Microbiol. Cult. Coll. Dec. 1999. p. 49-57 Vol. 15, No. 2

Reidentification of Yeast Strains Deposited as *Candida agrestis*, with a Description of *Candida kofuensis* sp. nov.

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IFO 10103 and IFO 10932 (=JCM 2321) deposited as *Candida agrestis* RIFY 4611^T (=No. 611, S. Goto) and IFO 10931 (=CBS 8058) deposited as *Candida* sp. RIFY 4841 formed spheroidal to ovoidal chlamydospores and were morphologically similar to *Candida pulcherrima*, the anamorph of *Metschnikowia pulcherrima*. However, these strains differed from *M. pulcherrima* in lactate assimilation, growth at 34°C, and pulcherrimin pigmentation. An analysis of domain D1/D2 26S rDNA sequences from currently accepted ascomycetous yeasts demonstrated that the strains represent a new species, which is described here as *Candida kofuensis* sp. nov. Phylogenetic placement of this new species in the *Metschnikowia* clade was further analyzed from 18S rDNA sequence divergence.

In additional comparisons, IFO 10933 (=JCM 10341=NRRL Y-17640=CBS 8055), which is believed to have originated from *C. agrestis* RIFY 4611^T has been reidentified as *Saturnispora zaruensis* from its morphological, physiological and molecular characters.

Key words : *Candida kofuensis* sp. nov., DNA/DNA hybridization, rRNA gene sequences

INTRODUCTION

In 1983, Goto and Oguri (6) described the new yeast *Candida agrestis* and designated RIFY 4611 (=No. 611) as the type strain. In 1979, before the species was described, this strain was sent to the Centraalbureau voor Schimmelcultures (CBS) where it has been maintained as CBS 8055 (=NRRL Y-17640). Yarrow (unpublished) found ascospores on the slant culture that he received from Goto. Barnett et al.(1) reported that the properties of *C. agrestis* CBS 8055 were very much like those of *Pichia saitoi*. Kurtzman (10) regarded *C. agrestis* as a synonym of *Saturnispora zaruensis* based on analysis of domain D1/D2 26S rDNA sequences.

Candida agrestis No. 611 (=RIFY 4611) was also sent to the Japan Collection of Microorganisms (JCM) in 1983 where it has been maintained as JCM 2321, and to the Institute for Fermentation, Osaka (IFO) in 1984 where it has been maintained as IFO 10103. Mikata (unpublished) noticed that IFO 10103 showed characteristics similar to *Candida pulcherrima*. Suzuki and Nakase (16) found that *C. agrestis* JCM 2321 (=IFO 10932) has Q-9 as the major ubiquinone in contrast to the original description by Goto and Oguri (6) where it was reported that *C.*

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Table 1. Yeast strains examined

Species	Strain[a)]	History[b)]
Candida agrestis Goto & Oguri	IFO 10103	← RIFY 4611[T]
	IFO 10932	← JCM 2321 ← No. 611[T], S. Goto (=RIFY 4611[T])
	IFO 10933	← JCM10341 ← NRRL Y-17640 ← CBS 8055[T]← RIFY 4611[T]
Candida sp.	IFO 10931	← CBS 8058 ← RIFY 4841

[a)] IFO, Institute for Fermentation, Osaka, Japan

[b)] CBS, Centraalbureau voor Schimmecultures, Delft, The Netherlands ; NRRL, Agricultural Research Service Culture Collection, Peoria, Illinois, USA ; JCM, Japan Collection of Microorganisms, Wako, Japan ; RIFY, Research Institute of Fermentation, Yamanashi University, Kofu, Japan ; T, Type strain

agrestis had Q-7 as the major ubiquinone.

These facts suggesed that strains maintained in CBS and NRRL may be different from those maintained in JCM and IFO. At about the same time as receiving *C. agrestis*, CBS received two additional strains from Goto. They were labeled *Candida* sp. RIFY 4841 (=CBS 8058) and *Candida* sp. RIFY 4933 (=CBS 8059). Yarrow identified these strains as *Metschnikowia pulcherrima*. Therefore, it is possible that one of these strains was sent to JCM and IFO as *C. agrestis*.

The present study was conducted to clarify the taxonomic problem of the *C. agrestis* strains maintained in CBS, NRRL, JCM and IFO, and describe a new species for the *C. agrestis* strains maintained in JCM and IFO.

