Pseudozyma pruni sp. nov., a novel ustilaginomycetous anamorphic fungus from flowers in Taiwan

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Two ustilaginomycetous anamorphic strains were isolated from flowers in Taiwan. Phylogenetic analysis based on the combined rRNA gene sequence of internal transcribed spacer 1 (ITS1)–5.8S–ITS2 and large-subunit D1/D2 domains indicated that the closest recognized species was Pseudozyma fusiformata. The results of DNA–DNA hybridization and physiological characteristics showed that the two strains represent a novel species within the genus Pseudozyma. The name Pseudozyma pruni sp. nov. is proposed, with FIRDI 005T (=BCRC 34227T =CBS 10937T) as the type strain.

Most species of the genus Ustilago parasitize monocotyledonous hosts and are economically important fungi. A number of anamorphs of Ustilago have been classified into diverse genera (Boekhout et al., 1998). These anamorphic species have been reclassified into the genus Pseudozyma Bandoni emend. Boekhout based on morphological, physiological and biochemical data and partial large-subunit (LSU) rRNA gene sequence analysis (Boekhout, 1995). Pseudozyma species are isolated most frequently from plant materials. Seven species were listed in the genus Pseudozyma by Boekhout & Fell (1998). More recently, six species were described by Sugita et al. (2003), Wang et al. (2006), Golubev et al. (2007) and Seo et al. (2007). During an investigation of yeast-like fungi in Taiwan, a novel species of the genus Pseudozyma was detected through rRNA gene sequencing of the internal transcribed spacer 1 (ITS1)–5.8S–ITS2 region and the large-subunit (LSU) D1/D2 domains, and confirmed by DNA–DNA hybridization.

Strains FIRDI 005T (=BCRC 34227T =CBS 10937T) and 167b (=BCRC 34379) were isolated from flowers in Taiwan by using a method described previously (Wei et al., 2005). Morphological and physiological characteristics were examined by using the methods described by Yarrow (1998). Nuclear DNA that was intended for use in PCR was extracted by using a DNeasy Plant Mini kit (Qiagen).

The ITS region and LSU D1/D2 domains of the rRNA gene were defined by using primers ITS5/ITS4 (White et al., 1990) and NL1/NL4 (O’Donnell, 1992). The reaction was performed in a GeneAmp PCR system 9700 (Applied Biosystems), using 30 cycles of the following steps: denaturation at 95 °C for 30 s, annealing at 55 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min following cycling. Sequencing reactions were performed by employing an ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing kit as directed by the manufacturer. Finally, the PCR products were sequenced by using an ABI PRISM 3730 DNA Analyzer.

Sequences of each strain were aligned by using CLUSTAL_X 1.83 (Thompson et al., 1997). Ambiguously aligned regions were excluded from the phylogenetic analyses. Phylogenetic evaluation was performed with the PHYLIP 3.63 package (Felsenstein, 2004). Related teleomorphs were selected based on the study by Wang et al. (2006). Phylogenetic trees were constructed by using the neighbour-joining and maximum-parsimony methods. Confidence values for the phylogenetic trees were estimated from bootstrap analyses with 1000 replicates. The trees were viewed by using TreeView 1.6.6 (Page, 1996).

Isolation and purification of DNA for determination of the DNA base composition (expressed as DNA G+C content, mol%) and for determination of the levels of DNA relatedness were performed with a Genomic DNA Buffer Set and Genomic-tip 500/G (Qiagen). The purified DNA

Abbreviations: ITS, internal transcribed spacer; LSU, large subunit.
was hydrolysed to nucleosides as described by Tamaoka & Komagata (1984) and the hydrolysate was analysed by reverse-phase HPLC to determine the DNA base composition (mol% G+C). DNA–DNA relatedness values were determined by the photobiotin-labelling microplate method (Lee et al., 1993, 1998). Hybridizations were performed at 47 °C for 24 h.

