**Vanderwaltozyma verrucispora** sp. nov., a new ascomycetous yeast species

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**Keywords**

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**Abstract**

A new yeast species, *Vanderwaltozyma verrucispora*, is proposed in this study based on two strains isolated from partially decayed leaves in Japan and one strain from soil in Taiwan. The species is characterized by the fermentation of glucose and galactose, formation of one to four spheroidal to ellipsoidal ascospores with warty surfaces in each ascus, and assimilation of a few carbon and nitrogen compounds. Genus assignment and distinction of the species from the other two recognized species of *Vanderwaltozyma* is based on the morphological and physiological characteristics, and phylogenetic analysis of nucleotide sequences of the D1/D2 domains of the large subunit (LSU) rRNA gene. From these comparisons, the name *V. verrucispora* sp. nov. is proposed. Sequence analysis of the D1/D2 domains of the LSU rRNA gene reveals that the phylogenetically closest relative of *V. verrucispora* is *Vanderwaltozyma yarrowii*. The type strain of the new species, which was isolated from a partially decayed leaf in Kagoshima Prefecture, Japan, is NBRC 1884T ( = CBS 10887T = BCRC 23141T).

**Introduction**

The genus *Vanderwaltozyma*, which is a member of the 'Saccharomyces complex', was first proposed based on the phylogenetic analysis of multiple gene sequences (Kurtzman, 2003; Kurtzman & Robnett, 2003). The genera of the *Saccharomyces* complex are difficult to differentiate based solely on either morphological and physiological criteria (van der Walt, 1970; Vaughan-Martini & Kurtzman, 1985) or a single molecular characteristic (Vaughan-Martini & Kurtzman, 1985; James et al., 1997). Kurtzman & Robnett (2003) resolved the species of the *Saccharomyces complex* into 11 well-supported clades based on combined multigene sequences. Following analysis, 11 genera were proposed, one for each clade (Kurtzman, 2003). The genus *Vanderwaltozyma* (clade 6) comprises two species *Vanderwaltozyma polyspora* and *Vanderwaltozyma yarrowii*, assigned previously to *Klyveromyces* (as *Klyveromyces polyspora* and *Klyveromyces yarrowii*, respectively) (Kurtzman, 2003). The genus is characterized by the fermentation of glucose and galactose, the production of spheroidal, oblong, or reniform ascospores, and an inability to assimilate nitrogen sources such as ethylamine, nitrate, l-lysine, and cadaverine.

During the investigation of yeast diversity from soil in Taiwan, one of the isolates, SM12S02, was identified as a member of the 'Saccharomyces complex' based on the morphological and physiological characteristics and the sequences of the D1/D2 domain of the large subunit (LSU) rRNA gene. The strain had a sequence identical to that of the D1/D2 domain of the LSU rRNA gene and physiological characteristics similar to those of strains NBRC 1884 and NBRC 1885, which were isolated from partially decayed leaves and identified as *Klyveromyces phaffii* by Banno & Mikata (1981), indicating that the three strains could be conspecific. Collectively, these three strains were found to be closely related to *V. yarrowii* and were identified as a new species belonging to the genus *Vanderwaltozyma*. The present study proposes a new species, *Vanderwaltozyma verrucispora*, to accommodate these three strains.

**Materials and methods**

**Yeast strains and growth conditions**

The two strains NBRC 1884T and NBRC 1885 were isolated from partially decayed leaves in Japan in 1975 (Banno &
The sequence data for *V. verrucispora* were deposited in GenBank or DDBJ (Table 1): D1/D2, AB041001 for NBRC 1884\(^T\), AB385386 for NBRC 1885 and EF460602 for SM12S02; and ITS, EU515871 for NBRC 1884\(^T\) and EU515872 for SM12S02. The reference sequences used in this study were retrieved from GenBank under the accession numbers indicated in Fig. 1.

