Spencermartinsiella ligniputridi sp. nov., a yeast species isolated from rotten wood

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Four strains of a novel heterothallic yeast species were isolated from rotten wood samples collected at different locations in Hungary. Analysis of sequences of the D1/D2 domain of the large subunit rRNA gene placed the novel species in the genus Spencermartinsiella. The novel species can be distinguished from Spencermartinsiella europaea, the single species of the genus, and from Candida cellulosicola, the only recognized anamorphic species of the Spencermartinsiella clade, on the basis of standard phenotypic characteristics. The relatedness among the four strains of the novel species and two closely related strains representing undescribed yeast species is discussed. The name Spencermartinsiella ligniputridi sp. nov. is proposed to accommodate the four novel strains. The type and isotype strains of Spencermartinsiella ligniputridi sp. nov. are NCAIM Y.01992T (=CBS 12585T =NRRL Y-48818) and NCAIM Y.01936T (=CBS 12586T =NRRL Y-48819), respectively. Two additional strains are NCAIM Y.01991 and NCAIM Y.01993.

The monotypic genus Spencermartinsiella can only be recognized from DNA sequence comparisons because the characteristic properties of its sexual reproduction, i.e. the formation of spheroid or ellipsoid, persistent asci with an attached apical cell, are shared by some members of the genera Trichomonascus and Sugiyamaella (Péter et al., 2011). In addition to Spencermartinsiella europaea, the only species of the genus, a related anamorphic species, Candida cellulosicola has recently been described (Guo et al., 2012). Both S. europaea and C. cellulosicola were isolated from rotten wood, and were reported to assimilate cellobiose and xylose (Péter et al., 2011; Guo et al., 2012). These carbohydrates can be derived during the degradation of polymers which are constituents of wood. In addition to these two recognized species, a BLAST search of the GenBank database reveals that the Spencermartinsiella clade also contains some undescribed yeast species.

During our studies investigating the yeast biodiversity of some natural habitats in Hungary, focusing mainly on methylotrophic species, four conspecific strains were recovered from rotten wood samples. Based on the analysis of their D1/D2 sequences, the strains are related to, but are clearly distinct from S. europaea. The haploid, heterothallic strains formed one ascospore in each persistent ascus with an attached apical cell, when mixed in compatible mating combinations. Their other phenotypical characteristics also fit to the diagnosis of the genus Spencermartinsiella (Péter et al., 2011).

The four S. ligniputridi sp. nov. strains investigated in this study were isolated from different rotten wood samples (sample size 10 g) of broad-leaved deciduous trees, collected at several locations in Hungary in 2004 and 2005 (Table 1). Three strains (NCAIM Y.01991, NCAIM Y.01992T, NCAIM Y.01993) were isolated after a two-step enrichment procedure (Dlauchy et al., 2003) in methanol-containing broth. One strain (NCAIM Y.01936T) was recovered following a similar procedure, but the enrichment was carried out in Yeast Carbon Base (YCB) supplemented with 0.005 % (w/v) imidazole, a nitrogen source suitable for selective isolation of members of the
Table 1. List of strains used in this study

<table>
<thead>
<tr>
<th>Strain accession number*</th>
<th>Mating type†</th>
<th>Source of isolation and year of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spencermartinsiella lignoniputridi sp. nov.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCAIM Y.01936† (=CBS 12586T=NRRRL Y-48819T)</td>
<td>hI</td>
<td>Rotten wood of hornbeam (Carpinus betulus), Pilis Mountains, Hungary, 2005</td>
</tr>
<tr>
<td>NCAIM Y.01991</td>
<td>hT</td>
<td>Rotten wood of oak (Quercus sp.), Pilis Mountains, Hungary, 2005</td>
</tr>
<tr>
<td>NCAIM Y.01992† (=CBS 12585T=NRRRL Y-48818T)</td>
<td>hT</td>
<td>Rotten wood of an unidentified tree, Pilis Mountains, Hungary, 2003</td>
</tr>
<tr>
<td>NCAIM Y.01993</td>
<td>hT</td>
<td>Rotten wood of beech (Fagus sylvatica), Bükk Mountains, Hungary, 2003</td>
</tr>
<tr>
<td>GY44S02</td>
<td></td>
<td>Soil, Taiwan</td>
</tr>
<tr>
<td>GA1S04</td>
<td></td>
<td>Soil, Taiwan</td>
</tr>
</tbody>
</table>

