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Taxonomic relationships among the taxa in the *Candida guilliermondii* complex, as revealed by comparative electrophoretic karyotyping

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Electrophoretic karyotypes of 15 type strains of the taxa in the *Candida guilliermondii* complex including *Candida fukuyamaensis* Nakase *et al.* and *Candida xestobii* Yarrow *et* S. A. Meyer were comparatively analysed by using the CHEF (contour-clamped homogeneous electric field) method of PFGE. Eighteen strains (isolated from various natural sources in China) which were originally identified as *C. guilliermondii* by conventional methods were also included. Six electrophoretic karyotype groups were recognized among the strains compared. The following type strains were grouped together with the type strains of *C. guilliermondii* (Castellani) Langeron *et* Guerra and *Pichia guilliermondii* Wickerham: *Blastodendrion arztii* Ota, *Blastodendrion krausi* Ota, *Candida amidovorans* Balloni *et al.*, *C. guilliermondii* var. *japonica* Sugiyama *et* Goto, *Candida mamillae* S. Goto, *Candida parapsilosis* (Ashford) Langeron *et* Talice var. *tokyoensis* Suzuki *et al.*, *C. parapsilosis* var. *tuxtlensis* Herrera *et al.* and six Chinese strains. The type strain of *Torulopsis kestonii* Scarr *et* Rose was classified into the group together with the type strain of *Candida fermentati* (Saito) Bai and seven Chinese strains. The group represented by the type strain of *C. fukuyamaensis* included five other strains isolated in China. The type strains of *Candida xestobii*, *C. guilliermondii* var. *carpophila* Phaff *et* M. W. Miller and *Trichosporon appendiculare* Batista *et al.* were separated into three different groups, respectively. Taxonomic relationships among the taxa studied are discussed.

Keywords: *Candida guilliermondii*, *Candida fermentati*, *Candida fukuyamaensis*, *Candida xestobii*, electrophoretic karyotype

INTRODUCTION

Candida guilliermondii (Castellani) Langeron *et* Guerra is a common species in both natural and clinical environments. Since its first description as *Endomyces guilliermondii* by Castellani (1912), a considerable number of new species and varieties with phenotypic characteristics similar to those of *C. guilliermondii* have been described. These taxa were listed in a series of monographs on yeast taxonomy as synonyms of *C. guillermondii* or its teleomorph *Pichia guilliermondii* Wickerham (Diddens & Lodder, 1942; Lodder & Kreger-van Rij, 1952; van Uden & Buckley, 1970; Barnet *et al.*, 1983, 1990; Meyer *et al.*, 1984, 1998).

Abbreviation: CHEF, contour-clamped homogeneous electric field.

Bai (1996) used DNA base composition and electrophoretic karyotyping to demonstrate that one of the synonyms, *Torula fermentati* Saito, represented a species distinct from *C. guilliermondii*. Consequently, a new combination, *Candida fermentati* (Saito) Bai, was proposed. Since *C. fermentati* and *C. guilliermondii* were indistinguishable by phenotypic criteria, the taxonomic status of the synonyms of *C. guilliermondii* that were not employed by Bai (1996) remained to be clarified.

San Millán *et al.* (1997) investigated isoenzyme and randomly amplified polymorphic DNA profiles of clinical isolates of *C. guilliermondii*, including the type strains of all synonyms of the species. In addition to confirming *C. fermentati* as a distinct species, their data implied that *C. guilliermondii* was still a heterogeneous species since additional groups were recognized. Kurtzman & Robnett (1997) predicted that *C.*

Table 1. Comparison of strains of *Candida guilliermondii* and related taxa

[T], Type strain; AS, Institute of Microbiology, Academia Sinica, Beijing, China; CBS, Centraalbureau voor Schimmelculture, Delft, The Netherlands; JCM, Japan Collection of Microorganisms, The Institute of Physical and Chemical Research, Japan.

