Pichia cellobiosa, *Candida cariosilignicola*, and *Candida succiphila*, New Species of Methanol-Assimilating Yeasts

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In the course of a taxonomic study of methanol-assimilating yeasts, a number of isolates were obtained from various natural habitats in Japan, and from these three new species, *Pichia cellobiosa*, *Candida cariosilignicola*, and *Candida succiphila*, have been described. *P. cellobiosa* differs from the related species *Pichia castillae* with respect to fermentation and assimilation of sugars, biotin requirement for growth, and lower maximum temperature for growth. *C. cariosilignicola* differs from the related species *Torulopsis apicola* and *Torulopsis bombicola* with respect to assimilation of sugars and proton magnetic resonance spectra of cell wall mannans. *C. succiphila* is similar to *C. cariosilignicola* but differs from it with respect to fermentation and assimilation of sugars, lower maximum temperature for growth, higher deoxyribonucleic acid base composition, and proton magnetic resonance spectra of cell wall mannans. The type strains of these new species are KL 1 (=IAM 12481), KL 24 (=IAM 12484), and KL 30 (=IAM 12489), respectively. Cultures of the type strains have been deposited in the Institute of Applied Microbiology Culture Collection Center, University of Tokyo, Tokyo, Japan, and the Institute for Fermentation, Osaka, Japan.

Since Ogata et al. (16) reported a methanol-assimilating yeast in 1969, a number of such yeasts have been described (1-3, 8, 9, 11, 14, 15, 17, 18, 21, 23, 28). Hazeu et al. (7) examined methanol-assimilation in type strains preserved at the Centraalbureau voor Schimmelcultures and reported assimilation by the type strains of 15 species in the genera *Hansenula*, *Pichia*, *Torulopsis*, and *Candida*. However, the systematics of methanol-assimilating yeasts have not been studied in detail. Therefore, we attempted to isolate methanol-assimilating yeasts from a wide variety of natural sources in Japan in order to compare them taxonomically with the presently described methanol-assimilating yeast species. During the investigation, 10 of the new isolates were identified as belonging to three new species, which are named and described here. A taxonomic study on methanol-assimilating yeasts, including the new species named and described here, will be reported in the future.

MATERIALS AND METHODS

Isolation of yeasts. Yeasts were isolated from various sources by using a methanol medium containing the following: $(\text{NH}_4)_2\text{SO}_4$, 3 g; KH_2PO_4 , 4 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g; NaCl , 0.2 g; ferric citrate, 3 mg; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 3 mg; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.5 mg; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 mg; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.05 mg; yeast extract, 0.2 g; methanol, 10 ml; chloramphenicol, 50 mg; and distilled water, 1,000 ml. The pH was 4.5 or was adjusted to 6.6 with Na_2HPO_4 . About 1 g of source material was

inoculated into 10 ml of medium; incubation was at 30°C with shaking. The enrichment culture technique was used for isolation, and pure cultures were obtained by the conventional streaking technique. The strains isolated and their sources are listed in Table 1.

Characterization of yeasts. The methods used to characterize the isolates were primarily those reported by Wickerham (24) and Lodder (12). Vitamin requirements were determined by the method of Komagata and Nakase (10). Deoxyribonucleic acid was isolated and purified by the procedure of Cryer et al. (4), and deoxyribonucleic acid base compositions were calculated from thermal denaturation temperatures by the formula of Marmur and Doty (13). The ubiquinone systems were determined by the method of Yamada and Kondo (25). Isolations and purifications of mannans from yeast cells were carried out as previously reported by Gorin and Spencer (5). Proton magnetic resonance (PMR) spectra of the mannans were obtained by using a JNM-4H 100 spectrometer equipped with a temperature control unit; these spectra were determined from 0.4 ml of a D_2O solution containing 80 mg of mannan at 70°C, using 3-(trimethylsilyl)propane sulfonic acid (sodium salt) as an internal standard.

