Mycoscience (2006) 47:36–40
DOI 10.1007/s10267-005-0266-4

FULL PAPER

Fumitoshi Yasuda · Daisuke Yamagishi
Hajime Akamatsu · Hiroki Izawa · Motoichiro Kodama
Hiroshi Otani

Meira nashicola sp. nov., a novel basidiomycetous, anamorphic yeastlike fungus isolated from Japanese pear fruit with reddish stain

Received: April 8, 2005 / Accepted: October 20, 2005

Abstract Three undescribed strains of basidiomycetous, anamorphic yeastlike fungi were isolated from Japanese pear fruits with a reddish stain collected in Tottori Prefecture, Japan. The strains are classified in a single group and assigned to the genus *Meira* by conventional and chemotaxonomic studies. Sequence analyses of the D1/D2 domain of 26S rDNA and internal transcribed spacer (ITS) regions indicate that the strains represent a novel species with a close phylogenetic relationship to *Meira geulakonigii* and *M. argovae*. The name *Meira nashicola* sp. nov. is proposed for the strains (type strain PFS 002 = MAFF 230028 = CBS 117161).

Key words Anamorphic yeastlike fungus · Japanese pear fruit stain · *Meira nashicola* sp. nov.

Introduction

In recent years, fruit stain disease on Japanese pear cvs. Nijisseiki and Gold Nijisseiki has been found frequently in Tottori Prefecture, Japan (Yasuda et al. 2005). The typical symptom of the disease is a reddish stain on the fruit surface accompanied by a fusty smell but no decay. In the course of a survey of fungi living on the surface of diseased fruits, we isolated hitherto undescribed anamorphic yeastlike fungus. A preliminary examination based on morphological and physiological characteristics and sequence data of the D1/D2 domain of the 26S rDNA and internal transcribed spacer (ITS) regions including 5.8S rDNA suggested that the fungus appeared to belong to the genus *Meira* (Yasuda et al. 2005), but the specific name was not identified. The genus *Meira* was proposed by Boekhout et al. (2003) as novel basidiomycetous, anamorphic yeastlike fungi as well as the genus *Acaromyces*. The genus *Meira* includes two species to date, and the type species is *M. geulakonigii* Boekhout, Scorzetti, Gerson & Sztejnberg. *Meira geulakonigii* and another species, *M. argovae* Boekhout, Scorzetti, Gerson & Sztejnberg, were isolated only from cadavers of mites collected in Israel and speculated to be acaropathogenic fungi (Boekhout et al. 2003; Sztejnberg et al. 2004) such as *Hirsutella thompsonii* F.E. Fisher and *Neozygites floridana* (Weiser & Muma) Remaud. & S.S. Kellar (Chandler et al. 2000). The present fungus from the Japanese pear fruit surface was distinguished from the two known species of the genus *Meira* in the morph of colonies on some media, physiological characteristics, and sequence analyses of the D1/D2 domain of 26S rDNA and ITS regions. Therefore, in this article, the fungus isolated from Japanese pear fruit surface with reddish stain is described as a new species in the genus *Meira*, and the phylogenetic position in the genus is discussed.

Materials and methods

Strains employed

Three strains (PFS 002, PFS 023, and PFS 034) of the genus *Meira* were obtained from lesions of Japanese pear cv. Gold Nijisseiki fruits with a reddish stain harvested from orchards in Tohaku-cho, Tottori Prefecture, Japan, in September 2001. Tissue pieces of the lesions were cut off and suspended with 10 ml sterilized distilled water. A small amount of suspension was streaked onto potato dextrose agar (PDA; Becton Dickinson, Sparks, MD, USA) plates. PDA plates were incubated in darkness at 25°C for 5 days. Single

F. Yasuda (✉) · H. Izawa
Tottori Horticultural Experiment Station, 2048 Yurasyuku, Hokuei-cho, Tohaku-gun, Tottori 689-2221, Japan
Tel. +81-858-37-4211; Fax +81-858-37-4822
e-mail: yasudaf@pref.tottori.jp

D. Yamagishi · H. Akamatsu* · M. Kodama · H. Otani
Faculty of Agriculture, Tottori University, Tottori, Japan

Present address:
*Department of Plant Pathology, Washington State University, Pullman, WA, USA

colonies were picked up and streaked onto fresh PDA plates again. This operation was repeated a few times, and finally single colonies were transferred to fresh PDA slants. Obtained strains were stocked at 25°C in darkness until used for experiments.

