Fatty Acid Composition as a Guide to the Classification of Selected Genera of Yeasts Belonging to the Endomycetales

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Gas chromatography was used to determine the long-chain fatty acid composition of 33 strains representing 17 genera of the Saccharomycetaceae, Endomycetaceae, Metschnikowiaceae and Saccharomycodaceae. The similarities between the strains were calculated on the basis of fatty acid composition, resulting in three groups. Group I strains contained oleic acid (C18:1) as the major fatty acid, while group II strains had substantial proportions of both oleic acid and linoleic acid (C18:2). Strains of group III were characterized by a high percentage of palmitoleic acid (C16:1). The one strain of *Metschnikowia reukaufii* studied fell within the range of variation of group II, but it contained a slightly higher proportion of palmitoleic acid than the other strains in this group.

INTRODUCTION

Lipid analyses have been successfully used to determine and classify various yeasts (Gangopadhyay *et al.*, 1979; Gunasekaran & Hughes, 1980; Kaneko *et al.*, 1976; Moss *et al.*, 1982). Recently a simple and rapid method for estimating the cellular long-chain fatty acids of yeasts was established in our laboratory (Kock *et al.*, 1985). In this paper the cellular long-chain fatty acids of 33 strains representing 17 selected genera of the families Saccharomycetaceae, Endomycetaceae, Metschnikowiaceae and Saccharomycodaceae are described to test the taxonomic value of this phenotypic character.

METHODS

Strains. Cultures were obtained from Professor J. P. van der Walt, Pretoria, South Africa (CSIR-Y) and the Centre of Biology, Gulbenkian Institute of Science, Oeiras, Portugal (IGC). The names used are those used by the culture collection of origin. The assignment to families is according to Von Arx (1981).

Saccharomycetaceae: *Saccharomycopsis capsularis* CSIR-Y447, CSIR-Y651; *Williopsis saturnus* CSIR-Y17; *Clavispora lusitanae* CSIR-Y484; *Schwanniomyces occidentalis* CSIR-Y937, CSIR-Y936, CSIR-Y938; *Schwanniomyces personii* CSIR-Y285; *Schwanniomyces castellii* CSIR-Y993; *Schwanniomyces alluvius* CSIR-Y828; *Lodderomyces elongisporus* CSIR-Y162; *Wingea robertsii* CSIR-Y208, CSIR-Y446; *Zygosaccharomyces bailii* CSIR-Y126; *Debaryomyces marama* CSIR-Y216; *Debaryomyces vanrijae* CSIR-Y960; *Schizosaccharomyces pombe* CSIR-Y457; *Lipomyces starkeyi* CSIR-Y205, CSIR-Y158, IGC 3944; *Lipomyces kononenkoae* CSIR-Y353, IGC 4052; *Lipomyces tetrasporus* CSIR-Y503.

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

Metschnikowiaceae: *Metschnikowia reukaufii* CSIR-Y13.

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

Cultivation. Stock cultures were maintained on yeast extract/malt extract (YM) (Wickerham, 1951) slants. The strains were cultured under standard conditions for 16 h at 30 °C on a rotary shaker at 160 r.p.m. (throw 50 mm) in 150 ml conical flasks containing 40 ml YNB medium [80 g glucose (Merck) and 6.7 g yeast/nitrogen base l⁻¹]

(Wickerham, 1951)]. Volumes (18 ml) of these cultures (Klett reading 150, 640 nm filter) were then inoculated into 400 ml glucose YNB liquid medium in 1 l conical flasks and cultured to stationary phase (48 h) on the rotary shaker (Dawson & Craig, 1965). This procedure was repeated three times. The cells were harvested by centrifugation at 8000 *g* for 5 min at 4 °C and washed three times with cold saline (0.85% NaCl), with centrifugation after each washing. The cells were stored at -15 °C.

Fatty acid analysis. The extraction and preparation of fatty acid methyl esters were done according to the method proposed by Gunasekaran & Hughes (1980). The final extraction of the fatty acid methyl esters was done with *n*-hexane. The fatty acid methyl esters were analysed by gas chromatography 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). Analyses were done using glass columns (4 mm i.d. × 1.5 m) packed with 5% diethylene glycol 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.

