Mycoscience (2008) 49:329–333
DOI 10.1007/s10267-008-0429-1

SHORT COMMUNICATION

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Isolation of basidiomycetous anamorphic yeast-like fungus *Meira argovae* found on Japanese bamboo

Received: November 26, 2007 / Accepted: June 5, 2008

Abstract A basidiomycetous anamorphic yeast-like fungus, isolated from new bamboo shoots collected in Japan, was assigned to *Meira argovae* by comparison of conidial morphology, physiological characteristics, rDNA sequences, and DNA–DNA relatedness with the ex-type strains of *Meira* species. This is the first record of the finding of *M. argovae* from other than mite cadavers and in regions other than Israel. Phylogenetic analysis based on the D1–D2 domain demonstrated that *Meira* species and teleomorphic *Dicellomyces* species, which include a bamboo leaf parasite, *D. gloeosporus*, formed sister clades.

Key words Bamboo in Japan · Basidiomycetous yeast-like fungus · *Dicellomyces gloeosporus* · DNA–DNA relatedness · *Meira argovae* · rDNA sequences

During a survey of bambusicolous endophytic fungi, two isolates of basidiomycetous yeast-like fungus were obtained. These strains were isolated from newly growing shoot tissues of bamboo (Poaceae), *Phyllostachys bambusoides* Sieb. & Zucc., with witches' broom disease caused by *Aciculosporium take* Miyake, which were collected in October 2004 from Omishima Island, Imabari-shi, Ehime Prefecture, Japan (34°15′16″ N, 133°1′6″ E). The shoots, which were enclosed by a young leaf, were peeled, and the tissues including the shoot growing points were put on an agar medium [0.1% (w/v) yeast extract, 0.1% (w/v) tryptone, 1% (w/v) glucose, and 1.5% (w/v) agar] containing chloramphenicol (100 μg/ml). After incubation at room temperature for a week, two yeast-like colonies developed. Each colony was picked up and streaked repeatedly to confirm purity. Single colonies of the respective isolates (OM1 and OM2) were retrieved and were maintained for further experiments.

Morphological characteristics of strains OM1 and OM2 showed an identical appearance on potato dextrose agar (PDA) (Difco, USA) and YPGA [1% (w/v) yeast extract, 0.5% (w/v) peptone, 4% (w/v) glucose, and 1.5% (w/v) agar]. Young colonies consisted of fusiform yeast cells [(5–)6–15(–20) × 1.5–2.2 μm] (Fig. 1), with polar budding on a sympodial rachis. Older colonies formed acropetal chains of fusiform conidia [(3–)4–10(–13) × 1.3–1.8 μm] originating from sterigma-like structures on narrow, hyaline, and septate hyphae. Thin aerial mycelia that were made up of these conidia gave the colony a somewhat velvety appearance. Brown pigment was exuded during growth on both agar media. On oatmeal agar medium [2% (w/v) oatmeal, 1% (w/v) glucose, and 1.5% (w/v) agar], the colonies continued to grow in yeast-like shapes. Diazonium Blue B and urease reactions were positive. Neither OM1 nor OM2 assimilated *myo*-inositol. Based on these morphological and some physiological characteristics, we assumed that the strains belong to the genus *Meira* or related basidiomycetous yeast-like fungi.

The genus *Meira* was proposed by Boekhout et al. (2003) as a novel basidiomycetous anamorphic yeast-like fungi, assigned to the Exobasidiomycetidae of the Ustilaginomycetes. Three species have been described to date in this genus: *M. geulakonigii* Boekhout et al., *M. argovae* Boekhout et al. (Boekhout et al. 2003), and *M. nashicola* Yasuda et al. (Yasuda et al. 2006). *Meira geulakonigii* and *M. argovae* were isolated from cadavers of the carmine spider mite on the leaves of castor beans or cadavers of citrus rust mites on citrus leaves in Israel (Boekhout et al. 2003), *M. nashicola* and *M. geulakonigii* were isolated from the

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surfaces of diseased pear fruit in Japan (Yasuda et al. 2005, 2006, 2007), and *M. geulakonigii* was detected by polymerase chain reaction (PCR) from the inner surface of grapefruit peels (Paz et al. 2007a). *Meira geulakonigii* and *M. argovae* exhibit capabilities as biocontrol agents against mites (Acari) and powdery mildew (Paz et al. 2007b; Sztejnberg et al. 2004).

