**Udeniomyces pannonicus sp. nov., a ballistoconidium-forming yeast isolated from leaves in Hungary**

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Fifteen ballistoconidium-forming yeasts, isolated from the leaves of plants in Hungary, showed morphological, physiological and biochemical characteristics similar to those of *Udeniomyces pyricola*. The identical sequences of internal transcribed spacer regions for selected strains (HY-16T, HY-29, HY-111 and HY-186) indicated that they should be classified as one species. Although a representative strain, HY-16T, showed a closer relationship to *Itersonilia perplexans* than to known *Udeniomyces* species in phylogenetic trees constructed using 18S rDNA and the D1/D2 region of the 26S rDNA sequence, this species was placed in the genus *Udeniomyces* on the basis of its morphological and chemotaxonomic characteristics. *Udeniomyces pannonicus* sp. nov. (type strain HY-16T = JCM 11145T = NCAIM Y 01556T = CBS 9123T) is proposed.

**Keywords:** yeast, *Udeniomyces pannonicus*, Hungary

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**INTRODUCTION**

The genus *Udeniomyces* Nakase & Takematsu was established in 1992 for three species, *Udeniomyces pyricola* (Stadelmann) Nakase & Takematsu, *Udeniomyces punicus* (Komagata & Nakase) Nakase & Takematsu and *Udeniomyces megalosporus* (Nakase & M. Suzuki) Nakase & Takematsu (Nakase & Takematsu, 1992). Prior to this paper, the genus *Bullera* Derx had been known as a hymenomycetous ballistoconidium-forming yeast, but it was reported to be divided into two groups, the typical *Bullera* group and the ‘*pyricola* group’, based on the morphology of the ballistoconidia and the colour of colonies (Nakase, 1987, 1989).

Typical *Bullera* species produce symmetrical ballistoconidia and pale-coloured or orange colonies, while species in the ‘*pyricola* group’ produce large, asymmetrical ballistoconidia and pinkish-white to pale-pink colonies. On the basis of the phylogenetic analyses of partial sequences of 18S rRNA, the ‘*pyricola* group’ was found to be phylogenetically far from *Bulleromyces albus* Boekhout & A. Fonseca [teleomorph of *Bullera alba* (Hanna) Derx, type species of the genus] and formed a single branch (Nakase et al., 1993). The genus *Udeniomyces* was established on the basis of this phylogenetic divergence (Nakase & Takematsu, 1992). Suh & Nakase (1995) also drew a phylogenetic tree based on almost complete 18S rDNA sequences, which supported this separation of the genus *Udeniomyces*. Fell et al. (2000) reported that this genus fits into the Cystofilobasidiomycetes Boekhout & Fell together with the non-ballistoconidium-forming yeasts *Cystofilobasidium* Oberwinkler & Bandoni and *Mrakia* Y. Yamada & Komagata in a phylogenetic tree based on the sequences of the D1/D2 region of the 26S rDNA. In the typical *Bullera* species, which produce symmetrical ballistoconidia, phylogenetic heterogeneity has also been reported (Suh & Nakase, 1995; Suh et al., 1996; Takashima & Nakase, 1999; Fell et al., 2000), and Takashima et al. (2001) recently restated the genus *Dioszegia* Zsolt for one phylogenetically distinct group.

In 1999, we isolated 204 ballistoconidium-forming yeasts from the leaves of plants collected in various places in Hungary. Fifteen strains were selected by their morphological, physiological, biochemical and...
chemotaxonomic characteristics and used in this study. They proliferate by means of budding cells and ballistoconidia, possess Q-10 as a major ubiquinone and contain xylose in the cells. Phylogenetically, they showed an affinity with the Cystofilobasidiomycetes on the basis of sequences of the 18S rDNA and the D1/D2 region of the 26S rDNA, indicating that they belonged to the genus Udeniomyces. A novel species, Udeniomyces pannonicus sp. nov. (type strain HY-16$^T$ = JCM 11145$^T$ = NCAIM Y 01557$^T$ = CBS 9123$^T$), is proposed in this paper.

**METHODS**

**Yeast strains.** The strains used in this study, and their sources, are shown in Table 1. They were isolated using the ballistoconidium-fall method with YM agar (Difco) plates (Nakase & Takashima, 1993).

