Candida gosingica sp. nov., an anamorphic ascomycetous yeast closely related to Scheffersomyces spartinae

Chin-Feng Chang,1 Cheng-Hsu Yao,1 Shuh-Sen Young,1 Savitree Limtong,2 Rungluk Kaewwichian,2 Nantana Srisuk2 and Ching-Fu Lee1

1Department of Applied Science, National Hsinchu University of Education, 521 Nanda Rd, Hsinchu 30014, Taiwan
2Department of Microbiology, Kasetsart University, Bangkok 10900, Thailand

During surveys on yeast diversity in forest soils from Taiwan and Thailand, ten yeast strains isolated from different samples were found to have similar molecular and physiological characteristics. Sequence analysis of small subunit (SSU) rDNA, the D1/D2 domain of large subunit (LSU) rDNA and internal transcribed spacer (ITS)-5.8S rDNA demonstrated that these strains were closely related to Scheffersomyces spartinae. The novel strains could be differentiated from S. spartinae by a 0.9 % sequence divergence (5 substitutions, 0 gaps) in the D1/D2 domain of LSU rDNA, a 1.5 % divergence (8 substitutions, 0 gaps) in the ITS-5.8S rDNA and a 0.7 % divergence (12 substitutions, 2 gaps) in the SSU rDNA. The novel strains also showed specific patterns of electrophoretic karyotypes that differed from that of S. spartinae. Therefore, a novel yeast species, Candida gosingica sp. nov., is proposed to accommodate these strains. The type strain SJ7S11T (=BCRC 23194T= CBS 11433T) was assigned and deposited in the Bioresource Collection and Research Center (BCRC), Food Industry Development and Research Institute, Hsinchu, Taiwan, and Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

Pichia spartinae, proposed by Ahearn et al. (1970), is characterized by the production of coenzyme Q-9 and the presence of hat-shaped ascospores. The species has since been transferred to a new genus, Yamadazyma, established by Billon-Grand (1989) and based on the production of coenzyme Q-9. Kurtzman & Robnett (1998) pointed out that the three Q-9-producing species assigned to the genus Yamadazyma by Billon-Grand, Pichia acaciae, Pichia guillermondii and P. spartinae, were clustered among several clades in the phylogenetic tree, and found that they were quite closely related to members of the genus Debaryomyces. Because of the uncertain circumscription of the genera Yamadazyma and Pichia and their unresolved relationships with other genera that produce coenzyme Q-9, Kurtzman & Suzuki (2010) resolved the species of these genera into 12 well-supported clades based on the sequences of the D1/D2 domain of the large subunit (LSU) and small subunit (SSU) rDNAs. According to this analysis, 12 genera were proposed, one for each clade. Pichia spartinae was transferred to the genus Scheffersomyces (Kurtzman & Suzuki, 2010). In this report, a novel anamorphic yeast closely related to Scheffersomyces spartinae is described.

During an investigation of the yeast community associated with soil in Taiwan, seven strains were isolated from forest soil samples in January, February, April, August, and October of 2006. The novel strains were found to possess similar morphological, physiological and molecular characteristics and were closely related to S. spartinae. Additional strains isolated from Thailand were confirmed to be conspecific to those from Taiwan. These strains, which form the basis for the proposal of a new species in this study, are listed in Table 1. Strain isolation was performed by the method described by Lee et al. (2008). Yeasts were grown on YM agar at 24 °C for 3 days, followed by preservation at −70 °C and/or on YM agar at 4 °C. The additional strains from Thailand (Table 1) were isolated according to the methods described by Limtong et al.
(2009). Isolated strains were morphologically, physiologically and biochemically characterized using standard methods for current yeast taxonomy (Yarrow, 1998). The strains used for the examination of ascospore production were incubated on McClary’s acetate agar, cornmeal agar, malt extract agar and V8 agar (Yarrow, 1998), individually or as pairwise mixtures on the sporulation medium. The cultures were observed for ascospore production every week for a month at 18 °C.

Yeast DNA was extracted using a Biokit Genome DNA Extraction kit according to the manufacturer’s protocol (Biokit Co.). The D1/D2 LSU rDNA, SSU rDNA and ITS regions were amplified by PCR and sequenced using the primer pairs NL1 and NL4 (Kurtzman & Robnett, 1998), P1 and NS8 (Suzuki & Nakase, 2002) and ITS1 and ITS4 (White et al., 1990), respectively. The resulting sequences were compared with those of reference organisms that were retrieved from the GenBank database. The sequences for phylogenetic analysis were aligned automatically by the CLUSTAL_X 1.83 multiple sequence alignment program (Kumar et al., 2004), and evolutionary distances were calculated using the neighbour-joining method with the Kimura two-parameter distance measure. Confidence values were estimated from bootstrap analyses of 1000 replicates (Felsenstein, 1985). Wickerhamia fluorescens NRRL YB-4819T was the designated outgroup in the analyses; other related sequences were obtained from GenBank. The full list of the GenBank/EMBL/DDBJ accession numbers for the LSU rDNA, SSU rDNA and ITS-5.8S sequences reported in this paper is given in Supplementary Table S1 (see IJSEM Online).

