American Journal of Biochemistry and Biotechnology

Original Research Paper

Effect of Adding Cellulose on Lipid Accumulation in Cellobiohydrolase and Delta-6 Desaturase Engineered *Mucor circinelloides* Strains

[1,2]Yao Zhang, [2]Yanxia Wang, [2]Qing Liu and [2]Yuanda Song

[1]Department of Food Science and Nutrition, Food Bioengineering and Technology Laboratory, College of Culture and Tourism, University of Jinan, 13 Shungeng Road, Jinan 250022, China
[2]Colin Ratledge Center for Microbial Lipids, School of Agricultural Engineering and Food Science, Shandong University of Technology, 266 Xincun West Road, Zibo 255000, China

Article history:
Received: 16-06-2023
Revised: 23-08-2023
Accepted: 01-09-2023

Corresponding Author:
Yao Zhang
Department of Food Science and Nutrition, Food Bioengineering and Technology Laboratory, College of Culture and Tourism, University of Jinan, 13 Shungeng Road, Jinan 250022, China
Email: shc_zhangy@ujn.edu.cn

Abstract: Despite microbial production of multifunctional gamma-linolenic acid (GLA) being an important and promising way, the high cost is still a major factor limiting industrial applications. Previously, we proposed a novel approach to promote the production of GLA from cellulose, a widely available and cheapest resource, by overexpression of the cellobiohydrolase (CBH2) and delta-6 desaturase (D6) in *Mucor circinelloides*. In the present study, an in-depth analysis of growth and lipid accumulation in engineered M. *circinelloides* strains using cellulosic substrate was to be explored. When cultivated in modified Kendrick and Ratledge (K and R) medium supplemented with the ratio of 1: 1 for glucose and carboxymethyl cellulose (CMC), the cellobiohydrolase and delta-6 desaturase overexpressing strains led to increases in the biomass (up to 10.4 g/L) and lipid yield (up to 2.6 g/L) of 68% and 2.2-fold, respectively, compared with that of the control strain. The yield of GLA in the cellobiohydrolase and delta-6 desaturase co-expression strains reached 415 mg/L, which was a remarkable increase of 3.1-fold compared to that of the control strain. These results proved that the introduction of CBH2 and D6 two key enzymes could significantly enhance the utilization of CMC and effectively convert cellulose into GLA by engineered *M. circinelloides*. This study provided a reference for the further potential application of the engineered *M. circinelloides* strains in the conversion of functional GLA from cellulose.

Keywords: Γ-Linolenic Acid, *Mucor circinelloides*, Cellulose, Cellobiohydrolase, Delta-6 Desaturase

Introduction

Gamma-linolenic acid (GLA, 18:3) is an essential n-6 polyunsaturated fatty acid, that has anti-cancer, anti-tumor, anti-atherosclerosis, and hypoglycemic and lipid-lowering effects (Miyake *et al*., 2020; Ide, 2023; Paredes *et al*., 2023; Zhang *et al*., 2022). Because of its unique physiological impacts, GLA will be increasingly in demand as a source of nutraceuticals and medicines. However, the traditional production cost of GLA is relatively high. Microbial fermentation to produce GLA is an important and promising way. *Mucor circinelloides* was one of the first strains to be used for industrial production of GLA, but it was discontinued due to cost concerns (Andrade *et al*., 2014; Fazili *et al*., 2022; Zhang and Song, 2021; Zhang *et al*., 2023a). Therefore, it is feasible to reduce the cost of GLA biosynthesis by using cheap raw materials, such as cellulose, the widely available and cheapest resources.

In general, the use of cellulosic raw materials includes the process of cellulose degradation and its subsequent fermentation and biotransformation (Singhania *et al*., 2022). Cellulosic raw materials have been used in many fields and some products (such as ethanol) have been successfully scaled up to industrial production levels (Ismail *et al*., 2022; Plioni *et al*., 2022). However, the production of microbial lipids from cellulosic raw materials is still in its infancy. The commonly used microbial production of lipids from cellulose must first degrade cellulose into small molecules of available monosaccharides and then transform into lipids by

microbial fermentation. Most of the cellulose treatment processes use the traditional acid-base method, but there are a series of problems such as toxic by-product inhibition, cumbersome steps, high process cost, and serious pollution (Vadivel *et al*., 2017). At present, most research has focused on screening suitable strains to produce lipids from cellulosic feedstock hydrolysates. In the long run, the direct use of microorganisms that not only have the ability to produce cellulase to degrade cellulose but also can accumulate a high content of lipids, can realize the synchronization of cellulose-monosaccharide-lipids in one step and greatly reduce the production cost.

