Ogataea kolombanensis sp. nov., Ogataea histrianica sp. nov. and Ogataea deakii sp. nov., three novel yeast species from plant sources

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Nine methanol-assimilating yeast strains isolated from olive oil sediments in Slovenia, extra virgin olive oil from Italy and rotten wood collected in Hungary were found to form three genetically separated groups, distinct from the currently recognized yeast species. Sequence analysis from genes of the small subunit (SSU) rRNA, internal transcribed spacer region/5.8S rRNA, large subunit (LSU) rRNA D1/D2 domains and translational elongation factor-1α (EF-1α) revealed that the three closely related groups represent three different undescribed yeast species. Sequence analysis of the LSU rRNA gene D1/D2 domains placed the novel species in the Ogataea clade. The three novel species are designated as Ogataea kolombanensis sp. nov. (type strain: ZIM Y.01896T = CBS 12778T = NRRL Y-63657T), Ogataea histrianica sp. nov. (type strain: ZIM Y.2322T = CBS 12779T = NRRL Y-63658T) and Ogataea deakii sp. nov. (type strain: NCAIM Y.01895T = CBS 12735T = NRRL Y-63656T).

INTRODUCTION

The latest edition of ‘The Yeasts a Taxonomic Study’ lists 31 species of the genus Ogataea (Kurtzman, 2011a). Several additional species, Ogataea cecidiorum (Glushakova et al., 2010), Ogataea phyllophila (Koowadjanakul et al., 2011), Ogataea parapolymorpha (Kurtzman, 2011b), Ogataea saltuana (Péter et al., 2011), Ogataea kanchanaburiensis and Ogataea walterdorffii (Limtong et al., 2013) were described too late for inclusion in this monograph or have had descriptions published since the monograph was printed. Some species of the genus Candida nested in the Ogataea clade (Candida rishirensis, Candida chumphonensis, Candida mattranensis and Candida malaysiensis) have also been described recently (Nakase et al., 2010; Koowadjanakul et al., 2011; Sub & Zhou, 2010). The vast majority of the members of the Ogataea clade share the ability to grow with methanol as a sole carbon source, which is primarily formed as a by-product of plant cell-wall metabolism (Fall & Benson, 1996). Indeed, numerous strains of species of the genus Ogataea and related methanol-assimilating species of the genus Candida have been recovered from plants or plant related substrates (Kurtzman, 2011a; Lachance et al., 2011).

Olive oil, together with the by-products of its manufacture and rotten wood are substrates known to be yeast habitats. In the case of olive oil, yeasts may be the predominant component of the microbial community (Ciafardini & Zullo, 2002) and are supposed to affect the quality of the olive oil either by improving or diminishing it (Ciafardini et al., 2006; Koidis et al., 2008; Romo-Sánchez et al., 2010; Vichi et al., 2011). Presumably methylotrophic yeasts reside on olive fruit as they are rich in pectic polysaccharides, the major components of their cell wall (Coimbra et al., 1994). In the case of rotten wood, lignocellulose is the major component of plant biomass and the organisms predominantly responsible for lignocellulose degradation are fungi (Sánchez, 2009). Although yeasts are unable to degrade intact cellulose, hemicellulose or lignin, they are able to utilize numerous constituents of these major components of wood, released during the degradation of wood by other organisms.

Recently we have described several novel yeast species from olive oil and its by-products and from rotten wood (e.g. Čadež et al., 2012; Dlauchy et al., 2012). In the present contribution, we report on the isolation of eight methylotrophic yeast strains from olive oil and its sediments and one from rotten wood, representing three novel species. The three novel species are placed by phylogenetic analyses...
in the *Ogataea* clade. Based on the above-noted nine strains we propose the following novel yeast species: *Ogataea kolombanensis* sp. nov., *Ogataea histrionica* sp. nov. and *Ogataea deakii* sp. nov.

