Long-chain Fatty Acid Compositions of Some Asporogenous Yeasts and Their Respective Ascosporogenous States

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Long-chain fatty acid compositions were determined for strains of seven species of *Candida* and their counterparts within the perfect genera: *Candida shehatae* and *Pichia stipitis*, *Candida kefyr* and *Kluyveromyces marxianus*, *Candida lipolytica* and *Yarrowia lipolytica*, *Candida pelliculosa* and *Hansenula anomala*, *Candida pseudotropicalis* and *Kluyveromyces fragilis*, *Candida utilis* and *Hansenula jadinii*, *Candida parapsilosis* and *Lodderomyces elongisporus*, *Candida shehatae* and *Pichia stipitis*. Close correlations were found between the fatty acid compositions of these pairs of strains, indicating that the analysis of long-chain fatty acids may be useful for studying the relationships between the perfect and imperfect states of the genus *Candida*.

INTRODUCTION

The genus *Candida* Berkhout is described by Van Uden & Buckley (1970) as a heterogeneous collection of asporogenous yeasts which cannot be classified conveniently elsewhere and is gathered for convenience into a single group, based on a few morphological and physiological criteria. The low relatedness observed within this group and the similarities of these imperfect species with the perfect genera *Kluyveromyces*, *Pichia*, *Hansenula*, *Yarrowia* or *Lodderomyces* highlighted the diversity of this genus (Leth Bak & Stenderup, 1969).

Long-chain fatty acid analysis can be a useful tool in the identification and classification of yeasts (Viljoen *et al.*, 1986; Kock *et al.*, 1985). In this study, the long-chain fatty acid compositions of seven species of *Candida* were compared with those of their supposed teleomorphs.

METHODS

Strains. Seven *Candida* species, two species of *Kluyveromyces*, two species of *Hansenula*, and one species each of *Lodderomyces*, *Yarrowia* and *Pichia* were used (Table 1).

Cultivation. The stock cultures were maintained on yeast extract/malt extract (YM) (Wickerham, 1951) slants. The yeast strains were cultured for 16 h at 30 °C on a rotary shaker at 160 r.p.m. (throw 50 mm) in 150 ml Erlenmeyer flasks containing 40 ml medium consisting of glucose (Merck) (80 g l⁻¹) yeast/nitrogen base (6.7 g l⁻¹) (YNB; Wickerham 1951). Then 18 ml of the above culture (Klett reading 150, 640 nm filter) was inoculated into 400 ml glucose/YNB liquid medium in 1 l Erlenmeyer flasks and cultured for 2 d (in order to reach stationary phase) on the rotary shaker. This procedure was repeated three times. The cells were harvested and washed three times with cold saline (0.85% NaCl) solution by centrifugation at 8000 g for 5 min at 4 °C. The cells were stored at -15 °C.

Extraction of fatty acids and preparation of methyl esters. The methods of Gunasekaran & Hughes (1980) were used. The final extraction of the fatty acid methyl esters was performed with the addition of n-hexane.

Gas chromatography. The fatty acid methyl esters were analysed and identified by methods described in detail by Viljoen *et al.* (1986) and Kock *et al.* (1985).

Numerical analysis. The Pearson product-moment correlation coefficient, *r*, was used to measure the similarity

Table 1. Mean percentage long-chain fatty acid composition of the strains representing *Candida* species and their supposed teleomorphs

Values are the means of three repetitions. The standard deviations were about 5% of the mean value (range 2-6%). Fatty acids are designated as number of carbon atoms: number of double bonds. UN1 and UN2 represent unidentified fatty acids with retention times of 13.36 and 14.13 min, respectively (retention time for C16:0 10.25 min).

