The Significance of Long-chain Fatty Acid Composition and Other Phenotypic Characteristics in Determining Relationships Among Some *Pichia* and *Candida* Species

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The long-chain fatty acid compositions of 22 species of *Candida* were determined, and compared with the fatty acid compositions of 10 species of the genus *Pichia* that contain coenzyme Q9. The long-chain fatty acid results were also compared with other phenotypic criteria (i.e. assimilation of carbon sources, coenzyme Q type, G + C content and proton magnetic resonance spectra) in order to establish possible anamorph/teleomorph relations. Close correlations were found between known perfect/imperfect states. The results suggest that *C. cacaoi* and *P. farinosa*, and *C. maltosa* and *P. etchellsii*, also have anamorph/teleomorph relationships.

INTRODUCTION

In previous papers (Viljoen *et al.*, 1986*a*, 1987) we have shown long-chain fatty acid analysis to be valuable in determining relations between teleomorphic and anamorphic yeasts. In this study, we investigated the relationships between species of the genus *Pichia* containing coenzyme Q type 9, and species of the genus *Candida*. Several possible anamorph/teleomorph relations are discussed on the basis of long-chain fatty acid compositions, coenzyme Q systems, G + C values and other phenotypic characteristics (i.e. carbon and nitrogen assimilation, and growth in vitamin-free media and at 37 °C). A new comparative model for determining anamorph/teleomorph relations is also demonstrated.

METHODS

Strains. Twenty-two *Candida* and ten *Pichia* species were examined (Table 1).

Cultivation. Stock cultures were maintained on yeast extract/malt extract (YM) (Wickerham, 1951) slants. The yeast strains were cultured under standard conditions in 40 ml of a liquid medium containing 80 g glucose (Merck) l⁻¹ and 6.7 g yeast nitrogen base (YNB: Wickerham, 1951) l⁻¹. The harvesting and time of growth were as described in previous papers (Viljoen *et al.*, 1986*b*; Kock *et al.*, 1985).

Extraction of fatty acids, preparation of methyl esters and gas chromatography. These procedures were described in detail by Viljoen *et al.* (1986*a*) and Koch *et al.* (1985).

The long-chain fatty acid composition of some species of *Pichia* (i.e. *P. carsonii*, *P. etchellsii*, *P. farinosa*, *P. ohmerii* and *P. sorbitophila*) are reproduced from Muller *et al.* (1987). The relative mean percentages are shown in Table 1 and Fig. 1.

Carbon-source assimilation. The assimilation results were obtained from Barnett *et al.* (1983) and Kreger-van Rij (1984) and are illustrated in Fig. 1. 'Positive', 'variable', and also descriptions such as 'seldom positive' or 'rarely positive', are indicated by black blocks while negative properties are indicated by open spaces.

Numerical analyses. Analyses were performed by comparing groups comprising 29 characters (assimilation of 26 carbon sources; nitrate utilization; growth in vitamin-free medium and at 37 °C). Both matched and unmatched pairs were equally weighted using the simple matching coefficient of association of Sokal & Michener (Sokal & Sneath, 1963). A dendrogram (Fig. 1) was prepared by single linkage cluster analysis.

The different *Candida* and *Pichia* species were positioned in Fig. 1 according to the results indicated on the horizontal and vertical scales. Numbers were allocated to each species to aid clarity of presentation.

Table 1. *Mean percentage long-chain fatty acid composition of the species representing Candida and Pichia*

Values are the means of three repetitions. Fatty acids are designated as number of carbon atoms : number of double bonds.

