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New Species of the Genus *Metschnikowia* Isolated from Flowers in Indonesia, *Metschnikowia cibodasensis* sp. nov.

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A novel species, *Metschnikowia cibodasensis*, is proposed to accommodate eight strains (ID03-0093^T, ID03-0094, ID03-0095, ID03-0096, ID03-0097, ID03-0098, ID03-0099, and ID03-0109) isolated from flowers of *Saurauia pendula, Berberis nepalensis,* and *Brunfelsia americana* in Cibodas Botanical Garden, West Java, Indonesia. The type strain of *M. cibodasensis* is ID03-0093^T (= NBRC 101693^T =UICC Y-335^T = BTCC-Y25^T). The common features of *M. cibodasensis* are a spherical to ellipsoidopedunculate shaped ascus, which contains one or two needle-shaped ascospores, and lyse at maturity. Asci generally develop directly from vegetative cells but sometimes from chlamydospores. The neighbor-joining tree based on the D1/D2 domain of nuclear large subunit (nLSU) ribosomal DNA sequences strongly supports that *M. cibodasensis* (eight strains) and its closest teleomorphic species, *M. reukaufii,* are different species by a 100% bootstrap value. The type strain of *M. cibodasensis,* ID03-0093^T, differed from *M. reukaufii* NBRC 1679^T by six nt (five substitutions and one deletion) in their D1/D2 region of nLSU rDNA, and by 18 nt (five deletions, four insertions, and nine substitutions) in their internal transcribed spacer regions of rDNA, respectively. Four strains representative of *M. cibodasensis* (ID03-0093^T, ID03-0095, ID03-0096, and ID03-0099) showed a mol% G+C content of 44.05 ± 0.25%, whereas that of *M. reukaufii* NBRC 1679^T was 41.3%. The low value of DNA-DNA homology (5–16%) in four strains of *M. cibodasensis* and *M. reukaufii* NBRC 1679^T strongly supported that these strains represent a distinct species.

Keywords: Ascomycetous yeast, flowers, *Metschnikowia cibodasensis*, taxonomy

Introduction

Metschnikowia is a well-documented genus of teleomorphic ascomycetous yeasts. Since its first report by Metschnikoff in 1884 as the genus *Mosnospora*, more than 40 species have been accommodated within the *Metschnikowia* lineage, including several species of *Candida*, which is known as an anamorphic genus of *Metschnikowia*. Species of *Metschnikowia* are characterized by the formation of long needle-shaped or acicular ascospores that lack appendages [25]. Most terrestrial species of *Metschnikowia* are found in the nectar or corolla of flowers and in decaying fruit or plant tissue. They are transmitted to new niches by insects, such as bees and drosophilids [16, 17, 25, 32].

Insects associated with ephemeral flowers harbor very specific yeast communities. The insects, principally nitidulid beetles and drosophilid flies, vector a highly specific yeast community that may serve as food for the larvae of the insects [14, 17]. The proliferation of terrestrial flowering plants and associated insects has markedly affected the

genetic diversification in *Metschnikowia* [6]. In fact, several newly described *Metschnikowia* and its anamorphic *Candida* species were isolated from a large numbers of fruit and flowers-associated species. These included *Metschnikowia koreensis* [7], *M. arizonensis, M. dekortorum* [14], *M. vanudenii*, *M. lachancei* [6], *Candida kunwiensis* [8], *M. santaceciliae, C. hawaiiana, C. kipukae* [18], *M. similis*, *M. colocasiae* [15], *M. hamakuensis, M. kamakouana*, *M. mauinuiana* [19], *M. aberdeeniae* [20], *M. orientalis* [21], and *M. shivogae* [22], which were recorded from flowers or their associated insects, and *M. viticola*, which was isolated from grapes [27].

On the other hand, there is no report on *Metschnikowia* isolated from Indonesia, although its prospect is likely because of the country's diversity of hosts and vectors. In a taxonomic study of fungi in Indonesia in July 2003, we took samples from flowers of several plants in the Cibodas Botanical Garden in West Java, Indonesia. Eight of the strains represented a novel, sister species to *M. reukaufii*. We describe them as *Metschnikowia cibodasensis* sp. nov.