* NCAIM, National Collection of Agricultural and Industrial Micro-organisms, Budapest, Hungary; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; NRRRL, ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL, USA.
† hT, mating type identical with the type strain; hI, mating type identical with the isotype strain.

genus Lipomyces from soils (Yurkov et al., 2011). In both cases, the yeast strains were isolated at 25 °C following serial dilution and plating on Rose-Bengal Chloramphenicol (RBC) agar. These strains represented the minor components of the enrichment liquid, accompanying the dominant methanol- and imidazole-assimilating strains, and they were unable to grow on methanol and imidazole as a sole carbon and nitrogen source, respectively. Two yeast strains, isolated from soil in Taiwan, were also included in this study (Table 1), because based on their D1/D2 domains of large subunit rRNA gene (D1/D2 LSU rRNA) and ITS sequences they are closely related to the novel species. The Taiwanese strains were isolated on Dichloran Rose-Bengal Chloramphenicol agar (DRBC; Merck) following the method described by Liu et al. (2008). Phenotypic characterization of the strains was performed by standard methods described by Yarrow (1998). Based on the results of our preliminary experiments, sexual reactivity was studied by mixing actively growing cultures on potato-dextrose agar (PDA). The four strains of the novel species were mixed in all possible combinations. Strains GY44S02 and GA1S04 as well as the type and isotype strains of S. europaea (NCAIM Y.01817T, NCAIM Y.01819I) were mixed pairwise with the type and isotype strains of the novel species (NCAIM Y.01992T and NCAIM Y.01993I). The mixtures were incubated at 25 °C and examined weekly by microscopy for up to 3 weeks except for mixtures containing strains NCAIM Y.01991, GY44S02, GA1S04, NCAIM Y.01817T and NCAIM Y.01819I which were incubated for 8 weeks. The type strain of S. ligniputridi sp. nov. (NCAIM Y.01992T) was designated hT and the isotype strain (NCAIM Y.01936T) as hI while the mating types of the other strains investigated were defined in reference to them.

The D1/D2 domain of the large subunit rRNA gene from all strains was sequenced as described by Kurtzman & Robnett (1998). The ITS regions (ITS1, 5.8S rRNA gene and ITS2) were amplified and sequenced from all strains as described by Péter et al. (2009). A 363 nt fragment of the mitochondrial small-subunit rRNA (mtSSU rRNA) gene was amplified with the primers 5’-GTGCCAGCAGCT-GCCGTTAGACA-3’ and 5’-ATTTAACGACATGTTCCA-CCTG-3’ which were also used for the sequencing reactions. The GenBank accession numbers of the DNA sequences deposited during this study are listed in Table S1, available in IJSEM online.

Sequence similarity searches were performed against the GenBank sequence database using the BLAST 2.2.26 database search program (Zhang et al., 2000). The D1/D2 sequences generated during this study along with sequences of related species retrieved from GenBank were aligned and a phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) using MEGA software version 5 (Tamura et al., 2011). Positions with gaps were excluded from the analysis. Bootstrap support (Felsenstein, 1985) for the tree was determined from 1000 replications.

 Parsimony network analysis was made from the aligned concatenated sequences for the ITS regions and the D1/D2 domains of the LSU rRNA gene, after the exclusion of gapped positions, with the TCS 1.21 program (Clement et al., 2000).

