europass Curriculum vitae

PERSONAL INFORMATION

Maria Mavrommati

35, Chrisostomou, 26226 PATRAS (Greece)

+306946546579 ☎ +302610338106

marimav1983@gmail.com

WORK EXPERIENCE

28 May 2017–Present

Biology researcher (preparing for a master's degree)

University of Ioannina, Ioannina (Greece)

Manipulation of molecular techniques:

- PCR
- Reverse transcription PCR
- Cloning
- Gel electrophorisis of Nucleic acids
- DNA- cutting with restriction enzymes
- Southern blotting

Manipulation of microbiological techniques:

- bacterial transformation
- replica plating
- bacterial conjugation
- cultivation and use of the bacterium Zymomonas mobilis

Apr 2012–Present

Translator

Patras (Greece)

Translating science texts from English to Greek to help university students post their theses in Greek.

Oct 2010–Present

Spanish teacher

Patras (Greece)

Teaching Spanish to adults in Greece, preparing them for DELE certificates.

Sep 2014–Present

Biology teacher

Coaching school "Logos", Patras (Greece)

Teaching Biology to Senior high school students. Also teaching high standard Biology to students who want to achieve admission to University.

Sep 2015–May 2016

Biology teacher

Coaching school "Mathiton Erisma", Patras (Greece)

Teaching high standard Biology to students, preparing them for the final exams in order to achieve admission to University.

Jul 2015–May 2016

Biology teacher

Coaching school "Dinamikon", Patras (Greece)

Teaching high standard Biology to students, preparing them for the final exams , in order to achieve admission to University

Jul 2013–Sep 2013

Biology teacher

Coaching school "Notis", Patras (Greece)

Teaching successfully biology to a student who failed the initial exams of Senior High school.

Sep 2012–Mar 2013

Biologist

Mental Health Association, University of Patras, Patras (Greece)

- Realization of a research about the condition of hygiene and safety of the University of Patras.

- Writing of two chapters of the New Hygiene and Safety manual of the University of Patras. These chapters refer to the biological hazards concerning exposure to radioactive elements, as well as to instructions on how to treat guinea- pigs properly.

Apr 2011–May 2011

Census- taker

Greek Association of Statistics, Patras (Greece)

Conducting a census of a Patras' (Greece) region for statistical purposes.

Oct 2007–Dec 2009

Microbiology researcher/ assistant

University of Patras, Patras (Greece)

- Cultivation of different types of bacteria, fungi and yeasts in solid and liquid state fermentation.

- Microscopic examination of the above micro-organisms in a daily basis.

- Observation of the oil production of the fungus *Mortierela isabellina.* Attempt to increase its oil production as much as possible.

EDUCATION AND TRAINING

11 Oct 2016–12 Dec 2018

Master's degree in Biotechnology

University of Ioannina, Ioannina (Greece)

Oct 2005–Jan 2010

Bachelor's degree in Biology

University of Patras, Patras (Greece)

PERSONAL SKILLS

Mother tongue(s): Greek

Foreign language(s)

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
English	C2	C2	C2	C2	C2
	ECPE (C2)				
Spanish	C2	C2	C2	C2	C2
	Diploma intermedio DELE (B2)				
French	B2	B2	B1	B1	B2
	DELF B2				
German	A1	A1	A1	A2	A2

Still learning

Levels: A1 and A2: Basic user - B1 and B2: Independent user - C1 and C2: Proficient user
Common European Framework of Reference for Languages

Communication skills

- Excellent communication skills obtained through working in a laboratory team as a biology researcher

- Very good communication skills obtained through collaborating with people of many different professions (civil engineers, chemists, medical visitors etc.) while working for the Menatl health Association.

- Great ability to communicate with adolescent students, obtained through biology teaching. Also, great communication with adults obtained through Spanish teaching.

Job-related skills

Mentoring skills (I have been responsible of training students to integrate and perform in a biological laboratory)

Digital skills

SELF-ASSESSMENT				
Information processing	Communication	Content creation	Safety	Problem solving
Independent user	Independent user	Basic user	Independent user	Independent user

Digital skills - Self-assessment grid

Other skills

Music: I used to play the drums at a music band.