Group	Strain	Taxon	Origin	Size of chromosomal DNA (Mb)
I	JCM 1539[T]	*Candida guilliermondii*	Sputum	0·45–2·17
	CBS 2030[T]	*Pichia guilliermondii*	Frass	0·45–2·17
	CBS 2024[T]	*Blastodendrion krausi*	Ulcer (horse)	0·45–2·19
	CBS 2083[T]	*Blastodendrion arztii*	Blood	0·45–2·17
	CBS 6021[T]	*Candida guilliermondii* var. *japonica*	Soil	0·44–2·17
	CBS 6557[T]	*Candida parapsilosis* var. *tuxtlensis*	Maize dough	0·45–2·17 (2·23)
	CBS 7099[T]	*Candida mamillae*	Nursing bottle	0·44–2·17
	CBS 7232[T]	*Candida amidovorans*	Soil	0·45–2·17
	CBS 8105	*Candida parapsilosis* var. *tokyoensis*	Unknown	0·45–2·17
	AS 2.1660		Soil	0·45–2·17
	AS 2.1674		Laver	0·45–2·17
	AS 2.1684		Leaf	0·45–2·17
	AS 2.1685		Flower	0·45–2·17
	AS 2.1687		Soil	0·45–2·17
	AS 2.1698		Mushroom compost	0·46–2·17
II	JCM 9569[T]	*Candida xestobii*	Beetle	0·53–2·22
III	CBS 5256[T]	*Candida guilliermondii* var. *carpophila*	Appendix (human)	0·69–2·07
IV	JCM 9396[T]	*Candida fukuyamaensis*	Pond water	0·68–2·02
	AS 2.1678		Slime flux of tree	0·68–2·04
	AS 2.1688		Ladybird	0·68–2·02
	AS 2.1689		Fruit	0·68–2·02
	AS 2.1690		Frass	0·68–2·02
	AS 2.1691		Slime flux of tree	0·68–2·02
V	CBS 2022[T]	*Candida fermentati*	Air	0·96–1·77
	CBS 5674[T]	*Torulopsis kestonii*	Air	0·98–1·78
	AS 2.1677		Fruit	1·05–1·77
	AS 2.1680		Soil	0·95–1·77
	AS 2.1681		Flower	0·95–1·78
	AS 2.1682		Mulberry fruit	0·94– 1·76
	AS 2.1686		Soil	0·94–1·77
	AS 2.1692		Jujube fruit	0·95–1·77
	AS 2.1693		Orange peel	0·98–1·78
VI	CBS 5265[T]	*Trichosporon appendiculare*	Fig wasp	1·20–2·23

fukuyamaensis Nakase *et al*. and *C. xestobii* Yarrow *et* S. A. Meyer were conspecific with *C. guilliermondii* on the basis of their similar or identical nucleotide sequences in the large-subunit rRNA gene region D1/D2.

In recent years, electrophoretic karyotyping has been increasingly employed in yeast systematics. Though chromosomal length polymorphisms were observed in some yeast species (Boekhout *et al*., 1993), a remarkable correlation between identical or similar karyotypes and high DNA homologies was shown among several species of *Candida* and other yeast genera (Lee *et al*., 1994, 1998; Vallini *et al*., 1997; Phaff *et al*., 1998). In the present study, we comparatively analysed the chromosomal DNA banding profiles of type strains of all the taxa that were considered or suspected to be synonymous with *C. guilliermondii*. The strains isolated from natural sources in China and which were originally identified as *C. guilliermondii* by conventional methods were also included. Taxonomic relationships among the taxa concerned were suggested on the basis of their electrophoretic karyotypes.