Strain	Strain no.*	C14:0	C14:1	C16:0	C16:1	UN1	UN2	C18:0	C18:1	C18:2	C18:3	r†
<i>C. kefyr</i>	CSIR-Y879	0.1	0.1	10.1	27.9	0.3	—	3.8	38.1	16.4	2.3	
<i>K. marxianus</i>	CBS 5669	0.5	0.1	14.1	26.1	0.2	0.5	3.8	37.2	14.1	2.9	0.992
<i>C. lipolytica</i> ‡	CBS 599	0.1	0.1	12.2	15.7	0.5	0.9	2.0	53.8	14.3	—	
<i>Y. lipolytica</i>	Y28	0.1	0.1	9.9	10.2	0.1	0.6	4.0	58.5	16.3	—	0.990
<i>C. pelliculosa</i>	CSIR-Y5	0.7	0.2	12.3	8.8	0.9	1.4	4.9	36.1	27.1	7.3	
<i>H. anomala</i>	CSIR-Y605	0.8	0.2	15.5	10.0	0.2	0.3	3.5	37.2	27.7	3.1	0.990
<i>C. pseudotropicalis</i>	CSIR-Y95	0.5	0.3	12.6	13.4	0.9	0.3	3.5	27.6	31.9	9.0	
<i>K. fragilis</i> §	CBS 1556	0.6	0.3	14.3	14.5	0.4	0.1	2.1	28.9	32.7	5.9	0.994
<i>C. utilis</i>	CBS 890	0.7	0.5	15.2	2.8	0.5	0.6	2.3	48.1	26.5	1.9	
<i>H. jadinii</i>	CSIR-Y227	0.6	0.4	16.3	2.0	0.3	0.5	3.2	51.0	23.2	2.5	0.996
<i>C. parapsilosis</i>	CSIR-Y685	0.2	—	11.0	6.5	0.3	1.7	5.3	50.3	20.3	0.4	
<i>L. elongisporus</i>	CSIR-Y162	0.2	—	11.9	7.4	0.7	1.8	5.2	52.5	19.1	0.6	0.998
<i>C. shehatae</i>	CSIR-Y493	0.9	0.1	16.9	9.4	0.9	1.9	4.8	36.7	23.8	2.1	
<i>P. stipitis</i>	CSIR-Y633	0.2	0.1	20.3	10.3	0.4	0.5	4.8	36.2	26.2	0.9	0.994

* CBS, Centraalbureau voor Schimmelcultures, Yeast Division, Delft, The Netherlands; CSIR-Y, cultures obtained from Professor J. P. van der Walt, Pretoria, South Africa.

† r = Pearson product correlation (Pearson & Hartley, 1958).

‡ Now called *Yarrowia lipolytica* in the CBS catalogue.

§ This strain is now also called *Kluyveromyces marxianus* in the CBS catalogue.

between any pair of long-chain fatty acids (Pearson & Hartley, 1958). The product-moment correlation coefficient is defined by the equation

$$r = \frac{(1/n) \sum d_x d_y}{S_x S_y}$$

The numerator is the mean product of the deviations of each paired member of the distribution. The denominator $S_x S_y$ is the product of the standard deviations of the two parts of the yeasts' long-chain fatty acid distributions considered separately.

RESULTS

Reproducible fatty acid compositions were obtained when the yeast strains were grown in triplicate under standard conditions (Table 1). The 14 species contained palmitic acid (C16:0), palmitoleic acid (C16:1), oleic acid (C18:1) and linoleic acid (C18:2) as major fatty acids. The following pairs of strains were recognized on the basis of similarities in long-chain fatty acid composition; their characteristic features are summarized.

Candida kefyr and *Kluyveromyces marxianus* both had a higher mean percentage of palmitoleic acid content than the other species investigated.

Candida lipolytica and *Yarrowia lipolytica* contained the highest mean percentage of oleic acid, whereas no linolenic acid (C18:3) was detected.

Candida pelliculosa and *Hansenula anomala* were both characterized by relatively high mean percentages of oleic acid, linoleic acid and linolenic acid.

Candida pseudotropicalis and *Kluyveromyces fragilis* contained the highest mean percentages of linoleic acid and linolenic acid.

Candida utilis and *Hansenula jadinii* contained the lowest mean percentages of palmitic acid, oleic acid and linoleic acid.