Morphology and physiology

Morphology of the strains was investigated by streak and line inoculations on 1% yeast extract/0.5% peptone/4% glucose agar (YPGA) and PDA plates. Plates were kept in darkness at 25°C for 3–14 days. Comparative nutritional tests were performed according to Boekhout (1991) and Yarrow (1998).

PCR amplification and sequencing

The strains were cultured in darkness at 25°C on PDA plates and harvested after 7 days. DNA from each strain was extracted using a FastDNA Kit (Bio101, Vista, CA, USA) according to the instructions supplied by the manufacturer. Amplifications were conducted using 25 µl polymerase chain reaction (PCR) mixtures each containing 0.4 µM of each primer, 0.625 U TaKaRa Ex Taq (TaKaRa Bio, Otsu, Japan), the supplied deoxyribonucleotide triphosphate (dNTP) mixture (containing 2.5 mM of each dNTP), and Ex Taq reaction buffer (containing 2 mM Mg^{2+}). PCR was carried out using a RoboCycler (Stratagene, La Jolla, CA, USA) under the following conditions: 94°C for 2.5 min, then 30 cycles of 94°C for 30 s, 56°C for 45 s, and 72°C for 1.5 min, and a final step of 72°C for 7 min. Primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (O'Donnell 1993) were used for PCR amplification of the D1/D2 domain of 26S rDNA, while the ITS regions including 5.8S rDNA were amplified using ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990). PCR products were purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and were sequenced directly using an ABI Prism BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the same primers used for PCR amplifications. Cycle sequencing reaction products were purified using a DyeEx 2.0 Spin Kit (Qiagen). Data were collected using an ABI 310 automated sequencer (Applied Biosystems) according to the standard protocol. The GenBank/EMBL/DDBJ accession numbers for the sequences of D1/D2 domain of 26S rDNA and ITS regions including 5.8S rDNA of the strain PFS 002 determined in this study are AB185157 and AB185159, respectively.

Phylogenetic analysis

The sequences were aligned using the CLUSTAL W version 1.83 computer program (Thompson et al. 1994). For phylogenetic analysis, the D1/D2 domain sequences of 26S rDNA of related fungi were obtained from international DNA databases. The phylogenetic tree was constructed from the evolutionary distance data according to Kimura (1980), using the neighbor-joining method (Saitou and Nei 1987) in the CLUSTAL W version 1.83 computer program. Bootstrap analysis (Felsenstein 1985) was performed with 1000 random resamplings. Phylogenetic trees were visualized with the TREEVIEW program (Page 1996).

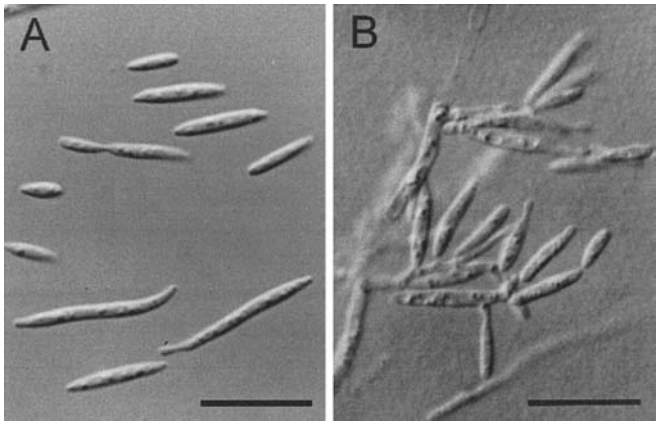

Fig. 1. Light micrographs of *Meira* sp. PFS 002. Initial yeastlike fusiform cells (**A**) produced on potato dextrose agar (PDA) after 3 days at 25°C and fusiform blastoconidia (**B**) produced on PDA after 7 days at 25°C. *Bars* 10 µm

