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Full Length Article

Effect of Temperature and Agitation Speed on Fatty Acid Accumulation in *Mortierella alpina*

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Abstract

The effect of temperature and agitation speed on cell growth and fatty acid accumulation by *Mortierella alpina* were investigated in the present study. The fungi were cultivated in a liquid medium at 6 different temperatures (10, 15, 20, 25, 30 and 35°C), and 7 agitation speeds (100, 125, 150, 175, 200, 225 and 250 rpm) for 7 days. The results revealed that temperature and agitation speed significantly influenced fungal growth and fatty acid accumulation. There was a temperature-threshold effect on mycelial biomass. The optimal temperature for cell growth, total fatty acid (TFA) accumulation, and polyunsaturated fatty acid (PUFA) accumulation, was 20°C. The major PUFAs in the mycelia were eicosapentaenoic acid (EPA), arachidonic acid (ARA), dihomo-γ-linolenic acid (DGLA) and eicosadienoic acid (C20:2). At temperatures 25°C and beyond, EPA and parinaric acid (C18:4) were not detected. The optimal agitation speed for cell growth, TFA accumulation, and PUFA accumulation was 175 rpm. Fungal growth ceased at 30°C or agitation speed of 250 rpm. In addition, mycelial morphologies under different agitation speeds were distinctly different. Pompon-like morphology favored ARA accumulation. © 2018 Friends Science Publishers

Keyword: *Mortierella alpina*; Temperature; Agitation speed; Arachidonic acid; Polyunsaturated fatty acids

Introduction

Polyunsaturated fatty acids (PUFAs) are linear-chain fatty acids with 16 - 22 carbon atoms and two or more double bonds. They have various physiological activities and positive effects on human health, especially 20-carbon (C20) PUFAs, such as ARA (C20:4, n-6), EPA (C20:5, n-3) and DGLA (C20:3, n-6). Arachidonic acid (ARA), one of the n-6 PUFAs, is effective in keeping vascular homeostasis, inhibiting platelet aggregation, and improving immunity (Harizi *et al.*, 2008). It is also necessary for the normal neurological development of both term and pre-term infants (Malaiwong *et al.*, 2016). Eicosapentaenoic acid (EPA), an n-3 PUFA, is beneficial in the treatment of cardiovascular diseases, decreases platelet aggregation and blood pressure (Sakuradani *et al.*, 2013). It has been reported that DGLA is used in combination with interferons to enhance their anti-virus, anti-cancer, and anti-inflammatory effects; and in combination with prostaglandin E1 for treating atopy of the skin and mucosa (Kikukawa *et al.*, 2016). Fish oil, animal tissues and algal cells are the most readily available lipid resources which are relatively rich in 20-carbon (C20) PUFAs, none of which is present in plants (Sakuradani *et al.*, 2009). However, some problems such as limited fishing quotas, remote geographical locations, man-made pollutants from the marine environment, and undesirable odors and flavors, are associated with these traditional sources (Asadi *et al.*, 2015). Therefore, due to health and dietary requirements, increasing attention has focused on novel rich sources, and microbial production of fatty acids has been suggested as an alternative source (Liang and Jiang, 2013).

Mortierella alpina, a member of the family Mortierellaceae, (order Mortierellales, and class Zygomycetes) has high capacity for lipid synthesis. It is the most promising organism for n-6 PUFA production, and the most important species for industrial production of ARA (Ho *et al.*, 2007). Moreover, the safety evaluation of *M. alpina* has been reported (Nisha *et al.*, 2009). The mycelial biomass and fatty acid accumulation of *M. alpina* can be increased by manipulating cultural conditions. For example, it has been reported that glucose concentration in the culture medium has a marked effect on ARA production in *M. alpina* (Zhu *et al.*, 2006), and that low nitrogen concentration could induce fatty acid accumulation in oleaginous micro-organisms (Economou *et al.*, 2011; Boyle *et al.*, 2012).

Temperature and agitation speed are also the most important environmental parameters that affect microorganism growth and metabolism. In studies on the effect of temperature on biomass, esterified fatty acid content and fatty acid production in *Scenedesmus acutus*, it was found that incubation temperature was a critical parameter for quantitative and qualitative fatty acid

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compositions (El-Sheekh *et al.*, 2017). Agitation speed and temperature significantly influenced cell growth and PUFA production in *Aspergillus oryzae*, with maximum unsaturated fatty acid content of 62.15% at 120 rpm and 25°C (Miranti *et al.*, 2018). However, only very few studies have focused on the effect of temperature and agitation speed on fatty acid accumulation by *M. alpina*.