MATERIALS AND METHODS

Yeast strains. The three strains identified as *Candida agrestis* and a strain of *Candida* sp. IFO 10931 (=CBS 8058=RIFY 4841) that were studied are listed in Table 1. *Metschnikowia pulcherrima* IFO 1678[T] and *Saturnispora zaruensis* IFO 1384[T] were also used for taxonomic comparisons.

Physiological, morphological and cultural characteristics. Physiological, morphological and cultural characteristics were investigated by the methods of Yarrow (19).

Coenzyme Q systems. The yeasts were grown at 28°C for 2 days as shake cultures in 1-*l* Erlenmeyer flasks containing 400 m*l* of YPD broth (1 % Bacto-yeast extract, 2 % Bacto-peptone and 2 % glucose). The extraction, purification and analysis of ubiquinone were performed as described by Mikata and Yamada (13).

DNA preparation. The yeast strains were grown at 28°C on a shaker for 10 to 24 h in 200-m*l* Erlenmeyer flasks containing 50 m*l* of YPD broth. Protoplasts were prepared and DNA was extracted following the protocol of Holm et al.(7) as modified by Kaneko and Banno (8).

Mol% G+C determination. The mol % G+C was determined by the method of Tamaoka and Komagata (17).

DNA/DNA hybridization. Extent of DNA/DNA hybridization between strains was determined by the photobiotin microplate method of Ezaki et al.(2, 3) as modified by Kaneko and Banno (8).

18S rRNA gene sequencing and phylogenetic analysis. 18S rRNA gene (rDNA) sequences were determined using the Thermo Sequenase fluorescence labeled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech) following the supplier's protocol. PCR for amplification of 18S rDNA and cycle sequencing using PCR products were performed as described by Ueda-Nishimura and Mikata (18). The 18S rDNA sequences from this study were deposited in DDBJ with the following accession numbers : IFO 10783, AB023465 ; IFO 1408, AB023466 ; IFO 1677, AB 023467 ; IFO 1680, AB023468 ; IFO 1679, AB023469 ; IFO 10103 (identical to 18S rDNA of IFO 10931), AB023471 ; IFO 10788, AB023472 ; IFO 1678, AB 023473 ; IFO 1605, AB023474 ; IFO 10860, AB023475 ; IFO 10791, AB023476. A phylogenetic tree was constructed by Kimura's 2-parameter method (9) and the neighbor-joining method (15) using clustal W. Bootstrap values (4) were calculated from 1,000 replicates. The 18S rDNA sequence of *Schizosaccharomyces pombe*, which was used as outgroup species in the analyses, was obtained from GenBank.

Domain D1/D2 26S rRNA gene sequencing and phylogenetic analysis. Methods for nuclear DNA

isolation, amplification of the 600-nucleotide 26S rDNA domain D1/D2 by the polymerase chain reaction (PCR), and sequencing with the ABI Taq-DyeDeoxy Terminator Cycle sequencing kit and the ABI Model 377 automated DNA sequencer (Applied Biosystems) were previously described (11). Sequence data were visually aligned with QEdit 2.15 (SemWare). Phylogenetic relationships were calculated with a Power Macintosh 8500/120 by the maximum parsimony program of PAUP* 4.0 (written by D. L. Swofford ; test version distributed by Sinauer Associates) with the simple heuristic search option. Relationships were further analyzed by the neighbor-joining program of PAUP* 4.0 with the Jukes-Cantor distance measure. *Schizosaccharomyces pombe* was the designated outgroup in all analyses. Confidence limits for phylogenetic trees were estimated from bootstrap analysis (1000 replications). The nucleotide sequences for the new species, *Candida kofuensis* NRRL Y-27226 (=IFO 10103=JCM 2321), reported in this study have been deposited in GenBank with accession number AF 158019, and the GenBank accession numbers for reference species were reported by Kurtzman and Robnett (12).

