Ogataea pignaliae sp. nov., the teleomorph of *Candida pignaliae*

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Six ascospore-forming *Candida pignaliae* strains were isolated from epigeal plant parts in Hungary. They share identical D1/D2 LSU rRNA gene sequences with the type strain of *C. pignaliae*, and the physiological characteristics investigated are also very similar to that of the type strain. The only substantial difference compared to the type strain of *C. pignaliae* is their ability to assimilate β -glucosides (cellobiose, salicin and arbutin). The majority of the isolation sources of the strains reported in this study have the common feature of containing tannic acid, while the type strain of *C. pignaliae* was recovered from tanning fluid. We were able to induce ascospore formation also in the type strain of *C. pignaliae*. Therefore, *Ogataea pignaliae* Péter, Tornai-Lehoczki & Dlačny sp. nov. is proposed as the teleomorph of *C. pignaliae* (F. H. Jacob) S. A. Meyer & Yarrow. The type strain is CBS 6071^T.

Torulopsis pignaliae was described by Jacob (1970) based on six strains isolated from tanning fluid in France and the species was later transferred to the genus *Candida* by Yarrow & Meyer (1978). From the six strains mentioned in the original description of *T. pignaliae*, only the type strain was deposited in CBS (Centraalbureau voor Schimmelcultures) and the latest editions of the yeast monographs treated only the type strain of this species (Meyer *et al.*, 1998; Barnett *et al.*, 2000). Jacob (1970) observed copulation but no ascospore formation in the *T. pignaliae* strains investigated.

In this contribution, we report the isolation of six ascospore-forming *Candida pignaliae* strains. In addition, we were able to induce sporulation also in the type strain of this species. Based on the type strain and the additional sporulating strains, we propose *Ogataea pignaliae* Péter, Tornai-Lehoczki & Dlačny sp. nov. as the teleomorph of *Candida pignaliae* (Jacob) Meyer & Yarrow.

The strains considered in this study are shown in Table 1. Tree exudates were sampled with sterile cotton swabs; leaves and mosses were collected with disposable gloves in sterile plastic bags. Yeast strains were isolated by plating on Rose-Bengal Chloramphenicol (RBC) agar at 25 °C following a two-step enrichment procedure in Yeast Nitrogen Base (YNB) supplemented with 0.5% (v/v) methanol as described by Dlačny *et al.* (2003).

Phenotypic characterization of the yeast strains (including the type strain of *C. pignaliae* as a reference) was carried out according to Yarrow (1998). The utilization of carbon sources was examined at 25 °C on a rotary shaker (30 r.p.m.) for three weeks. Two different products (Sigma G4750, Merck Art. 288) of glucono- δ -lactone were tested as a sole carbon source. The utilization of nitrogen sources was

examined by using the conventional auxanographic technique for 7 days. Sporulation was investigated on acetate agar (1.4% sodium acetate, 0.04% glucose), cornmeal agar, 'spezieller nährstoffarmer agar' (SNA; 0.1% KH₂PO₄, 0.1% KNO₃, 0.05% MgSO₄·7H₂O, 0.05% KCl, 0.02% glucose, 0.02% sucrose and 2% agar), potato dextrose agar, 2% malt extract agar, glucose–peptone–yeast extract agar, yeast extract–malt extract agar, V8 agar, Gorodkova agar, modified KNO₃ agar [0.14% KNO₃, 0.16% yeast carbon base (Difco), 0.012% bromothymol blue, 1.6% agar, pH: 5.9–6.0] (Pincus *et al.*, 1988) and modified KNO₃ agar with double (0.28%) KNO₃ content at 15 °C and 25 °C for 3 weeks.

The D1/D2 domain of the large subunit (26S) rRNA gene from the newly isolated strains was sequenced as described by Kurtzman & Robnett (1998). The corresponding sequence of *C. pignaliae* was retrieved from the GenBank database and alignment of the sequences was made with the CLUSTAL X 1.83 program (Thompson *et al.*, 1997).

The six strains reported in this manuscript share identical D1/D2 LSU rRNA gene sequences which are also identical with that of the type strain of *C. pignaliae*, strongly suggesting that they are conspecific (Kurtzman & Robnett, 1998). The conspecificity of the six new isolates and the type strain of *C. pignaliae* is further substantiated by their phenotypic similarity. However, unlike the type strain of *C. pignaliae*, two strains (NCAIM Y.01825 and NCAIM Y.01826) isolated during this study formed 1–2 hat-shaped ascospores in persistent conjugated or unconjugated asci on V8 agar at 15 °C after a two- or three-week incubation period, although the sporulation was extremely rare. After a series of unsuccessful attempts not detailed here, finally, we were able to induce sporulation in the remaining strains

Table 1. List of *Ogataea pignaliae* sp. nov. strains considered in this study

NCAIM, National Collection of Agricultural and Industrial Micro-organisms, Budapest, Hungary; IFO, Institute for Fermentation, Osaka, Japan; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