The aim of the present study was to characterize the effect of temperature and agitation speed on the mycelial growth and fatty acid accumulation of *M. alpina*. The effect of temperature and agitation speed on mycelial biomass, fatty acid composition, total fatty acid (TFA) and PUFA contents in fungal cells of *M. alpina* were investigated. The effect of different agitation speeds on mycelial morphology was also investigated.

Materials and Methods

Fungal Strain

M. alpina D36 was isolated from the soil collected from Kunming, Yunnan province, Southwest China, and identified by both morphological and molecular techniques. The fungal strain was maintained on potato dextrose agar (PDA) slants at 4°C.

Fermentation Liquid Medium

The inoculum medium contained 30 g/L glucose, 6 g/L yeast extract 3 g/L KH_2PO_4, 3 g/L $NaNO_3$, and 0.5 g/L $MgSO_4 \cdot 7H_2O$. The fermentation medium had 80 g/L glucose, 11 g/L yeast extract, 3.8 g/L KH_2PO_4, 3.4 g/L $NaNO_3$ and 0.5 g/L $MgSO_4 \cdot 7H_2O$.

Fermentation Methods

The medium was autoclaved at 115°C for 15 min. Inoculum was prepared in 250-mL Erlenmeyer flasks, each containing 50 mL of the pre-culture medium for 3 days at 20°C with shaking at 175 rpm. Erlenmeyer flasks of 500 mL, each containing 100 mL of fermentation medium, were inoculated with 10 - 15 small pellets (1 - 2 mm) of an exponentially growing inoculum at natural pH for 7 days.

In the studies on the effect of temperature, *M. alpina* was cultivated at six different temperatures (10, 15, 20, 25, 30 and 35°C) with shaking at 200 rpm, while in the studies on the effect of agitation speed, *M. alpina* was cultivated at seven agitation speeds (100, 125, 150, 175, 200, 225, 250 rpm) at a temperature of 25°C.

Determination of Cell Dry Weight

The biomass of *M. alpina* was determined gravimetrically. The mycelia from the fermentation broth were harvested by vacuum filtration, washed twice with distilled water, and then vacuum freeze-dried to a constant weight.

Analysis of Fatty Acids

Freeze-dried mycelia (0.05 g) was ground to powder and transferred to a glass test tube with caps; 1 mL toluene, 2 mL sulfuric acid in methanol (v/v =1:99) and 0.8 mL internal standard (C17:0, 1 mg/mL hexane) were added, with thorough mixing. The tube was left overnight in a water bath at 50°C. After cooling to room temperature, 5 mL of 5% (w/v) Sodium chloride was added, mixed well and the mixture was extracted with hexane twice. The hexane layer was collected and washed with 4 mL of 2% (w/v) $KHCO_3$-water solution. Finally, the upper lipid phase was evaporated with N_2, and the residue was dissolved in 1 mL hexane and subjected to gas chromatography-mass spectroscopic (GC-MS) analysis (Agilent 5975C-7890A with a capillary column (30 m × 0.25 mm). Helium was used as the carrier gas. Initial column temperature was set at 80°C for 3 min, which was subsequently raised to 180°C at the rate of 20°C/min, and kept for 1 min, then raised to 210°C at the rate of 1°C/min, and kept for 5 min. Finally, it was raised to 240°C at the rate of 5°C/min, and kept for 8 min. The injector was kept as 250°C with an injection volume of 1 μL, split 50:1.

Statistical Analysis

Analysis of variance (ANOVA) was carried out with SPSS software package (version 19.0). Data were tested for normal distribution and homogeneity of variance prior to ANOVA. One-way ANOVA was performed to test the differences between fermentation temperatures, or between agitation speeds. Multiple mean comparisons were performed using Duncan's multiple range test at the 0.05 level of probability within one-way ANOVA. The relationship between mycelial biomass and TFA, PUFA was determined by Pearson's correlation analysis. And the regression equation of temperatures and agitation speed with mycelial biomass was performed by SPSS software.

