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LONG-CHAIN FATTY ACIDS FROM *MONASCUS PURPUREUS*

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Abstract—Long-chain fatty acids from red and albino mutants of the filamentous fungus, *Monascus purpureus*, were investigated. Fatty acids from C_{14} to C_{24} were identified and quantified by means of gas chromatography–mass spectrometry. Thirty-nine fatty acids were identified, 22 saturated (including iso and anteiso), 14 monoenoic, two dienoic and α-linolenic acid (up to *ca* 8%). The difference in fatty acid composition between the two mutants is discussed. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The fungus *Monascus* has been traditionally used in east Asia for the production of fermented rice, which served as a red food colourant. Nowadays, this natural dye is also used in European countries. Most studies have addressed the production of red–orange oligoketides by the genus *Monascus* [1–5] and their biological activities [6]. Literature concerning the production of the secondary metabolites by *Monascus* has been recently reviewed [7].

Fungi of the class Ascomycetes are very important producers of various compounds, such as organic acids, antibiotics and enzymes, as well as a number of oriental fermented foods [8]. In contrast, to the more thoroughly investigated yeasts, fatty acid (FA) profiles are available for only a few strains of Ascomycetes [9]. Only a single study of the FA profile of the genus *Monascus* has so far been published [10]. The authors did not document FAs other than those containing C_{16} and C_{18} chains. 16:0, 18:0, 18:1 and 18:2 were identified as the major acids, 16:1 being a minor component. Other FAs, such as those containing odd numbers of carbon atoms or those with chains longer than C_{18} were found only in traces in two strains from the 36 examined. Surprisingly, the presence of FAs with more than 18 carbon atoms was not demonstrated in *Aspergillus* and *Penicillium* [10], genera where such acids have been reported [11, 12]. Therefore, we suppose that the possible production of these FAs by *Monascus* could not be detected by the analytical method used. By using advanced methods developed by us [13, 14] many more FAs were identified in the genus *Monascus*.

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RESULTS AND DISCUSSION

The composition of FAs in the red and albino mutant of *M. purpureus* is shown in Table 1. The albino mutant did not produce any of the major pigments (ankaflavin, monascin, monascorubrin, rubropunctatin, monascorubramine and rubropunctamine) as indicated by TLC (data not shown). Both strains contained similar proportions of palmitic, oleic, linoleic and linolenic acid as other Ascomycetes. In comparison with the only published data [10], the strains examined by us produced less linoleic acid (11.4–15.9% compared to 26.8–53.1%) and oleic acid (9.6–19.3% compared to 21.2–44.6%). The concentration of palmitic acid was nearly the same (9.6–18.2% compared to 12.9–21.6%). Stearic and palmitoleic acids were present in far higher amounts, 9.6–14.0% and 2.3–8.9%, compared to 9.6–19.3% and 0.8–2.4%, respectively. Moreover, we found 1.7–7.9% of α-linolenic acid, which was not previously identified in *Monascus*. This acid, however, is a common component of lipids of other Ascomycetes, e.g. of the genera *Aspergillus* and *Penicillium* [15]. In general Ascomycetes, FAs with chains longer than C_{18} have been identified; in the genera *Aspergillus* and *Penicillium*, these compounds form *ca* 3.5–3.7% of the total amount of fatty acids [11, 12].

In the two mutants examined, FAs with chains containing more than 18 carbon atoms were found. These were mainly FAs with even numbers of carbons, prevalently saturated and also monoenoic ones. Positional isomers of some monoenoic acids were also identified; this is a common feature of the FA profiles of many Ascomycetes [11, 12, 15]. Minor FAs with odd numbers of carbons were also detected. These were mainly 15:0, 17:0, 19:0 and 21:0, and their unsaturated

Table 1. Fatty acids from *Monascus purpureus* (relative %)

