

NOTE

***Kockovaella barringtoniae* sp. nov., a new basidiomycetous yeast species isolated from a plant leaf collected in a tropical rain forest in Thailand**Bundit Fungsin,^{1,2} Makiko Hamamoto,¹ Vullapa Arunpairojana,² Jiraporn Sukhumavasi,² Poonsook Atthasampunna² and Takashi Nakase¹¹ Japan Collection of Microorganisms, RIKEN (The Institute of Physical and Chemical Research), Wako, Saitama 351-0198, Japan² Thailand Institute of Scientific and Technological Research, Chatuchak, Bangkok 10900, Thailand

Author for correspondence: Makiko Hamamoto. Tel: +81 48 467 9560. Fax: +81 48 462 4617. e-mail: hamamoto@jcm.riken.go.jp

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 diazotium 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-278^T (= JCM 10998^T = TISTR 5770^T).

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 DDBJ accession numbers for the 18S rDNA-ITS regions–5.8S rDNA sequences are AB052631 (*Kockovaella barringtoniae* TY-278^T), AB052560 (*Kockovaella sacchari* TY-210) and AB052559 (*Kockovaella thailandica* TY-208).

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^T was isolated from *Barringtonia* sp. Six known species of the genus *Kockovaella*, *Kockovaella imperatae* JCM 7826^T, *Kockovaella machilophila* JCM 10052^T, *Kockovaella phaffii* JCM 10073^T, *Kockovaella sacchari* JCM 9858^T, *Kockovaella shimae* JCM 10051^T and *Kockovaella thailandica* JCM 7824^T, 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

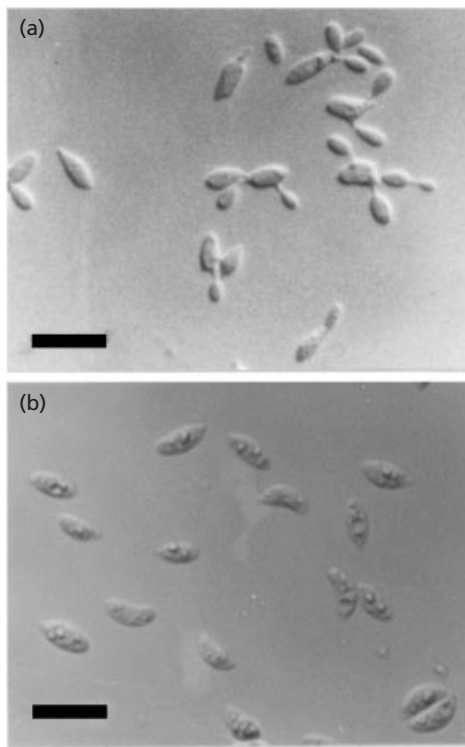


Fig. 1. Photomicrographs of *Kockovaella barringtoniae* TY-278^T (= JCM 10998^T = TISTR 5770^T). (a) Vegetative cells grown on YM agar after 5 d at 25 °C. (b) Ballistoconidia produced on corn meal agar after 7 d at 17 °C. Bars, 10 µm.

