Ogataea ganodermae sp. nov., a methanol-assimilating yeast species isolated from basidiocarps of Ganoderma sp.

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Three methanol-utilizing yeast strains were isolated from basidiocarps of Ganoderma sp. collected from a tree trunk in Mangshan Mountain, Hunan Province, southern China. These strains formed hat-shaped ascospores in unconjugated and deliquescent asci. Sequence analysis of the large-subunit rRNA gene D1/D2 domain and internal transcribed spacer (ITS) region, electrophoretic karyotype comparison and phenotypic characterization demonstrated that the three strains represent a novel species of the genus Ogataea, which is described as Ogataea ganodermae sp. nov. (type strain SHS 2.1$^T$ = CGMCC AS 2.3435$^T$ = CBS 10646$^T$). Phylogenetically, the novel species was closely related to Ogataea pini and Ogataea henricii. The latter two taxa with similar D1/D2 sequences were confirmed to represent separate species by ITS sequence and electrophoretic karyotype comparisons.

Based on partial sequence analysis of small (18S) and large (26S) subunit rRNA genes, Yamada et al. (1994) proposed the genus Ogataea to accommodate a group of yeast species formerly classified in the genus Pichia. The species included in Ogataea were characterized by forming hat-shaped ascospores and utilizing methanol and nitrate as sole sources of carbon and nitrogen, respectively (Yamada et al., 1994; Mikata & Yamada, 1995). The genus Ogataea was not accepted by Kurtzman (1998) because of the absence of a more robust dataset to substantiate the circumscription of the genus. Kurtzman & Robnett (1998) showed that the genus Pichia was remarkably polyphyletic and that most methanol-assimilating yeasts appeared closely related. Ogataea tends to be accepted as a currently recognized genus (Suh et al., 2006) and several novel species of the genus have been described (Morais et al., 2004; Péter et al., 2007; Limtong et al., 2008). The described Ogataea and related species are usually from tree exudates, bark and rotten wood material or associated with bark beetles. Recently, three hat-shaped ascospore-forming yeast strains isolated from basidiocarps of a polypore fungus, Ganoderma sp., were found to be closely related to Ogataea species. Comparison of the large-subunit rRNA gene D1/D2 domain and internal transcribed spacer (ITS) region sequences and electrophoretic karyotypes of the strains with those of their closest relatives showed that the three strains represent a novel species of the genus Ogataea.

Two basidiocarps of Ganoderma sp. were collected from a tree trunk in Mangshan Mountain, Hunan Province, southern China, in October 2006. Pieces of the two samples were transferred into two microfuge tubes each containing 1 ml sterile water. The tubes were put at room temperature for several hours. After vigorous shaking for 2 min, 0.5 ml aliquots of the water suspension were plated onto YM agar (Difco) plates containing chloramphenicol (100 μg ml$^{-1}$). Petri dishes were incubated at 25°C and yeast colonies were isolated and purified by using the conventional streaking technique. On the basis of morphological characterization, strains SHS 2.1$^T$ and SHS 2.2 isolated from one basidiocarp and SHS 2.3 from the other were selected for further study.

Most morphological, physiological and biochemical characteristics were examined according to standard methods (Yarrow, 1998). Nuclear DNA was extracted from yeast cells by using the method of Makimura et al. (1994). Sequences of the ITS region (including the 5.8S rRNA gene) and D1/D2 domain were determined by methods described previously (Bai et al., 2002). Sequences were aligned with the program CLUSTAL_X (Thompson et al., 1997). Phylogenetic trees were constructed from the evolutionary distance data calculated from Kimura’s two-parameter model (Kimura, 1980) using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analyses (Felsenstein, 1985) were performed on 1000 random resamplings.
Intact yeast chromosomal DNA was prepared for pulsed-field gel electrophoresis (PFGE) by the method of Bai et al. (2000). Chromosomal DNA bands were separated on a 1.0 % agarose gel in 0.5 × TBE buffer in a contour-clamped homogeneous electric field (CHEF) electrophoresis apparatus (CHEF Mapper XA system; Bio-Rad). Electrophoresis was performed at 100 V for 24 h with a switch time of 300 s and then for 28 h with a switch time of 600 s. The temperature of the running buffer was maintained at 12–14 °C. After electrophoresis, the gel was stained in ethidium bromide solution (0.5 μg ml⁻¹) for 30 min, destained in distilled water and imaged under UV light (302 nm) with an Alphalager 2200 gel documentation system (Alpha Innotech).

