Kazachstania siamensis sp. nov., an ascomycetous yeast species from forest soil in Thailand

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Two strains (S-34T and S-35) of a novel ascomycetous yeast species belonging to the genus Kazachstania were isolated from soil from a mixed deciduous forest in Amphoe Wang Nam Khiao, Nakhon Ratchasima Province, Thailand. The D1/D2 domains of the large-subunit rDNA sequences of the two strains were identical and also indicated a close relationship with respect to Kazachstania aquatica, Kazachstania unispora, Kazachstania aerobia, Kazachstania servazzii and Kazachstania solicola. The most closely related species, K. aquatica, has 14 nucleotide substitutions and three gaps in 566 nt. The phenotypic characteristics of the two strains were typical of those of members of the genus Kazachstania. These characteristics include the formation of a single globose ascospore in an unconjugated and persistent ascus, multilateral budding, the typical of those of members of the genus Kazachstania. The novel species is closely related to Kazachstania aquatica, Kazachstania aerobia, Kazachstania solicola, Kazachstania servazzii and Kazachstania unispora; the first three of these were described only recently (Lu et al., 2004; Wu & Bai, 2005) and the last two were transferred from the genus Saccharomyces (Kurtzman, 2003; Kurtzman & Robnett, 2003). K. aquatica and K. solicola were identified as novel species by sequence analysis of the SSU rDNA, the internal transcribed spacer region (including 5.8S rDNA) and the large-subunit (LSU) D1/D2 domain (Wu & Bai, 2005), whereas K. aerobia was circumscribed on the basis of the internal transcribed spacer and D1/D2 sequences and electrophoretic karyotypes (Lu et al., 2004). K. servazzii and K. unispora were transferred from the genus Saccharomyces on the basis of a multigene sequence analysis (Kurtzman, 2003; Kurtzman & Robnett, 2003).

Strains S-34T and S-35 were isolated from two soil samples collected from a mixed deciduous forest in Amphoe Wang Nam Khiao, Nakhon Ratchasima Province, Thailand, by using an enrichment technique. Each soil sample (5 g) was added to 50 ml acidified yeast extract/malt extract (YM) broth (0.3 % yeast extract, 0.3 % malt extract, 0.5 % peptone, 1 % glucose; adjusted to pH 3.7–3.8 with 1 M

The genus Kazachstania, which is a member of the family Saccharomycetaceae, was first described on the basis of the single species, Kazachstania viticola (Zubkova, 1971). In 2003, several species previously assigned to Saccharomyces and Kluyveromyces were transferred to Kazachstania on the basis of a multigene sequence analysis that included genes of the rDNA repeat (18S, 26S, internal transcribed spacer), single-copy nuclear genes (translation elongation factor 1a, actin-1, RNA polymerase II) and mitochondrially encoded genes [small-subunit (SSU) rDNA, cytochrome oxidase II] (Kurtzman, 2003; Kurtzman & Robnett, 2003). Recently, three non-pathogenic species (Lu et al., 2004; Wu & Bai, 2005) and four pathogenic species (Kurtzman et al., 2005) of the genus Kazachstania were described. During an investigation of yeasts in soil, two novel strains, S-34T and S-35, were isolated and found to represent a novel species of the genus Kazachstania. The novel species is closely related to Kazachstania aquatica, Kazachstania aerobia, Kazachstania solicola, Kazachstania servazzii and Kazachstania unispora; the first three of these were described only recently (Lu et al., 2004; Wu & Bai, 2005) and the last two were transferred from the genus Saccharomyces (Kurtzman, 2003; Kurtzman & Robnett, 2003). K. aquatica and K. solicola were identified as novel species by sequence analysis of the SSU rDNA, the internal transcribed spacer region (including 5.8S rDNA) and the large-subunit (LSU) D1/D2 domain (Wu & Bai, 2005), whereas K. aerobia was circumscribed on the basis of the internal transcribed spacer and D1/D2 sequences and electrophoretic karyotypes (Lu et al., 2004). K. servazzii and K. unispora were transferred from the genus Saccharomyces on the basis of a multigene sequence analysis (Kurtzman, 2003; Kurtzman & Robnett, 2003).