RESULTS AND DISCUSSION

Three strains (KL 1, KL 2, and KL 5) form hat-shaped ascospores and assimilate galactose, L-sorbose, cellobiose, and a considerable number of other sugars. They resemble *Pichia castillae* (19) in these characteristics but differ from it in that they ferment glucose and cellobiose, do not assimilate maltose, melibiose, or raffinose, re-

TABLE 1. Sources of the strains studied

Strain	Source
KL 1	Soil, Chiba Prefecture
KL 2	Soil, Chiba Prefecture
KL 5	Soil, Metropolitan Tokyo
KL 24	Decayed timbers, Yamanashi Prefecture
KL 14	Soil, Yamanashi Prefecture
KL 16	Sap of peach tree, Yamanashi Prefecture
KL 21	Sap of peach tree, Yamanashi Prefecture
KL 22	Peach, Yamanashi Prefecture
KL 30	Sap of peach tree, Yamanashi Prefecture
KL 31	Peach, Yamanashi Prefecture

quire biotin for growth, and have a lower maximum temperature for growth. Since most *Pichia* species with hat- or Saturn-shaped ascospores possess Q8 (26) and most of the species in the genus *Hansenula* possess Q7 (26), strains KL 1, KL 2, and KL 5 seem to be unique in that they possess a ubiquinone system composed of both Q8 (major component) and Q7 (minor component) and thus appear to be intermediate between *Pichia* and *Hansenula*. These three strains are here regarded as belonging to a new species, for which the name *Pichia cellobiosa* is proposed.

Strain KL 24 is asporogenous, has globose or oval cells, does not form pseudomycelia or true mycelia, and does not produce manifest carotenoid pigments; furthermore, it does not assimilate inositol and produces no starch. These characteristics fit those of the genus *Torulopsis* Berlese (22). Yarrow and Meyer (27) proposed an amendment of the description of the genus *Candida* Berkhout to allow for nonhyphal species. In accordance with the amended description, strain KL 24 was classified in the genus *Candida*. With respect to the fermentation of sugars, strain KL 24 resembles *Torulopsis apicola* (6) and *Torulopsis bombycolia* (20), but it differs from them by its assimilation of trehalose, D-xylose, D-arabinose, and L-arabinose and its PMR spectrum of cell wall mannan (20). Strain KL 24 is here regarded as a member of a new species, *Candida cariosilignicola*.

Six strains (KL 14, KL 16, KL 21, KL 22, KL 30, and KL 31) resemble strain KL 24 in morphological and cultural characteristics, but they differ from it and other related strains in the fermentation and assimilation of sugars. These six strains cannot grow at 37°C, their deoxyribonucleic acid base compositions are about 5% higher than that of strain KL 24, and the PMR spectra of their cell wall mannans are different from the PMR spectrum of strain KL 24 cell

wall mannan. Mating was not found in combinations of these six strains, which are here placed in a new species, *Candida succiphila*. Descriptions of these three newly recognized species follow.

***Pichia cellobiosa* sp. nov.** (cel.lo.bi.o'sa. M.L. adj. *cellobiosus* pertaining to cellobiose, a sugar derived from cellulose.) This description is based on strains KL 1, KL 2, and KL 5, which possess the same characteristics.

Latin diagnosis. In medio liquido cellulae subovoideae aut ovoideae, (1.8–4.5) × (2.7–5.4) μm, singulae aut binae. Sedimentum formatum. Cultura in agar flavoglaucia, glabra, butyrosa, margine undulato. Pseudomycelium non formatum. Asci inconjugati fiunt, habentes 3 aut 4 sporos pileiformos in quoque asco, asci rumpuntur. Species homothallica.

D-Glucosum et cellobiosum (lente) fermentantur at non D-galactosum, saccharosum, maltosum, lactosum, raffinose, melibiosum, nec trehalosum.

D-Glucosum, D-galactosum, L-sorbose, cellobiosum, trehalosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, methanolum, ethanolum, glycerolum, erythritolum, ribitolum, galactitolum, mannitolum, glucitolum, salicinum, acidum succinicum (variabile), et acidum citricum (variabile) assimilantur at non saccharosum, maltosum, lactosum, melibiosum, raffinose, melezitolum, inulinum, amyllum solubile, L-rhamnosum, α-methyl-D-glucosidum, DL-acidum lacticum, nec inositolum.

