this intron and ambiguously aligned regions of the sequences, the Cambodian isolate C5b^T appeared as a distinct branch of Microstromatales again, although not as basal as in the D1/D2 and ITS trees. We also constructed a Fitch–Margoliash tree from combined D1/D2 + 18S + ITS1–5.8S–ITS2 sequences of species shown in Fig. 3, for which all three regions were available. Strain C5b^T grouped with the Microstromatales species as a basal branch with a bootstrap value of 100 %. Although the molecular phylogenetic analyses unanimously indicate its association with the Microstromatales, the exact taxonomic placement of *Jaminaea* (e.g. relationship to families within the Microstromatales) requires a comprehensive cytological and molecular biological investigation involving representatives of the major genera of the Microstromatales, Entylomatales and Exobasidiales. Sequencing of genes

encoding phylogenetically conserved proteins such as β -tubulin (Begerow *et al.*, 2004), the RNA polymerase II subunit Rpb2, and the translation elongation factor Tef1 (Matheny *et al.*, 2007), etc. could be particularly useful. Electron microscopic examination of the septal structures in *Jaminaea* hyphae would provide cytological data important for the elucidation of the relationship of the new genus to the Exobasidiales, in which the septal pores and the caps enclosing the pores are larger than those in the Microstromatales (Bauer *et al.*, 1997; Begerow *et al.*, 2001).

Sequence analysis of the intron of the 18S rRNA gene

When the intron was used as query sequence, a BLASTN search in the NCBI database identified similarity with many group I introns. All but one of the most similar 15 hits (similarity: 74–86 %; E-value: e^{-31} – e^{-54}) were sequences of Ustilagomycotina species. Supplementary Fig. S1 (available in IJSEM Online) shows the CLUSTAL alignment of the C5b^T intron with the intron of *Ustilago maydis*, for which a secondary structure has been published (De Wachter *et al.*,

Fig. 3. Phylogenetic analysis of the chromosomal sequences coding for the D1/D2 domains of the large-subunit (26S) RNA gene by using the Fitch–Margoliash distance method. Outgroup: *Filobasidiella neoformans* (*Cryptococcus neoformans*). GenBank accession numbers are given in parentheses. Do., Doassantiales; En., Entylomatales; Ex., Exobasidiales; Exis., Exobasidiomycetidae incertae sedis; Mi., Microstromatales; Ti., Tilletiales; Ur., Urocystales; Us., Ustilaginales. Numbers at branch points are bootstrap probability values (1000 replicates). Bootstrap values of less than 50% are not given. Bar, 1 substitution per 10 nucleotides.

1992). It contains all conserved elements (P, Q, R and S) characteristic of group I introns (Cech, 1988). It can be classified as belonging to the subgroup 1B because its R element is immediately followed by P3.

Group I introns are widespread in fungal genomes (Bhattacharya *et al.*, 2005), but have not yet been reported in the Microstromatales. In view of this, the presence of the 1B intron in the 18S rRNA gene of strain C5b^T can be interpreted as reinforcing the basal location of this fungus in the rRNA gene phylogenetic trees. It might represent an early stage of Microstromatales evolution characterized by the presence of an intron in the 18S rRNA gene. The absence of sequences encoding homing endonucleases in strain C5b^T and related introns makes it likely that these introns are vertically inherited, and thus their phylogenetic analysis might give a clue for the origin of the C5b^T intron. Therefore we inferred trees from the most similar intron sequences. The intron of strain C5b^T generated a distinct branch located basically to the common root of the Ustilaginales, Exobasidiales and Entylomatales in the MAFFT-maximum-likelihood tree (Supplementary Fig. S2 in IJSEM Online) and on a common branch with Exobasidiales, close to the node separating Exobasidiales from Entylomatales in the CLUSTAL-Fitch–Margoliash tree. The latter result suggests that it might have diverged from

them after their separation from the Ustilaginales introns. However, because of the weak bootstrap support for certain branches (typical for these short sequences; Haugen *et al.*, 2004), its history cannot be unambiguously reconstructed.

Latin diagnosis of *Jaminaea* Sipiczki et Kajdacsi gen. nov.

Genus anamorphicum e Microstromatales (Basidiomycota). Coloniis dimorphicis, primum zymoideis, cellulis fusiformibus, ad apicem e rachide acropetali proliferantibus, deinde hyphae septatae, hyalinae, ramosae, immersae et superficiales. Protuberantiae sterigmatideae saepe ad apicem formatae, e quibus catenae ramosae cellularum zymoticarum oriuntur. Ascosporae, basidiosporae, teliosporae, ballistosporae nullae. Fermentatio nulla.

Typus Jaminaea angkorensis Sipiczki & Kajdacsi.

Description of *Jaminaea* Sipiczki & Kajdacsi gen. nov.

Jaminaea (Ja.mi.na.e'a. N.L. fem. n. *Jaminaea* pertaining to Jamina, the name of the township where Matthias Sipiczki stayed when writing this paper).