**METHODS**

The origins of the investigated yeast strains are shown in Table 1. The majority of the strains were isolated from olive oil sediments. After decantation of olive oil, the sediments were diluted and transferred onto YPD (Sigma) agar plates. The agar plates were incubated at 26 °C for 4 days. Strain NCAIM Y.02026 was isolated from extra virgin olive oil, purchased in Hungary, but originating from Italy. From the sample, 0.1 ml olive oil was transferred to the surface of Rose-Bengal chloramphenicol (RBC) agar (Merck) and streaked with a glass loop. Strain NCAIM Y.01896T was recovered from a rotten wood sample following a two-step enrichment procedure in broth containing methanol (Dlauchy et al., 2003), serial dilution and surface plating on RBC agar. In both cases, the RBC agar plates were incubated at 25 °C for 5 days in darkness. In each case morphologically distinct colonies were picked and purified by repeated streaking. The strains were phenotypically characterized by standard methods according to the protocols of Kurtzman et al. (2011). Sporulation was investigated on acetate (1.4 % sodium acetate, 0.04 % glucose), corn meal (CM), 'special nutrient-poor' (SNA) (0.1 % KH2PO4, 0.1 % KNO3, 0.05 % MgSO4·7H2O, 0.05 % KCl, 0.02 % glucose, 0.02 % sucrose, 2 % agar), potato–glucose (PD), 2 % malt extract (ME), 3 % malt extract (Difco, BD), glucose–peptone–yeast extract (GPY), yeast extract–malt extract (YM) and V8 agar at 25 °C for 3 weeks. If no sporulation was observed in 3 weeks, the incubation time was extended up to 5 weeks and in case of failure of ascospore formation at 25 °C, the sporulation tests were repeated with 15 °C incubation temperature. Lipolytic activity was investigated on tributyrin agar (Fluka) incubated for 7 days at 25 °C and following the method described by Pfaff et al. (1997).

The large subunit (LSU) D1/D2 domains and the internal transcribed spacer (ITS)/5.8S of the rRNA gene of the Slovenian isolates were amplified and sequenced by using primer pair ITS1 and NL4 as described previously (Cadez et al., 2003). These DNA fragments for strains NCAIM Y.01896T and NCAIM Y.02026 were amplified and sequenced according to the methods of Kurtzman & Robnett (1998) & Péter et al. (2009), respectively. The small subunit (SSU) rRNA and elongation factor-1α (EF-1α) genes were amplified and sequenced by primers NS1 and NS8 (White et al., 1990) and YTEF-1G and YTEF-6G (Kurtzman & Robnett, 2003), respectively. The majority of sequences were determined by a commercial sequencing facility (Macrogen, Seoul, Korea), while the D1/D2 and ITS sequences of strains NCAIM Y.01896T and NCAIM Y.02026 were amplified and sequenced according to the methods of Kurtzman & Robnett (1998) & Péter et al. (2009). The small subunit (SSU) rRNA and elongation factor-1α (EF-1α) genes were amplified and sequenced by primers ITS1 and NS8 (White et al., 1990) and YTEF-1G and YTEF-6G (Kurtzman & Robnett, 2003), respectively. The majority of sequences were determined by a commercial sequencing facility (Macrogen, Seoul, Korea), while the D1/D2 and ITS sequences of strains NCAIM Y.01896T and NCAIM Y.02026 were determined by another commercial sequencing facility (Bay Zoltán Nonprofit Ltd for Applied Research, Szeged, Hungary). The sequences were aligned using CLUSTAL X (Thompson et al., 1997). A neighbouring-joining tree using the Kimura two-parameter model was generated with the PAUP* 4.0b10 software package (Swofford, 2002). The stability of the branches was assessed by bootstrap analysis (Felsenstein, 1985) in which 1000 replicates were set in PAUP*. Relationships among strains were determined by the minimum spanning tree (MST) network creation method, based on the number of nucleotide changes in the SSU, ITS/5.8S and LSU D1/D2 domains of the rRNA gene and the EF-1α gene, using BioNumerics 6.5. For PCR fingerprinting, a microsatellite primer (GTG)5 was used in PCR amplification reactions as described previously (Cadez et al., 2002). Cluster analysis was performed using BioNumerics 6.5.