Sequence and electrophoretic karyotype analyses

Strains SHS 2.1⁷, SHS 2.2 and SHS 2.3 had identical D1/D2 and ITS sequences, indicating their conspecificity. In the phylogenetic tree constructed from D1/D2 sequences, strain SHS 2.1⁷ was clustered together with *Ogataea pini*, *Ogataea henricii* and *Ogataea glucozyma* with strong bootstrap support (Fig. 1). Strain SHS 2.1⁷ differed from the type strains of the latter three described species by 6 (1.1 %), 7 (1.2 %) and 14 (2.5 %) substitutions, respectively, in the D1/D2 domain and by 14, 16 and 12 mismatches, respectively, in the ITS 1 and 2 regions (497 bp overall). Kurtzman & Robnett (1998) predicted that *O. pini* (*Pichia pini*) and *O. henricii* (*Pichia henricii*) were the same or sister species because they differed by only 3 substitutions in the D1/D2 domain. ITS sequence comparison made in the present study showed that the two species differed by 16 substitutions and 3 indels in the ITS 1 and 2 sequences, suggesting that they may not be conspecific.

The chromosomal banding patterns of strains SHS 2.1⁷ and SHS 2.2 seemed identical (Fig. 2), suggesting that the two strains from the same basidiocarp may be subcultures of the same strain. Strain SHS 2.3 from the other basidiocarp differed slightly from the former two in the relative positions of three of the four chromosomal bands resolved, indicating its genotypic differentiation. The three strains were clearly differentiated from the closely related species *O. pini* and *O. henricii* by comparative electrophoretic karyotyping. The latter two species also exhibited different electrophoretic karyotypes, supporting their separation as distinct species (Fig. 2).

**Morphology and physiology**

Strains SHS 2.1⁷, SHS 2.2 and SHS 2.3 produced two to four hat-shaped ascospores in deliquescent asci on 5 % malt extract agar after 5 days at 25 °C (Fig. 3b). Conjugation before spore formation was not observed. They were able to utilize methanol as the sole source of carbon. These characters are typical for the genus *Ogataea* (Yamada et al., 1994). Only delayed and very weak growth was observed in repeated nitrate assimilation tests of the three strains; thus, it was difficult to give a clear interpretation of the results. The reliability of the positive nitrate assimilation reaction as one of the diagnostic characters of the genus *Ogataea* (Yamada et al., 1994) should be evaluated further. The two closely related sister species *O. pini* and *O. henricii* showed opposite nitrate assimilation reactions (Kurtzman, 1998; Barnett et al., 2000). Kurtzman (1984) has demonstrated that strains with different nitrate utilization abilities may have a quite high degree of DNA–DNA relatedness.

Physiologically, strain SHS 2.1⁷, SHS 2.2 and SHS 2.3 differed from the closely related species *O. pini*, *O. henricii* and *O. glucozyma* in the assimilation reactions of several carbon and nitrogen compounds, notably including sucrose, maltose, melibiose, raffinose and nitrite (Table 1). The molecular and phenotypic comparisons made above clearly indicated that strains SHS 2.1⁷, SHS 2.2 and SHS...
2.3 represent a novel species of *Ogataea*, for which the name *Ogataea ganodermae* sp. nov. is proposed.

**Latin diagnosis of *Ogataea ganodermae* F. Y. Bai & Z. H. Ji sp. nov.**


**Description of *Ogataea ganodermae* F. Y. Bai & Z. H. Ji sp. nov.**

*Ogataea ganodermae* (ga.no.der’mae. N.L. fem. adj. *ganodermae* of *Ganoderma*, referring to the genus of the polypore fungus from which the type strain was isolated).