Phylogenetic placement and species delineation

The four strains isolated from rotten wood samples in Hungary (NCAIM Y.01936T, NCAIM Y.01991, NCAIM Y.01992T, NCAIM Y.01993) shared identical D1/D2 LSU rRNA gene sequences, and three of them (NCAIM Y.01936T, NCAIM Y.01992T, NCAIM Y.01993) also had identical ITS sequences. Strain NCAIM Y.01991 exhibited five substitutions and one indel in the ITS region (515 nt). While the identical D1/D2 sequences clearly suggest conspecificity, the almost 1.2% divergence in the ITS sequences is on the borderline of divergence observed among conspecific yeast strains. Although no single unifying yet stringent upper limit for intraspecific ITS variability can be provided throughout the fungi (Nilsson et al., 2008), there are some orientation points for yeasts. It has been proposed that conspecific
strains generally demonstrate 99% or more overall ITS sequence similarity (Sugita et al., 1999; Chen et al., 2001); however the possibility exists for exceptions to this benchmark (Chen et al., 2001). Conspecificity of strain NCAIM Y.01991 and the other three strains was further supported by the mating experiments. All strains formed ascospores on PDA agar at 25 °C in 3 weeks if mixed with the proper mating partner, but strain NCAIM Y.01991 was reluctant to sporulate when mixed with strain NCAIM Y.01936; very few asci and even fewer ascospores were observed after 5–8 weeks incubation. From the evidence presented, we suggest that the four strains, including the somewhat divergent NCAIM Y.01991, are conspecific although speciation may be in progress. This hypothesis is also supported by the comparisons of mtSSU rRNA gene sequences (see below). The four novel strains are placed in the well-supported Spencermartinsiella clade (bootstrap 98%) based on analysis of D1/D2 sequences (Fig. 1). They are well-separated from S. europaea and C. cellulosicola, the two recognized species in the Spencermartinsiella clade, as they exhibit more than 2% and more than 15% sequence divergences in the D1/D2 and ITS regions, respectively, compared to these recognized species. In addition, no ascospore formation was detected in the pairwise mixtures of the four strains of the novel species and the type and isotype strains of S. europaea.

The estimation of relatedness of two undescribed yeast strains isolated from soil in Taiwan (GY44S02 and GA1S04) to S. ligniputridi sp. nov. was problematic. They differed only by 3 nt (two substitutions and one indel) from the strains of S. ligniputridi sp. nov., which is usually considered to be the borderline of divergence among conspecific strains, and according to the prediction of Kurtzman & Robnett (1998) they are either conspecific or sister species. The parsimony network analysis (with 95% connection limit) based on the concatenated ITS regions and the D1/D2 LSU rRNA gene sequences placed all six strains in a single network (data not shown), and no clear-cut physiological differences were found among the strains. To further investigate their relatedness, the ITS and the partial mtSSU rRNA gene sequences were also compared, and significantly higher differences were found among GY44S02, GA1S04 and strains of S. ligniputridi sp. nov. than among the divergent strain NCAIM Y.01991 and the other strains of S. ligniputridi sp. nov. (Table 2). The overall ITS sequence divergence among strains GY44S02, GA1S04 and NCAIM Y.01992 exceeded 3%, and the rate of substitutions was also above 1.5%. In addition, no ascospore formation was observed in pairwise mixtures of strains GY44S02 or GA1S04 and the four strains of S. ligniputridi sp. nov. Considering all the above described data, we conclude that strains NCAIM Y.01936, NCAIM Y.01991, NCAIM Y.01992 and NCAIM Y.01993 represent a single undescribed yeast species, while GY44S02 and GA1S04 belong to (most likely two) very closely related but distinct species, despite the fact that parsimony network analysis failed to separate them. The parsimony network analysis of the concatenated ITS and the D1/D2 sequences advocated by Lachance et al. (2010, 2011a) for detecting interspecific discontinuities among yeast species was demonstrated to be a useful tool in the delineation of the haplontic, heterothallic yeast species Metschnikowia agaves and Starmernessella bombicola (Lachance et al., 2011a). However, according to our experiences in cases of some closely related species, like members of the genus Debaryomyces, it may fail to resolve them (G. Péter & D. Dlauchy, unpublished results).