Strain accession number	Source of isolation
IFO 10307 ^T (=CBS 6071 ^T)	Tanning fluid, France
NCAIM Y.01825	Exudate of sessile oak (<i>Quercus petraea</i>), Pilis Mountains, Hungary, 2002
NCAIM Y.01826	Exudate of sessile oak (<i>Quercus petraea</i>), Pilis Mountains, Hungary, 2003
NCAIM Y.01942	Leaf of agrimony (<i>Agrimonia eupatoria</i>), Pilis Mountains, Hungary, 2006
NCAIM Y.01943	Leaf of maple (<i>Acer</i> sp.), Pilis Mountains, Hungary, 2006
NCAIM Y.01944, NCAIM Y.01945	Unidentified moss, Pilis Mountains, Hungary, 2006

as well, including the type strain of *C. pignaliae*. It was recognized that all investigated strains formed ascospores on modified KNO₃ agar, at 15 °C within 3 weeks, although still at low or very low frequency. The modified KNO₃ agar was suggested by Pincus *et al.* (1988) for the quick testing of the potassium nitrate assimilation of clinically important yeasts. We also investigated the performance of modified KNO₃ agar with twofold elevated KNO₃ content to see if it supported the sporulation, but no significant difference was observed.

Considering their physiological characteristics, the newly isolated strains form a fairly uniform group and differ from the type strain of *C. pignaliae* substantially only by their ability to assimilate three β -glucosides (cellobiose, salicin and arbutin). A further difference observed compared to the data published by Barnett *et al.* (2000) is that our isolates assimilated glucono- δ -lactone. However, using two different glucono- δ -lactones from two different suppliers gave invariably positive results for all strains, including the type strain of *C. pignaliae*, indicating that the difference originates in methodology rather than reflecting a real variance.

The type strain of *C. (Torulopsis) pignaliae* was isolated from tanning fluid (Jacob, 1970). Tannic acid is a common phenolic compound, frequently accumulated in various tissues of plants belonging to diverse taxonomic groups (Loureiro *et al.*, 2006). Among the isolation substrates of the novel *O. pignaliae* strains reported in this study (Table 1), oaks and agrimony are known to have high tannic acid contents (Zoldoš *et al.*, 1998; Drozd *et al.*, 1983) and all maple species contain tannins in greater or lesser amounts (Bate-Smith, 1977). Thus, the tannic acid content of at least the majority of the isolation sources of the strains listed in Table 1 indicates that they are tolerant to this compound.

Considering the ability of the type strain of *C. pignaliae* to form ascospores and the additional six conspecific ascosporulating strains, we propose *Ogataea pignaliae* sp. nov. as the teleomorph of *C. pignaliae*. The phylogenetic position of *O. pignaliae* sp. nov. based on analysis of the D1/D2 large subunit rRNA gene sequences is shown in Fig. 1. When selecting the genus *Ogataea* to accommodate

the teleomorph of *C. pignaliae*, we interpreted the genus *Ogataea* in a broader sense than Nagatsuka *et al.* (2008). They even suggested the exclusion of *O. nitratoaversa*, a species which on some phylogenetic trees clusters near to *C. pignaliae* [see Fig. 1 and Fig. 1 of Nagatsuka *et al.* (2008)], from the genus. It should also be considered that the genus *Pichia* is already not available as an alternative taxonomic placement for the teleomorph of *C. pignaliae* because based on multigene sequence analysis Kurtzman *et al.* (2008) restricted *Pichia* to those species that are phylogenetically closely related to *Pichia membranifaciens*, the type species of the genus. A future multigene sequence analysis may more firmly establish the taxonomic designation of the teleomorph of *C. pignaliae*. Because the number of physiological tests performed in this study exceeds that in the original description of *C. (Torulopsis) pignaliae* (Jacob, 1970), and in addition some characteristics became variable due to the inclusion of additional strains, a detailed description of its teleomorph is given.

Latin diagnosis of *Ogataea pignaliae* Péter, Tornai-Lehoczki & Dlačny, sp. nov.

Status ascigerus Candida pignaliae (F. H. Jacob) S. A. Meyer & Yarrow. In extracto malti post dies tres in 25 °C cellulase sunt globosae vel subglobosae 3–7.5 μ m, singulae, binae, catenis curtis vel racemis parvis conexae, undique gemmantes. Pellicula non formatur, sedimentum formatur. In agar malti post dies tres in 25 °C cultura butyrosa, alba vel crenea, glabra et nitida est. Colonia leviter sublata est, margine integro. In agar Zea maydis confecto post dies 7 in 25 °C nec pseudohyphae nec hyphae formantur. Asci conjugati vel inconjugati, (1)–2 ascoparas petasoformes, non liberas habent. Species est homothallica. D-Glucosum et trehalosum (fortasse lente) fermentantur. D-Galactosum, maltosum, sucrosum, lactosum, raffinose non fermentantur. D-Glucosum, L-sorbose (fortasse lente), D-ribosum (fortasse lente), D-xylosum, L-arabinosum, trehalosum, cellobiosum (variabile), salicinum (variabile), arbutinum (variabile), glycerolum, meso-erythritolum (exiguum, lente, variabile), ribitolum, xylitolum, L-arabinitolum, D-glucitolum, D-mannitolum, glucono- δ -lactonum, D-gluconicum (lente, variabile), succinatum, methanolum, ethanolum, propane-1,2-diolum (fortasse lente) assimilantur, at non

Fig. 1. Phylogenetic tree showing the placement of *Ogataea pignaliae* sp. nov. (bold) and some related species based on analysis of the D1/D2 domain of the large subunit rRNA gene. Sequences were obtained from GenBank. The tree was reconstructed by neighbour-joining analysis of aligned sequences. Numbers at branches indicate the bootstrap values from 1000 replications (frequencies of less than 50 % are not shown). Bar, proportional sequence divergence.

