Proposal of *Zygosaccharomyces parabailii* sp. nov. and *Zygosaccharomyces pseudobailii* sp. nov., novel species closely related to *Zygosaccharomyces bailii*

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Twenty-three yeast strains traditionally identified as *Zygosaccharomyces bailii* were studied in order to clarify their taxonomy and phylogenetic relationships. The molecular phylogeny from rRNA gene sequences showed that these yeasts were well divided into three major groups, and two of the groups could be clearly distinguished from the type strain of *Z. bailii* at the species level. Therefore, we propose *Zygosaccharomyces parabailii* sp. nov. (type strain ATCC 56075T =NBRC 1047T =NCYC 128T =CBS 12809T) and *Zygosaccharomyces pseudobailii* sp. nov. (type strain ATCC 56074T =NBRC 0488T =CBS 2856T) to accommodate the yeasts belonging to the two groups. By conventional physiological tests, *Z. bailii* and the two novel species are not clearly distinguished from one another, as variations exist more frequently between individual strains and are not species-specific. However, the conclusions from rRNA gene sequence analyses are well supported by genome fingerprinting patterns as well as other protein-coding gene sequence comparisons.

**INTRODUCTION**

*Zygosaccharomyces bailii* is a widely distributed yeast species that is often associated with food spoilage, particularly of acidified, preserved foods containing high concentrations of fermentable sugars (Thomas & Davenport, 1985; Cole & Keenan, 1987; Makdesi & Beuchat, 1996; James & Stratford, 2011). The yeast has been proposed as a new host for several biotechnological processes (e.g. Branduardi *et al.*, 2004) due to its ability to tolerate such environments at relatively high temperatures, which could improve the efficiency of these processes under restrictive conditions. Moreover, the high growth rate of *Z. bailii* and its high biomass yield make this yeast particularly attractive for heterologous protein and metabolite production (e.g. Sousa *et al.*, 1996, 1998).

Despite their well-known role in food/beverage spoilage, accurate identification of *Z. bailii* and related yeasts to the species level using conventional taxonomic tests remains problematic. An inability to ferment and assimilate many of the carbon compounds typically used in yeast identification, as well as ambiguous tests results due to strain variability, often hampers identification (James & Stratford, 2011). Furthermore, significant intraspecific variation in internal transcribed spacer (ITS) sequences was also reported among some strains of the species (James *et al.*, 1996), which may cause difficulties for the use of this barcode region for identifying the species (Schoch *et al.*, 2012). We hypothesized that polyphasic analyses of the yeasts encompassed by *Zygosaccharomyces bailii sensu lato* may lead to a more accurate understanding of their phylogenetic relationship and taxonomic status. Here we report the molecular, physiological and morphological characterization of these yeasts, and propose two novel species near *Z. bailii*.

**METHODS**

**Yeast strains and characterization.** Strains of *Z. bailii sensu lato* and related taxa were selected from the ATCC Mycology Collection or were provided by bioMérieux, Inc. (Table 1). Morphological observations and metabolic tests comprising the yeast standard...
Table 1. Strains of *Z. bailii* and related yeasts investigated in this study