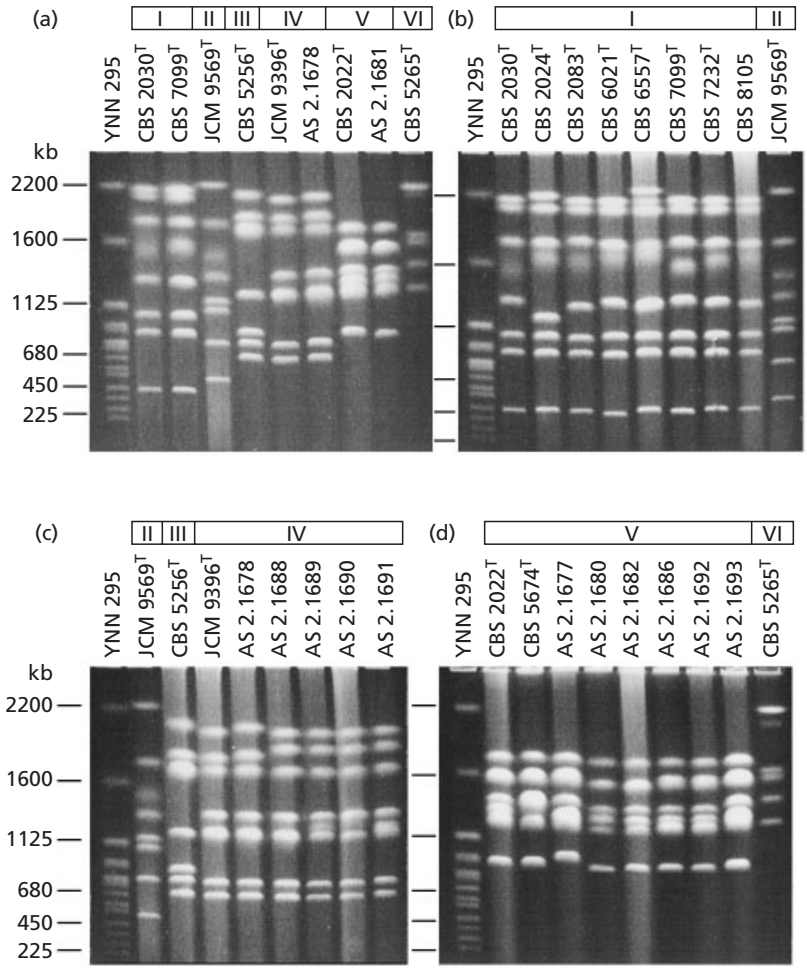

Fig. 1. Electrophoretic karyotypes of the strains in the *Candida guilliermondii* complex. (a) Comparison among representative strains of the six groups; (b) comparison between the strains in groups I and II; (c) comparison among the strains in groups II, III and IV; (d) comparison between the strains in groups V and VI.

METHODS

Organisms and conventional identification. The strains employed are listed in Table 1. Type strains of the taxa studied were obtained from the CBS (Centraalbureau voor Schimmelculture), Delft, The Netherlands and the JCM (Japan Collection of Microorganisms), The Institute of Physical and Chemical Research (RIKEN), Japan. The strains isolated from natural sources in China were characterized using the methods described by van der Walt & Yarrow (1984) and were identified according to Meyer *et al.* (1984).

Preparation of chromosomal DNA and PFGE. Intact yeast chromosomal DNA was prepared for PFGE by using a modified version of the method of Schwartz & Cantor (1984): yeast cells were grown overnight in 5 ml YPD broth (1% yeast extract, 1% peptone, 2% dextrose, all w/v) on a rotary shaker (180 r.p.m.) at 25 °C, harvested with a refrigerated centrifuge and then washed twice with 50 mM EDTA (pH 8·0). Pellets were resuspended in 300–400 µl SPG buffer [10 mM NaH_2PO_4 in 50% (v/v) glycerol, pH 6·2]. A 50 µl aliquot of the cell suspension was mixed with 25 µl Lyticase (Sigma) solution (1000 units ml^{-1} in SPG) and incubated at 40 °C for 3–5 min. The preparation was then gently mixed with 225 µl molten 1% (w/v) LMP agarose (BRL) in 125 mM EDTA (pH 7·5). The mixture was pipetted into a mould chamber (20 × 9 × 1·2 mm; Bio-Rad). After solidification, the plug was incubated in 2 ml LET buffer [500 mM EDTA, 10 mM Tris (pH 8·5), 7·5% (v/v) 2-mercaptoethanol] at 37 °C overnight. After being washed in 50 mM EDTA (pH 8·0), the plug was incubated in 2 ml NDS buffer [500 mM EDTA, 10 mM Tris (pH 8·5), 1% laurylsarcosine] with 0·5 mg proteinase K (Merck) ml^{-1} at 50 °C for 24 h, then rinsed three times in 50 mM EDTA (pH 8·0) and stored in 100 mM EDTA, 10 mM Tris (pH 9·0) at 4 °C.

