Long-chain fatty acid composition of selected genera of yeasts belonging to the Endomycetales

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Abstract. The cellular long-chain fatty acids of 36 strains representing 18 genera of the Saccharomycetaceae, Endomycetaceae, Metchnikowiaceae, Saccharomycodaceae, Schizosaccharomycetaceae and Dipodascaceae were extracted and analyzed as methyl esters by gas chromatography. On the basis of their fatty acid content the set of strains was divided into 6 groups, coinciding with the above families. The members of the Saccharomycetaceae (group I) had a high percentage of oleic acid while the strains classified under the Endomycetaceae (group II) and Metchnikowiaceae (group III) were characterized by oleic acid and linoleic acid as major fatty acids. The Saccharomycodaceae (group IV) had the highest percentage of palmitoleic acid. The Schizosaccharomycetaceae (group V) had the highest percentage of oleic acid, while the Dipodascaceae (group VI) were characterized by a high percentage of linoleic acid.

INTRODUCTION

The possibility of classifying microorganisms on the basis of their lipid composition has been demonstrated by Abel et al. (1963). This has since been successfully used by various workers to identify and classify bacteria and yeasts from mainly clinical specimens (Dees & Moss 1975; Gunasekaran & Hughes 1980; Moss & Dees 1976). The use of cellular long-chain fatty acid composition for the identification of various yeasts has also been investigated (Kaneko et al. 1976; Moss et al. 1982; Ratray et al. 1975; Shaw 1974).

In this investigation the cellular long chain fatty acid composition of 36 strains representing 18 selected genera of the families Saccharomycetaceae, Endomycetaceae, Metchnikowiaceae, Saccharomycodaceae, Schizosaccharomycetaceae and Dipodascaceae grown under standard conditions is described. The value of these phenotypic characteristics in taxonomy is considered as well as its feasibility for identification purposes.

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MATERIALS AND METHODS

Strains studied. Cultures were obtained from Prof. J. P. van der Walt, Pretoria, South Africa (CSIR-Y) and the Centre of Biology, Gulbenkian Institute of Science, Oeiras, Portugal (IGC).

Saccharomycetaceae:

Saccharomycopsis capsularis, CSIR-Y447, CSIR-Y651; *Williopsis saturnus*, CSIR-Y17; *Clavispora lusitaniae*, CSIR-Y484; *Schwanniomyces occidentalis* var. *occidentalis*, CSIR-Y937, CSIR-Y936, CSIR-Y938, var. *persoonii*, CSIR-Y285, var. *castellii*, CSIR-Y993, var. *alluvius*, CSIR-Y828; *Lodderomyces elongisporus*, CSIR-Y162, *Wingea robertsii*, CSIR-Y208, CSIR-Y446; *Zygosaccharomyces bailii*, CSIR-Y126; *Debaryomyces marama*, CSIR-Y216; *D. vanrijiae*, CSIR-Y960; *Lipomyces starkeyi*, CSIR-Y205, CSIR-Y158, IGC 3944; *L. kononenkoae*, CSIR-Y353, IGC 4052; *L. tetrasporus*, CSIR-Y503.

Endomycetaceae:

Endomyces fibuliger, CSIR-Y643; *Pichia fluxuum*, CSIR-Y183; *Botryosascus synnaedendrus*, CSIR-Y406; *Hansenula dryadoides*, CSIR-Y649, CSIR-Y650.

Metchnikowiaceae:

Metchnikowia reukaufii, CSIR-Y13.

Saccharomycodaceae:

Hanseniaspora valbyensis, CSIR-Y895; *H. uvarum*, CSIR-Y898; *Saccharomyces ludwigii*, CSIR-Y22, CSIR-Y8.

Schizosaccharomycetaceae:

Schizosaccharomyces malidevorans, CSIR-Y933; *S. pombe*, CSIR-Y457.

Dipodascaceae:

Dipodascus australiensis, CSIR-Y584, *D. reessii*, CSIR-Y585.