Sequence analyses of the D1–D2 domain and internal transcribed spacer (ITS) region indicated that the strains OM1 and OM2 were clonal. Nuclear DNAs from both strains were extracted using a QuickGene DNA extraction kit (Fujifilm, Japan). The ITS, 5.8S rDNA, and D1/D2 domain of the 26S rDNA were amplified using primer pairs ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG) and NL4 (5′-GGTCCGTGTTTCAAGACGG), and were directly sequenced by the Sanger method using an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequencing reaction was completed using primers ITS4 (5′-TCCTCCGCTTATT GATATGC), ITS5, NL1 (5′-GCATATCAATAAGCG GAGGAAAAG), and NL4 with a BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems). This analysis revealed that both strains had identical nucleotide sequences. The sequences were deposited in the DDBJ database under accession numbers AB367528 (ITS1-5.8S rDNA-ITS2 region) and AB367529 (D1–D2 domain).

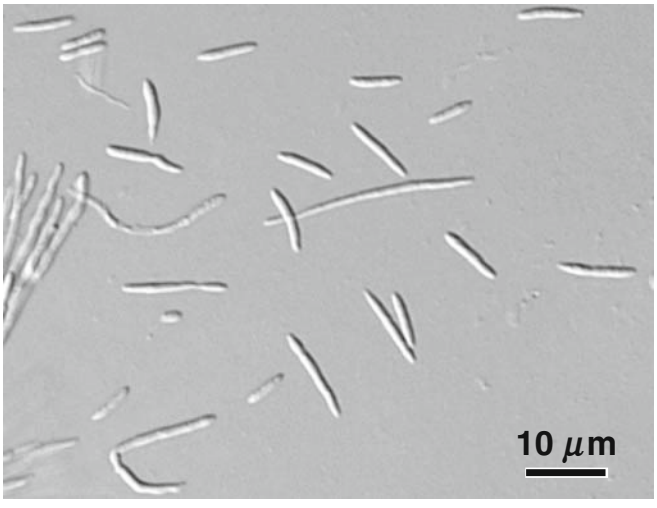

Fig. 1. Fusiform conidia of *Meira* sp. strain OM1

Sequence analyses of rDNA suggested that strain OM1 was conspecific with *M. argovae*. In basidiomycetous yeasts, combined sequence analysis of the D1–D2 domain and ITS regions is recommended for species identification (Scorzetti et al. 2002). Furthermore, detailed examination of the variation of the D1–D2 domain across approximately 500 species of ascomycetous yeast reveals that differences of 0 to 3 nucleotides are generally indicative of conspecific or sister species (Kurtzman and Robnett 1998; Kurtzman 2006). The rDNA sequence of the strain OM1 was compared with the ex-type strains of the three known *Meira* species. ITS1 and ITS2 sequences of the strains showed similarities of 70.4%–95.7% (ITS1) and 82.8%–95.7% (ITS2) (Table 1). The sequences for strains OM1 were the closest to those of *M. argovae* isolate AS005 (Table 1). Comparison between strain OM1 and *M. argovae* AS005, of the D1–D2 domain and ITS1-5.8S rDNA-ITS2 region sequences, showed similarities of 99.5% (D1–D2) and 96.9% (ITS1-5.8S rDNA-ITS2) (Table 2). The total sequences for ITS1-5.8S rDNA-ITS2 regions of strain OM1 differ in eight substitutions and nine gaps (17 nucleotides) from that of the *M. argovae* AS005. The D1–D2 domain of strain OM1 differs in two nucleotide substitutions and one gap (3 nucleotides) from that of the *M. argovae* AS005. These nucleotide variations are exactly higher than the expected intraspecific variations of ascomycetous yeasts. However, there is little information about intraspecific variations in the nucleotide sequence of basidiomycetous yeast-like fungus. The strain OM1 would be affiliated rather with *M. argovae* than with undescribed new species.

