Kockovaella barringtoniae sp. nov., a new basidiomycetous yeast species isolated from a plant leaf collected in a tropical rain forest in Thailand

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One yeast strain characterized by the proliferation of non-ballistosporous stalked conidia, budding cells and ballistoconidia, the presence of xylose in whole-cell hydrolysates, the presence of Q-10 as the major ubiquinone isoprenologue, the inability to ferment sugars and positive diazonium blue B (DBB) and urease reactions was isolated from a plant sample collected in a tropical rain forest in Thailand. The isolate clustered with Kockovaella species in the 18S rDNA-based tree. On the basis of the morphological, biochemical and molecular phylogenetic characteristics, the isolate was assigned to the genus Kockovaella. DNA complementarity experiments showed that the isolate was genetically distinct from known species of the genus Kockovaella. The isolate is described as Kockovaella barringtoniae sp. nov. The type strain is strain TY-278T (= JCM 10998T = TISTR 5770T).

Keywords: Kockovaella barringtoniae sp. nov., ballistoconidium-forming yeasts, basidiomycetous yeasts, Hymenomycetes

In the course of a survey of yeasts living in the natural environment in Thailand, one of the authors (T. Nakase) and his coworkers isolated numerous strains of ballistoconidium-forming yeasts and described two new species of the genus Bensingtonia Ingold emend. Nakase & Boekhout (Takashima et al., 1995; Fungsin et al., 2001), one new species of the genus Bullera Derx (Takashima & Nakase, 1998), three new species of the genus Kockovaella Nakase, Banno & Y. Yamada (Nakase et al., 1991; Takashima & Nakase, 1998) and four new species of the genus Sporobolomyces Kluyver & van Niel (Takashima & Nakase, 2000). In this study, we isolated three strains of the genus Kockovaella from leaves collected in a tropical rain forest in Thailand. Two of them were assigned to already known species and one isolate was found to represent an undescribed species. The identification of these two isolates and the description of a new species in the genus Kockovaella are reported here.

The three strains were isolated from plant leaves collected in a tropical rain forest in the Sakearat Environmental Research Station, Nakhon Ratchasima Province, Thailand, in November 1996 by the procedure described previously (Nakase & Suzuki, 1985; Nakase & Takashima, 1993). Strains TY-208 and TY-210 were isolated from Dichanthium caricosum and strain TY-278 was isolated from Barringtonia sp. Six known species of the genus Kockovaella, Kockovaella imperatae JCM 7826T, Kockovaella machilophila JCM 10052T, Kockovaella phaffii JCM 10073T, Kockovaella sacchari JCM 9858T, Kockovaella shinae JCM 10051T and Kockovaella thailandica JCM 7824T, were used in a comparative study. All strains were grown at 25 °C in yeast extract/malt extract broth or on yeast extract/malt extract agar (Difco). The isolates were characterized morphologically, physiologically, biochemically and chemotaxonomically as described by Fungsin et al. (2001). Nucleotide sequence analyses of 18S rDNA and the internal transcribed spacer (ITS) region were performed as described previously (Hamamoto & Nakase, 2000a, b).

The three isolates were characterized by propagation

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with stalked conidia (Fig. 1a), formation of ovoidal to ellipsoidal ballistoconidia (Fig. 1b), inability to assimilate inositol and to ferment sugars, positive diazonium blue B (DBB) and urease reactions, presence of xylose in whole-cell hydrolysates and presence of Q-10 as the major ubiquinone isoprenologe. The 18S rDNA sequence data for these isolates were aligned with 19 published sequences and a total of 1709 nt, present in all species between positions 39 and 1768 (Saccharomyces cerevisiae numbering), were used for analysis. In the 18S rDNA-based tree (Fig. 2), our three isolates were grouped with the six known species of the genus Kockovaella (Kockovaella imperatae, Kockovaella machilophila, Kockovaella phaffii, Kockovaella sacchari, Kockovaella thailandica and Kockovaella thailandica) and with seven species of the genus Fellomyces (Fellomyces chinesis, Fellomyces distylus, Fellomyces fuzhouensis, Fellomyces lichenicola, Fellomyces ogasawarenisis, Fellomyces sichuanensis and Fellomyces thailandicus) in a statistically well-supported cluster (bootstrap 94%). On the basis of the phenotypic and genealogical characteristics described above, these isolates were assigned to the genus Kockovaella. In the ITS-based dendrogram (Fig. 3), strain TY-208 clustered with the type strain of Kockovaella thailandica with good statistical support (bootstrap 100%). The G+C contents of strain TY-208 and the type strain of Kockovaella thailandica were 50.5 and 49.5 mol%, respectively. The degrees of relative binding between strain TY-208 and the type strain of Kockovaella thailandica ranged from 70 to 100%. Based on the similar G+C content, the high degrees of DNA complementarity and the ITS analysis, strain TY-208 was considered to belong to Kockovaella thailandica. Strain TY-210 formed a tight phylogenetic cluster with the type strain of Kockovaella sacchari in the ITS-based dendrogram (Fig. 3). Only one base difference in ITS1 (121 bp compared) was detected between strain TY-210 and the type strain of Kockovaella sacchari, and also one base difference in ITS2 (171 bp compared). The G+C contents of strain TY-210 and the type strain of Kockovaella sacchari were both 48.1 mol%, Based on the same G+C content and the analysis of ITS, strain TY-210 was assigned to Kockovaella sacchari. Strain TY-278 clustered with the type strains of Kockovaella machilophila, Kockovaella phaffii and Kockovaella shimaie in the ITS-based dendrogram (Fig. 3). The G+C contents of strain TY-278 and the type strains of Kockovaella machilophila, Kockovaella phaffii and Kockovaella shimaie were 48.5, 52.1, 51.4 and 53.0 mol%, respectively. Strain TY-278 showed low degrees of DNA complementarity with the type strains of Kockovaella machilophila, Kockovaella phaffii and Kockovaella shimaie (1–10%). These results indicated that strain TY-278 represented a new species of the genus Kockovaella. Table 1 shows the phenotypic and chemotaxonomic properties of the seven species of the genus Kockovaella. We propose the name Kockovaella barringtoniae sp. nov. for the new species which is described below.