RESULTS

Physiological and morphological characteristics

Physiological and morphological characteristics of *C. agrestis* IFO 10103, IFO 10932, and IFO 10933, *Candida* sp. IFO 10931, *Saturnispora zaruensis* IFO 1384^T, and *Metschnikowia pulcherrima* IFO 1678^T were compared with those from the description of *C. agrestis* (6) (Table 2). Three strains, IFO 10103, IFO 10932 (*C. agrestis*), and IFO 10931 (*Candida* sp.) had identical physiological characteristics. They were also morphologically indistinguishable and formed spheroidal to ovoidal chlamydospores (pulcherrima cells) (14) after 30 days culture at 15°C on corn-meal agar, dilute (1 : 9) corn-meal agar, V-8 agar, and dilute (1 : 9) V-8 agar (Fig. 1). The strains did not form airplane-type vegetative cells, which are characteristic of *Metschnikowia gruessii* (5). The characteristics observed were quite different from the description of *C. agrestis* (6), but similar to that of *M. pulcherrima* IFO 1678^T, except for the absence of lactate assimilation, growth at 34°C, pulcherrimin pigmentation, and an ascosporic state.

Physiological and morphological characteristics of IFO 10933 (*C. agrestis*) were quite different from those of the above-mentioned three strains. These characteristics were not identical to the description of *C. agrestis* (6), but ubiquinone type and G+C content were similar (Table 2). The strain formed saturn-shaped ascospores after 7 days culture at 24°C on YM agar and corn-meal agar and all physiological characteristics were identical to those of *Saturnispora zaruensis* IFO 1384^T. Therefore, IFO 10933 (=JCM 10341=NRRL Y-17640=CBS 8055) can be identified as *S. zaruensis*.

G+C content and DNA/DNA hybridization

The mol % G+C contents and DNA relatedness among *Candida agrestis* IFO 10103, IFO 10932 and IFO 10933, *Candida* sp. IFO 10931, *Metschnikowia* species, and the reference species examined are shown in Table 3.

Because of similar mol % G+C contents (46.1～46.5) and high DNA relatedness (88～105 %), IFO 10103, IFO 10932 and IFO 10931 are identified as the same species. Furthermore, their DNA relatedness with other *Metschnikowia* species, including *M. pulcherrima*, was low (4～20 %). Based on these determinations, the new species, *Candida kofuensis* sp. nov., is proposed for these strains.

In contrast to the preceding strains, IFO 10933 and *S. zaruensis* IFO 1384^T have mol % G+C contents of 36.7 and show high DNA relatedness (92 %), which demonstrates that they are conspecific.

Candida kofuensis Mikata, Ueda-Nishimura, Goto, Kurtzman, Suzuki, Yarrow et Nakase sp. nov. (Candidaceae)

In liquid YM post dies 3 ad 25°C, cellulae globosae ad ellipsoideae 3.9～8.0 × 5.4～9.1μm singulae aut binae ; chlamydosporae absentes. Post unum mensem chlamydosporae praesentes plerumque, globosae 5.4～10.7 × 7.5～14.5μm vel subglobosae, uninucleolatae typice ; sedimentum et annulus formantur.

Cultura in agaro YM post unum mensem ad 25°C, color cremeus vel suffuscus. Pulcherrimin pigmentum non praesens, pagina laevis et nitens, margo integer. Pseudomycelium primitivum formantur.

Glucosum fermentatur at non galactosum, sucrosum, maltosum, cellobiosum, trehalosum,

Table 2. Characteristics differentiating *Candida* sp., *C. agrestis*, *Saturnispora zaruensis*, and *Metschnikowia pulcherrima*

	Candida sp. IFO 10931	*C. agrestis* IFO 10103	*C. agrestis* IFO 10932	*C. agrestis* IFO 10933	*C. agrestis* RIFY 4611[c]	*S. zaruensis* IFO 1384[T]	*M. pulcherrima* IFO 1678[T]
Assimilation[a] of :							
Galactose	+[b]	+	+	−	−	−	+
D-Sorbose	+	+	+	−	−	−	+
Sucrose	+	+	+	−	+	−	+
Maltose	+	+	+	−	−	−	+
Cellobiose	+	+	+	−	+	−	+
Raffinose	−	−	−	−	+	−	−
Melezitose	+	+	+	−	+	−	+
D-Xylose	w	w	w	w	−	w	+
D-Ribose	+	+	+	−	−	−	+
Glycerol	+	+	+	−	−	−	+
α-MGS	+	+	+	−	vw	−	+
Salicin	+	+	+	−	−	−	+
DL-lactate	−	−	−	+	w	+	+
Citrate	+	+	+	−	−	−	+
Glucosamine	+	+	+	−	NT	−	+
Arbutin	+	+	+	−	NT	−	+
10 % NaCl	w	w	w	−	NT	−	+
Growth at 34°C	−	−	−	NT	+	NT	+
G+C mol %	46.5	46.3	46.1	36.7	37.8	36.7	45.1
Ubiquinone	Q-9	Q-9	Q-9	Q-7	Q-7	Q-7	Q-9
Ascospores	−	−	−	saturn	−	saturn	acicular
Chlamydospore	+	+	+	−	−	−	+
Pulcherrimin Pigment	−	−	−	−	−	−	+

