**Tetrapisispora namnaonensis** sp. nov., a novel ascomycetous yeast species isolated from forest soil of Nam Nao National Park, Thailand

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Twenty-one strains of a novel ascomycetous yeast species were isolated from soil collected in three kinds of natural forest, namely a dry dipterocarp forest, a mixed deciduous forest and a pine forest, in Nam Nao National Park, Phetchabun province, Thailand. The strains formed ascii containing one to four ovoid to reniform ascospores, assimilated glucose, galactose and glycerol, fermented glucose and galactose vigorously and contained ubiquinone Q-6, indicating that they belonged to the genus *Tetrapisispora*. A comparative analysis of the small subunit rDNA (SSU rDNA) and the D1/D2 domain of the large subunit rDNA (LSU rDNA) of all available sequences for ascomycetous yeasts confirmed that the strains were phylogenetically related to the genus *Tetrapisispora*. All strains had identical nucleotide sequences in the D1/D2 domain of the LSU rDNA and differed from the nearest species, *Tetrapisispora arboricola* IFO 10925T, by 6-4 % nucleotide substitutions. The strains differed from *Tetrapisispora arboricola* by the ability to assimilate D-gluconic acid, the inability to grow on 50 % glucose medium, the nuclear DNA base composition and deliquescent ascii. The strains were differentiated from the other four species of *Tetrapisispora* on the basis of trehalose assimilation, the ability to grow on 50 % glucose or 10 % NaCl plus 5 % glucose, vitamin requirement, the nuclear DNA base composition and the type of ascus. Based on the characteristics mentioned above, the strains are recognized as a single novel species of the genus *Tetrapisispora* and the name *Tetrapisispora namnaonensis* sp. nov. is proposed. The type strain is TN1-01T (= TISTR 5828T = JCM 12664T = CBS 10093T).

The ascomycetous yeast genus *Tetrapisispora* Ueda-Nishimura & Mikata 1999 was established on the basis of four species, *Tetrapisispora arboricola*, *Tetrapisispora iriomotensis*, *Tetrapisispora nanseiensis* and *Tetrapisispora phaffii*. *T. phaffii* was reclassified from *Kluyveromyces phaffii* van der Walt 1971 based on phylogenetic analysis of the small subunit (SSU) rDNA sequence. These yeasts had similar physiological and biochemical characteristics and nuclear DNA base composition, but, on the basis of DNA-DNA hybridization and karyotyping, they were assigned to four different species. Three came from soil collected in Japan (*T. iriomotensis* and *T. nanseiensis*) and South Africa (*T. phaffii*), and *T. arboricola* was recovered from a flower of *Elaeocarpus decipiens* and leaves of *Bruguiera conjugata* and *Rhizophora mucronata* in Japan (Ueda-Nishimura & Mikata, 1999). Recently, Kurtzman (2003) reclassified *Kluyveromyces blattae* to *Tetrapisispora blattae* on the basis of comparative sequence analysis of SSU, 5S/8S/alignable internal transcribed spacer (ITS) and large subunit (LSU) rDNAs, EF-1α, mitochondrial SSU rDNA and COXII. While investigating the yeast diversity of forest soil collected in Nam Nao National Park, Phetchabun province, Thailand, we have found 21 yeast strains that represent a novel species of the genus *Tetrapisispora*. In this study we report on these strains and describe the novel species *Tetrapisispora namnaonensis*.

Twenty-one strains were isolated from soil collected in a pine forest (TN1-01T to TN1-10), a dry dipterocarp forest (TN1-11 to TN1-15) and a mixed deciduous forest (TN1-16 to TN1-21) in Nam Nao National Park. Isolation of the strains was carried out by the enrichment technique using yeast extract/malt extract (YM) broth containing 0·3 % glucose.

**Abbreviations**: ITS, internal transcribed spacer; LSU, large subunit; SSU, small subunit.

The GenBank/EMBL/DDBJ accession numbers for the SSU rDNA and the D1/D2 domain sequence of the LSU rDNA of *Tetrapisispora namnaonensis* strain TN1-01T are AB180479 and AB180480, respectively.
yeast extract, 0·3 % malt extract, 0·5 % peptone and 1 % glucose supplemented with 0·1 % sodium propionate and 200 mg chloramphenicol l⁻¹ to prevent growth of moulds and bacteria, respectively.

