Appl Biochem Biotechnol (2012) 167:536–551
DOI 10.1007/s12010-012-9713-9

Mushroom Polysaccharides and Lipids Synthesized in Liquid Agitated and Static Cultures. Part I: Screening Various Mushroom Species

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Received: 5 November 2011 / Accepted: 13 April 2012 /
Published online: 10 May 2012

Abstract The effect of four synthetic media containing glucose (initial concentration 30 g l^{-1}) on mycelial growth, exopolysaccharides (EPS) and cellular lipids production was examined in 11 mushroom species after 12 and 16 days of culture in static- and shake-flasks. Fatty acid analysis of cellular lipids produced was also performed. Agitation had a positive effect on biomass production, glucose consumption and lipid biosynthesis. Media that favoured the production of biomass were not suitable for EPS biosynthesis and vice versa. Biomass values varied from ~1.0 gl^{-1} (*Lentinula edodes*) to ~19 gl^{-1} (*Pleurotus ostreatus*), while the highest EPS quantity achieved ranged between 1.6 and 1.8 gl^{-1} (for *Ganoderma lucidum* and *L. edodes*, respectively). Quantities of total cellular lipids varied between 2.5 and 18.5 % *w*/*w*, in dry mycelial mass for the fungi tested. Lipid in dry weight values were influenced by the medium composition. Cellular lipids presented noticeable quantities of poly-unsaturated fatty acids like linoleic acid. Compared to most of the mushrooms tested, lipids of *Volvariella volvacea* were more saturated. The ability of several mushroom species of our study to produce in notable quantities the above-mentioned added-value compounds renders these fungi worthy for further investigations.

Keywords Ascomycetous · Basidiomycetous · Biomass · Cellular lipids · Exopolysaccharides · Submerged cultures

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Introduction

Cultivation of edible mushrooms in solid substrates has been widely studied since it represents the traditional process of producing mushrooms, a food with well-known high nutritional value [1, 2]. Moreover, non-edible species (e.g. *Ganoderma* spp.) are cultivated for their outstanding medicinal properties [3]. In fact, many Basidiomycetous (*Ganoderma* spp., *Pleurotus* sp., *Flammulina velutipes*, *Lentinula edodes*, *Auricularia auricula*, *Volvariella volvacea*) and Ascomycetous (*Morchella* spp.) fungi have been studied for their medicinal and therapeutic actions with antitumor and immunological activities, which are associated with substances such as hetero-β-glucans (e.g. lentinan), ganoderic acid and intra- or extra-cellular polysaccharides [4, 5]. Fungal biomass and exopolysaccharide (EPS) production have already drawn much attention in numerous studies because of their various biological and pharmacological activities, in contrast to mushroom lipids, which despite their relatively small quantity in basidiocarp or mycelium, they contain poly-unsaturated fatty acids (e.g. α-linoleic acid) essential for human nutrition [6, 7]. As in submerged cultures, biomass, EPS and lipid synthesis are strongly influenced by the culture conditions, especially medium composition [8–10], its knowledge is necessary for the direction of fungal metabolism towards the production of the desirable metabolites. Glucose constitutes one of the most suitable carbon sources for the culture of edible fungi, whereas peptone and yeast extract have been commonly selected as they enhance mycelial growth in a variety of mushrooms [11, 12]. EPS production is also favoured by glucose and organic nitrogen sources (e.g. yeast extract, peptone, corn steep liquor) as it has been reported by Gern et al. [13], Meng et al. [12], or Fang and Zhong [14]. Likewise, non-nutritional factors such as the initial pH, the incubation temperature and the aeration play an important role upon the biosynthesis of EPS [5, 15]. However, culture conditions that favour the production of biomass may not favour the accumulation of EPS [5, 6]. On the other hand, the knowledge of fatty acid composition in cellular lipids produced would serve as a useful database for taxonomical, nutritional and medicinal evaluation of mushroom species [16–18].

In an attempt to select mushrooms with elevated mycelial growth and adequate production of EPS, various mushroom species were screened in submerged glucose-based agitated and non-agitated cultures. At the same time, four semi-synthetic media widely used for the cultivation of higher fungi were employed in order to select the most appropriate for their nutritional requirements and mycelium growth. The fatty acid composition of the total cellular lipids produced in some of the performed trials was also studied.

Materials and Methods

Organisms, Media and Culture Conditions

The 11 screened mushroom species/strains (representative of 11 genera) deposited at the Athens Mushroom Research Laboratory fungal culture collection of NAGREF are depicted in Table 1. The biological material is maintained on potato dextrose agar (PDA, Merck, Germany) in agar slants and/or submerged into distilled water at 2.0±0.1 °C, except for *V. volvacea* that is maintained at room temperature. Four [4] different liquid growth media, all presenting the same initial C/N molar ratio (~20), were used. All media contained analytical-grade D(+)glucose (Alfa-Aesar, Germany) as the main carbon source, peptone (Fluka, Germany) and yeast extract (Serva, Germany) as the main nitrogen sources, macro- and microelements in various concentrations as described at Table 2. pH values, measured with a

Table 1 Mushroom species/strains examined in the present study

Taxa/order	Mushroom species	AMRL	Origin
Ascomycetes			
Pezizales	*Morchella esculenta* (L.) Pers.	36	Wild, Greece (ATCC 200336)
	M. elata Fr.	63	Wild, Greece (ATCC 200328)
Basidiomycetes			
Polyporales	*Ganoderma lucidum* (Curtis) P. Karst.	330	Commercial, Taiwan
	G. applanatum (Pers.) Pat.	341	Wild, Greece
Auriculariales	*Auricularia auricula-judae* (Bull.) Quél.	111	Commercial, China
Agaricales	*Agrocybe aegerita* (V. Bring.) Singer	104	Commercial, France
	Pleurotus ostreatus (Jacq.) P. Kumm.	135	Commercial, Italy
	P. pulmonarius (Fr.) Quél.	177	Commercial, France
	Flammulina velutipes (Curtis) Singer	271	Commercial, China
	Lentinula edodes (Berk.) Pegler	121	Commercial, France
	Volvariella volvacea (Bull.:Fr.) Singer	190	Commercial, Taiwan

AMRL Athens Mushroom Research Laboratory, *ATCC* American Type Culture Collection