[a] α-MGS, α-Methyl-D-glucoside ; 10 % NaCl, 10 % NaCl+5 % glucose

[b] +, Positive ; w, Weak growth ; vw, Very weak ; −, Negative ; NT, Not tested

[c] Reference (6)

lactosum nec raffinosum. Glucosum, galctosum, L-sorbosum, sucrosum, maltosum, cellobiosum, trehalosum, melezitosum, D-xylosum (lente), D-ribosum, D-glucosaminum, ethanolum, glycerolum, ribitolum, D-mannitolum, D-glucitolum, α-methylum-D-glucosidum, salicinum, acidum gluconicum, acidum succinium et acidum citricum assimilantur, at non lactosum, melibiosum, raffinosum, inulinum, amylum solubile, L-arabinosum, D-arabinosum, L-rhamnosum, methanolum, erythritolum, galactitolum, acidum DL-lacticum nec inositolum. Ethylaminum, L-lysinum et cadaverinum assimilantur at non kalium nitricum. Ad crescentiam vitaminae necessariae sunt. In 10 % NaCl/5 % glucoso crescentiae (exiguam) at non 0.01 % cycloheximido. Ureum non hydrolysatur. Diazonium caeruleum B non respondens. Maxima temperatura crescentiae 32～33°C. Proportio molaris guanini+cytosini in acido deoxyribonucleinico 46.1～46.4 mol % per HPLC. Ubiquinonum majus Q-9.

Typus depositus in collectione Centraalbureau voor Schimmelcultures, Delphi, Nederlandia (CBS 8058), et Institute for Fermentation, Osaka, Japonia (IFO 10931).

Candida kofuensis (ko.fu. en'sis. L. adj. kofuensis,

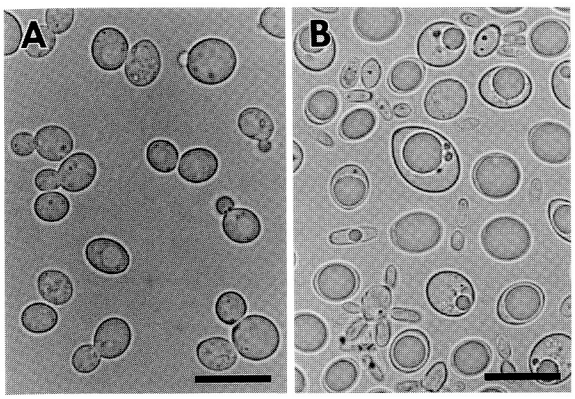

Fig. 1. Photomicrographs of *Candida kofuensis* IFO 10931 (=CBS 8058)

A : Vegetative cells in YM broth after 3 days at 25°C,
B : Chlamydospores on dilute corn-meal agar (1 : 9) 30 days at 15°C
Bar=10μm

pertaining to Kofu in Yamanashi Pref., where the yeast was originally isolated).

Growth in YM medium : after three days at 25°C, vegetative cells are globose to ellipsoidal, (3.9~8.0) × (5.4~9.1) μm, single, and reproduce by multilateral budding. Chlamydospores (pulcherrima cells) are not present. After one month at 25°C, pulcherrima cells are usually present, highly refractile, globose (5.4~10.7) × (7.5~14.5) μm, or subglobose, and contain one or more lipid globules. A thin ring and abundant sediment are present ; a pellicle is absent.

Growth on YM agar : after one month at 25°C, growth is cream or tan-colored. Pulcherrimin pigment is not present. The surface is smooth and glistening, slightly raised and convex with an entire margin. The texture is butyrous.

Dalmau plate cultures on corn-meal agar after seven days at 25 °C : a rudimentary pseudomycelium is formed.