Anamorphic genus, belonging phylogenetically to the Microstromatales (Basidiomycota). Colonies are dimorphic with initial yeast-like growth. Cells are fusiform showing polar budding on an acropetal rachis. Hyphae are septate, hyaline, branched, and grow both into the substrate and on its surface. Sterigma-like outgrowths, frequently formed on the tips of hyphae, give rise to branched chains of cells.

The type species is *Jaminaea angkorensis* Sipiczki & Kajdacsi.

Latin diagnosis of *Jaminaea angkorensis* Sipiczki et Kajdacsi sp. nov.

In medio liquido YPG post 2 dies ad 30 °C, cellulae zymosae ovoidae vel fusiformae, 5–7 × 2–3 µm, singulae aut binae, utrinque sympodialiter proliferantes. Annulus et sedimentum exiguum formantur. Coloniae in agar YPGA et malti post 5 dies ad 30 °C, subroseae, nitidae, planae, molles, et margine glabrae; post 3 hebdomades roseae, convexae, superficie venosa vel cerebriformi, marginem versus sulcatae, partim synnematis angustatis obiectae, sed etiam in sectoribus leves; margo integra vel modice erosa et sectoribus divisa. In agar malti et in agar farina Zea maydi confecto, post 2 hebdomades ad 25 °C hyphae formantur. Mycelium partim superficiale et partim in substrato immersum. Hyphae 2–3 µm hyalinae, septatae. Fermentatio nulla. Proprietate physiologicae in Tabella 1 compositae. Typus C5b^T (=CBS 10918^T=CCY 88-1-1^T), isolatus ex folium putridum in Angkor World Heritage Site, Cambodia.

Description of *Jaminaea angkorensis* Sipiczki & Kajdacsi sp. nov.

Jaminaea angkorensis (ang.ko.ren'sis. N.L. fem. adj. *angkorensis* pertaining to Angkor).

In liquid medium YPG after 2 days of incubation at 30 °C, cells are oval or fusiform, 5–7 × 2–3 µm, single or in pairs, with polar sympodial budding. Ring and a sparse sediment are present. On YPG agar, after 5 days at 30 °C, colonies are light-pink coloured, glistening, flat, soft and have an entire margin. After 3 weeks, colonies are rose-red, convex, with the surface venose to cerebriform, radially furrowed near the margin, partly covered with tapered synnemata, but also with smooth sectors; margin entire or somewhat eroded and with sectors. On malt agar and on CMA after 2 weeks at 25 °C, hyphae are formed. The mycelium grows on the surface but also invades the medium. Hyphae are 2–3 µm in diameter, white and septate. Physiological characteristics are presented in Table 1.

The type strain is C5b^T (=CBS 10918^T=CCY 88-1-1^T), which was isolated from fallen and decaying leaves collected at the Angkor World Heritage Site, Cambodia.

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References

- Bauer, R., Oberwinkler, F. & Vanky, K. (1997). Ultrastructural markers and systematics in smut fungi and allied taxa. *Can J Bot* **75**, 1273–1314.
- Begerow, D., Bauer, R. & Oberwinkler, F. (2001). Muribasidioidespora: microstromatales or exobasidiales? *Mycol Res* **105**, 798–810.
- Begerow, D., John, B. & Oberwinkler, F. (2004). Evolutionary relationships among β -tubulin gene sequences of basidiomycetous fungi. *Mycol Res* **108**, 1257–1263.
- Begerow, D., Stoll, M. & Bauer, R. (2006). A phylogenetic hypothesis of Ustilaginomycotina based on multiple gene analyses and morphological data. *Mycologia* **98**, 906–916.
- Bhattacharya, D., Reeb, V., Simon, D. M. & Lutzoni, F. (2005). Phylogenetic analyses suggests reverse splicing spread of group I introns in fungal ribosomal DNA. *BMC Evol Biol* **5**, 68.
- Boekhout, T., Theelen, B., Houbraken, J., Robert, V., Scorzetti, G., Gafni, A., Gerson, U. & Sztejnberg, A. (2003). Novel anamorphic mite-associated fungi belonging to the Ustilaginomycetes: *Meira geulakonigii* gen. nov., sp. nov., *Meira argovae* sp. nov. and *Acaromyces ingoldii* gen. nov., sp. nov. *Int J Syst Evol Microbiol* **53**, 1655–1664.
- Boekhout, T., Gildemacher, P., Theelen, B., Muller, W. H., Heijne, B. & Lutz, M. (2006). Extensive colonization of apples by smut anamorphs causes a new post-harvest disorder. *FEMS Yeast Res* **6**, 63–76.
- Cech, T. R. (1988). Conserved sequences and structures of group I introns: building an active site for RNA catalysis – a review. *Gene* **73**, 259–271.
- De Wachter, R., Neefs, J.-M., Goris, A. & Van der Peer, Y. (1992). The gene coding for small ribosomal subunit RNA in the basidiomycete *Ustilago maydis* contains a group I intron. *Nucleic Acids Res* **20**, 1251–1257.
- Fell, J. W., Boekhout, T., Fonseca, A., Scorzetti, G. & Statzell-Tallman, A. (2000). Biodiversity and systematics of basidiomycetous yeasts as determined by large-subunit rDNA D1/D2 domain sequence analysis. *Int J Syst Evol Microbiol* **50**, 1351–1371.
- Felsenstein, J. (2007). PHYLIP (phylogeny inference package), version 3.67. Department of Genome Sciences, University of Washington, Seattle, USA.
- Haugen, P., Reeb, V., Lutzoni, F. & Bhattacharya, D. (2004). The evolution of homing endonuclease genes and group I introns in nuclear rDNA. *Mol Biol Evol* **21**, 129–140.
- Katoh, K. & Toh, H. (2008). Recent developments in the MAFFT multiple sequence alignment program. *Brief Bioinform* **9**, 286–298.
- Kulakovskaya, T. V., Shashkov, A. S., Kulakovskaya, E. V. & Golubev, W. I. (2004). Characterization of an antifungal glycolipid secreted by the yeast *Sympodiomyces paphiopedili*. *FEMS Yeast Res* **5**, 247–252.
- Kurtzman, C. P. & Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Antonie van Leeuwenhoek* **73**, 331–371.
- Matheny, P. B., Wang, Z., Binder, M., Curtis, J. M., Lim, Y. W., Nilsson, R. H., Hughes, K. W., Hofstetter, V., Ammirati, J. F. & other authors (2007). Contributions of *rpb2* and *tefl* to the phylogeny of mushrooms and allies (Basidiomycota, Fungi). *Mol Phylogenet Evol* **43**, 430–451.
- O'Donnell, K. (1993). *Fusarium* and its near relatives. In *The Fungal Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics*, pp. 225–233. Edited by D. R. Reynolds & J. W. Taylor. Wallingford, UK: CAB International.