The rRNA gene ITS region and the LSU D1/D2 domain sequences of strains FIRDI 005T and 167b were identical. The maximum-parsimony tree (see Supplementary Fig. S1, available in IJSEM Online) has the same topology as the neighbour-joining tree (Fig. 1). Phylogenetic analyses revealed that strain FIRDI 005T belongs to the genus Pseudozyma and is located in the anamorphic clade of Ustilago sensu stricto (Stoll et al., 2005; Wang et al., 2006). The closest recognized species was Pseudozyma fusiformata, with strong bootstrap support based on combined alignments of the ITS region and the LSU D1/D2 domain of the rRNA gene. In the LSU D1/D2 domain, strain FIRDI 005T exhibited <2 % divergence from seven species, namely Pseudozyma flocculosa (0.9 %), Pseudozyma graminicola (1.4 %), P. fusiformata (1.8 %), Pseudozyma shanxiensis (1.8 %), Pseudozyma prolifica (1.8 %), Pseudozyma rugulosa (1.8 %) and Pseudozyma aphidis (1.8 %). In the ITS region, the sequence of strain FIRDI 005T differed by 11.3 % from that of P. fusiformata, whereas the differences in the sequences between strain FIRDI 005T and other members of the genus Pseudozyma occurred over >14 % of the sequence. The sequence difference of the ITS region within a single species is <1 % in some species of the genus Trichosporon (Sugita et al., 1999) and in Pseudozyma antarctica (Sugita et al., 2003; Wei et al., 2005). The sequence analyses support the distinction between strain FIRDI 005T and the recognized species in the genus Pseudozyma.

Strain FIRDI 005T differed from strain 167b in terms of colony morphology and physiological characteristics.

**Fig. 1.** Neighbour-joining phylogenetic tree of members of the genus Pseudozyma and related teleomorphs, based on the combined sequences of the ITS and LSU rRNA gene D1/D2 domain. The phylogenetic tree was rooted with Cintractia axicola and Trichocintractia utriculicola (Fell et al., 2000). GenBank accession numbers for the sequences used in the analysis are given in parentheses (ITS/LSU rRNA gene D1/D2 domain; single accession numbers indicate sequences that cover both regions). Numbers on branches indicate the confidence level from 1000 bootstrap replicates. Only bootstrap values >50 % are indicated. Bar, 0.1 substitutions per site.
Colonies of strain FIRDI 005<sup>T</sup> were pale orange and friable, whereas colonies of strain 167b were white and velutinous. In terms of physiological characteristics, strain FIRDI 005<sup>T</sup> differed from strain 167b in its ability to assimilate ribitol. Strain FIRDI 005<sup>T</sup> differed from <i>P. fusiformata</i> in the assimilation of galactose, lactose and soluble starch and its growth at 37 °C. Strain FIRDI 005<sup>T</sup> differed from <i>P. flocculosa</i> in the assimilation of lactose and ethanol and its growth at 37 °C (Table 1). Furthermore, the DNA G+C content of strain FIRDI 005<sup>T</sup> was 56.2 mol%, very close to that of <i>P. fusiformata</i> (56.1 mol%). To confirm the relationship of strain FIRDI 005<sup>T</sup> to <i>P. fusiformata</i> and <i>P. flocculosa</i>, a DNA–DNA hybridization experiment was performed (see Supplementary Table S1, available in IJSEM Online). Low DNA–DNA hybridization values (13–27 %) were observed between strain FIRDI 005<sup>T</sup>, <i>P. fusiformata</i> BCRC 33869<sup>T</sup> (=CBS 423.96<sup>T</sup>) and <i>P. flocculosa</i> BCRC 33999<sup>T</sup> (=CBS 167.88<sup>T</sup>). Therefore, strain FIRDI 005<sup>T</sup> is considered to represent a novel species of the genus <i>Pseudozyma</i>. This study indicated that the ITS region was a better molecular marker than the LSU D1/D2 domain for the identification of the genus <i>Pseudozyma</i> at the species level.