Table 3. DNA base compositions and levels of DNA relatedness between species belonging to *Candida* sp., *C. agrestis*, *Saturnispora zaruensis*, and *Metschnikowia* species

Species[a]	Strain	G+C content (mol %)	% DNA Relatedness[b]		
			IFO 10103	IFO 1384^T	IFO 1678^T
Candida sp.	IFO 10931	46.1	88.1	15.8	17.6
C. agrestis	IFO 10103	46.5	100	17.5	12.4
C. agrestis	IFO 10932	46.3	105.5	14.6	17.0
C. agrestis	IFO 10933	36.7	16.0	92.1	8.2
S. zaruensis	IFO 1384^T	36.7	12.0	100	5.8
M. agaves	IFO 10860^T	43.4	10.2	NT	NT
M. australis	IFO 10783^T	45.5	20.0	NT	NT
M. bicuspidata	IFO 1408^T	44.7	18.5	14.5	18.8
M. bicuspidata	IFO 10785	45.3	10.2	NT	NT
M. bicuspidata	IFO 10786	43.9	5.0	NT	NT
M. gruessii	IFO 10788^T	41.8	8.6	NT	NT
M. hawaiiensis	IFO 10791^T	42.8	4.7	NT	NT
M. krissii	IFO 1677^T	43.6	16.7	NT	NT
M. lunata	IFO 1605^T	42.9	9.1	NT	NT
M. pulcherrima	IFO 1678^T	45.1	14.6	12.5	100
M. pulcherrima	IFO 10796	45.2	16.5	17.1	91.2
M. pulcherrima	IFO 10797	45.0	19.4	NT	NT
M. reukaufii	IFO 1679^T	40.6	18.7	NT	NT
M. zobellii	IFO 1680^T	47.7	16.9	NT	NT
Sac. cerevisiae	IFO 10217^T	38.0	11.7	15.0	4.4

[a] *C.*, *Candida* ; *M.*, *Metschnikowia* ; *S.*, *Saturnispora* ; *Sac.*, *Saccharomyces*
[b] NT, not tested

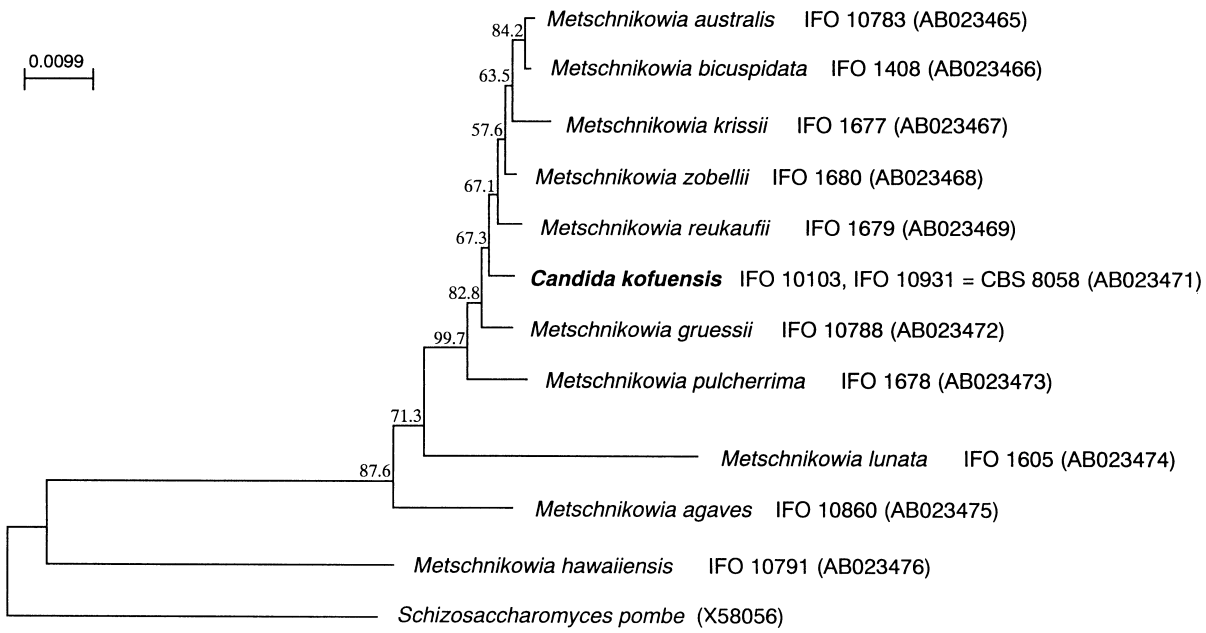

Fig. 2. Phylogenetic tree showing placement of *Candida kofuensis* among near relatives as represented by the neighbor-joining method of 18S rDNA sequence