Since most oleaginous microorganisms cannot directly synthesize functional lipids from cellulose due to their lack of cellulases, the cellobiohydrolase (CBH2) from *T. longibrachiatum* was previously selected to be overexpressed in *M. circinelloides* for the efficient use of cellulose to accumulate lipid (Zhang *et al*., 2022). In order to further enhance the content of GLA, the homologous Delta-6 desaturase (D6) was also co-overexpressed in *M. circinelloides*, which finally led to an increase in the GLA content from cellulose (Zhang *et al*., 2022). Generally, in our preliminary study, multiple engineering strains with overexpression or co-expression of cellobiohydrolase and delta-6 desaturase were constructed through homologous recombination technology (Zhang *et al*., 2022). However, only a few studies have focused on the GLA synthesis from cellulosic substrates in this cellobiohydrolase and delta-6 desaturase-engineered *M. circinelloides* strains.

In the present study, an in-depth survey of GLA accumulation by cellobiohydrolase and delta-6 desaturase overexpressing *M. circinelloides* strains using cellulosic substrate was to be pursued. The differences in cell biomass, lipid content, lipid composition, and GLA yield in engineered *M. circinelloides* strains were compared when grown on the base medium with cellulose. In addition, the effects of adding different proportions of cellulose on the growth, lipid accumulation, and GLA synthesis of engineered *M. circinelloides* strains were also analyzed. This study provided a reference for the further potential use of the engineered *M. circinelloides* strains in the conversion of functional GLA from cellulose.

Materials and Methods

Strains and Media

The recombinant strains Mc-2075 (control strain with empty plasmid), Mc-C2 (cellobiohydrolase overexpression strain), Mc-D6 (delta-6 desaturase overexpression strain), Mc-C2TD6 and Mc-C2PD6 (cellobiohydrolase and delta-6 desaturase coexpression strains with different linkers) were previously constructed and stocked in our laboratory (Zhang *et al*., 2022). The strains used in this study are summarized in Table 1.

Table 1: The strains used in this study

Strains	Relevant description	Reference
Mc-2075	Control strain with a empty plasmid	Zhang *et al*. (2022)
Mc-C2	CBH2 overexpressing strain	
Mc-D6	D6 overexpressing strain	
Mc-C2TD6	CBH2 and D6 coexpressing strain linked by T2A	
Mc-C2PD6	CBH2 and D6 coexpressing strain linked by T1 and Pgpd1	

Keeping other components unchanged, the modified Kendrick and Ratledge (K and R) mediums (Kendrick and Ratledge, 1992) with glucose and different proportions of carboxymethyl cellulose (CMC) as carbon sources were prepared. For seed growth, the modified K and R medium consisted of (g/L): glucose with different proportions of CMC 30, ammonium tartrate 2.0, KH_2PO_4 7.0, and $MgSO4{\cdot}7H_2O$ 1.5, Na_2HPO_4 2.0, yeast extract 1.5, $CaCl_2{\cdot}2H_2O$ 0.1, $CuSO_4{\cdot}5H_2O$ 0.0001, $ZnSO_4{\cdot}7H_2O$ 0.001, $MnSO_4{\cdot}5H_2O$ 0.0001, Co $(NO_3)_2{\cdot}6H_2O$ 0.0001, $FeCl_3{\cdot}6H_2O$ 0.008 and agar 20 for solid medium. The nitrogen-limited medium for lipid accumulation is consisting of the following (g/L): glucose with different proportions of CMC 50, ammonium tartrate 2.0, KH_2PO_4 7.0, $MgSO_4{\cdot}7H_2O$ 1.5, Na_2HPO_4 2.0, yeast extract 1.5, $CaCl_2{\cdot}2H_2O$ 0.1, $CuSO_4{\cdot}5H_2O$ 0.0001, $ZnSO_4{\cdot}7H_2O$ 0.001, $MnSO_4{\cdot}5H_2O$ 0.0001, $CO(NO_3)_2{\cdot}6H_2O$ 0.0001 and $FeCl_3{\cdot}6H_2O$ 0.008 (Kendrick and Ratledge, 1992).

The reagents used in the fermentation culture were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All of them were analytical-grade standard products unless otherwise stated.

Culture Conditions

Solid Culture

To investigate the effects of cellulose addition on the growth of engineered *M. circinelloides* strains, solid culture was adopted. Fresh spore suspensions with a concentration of 20~30/μL (10 μL) were inoculated and coated on seed solid medium with different proportions of CMC and placed in a constant temperature incubator under 30℃ for culture 24-48 h to observe the growth of strains.

Liquid Culture

The liquid culture was adopted to verify the growth and lipid production of engineered *M. circinelloides* strains at the optimal ratio of glucose: CMC. The spore's suspension (approx. 10^7 spores/mL) of the constructed strains was cultured in K&R medium with shaking at 150 rpm at 28°C for 24 h. The above cultures were then inoculated at 10% (v/v) into a 2 L fermenter (BioFlo 110, New Brunswick Scientific Co., Ltd) of the modified K and R medium supplemented with CMC. The fermenter was controlled at 28°C, stirred at 600 rpm, and aerated

at 0.5 v/v min^{-1}. The pH was kept at 6.0 by automatically adding 2 mol/L HCl or 4 mol/L NaOH. The following indicators were detected by regular sampling during the culture process.

