GLA synthesis in *Mortierella isabellina* in solid state fermentation

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Introduction

Single cell oils (SCOs) containing polyunsaturated fatty acids (PUFAs), such as γ-linolenic acid (GLA), are widely used in pharmaceutical and food industry. Profound knowledge of the regulation of the metabolic pathways that lead to GLA formation is required, in order to design a successful bioprocess for GLA production. According to current data GLA is synthesized mostly during the growth phase indicating a correlation between mycelial age and fatty acid biosynthesis [1]. In this study, we investigated lipid composition of hyphae of different age of *Mortierella isabellina* ATHUM 2935, in an effort to establish a correlation between GLA synthesis and mycelial growth. For this purpose, the fungus was cultivated on PDA medium in solid state cultures where fungal growth takes place mostly in the substrate surface and hyphae of different age can be excised lengthwise the mycelium and analysed individually. What is more, we used *M. isabellina* to produce GLA-rich SCOs from cultures on pear pomace, an agro-industrial residue produced in large amounts in several Mediterranean countries.

Materials and Methods

Microorganism: *Mortierella isabellina* ATHUM 2935. **Growth on potato dextrose agar (PDA):** 2 lots of 40 Petri dishes with PDA were inoculated with circular portions of 0.2 cm radius (inoculating cultures were produced by growing the fungus on PDA for eight days at 28 ± 1 °C and inocula were obtained from the peripheral ring) and incubated at 28 ± 1 °C until the fungal colony occupied the 3/4 of the dish. **Growth on pear pomace:** 9 cm diameter glass Petri dishes were filled with 25 g pear pomace homogenate (pH 6.5), were sterilized by autoclaving and then were inoculated with 1 ml of spore suspension (4 x 10^4 spores). Inoculated pear pomace dishes were incubated at 28 ± 1 °C in a water saturated atmosphere under constant aeration for 10 days. **Analytical methods:** Fungal mycelia from PDA cultures were harvested as follows: the fungal colonies were divided into three circular rings having different ages: the outer ring aged from 0 to 87 h, the middle ring aged from 87 to 160 h, and the inner ring aged from 160 to 215 h. Then, the mycelia were meticulously separated from the solid substrate, washed thoroughly with cold distilled water, and dried at 80 °C until constant weight (usually 20–30 min) for dry mass estimation. Reducing sugars (RS) in pear pomace were measured by the DNS method [2], while total sugars by the method of Dubois [3]. Reducing and total sugars were expressed as glucose. For lipid extraction, a portion of the fermented mass was dried at 80 °C until constant weight and then extracted three times with hexane. **Lipid analysis** was performed according to Fakas et al., 2006 [4].

		C16:0	C16:1	C18:0	C18:1	C18:2	GLA	Others*
0-87h	NL	22.8±1.0	2.6±0.2	3.9±0.3	37.2±1.6	22.9±0.5	7.9±0.1	2.7±0.2
	G+S	18.4±0.2	2.2±0.1	2.9±0.1	34.9±0.3	28.7±0.4	9.0±0.5	3.9±0.5
	P	27.2±0.3	4.1±0.5	1.1±0.1	24.6±0.8	28.7±0.4	10.2±0.3	4.1±0.7
87-160 h	NL	19.7±1.1	2.6±0.1	3.4±0.5	38.0±0.1	24.4±1.8	9.2±0.6	2.7±0.6
	G+S	19.5±3.9	2.3±0.4	2.4±0.1	41.0±2.2	24.6±0.1	6.7±0.8	3.5±0.6
	P	26.3±0.3	3.9±0.4	1.1±0.1	32.9±0.8	23.0±0.8	8.4±0.6	4.4±0.9
160-215h	NL	21.9±0.9	3.5±0.3	3.6±0.2	41.3±2.1	20.5±1.2	7.1±0.4	2.2±0.1
	G+S	17.7±1.5	2.2±0.1	2.6±0.1	47.2±3.6	21.3±0.9	6.6±0.4	2.3±0.1
	P	27.3±0.5	3.8±0.5	0.9±0.2	36.0±2.2	21.3±0.5	6.8±0.1	3.9±0.3

Table 1 Fatty acid composition in *M. isabellina* mycelia of different age. Cultures were prepared in duplicate and data are presented as mean ± standard deviation. NL: neutral lipids; G + S: glycolipids plus sphingolipids; P: phospholipids (Reprinted from [7]. Copyright 2009, with permission from Elsevier).