Numbers given at nodes are the percentage frequencies with which a given branch appeared in 1,000 bootstrap replicates. GenBank, EMBL, and DDBJ accession numbers are shown in parentheses. Bar, sequence dissimilarity value of 0.0099

Glucose is fermented. Galactose, sucrose, maltose, cellobiose, trehalose, lactose and raffinose are not fermented.

Glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, melezitose, D-xylose (weak), D-ribose, D-glucosamine, ethanol, glycerol, ribitol, D-mannitol, D-glucitol, α-methyl-D-glucoside, salicin, D-gluconate, succinate and citrate are assimilated. Not assimilated are lactose, melibiose, raffinose, inulin, soluble starch, L-arabinose, D-arabinose, L-rhamnose, methanol, erythritol, galactitol, DL-lactate and inositol.

Ethylamine, L-lysine and cadaverine are assimilated ; nitrate is not assimilated. No growth occurred in 100 ppm cycloheximide and vitamin-free medium. Growth developed in 10 % NaCl-5 % glucose (weak). Urease is negative. The diazonium blue B reaction is negative. Maximum growth temperature is 32～33°C.

The G+C content of the nuclear DNA is 46.1～46.3 mol %, as determined by HPLC. The major ubiquinone is Q-9.

Cultures of the type strain from wild grapes have been deposited in the Centraalbureau voor Schimmelcultures, Delft, The Netherlands (CBS 8058), and the Institute for Fermentation, Osaka, Japan (IFO 10931).

Phylogenetic analysis

18S rDNA and 26S rDNA domain D1/D2 sequences of *Candida kofuensis* and *Metschnikowia* species were determined by the direct method.

The phylogenetic tree from 18S rDNA was determined by the neighbor-joining method and is shown in Fig. 2. The 18S rDNA sequences for *Candida kofuensis* IFO 10103 and IFO 10931^T are identical. The single most parsimonious tree derived from maximum parsimony analysis of domain D1/D2 26S rDNA is shown in Fig. 3. Placement of *Candida kofuensis* differs somewhat in the two gene trees, probably because of weak internal bootstrap support, but both analyses clearly demonstrate that this new species is well nested within the *Metschnikowia* clade.

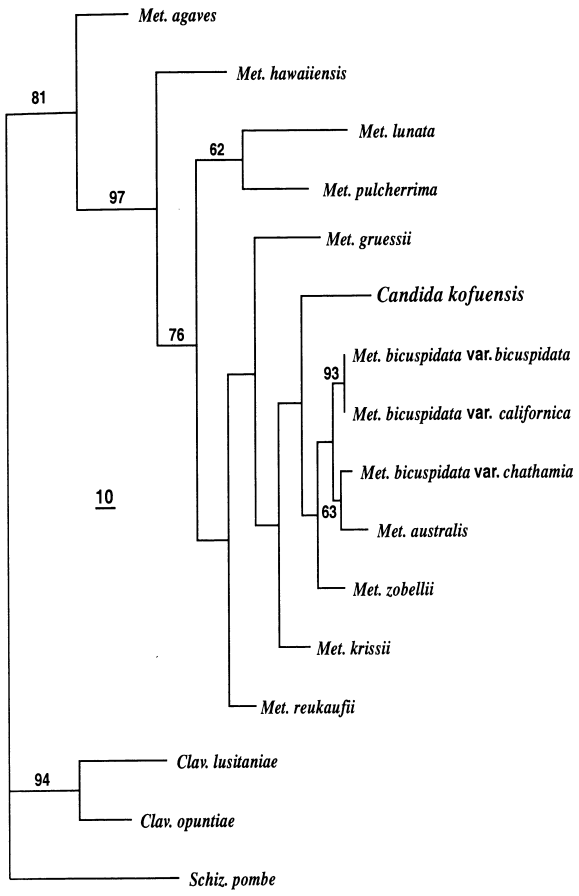

Fig. 3. Phylogenetic tree showing placement of *Candida kofuensis* among near relatives as represented by the single most parsimonious tree derived from maximum parsimony analysis of domain D1/D2 26S rDNA