Results and discussion

Morphology and physiology

Three strains (PFS 002, PFS 023, and PFS 034) isolated from Japanese pear fruits with reddish stain were demonstrated to have morphological and physiological characteristics identical to each other. Colonies of the strains on PDA after 14 days at 25°C showed a grayish-brown velvety appearance with the surface venose to cerebriform. In contrast, colonies on YPGA after 14 days at 25°C were dull, creamy white, somewhat raised to pulvinate, and tough. The strains formed a brownish pigment on PDA and YPGA. Initial yeastlike growth with fusiform cells [(4–)6–12(–17) × 2–3 µm] (Fig. 1A) showed polar budding on an acropetal rachis. Sterigma-like outgrowths frequently occurred near the septa and gave rise to acropetally short chains of fusiform conidia [(4–)5–15 × 2–3 µm] (Fig. 1B). The strains produced no teleomorph. Detailed physiological characteristics of representative strain PFS 002 were previously reported (Yasuda et al. 2005). The strains did not assimilate *myo*-inositol and did not produce extracellular starch. Diazonium Blue B (DBB) and urease reactions were positive. These morphological and physiological characteristics support that the strains belong to the genus *Meira*. The strains assimilated melezitose, potassium nitrate, sodium nitrite, and L-lysine and did not assimilate inulin, glycerol,