DNA–DNA reassociation analyses also confirmed the conspecificity of the strain OM1 with *M. argovae*. For comparison with the strain OM1, the ex-type strains of *M. geulakonigii* AS004 (CBS 110052; Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands), *M. argovae* AS005 (CBS 110053), and *M. nashicola* PFS002 (CBS 117161) were used. *Dicellomyces gloeosporus* TMIC 50099 (Tottori Mycological Institute, Kokoge, Tottori, Japan; Nagasawa 1987) was also used as a control for sister generic basidiomycetous fungus (Fig. 2). A loopful of cells was inoculated into 10 ml potato dextrose broth and grown at 25°C overnight. Cells were collected in a 15-ml test tube and

Table 1. Pairwise sequence comparison (%) in ITS1 and ITS2 regions among *Meira* species[a]

	Meira sp. OM1	*M. argovae* AS005^T	*M. geulakonigii* AS004^T	*M. nashicola* PFS002^T
Meira sp. OM1 (MAFF 240320)	–	95.7	79.0	70.4
M. argovae AS005^T (CBS 110053)	95.7	–	78.3	71.4
M. geulakonigii AS004^T (CBS 110052)	83.5	84.5	–	71.6
M. nashicola PFS002^T (CBS 117161)	86.7	88.3	82.8	–

ITS, internal transcribed spacer region; MAFF, GeneBank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

Accession numbers of the ITS region sequence data were as follows: *Meira* sp. OM1 (AB367528), *M. argovae* AS005 (AY158675), *M. geulakonigii* AS004 (AY158674), and *M. nashicola* PFS002 (AB185159)

Values were calculated from pairwise alignment using Stretcher algorithm of the EMBOSS package (Rice et al. 2000)

[a] Data in the upper right portion of the table are ITS1 similarity, and those in the lower left portion are ITS2 similarity

^{T} Ex-type strain

Table 2. Pairwise sequence comparison (%) in ITS1-5.8S rDNA-ITS2 region and D1–D2 domain of the 26S rDNA among *Meira* species[a]

	Meira sp. OM1	*M. argovae* AS005[T]	*M. geulakonigii* AS004[T]	*M. nashicola* PFS002[T]
Meira sp. OM1 (MAFF 240320)	–	99.5	98.8	98.0
M. argovae AS005[T] (CBS 110053)	96.9	–	99.0	98.5
M. geulakonigii AS004[T] (CBS 110052)	86.7	86.7	–	98.5
M. nashicola PFS002[T] (CBS 117161)	85.1	86.0	83.8	–

MAFF, GeneBank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

Accession numbers of the D1–D2 region sequence data were as follows: *Meira* sp. OM1 (AB367529), *M. argovae* AS005 (AY158669), *M. geulakonigii* AS004 (AY158668), and *M. nashicola* PFS002 (AB185157)

Values were calculated from pairwise alignment using Stretcher algorithm of the EMBOSS package (Rice et al. 2000)

[a] Data in the upper right portion of the table are D1–D2 similarity, and those in the lower left portion are ITS similarity