Reading: I enjoy reading books in my free time, especially literature, politics and science.

Exercise: I try to exercise physically as much as possible to keep in shape. I can also perform the basics of tae kwon do and kick boxing.

Theater: I am still participating at a theatre team.

ADDITIONAL INFORMATION

Publication: Fatty acid composition in lipid lengthwise the mycelium of *Mortierella isabellina* and lipid production by solid fermentation. (December 2009)

ANNEXES

- Fatty acid composition in lipid fractions lengthwise the mycelium of Mortierella isabellina and lipid production by solid state fermentation [1] (δημοσίευσή μου).pdf

Fatty acid composition in lipid fractions lengthwise the mycelium of Mortierella isabellina and lipid production by solid state ferm

Bioresource Technology xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Fatty acid composition in lipid fractions lengthwise the mycelium of *Mortierella isabellina* and lipid production by solid state fermentation

Stylianos Fakas [1], Anna Makri, Maria Mavromati [2], Maria Tselepi [2], George Aggelis *

Unit of Microbiology, Division of Genetics, Cell and Development Biology, Department of Biology, University of Patras, Patras, 265 04 – GR, Greece

ARTICLE INFO

Article history:
Received 7 April 2009
Received in revised form 2 June 2009
Accepted 4 June 2009
Available online xxxx

Keywords:
Mycelial lipid fractions
Fatty acid composition
Mortierella isabellina
Solid state fermentation

ABSTRACT

This paper investigates the correlation between mycelial age and fatty acid biosynthesis. The correlation was investigated by analyzing the lipid composition lengthwise the mycelium of the oleaginous fungus *Mortierella isabellina*, a potential producer of γ-linolenic acid (GLA). Young mycelia were rich in polar lipids (glycolipids plus sphingolipids and phospholipids), while neutral lipid content increased in aged mycelia. In young mycelia, each polar lipid fraction contained almost 40% (w/w) polyunsaturated fatty acids (PUFAs), but this content decreased to less than 30% (w/w) in aged mycelia. On the other hand, PUFA content in neutral lipids fluctuated slightly with age. These results indicate that PUFA biosynthesis is favored in young, fast growing mycelia, while it decreases significantly in aged mycelia. This trend was also observed when we grew *M. isabellina* on pear pomace, an agro-industrial waste. Pear pomace cultures yielded significant amounts of lipid, which reached 12% (w/w) in dry fermented mass. The produced lipid was rich in GLA and the maximum GLA content in dry fermented mass was 2.9 mg/g.

1. Introduction

Single cell oils (SCOs) containing the PUFA γ-linolenic acid (GLA) are emerging as potential targets for industrial development since it was shown that GLA could be used in cancer treatment (Kenny et al., 2000). Thus, up to date, much effort has been put to the development of economical bioprocesses for GLA production (Certik et al., 2006; Fakas et al., 2008). It can be realized, however, that designing a successful bioprocess for GLA production requires profound knowledge of the regulation of the metabolic pathways that lead to GLA formation. To this end, there has been some evidence showing that GLA is synthesized mostly during the growth phase (Fakas et al., 2007), indicating a correlation between GLA synthesis and mycelial growth. This conclusion, however, was drawn from submerged cultures, where mycelial age is hard to be defined due to the formation of mycelial agglomerates comprising mycelia having different ages. In solid state cultures, however, fungal growth takes place mostly in the substrate surface, where mycelia of different ages can be discriminated. This discrimination then allows for the investigation of the potential correlation between GLA synthesis and mycelial growth.

In this paper, we investigated how lipid composition in mycelia of *Mortierella isabellina* changes with age in an effort to establish a correlation between GLA synthesis and mycelial growth. This knowledge will substantially add to the current understanding of the physiological role that GLA plays in fungi. On the side, we used *M. isabellina* to produce GLA-rich SCO from pear pomace, an agro-industrial waste accumulating in large amounts in several Mediterranean countries.