Cultivation of strains. 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. Each flask contained 40 ml medium consisting of 80 g·l⁻¹ glucose (Merck) and 6.7 g·l⁻¹ yeast/nitrogen base (YNB) (Wickerham 1951). Eighteen ml of the above culture (Klett = 150, filter = 640 nm) was then inoculated into 400 ml of glucose YNB liquid medium in 1 l Erlenmeyer flasks and cultured for 2 days (in order to reach stationary phase) on the rotary shaker. This procedure was repeated 3 times. The cells were harvested by centrifugation at 8000 g for 5 minutes at 4°C and washed 3 times with cold saline (0.85% NaCl) solution. Centrifugation was performed after each washing. The cells were stored at -15°C.

Extraction of the fatty acids and preparation of methyl esters. The extraction

and preparation of fatty acid methyl esters were carried out according to the method proposed by Gunasekaran and Hughes (1980). The final extraction of the fatty acid methyl esters was performed with the addition of n-hexane.

Gas chromatography (GC). The fatty acid methyl esters were analysed by GC on a Hewlett Packard Model 5830A gas chromatograph equipped with dual flame-ionization detectors. Identification of the esters was based on the comparison of retention times with known standards of C14:0 (myristic acid), C14:1 (myristoleic acid), C16:0 (palmitic acid), C16:1 (palmitoleic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid) and C18:3 (linolenic acid) (Serva, Heidelberg, FRG). Analyses were carried out using glass columns (4 mm ID $\times$ 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 $\cdot$ min⁻¹ at a column temperature of 160°C.

RESULTS AND DISCUSSION

Reproducible results were obtained when the 36 strains were grown in triplicate under standard conditions. The mean fatty acid composition with standard deviation for each group is shown (Table 1). The statistical interpretation of the results was performed by the Student's *t*-test (Scheffler 1979). The selected genera representing the Endomycetales contained mainly saturated and unsaturated fatty acids with chain lengths from 16 to 18 carbon atoms.

On the basis of long-chain fatty acid composition, the 18 genera could be divided into 6 groups. The strains representing group I had a high percentage of oleic acid, while group II and group III were characterized by oleic acid and linoleic acid. The strains included in group IV had by far the highest percentage palmitoleic acid ($P < 0.01$). Group V included strains which contained the highest amount of oleic acid ($P < 0.01$) while the strains present in group VI were characterized by the highest percentage linoleic acid ($P < 0.01$).

Group I. Nine genera were included and comprised 22 strains representing 12 species (Table 1) which are at present classified under the Saccharomycetaceae. These strains were divided into 2 subgroups according to their palmitic acid contents. The strains of subgroup 1 contained almost half the percentage palmitic acid compared to the strains in subgroup 2 and include *S. capsularis*, *W. saturnus*, *C. lusitaniae*, *S. occidentalis*, *L. elongisporus*, *W. robertsiae*, *Z. bailii*, *D. marama* and *D. vanrijiae*. Subgroup 2, which is characterized by a higher percentage palmitic acid includes three species of *Limpomyces* i.e. *L. starkeyi*, *L. konoenenkoae* and *L. tetrasporus*.

Table 1. The percentage long chain fatty acid composition^a of the strains representing Saccharomycetaceae, Endomycetaceae, Metchnikowiaceae and Saccharomycodaceae

