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

Saviteer Limtong,1 Wichien Yongmanitchai,1 Moe Moe Tun,1,2 Hiroko Kawasaki3 and Tatsuji Seki3

1Department of Microbiology, Faculty of Science, Kasetsart University, 50 Paholyothin Rd, Bangkok 10900, Thailand
2Department of Botany, University of East Yangon, Thanayin Township, Myanmar
3The International Center for Biotechnology, Osaka University, 2-1 Yamada-oka, Suita-City, Osaka 565-0871, Japan

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 absence of arthrospores and ballistospores, the fermentation of glucose, the inability to assimilate nitrate, negative diazonium blue B and urease reactions, and the presence of ubiquinone Q-6. The novel strains can be distinguished from *K. aquatica* on the basis of a number of phenotypic characteristics and represent a novel species in the genus *Kazachstania*, for which the name *Kazachstania siamensis* sp. nov. is proposed. The type strain is S-34T (= CBS 10361T = NBRC 101968T = BCC 21230T).

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 *Kluveromyces* 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 1α, 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).

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

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

The GenBank/EMBL/DDJB 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.
HCl) supplemented with 0.025% sodium propionate and 200 mg chloramphenicol l−1 in a 250 ml Erlenmeyer flask and incubated at room temperature for 3–4 days on a rotary shaker. The enrichment culture was then spread out on YM agar supplemented with 0.025% sodium propionate and 200 mg chloramphenicol l−1. When necessary, colonies were restreaked onto YM agar.

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 (SC18; 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 pintolopessii and Kazachstania slooffiae) 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

![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)
the major ubiquinone. These characteristics fit well with those of species of the genus Kazachstania. The two strains also shared the same conventional taxonomic characteristics, as shown in Table 1. We conclude, therefore, that the two strains represent a single novel species of the genus Kazachstania, for which the name Kazachstania siamensis is proposed.

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.

**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 multipolarem reproducentes. Cultura cremea, butyrosa, initiata, glabra, nitida et margine undulato. 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 agaro Gorodkowa post dies 7 ad 28 °C asci formantur. Asci inconjugatio fiunt. Ascopora globosae, 1 in quoque asco. In medio liquido YM post unum mensem ad 28 °C, sedimentum formantur et pellicula non formantur. Glucosum et galactosum (infrimae) fermentatur at non malto- sum, sucrosum, trehalosum, lactosum nec raffinosum. Glucosum, galactosum et trehalosum assimilatur at non L-sorbosum, D-ribosem, D-xylosum, L-arabinosum, D-arabinosum, L-rhamnosum, sucrosum, maltosum, cellobiosum, salicinum, melezitosum, inulinum, amyllum solubile, glyceroolum, erythritolum, ribitol, D-glucozil, D-mannitolum, galactitol, myo-inositol, DL-lactate, succinicum acidum, citricum acidum, 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 diazonia 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>
</tr>
</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>

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-Sorbitosum, D-ribosem, D-xylosum, L-arabinosum, D-rhamnosum, 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 diazonia blue B colour and urease reactions were negative. +, Positive; −, negative; D, delayed positive; ND, no data available; W, weak.

**Tupus 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 Limtong, 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 Forwell’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.

Acknowledgements

We are grateful to ICBiotech, Osaka University, Japan, and the National Research Council, Thailand, for financial and technical support. We thank Dr Tawatchai Sumpradit, Dr Sasitorn Jindamorakot, Ms Chutima Sringiew, Ms Suthida Tuntigumton and Ms Somjit Am-in for help with some of the experiments.

References


