Synonymy of *Candida methylica* with *Candida boidinii* and of *Candida methanolophaga* with *Candida succiphila*

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Some *Candida* species which can use methanol as a sole carbon source were studied by performing chemotaxonomic tests; we determined the ubiquinone systems of these organisms, their DNA base compositions, their electrophoretic karyotypes, and their DNA relatedness values. The type strains of *Candida methanolophaga* and *Candida succiphila* had similar DNA base compositions and exhibited 90% DNA relatedness. All *Candida boidinii* and *Candida methylica* strains had DNA G+C contents of 30.8 to 31.0 mol%, and these organisms exhibited 87 to 101% DNA relatedness to the type strain of *C. boidinii*. We propose that *C. methanolophaga* and *C. methylica* should be synonyms of *C. succiphila* and *C. boidinii*, respectively.

Identification of methanol-assimilating yeasts is based on several characteristics, including DNA base composition, ubiquinone system, proton magnetic resonance spectra of cell wall mannans, and electrophoretic patterns of enzymes (8, 10–12). Several new methanol-assimilating *Candida* species have been described, including *Candida methylica* (17), *Candida cariosilignicola* and *Candida succiphila* (11), *Candida methanolophaga* (9), and *Candida nanaspora* (14). Among these species, *C. cariosilignicola* and *C. nanaspora* can be easily differentiated from other *Candida* species on the basis of physiological characteristics, while *C. methanolophaga* and *C. methylica* differ from *C. succiphila* and *C. boidinii*, respectively, in only a few phenotypic characteristics (1).

In this study, we compared the electrophoretic karyotypes and DNA base compositions of strains belonging to several methylotrophic *Candida* species. In addition, we investigated the levels of DNA relatedness between pairs of strains to verify the relationships among the *Candida* species studied.

Ten strains belonging to nine *Candida* species were used in this study. The sources of these strains are shown in Table 1. Morphological and physiological characteristics were investigated by the methods described by van der Walt and Yarrow (18).

To determine ubiquinone systems, cells were grown in the liquid medium of Billon-Grand (2) and were collected at the early stationary phase of the growth. The ubiquinones were extracted and purified by the method of Collins et al. (4). The purified quinones were analyzed by high-performance liquid chromatography (HPLC).

In addition, all of the strains examined were grown in YM (0.3% yeast extract, 0.3% malt extract, 0.5% Bacto-Peptone, 1% glucose; Difco) agar slants at 25°C for 1 to 2 days. The cells were harvested, washed twice with 0.05 M EDTA (pH 7.5), and then used to prepare agarose blocks as described by Carle and Olson (3). Electrophoresis was performed by using a contour-clamped homogeneous electric field system with a hexagonal electrode array (Bio-Rad Laboratories) as described by Vollrath and Davis (19).

For DNA studies cells were grown in YM broth and harvested in the late exponential phase of growth. DNA was isolated and purified by the method of Kaneko and Banno (7). Each purified DNA was hydrolyzed to nucleosides as described by Tamaoka and Komagata (16), and the hydrolysate was analyzed by reverse-phase HPLC to determine its DNA base composition (G+C content). Levels of DNA relatedness were determined by the photobiotin labeling microplate method, as

TABLE 1. Yeast strains studied

Species	Strain designations ^a		Source
	CCRC	CBS	
<i>Candida boidinii</i> Ramirez 1953	20464 ^T	2428 ^T	Tanning liquor
<i>Candida boidinii</i>	21758	8051	Soil
<i>Candida cariosilignicola</i> Lee et Komagata 1980	21405 ^T	8001 ^T	Decayed timber
<i>Candida methanolophaga</i> Kumamoto et Yamamoto 1986	22418 ^T	7297 ^T	Oil field
<i>Candida methylica</i> Trotsenko et Bykovskaya 1974	21757 ^T	8030 ^T	Soil
<i>Candida nanaspora</i> Saëz et Rodrigues de Miranda 1988	22419 ^T	7200 ^T	Male ape
<i>Candida nitratophila</i> Meyer et Yarrow 1978	21583 ^T	2027 ^T	Bark beetle
<i>Candida pignaliae</i> Meyer et Yarrow 1978	21625 ^T	6071 ^T	Tanning liquor
<i>Candida pinus</i> Meyer et Yarrow 1978	22437 ^T	970 ^T	Pine
<i>Candida succiphila</i> Lee et Komagata 1980	21410 ^T	8003 ^T	Sap of peach tree

^a CCRC, Culture Collection and Research Center, Food Industry Research and Development Institute, Taiwan, Republic of China; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands.