		C 18:1/ 18:0	C 18:2/ 18:1	GLA/ 18:2
0-87h	NL	9.56 ± 1.22	0.62 ± 0.01	0.35 ± 0.03
	G+S	12.24 ± 0.01	0.82 ± 0.02	0.31 ± 0.01
	P	23.22 ± 1.56	1.16 ± 0.06	0.36 ± 0.01
87-160h	NL	11.42 ± 1.78	0.64 ± 0.05	0.38 ± 0.01
	G+S	17.30 ± 1.59	0.60 ± 0.04	0.27 ± 0.01
	P	29.64 ± 1.88	0.70 ± 0.04	0.36 ± 0.07
160 -215h	NL	11.39 ± 1.89	0.50 ± 0.01	0.35 ± 0.01
	G+S	18.01 ± 2.01	0.45 ± 0.01	0.31 ± 0.03
	P	40.66 ± 3.45	0.59 ± 0.02	0.32 ± 0.01

Table 2 Desaturation ratios in *M. isabellina* lipids. Data are presented as mean ± standard deviation. NL: neutral lipids; G + S: glycolipids plus sphingolipids; P: phospholipids (Reprinted from [7]. Copyright 2009, with permission from Elsevier).

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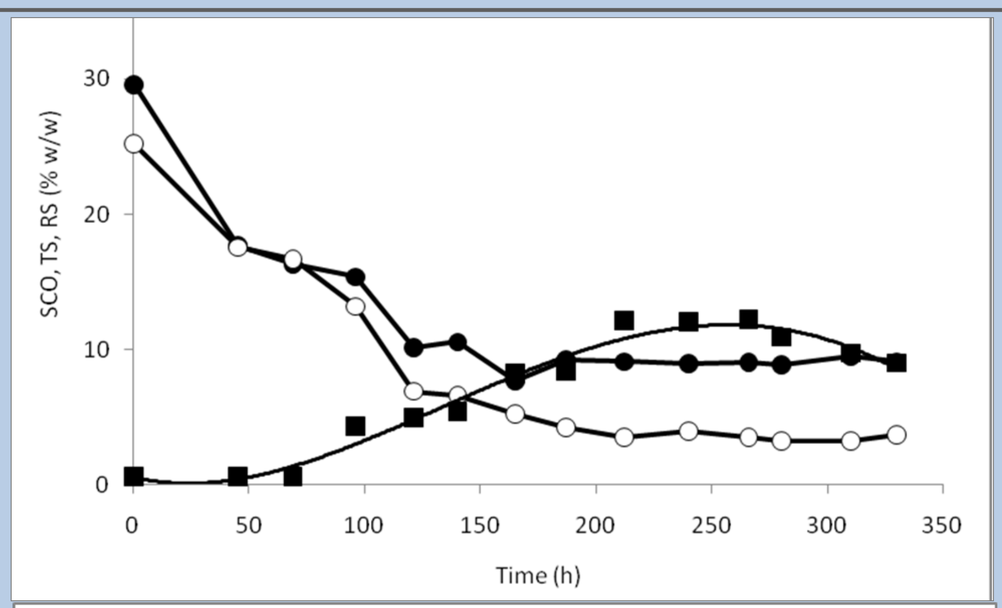
Results and Discussion

