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Three new species of anamorphic yeasts phenotypically and phylogenetically related to *Candida mesenterica*: the description of *Candida fungicola* sp. nov., *Candida sagamina* sp. nov., and *Candida fukazawae* sp. nov. isolated from fruit bodies of mushrooms

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Four strains of anamorphic yeasts isolated from fruit bodies of mushrooms collected in Japan were found to represent three new species of the genus *Candida*. These species resemble *Candida mesenterica* in characteristics commonly employed in the classification of yeasts. On the basis of DNA-DNA reassociation, however, they were clearly distinguished from *C. mesenterica* and from one another. Three new species, *Candida fungicola*, *Candida sagamina*, and *Candida fukazawae*, are proposed for these yeasts. The analysis of SSU rDNA sequences suggested that these three species were closely related to each other and to *C. mesenterica* and *C. suecica*.

Key Words——*Candida fukazawae*; *Candida fungicola*; *Candida sagamina*; new yeast species; SSU rDNA sequence.

In the course of a survey of yeasts living in the natural environment in Japan, four strains of undescribed yeasts were isolated from fruit bodies of mushrooms which were collected in the Tanzawa Mountains, Kanagawa Pref. They showed characteristics similar to *Candida mesenterica* (Geiger) Diddens & Lodder but not identical. DNA-DNA reassociation experiments revealed that these strains represent three heretofore undescribed species in the genus *Candida*. This paper describes these new species and discusses their phylogenetic positions among the species of *Candida*.

Materials and Methods

Strains employed The strains employed in the present study were isolated from fruit bodies collected at Mt. Tonotake in the Tanzawa Mountains, Kanagawa Pref., Japan, in July 1966, by direct streaking on YM agar (Difco Labs, Detroit, USA) plates containing 100 μm/ml of chloramphenicol and 2 mg/ml of sodium propionate. The pH of the medium was adjusted to 4.5. Authentic strains of *Candida mesenterica* including the type strain were employed for comparative study with the isolates. Strains employed are shown in Table 1. *Candida suecica* Rodrigues de Miranda & Norkrans JCM 7530 was also employed for molecular phylogenetic analysis.

Traditional taxonomic criteria Most of the methods employed for the examination of morphological, physiological, and biochemical characteristics were those described by van der Walt and Yarrow (1984). Assimilation of nitrogen compounds was investigated on solid media with starved inocula. Vitamin requirements were investigated according to Komagata and Nakase (1967) with starved inocula. The maximum growth temperature was determined in YM broth using metal block baths.

Chemotaxonomic criteria Extraction and purification of ubiquinones were carried out according to Nakase and Suzuki (1986) using cells harvested in the stationary phase. Ubiquinone isoprenologues were identified by high performance liquid chromatography (HPLC). DNAs were isolated and purified according to Nakase and Suzuki (1985). DNA base composition was analyzed by HPLC after the hydrolysis of DNA with nuclease and phosphatase as described by Hamamoto and Nakase (1995). DNA-DNA reassociation experiments were carried out by the membrane filter technique reported by Kaneko (1982) as described by Hamamoto and Nakase (1995). Cellular monosaccharides were analyzed by the

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Table 1. Strains employed.

Species	Strain	Source or other designations
Candida sp.	AJ 4917	Fruit body of a mushroom*
〃	AJ 4918	〃
〃	AJ 4919	〃
〃	AJ 1922	〃
Candida mesenterica	IFO 0969	→JCM 8892
〃	CBS 602^T	→JCM 7531
〃	CBS 2096	→JCM 8899
〃	IFO 1210	→JCM 8893
〃	CBS 2095	→JCM 10566
〃	CBS 6299	→JCM 8901
〃	CBS 2210	→JCM 8900
〃	IFO 1299	→JCM 10567 (=CBS 2094)
〃	IFO 1302	→JCM 10568 (=IBL 3331)

* collected in July 1966 at Mt. Tonotake in the Tanzawa Mountains, Kanagawa Pref., Japan.
^{T} type strain.

method reported previously (Suzuki and Nakase, 1988).

Extraction and purification of polysaccharide and determination of component monosaccharides were carried out according to the procedures reported in a previous paper (Suzuki et al., 1992). Yeasts were cultivated in YM broth (Difco Labs) on a rotary shaker and harvested by centrifugation at the logarithmic phase. After washing with purified water, the cells were suspended in 5 volumes of 2% KOH solution. Extraction was carried out by heating the cell suspensions at 100°C for 2 h. The succeeding extraction and purification of polysaccharides followed the method of Gorin and Spencer (1970). Component sugars were analyzed by HPLC as described by Suzuki and Nakase (1988). The proton magnetic resonance (PMR) spectra were determined in deuterium oxide solution at 70°C with a JNM-GX 500 MHz nuclear magnetic resonance spectrometer (JEOL Ltd, Tokyo, Japan) as described previously (1992).

Molecular phylogengetic analysis The nucleotide sequences of small subunit ribosomal DNA (SSU rDNA) containing the internal transcribed spacer (ITS) region was directly determined using PCR products according to the method of Sugita and Nakase (1999). The PCR product was sequenced using an *ALF*express DNA Sequencer (Pharmacia Biotech) and a SequiTherm Long Read Cycle Sequencing Kit (Epicentre Technologies, Wisconsin, USA). Primers for amplifying and sequencing of rDNAs were the same as those employed by Sugita and Nakase (1999). The sequences obtained have been deposited at the DDBJ data library with following accession numbers: *Candida fungicola* JCM 10142 (SSU rDNA, AB027754; ITS1/5.8S/ITS2, AB028031), *Candida sagamina* JCM 10144 (SSU rDNA, AB 027755; ITS1 /5.8S/ITS2, AB028032), *Candida fukazawae* JCM 1614 (SSU rDNA, AB027756; ITS1/5.8S/ITS2, AB028033), *C. mesenterica* JCM 7531 (ITS1, AB028029), and *C. suecica* JCM 7530 (ITS1, AB028030). The sequences were aligned with the reference DNA sequences using the Clustal W program. The phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987), and the distances between the sequences were calculated using Kimura's two parameter model (Kimura, 1980). Sites where gaps existed in any of the sequences were excluded.

Results and Discussion

Morphological, physiological and biochemical charactersitics Four strains isolated from fruit bodies of mushrooms produced no sexual stages. They reproduced by multilateral budding, produced well-developed pseudomycelia, and did not produce any characteristic conidia. They did not produce carotenoid pigments, amyloid substances, or excess amount of acids. Urease and DBB color reactions were negative. These characteristics suggest that the strains belong to the genus *Candida*.