Materials and Methods

Yeast Strains

The eight strains of *M. cibodasensis* were isolated from flowers in Cibodas Botanical Garden in West Java, Indonesia. The flower samples were collected by Chiharu Nakashima and Ariyanti Oetari on 23rd July 2003. Three strains (ID03-0093^T, ID03-0094, and ID03-0095) were isolated from *Saurauia pendula* Blume, four strains (ID03-0096, ID03-0097, ID03-0098, and ID03-0099) were isolated from *Berberis nepalensis* Spreng., and strain ID03-0109 was isolated from *Brunsfelsia americana* L. All parts of the flower were weighed and cut into pieces. The flower pieces were washed with 30 ml of sterile distilled water and vortexed for 5 min. Washed materials were placed directly onto YM agar (1% glucose, 0.3% yeast extract, 0.3% malt extract, and 1.5% agar) containing 0.05% chloramphenicol. The suspension was filtered using a 0.45 μm membrane filter (Merck Millipore, USA), and the membrane filter was put onto the YM agar medium. Incubation of the plates was done for 5 days at room temperature.

The type strain ID03-0093^T (NBRC 101693^T = UICC Y-335^T = BTCC-Y25^T) and another seven strains were deposited (living and dried) at three culture collections: the NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Japan under the numbers NBRC 101693^T–NBRC 101700; University of Indonesia Culture Collection (UICC), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, Indonesia under the numbers UICC Y-335^T–UICC Y-341 and UICC Y-351; and the Research Center for Biotechnology Culture Collection (BTCC), Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia under the numbers BTCC-Y25^T–BTCC-Y32. The strains studied and their accession numbers of sequences of the D1/D2 domain of nuclear LSU ribosomal DNA and the ITS regions (including ITS1, 5.8S, and ITS2) are shown in Table 1.

Morphological and Physiological Characterization

The morphological and physiological characteristics of the eight isolates were determined by the standard method described by Yarrow [40]. The observations of asci and ascospores were performed on diluted V8 agar (1:9 and 1:29), diluted Corn Meal agar (1:9), and Yeast Carbon Base agar at 15–20°C for up to 2 months.

Ultrastructure of Ascospores

The mode of ascogenesis was observed by scanning electron microscopy (SEM) following the methods of Talens *et al*. [35, 36] and Mikata [24], with a slight modification. One-week-old cultures on diluted V8 agar (1:9) were scraped and suspended in 2 ml of 67 mM phosphate buffer (pH 7.0). After the cells were collected by centrifugation, they were washed with distilled water. The cells were then suspended in 0.5 ml of 67 mM phosphate buffer (pH 7.0) and 0.25 ml of Zymolyase 100T (3 mg/ml) containing 2-mercaptoethanol, and 10 μl of RNase (100 mg/ml). The samples were subsequently washed with 67 mM phosphate buffer (pH 7.0) before they were dehydrated in a graded ethanol series (30 min each), which was followed by washes in isoamyl acetate in absolute ethanol (1:1) (1 h) and then in absolute isoamyl acetate for 1 h. After treatment

Table 1. Origin of the *Metschnikowia cibodasensis* strains sequenced in this study.

Strain	Other designation(s)	Isolation sources	Sample code	DDBJ/GenBank/EMBL Accession No. (D1/D2 of LSU rDNA, ITS)
ID03-0093^T	UICC Y-335^T, NBRC 101693^T, BTCC-Y25^T	Flowers of *Saurauia pendula*	182	AB236910, AB236918
ID03-0094	UICC Y-336, NBRC 101694, BTCC-Y26	Flowers of *Saurauia pendula*	182	AB236911, AB236919
ID03-0095	UICC Y-337, NBRC 101695, BTCC-Y27	Flowers of *Saurauia pendula*	182	AB236912, AB236920
ID03-0096	UICC Y-338, NBRC 101696, BTCC-Y28	Flowers of *Berberis nepalensis*	183	AB236913, AB236921
ID03-0097	UICC Y-339, NBRC 101697, BTCC-Y29	Flowers of *Berberis nepalensis*	183	AB236914, AB236922
ID03-0098	UICC Y-340, NBRC 101698, BTCC-Y30	Flowers of *Berberis nepalensis*	183	AB236915, AB236923
ID03-0099	UICC Y-341, NBRC 101699, BTCC-Y31	Flowers of *Berberis nepalensis*	183	AB236916, AB236924
ID03-0109	UICC Y-351, NBRC 101700, BTCC-Y32	Flowers of *Brunsfelsia americana*	204	AB236917, AB236925

with a critical point dryer (Hitachi HCP-2), the materials were coated with Pt-Pd and examined at 20 kV under a JEOL scanning electron microscope.