Group	Species	Strain no.*	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
I	<i>C. oregonensis</i>	CBS 5036	0.2	0.7	15.2	14.8	1.0	23.7	38.1	6.3
IIA.	<i>C. catenulata</i>	CBS 564	0.4	0.6	14.0	9.8	2.4	41.4	27.3	1.8
	<i>C. santamariae</i>	CBS 4515	0.1	0.1	11.2	8.3	1.2	43.7	31.3	3.3
	<i>C. zeylanoides</i>	CSIR-Y979	0.1	0.1	10.0	2.7	1.7	43.8	29.4	10.4
	<i>C. glabrosa</i>	CSIR-Y581	0.2	0.1	13.0	4.0	3.7	33.4	33.1	12.8
	<i>P. stipitis</i>	CSIR-Y633	0.2	0.1	20.3	10.3	4.8	36.2	26.2	0.9
	<i>C. shehatae</i>	CSIR-Y492	0.9	0.1	16.9	9.4	4.8	36.7	23.8	2.1
	Mean		0.32	0.18	14.23	7.41	3.10	39.20	28.50	5.21
	Standard deviation		0.31	0.20	3.81	3.24	1.56	4.36	3.43	5.06
IIB.	<i>C. rugosa</i>	CSIR-Y299	0.3	0.2	14.5	3.8	0.4	55.0	20.3	3.6
	<i>P. scolyti</i>	CBS 4802	0.2	0.1	17.7	4.8	3.2	40.3	27.4	6.4
	<i>C. silvicultrix</i>	CSIR-Y481	0.2	0.1	15.8	3.6	2.3	41.9	20.5	10.5
	<i>C. entomophila</i>	CSIR-Y479	0.2	0.1	12.5	3.8	3.2	49.3	26.0	3.1
	<i>C. dendronema</i>	CBS 6270	0.1	0.2	27.8	8.3	4.4	35.3	15.3	5.1
	<i>P. mexicana</i>	CBS 7066	0.1	0.1	25.9	9.5	2.0	37.5	18.9	4.1
	Mean		0.18	0.13	19.03	5.63	2.58	43.21	21.4	5.46
	Standard deviation		0.07	0.05	6.31	2.59	1.36	7.50	4.50	2.73
IIC.	<i>C. quercitrusa</i>	CBS 4412	0.3	0.1	19.9	3.7	2.0	53.2	14.6	4.6
	<i>P. ohmerii</i>	CBS 568	0.2	0.1	12.3	4.7	4.4	63.7	12.0	2.4
	<i>C. tenuis</i>	CSIR-Y566	0.3	0.1	13.9	11.5	3.2	54.8	11.9	3.8
	<i>C. guilliermondii</i>	CSIR-Y47	0.1	0.1	18.5	8.3	5.8	54.7	10.6	1.5
	<i>P. guilliermondii</i>	CBS 2030	0.1	0.1	19.1	9.6	4.6	50.1	14.3	1.8
	Mean		0.20	0.10	16.75	7.56	4.00	55.30	12.68	2.82
III	Standard deviation		0.10	0.00	3.38	3.29	1.44	5.06	1.71	1.33
	<i>P. haplophila</i>	CBS 2028	0.2	0.3	17.1	7.8	1.5	26.6	46.8	—
	<i>C. maltosa</i>	CBS 5611	0.2	0.2	16.6	8.7	4.6	28.9	40.7	—
	<i>P. etchellsii</i>	CBS 2011T	0.2	—	14.0	7.8	2.9	31.1	43.7	—
	Mean		0.20	0.16	15.90	8.10	3.00	28.86	43.73	—
	Standard deviation		0.00	0.15	1.66	0.51	1.55	2.25	3.05	—
IV	<i>C. schatavii</i>	CBS 6452	0.2	0.1	18.7	2.6	10.4	48.4	18.0	—
	<i>P. sorbitophila</i>	CBS 7064T	0.2	—	13.3	3.6	0.6	69.3	12.7	—
	<i>C. cacaui</i>	CBS 202	0.1	0.1	9.3	3.0	0.9	53.7	25.9	—
	<i>P. farinosa</i>	NRRL Y-7553	0.1	—	9.4	4.0	1.0	56.3	22.8	—
	<i>C. blankii</i>	CBS 1898	0.1	—	16.2	2.4	3.7	49.2	26.9	—
	<i>P. carsonii</i>	NRRL Y-4275	0.1	—	16.3	2.7	2.4	51.7	26.5	—
	<i>C. hydrocarbofumarica</i>	CBS 6734	0.2	0.1	16.8	3.0	8.5	49.8	19.7	—
	Mean		0.14	0.04	14.28	3.04	3.92	54.05	21.78	—
V	Standard deviation		0.05	0.05	3.72	0.57	3.95	7.26	5.28	—
	<i>C. gropengiesseri</i>	CBS 156	0.8	0.6	5.1	11.0	8.2	70.3	—	—
	<i>C. magnoliae</i>	CSIR-Y449	0.2	0.2	8.8	15.9	2.3	71.4	—	—
	<i>C. versatilis</i>	CBS 1731	0.1	0.2	6.9	22.7	5.9	62.7	—	—
	<i>C. intermedia</i>	CSIR-Y78	0.1	0.1	2.9	15.9	4.9	73.1	—	—
	Mean		0.30	0.27	5.92	16.37	5.32	69.37	—	—
	Standard deviation		0.33	0.22	2.51	4.80	2.44	4.59	—	—

* CBS, Centraalbureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands; CSIR-Y, cultures obtained from Professor J. P. van der Walt, Pretoria, South Africa; NRRL, Northern Regional Research Laboratories, Peoria, Illinois, USA.