The four strains isolated from mushrooms and nine strains of *C. mesenterica* including the type strain demonstrated similar physiological and biochemical charactersitics to each other. All of them assimilated ammonium sulphate, ethylamine hydrochloride, L-lysine, and cadaverine dihydrochloride but not nitrate or nitrite as a source of nitrogen, and required biotin for their growth. However, they showed several differences in the assimilation of carbon compounds and the maximum growth temperature (Table 2) and alcoholic fermentative ability. *Candida* sp. AJ 4917 and AJ 4918 assimilated melezitose but not L-sorbose, D-xylose, ethanol, and erythritol, had maximum growth temperatures of 28–29°C and 29–30°C, respectively, and demonstrated alcoholic fermentative ability. *Candida* sp. AJ 4919 assimilated melezitose, D-xylose, and ethanol but not L-sorbose and erythritol, had a maximum growth temperature of 29–30°C, and did not demonstrate alcoholic fermentative ability. *Candida* sp. AJ 4922 assimilated D-xylose (delayed), ethanol (delayed), and erythritol but not L-sorbose and melezitose, had a high maximum growth temperature of 36–37°C, and demonstrated alcoholic fermentative ability. Nine strains of *C. mesenterica* assimilated L-sorbose, ethanol, and erythritol and showed variable assimilation of melezitose and D-xylose. Their maximum growth temperatures were below 28°C. According to Meyer et al. (1998), glucose fermentation of this species is negative or slow. However, glucose fermentation was not detected in the present experiment.

Cellular monosaccharides and PMR spectra of mannans The thirteen strains examined in the present study had glucose and mannose as neutral monosaccharides in whole cell hydrolysates.

The patterns of H-1 regions of PMR spectra of alkali-extracted purified mannans were separated into six types (Fig. 1). The spectra of mannans from strains of *C. mesenterica* had common signals of 5.05–5.06, 5.09–5.10, 5.24 and 5.26 ppm, and were separated into three groups, IV, V, and VI, depending on the presence of additional signals of 4.83–4.84, 4.90, 5.05–5.06 and 5.14–

Table 2. Assimilation of carbon compounds and maximum growth temperatures of *Candida mesenterica* and related species.

Species	Strain	Glucose	Galactose	L-Sorbose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Melibiose	Raffinose	Melezitose	Inulin	Sol. starch	D-Xylose	L-Arabinose	D-Arabinose	D-Ribose	L-Rhamnose	Ethanol	Glycerol	Erythritol	Ribitol	Galactitol	Mannitol	Dulcitol	α-Me-D-glucoside	Salicin	Guluco-δ-lactone	2-Ketogluconic acid	5-Ketogluconic acid	DL-Lactic acid	Succinic acid	Citric acid	Inositol	D-Glucuronic acid	D-Galacturonic acid	Maximum growth temp. (°C)
Candida sp.	AJ 4917	+	−	−	+	+	+	+	−	−	−	+	−	+	−	−	−	w	−	−	+	−	+	−	+	+	+	+	d/−	+	−	−	+	+	−	−	−	28–29
〃	AJ 4918	+	−	−	+	+	+	+	−	−	−	+	−	+	−	−	−	d/dw	−	−	+	−	+	−	+	+	+	+	d/−	+	−	−	+	+	−	−	−	29–30
〃	AJ 4919	+	−	−	+	+	+	+	−	−	−	+	−	+	+	−	+/−	−	−	+	+/s	−	+	−	+	+	+	+	+	+	−	−	+	+/w	−	−	−	29–30
〃	AJ 4922	+	−	−	+	+	+	+	−	−	−	−	−	+	d	−	−	d	−	d	d	+	+	−	+	+	+	+	+	+	−	−	+	w	−	−	−	36–37
C. mesenterica	IFO 0969	+	−	+	+	+	+	+	−	−	−	d	−	w	dw	−	−	+	−	+	+	+	+	−	+	−	+	+	+	+	−	−	+	w	−	−	−	25>
〃	CBS 602ᵀ	+	−	+	+	+	+	+	−	−	−	d	−	w	−	−	−	+	−	+	+	+	+	−	+	+	+	+	+	+	−	−	+	w	−	−	−	25>
〃	CBS 2096	+	−	+	+	+	+	+	−	−	−	+	−	w	dw	−	−	+	−	+	+	+	+	−	+	+	+	+	+	+	−	w	w	w	−	−	−	26–27
〃	IFO 1210	+	−	+	+	+	+	+	−	−	−	−	−	w	s	−	−	w	−	+	+	+	+	−	+	+	+	+	+	+	−	−	+	w	−	−	−	27–28
〃	CBS 2095	+	−	+	+	+	+	+	−	−	−	−	−	w	−	−	−	d	−	+	+	+	+	−	+	+	+	+	−	+	−	d	s	s	−	−	−	27–28
〃	CBS 6299	+	−	+	+	+	+	+	−	−	−	w	−	s	−	−	−	+	−	+	+	+	s	−	+	+	+	+	w	+	−	w	s	w	−	−	−	27–28
〃	CBS 2210	+	−	+	+	+	+	+	−	−	−	w	−	w	s	−	−	−	−	+	+	+	+	−	+	+	+	+	+	+	−	w	+	w	−	−	−	25>
〃	IFO 1299	+	−	+	+	+	+	+	−	−	−	dw	−	w	−	−	−	+	−	+	+	+	+	−	+	+	+	+	dw	+	−	−	+	+	−	−	−	27–28
〃	IFO 1302	+	−	+	+	+	+	+	−	−	−	dw	−	w	−	−	−	d	−	+	+	+	d	−	+	+	+	+	dw	+	−	w	+	w	−	−	−	27–28