In YM broth, after 7 days at 25 °C, cells are globose to short-ovoidal, 2.5–5.5 x 2.5–5.0 μm, single or in pairs, reproducing by multilateral budding (Fig. 3a). Sediment is formed. After 1 month at 25 °C, sediment is present; ring and pellicle are not formed. On YM agar, after 1 month at 25 °C, the streak culture is white to cream, dull, smooth and slimy. The margin is entire. In Dalmau plate culture on cornmeal agar, pseudomyccelium is not formed. On 5 % malt extract agar after 5 days at 25 °C, unconjugated asci are formed and each ascus contains two to four hat-shaped ascospores. Asci are deliquescent (Fig. 3b). Starch-like substances are not produced. *Diazonium blue B* reaction is negative. Urea is not hydrolysed. Weak growth occurs on 50 % glucose agar. The maximum temperature for growth is 37–39 °C on YM agar. Splitting of arbutin is detected. Glucose is fermented, but not galactose, sucrose, maltose, lactose or raffinose. The following carbon compounds are assimilated: glucose, l-sorosum (delayed), sucrose, maltose (delayed), cellobiosum, trehalosum, melibiosum (weak), raffinosum (delayed), D-xilosum (delayed), D-ribosum (delayed), L-rhamnosum, methanol (delayed), ethanol, glycerol, erythritol (delayed), ribitolum, D-glucitolum, D-mannitolum, salicinum, succinic acid (delayed) and inositol (delayed). The following are not assimilated: galactose, lactose, melezitose, inulins, soluble.
Sporobolomyces phaffii

the

Sporidiobolus pararoseus

Ganoderma

sp. collected from Mangshan Mountain, Hunan Province, China, in October 2006.

References


Table 1. Physiological characteristics that distinguish Ogataea ganodermae sp. nov. from closely related species

Data for reference species were taken from Kurtzman (1998) and Barnett et al. (2000). +, Positive; –, negative; D, delayed positive; V, variable; W, weakly positive.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>O. ganodermae sp. nov.</th>
<th>O. pini</th>
<th>O. henricii</th>
<th>O. glucozyma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermentation of glucose</td>
<td>+</td>
<td>V</td>
<td>–</td>
<td>+</td>
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<td>Assimilation of:</td>
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<tr>
<td>L-Sorbitol</td>
<td>D</td>
<td>+/–</td>
<td>–</td>
<td>D/–</td>
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<tr>
<td>Sucrose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Maltose</td>
<td>D</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Melibiose</td>
<td>W</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Raffinose</td>
<td>D</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>D-Xylose</td>
<td>D</td>
<td>+/–</td>
<td>+</td>
<td>+/–</td>
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<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>+/–</td>
<td>D</td>
<td>D/–</td>
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<tr>
<td>D-Arabinose</td>
<td>–</td>
<td>+/–</td>
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<td>+/–</td>
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<tr>
<td>D-Glucosamine</td>
<td>–</td>
<td>+/–</td>
<td>?</td>
<td>–</td>
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<tr>
<td>Galactitol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>D/–</td>
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<tr>
<td>DL-Lactate</td>
<td>–</td>
<td>D/–</td>
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<td>Succinic acid</td>
<td>D</td>
<td>+/–</td>
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<td>+/–</td>
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<td>Citric acid</td>
<td>–</td>
<td>+/–</td>
<td>+</td>
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<td>Inositol</td>
<td>D</td>
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<tr>
<td>Nitrite</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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starch, D-arabinose, L-arabinose, D-glucosamine, galactitol, methyl alpha-D-glucoside, DL-lactic acid, citric acid and hexadecane. Ammonium sulfate, L-lysine, ethylamine hydrochloride and cadaverine dihydrochloride are assimilated. Potassium nitrate is not utilized or utilization is delayed and weak; sodium nitrate is not utilized.

The type strain is SHS 2.1T (=CGMCC AS 2.3435T =CBS 10646T), which was isolated from a basidiocarp of Ganoderma sp. collected from Mangshan Mountain, Hunan Province, China, in October 2006.

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