RESULTS AND DISCUSSION

Cellular long-chain fatty acid compositions

Reproducible results were obtained when the 32 strains representing the genera *Candida* and *Pichia* were grown in triplicate under standard conditions. The mean fatty acid compositions are shown in Table 1. The species were mainly characterized by the presence of palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). They could be divided into five groups according to the presence of linoleic acid (C18:2) and linolenic acid (C18:3) and the mean percentage oleic acid (C18:1) (Table 1).

Group I, characterized by a pronounced high mean percentage linoleic acid (C18:2), the presence of linolenic acid (C18:3) and a low mean percentage oleic acid (C18:1) consisted of the single species *Candida oregonensis*.

Group II comprised 12 *Candida* species and five *Pichia* species; it was subdivided into three subgroups based on the ratios between oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). Subgroup A comprised those species which had high mean percentages of oleic acid (C18:1) and linoleic acid (C18:2); subgroup B included species with a lower mean percentage of linoleic acid (C18:2) and a higher mean percentage of oleic acid (C18:1); and subgroup C comprised species which had much higher mean percentages of oleic acid (C18:1) and a lower mean percentage of linoleic acid (C18:2).

Group III, characterized by the absence of linolenic acid (C18:3) and a higher mean percentage of linoleic acid (C18:2) than oleic acid (C18:1), included *P. etchellsii*, *C. maltosa* and *P. haplophila*.

Group IV comprised species containing no linolenic acid (C18:3) and a higher mean percentage of oleic acid (C18:1) than linoleic acid (C18:2).

Group V included species which produced pronounced high mean percentages of oleic acid (C18:1) and no linoleic acid (C18:2) or linolenic acid (C18:3).

Anamorph/teleomorph relations

Several criteria should be met before an anamorph non-spore forming yeast is ascribed to a specific ascosporeogenous teleomorph yeast. Anamorph/teleomorph relations are mainly based on morphological and physiological properties (Van Uden & Buckley, 1970; Meyer *et al.*, 1984). Other criteria, however, such as the coenzyme Q type (Phaff, 1984; Yamada & Kondo, 1972), the degree of DNA relatedness (LethBak & Stenderup, 1969; Nakase & Komagata, 1971), cellular long-chain fatty acid compositions (Viljoen *et al.*, 1987), proton magnetic resonance spectra (Manachini, 1979; Spencer & Gorin, 1969), serological studies (Montrocher, 1982; Tsuchiya *et al.*, 1965) and electrophoretic comparison of enzymes (Yamazaki & Komagata, 1982), are all useful methods in correlating the relationships between imperfect and perfect yeasts. Most investigators agree that these phenotypic and genetic characteristics should be applied coordinately in order to correctly assign imperfect yeasts to perfect yeast forms.

In this study the similarities between 32 species, representing the genera *Pichia* and *Candida*, were determined on the basis of cellular long-chain fatty acid compositions, carbon-source assimilation, and the ability to utilize nitrate and to grow in a vitamin-free medium and at 37 °C. These characteristics were used coordinately in a new model (Fig. 1). The results obtained were compared with other criteria, viz. G + C values, proton magnetic resonance spectra and enzyme studies, in order to evaluate possible new teleomorph/anamorph relations.

Anamorphic yeasts having a known perfect stage. The known anamorph/teleomorph-related yeasts are all closely positioned in Fig. 1 (enclosed in boxes), which indicates a high similarity in cellular long-chain fatty acid composition and other phenotypic characteristics.

(a) *C. shehatae* and *P. stipitis*. *C. shehatae* and *P. stipitis*, the species regarded as its perfect counterpart (Buckley & Van Uden, 1967), have similar long-chain fatty acid compositions (Viljoen *et al.*, 1987), carbon-source assimilation patterns (>90% similarity) and G + C values [42.5 mol % for *P. stipitis* (Miranda *et al.*, 1982) and 41.2 mol % for *C. shehatae* (Nakase & Komagata, 1971)], and both have coenzyme Q type 9 (Yamada & Kondo, 1972).