+, positive; − negative, s, slow; w, wesk; d, delayed; dw, delayed and weak

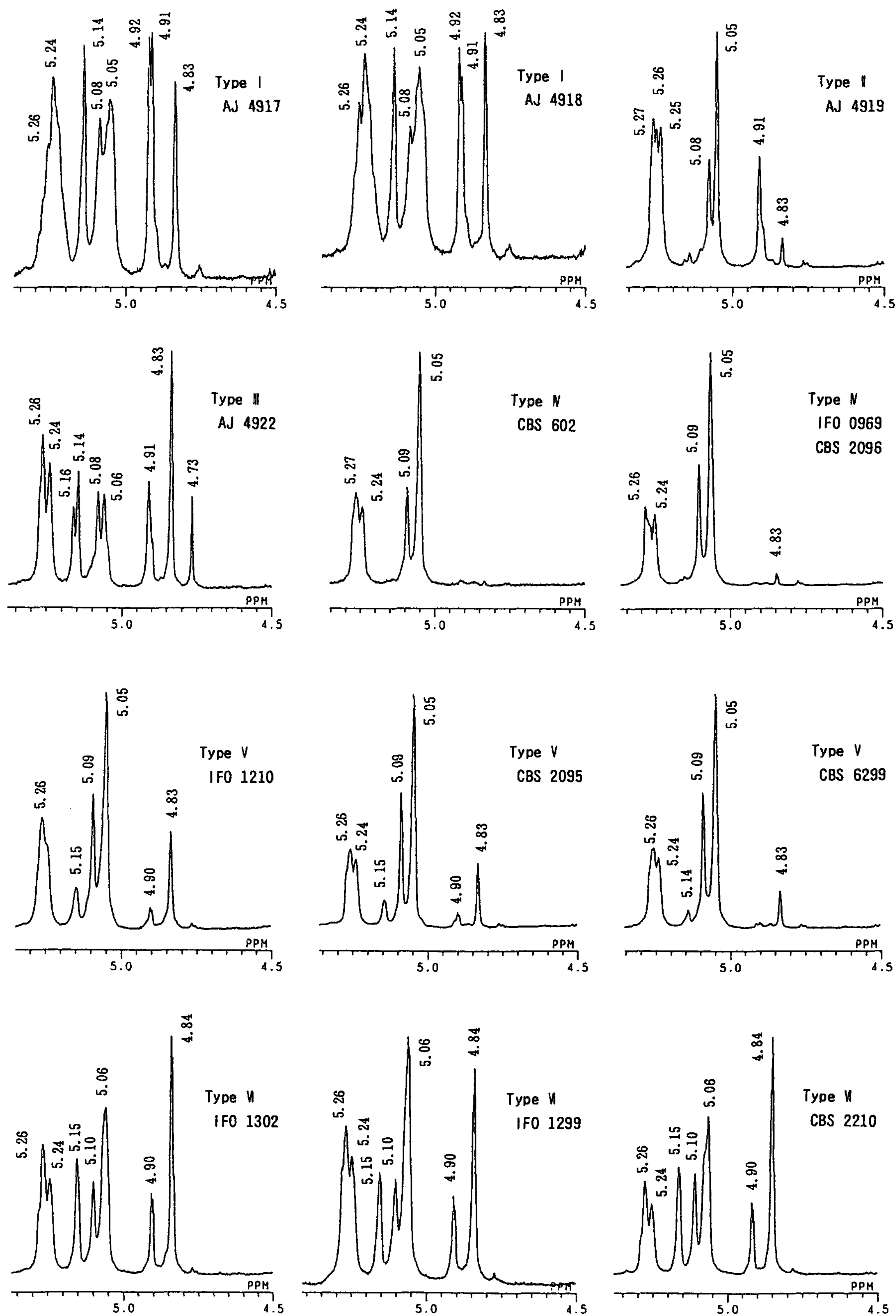

Fig. 1. PMR spectra (H-1 region) of mannans of *Candida mesenterica* and related yeasts. Arabic numerals indicate chemical shift of each signal (ppm).

5.15ppm, and their relative intensities.

The PMR spectra of mannans of *Candida* sp. AJ 4917 and AJ 4918 (type I) were similar to each other and clearly differentiated from those of the other strains examined. The mannan PMR spectrum of *Candida* sp. AJ 4922 (type II) was also differentiated from those of the remaining strains. The mannan spectrum of *Candida* sp. AJ 4919 (type III) resembled the type V spectrum of *C. mesenterica*, but the relative intensities of signals of 4.83 and 4.90 ppm were quite different (Fig. 1).

Uniquinones In all strains examined, Q-9 was the major component of ubiquinones and, accounting for 77.9–95.5 mol% of isoprenologues; and Q-8 was the minor component, accounting for 2.9–18.5 mol% (Table 3). Very small amount of Q-6 and Q-7 were detected in all strains.

DNA base composition and DNA homology The mol% G+C of DNAs of strains examined distributed in the range from 38.5 to 45.1 (Table 4). *Candida* sp. AJ 4922 had a high G+C of 45.1 mol% and was clearly differentiated from other strains in this respect. The remaining strains showed G+C values in the range of

Table 3. PMR spectral type of mannans and molar ratio of ubiquinones in *Candida mesenterica* and related species.

Species	Strain	PMR spectral type*	Molar ratio of ubiquinones (%)				
			Q-6	Q-7	Q-8	Q-9	Q-10
Candida sp.	AJ 4917	I	tr	tr	3.6	94.2	tr
〃	AJ 4918	I	tr	tr	2.9	95.5	tr
〃	AJ 4919	II	tr	1.0	4.3	93.8	tr
〃	AJ 4922	III	1.1	tr	11.8	85.9	tr
Candida mesenterica	IFO 0969	IV	tr	tr	9.5	90.5	tr
〃	CBS 602ᵀ	IV	tr	tr	6.9	90.3	tr
〃	CBS 2096	IV	tr	tr	11.1	87.0	tr
〃	IFO 1210	V	tr	1.1	11.7	85.6	tr
〃	CBS 2095	V	tr	1.2	13.2	85.1	tr
〃	CBS 6299	V	tr	1.4	15.6	81.6	tr
〃	CBS 2210	VI	1.0	2.6	18.5	77.9	tr
〃	IFO 1299	VI	tr	tr	9.7	88.3	tr
〃	IFO 1302	VI	tr	1.3	9.9	87.8	tr

* PMR spectral type of mannans: See Fig. 1

Table 4. DNA homology among strains of *Candida mesenterica* and related species.

Species	Strain	Mol% G+C	% Relative binding of labeled DNA from				
			C. mesenterica		*Candida* sp.		
			CBS 602ᵀ	CBS 2210	AJ 4917	AJ 4919	AJ 4922
Candida sp.	AJ 4917	39.4	20	18	100	25	23
〃	AJ 4918	38.5	15	22	90	21	21
〃	AJ 4919	40.5	18	19	24	100	22
〃	AJ 4922	45.1	22	21	26	26	100
C. mesenterica	IFO 0969	41.8	84	82	25	28	21
〃	CBS 602ᵀ	43.1	100	103	16	17	18
〃	CBS 2096	42.4	96	102	28	29	28
〃	IFO 1210	42.1	89	102	23	26	26
〃	CBS 2095	42.6	84	92	24	23	21
〃	CBS 6299	42.4	104	101	31	28	31
〃	CBS 2210	42.6	83	100	26	23	20
〃	IFO 1299	43.0	86	102	28	25	28
〃	IFO 1302	42.7	82	96	29	21	23
C. parapsilosis	JCM 1785ᵀ		13	20	21	20	18
Calf thymus			3	8	6	5	6

ᵀ type strain

38.5–43.0 mol%, so the grouping of strains is difficult based on this property.