2. Methods

2.1. Microorganism and culture conditions

Mortierella isabellina ATHUM 2935 was maintained on potato dextrose agar (PDA) at 6 ± 1 °C.

2.1.1. Growth on potato dextrose agar

Inoculating cultures were produced by growing the fungus on PDA for eight days at 28 ± 1 °C. Inocula (circular portions of 0.2 cm radius) were obtained from the peripheral ring (that consists of young mycelia) of the inoculating cultures and implanted to the center of new PDA dishes, from which the respective 0.2 cm radius piece had been removed. PDA dishes (2 lots of 40 Petri dishes) were incubated at 28 ± 1 °C until the fungal colony occupied the 3/4 of the dish, while the radial growth of the fungal colony was estimated by measuring its radius at least twice daily.

2.1.2. Growth on pear pomace

Pear pomace was homogenized using a food processor, and the pH of the pomace homogenate was adjusted to 6.5 with a saturated

* Corresponding author. Tel./fax: +30 2610969260.
E-mail address: George.Aggelis@upatras.gr (G. Aggelis).
[1] Present address: Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901, USA.
[2] Equal contribution.

0960-8524/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2009.06.015

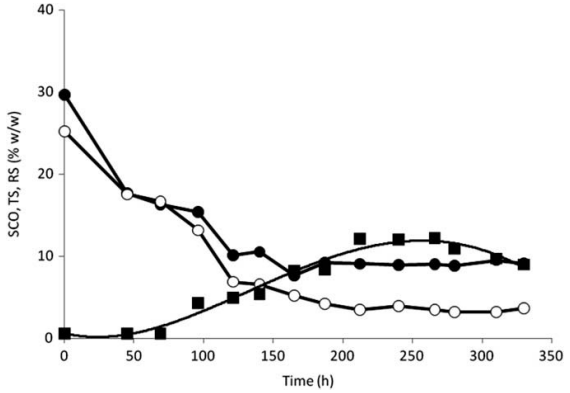

Fig. 1. Kinetics of sugar consumption and single cell oil production by *M. isabellina* growing on pear pomace. (■) Single cell oil (% w/w), (●) total sugars (TS, % w/w), and (○) reducing sugars (RS, % w/w).

KOH solution. After, 25 g aliquots of the homogenate were transferred to 9 cm diameter glass Petri dishes, which were then sterilized by autoclaving (121 °C/20 min) and inoculated with 1 ml of spore suspension containing 4×10^4 spores. Inoculated pear pomace dishes were incubated at 28 ± 1 °C in a water saturated atmosphere under constant aeration for 10 days.

2.2. Analytical methods

Fungal mycelia (from about 40 Petri dishes) 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. Lipid extraction, lipid fractionation, and fatty acid analysis were done as described by Fakas et al. (2006). Reducing sugars (RS) in pear pomace were measured by the DNS method (Miller, 1959), while total sugars by the method of Dubois (Dubois et al., 1956). 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.

3. Results

3.1. Lipid content and composition in mycelia of M. isabellina

Lipid content in *M. isabellina* mycelia of different maturities fluctuated between 3.5% and 4% (w/w). However, mycelia contained higher amounts of neutral lipids than polar lipids. More specifically, neutral lipid content was 1.9% (w/w) in young mycelia, glycolipid plus sphingolipid content was somehow lower (1.4% (w/w)), but phospholipid content was much lower, being 0.6% (w/w). Phospholipid content, however, decreased slightly with age. On the other hand, neutral lipid content increased to 2.4% (w/w) in the mycelia aged 87–160 h, but dropped again to 1.9% (w/w) in mycelia aged 160–215 h. Glycolipid plus sphingolipid content showed the greatest decrease, dropping by 35% in aged mycelia.

3.2. Fatty acid composition in lipid fractions

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 γ-linolenic (GLA, C18:3$^{\Delta 6,9,12}$), stearic (C18:0), and palmitoleic (C16:1) acids were found in lower amounts.

