

Research Article

ARTP Mutagenesis to Improve Mycelial Polysaccharide Production of *Grifola frondosa* Using a Mixture of Wheat Bran and Rice Bran as Substrate

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Mycelial polysaccharides from *Grifola frondosa* have shown potential for the prevention of chronic diseases. Atmospheric and room temperature plasma (ARTP) technology was used to enhance the ability of *G. frondosa* to efficiently utilize a mixture of rice bran and wheat bran in the production of mycelial polysaccharides. The ARTP-mutant *G. frondosa* GFA2 had an improved growth rate of 6.0 mm/d and polysaccharide yield of 2.65 g/L and showed stable genetic characteristics. Uniform design experiments showed that polysaccharide yield could be increased to 5.90 g/L using the optimized conditions of 10.0 g/L rice bran and 110.0 g/L wheat bran while omitting KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Gas chromatography demonstrated that GFA2 polysaccharides were composed of the monosaccharides rhamnose, arabinose, fucose, xylose, mannose, glucose, and galactose. This study provides an effective strategy for improving polysaccharide production in edible fungi while proposing the added-value utilization of rice and wheat brans.

1. Introduction

Grifola frondosa (*G. frondosa*) is a widely used medicinal and edible fungus in China, Japan, and Korea, having good texture, delicious taste, and excellent aroma [1]. Over the past three decades, many studies have shown that *G. frondosa*'s main functional components are polysaccharides. It has potential applications in the prevention of chronic diseases due to its antioxidative [2], immunoregulation [3, 4], antitumor [5, 6], antiviral [7], and blood glucose and lipid regulating bioactivities [8]. The polysaccharides from different sources and extraction processes might display different structures. It could result in the

polysaccharides varying in the biological activities significantly. *G. frondosa* polysaccharides can be obtained from submerged culture broth or cultivated fruiting bodies. For example, the mycelial polysaccharides extracted by Zhao et al. from *G. frondosa* submerged culture could block EV71 virus replication and has certain antiviral activity [9]. Guo et al. extracted polysaccharides from *G. frondosa* fruiting body that could be used as a potential functional food component for the prevention and treatment of hyperglycemia and hyperlipidemia [10]. Generally, the cultivation of *G. frondosa* fruiting bodies takes several months using composts or lignocellulosic wastes such as straw or wood. Submerged culturing has the advantages of a shorter

cultivation period and consistent quality of the product. Moreover, agricultural by-products such as rice and wheat brans can be used as the substrate for this fungal fermentation.

Rice and wheat are the most important grains in China, with annual yields of 206 million and 100 million tons, respectively. After processing, 16 million tons of rice bran and 20 million tons of wheat bran are produced per year as primary by-products and are mainly used as feed, fermentation additives, or for extracting usable components. However, their overall utilization rate is below 20% [11, 12]. Rice bran contains carbohydrates (18–23%), proteins (16–20%), and other valuable components such as dietary fiber (20–35%) [13]. Wheat bran is rich in proteins (12–18%) and carbohydrates (45–65%). [14]. The proteins in rice and wheat brans have been thoroughly investigated [15, 16]. For example, rice bran protein extracted with alkali by Han et al. had a pepsin digestibility of 89.8%, while its true digestibility (94.8%) was significantly higher than soy protein, rice endosperm protein, and whey protein [17]. Recently, various studies have shown that rice and wheat brans can be used as carbon and nitrogen sources for microorganisms [18–20]. This increases the value of the bran and reduces wastage of agricultural resources.

G. frondosa strains show significant differences in their production of biomass, mycelial polysaccharides, and other metabolites. For instance, Quan et al. [21] screened a *G. frondosa* strain by the protoplast ultraviolet method and achieved a biomass yield of 11.5 g/L and intracellular polysaccharide yield of 0.78 g/L after 7 days of fermentation. Liu [22] used ultraviolet and microwave irradiation to obtain two *G. frondosa* mutants with improved fermentation performance using rice bran and wheat bran as substrates. However, *G. frondosa* strains with high fermentation performance using rice and wheat brans need to be continually improved. Recently, a new microbial mutation technique has been developed: atmospheric and room temperature plasma (ARTP) mutation. ARTP has proven to be a reliable and effective microbial breeding method with a high frequency of random mutations induced by reactive chemical species produced by the helium-based atmospheric and room temperature plasmas [23]. Compared to traditional mutation methods, ARTP can lead to the generation of multiple mutation mechanisms and types of genetic material. It has the advantages of high-throughput mutagenesis, high positive mutation rate, and good mutation stability [24]. Liu et al. [25] obtained a *Cryptocodium cohnii* mutant (M7) with a volume yield of 1.02 g/L EPS and a biological yield of 0.39 g/g EPS, which are 33.9% and 85.4% higher than the native microalgae, respectively. The T31 mutant of *Candida tropicalis* yeast bred by ARTP showed a 22% increase in xylitol yield (0.61 g/g) [26]. Zhuang et al. [27] used the *Phaffia rhodozyma* mutant Y1 obtained by ARTP to hydrolyze bagasse for 96 h, achieving biomass and carotenoid concentrations of 12.65 g/L and 88.57 mg/L, respectively. There have been few reports on the use of ARTP to improve the fermentation performance of *G. frondosa*.