**Results and discussion**

**Sequence comparison and species delineation**

Type strain NBRC 1884\(^T\) of the species isolated from a partially decayed leaf in Japan, and strain SM12S02 isolated from a soil sample in Taiwan, have identical sequences in the D1/D2 domain of the LSU rRNA gene and the ITS. Strain

<table>
<thead>
<tr>
<th>Species</th>
<th>Fermentation</th>
<th>Assimilation</th>
<th>Growth</th>
<th>Cycloheximide Ascospore</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. verrucispora</em></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Short-ellipsoidal</td>
</tr>
<tr>
<td><em>V. polyspora</em></td>
<td>+</td>
<td>s</td>
<td>+</td>
<td>Oblong to reniform</td>
</tr>
<tr>
<td><em>V. yarrowii</em></td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>Spherical</td>
</tr>
</tbody>
</table>

Data:

\(^a\)Lachance (1998).

+ , positive; – , negative; W, weak; V, variable; D, delayed growth; s, slow growth.

**Examination of morphological, physiological, and biochemical characteristics**

The morphological, physiological, and biochemical characteristics of the species were determined by the methods described by Yarrow (1998). Yeast vegetative cells and ascospores were examined by a scanning electron microscope using the method described previously by Lee et al. (1994). The strains used for the examination of ascospores were incubated on Fowell’s acetate agar (Yarrow, 1998) for 7 days at 25 \(^\circ\)C.

**Phylogenetic analysis**

The sequencing of the D1/D2 domain of the LSU rRNA gene and the internal transcribed spacer (ITS) fragments of the strains NBRC 1884 and SM12S02 was carried out using PCR products of genomic DNA extracted from yeast cells using a Biokit Genome DNA Extraction Kit (Biokit Co., Taiwan). The D1/D2 rRNA gene and ITS fragments were amplified using primers NL1 and NL4 (Kurtzman & Robnett, 1998) and the internal transcribed spacer (ITS) fragments of the strains NBRC 1884 and SM12S02 was carried out using PCR and the internal transcribed spacer (ITS) fragments of the strains NBRC 1884 and SM12S02 was carried out using PCR. The sequencing of the D1/D2 domain of the LSU rRNA gene was performed using a Dye Terminator cycle sequencing kit (Beckman Coulter). The sequencing of the ITS1 and ITS4 (White et al., 1990) using a Peltier thermal cycler (PTC-200, MJ Research), respectively. Successful amplification was confirmed by agarose gel electrophoresis. Sequencing of the fragments was performed using an automated DNA sequencer (model CEQ 2000 DNA Analysis System, Beckman, Fullerton, CA). Both DNA strands were sequenced, and the reactions were carried out using a Dye Terminator cycle sequencing kit (Beckman Coulter). The sequencing of the D1/D2 domain of strain NBRC 1885 was carried out as described by Nakase et al. (2007). The sequences were initially aligned using the multiple alignment program Clustal X 1.83 (Thompson et al., 1997). A phylogenetic tree was constructed using the neighbor-joining method with the MEA version 4.0 software package (Kumar et al., 2004). *Schizosaccharomyces pombe* NRRL Y- 12796\(^a\) was used as an outgroup. Bootstrap analysis was performed from 1000 bootstrap replications (Felsenstein, 1995).

.Mikata, 1981), while strain SM12S02 was isolated from a Taiwanese forest soil sample that was collected in 2006. The isolation of strain SM12S02 was performed by the method described by Lee et al. (2008). The yeasts were grown on yeast extract–malt extract (YM) agar at 24 \(^\circ\)C for 3 days, followed by preservation on YM agar at 4 \(^\circ\)C and/or in the freezer at –70 \(^\circ\)C. The type strain of the species NBRC 1884\(^T\) ( = CBS 10887 = BCRC 23141\(^T\)) was deposited in the NITE Biological Resource Center (NBRRC), National Institute of Technology and Evaluation (NITE), Japan; Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands; and Bioresources Collection and Research Center (BCRC), Food Industry Research and Development Institute, Taiwan.
NBRC 1885 possesses a D1/D2 sequence identical to the other two strains, and all three strains exhibit similar physiological and morphological characteristics, indicating they are conspecific. Based on the morphological, physiological, biochemical, and molecular characteristics, we classified these three strains into the genus *Vanderwaltozyma*, because their D1/D2 sequences of the LSU rRNA genes are closely related to that of *V. yarrowii* (Kurtzman, 2003) and they demonstrate similar morphological characteristics compared with those of the genus.