The genotypes were designated LSU1–LSU7 for the D1/D2 region of the LSU rRNA gene and ITSa–ITSn for the ITS region including the 5.8S rRNA gene. The identification test with the VITEK 2 system was performed at least twice for each strain. If the result was not consistent for a strain, all the identifications from each test are listed.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source and comments</th>
<th>Genotype from DNA sequences</th>
<th>VITEK 2 result with YST identification card (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Z. bailii</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 58445T (=CBS 680T =NCYC 1416T =NRRL Y-2227T)</td>
<td>Brewery, Tokyo; type strain of <em>Z. bailii</em></td>
<td>LSU1/ITSa</td>
<td>Unidentified organism; low discrimination</td>
</tr>
<tr>
<td>ATCC 38924</td>
<td>Deposited at ATCC as <em>Saccharomyces acidifaciens</em></td>
<td>LSU1/ITSb</td>
<td><em>Z. bailii</em> (93%); <em>Rhodotorula glutinis</em>/<em>R. mucilaginosa</em> (91%)</td>
</tr>
<tr>
<td>ATCC 8766 (=CBS 749 =NCYC 573 =NRRL Y-1011)</td>
<td>Domestic wine; type strain of <em>Zygosaccharomyces acidifaciens</em></td>
<td>LSU1/ITSc</td>
<td><em>R. glutinis</em>/<em>R. mucilaginosa</em> (94–95%); low discrimination</td>
</tr>
<tr>
<td>ATCC 11486 (=CBS 1170 =NRRL Y-1404)</td>
<td>Vinegar, Spain; deposited as <em>S. mestris</em></td>
<td>LSU1/ITSd</td>
<td><em>Z. bailii</em> (99%)</td>
</tr>
<tr>
<td>ATCC 66825 (=PYCC 4267)</td>
<td>Bottled white wine, Portugal</td>
<td>LSU1/ITSf</td>
<td>Unidentified organism/<em>Candida sphaerica</em> (86%)</td>
</tr>
<tr>
<td>ATCC 38923</td>
<td>Used for degradation of malic acid in grape must</td>
<td>LSU1/ITSe</td>
<td><em>Z. bailii</em> (99%)</td>
</tr>
<tr>
<td>ATCC 42476</td>
<td>Used for production of sherry wine</td>
<td>LSU2/ITSd</td>
<td><em>R. glutinis</em>/<em>R. mucilaginosa</em> (98%); low discrimination</td>
</tr>
<tr>
<td>ATCC 42477</td>
<td>Used for production of sherry wine</td>
<td>LSU2/ITSd</td>
<td><em>Candida sake</em> (96%); <em>R. glutinis</em>/<em>R. mucilaginosa</em> (98%); low discrimination</td>
</tr>
<tr>
<td><em>Zygosaccharomyces parabailii</em> sp. nov.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 56075T (=NBRC 1047T =NCYC 128T =CBS 12809T)</td>
<td>Wild-type isolate associated with fermentation</td>
<td>LSU4/ITSj</td>
<td><em>C. sake</em> (91%); low discrimination</td>
</tr>
<tr>
<td>ATCC MYA-4549 (=MUCL 51627)</td>
<td>Quality control strain for VITEK 2 yeast identification card for <em>Z. bailii</em></td>
<td>LSU3/ITSj</td>
<td><em>Z. bailii</em> (96–97%)</td>
</tr>
<tr>
<td>ATCC 56535 (=NCYC 1520)</td>
<td>Netherlands</td>
<td>LSU4/ITSi</td>
<td><em>Z. bailii</em> (96%)</td>
</tr>
<tr>
<td>ATCC 60483</td>
<td>Imported citrus concentrate, Netherlands</td>
<td>LSU4/ITSh</td>
<td><em>Z. bailii</em> (96%)</td>
</tr>
<tr>
<td>ATCC 60484</td>
<td>Citrus paste, Netherlands</td>
<td>LSU4/ITSk</td>
<td><em>Z. bailii</em> (96%)</td>
</tr>
<tr>
<td>ATCC 36947</td>
<td>Salad dressing, USA; caused spoilage outbreak of salad dressing</td>
<td>LSU5/ITSi</td>
<td><em>Z. bailii</em> (92–93%)</td>
</tr>
<tr>
<td>ATCC 8099 (=NCYC 580)</td>
<td>Not known</td>
<td>LSU6/ITSg</td>
<td><em>Z. bailii</em> (97%); <em>R. glutinis</em>/<em>R. mucilaginosa</em> (95%)</td>
</tr>
<tr>
<td>304978</td>
<td>Provided by bioMérieux Inc.</td>
<td>LSU3/ITSh</td>
<td><em>Z. bailii</em> (99%)</td>
</tr>
<tr>
<td>304979</td>
<td>Provided by bioMérieux Inc.</td>
<td>LSU4/ITSi</td>
<td><em>Z. bailii</em> (99%)</td>
</tr>
<tr>
<td>302778</td>
<td>Provided by bioMérieux Inc.</td>
<td>LSU3/ITSh</td>
<td><em>Z. bailii</em> (99%)</td>
</tr>
<tr>
<td><em>Zygosaccharomyces pseudobailii</em> sp. nov.</td>
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<td></td>
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</tr>
<tr>
<td>ATCC 56074T (=CBS 2856T =NBRC 0488T)</td>
<td>Worcestershire sauce, Japan</td>
<td>LSU7/ITSi</td>
<td><em>Z. bailii</em> (99%)</td>
</tr>
<tr>
<td>ATCC 2602 (=NRRL Y-54)</td>
<td>Deposited as <em>Zygosaccharomyces mandshuricus</em></td>
<td>LSU7/ITSm</td>
<td><em>Z. bailii</em> (96%); <em>R. glutinis</em>/<em>R. mucilaginosa</em> (98%)</td>
</tr>
<tr>
<td>ATCC 2333 (=NRRL Y-125)</td>
<td>Deposited as <em>Z. mandshuricus</em></td>
<td>LSU7/ITSm</td>
<td><em>Z. bailii</em> (97%); <em>R. glutinis</em>/<em>R. mucilaginosa</em> (97%)</td>
</tr>
<tr>
<td>ATCC 36946</td>
<td>Pickle relish; deposited as <em>S. acidifaciens</em></td>
<td>LSU7/ITSn</td>
<td><em>Z. bailii</em> (96%)</td>
</tr>
<tr>
<td>ATCC 11003</td>
<td>Deposited as <em>Zygosaccharomyces naniwaensis</em></td>
<td>LSU7/ITSi</td>
<td><em>Z. bailii</em> (96–97%)</td>
</tr>
<tr>
<td><em>Z. bisporus</em> 302815</td>
<td>Provided by bioMérieux Inc.</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
description were performed according to established methods (Barnett et al., 2000; Kurtzman et al., 2011). Assaymitations tests for carbon and nitrogen compounds were conducted in liquid media. Automated identification by the VITEK 2 system was performed with the YST card (bioMérieux) by following the manufacturer’s instructions. Ascospore formation was tested on YM agar, 2% malt agar, cornmeal agar, V8 juice agar and V8 Ray agar (ATCC medium 1970; 0.2 g CaCO₃, 50 ml V-8 juice and 20 g agar in 1 l Ray broth) at 25 °C for up to 4 weeks.

