NOTE

DNA hybridization and electrokaryotype study of some *Candida* species

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Some *Candida* species which were similar to each other in phenotypic characteristics were studied by chemotaxonomic and molecular taxonomic comparison including the ubiquinone systems, electrophoretic karyotypes, DNA base composition and DNA relatedness. *Candida viswanathii* and *Candida lodderae* showed similar electrophoretic karyotypes and DNA base composition and demonstrated 89 to 91% DNA relatedness, confirming their synonymy.

Keywords: DNA hybridization, electrokaryotype, *Candida viswanathii*, *Candida lodderae*, DNA base composition

In the course of a culture authentication in our Culture Collection and Research Center (CCRC), it was found that some *Candida* species had similar phenotypic characteristics. *Candida viswanathii* and *Candida lodderae* could only be discriminated by fermentation of sucrose and growth at 40 °C, but other taxonomic criteria, including phenotypic characteristics (Meyer *et al.*, 1984) and DNA base composition (Meyer & Phaff, 1972; Nakase & Komagata, 1971) were very similar. It is therefore necessary to clarify the relationship between these species using other reliable methods. In addition, strain CBS 433 is listed as *C. lodderae* (CBS, Centraalbureau voor Schimmelcultures, 1996), but the same strain NRRL-Y1552 was identified as *Candida tropicalis* (C. P. Kurtzman, personal communication). Strains ATCC 28777 and ATCC 22982 are listed as *C. viswanathii* (ATCC, 1995), but the same strains CBS 434 and CBS 2780 were listed as *Candida* sp. by the CBS (CBS, 1996).

In this study, we selected those *Candida* strains with similar phenotypic characteristics and compared their electrophoretic karyotypes and DNA base composition. Furthermore, we investigated DNA relatedness between pairs of strains to verify their interrelationships. Eight strains of *Candida* species representing three species were used. The sources of these strains are listed in Table 1. Morphological and physiological characteristics were investigated by the methods described by van der Walt & Yarrow (1984).

To determine the ubiquinone system, cells were grown in the liquid medium of Billon-Grand (1989) and

collected at the early stationary phase of the growth. The ubiquinones were extracted and purified by the method of Collins *et al.* (1977). The purified quinones were analysed by HPLC.

All of the strains examined were grown in YM broth (0.3% yeast extract, 0.3% malt extract, 0.5% Bacto-Peptone, 1% glucose; Difco) at 25 °C for 1–2 d and harvested at the late-exponential phase of growth. The cells were washed twice with 0.05 M EDTA (pH 7.5), and were used to prepare agarose blocks according to the method of Carle & Olson (1985). Electrophoresis was carried out using the contour-clamped homogeneous electric field (CHEF) system with a hexagonal electrode array (Bio-Rad) as described by Vollrath & Davis (1987).

The isolation and purification of DNA were performed by the method of Kaneko & Banno (1991). The purified DNA was hydrolysed to nucleosides as described by Tamaoka & Komagata (1984), and the hydrolysate was analysed by reverse-phase HPLC to determine DNA base composition (mol% G+C). Levels of DNA relatedness were determined by the photobiotin-labelling microplate method (Lee *et al.*, 1994a, b). The method was modified by Kaneko & Banno (1991) from that of Ezaki *et al.* (1988, 1989). The main modification is described as follows. A heat-denatured yeast DNA (400 ng) was immobilized in a microplate well at 28 °C overnight. Hybridization was performed at 42 °C for 24 h.

The cells of all strains studied were globose, ovoidal to ellipsoidal, even elongated, produced abundant pseudomycelium, and showed somewhat similar physiological characteristics. The phenotypic characteristics

Abbreviation: CHEF, contour-clamped homogeneous electric field.

Table 1. Yeast strains studied

Strain	Strain designation*					Source
	CCRC	UCD	CBS	NRRL	ATCC	
<i>Candida viswanathii</i> Sandhu and Randhawa 1962	21330 ^T		4024 ^T	Y-6660 ^T	22981 ^T	Cerebrospinal fluid
<i>Candida viswanathii</i>	22554		5362			Sputum
<i>Candida lodderae</i> (Phaff <i>et al.</i>) Meyer and Ahearn 1983	22270 ^T	48-231 ^T	1924 ^T	Y-17317 ^T	28774 ^T	Shrimp
<i>Candida tropicalis</i>	22705		433†			Rotten pineapples
<i>Candida tropicalis</i>	22555			Y-1552‡	10610	Rotten pineapples
<i>Candida tropicalis</i> (Castellani) Berkhaut 1923	20520 ^T		94 ^T	Y-12968 ^T	750 ^T	Bronchitis
<i>Candida</i> sp.	22688		434		28777	Pressed yeast
<i>Candida</i> sp.	22689		2780		22982	Soil

*^T = Type strain. CCRC, Culture Collection and Research Center, Food Industry Research and Development Institute, Taiwan, ROC; UCD, Department of Food Science and Technology, University of California, Davis, CA, USA; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, USDA, Peoria, IL, USA; ATCC, American Type Culture Collection, Rockville, MD, USA.

