Sporobolomyces koalae sp. nov., a basidiomycetous yeast isolated from nasal smears of Queensland koalas kept in a Japanese zoological park

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Three strains (JCM 15063^T, JCM 15098 and JCM 15099) of a novel basidiomycetous yeast species belonging to the genus *Sporobolomyces* were isolated from nasal smears of Queensland koalas kept in a Japanese zoological park. Analyses of sequences of the nuclear rDNA internal transcribed spacer region and the 26S rDNA D1/D2 domain and morphological studies indicated that these strains represent a novel species with a close phylogenetic relationship to *Sporobolomyces carnicolor* and *Sporobolomyces japonicus* in the *Sporidiobolus* lineage, for which the name *Sporobolomyces koalae* sp. nov. is proposed (type strain JCM 15063^T = CBS 10914^T = DSM 19992^T).

Yeast species belonging to the polyphyletic genus *Sporobolomyces* are anamorphic basidiomycetes that produce bilaterally symmetrical ballistoconidia and have CoQ-10 or CoQ-10(H₂) as their major ubiquinone. This group is also characterized by the inability to ferment sugars, the absence of xylose in whole-cell hydrolysates and positive diazonium blue B and urease reactions (Boekhout & Nakase, 1998). In the course of a study of the yeast microbiota of koalas, we isolated a number of basidiomycetous yeasts from Queensland koalas (*Phascolarctos cinereus*). In this study, we characterized three strains of basidiomycetous yeast isolated from the nasal smears of Queensland koalas kept in a Japanese zoological park (Kobe Oji Zoo, Kobe). Phenotypic, morphological and phylogenetic analyses indicated the strains to be affiliated with the genus *Sporobolomyces*. The data obtained also suggest that the isolates represent a novel species.

Most of the morphological, biochemical and physiological characteristics of the isolates were determined using methods described by Yarrow (1998). Nasal smears of five koalas and three zoological keepers were collected with swabs and inoculated onto CHROMagar Candida (CHROMagar) spread with micafungin (30 µg per plate).

The nasal smears of the three zoological keepers were gathered with informed consent. Yeast DNA was extracted and purified according to the protocol recommended for the FTA Classic Card (Whatman). DNA fragments covering the nuclear internal transcribed spacer (ITS) region and the 26S rDNA D1/D2 domain were amplified with primers ITS1, ITS4, 28SF1 and 635 as described previously (Mirhendi *et al.*, 2005; Sugita *et al.*, 2003; White *et al.*, 1990). DNA sequencing was performed with an Applied Biosystems model 310 sequencer. The neighbour-joining tree (Saitou & Nei, 1987) was made using MEGA3.1 (Kumar *et al.*, 2004). Data consistency was tested by bootstrapping the alignments 1000 times with corrections for multiple substitutions. The quinone profile was determined by TechnoSuruga Laboratory Co. (Shizuoka, Japan).

Three strains (JCM 15063^T, JCM 15098 and JCM 15099) of red yeast were isolated from an individual koala. In phylogenetic trees drawn from D1/D2 sequences (Fig. 1) and ITS sequences (Supplementary Fig. S1, available in IJSEM Online), these strains clustered in the Johnsonii clade of the *Sporidiobolus* lineage (Scorzetti *et al.*, 2002). The D1/D2 sequences showed single-base substitutions and the ITS sequences were identical. The D1/D2 domains of these koala-isolated strains showed 98 % (9 substitutions, without gaps) and 97 % (13 substitutions, two gaps) similarity to those of *Sporobolomyces carnicolor* CBS 4215^T and *Sporobolomyces japonicus* CBS 5744^T. The ITS1–5.8S rDNA–ITS2 regions of the koala-isolated strains showed 93 and 91 % similarity, respectively, to those of *S. carnicolor* CBS 4215^T and *S. japonicus* CBS 5744^T. The koala-isolated

Abbreviation: ITS, internal transcribed spacer.

The GenBank/EMBL/DBJ accession numbers for the 26S rDNA D1/D2 domain sequence and ITS sequence of strain JCM 15063^T are EU276011 and EU276008, respectively.

A phylogenetic tree based on ITS sequences is available as supplementary material with the online version of this paper.

Fig. 1. Phylogenetic tree drawn from neighbour-joining analysis of 26S rDNA D1/D2 domain sequences, depicting the relationships of strains of *Sporobolomyces koalae* sp. nov. with closely related species. Bootstrap percentages over 60% from 1000 bootstrap replicates are shown. The outgroup was *Bensingtonia yamatoana* CBS 7243^T. Bar, 0.01 substitutions per nucleotide position.

strains were classified as belonging to the genus *Sporobolomyces* based on molecular systematics and culture properties.

In liquid culture (YM broth, 28 °C, 150 strokes min⁻¹), cells germinated with pseudohyphae (Fig. 2a). No pseudohyphae formed on cornmeal agar, malt extract agar or in broth. *S. carnicolor* does not form pseudohyphae (Bai *et al.*, 2002), while *S. japonicus* developed pseudohyphae of the 'Candida' or 'Mycotoruloides' type (Iizuka & Goto, 1965). The koala-isolated strains therefore differed from these species in pseudohypha formation. These results indicated that the three strains from nasal smears of Queensland koalas represent a novel *Sporobolomyces* species, for which the name *Sporobolomyces koalae* sp. nov. is proposed.