**DNA sequencing and molecular phylogenetic analyses.** Nucleic acids were extracted and purified following the procedures of Lee & Taylor (1990). The primer sets ITS5/ITS4 and LR0R/LR3 were used for PCR amplification of nuclear rRNA gene repeats (White et al., 1990; Hausner et al., 1993). Six additional genes were also sequenced for representatives of each major group from the RNA gene analyses. These genes and the primers used for PCR and sequencing were: the cytochrome oxidase subunit 2 gene (coxII) with primers COII-3 and COII-5 (Belloch et al., 2000); the mitochondrial small-subunit (mSSU) rRNA gene with MS-1A and MS-2 (Kurtzman & Robnett, 2003); the β-tubulin gene with βtub3 and βtub4r (Einax & Voigt, 2003); the translation elongation factor 1-α gene (EF-1α) with primers EF1-983F and EF1-2218R (Rehner & Buckley, 2005); the RNA polymerase II largest subunit gene (RPB1) with primers RPB1-Af and RPB1-Cr (Stiller & Hall, 1997; Matheny et al., 2002); and the RNA polymerase II second-largest subunit gene (RPB2) with primers RRPB2-5F and RRPB2-7C (Kurtzman & Robnett, 2003).

PCR conditions for the additional genes were those recommended in the references cited for each primer. PCR products were purified using Quantum Prep PCR Kleen spin columns (Bio-Rad Laboratories), and the purified PCR products were used as templates for sequencing with the purified PCR products were used as templates for sequencing with the purified PCR products were used as templates for sequencing with the purified PCR products were used as templates for sequencing. DNA sequencing and molecular phylogenetic analyses.