**RESULTS AND DISCUSSION**

**Species delineation and phylogenetic placement**

Based on the comparisons of the gene sequences from the SSU, ITS/5.8S and LSU D1/D2 domains of the rRNA and the translation elongation factor-1α, the nine strains listed in Table 1 formed three well-separated groups. Members of the first group (ZIM 2322T and ZIM 2470) shared identical D1/D2, SSU rRNA and EF-1α gene (816 bp) sequences suggesting they are conspecific. They differed by two nucleotide substitutions in their ITS region. The conspecificity of strains ZIM 2322T and ZIM 2470 was also supported by their similar phenotypic characteristics. The second group contained six strains: ZIM 2463T, ZIM 2466, ZIM 2467, ZIM 2476, ZIM 2477 and NCAIM Y.02026. Five of them shared identical D1/D2 sequences, while strain NCAIM Y.02026 differed by one substitution. The six strains also had identical SSU rRNA genes and up to one nucleotide difference in the ITS region. Among the strains there was also up to one base difference among the 816 bp EF-1α gene sequences with one exception. Strain NCAIM Y.02026 differed by ten nucleotide substitutions from most group 2 strains. Therefore, with the aim of confirming the conspecificity of strain NCAIM Y.02026 with group 2 we used PCR fingerprinting (see Fig. S1 available in IJSEM Online). The very similar banding patterns clearly confirmed the conspecificity of strains included in group 2. Interestingly, the physiological characteristics of the strains belonging to group 2 (ZIM 2463T, ZIM 2466, ZIM 2467, ZIM 2476 and ZIM 2477) were very similar, while strain NCAIM Y.02026 was slightly divergent. Unlike the other five strains assigned to this group, this strain utilized trehalose and L-arabinose (slowly) as a sole carbon source and was unable to grow at 35 °C. The third group included the single strain NCAIM Y.01896T. The members of the three above-noted groups are closely related to each other. The D1/D2 sequence divergences among the groups are six substitutions and one or two indels, while the ITS sequences differ among the members of the groups by 3–5 %. Considering the guidelines of Kurtzman & Robnett (1998), Sugita et al. (1999) & Chen et al. (2001), these sequence divergences are interpreted here as evidence for the existence of three different, closely related species. In order to confirm that the sequence variations between strains are a consequence of speciation, we used the network creation method MST to demonstrate that the intraspecific and interspecific sequence divergence are significantly different. The MST based on the concatenated sequences of the SSU, ITS/5.8S, LSU D1/D2 domains of the rRNA gene and the EF-1α gene is depicted in Fig. 1. The nine strains segregated in three groups, and the number of nucleotide substitutions in each group was significantly lower than the number among the groups, supporting the concept of three distinct species. The three groups are also sufficiently separated from their closest neighbour species based on pairwise comparisons of D1/D2 sequences. The D1/D2 sequence of strain ZIM 2322T differs from that of

N. Čadež and others
Table 1. List of strains considered in this study, their origin and the GenBank/EMBL/DDBJ accession numbers

<table>
<thead>
<tr>
<th>Strain accession number*</th>
<th>Origin</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SSU</td>
</tr>
<tr>
<td>Ogataea kolombanensis sp. nov.</td>
<td>ZIM 2323 = CBS 12778 = NRRL Y-63657</td>
<td>Olive oil sediment of 'Istrska Belica' variety, Koper, Slovenia, 2009</td>
</tr>
<tr>
<td></td>
<td>ZIM 2470</td>
<td>Olive oil sediment of mixed varieties, Koloban, Slovenia, 2011</td>
</tr>
<tr>
<td>Ogataea histrianica sp. nov.</td>
<td>ZIM 2463 = CBS 12779 = NRRL Y-63658</td>
<td>Olive oil sediment of mixed varieties, Ascolana, Ancharan, Slovenia, 2011</td>
</tr>
<tr>
<td></td>
<td>ZIM 2466</td>
<td>Olive oil sediment of variety Ascolana, Ancharan, Slovenia, 2011</td>
</tr>
<tr>
<td></td>
<td>ZIM 2467</td>
<td>Olive oil sediment of variety Ascolana, Ancharan, Slovenia, 2011</td>
</tr>
<tr>
<td></td>
<td>ZIM 2476</td>
<td>Olive oil sediment of mixed varieties, Ancharan, Slovenia, 2011</td>
</tr>
<tr>
<td></td>
<td>ZIM 2477</td>
<td>Olive oil sediment of mixed varieties, Ancharan, Slovenia, 2011</td>
</tr>
<tr>
<td></td>
<td>NCAIM Y.02026</td>
<td>Unfiltered extra virgin olive oil, Sicily, Italy, 2012</td>
</tr>
<tr>
<td>Ogataea deakii sp. nov.</td>
<td>NCAIM Y.01896 = CBS 12735 = NRRL Y-63656</td>
<td>Brown rotten wood of beech (Fagus sylvatica), Pilis mountains, Hungary, 2003</td>
</tr>
</tbody>
</table>

*ZIM, Collection of Industrial Micro-organisms, Ljubljana, Slovenia; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; NRRL, ARS Culture Collection, National Centre for Agricultural Utilization Research, Peoria, IL, USA; NCAIM, National Collection of Agricultural and Industrial Micro-organisms, Budapest, Hungary.