Neutral lipids maintained a somehow constant fatty composition with age, but in the glycolipid plus sphingolipid fraction PUFA content decreased gradually with age (Table 1). This decrease was mainly due to the drop in linoleic acid content, which was more pronounced during the transition from young to middle-aged mycelia. Phospholipids' fatty acid profile and changes in composition with age resembled those of glycolipids plus sphingolipids (Table 1). More specific, PUFA content in phospholipids was high in young mycelia but it decreased with age.

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. Desaturation rate changes can be evaluated by measuring the ratios of desaturase product to substrate, as described by Dimou et al. (2002). Thus, changes in the ratios C18:1$^{\Delta 9}$/C18:0, C18:2$^{\Delta 9,12}$/C18:1$^{\Delta 9}$, GLA/C18:2$^{\Delta 9,12}$ with age reflect the respective changes in fatty acid desaturation rate with age. Calculation of desaturation ratios in *M. isabellina* lipids showed that, at all times, Δ9 desaturation proceeds much more efficiently than any other (Table 2). Δ12 desaturation comes next to Δ9, but is much lower than the latter, while Δ6 desaturation efficiency was the lowest throughout growth. These differences in desaturation ratios with age were verified by statistical analysis using one-way ANOVA and LSD tests ($a = 0.005$).

3.3. Growth and lipid production on pear pomace

Fig. 1 shows the time course of pear pomace fermentation by *M. isabellina*. Total sugar consumption was paralleled by reducing sugar consumption, and calculations showed that almost all sugars consumed by the fungus were reducing sugars. Oil content in fermented mass reached its maximum value (12% w/w) at 212 h after inoculation. However, oil content started to decrease after 280 h

Table 1
Fatty acid composition in *M. isabellina* mycelia of different maturities. Cultures were prepared in duplicate and data are presented as mean ± standard deviation. NL: neutral lipids; G + S: glycolipids plus sphingolipids; P: phospholipids.

		C16:0	C16:1	C18:0	C18:1	C18:2	GLA	Others[a]
0–87 h	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	10.6 ± 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	12.9 ± 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	14.3 ± 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	11.9 ± 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	10.3 ± 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	12.8 ± 0.9
160–215 h	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

[a] Others are: C12:0, C14:0, C14:1, C20:0, C20:1, C22:0, C22:1.

Please cite this article in press as: Fakas, S., et al. Fatty acid composition in lipid fractions lengthwise the mycelium of *Mortierella isabellina* and lipid production by solid state fermentation. Bioresour. Technol. (2009), doi:10.1016/j.biortech.2009.06.015

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.

		C18:1/C18:0	C18:2/C18:1	GLA/C18:2
0–87 h	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
87–160 h	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
160–216 h	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

cultivation reaching 9% w/w at 330 h; after that time point oil content remained constant (data not shown).

3.4. Fatty acid composition during growth on pear pomace

Fatty acid composition in *M. isabellina* grown on pear pomace resembled closely that of PDA cultures. This trend was somehow expected since the inherent oil content in pear pomace was very low (0.5% w/w), and thus fatty acid composition of fermented mass reflected that of *M. isabellina* lipids. GLA percentage 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).

4. Discussion

Lipid composition during growth of oleaginous fungi has rarely been studied, while all studies so far were done in submerged cultures. In these cultures, however, mycelia of different ages intertwine to form either pellets or mats or both, depending on culture conditions. Therefore, lipid analysis during growth in submerged cultures involves an inherent error caused by the inevitable agglomeration of young and aged mycelia. On the other hand, solid state cultures provide an experimental system where hyphae of different age belonging to the same thallus can be separated and studied.

Mycelial lipid composition in *M. isabellina* showed specific trends with age which largely reflect the physiological role of individual lipids. More specifically, the increase in neutral lipid content in middle-aged mycelia could be indicative of the accumulation of some storage lipid with time. This may then explain the subsequent decrease in neutral lipid content in aged mycelia, where it seems that some of the accumulated lipid was degraded. This series of events (accumulation and subsequent degradation) is in line with the physiological role of neutral lipids, which is energy storage in times of plenty and energy provision in times of shortage (Holdsworth and Ratledge, 1988; Fakas et al., 2007). Overall, lipid composition on solid substrates shows some of the major trends that are previously reported for submerged cultures, but reveals a more specific role for some lipids.