(b) *C. guilliermondii* and *P. guilliermondii*. *C. guilliermondii* and its assumed perfect state, *P. guilliermondii* (Van Uden & Buckley, 1970) have similar long-chain fatty acid profiles (Table 1), carbon-source assimilation patterns (>90% similarity) and G + C values [44.1–44.4 mol % for *C. guilliermondii* (Nakase & Komagata, 1971) and 44.4 mol % for *P. guilliermondii* (Fuson *et al.*, 1980)], and both have coenzyme Q type 9 (Yamada & Kondo, 1972).

Yeasts having possible teleomorph/anamorph relations. (a) *C. cacaoi* and *P. farinosa*. These species produce similar long-chain fatty acid profiles (Fig. 1), have ovoid cells and moderate to well-developed pseudomycelium, and show 82% similarity in the assimilation of carbon sources. The two species are differentiated by the ability of *P. farinosa* to assimilate L-arabinose and L-arabinitol (Fig. 1). They have similar G + C values [42.5 mol % for *P. farinosa* and 41.7 mol % for *C. cacaoi* (Meyer & Phaff, 1972)] and proton magnetic resonance spectra (Gorin & Spencer, 1970).

(b) *C. maltosa* and *P. etchellsii*. These species have similar long-chain fatty acid profiles (Table 1), morphology [short ovoidal cells of equal size, and well-developed pseudomycelium] (Meyer *et al.*, 1984; Kurtzman, 1984)] and physiological properties (>90% similarity), and both have coenzyme Q type 9 (Yamada & Kondo, 1972). According to the literature they are differentiated only by the ability of *C. maltosa* to assimilate L-arabinose and by the fermentation of galactose and trehalose (Meyer *et al.*, 1984). A close relationship between these two yeasts is further indicated by their similar G + C values [36.3 mol % for *C. maltosa* (Nakase & Komagata, 1971) and 38.5 mol % for *P. etchellsii* (Nakase & Komagata, 1970)].

Other Candida and Pichia species with similar long-chain fatty acid profiles. (a) *C. blankii* and *P. carsonii*. These two yeast species have almost identical long-chain fatty acid compositions (Table 1), show 85% similarity in the assimilation of carbon sources (Fig. 1), and produce similar cell shapes and abundant pseudohyphae. However, their G + C values are very different [55.1 mol % for *C. blankii* (Meyer *et al.*, 1984) and 39.6 mol % for *P. carsonii* (Kurtzman & Smiley, 1979)].

(b) *C. dendronema* and *P. mexicana*. These species have similar long-chain fatty acid compositions (Table 1), and show 85% similarity in the assimilation of carbon sources (Fig. 1). Their G + C values are 42.2 mol % for *P. mexicana* (Miranda *et al.*, 1982) and 40.4 mol % for *C. dendronema* (Stenderup *et al.*, 1972).

(c) *C. silvicultrix*, *C. entomophila* and *P. scolyti*. These yeasts all have similar long-chain fatty acid profiles. *C. silvicultrix* and *C. entomophila* show 90% similarity in the assimilation of carbon sources and 85% similarity with *P. scolyti*. *C. silvicultrix* and *P. scolyti* both produce well-developed pseudomycelium with blastospores and ovoidal cells, of equal size. *C. entomophila*, on the other hand, produces true mycelium and cells of various shapes. There are major differences in G + C values, however, between *P. scolyti* [40.1 mol % (Fuson *et al.*, 1980)] and *C. entomophila* [54.0 mol % (Stenderup *et al.*, 1972)] while *C. silvicultrix* has a lower value [36.6 mol % (Meyer *et al.*, 1984)].

