Geotrichum vulgare sp. nov., a novel asexual arthroconidial yeast

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Two strains of a novel yeast species were isolated from different habitats, from soil in an alluvial zone national park in Austria and from a drain in a Turkish soft drinks factory. Analysis of the nucleotide sequences of the D1/D2 region of their large-subunit rDNAs and PCR fingerprints show that the strains are members of the same species, described as *Geotrichum vulgare* sp. nov. Analysis of nucleotide sequences showed that this species is related to the ascogenous genus *Galactomyces*. The closest phylogenetic relative is *Geotrichum silvicola*, a recently described species. The type strain of *Geotrichum vulgare* is HA1379^T (= CBS 10073^T = NRRL Y-27915^T).

The anamorphic genus *Geotrichum* Link: Fries consists of 12 species having their teleomorphs in the genera *Dipodascus* Lagerheim and *Galactomyces* Redhead and Malloch (Barnett *et al.*, 2000; de Hoog *et al.*, 1986, 1998a, b; Kurtzman & Robnett, 1998; Pimenta *et al.*, 2005). One species, *Geotrichum silvicola* Pimenta, Prasad, Lachance et Rosa, was described recently (Pimenta *et al.*, 2005). Four strains of this species were isolated from flies of the genus *Drosophila* and two strains were found on larvae of the oak tasar silk worm, *Antheraea proylei*. The D1/D2 region of the large-subunit rDNA of *Geotrichum silvicola* differs by 15 substitutions from that of an undescribed species, designated by Kurtzman & Robnett (1998) as *Galactomyces* sp. NRRL Y-6418.

In this paper, a novel species of *Geotrichum* is described consisting of two strains from different habitats. One strain was isolated from soil in an alluvial zone forest in Austria and the other originated from a drain in a soft drinks factory in Turkey.

Yeast isolation and characterization

Strain HA1379^T was collected in November 1999 from soil in an alluvial zone forest national park ('Nationalpark

Donauauen') along the river Danube downstream from Vienna, Austria, near Mannswörth, as part of a biodiversity study on microfungi and yeasts (Wuczkowski *et al.*, 2003a, b). The sampling and isolation procedures are described in Wuczkowski *et al.* (2003a, b). Strain NCYC 3138 was isolated from a drain in a soft drinks factory in Turkey and deposited in the NCYC by Dr M. Stratford (Unilever Research, UK). Both strains were characterized by standard methods (Yarrow, 1998).

Sequencing and PCR-fingerprinting

Sequencing of the D1/D2 region of the 26S rDNA and PCR fingerprinting were performed as described in Wuczkowski *et al.* (2003a, b).

Species delineation and identification

Strains HA1379^T and NCYC 3138 show identical nucleotide sequences in the D1/D2 region of the 26S rDNA. The results of PCR fingerprinting (Fig. 1) show a high degree of similarity between the two strains and therefore it is highly likely that these strains belong to the same species. After growth on sporulation media (potato-dextrose agar, cornmeal agar, malt extract agar), asci were not found.

The nearest relative to the novel species is *Geotrichum silvicola*, a recently described species which belongs to a clade of several *Galactomyces* species (Pimenta *et al.*, 2005). *Geotrichum silvicola* shows 13 substitutions within the D1/D2 region of the 26S rDNA when compared with the strain HA1379^T.

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The GenBank/EMBL/DDBJ accession number for the large-subunit rRNA gene sequence of *Geotrichum vulgare* sp. nov. HA1379^T is AJ511334.

A supplementary figure showing the appearance of arthroconidia of *Geotrichum vulgare* sp. nov. HA1379^T is available in IJSEM Online.

Fig. 1. PCR fingerprints of the two isolates of *Geotrichum vulgare* with three different primers. M, Length marker.

The sequence was compared with already published sequences retrieved from GenBank via the CLUSTAL_X program (Thompson *et al.*, 1997). A neighbour-joining tree was constructed with the TREECON software package (Van de Peer & De Wachter, 1994), using the Kimura model (Kimura, 1980). Bootstrap values were calculated by 500 replications (Fig. 2). On the basis of the results of this study, it is evident that the new strains represent a novel species for which the name *Geotrichum vulgare* is proposed with strain HA1379^T as the type strain.

Typical characteristics for the members of this clade and also for *Geotrichum vulgare* are the assimilation of D-glucose, D-galactose, L-sorbose, glycerol, DL-lactate, succinate and ethanol as the carbon source. Some differences were observed when the novel species was compared with *Galactomyces geotrichum*. The novel species does not use ribitol, D-mannitol, D-glucono-1,5-lactone or citrate. In order to separate *Geotrichum vulgare* from *Geotrichum silvicola*, its closest relative, assimilation of D-mannitol and citrate can be used, as *Geotrichum vulgare* does not assimilate these substrates. In addition, *Geotrichum vulgare* does not grow at 37 °C.