Strain Growth and Biomass Determination

Three different directions were selected to read the growth diameter of the colony and calculate the colony area of the petri dish under different culture times. Cell dry weight (CDW) was obtained by the differential weight method. The mycelium was harvested at different times, filtered, and washed three times with distilled water, and then freeze-dried for 48 h. The biomass of each sample was gravimetrically determined.

Determination of Glucose and Ammonium Concentrations

Glucose content was measured by the glucose oxidase-peroxidase (GOD) method with a glucose measurement kit (Rongsheng Co. Ltd, China). The depletion of ammonium was determined by indophenol blue spectrophotometric method (Zhang *et al*., 2022).

Determination of Lipid Content and Fatty Acid Composition

The total lipids were extracted referring to the Folch method with minor modifications as follows: Approximately 20 mg of dried mycelium was taken with chloroform/methanol (2:1, v/v) and methylated with 10% HCl/methanol (v/v) at 60°C for 3 h. Pentadecanoic acid (C15:0, Sigma) was added to the freeze-dried cells as an internal standard. Fatty acid methyl esters were separated by n-hexane and analyzed by gas Chromatography (GC) equipped with a 30 m ×0.32 mm DB-waxetr column with a film thickness of 0.25 μm. The program was set as follows: 120°C for 3 min, rising to 200°C at a rate of 5°C per min, and then rising to 220°C at a rate of 4°C per min and holding for 2 min (Wang *et al*., 2022; Zhang *et al*., 2017; 2022).

Statistical Analysis

SPSS Statistics 22 software was used for statistical analysis. All data were expressed as means ± S.D. The mean value and the standard error of the mean value were calculated from three independent experiments. Student's t-test was used for differences between test means and $p<0.05$ was considered statistically significant.

Results and Discussion

Effect of Cellulose Addition on the Growth of Engineered M. circinelloides Strains

Carbon sources are very important for strain growth and metabolism, so the utilization of cheap and widespread carbon sources such as cellulose is a prerequisite for reducing GLA production cost (Lynd *et al*., 2002; Zhang and Song, 2021; Zhang *et al*., 2022; 2023b). Previously, the cultivation of engineered *M. circinelloides* strains on the modified K and R medium supplemented with microcrystalline cellulose was preliminarily verified (Zhang *et al*., 2022). In this study, another cellulosic substrate, carboxymethyl cellulose (CMC) was selected as an additive carbon because it is a kind of water-soluble cellulose ether obtained by chemical modification of natural cellulose (Kanikireddy *et al*., 2020). Remaining the other nutrients in the medium unchanged, a complex of glucose and CMC with different ratios were respectively used as carbon sources to investigate the effects of cellulose addition on the growth of engineered *M. circinelloides* strains and the results were shown in Fig. 1. As can be seen from Fig. 1a, except for CMC as the sole carbon source, there was no significant difference in the growth of each engineered strain when glucose and CMC were mixed with different proportions at the beginning of 24 h culture. However, when cultured to 48 h, the growth of the strains with different proportions of glucose and CMC as carbon sources showed significant differences (Fig. 1b). With the increasing proportion of CMC, the colony diameter of the strains gradually decreased. Especially when CMC was completely used as the carbon source, the growth of the strains was the worst. This is consistent with our previous result that the engineered strains did not grow well in the medium containing only microcrystalline cellulose as the carbon source (Zhang *et al*., 2022). This indicated that a small number of strains could not secrete enough cellulases to degrade cellulose and provide available sugars for strain growth. When using a combined carbon source of glucose and CMC with different ratios, the growth of strains is much better than only using CMC as the carbon source, but lower than the growth of that using glucose alone. Based on the growth results of the different mixing ratios of glucose and CMC (Fig. 1), the ratio of 1:1 for glucose and CMC was adopted for the growth and lipid accumulation of engineered *M. circinelloides* strains in the following study.

Growth and Lipid Accumulation of Engineered M. circinelloides Strains from Cellulose

Based on the above results, the cell growth and lipid production in engineered *M. circinelloides* strains were investigated when cultivated with the ratio of 1:1 for glucose and CMC in the modified K and R medium (Fig. 2). Since nitrogen limitation is a prerequisite for lipid accumulation (Ratledge and Wynn, 2002; Zhang *et al*., 2023a) and a low nitrogen source concentration was used in the modified medium. As shown in Fig. 2a, ammonium was completely exhausted at about 12 h in all of the engineered *M. circinelloides* strains. Similarly, the glucose contents in the engineered *M. circinelloides*

strains decreased gradually with the extension of fermentation time. However, the glucose consumption in the cellobiohydrolase overexpression strains (Mc-C2, Mc-C2TD6, and Mc-C2PD6) slowed down and it was not completely consumed until 60 h, while that of other cellobiohydrolase deficient strains (Mc-D6 and Mc-2075) was depleted after only 36 h. This was consistent with the results of our previous study (Zhang *et al*., 2022) and the reason was probably because the lead of cellobiohydrolase contributed to the efficient degradation of CMC thus providing more carbon sources for the strains. It could also be inferred that the foreign cellobiohydrolase and the internal cellulases in *M. circinelloides* can act synergistically on CMC.