DNA-DNA hybridization experiments clearly demonstrated that the strains examined were classified into four different species (Table 3). *Candida* sp. AJ 4917 and AJ 4918 demonstrated a high homology value of 90–100% with each other and are considered to represent a single new species. *Candida* sp. AJ 4919 and *Candida* sp. AJ 4922 demonstrated low homology values with each other and with the remaining strains, and were considered to represent two distinct new species. Strains of *C. mesenterica* demonstrated high homology values of 82–104% with one another and were considered to be a well-defined species.

Description of new species As discussed above, four strains isolated from fruit bodies of mushrooms were found to represent three new species of the genus *Candida*. They are described below as *Candida fungicola*, *Candida sagamina*, and *Candida fukazawae*. Practical differential characteristics of these species are summarized in Table 5.

1) *Candida fungicola* Nakase, M. Suzuki, Sugita, Suh & Komagata, sp. nov. Fig. 2

Coloniae in agaro YM post 1 mensem ad 17°C flavidae vel flavido-griseae, glabratae, rugulosae, butyraceae vel tenaces. In liquido YM post 3 dies ad 25°C pellicula et sedimentum formantur; cellulae in sedimento ellipsoideae, cylindricae vel elongatae, 1.8–3.5×4.5–15–25 μm, singulae, binae aut catenatae. Pseudomycelium formatur. Fermentatio nulla. Glucosum, sucrosum, maltosum, cellobiosum, trehalosum, melezitosum, amylum solubile, D-ribosum (lente vel lente & infirme), glycerolum, ribitolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum, salicinum, glucono-δ-lactonum (lente vel non), acidum 2-ketogluconicum, acidum succinicum et acidum citricum assimilantur, autem galactosum, L-sorbosum, lactosum, melibiosum, raffinosum, inulinum, D-xylosum, L-arabinosum, D-arabinosum, L-rhamnosum, ethanolum, erythritolum, galactitolum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum, acidum DL-lacticum et inositolum non assimilantur. Kalium nitricum non assimilatur. Maxima temperatura cresentiae: 28–30°C est. Ad crescentiam biotina necessaria est. Materia amyloidea iodophila non formatur. Ureum non hydrolysatur. Commutatio colori per diazonium caeruleum B negativa est. Proportio molaris guanini+ cytosini in acido deoxyribonucleinico 38.5–39.4 (per HPLC) est. Ubiquinonum majus Q-9 adest. Teleomorphosis ignota.

Holotypus: Colonia in cultura ex fructificatione ex fungi, Mt. Tonotake, Kanagawa Pref., Japonia, vii, 1966, a T. Nakase isolata (originaliter ut AJ 4917) et in Collectione Culturarum Japonensium qua 'Japan Collection of Microorganisms', Wako, Saitama conservatur (JCM 10142).

Growth in YM broth: After 3 d at 25°C, a pellicle and a sediment are produced. Cells in the sediment are ellipsoidal, cylindrical, elongate and slender, 1.8–3.5×4.5–15–25 μm, single, in pairs or in chains (Fig. 2A). Pseudomycelia are observed. After 1 mo at 17°C, a thick, wrinkled pellicle and a sediment are present. The first pellicle often falls into the medium, then a second pellicle is produced.

Growth on YM agar: After 1 mo at 17°C, the streak culture is pale yellow, yellowish-grey or light yellow, smooth, finely or roughly wrinkled, dull, butyrous to tough or tough, margin ciliate.

Slide culture on potato dextrose agar: Pseudomycelia are produced abundantly (Fig. 2B). They frequently branch and usually bear few blastoconidia.

Production of sexual spores: Not observed.

Fermentation: Negative.

Assimilation of carbon compounds:

Glucose	+	Ethanol	−
Galactose	−	Glycerol	+
L-Sorbose	−	Erythritol	−
Sucrose	+	Ribitol	+
Maltose	+	Galactitol	−
Cellobiose	+	D-Mannitol	+
Trehalose	+	D-Glucitol	+
Lactose	−	α-Methyl-D-glucoside	+
Melibiose	−	Salicin	+
Raffinose	−	Glucono-δ-lactone	+ (latent) or −
Melezitose	+	2-Ketogluconic acid	+
Inulin	−	5-Ketogluconic acid	−
Soluble starch	+	D-Glucuronic acid	−
D-Xylose	−	D-Galacturonic acid	−
L-Arabinose	−	DL-Lactic acid	−
D-Arabinose	−	Succinic acid	+
D-Ribose	+ (latent or latent & weak)	Citric acid	+
		Inositol	−
L-Rhamnose	−		

Assimilation of nitrogen compounds:

Ammonium sulfate	+	Ethylamine hydrochloride	+
Potassium nitrate	−	L-Lysine hydrochloride	+
Sodium nitrite	−	Cadaverine dihydrochloride	+

Maximum growth temperature: 28–30°C.

Vitamin required: Biotin.

Production of starch-like substances: Negative.

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

Hydrolysis of fat: Negative.

Diazonium blue B color reaction: Negative.

Urease: Negative.

Liquefaction of gelatin: Negative.

Acid production on chalk agar: Positive (delayed and weak).

G+C content of nuclear DNA: 38.5–39.4 mol% (by HPLC).

Major ubiquinone: Q-9 (Table 3).