To examine the electrophoretic karyotypes of the yeasts by PFGE, agarose plugs containing intact chromosomal DNA were prepared according to the method described by Carle & Olson (1985) and modified by Lee et al. (2009a, b). Agarose blocks were cut to fit the wells of a 1 % agarose gel (Amresco) and the plug pieces were inserted and sealed in place with 1 % agarose. Gels were equilibrated in 0.5 × TBE (45 mM Tris/borate, 1 mM EDTA, pH 8.0) for 1 h before electrophoresis.

Chromosomal DNA bands were separated on 1 % agarose gel in 0.5 × TBE buffer in a rotating-field electrophoresis machine (Rotaphor 6.0; Biometra). The electrophoresis program was completed in two steps, with a total running time of 94 h: Step 1, field switch time of 120–360 s for 24 h at 120 V; Step 2, 360–1200 s for 70 h at 70 V. The temperature of the running buffer was maintained at 13 °C throughout electrophoresis. After electrophoresis, the gels were stained with 0.5 μg ethidium bromide ml⁻¹ for 30 min, destained with tap water for 30 min and then photographed under UV illumination. Intact chromosomal DNA of Saccharomyces cerevisiae YPH 80 purchased from Biometra was used as a DNA marker.

**Sequence comparison**

All the strains belonging to the proposed novel species were found to share identical ITS and SSU rDNA sequences. Strain SF5S11 differed by only two substitutions (2 gaps) in the D1/D2 LSU rDNA sequence from the other strains, indicating that these strains are conspecific. Phylogenetic analysis based on the sequences of D1/D2 LSU rDNA showed that S. spartinae was the most closely related species (Fig. 1) to the novel strains, which differed from S. spartinae by a 0.9 % sequence divergence (5 substitutions, 0 gaps), which is at the high end of the divergence range usually observed for members of the same species (Kurtzman & Robnett, 1998). The ITS-5.8S and SSU rDNA sequences of the novel strains showed a 1.5 % divergence (8 substitutions, 0 gaps) and a 0.7 % divergence (12 substitutions, 2 gaps), respectively, from those of S. spartinae NRRL Y-7322T. These results clearly indicated that the new strains were representatives of a novel species closely related to S. spartinae.

**Electrophoretic karyotypes**

In order to further elucidate the taxonomic relationships of the novel species with S. spartinae, the electrophoretic karyotype of each novel strain was determined and compared with that of S. spartinae NRRL Y-7322T. The strains representing the novel species were found to be quite different to S. spartinae NRRL Y-7322T in terms of electrophoretic karyotypes, with 6–8 chromosomal bands ranging from 745–3000 kb (Fig. 2). Strains SC10S07, SG4S01 and SA8S03 exhibited polymorphisms in chromosomal DNA size and band number (Fig. 2, lanes 10, 11 and 12) when compared with the other novel strains. It is possible that strains in the same species with different PFGE patterns could be differentiated between strains at the species level (Querol et al., 1992; Stoltenburg et al., 1992). The electrophoretic karyotypes of these strains were very similar or identical to each other but were distinctly different from those of their closest relative S. spartinae.

**Table 1. Yeast strains examined in this study**

<table>
<thead>
<tr>
<th>Strains</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. spartinae NRRL Y-7322T</td>
<td>Spartina alterniflora, Louisiana, USA</td>
</tr>
<tr>
<td>Candida gosingica sp. nov.</td>
<td></td>
</tr>
<tr>
<td>SJ7S11T</td>
<td>Soil, Nanto, Taiwan</td>
</tr>
<tr>
<td>SF5S11</td>
<td>Soil, Hualien, Taiwan</td>
</tr>
<tr>
<td>SC2S03</td>
<td>Soil, Taoyuan, Taiwan</td>
</tr>
<tr>
<td>SC10S07</td>
<td>Soil, Taoyuan, Taiwan</td>
</tr>
<tr>
<td>SG2S06</td>
<td>Soil, Nanto, Taiwan</td>
</tr>
<tr>
<td>SG4S01</td>
<td>Soil, Nanto, Taiwan</td>
</tr>
<tr>
<td>SA8S03</td>
<td>Soil, Miaoli, Taiwan</td>
</tr>
<tr>
<td>CP1</td>
<td>Soil, Thep Sathit, Chaiyaphum, Thailand</td>
</tr>
<tr>
<td>LY20</td>
<td>Soil, Phu Rueba, Leci, Thailand</td>
</tr>
<tr>
<td>LY24</td>
<td>Soil, Phu Rueba, Leci, Thailand</td>
</tr>
</tbody>
</table>

Downloaded from www.microbiologyresearch.org by IP: 54.70.40.11 On: Mon, 19 Nov 2018 22:39:41
NRRL Y-7322<sup>T</sup> (Fig. 2). The results described above indicate that these strains represent a separate species closely related to <i>S. spartinae</i>.