Branch lengths are proportional to nucleotide differences as indicated by the marker bar. Numbers given at nodes are the percentage of frequencies with which a given branch appeared in 1,000 bootstrap replicates. Frequencies under 50 % are not given. Tree length = 658, consistency index = 0.639, retention index = 0.489, rescaled consistency index = 0.321, homoplasy index = 0.362, parsimony informative characters = 139. *Schizosaccharomyces pombe* served as outgroup species for the analysis. Abbreviations : *Clav.* = *Clavispora*, *Met.* = *Metschnikowia*, *Schiz.* = *Schizosaccharomyces*

DISCUSSION

In this study, three strains designated as *C. agrestis* were examined and were separated into two species, *S. zaruensis* and *C. kofuensis* sp. nov. Physiological and morphological characteristics of *C. agrestis* IFO 10933 (=CBS 8055=NRRL Y-17640) were similar to those of *S. zaruensis* IFO 1384^T (=NRRL Y-7008^T) (Table 3). Furthermore, the DNA/DNA reassociation studies reported here, and the domain D1/D2 rDNA comparison (12) clearly demonstrated the two taxa to be conspecific. Although it is impossible to reexamine *C. agrestis* RIFY 4611^T (=No. 611) because it is now lost, *S. zaruensis* IFO 10933 (=CBS 8055=NRRL Y-17640) was compared with the original description of *C. agrestis* (6). The major ubiquinone type Co Q-7 and the mol % G+C content of 37.8 reported in the original description of *C. agrestis* (6) corresponded to those of *S. zaruensis*. However, assimilation of sucrose, cellobiose, raffinose and melezitose were not identical. Because of these differences, the identity of RIFY 4611 and IFO 10933 (=CBS 8055=NRRL Y-17640) as *C. agrestis* is uncertain, and the proposed synonymy with *S. zaruensis* (10) cannot be verified with certainty.

Physiological characteristics of *C. agrestis* strains IFO 10103 and IFO 10932 (=JCM 2321) were quite different from the original description of *C. agrestis* (6) but were similar to those of *Candida* sp. IFO 10931 (=CBS 8058=RIFY 4841). On the bases of phylogenetic analysis (Figs. 2, 3) and DNA/DNA hybridization tests (Table 3), *C. agrestis* IFO 10103, IFO 10932 (=JCM 2321) and *Candida* sp. IFO 10931 (=CBS 8058=RIFY 4841) represent a new anamorphic species of the *Metschnikowia* clade, which is described as *Candida kofuensis* sp. nov. *C. agrestis* RIFY 4611^T (=CBS 8055), *Candida* sp. RIFY 4841 (=CBS 8058) and *Candida* sp. RIFY 4933 (=CBS 8059) were sent by Goto to CBS. *Candida* sp. IFO 10931 (=CBS 8058=RIFY 4841) was reidentified as *C. kofuensis* sp. nov. *Candida* sp. CBS 8059 (=RIFY 4933) was observed to form needle-shaped ascospores by Yarrow (unpublished), which is characteristic of *Metschnikowia* species, however, the culture is lost now and so it is impossible to reexamine it. On the basis of these facts, it is assumed that *Candida* sp. RIFY 4841 or *Candida* sp. RIFY 4933 was sent to JCM and IFO as *C. agrestis* RIFY 4611^T (=No. 611).

Acknowledgements. Thanks are given to Christie J. Robnett for determining the domain D1/D2 26S rDNA sequences.