Fig. 1 (on facing page). Relationships between species of *Candida* and *Pichia*, arranged according to carbon source utilization (vertical scale, left) and long-chain fatty acid composition (horizontal scale, top). Species known or believed to have anamorph/teleomorph relationships are enclosed in boxes. 1. *C. hyd.*, *Candida hydrocarbofumarica*; 2. *C. ten.*, *Candida tenuis*; 3. *P. guil.*, *Pichia guilliermondii*; 4. *C. guil.*, *Candida guilliermondii*; 5. *C. ent.*, *Candida entomophila*; 6. *P. scol.*, *Pichia scolyti*; 7. *C. sil.*, *Candida silvicultrix*; 8. *C. glae.*, *Candida glaeobosa*; 9. *P. stip.*, *Pichia stipitis*; 10. *C. she.*, *Candida shehatae*; 11. *C. bla.*, *Candida blankii*; 12. *P. mex.*, *Pichia mexicana*; 13. *C. den.*, *Candida dendronema*; 14. *C. int.*, *Candida intermedia*; 15. *P. car.*, *Pichia carsonii*; 16. *P. etc.*, *Pichia etchellsii*; 17. *C. mal.*, *Candida maltosa*; 18. *P. ohm.*, *Pichia ohmerii*; 19. *C. oreg.*, *Candida oregonensis*; 20. *P. far.*, *Pichia farinosa*; 21. *C. ver.*, *Candida versatilis*; 22. *P. sor.*, *Pichia sorbitophila*; 23. *C. cac.*, *Candida cacaoi*; 24. *C. schat.*, *Candida schataui*; 25. *C. san.*, *Candida santamariae*; 26. *C. rug.*, *Candida rugosa*; 27. *C. zey.*, *Candida zeylanoides*; 28. *P. hap.*, *Pichia haplophila*; 29. *C. cat.*, *Candida catenulata*; 30. *C. quer.*, *Candida quericitrusa*; 31. *C. mag.*, *Candida magnoliae*; 32. *C. grop.*, *Candida gropengiesseri*. Key to lipid histograms: ■■■, C16:0 (palmitic acid); ■■■■, C16:1 (palmitoleic acid); ■■■■■, C18:0 (stearic acid); ■■■■■■, C18:1 (oleic acid); ■■■■■■■, C18:2 (linoleic acid); ■■■■■■■■, C18:3 (linolenic acid). For the carbon-source utilization data, black blocks denote positive, and white spaces denote negative.

(d) *C. schatavii* and *P. sorbitophila*. Both species are characterized by the absence of linolenic acid (C18:3), and have similar fatty acid profiles and carbon-source utilization patterns (82% similarity).

(e) *C. tenuis* and *P. guilliermondii*. These species have closely similar long-chain fatty acid compositions (Table 1), coenzyme Q type 9 (Yamada & Kondo, 1972), 85% similarity in the assimilation of carbon sources (Fig. 1) and G + C values of 44.0 mol % for *C. tenuis* (Stenderup & LethBak, 1968) and 44.4 mol % for *P. guilliermondii*. Furthermore, both species have ovoid to elongate cells of equal sizes and produce pseudomycelium and blastospores.

Teleomorph relations

Based on the results in Fig. 1 we detected two distinct groups in the genus *Pichia*. The first group comprises all the species with hat-shaped ascospores (Kurtzman, 1984), i.e. *P. stipitis*, *P. scolyti*, *P. mexicana*, *P. guilliermondii* and *P. ohmeri*, characterized by the presence of linolenic acid (C18:3). The second group comprises those species which form spheroidal ascospores (except *P. haplophila*) and are characterized by the absence of linolenic acid (C18:3), i.e. *P. sorbitophila*, *P. farinosa*, *P. etchellsii*, *P. carsonii* and *P. haplophila* (Muller *et al.*, 1987).

Anamorph relations

The highly heterogeneous nature of the genus *Candida* is reflected in Fig. 1. Species without linoleic (C18:2) and linolenic (C18:3) acids (*C. gropengiesseri*, *C. magnoliae*, *C. versatilis* and *C. intermedia*) showed no resemblance to *Pichia* species on the basis of long-chain fatty acid compositions.

CONCLUSIONS

According to the results the following conclusions are drawn.

1. The coordinate use of long-chain fatty acid composition and other phenotypic characters, as illustrated in Fig. 1, provides a useful model to determine similarities between yeasts (i.e. anamorph/teleomorph, anamorph and teleomorph relations). Imperfect yeasts of the genus *Candida* and their known perfect forms were similar in their long-chain fatty acid compositions and in other phenotypic characteristics. These yeasts were in all cases closely positioned in the coordinate scheme (Fig. 1).
2. The following anamorph/teleomorph associations are proposed: *C. cacaoi* and *P. farinosa*, *C. maltosa* and *P. etchellsii*.
3. A correlation seems to exist between the coenzyme Q type 9 *Pichia* species investigated which produce hat-shaped ascospores and the presence of linolenic acid (C18:3), and the coenzyme Q type 9 *Pichia* species which produce spheroidal ascospores (except for *P. haplophila*) and the absence of linolenic acid (C18:3). More strains and species should however be investigated before any generalization is made.
4. The heterogeneous nature of the genus *Candida* is clearly illustrated in the coordinate scheme (Fig. 1).

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