Molecular Characterization

DNA preparation for DNA base composition and DNA-DNA hybridization

Overnight cultures of strains ID03-0093^T (NBRC 101693^T), ID03-0095, ID03-0096, ID03-0099, and *Metschnikowia reukaufii* NBRC 1679^T on 50 ml of YPD at 25°C with shaking at 180 rpm were harvested by centrifugation. The DNA was extracted by the phenol-extraction following the method of Burke *et al*. [2] with a slight modification. The obtained DNA was then used for determination of the DNA base composition (molar percentage of guanine + cytosine) and DNA-DNA hybridization.

DNA base composition and DNA-DNA hybridization

Determination of the DNA base composition (molar percentage of guanine + cytosine) was carried out by high performance liquid chromatography according to the method in Tamaoka and Komagata [37]. DNA-DNA hybridization was performed using the photobiotin method [3].

rDNA Sequence Analysis

Nuclear DNA from the isolates was extracted using the method in Sjamsuridzal and Oetari [30]. The D1/D2 domain of nuclear LSU rDNA and the ITS regions of rDNA (including 5.8S rDNA region) were amplified and sequenced using the primer set NL1 (forward: GCATATCAATAAGCGGAGGAAAAG) and NL4 (reverse: GGTCCGTGTTCAAGACGG) for the D1/D2 domain, and ITS5 (forward: GGAAGTAAAAGTCGTAACAAGG) and ITS4 (reverse: TCCTCCGCTTATTGATATGC) for the ITS regions, as described by White *et al*. [39]. The D1/D2 domain of nuclear LSU rDNA and the ITS regions of the rDNA unit were sequenced directly from polymerase chain reaction (PCR) products using the primer pairs NL1 and NL4, and ITS4 and ITS5. PCR amplifications were conducted under the condition described in Sjamsuridzal *et al*. [31]. The nucleotide sequences were determined by using a Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA) according to the manufacturer's instructions. The gel electrophoresis and data collection were performed using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The sequences of the D1/D2 domain of nLSU rDNA and the ITS regions were aligned using CLUSTAL X [38] with other sequences of *Metschnikowia* species retrieved from GenBank. The tree topology was reconstructed using the neighbor-joining method [29] with Kimura's two-parameter distance correction [9] in the PHYLIP 3.05 package [5]. The gaps were not included in our phylogenetic analyses. *Schizosaccharomyces pombe* (U40085) was used as an outgroup taxon. Confidence levels for the individual branches of the resulting tree were assessed by bootstrap analysis [4] with 1,000 bootstrap resamplings. The GenBank/EMBL/DDBJ accession numbers for the D1/D2 domain of nLSU rDNA and ITS region sequences of the strains newly obtained in this study are shown in Table 1.

Results and Discussion

Phenotypic Affiliation of Strains

Morphologically, the strains of *M. cibodasensis* are similar to the teleomorphic genus *Metschnikowia*, which is classified in the phylum Ascomycota, order Saccharomycetales, belonging to the Family Metschnikowiaceae. All these strains produce needle-shape ascospores, which is the specific character of the genus *Metschnikowia*. The morphological differences between the strains of *M. cibodasensis* and its morphologically closest species, *Metschnikowia reukaufii*, are as follows: the former has asci that arise from vegetative cells and rarely from chlamydospores, which are spherical to ellipsoid-pedunculate in shape, containing one or two ascospores, that lyse at maturity. On the other hand, *M. reukaufii* has the following characteristics: asci arising from chlamydospores that are ellipsoid-pedunculate to clavate in shape, and it do not lyse at maturity [25].

Two types of colonies were observed within the strains through repeated subculturing on Malt Extract Agar (MEA; Difco, Becton Dickson and Company, USA). One colony has a somewhat rough and dull surface and is dominated by vegetative cells; the other colony is smooth with a slightly glistening surface and is dominated by subglobose to ellipsoidal chlamydospores. Two types of cells were observed in one colony on slide cultures after 10 days at 25°C. One type consists of vegetative cells (2-3 × 6-9 μm), and the other type consists of subglobose to ellipsoidal chlamydospores (4-7 × 9-13 μm). The same phenomenon was observed in *M. koreensis* as reported by Hong *et al*. [7].

