FOUR NEW YEASTS FOUND IN JAPAN

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Four new yeasts, Pichia nonfermentans, Candida boleticola, Candida butyri, and Candida quercuum, were described on the basis of taxonomic characteristics commonly employed and the base composition in DNA.

During a survey on wild yeasts in Japan, 15 strains of hitherto undescribed yeasts were isolated from various sources and classified into 4 species. This paper describes these new yeasts.

MATERIALS AND METHODS

Isolation of yeasts. Yeasts were isolated from various sources and purified by the conventional streaking technique using yeast extract-malt extract (YM) agar plates. Source of cultures and date of isolation are shown in Table 1.

Determinative methods. Determinative methods used were mainly those described by WICKERHAM (1), LODDER and KREGER-VAN RIJ (2), and KREGER-VAN RIJ (3). Tests for maximum growth temperature and vitamin requirement followed the procedures described by KOMAGATA and NAKASE (4). Urease was detected by the method of SEELIGER (5).

Determination of DNA base composition. DNAs were isolated and purified by the procedures previously described (6). The DNA base composition (GC content) was calculated from the thermal denaturation temperature of DNA (Tm) according to the procedure of MARMUR and DOTY (7). Tm was measured by the apparatus described by YAMADA and KOMAGATA (8).

RESULTS AND DISCUSSION

1. Pichia nonfermentans NAKASE sp. nov.

Strain: AJ 4147

In medio liquido cellulae longae et tenuis, (2–3.5)×(5.5–55)μ, singulae, binae aut catenatae. Sedimentum formatur. Cultura in agaro (post unum mensem, 17°) glaucofusca, glabra aut crispulata, margine glabra. Pseudomy-

Growth in YM broth: After 3 days at 25°, growth is poor. Cells are elongate and slender, (2–3.5)×(8.5–55) μ, and occur singly, in pairs, or in chains. Only a sediment is formed. After one month at 17°, a trace of ring and a scanty sediment are present.

Growth on YM agar: After one month at 17°, the streak culture is light brownish gray, smooth or sometimes slightly wrinkled, dull-shining, butyrous, and has an entire margin.

Dalmau plate culture on potato-dextrose agar: Well-developed pseudomycelia are formed abundantly. Pseudomycelial cells are slender and often curved. The difference between pseudomycelial cells and blastospores is scanty.

### Table 1. Source of new yeasts.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Source</th>
<th>Date of isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pichia nonfermentans</em></td>
<td>AJ 4147</td>
<td>Soybean protein-manufacturing factory</td>
<td>Feb. 1962</td>
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<tr>
<td><em>Candida boleticola</em></td>
<td>AJ 4703</td>
<td>Fruiting body of <em>Astraeus hygrometricus</em></td>
<td>Oct. 1965</td>
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<tr>
<td></td>
<td>AJ 4704</td>
<td>Fruiting body of a mushroom</td>
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<td>AJ 4705</td>
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<td>AJ 4706</td>
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<td>AJ 4707</td>
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<td></td>
<td>AJ 4920</td>
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<td>Jun. 1966</td>
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<td></td>
<td>AJ 4921</td>
<td></td>
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<tr>
<td></td>
<td>AJ 4927</td>
<td>Fruiting body of <em>Cortinarius</em> sp.</td>
<td>Nov. 1966</td>
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<td><em>Candida butyri</em></td>
<td>AJ 4668</td>
<td>Butter</td>
<td>Aug. 1961</td>
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<tr>
<td></td>
<td>AJ 4669</td>
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<td></td>
<td>AJ 4671</td>
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<tr>
<td></td>
<td>AJ 4674</td>
<td>Onion</td>
<td>Apr. 1962</td>
</tr>
<tr>
<td><em>Candida quercum</em></td>
<td>AJ 4781</td>
<td>Exudate of <em>Quercus serrata</em></td>
<td>May 1965</td>
</tr>
</tbody>
</table>
Sporulation: Ascii are formed parthenogenetically. Ascospores are saturn-shaped, 1-4, usually 4 in the ascus.

Fermentation: Absent.

Assimilation of carbon compounds: D-Glucose, L-sorbose (latent), trehalose, ethanol, glycerol, and succinic acid are assimilated. D-Galactose, saccharose, maltose, lactose, cellobiose, melibiose, raffinose, melezitose, inulin, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, erythritol, adonitol, dulcitol, D-mannitol, D-sorbitol, α-methyl-D-glucoside, salicin, potassium gluconate, calcium 2-ketogluconate, DL-lactic acid, citric acid, and inositol are not assimilated.

Potassium nitrate is not assimilated as the sole nitrogen source.