†Sequence identical with HE799677.

The type strain of *Ogataea nonfermentans* by ten substitutions and one indel; ZIM 2463 differs from the type strain of *O. phyllophila* by seven substitutions and two indels; finally, NCAIM Y.01896 differs from the type strain of *O. phyllophila* by eight substitutions and one indel. The D1/D2-sequence-based phylogenetic placement of the investigated strains among related species, is shown in Fig. 2. The taxa included in the analysis were selected based on sequence similarity searches against the GenBank database and from the phylogenetic tree presented by Koowadjanakul *et al.* (2011). *Ogataea cecidiorum* and *O. trehalophila* formed a peripheral branch and were excluded from the final analysis. The three novel species are placed in a well-supported (bootstrap 98%) clade (Fig. 2), which also contains *Ogataea minuta*, the type species of the genus. In addition, the phenotypic characteristics of the investigated strains match the current diagnosis of the genus *Ogataea* (Kurtzman, 2011a) and all strains except ZIM 2470 formed ascospores. Based on the data presented above, we propose three novel yeast species to accommodate the three groups of strains; *Ogataea kolombanensis* sp. nov. (MycoBank no. MB 803607) for group 1, *Ogataea histrianica* sp. nov. (MycoBank no. MB 803608) for group 2 and *Ogataea deakii* sp. nov. (MycoBank no. MB 803672) for NCAIM Y.01896. The proposal of *O. deakii* sp. nov. is based on a single strain, which is not supported by several yeast taxonomists (see e.g. Lachance *et al.*, 2010). The reason for our decision was that NCAIM Y.01896, the only known strain of *O. deakii* sp. nov. is closely related to the other two novel *Ogataea* species proposed here and, furthermore, we were unable to isolate additional strains of the species despite our extensive efforts. At the time of writing, no additional *O. deakii* strain has been recovered from hundreds of rotten wood samples during our methylotrophic yeast isolation program.

The above-noted intraspecific EF-1α gene sequence divergence (up to 10 substitutions) and the 51–57 substitutions counted among the type strains of the three novel species are significantly higher than those reported by Kurtzman & Robnett (2010) for the species *Candida cariosilignicola* and *Ogataea methylovora*, which are currently considered to be conspecific, or for the very closely related species *O. minuta* and *O. nonfermentans*. The differing results can partly be explained by the fact that the fragments of the EF-1α gene analysed by Kurtzman & Robnett (2010) and in the current study are not exactly the same. Approximately 930 and 820 nt were amplified by Kurtzman & Robnett (2010) and in the current study, respectively, and about 640 bp are overlapping. The other reason might be that counts by Kurtzman & Robnett (2010) were apparently made from hundreds of rotten wood samples during our methylotrophic yeast isolation program.
Kurtzman, personal communication). In our case, third positions contained from 0.25% to 3.4% ambiguously called bases observed as double peaks on sequencing chromatograms (data not shown). This phenomenon suggests the presence of heterozygous diploids (Clark, 1990) or the presence of a paralogue of the gene that amplifies with the presently used primer sequences. If the third positions are not considered in our analysis, intraspecific sequence differences disappear and the interspecific divergences become very similar to those reported by Kurtzman & Robnett (2010). Nevertheless, further studies are needed to reveal the distribution of the EF-1α alleles among the conspecific strains of the novel species of the genus *Ogataea*.

**Occurrence and identification**

The strains of *O. kolombanensis* sp. nov. and *O. histrianica* sp. nov. were isolated from olive oil sediments collected in Slovenia and one strain (NCAIM Y.02026) from extra virgin olive oil originating from Italy. It is important from the point of view of olive oil production that none of the investigated *O. kolombanensis* sp. nov. and *O. histrianica* sp. nov. strains were able to split fat. We hypothesize that they are part of a yeast community residing on olive fruits rich in pectic polysaccharides (Coimbra et al., 1994). As methanol is a normal product of pectin metabolism during cell wall synthesis (Fall & Benson, 1996) the occurrence of methylo trophic micro-organisms on olives may be a consequence of that. However, as reported by Romo-Sánchez *et al.*, 2010 *Candida molendinoi, Lachancea fermentati* and *Meyerozyma caribbica* predominated on olive fruits. Nevertheless, none of these species are either methylo trophic or pectinolytic (Vaughn *et al.*, 1972; Golomb *et al.*, 2013).