- Page, R. D. M. (1996).** TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.
- Scorzetti, G., Fell, J. W., Fonseca, A. & Statzell-Tallman, A. (2002).** Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Res* **2**, 495–517.
- Sipiczki, M. (2003).** *Candida zemplinina* sp. nov., an osmotolerant and psychrotolerant yeast that ferments sweet botrytized wines. *Int J Syst Evol Microbiol* **53**, 2079–2083.
- Sipiczki, M. (2006).** *Metschnikowia* strains isolated from botrytized grapes antagonize fungal and bacterial growth by iron depletion. *Appl Environ Microbiol* **72**, 6716–6724.
- Sipiczki, M., Takeo, K., Yamaguchi, M., Yoshida, S. & Miklos, I. (1998).** Environmentally controlled dimorphic cycle in a fission yeast. *Microbiology* **144**, 1319–1330.
- Spadaro, D. & Gullino, M. L. (2004).** State of the art and future prospects of the biological control of postharvest fruit disease. *Int J Food Microbiol* **91**, 185–194.
- Sugiyama, J., Tokuoka, K., Suh, S.-O., Hirata, A. & Komagata, K. (1991).** *Sympodiomyces*: a new yeast-like anamorph genus with basidiomycetous nature from orchid nectar. *Antonie van Leeuwenhoek* **59**, 95–108.
- Suh, S.-O. & Sugiyama, J. (1994).** Phylogenetic placement of the basidiomycetous yeasts *Kondoa malvinella* and *Rhodospiridium dacryoidum*, and the anamorph yeast *Sympodiomyces paphiopedili* by means of 18S rRNA gene sequence analysis. *Mycoscience* **35**, 367–375.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994).** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- van der Walt, J. P. & Yarrow, D. (1984).** Methods for the isolation, maintenance, classification and identification of yeasts. In *The Yeasts: a Taxonomic Study*, pp. 45–104. Edited by N. J. W. Kreger-van Rij. Amsterdam: Elsevier.
- Weijman, A. C., Rodrigues de Miranda, L. & van der Walt, J. P. (1988).** Redefinition of *Candida* Berkhout and the consequent emendation of *Cryptococcus* Kutzing and *Rhodotorula* Harrison. *Antonie van Leeuwenhoek* **54**, 545–553.
- White, T. J., Burns, T., Lee, S. & Taylor, J. (1990).** Amplification and sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols. A Guide to Methods and Applications*, pp. 315–322. Edited by M. A. Innis, D. H. Gelfand, J. J. Snisky & T. J. White. San Diego, CA: Academic Press.
- Xu, J., Saunders, C. W., Hu, P., Grant, R. A., Boekhout, T., Kuramae, E. E., Kronstad, J. W., Deangelis, Y. M., Reeder, N. L. & other authors (2007).** Dandruff-associated *Malassezia* genomes reveal convergent and divergent virulence traits shared with plant and human fungal pathogens. *Proc Natl Acad Sci U S A* **104**, 18730–18735.
- Zimmer, C., Platz, T., Cadez, N., Giffhorn, F. & Kohring, G. W. (2006).** A cold active (2R,3R)-(-)-di-O-benzoyl-tartrate hydrolyzing esterase from *Rhodotorula mucilaginosa*. *Appl Microbiol Biotechnol* **73**, 132–140.