Results

Effect of Fermentation Temperature on Mycelial Growth

The effect of fermentation temperature on mycelial growth is shown in Fig. 1. Mycelial biomass was highest at 25°C (15.59 g/L), followed by 20°C (13.87 g/L), while mycelial biomass was significantly reduced at fermentation temperatures of 30 and 35°C ($p < 0.05$). Statistical results showed there was a temperature-threshold effect on mycelial biomass. The regression equation for mycelial biomass fluctuating with temperatures was $Y = -0.138X + 0.569X*D + 5.29$ ($R^2=0.986$, $p < 0.01$). (Where Y is mycelial biomass and X is the temperature. When temperature ≤25°C, D=1; when temperature is >25°C, D=0).

Effect of Fermentation Temperature on the Contents of TFAs and PUFAs

The TFA and PUFA contents were gradually increased

initially, and then reduced with increase in fermentation temperature (Fig. 2). The TFA and PUFA contents in mycelial cells were highest at 20°C (161.05 and 96.78 mg/g of cell dry weight, respectively). These values were 8.2 and 9.4 times, respectively higher than the corresponding values at 10°C.

Effect of Fermentation Temperature on Compositions of Various Fatty Acids

The composition of fatty acids in mycelial cells differed with fermentation temperature (Table 1). The most variation in fatty acid profiles was detected at 20°C, and the highest degree of unsaturation (2.13) was also obtained at 20°C, followed by 10°C (2.10). Docosanoic acid (C22:0), eicosenoic acid (C20:1), and eicosadienoic acid (C20:2) were absent at 10°C, when temperature rising to 15°C, C20:2 began to appear, while C22:0 and C22:1 were not also detectable. Since 35°C is not conducive to the growth of *M. alpina*, there were reductions in fatty acid types at this temperature: only the fatty acids synthesized by n-6 pathway were detected. When fermentation temperature was 25°C or above 25°C, parinaric acid (C18:4) and eicosapentaenoic acid (C20:5; EPA) were not synthesized. The proportion of C18:4 in total fatty acids was highest at 10°C, while EPA was highest at 10°C and 20°C. The levels of various C20 fatty acids (especially ARA and dihomo-γ-linolenic acid) were significantly higher at 20°C and 25°C, when compared with their proportions at other fermentation temperatures. In contrast, the proportion of γ-linolenic acid (C18:3, LA) was relatively lower.

Effect of Agitation Speed on Mycelial Growth

Cultivation of *M. alpina* for 7 days at seven agitation speeds (100, 125, 150, 175, 200, 225, 250 rpm) produced remarkable effect on mycelial growth (Fig. 3). It was obvious that agitation speeds of 150, 175 and 200 rpm were favorable to *M. alpina* growth, resulting in mycelial biomass levels of 17.96, 17.70, 16.02 g/L, respectively. At 250 rpm, *M. alpina* barely grew, and mycelial biomass was only 1.72 g/L. The regression equation for mycelial biomass fluctuating with agitation speed was $Y= -0.002X^2 + 0.6693X - 36.577$ ($R^2 = 0.935$, $p < 0.01$). (Where Y is mycelial biomass and X is agitation speed).

Effect of Different Agitation Speed on TFA and PUFA Contents

The TFA and PUFA contents of *M. alpina* varied with different agitation speeds (Fig. 4). The TFAs contents were significantly higher at 150, 175, 200 rpm than at other agitation speeds, with values of 158.27, 161.56, 146.17 mg/g, respectively. Similarly, the PUFA contents of mycelia at 150, 175, 200 rpm were 3.9, 4.3 and 3.4 times, respectively higher than PUFA content obtained at 225 rpm

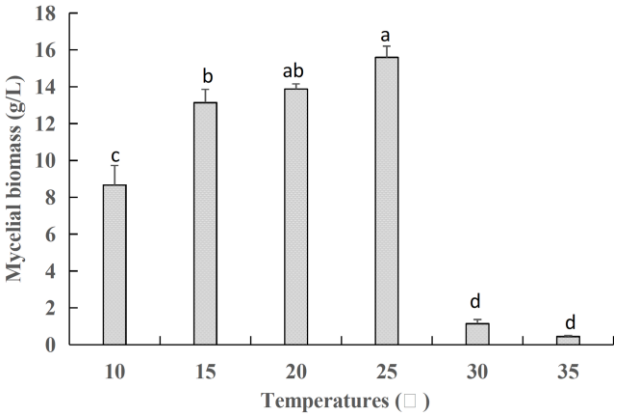

Fig. 1: Mycelial biomass of *M. alpina* cultivated at different temperatures. Data are expressed as mean ± standard error of four replicates. Values of mycelial biomass with different letters differ significantly ($p < 0.05$)