Fatty acid	Red mutant	Albino mutant
i-14:0	0.18±0.04	0.14±0.04
14:0	1.76±0.19	0.87±0.16
7–14:1	0.35±0.12	0.22±0.05
i-15:0	0.00	0.19±0.04
ai-15:0	0.00	0.06±0.02
15:0	1.29±0.13	0.71±0.15
i-16:0	0.00	0.44±0.14
16:0	18.19±0.27	9.54±0.27
7–16:1	8.07±0.16	6.12±0.22
9–16:1	0.00	1.44±0.20
16:2	0.23±0.08	0.54±0.14
i-17:0	1.68±0.20	0.60±0.13
ai-17:0	3.03±0.22	0.80±0.12
17:0	0.99±0.10	0.84±0.13
8–17:1	0.39±0.09	0.60±0.12
10–17:1	0.41±0.12	0.80±0.15
18:0	14.03±0.26	9.62±0.27
9–18:1	19.28±0.30	9.60±0.29
9, 12–18:2	15.89±0.28	11.42±0.26
9, 12, 15–18:3	1.67±0.15	7.91±0.23
i-19:0	0.20±0.08	0.34±0.06
ai-19:0	0.59±0.13	0.25±0.04
19:0	0.31±0.11	0.33±0.06
10–19:1	0.68±0.12	0.25±0.05
12–19:1	0.30±0.10	0.19±0.05
i-20:0	0.67±0.15	1.59±0.17
20:0	2.15±0.20	6.85±0.28
i-20:1	0.47±0.14	5.69±0.25
11–20:1	0.89±0.18	5.58±0.23
i-20:0	1.29±0.19	2.10±0.19
21:0	1.51±0.21	1.47±0.18
21:1	1.47±0.20	1.43±0.16
i-22:0	0.00	0.38±0.12
22:0	0.92±0.21	4.47±0.20
13–22:1	0.16±0.07	1.96±0.19
15–22:1	0.00	1.82±0.20
23:0	0.08±0.02	1.04±0.18
24:0	0.73±0.13	0.92±0.16
15–24:1	0.14±0.03	0.88±0.17

Each value represents the mean ± S.D. from three independent cultivations, extractions and chromatographic analyses.

homologues. In general, the FA composition in *Monascus* is typical of the Ascomycetes.

Comparison of the quantitative composition of the FAs in the two mutants examined (Table 2) seems to be more interesting. The FAs are presented according to their biosynthetic precursors, i.e. the respective acyl-coenzymes A. Acids having acetate, propionate or isovalerate as the starter unit, are present in nearly equal proportions. On the other hand, the proportions of acids having isobutyrate (i.e. iso-acids, designated i) and those having 2-methyl butyrate as precursor (i.e. anteiso-acids, designated ai) are different. In the red mutant, the relation of $\Sigma i:\Sigma ai = 1.3:3.6$, but in the albino mutant this relation is $\Sigma i:\Sigma ai$–8.2:1.1. In order to explain this observation, it is possible to produce two hypotheses. First, we assume that the albino mutant is blocked in the biosynthesis of some precursors of

Table 2. Fatty acid classes in mutants of *Monascus purpureus* (relative %)

	Red mutant	Albino mutant
iso-Even	1.32	8.24
iso-Odd	3.17	3.23
anteiso	3.62	1.11
Even	84.46	79.76
Odd	7.43	7.66

2-methyl butyric acid and, on the other hand, biosynthesis of isobutyric acid is stimulated. It is evident that the biosynthetic pathway may be blocked in many steps from oxobutyrate synthesis to 2-methyl butyric acid. The second hypothesis considers the possibility that some still unknown metabolite (or metabolites) utilizes isobutyric or 2-methyl butyric acid as a precursor. It is known that many fungal secondary metabolites, e.g. the group of aspergillic acids from *Aspergillus flavus* [16] or even cyclosporines from *Tolypocladium terricola* [17] are built from branched-chain amino acids or their precursors. On the basis of our preliminary results (many unknown spots on TLC) we assumed that this hypothesis was more plausible.

EXPERIMENTAL

Cultivation. Monascus purpureus CCM 8152 and its albino mutant (obtained after UV irradiation), maintained on malt agar, were used throughout this study. Both fungi were cultivated as described previously [5] in 300 ml Erlenmeyer flasks containing 50 ml of culture medium on a reciprocal shaker at 30° for 10 days. The culture medium, which was modified production medium [18], consisted of (in $g\,l^{-1}$): glucose 30, $(NH_4)_2SO_4$ 1.2, $MgSO_4.7H_2O$ 1.0, KH_2PO_4 2.5 dissolved in H_2O. The pH after sterilization was *ca.* 5.

Isolation and identification. FA Me esters were prepd according to ref. [14]. GC analysis was performed using a capillary column 15 m × 0.53 mm × 1 μm coated with DBWAX phase (J & W Scientific). The oven temp. was programmed from 130 to 210° with N_2 as carrier gas. Me esters were identified and quantified (by total ion current) by GC-MS using a fused-silica capillary column (60 m × 0.32 mm × 0.25 μm; Supelcowax 10) using splitless injection with He as carrier gas. The oven temp. was programmed from 100 to 250° at 4° min^{-1}. The ionization energy was 70 eV. Identification of all FA Me esters was confirmed by means of co-eluting with standards and by MS. Identification of double bond positions was based on ref. [19], viz. for unsaturated Me esters having at least three double bonds the ion at m/z 108 is characteristic for n-3 acids (e.g. α-linolenic acid) and the ion at m/z 150 is characteristic for n-6 acids (e.g. γ-linolenic acid).

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