Fig. 2. Neighbour-joining tree based on nucleotide sequences of the D1/D2 region of the 26S rDNA of *Geotrichum vulgare* and its relatives. Percentage bootstrap values (shown at branch nodes) were obtained from 500 replications. Bar, 5% sequence divergence.

Latin diagnosis of *Geotrichum vulgare* Wuczkowski, Bond et Prillinger sp. nov.

Cultura in agar malti post dies 7 (24 °C) plana, sicca, capillata et candida. In agar Solanum tuberosum et glucosum post dies 7 mycelium verum et arthroconidia formantur. Asci nec ascospores non formantur. Glucosum non fermentatur. D-Glucosum, D-galactosum, L-sorbosum, D-xylosum, sucrosus (var.), glycerolum, D-glucitolum, 2-keto-D-gluconatum (lente), DL-lactatum (lente), succinatum, ethanolum, propan-1,2-diolum, butan-2,3-diolum et assimilantur, at non D-glucosaminum, D-ribosum, L-arabinosum, D-arabinosum, L-rhamnosum, maltosum, α,α-trehalosum, methyl α-D-glucosidum, cellobiosum, salicinum, arbutinum, melibiosum, lactosum, raffinatum, melezitolum, inulinum, amyllum solubile, erythritolum, ribitolum, xylitolum, L-arabinitolum, D-mannitolum, galactitolum, myo-inositolum, D-glucono-1,5-lactonum, 5-keto-D-gluconatum, D-gluconatum, D-glucuronatum, citratum et methanolum. Ethylaminum et cadaverinum assimilantur et non natrium nitricum, natrium nitrosus et lysinum. Ad crescentiam vitaminarum externarum non necessariae sunt. Augmentum in 30 °C, at non 37 °C. Typus HA1379^T, collectione zymotica Austrian Center of Biological Resources and Applied Mycology (ACBR) (= CBS 10073^T = NRRL Y-27915^T).

Description of *Geotrichum vulgare* Wuczkowski, Bond & Prillinger sp. nov.

After 7 days on malt extract-yeast extract agar at 24 °C, colonies are white, flat, dry and powdery to finely hairy. Arthroconidia are abundantly present (see Fig. 3 and Supplementary Fig. S1. in IJSEM Online). Hyphae are 3–7 µm wide with early disarticulation into cubic arthroconidia. Arthroconidia are 3–5 µm wide and 5–70 µm long.

Fig. 3. Differential interference contrast micrograph of *Geotrichum vulgare* HA1379^T. Vegetative cells and hyphae after 1 week growth on potato-dextrose agar at 25 °C. Bar, 20 µm.

On slide cultures with potato-dextrose agar after 1 week, abundant true mycelia and arthroconidia are formed. Glucose is not fermented. The following carbon compounds are assimilated: D-glucose, D-galactose, L-sorbose, D-xylose, sucrose (variable), glycerol, D-glucitol, 2-keto-D-gluconate (weak), DL-lactate (weak), succinate, ethanol, propane-1,2-diol and butane-2,3-diol. No growth occurs on D-glucosamine, D-ribose, L-arabinose, D-arabinose, L-rhamnose, maltose, α,α -trehalose, methyl α -D-glucoside, cellobiose, salicin, arbutin, melibiose, lactose, raffinose, melezitose, inulin, starch, erythritol, ribitol, xylitol, L-arabinitol, D-mannitol, galactitol, *myo*-inositol, D-glucono-1,5-lactone, 5-keto-D-gluconate, D-gluconate, D-glucuronate, citrate or methanol. The following nitrogen compounds are assimilated: ethylamine and cadaverine but not nitrate, nitrite or lysine. Growth in vitamin-free medium is positive. Growth at 30 °C and weak growth at 35 °C; no growth at 37 °C. No growth in 10 % sodium chloride or in 50 % glucose. Starch is not produced. Growth occurs at 0.01 % cycloheximide, growth at 0.1 % is variable. Urease activity is negative, Diazonium blue B test is negative. No production of extracellular amyloid compounds. Strain habitats include soil from an alluvial zone forest (Austria) and a drain in a Turkish soft drinks factory.

The type strain of *Geotrichum vulgare*, HA1379^T (=CBS 10073^T=NRRL Y-27915^T), was isolated from soil. The epithet *vulgare* (common) refers to the diverse habitats from where the strains have been isolated.

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