Arbutin is not split.

Starch-like compounds are not produced.

Gelatin is not liquefied.

Maximum growth temperature is 34-35°C.

Biotin, pyridoxine, and thiamine are required at 25°C.

Urease is not detected on Christensen’s medium.

Source: See Table 1.

Although ascospores of this yeast often look round, especially when
Fig. 2. Pseudomycelia of *Candida boleticola* AJ 4703, Dalmau plate culture on potato-dextrose agar for 7 days at 25°C.

Fig. 3. Pseudomycelia of *Candida butyri* AJ 4568, Dalmau plate culture on potato-dextrose agar for 7 days at 25°C.

Fig. 4. Pseudomycelia of *Candida quercuum* AJ 4781, Dalmau plate culture on potato-dextrose agar for 7 days at 25°C.
pseudomycelial cells are diverted into asci, they are considered to be saturn-shaped (Fig. 1). Sometimes they also resemble those found in the genus *Wingea* (9, 10) but detailed examination could not be made due to relatively poor sporulation of this yeast. Ascospores of this species are formed parthenogenetically, and differ from *Wingea robertsii*, the only species described in the genus *Wingea*, in this point. Physiologically, this species resembles *Pichia membranaefaciens* in many respects (11). As shown in Table 2, the GC content of this species is much lower than that of *Pichia membranaefaciens* (12–14). The strain AJ 4147 is the type strain of this species. This strain was deposited in the Institute for Fermentation, Osaka, as IFO 1579.

2. *Candida boleticola* NAKASE *sp. nov.*

Strains: AJ 4703, 4704, 4705, 4706, 4707, 4920, 4921, and 4927


Growth in YM broth: After 3 days at 25°, cells are round, short oval to oval, or elongate, (2.5–5)×(2.5–14) μ, and occur singly, in pairs, or in chains. A ring and a sediment are present. Some strains also form thick pellicles.

Growth on YM agar: After one month at 17°, the streak culture is yellowish white to light brownish gray, smooth or delicately wrinkled, soft to butyrous, and has an entire or ciliate margin.

Dalmau plate culture on potato-dextrose agar: Pseudomycelia are primitive to well developed. They are often tree-like. In some strains, blastospores are oval and occur in clusters.

Sporulation: Absent.

Fermentation: D-Glucose is fermented. D-Galactose, saccharose, maltose, lactose, raffinose, and melibiose are not fermented.

Assimilation of carbon compounds: D-Glucose, D-galactose, L-sorbose (sometimes latent), trehalose, D-xyllose (sometimes latent), D-arabinose (some-
times latent), D-ribose (sometimes latent), ethanol, glycerol, erythritol, adonitol, D-mannitol, D-sorbitol, potassium gluconate (sometimes latent), calcium 2-ketogluconate, succinic acid, and citric acid are assimilated. Cellobiose and salicin are assimilated or not. Saccharose, maltose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, L-arabinose, L-rhamnose, dulcitol, a-methyl-D-glucoside, DL-lactic acid, and inositol are not assimilated.

Potassium nitrate is not utilized as the sole nitrogen source.

Arbutin is split or not.

Starch-like compounds are not produced.

Gelatin is not liquefied.

Maximum growth temperature is 34–38°C.

Biotin is required at 25°C. Thiamine is also required in some strains.

Urease is not detected on Christensen’s medium.

Source: See Table 1.

This yeast resembles Candida conglobata in many respects. Morphologically, this yeast forms somewhat shorter cells than Candida conglobata but this difference is very small since the cell shape of the yeast tends to elongate during the long period of preservation (2). Physiologically, these two species differ in the assimilation of only two carbon compounds; Candida conglobata assimilates L-arabinose but not citric acid, while Candida boleticola assimilates citric acid but not L-arabinose. A clear difference, however, was found in the GC content in DNA (Table 2). Candida boleticola had a GC content of 43.2–44.1% which is about 3–4% higher than that of Candida conglobata (40.2%). The specific epithet is derived from the Latin “boletus” meaning “mushroom”, and “cold” meaning “inhabit”. The strain AJ 4703 is the type strain of this species. This strain was deposited in the Institute for Fermentation, Osaka, as IFO 1570.
3. *Candida butyri* NAKASE *sp. nov.*

Strains: AJ 4668, 4669, 4670, 4671, and 4674


Growth in YM broth: After 3 days at 25°, cells are short oval, oval or elongate, (1.5–7.5) × (2.5–17.5) μ, usually occur in chains or in groups. A ring and a sediment are formed. After one month at 17°, a ring and a sediment are present.