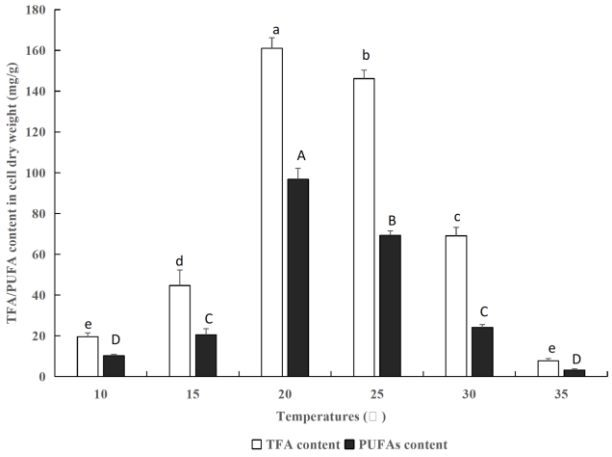

Fig. 2: Total fatty acid (TFA) and PUFA contents (mg/g of cell dry weight) after 7 days of cultivation at different temperatures. Data are expressed as mean ± standard error of four replicates. Values with different letters differ significantly ($p < 0.05$)

($p < 0.05$). However, the PUFA contents were dramatically decreased when agitation speeds were increased to 225 rpm or higher. There was no significant difference in PUFA contents at 100 rpm and 225 rpm, whereas TFA content was significantly lower at 225 rpm ($p < 0.05$).

Effect of Agitation Speed on Various Fatty Acid Compositions of *M. alpina*

The fatty acid composition of *M. alpina* varied with different agitation speeds (Table 2). When the speeds were 100 - 200 rpm, similar types of fatty acids were seen in *M. alpina*, but the content of each fatty acid was different. However, at agitation speeds higher than 200 rpm, some types of fatty acids were not detected.

Table 1: Fatty acid compositions of *M. alpina* cultivated at different temperatures for 7 days

Temp (°C)	Proportion of fatty acids (%)													
	Saturated fatty acids				Monounsaturated fatty acids		polyunsaturated fatty acids							
	C14:0	C16:0	C18:0	C22:0	C18:1	C20:1	LA (C18:2)	C20:2	GLA (C18:3)	DGLA (C20:3)	C18:4	ARA (C20:4)	EPA (C20:5)	Δ/mol
10	2.09^{b}± 0.66	12.84^{d}± 1.94	5.98^{cd}± 1.78	-	23.46^{c}± 2.25	-	8.62^{a}± 1.68	-	16.81^{a}± 1.90	2.53^{c}± 0.56	4.24^{a}± 1.19	14.62^{c}± 2.03	7.36^{a}± 1.54	2.10
15	2.77a± 0.15	16.84bc± 1.23	4.76d± 0.46	-	28.98b± 1.65	-	8.85a± 1.56	0.77a± 0.46	9.59c± 2.08	3.87b± 1.46	1.99b± 0.31	18.87b± 1.93	3.69b± 0.70	1.89
20	1.52bc± 0.11	14.98c± 0.95	7.98b± 1.00	1.09b± 0.10	21.37c± 1.40	0.55a± 0.17	6.06b± 0.53	1.15a± 0.16	5.29d± 0.49	7.04a± 0.46	0.96b± 0.32	26.53a± 2.58	5.97a± 0.72	2.13
25	1.26c± 0.07	18.10b± 0.66	11.33a± 0.65	1.86a± 0.15	21.47c± 1.30	0.65a± 0.14	8.34a± 0.34	1.18a± 0.23	6.83d± 0.43	6.12a± 0.20	-	26.83a± 1.16	-	1.87
30	1.58bc± 0.48	17.51b± 1.09	7.18bc± 0.55	0.82b± 0.56	35.43a± 1.31	0.36a± 0.25	5.83b± 0.51	0.81a± 0.08	10.71c± 0.67	3.17bc± 0.40	-	16.60bc± 0.74	-	1.58
35	-	23.18a± 1.69	8.45b± 1.88	-	26.91b± 1.43	-	8.19a± 1.26	-	13.84b± 0.40	3.31bc± 0.737	-	16.56bc± 1.13	-	1.51