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Abbreviations: LSU, large subunit; SSU, small subunit.

The GenBank/EMBL/DBJ accession numbers for the D1/D2 domains of the LSU rDNA sequences of strains S-34T and S-35 are AB258462 and AB258463, respectively.

A neighbour-joining phylogenetic tree based on SSU rDNA sequences is available as a supplementary figure in IJSEM Online.
The sequences of the D1/D2 domain of the LSU rDNA and the SSU rDNA were determined from PCR products from genomic DNA extracted from yeast cells by using a slightly modified version of the method described by Lachance et al. (1999). The D1/D2 domain of the LSU rDNA was amplified by a PCR with the forward primer NL-1 and the reverse primer NL-4 (O'Donnell, 1993); amplification of the SSU rDNA was done with the forward primer P1 and the reverse primer P2 (Sjamsuridzal et al., 1997). The PCR product was checked by agarose gel electrophoresis, purified using the QiAquick purification kit (Qiagen) and cycle-sequenced using the ABI BigDye terminator cycle sequencing kit, version 3.1 (Applied Biosystems), with the external primers NL-1 and NL-4 for the D1/D2 domain (Kurtzman & Robnett, 1998) and eight primers, P1–P8, for the SSU rDNA (Yamada et al., 1999). The sequences were determined with an ABI PRISM 3100 automated DNA sequencer (Applied Biosystems) according to the instructions of the manufacturer. The sequences were compared pairwise by the BLASTN homology search program (Altschul et al., 1990) and were aligned with the sequences of related species retrieved from GenBank using the multiple alignment program CLUSTAL X, version 1.81 (Thompson et al., 1997). A phylogenetic tree was constructed from the evolutionary distance data according to the two-parameter method of Kimura (1980) and the neighbour-joining method (Saitou & Nei, 1987). The robustness of the phylogenetic trees was estimated using bootstrap analysis (1000 replicates) (Felsenstein, 1985).

The strains were characterized morphologically, biochemically and physiologically according to the standard methods described by Yarrow (1998). Assimilation of nitrogen compounds was examined on solid media with starved inocula, using the method of Nakase & Suzuki (1986). Growth at various temperatures was determined by cultivation on yeast extract/peptone/dextrose (YPD) agar (1 % yeast extract, 2 % peptone, 2 % glucose and 2 % agar). Ubiquinones were extracted from intact packed cells cultivated in YPD broth on a rotary shaker at 28 C for 24–48 h and then purified according to the method described by Yamada & Kondo (1973). The isoprenologues were identified by HPLC using a Cosmosil (5C18; Waters) 4.6 × 250 mm column and methanol/2-propanol (2:1) at 1 ml min⁻¹ as the elution system, with spectrophotometric detection (275 nm wavelength), according to the method of Kuraishi et al. (1985).

The sequences of the D1/D2 domains of the LSU rDNAs of strain S-34T and S-35 were identical. The two strains clustered with *K. aquatica*, *K. unispora*, *K. aerobia*, *K. servazzii* and *K. solicola* with high bootstrap values and were separate from *Saccharomyces* species (Fig. 1) and as well as from the pathogenic species of the *Kazachstania telluris* complex (*Kazachstania bovina*, *Kazachstania heterogenica*, *Kazachstania pintolopese* and *Kazachstania sloffiae*) recently described by Kurtzman et al. (2005) (data not shown). Strain S-34T and S-35 showed 3 % divergence (14 nucleotide substitutions and three gaps out of 566 nt) from *K. aquatica*, the closest species in terms of pairwise sequence similarity, indicating that the two strains could represent a novel species. According to Kurtzman & Robnett (1998), yeast strains that show nucleotide substitution greater than 1 % in the D1/D2 domain of the LSU rDNA usually represent different species. However, to confirm the novelty of the two strains, their SSU rDNA sequences were determined: they were found to be identical. The closest relative of the two strains in terms of pairwise sequence similarity was *K. aquatica*, which differed by nine nucleotide substitutions and three gaps out of 1781 nt. In the phylogenetic analysis based on the SSU rDNA sequences, the two novel strains clustered with *K. aquatica*, *K. unispora*, *K. aerobia*, *K. servazzii* and *K. solicola* (see Supplementary Fig. S1 available in IJSEM Online), which is similar to what is seen in the tree based on the D1/D2 LSU rDNA sequences. These results lend further support to the conclusion that the two strains represent a novel species closely related to *K. aquatica*.