This study, therefore, focused on (i) using ARTP to produce a new *G. frondosa* mutant strain which could use

rice and wheat brans to produce high-yield mycelial polysaccharides, (ii) optimizing the cultivation conditions to maximize production of mycelia and mycelial polysaccharides by this ARTP-mutated strain, and (iii) analyzing the components of the mycelial polysaccharides before and after mutation of *G. frondosa*.

2. Materials and Methods

2.1. Materials. Rice bran was purchased from Zhenjiang Sanshan Rice Factory Co., Ltd. and wheat bran from Zhenjiang Fuhua Flour Co., Ltd. (Zhenjiang, Jiangsu, China). The brans were hot-air-dried, ground to 60 mesh, and stored at 4°C for further investigation.

2.2. Fungal Strain and Media. *G. frondosa* ZJQYLIU2017 was isolated from *G. frondosa* fruiting body (Qinghui151, national certification of edible fungi variety for cultivation and food) from Qingyuan Scientific Research Center of Edible Fungi, Lishui, Zhejiang, China.

Culture media were prepared as follows: potato dextrose agar medium (PDA, g/L) contained potato 200, glucose 20, peptone 5, KH₂PO₄ 1.5, MgSO₄·7H₂O 0.75, and agar 20; potato dextrose medium (PD, g/L) consisted of potato 200, glucose 20, peptone 5, KH₂PO₄ 1.5, and MgSO₄·7H₂O 0.75; screening plate medium (g/L) contained rice bran 20, wheat bran 30, KH₂PO₄ 1.5, MgSO₄·7H₂O 0.75, and agar 20; and screening fermentation medium (g/L) included rice bran 40, wheat bran 60, KH₂PO₄ 1.5, and MgSO₄·7H₂O 0.75.

2.3. ARTP Mutagenesis. The parent strain *G. frondosa* ZJQYLIU2017 was incubated on PDA plates, brushed with 10 mL of sterile normal saline to produce a liquid suspension, then diluted for ARTP mutagenesis. With pure helium as the working gas, the parameters of the ARTP mutation instrument (ARTP-IIS; Wuxi Yuanqing Tianmu Biotechnology Co., Ltd., Jiangsu, China) were 120 W radio frequency power input, 10 L/min gas flow, and 2 mm irradiation distance [28]. Suspension samples (20 μL) were exposed to the ARTP plasma jet for 0 to 130 s, spread on screening media plates, and incubated for 15 days at 23°C, observing colony growth (Figure 1). Lethality was calculated under various treatment times according to the following equation:

$$\text{Lethality (\%)} = \frac{(\text{Control colonies} - \text{Survival colonies})}{\text{Control colonies} \times 100} \quad (1)$$

Surviving ARTP-mutated strains were inoculated onto screening media to compare their colony morphologies and sizes. Eleven positive mutants with high growth rate were identified and transferred to screening medium plates to determine their growth rate. The mycelia were inoculated into PD medium to obtain a seed solution of *G. frondosa*. This seed solution was then inoculated (10% v/v) into fermentation media at 23°C and 150 rpm for 10 days. Samples of the fermentation broth were filtered and washed with distilled water through a 40-mesh filter to separate mycelia