Nitras kalicus non assimilatur.

Biotinae necessariae ad crescentiam.

Maxima temperatura crescentiar: 35–36°C.

Proportio molaris G + C in acido deoxyribonucleico: 36.9 mol%.

Typus isolatus ex solum Chibaensis, depositus in Institute of Applied Microbiology Culture Collection Center, University of Tokyo (IAM 12481), et Institute for Fermentation, Osaka, Japan (IFO 1909).

Description. Growth in glucose-yeast extract-peptone water: After 3 days at 25°C, the cells are oval or ovoid, 1.8 to 4.5 by 2.7 to 5.4 μm (Fig. 1A). A pellicle is not formed.

Growth on YM agar: After 1 month at 20°C, a streak culture is brownish white, smooth, and butyrous and has an undulate margin.

Growth on yeast morphology agar: After 1 month at 20°C, a streak culture is yellowish white, smooth with slight radial striations, and butyrous and has an undulate margin.

Dalmau plate culture on potato dextrose agar: Pseudomycelia are not formed either aerobically or anaerobically.

Formation of ascospores: Ascospores are formed readily on V-8 vegetable agar and YM

FIG. 1. Cells of *P. cellobiosa*, *C. cariosilignicola*, and *C. succiphila*. (A) Vegetative cells of *P. cellobiosa* grown in glucose-yeast extract-peptone water for 3 days at 25° C. (B) Ascospores of *P. cellobiosa* grown on YM agar for 7 days at 25° C. Arrow indicates hat-shaped ascospores. (C) Vegetative cells of *C. cariosilignicola* grown in glucose-yeast extract-peptone water for 3 days at 25° C. (D) Vegetative cells of *C. succiphila* grown in glucose-yeast extract-peptone water for 3 days at 25° C. The bars represent 10 μ m in all illustrations.

agar after 5 to 7 days at 25° C. Strongly sporulating cultures appear brownish red, presumably because of the pigmentation of the ascospores. Ascus formation is usually preceded by the fusion of independent cells, which frequently form conjugation tubes, or less frequently by somatogamous autogamy. The asci contain three to four rather small hat-shaped ascospores with short brims (Fig. 1B). The asci rupture at maturity, releasing the spores soon after formation. The strains examined are homothallic.

Fermentation: D-Glucose and cellobiose (latent) are fermented. D-Galactose, saccharose, maltose, lactose, raffinose, and melibiose are not fermented.

Assimilation of carbon compounds: D-Glucose, D-galactose, L-sorbose, cellobiose, trehalose, D-xylose, L-arabinose, D-arabinose, D-ribose, methanol, ethanol, glycerol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, and salicin are assimilated. Assimilations of succinate and citrate are variable. Saccharose, maltose, lactose, melibiose, raffinose, melezitose, inulin, soluble

starch, L-rhamnose, α -methyl-D-glucoside, DL-lactate, and inositol are not assimilated.

Assimilation of potassium nitrate: Negative.

Growth in vitamin-free medium: Negative. Biotin is required for growth.

Growth on 50% (wt/wt) glucose yeast extract agar: Negative.

Sodium chloride tolerance: Tolerates 9 to 10% NaCl.

Maximum temperature for growth: 35 to 36° C.

Production of starchlike substance: Negative.

Gelatin liquefaction: Negative.

Urease and extracellular deoxyribonuclease activity: Negative.

Guanine plus cytosine content of nuclear deoxyribonucleic acid: 36.9 mol%.

Ubiquinone system: Q8 (minor component, Q7).

PMR spectrum of cell wall mannan: Group 9-d of Spencer and Gorin (Fig. 2).

Type strain: KL 1, which was isolated in 1977 from soil from Chiba Prefecture, Japan. Cultures

FIG. 2. PMR spectra of cell wall mannans. (A) *P. cellobiosus*. (B) *C. cariosilignicola*. (C) *C. succiphila*.

of this strain have been deposited in the Institute of Applied Microbiology Culture Collection Center, University of Tokyo, with the accession number 12481 and in the Institute for Fermentation, Osaka, under the number 1909.