PCR conditions for the additional genes were those recommended in the references cited for each primer. PCR products were purified using Quantum Prep PCR Kleen spin columns (Bio-Rad Laboratories), and the purified PCR products were used as templates for sequencing with the purified PCR products were used as templates for sequencing. DNA sequencing and molecular phylogenetic analyses. A total of 23 yeast strains that have been identified as Z. bailii were sequenced for the D1/D2 region of the large-subunit (LSU) rRNA gene and the ITS region, including the 5.8S rRNA gene. As a result, the yeasts were divided into seven genotypes by their D1/D2 sequences (LSU1–LSU7; 606 nt in total), which showed one to five nucleotide variations between different genotypes (Table 1; Fig. S1). Strain ATCC MYA-4549 and two bioMérieux strains, 304978 and 302778, in genotype LSU3 were the most distinct group from the type strain of Z. bailii (ATCC 58445T; genotype LSU1), with four substitutions and gaps in the D1/D2 sequence. Strains of LSU2 and two other genotypes, LSU3 and LSU5, were distinct from each other by five nucleotide substitutions. On the other hand, the ITS region showed more significant variations among the test strains. Fourteen genotypes (ITSa–ITSn) were recognized from the 23 strains, which could be distinguished from each other by 1 to 71 nucleotide substitutions and gaps in the 806-bp aligned sequences (Table 1; Fig. S2). ATCC 56074 and ATCC 11003 in genotype ITSl were the most distinct strains from the type strain of Z. bailii, ATCC 58445T (ITSa), which showed 71 nucleotide differences including gaps. Genotypes ITSl, ITSm and ITSn were highly divergent from all other ITS types based on the sequence comparison (Fig. S2). The pattern of ITS genotyping in this study was somewhat similar to that of James et al. (1996), which was performed with only seven Z. bailii strains stored in the National Collection of Yeast Cultures (NCYC). They found three ITS types, designated A, B and C, from ITS1 and ITS2 region sequences. The sequence of ITS type A is similar to those of ITSa–f in this study (James et al., 1996; Fig. S2). Their ITS types B and C correspond to our groups ITSl–k and ITSn–n, respectively, based on sequence similarity (James et al., 1996; Fig. S2). Both studies clearly indicate that there are at least three major ITS groups among the yeasts that have been identified as Z. bailii by traditional taxonomic criteria. Sequence variations in the ITS region among the three major groups in this study were 40–45 bp between ITSa–f and ITSl–k, 68–71 bp between ITSa–f and ITSn–n and 67–71 bp between ITSl–k and ITSn–n. Genotypes from the D1/D2 region and the ITS region were correlated, i.e. the strains in LSU1 and LSU2 have ITS sequences of ITSa–f, while those in LSU3–6 share the sequences of ITSl–k. Strains in LSU7 showed three ITS genotypes, ITSl–n (Table 1). This close genotype correlation between the two regions implies that these 23 Z. bailii sensu lato strains have evolved distinctly along three lineages since they diverged from a common ancestor. As the ITS and D1/D2 regions have been widely accepted as fungal DNA barcodes (e.g. Kurtzman, 2006; Kurtzman & Robnett, 1998; Scorzzetti et al., 2002; Schoch et al., 2012), the significant variations found in these two genetic loci are strong evidence of the
Two novel species near *Zygosaccharomyces bailii*

need for taxonomic revision among traditionally recognized *Z. bailii* strains. Similar sequence variations were also reported in the two loci among strains of *Zygosaccharomyces rouxii* isolated from miso and soy sauce (Suezawa et al., 2008). However, as some of the genotypes in *Z. rouxii* are homologous in one or other of the two loci, it is difficult to distinguish them clearly as two or more species based on sequence comparison.