It can be seen from Fig. 2b that the biomass in the cellobiohydrolase overexpression strains increased rapidly during the first 72 h and then the growth entered into a stable period. The cellobiohydrolase overexpression strains have similar amounts of biomass (up to 10.4 g/L at 72 h), which were improved by 68% (for Mc-C2), 59% (for Mc-C2TD6) and 64% (for Mc-C2PD6), respectively, compared to that of the control strain (Mc-2075), (Fig. 2b). Due to the inability to obtain sufficient carbon sources, other strains lacking cellobiohydrolase grew slowly and stopped growing after 48 h, which resulted in a low biomass.

Figure 2c, the lipid accumulation patterns in engineered *M. circinelloides* strains were similar: The lipid content increased rapidly in the first 48 h and then it slowed down and leveled off. The maximum lipid contents of the engineered strains (except for Mc-D6) were improved by 17-34% when compared with that of the control strain (Fig. 2c). It should be noted that the lipid yields of the engineered strains (excluding Mc-D6) were also dramatically improved, which were enhanced 2.0-fold for Mc-C2, 2.1-fold for Mc-C2TD6 and 2.2-fold for Mc-C2PD6, respectively (Fig. 2d). Thus, it could be concluded that the strains containing cellobiohydrolase could effectively decompose CMC to provide more carbon sources for the growth and lipid production.

GLA Accumulation of Engineered M. circinelloides Strains from Cellulose

Generally, GLA biosynthesis requires a series of fatty acid desaturases to introduce double bonds at the 9, 12, and 6 carbon atoms of the stearic acid chain (Nykiforuk *et al*., 2012; Ratledge, 2005; Wang *et al*., 2022; Zhang *et al*., 2017). The final step in GLA formation is catalyzed by delta-6 fatty acid desaturase, which is the rate-limiting enzyme and a crucial regulatory point of the GLA biosynthetic pathway (Wang *et al*., 2023; Zhang *et al*., 2017; 2022). The fatty acid profiles of engineered *M. circinelloides* strains using CMC are shown in Table 2. Except that Mc-C2 had similar GLA content with that of the control strain, the intracellular GLA contents of other recombinant stains with overexpression of delta-6 desaturase were improved by 42-47% compared to that of the control strain, which indicated that delta-6 desaturase indeed played an important role in the synthesis of GLA. Many previous studies and ours have confirmed that the introduction of delta-6 desaturase has a crucial effect on improving GLA content.

Combined with the biomass and total lipid data of the engineered strains, the final GLA yields showed that the cellobiohydrolase and delta-6 desaturase coexpression strains had an advantage in producing GLA from CMC. When compared with the control strain Mc-2075, the GLA yields of Mc-C2TD6 and Mc-C2PD6 were significantly increased by 3.0- and 3.1- -fold, respectively, which were much higher than that of the single gene overexpression strains for their inability to effectively utilize CMC (for Mc-D6) or enhance the synthesis of GLA (for Mc-C2). This is consistent with our previous findings that the engineered *M. circinelloides* strains possess the ability to decompose cellulose for GLA production (Zhang *et al*., 2022).

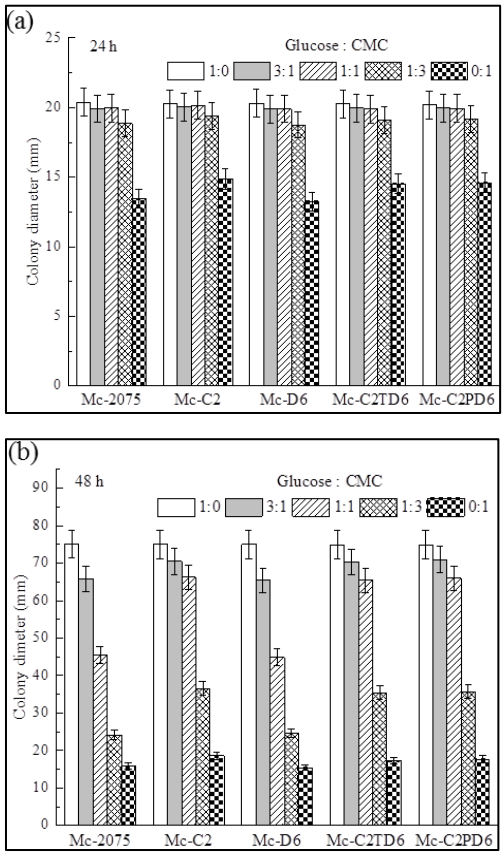

Fig. 1: Effect of adding different ratios of CMC on the colony diameters of engineered *M. circinelloides* strains. The ratios of glucose: CMC (w/w) were 1:0, 3:1, 1:1, 1:3 and 0:1; (a) The colony diameter of 24 h; (b) The colony diameter of 48 h values