Jenway 3310 (UK) pH-meter, were regulated after autoclaving to 6.2±0.2, by adding calcium carbonate. The final pH values after 12 or 16 days of cultivation ranged between 5.6 and 7.2 depending on the strain and medium used. Experiments were performed in 100-ml Erlenmeyer flasks containing 30±1 ml of growth medium. They were autoclaved for 20 min at 121 °C, allowed to cool and inoculated with 7 mm agar plugs cut from a growing

Table 2 Composition of the four culture media (GPY, GPYS, GYS, MCM) used for the cultivation of 11 edible fungi of this study

Component	Concentration (g l^{-1})			
	GPY[a]	GPYS[b]	GYS[c]	MCM[d]
Glucose	30.0	30.0	30.0	30.0
Yeast extract	3.0	2.5	3.0	3.0
Peptone	3.0	3.5	–	3.0
$CaCO_3$	2.0	2.0	2.0	2.0
KH_2PO_4	–	1.0	7.0	0.46
K_2HPO_4	–	–	2.5	1.0
$MgSO_4$ $7H_2O$	–	0.5	1.5	0.5
$(NH_4)_2SO_4$	–	–	1.3	–
$CaCl_2$ $2H_2O$	–	0.3	0.15	–
$MnSO_4$ H2O	–	0.04	0.06	–
$ZnSO_4$ $7H_2O$	–	0.02	0.02	–
$FeCl_3$ 6 H_2O	–	0.08	0.15	–

[a] Glucose, peptone, yeast extract medium

[b] Glucose, peptone, yeast extract, supplements medium

[c] Glucose, yeast extract, supplements medium

[d] Mushroom complete medium

colony in a PDA Petri dish, aged 5–10 days depending of the fungus [19]. Cultures were then incubated at T=26±0.2 °C [20], except for *V. volvacea* that was incubated at T=32±0.2 °C [21], in agitated (ZHICHENG ZHWY 211 C, China) at 120±5 rpm and non-agitated conditions.

Analytical Techniques

Biomass concentration was determined from dry weight; fungal mycelia were harvested by filtration under vacuum (using a Whatman No 2 filter, England), washed at least twice with distilled water, dried (at 65±2 °C until constant weight, usually within 3 days) and weighed (in a four digits Kern AGB balance, Germany). The resulting culture filtrate containing the EPS was mixed with four volumes of 95 % (*v/v*) ethanol (Merck) and maintained at 4±0.2 °C for 12 h. The precipitated EPS was centrifuged (Micro 22R, Hettich, Germany) at 10,000×*g* for 20 min at 4 °C, the supernatant was discarded and the remaining was dried at 60±2 °C to remove residual ethanol. The EPS content was determined by phenol–sulphuric acid assay according to Dubois et al. [22] method, using glucose as standard. The residual glucose level in the culture medium was estimated by 3,5-dinitro-2-hydroxybenzoic acid method reported by Miller [23]. Total cellular lipids were determined gravimetrically using a mixture of chloroform/methanol 2/1 (*v/v*; "Folch" extract) as the extracting solvent. Cellular lipids were converted to their methyl-esters in a two-step reaction with methanolic sodium and hydrochloric methanol in order to avoid generation of trans fatty acid isomers and fatty acid methyl-esters were analysed in a Fisons 8060 (USA) device equipped with a capillary column CP-WAX 52 CB (Chrompack; 60 m× 0.25 mm, film thickness 0.25 μm; JK&W Scientific, USA) and a flame ionization detector. Analysis conditions were as follows: oven temperature 200 °C for 13 min, increase to 220 °C (2 °C per min) for 4 min and further increase to 240 °C for 3 min; injector temperature 250 °C; detector temperature 300 °C; carrier gas helium; flow 1.5 ml min^{-1}. Identification of methyl-esters was based on the comparison of retention times with known standards.

All experiments were repeated at least twice and within experiments triplicate flasks were used to generate each data point. In the tables, values are given as the mean and the standard deviations are calculated.

Results

Biomass, EPS Production and Glucose Consumption

The results concerning biomass production (X, in grams per liter), glucose consumption (Gl_c, in grams per liter) and exopolysaccharides (EPS, in grams per liter) are given for the 12th and 16th day of cultivation in non-agitated and agitated cultures at Tables 3, 4, 5 and 6, respectively. GPYS was the medium that better promoted growth of numerous mushrooms (*Morchella elata, Morchella esculenta*, *Ganoderma lucidum*, *A. auricula, Agrocybe aegerita, Pleurotus ostreatus, Pleurotus pulmonarius, F. velutipes, V. volvacea*), followed by MCM (for *L. edodes*) and GYS (for *Ganoderma applanatum*) and this was observed the 16th day of cultivation (Tables 5 and 6). Likewise, agitation favoured biomass production in most cultures performed (Tables 3, 4, 5 and 6). Actually, three- to fourfold increase was observed in *P. ostreatus*, *G. lucidum*, *A. auricula* and *L. edodes* agitated cultures compared to the static ones the 12th day of cultivation. Agitation also favoured the production of biomass by the fungi *M. elata* and *M. esculenta*, but to a lesser extent than in the previous

Table 3 Mean values of biomass (X, g l^{-1}), exopolysaccharides (EPS, g l^{-1}) and glucose consumption (Gl_c, g l^{-1}) of strains from 11 mushrooms cultivated on GPYS, GPY, GYS and MCM media for 12 days at 26±2 °C (32±2 °C for *V. volvacea*) in static-flask cultures