**Morphological, physiological and biochemical characteristics.** Most of the methods used to examine morphological, physiological and biochemical characteristics were those described by Yarrow (1998). Assimilation of nitrogen compounds was investigated on solid media, using starved inocula according to the method of Nakase & Suzuki (1986). Vitamin requirements were investigated by following the method of Komagata, 1984). A DNA-GC kit (Yamasa Shoyu) was used as a quantitative standard.

For analysis of the ubiquinone system, cells were grown in 500 ml Erlenmeyer flasks containing 250 ml YM broth on a rotary shaker at 100 r.p.m. at 17°C and were harvested in the early stationary growth phase. The cells were washed with distilled water. The extraction and purification of ubiquinone was carried out according to the method of Nakase & Suzuki (1986).

**Xylose in the cells.** Cells were grown in 500 ml Erlenmeyer flasks containing 250 ml YM broth on a rotary shaker at 100 r.p.m. at 17°C and were harvested in the early stationary growth phase; they were then washed with distilled water. Acid hydrolysis of whole cells was performed as described by Suzuki & Nakase (1988). The presence of xylose in the whole-cell hydrolysate was analysed with an HPLC reducing-sugar analysis system (Shimadzu) according to the instructions of the manufacturer.

**DNA base composition.** Cells were grown in 500 ml Erlenmeyer flasks containing 250 ml YM broth on a rotary shaker at 100 r.p.m. at 17°C and were harvested in the exponential growth phase and then washed with distilled water. Isolation and purification of DNA were carried out according to Takashima & Nakase (2000). The DNA base composition was determined by HPLC after enzymic digestion of DNA to deoxyribonucleosides (Tamaoka & Komagata, 1984). A DNA-GC kit (Yamasa Shoyu) was used as a quantitative standard.

**Nucleotide sequences and phylogenetic analysis.** Nuclear DNA was extracted by the method of Makimura et al. (1994). The 18S rDNA and internal transcribed spacer (ITS)
regions including 5.8S rDNA were amplified by using a PCR according to Sugita & Nakase (1999). The D1/D2 region of the 26S rDNA was amplified according to the method of Kurtzman & Robnett (1997). The PCR products were sequenced directly using an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems) and analysed by using an Applied Biosystems sequencer (model 310) according to the instructions of the manufacturer. The sequences generated were aligned with sequences of other unicellular and filamentous basidiomycetes (see Fig. 2) by using the program Clustal X (version 1.8) (Thompson et al., 1997). Bulleromyces albus was used as an outgroup. The phylogenetic trees were constructed by two methods: the neighbour-joining method (Saitou & Nei, 1987) based on Kimura’s distance (Kimura, 1980) in clustal x and the maximum-parsimony method in the program DAMBE version 4.0.43 (Xia & Xie, 2001). In making phylogenetic trees, sites where gaps existed in any sequence were excluded and bootstrap analyses (Felsenstein, 1985) were performed from 100 random resamplings.

RESULTS AND DISCUSSION

Fifteen strains isolated from the leaves of plants in Hungary in June 1999 showed similar morphological, physiological and biochemical properties: vegetative cells were spherical, ellipsoidal or cylindrical (Fig. 1); ballistospores were apiculate, ellipsoidal or pyriform (Fig. 1); mycelium and pseudomycelium were not

formed; fermentation was absent; the diazonium blue B colour test was positive; the major ubiquinone was Q-10; and xylose was present in the cells. The strains were assumed to belong to one of the genera Udeniomyces or Bullera, and an especially close relationship with U. pyricola was suspected on the basis of physiological and biochemical properties.

Four strains (HY-16T, HY-29, HY-111 and HY-186) were selected on the basis of their isolation sources and the sequences of their ITS regions were determined. The identity of the sequences (AB072229–AB072232) indicated that the strains are conspecific (Sugita et al., 1999). From the physiological and biological properties and the result of sequence analyses of ITS regions, we concluded that these 15 strains should be included in one species.