***Candida cariosilignicola* sp. nov.** (ca.ri.o.si.lig.ni' co.la. L. adj. *cariosus* decayed, rotted; L. noun *lignum* wood; L. *-cola* dweller; M.L. fem. n. *cariosilignicola* decayed-wood dweller or inhabitant.) The description of this species is based on a single strain, KL 24.

Latin diagnosis. In medio liquido cellulae rotundae aut ovoideae, (1.8–2.7) × (1.8–3.0) μm, singulae, binae, aut in catenis brevis. Sedimentum formatur. Cultura in agar flavoglaucula, glabra, butyrosa, vel semimucosa, nitida, margine undulato. Pseudomycelium non formatur.

D-Glucosum, saccharosum, et raffinsum (1/3) fermentantur at non D-galactosum, maltosum, lactosum, cellobiosum, melibiosum, nec trehalosum.

D-Glucosum, L-sorbose, saccharosum, trehalosum, raffinsum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, methanol, ethanol, glycerol, erythritol, ribitol, galactitol (tarde et exigue), D-mannitol, D-glucitol, et acidum citricum assimilantur at non D-galactosum, maltosum, cellobiosum, lactosum, melibiosum, melezitum, inulinum, amyllum solubile, α-methyl-D-glucosidum, salicinum, DL-acidum lacticum, acidum succinicum, nec inositolum.

Nitras kalicus assimilatur.

Biotinae necessariae ad crescentiam.

Maxima temperatura crescentiar: 41–42°C.

Proportio molaris G + C in acido deoxyribonucleico: 35.1 mol%.

Typus isolatus ex calinoso lignum Yamanash-

ensis, depositus in Institute of Applied Microbiology Culture Collection Center, University of Tokyo (IAM 12484), et Institute for Fermentation, Osaka (IFO 1910).

Description. Growth in glucose-yeast extract-peptone water: After 3 days at 25°C, the cells are globose or spherical, 1.8 to 2.7 by 1.8 to 3.0 μm (Fig. 1C). A pellicle is not formed.

Growth on YM agar and yeast morphology agar: After 1 month at 20°C, a streak culture is yellowish white, smooth, butyrous to slightly mucoid, and glistening and has an undulate margin.

Dalmau plate culture on potato dextrose agar: Pseudomycelia are not formed either aerobically or anaerobically.

Formation of ascospores: Ascospores are not formed on YM agar, Gorodkova agar, V-8 vegetable agar, or Kleyn acetate agar.

Fermentation: D-Glucose, saccharose, and raffinose (1/3) are fermented. D-Galactose, maltose, cellobiose, lactose, and melibiose are not fermented.

Assimilation of carbon compounds: D-Glucose, L-sorbose, saccharose, trehalose, raffinose, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, methanol, ethanol, glycerol, erythritol, ribitol, galactitol (slow and weak), D-mannitol, D-glucitol, and citrate are assimilated. D-Galactose, maltose, cellobiose, lactose, melibiose, melezitose, inulin, soluble starch, α-methyl-D-glucoside, salicin, DL-lactate, succinate, and inositol are not assimilated.

Assimilation of potassium nitrate: Positive.

Growth in vitamin-free medium: Negative. Biotin is required for growth.

Growth on 50% (wt/wt) glucose yeast extract agar: Negative.

Sodium chloride tolerance: Tolerates 6 to 7% NaCl.

Maximum temperature for growth: 41 to 42°C.

Production of starchlike substance: Negative.

Gelatin liquefaction: Negative.

Urease and extracellular deoxyribonuclease activity: Negative.

Guanine plus cytosine content of nuclear deoxyribonucleic acid: 35.1 mol%.

Ubiquinone system: Q7.

PMR spectrum of cell wall mannan: Group 9-b of Spencer and Gorin (Fig. 2).

Type strain: KL 24, which was isolated in 1977 from decayed timber in Yamanashi Prefecture, Japan. Cultures of this strain have been deposited in the Institute of Applied Microbiology Culture Collection Center, University of Tokyo, with the accession number 12484 and in the Institute for Fermentation, Osaka, under the number 1910.