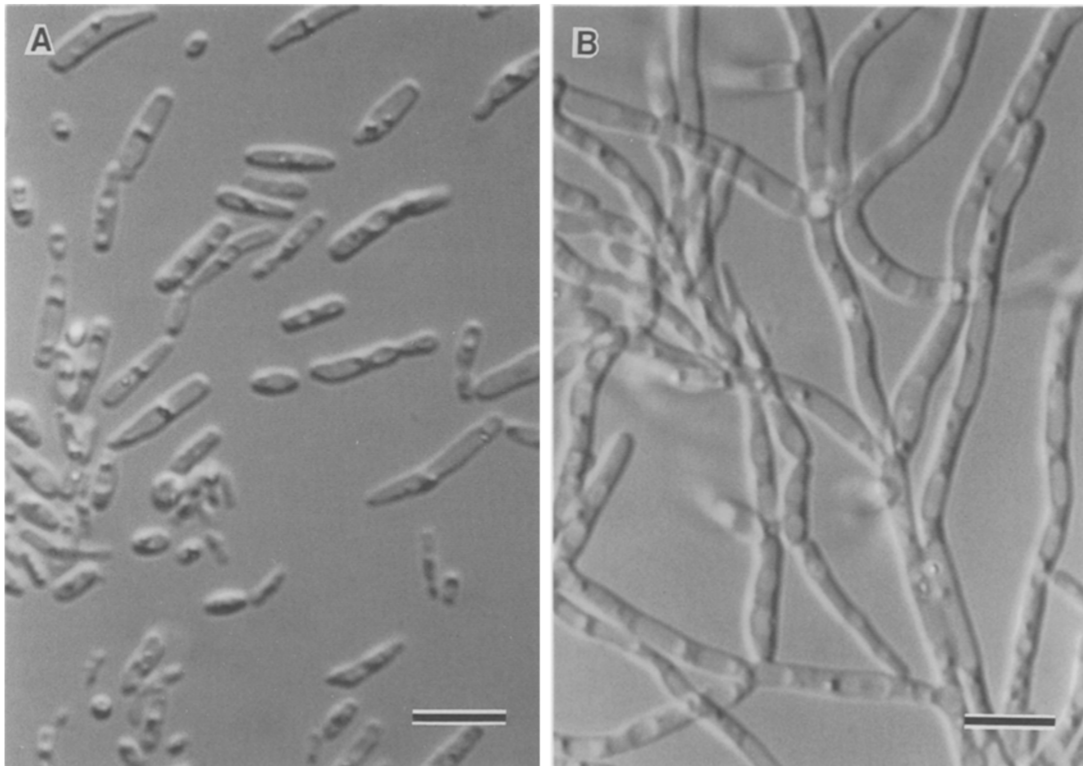

Fig. 2. Vegetative cells and pseudomycelia of *Candida fungicola* JCM 10142. A: Vegetative cells in the sediment grown in YM broth for 3 d at 25°C. B. Pseudomycelia produced on slide culture on PDA after 4 d at 25°C. Scales = 10 μm.

Type strain: AJ 4917 was designated as the type strain of the species. This strain was isolated from a fruit body of an unidentified mushroom collected at Mt. Tonotake in the Tanzawa Mountains, Kanagawa Pref., Japan by T. Nakase in July 1966. It is maintained in the Japan Collection of Microorganisms, Wako, Saitama, as JCM 10142. Another strain, AJ 4918, is maintained as JCM 10143.

Etymology: The specific epithet "*fungicola*" was chosen since this species was isolated from fruit bodies of mushrooms.

2) *Candida sagamina* Nakase, M. Suzuki, Sugita, Suh & Komagata, sp. nov. Fig. 3

Coloniae in agaro YM post 1 mensem ad 17°C albae vel flavido-albae, rugulosae, infime byssoideae, tenaces, margine ciliatae. In liquido YM post 3 dies ad 25°C pellicula tenuis rugosaque et sedimentum formantur; pseudomycelium predominans cellulas; cellulae ellipsoideae, longe-ellipsoideae vel elongatae, 2.5–5.5 × 6–15–20 μm. Fermentatio nulla. Glucosum, sucrosum, maltosum, cellobiosum, trehalosum, melezitosum, amylum solubile, D-xylosum, D-arabinosum (fortasse infirme), ethanolum, glycerolum, ribitolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum, salicinum, glucono-δ-lactonum, acidum 2-ketogluconicum, acidum succinicum et acidum citricum (fortasse infirme) assimilantur, autem galactosum, L-sorbosum, lactosum, melibiosum, raffinosum, inulinum, L-arabinosum, D-ribosum, L-rhamnosum, erythritolum, galactitolum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum, acidum DL-lacticum et inositolum non assimilantur. Kalium nitricum non assimilatur. Maxima temperatura cresentiae: 29–30°C est. Ad crescentiam biotina necessaria est. Materia amyloidea iodophila non formatur. Ureum non hydrolysatur. Commutatio colori per diazonium caeruleum B negativa est. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 40.5 (per HPLC) est. Ubiquinonum majus Q-9 adest. Teleomorphosis ignota.

Holotypus: Colonia in cultura ex fructificatione ex fungi, Mt. Tonotake, Kanagawa Pref., Japonia, vii, 1966, a T. Nakase isolata (originaliter ut AJ 4919) et in Collectione Culturarum Japonensium qua 'Japan Collection of Microorganisms', Wako, Saitama conservata (JCM 10144).

Growth in YM broth: After 3 d at 25°C, a thin, wrinkled pellicle and a sediment are produced. Pseudomycelia predominate. Yeast cells are usually elongate, sometimes ellipsoidal or long ellipsoidal, 2.5–5.5 × 6–15–20 μm (Fig. 3A). Pseudomycelial cells are often very long. After 1 mo at 17°C, a thick pellicle with cottony surface and a sediment are present.

Growth on YM agar: After 1 mo at 17°C, the streak culture is white to yellowish-white, wrinkled, cottony near the bottom, tough, margin ciliate.

Slide culture on potato dextrose agar: Pseudomycelia are produced abundantly. They are well-deve-