Growth on YM agar: After one month at 17°, the streak culture is grayish white to white, smooth or wrinkled, mat, and has a ciliate margin.

Dalmau plate culture on potato-dextrose agar: Pseudomycelia develop abundantly. Blastospores are round to short oval, usually occur in chains or in verticils.

Sporulation: Absent.

Fermentation: D-Glucose and D-galactose (latent) are fermented. Some strains ferment saccharose and maltose latently and very weakly. Lactose, raffinosum, and melibiosum are not fermented.


Potassium nitrate is not utilized as the sole nitrogen source.

Arbutin is split.

Starch-like compounds are not produced.

Gelatin is not liquefied.

Maximum growth temperature is 38–40°.

Biotin and thiamine are required at 25°.
Urease is not detected on Christensen's medium.
Source: See Table 1.

Although the characteristics of this yeast agreed with the standard description of *Candida tenuis* by VAN UDEN and BUCKLEY (15), this yeast is clearly differentiated from the latter in the GC content in DNA. As shown in Table 2, *Candida butyri* showed a GC content of 34.4–34.9%. Meanwhile, STENDERUP and BAR (16) reported that *Candida tenuis* CBS 615 (type strain) had a GC content of 44%. Further, NAKASE and KOMAGATA (17) found that one strain of this species had a GC content of 43.2%. This strain (IFO 0716) assimilates L-rhamnose and potassium gluconate and differed from *Candida butyri* in this point. VAN UDEN and BUCKLEY (15) reported that assimilation reactions of several carbon compounds including L-rhamnose are variable in *Candida tenuis*. It is assumed that *Candida tenuis* studied by them contains heterogeneous members, and some of them may be included in *Candida butyri*. The specific epithet is derived from the Latin "butyrum" meaning "butter" since this yeast was first isolated from butter. The strain AJ 4668 is the type strain of this species. This strain was deposited in the Institute for Fermentation, Osaka, as IFO 1571.

4. *Candida quercuum* NAKASE sp. nov.

Strain: AJ 4781


Growth in YM broth: After 3 days at 25°, cells are long oval, elongate, or cylindrical, (1.5–4) × (2–7) μ, and occur singly, in pairs, or in chains. A ring and a sediment are formed. After one month at 17°, a ring and a sediment are present. Sometimes a thin pellicle is formed.

Growth on YM agar: After one month at 17°, the streak culture is grayish white, smooth, shining, soft, and has an entire margin.

Dalmau plate culture on potato-dextrose agar: Pseudomycelium develop abundantly. Pseudomycelial cells are often curved. Blastospores are oval to long oval or elongate, and occur singly, in chains, or in verticils.
Sporulation: Absent.

Fermentation: D-Glucose is slowly fermented. D-Galactose, saccharose, maltose, lactose, melibiose, and raffinose are not fermented.

Assimilation of carbon compounds: D-Glucose, saccharose, maltose, cellobiose, trehalose, melezitose, D-xyllose, ethanol, glycerol, D-mannitol, D-sorbitol, α-methyl-D-glucoside, salicin, potassium gluconate (latent), DL-lactic acid, succinic acid, and citric acid are assimilated. D-Galactose, lactose, L-sorbose, melibiose, raffinose, inulin, soluble starch, L-arabinose, D-arabinose, D-ribose, L-rhamnose, erythritol, adonitol, dulcitol, calcium 2-ketogluconate, and inositol are not assimilated.

Potassium nitrate is not utilized as the sole nitrogen source.

Arbutin is split.

Starch-like compounds are not produced.

Gelatin is not liquefied.

Maximum growth temperature: 39–40°C.

Biotin, pyridoxine, and thiamine are required at 25°C.

Urease is not detected on Christensen’s medium.

Source: See Table 1.

*Candida quercuum* resembles *Candida oregonensis* and *Candida solani* in several respects but differs from these species in the GC content in DNA. *Candida quercuum* showed a GC content of 38.3% (Table 2), while 48% (17–18) and 42% (19) of GC values were reported for *Candida oregonensis* and *Candida solani*, respectively. *Candida quercuum* is discriminated from *Candida oregonensis* in assimilation of glycerol, and citric acid, lack of assimilation of soluble starch, L-rhamnose, and adonitol, and higher maximum growth temperature, and from *Candida solani* in assimilation of D-mannitol, D-sorbitol, and citric acid, lack of assimilation of L-sorbose, and higher maximum growth temperature. The specific epithet “quercuum” shows the source of this yeast. The strain AJ 4781 is the type strain of this species. This strain was deposited in the Institute for Fermentation, Osaka, as IFO 1576.

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REFERENCES