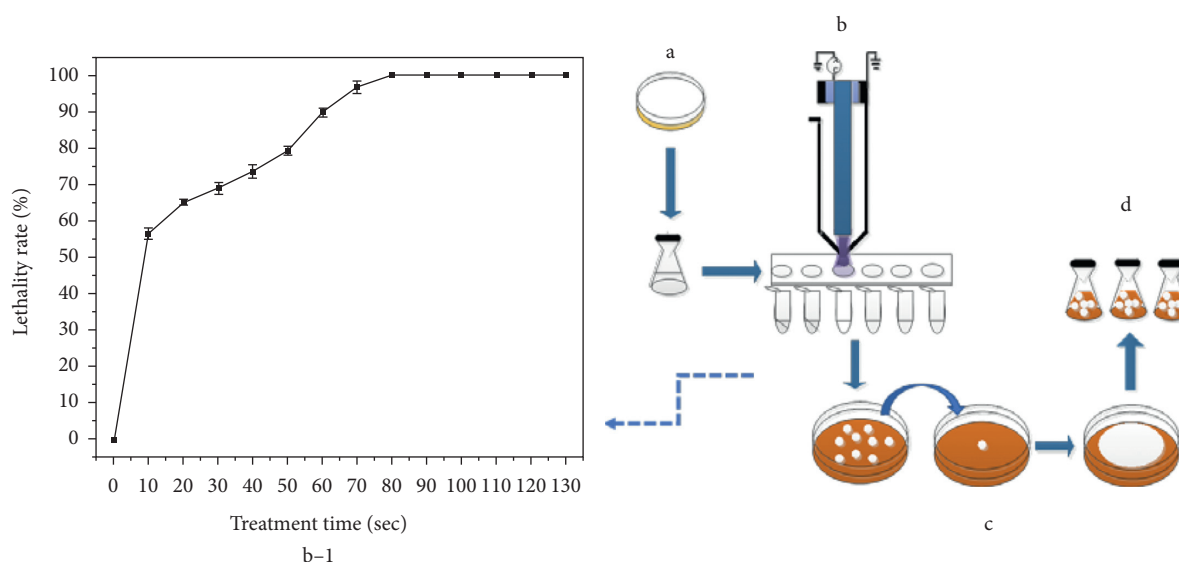


FIGURE 1: Flow chart of ARTP screening. (a) Cultivation of *G. frondosa*; (b) ARTP mutagenesis, and b-1: lethality of *G. frondosa* at different irradiation times; (c) positive mutants transfer to rice bran and wheat bran for growth tests, and (d) dominance mutants performance in rice bran and wheat bran submerged culture. The results were calculated from three independent tests.

and residues, then dried at 50°C, ground to a powder and extracted in 90°C water for 2 h at 1:30 (*w/v*) [29]. The concentration of mycelial polysaccharides was determined by the phenol-sulfuric acid method [30].

2.4. Preparation of Crude Polysaccharide Samples. Mycelial polysaccharides were precipitated by adding four volumes of 95% ethanol to the polysaccharide extract solution, protein was removed three times with Sevag reagent (4:1 chloroform: *n*-butyl alcohol), the solution was dialyzed against distilled water in a 3500 Da molecular weight cut-off dialysis bag for 72 h, and the retained crude *G. frondosa* polysaccharides were freeze-dried.

2.5. Strain Identification. The parent strain *G. frondosa* ZJQYLIU2017 and mutant strain *G. frondosa* JSULIU-WANG2019 (GFA2) were sequenced by Sangon Biotech Co. (Shanghai, China) via PCR amplification using an ITS primer [31]. NCBI BLAST was used for homology comparison and phylogenetic analysis was performed using Mega 7.0 software to identify the mutant.

2.6. Optimization of Fermentation Medium for Mutant GFA2. One-factor-at-a-time experiments were carried out to investigate the influence of rice bran (10, 30, 50, 70, 90, and 110 g/100 L), wheat bran (10, 30, 50, 70, 90, and 110 g/L), KH_2PO_4 (0, 0.5, 1, 1.5, 2, and 2.5 g/L), and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0, 0.5, 1, 1.5, 2, and 2.5 g/L) on the *G. frondosa* mycelial biomass and polysaccharides. A seed solution of 10% (*v/v*) was inoculated into 100 mL fermentation media (250 mL flask) and cultured at 23°C and 150 rpm for 10 days.