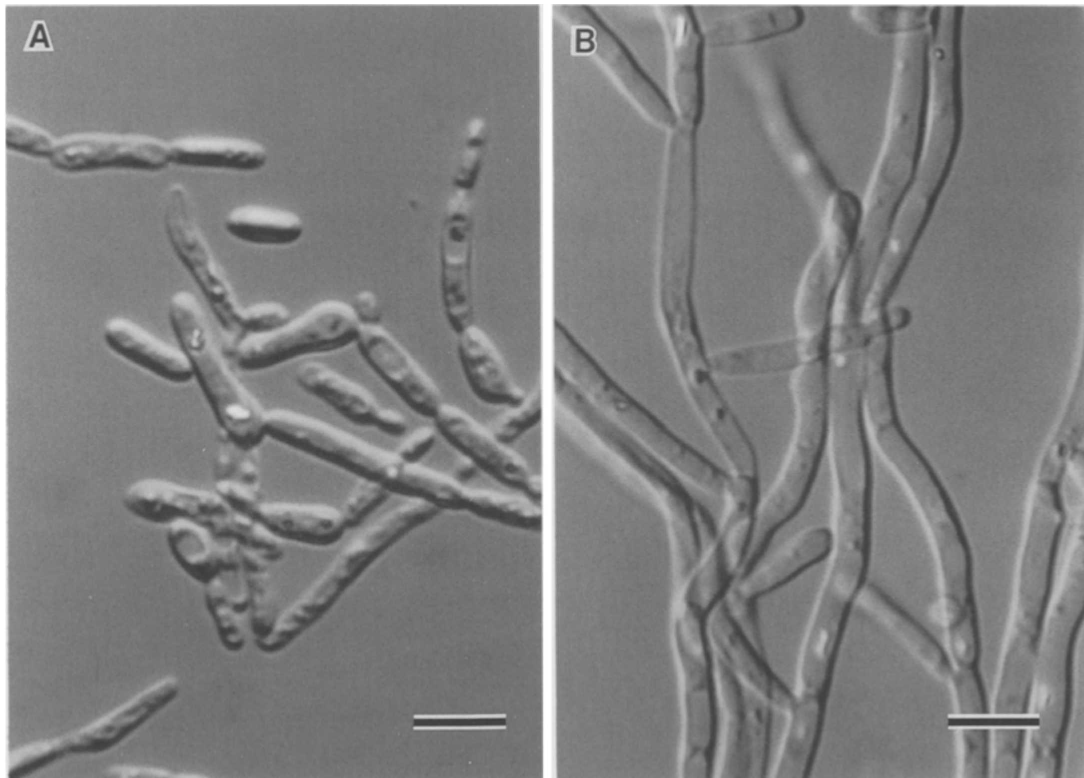

Fig. 3. Vegetative cells and pseudomycelia of *Candida sagamina* JCM 10144. A: Vegetative cells in the sediment grown in YM broth for 3 d at 25°C. B: Pseudomycelia produced on slide culture on PDA after 4 d at 25°C. Scales = 10 μm.

loped and bear few blastoconidia (Fig. 3B).

Production of sexual spores: Not observed.

Fermentation: Negative.

Assimilation of carbon compounds:

Glucose	+	Ethanol	+
Galactose	−	Glycerol	+
L-Sorbose	−	Erythritol	−
Sucrose	+	Ribitol	+
Maltose	+	Galactitol	−
Cellobiose	+	D-Mannitol	+
Trehalose	+	D-Glucitol	+
Lactose	−	α-Methyl-D-glucoside	+
Melibiose	−	Salicin	+
Raffinose	−	Glucono-δ-lactone	+
Melezitose	+	2-Ketogluconic acid	+
Inulin	−	5-Ketogluconic acid	−
Soluble starch	+	D-Glucuronic acid	−
D-Xylose	+	D-Galacturonic acid	−
L-Arabinose	−	DL-Lactic acid	−
D-Arabinose	+ (may be weak)	Succinic acid	+
D-Ribose	−	Citric acid	+ (may be weak)
L-Rhamnose	−	Inositol	−

Assimilation of nitrogen compounds:

Ammonium sulfate	+	Ethylamine hydrochloride	+
Potassium nitrate	−	L-Lysine hydrochloride	+
Sodium nitrite	−	Cadaverine dihydrochloride	+

Maximum growth temperature: 29–30°C.

Vitamin required: Biotin.

Production of starch-like substances: Negative.

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

Hydrolysis of fat: Negative.

Diazonium blue B color reaction: Negative.

Urease: Negative.

Liquefaction of gelatin: Negative.

Acid production on chalk agar: Positive (delayed and weak).

G+C content of nuclear DNA: 40.5 mol% (by HPLC).

Major ubiquinone: Q-9 (Table 3).

Type strain: AJ 4919 is the type strain of the species. It was isolated from a fruit body of an unidentified mushroom collected at Mt. Tonotake in the Tanzawa Mountains in Kanagawa Pref., Japan by T. Nakase in July 1966. This strain is maintained in the Japan Collection of Microorganisms, Wako, Saitama, as JCM 10144.

Table 5. Differential characteristics of *Candida fungicola*, *C. sagamina*, *C. fukazawae*, and *C. mesenterica*.

Characteristics	*C. fungicola*	*C. sagamina*	*C. fukazawae*	*C. mesenterica*
Fermentation of				
Glucose	−	−	+	−/s
Assimilation of				
L-Sorbose	−	−	−	+
Melezitose	+	+	−	v
D-Xylose	−	+	d	−/s
Ethanol	−	+	d	+
Erythritol	−	−	+	+
Maximum Growth				
Temp. (°C)	28–30	29–30	36–37	~28
Mol% G+C of Nuclear DNA	38.5–39.4	40.5	45.1	41.8–43.0

+, positive; −, negative; s, slow; d, delayed; v, variable

Etymology: The specific epithet "*sagamina*" was chosen since this species was isolated in the western area of Kanagawa Prefecture, Japan. Sagami is the old name of this area.

3) ***Candida fukazawae*** Nakase, M. Suzuki, Sugita, Suh & Komagata, sp. nov. Fig. 4

Coloniae in agaro YM post 1 mensem ad 17°C albae vel flavido-albae, delicate rugulosae, butyraceae vel tenaces, margine ciliatae. In liquido YM post 3 dies ad 25°C pellicula et sedimentum formatur; cellulae in sedimento ovoideae, longe ovoideae, ellipsoideae, cylindricae vel elongatae, singulae, binae aut catenatae, 2.5–4–7 × 7–15–25 μm. Pseudomycelium formatur. L-Glucosum fermentatur, autem galactosum, sucrosum, maltosum, lactosum, melibiosum et raffinosum non fermentantur. Glucosum, sucrosum, maltosum, cellobiosum, trehalosum, amylum solubile, D-xylosum (lente), D-ribosum (lente), ethanolum (lente), glycerolum (lente), erythritolum, ribitolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum, salicinum, glucono-δ-lactonum, acidum 2-ketogluconicum, acidum succinicum et acidum citricum (infirme) assimilantur, autem galactosum, L-sorbosum, lactosum, melibiosum, raffinosum, melezitosum, inulinum, L-arabinosum, D-arabinosum, D-rhamnosum, galactitolum, acidum 5-ketogluconicum, acidum D-glucuronicum, acidum D-galacturonicum, acidum DL-lacticum et inositolum non assimilantur. Kalium nitricum non assimilatur. Maxima temperatura crescentiae 36–37°C est. Ad crescentiam biotina necessaria est. Materia amyloidea iodophila non formatur. Ureum non hydrolysatur. Commutatio colori per diazonium caeruleum B negativa est. Proportio molaris guanini+ cytosini in acido deoxyribonucleinico 45.1 (per HPLC) est. Ubiquinonum majus Q-9 adest. Teleomorphosis ignota.

Holotypus: Colonia in cultura ex fructificatione ex fungi, Mt. Tonotake, Kanagawa Pref., Japonia, vii, 1966, a T. Nakase isolata (originaliter ut AJ 4922) et in Collectione Culturarum Japonensium qua 'Japan Collection of Microorganisms', Wako, Saitama conservata (JCM 1641).