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 isoprenologue. 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 shimae* and *Kockovaella thailandica*) and with seven species of the genus *Fellomyces* (*Fellomyces chinensis*, *Fellomyces distylii*, *Fellomyces fuzhouensis*, *Fellomyces lichenicola*, *Fellomyces ogasawarensis*, *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^T clustered with the type strains of *Kockovaella machilophila*, *Kockovaella phaffii* and *Kockovaella shimae* in the ITS-based dendrogram (Fig. 3). The G+C contents of strain TY-278^T and the type strains of *Kockovaella machilophila*, *Kockovaella phaffii* and *Kockovaella shimae* were 48.5, 52.1, 51.4 and 53.0 mol%, respectively. Strain TY-278^T showed low degrees of DNA complementarity with the type strains of *Kockovaella machilophila*, *Kockovaella phaffii* and *Kockovaella shimae* (1–10%). These results indicated that strain TY-278^T 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 vegetativae ovoideae vel ellipsoideae, 3.0–4.0 × 4.0–10.0 µm, singulae aut binae, propagantes formatione conidiorum stipitatae et gemmarum. Post unum mensem pellicula imperfecta et sedimentum formatur. Cultura in agaro YM, albocrema, non-nitida, mollis et margine glabra. Hyphae et pseudohyphae non formantur. Ballistosporae reniformes aut ellipsoideae, 4.0–5.0 × 6.0–10.0 µm. Fermentatio nulla. Glucosum, galactosum, L-sorbosum (exiguum), saccharosum, maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitosum, amyllum solubile, D-xylosum, L-arabiosum, D-arabiosum, D-ribosum, L-rhamnosum, ethanolum, glycerolum, erythritolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, methyl-α-D-glucosidum (lente et exiguum), salicinum (lente et exiguum), glucono-δ-lactonum, acidum 2-keto-gluconicum, acidum 5-keto-gluconicum, inositolum (lente et exiguum), acidum D-glucuronicum, acidum D-galacturonicum, acidum succinicum et acidum citricum (exiguum) assimilantur, at non inulinum, nec acidum DL-lacticum. L-lysinum assimilantur at non kalium nitricum, natrium nitricum, ethylaminum nec cadaverinum. Ad crescentiam thiaminum necessarium est. G+C acidi deoxyribonucleati 48.5 mol% (per HPLC). Ubiquinonum majus Q-10. Typus stirpis TY-