Group	Strain	Strain Number	Fatty acid ^b									
			C14:0	C14:1	C16:0	C16:1	UN ^c	UN ^c	C18:0	C18:1	C18:2	C18:3
I	<i>Saccharomycopsis capsularis</i>	CSIR-Y447	0.4	0.1	19.9	11.0	0.1	0.1	5.0	48.3	10.2	4.1
	<i>Saccharomycopsis capsularis</i>	CSIR-Y651	0.2	0.1	19.3	10.9	—	0.2	2.7	47.0	13.0	5.7
	<i>Williopsis saturnus</i>	CSIR-Y17	0.1	0.4	15.6	16.4	0.2	0.4	—	44.8	15.5	5.5
	<i>Clavispora lusitanae</i>	CSIR-Y484	—	—	14.7	9.5	0.3	0.5	—	45.8	22.5	6.2
	<i>Schwanniomyces occidentalis</i>											
	var. <i>occidentalis</i>	CSIR-Y937	0.1	0.2	15.5	10.7	0.3	0.3	1.0	42.2	20.6	7.9
	var. <i>occidentalis</i>	CSIR-Y936	—	0.1	14.2	4.4	0.2	0.2	3.4	41.1	21.9	14.0
	var. <i>occidentalis</i>	CSIR-Y938	—	0.1	14.5	4.5	0.2	0.2	3.6	43.6	22.9	9.5
	var. <i>persoonii</i>	CSIR-Y285	—	—	13.0	6.2	0.3	0.2	1.0	41.2	24.0	13.3
	var. <i>castellii</i>	CSIR-Y993	0.1	0.1	14.8	4.0	0.1	0.1	3.5	40.6	22.0	14.0
	var. <i>altivius</i>	CSIR-Y828	0.1	0.1	16.0	4.7	0.1	0.2	3.5	41.6	19.5	12.4
	<i>Lodderomyces elongisporus</i>	CSIR-Y162	0.2	—	11.9	7.4	0.7	1.8	5.2	52.5	19.1	0.6
	<i>Wingea roberisiae</i>	CSIR-Y208	0.2	—	12.3	4.6	0.9	2.0	4.4	47.3	25.2	2.0
	<i>Wingea roberisiae</i>	CSIR-Y446	0.2	—	12.6	3.4	0.6	1.0	4.6	48.8	24.1	3.7
	<i>Zygosaccharomyces bailii</i>	CSIR-Y126	0.1	0.1	6.5	11.8	0.2	1.6	0.2	48.3	26.7	3.5
	<i>Debaryomyces nanama</i>	CSIR-Y216	0.1	—	12.7	4.3	0.5	1.0	14.9	55.4	7.9	3.2
	<i>Debaryomyces vanrijae</i>	CSIR-Y960	0.2	0.1	14.1	1.1	0.5	0.2	25.8	40.7	15.6	0.7
	<i>Lipomyces kononenkoae</i>	CSIR-Y353	0.1	—	31.7	8.5	0.4	0.5	8.5	41.4	7.7	0.5
	<i>Lipomyces kononenkoae</i>	IGC 4052	0.1	0.1	22.5	2.5	0.3	0.7	11.4	51.5	10.5	0.1
	<i>Lipomyces kononenkoae</i>	CSIR-Y205	0.1	—	28.1	7.6	0.4	0.9	8.5	46.4	7.9	—
	<i>Lipomyces starkeyi</i>	CSIR-Y158	—	0.1	33.0	4.5	0.5	0.9	5.0	52.6	3.5	0.1
	<i>Lipomyces starkeyi</i>	IGC 3944	0.1	0.1	33.9	5.8	0.5	0.9	5.1	50.6	3.4	0.2
	<i>Lipomyces starkeyi</i>	CSIR-Y503	0.1	0.2	31.0	3.5	0.4	0.8	14.5	42.5	5.5	0.5
	<i>Lipomyces tetrasporus</i>		0.1	0.1	18.5	6.7	0.4	0.7	6.0	46.1	15.9	4.9
	Mean		0.1	0.1	18.5	6.7	0.4	0.7	6.0	46.1	15.9	4.9
	Standard deviation		0.1	0.1	7.9	3.7	0.2	0.6	6.1	4.5	7.7	4.9