Candida parapsilosis and *Lodderomyces elongisporus* contained the highest mean percentage of stearic acid (C18:0), whereas no myristoleic acid (C14:1) was detected.

Candida shehatae and *Pichia stipitis* contained a higher mean percentage of palmitic acid than the other yeasts studied.

DISCUSSION

The genus *Candida* is composed of species with different morphological, biochemical and ecological characteristics (Montrocher & Claisse, 1984). This heterogeneity was reflected in the large variation in long-chain fatty acid compositions between the seven *Candida* species we studied.

Yeasts which produce similar long-chain fatty acids are more closely related than those which differ (Kock *et al.*, 1985; Cottrell *et al.*, 1985; Viljoen *et al.*, 1986). Since all the assumed pairs of perfect and imperfect strains had almost identical long-chain fatty acid compositions (Table 1), these pairings would appear to be correct.

Candida kefir and *Kluyveromyces marxianus* produce similar electrophoretic isoenzyme patterns (Sidenberg & Lachance, 1986) and have similar carbon and nitrogen assimilation profiles (Van Uden & Buckley, 1970). The similarity in long-chain fatty acid composition between these two yeasts ($r = 0.992$) is in agreement with these results.

Spencer & Gorin (1969) found that most strains of *Candida pelliculosa* differed from *Hansenula anomala* (the supposed ascosporogenous form) since they yielded galactomannans with different proton magnetic resonance spectra. Wickerham & Burton (1954) found that strains of *Candida pelliculosa* differed from *Hansenula anomala* in their physiology. However, Leth Bak & Stenderup (1969) found close DNA homology between these species. Our data support the latter results since the long-chain fatty acids compositions of these two yeasts were very similar ($r = 0.990$).

Candida lipolytica, which has been designated as the imperfect form of the ascosporogenous yeast *Yarrowia lipolytica* (Diddens & Lodder, 1939) showed a close relationship ($r = 0.990$), as did *Candida pseudotropicalis* and its imperfect form *Kluyveromyces fragilis* ($r = 0.994$). Spencer & Gorin (1969), in agreement with the latter result, found that '*Saccharomyces fragilis*' and *Candida pseudotropicalis* (its presumed imperfect form) formed mannose-containing polysaccharides having identical proton magnetic resonance spectra. ['*Saccharomyces fragilis*' has since been renamed as *Kluyveromyces marxianus* var. *fragilis* Johansen (Sidenberg & Lachance, 1986; Barnett *et al.*, 1983).] Leth Bak & Stenderup (1969) also found high DNA homology between *Candida pseudotropicalis* and *Kluyveromyces fragilis*.

Candida utilis, which is assumed to be the imperfect form of the ascosporogenous yeast *Hansenula jadinii* (Lodder & Kreger-van Rij, 1952), produced long-chain fatty acids similar ($r = 0.996$) to those of its perfect state. *Lodderomyces elongisporus* is regarded as the ascosporogenous perfect form of *Candida parapsilosis* (Van der Walt, 1966). Although Meyer & Phaff (1972) found similar DNA base ratios (39.5–41.2 mol % G + C) between these two yeasts, Montrocher & Claisse (1984) stated that *Lodderomyces elongisporus* and *Candida parapsilosis* differed in their serological and biophysical properties. We found similar long-chain fatty acid compositions ($r = 0.998$) between these two yeasts. A close relationship ($r = 0.994$) was also found between *Candida shehatae* and its assumed imperfect counterpart *Pichia stipitis* (Buckley & Van Uden, 1967).

The long-chain fatty acid compositions of the *Candida* species studied coincided with those of their supposed ascosporogenous states. Analysis of long-chain fatty acid composition may therefore prove to be a useful technique for studying the relationships of ascosporogenous and sporogenous yeast species, and may allow the relation between a teleomorph species and its corresponding anamorphic state to be more clearly determined. However, further work is needed on more strains in order to establish the validity of this method.

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