A parsimonious tree was generated from the combined dataset of ITS and D1/D2 sequences from the *Z. bailii sensu lato* strains and other *Zygosaccharomyces* species using *Lachancea thermotolerans* NRRL Y-8284\(^T\) as an outgroup (Fig. 1). *Z. bailii* and related yeasts were well divided into three major clades on the tree: (i) the type strain ATCC 58445\(^T\) and seven other strains belong to genotypes LSU1–2/ITSa–f, (ii) ten strains belong to genotypes LSU3–6/ITSg–k and (iii) five strains belong to LSU7/ITSl–n (Fig. 1). These three groups could be distinguished from each other by approximately 3–5% sequence variation in the ITS and D1/D2 regions, while the variation among strains within each group was less than 0.5% in the same region. The degree of sequence variation in the two loci among the groups is significant enough to separate them at the species level, as concluded in many studies (e.g. Kurtzman, 2006; Kurtzman & Robnett, 1998; Scorzetti et al., 2002). Therefore, we propose two novel species near *Z. bailii*, *Zygosaccharomyces parabailii* sp. nov. and *Zygosaccharomyces pseudobailii* sp. nov., to accommodate the yeast strains that belong to the two major groups distinct from the type strain of *Z. bailii* (Fig. 1).

*Zygosaccharomyces bisporus* is the taxon closest to *Z. bailii* and the two novel species in the tree reconstructed from the combined ITS and D1/D2 region sequences (Fig. 1), as well as in those reconstructed from the individual sequences (Fig. S3; Kurtzman et al., 2001; Rosa & Lachance, 2005; Saksinchai et al., 2012; James & Stratford, 2011; Solieri et al., 2013). Interestingly, some intragenomic polymorphism in the ITS region has been reported within individual strains of *Zygosaccharomyces* species (Gordon & Wolfe, 2008; Solieri et al., 2007, 2013). *Zygosaccharomyces sapae* CBS 12607\(^T\) is a good example, which has three different ITS types in the genome (Figs 1 and S3). Among the 23 strains of *Z. bailii* and the two novel species tested in this study, however, we did not find any intragenomic polymorphism in the region, although some poly(A) or poly(T) repeats often caused difficulties in sequencing the gene (Fig. S2).

![Fig. 1. Consensus of 1 690 800 parsimonious trees obtained from sequence data of the ITS region and the D1/D2 region of the LSU rRNA gene from *Z. bailii* and related yeasts. *Lachancea thermotolerans* NRRL Y-8284\(^T\) was used as the outgroup taxon. GenBank accession numbers after the names of yeast species are for the sequences of ITS and D1/D2 regions, respectively, used in the analyses. Numbers on tree branches indicate percentages of bootstrap support derived from 1000 samples (upper) and the probability of nodes from Bayesian Markov chain Monte Carlo analysis (lower).](http://ijs.sgmjournals.org)
Z. bailii and the two novel species were also well distinguished from one another by the sequences of additional genes other than those in the nuclear rRNA gene repeat (Table S1). Although the two mitochondrial genes, coxII and the mSSU rRNA gene, were not variable enough to distinguish the three species, the other four protein-coding genes showed significant sequence variations among the species: i.e. 37–42 (4–4.8 %) nucleotide mismatches in the β-tubulin gene, 19–31 (2–3.3 %) in EF-1α, 22–37 (3.3–5.6 %) in RPB1 and 41–74 (4–7.2 %) in RPB2. Intraspecific variations of RPB1 and RPB2 were less than 0.6 % in both genes (Table S1).

The results from genome-wide DNA fingerprinting were consistent with the genetic heterogeneity in the Z. bailii sensu lato strains and the existence of three major clades revealed by rRNA gene sequence analyses. The PCR amplification patterns with Diversilab clearly grouped the 23 strains into three clusters with significant distances in similarity scores on the dendrogram (Fig. 2): Z. bailii and Z. parabailii were separated from each other at approximately 80 %, while Z. pseudobailii was distinguished from the other two species at a similarity of 60 % (Fig. 2). The result from microsatellite-primed PCR was similar to that for the Diversilab test, and clearly showed three distinct PCR amplification patterns (data not shown).

The ecology of the three species could be similar, based on information from isolation sources, often being associated with food spoilage, especially in acidified preserved foods. Phenotypic variations were confirmed among Z. bailii and related yeasts from automated identification tests by the VITEK 2 system, although variation was more frequent among individual strains and was not species-specific (Table 1). As such, some of the Z. bailii strains, including the type strain ATCC 58445T, were not correctly identified as the species by the current VITEK 2 system, while most strains in Z. parabailii and Z. pseudobailii were identified as Z. bailii. It appears that these plasmids are common among strains of Z. bailii and related species, which may help exchange of genetic information across these yeasts.