Growth in YM broth: After 3 d at 25°C, a thick, wrinkled pellicle and a sediment are produced. Yeast cells in the sediment are long ovoid, ellipsoidal, cylindrical, or elongate, single, in pairs or in chains (Fig. 4A). Ovoid, long ovoid and ellipsoidal cells measure 4–7 × 8–15 μm, and cylindrical to elongate cells usually measure 2.5–8 × 7–25 μm. Pseudomycelial production is abundant. After 1 mo at 17°C, a thick, wrinkled pellicle and a scanty sediment are present. The first pellicle often falls into the medium, then a second pellicle is produced.

Growth on YM agar: After 1 mo at 17°C, the streak culture is white to yellowish-white, delicately wrinkled, butyrous to tough, margin ciliate.

Slide culture on potato dextrose agar: Pseudomycelia are produced abundantly. They are well developed (Fig. 4B). Blastoconidia are ovoid, long ovoid or elongate, in chains or in verticils. Pseudomycelial cells are often very long.

Production of sexual spores: Not observed.

Fermentation:

Glucose	+	Lactose	−
Galactose	−	Melibiose	−
Sucrose	−	Raffinose	−
Maltose	−		

Assimilation of carbon compounds:

Glucose	+	Ethanol	+ (latent)
Galactose	−	Glycerol	+ (latent)
L-Sorbose	−	Erythritol	+
Sucrose	+	Ribitol	+
Maltose	+	Galactitol	−
Cellobiose	+	D-Mannitol	+
Trehalose	+	D-Glucitol	+
Lactose	−	α-Methyl-D-glucoside	+
Melibiose	−	Salicin	+
Raffinose	−	Glucono-δ-lactone	+
Melezitose	−	2-Ketogluconic acid	+

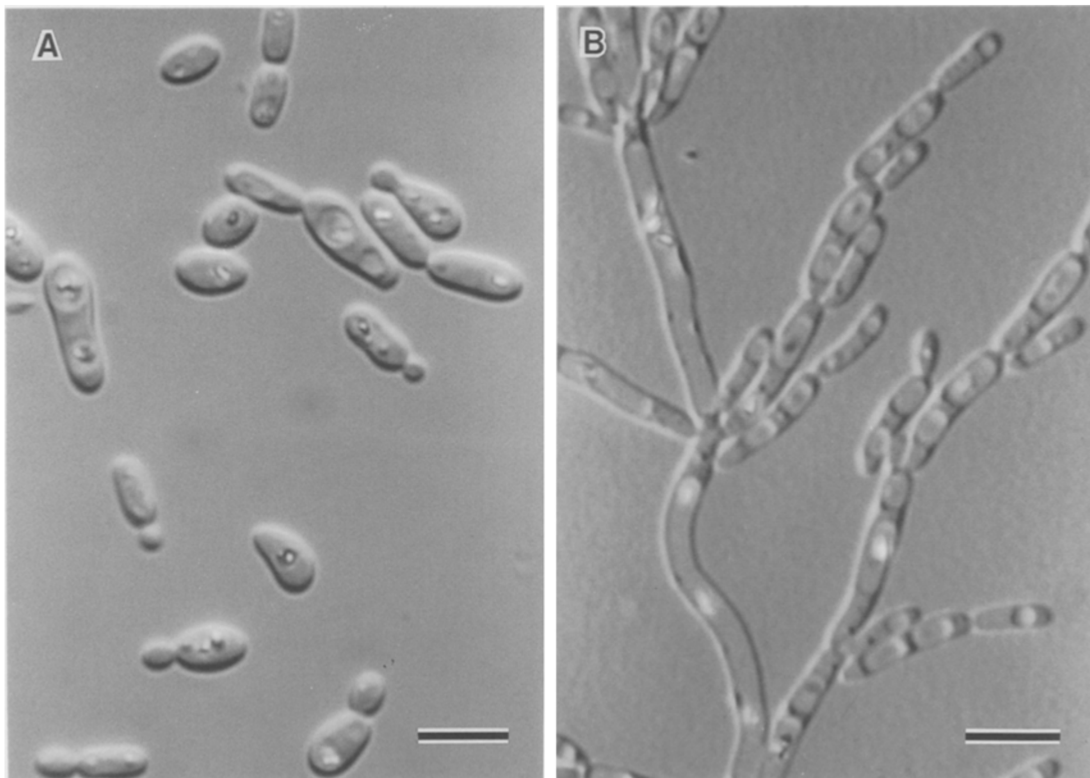

Fig. 4. Vegetative cells and pseudomycelia of *Candida fukazawae* JCM 1641. A: Vegetative cells in the sediment grown in YM broth for 3 d at 25°C. B: Pseudomycelia produced on slide culture on PDA after 4 d at 25°C. Scales = 10 μm.

Inulin	−	5-Ketogluconic acid	−
Soluble starch	+	D-Glucuronic acid	−
D-Xylose	+ (latent)	D-Galacturonic acid	−
L-Arabinose	−	DL-Lactic acid	−
D-Arabinose	−	Succinic acid	+
D-Ribose	+ (latent)	Citric acid	+ (weak)
L-Rhamnose	−	Inositol	−

Assimilation of nitrogen compounds:

Ammonium sulfate	+	Ethylamine hydrochloride	+ (latent)
Potassium nitrate	−	L-Lysine hydrochloride	+
Sodium nitrite	−	Cadaverine dihydrochloride	+

Maximum growth temperature: 36–37°C.

Vitamin required: Biotin.

Production of starch-like substances: Negative.

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

Hydrolysis of fat: Negative.

Diazonium blue B color reaction: Negative.

Urease: Negative.

Liquefaction of gelatin: Negative.

Acid production on chalk agar: Negative.

G+C content of nuclear DNA: 45.1 mol% (by HPLC).

Major ubiquinone: Q-9 (Table 3).

Type strain: AJ 4922, isolated from a fruit body of an unidentified mushroom collected at Mt. Tonotake in the Tanzawa Mountains, Kanagawa Pref., Japan by T. Nakase in July 1966. This strain is maintained in the Japan Collection of Microorganisms, The Institute of Physical and Chemical Research, Wako, Saitama, as JCM 4614.

Etymology: The specific epithet "*fukazawae*" for this species was given in honor of Prof. Yoshimura Fukazawa, who contributed greatly to the progress of antigenic analysis and the serological classification system of yeasts.