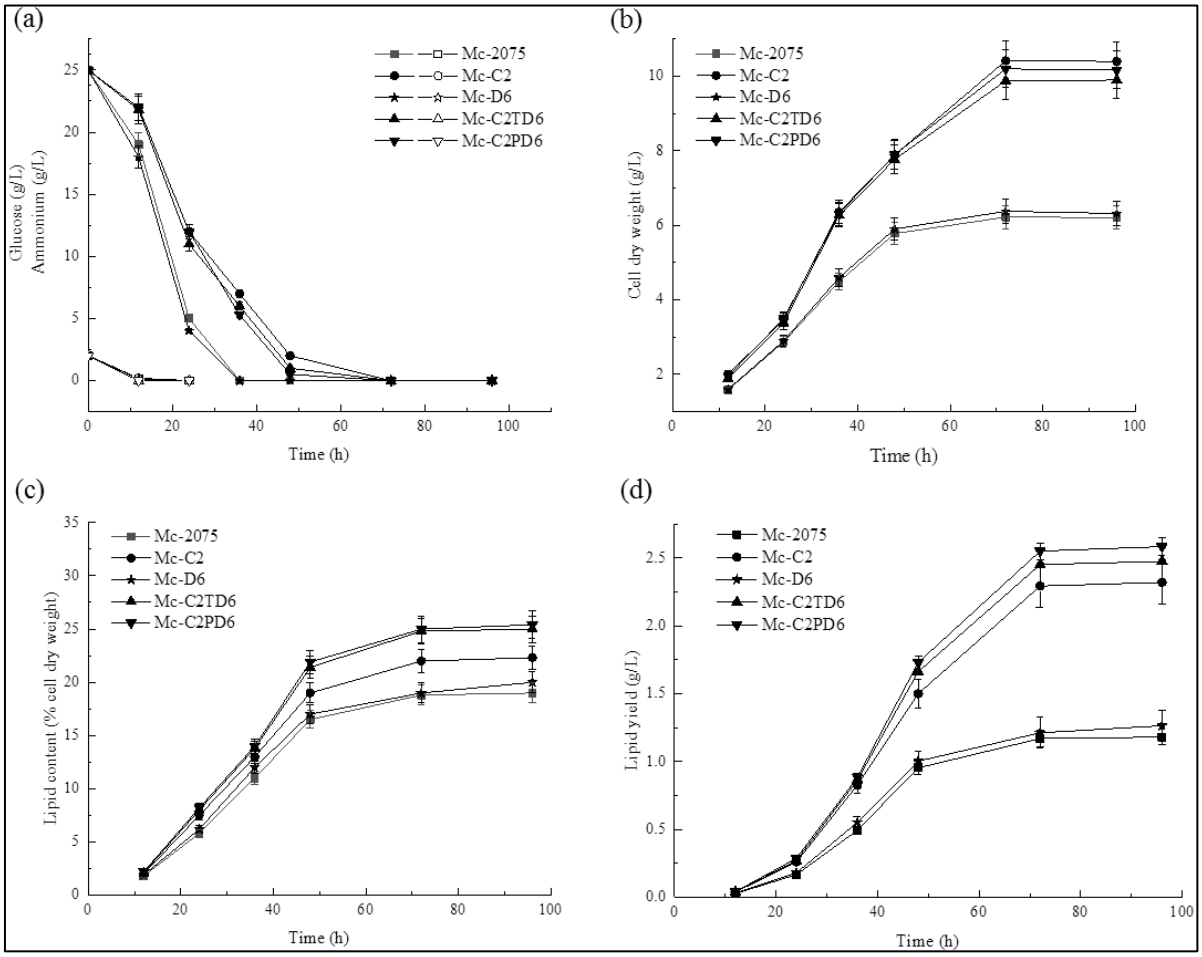

Fig. 2: Cell growth and lipid accumulation of engineered *M. circinelloides* strains. Strains were cultured in a 1.5 L fermenter with modified K and R medium containing the ratio of glucose: CMC as 1:1 during 96 h at pH 6.0 and 28°C, with aeration at 2.0 v/v min 1 and stirring at 600 rpm. The samples were taken from the fermenter at the specified times; (a) Glucose (solid marks) and ammonium (empty marks) concentration; (b) Cell dry weight; (c) Lipid content of cell dry weight; (d) Lipid yield. The value was the mean of three independent experiments. The error bar represented the standard error of the mean