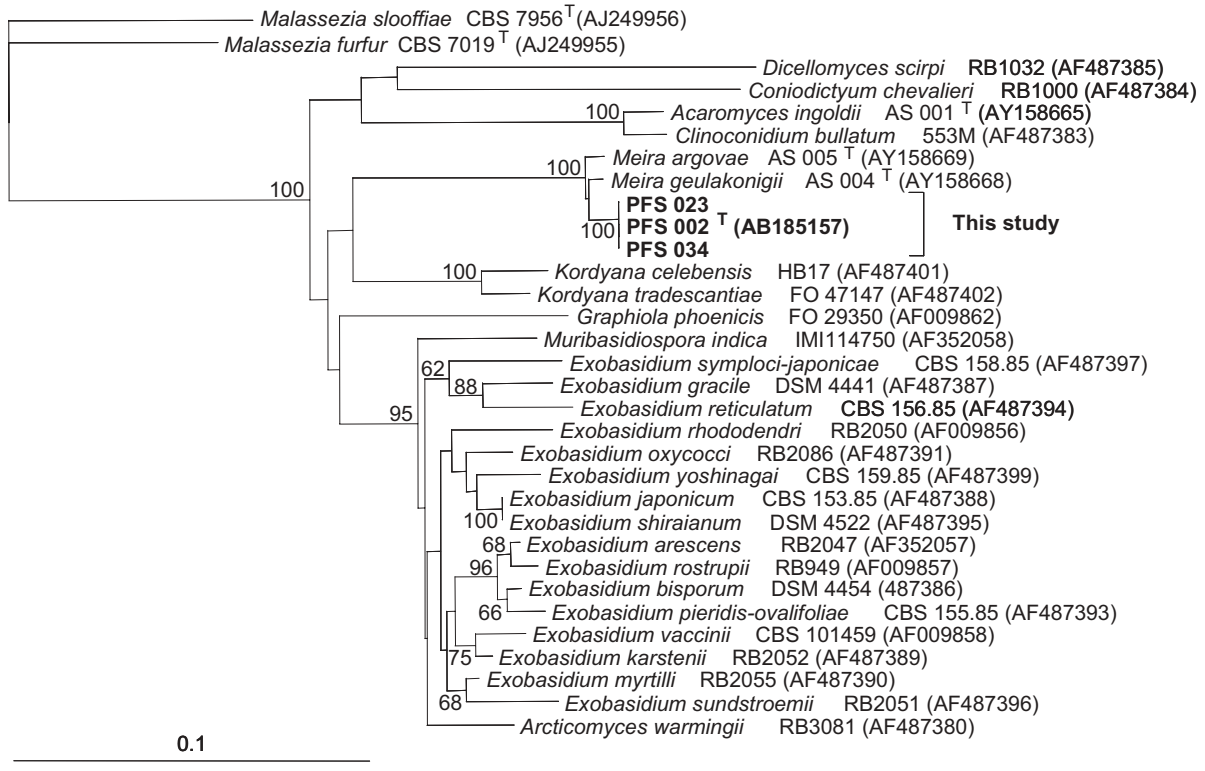

Fig. 2. Phylogenetic tree drawn from neighbor-joining analysis of 26S rDNA D1/D2 domain sequences, depicting the relationship of the strains of *Meira* spp. with closely related species. *Malassezia slooffiae* CBS 7956^T (AJ249956) and *Malassezia furfur* CBS 7019^T (AJ249955) are the relevant outgroup species. *Numbers* represent percentages from 1000 replicate bootstrap sampling (frequencies less than 50% are not shown). *Bar* indicates 0.1 substitutions per site

Table 1. Sequence similarity (%) of internal transcribed spacer (ITS) regions of rDNA among *Meira* spp.[a]

Scientific name	Strain	PFS 002^T	PFS 023	PFS 034	AS 004^T	AS 005^T
Meira sp.	PFS 002^T	–	100	100	79	85
	PFS 023	100	–	100	79	85
	PFS 034	100	100	–	79	85
M. geulakonigii	AS 004^T	76	76	76	–	82
M. argovae	AS 005^T	74	74	74	80	–

^{T}Type strain
[a]Lower left triangle shows ITS1 similarity, and upper right triangle shows ITS2 similarity; the sequences were aligned including gaps

ribitol, citrate, and glucono-δ-lactone. These biochemical characteristics of the present fungus differed from known species of the genus *Meira*. Morphological and physiological characteristics of the additional strains, PFS 023 and PFS 034, were identical with those of PFS 002.

Molecular phylogenetic analysis

The D1/D2 domain of 26S rDNA and the ITS regions including 5.8S rDNA were completely sequenced in both directions. The D1/D2 domain sequences of the three strains (PFS 002, PFS 023, and PFS 034) were either identical or differed in only one position. DNA sequences of D1/D2 domain of three strains and presumed relatives ranged from 519 to 633 bp in length. A total alignment of 655 bases (364 variable sites) was obtained and used in the comparisons among relative strains. In a phylogenetic tree drawn from the D1/D2 domain sequences, three strains clustered in the genus *Meira* (Fig. 2). Furthermore, the sequences of ITS1 of three strains and relative species of the genus *Meira* ranged from 173 to 184 bp in length, whereas the sequences of ITS2 ranged from 227 to 256 bp, respectively. These sequences of ITS regions were aligned, including gaps. ITS1 and ITS2 sequences of three strains were identical completely, but showed similarity of 74%–76% and 79%–85% in comparison with the two known *Meira* species, respectively (Table 1). These results of sequence analyses indicate that three strains from Japanese pear fruits differ from the known *Meira* species.

Based on conventional and chemotaxonomic studies and molecular phylogenetic analyses, we concluded that the

three strains (PFS 002, PFS 023, and PFS 034) were identical with each other and represented a new species of the genus *Meira*.