In this study, all five koalas from Kobe Oji Zoo and three zoological keepers were examined. We isolated *S. koalae* from three koalas, but not from any of the keepers. As cryptococcosis is a fatal disease in koala, *Cryptococcus* species are major targets for microbiota analysis in koalas (Makimura *et al.*, 2002). To our knowledge, this is first report of isolation of members of the genus *Sporobolomyces* from koalas in Japan. Not only *Cryptococcus* species but also *S. koalae* were detected in nasal smears from the koalas. Members of the genus *Sporobolomyces* are widespread in various environments, e.g. the phyllosphere, air and also deep-sea environments (Boekhout & Nakase, 1998; Nagahama *et al.*, 2001). They were presumably transferred readily to nasal smears of the koala and may adapt to this environment. The pathogenicity of *S. koalae*

Fig. 2. *Sporobolomyces koalae* JCM 15063^T. (a) Vegetative cells and pseudohyphae grown in YM broth overnight at 28 °C. (b) Ballistoconidia-producing cell and vegetative cells. (c) Ballistoconidia produced on cornmeal agar after 3 days at 17 °C. Bars, 10 µm.

was not determined, but it appeared to be a normal indigenous yeast, because the hosts were healthy. Further studies to determine the pathogenic presence of this yeast will be performed in future.

Latin diagnosis of *Sporobolomyces koalae* Satoh et Makimura sp. nov.

In liquido malti post dies 3 ad 20 °C, cellulae vegetativae, ovoideae, ellipsoideae vel elongatae, 2.5–5.0 × 5.0–15.0 µm, singulae, binae aut tetradium. Post unum mensem ad 20 °C, annulus, pelliculum et sedimentum formantur. In agar malti post unum mensem ad 20 °C, cultura rosea, glabra, butyracea vel viscida, margine glabra. In liquido YM pseudomycelium formantur. In agar farinae zae pseudomycelium non formantur. Ballistosporae ellipsoideales vel reniformes, 2.0–5.0 × 3.0–7.0 µm. Fermentatio nulla. Assimilat sic: glucosum, saccharosum, maltosum, cellobiosum, trehalosum (exigue), raffinsum, melezitsum (exigue), inulinum (exigue), amyllum solubile, L-arabiosum (exigue), glycerolum (exigue), D-mannitolum, D-glucitolum (exigue), salicinum (exigue), acidum gluconicum (exigue) et acidum succinicum; non respondent D-galactosum, L-sorboseum, lactosum, melibiosum, D-xylosum, D-arabiosum, D-ribosum, L-rhamnosum, D-glucosaminum, N-acetyl-D-glucosaminum, methanolum, ethanolum, erythritolum, ribitolum, galactitolum, methyl α-D-glucosidum, acidum DL-lacticum, acidum citricum, inositolum, hexadecanum, 2-ketogluconatum, nec xylitolum. Ammonium sulfatum et kalium nitricum assimilantur at non natrium nitrosum, L-lysinum, ethylaminum nec cadaverinum. Ad crescentiam vitaminum non necessarium est. Augmentum in 28–30 °C, non-crescit in 32 °C. Non respondet 50 % (w/w) GY liquido. Materia amyloidea iodophila non formantur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: Q-10. Typus stirps JCM 15063^T ex nasus proluvius Phascolarctos cinereus, Japonia, isolata est. In collectionibus culturarum quas Japan Collection of Microorganisms, Wako, Saitama, sustentant, no. JCM 15063^T, Centraalbureau voor Schimmelcultures, Delphi Batavorum, Hollandia, sustentant, no. CBS 10914^T, et Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germania, sustentant, no. DSM 19992^T deposita est.

Description of *Sporobolomyces koalae* Satoh and Makimura sp. nov.

Sporobolomyces koalae (ko.a'lae. N.L. fem. gen. n. *koalae* of a koala, *Phascolarctos cinereus*, from which the first strains were isolated).

After 3 days in malt extract at 20 °C, cells are ovoid, ellipsoidal to elongate, 2.5–5.0 × 5.0–15.0 µm, single, in pairs or groups of four. After 1 month on malt extract agar at 20 °C, the streak culture is butyrous to viscous, pink to orange-red, smooth and glistening with an entire margin. Pseudohyphae are formed in YM broth on overnight cultivation at 28 °C. Pseudohyphae are not formed in plate culture on malt extract agar. On cornmeal agar, ballisto-

conidia are formed on short sterigmata and are asymmetrical, ellipsoidal to reniform, 2.0–5.0 × 3.0–7.0 µm (Fig. 2b, c). Fermentation of glucose is negative. The following carbon compounds are assimilated: glucose, sucrose, maltose, cellobiose, trehalose (weak), raffinose, melezitose (weak), inulin (weak), soluble starch, L-arabinose (weak), glycerine (weak), D-mannitol, D-sorbitol (weak), salicin (weak), D-gluconate (weak) and succinic acid. The following are not assimilated: D-galactose, L-sorbose, lactose, melibiose, D-xylose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, N-acetyl-D-glucosamine, methanol, ethanol, erythritol, adonitol, galactitol, methyl α-D-glucoside, DL-lactic acid, citric acid, inositol, hexadecane, 2-keto-D-gluconate and xylitol. Ammonium sulphate and potassium nitrate are utilized as sole sources of nitrogen; sodium nitrite, L-lysine, ethylamine and cadaverine are not utilized. Growth in vitamin-free medium is positive. Optimum growth temperature is 28–30 °C; growth is negative at 32 °C. Growth does not occur in 50 % (w/w) glucose/yeast extract broth. No starch-like substrate is produced. Urease activity is positive. Diazonium blue B reaction is positive. The major ubiquinone is Q-10.

The type strain, JCM 15063^T (=CBS 10914^T =DSM 19992^T), was isolated from a nasal smear from a captive Queensland koala (Kobe Oji Zoo, Kobe, Japan).

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