12-day, static		*M. elata* 63	*M. esculenta* 36	*G. lucidum* 330	*G. applanatum* 341	*A. auricula* 111	*A. aegerita* 104	*P. ostreatus* 135	*P. pulmonarius* 177	*F. velutipes* 271	*L. edodes* 121	*V. volvacea* 190
GPYS	X (g l^{-1})	5.4±0.2	9.8±0.5	1.4±0.2	6.1±0.4	0.9±0.0	0.3±0.0	1.5±0.1	2.3±0.4	2.7±0.8	0.7±0.4	10.2±1.5
	Gl_c (g l^{-1})	17.8±1.3	22.7±1.3	1.9±0.4	10.0±1.1	1.5±0.2	0.5±0.1	3.3±0.4	4.4±0.5	5.1±0.7	1.0±0.3	16.5±1.0
	EPS (g l^{-1})	0.45±0.08	0.84±0.22	1.08±0.13	0.87±0.21	0.98±0.32	0.60±0.05	1.16±0.24	0.71±0.20	0.70±0.21	1.31±0.30.3	1.14±0.21
GPY	X (g l^{-1})	3.0±0.3	1.4±0.2	0.8±0.1	4.9±0.4	1.7±0.1	0.3±0.1	1.1±0.1	3.5±0.2	0.5±0.2	0.6±0.3	5.2±0.4
	Gl_c (g l^{-1})	9.9±0.6	7.8±0.5	2.8±0.1	7.9±0.6	1.9±0.1	2.9±0.1	1.7±0.1	4.1±0.2	1.9±0.1	0.7±0.2	5.8±±0.4
	EPS (g l^{-1})	0.80±0.09	0.77±0.04	1.04±0.05	0.72±0.06	1.17±0.20	0.30±0.05	1.09±0.09	0.84±0.10	0.89±0.12	1.01±0.13	0.96±0.13
GYS	X (g l^{-1})	5.7±0.4	5.5±0.4	0.2±0.1	6.7±0.5	0.5±0.1	0.9±0.2	0.9±0.1	1.1±0.2	1.9±0.3	0.3±0.1	10.0±0.7
	Gl_c (g l^{-1})	18.1±1.0	17.6±0.9	1.0±0.2	9.1±0.7	0.6±0.2	0.5±0.1	1.7±0.1	2.1±0.3	4.2±0.3	0.5±0.1	12.9±0.8
	EPS (g l^{-1})	0.41±0.06	0.56±0.07	0.93±0.05	0.59±0.14	0.47±0.06	0.40±0.02	0.80±0.08	1.04±0.06	0.79±0.12	0.50±0.03	0.88±0.01
MCM	X (g l^{-1})	5.6±0.4	2.8±0.1	1.7±0.1	5.7±0.4	0.4±0.1	0.5±0.1	1.6±0.1	0.3±0.0	2.3±0.1	0.1±0.0	9.2±0.6
	Gl_c (g l^{-1})	17.5±0.8	7.7±0.3	2.1±0.1	9.7±0.5	1.1±0.1	2.0±0.2	2.5±0.1	0.7±0.2	6.1±0.4	1.0±0.1	11.9±0.8
	EPS (g l^{-1})	0.47±0.03	0.55±0.03	1.06±0.06	0.73±0.05	0.46±0.04	0.50±0.04	0.94±0.06	0.58±0.04	0.96±0.07	0.25±0.03	0.83±0.02

Table 4 Mean values of biomass (X, g l^{-1}), exopolysaccharides (EPS, g l^{-1}) and glucose consumption (Gl_c, g l^{-1}) of strains from 11 mushrooms cultivated on GPYS, GPY, GYS and MCM media for 12 days at 26±2 °C (32±2 °C for *V. volvacea*) in agitated-flask (120±5 rpm) cultures

12-day, agitated		*M. elata* 63	*M. esculenta* 36	*G. lucidum* 330	*G. applanatum* 341	*A. auricula* 111	*A. aegerita* 104	*P. ostreatus* 135	*P. pulmonarius* 177	*F. velutipes* 271	*L. edodes* 121	*V. volvacea* 190
GPYS	X (g l^{-1})	7.9±0.6	10.5±0.7	8.3±0.6	4.1±0.2	6.5±0.4	2.0±0.1	15.7±0.9	9.0±0.6	7.0±0.5	1.9±0.1	11.2±0.8
	Gl_c (g l^{-1})	13.5±0.8	25.1±1.0	14.6±0.8	6.5±0.4	10.1±0.7	3.0±0.1	18.4±0.8	10.2±0.7	17.5±0.8	2.5±0.1	13.0±0.8
	EPS (g l^{-1})	0.82±0.13	0.84±0.07	1.04±0.10	0.50±0.10	1.03±0.11	0.60±0.04	1.25±0.13	0.62±0.12	1.0±0.11	0.98±0.10	0.94±0.10
GPY	X (g l^{-1})	5.5±0.4	3.0±0.2	3.5±0.2	0.9±0.1	2.9±0.1	0.9±0.1	1.9±0.1	4.0±0.2	3.9±0.2	0.8±0.1	6.1±0.5
	Gl_c (g l^{-1})	7.9±0.6	6.5±0.4	3.9±0.2	1.1±0.1	3.8±0.1	2.9±0.1	2.1±0.1	4.5±0.1	8.5±0.6	1.9±0.1	6.6±0.2
	EPS (g l^{-1})	1.06±0.12	0.93±0.10	1.64±0.14	1.06±0.13	1.45±0.15	0.38±0.17	1.14±0.36	0.86±0.40	1.29±0.12	1.62±0.17	0.76±0.15
GYS	X (g l^{-1})	6.9±0.5	9.9±0.6	6.7±0.4	4.9±0.2	4.5±0.2	1.1±0.1	14.5±0.8	12.8±0.7	5.2±0.3	1.9±0.1	10.6±0.8
	Gl_c (g l^{-1})	14.5±0.8	25.5±0.9	16.8±0.8	5.4±0.2	5.6±0.2	1.5±0.1	17.4±0.8	15.9±0.8	8.7±0.6	2.9±0.1	12.7±0.9
	EPS (g l^{-1})	0.40±0.04	1.00±0.04	1.25±0.13	0.40±0.05	0.76±0.02	1.29±0.12	0.73±0.12	1.00±0.13	1.39±0.12	1.77±0.14	0.97±0.12
MCM	X (g l^{-1})	7.8±0.6	6.2±0.5	5.9±0.4	1.7±0.1	2.4±0.1	2.9±0.1	3.9±0.2	15.9±0.8	4.9±0.4	2.9±0.1	9.9±0.5
	Gl_c (g l^{-1})	14.2±0.8	11.6±0.8	6.8±0.3	2.9±0.1	3.1±0.1	3.7±0.2	5.1±0.4	20.1±0.9	6.6±0.4	3.1±0.1	12.9±0.6
	EPS (g l^{-1})	0.38±0.02	0.81±0.04	1.50±0.09	1.00±0.04	1.16±0.07	0.40±0.03	1.67±0.15	0.84±0.10	1.24±0.11	1.17±0.15	0.62±0.12