### Latin diagnosis of <i>Pseudozyma pruni</i> G.-Y. Liou, Y.-H. Wei & F.-L. Lee sp. nov.

<i>In agaro multi post dies 5 ad 20 °C, cellae vegetativae ellipsoideae, cylindraceae aut fusiformae, 1.1–3.5 × 3.1–10.0 μm. Pseudozymella fermentans. Fermentatio nulla. Assimilantur glucosum, galactosum, L-sorbitum (infirme), D-ribosum, D-xulosum, L-arabinosum (infirme), L-rhamnosum (infirme), sucrosum, maltosum, trehalosum, α-methyl-D-glucosidum, cellobiosum, salicinum, arbutinum, melibiosum, lactosum, raffinosum, melitizosum, inulinum (infirme), amyllum solubile, glycerolum, erythritolum, ribitolum (variabile), xylitolum (lente), L-arabininitolum (lente), D-glucitolum, D-mannitolum, inositolum, D-glucono-δ-lactonum, 2-ketoglucuronatum (infirme), D-glucuronatum, galacturonatum (infirme), DL-lactatum (infirme), succinatum, citratum (infirme), ethanolum, propanum 1,2 diolium, butanum 2,3 diolium (infirme), quinatum, kalium nitricum, natrium nitrosum, ethylaminum (infirme), L-lysinum, cadaverinunum (infirme), creatinunum (infirme), creatininitum (infirme), D-glucosaminum, imidazolum (infirme), ad crescentiam vitaminum non necessarium est, crescit in medio 0.01 % cycloheximidi addito. Non assimilantur galactitolum, methanolum, saccharatum.

### Table 1. Physiological characteristics that differentiate species of the genus <i>Pseudozyma</i>

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<tr>
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| Growth:                 |    |    |    |    |    |    |    |    |    |    |    |    |    |    |
| Without vitamins        | +  | -  | +  | +  | +  | +  | +  | +  | w  | -  | +  | -  | s  |
| At 37 °C                | +  | v  | +  | -  | -  | w  | -  | +  | +  | +  | +  | +  | +  | +  |
| At 40 °C                | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |

*Data from this study.
B. galactonatum. In medio 0.1 % cycloheximidio addito non crescit. In medio 50 % glucosum continente non crescit. Augmentum fiunt in temperatura 37 °C. Urea finditur. Amylum non formantur. Diazonium caeruleum B positivum. Proportio molaris guanini + cytosine in acido deoxyribonucleico: 56.2 mol% (per HPLC).

Typus: isolatus ex flos Prunus mume Sieb. & Zucc. ad Tianwei, Changhua, Taiwan, FIRDI 005T (=BCRC 34227T =CBS 10937T), deponitus in BCRC, FIRDI, Hsinchu, Taiwan.

**Description of Pseudozyma pruni** G.-Y. Liou, Y.-H. Wei & F.-L. Lee sp. nov.

*Pseudozyma pruni* (pru’ni. L. n. prunus a plum tree, also a botanical genus name; L. gen. n. pruni of Prunus, from which the type strain was isolated).

After 5 days growth on 5% malt extract agar at 20 °C, colonies are smooth to irregularly wrinkled, somewhat friable or velutinous, pale orange or white and have entire or somewhat eroded margins. Cells are ellipsoidal, cylindrical or fusiform and variable in size (1.1–3.5 × 3.1–10.0 μm), some have denticles up to 4.2 μm, they contain oil droplets (Fig. 2) and pseudoophyrae are present. Fermentation is negative. The following compounds are assimilated: glucose, galactose, L-sorbose (weak), D-ribose, D-xyllose, L-arabinose, D-arabinose (slow), L-rhamnose (weak), sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose, salicin, arbutin, melibiose, lactose, raffinose, melezitose, inulin (weak), soluble starch, glyceral, erythritol, xylitol (variable), yxylitol (slow), L-arabinitol (slow), D-glucitol, D-mannitol, inositol, D-glucono-δ-lactone, 2-ketogluconate (weak), D-gluconate (weak), D-glucuronate, D-galacturonate (weak), DL-lactate (weak), succinate, citrate (weak), ethanol, propane 1,2 diol, butane 2,3 diol (weak), quinate, potassium nitrate, sodium nitrite, ethylamine (weak), L-lysine, cadaverine (weak), creatine (weak), creatinine (weak), D-glucosamine, imidazole (weak) and 0.01 % cycloheximide. Vitamins are not necessary for growth. Galactitol, methanol, saccharate, D-galactonate and 0.1 % cycloheximide are not assimilated. No growth is observed in the presence of 50 % glucose. Maximum growth temperature is 37 °C. Urease acivity is positive. Starch-like substance is not produced. Diazonium blue B reaction is positive. G+C content of nuclear DNA: 56.2 mol% (by HPLC).

The type strain, FIRDI 005T (=BCRC 34227T =CBS 10937T), was isolated from flowers of *Prunus mume* Sieb. & Zucc. that were collected at Tianwei, Changhua, Taiwan, on 17 April 1998.

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**References**


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