Table 2: Fatty acid composition in engineered *M. circinelloides* strains cultured with CMC

		Fatty acid composition (relative %, w/w)[a]							
Strains	Time (h)	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3 (GLA)	% mg/L
Mc-2075	12	2.04±0.10	22.12±1.11	2.71±0.13	6.54±0.31	29.97±1.48	15.72±0.78	20.90±1.04	5.94±0.30
	24	1.32±0.04	20.14±0.98	1.85±0.10	7.12±0.40	32.33±1.26	15.59±0.69	16.62±0.83	27.67±1.25
	48	1.45±0.06	22.65±1.21	1.82±0.07	7.63±0.25	36.88±1.43	14.79±0.45	14.78±0.56	140.96±4.23
	72	1.65±0.08	22.62±1.07	2.29±0.14	6.38±0.33	38.86±1.55	14.03±0.74	12.74±0.63	148.98±5.47
	96	1.90±0.12	23.63±1.47	2.72±0.12	5.44±0.17	40.89±1.83	14.22±0.61	11.19±0.56	132.03±4.88
Mc-C2	12	1.68±0.08	19.15±0.96	1.53±0.08	4.57±0.23	30.55±1.53	15.52±0.78	21.52±1.08	8.61±0.43
	24	1.30±0.04	19.36±0.84	1.40±0.05	6.69±0.32	31.15±1.36	14.59±0.64	18.24±0.91	47.47±1.74
	48	1.35±0.05	20.39±1.01	1.62±0.06	7.02±0.35	34.15±1.19	14.87±0.76	16.98±0.85	254.55±6.27
	72	1.28±0.02	20.55±0.97	1.34±0.04	7.28±0.34	34.49±1.27	15.89±0.81	14.60±0.73	334.69±7.48
	96	1.38±0.04	20.25±1.02	1.53±0.07	7.92±0.44	35.40±1.64	15.52±0.79	12.48±0.62	289.44±4.91
Mc-D6	12	1.81±0.09	19.60±0.98	2.23±0.11	5.16±0.26	29.24±1.46	17.53±0.88	21.00±1.05	6.38±0.14
	24	1.32±0.07	19.46±0.97	1.56±0.07	6.56±0.33	31.87±1.58	17.24±0.81	16.37±0.83	29.43±1.20
	48	1.28±0.06	20.87±0.99	1.38±0.06	7.73±0.39	33.51±1.67	15.71±0.79	14.84±0.74	148.85±5.88
	72	1.26±0.05	21.31±1.03	1.21±0.06	8.17±0.41	34.35±1.72	15.53±0.76	15.50±0.78	187.89±5.52
	96	1.37±0.06	21.08±1.05	1.52±0.08	7.30±0.37	35.24±1.76	14.88±0.70	16.39±0.82	207.17±7.33
Mc-C2TD6	12	1.79±0.09	20.70±1.04	3.49±0.17	1.74±0.09	21.66±1.08	19.15±0.96	25.48±1.27	10.11±0.34
	24	1.14±0.06	21.17±1.05	1.28±0.06	7.02±0.35	30.77±1.54	16.92±0.85	16.10±0.80	43.53±1.73
	48	1.18±0.06	21.87±1.06	1.14±0.05	8.34±0.41	33.54±1.68	15.61±0.78	14.61±0.73	242.93±6.24

Table 2: Continue

	72	1.10±0.05	21.53±1.01	0.86±0.04	8.77±0.44	32.63±1.63	14.97±0.75	14.84±0.74	363.62±7.97
	96	1.08±0.05	21.45±1.07	0.82±0.04	8.54±0.42	34.03±1.70	15.27±0.76	15.83±0.79	391.79±8.20
Mc-C2PD6	12	1.94±0.10	17.66±0.88	3.60±0.18	4.08±0.20	25.99±1.30	16.69±0.83	19.08±0.95	8.19±0.41
	24	1.32±0.06	20.50±1.03	2.27±0.11	6.88±0.34	29.89±1.49	18.55±0.93	14.56±0.73	41.79±2.02
	48	1.29±0.06	20.90±1.05	1.65±0.08	7.68±0.38	33.76±1.69	16.56±0.83	13.99±0.70	242.04±7.63
	72	1.19±0.06	20.70±1.02	1.39±0.07	8.04±0.40	33.78±1.69	15.78±0.79	14.73±0.74	375.62±6.46
	96	1.11±0.05	20.13±1.04	1.21±0.06	7.87±0.39	32.87±1.64	15.26±0.77	16.07±0.80	415.12±8.08

strains were cultured in a 1.5 L fermenter with modified K&R medium containing CMC for 96 h. The fatty acid composition was displayed at different points in time. The values are means ± standard deviations of three independent experiments