Morphological, biochemical and physiological characteristics of the strains were examined by standard methods as described by Yarrow (1998). Assimilation of nitrogen compounds was tested by the method of Nakase & Suzuki (1986) using solid media with starved inocula. Determination of the ubiquinone system was done by the methods of Yamada & Kondo (1984) and Kuraishi et al. (1985). Analysis of the nuclear DNA base composition was carried out by the method of Marmur (1961). The remaining RNA, mitochondrial DNA and polysaccharides were removed from the nuclear DNA by the method of Hamamoto & Nakase (1995) using equilibrium caesium chloride and ethidium bromide ultracentrifugation.

Analysis of the sequences of the SSU rDNA and the D1/D2 domain of the LSU rDNA were carried out from PCR products of genomic DNA fragment extracted from cells by the method of Lachance et al. (1999) with some modifications. The D1/D2 domain of the LSU rDNA was amplified with universal primers NL1 and NL4 (O’Donnell, 1993) and amplification of the LSU rDNA was done with the universal primers P1 and P2 (Sjamsuridzal et al., 1997). The PCR product was confirmed by agarose gel electrophoresis, purified using the QIAquick purification kit (Qiagen) and cycle-sequenced using a BigDye Terminator cycle sequencing kit version 3.1 (Applied Biosystems) with primers NL1 and NL4 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 Genetic Analyzer (PE Applied Biosystems).

These sequences were aligned with representative sequences retrieved from GenBank using CLUSTAL X 1.81 (Thompson et al., 1997). Phylogenetic trees were constructed from the evolutionary-distance data (Kimura, 1980) using the neighbour-joining method as described by Saitou & Nei (1987) in CLUSTAL X 1.81. Candida albicans NRRL Y-12983NT was used as an outgroup in the phylogenetic tree. Bootstrap analysis was performed from 1000 random resamplings (Felsenstein, 1985).

Based on morphological, biochemical, physiological and chemotaxonomic characteristics, the strains were assigned to the genus Tetrapisispora. The strains were essentially identical in conventionally employed taxonomic characteristics. In phylogenetic analyses based on the sequences of the SSU rDNA (data not shown) and the D1/D2 domain of the LSU rDNA (Fig. 1), the strains were clustered with the four known species of Tetrapisispora, confirming that the strains are the member of this genus. The D1/D2 domains of the LSU rDNA sequences of the 21 strains were identical and differed from that of T. arboricola IFO 10925T, the closest species, by 6-4 % nucleotide substitutions (37 substitutions and 1 gap), confirming that the strains belonged to a novel species in the genus Tetrapisispora. The novel strains were similar to the other species of Tetrapisispora, although the ability to assimilate D-gluconic acid, the inability to grow on 50 % glucose medium and the liberation of ascospores can be used to distinguish the novel species from the type strain of T. arboricola. Other phenotypic characteristics useful in separation from the other four species of Tetrapisispora are given in Table 1. The nuclear DNA base composition confirmed that the strains differed from other species of Tetrapisispora (Table 1). The novel strains had G+C contents of 36-2–37-2 mol%, which is higher than in the other species. On the basis of phenotypic and molecular characters, including phylogenetic relationships based on the SSU rDNA and the D1/D2 domain of the LSU rDNA, we

**Fig. 1.** Neighbour-joining phylogenetic tree based on the D1/D2 domain of the LSU rDNA showing the relationship of T. namaenonis sp. nov. and closely related ascomycetous yeast species. Bar, 0·1 substitutions per nucleotide position.
**Table 1.** Characteristics that differentiate *T. namnaonensis* sp. nov. from described species


Data were determined in this study unless indicated as follows: 
a, data from Lachance (1998); b, data from Ueda-Nishimura & Mikata (1999).