The results of the physiological characterizations showed that the strains of novel species *M. cibodasensis* are typical of many members of the Metschnikowiaceae and are practically indistinguishable from those of the closest species. The strains of *M. cibodasensis* have fermentation and assimilation patterns that are almost identical to those of *M. reukaufii*. *Metschnikowia cibodasensis* can be differentiated from *M. reukaufii* only by its inability to assimilate L-lysine and cadaverine, and its inability to grow on 1 μg/ml cycloheximide. In view of the similar nutritional profiles of *M. cibodasensis* and *M. reukaufii*, a definitive identification of the species should rely on the determination of genotypic characteristics by sequence differences in the D1/D2 region of LSU and ITS regions of rDNA, the differences in the mol% G+C content, and in the values of their DNA-DNA hybridization.

Genotypic Affiliation of Strains

Phylogenetic placement

The phylogenetic analysis of the type strain *M. cibodasensis* ID03-0093^T (NBRC 101693^T), based on the sequence of D1/D2 of nLSU rDNA, revealed that it was clustered with an anamorphic yeast, *Candida* sp. CBS 10645 (EU117200), which was isolated in 2007 by Ji, Z.-H. and Bai, F.-Y. in China. Although they are closely related, the lengths of D1/D2 of the nLSU rDNA sequences of *M. cibodasensis* and *Candida* sp. CBS 10645 are different. Eight strains of *M. cibodasensis* vary in the length of their sequences from 518-530 nt; however, *Candida* sp. CBS 10645 has a shorter sequence, 512 nt. Nevertheless, the homology of the overlapping sequences between *M. cibodasensis* strains and *Candida* sp. CBS 10645 are highly similar or identical (99–100%). The highly similar or identical sequence data of D1/D2 of the nLSU rDNA in *M. cibodasensis* strains and *Candida* sp. CBS 10645 led us to assume that they may have an anamorph-teleomorph connection. These species were isolated from similar habitats: strains of *M. cibodasensis* were isolated from flowers in Indonesia, and *Candida* sp. CBS 10645 was isolated from flowers and pollinating bees in China.

The strains of *M. cibodasensis* were clustered with its morphologically and physiologically closest teleomorphic species, *M. reukaufii*, and some *Candida* spp. (EU11720 & U44821) with 100% bootstrap support under the genus *Metschnikowia* clade. These strains were then divided into two different clusters with high bootstrap values (BS: 85% & 95%, respectively) (Fig. 1).

In general, strains of "yeast" are considered separate species if they show six or more noncontiguous substitutions (1%) in their D1/D2 region of LSU rDNA [13, 12] or by sequence differences of at least 1% in their ITS regions [33]. Based on the D1/D2 of nLSU rDNA sequence data of the type strain of *M. cibodasensis* NBRC 101693^T, it differed from *M. reukaufii* NBRC 1679^T by six nucleotides (five substitutions and one deletion) (1.17%).

The ITS sequence yields a greater resolution than that obtained from large subunit domains D1/D2. Significant differences in the ITS regions sequence (18 nt) (5.0%) are found between *M. cibodasensis* NBRC 101693^T and *M. reukaufii* NBRC 1679^T, which supports that they are separate species. Because the sequence data of the ITS region of *Candida* sp. CBS 10645 was not available in the database, we could not compare it with those of *M. cibodasensis*. On the other hand,