As shown in Fig. 2, strain HY-16T was positioned in the Cystofilobasidiales on the basis of the 18S rDNA. It is interesting that the novel strains showed a closer relationship with Itersonilia perplexans Derx than with Udeniomyces species, in spite of the physiological and biochemical resemblance to U. pyricola. The same topologies were obtained by the maximum-parsimony method. In the D1/D2 region of the 26S rDNA, only four base substitutions and one base deletion or insertion were detected between strain HY-16T (accession no. AB077382) and I. perplexans (accession no. AJ235274). Although the results showed that our isolates and I. perplexans were very closely related, the sequence similarities of ITS1 and ITS2 in our isolates and I. perplexans were respectively 94-9 and 96-1%, indicating that they are two distinct species.

The genus Itersonilia was initially thought to contain three species, I. perplexans Derx (Derx, 1948), Itersonilia pyriformis Nyland (Nyland, 1949) and Itersonilia pastinacae Channon (Channon, 1963). Because of mating compatibility, rather high DNA similarities and an intergrading morphology, however, the existence of separate species was later questioned and the latter two are considered synonyms of I. perplexans (Bockhout, 1991; Bockhout et al., 1991). I. perplexans is characterized by the presence of hyphae with clamp connections, inflated cells, ballistoconidia and appressoria formed from germinating ballistoconidia. The yeast phase is formed under conditions of high humidity or submerged growth and has never been isolated from nature. Furthermore, the yeast phase of I. perplexans produces monokaryotic hyphae with pseudoclamps. We have observed no hyphae with clamp connections, inflated cells or appressoria in our isolates. The major ubiquinone of the genus Itersonilia is Q-9 (Yamada & Konda, 1984) and that of our isolates was Q-10, the same as that of the genus Udeniomyces. From these morphological and chemotaxonomic characteristics, we decided to treat our isolates as a member of the genus Udeniomyces and to propose the name Udeniomyces pannonicus sp. nov. As the physiological and biochemical properties of U. pannonicus are very similar to those of U. pyricola as described above, only a few characteristics can be used

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Fig. 1. Udeniomyces pannonicus sp. nov. JCM 11145T. (a) Vegetative cells grown in YM broth for 3 days at 17°C. (b) Vegetative cells with sterigma and ballistoconidia grown on YM agar for 10 days at 17°C and 3 days at room temperature. (c) Ballistoconidia produced on cornmeal agar after 7 days at 17°C. Bars, 10 μm.
Itersonilia perplexans

Table 2. Differential characteristics among *Udeniomyces pannonicus* sp. nov., other *Udeniomyces* species and *Itersonilia perplexans*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>U. pannonicus</em></th>
<th><em>I. perplexans</em></th>
<th><em>U. megalosporus</em></th>
<th><em>U. puniceus</em></th>
<th><em>U. pyricola</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Assimilation of:*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-d-glucosamine</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Melibiose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lactose</td>
<td>–/LW</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Inositol</td>
<td>–</td>
<td>+/W</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vitamin requirement</td>
<td>Biotin</td>
<td>None</td>
<td>Biotin and thiamin</td>
<td>Biotin and thiamin</td>
<td>Biotin and thiamin</td>
</tr>
<tr>
<td>Major ubiquinone</td>
<td>10</td>
<td>9/11</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>58/9</td>
<td>61/8</td>
<td>49/8</td>
<td>53/9</td>
<td>51/7</td>
</tr>
<tr>
<td>Colour of colony</td>
<td>Yellowish white</td>
<td>Yellowish white</td>
<td>Pinkish white</td>
<td>Pinkish white</td>
<td>Pinkish white</td>
</tr>
<tr>
<td>Sequence accession numbers:*</td>
<td>AB077332 (JCM 11145)</td>
<td>AB255274 (CIBS 363.855)</td>
<td>AF075510 (CBS 37236)</td>
<td>AF075519 (CBS 35895)</td>
<td>AF075507 (CBS 67547)</td>
</tr>
</tbody>
</table>

*† Data taken from this study unless indicated by: a, Yamada & Konda (1984); b, Boekhout et al. (1991); c, Nakase & Suzuki (1986); d, Nakase & Komagata (1971).¶ Source strains in parentheses.

to distinguish them (Table 2). The G+C contents (mol%) of nuclear DNA and the ITS sequence data, however, show their separation clearly. *U. pannonicus* is distinguishable from *I. perplexans* by a combination of biological properties, in particular the assimilation of N-acetyl d-glucosamine and inositol, vitamin requirements and the major ubiquinone (Table 2).