Conclusion

GLA is an important polyunsaturated fatty acid with nutritional and therapeutic applications. The oleaginous fungus *M. circinelloides* was one of the first strains to be used for industrial production of GLA, but it was discontinued due to cost concerns. Making full use of abundant cellulose resources for GLA biosynthesis is an effective way to reduce the cost. In the present study, the effects of adding different proportions of carboxymethyl cellulose on the growth, lipid accumulation, and GLA synthesis of engineered *M. circinelloides* strains were investigated in detail. When cultivated in a modified K&R medium supplemented with the ratio of 1:1 for glucose and cellulose (CMC), the maximum biomass, lipid content, and lipid yield of engineered *M. circinelloides* strains achieved 10.4 g/L, 25.4%, and 2.6 g/L, respectively, which were 68, 34% and 2.2-fold higher than that of the control strain, respectively. It was worth noting that the maximum GLA yield of the cellobiohydrolase and delta-6 desaturase coexpression strains reached 415 mg/L, which was 3.1-fold higher than that of the control strain. This study proved the ability of engineered *M. circinelloides* strains to produce GLA from CMC. It has also manifested the potential use of the constructed strains as biocatalysts in cellulosic biorefinery. This study is expected to achieve a one-step efficient conversion of cellulosic raw materials to functional lipids and play a positive role in promoting the sustainable development of the biological industry.

Acknowledgment

We would like to thank the persons who helped with this research.

Funding Information

This study was supported by the National Natural Science Foundation of China (32101927), the Shandong Provincial Natural Science Foundation (ZR2020MC007), and the school-city integration project in Zhangdian district (2021JSCG0016).

Author's Contributions

Yao Zhang: Involved in the study conception, experimental design, figures and tables' arrangement, data analysis, result interpretation, manuscript written, and reviewed of the final manuscript.

Yanxia Wang: Carried out the experiments and collected data.

Qing Liu: Participated in the experimental research.

Yuanda Song: Conceived the study and reviewed the original manuscript.

Ethics

All authors read and approved the final version of this manuscript. There are not any ethical issues to declare that could arise after the publication of this manuscript.

References

Andrade, G. S. S., A. Carvalho, K. F., Romero, C. M., Oliveira, P. C., & de Castro, H. F. (2014). *Mucor circinelloides* whole-cells as a biocatalyst for the production of ethyl esters based on babassu oil. *Bioprocess and Biosystems Engineering*, *37*, 2539-2548. https://doi.org/10.1007/s00449-014-1231-4

Fazili, A. B. A., Shah, A. M., Zan, X., Naz, T., Nosheen, S., Nazir, Y., & Song, Y. (2022). *Mucor circinelloides*: A model organism for oleaginous fungi and its potential applications in bioactive lipid production. *Microbial Cell Factories*, *21*(1), 1-19. https://doi.org/10.1186/s12934-022-01758-9

Ide, T. (2023). γ-Linolenic Acid-Rich Oil-and Fish Oil-Induced Alterations of Hepatic Lipogenesis, Fatty Acid Oxidation and Adipose Tissue mRNA Expression in Obese KK-A y Mice. *Journal of Oleo Science*, *72*(3), 313-327. https://doi.org/10.5650/jos.ess22341

Ismail, K. S. K., Matano, Y., Sakihama, Y., Inokuma, K., Nambu, Y., Hasunuma, T., & Kondo, A. (2022). Pretreatment of extruded Napier grass by hydrothermal process with dilute sulfuric acid and fermentation using a cellulose-hydrolyzing and xylose-assimilating yeast for ethanol production. *Bioresource Technology*, *343*, 126071. https://doi.org/10.1016/j.biortech.2021.126071

Kanikireddy, V., Varaprasad, K., Jayaramudu, T., Karthikeyan, C., & Sadiku, R. (2020). Carboxymethyl cellulose-based materials for infection control and wound healing: A review. *International Journal of Biological Macromolecules*, *164*, 963-975. https://doi.org/10.1016/j.ijbiomac.2020.07.160

Kendrick, A., & Ratledge, C. (1992). Desaturation of polyunsaturated fatty acids in *Mucor circinelloides* and the involvement of a novel membrane-bound malic enzyme. *European Journal of Biochemistry*, *209*(2), 667-673. https://doi.org/10.1111/j.1432-1033.1992.tb17334.x

Lynd, L. R., Weimer, P. J., Van Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews*, *66*(3), 506-577. https://doi.org/10.1128/MMBR.66.3.506-577.2002

Miyake, J. A., Gomes, R. N., & Colquhoun, A. (2020). Gamma-Linolenic acid alters migration, proliferation and apoptosis in human and rat glioblastoma cells. *Prostaglandins & Other Lipid Mediators*, *150*, 106452. https://doi.org/10.1016/j.prostaglandins.2020.106452