**Occurrence and identification**

The four strains of the novel species of the genus Spencermartinsiella were recovered from independent rotten wood samples collected in Hungary. S. europaea and C. cellulosicola were also isolated from similar substrates (Péter et al., 2011; Guo et al., 2012), like numerous other strains of the neighbouring genera Blastobotrys, Sugiyamaella and related species of the genus Candida (Smith et al., 2011b; Kurtzman, 2011; Lachance et al., 2011b). S. ligniputridi sp. nov., S. europaea, C. cellulosicola and many other hyphal species of the Trichomonascaceae family are able to grow on cellulose and xylene (Smith et al., 2011a, b; Kurtzman, 2011; Lachance et al., 2011b), the building blocks of cellulose,

![Fig. 1. Phylogenetic tree showing the placement of Spencermartinsiella ligniputridi sp. nov. and some related species based on analysis of the D1/D2 domain of the large subunit rRNA gene. Sequences not generated during this study were obtained from GenBank. The tree was constructed by neighbour-joining analysis of aligned sequences. Wickerhamiella domercqiae was used as an outgroup. Numbers indicate bootstrap values of ≥ 50% based on 1000 replications. Bar, 2 bases substitution per 100 nt.](http://ijs.sgmjournals.org)
Table 2. Sequence divergences of strains NCAIM Y.01991, GY44S02 and GA1S04 compared to strains NCAIM Y.01936, NCAIM Y.01992, NCAIM Y.01993, which share identical sequences in all investigated regions (identical nucleotides/all nucleotides/indels)

<table>
<thead>
<tr>
<th>Strain</th>
<th>D1/D2</th>
<th>ITS</th>
<th>mtSSU tRNA gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCAIM Y.01991</td>
<td>574/574</td>
<td>510/516/1</td>
<td>360/365/2</td>
</tr>
<tr>
<td>GY44S02</td>
<td>571/574/1</td>
<td>501/518/9</td>
<td>353/365/6</td>
</tr>
<tr>
<td>GA1S04</td>
<td>571/574/1</td>
<td>496/516/11</td>
<td>349/363/0</td>
</tr>
</tbody>
</table>

and hemicellulose, two major constituents of wood. It is probable that these yeasts are beneficiaries of the degradation of wood and are vectored by insects. Many strains of the hyphal species of the Trichomonacidadae family were isolated from insects or from their frass (Smith et al., 2011a, b; Kurtzman, 2011; Lachance et al., 2011b). The strains GY44S02 and GA1S04, closely related to S. ligniputridi sp. nov. were isolated from soil, a habitat which often has direct physical interface with rotten wood.

S. ligniputridi sp. nov. can be distinguished from its phylogenetically closest recognized species S. europaea and C. cellulosica by its (weak) fermenting ability, its ability to grow on starch, and its inability to grow on L-rhamnose; from S. europaea by its inability to utilize D-arabinose; and from C. cellulosica by its ability to utilize L-arabinose, methyl D-glucoside and its inability to assimilate inulin. However, phenotypic identification is impractical, because there are many species in the genera Trichomonascus, Blastobotrys, Sugiyamaella and among related Candida spp., which have similar assimilation spectra and morphological characteristics. Furthermore, as a result of recovering related novel species keys may be rendered inaccurate (Kurtzman & Robnett, 2007), and revealing physiologically divergent strains of recognized species often has the same consequence. An example of the possible difficulties with phenotype-based identification is found in the novel strains with inositol assimilation, which has often been considered a reliable character in identification of yeast species; three strains of the novel species were unable to grow with inositol as the sole carbon source, while the fourth one (NCAIM Y.01993) was variable (usually positive) for this test.

Description of Spencermartinsiella ligniputridi
Péter & Dlauchy sp. nov.

