SHORT COMMUNICATION

A Rapid Method to Differentiate between Four Species of the *Endomycetaceae*

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The cellular long-chain fatty acids of seven strains representing four species of the *Endomycetaceae* were extracted from yeast cells by saponification and analysed as their methyl esters by GLC. Each of these species produced a distinctive mean fatty acid 'fingerprint'. It was possible to distinguish between the four species within 4 h after they were obtained from 48 h cultures as compared with 7 to 10 d for more conventional methods.

INTRODUCTION

The conventional identification of *Endomycetaceae* is mainly based on microscopical appearance, mode of sexual reproduction, and physiological and certain biochemical features (Barnett *et al.*, 1983). Although these methods provide information to differentiate between yeasts, they are often time consuming. Abel *et al.* (1963) demonstrated that micro-organisms can be classified into genera and species on the basis of their fatty acid composition as determined by GLC. This technique has since been successfully used by workers to identify, differentiate and classify bacteria and yeasts from clinical specimens (Dees & Moss, 1975; Gunasekaran & Hughes, 1980). Ratray *et al.* (1975) and Shaw (1974) have discussed the application of cellular fatty acid composition to the identification of some yeast species, but no standard cultivation procedure had been applied and therefore no direct comparisons between previous studies on yeasts could be made. Here we describe the fatty acid composition of seven yeast strains representing four species of the *Endomycetaceae* grown under standard conditions and assess whether this method is reliable and more rapid than the conventional methods for the identification of these yeasts.

METHODS

Yeast species and growth conditions. Cultures of the four species examined (Table 1) were obtained from Professor J. P. van der Walt, Pretoria, South Africa (CSIR-Y); they were maintained on glucose YM (yeast extract-malt extract) agar slopes and then cultured in triplicate for 16 h at 30 °C on a rotary shaker at 60 r.p.m. (throw = 50 mm) in 150 ml Erlenmeyer flasks. Each flask contained 40 ml medium [80 g glucose l⁻¹ (Merck) and 6.7 g yeast nitrogen base l⁻¹ (Difco)]. A 10 ml portion of this suspension (200 Klett units 640 nm filter) was inoculated into 400 ml of the same medium in 1 litre Erlenmeyer flasks and cultured for 2 d. The organisms were harvested by centrifugation at 8000 g for 5 min at 4 °C, washed three times in cold saline, and then freeze dried.

Fatty acid analysis. The extraction of fatty acids, the preparation of their methyl esters and the extraction of these with n-hexane was done according to the method proposed by Gunasekaran & Hughes (1980). The methyl esters of the total fatty acids were analysed by GLC on a Hewlett-Packard Model 5830A gas chromatograph equipped with dual flame-ionization detectors. Identification of the esters was based on a comparison of retention times with those of known standards of C14:0 (myristic acid), C14:1 (myristoleic acid), C16:0 (palmitic acid),

Table 1. Percentage long-chain fatty acid composition of seven strains representing four species of *Endomycetaceae*

Species	Strain	Percentage fatty acid composition*					
		C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
<i>Ambrosiozyma monospora</i>	CSIR Y-355	17.07 ± 0.91	3.48 ± 0.07	2.58 ± 0.04	39.66 ± 1.61	19.24 ± 0.70	17.97 ± 0.74
	Mean	21.32	4.58	3.20	34.65	36.28	—
<i>Endomyces fibuliger</i>	CSIR Y-269	25.12 ± 1.20	5.32 ± 1.10	3.28 ± 0.09	35.11 ± 1.72	31.18 ± 1.31	—
	CSIR Y-643	17.51 ± 0.81	3.83 ± 0.01	3.11 ± 0.15	34.18 ± 2.01	41.37 ± 1.61	—
<i>Botryosaurus symmaedendrus</i>	Mean	21.32	4.58	3.20	34.65	36.28	—
	SD	5.38	1.05	0.12	0.66	7.21	—
<i>Pichia stipitis</i>	CSIR Y-406	13.31 ± 0.51	13.22 ± 0.30	2.70 ± 0.01	40.30 ± 1.61	30.50 ± 1.34	—
	Mean	23.70 ± 1.13	4.70 ± 0.03	8.40 ± 0.73	22.10 ± 1.00	35.50 ± 1.33	5.60 ± 0.34
<i>Pichia stipitis</i>	CSIR Y-632	25.13 ± 1.21	5.21 ± 0.61	9.21 ± 0.81	21.31 ± 1.01	37.60 ± 1.21	1.54 ± 0.76
	CSIR Y-633	20.81 ± 1.00	6.30 ± 0.33	9.00 ± 0.90	20.20 ± 1.11	36.35 ± 1.03	7.34 ± 1.61
<i>Pichia stipitis</i>	Mean	23.21	5.40	8.87	21.20	36.48	4.83
	SD	2.20	0.82	0.42	0.95	1.06	2.98

* Values are means ± SD of three or more repetitions; fatty acids are designated as number of carbon atoms: number of double bonds.

Fig. 1. Mean percentage fatty acid composition of *A. monospora* (1), *E. fibuliger* (2), *B. synnaedendrus* (3) and *P. stipitis* (4).

C16:1 (palmitoleic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid) and C18:3 (linolenic acid) (Serva). All analyses were done on glass columns (4 mm i.d. $\times$ 1.5 m) packed with 5% diethyleneglycol succinate on Chromosorb W (80–100 mesh: Anatech). The flow rate of the carrier gas (nitrogen) was 40 ml min⁻¹ at a column temperature of 160 °C. Relative amounts of given fatty acids were calculated from their respective peak areas.

RESULTS AND DISCUSSION

The results obtained were reproducible when the individual strains were grown three times under standard conditions (Table 1). The fatty acid composition of the strains representing each species is summarized in Fig. 1. Since a negligible amount of myristic acid and myristoleic acid was produced by the species under investigation, it was decided to omit these fatty acids from Table 1 and Fig. 1. The percentage of linolenic acid was higher in *Ambrosiozyma monospora* than in *Endomyces fibuliger*, *Botryosascus synnaedendrus* and *Pichia stipitis*, but the percentage of linoleic acid was lower. The strains of *E. fibuliger* had a significantly higher mean oleic acid content than did *P. stipitis* ($P < 1\%$ – Student's *t*-test). Linolenic acid was absent from *E. fibuliger*. *B. synnaedendrus* had a higher palmitoleic acid content than did the other three species while linolenic acid could not be detected. The mean percentage of stearic acid in the *P. stipitis* strains was higher than in the other species while the mean percentage of oleic acid was less. *P. stipitis* contained less linolenic acid than *A. monospora*.

With GLC, it was therefore possible to differentiate between the four *Endomycetaceae* yeast species within 4 h, once they had been cultivated for 48 h on glucose yeast nitrogen base medium. This is a marked improvement over the usual 7 to 10 d required with conventional methods (Barnett *et al.*, 1983; Lennette *et al.*, 1974). Further work is needed on more yeasts in order to construct not only a rapid differentiating technique but also a sensitive and rapid identification method which would afford obvious advantages over the conventional morphological and physiological tests now being used for the identification of yeasts.

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