Data are expressed as mean ± standard error of four replicates. Values sharing same letters differ non-significantly (p >0.05). The degree of unsaturation (Δ/mol) was calculated by the method of Ho and Chen (2008), according to the formula: Δ/mol = [1.0 (% monoene) + 2.0 (% diene) + 3.0 (% triene) + 4.0 (% tetraene) 5.0 (% pentaene)]/100. The "-" in the table indicates that it is not detected

Table 2: Fatty acid composition of *M. alpina* grown at different agitation speeds for 7 days

Agigation speeds (rpm)	Proportion of fatty acids (%)											
	Saturated fatty acids				Monounsaturated fatty acids		polyunsaturated fatty acids					
	C14:0	C16:0	C18:0	C22:0	C18:1	C20:1	LA(C18:2)	C20:2	GLA(C18:3)	DGLA(C20:3)	ARA(C20:4)	Δ/mol
100	1.41^{a}±0.07	15.63^{c}±0.55	6.64^{d}±0.77	1.05^{d}±0.31	41.89^{a}±0.47	0.35^{b}±0.09	4.15^{c}±0.45	0.90^{b}±0.21	6.43^{b}±0.60	2.82^{d}±0.18	17.25^{e}±0.94	1.49
125	1.18^{ab}±0.03	16.55^{c}±0.43	8.99^{c}±0.19	1.43^{c}±0.06	27.93^{b}±1.40	0.57^{ab}±0.11	8.61^{a}±0.16	1.21^{ab}±0.10	6.25^{b}±0.19	3.66^{c}±0.32	24.51^{cd}±0.69	1.76
150	1.43^{a}±0.36	16.53^{c}±0.25	7.88^{cd}±0.97	1.30^{cd}±0.24	18.67^{d}±1.22	0.82^{a}±0.53	8.16^{a}±0.65	1.30^{a}±0.07	6.15^{b}±1.39	5.29^{b}±0.39	33.27^{b}±2.50	1.95
175	1.11^{b}±0.16	20.45^{a}±0.43	13.06^{a}±0.73	2.34^{a}±0.12	11.56^{f}±1.85	0.90^{a}±0.10	7.04^{b}±0.27	1.01^{ab}±0.33	4.69^{c}±0.18	5.91^{ab}±0.57	39.30^{a}±2.92	2.18
200	1.26^{ab}±0.07	18.10^{b}±0.67	11.33^{b}±0.65	1.86^{b}±0.15	21.47^{c}±1.31	0.65^{ab}±0.14	8.34^{a}±0.34	1.18^{ab}±0.23	6.83^{b}±0.43	6.12^{a}±0.20	26.83^{c}±1.16	1.87
225	-	11.34^{d}±0.89	3.85^{e}±0.48	-	14.65^{e}±2.91	-	8.19^{a}±1.27	-	9.57^{a}±0.73	4.10^{c}±0.75	22.76^{d}±2.43	1.63
250	-	6.88^{e}±1.79	3.23^{e}±1.63	-	6.88^{g}±0.74	-	2.67^{d}±0.24	-	3.35^{d}±0.50	1.20^{e}±0.12	6.73^{f}±1.80	0.53

Data are expressed as mean ± standard error of four replicates. Values sharing same letters differ non-significantly (p >0.05). The degree of unsaturation (Δ/mol) was calculated by the method of Ho and Chen (2008), according to the formula: Δ/mol = [1.0 (% monoene) + 2.0 (% diene) + 3.0 (% triene) + 4.0 (% tetraene) + 5.0 (% pentaene)]/100. The "-" in the table indicates that it is not detected

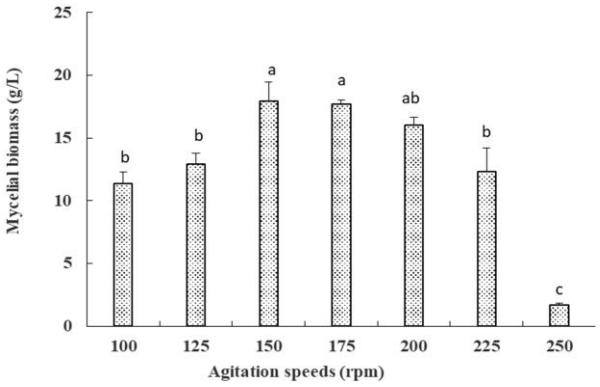

Fig. 3: Mycelial biomass of *M. alpina* cultivated at different agitation speeds. Data are expressed as mean ± standard error of four replicates. Values with different letters on mycelial biomass differ significantly ($p < 0.05$)