**Taxonomy**

None of the novel strains examined in this study produced ascospores or exhibited conjugation in common sporulation medium either alone or in pairwise mixtures. In the absence of a sexual cycle, the molecular characteristics described above were used for species delineation of the proposed novel species. As evidenced by analysis of DNA sequences and electrophoretic karyotypes, these strains represent a new species closely related to <i>S. spartinae</i>. In addition, the novel strains all exhibit similar assimilation patterns. The strains of the novel species could be differentiated from <i>S. spartinae</i> by their inability to grow on citrate, N-acetyl-D-glucosamine or 10% NaCl/5% glucose at 37°C and by their ability to grow on glycerol, D-glucanate, and vitamin-free medium. Based on this evidence, a novel species, <i>Candida gosingica</i> sp. nov., is proposed to accommodate these strains with the type strain SJ7S11<sup>T</sup>.

**Latin diagnosis of Candida gosingica Lee sp. nov.**


**Typus SJ7S11<sup>T</sup> (=BCRC 23194<sup>T</sup>=CBS 11433<sup>T</sup>) isolatus ex tere in Nanto, Taiwan. Conservaturn in Bioresource Collection and Research Center (BCRC), Food Industry Development and Research Institute, Hsinchu, Taiwan; et Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.**

![Fig. 2. Electrophoresis karyotypes of strains of Candida gosingica sp. nov. and related type strains. Lanes: 1 and 13, marker (Saccharomyces cerevisiae YPH80); 2, Scheffersomyces stiptis NRRL Y-7322<sup>T</sup>; 3, Candida gosingica sp. nov. SJ7S11<sup>T</sup>; 4, SF5S11; 5, SC25S03; 6, SG2S06; 7, CP1; 8, LY20; 9, LY24; 10, SC10S07; 11, SG4S01; 12, SA8S03.](image-url)
**Description of Candida gosingica** Lee sp. nov.

*Candida gosingica* (go.sin.gi’ca. N.L. fem. adj. gosingica of or belonging to Gosing, Nanto, Taiwan, where the yeast was originally isolated).

After growth on YM agar for 3 days at 25 °C, cells are subglobose to ellipsoidal, 3.1–5.5 × 3.8–6.7 μm long, and occur singly or in pairs (Fig. 3a). Asexual reproduction is by multilateral budding. Streak culture is smooth, glistening and white in colour, with an entire margin. After 1 month of incubation in YM broth at 25 °C, sediment is observed. Pseudomycelia are formed in Dalmau plate cultures on cornmeal agar after 2 weeks incubation (Fig. 3b). Glucose, maltose, methyl α-D-glucoside, sucrose, cellobiose and melezitose are fermented, but galactose, trehalose, melibiose, lactose, raffinose, inulin, starch and xylose are not fermented. D-Glucose, L-sorbitose, sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose, salicin, arbutin, melezitose, ribitol, xylitol, D-glucitol, D-mannitol, D-glucono-1,5-lactone (weak), 2-keto-D-gluconate, DL-lactate, succinate, ethanol, propane-1,2-diol and butane-2,3-diol are assimilated, but D-galactose, D-glucomamine, D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, melibiose, lactose, raffinose, inulin, soluble starch, glycerol, erythritol, L-arabinitol, galactitol, myo-inositol, 5-keto-D-gluconate, D-gluconate, D-gluconuronate, D-galacturonic acid, citrate, methanol and N-acetyl-D-glucosamine are not assimilated. Ethylamine, L-lysine and cadaverine are utilized, while potassium nitrate, sodium nitrate and creatine are not utilized. Growth occurs on YM agar at 25 °C, 30 °C and 35 °C, but not at 37 °C. Growth in the presence of 0.01 % cycloheximide is negative, as is growth on 1 % acetic acid, 50 % glucose and 10 % sodium chloride plus 5 % glucose agar. Growth in vitamin-free medium is positive. Production of starch-like compounds is absent. Acetic acid production on chalk agar is negative. Diazonium blue B reaction and urease activity are negative.

The type strain, SJ7S11T (=BCRC 23194T=CBS 114833T), was isolated from soil in Nanto, Taiwan and deposited in the Bioreource Collection and Research Center (BCRC), Food Industry Development and Research Institute, Hsinchu, Taiwan, and Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands.

**Acknowledgements**

This work was supported by the National Science Council, Executive Yuan, Taiwan, by means of a Grant-in-Aid (NSC 97-2621-B-134-001-MY3).

**References**