Group	Strain	Strain Number	Fatty acid ^b									
			C14:0	C14:1	C16:0	C16:1	UN ^c	UN ^c	C18:0	C18:1	C18:2	C18:3
II	<i>Endomyces fibuliger</i>	CSIR-Y643	1.5	2.7	15.4	3.6	1.4	1.4	5.0	30.0	38.5	—
	<i>Pichia fluxuam</i>	CSIR-Y183	0.5	—	13.7	9.7	1.5	3.1	2.4	30.3	37.0	0.6
	<i>Botryosaurus synnaedendrus</i>	CSIR-Y406	0.4	0.3	13.4	6.5	—	4.5	4.5	33.4	33.7	2.4
	<i>Hansenula dryadoides</i>	CSIR-Y649	0.5	0.2	17.4	12.4	0.3	1.4	4.0	20.5	29.4	12.4
	<i>Hansenula dryadoides</i>	CSIR-Y650	0.4	0.4	10.6	9.6	—	0.6	3.1	27.1	30.4	17.5
	Mean		0.7	0.7	14.1	8.4	0.6	2.2	3.8	28.3	33.8	6.6
	Standard deviation		0.5	1.1	2.5	3.4	0.8	1.6	1.1	4.9	4.0	7.9
III	<i>Metchnikowia reukauffii</i>	CSIR-Y13	0.1	—	14.2	14.3	0.8	0.5	0.1	31.6	33.9	4.2
IV	<i>Hanseniaspora valbyensis</i>	CSIR-Y895	0.2	—	13.5	66.5	—	—	0.7	18.5	0.2	—
	<i>Hanseniaspora uvarum</i>	CSIR-Y898	0.1	—	14.4	59.3	—	—	1.3	22.3	1.4	0.5
	<i>Saccharomycodes ludwigii</i>	CSIR-Y22	0.5	—	17.2	55.4	—	—	2.0	23.4	—	—
	<i>Saccharomycodes ludwigii</i>	CSIR-Y8	0.3	—	12.0	54.8	0.1	—	2.9	29.0	—	—
	Mean		0.3	0.0	14.3	59.0	0.0	0.0	1.7	23.3	0.4	0.1
	Standard deviation		0.2	0.0	2.2	5.4	0.1	0.0	0.9	4.3	0.7	0.3
V	<i>Schizosaccharomyces malidevorans</i>	CSIR-Y933	0.3	—	7.3	2.1	0.1	—	5.4	83.4	0.7	0.7
	<i>Schizosaccharomyces pombe</i>	CSIR-Y457	0.3	—	8.5	1.1	0.1	—	10.8	77.1	0.9	0.8
	Mean		0.3	0.0	7.9	1.6	0.1	0.0	8.1	80.3	0.8	0.8
	Standard deviation		0.0	0.0	0.8	0.7	0.0	0.0	3.8	4.5	0.1	0.1
IV	<i>Dipodascus australiensis</i>	CSIR-Y584	—	0.3	14.5	2.7	0.4	1.0	1.1	31.5	48.4	—
	<i>Dipodascus reessii</i>	CSIR-Y585	0.4	0.8	17.2	—	0.7	0.9	1.3	25.1	53.6	—
	Mean		0.2	0.6	15.9	1.4	0.6	1.0	1.2	28.3	51.0	0.0
	Standard deviation		0.3	0.4	1.9	1.9	0.2	0.1	0.1	4.5	3.7	0.0

^a Values are the means with standard deviation of 3 and more repetitions. The standard deviation of the values were about 5% of the mean value (range 2% to 6%). ^b Fatty acids designated as number of carbon atoms; number of double bonds; ^c UN = Unidentified fatty acids

Subgroup 1. The *S. capsularis* strains had the highest palmitic acid percentage as compared to the other strains. The highest percentage palmitoleic acid was present in *W. saturnus* while *C. lusitaniae* contained no stearic acid.

The palmitoleic acid content in *C. lusitaniae* was also lower compared to *W. saturnus*. The fatty acid compositions of the *S. occidentalis* strains were similar with the exception of *S. occidentalis* var. *occidentalis* CSIR-Y937, which had a higher percentage palmitoleic acid and a lower percentage linolenic acid. *S. occidentalis* strains had the highest percentage linolenic acid, while *L. elongisporus* showed an abundance of oleic acid and had the lowest percentage of linolenic acid. The strains of *W. robertsiae* had a high percentage of oleic acid and linoleic acid as compared to the other strains in this subgroup. *Z. bailii* had relatively much palmitoleic but little palmitic acid. The oleic acid percentage of *D. marama* was the highest, while *D. vanrijiae* had the highest percentage stearic acid.