† This strain was transferred from CBS 433, where it was listed as *Candida lodderae*.

‡ This strain was transferred from NRRL Y-1552, where it was listed as *Candida tropicalis*.

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

Abbreviations: Glc, Glucose; Gal, Galactose; Mal, Maltose; Suc, Sucrose; Lac, Lactose; Raf, Raffinose; Tre, Trehalose; Cel, Cellobiose; Rha, Rhamnose; Mel, Melibiose; Sta, Starch; Nit, Nitrate. +, Positive; —, negative; w, weak positive; D, delayed for longer than 7 d.

Strain	Fermentation of:						Assimilation of:											Growth at 40 °C
	Glc	Gal	Mal	Suc	Lac	Raf	Gal	Mal	Suc	Tre	Cel	Lac	Rha	Mel	Sta	Raf	Nit	
<i>C. viswanathii</i> CCRC 21330 ^T	+	w	+	—	—	—	+	+	w	+	+	—	—	—	+	—	—	w
<i>C. viswanathii</i> CCRC 22554	+	D	+	w	—	—	+	+	w	+	+	—	—	—	+	—	—	w
<i>C. lodderae</i> CCRC 22270 ^T	+	—	+	D	—	—	+	+	D	+	+	—	—	—	D	—	—	—
<i>C. tropicalis</i> CCRC 22705	+	—	+	+	—	—	+	+	+	+	+	—	—	—	D	—	—	+
<i>C. tropicalis</i> CCRC 22555	+	w	+	w	—	—	+	+	+	+	D	—	—	—	D	—	—	+
<i>C. tropicalis</i> CCRC 20520 ^T	+	+	+	+	—	—	+	+	+	+	+	—	—	—	+	—	—	+
<i>Candida</i> sp. CCRC 22688	+	—	+	—	—	—	+	+	w	+	w	—	—	—	w	—	—	—
<i>Candida</i> sp. CCRC 22689	w	—	—	—	—	—	+	+	+	+	D	—	—	—	D	—	—	—

which we determined were the same as those described in the monograph by Barnett *et al.* (Barnett *et al.*, 1990). On the basis of the results of our phenotypic characterization of *Candida* species we identified three groups; group I contained *C. viswanathii* and *C.*

lodderae, group II contained *C. tropicalis*, and group III contained *Candida* sp. Selected phenotypic characteristics are shown in Table 2. The phenotypic characteristics of the members of each group were quite similar. *C. viswanathii* and *C. lodderae* differed only in

Table 3. DNA base composition and DNA reassociation of *Candida* species studied

Source of unlabelled DNA	G + C content (mol%)	Percentage DNA relatedness to:		
		CCRC 21330 ^T	CCRC 22270 ^T	CCRC 20520 ^T
<i>C. viswanathii</i> CCRC 21330 ^T	43.7	100	89	18
<i>C. viswanathii</i> CCRC 22554	43.6	97	92	15
<i>C. lodderae</i> CCRC 22270 ^T	43.6	91	100	20
<i>C. tropicalis</i> CCRC 22705	33.8	13	13	86
<i>C. tropicalis</i> CCRC 22555	33.8	12	13	84
<i>C. tropicalis</i> CCRC 20520 ^T	34.4	13	12	100
<i>Candida</i> sp. CCRC 22688	34.4	12	12	15
<i>Candida</i> sp. CCRC 22689	36.7	9	9	11

Fig. 1. PFGE banding patterns of chromosomal DNA from *Candida* species. Conditions for CHEF: hexagonal electrode array, pulsed time 60–200 s for 24 h, then 200–600 s for 48 h, constant voltage of 100 V. *Saccharomyces cerevisiae* YNN 295 and *Pichia canadensis* (Hansenula wingei) YB-4662-VIA were used as references (lanes 1 and 10, respectively). Lanes: 2, *C. viswanathii* CCRC 21330^T; 3, *C. viswanathii* CCRC 22554; 4, *C. lodderae* CCRC 22270^T; 5, *C. tropicalis* CCRC 22705; 6, *C. tropicalis* CCRC 22555; 7, *C. tropicalis* CCRC 20520^T; 8, *Candida* sp. CCRC 22688; 9, *Candida* sp. CCRC 22689.

fermentation of sucrose and growth at 40 °C. All strains of the *Candida* species which we studied had Q-9 as the major component of the ubiquinone system (Table 3), as reported previously (Barnett *et al.*, 1990).