Spencermartinsiella ligniputridi (lig.ni,p'u'tri.di. L. n. lignum wood; L. adj. putridus -a -um rotten, decayed; N.L. gen. n. ligniputridi of/from decayed wood, referring to the isolation substrate of the strains).

On 5% malt extract agar after 3 days at 25 °C, the streak culture is butyrous, smooth or folded or mycelial, moderately raised, tannish-white, semi-glossy or dull. The edge is entire and fringed with filaments. Cells are spheroid, ovoid, drop-shaped or elongated; 2–6 × 3–9 μm in size. Some cells form by multilateral budding and occur singly or in pairs, but the majority are formed on hyphae, some on small denticles. In 5% malt extract after 3 days at 25 °C, a ring is present. On slide culture with cornmeal agar after 7 days at 25 °C, abundant true hyphae and simple pseudohyphae are present. True hyphae give rise to mostly solitary blastoconidia (Fig. 2), some of them on small denticles. The species is heterothallic. Individual strains do not form ascospores after 3 weeks incubation at 25 °C on PDA. When compatible mating types are mixed, ascosporulation is observed on PDA at 25 °C within 3 weeks. Ascii (3–5 × 4–6 μm) are persistent; ellipsoid, ovoid or subspherical; and are formed on hyphae. They usually bear an ellipsoid to cylindrical apical cell, and contain one hemispherical or helmet-shaped ascospore, which from an upper view appears spheroid or ellipsoid (Fig. 3). Ferments D-glucose (slow or weak and slow), but not D-galactose, sucrose, maltose, lactose, raffinose or α,β-trehalose. Assimilates the following carbon compounds: D-glucose, sucrose, raffinose, melibiose, D-galactose, lactose (slow and variable), α,β-trehalose, maltose, melezitose, methyl α-D-glucoside, starch, cellobiose, salicin, arbutin, L-sorbose, D-xylose, L-arabinose, D-ribose (variable), ethanol (weak, slow and variable), glycerol (positive or slow), meso-erythritol, ribitol (slow or latent), xylitol (positive or slow), D-mannitol (slow and variable), D-glucitol (positive or slow), myo-inositol (variable), L-arabinobitol, DL-lactate (slow and variable), succinate (slow), citrate (positive or slow), 2-keto-D-gluconate (positive or slow, or weak and slow), glucono-δ-lactone (positive or slow), D-gluconurate, D-galacturonate, D-glucosamine, N-acetyl-D-glucosamine, and hexadecane (slow or latent). No growth occurs on inulin, L-rhamnose,

Fig. 2. Septate hyphae and blastoconidia of Spencermartinsiella ligniputridi sp. nov. NCAIM Y.01992 grown on cornmeal agar for 7 days at 25 °C. Bar, 10 μm.
D-arabinose, methanol, galactitol, D-glucuronate, saccharate, propane-1,2-diol or butane-2,3-diol. Assimilates ethylamine hydrochloride (variable), l-lysine, cadaverine dihydrochloride and glucosamine as nitrogen sources, but not potassium nitrate, sodium nitrite, creatine, creatinine or imidazole. Amyloid material is not formed. Growth occurs at 30 °C but not at 37 °C. Does not grow in vitamin-free medium or on 50 % (w/w) glucose yeast extract agar, with 10 % NaCl, or with 1 % acetic acid. Growth with 0.1 % cycloheximide is 50 % (w/w) glucose yeast extract agar, with 10 % NaCl, or

The type and isotype strains were recovered, respectively, is detected on chalk agar. No colour reaction with Diazonium Blue B. No acid production with 1 % acetic acid. Growth with 0.1 % cycloheximide is 50 % (w/w) glucose yeast extract agar, with 10 % NaCl, or


Fig. 3. Ascosporulating culture of Spencermartinsiella ligniputridi sp. nov. NCAIM Y.01992T NCAIM Y.011936I grown on potato-dextrose agar for 21 days at 25 °C. Bar, 10 μm.

References


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