Sequence analysis of D1/D2 domain of 26S rDNA demonstrated that the genus *Meira* cluster within the Exobasidiomycetidae of the Ustilaginomycetes (Boekhout et al. 2003). The closest relatives of the genus *Meira* appeared to be *Kordyana celebensis* Gäum. and *Kordyana tradescantiae* (Pat.) Rac., although the bootstrap values were lower than 50%. These species were classified in the Brachybasidiaceae of the Exobasidiales (Exobasidiomycetidae, Ustilaginomycetes) (Begerow et al. 2002). The systematic position of the genus *Meira* within the Exobasidiomycetidae is not clear because of the low bootstrap values of the phylogenetic analysis. The Brachybasidiaceae may be a prospective family to accommodate the genus *Meira*, but Graphiolaceae or Exobasidiaceae are possible alternatives (Boekhout et al. 2003). Many other basidiomycetous yeastlike fungi belonging phylogenetically to the Ustilaginomycetes, such as the genus *Pseudozyma* Bandoni emend. Boekhout (Boekhout 1995) and the genus *Acaromyces* (Boekhout et al. 2003), are morphologically similar to the genus *Meira*. They cannot be differentiated easily from one another, because these fungi are characterized by the presence of acropetal chains of fusiform conidia originating from sterigma-like structures occurring on narrow, hyaline, and septate hyphae.

However, sequence analysis of the D1/D2 domain of 26S rDNA clearly revealed the phylogenetic positions of these basidiomycetous yeastlike fungi (Begerow et al. 2000; Fell et al. 2000; Boekhout et al. 2003). Most species of yeasts and yeastlike fungi can be directly identified by sequence analysis of the D1/D2 domain of 26S rDNA, alignment of the sequence data of the DNA databases, and placement within the appropriate phylogenetic tree at present. Alternatively, species can be identified via the ITS regions; because the ITS regions have a higher rate of divergence than the D1/D2 domain, their sequence analysis is generally considered to be a useful identification tool for species (Sugita et al. 1999). As previously reported, there were few differences (less than 1%) in sequences of D1/D2 domain among three species of the genus *Meira* (Yasuda et al. 2005). However, sequences of ITS regions were apparently distinguished from one another among species of the genus *Meira*. In addition, *M. geulakonigii* and *M. argovae* were isolated only from cadavers of dead mites and speculated to be acaropathogenic fungi (Boekhout et al. 2003; Sztejnberg et al. 2004). More studies are necessary to reveal the relationship between the present new species and mites in orchards in view of a potential acaropathogenic fungus.

Taxonomy

Meira nashicola F. Yasuda & H. Otani, sp. nov.

Coloniae in YPGA post 14 dies ad 25°C valde convexae, cremeo-albae, superficie venosa vel cerebriformi, synnematibus obtegentes, margine integra. Coloniae in PDA post 14 dies ad 25°C rigidae, planae, centro nitide cinereo-brunneae, sulcatae vel reticulatae, cum synnematibus sursum attenuatis versus margo prostratis obtegentes. Reversum in YPGA et PDA brunneum, pigmento brunneo in agaros diffluenti. Cellulae initiae zymoideae, ellipsoideae, (4–)6–12(–17) × 2–3 µm, blastosporis acrogenis pullulantibus formantes. Hyphae ~ 1.5–3 µm latae, plerumque partim strictura cytoplasmatis orientes, ad septum aliquot constrictae; protuberantiones sterigmatoideae, sympodialiter ramificantes, plerumque juxta septum hyphae formatae, catenas conidiorum proferentes; blastoconidia fusiformia, (4–)5–15 × 2–3 µm. Fermentatio pro glucosum nulla. Assimilatio melezitosum, potassium nitratum, sodium nitritum et L-lysinum positiva; assimilatio inulinum, glycerolum, ribitolum, citratum et glucono-δ-lactonum negativa.

Holotypus: MAFF 230028 (originaliter ut PFS 002), cultura viva ex fructu *Pyri pyrifoliae* Nakai var. *cultae* Nakai, Tohaku-cho, Tottori Pref. in Japonia, Sept. 2001, a F. Yasuda leg. et isolata et ea in Herbario "Genebank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan" conservatus. Isotypus: CBS 117161.