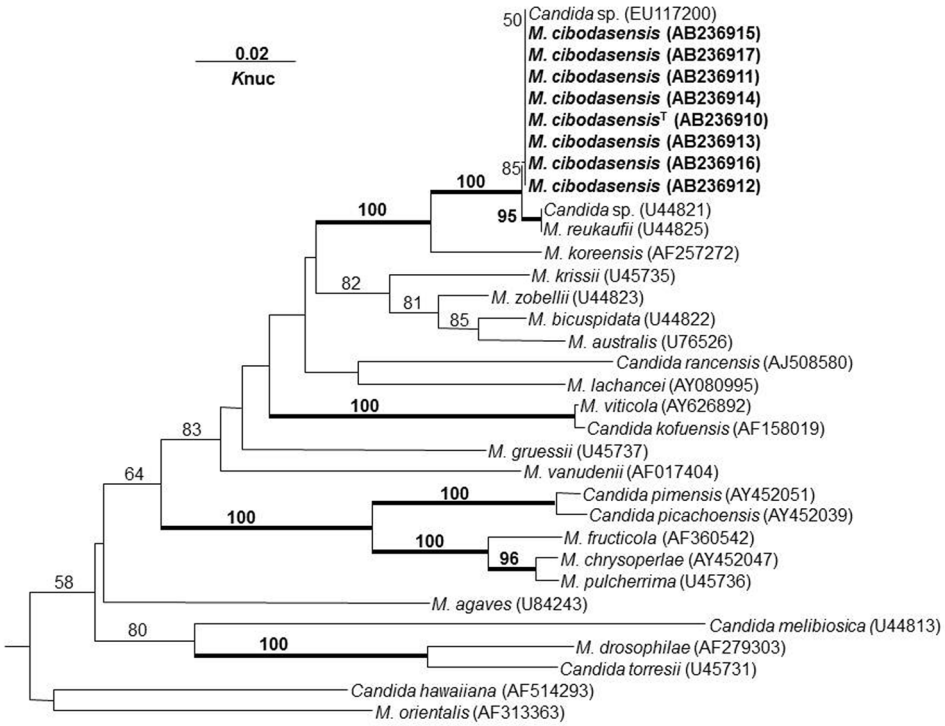

Fig. 1. Neighbor-joining tree constructed using the D1/D2 region of the nLSU rDNA sequences of a new *Metschnikowia* species and its related species.

Only closely related species are shown. The DDBJ/GenBank/EMBL accession numbers are indicated in parentheses. The numerals indicate the confidence level from 100 replicate bootstrap samplings (frequencies below 50% are not indicated). *K*nuc, Kimura's parameter (1980).

the sequence differences of the D1/D2 region of LSU and the ITS region of rDNA between *M. cibodasensis* NBRC 101693^T and *M. reukaufii* NBRC 1679^T were 1.17% and 5.0%, respectively, which indicates that they are distinct species.

G+C Content and DNA Relatedness

Determination of the mol% G+C content revealed that this species differs by almost 3% from its closely related species, *M. reukaufii*. Four strains of *M. cibodasensis* (ID03-0093, ID03-0095, ID03-0096, and ID03-0099) showed mol% G+C content of 44.05 ± 0.25 %, whereas *M. reukaufii* NBRC 1679^T showed 41.3%. Kurtzman [11] mentioned that strains showing a difference in G+C content of more than 1.5% might be expected to represent different species.

We performed DNA-DNA hybridization experiments on four representative strains of *M. cibodasensis* and the type strain of *M. reukaufii* NBRC 1679 ^{T} (Table 2). These four strains of *M. cibodasensis* (ID03-0093^T, ID03-0095, ID03-0096, and ID03-0099) have a high similarity value, ranging from 84% to 100% on each pairing. According to Martini and Phaff [23] and Price *et al.* [28], strains having 80% or greater DNA relatedness are conspecific. Therefore, the similarity of 84% to 100% found in the DNA-DNA hybridization experiments indicated that the four strains (ID03-0093, ID03-0095, ID03-0096, and ID03-0099) are conspecific. The low values of DNA relatedness (5–16%) between the four strains of *M. cibodasensis* and *M. reukaufii* NBRC 1679^T coincide well with the results of phylogenetic analysis based on the D1/D2 domain of nLSU rDNA sequences (Fig. 1), which showed that they are distinct species.

The phylogenetic tree based on the D1/D2 domain of nLSU rDNA (Fig. 1) showed that the novel species *M. cibodasensis* was grouped together with other species isolated from flowers, such as *M. reukaufii* [25] and *M. koreensis* [7]. The physiological characters of *M. cibodasensis* showed similarity with other species isolated from flowers, such as the ability of all strains to grow in 50% and 60% glucose. This physiological characteristic is common in flower-associated yeasts. The eight strains of new species did not show host specificity, as they were isolated from three different species of flowering plants (*Berberis nepalensis, Brunsfelsia americana,* and *Saurauia pendula*). Antonovics [1] mentioned that pollinators are major vectors for nectar-inhabiting organisms. We suggest that *M. cibodasensis* might be transmitted by insect pollinator from flower to flower of different plant species.