Table 5 Mean values of biomass (X, g l^{-1}), exopolysaccharides (EPS, g l^{-1}) and glucose consumption (Gl_c, g l^{-1}) of strains from 11 mushrooms cultivated on GPYS, GPY, GYS and MCM media for 16 days at 26±2 °C (32±2 °C for *V. volvacea*) in static-flask cultures

16-day, static		*M. elata* 63	*M. esculenta* 36	*G. lucidum* 330	*G. applanatum* 341	*A. auricula* 111	*A. aegerita* 104	*P. ostreatus* 135	*P. pulmonarius* 177	*F. velutipes* 271	*L. edodes* 121	*V. volvacea* 190
GPYS	X (g l^{-1})	8.6±0.5	10.7±0.9	2.3±0.1	9.2±0.6	3.3±0.1	0.8±0.1	3.8±0.2	8.6±0.7	5.5±0.2	1.1±0.1	14.5±0.8
	Gl_c (g l^{-1})	29.4±1.2	27.7±1.3	2.9±0.1	15.7±0.8	4.1±0.2	1.0±0.1	4.5±0.2	9.9±0.6	7.0±0.5	1.5±0.1	24.7±0.9
	EPS (g l^{-1})	0.26±0.07	0.29±0.08	0.64±0.05	0.40±0.04	0.65±0.06	0.60±0.05	0.69±0.06	0.35±0.06	0.50±0.1	0.68±0.07	0.76±0.06
GPY	X (g l^{-1})	5.2±0.3	3.2±0.2	1.3±0.6	7.9±0.6	2.8±0.1	0.6±0.1	2.0±0.1	4.4±0.2	5.8±0.4	1.1±0.1	7.2±0.6
	Gl_c (g l^{-1})	13.6±0.7	9.0±0.8	4.1±0.2	11.1±0.8	3.3±0.2	4.1±0.2	3.3±0.2	6.1±0.4	7.0±0.6	1.8±0.1	8.6±0.4
	EPS (g l^{-1})	0.35±0.05	0.30±0.04	0.53±0.06	0.56±0.04	0.81±0.06	0.23±0.04	0.85±0.05	0.61±0.07	0.79±0.1	0.71±0.06	0.50±0.03
GYS	X (g l^{-1})	6.9±0.5	7.5±0.5	0.5±0.0	15.1±0.7	3.7±0.2	2.1±0.1	4.2±0.1	8.8±0.6	4.0±0.2	0.8±0.1	12.4±0.6
	Gl_c (g l^{-1})	24.1±0.8	23.1±0.9	1.0±0.1	19.2±0.6	4.5±0.2	3.5±0.1	4.9±0.2	10.5±0.7	8.3±0.6	1.7±0.1	14.6±0.7
	EPS (g l^{-1})	0.20±0.05	0.27±0.04	0.68±0.07	0.17±0.03	0.28±0.04	0.69±0.06	0.37±0.05	0.52±0.06	0.66±0.06	0.31±0.02	0.77±0.03
MCM	X (g l^{-1})	7.2±0.5	4.4±0.2	2.7±0.1	12.6±0.7	1.1±0.1	0.6±0.0	2.2±0.1	4.1±0.2	4.8±0.4	0.1±0.0	12.7±0.6
	Gl_c (g l^{-1})	22.7±0.8	10.1±0.5	3.3±0.2	18.2±0.8	2.7±0.1	2.5±0.2	5.0±0.2	7.9±0.6	7.8±0.6	1.0±0.1	14.8±0.8
	EPS (g l^{-1})	0.21±0.06	0.29±0.03	0.65±0.04	0.44±0.03	0.40±0.04	0.35±0.03	0.84±0.05	0.49±0.03	0.72±0.05	0.13±0.05	0.51±0.02

Table 6 Mean values of biomass (X, g l^{-1}), exopolysaccharides (EPS, g l^{-1}) and glucose consumption (Gl_c, g l^{-1}) of strains from 11 mushrooms cultivated on GPYS, GPY, GYS and MCM media for 16 days at 26±2 °C (32±2 °C for *V. volvacea*) in agitated -flask (120±5 rpm) cultures

16-day, agitated		*M. elata* 63	*M. esculenta* 36	*G. lucidum* 330	*G. applanatum* 341	*A. auricula* 111	*A. aegerita* 104	*P. ostreatus* 135	*P. pulmonarius* 177	*F. velutipes* 271	*L. edodes* 121	*V. volvacea* 190
GPYS	X (g l^{-1})	9.6±0.4	12.2±0.8	10.9±0.7	8.0±0.6	10.6±0.6	7.5±0.6	19.2±0.9	17.7±0.6	10.9±0.6	3.7±0.3	13.9±0.7
	Gl_c (g l^{-1})	23.1±1.1	29.7±1.1	18.2±0.9	13.1±0.7	12.9±0.7	8.8±0.6	23.0±0.8	21.2±0.7	20.0±1.2	6.0±0.5	23.6±1.2
	EPS (g l^{-1})	0.51±0.03	0.44±0.05	0.65±0.06	0.54±0.1	0.65±0.08	0.80±0.05	0.78±0.1	0.40±0.06	0.40±0.1	0.92±0.1	0.35±0.04
GPY	X (g l^{-1})	7.1±0.4	6.0±0.3	6.9±0.4	2.9±0.1	5.8±0.5	1.4±0.1	3.9±0.1	4.9±0.2	7.6±0.5	1.3±0.1	8.0±0.6
	Gl_c (g l^{-1})	12.8±0.6	9.2±0.5	8.5±0.5	4.2±0.2	7.5±0.5	4.0±0.2	5.1±0.2	6.4±0.2	12.2±0.6	1.8±0.07	10.1±0.6
	EPS (g l^{-1})	0.47±0.07	0.70±0.07	0.61±0.03	0.80±0.10	0.29±0.13	0.29±0.05	1.04±0.12	0.80±0.12	0.80±0.12	0.82±0.12	0.47±0.05
GYS	X (g l^{-1})	9.0±0.6	12.2±0.8	9.4±0.6	7.5±0.5	6.8±0.4	3.0±0.1	16.9±0.8	16.2±0.8	6.9±0.3	3.6±0.1	11.9±0.6
	Gl_c (g l^{-1})	20.9±0.8	28.8±1.4	18.1±0.6	9.0±0.6	8.6±0.5	4.2±0.1	20.1±0.7	19.3±0.9	13.1±0.8	4.4±0.2	15.1±0.5
	EPS (g l^{-1})	0.46±0.05	0.36±0.06	0.49±0.04	0.35±0.03	0.39±0.04	1.67±0.18	0.75±0.14	0.64±0.06	0.35±0.1	0.47±0.05	0.35±0.04
MCM	X (g l^{-1})	9.1±0.6	7.2±0.6	8.5±0.5	3.9±0.2	4.0±0.2	4.4±0.2	5.9±0.4	18.8±0.8	6.5±0.4	4.6±0.2	13.4±0.7
	Gl_c (g l^{-1})	20.1±1.0	16.6±0.8	10.5±0.5	6.1±0.3	5.0±0.2	5.1±0.3	6.9±0.4	21.6±0.8	9.8±0.5	5.9±0.2	17.7±0.7
	EPS (g l^{-1})	0.45±0.08	0.56±0.06	0.62±0.04	0.61±0.06	0.44±0.05	0.47±0.03	0.88±0.12	0.53±0.04	0.71±0.06	0.83±0.11	0.38±0.05