[T] Ex-type strain

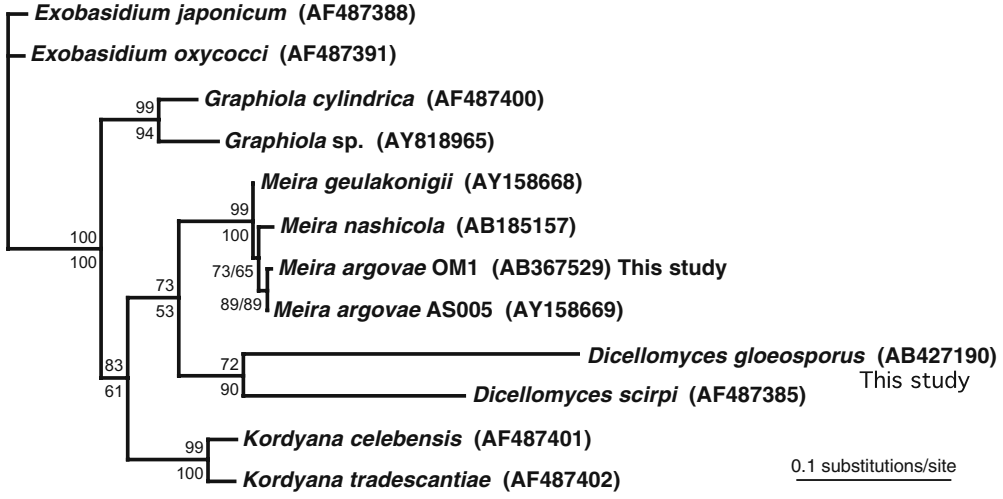

Fig. 2. Maximum-likelihood tree from D1–D2 domain of the 26S rDNA sequences of *Meira* species and closely related species under the best fitting model GTR + I (–ln L = 2538.3567). The parameters were as follows: base frequencies, A = 0.2582, C = 0.1939, G = 0.2812, T = 0.2667; substitution model rate matrix, [A–C] = 0.8295, [A–G] = 2.3477, [A–T] = 0.9027, [C–G] = 0.3304, [C–T] = 4.4605, [G–T] = 1.0000; proportion of invariable sites = 0.5219. Sequence data of other *Meira* species, allied genera, and *Exobasidium* species as outgroup were obtained from DDBJ/GenBank/EMBL DNA databases. In addition to this, the rDNA sequence of *Dicellomyces gloeosporus* (TMIC 50099; Nagasawa 1987) was analyzed. The accession numbers are indicated in *parentheses*. The sequences were aligned with CLUSTAL W (Thompson et al. 1994). The alignment was deposited in TreeBASE (M3921). Phylogenetic analyses using maximum likelihood with heuristic searches and maximum parsimony were implemented with PAUP* version 4.0 b10 (Swofford 2002). Each analysis gave the same topology. Maximum-likelihood analysis under the GTR + I model, which was determined to be the best model for evolution to this data set by Modeltest ver. 3.7 (Posada and Crandall 1988), resulted in a single tree. Bootstrap values (1000 replicates; Felsenstein 1985) are given *above the branches* for maximum likelihood and *below the branches* for maximum parsimony

used for genomic DNA preparation. Genomic DNA samples were purified by repeated phenol/chloroform extractions followed by ethanol precipitation, and their purity was confirmed by GeneQuant pro (Amersham Pharmacia). The DNA base composition (mol% G + C) was determined by high pressure liquid chromatography (HPLC) as described previously by Tamaoka and Komagata (1984). DNA–DNA hybridization was performed by the photobiotin microplate hybridization method of Kaneko and Banno (1991). All four isolates showed similar G + C contents of 41.2–43.7 mol% (Table 3). DNA–DNA relatedness values were 26.1%–42.9% among *Meira* species. When OM1 and *M. argovae* AS005 were compared, the values were 69.1%–79.2%, whereas those between OM1 and *M. geulakonigii* AS004 or *M. nashicola* PSF002 were 35.8%–49.5%. As conspecific ascomycetous yeasts generally demonstrate 70% or greater DNA relatedness (Kurtzman 2006), and the DNA relatedness values between OM1 and AS005 are nearly or greater than 70%, OM1 should be considered conspecific with *M. argovae*.

Physiological characteristics of strain OM1 mostly coincided with those of *M. argovae*. Carbohydrate and nitrogen assimilation tests were performed as described previously (Yarrow 1998). These physiological characteristics revealed only a slight difference between the strain OM1 and *M. argovae* AS005 (Table 4). Taken together, the strain OM1 was identified as one strain of *M. argovae*. Thus, *M. argovae* is added to the Japanese mycoflora.

Meira argovae Boekhout, Scorzetti, Gerson & Sztejnberg, 2003. Int. J. Syst. Evol. Microbiol. 53: 1661–1662. Fig. 1

Specimens from newly growing bamboo shoots were collected in Omishima Island, Imabari-shi, Ehime Prefecture,

Table 3. DNA base composition (G + C content) and DNA–DNA hybridization among *Meira* species

Strains	G + C content (mol%)	% DNA relatedness				
		OM1	AS005^T	AS004^T	PSF002^T	TMIC50099
Meira sp. OM1 (MAFF 240320)	41.9	100	79.2	49.5	44.2	6.66
M. argovae AS005^T (CBS 110053)	41.2	69.1	100	41.4	42.9	7.26
M. geulakonigii AS004^T (CBS 110052)	43.7	35.8	26.1	100	42.7	7.48
M. nashicola PSF002^T (CBS 117161)	41.8	41.3	29.4	42.5	100	11.4
Dicellomyces gloeosporus (TMIC 50099)	N.T.	2.49	0.54	4.85	9.68	100