In the course of our taxonomic studies of fungi in Indonesia, we pointed out the diversity of entomophagous fungi [10, 34], plant parasitic fungi [26], and soilborne fungi [34], based on the modern taxonomic criteria. Likewise, Sjamsuridzal *et al.* [31] reported the yeast population in the flowers of several plants in Indonesia to be rich in those diversity. They found genera of yeasts and yeast-like fungi, such as *Anomalomyces, Aureobasidium, Bullera, Candida, Cryptococcus, Metschnikowia, Pseudozyma, Rhodotorula*, and *Rhodosporidium*. Their report indicates that flower surfaces are an important habitat for yeasts. The present study supports their outlook. However, more studies are required to reveal the diversity of "yeasts" in Indonesia.

Description of *Metschnikowia cibodasensis* sp. nov.

Metschnikowia cibodasensis W. Sjamsuridzal, A. Oetari, C. Nakashima, A. Kanti, R. Saraswati, Y. Widyastuti & K. Ando, sp. nov. Figs. 1, 2

MycoBank no.: MB 563762

Latin diagnosis. *Coloniae in agaro farinae zeae post dies 10 ad 25°C ellipsoideae, cylindratae, subglobosae vel ovoideae (2–3 × 6–9 μm), singulae, binae vel in catenis brevibus; pseudohyphae fiunt. Post unum mensem sedimentum formatur (After one month sediments are formed). Cultura in agaro malti (Culture on Malt extract agar) (after 3 days) post dies 3 ad 25°C cremea (cream) vel lutea (yellow)et butyrosa (butter like). In agaro farinae Zea mays (on corn meal agar) post dies 10 pseudomycelium nullum vel rudimentarium (absent or rudimentary). Chlamydosporae subglobosae ad ellipsoideae formantur. Asci globosae ad ellipsoidopedunculati (2–3 × 6–8 μm), 1 ad 2-spori, ascosporae aciculares vel acerosae (0.7–1.1 × 9–105 μm). Glucosum fermentatur, galactosum (lente), at non saccharum, maltosum, lactosum,*

Table 2. DNA relatedness (%) among strains of *M. cibodasensis* and the type strain of *M. reukaufii* (NBRC 1679^T).

Strain	DNA relatedness (%)				
	NBRC 1679^T	ID03-0093^T	ID03-0095	ID03-0096	ID03-0099
NBRC 1679^T	100	5	14	16	15
ID03-0093^T		100	88	84	85
ID03-0095			100	96	94
ID03-0096				100	94
ID03-0099					100

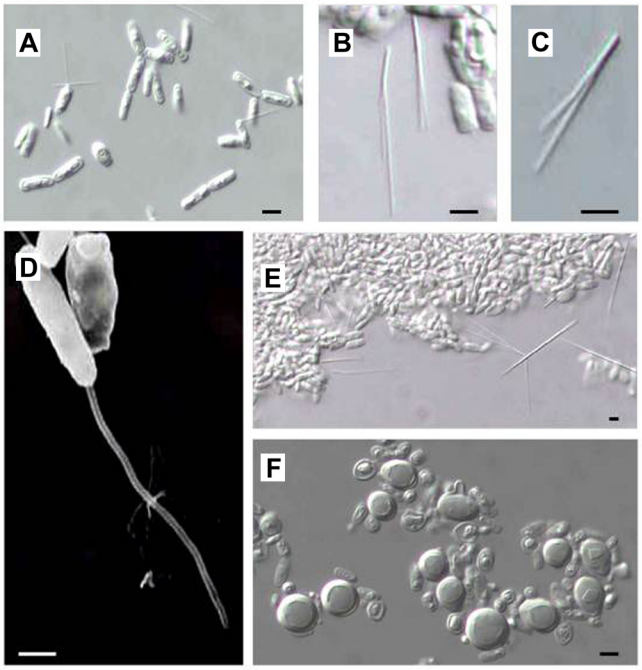

Fig. 2. Ascus and ascospores of *M. cibodasensis* after 1 week of growth on V8 diluted (1:9) agar.