***Candida succiphila* sp. nov.** (suc.ci.phi'la. L. noun *succus* sap; Gr. adj. *philus* loving; M.L. adj. *succiphilus* sap-loving.) The description of this species is based on strains KL 14, KL 16, KL 21, KL 22, KL 30, and KL 31, which possess the same characteristics.

Latin diagnosis. In medio liquido cellulae rotundae aut ovoideae, (1.8–2.7) × (1.8–3.0) μm, singulae, binae aut in catenis brevis. Sedimentum formatur. Cultura in agar flavoglaucia, glabra, butyrosa, nitida vel seminitida, margine undulato. Pseudomycelium non formatur.

D-Glucosum, D-galactosum, et cellobiosum (lente) fermentantur at non saccharosum, maltosum, lactosum, raffinose, melibiosum, nec trehalosum.

D-Glucosum, D-galactosum, L-sorbosum, cellobiosum, trehalosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, methanol, ethanol, glycerolum, erythritolum, ribitol, galactitol (lente aut exigue), D-glucitol, et salicinum assimilantur at non saccharosum, maltosum, lactosum, melibiosum, raffinose, melezitose, inulinum, amyllum solubile, α-methyl-D-glucosidum, DL-acidum lacticum, acidum succinicum, acidum citricum, nec inositolum.

Nitras kalicus non assimilatur.

Biotinae necessariae ad crescentiam.

Maxima temperatura crescentiar: 35–36°C.

Proportio molaris G + C in acido deoxyribonucleico: 40.9 mol%.

Typus isolatus ex cerasus stemma Yamanashiensis, depositus in Institute of Applied Microbiology Culture Collection Center, University of Tokyo (IAM 12489), et Institute for Fermentation, Osaka (IFO 1911).

Description. Growth in glucose-yeast extract-peptone water: After 3 days at 25°C, cells are globose or spherical, 1.8 to 2.7 by 1.8 to 3.0 μm (Fig. 1D). A pellicle is not formed.

Growth on YM agar and yeast morphology agar: After 1 month at 20°C, a streak culture is yellowish white, smooth, butyrous, and slightly glistening with an undulate margin.

Dalmau plate culture on potato dextrose agar: Pseudomycelia are not formed either aerobically or anaerobically.

Formation of ascospores: Ascospores are not formed on YM agar, Gorodkova agar, V-8 vegetable agar, or Kleyn acetate agar.

Fermentation: D-Glucose, D-galactose, and cellobiose (latent) are fermented. Saccharose, maltose, lactose, raffinose, and melibiose are not fermented.

Assimilation of carbon compounds: D-Glucose, D-galactose, L-sorbose, cellobiose, trehalose, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, methanol, ethanol, glycerol, erythritol, ribitol, galactitol (latent or weak), D-mannitol, D-glucitol, and salicin are assimilated. Saccharose, maltose, lactose, melibiose, raffinose, melezitose, inulin, soluble starch, α-methyl-D-glucoside, DL-lactate, succinate, citrate, and inositol are not assimilated.

Assimilation of potassium nitrate: Negative.

Growth in vitamin-free medium: Negative. Biotin is required for growth.

Growth on 50% (wt/wt) glucose yeast extract agar: Negative.

Sodium chloride tolerance: Tolerates 7 to 8% NaCl.

Maximum temperature for growth: 35 to 36°C.

Production of starchlike substance: Negative.

Gelatin liquefaction: Negative.

Urease and extracellular deoxyribonuclease activity: Negative.

Guanine plus cytosine content of nuclear deoxyribonucleic acid: 40.9 mol%.

Ubiquinone system: Q7.

PMR spectrum of cell wall mannan: Group 9-a of Spencer and Gorin (Fig. 2).

Type strain: KL 30, which was isolated in 1977 from the sap of a peach tree in Yamanashi Prefecture, Japan. Cultures of this strain have been deposited in the Institute of Applied Microbiology Culture Collection Center, University of Tokyo, with the accession number 12489 and in the Institute for Fermentation, Osaka, under the number 1911.

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REPRINT REQUESTS

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