Phylogenetic position of new *Candida* species In the phylogenetic tree constructed by the neighbor-joining method based on the sequences of SSU rDNAs, *C. fungicola*, *C. sagamina*, and *C. fukazawae* consitituted a cluster (Fig. 5). This cluster is connected with a cluster comprising *C. mesenterica* and *C. suecica*. The phylogenetic distances between the cluster containing *C. fungicola*, *C. sagamina*, *C. fukazawae*, *C. mesenterica*, and *C. suecica* and the clusters containing other *Candida* species are fairly long, so that the former cluster may represent a taxonomic rank of genus. However, there is no clear phenotypic taxonomic characteristics to separate the cluster represented by *C. mesenterica* from other *Candida* species. The genus *Candida* is apparently heterogeneous, including more than 10 corresponding teleomorphic genera. The heterogeneity of *Candida* is also clearly indicated by molecular phylogenetic studies

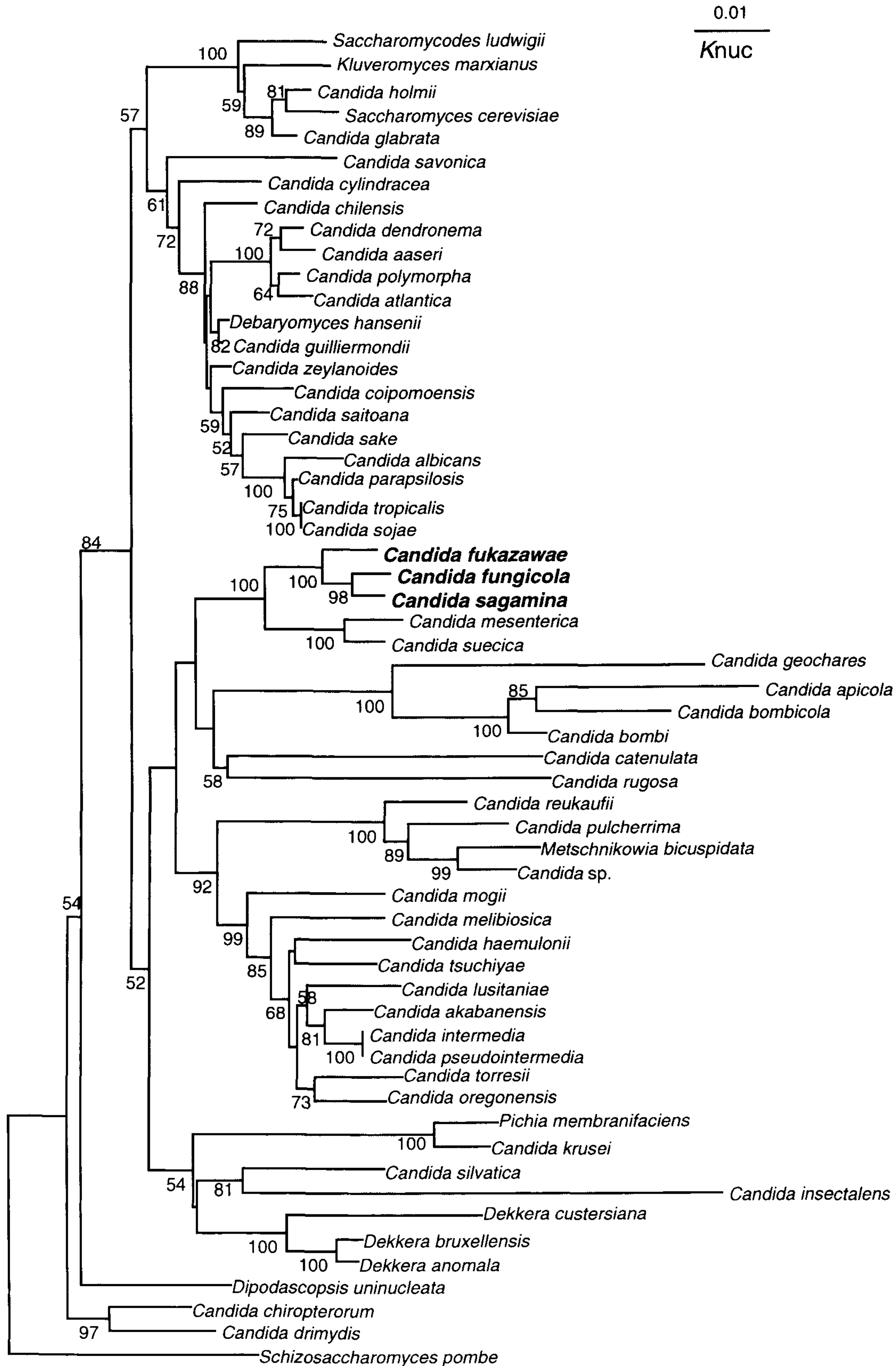

Fig. 5. Neighbor-joining tree based on the SSU rDNA sequences of *Candida fungicola*, *C. sagamina*, *C. fukazawae*, *C. mesenterica*, and related taxa. The numerals represent the confidence level (%) from 1,000 replicate bootstrap sampling (the frequency level less than 50% is not indicated). The distance corresponding to one base change per hundred nucleotide positions is indicated by a bar.

using the D1/D2 region of large subunit rDNAs (Kurtzman & Robnett, 1998) and complete sequences of SSU rDNAs (Sugita & Nakase, 1999). The genus *Candida* should be separated into several genera in the future. However, it is difficult to find phenotypic characteristics reflecting molecular phylogenetic data at present. It will probably be necessary to use specific sequence data for the separation of the taxonomic rank of genus, as is already done in the systematics of bacteria (Shida et al., 1996).

Comparison of ITS1 of rDNA In the phylogenetic tree based on SSU rDNA sequences, *C. suecica* was located in the cluster of *C. mesenterica*. In the present study, *C. suecica* was not included in the DNA-DNA hybridization experiment with the three new species and *C. mesenterica* because it differs from them in the phenotypical characteristics commonly employed in yeast taxonomy. The ITS1 sequences of rDNA from *C. fungicola*, *C. sagamina*, *C. fukazawae*, *C. mesenterica*, and *C. suecica* were determined and examined for their similarity. The ITS1 sequences of these five species differed greatly from one another, and their alignment was impossible. Sugita et al. (1999) reported that between conspecific strains nucleotide differences accounted for less than 1% of nucleotides in the ITS1 and 2 regions when they examined the relationship between nuclear DNA relatedness values and ITS sequence similarity among the species of *Trichosporon*, an anamorphic yeast genus. This fact clearly indicates that these five *Candida* species are different from one another.

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