Chromosomal DNA bands were separated on 0·8% agarose gels in 0·5 × TBE buffer in contour-clamped homogeneous electric field (CHEF) electrophoresis apparatus (CHEF-DR II; Bio-Rad). Electrophoresis was performed at 150 V for 24 h with a 100–200 s ramping switch interval and then at 100 V for 30 h with a 300–400 s ramping switch interval. The temperature of the running buffer was maintained at 12–14 °C. After electrophoresis the gel was stained in ethidium bromide solution (0·5 µg ml^{-1}) for 30 min, destained in distilled water and imaged under UV light (302 nm) with the UVP's GDS 5000 gel documentation system. *Saccharomyces cerevisiae* (YNN 295) chromosomal DNA (Bio-Rad) was used as the marker.

RESULTS

The CHEF electrophoretic karyotypes of a total of 33 strains of the *C. guilliermondii* complex and related species were determined. Six groups were recognized among these strains. The group including the type

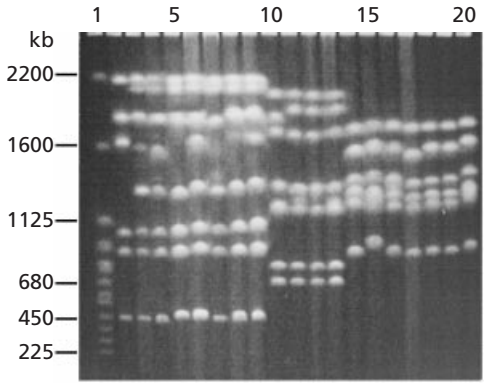

Fig. 2. Electrophoretic karyotypes of the strains in the *Candida guilliermondii* complex isolated from natural sources in China. Lane 1, YNN 295; lanes 2–9, *C. guilliermondii* strains JCM 1539^T, CBS 2030^T, AS 2.1660, AS 2.1674, AS 2.1684, AS 2.1685, AS 2.1687 and AS 2.1698, respectively; lanes 10–13, *Candida fukuyamaensis* strains JCM 9396^T, AS 2.1689, AS 2.1690 and AS 2.1691, respectively; lanes 14–20, *Candida fermentati* strains CBS 2022^T, AS 2.1677, AS 2.1680, AS 2.1682, AS 2.1686, AS 2.1692 and AS 2.1693, respectively.

strains of *C. guilliermondii* and *P. guilliermondii* was designated as group I. The other groups were designated as groups II–VI in decreasing order of chromosomal DNA-banding-pattern similarity to group I. The karyotypes of representative strains of the six groups were compared, as shown in Fig. 1(a).

The following type strains were included, along with the type strains of *C. guilliermondii* (JCM 1539^T) and *P. guilliermondii* (CBS 2030^T), in group I: *Blastodendrion arztii* Ota (CBS 2083^T), *Blastodendrion krausi* Ota (CBS 2024^T), *Candida amidovorans* Balloni *et al.* (CBS 7232^T), *Candida guilliermondii* var. *japonica* Sugiyama *et* Goto (CBS 6021^T), *Candida mamillae* S. Goto (CBS 7099^T), *Candida parapsilosis* var. *tuxtlensis* Herrera *et al.* (CBS 6557^T), the authentic strain (CBS 8105) of *C. parapsilosis* (Ashford) Langeron *et* Talice var. *tokyoensis* Suzuki *et al.* and six Chinese strains. Most of the strains in this group contained eight chromosomal DNA bands in their electrophoretic karyotypes, with molecular sizes ranging from approximately 0·45 to 2·17 Mb (Figs 1b and 2). No bands were found at positions representing 1·15–1·25 Mb and 1·80 Mb for JCM 1539^T (= CBS 566^T), the type strain of *C. guilliermondii* (Fig. 2). Strain CBS 6557^T, the type strain of *C. parapsilosis* var. *tuxtlensis*, contained one additional band representing a molecular size larger than 2·20 Mb (Fig. 1b).