D-galactosum, D-glucosaminum, N-acetyl-D-glucosaminum, D-arabinosum, L-rhamnosum, sucrosum, maltosum, methyl α -D-glucosidum, melibiosum, lactosum, raffinose, melezitose, inulinum, amyllum solubile, galactitolum, myo-inositolum, 2-ketogluconicum, D-glucuronicum, D-galacturonicum, DL-lactatum, citratum, butane-2,3-diolum, hexadecanum et saccharatum. Kalium nitricum, natrium nitrosum, ethylaminum hydrochloricum, lysinum et cadaverinum dihydrochloricum assimilantur, at non creatinum, creatininum, glucosaminum, imidazolum. Materia amyloidea iodophila non formatur. Vitamina externa crescentiae sunt necessaria. Crescere potest in 30 °C, at non in 35 °C. Non crescit in agaro extracto fermenti confecto 50 partes glucosi per centum. Parte una cycloheximidi per mille crescit. Ureum non finditur. Diazonium caeruleum B est negativum. Typus stirps CBS 6071^T. Isolata ex liquore coriario, Gallia.

Description of *Ogataea pignaliae* Péter, Tornai-Lehoczki & Dlačhy sp. nov.

Ogataea pignaliae (pig.nali'ae. N.L. gen. fem. sing. n. *pignaliae* of Pignal, named after F. Pignal, and also to indicate that this species is the teleomorph pair of *Candida pignaliae*).

Anamorph: *Candida pignaliae* (F. H. Jacob) S. A. Meyer & Yarrow. [Yarrow & Meyer, *Int J Syst Evol Bacteriol* **28** (1978), 611–615].

In 5 % malt extract after 3 days at 25 °C, cells are globose or subglobose and 3–7.5 μ m. Cell division proceeds by multilateral budding. Cells occur singly, in pairs, in short chains and in small clusters. Pellicle is absent; a compact sediment is present. On 5 % malt extract agar after 3 days at 25 °C, the streak culture is butyrous, slightly raised, white to cream-coloured, smooth and glistening. The margin is entire. On cornmeal agar after 10 days at 25 °C, neither pseudohyphae nor septate hyphae are formed. Asci are either unconjugated, or conjugation of independent cells or parent cell-bud conjugation precedes ascospore formation. One (rarely) or two hat-shaped ascospores are formed in each ascus (Fig. 2). The great majority of the asci are persistent. The presence of asci showing heterogamous conjugation suggests that the species is homothallic. Sporulation is observed in all strains on modified KNO₃ agar or on modified KNO₃ agar with double KNO₃ content within three weeks at 15 °C. D-Glucose and α , α -trehalose (positive or slow) are fermented; D-galactose, maltose, sucrose, lactose and raffinose are not fermented. Carbon compounds D-glucose, L-sorbose (positive or slow), D-ribose (positive or slow), D-xylose, L-arabinose, α , α -trehalose, cellobiose (variable), salicin (variable), arbutin (variable), glycerol, meso-erythritol (weak, slow and variable), ribitol, xylitol, L-arabinitol, D-glucitol, D-mannitol, glucono- δ -lactone, D-gluconate (slow and variable), succinate, methanol, ethanol and propane-1,2-diol (positive or slow) are assimilated; no growth occurs on D-galactose, D-glucosamine, N-acetyl-D-glucosamine, D-arabinose, L-rhamnose, sucrose, maltose, methyl α -D-glucoside,

Fig. 2. Ascospore cultures of *Ogataea pignaliae* sp. nov. CBS 6071^T (modified KNO₃ agar with double KNO₃ content, 21 days, 15 °C). Bar, 10 μ m.

melibiose, lactose, raffinose, melezitose, inulin, starch, galactitol, myo-inositol, 2-keto-D-gluconate, D-glucuronate, D-galacturonate, DL-lactate, citrate, butane-2,3-diol, hexadecane or saccharate. Potassium nitrate, sodium nitrite, ethylamine hydrochloride, L-lysine and cadaverine dihydrochloride are assimilated; creatine, creatinine, glucosamine (as nitrogen source) and imidazole are not assimilated. The formation of amyloid material is negative. Growth in vitamin-free medium is negative. Growth occurs at 30 °C and is negative at 35 °C. No growth occurs on 50 % (w/w) glucose yeast extract agar and with 10 % NaCl or with 1 % acetic acid. Growth with 0.1 % cycloheximide is positive. Urea hydrolysis, and colour reaction with Diazonium blue B are negative.

The type strain was recovered from tanning fluid (Jacob, 1970) in France and is maintained as CBS 6071^T in Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

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