In the neighbor–joining phylogenetic tree based on the D1/D2 domain of the LSU rRNA gene (Fig. 1), these three strains clustered with *V. yarrowii* and *V. polyspora*, confirming that the new species is a member of this genus. They were found to differ from their phylogenetically closest relatives, *V. yarrowii* and *V. polyspora*, by 7 and 14 nucleotide substitutions, respectively, in the D1/D2 domains of the LSU rRNA gene (Fig. 1). Further, strains NBRC 1884 and SM12S02 differed from these species by 26 and 84 nucleotide substitutions in the ITS region, respectively. These results clearly indicate that the three strains are representatives of a novel species of *Vanderwaltozyma*. In addition, the strains differ from the two currently recognized *Vanderwaltozyma* species in terms of their morphological and physiological characteristics, including carbon and nitrogen assimilation (Table 1). The two currently recognized *Vanderwaltozyma* species, *V. polyspora* and *V. yarrowii*, produce ascospores with smooth surfaces, whereas the strains examined in this study produce ascospores with warty surfaces. These strains can be distinguished from *V. yarrowii* in assimilation reactions with xylitol, D-glucono-1,5-lactone, and cycloheximide resistance (Table 1). Further, they can be easily separated from *V. polyspora* on the basis of carbon assimilation and other physiological characteristics (Table 1). Based on the evidence described above, a new species, *V. verrucispora*, is proposed.

**Latin diagnosis of *V. verrucispora* sp. nov.**

In medio liquido cum glucoso et peptono et extracto levidinis post dies 3 ad 25 °C, cellulae ovoideae vel ellipsoidae (1.5–4.8 μm × 3.2–6.7 μm), singulae aut binae, per gemmationem reproducentes. Post 1 mensem sedimentum formatur. Cultura in agaro cum glucoso et peptono et extracto levidinis post dies 7 ad 25 °C, cremea, butyrosa, glabra et nitida. In agaro farinae Zea mays post dies 10 ad 25 °C, mycelium et pseudomycelium nulla. Asci per transformationem cellularum vegetativarum diploidearum, 1–4 ascosporus continentes. Ascospores spheroidae vel
ellipsoideae (1.2–1.8 μm × 1.5–2.4 μm), tuberculatae. Ascosporeae ex ascis liberantur et agglutinantes post 1 mensem ad 25 °C. Glucosum et galactosum fermentantur, at non maltosum, methyl α-D-glucosidum, sucrosum, α,α-trehalosum, melibussum, lactosum, cellobiosum, melezitosum, raffinosum, inulimum, amyllum, nec D-xylosum. Glucosum, galactosum, glycerolum (infirme, variabile) et D-glucono-1,5-lactonum (infirme, variabile) assimilantur, at non 1-sorbosum, D-glucosaminum, D-ribosum, D-xylolsum, L-arabinosum, D-arabinosum, L-rhamnosum, sucrosum, maltosum, α,α-trehalosum, Methyl α-D-glucosidum, cellobiosum, salicinum, arbutinum, melibiosum, lactosum, raffinosum, melezitosum, inulimum, amyllum, erythritolum, ribotolum, xylotolum, L-arabinotolum, D-glucitolum, D-mannitolum, galactitolum, myo-inositolum, acidum 2-keto-D-gluconicum, acidum 5-keto-D-gluconicum, acidum D-gluconicum, acidum D-gluconuronicum, acidum D-galacturonicum, acidum Dl-lacticum, acidum succinicum, acidum citricum, methanolum, ethanolum, propanum 1,2 diolum, butane 2,3 diolum nec N-acetylglucosaminum. Natrium nitrosum, natrium nitricum, L-lysinum, ethylaminum, nec cadaverinum non assimilantur. Non crescit in substrato 10% sal/5% glucose continente. Non crescit in 50% glucose addito. Crescere potest in 0.1% cycloheximido. In 29 °C crescere potest at non in 30 °C. Materia amyloidea iodophila non format. Ureum non hydrolysatur. Diazonium caeruleum B est negativum. Guaninum et cytosinium in acidi deoxyribonucleati 27.2–27.6 mol%.