Cells of strains S-34T and S-35 formed single globose ascospores in unconjugated and persistent asci (Fig. 2), proliferated by multilateral budding (Fig. 2), lacked arthrospores and ballistospores, fermented glucose but did not assimilate nitrate, gave negative reactions for the diazonium blue B and urease tests and contained Q-6 as the predominant ubiquinone (Table 1). The production of arthrospores was observed in strain S-34T, but not in strain S-35. The two strains showed a similar pattern of assimilation of carbon sources (Table 1). The pair of species *K. unispora* and *K. aquatica* is the closest species in terms of pairwise sequence similarity (14 nucleotide substitutions and three gaps out of 566 nt). Strain S-34T and S-35 showed 3 % divergence from *K. aquatica*, the closest species in terms of pairwise sequence similarity, indicating that the two strains could represent a novel species. According to Kurtzman & Robnett (1998), yeast strains that show nucleotide substitution greater than 1 % in the D1/D2 domain of the LSU rDNA usually represent different species. However, to confirm the novelty of the two strains, their SSU rDNA sequences were determined: they were found to be identical. The closest relative of the two strains in terms of pairwise sequence similarity was *K. aquatica*, which differed by nine nucleotide substitutions and three gaps out of 1781 nt. In the phylogenetic analysis based on the SSU rDNA sequences, the two novel strains clustered with *K. aquatica*, *K. unispora*, *K. aerobia*, *K. servazzii* and *K. solicola* (see Supplementary Fig. S1 available in IJSEM Online), which is similar to what is seen in the tree based on the D1/D2 LSU rDNA sequences. These results lend further support to the conclusion that the two strains represent a novel species closely related to *K. aquatica*.

![Fig. 1. Phylogenetic tree, based on the D1/D2 LSU rDNA, showing the placement of strains S-34T and S-35 with respect to closely related species. The tree was constructed with the neighbour-joining method, based on approximately 600 nt, using Kimura's two-parameter distance correction. Numbers at branch points are bootstrap percentages derived from 1000 pseudoreplicates.](image-url)
galactosum et trehalosum assimilatur at non L-
sum farinae Zea mays post dies butyrosa germinationem multipolarem reproducentes. Cultura cremea In agaro aceti et agaro YM sp. nov. Limtong, Yongmanitchai, Tun, Kawasaki

Latin diagnosis of Kazachstania siamensis

Limtong, Yongmanitchai, Tun, Kawasaki et Seki sp. nov.

In agaro YM post dies 3 ad 28 °C cellulae ovoideae aut ellipsoideae, (2.4–4.8 × 2.9–8.1 μm), singulae, aut binae, per germinationem multipolarens reproducentes. Cultura cremea, butyrosa, initita, glabra, nitida et margine undulata. In agaro farinae Zea mays post dies 21 ad 28 °C et agaro YM post dies 21 ad 25 °C pseudomycelium nec mycelium non formantur. In agaro aceti et agar Gorodkowa post dies 7 ad 28 °C asci formantur. Asci inconjugatio fiunt. Ascospores globosae, 1 in quoque asco. In medio liquido YM post unam mensem ad 28 °C, sedimentum formantur et pellicula non formantur. Glucosum et galactosum (infrim) fermentatur at non maltosum, sucrosum, trehalosum, lactosum nec raffinosum. Glucosum, galactosum et trehalosum assimilantur at non L-sorbosum, D-ribosum, D-xyllosum, L-arabinosum, D-arabinosum, L-rhamnosum, sucrosum, maltosum, cellobiosum, salicinum, melezitosum, inulinum, amyllum solubile, glucosum, erythritolum, ribitol, glucitol, D-mannitol, galactitolum, myo-inositol, DL-lactate, succinic acid, citric acid, methanol, potassium nitrate and sodium nitrite were not assimilated by either taxon. For both taxa, growth did not occur in vitamin-free medium, growth occurred at 25, 30, 35 and 37 °C and growth did not occur at 42 °C. For both taxa, the results for diazonium blue B colour and urease reactions were negative. +, Positive; −, negative; D, delayed positive; ND, no data available; W, weak.