(**A**) Ascus (asci) of *M. cibodasensis* strain ID03-0093^T (NBRC 101693 ^{T}); (**B**) asci and ascospores of strain ID03-0095; (**C**) ascospores of *M. cibodasensis* strain ID03-0095; (**D**) ascus of *M. cibodasensis* strain ID03-0093 as observed on a scanning electron micrograph; (**E**) release ascospores of *M. cibodasensis* strain ID03-0095; (**F**) vegetative cells and chlamydospores of *M. cibodasensis* strain ID03-0093. Bars, 1 μm.

raffinosum nec trehalosum. Glucosum, galactosum (variabile), sucrosum, maltosum, cellobiosum, trehalosum, melezitosum, D-xylosum (variabile), N-acetylglucosaminum, D-glucosaminum, glycerolum, D-mannitolum methyl a-D-glucosidum, D-ribosum (variabile), D-sorbitolum et 2-ketogluconatum assimilantur at non lactosum, D-melibiosum, raffinosum, D-arabinosum, L-rhamnosum, methanolum, acidum DL-lacticum, acidum citricum, erythritolum et inositolum. Assimilatio ethylamini et casein ad non kalii nitratis, sodii nitrosinum, hypoxanthinum, L-lysinum, cadaverinum et D-glucosaminum. Vitaminae externae ad crescentiam necessariae sunt. Ureum non finditur. Crescit in 28°C sed non in 37°C. Materia amyloidea non formantur. Proportio molaris guanini plus cytosini in acidideoxyribonucleati 44.05 ± 0.25 mol %.

Holotypus (as designated here). Dried specimens of NBRC 101693^T (=UICC Y-335^T = BTCC-Y25^T), from the Cibodas Botanical Garden in West Java, Indonesia were isolated from the flowers of *Saurauia pendula* Blume on 23rd July 2003 by Nakashima and Oetari. The ex-holotype strain was deposited in three culture collections: NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Japan under the number NBRC 101693^T; University of Indonesia Culture Collection (UICC), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, Indonesia under the number UICC Y-335^T; and the Research Center for Biotechnology Culture Collection (BTCC), Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia under the number BTCC-Y25^T.

rDNA sequences ex-holotype: AB236910, AB236918

Etymology: *cibodasensis* (ci.bo.das.en'sis. L. nom. sing. f. adj.) or cibodasensis of Cibodas, refers to the Cibodas Botanical Garden, West Java, Indonesia, where all strains of this species were discovered.

Description. In slide culture on corn meal agar after 10 days at 25°C, the cell shapes were elliptical, cylindrical, subglobose, or ovoidal. The cell arrangements were solitary, two-fold, or in short chains. Pseudomycelium was rudimentary or absent. Two types of cells were observed in one colony: vegetative cells (2–3 × 6–9 μm) with dull texture under the microscope, and cells consisting of subglobose to ellipsoidal chlamydospores (4–7 × 9–13 μm) that showed a glistening texture under the microscope. Sporulation was observed in all strains on diluted (1:9, 1:29) V8 agar, malt extract agar, and diluted (1:9) CMA medium after 3 days to 1 week incubation at 25°C. The asci were spherical to ellipsoidopedunculate (2–3 × 6–8 μm) (Fig. 2A) and contained one or two ascospores (Figs. 2B, 2C). SEM examination of asci of *M. cibodasensis* showed an ellipsoidopedunculate ascus (Fig. 2D). The ascospores were acicular or acerose (0.7–1.1 × 9–105 μm). Asci mainly arise from vegetative cells, rarely from chlamydospores (4–7 × 9–13 μm) and lyse at maturity. Formation of asci was not frequent in all strains examined; however, some strains produced abundant free ascospores (Fig. 2E). Chlamydospores of *M. cibodasensis* strain ID03-0093^T grew on V8 diluted (1:9) agar after 1 week. D-Glucose and galactose were fermented (weak); sucrose, maltose, lactose, raffinose, and trehalose were not fermented. The following carbon compounds were assimilated: D-glucose, D-galactose, D-sorbose (variable), sucrose, D-maltose, D-cellobiose, trehalose, D-melezitose, D-xylose (variable), D-glucosamine, *N*-acetylglucosamine, glycerol, D-mannitol, methyl α-D-glucoside, D-ribose (variable), D-sorbitol, and 2-ketogluconate. Lactose, melibiose, raffinose, D-arabinose, L-rhamnose, methanol, DL-lactic acid, citric acid, erythritol, and inositol were not assimilated. The nitrogen compounds ethylamine and casein were assimilated; nitrate, nitrite, hypoxanthine, cadaverine, L-lysine, and D-glucosamine were not assimilated. It grew in the presence of 50% and 60% glucose, and at 28°C. It did not grow in the presence of

0.01% cycloheximide, in a vitamin-free medium, and at 37°C. Starch was not form. Urease test was negative.

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