It is difficult to distinguish Z. bailii and the two novel species from one another with conventional physiological tests, because many assimilation or fermentation abilities of the yeasts are variable at the strain level, e.g. those for sucrose, inulin or xylitol. Phenotypic variations were confirmed among Z. bailii and related yeasts from automated identification tests by the VITEK 2 system, although variation was more frequent among individual strains and was not species-specific (Table 1). As such, some of the Z. bailii strains, including the type strain ATCC 58445T, were not correctly identified as the species by the current VITEK 2 system, while most strains in Z. parabailii and Z. pseudobailii were identified as Z. bailii. In light of our new understanding of the taxonomic and phylogenetic relationships among strains of Z. bailii sensu lato, an updated VITEK system profile that reflects this taxonomic revision is desirable. Strain ATCC MYA-4549 (=MUCL 51627) has been designated the quality control strain for Z. bailii for the VITEK 2 system based on its biochemical profile. Taxonomically, the strain was clearly distinguished from the type strain of Z. bailii by molecular analyses and was reclassified as a strain of Z. parabailii in this study (Table 1, Fig. 1). Data from this study may be used to update the nomenclature of the Z. bailii quality control strain for the VITEK 2 system.

**Description of Zygosaccharomyces parabailii sp. nov. Suh, Gujjari, Beres, Beck & Zhou**

*Zygosaccharomyces parabailii* (pa.ra.ba’il’li). Gr. prep. para alongside of, resembling, like, beside; N.L. gen. n. bailii a specific epithet; N.L. gen. n. parabailii bailii-like, referring to its phylogenetic closeness to Z. bailii).

After 7 days of growth on YM agar at 25 °C, cells are ellipsoidal to ovoid (2.0–4.0 × 2.5–7.5 μm) and occur singly, in pairs or in small clusters (Fig. 3a). Pseudohyphae and true

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**Fig. 2.** Dendrogram and genome fingerprinting profiles of strains in Z. bailii and the two novel species generated by Diversilab. Similarity scores on the dendrogram were calculated by Kullback–Leibler analysis. Z. bisporus 302815 was used as a reference strain.
hyphae are not present. Colonies are cream-coloured, butyrous and smooth and have a somewhat uneven margin. After 11 days of growth in Dalmau plate culture on cornmeal agar at 25 °C, pseudohyphae and true hyphae are not present. Aerobic growth is white to cream-coloured and smooth. Sporulation occurs on 2 % malt extract agar and V8 Ray agar after 7–10 days at 25 °C. Vegetative cells may differentiate after conjugation between individual cells into persistent ascii containing up to two ascospores, which are spherical to ovoid (1.5–2.0 × 1.5–2.0 μm; Fig. 3b). Glucose and sucrose (variable) are fermented; galactose, maltose, methyl α-D-glucoside, trehalose, melibiose, lactose, cellobiose, melezitose, raffinose, inulin, starch and D-xylose are not fermented. Glucose, galactose (variable), L-sorbose (variable), sucrose (variable), trehalose, glycerol, ribitol, xylitol (variable), D-glucitol, D-mannitol, D-glucono-1,5-lactone (variable), 2-keto-D-gluconate (variable) and ethanol (variable) are assimilated; D-glucosamine, D-ribose, D-xylose, L- and D-arabinose, L-rhamnose, maltose, methyl α-D-glucoside, cellobiose, salicin, arbutin, melibiose, lactose, raffinose, melezitose, inulin, soluble starch, erythritol, L-arabinitol, galactitol, myo-inositol, D-glucurate, D-galacturonic acid, succinate, DL-lactate, citrate, methanol, propane-1,2-diol, butane-2,3-diol and quinic acid are not assimilated. L-Lysine (variable), ethylamine, cadaverine and D-tryptophan (variable) are assimilated; potassium nitrate, sodium nitrite, creatine, creatinine, D-glucosamine (as nitrogen source) and imidazole are not assimilated. Growth without vitamins is negative. Growth in 0.01 % cycloheximide is negative. Growth in 0 % acetic acid, 50 % D-glucose, 60 % D-glucose (variable) and 10 % NaCl is positive, but growth on 16 % NaCl is negative. Growth at 30 °C is positive, while growth at 37 °C is negative. Starch-like compounds are not produced. Acetic acid production, the diazoin blue B reaction and urease activity are absent.