The U_{11} (11^3) uniform experimental design allowed more factors and levels to be assessed in a limited number of

experiments [32] and the regression equation enabled the selection of the optimum fermentation media composition based on the ratio of rice bran to wheat bran, KH_2PO_4 , and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

2.7. Monosaccharide Composition Analysis. Monosaccharide composition of the freeze-dried polysaccharides was determined by gas chromatography using an Agilent 7890A (DB-5MS quartz capillary column, 30 m × 0.25 mm × 0.25 μm) with flame ionization detector. The sample derivatization procedure was based on Yan et al. [33]. Briefly, 10 mg of polysaccharide was hydrolyzed with 5.0 mL TFA (2 M) at 110°C for 8 h. Hydroxylamine hydrochloride (10 mg) and pyridine (1 mL) were heated at 90°C for 30 min, then the hydrolysate was derivatized with 1 mL acetic anhydride at 90°C for 30 min. The detection conditions were as follows: column temperature started at 120°C, rose at 5°C/min to 200°C, then at 2°C/min to 215°C, and finally 20°C/min to 270°C; inlet and detector temperatures were 250°C; the split ratio was 30:1; flow rates of H_2 , N_2 and air were 35, 30 and 350 mL/min, respectively.

2.8. Fourier Transform Infrared Spectroscopy. Fourier transform infrared (FT-IR) spectra were acquired using a Bruker Vector33 FT-IR spectrophotometer in the wavelength region 4000 to 400 cm^{-1} [34].

2.9. Statistical Analysis. All experiments were performed in triplicate and the results expressed as mean ± standard deviation. Correlation and regression analyses were performed using Origin 9.0. Student's *t*-test was used to determine significant differences between samples with $p < 0.05$ considered to indicate significance (SPSS 22.0; SPSS Inc., USA).

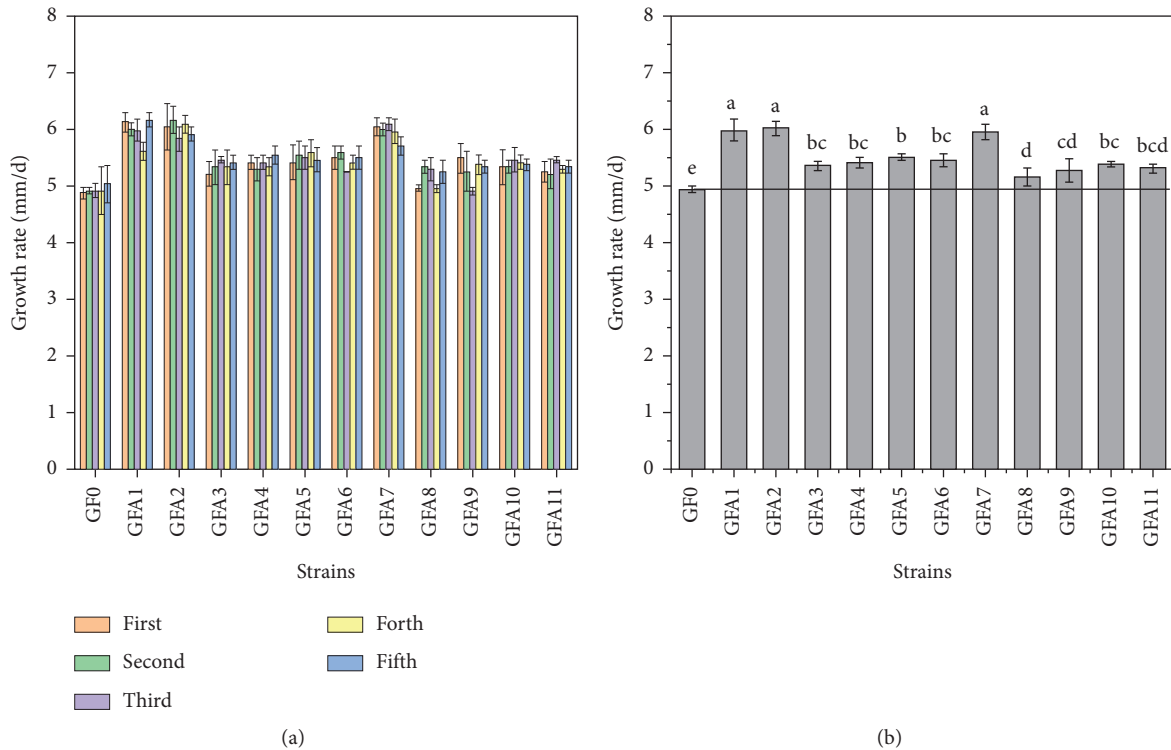


FIGURE 2: Growth rate of the parent strain (GF0) and mutants (GFA1–GFA11) of *G. frondosa* on screening media at 23°C. The growth rate in five generations (a). The average growth rate of five generations (b). Each data point is the mean of three replicate samples. (a), (b), (c), and so forth indicate significant difference at $p < 0.05$.