Typus stirpis NBRC 1884 (=CBS 10887 = BCRC 23141) isolatus ex leaf, Kagoshima Prefecture, Japan. NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), Japan; Bioreresources Collection and Research Center (BCRC), Food Industry Research and Development Institute, Taiwan; et Centraalbureau voor Schimmelcultures (CBS), Utrecht, the Netherlands, deposita est.

Description of V. verrucispora sp. nov.

Vanderwaltozyma verrucispora (ver.ru.ci spo’ra. L. adj. verrucispora, verrucose referring to the verrucous ascospores produced by the yeast species). After growth in YM broth at 25 °C for 3 days, the cells are ovoidal to ellipsoidal, 1.5–4.8 μm × 3.2–6.7 μm, single or in pairs (Fig. 2a). Vegetative reproduction is by multilateral budding. Sediment is present. After 7 days at 25 °C on YM agar, streak cultures are creamy, butyrous, smooth, and glistening. On Dalmau plate cultures on corn meal agar after 10 days at 25 °C, pseudomycelium and mycelium are absent. Sporulation occurs on YM agar, Fowell’s acetate agar, and malt extract agar after incubation at 25 °C for 7 days. Diploid cells are transformed directly into asci containing one to four spheroidal to ellipsoidal ascospores (1.2–1.8 μm × 1.5–2.4 μm) with warty surfaces (Fig. 2b and c). Glucose and galactose are fermented, but maltose, methyl α-D-glucoside, sucrose, α,α-trehalose, melibiose, lactose, cellobiose, melezitose, raffinose, inulin, starch, and D-xylose are not fermented. Only glucose, galactose, glycerol (weak, variable), and D-glucono-1,5-lactone (weak, variable) are assimilated. Other carbon and nitrogen compounds tested

![Fig. 2. Morphology of Vanderwaltozyma verrucispora NBRC 1884T as determined by light microscopy and scanning electron microscopy (SEM). The strain was cultivated in YM broth for 3 days at 25 °C (a). Ascospores produced on Fowell’s acetate agar after 7 days at 25 °C (b) and the surface of the ascospores under SEM (c). Scale bars: 10 μm in (a) and (b); 1 μm in (c).](image-url)
DNA was 27.2–27.6 mol%.

in this study, including L-sorbose, D-glucosamine, D-ribose, D-xylene, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, α,β-trehalose, methyl α-D-glucoside, cellobiose, salicin, arbutin, melibiose, lactose, raffinose, melezitose, inulin, starch, erythritol, ribitol, xylitol, L-arabinitol, D-glucitol, D-mannitol, galactitol, myo-inositol, 2-keto-D-gluconate, 5-keto-D-gluconate, D-gluconate, D-glucuronate, D-galacturonic acid, D-lactate, succinate, citrate, methanol, ethanol, propane 1,2 diol, butane 1,2 diol, N-acetylglucosamine, nitrate, nitrite, L-lysine, cadaverine, and creatine, are not assimilated. Growth on 50% or 60% glucose and 10% NaCl plus 5% glucose is negative. Growth occurs in the presence of 0.1% cycloheximide. Growth occurs at 29 °C but not at 30 °C. No starch-like substance is produced. Acid production on 0.1% cycloheximide. Growth occurs in the presence of 0.1% cycloheximide. Growth occurs at 29 °C but not at 30 °C.

The type strain of V. verrucispora, strain NBRC 1884T (= CBS 10887T = BCRC 23141T), was isolated from a partially decayed leaf in Japan in 1980.

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References