The type strain is ATCC 56075 T (=NBRC 1047 T =NCYC 128 T =CBS 12809 T), a wild-type isolate associated with fermentation. GenBank accession numbers for the DNA barcode sequences of the type strain are JX458094 and JX458113 (ITS and D1/D2 region of LSU rRNA gene, respectively). The Mycobank number for Zygosaccharomyces parabailii is MB 803596.

**Description of *Zygosaccharomyces pseudobailii* sp. nov. Suh, Gujjari, Beres, Beck & Zhou**

Zygosaccharomyces pseudobailii (pseu.do.bail’i.i. Gr. adj. pseu’des false; N.L. gen. n. bailii a specific epithet; N.L. gen. n. pseudobailii false bailii, referring to its phylogenetic closeness to *Z. bailii*).

After 7 days of growth on YM agar at 25 °C, cells are ellipsoidal to ovoid (2.0–3.0 × 2.5–7.5 μm) and occur singly, in pairs or in small clusters (Fig. 3c). Pseu-dohyphae and true hyphae are not present. Colonies are cream-coloured, butyrous and smooth and have a somewhat clear margin. Colonies on 2 % malt extract agar under the same conditions are slightly wrinkled on top. After 11 days of growth in Dalmau plate culture on cornmeal agar at 25 °C, pseudohyphae and true hyphae are not present. Aerobic growth is white to cream-coloured and smooth. Sporulation occurs on 2 % malt extract agar and V8 Ray agar after 9 days at 25 °C. Vegetative cells may differentiate after conjugation between individual cells into persistent ascii containing up to two ascospores that are spherical to ovoid (1.5–2.0 × 1.5–2.0 μm; Fig. 3d). Glucose is fermented; galactose, maltose, methyl α-D-glucoside, sucrose, trehalose, melibiose, lactose, cellobiose, melezitose, raffinose, inulin, starch and D-xylose are not fermented. Glucose, galactose (variable), L-sorbose (variable), trehalose, glycerol, ribitol, D-glucitol, D-mannitol, D-glucono-1,5-lactone (variable), 2-keto-D-gluconate (weak), DL-lactate (weak) and ethanol are assimilated; sucrose, D-glucosamine, D-ribose, D-xylose, L- and D-arabinose, L-rhamnose, maltose, methyl α-D-glucoside, sucrose, trehalose, melibiose, lactose, cellobiose, melezitose, raffinose, inulin, starch and D-xylose are not fermented. Growth without vitamins is negative. Growth in 0.01 % cycloheximide is negative. Growth in 0 % acetic acid, 50 % D-glucose, 60 % D-glucose (variable) and 10 % NaCl (weak) is positive, but growth on 16 % NaCl (weak) is negative. Growth at 37 °C is positive. Starch-like compounds are not produced. Acetic acid production, the diazoin blue B reaction and urease activity are absent.

**Fig. 3.** Cells of *Z. parabailii* sp. nov. ATCC 56075 T (a, b) and *Z. pseudobailii* sp. nov. ATCC 56074 T (c, d). Vegetative yeast cells (a, c) after 7 days on YM agar at 25 °C, and asci with two ascospores (arrows) on V8 Ray agar (b) or 2 % malt extract agar (d) after 9 days at 25 °C. Bar, 10 μm.
production, the diazonium blue B reaction and urease activity are absent.

The type strain is ATCC 56074T (=NBRC 0488T =CBS 2856T), isolated from Worcestershire sauce in Japan. Gen-Bank accession numbers for the DNA barcode sequences of the type strain are JX458109 and JX458128 (ITS and D1/D2 regions of LSU rRNA gene, respectively). The Mycobank number for Zygosaccharomyces pseudobailii is MB 803597.

ACKNOWLEDGEMENTS

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REFERENCES