3. Results and Discussion

3.1. Mutation of *G. frondosa* by ARTP. ARTP breaks DNA strands and induces mutations using high purity helium gas in a high-frequency electrical field at atmospheric pressure and room temperature [27, 35]. Figure 1 shows the dose- and time-dependent lethality, based on equation (1), induced by ARTP mutagenesis of the parent *G. frondosa* strain (GF0). When GF0 was treated for 10 s, about 55% of cells were killed, indicating that many cells were sensitive to the treatment. The lethality reached 89.9% at 60 s and 96.9% at 70 s. Substantial DNA damage may cause cells to initiate their SOS emergency repair mechanism. This repair process can generate various types of mismatch site, significantly raising the mutation rate [36]. Longer treatment time increases the lethality, DNA damage, and mutation rate. The positive mutation rate was the highest when lethality was 90–95% [37], so 60 s was considered to be the optimum ARTP treatment time in this study.

3.2. Screening of Mutant Strains. As an effective method to generate mutant bacterial and fungal strains with improved properties such as tolerance to growth conditions and increased production of biomass, polysaccharides, enzymes, and other components [38], ARTP was adopted to produce an improved mutant strain of *G. frondosa*. After ARTP mutagenesis, the parent strain GF0 and 11 mutant strains (GFA1, GFA2, ..., GFA11) from large colonies were

screened by culturing on screening media plates for five generations. The mycelial growth rates per generation of these 12 strains are shown in Figure 2(a). Figure 2(b) shows that the daily mycelial growth rate of GF0 was 4.93 ± 0.05 mm/d, while the mutants GFA1, GFA2, and GFA7 were 5.98 ± 0.19 mm/d, 6.00 ± 0.12 mm/d, and 5.96 ± 0.43 mm/d, which increases of 21.5%, 22.2%, and 21.1%, respectively. These mutant strains showed significant improvement in mycelia growth on rice and wheat brans media compared with GF0.

After five generations, GFA1, GFA2, and GFA7 were selected for fermentation performance testing (Figure 3). As shown in Figure 3(c), GFA1, GFA2, and GFA7 had average mycelial biomass yields of 21.81 ± 0.61 g/L, 22.26 ± 0.43 g/L, and 22.06 ± 0.23 g/L, respectively (GF0 was 20.02 ± 0.29 g/L). GFA2 had the highest mycelial polysaccharide concentration of 2.65 ± 0.02 g/L, with the parent strain GF0 having 2.43 ± 0.06 g/L. Thus, biomass and polysaccharide concentration increased by 11.2% and 9.2%, respectively, in GFA2. However, notwithstanding the increases shown in Figure 3(c), the weight-for-weight yields of polysaccharides from mutants GFA1, GFA2, and GFA7 were not significantly different from the parent strain GF0 in Figure 3(d), indicating that ARTP mutation could improve the ability to utilize wheat and rice brans, but not significantly change the synthesis of mycelial polysaccharides. Nevertheless, GFA2 was the most efficient strain to use with rice and wheat brans for *G. frondosa* polysaccharide production.