Group II included a single strain, JCM 9569^T, the type strain of *Candida xestobii*. Eight chromosomal DNA bands with molecular sizes ranging from 0·53 to 2·22 Mb were resolved. The pattern was similar to that for group I but could be easily differentiated from the latter. The bands representing 0·45, 0·90, 1·10, 2·08 and 2·17 Mb, shared by the strains in group I, were not found in group II (Fig. 1a, b).

Group III was also composed of a single strain, CBS 5256^T, the type strain of *C. guilliermondii* var. *carpophila* Phaff *et* M. W. Miller. Its karyotype contained seven chromosomal DNA bands with molecular sizes ranging from 0·69 to 2·07 Mb (Fig. 1a, c). Group IV consisted of the type strain of *C. fukuyamaensis*, JCM 9396^T, and five other strains isolated in China. Seven to eight bands in the 0·68–2·02 Mb size range were found in their chromosomal DNA banding profiles (Figs 1c and 2).

Group III and group IV were similar in their chromosomal DNA banding patterns in that they contained a similar number of bands over a similar size range. In view of the fact that strain CBS 5256^T contained a 0·93 Mb band that was absent from all the strains of group IV and that every strain in group IV contained a 1·30 Mb band that was absent from strain CBS 5256^T (Fig. 1c), we tentatively classified strain CBS 5256^T as representing a separate group.

The type strain of *Torulopsis kestonii* Scarr *et* Rose was grouped together with the type strain of *C. fermentati* in group V. A total of seven Chinese strains fell into this group. This group could be easily distinguished from all the other ones. The electrophoretic karyotypes of the strains in this group contained five to six bands with molecular sizes ranging from approximately 0·9 to 1·8 Mb (Figs 1d and 2).

The type strain of *Trichosporon appendiculare* Batista *et al.*, CBS 5265^T, was found to have a unique karyotype. In the present study, six chromosomal DNA bands with molecular sizes ranging from 1·20 to 2·23 Mb were resolved (Fig. 1a, d). However, its obviously much heavier density compared to other bands in the same lane suggests that the band at the 2·23 Mb position may contain more than one chromosomal DNA molecule not resolved under the electrophoresis conditions employed in the present study. The unresolved DNA molecules may be larger than 2·23 Mb in size.

DISCUSSION

In recent years, we have accumulated hundreds of *Candida* strains isolated from various natural sources in China. A considerable number of them were identified as *C. guilliermondii* by conventional methods commonly employed in yeast taxonomy (van der Walt & Yarrow, 1984). After separating *C. fermentati* from *C. guilliermondii* on the basis of the type strain of *Torula fermentati* Saito and three of the Chinese strains (Bai, 1996), we analysed the chromosomal DNA banding patterns of the remaining 18 *C. guilliermondii* strains isolated in China. As a result, three distinct groups were recognized (Fig. 2). Six of the strains were included in the *C. guilliermondii* group, seven were included in the *C. fermentati* group and the remaining five strains were included in the *Candida fukuyamaensis* group. This result suggests that *C. fermentati* is a common species in nature as well as in clinical

environments, as demonstrated by San Millán *et al.* (1997). Furthermore, the data obtained indicate that the species *C. fukuyamaensis* is not uncommon in nature, as are *C. guilliermondii* and *C. fermentati*. In view of the results of electrophoretic karyotyping, each of the three species, *C. guilliermondii*, *C. fermentati* and *C. fukuyamaensis*, represents a genetically homogeneous population, since the chromosomal DNA banding profiles of individual strains with different origins in the same species are either very similar or identical.

The following taxa were confirmed as synonymous with *C. guilliermondii* on the basis of the similarities in their chromosomal DNA banding profiles: *B. arztii*, *B. krausi*, *C. amidovorans*, *C. guilliermondii* var. *japonica*, *C. mamillae*, *C. parapsilosis* var. *tokyoensis* and *C. parapsilosis* var. *tuxtlensis*. This result conformed with the conclusion of San Millán *et al.* (1997), who included these taxa in the same group with the type strain of *C. guilliermondii* on the basis of isoenzyme- and randomly amplified polymorphic DNA profiles. The synonymy of *T. kestonii* with *C. fermentati* demonstrated in the present study also conforms with the work of San Millán *et al.* (1997).