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TABLE 2. Selected phenotypic characteristics of the *Candida* strains studied

Strain	Fermentation of:					Assimilation of:										Growth at 37°C
	Glucose	Galactose	Trehalose	Cellobiose	Raffinose	Galactose	Galactitol	Sucrose	Rhamnose	Trehalose	Cellobiose	Lactate	Raffinose	Ribose	Nitrate	
<i>C. boidinii</i> CCRC 20464 ^T	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—
<i>C. boidinii</i> CCRC 21758	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—
<i>C. methylica</i> CCRC 21757 ^T	+	—	—	—	—	—	—	—	+	—	—	—	—	—	+	—
<i>C. methanolophaga</i> CCRC 22418 ^T	+	+	+	+	—	+	—	—	+	+	+	—	—	+	—	w
<i>C. succiphila</i> CCRC 21410 ^T	+	+	+	+	—	+	+	—	+	+	+	+	—	+	—	+
<i>C. cariosilignicola</i> CCRC 21405 ^T	+	—	+	—	+	—	—	+	+	+	—	—	+	+	+	+
<i>C. nanaspora</i> CCRC 22419 ^T	+	+	+	—	—	+	—	—	+	+	—	—	—	+	+	+
<i>C. nitratophila</i> CCRC 21583 ^T	+	+	+	—	—	+	—	—	+	+	—	—	—	+	+	—
<i>C. pignaliae</i> CCRC 21625 ^T	+	—	+	—	—	—	—	—	—	+	—	—	—	—	+	—
<i>C. pinus</i> CCRC 22437 ^T	+	—	+	—	—	—	—	—	—	+	—	—	—	+	—	—

^a +, positive; —, negative.

modified by Kaneko and Banno (7) from the method of Ezaki et al. (5, 6). The main modification was as follows: 400 ng of heat-denatured yeast DNA was immobilized in a well of a microplate at 28°C overnight, and hybridization was performed at 42°C for 24 h.

The phenotypic characteristics which we determined were the same as those described in the monograph of Barnett et al. (1). On the basis of the results of our phenotypic characterization of *Candida* species we identified three groups; group I contained *C. boidinii* and *C. methylica*, group II contained *C. methanolophaga* and *C. succiphila*, and group III contained *Candida pignaliae*, *Candida pinus*, *Candida nitratophila*, and *Candida nanaspora*. Selected phenotypic characteristics are shown in Table 2. The phenotypic characteristics of the members of each group were quite similar. *C. methanolophaga* and *C. succiphila* differed only in assimilation of galactitol and DL-lactate and growth at 37°C, whereas *C. boidinii* and *C. methylica* differed only in assimilation of D-ribose and L-rhamnose.

All strains of the *Candida* species which we studied had Q-7 as the major component of the ubiquinone system (Table 3), as reported previously (8, 10–12).

The chromosomal DNA banding patterns of the *Candida* species studied were examined by pulsed-field gel electrophoresis (Fig. 1). In group I (*C. boidinii* and *C. methylica*) the electrophoretic karyotypes of the two strains of *C. boidinii* exhibited polymorphisms in chromosomal DNA size and band number (Fig. 1, lanes 2 and 3). This is probably an example of

chromosome length polymorphism, which has also been reported for *Saccharomyces cerevisiae* (3), and *Kluyveromyces* spp. (15). The pattern of *C. methylica* CCRC 21757^T (T = type strain) was rather similar to that of *C. boidinii* CCRC 21758, but differed in DNA band number from the pattern of *C. boidinii* CCRC 20464^T. In group II, *C. methanolophaga* and *C. succiphila* (lanes 8 and 9) produced identical chromosomal DNA patterns; it was obvious that these two species are closely related. In group III, every species produced a distinct chromosomal band pattern.

The DNA base compositions of the *Candida* species studied are shown in Table 3. The DNA G+C contents of the group I species *C. boidinii* and *C. methylica* were 30.8 to 31.0 mol%, and the DNA G+C contents of the group II species *C. methanolophaga* and *C. succiphila* were 39.5 and 40.0 mol%, respectively. Our values were somewhat lower than the values determined previously by the thermal denaturation method (1, 8, 9, 11). In group III, the DNA base compositions of the species described in the references cited by Barnett et al. (1) ranged from 35.1 to 42.7 mol%.