Colonies on YPGA after 14 days at 25°C are highly convex, creamy white, with the surface venose to cerebriform, covered with synnemata, with entire margins. Colonies on PDA after 14 days at 25°C are ridged, smooth, with the center shiny grayish-brown, furrowed to reticulate, and covered with tapered synnemata that prostrate toward the margin. Reverse brown, with brown pigment exuding into YPGA and PDA. Initial growth with ellipsoidal yeast cells, (4–)6–12(–17) × 2–3 µm, with polar acropetal budding; hyphae approximately 1.5–3 µm in diameter, somewhat constricted near the septa; acropetal chains of fusiform conidia originate on sterigma-like structures, which may be sympodially branched and usually occur near the hyphal septa; conidia are (4–)5–15 × 2–3 µm in size. Fermentation of D-glucose is negative. The following compounds are assimilated: melezitose, potassium nitrate, sodium nitrite, and L-lysine. The following are not assimilated: inulin, glycerol, ribitol, citrate, and glucono-δ-lactone.

The type strain of *Meira nashicola*, PFS 002, was isolated from Japanese pear (*Pyrus pyrifolia* Nakai var. *culta* Nakai) fruits with reddish stain that were harvested from orchards in Tottori Prefecture, Japan, in September 2001. This strain has been deposited as the holotype in Genebank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan, as MAFF 230028. Isotype was deposited in Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, as CBS 117161.

Etymology: *nashicola* means a dweller of *nashi*, Japanese name of the substratal plant.

Acknowledgments We thank Dr. K. Katumoto, formerly professor of Yamaguchi University, Japan, Dr. T. Nakase, Central Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Thailand, Dr. N. Maekawa, professor of Tottori University, Japan, and Dr. H. Nakamura, National Institute of Fruit Tree Science, Japan, for their kind advice in carrying out this study.

References

Begerow D, Bauer R, Boekhout T (2000) Phylogenetic placements of ustilaginomycetous anamorphs as deduced from nuclear LSU rDNA sequences. Mycol Res 104:53–60

Begerow D, Bauer R, Oberwinkler F (2002) The Exobasidiales: an evolutionary hypothesis. Mycol Prog 1:187–199

Boekhout T (1991) A revision of ballistoconidia-forming fungi. Stud Mycol 33:1–194

Boekhout T (1995) *Pseudozyma* Bandoni emend. Boekhout, a genus for yeast-like anamorphs of Ustilaginales. J Gen Appl Microbiol 41:359–366

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

Chandler D, Davidson G, Pell JK, Ball BV, Shaw K, Sunderland KD (2000) Fungal biocontrol of acari. Biocontrol Sci Technol 10:357–384

Fell JW, 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 (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

O'Donnell K (1993) *Fusarium* and its near relatives. In: Reynolds, DR, Taylor JW (eds) The fungal holomorph: mitotic, meiotic, and pleomorphic speciation in fungal systematics. CAB International, Wallingford, pp 225–236

Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sugita T, Cañete-Gibas CF, Takashima M, Nakase T (1999) Three new species of *Bullera* isolated from leaves in the Ogasawara Islands. Mycoscience 40:491–501

Sztejnberg A, Paz Z, Boekhout T, Gafni A, Gerson U (2004) A new fungus with dual biocontrol capabilities: reducing the numbers of phytophagous mites and powdery mildew disease damage. Crop Protect 23:1125–1129

Thompson JD, Higgins DG, Gibson TJ (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

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp 315–322

Yarrow D (1998) Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP, Fell JW (eds) The yeasts: a taxonomic study, 4th edn. Elsevier, Amsterdam, pp 77–100

Yasuda F, Yamagishi D, Akamatsu H, Izawa H, Kodama M, Otani H (2005) Fruit stain of Japanese pear caused by basidiomycetous, yeast-like fungi, *Acaromyces ingoldii* and *Meira* sp. (in Japanese) Jpn J Phytopathol 71:156–165