Subgroup 2. The *Lipomyces starkeyi* strains were characterized by the highest mean percentage of oleic acid, while the *L. kononenkoae* strains had the highest mean percentage linoleic acid. *L. tetrasporus* had the highest percentage stearic acid (C18:0).

Group II. This group included 4 species (Table 1) which are classified under the Endomycetaceae. This include *E. fibuliger*, *P. fluxuum*, *B. synnaedendrus* and *H. dryadoides*. *E. fibuliger* had a high percentage of linoleic acid while no linolenic acid could be detected. Stearic and linolenic acid were present in low abundance in *P. fluxuum*. *B. synnaedendrus* was characterized by the highest percentage oleic acid as compared to the strains in group II. The strains of *H. dryadoides* had low percentages of oleic and linoleic acid.

Group III. Only *M. reukaufii* was included which contained oleic acid and linoleic acid as major fatty acids.

Group IV. Three species (Table 1) were included which are at present classified under the Saccharomycodaceae. *H. valbyensis* had the highest percentage palmitoleic acid while *H. uvarum* had the highest percentages linoleic and linolenic acids. The strains of *S. ludwigii* contained little palmitoleic acid but much oleic acid compared to the other strains in this group.

Group V. This group included 2 species belonging to the Schizosaccharomycetaceae. *S. malidevorans* had a higher percentage of oleic acid than *S. pombe*.

Group VI. The strains belonging to this group are included in the Dipodascaceae. *D. australiensis* had lower percentages of palmitic and linoleic acids and a higher percentage oleic acid than *D. reessii*.

Conclusion. With the aid of this method, it was possible to differentiate between the 6 yeast families as well as between the 18 representative genera on the basis of their fatty acid composition. Further research work is needed on more strains in order to construct not only a reliable rapid differentiating technique, but also to prove the general validity of this method as an additional phenotypic aid in classification.

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REFERENCES

- Abel, K., Schmertzling, H. & Peterson, J. I. (1963) Classification of micro-organisms by analysis of chemical composition. I: Feasibility of utilizing gas chromatography. *J. Bact.* 85: 1039–1044
- Dees, S. B. & Moss, C. W. (1975) Cellular fatty acids of *Alcaligenes* and *Pseudomonas* species isolated from clinical specimens. *J. Clin. Microbiol.* 1: 414–419
- Gunasekaran, M. & Hughes, W. T. (1980) Gas-Liquid Chromatography: A rapid method for identification of different species of *Candida*. *Mycologia* 72: 505–511
- Kaneko, H., Hosohara, M., Tanaka, M. & Itoh, T. (1976) Lipid composition of 30 species of yeasts. *Lipids* 11: 837–844
- Moss, C. W. & Dees, S. B. (1976) Cellular fatty acids and metabolic products of *Pseudomonas* species obtained from clinical specimens. *J. Clin. Microbiol.* 4: 492–502
- Moss, C. W., Shinoda, T. & Samuels, J. W. (1982) Determination of cellular fatty acid compositions of various yeasts by gas liquid chromatography. *J. Clin. Microbiol.* 16: 1073–1079
- Ratray, J. B. M., Schibeci, A. & Kidby, D. K. (1975) Lipids of yeasts. *Bact. Rev.* 39: 197–231
- Scheffler, W. C. (1979) Statistics for the Biological Sciences. Second edition. Addison-Wesley, London
- Shaw, N. (1974) Lipid composition as a guide to the classification of bacteria. In: D. Perlman (ed), *Advances in Applied Microbiology*. Academic Press, New York
- Wickerham, L. J. (1951) Taxonomy of Yeasts. Technical Bulletin No. 1029, United States Department of Agriculture

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