*U. pannonicus* was isolated from 10 of 25 plants that we sampled, indicating that this species is widely distributed in Hungary. We also isolated *U. pyricola* from the same number of plants in the course of this study (our unpublished data). Over 10% of the total isolates belonged to the genus *Udeniomyces*. This is rather intriguing, as these yeasts were not isolated in Thailand (Nakase et al., 2001), even though *U. pyricola* was isolated from plants collected in Japan, New Zealand and the islands of Tasmania, Australia, *U. megalosporus* from plants in Japan and *U. puniceus* from a frozen fish in Japan (Nakase, 2000).

**Latin diagnosis of *Udeniomyces pannonicus* Niwata, Takashima, Tornai-Lehoczki, Deak & Nakase sp. nov.**


Description of *Udeniomyces pannonicus* Niwata, Takashima, Tornai-Lehoczki, Deak & Nakase sp. nov.

Udeniomyces pannonicus (pan.non.i.cus. L. adj. pannonicus pertaining to Pannonia, the Roman name for the area of modern Hungary, the country in which the type strain was isolated).

In YM broth, after 5 days at 17 °C, the vegetative cells are spherical, ellipsoidal or cylindrical, 4.10×6–14 μm, single or in pairs. A sediment is formed. After 1 month at 17 °C, an incomplete or complete fragile ring and sediment are present. On YM agar, after 1 month at 17 °C, the streak culture is greyish yellow, semi-shining, smooth, soft and has an entire margin. On slide culture on cornmeal agar, after 14 days at 17 °C, mycelia and pseudomyelia are not observed. Ballistoconidia are produced abundantly on cornmeal agar. They are apiculate, ellipsoidal or pyriform and 2.6–6.5×4.3–10.4 μm. Does not ferment glucose. Assimilates glucose, galactose, L-sorbosce (may be latent or latent and weak), sucrose, maltose, cellobiose, trehalose, melibiose, raffinose, melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose (latent, latent and weak or negative), D-ribose (weak, latent and weak or negative), L-rhamnose (may be latent), methanol (weak or latent and weak), ethanol (may be latent), glycerol (may be latent or latent and weak), D-mannitol (may be latent), D-glucitol (may be latent or weak), methyl α-D-glucoside, salicinum, glucono-δ-lactone (may be latent or negative), D-glucuronate (latent, latent and weak or negative), 2-ketogluconic acid, 5-ketogluconic acid, succinic acid, citric acid (may be weak or latent and weak), succinate (latent and weak), xylitol, propan-1,2-diol (weak), D-glucuronic acid and D-galacturonic acid. Does not assimilate lactose (may be latent and weak), inulin, D-glucoseamine, N-acetyl D-glucosamine, erythritol (may be latent and weak), ribitol (may be weak or latent and weak), galactitol (may be latent and weak), DL-lactate (may be weak or latent and weak), inositol, hexadecane, L-arabitol (may be weak or latent and weak) or butane-2,3-diol. Assimilates potassium nitrate and sodium nitrite. Does not assimilate ethylamine hydrochloride, L-lysine (may be latent and weak) or cadaverine hydrochloride. Biotin is required for growth. The maximum growth temperature is 24–25 °C. Starch-like substances are produced. Growth does not occur on 50% (w/w) glucose/yeast extract agar. Urease is positive. May or may not liquefy gelatin. Does not hydrolyse fat. The diazonium blue B reaction is positive. The G+C content of nuclear DNA is 58.9 mol% as determined by HPLC. The major ubiquinone is Q-10. Xylose is present in the cell.

The type strain, HY-16T, was isolated from a leaf of *Angelica sylvestris* collected in June 1999 in Hungary by T. Nakase, J. Tornai-Lehoczki and M. Takashima. This strain has been deposited in the Japan Collection of Microorganisms (JCM), Wako, Saitama, as JCM 11145T, in the National Collection of Agricultural and Industrial Microorganisms (NCAIM), Szent István University, Budapestinum, Pannonia, et Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands, as CBS 9123T.

REFERENCES


Y. Niwata and others