The degree of unsaturation gradually increased with the agitation speeds, and then decreased rapidly. Similarly, maximum percentage of ARA was obtained at 175 rpm (39.30%), which was 2.2 times of that obtained at 100 rpm. The level of DGLA followed the same pattern, reaching maximum values at 175 and 200 rpm (5.91 and 6.12%, respectively). When agitation speed was 175 rpm, palmitic acid (C16:0) and stearic acid (C18:0) also reached maximum values, while oleic acid (C18:1) and methyl alpha-linolenate (C18:3) were relatively lower. Interestingly, the contents of C18:1 and C18:3 decreased as agitation speed increased when agitation speed ≤ 175 rpm, as opposed to DGLA and ARA. Although *M. alpina* could grow normally at 225 rpm, there were decreases in the types of fatty acids produced. The only fatty acids detected were those synthesized by the n-6 pathway.

Mycelial Morphology at Different Agitation Speeds

The mycelial morphology of *M. alpina* was greatly influenced by agitation speed, and the mycelial pellets were different at different agitation speeds (Fig. 5). Slippery large pellets (3 - 5 mm) were obtained at 100 rpm, while large pellets with mycelial bulges at the edges were obtained at 125 rpm; slippery small pellets (2 - 3 mm) were obtained at 150 rpm; pompon-like morphology was obtained at 175 rpm; free dispersed mycelia were obtained at 200 rpm, while broken hyphal fragments were obtained at 225 rpm. At agitation speed of 250 rpm, *M. alpina* stopped growing.

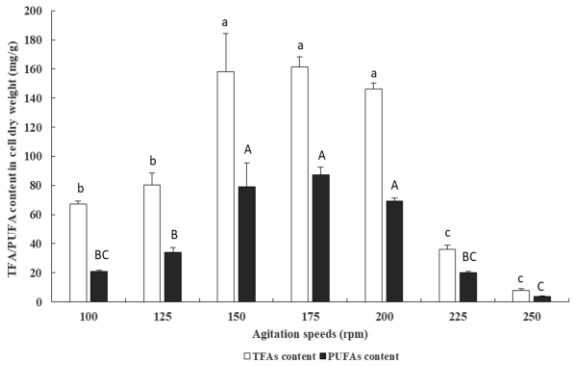

Fig. 4: Total fatty acid (TFA) and PUFA contents (mg/g of cell dry weight) after 7 days of cultivation at different agitation speeds. Data are expressed as mean ± standard error of four replicates. Values with different letters differ significantly ($p < 0.05$)

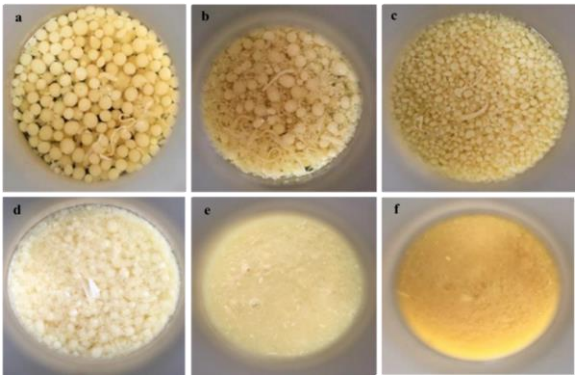

Fig. 5: Morphology of *M. alpina* after cultivation for 7 days at different agitation speeds. (a)100 rpm, (b) 125 rpm, (c)150 rpm, (d)175 rpm, (e) 200 rpm, (f) 225 rpm

Relationship between Mycelial Biomass, TFA Content and PUFA Content

Mycelial growth was a positive correlation between TFA content, and PUFA content. At different temperatures, the correlation coefficient between mycelial biomass on the one hand, and between TFA and PUFA, on the other were 0.835 and 0.802, respectively (n = 25, $p < 0.001$). The correlation coefficient between TFA content and PUFA content was 0.984 (n = 25, $p < 0.001$). At different agitation speeds, the correlation coefficient between mycelial biomass and TFA contents was 0.647 (n = 25, $p < 0.01$), and the correlation coefficient between mycelial biomass and PUFA content was 0.662 (n = 25, $p < 0.01$), while that between TFA content and PUFA content was 0.976 (n = 25, $p < 0.001$).