M. isabellina lipids contained mostly oleic acid (C18:1$^{\Delta 9}$), followed by palmitic (C16:0) and linoleic (C18:2$^{\Delta 9,12}$) acids, while GLA, (C18:3$^{\Delta 6,9,12}$), stearic (C18:0), and palmitoleic (C16:1$^{\Delta 9}$) acids were found in lower amounts. Neutral lipids-NL maintained a somehow constant fatty acid composition with age, but in the glycolipid plus sphingolipid-G+S and phospholipids-P fractions PUFAs content decreased gradually with age (Table 1). Changes in fatty acid profile with age reflect changes in fatty acid biosynthetic machinery. In particular, changes in unsaturated fatty acid content in lipids reflect changes in fatty acid desaturation rate with age [5]. According to our data (Table 2) oleic acid desaturation and incorporation to polar lipids proceed with high efficiency at all ages, but oleic acid conversion to linoleic acid decreases with age, resulting in the accumulation of oleic acid in P and G+S. Δ6 desaturation is heavily regulated by substrate availability (i.e. linoleic acid), which in turn depends on the action of Δ12 desaturase on oleic acid. Therefore, it seems that the rate limiting step for GLA formation is the reaction catalyzed by Δ12 desaturase, the activity of which decreases with age. This would then mean that GLA biosynthesis is favoured in young, fast growing mycelia.

Fatty acid composition in *M. isabellina* grown on pear pomace resembled closely that of PDA cultures. SCOs and GLA yields obtained on pear pomace were considered satisfactory in comparison with the yields reported in the literature [6]. Oil content in fermented mass reached its maximum value (12% w/w) at 212 h after inoculation (Fig. 1a). As far as GLA percentage was concerned, this was highest at the beginning of the growth (3.8% w/w), decreasing thereafter to 2.2% (w/w). At the end of the growth, however, when the oil content in fermented mass started to decrease, GLA percentage increased a little (2.9% w/w) (Fig. 1b).

a.



b.

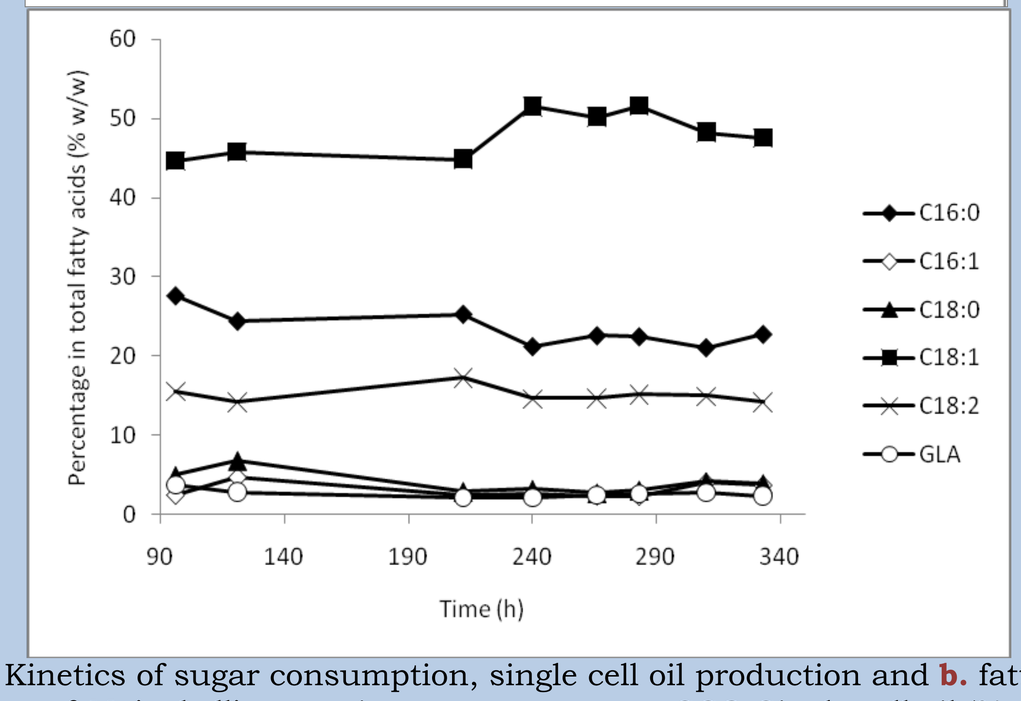


Fig. 1. a. Kinetics of sugar consumption, single cell oil production and **b.** fatty acid composition of *M. isabellina* growing on pear pomace. SCO-Single cell oil (% w/w), TS-total sugars % w/w, RS-reducing sugars % w/w, (♦) C16:0, (◊) C16:1, (▲) C18:0, (■) C18:1, (×) C18:2, and (○) GLA (Reprinted from [7]. Copyright 2009, with permission from Elsevier).

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