cases (Tables 3 and 4). In contrast, agitation had little effect upon biomass production of *V. volvacea*, while static cultures of *G. applanatum* were clearly favoured in comparison with the agitated ones. In addition, most X_{max} values were recorded in agitated cultures the 16th day of cultivation (Table 6), whereas different mushroom species were not equally affected by agitation. *P. ostreatus* and *P. pulmonarius* produced considerably higher X_{max} quantities than the other studied mushrooms; therefore, at the 16th day of agitated cultures, *P. ostreatus* presented a X_{max} of 19.2 gl^{-1} (GPYS medium), *P. pulmonarius* a X_{max} of 18.8 gl^{-1} (MCM medium) with the maxima of mycelial biomass produced being recorded in the order of *P. pulmonarius*>*P. ostreatus*>*V. volvacea*>*M. esculenta*>*G. lucidum* or *M. elata*>*F. velutipes*>*A. auricula*>*G. applanatum*>*A. aegerita* or *L. edodes*. It can be assumed therefore that mycelial biomass production was depended on mushroom strain and agitation.

A significant portion of the available sugar quantity remained non-assimilated after 12 or 16 days of growth in non-agitated flasks (Tables 3 and 5). For instance, in *L. edodes*, *A. auricula*, *A. aegerita*, or *G. lucidum*, glucose was consumed at a rate of 2.5 to 9.5 % only. On the other hand, *M. elata*, *M. esculenta*, *G. applanatum* and *V. volvacea* fungi (the growth of which was not particularly favoured by agitation), combined a high biomass production with satisfactory glucose consumption (60–90 %), especially at GPYS and GYS substrates. Specifically for *G. applanatum*, mycelial mass production was noticeable in the static cultures (X_{max}=15.1 gl^{-1} in the GYS medium at the 16th day of growth), while in all the above-mentioned cases the conversion yield $Y_{X/Gl}$ (yield of mycelial dry biomass produced per unit of glucose consumed) ranged between 0.3–0.4 gg^{-1} for *M. elata* and *M. esculenta* and 0.5–0.7 gg^{-1} for *G. applanatum* and *V. volvacea* fungi. Glucose was not totally consumed by the majority of mushrooms in agitated cultures too (Tables 4 and 6), but assimilation increased in GPYS and then GYS media, particularly the 16th day. *M. elata*, *M. esculenta*, *V. volvacea*, *P. ostreatus*, *P. pulmonarius* and *F. velutipes* were the fungi that consumed most of the available glucose, in quantities ranging between 67 % (*F. velutipes* and *P. ostreatus*) and ~100.0 % *w*/*w* (*M. esculenta*). In various cases, biomass synthesis was accompanied by yield $Y_{X/Gl}$ values that were indeed very high (e.g. 0.6–0.9 gg^{-1} for *P. pulmonarius*, *P. ostreatus* and *V. volvacea* on GPYS medium).

Concerning EPS production, in contrast to mycelial mass accumulation, notably higher quantities were detected in the 12th day after inoculation, since probably EPS were subjected to degradation when the fermentation was extended. Also, comparing EPS biosynthesis performed in the different media, it seems that the most suitable substrate for the majority of the tested strains was GPY followed by GYS. Likewise agitation seemed to have a positive effect upon EPS production, as the maximum EPS values were obtained in agitated cultures (with the exception of *P. pulmonarius*). The maximum EPS values that were recorded in the current study were achieved in GPY medium by *G. lucidum* (1.64 gl^{-1}), *L. edodes* (1.62 g l^{-1}) and *A. auricula* (1.45 gl^{-1}), in GYS medium by *L. edodes* (1.77 gl^{-1}), *F. velutipes* (1.39 gl^{-1}), *A. aegerita* (1.29 gl^{-1}) and *G. lucidum* (1.25 gl^{-1}) and in MCM medium by *P. ostreatus* (1.67 gl^{-1}), *G. lucidum* (1.5 gl^{-1}) and *F. velutipes* (1.24 gl^{-1}).

Fungal Lipid Synthesis and Analysis

From the obtained results, it seems that the most suitable medium amenable for lipid biogenesis (in terms of grams of lipid per liter of medium) was GPYS followed by MCM and GYS (data not presented). As regards total lipid in dry weight values ($Y_{L/X}$, in % *w*/*w*), GPY and MCM media presented the best results. Cultures of 12th day after inoculation produced total cellular lipids in relatively small quantities and increment of biomass production at 16th day resulted in higher quantities of total cellular lipophilic compounds,

whereas agitation increased lipid synthesis in all media used. Among mushrooms tested, *M. esculenta*, *G. lucidum* and *P. pulmonarius* gave the best results (1.5–2 gl^{-1}) in both static and agitated cultures. Figure 1a,b shows the concentration (%, *w*/*w*) of total lipophilic compounds from mycelia grown in the four media at the 16th day of cultivation in static and agitated cultures, where values of 2.5–18.5 %, *w*/*w*, were depicted. It seems that some mushrooms, e.g. *G. lucidum*, *G. applanatum* were particularly favoured by agitation, as their lipid in dry weight values were almost doubled compared to the static ones, whereas others, e.g. *M. elata*, *F. velutipes* were not. Also, *L. edodes*, with small biomass production, seemed to produce non-negligible quantities of lipophilic compounds (e.g. ranging between 14 % and 16 %, *w*/*w*, in almost all media irrespective of the agitation or non-agitation imposed), whereas *M. esculenta* that presented good biomass production (X=12.2 gl^{-1}) was also accompanied by non-negligible biogenesis of total cellular lipids ($Y_{L/X}$~18.0 %, *w*/*w*) when shake-flask cultures were performed after 16 days of incubation.