The single strain of *O. deakii* sp. nov. was isolated from rotten beech wood, in Hungary. The association of certain methanol-assimilating yeasts with decaying wood is well known (Suh *et al.*, 2006) and it may correspond to the fact that methanol can be derived from the methoxy groups present in wood lignin (de Koning & Harder, 1992). Further studies are needed to reveal the distribution and substrate relatedness of the three novel yeast species.

Although the ability of *O. deakii* sp. nov. to assimilate galactitol (slowly) is a rather uncommon characteristic among methylo trophic yeasts species, phenotype-based identification of the three novel species is impractical, because there are hardly any definitive physiological differences among the three novel species. As in case of *O. deakii* only one strain was available, it is unclear whether galactitol assimilation is a species- or strain-specific feature. The existence of physiologically divergent strains, like the above-noted strain NCAIM Y.02026, makes identification

![Fig. 1. Minimum spanning tree based on the analysis of concatenated sequences of the SSU–ITS–LSU rRNA gene and the EF-1α gene (length 3715 nt) of strains isolated from sediments of olive oils, olive oil and brown rotten wood of beech in Slovenia and Hungary. The numbers on the connecting lines show number of substitutions between two strains. Levels of bootstrap support for the branches as percentages of 1000 replicates are shown, except for Group 2. In the case of Group 2, the bootstrap values of the branches connecting the strains are between 61 and 100%. The distances between the strains are not proportional to phylogenetic distance.](image-url)
from phenotype even more problematic. However, the three novel species can be reliably distinguished from other phylogenetic relatives (Table 2).

**Description of *Ogataea kolombanensis* Čadež & Péter sp. nov.**

Mycobank no.: MB 803607

*Ogataea kolombanensis* (ko.lom.ba.nen’sis. N.L. fem. adj. *kolombanensis* referring to the village of Kolomban, Slovenia, the collection site of one strain of the novel species).

In 5% malt extract after 3 days at 25°C, compact sediment is present. Asexual reproduction proceeds by multilateral budding. Cells are subspheroid, ovoid or ellipsoid, they measure 2–4 x 2.5–5 μm and occur singly, in pairs and in small clusters. On 5% malt extract agar after 3 days at 25°C, the streak culture is butyrous, cream-coloured, smooth, flat and glistening. The edge is entire. On slide culture with corn meal agar after 7 days at 25°C, neither pseudohyphae nor septate hyphae are formed. One of the two investigated strains (ZIM 2322T) forms ascospores.

**Fig. 2.** Phylogenetic tree showing the placement of novel species of the genus *Ogataea* (bold type) and closely related species based on D1/D2 sequences of the LSU rRNA gene. The tree was reconstructed by neighbour-joining analysis using the Kimura two-parameter model. Bootstrap percentages ≥ 50% for 1000 replicates are shown. *Saccharomyces cerevisiae* was used as the outgroup. Bar indicates one change per 100 nt positions.
Table 2. Physiological characteristics that differentiate Ogataea kolombanensis sp. nov., Ogataea histrianica sp. nov. and Ogataea deakii sp. nov. from each other and from related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Fermentation of:</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Trehalose</td>
<td>s or ws</td>
<td>s</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>+ or s</td>
<td>v</td>
<td>+</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>Erythritol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Galactitol</td>
<td>–</td>
<td>–</td>
<td>s</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DL-Lactate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>Gluco-δ-lactone</td>
<td>+ or s</td>
<td>+</td>
<td>–</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Growth at 37 °C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth with 0.1 % cycloheximide</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth with 10 % NaCl</td>
<td>+ or s</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Species: 1, O. kolombanensis sp. nov.; 2, O. histrianica sp. nov.; 3, O. deakii sp. nov.; 4, O. nonfermentans; 5, O. phyllophila. +, Positive; –, negative; s, slow; w, weak; v, variable; N, no data. Data for O. nonfermentans are from Kurtzman (2011a) and for O. phyllophila from Koowadjanakul et al. (2011).