Levels of DNA relatedness were determined by the microplate hybridization method (5–7), and the results are shown in Table 3. The DNAs of the type strains of *C. boidinii*, *C. succiphila*, and *C. pinus* were labeled with photobiotin to determine their levels of relatedness to other DNAs. In group I, *C. boidinii* CCRC 21758 and *C. methylica* exhibited high levels (87 to 101%) of DNA relatedness to the type strain of *C. boidinii*; it was obvious that there is a close relationship

TABLE 3. Major ubiquinones, DNA base compositions, and DNA reassociation values for the *Candida* strains studied

Source of unlabeled DNA	Major ubiquinone	G+C content (mol%)	% DNA relatedness to:		
			<i>C. boidinii</i> CCRC 20464 ^T	<i>C. succiphila</i> CCRC 21410 ^T	<i>C. pinus</i> CCRC 22437 ^T
<i>C. boidinii</i> CCRC 20464 ^T	Q-7	31.0	100	7	12
<i>C. boidinii</i> CCRC 21758	Q-7	31.0	87	6	9
<i>C. methylica</i> CCRC 21757 ^T	Q-7	30.8	101	6	15
<i>C. succiphila</i> CCRC 21410 ^T	Q-7	40.0	9	100	4
<i>C. methanolophaga</i> CCRC 22418 ^T	Q-7	39.5	13	90	9
<i>C. pinus</i> CCRC 22437 ^T	Q-7	37.3 ^a	32	3	100
<i>C. nitratophila</i> CCRC 21583 ^T	Q-7	36.6 ^a	5	3	5
<i>C. pignaliae</i> CCRC 21625 ^T	Q-7	42.7 ^a	23	10	12
<i>C. nanaspora</i> CCRC 22419 ^T	Q-7	—	13	11	5
<i>C. cariosilignicola</i> CCRC 21405 ^T	Q-7	35.1 ^a	30	8	11

^a Data from references cited by Barnett et al. (1).

FIG. 1. Pulsed-field gel electrophoresis banding patterns of chromosomal DNAs isolated from the *Candida* species studied. The following conditions were used for contour-clamped homogeneous electric field analysis: hexagonal electrode array; pulse time, 60 to 200 s for 18 h and then 200 to 600 s for 60 h; and a constant voltage of 100 V. *S. cerevisiae* YNN 255 was used as a reference (lane 1). Lane 2, *C. boidinii* CCRC 20464^T; lane 3, *C. boidinii* CCRC 21758; lane 4, *C. methylica* CCRC 21757^T; lane 5, *C. nitratophila* CCRC 21583^T; lane 6, *C. pinus* CCRC 22437^T; lane 7, *C. pignaliae* CCRC 21625^T; lane 8, *C. succiphila* CCRC 21410^T; lane 9, *C. methanolophaga* CCRC 22418^T; lane 10, *C. cariosilignicola* CCRC 21405^T; lane 11, *C. nanaspora* CCRC 22419^T.

between these two species. In group II, the level of DNA relatedness between *C. methanolophaga* and the type strain of *C. succiphila* was 90%, supporting the hypothesis that these two species are closely related. All of the species in group III exhibited low levels of DNA relatedness to the type strains of *C. pinus*, *C. boidinii*, and *C. succiphila* (Table 3).

On the basis of the results described above, we concluded that the members of each of two *Candida* species pairs (*C. boidinii* and *C. methylica*; *C. methanolophaga* and *C. succiphila*) do not differ significantly in their phenotypic characteristics. The organisms were also found to be closely related on the basis of ubiquinone system, DNA base composition, and DNA relatedness data. It seems reasonable to combine the members of each species pair into the same taxon. Because the names *C. boidinii* and *C. succiphila* have priority over *C. methylica* and *C. methanolophaga*, respectively (9, 11, 13, 17), *C. methylica* is considered a synonym of *C. boidinii*, and *C. methanolophaga* is considered a synonym of *C. succiphila*.

All of the group III (*C. pinus*, *C. nitratophila*, *C. pignaliae*, *C. nanaspora*, and *C. cariosilignicola*) exhibited low levels of DNA relatedness with one another, so the organisms in this group should be maintained in their original separate taxa.

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