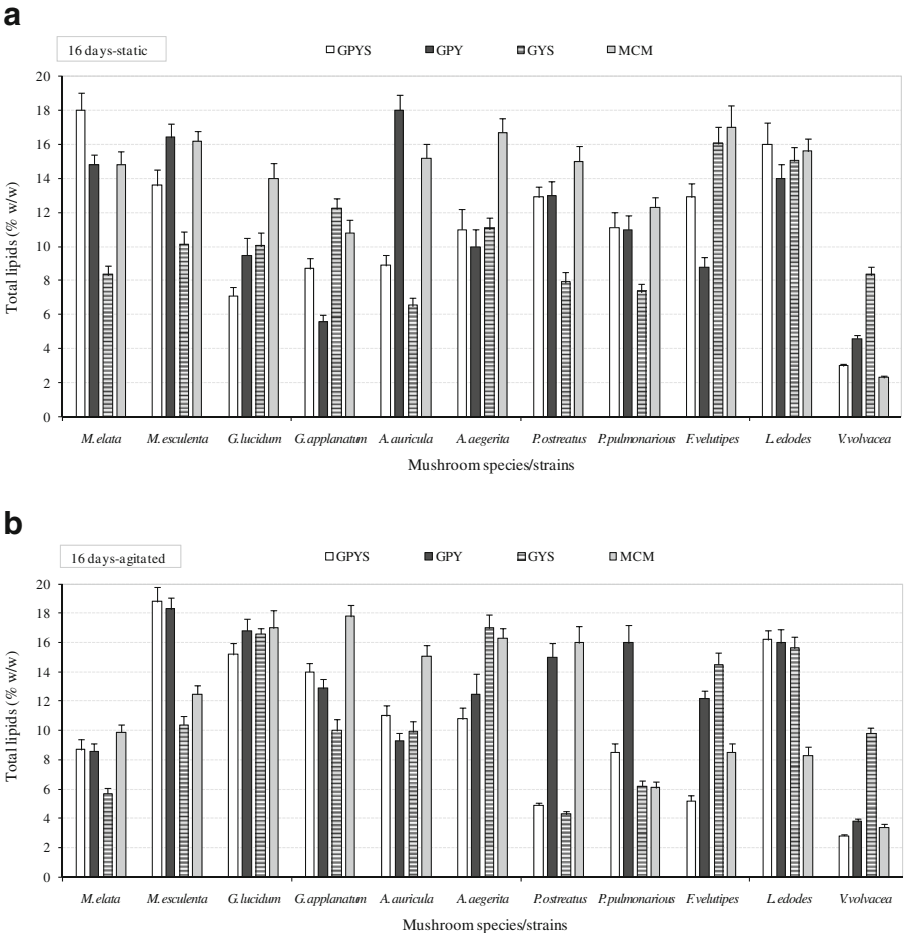

Fig. 1 Mean values of total lipophilic compounds (%, *w*/*w* in dry weight) of mycelia produced from 11 mushroom strains cultivated in GPYS, GPY, GYS and MCM media for 16 days at **a** static and **b** agitated (120 ±5 rpm) cultures. Data are means of three replicates with *error bars* indicating standard deviations

In addition, as shown in Table 7, in all cultures performed by the mushroom genera, the 16th day of cultivation in GPYS medium (static and agitation conditions), unsaturated fatty acids (FAs) accounted for an average of 74 %, *w*/*w*, of the total FAs, whereas saturated only for 26 %, *w*/*w*. The genera with the highest unsaturated FA content were *M. elata*, *G. applanatum*, *P. pulmonarius* (cultures both agitated and non-agitated), *L. edodes, A. aegerita* and *A. auricula* (agitated) and *P. ostreatus* (non-agitated cultures). On the opposite, *V. volvacea*'s lipids contained nearly 49 % saturated FAs, *M. esculenta* 38 % and *F. velutipes* 33 %, respectively. As a consequence, the ratios of unsaturated: saturated fatty acids ranged from around 1.0 (case of *V. volvacea*) to around 4.0 (*M. elata*, *A. aegerita*, *A. auricula*, *P. ostreatus*) and the maximum value of 9.20 (*L. edodes*).

Linoleic acid ($^{\Delta 9,12}$C18:2) was the predominant cellular FA. Palmitic (C16:0) and oleic acid ($^{\Delta 9}$C18:1) were also found in remarkable quantities, while stearic acid (C18:0) was detected in much lower concentrations (e.g. <4 %, *w*/*w*), with the exception of the fungal lipids produced by *M. elata, M. esculenta, G. lucidum* and *A. auricula*, in which higher amounts were detected. Palmitoleic acid ($^{\Delta 9}$C16:1) was also produced but in insignificant amounts (0.3–4.6 %, *w*/*w*, of total lipids). Other FAs including caprilic (C8:0), capric (C10:0), lauric (C12:0) and arachidic (C20:0) comprised a percentage that varied from 2 to 5.5 %, *w*/*w*, in the majority of mushrooms examined, with the exception of *V. volvacea* where an amount of 16.0–0.0 %, *w*/*w*, of these FAs in total produced lipids was observed. Regarding unsaturated FAs, lipid analysis showed that $^{\Delta 9,12}$C18:2 content reached 74.1 to 82.5 % in *L. edodes*, 73.4 % in *P. ostreatus*, 72.9 % in *G. lucidum*, 70.0 % in *A. auricula* and 67.4 % in *G. applanatum*. When $^{\Delta 9}$C18:1 was produced in significant quantities as in agitated cultures of *M. esculenta* and *A. aegerita*, $^{\Delta 9,12}$C18:2 reduced its percentages down to 27.3 and 38.2 % respectively. On the other hand, $^{\Delta 9}$C18:1 in *L. edodes, P. ostreatus* and *G. lucidum* was presented in indeed low concentrations (1.4–7.4 %, *w*/*w*). Also, the saturated FAs were dominated by C16:0, with its content varying between 12.4 and 28.4 %, *w*/*w*, depending on the mushroom strain and only in agitated cultures of *L. edodes* was reduced to 4.5 %. Interesting and different from the other mushroom strains was the profile of *V. volvacea*, as non-negligible quantities of saturated low or high chain FAs (e.g. C8:0, C10:0, C20:0) were produced. Finally, FA analysis of total lipids showed that total cellular FA composition was genus and/or strain dependent, since a differentiation in the distribution of fatty acids was observed. Data also indicate that agitation promoted unsaturation of lipids, as significant increase of around 10 % (or more) of unsaturated fatty acids was observed, e.g. in *L. edodes*, *G. lucidum*, *A. auricula* and *A. aegerita*. Only in *M. esculenta*, *V. volvacea* and *F. velutipes* more saturated fatty acids were produced when agitation was imposed in the cultures.