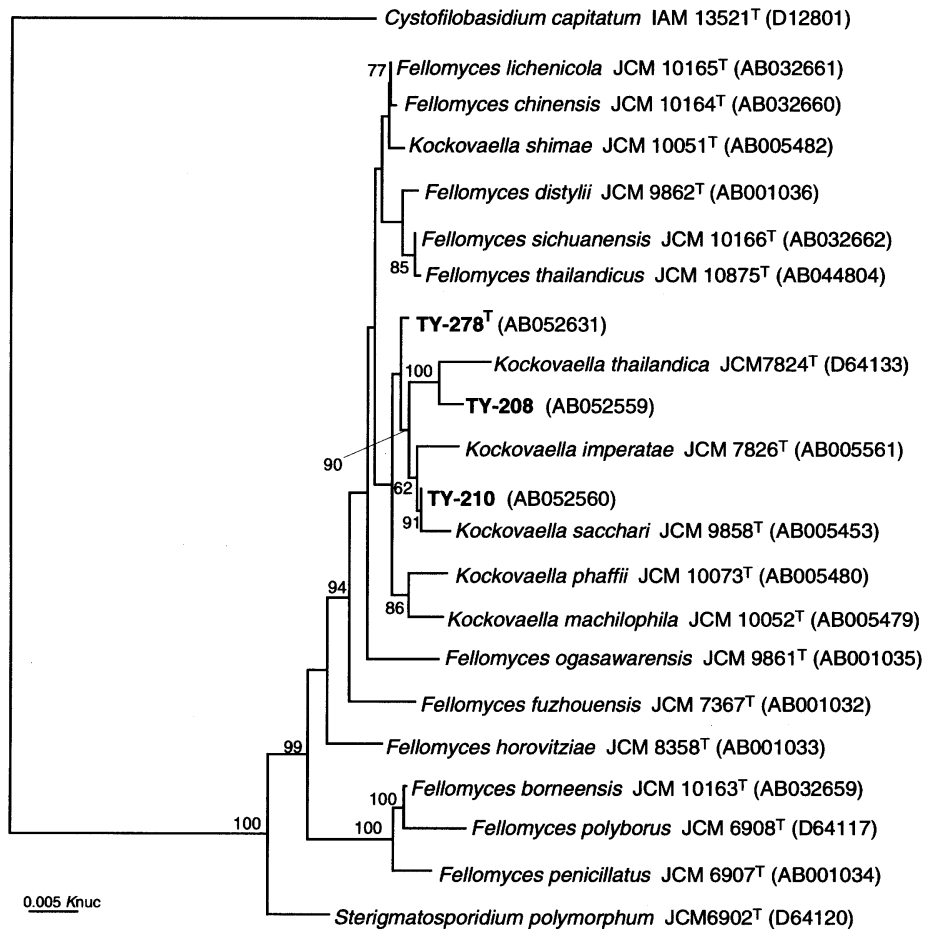


Fig. 2. Phylogenetic positions of the three *Kockovaella* isolates (TY-208, TY-210 and TY-278^T) based on 18S rDNA. The branching pattern was generated by the neighbour-joining method. Numbers by the nodes are bootstrap frequencies derived from 1000 replicates (values < 50 % are not shown).

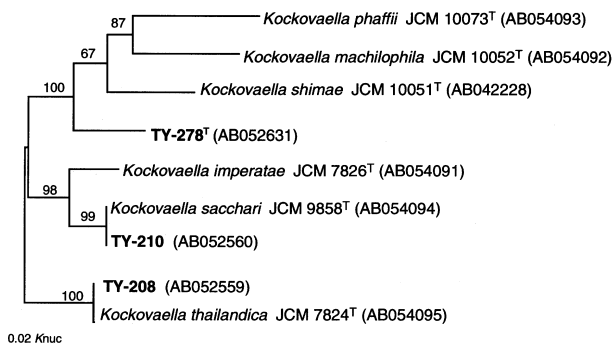


Fig. 3. Unrooted dendrogram of six species of the genus *Kockovaella* and the three isolates (TY-208, TY-210 and TY-278^T). The trees were constructed using the neighbour-joining method for ITS1 and ITS2. Numbers by the nodes are bootstrap frequencies derived from 1000 replicates (values < 50 % are not shown).

278^T *ex folio Barringtonia* sp., Nakhon Ratchasima Province, Thailand, *isolata est*. In *collectionibus culturarum quas* Japan Collection of Microorganisms,

Wako, Saitama *sustentant*, no. JCM 10998^T *deposita et in collectionibus culturarum quas* Thailand Institute of Scientific and Technological Research, Chatuchak, Bangkok *sustentant*, no. TISTR 5770^T *sunt*.

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

Kockovaella barringtoniae (bar.ring.ton'i.ae. 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 (3.0–4.0 × 4.0–10.0 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 (4.0–5.0 × 6.0–10.0 µm) (Fig. 1b). D-Glucose

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

L, Latent; LW, latent and weak.

Assimilation of:	<i>K.</i> <i>barringtoniae</i>	<i>K.</i> <i>imperatae</i>	<i>K.</i> <i>machilophila</i>	<i>K.</i> <i>phaffii</i>	<i>K.</i> <i>sacchari</i>	<i>K.</i> <i>shimae</i>	<i>K.</i> <i>thailandica</i>
Melibiose	+	+	L	–	+	–	+
L-Arabinose	+	+	L	–	+	+	+
Ethanol	+	–	–	–	–	–	–
Erythritol	+	–	LW	–	–	–	–
Galactitol	+	L	+	–	L	+	–
Methyl α -D-glucoside	LW	–	–	–	+	–	L
2-Ketogluconic acid	+	LW	+	LW	–	–	+
5-Ketogluconic acid	+	LW	+	L	–	–	+
G+C content (mol%)	48.5	49.5	52.8	50.8	48.1	53.0	48.9–49.5

is not fermented. Assimilation of D-glucose, galactose, L-sorbose (weak), sucrose, maltose, cellobiose, 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- α -D-glucoside (latent and weak), salicin (latent and weak), glucono- δ -lactone, 2-ketogluconic acid, 5-ketogluconic acid, inositol (latent and weak), D-glucuronic acid, D-galacturonic acid, succinic acid and citric acid (weak) (carbon sources). Absence of growth with inulin and DL-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^T, 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 10998^T and also deposited in the Thailand Institute of Scientific and Technological Research (TISTR), Chatuchak, Bangkok, Thailand, as strain TISTR 5770^T. 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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