The type strain of *Trichosporon appendiculare* was grouped with the type strains of *C. fermentati* and *T. kestonii* by San Millán *et al.* (1997). However, we found that the chromosomal DNA banding pattern of the former differed markedly from those of the latter two and all the other strains in group II (Fig. 1d). In our opinion, *T. appendiculare* should be treated as representing a separate species rather than as a synonym of *C. fermentati*.

Kurtzman & Robnett (1997) indicated that the nucleotide sequences in the large-subunit rDNA region D1/D2 of *C. fukuyamaensis* and *C. xestobii* were identical and that they differed from that of *C. guilliermondii* by only one nucleotide. Consequently, they predicted that the three species were conspecific. However, their prediction is not supported by the present study. The difference between *C. guilliermondii* and *C. fukuyamaensis* in electrophoretic karyotyping is clearly shown in Fig. 2, as discussed above. The similarity value of 35–42 % obtained from a reciprocal DNA–DNA reassociation experiment with the two species indicates their distinction at species level (Nakase *et al.*, 1994).

The karyotype of *C. xestobii* differed notably from that of *C. fukuyamaensis* (Fig. 1c). Although the chromosomal DNA banding profile of *C. xestobii* was relatively similar to that of *C. guilliermondii*, it was easy to differentiate the former from the latter (Fig. 1b). In view of the homogeneous karyotypes of the 15 strains in the *C. guilliermondii* group and the six strains in the *C. fukuyamaensis* group, it seems reasonable to separate *C. xestobii* from the former two species.

The taxonomic position of *C. guilliermondii* var. *carpophila* remains to be clarified. Although Meyer & Phaff (1972) showed that there was a significant degree of DNA–DNA relatedness between the type strain of *C. guilliermondii* var. *guilliermondii* and two strains of the variety *carpophila*, the type strain of the latter differed markedly from all the strains of the *C. guilliermondii* group in electrophoretic karyotyping. This result conforms with the results of San Millán *et al.* (1997), who separated *C. guilliermondii* var. *carpophila* from the *C. guilliermondii* var. *guilliermondii* group on the basis of different isoenzyme patterns.

C. guilliermondii var. *carpophila* may not be conspecific with *C. guilliermondii* var. *guilliermondii*, as discussed above; however, its relationship with *C. fukuyamaensis* should be investigated further. Although we tentatively allocated *C. guilliermondii* var. *carpophila* to a different group from that of *C. fukuyamaensis*, the similarities in chromosomal DNA size range and banding pattern between these two groups indicated a close genetic relationship.

Among the taxa studied, *C. xestobii* can be distinguished not only by electrophoretic karyotyping, but also by conventional methods. *C. xestobii* can only ferment glucose slowly, while all the other taxa compared with it can strongly ferment glucose and sucrose, can slowly or weakly ferment raffinose and can (in most cases) slowly or weakly ferment galactose. *C. xestobii* also differs from the others by its inability to use D-arabinose or D-mannitol as the sole source of carbon. Furthermore, the type strain of *C. xestobii* cannot grow at temperatures above 30 °C, whereas all the other strains studied can grow at 37 °C or above.

However, the remaining taxa of this study are indistinguishable according to the conventional criteria commonly employed in yeast taxonomy (van der Walt & Yarrow, 1984; Barnett *et al.*, 1990; Yarrow, 1998). Further study is required in order to differentiate these genetically separate taxa in practice. Although Nakase *et al.* (1994) indicated that *C. fukuyamaensis* could be distinguished from *C. guilliermondii* by means of a soluble starch-assimilation test (the former gives a positive result but the latter is negative), none of the five Chinese strains in the *C. fukuyamaensis* group could assimilate soluble starch. This implies that the phenotypic demarcation of the species *C. fukuyamaensis* requires revision.

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