Discussion

The results obtained in this study revealed that maximum biomass production occurred at 20 - 25°C. Cell growth was inhibited when temperature was below 20°C, possibly because relatively lower temperatures did not favor glucose consumption, although cell growth was slow during the whole fermentation, and the lag time of cell growth was longer than the value of higher temperature (Peng *et al.*, 2010). The cessation of fungal growth at temperatures above 30°C may be as a result of inactivation of vital enzymes involved in *M. alpina* metabolism. At temperatures below 20°C, C18:4 and EPA were synthesized by *M. alpina*, indicating that the enzymes involved in C18:4 and EPA synthesis were low-temperature dependent. The degree of unsaturation was relatively higher at low temperatures, most likely due to the fact that desaturation is an aerobic process. It has been reported that the concentration of dissolved oxygen is increased in low-temperature culture (Nisha and Venkateswaran, 2011). Another reason may be that PUFAs play an important role in regulating membrane fluidity in this fungus, thereby increasing the degree of unsaturation as a compensation for the decreased functionality of the bio-membranes under cold stress conditions (Gao *et al.*, 2016). The highest ARA percentage was obtained at 20 - 25°C due to the shortening of lag time at higher temperatures, leading to early entry into the stationary phase which mainly favors ARA formation (Jin *et al*., 2007).

Most of the fungi employed in ARA production are aerobic, and fungi are also physically weak. Thus, the agitation speed has to be controlled within a certain range. The results obtained in this study showed that *M. alpina* grew well between 150 – 200 rpm. Smooth pellets with large sizes obtained at 100 rpm had dense outer layers, which could limit the transfer of oxygen and nutrients and lead to mycelial autolysis in the core of the pellets (Gao *et al.*, 2014). With increase in dissolved oxygen concentration, mycelium bulges with large-pellet edges appeared, which could enhance transmission speed and mitigate autolysis. When agitation speed reached 150 rpm, smooth pellet formation resulted, and became smaller with exposure to high oxygen concentration. Higher surface area and smaller pellet size would favor the supply of oxygen and nutrients to mycelia in the inner region (Higashiyama *et al.*, 1999). Mycelial aggregate and free, dispersed mycelia are desired morphological forms of mycelial growth. The fragmentation of mycelia at 225 rpm might result from high shear stress, causing mycelial damage and inhibiting cell growth.

As an end-production of fatty acid desaturation, ARA accumulation was significantly different at 150 - 200 rpm, possibly because the oxygen requirement for desaturation was higher than that for cell growth and TFA accumulation (Higashiyama *et al.*, 1999). Compared with small pellets, there were more mycelia exposed to oxygen-rich liquid medium with formation of mycelial aggregates due to more convenient oxygen supply. In the free dispersed mycelia, the high viscosity of the liquid medium due to the high mycelial density influenced the transfer of oxygen. When agitation speed reached 225 rpm, there was high oxygen concentration in the liquid. Thus, filamentous mycelia could

not maintain their usual metabolic activity and had to β-oxidize TFA to obtain more energy required to adapt to the high oxygen concentration (Higashiyama *et al.*, 1999). This was the reason for the significantly lower TFA contents at 225 rpm, while mycelial biomass and PUFA contents were not significantly different at 100, 125 and 225 rpm.

The rate-limiting step in ARA biosynthesis in *M. alpina* is catalyzed by Δ6 elongase, resulting in the conversion of C18:3 to DGLA (Wynn and Ratledge, 2000). The level of C18:3 showed an opposite trend to that of DGLA or ARA in response to different temperatures and agitation speeds, which indicates that fermentation temperatures and agitation speeds influenced the activity of Δ6 elongase and hence, the levels of ARA in TFAs.

Conclusion

The effect of fermentation temperature and agitation speed on mycelial growth and fatty acid accumulation in *M. alpina* were investigated in the present study. The results indicate that mycelial biomass, TFA and PUFA contents, and the percentages of various fatty acids are influenced by temperature and agitation speed. The most suitable temperature and agitation speed for ARA, DGLA and EPA production were 20°C and 175 rpm, respectively. These findings are useful for understanding the changes in *M. alpina* metabolites under different fermentation temperatures and agitation speeds, and are expected to facilitate further optimization of PUFA production.

Acknowledgements

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