Basidiomycetous yeast biota and dynamics of koala nasal cavity

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Objectives: As cryptococcosis is a fatal disease in koalas (*Phascolarctos cinereus*), *Cryptococcus* spp. and related basidiomycetous yeasts are major targets for yeast microbiota analysis in koalas. In the course of a study of the yeast microbiota of koalas, we isolated a number of basidiomycetous yeasts from koalas and the breeding environments in Japanese zoological parks. In this study, further new species and the basidiomycetous yeast biota of the koala from previous presentation (ISHAM 2012, Berlin) to 2017 is reported.

Methods: Most of the morphological, biochemical, and physiological characteristics were investigated using the methods described by Kurtzman et al. (2011). Nasal smears of several healthy koalas and the breeding environments were collected with swabs and inoculated onto CHROMagar Candida (CHROMagar) spread with micafungin (30 mg per plate). The isolated strains were identified by comparison with DNA sequences registered in GenBank/EMBL/DDBJ as described previously¹. Since 2016, we have checked all colonies using MALDI Biotyper.

Sampling for nasal smear

Culture and Isolation. In this plate, count as 2 strains (purple: 14 CFU and milky white: 3 CFU)

Identification. DNA sequencing, Real time PCR or MALDI Biotyper

Results: Over 500 strains of yeast were isolated from nasal smears of koala and the breeding environments. The dominant genus was *Cryptococcus* (mainly *C. neoformans*). *Rhodotorula* was the next most dominant genus by culture methods. Furthermore, new species of *Cryptococcus lacticolor* sp. nov. and *Rhodotorula oligophaga* sp. nov. were discovered².

Cryptococcus lacticolor TIMM 10013^T.

Rhodotorula oligophaga TIMM 10017^T.

Description of *Cryptococcus lacticolor* Satoh and Makimura sp. nov.
Cryptococcus lacticolor (la.cti'color. L. n. fem adj. lacticolor, milky white, referring to the milky white color of colonies). After three days in yeast and mold (YM) broth at 28°C, cells globose, ovoid, ellipsoid, 2.0–2.5 × 2.0–3.0 µm, single or in parent–bud pairs. Sediment formation observed. Ballistospores not observed after 2 weeks on corn meal agar at 17°C. After 1 month on malt extract agar at 25°C, streak cultures butyrous to viscous, milky white, smooth, and glistening with entire margins. Pseudohyphae not produced on cornmeal agar slide cultures after 59 days at 17°C. Fermentation of glucose absent. Glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, lactose (weak), melibiose (weak), raffinose, melezitose, inulin (weak), soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, N-acetyl-D-glucosamine, ethanol (weak), glycerol (weak), erythritol, ribitol, galactitol, D-mannitol, sorbitol, α-methyl-D-glucoside, salicin, D-gluconate, succinate (weak), citrate (weak), and myo-inositol assimilated. Methanol, DL-lactate, hexadecane, 2-keto-D-gluconate, and xylitol not assimilated. Ammonium sulfate and L-lysine utilized as sole sources of nitrogen, but not sodium nitrite, potassium nitrate, ethylamine, or cadaverine. Growth in vitamin-free medium present. Growth in 50% glucose medium absent. Optimum growth temperature 28–30°C; no growth at 35°C. Starch formation present. Urease activity present. Diazonium blue B reaction present. The major ubiquinone Q-10. Mol% G+C = 49.3 %. The type strain, TIMM 10013^T, was isolated from a nasal smear from a Queensland koala kept in a Japanese zoological park (Saitama Children's Zoo, Saitama, Japan). This strain has been deposited in the Teikyo University Institute of Medical Mycology, Tokyo, Japan, as TIMM 10013^T; the Japan Collection of Microorganisms, Saitama, Japan, as JCM 15449^T; the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, as CBS 10915^T; and the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, as DSM 21093^T. The ITS sequence is deposited in DDBJ/EMBL/Genbank under accession number AB375774. The 26S rDNA D1/D2 region sequence is deposited in DDBJ/EMBL/Genbank under accession number AB375775. The type strain is registered in MycoBank (<http://www.mycobank.org/>) under MB 802688. The type strain is registered in the Fungal Barcoding Database (<http://www.fungalbarcoding.org/>) under 3174.

Description of *Rhodotorula oligophaga* Satoh and Makimura sp. nov.
Rhodotorula oligophaga (oli'.go.pha.ga L. n. fem adj. oligophaga, eating little; referring to the species characteristic of restricted utilization of carbon sources). After 3 days in YM broth at 28°C, cells ovoid to ellipsoidal (2–4 × 3–5 µm) and occurring singly or in parent–bud pairs. After 1 month, sediment formation and incomplete fragile pellicles. After 1 month on YM agar at 25°C, streak cultures pale pink, glistening, soft to slimy, and with entire margins. In Dalmau plate cultures on cornmeal agar, branching hyphae or pseudohyphae not formed. Fermentation absent. Glucose, sucrose, maltose, trehalose, melezitose, inulin, soluble starch, xylose, L-arabinose, ribose, N-acetyl-D-glucosamine, ethanol, erythritol (weak), ribitol, galactitol (weak), mannitol (weak), sorbitol (weak), α-methyl-D-glucoside (weak), DL-lactate, succinate (weak), hexadecane (weak), xylitol, p-hydroxybenzoic acid, m-hydroxybenzoic acid, vanillic acid, ferulic acid, and glucuronate assimilated. Galactose, L-sorbose, cellobiose, lactose, melibiose, raffinose, D-arabinose, L-rhamnose, D-glucosamine, methanol, glycerol, salicin, D-gluconate, citrate, myo-inositol, veratric acid, gentisic acid, gallic acid, saccharic acid, and tartaric acid not assimilated. Ammonium sulfate utilized as a sole source of nitrogen, but not sodium nitrite, potassium nitrate, ethylamine, L-lysine, or cadaverine. Growth in the presence of 0.1 % cycloheximide absent, and in the presence of 0.01% cycloheximide present. Growth in vitamin-free medium and 50% glucose medium absent. Optimum growth temperature 28–30°C; growth weak at 37°C, absent at 40°C. Starch formation absent. Urease activity present. Diazonium blue B reaction present. Major ubiquinones Q-9 and Q-10. Mol% G+C = 52.7%. The type strain, TIMM 10017^T, was isolated from the perch of a room housing a single koala (Kanazawa Zoo, Kanagawa, Japan). This strain has been deposited in the Teikyo University Institute of Medical Mycology, Tokyo, Japan, as TIMM 10017^T; the Japan Collection of Microorganisms, Wako, Saitama, as JCM 18398^T; the Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands, as CBS 12623^T; and the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, as DSM 25814^T. The ITS sequence is deposited in DDBJ/EMBL/Genbank under accession number AB702967. The 26S rDNA D1/D2 region sequence is deposited in DDBJ/EMBL/Genbank under accession number AB702967. The type strain is registered in MycoBank (<http://www.mycobank.org/>) under MB 802689. The type strain is registered in the Fungal Barcoding Database (<http://www.fungalbarcoding.org/>) under 3175.

Conclusion: The most frequent species in the basidiomycetous yeast biota isolated from koala nasal passages was *Cryptococcus neoformans*, followed by *Rhodotorula minuta*. *R. minuta* was the most frequent species in the breeding environments, while *C. neoformans* was rare.

References

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