Fig. 3. Phase-contrast image of ascosporulating culture of Ogataea kolombanensis sp. nov. ZIM 2322T, PDA, 10 days, 25 °C. Bar, 10 μm.

independent cells, precedes ascospore formation. Two to four hat-shaped ascospores are formed in each deliquescent ascus (Fig. 3). The presence of heterogamous conjugation suggests homothallism. Ascospores were observed after 7–21 days at 25 °C on CM, PD and ME agars. D-Glucose and α,α-trehalose (slowly or weakly and slowly) are fermented, D-galactose, sucrose, maltose, lactose and raffinose are not fermented. The carbon compounds assimilated are D-glucose, D-ribose (positive or slowly), D-xylitol (slowly or latent), D-arabinose (positive or slowly), L-rhamnose (variable), α,α-trehalose, cellobiose (variable), salicin, arbutin, glycerol, ribitol (positive or slowly), xylitol, D-glucitol, D-mannitol, glucono-δ-lactone (positive or slowly), D-gluconate (weakly or weakly and slowly), succinate (positive or latent), citrate, methanol (positive or weakly) and ethanol; no growth occurs on D-galactose, L-sorbose, D-glucosamine, N-acetyl-D-glucosamine, L-arabinose, sucrose, maltose, methyl α-D-glucoside, melibiose, lactose, raffinose, melezitose, inulin, starch, meso-erythritol, L-arabininitol, galactitol, myo-inositol, 2-keto-D-glucurate, D-galacturionate, D,L-lactate, saccharate, propane-1,2-diol, butane-2,3-diol or hexadecane. Potassium nitrate, sodium nitrite, ethyamine hydrochloride, L-lysine and cadaverine dihydrochloride are assimilated; creatine, creatinine, glucosamine (as a nitrogen source) and imidazole are not assimilated. Amyloid material is not formed. Growth in vitamin-free medium is absent. Growth occurs at 30 °C but is absent at 35 °C. Growth with 10 % NaCl and with 0.1 % cycloheximide is positive, but growth is absent with 16 % NaCl, on 50 %, w/w, glucose-yeast extract agar and with 1 % acetic acid. Urea hydrolysis, lipase activity and colour reaction with Diazonium Blue B are negative.

The type strain (ZIM 2322T=CBS 12778T=NRRL Y-63657T) was isolated from olive oil sediment of the ‘Istrska Belica’ variety, from Koper, Slovenia, in 2009.

Description of Ogataea histrianica Čadež & Péter sp. nov.

MycoBank no.: MB 803608

Ogataea histrianica (his.tri.an’i.ca. N.L. fem. adj. histrianica of belonging to the Istrian peninsula, the collection site of the majority of the strains).

In 5 % malt extract after 3 days at 25 °C, compact sediment is present. Asexual reproduction proceeds by multilateral budding and cells occur singly, in pairs and in small clusters. They are spheroid and subspheroid and measure 2.5–5 μm, or are ellipsoid and measure 2.5–5 μm. On 5 % malt extract agar after 3 days at 25 °C, the streak culture is butyrous, cream-coloured, smooth, flat and glistening. The edge is entire. On slide culture with corn meal agar after 7 days at 25 °C, neither pseudohyphae nor septate hyphae are formed. Conjugation between a cell and its bud, or between independent cells, precedes ascospore formation. Usually two to four hat-shaped ascospores are formed in each deliquescent ascus (Fig. 4), but very rarely single-spored asci are also formed. The presence of heterogamous conjugation suggests homothallism. Ascospores were observed following 7–21 days of incubation at 25 °C on different media, depending on the strains. Sporulation was detected on CM, SNA and ME agars for each strain, and PD agar also supported spore formation by all but one (NCAIM Y.02026) strain. D-Glucose is fermented, D-galactose, sucrose, maltose, lactose, raffinose and α,α-trehalose are not fermented. The carbon compounds assimilated are D-glucose, L-sorbose (variable),...
D-ribose, D-xylose (positive or slowly or latent), L-arabinose (variable), D-arabinose (variable), α,α-trehalose (variable), cellobiose (positive or slowly or latent), salicin, arbutin, glycerol (positive or latent), ribitol, xylitol, L-arabininol (slowly or weakly and slowly, variable), D-glucitol, D-mannitol, glucono-δ-lactone, succinate (positive or slowly), citrate, methanol (positive or weakly) and ethanol; no growth occurs on D-galactose, D-glucosamine, N-acetyl-D-glucosamine, L-rhamnose, sucrose, maltose, methyl α-D-glucoside, melibiose, lactose, raffinose, melezitose, inulin, starch, meso-erythritol, galactitol, myo-inositol, 2-keto-D-gluconate, D-gluconate, D-glucuronate, D-galacturonate, D-lactate, saccharate, propionate, ethanol; no growth occurs on D-galactose, D-glucuronate (weakly or weakly and slowly), succinate, citrate, methanol (positive or weakly) and ethanol; no growth extract agar and with 1% acetic acid. Urea hydrolysis, lipase activity and colour reaction with Diazonium Blue B are negative.