**Latin diagnosis of Kockovaella barringtoniae**

Fungsin, Hamamoto & Nakase sp. nov.

_In medio liquido post dies 3 cellulae vegetatiae ovoideae vel ellipsoidae, 3–40 × 40–100 μm, singulae aut binae, propagantes formatione conidiorum stipitatorum et gemmarum. Post unum mensem pellicula imperfecta._

_Cultura in agaro YM, albocremea, non-nitida, mollis et marginie glabra. Hyphae et pseudohyphae non formantur._

_Ballistosporae reniformes._

_C in medio liquido post dies 3._

_In YM agar after 5 d at 25 °C._

_Ballistoconidia produced on corn meal agar after 7 d at 17 °C._

_Bars, 10 μm._

_Fig. 1. Photomicrographs of Kockovaella barringtoniae TY-278T (= JCM 10998T = TISTR 5770T). (a) Vegetative cells grown on YM agar after 5 d at 25 °C. (b) Ballistoconidia produced on corn meal agar after 7 d at 17 °C. Bars, 10 μm._

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After 3 d at 25 °C in YM broth (Difco), the cells are ovoidal to ellipsoidal (3–40 × 40–100 µm), single or in pairs, and reproduce by multilateral budding and formation of stalked conidia (Fig. 1a). A sediment and an incomplete ring are formed after 1 month. After 1 month at 17 °C on YM agar, streak cultures are pale yellowish cream, dull–shining and smooth. The margin is entire. True hyphae or pseudohyphae are not formed in Dalmau plate cultures on cornmeal agar (Difco). Ballistoconidia are produced abundantly on cornmeal agar and YM agar. They are reniform, allantoidal or ellipsoidal (4–50 × 60–100 µm) (Fig. 1b). d-Glucose

278T ex folio Barringtonia sp., Nakhon Ratchasima Province, Thailand, isola est. In collectionibus culturarum quas Japan Collection of Microorganisms, Wako, Saitama sustentant, no. JCM 10998T deposita et in collectionibus culturarum quas Thailand Institute of Scientific and Technological Research, Chatuchak, Bangkok sustentant, no. TISTR 5770T sunt.

Description of Kockovaella barringtoniae Fungsin, Hamamoto & Nakase sp. nov.

Kockovaella barringtoniae (bar.ring.ton′iae. M.L. gen. n. barringtoniae of Barringtonia sp., the plant from which the type strain was isolated).

After 3 d at 25 °C in YM broth (Difco), the cells are ovoidal to ellipsoidal (3–40 × 40–100 µm), single or in pairs, and reproduce by multilateral budding and formation of stalked conidia (Fig. 1a). A sediment and an incomplete ring are formed after 1 month. After 1 month at 17 °C on YM agar, streak cultures are pale yellowish cream, dull–shining and smooth. The margin is entire. True hyphae or pseudohyphae are not formed in Dalmau plate cultures on cornmeal agar (Difco). Ballistoconidia are produced abundantly on cornmeal agar and YM agar. They are reniform, allantoidal or ellipsoidal (4–50 × 60–100 µm) (Fig. 1b). d-Glucose
is not fermented. Assimilation of D-glucose, galactose, L-sorbose (weak), succrose, maltose, cellubiose, trehalose, lactose, melibiose, raffinose, melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, ethanol, glycerol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, methyl-D-glucoside (latent and weak), salicin (latent and weak), gluconolactone, D-glucuronic acid, succinic acid and citric acid (weak) (carbon sources). Absence of growth with inulin and D-lyxose. Assimilation of D-glucuronic acid, succinic acid and citric acid (weak) (carbon sources). Absence of growth with inulin and D-lactic acid (carbon sources), and with nitrate, nitrite, ethylamine and cadaverine. Assimilation of L-lysine is positive. Thiamine is required for growth. Growth does not occur on 50% (w/w) glucose/yeast extract agar. Starch-like compounds are not produced. Does not liquefy gelatin. Urease activity is positive. The DBB reaction is positive. The major ubiquinone is Q-10. The G+C content of the nuclear DNA is 48.5 mol%, as determined by HPLC. The type strain of Kockovaella barringtoniae, strain TY-278, was isolated from a dead leaf of Barringtonia sp., in a tropical rain forest of Sakearat Environmental Research Station in Nakhon Ratchasima Province, Thailand. This strain has been deposited in the Japan Collection of Microorganisms (JCM), RIKEN, Wako, Saitama, Japan, as strain JCM 10998T and also deposited in the Thailand Institute of Scientific and Technological Research (TISTR), Chatuchak, Bangkok, Thailand, as strain TISTR 5770. The other two species isolated from leaves collected in a tropical rain forest in Thailand, Kockovaella sacchari TY-210 and Kockovaella thailandica TY-208, were also deposited in the JCM and TISTR as JCM 10999 (= TISTR 5771) and JCM 11000 (= TISTR 5772), respectively.

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References


Table 1. Differential phenotype and chemotaxonomic properties of Kockovaella barringtoniae and known Kockovaella species

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<td>Methyl α-D-glucoside</td>
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<td>2-Ketogluconic acid</td>
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<td>LW</td>
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<td>5-Ketogluconic acid</td>
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<td>G + C content (mol%)</td>
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