Nykiforuk, C. L., Shewmaker, C., Harry, I., Yurchenko, O. P., Zhang, M., Reed, C., ... & Moloney, M. M. (2012). High level accumulation of gamma linolenic acid (C18: 3Δ6. 9, 12 cis) in transgenic safflower (*Carthamus tinctorius*) seeds. *Transgenic Research*, *21*, 367-381. https://doi.org/10.1007/s11248-011-9543-5

Paredes, A., Justo-Méndez, R., Jiménez-Blasco, D., Núñez, V., Calero, I., Villalba-Orero, M., & Ricote, M. (2023). Γ-Linolenic acid in maternal milk drives cardiac metabolic maturation. *Nature*, 1-9. https://doi.org/10.1038/s41586-023-06068-7

Plioni, I., Kalogeropoulou, A., Dimitrellou, D., Kandylis, P., Kanellaki, M., Nigam, P. S., & Koutinas, A. A. (2022). Effect of cellulose crystallinity modification by starch gel treatment for improvement in ethanol fermentation rate by non-GM yeast cell factories. *Bioprocess and Biosystems Engineering*, *45*(4), 783-790. https://doi.org/10.1007/s00449-022-02706-y

Ratledge, C. (2005). Microbial production of gamma-linolenic acid. *Handbook of Functional Lipids. Edited by C. Akoh, CRC Press, Boca Raton, FL, USA*, 19. https://doi.org/10.1201/9781420039184.ch2

Ratledge, C., & Wynn, J. P. (2002). The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Advances in Applied Microbiology*, *51*, 1-52. https://doi.org/10.1016/S0065-2164(02)51000-5

Singhania, R. R., Patel, A. K., Tseng, Y. S., Kumar, V., Chen, C. W., Haldar, D., ... & Dong, C. D. (2022). Developments in bioprocess for bacterial cellulose production. *Bioresource Technology*, *344*, 126343. https://doi.org/10.1016/j.biortech.2021.126343

Vadivel, V., Moncalvo, A., Dordoni, R., & Spigno, G. (2017). Effects of an acid/alkaline treatment on the release of antioxidants and cellulose from different agro-food wastes. *Waste Management*, *64*, 305-314. https://doi.org/10.1016/j.wasman.2017.03.010

Wang, C., Hucik, B., Sarr, O., Brown, L. H., Wells, K. R., Brunt, K. R., ... & Mutch, D. M. (2023). Delta-6 desaturase (Fads2) deficiency alters triacylglycerol/fatty acid cycling in murine white adipose tissue. *Journal of Lipid Research*, *64*(6). https://doi.org/10.1016/j.jlr.2023.100376

Wang, X., Yang, J., Mohamed, H., Shah, A. M., Li, S., Pang, S., ... & Song, Y. (2022). Simultaneous overexpression ofΔ 6-,Δ 12-andΔ 9-desaturases enhanced the production of γ-linolenic acid in *Mucor circinelloides* WJ11. *Frontiers in Microbiology*, *13*, 1078157. https://doi.org/10.3389/fmicb.2022.1078157

Zhang, Y., & Song, Y. (2021). Lipid accumulation by xylose metabolism engineered *Mucor circinelloides* strains on corn straw hydrolysate. *Applied Biochemistry and Biotechnology*, *193*, 856-868. https://doi.org/10.1007/s12010-020-03427-2

Zhang, Y., Luan, X., Zhang, H., Garre, V., Song, Y., & Ratledge, C. (2017). Improved γ-linolenic acid production in *Mucor circinelloides* by homologous overexpressing of delta-12 and delta-6 desaturases. *Microbial Cell Factories*, *16*(1), 1-9. https://doi.org/10.1186/s12934-017-0723-8

Zhang, Y., Wang, Y., Yang, J., Yang, W., Wang, X., Wu, C., & Song, Y. (2022). Improved γ-Linolenic acid production from cellulose in *Mucor circinelloides* via coexpression of cellobiohydrolase and delta-6 desaturase. *Journal of Agricultural and Food Chemistry*, *70*(14), 4373-4381. https://doi.org/10.1021/acs.jafc.2c00359

Zhang, Y., Yang, Y., Zhang, H., Liu, Q., & Song, Y. (2023a). Effect of Different Carbons on Lipid Production and SNF1 Transcription in *Mucor circinelloides*. *Indian Journal of Microbiology*, *63*(1), 146-151. https://doi.org/10.1007/s12088-023-01070-z

Zhang, Y., Yang, Y., Zhang, S., Liu, Q., Dang, W., & Song, Y. (2023b). Lipid accumulation and SNF1 transcriptional analysis of *Mucor circinelloides* on xylose under nitrogen limitation. *Antonie Van Leeuwenhoek*, *116*(4), 383-391. https://doi.org/10.1007/s10482-023-01810-7