The type strain (ZIM 2463^T = CBS 12779^T = NRRL Y-63658^T) was isolated from olive oil sediment of mixed varieties, from Ankaran, Slovenia, in 2011.

**Description of *Ogataea deakii* Péter, Dlauchy & Čadež sp. nov.**

MycoBank no.: MB 803672

*Ogataea deakii* (de.a'k.ii N.L. gen. masc. sing. n. *deakii* of Deák, in honour of the late Professor Tibor Deák, Department of Microbiology and Biotechnology, Faculty of Food Science, Corvinus University of Budapest, Budapest, Hungary, for his contributions to yeast research).

In 5% malt extract after 3 days at 25 °C, compact sediment is present. Asexual reproduction proceeds by multilateral budding and cells occur singly, in pairs and in small clusters. They are spheroid and measure 2.5–5 μm, or ellipsoid and then they measure 2–5 × 2.5–6 μm. On 5% malt extract agar after 3 days at 25 °C, the streak culture is butyrous, cream-coloured, smooth, flat and glistening. The edge is entire. On slide culture with corn meal agar after 7 days at 25 °C, neither pseudohyphae nor septate hyphae are formed. Conjugation between a cell and its bud, or between independent cells, usually precedes ascospore formation, but a few unconjugated asci occur as well. Two to four hat-shaped ascospores are formed in each deliquescent ascus (Fig. 5). The presence of heterogamous conjugation suggests homothallism. Ascospores were observed after 7–21 days at 25 °C on CM, SNA, PD and ME agars. D-Glucose and α,α-trehalose (slowly) are fermented, D-galactose, sucrose, maltose, lactose and raffinose are not fermented. The carbon compounds assimilated are D-glucose, D-ribose, D-xylose (latent), D-arabinose, α,α-trehalose, cellobiose (weakly or weakly and slowly), salicin, arbutin, glycerol, ribitol, xylitol (positive or slowly), D-glucitol, D-mannitol, galactitol (slowly), D-gluconate (weakly or weakly and slowly), succinate, citrate, methanol (positive or weakly) and ethanol; no growth
occurs on D-galactose, L-sorbose, D-glucosamine, N-acetyl-D-glucosamine, L-arabinitol, L-rhamnose, sucrose, maltose, methyl a-D-glucoside, melibiose, lactose, raffinose, melezitose, inulin, starch, meso-erythritol, L-arabinitol, myoinositol, glucono-δ-lactone, 2-keto-D-gluconate, D-glucuronate, D-galacturonate, DL-lactate, saccharate, propane-1,2-diol, butane-2,3-diol or hexadecane. Potassium nitrate, sodium nitrite, ethylamine hydrochloride, L-lysine and cadaverine dihydrochloride are assimilated; creatine, creatinine, glucosamine (as nitrogen source) and imidazole are not assimilated. Amyloid material is not formed. Growth in vitamin-free medium is absent. Growth occurs at 30 °C but is absent at 35 °C. Growth with 10 % NaCl and with 0.1 % cycloheximide is positive, but is absent with 16 % NaCl, on 50 %, w/w, glucose–yeast extract agar and with 1 % acetic acid. Urea hydrolysis, lipase activity and colour reaction with Diazonium Blue B are negative.

The type strain (NCAIM Y.01896T=CBS 12735T=NRRL Y-63656T) was isolated from brown rotten wood of beech (Fagus sylvatica), from the Pilis mountains, Hungary, in 2003.

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