MAFF, GeneBank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; TMIC, Tottori Mycological Institute, Kokoge, Tottori, Japan; N.T., not tested
^{T}Ex-type strain

Table 4. Physiological characteristics differentiating *Meira* sp. OM1[a] and *M. argovae* AS005

	Assimilation of carbon compounds[b]			
	Arbutin	Melibiose	Glucono-σ-lactone	D-Glucuronate
Meira sp. OM1 (MAFF 240320)	–	+	D	W
M. argovae AS005^{T,c} (CBS 110053)	–, W, D	+, –	+	–

MAFF, GeneBank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands
[a] *Meira* sp. OM1 is positive for assimilation of the carbon compounds D-galactose, D-ribose, L-arabinose, D-arabinose, cellobiose, melezitose, soluble starch, *meso*-erythritol, citrate, ethanol, quinic acid, D-glucose, D-xylose, sucrose, maltose, D-trehalose, D-raffinose, D-glucitol, and D-mannitol; delayed growth for salicin, ribitol, and xylitol; weak growth for inulin, glycerol, and DL-lactate; negative for assimilation of lactose, L-arabinitol, galactitol, *myo*-inositol, D-gluconate, L-sorbose, D-glucosamine, L-rhamnose, methyl α-glucoside, 2-keto-D-gluconate, *N*-acetyl-D-glucosamine, and D-psicose; positive for assimilation of nitrogen compounds nitrate, nitrite, and ethylamine; and negative for assimilation of L-lysine. Growth in 0.01% cycloheximide is positive; growth in 0.1% cycloheximide is negative; growth at 35° and 37°C is negative. Diazonium Blue B and ureae reactions are positive
[b] Differentiating characteristics were scored as +, growth; –, no growth; W, weak growth; D, delayed growth (after 2 or more weeks)
[c] Data from Boekhout et al. (2003)
^{T}Ex-type strain

Japan, October 2004, by E. Tanaka. The strain OM1 was deposited in GeneBank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan, as the accession number MAFF 240320.

Phylogenetic analysis of the D1–D2 domain demonstrated that *Meira* species were closely related to but distinct from teleomorphic *Dicellomyces* species (Brachybasidiaceae, Exobasidiales) (Fig. 2). Previously, Boekhout et al. (2003) showed that *Meira* species were phylogenetically related to *D. scirpi* Raitiviir, which was the only *Dicellomyces* species having available molecular data at that time. *Dicellomyces* species are leaf parasites in monocotyledonous hosts. They protrude to form gelatinous basidiocarps from leaf epidermis and have two-sterigmate basidia. In this genus, *D. gloeosporus* Olive was reported as a leaf parasite of bamboo in the United States and Japan (Olive 1945; Nagasawa 1987). It is noteworthy that *M. argovae* was isolated from bamboo. Although no teleomorph is known for *Meira* species, *D. gloeosporus* produces blastconidia (Nagasawa 1987) that resemble those of *Meira* species. To investigate the possible anamorph-teleomorph connection, phylogenetic analysis was performed using four *Meira* species, two *Dicellomyces* species, and allied genera including two *Exobasidium* species as an outgroup (Fig. 2). This phylogenetic study revealed that *Meira* species and *Dicellomyces* species formed separate sister clades. Further work is needed to determine the teleomorph connection of *Meira* species.

This is the first report of *M. argovae* isolated from a plant. The other *Meira* species were also found on plants; *M. geulakonigii* from grapefruit or pear fruit (Paz. et al. 2007a; Yasuda et al. 2007), and *M. nashicola* from pear fruit (Yasuda et al. 2006). However, *Meira* species were originally reported as mite parasites (Boekhout et al. 2003; Sztejnberg et al. 2004; Paz et al. 2007b). In view of these facts, the mite-associated phase of *Meira* species probably derived from a plant-parasitic life cycle.

Acknowledgments The authors thank Dr. E. Nagasawa, Tottori Mycological Institute, for providing us with the strain of *Dicellomyces gloeosporus*.

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