Numerical analysis. The Pearson product-moment correlation coefficient, *r*, between any pair of long-chain fatty acids was computed (Pearson & Hartley, 1958). The resulting matrix of correlation coefficients was converted into a *z* matrix by Fischer transformation (Rohlf & Sokal, 1962), and the *z* values were clustered by the unweighted average pair group method. The *z* values of all clustering levels were again transformed into *r* values (Sokal & Sneath, 1963). All the computations were done at the University of the Orange Free State on a Sperry Univac 1100 computer.

RESULTS

The similarities of the cellular long-chain fatty acid composition of the 33 strains are presented in Fig. 1. The strains clustered into three major groups. Groups I and II had a similarity (Sokal & Sneath, 1963) of *r* = 0.82 and groups II and III a similarity of *r* = 0.42. The mean fatty acid composition with standard deviation for each group is shown in Fig. 2. The statistical interpretation of the results was performed by Student's *t*-test (Scheffler, 1979).

Group I. The percentage of oleic acid (C18:1) was higher in this group than in the other groups, while the percentage of linoleic acid (C18:2) was significantly lower than in group II (*P* < 0.01).

Group II. The four species included in this group were characterized by the presence of substantial amounts of both oleic acid (C18:1) and linoleic acid (C18:2). The percentage of oleic acid was significantly higher in group I (*P* < 0.01) and that of linoleic acid significantly lower in groups I and III (*P* < 0.01) than in group II.

Group III. This group was characterized by a significantly higher percentage of palmitoleic acid (C16:1) than the other groups (*P* < 0.01), and a low percentage of linoleic acid (C18:2).

DISCUSSION

The species in group I (excluding *Schizosaccharomyces pombe* CSIR-Y457) are placed in the family Saccharomycetaceae by Von Arx (1981). Three subgroups, (*a*), (*b*) and (*c*) could be recognized in group I (Fig. 1). Subgroup (*a*) consists of species which can ferment glucose (Kreger-van Rij, 1984), while the species in subgroup (*b*) are not capable of fermenting glucose, with the exception of *Schizosaccharomyces pombe* CSIR-Y457 (Kreger-van Rij, 1984). Subgroup (*b*) comprises species containing coenzymes Q-9 and Q-10 (Yamada *et al.*, 1973, 1976). Subgroup (*a*) includes four interbreeding species of *Schwanniomyces* which were placed in a single taxon, *Schwanniomyces occidentalis*, by Barnett *et al.* (1983) and Price *et al.* (1978). Subgroup (*c*) comprises three *Lipomyces* species which contain coenzyme Q-9 (Yamada *et al.*, 1977) and have no fermentation abilities (Kreger-van Rij, 1984).

The species representing group II are, with one exception, grouped under the Endomycetaceae as defined by Von Arx (1981). They contain coenzymes Q-7 and Q-8 (Yamada *et al.*, 1973, 1976). The single strain of *Metschnikowia reukaufii*, placed in the Metschnikowiaceae by Von Arx (1981), also fell into this group but it had a somewhat higher proportion of palmitoleic acid (C16:1) than the other members of the group.

Group III comprised *Hanseniaspora valbyensis* (CSIR-Y895), *Hanseniaspora uvarum* (CSIR-Y898) and *Saccharomycodes ludwigii* (CSIR-Y22, CSIR-Y8). Von Arx (1981) placed these

Fig. 1. A dendrogram showing similarity between 33 yeast strains of the order Endomycetales.

Fig. 2. Mean percentage fatty acid composition and standard deviation (bars) of the strains in groups I, II and III (see Fig. 1). UN1, UN2, unidentified long-chain fatty acids, having retention times of 13.36 and 14.13 min respectively (the retention time of C16:0 was 10.25 min).

species under the Saccharomycodaceae. The species in this group contain coenzyme Q-6 (Yamada *et al.*, 1980) and can ferment glucose (Kreger-van Rij, 1984). They all reproduce asexually by polar budding (Barnett *et al.*, 1983).

This study has shown that fatty acid composition can be used to classify yeast strains into groups and subgroups which correspond with the classification system proposed by Von Arx (1981). This work should be extended to include more yeasts to prove the general validity of this method as an additional phenotypic aid in classification.

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