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>Asci persistent</td>
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<td>Assimilation of:</td>
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<td>Trehalose</td>
<td>W</td>
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<td>D-Gluconic acid</td>
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<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<td>Growth on:</td>
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<td>50% Glucose</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>10% NaCl + 5% glucose</td>
<td>+</td>
<td>S</td>
<td>S</td>
<td>−</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>Vitamin-free medium</td>
<td>−</td>
<td>−</td>
<td>W</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>36–2–37-2</td>
<td>31b</td>
<td>32b</td>
<td>30b</td>
<td>35-3-3a</td>
<td>34-2a</td>
</tr>
</tbody>
</table>

describe a novel species within the genus *Tetrapisispora*, for which the name *Tetrapisispora namnaonensis* is proposed.

**Latin diagnosis of Tetrapisispora namnaonensis**

*Sumpradit, Limtong, Yongmanitchai, Kawasaki & Seki sp. nov.*

_Cultura in agaro YM post dies 3 ad 25 °C, cellulae ellipsoidae, ovoideae vel cylindraceae (1-7-3-3 x 5-0-6-8 μm), singulae aut binae, per gemmationem multipolarem reproducte._


G + C acidi deoxyribonucleati 36-2–37-2 mol% per HPLC.

Tetrapisispora namnaonensis sp. nov. **Typus stirpis TN1-01** (= TISTR 5828 = JCM 12664 = CBS 10093) _isolatus e terea, Nam Nao National Park, Phetchabun Provincia, Thailandia_, Thailand Institute of Scientific and Technological Research (TISTR) et Japan Collection of Microorganisms (JCM) et Centraalbureau voor Schimmelcultures (CBS) _deposita est._

In YM agar after 3 days at 25 °C, the cells are ellipsoid, ovoid to cylindrical (1-7-3-3 x 5-0-6-8 μm), single or in pairs (Fig. 2). Vegetative reproduction is by multilateral budding. Streak culture on YM agar after 7 days at 25 °C is butyrous, smooth, glistening and cream-coloured. In YM broth after 3 days at 25 °C, sediment is formed. In Dalmau plate culture on potato dextrose agar after 5 days incubated at 25 °C, pseudomyelium and true mycelium are not present. Asci containing one to four ovoid to reniform ascospores (1-7-2-1 x 2-1-2-9 μm) are formed directly from diploid...

**Fig. 2.** Vegetative cells of *T. namnaonensis* sp. nov. TN1-01T on YM medium for 3 days at 25 °C (a) and ascospores on Fowell’s acetate agar after 7 days at 25 °C (b). Bars, 10 μm.

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vegetative cells after incubation for 1 week at 25 °C on Fowell’s acetate agar. Asci are deliquescent. Glucose and galactose are fermented vigorously. Sucrose, maltose, lactose, raffinose, melibiose, trehalose, methyl α-D-glucoside, cellobiose, soluble starch, inulin, melezitose and D-xylose are not fermented. Glucose, galactose, glycerol, trehalose and D-gluconic acid are assimilated. No growth occurs on L-sorbose, sucrose, maltose, cellobiose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, N-acetyl-D-glucosamine, methanol, ethanol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, methyl α-D-glucoside, salicin, D-lactate, succinate, citrate, inositol and hexadecane. Nitrate, nitrite, ethylamine, L-lysine and cadaverine are not assimilated. No growth on 50 or 60% glucose. Growth on 10% NaCl plus 5% glucose is positive. No growth in the presence of 0-01% cycloheximide. Grows at 34 °C, but not at 35 °C. Urea hydrolysis, Diamzonium blue B test and amyloid compound formation are negative. The major ubiquinone is Q-6. The G+C content of the nuclear DNA is 36.2–37.2 mol%, as determined by HPLC.

The type strain is TN1-01T (=TISTR 5828T = JCM 12664T = CBS 10093T). Twenty-one strains, TN1-01T to TN1-21, were isolated from forest soil collected in a dry dipterocarp forest, a mixed deciduous forest and a pine forest of Nam Nao National Park, Phetchabun province, Thailand.

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References