The chromosomal DNA banding patterns of the *Candida* species studied were examined by PFGE (Fig. 1). *Saccharomyces cerevisiae* YNN295 and *Pichia canadensis* (Hansenula wingei) YB-4662-VIA were used as references (lanes 1 and 10, respectively). In group I, *C. viswanathii* and *C. lodderae* strains (lanes 2, 3 and 4), the electrophoretic karyotypes of the three strains were rather similar. It was likely that these two species would show a close relationship as described in previous reports (Viljoen & Kock, 1989; Viljoen *et al.*, 1988). In group II, *C. tropicalis* strains (lanes 5, 6 and 7) produced identical chromosomal DNA patterns; it also showed that these three strains are closely related.

In group III, each strain produced a distinct chromosomal band pattern.

Comparisons of whole genomes have yielded valuable taxonomic information about relationships among yeasts (Kurtzman & Phaff, 1987). The method was applied to ascertain relationships among the *Candida* species in this study. 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. viswanathii* and *C. lodderae* were 43.6–43.7 mol %, and the DNA G+C contents of the group II species *C. tropicalis* were 33.8–34.4 mol %. Our values were somewhat lower than the values determined previously by the thermal denaturation method (Barnett *et al.*, 1990; Meyer *et al.*, 1984; Meyer & Phaff, 1972). In group III, *Candida* sp., the DNA base composition of the two strains was 34.4 and 36.7 mol %, respectively.

Level of DNA relatedness was determined by the microplate hybridization method (Ezaki *et al.*, 1988; Lee *et al.*, 1994a, b), and the results are shown in Table 3. The DNAs of the type strains of *C. viswanathii*, *C. lodderae* and *C. tropicalis* were labelled with photobiotin to determine their levels of relatedness to other DNAs. In group I, *C. viswanathii* CCRC 22554 and *C. lodderae* showed high levels (91–97%) of DNA relatedness to the type strain of *C. viswanathii*. This close relationship or synonymy between *C. viswanathii* and *C. lodderae* was recently predicted based on nucleotide divergence in the 5' end of the large-subunit (26S) rRNA gene (Kurtzman & Robnett, 1997). In group II, the level of DNA relatedness among strains CCRC 22705, CCRC 22555 and *C. tropicalis* CCRC 20520^T was 84–86 %, supporting the hypothesis that these three strains are closely related. Both strains in group III exhibited low levels of DNA relatedness to the type strains of *C. viswanathii*, *C. lodderae* and *C. tropicalis* (Table 3).

On the basis of the results described above, we concluded that the members of each of groups I and II do not differ significantly in their phenotypic characteristics, their ubiquinone system, DNA base composition or DNA relatedness data. It seems reasonable to combine the members of group I (*C. viswanathii* and

C. lodderae) into the same taxon as was done by Kurtzman & Robnett (1997). Because the name *C. viswanathii* has priority over *C. lodderae* (Meyer & Ahearn, 1983; Sandhu & Randhawa, 1962), *C. lodderae* is considered to be a synonym of *C. viswanathii*. Strains CCRC 22705 (=CBS 433) and CCRC 22555 (=NRRL Y-1552) were identified as *C. tropicalis*.

In group III, *Candida* sp. CCRC 22688 (=ATCC 28777) and CCRC 22689 (=ATCC 22982) exhibited low levels of DNA relatedness with *C. viswanathii*, *C. lodderae* and *C. tropicalis*, so the organisms in this group should be maintained in their original separate taxon *Candida* sp. It will be necessary to clarify the relationship between these strains and other species using the reliable methods described above.

Acknowledgements

We thank H. J. Phaff, University of California, Davis, for critical reading, K. Komagata, Tokyo University of Agriculture, and I. Banno, Institute for Fermentation, Osaka, for their invaluable suggestions, and T. Y. Liu and C. C. Liao, Food Industry Research and Development Institute, Taiwan, ROC, for their encouragement. We thank C. P. Kurtzman, Agricultural Research Service Culture Collection, USDA, for kindly supplying a strain. We also thank the Ministry of Economic Affairs, Republic of China, for financial support.

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