Discussion

In the present study, four culture media were employed to select a suitable medium through the screening of 11 mushroom species for biomass production, as well as for EPS and lipid biosynthesis. Biomass synthesis, production of EPS and lipogenesis were found to depend upon the composition of the medium and on the fungus itself, in agreement with literature [11, 24, 25]. Agitation had a significant impact upon biomass and metabolite's synthesis in mushroom cultures too. Glucose and the combination of peptone, yeast extract and macro/microelements (calcium, magnesium, manganese, sodium, iron, zinc) in GPYS medium seemed to favour biomass production compared to the other nutrient media used, as other authors have already stated [12, 26]. Significant mycelial mass production, in terms of both

Table 7 Fatty acid composition of total lipids produced by strains from 11 mushrooms the 16th day of cultivation on GPYS medium, on static and agitated (120 rpm) cultures

Genera/fatty acids %		*M. elata*	*M. esculenta*	*G. lucidum*	*G. applanatum*	*A. auricula*	*A. aegerita*	*P. ostreatus*	*P. pulmonarius*	*F. velutipes*	*L. edodes*	*V. volvacea*
Static cultures	C14:0	–	–	1.0	1.0	–	–	–	–	0.3	–	1.5
	C16:0	12.4	18.4	16.5	16.4	15.4	23.0	12.9	18.8	15.6	17.5	14.8
	C16:1	0.3	–	–	–	–	–	1.7	–	0.3	–	–
	C18:0	5.6	7.2	12.5	1.1	11.0	0.8	2.6	3.4	4.1	1.6	5.0
	C18:1	28.0	29.0	11.6	9.0	10.3	24.5	5.7	19.7	14.7	1.4	3.3
	C18:2	51.5	41.9	56.1	67.4	60.6	49.8	73.4	55.7	61.3	74.1	54.6
	Others[a]	2.2	3.5	2.3	5.1	2.7	1.9	3.7	2.4	3.7	5.4	20.3
Agitated cultures	C14:0	–	–	0.8	0.8	0.3		0.3	–	–	0.7	2.1
	C16:0	16.6	23.8	14.6	14.8	13.0	17.2	13.4	14.3	28.4	4.5	23.1
	C16:1	1.1	–	–	4.6	–		–	–	–	–	–
	C18:0	4.5	9.0	1.0	1.6	5.2	1.2	4.9	5.8	1.0	0.3	6.2
	C18:1	17.3	35.1	5.6	8.4	7.6	41.8	11.3	16.2	17.2	7.4	3.2
	C18:2	58.4	27.3	72.9	66.3	70.0	38.2	65.2	61.1	49.4	82.8	48.2
	Others	2.1	4.8	5.1	3.5	3.9	1.6	4.9	2.6	4.0	4.3	17.2

[a] Mainly C8:0, C:10, C12:0, C20:0

dry weight and conversion yield of biomass produced per glucose consumed was obtained in several of the performed experiments (X_{max} 15–19 gl^{-1}, values comparable to those of the international literature); according to Kim et al. [19] the mycelial mass production of *F. velutipes* was 4.8 gl^{-1}, of *L. edodes* 0.4 gl^{-1}, of *P. ostreatus* ~7.0 gl^{-1} and of *G. lucidum* 6.9 gl^{-1}, the last being 2 times higher when trials were conducted in 3-l bioreactor. Maziero et al. [27] recorded similar to our data values of biomass in agitated cultures of *F. velutipes*, *L. edodes* and *P. ostreatus* with glucose used as substrate. For *M. esculenta*, biomass of 9.2 g l^{-1} was produced in an optimised medium containing glucose and yeast extract [12]. Lee et al. [28] recorded that X_{max} value for a *G. applanatum* strain cultivated in agitated cultures with glucose and yeast extract was 7 gl^{-1}. Tang and Zhong [5] reported X_{max} quantities of ~22 g l^{-1} in shake-flask and bioreactor cultures of *G. lucidum*. Likewise, with the same fungus, Tang et al. [29] indicated X_{max} values ranging between 17 and 22 gl^{-1} in shake-flask and large-scale stirred-tank bioreactors, whereas according to Stajić et al. [30] different *G. lucidum* strains produced 3–28 gl^{-1} biomass in agitated cultures. On the other hand, lower X_{max} quantities (1.5–3.0 gl^{-1}) have been recorded during growth of *V. volvacea* strains on water extracts of several agro-industrial residues in shake-flask submerged cultures [21], while equally optimisation of culture conditions for mycelial growth of *V. esculenta* resulted in X_{max} concentration of ~5.0 g l^{-1} [31]. *L. edodes* strains growing in shake-flasks with glucose and yeast extract, the optimisation of culture conditions increased the biomass from 2.75 to 6.88 gl^{-1} [32]. As for the yield $Y_{X/Gl}$, in some of the above-mentioned cultures, $Y_{X/Gl}$ presented remarkably high values (0.7–0.9 gg^{-1}). Given that the maximum theoretical yield of dry biomass produced per sugar consumed is ~0.8 gg^{-1} [33], it is evident that some of the organic compounds added into our culture medium (like peptone and yeast extract) were also utilized as carbon sources, resulting, thus, in some overestimation of this yield. In any case, the simultaneous high values of both X and $Y_{X/Gl}$ suggests the potentiality of utilization of several of the currently tested mushroom species as cell factories in sugar-based biorefineries.