Desaturation of fatty acids in *M. isabellina* lipids seemed to proceed with different rates in mycelia having different ages. Interpretation of the data, however, requires consideration of the different substrate requirements of desaturases: Δ9 desaturase acts on stearoyl-CoA, while Δ6 and Δ12 desaturases act on phospholipid bound fatty acids (Ratledge and Wynn, 2002). The data in our study show that 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 phospholipids and glycolipids plus sphingolipids. Thus, 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 favored in young, fast growing mycelia, while it is known that oil biosynthesis is favored under non-growth conditions (Ratledge and Wynn, 2002).

SCO and GLA yields obtained on pear pomace were considered satisfactory in comparison with the yields reported in the literature. For example, Certik et al. (2006) used a mixture of wheat flakes and spent malt grains as substrate for growth of *Thamnidium elegans*, which produced 7.2 mg/g GLA. In addition, the use of pear pomace as is has the advantage that it does not add to the overall fermentation costs.

5. Conclusions

Data from solid state cultures revealed a correlation between lipid composition and mycelial age. Our results clearly indicate that the rates of desaturation reactions are a function of age and lipid nature. Thus, it seems that desaturation reactions leading to GLA proceed faster in young, growing mycelia, and that the phospholipids are more actively involved in these reactions. Given that lipids accumulate under non-growth conditions and that the accumulated lipid are mostly comprised of neutral lipids, it follows that lipid accumulation and GLA synthesis vary in opposite directions. This means that when designing GLA production processes some compromise between lipid content in biomass and GLA content in the lipid must be reached.

Acknowledgements

Financial support was provided by the project "Kinetics of growth of oleaginous microorganisms and dynamics of biosynthesis of polyunsaturated fatty acids" funded by the University of Patras (Project K. Karatheodori).

References

Certik, M., Slavikova, L., Marsnova, S., Sajbidor, J., 2006. Enhancement of nutritional value of cereals with γ-linolenic acid by fungal solid-state fermentations. Food Technol. Biotechnol. 44, 75–82.
Dimou, D.M., Georgala, A., Komaitis, M., Aggelis, G., 2002. Mycelial fatty acid composition of *Pleurotus* spp. and its application in the intrageneric differentiation. Mycol. Res. 106, 925–929.
Dubois, M., Gilles, K., Hamilton, J., Rebers, P., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Fakas, S., Certik, M., Papanikolaou, S., Aggelis, G., Komaitis, M., Galiotou-Panayotou, M., 2008. [gamma]-Linolenic acid production by *Cunninghamella echinulata* growing on complex organic nitrogen sources. Bioresour. Technol. 99, 5986–5990.
Fakas, S., Galiotou-Panayotou, M., Papanikolaou, S., Komaitis, M., Aggelis, G., 2007. Compositional shifts in lipid fractions during lipid turnover in *Cunninghamella echinulata*. Enzyme Microb. Technol. 40, 1321–1327.
Fakas, S., Papanikolaou, S., Galiotou-Panayotou, M., Komaitis, M., Aggelis, G., 2006. Lipids of *Cunninghamella echinulata* with emphasis to γ-linolenic acid distribution among lipid classes. Appl. Microbiol. Biotechnol. 73, 676–683.
Holdsworth, J., Ratledge, C., 1988. Lipid turnover in oleaginous yeasts. J. Gen. Microbiol. 134, 339–346.
Kenny, F., Pinder, S., Ellis, I., Gee, J., Nicholson, R., Bryce, R., Robertson, J., 2000. Gamma linolenic acid with tamoxifen as primary therapy in breast cancer. Int. J. Cancer 85, 643–648.
Miller, G., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.
Ratledge, C., Wynn, J., 2002. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. Adv. Appl. Microbiol. 51, 1–51.

Please cite this article in press as: Fakas, S., et al. Fatty acid composition in lipid fractions lengthwise the mycelium of *Mortierella isabellina* and lipid production by solid state fermentation. Bioresour. Technol. (2009), doi:10.1016/j.biortech.2009.06.015