REFERENCES

1. Barnett, J.A., Payne, R.W. and Yarrow, D. Yeasts : characteristics and identification. 2nd ed., p. 110, Cambridge University Press, Cambridge (1990).
2. Ezaki, T., Hashimoto, Y., Takeuchi, N., Yamamoto, H., Liu, S.-L., Miura, H., Matsui, K. and Yabuuchi, E. Simple genetic method to identify viridans group streptococci by colorimetric dot hybridization and fluorometric hybridization in microdilution wells. J. Clin. Microbiol. **26** : 1708-1713 (1988).
3. Ezaki, T., Hashimoto, Y. and Yabuuchi, E. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int. J. Syst. Bacteriol. **39** : 224-229 (1989).
4. Felsenstein, J. Confidence limits on phylogenies : an approach using the bootstrap. Evolution **39** : 783-791 (1985).
5. Giménez-Jurado, G. *Metschnikowia gruessii* sp. nov., the teleomorph of *Nectaromyces reukaufii* but not of *Candida reukaufii*. Syst. Appl. Microbiol. **15** : 432-438 (1992).
6. Goto, S. and Oguri, H. Two new species of the genus *Candida* from wild grapes. J. Gen. Appl. Microbiol. **29** : 85-90 (1983).
7. Holm, C., Meeks-Wagner, D.W., Fangman, W.L. and Botstein, D. A rapid efficient method for isolating DNA from yeast. Gene **42** : 169-173 (1986).
8. Kaneko, Y. and Banno, I. Reexamination of *Saccharomyces bayanus* strains by DNA-DNA hybridization and electrophoretic karyotyping. IFO Res. Comm. **15** : 30-41 (1991).
9. Kimura, M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16** : 111-120 (1980).
10. Kurtzman, C.P. *Saturnispora* Liu & Kurtzman, *In* Kurtzman C.P. and Fell J.W.(eds.), The yeasts. a taxonomic study, 4th ed., pp. 387-390, Elsevier, Amsterdam (1998).
11. Kurtzman, C.P. and Robnett, C.J. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5' end of the large subunit (26S) ribosomal RNA gene. J. Clin. Microbiol. **35** : 1216-1223 (1997).
12. Kurtzman, C.P. and Robnett, C.J. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek **73** : 331-371 (1998).
13. Mikata, K. and Yamada, Y. The ubiquinone system in *Hasegawaea japonica* (Yukawa et Maki) Yamada et Banno : a new method for identifying ubiquinone homologs from yeast cells. IFO Res. Comm. **19** : 41-46 (1999).
14. Pitt, J.I. and Miller, M.W. Sporulation in *Candida pulcherrima*, *Candida reukaufii* and *Chlamydozyma* species : their relationships with *Metschnikowia*. Mycologia **60** : 663-685 (1968).
15. Saitou, N. and Nei, M. The neighbor-joining method : a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4** : 406-425 (1987).
16. Suzuki, M. and Nakase, T. Cellular neutral sugar compositions and ubiquinone systems of the genus *Candida*. Microbiol. Cult. Coll. **14** : 49-62 (1998).
17. Tamaoka, J. and Komagata, K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol. Lett. **25** : 125-128 (1984).
18. Ueda-Nishimura, K. and Mikata, K. A new yeast genus, *Tetrapisispora* gen. nov. : *Tetrapisispora iriomotensis* sp. nov., *Tetrapisispora nanseiensis* sp. nov. and *Tetrapisispora arboricola* sp. nov., from the Nansei Islands, and reclassification of *Kluyveromyces phaffii* (van der Walt) van der Walt as *Tetrapisispora phaffii* comb. nov. Int. J. Syst. Bacteriol. **49** : 1915-1924 (1999).
19. Yarrow, D. Methods for the isolation, maintenance and identification of yeasts, *In* Kurtzman, C.P. and Fell, J.W.(eds.), The Yeasts. a Taxonomic Study, 4th ed., pp. 77-100, Elsevier, Amsterdam (1998).

Candida agrestis として寄託された酵母の再同定，および山梨県から分離された酵母の新種，*Candida kofuensis*

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Candida agrestis RIFY 4611^T として寄託されている IFO 10103 および IFO 10932（＝JCM 2321），および *Candida* sp. RIFY 4841 として寄託されている IFO 10931（＝CBS 8058）は厚膜胞子を形成し子嚢胞子は形成しないことから，形態的に *Candida pulcherrima*（*Metschnikowia pulcherrima* のアナモルフ）に類似する．しかし，プルチェリミン色素生産性，乳酸資化性，G+C 含量，および 34°C での生育において相違することから，新種 *Candida kofuensis*（基準株 IFO 10931＝CBS 8058）と命名することを提案した．一方，同じく *C. agrestis* RIFY 4611^T として寄託された IFO 10933（＝JCM 10341＝NRRL Y-17640＝CBS 8055）は，形態および生理性状から *Saturnispora zaruensis* と同定した．RIFY 4611 として寄託された株は，*C. kofuensis* および *S. zaruensis* の 2 系統あることが明らかとなった．