Although our experiments were not optimised towards the production of EPS, a good level of EPS was achieved in the medium with both peptone and yeast extract (GPY), then in GYS, implying that supplements would be unnecessary for maximum EPS production of mushroom genera examined. Literature indicates that glucose and organic nitrogen sources present the best results in fungi EPS production [12, 13]. Zhou et al. [34] suggested that peptone had more pronounced effect on EPS production of *G. umbellate* than yeast extract, whereas Meng et al. [12] obtained the maximum EPS values with yeast extract as main nitrogen source in *M. esculenta* cultures. A combination of yeast extract and peptone used by Fang and Zhong [14] improved the EPS production of *G. lucidum* up to 0.81 gl^{-1}. Also, in Elisashvili et al. [11] study of several basidiomycetes, organic nitrogen sources enhanced the production of EPS (max values 2.5–3.0 gl^{-1}). Among our 11 mushrooms tested, maximum EPS values ranged from to 1.24 (*F. velutipes*) to 1.77 gl^{-1} (*L. edodes*). Feng et al. [32] under optimised conditions of *L. edodes* cultivation (in shake-flasks with glucose and yeast extract as main substrates) produced 0.21–0.75 gl^{-1} of EPS, values much lower to those of our study. For *G. lucidum*, the maximum concentration of the EPS obtained in batch fermentation was ~1.5 gl^{-1} [15, 30]. On the other hand, Wu et al. [9] achieved great amounts of EPS during the cultivation of *A. auricula* both in shake-flasks (2.0–2.5 gl^{-1}) and in a 7-l stirred fermentor (4.5 gl^{-1}) with glucose, soybean, yeast extract or peptone. Also, in several cases, EPS accumulation was remarkable although the respective production of biomass was restricted, e.g. our data of *G. lucidum* or *L. edodes* on GPY medium (0.8 and 3.5 gl^{-1} biomass produced ~1.62 gl^{-1} EPS respectively), in *G. lucidum* on sucrose [5], or the case of *T. sinense* on lactose [6].

In our study, lipid in dry weight values ranging from 2.5 to 18.5 %, *w*/*w*, have been recorded. In some strains, maximum total lipid quantities achieved (e.g. 15–18 %, *w*/*w*) were

higher than those reported in the literature for the mycelia or carposomes of edible/pharmaceutical fungi, as mushrooms like *P. ostreatus*, *P. pulmonarius*, *Pleurotus cornucopiae*, *Pleurotus sajor-caju*, *Stropharia aeruginosa*, *Phellinus* spp., *F. velutipes*, *Boletus* spp., *L. edodes*, *M. esculenta*, *Grifola frondosa*, etc., contained lipid quantities ≤10 %, *w*/*w*, in dry mycelial mass [10, 16, 35–38]. However, in some other cases, total lipid quantities ranging between 14.2 and 16.3 %, *w*/*w*, have been reported for the mycelia of *Polyporus hirsutus* and *Calvatia caelata* [39], whereas in other studies even higher lipid content values in the mycelia of *G. frondosa* (~25 %, *w*/*w*) [40], *G. tsugae* (~22 %, *w*/*w*) [41] and *L. edodes* (~20 %, *w*/*w*) [42] have been indicated, respectively. Finally, lipid quantities of ~18 %, *w*/*w*, in dry matter, have been recorded for the carposomes of *V. esculenta* [43], while elevated quantities of lipids (~32 %, *w*/*w*) were reported for *Antrodia camphorata* [44]. In any case, in the current investigation cellular lipids were extracted by using the mixture chloroform/methanol 2/1 *v*/*v* ("Folch" extract), therefore, lipophilic compounds other than triglycerides (e.g. pigments, lipoproteins, etc.) might have also been co-extracted with lipid compounds.

Fatty acid composition of fungal lipids demonstrated significant predominance (ranging between 67 % and 83 %, *w*/*w*, of total lipids) for the fatty acid $^{\Delta 9,12}$C18:2 in *L. edodes*, *P. ostreatus*, *G. lucidum*, *A. auricula* and *G. applanatum*. The second unsaturated fatty acid, namely, $^{\Delta 9}$C18:1, was produced in significant quantities in *M. esculenta* and *A. aegerita*. The fatty acid $^{\Delta 9,12}$C18:2 was the most abundant for the case of mushrooms belonging to the genera *Boletus* [45], *Pleurotus* [16, 35, 37], *Leccimum* and *Tylopilus* [38], *Daedaleopsis* [36], *Lentinus* (*Lentinula*) [7, 17]. On the other hand, the fatty acid $^{\Delta 9}$C18:1 represented the major cellular fatty component in strains of the mushroom species *P. eryngii* [16], *P. ostreatus* [46], *Suillus grevillei* [38], *Russula brevipes* and *Termitomyces microcarpus* [17]. On the opposite, lipids of *V. volvacea* contained non-negligible quantities of saturated fatty acids, while fungi like *M. esculenta, A. aegerita*, *P. pulmonarius* and *F. velutipes* produced more unsaturated cellular fatty acids during growth on static flask cultures as compared with the shake-flask experiments.

As a conclusion, several of the mushroom strains tested in the current work presented interest as potential producers of mycelial biomass containing fatty acids of nutritional value and bioactive polysaccharides, although their production was only partially optimised. Nevertheless, in some cases, both mycelial biomass and EPS synthesis was significant and comparable with literature values, although the current investigation aimed to test the capacity of four media to support growth of edible and medicinal mushrooms and to screen their potentialities of producing biomass, EPS and not to maximise the above-mentioned compounds at this stage. The ability of mushroom species such as *P. pulmonarius*, *F. velutipes*, *L. edodes*, *V. volvacea*, *G. applanatum*, *M. esculenta* to produce in notable quantities of EPS and biomass rich in unsaturated fatty acids of nutritional importance, renders these fungi worthy for further investigations.

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