Table 1. Growth characteristics of the novel strains and K. aquatica

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain S-34T</th>
<th>K. aquatica</th>
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</thead>
<tbody>
<tr>
<td>Fermentation of D-galactose</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>W, D</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>W, D</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>W, D</td>
</tr>
<tr>
<td>2-Ketogluconic acid</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>D-Gluconic acid</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>D-Glucuronic acid</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>D-Galacturonic acid</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>Ethanol</td>
<td>−</td>
<td>W, D</td>
</tr>
<tr>
<td>Ethylamine hydrochloride</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>l-Lysine hydrochloride</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Growth at/with:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 °C</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>0.01 % Cycloheximide</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>0.1 % Cycloheximide</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>50 % Glucose</td>
<td>W</td>
<td>ND</td>
</tr>
<tr>
<td>60 % Glucose</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>10 % NaCl + 5 % glucose</td>
<td>−</td>
<td>ND</td>
</tr>
</tbody>
</table>

K. siamensis can be distinguished from K. aquatica, the species closest to it in the phylogenetic tree, on the basis of a number of phenotypic characteristics, as shown in Table 1.

Results for strain S-35 were identical to those given for strain S-34T. Data for K. aquatica were taken from Wu & Bai (2005). Both taxa were positive for the fermentation of D-glucose but negative for the fermentation of D-maltose, sucrose, lactose and raffinose. L-Sorbose, D-ribose, D-xyllose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, cellobiose, salicin, melibiose, lactose, raffinose, melezitose, inulin, soluble starch, glycerol, erythritol, ribitol, D-glucitol, D-mannitol, galactitol, myo-inositol, DL-lactate, succinic acid, citric acid, methanol, potassium nitrate and sodium nitrite were not assimilated by either taxon. For both taxa, growth did not occur in vitamin-free medium, growth occurred at 25, 30, 35 and 37 °C and growth did not occur at 42 °C. For both taxa, the results for diazonium blue B colour and urease reactions were negative. +, Positive; −, negative; D, delayed positive; ND, no data available; W, weak.

Typus stirpis S-34T (= CBS 10361T = NBRC 101968T = BCC 21230T) isolatus e terea, Nakhon Ratchasima Provincia,
Thailandia, conservatur in collectionibus culturarum quas Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands), NITE Biological Resource Center, National Institute of Technology and Evaluation (Chiba, Japan) et BIOTEC Culture Collection, National Center for Genetic Engineering and Biotechnology, Thailand (Pathumthani, Thailand) deposita est.

Description of Kazachstania siamensis Lim tong, Yongmanitchai, Tun, Kawasaki & Seki sp. nov.

Kazachstania siamensis (si.am.en’ sis. N.L. fem. adj. siamen-sis referring to Siam, the old name of Thailand, where the two strains were isolated).

After 3 days growth on YM agar at 28 °C, cells are ovoid to ellipsoid (2.4–4.8 × 2.9–8.1 μm) and occur singly or in pairs (Fig. 2). Budding is multilateral. Streak culture on YM agar after 3 days at 28 °C is butyrous, cream-coloured, glossy, smooth and raised with undulate margins. In Dalmau plate culture on cornmeal agar at 28 °C and YM agar at 25 °C after 3 weeks, pseudohyphae and true hyphae are not formed. Neither arthrospores nor ballistospores are produced. Ascospores form on Forrest’s acetate agar and Gorodkowa agar after 7 days at 28 °C; asci are unconjugated, persistent and each contain a single globose ascospore (Fig. 2). After 1 month in YM broth at 28 °C, sediment is present. A pellicle is not present during growth on the surface of assimilation medium. The major ubiquinone is Q-6. Phenotypic characteristics of the species are shown in Table 1.

The type strain, S-34T (CBS 10361T = NBRC 101968T = BCC 21230T), was isolated from soil collected in a mixed deciduous forest in Amphoe Wang Nam Khiao, Nakhon Ratchasima Province, Thailand.

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References