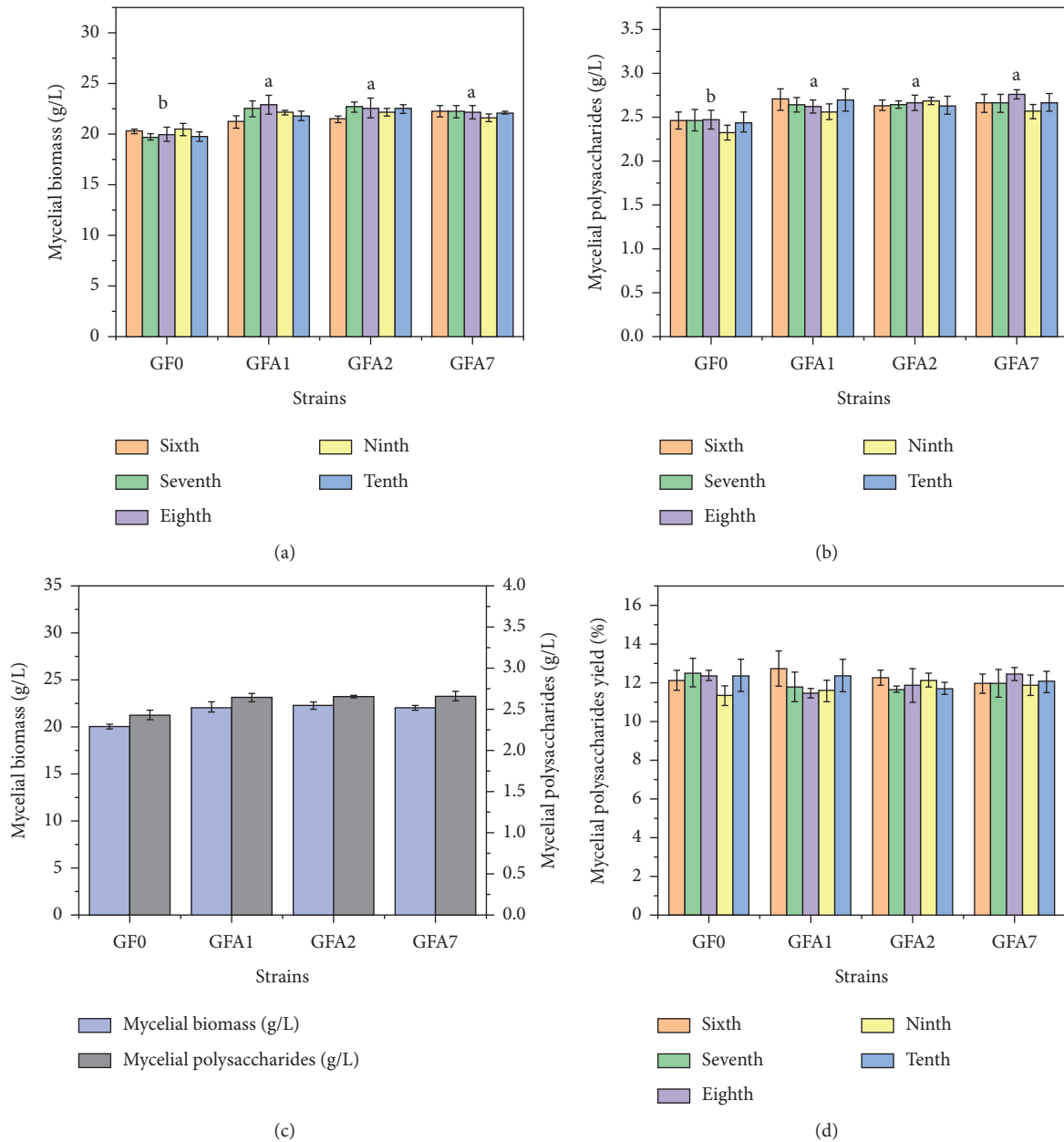


FIGURE 3: Five generations of fermentation of the parent strain and three mutant strains (GFA1, GFA2, GFA7) during bioconversion of wheat bran and rice bran at 23°C and 150 r/min. The mycelial biomass concentration in five generations (a), the mycelial polysaccharides concentration in five generations (b), the average mycelial biomass and mycelial polysaccharides of five generations (c), the weight-for-weight yields of mycelial polysaccharides (d). The mycelial biomass was calculated as the dry weight of mycelia. Each data point is the mean of three replicate samples. (a) and (b) indicate significant difference at $p < 0.05$ in average five generations.

The growth and fermentation performances of the mutant strains were investigated by culturing for ten generations, including five generations on screening plate media and five on screening fermentation media to measure fermentation performance. GFA1 had the lowest biomass production, 21.81 g/L (Figure 3), and the growth rate of GFA7 decreased significantly from 6.04 mm/d to 5.71 mm/d by the 5th generation ($p < 0.05$) (Figure 2), whereas GFA2 was genetically stable ($p > 0.05$) and its bran utilization rate was higher than the parent strain. Hence, GFA2 was selected as the preferred polysaccharide-producing strain for subsequent studies.

3.3. DNA Sequence Analysis of the GFA2. Partial sequencing of the internal transcribed spacers and the 5.8S gene of the parent *G. frondosa* strain ZJQYLIU2017 (NCBI accession no. MT830899) and mutant strain *G. frondosa* JSULIU-WANG19 (NCBI accession no. MT830900) were uploaded to the NCBI database. GFA2 (*G. frondosa* JSULIU-WANG19) was identified by partial sequence analysis and a neighbor-joining phylogenetic tree was constructed to compare strains. The phylogenetic tree (Figure 4) demonstrates the very close genetic relationship between GFA2 and the parent strain.

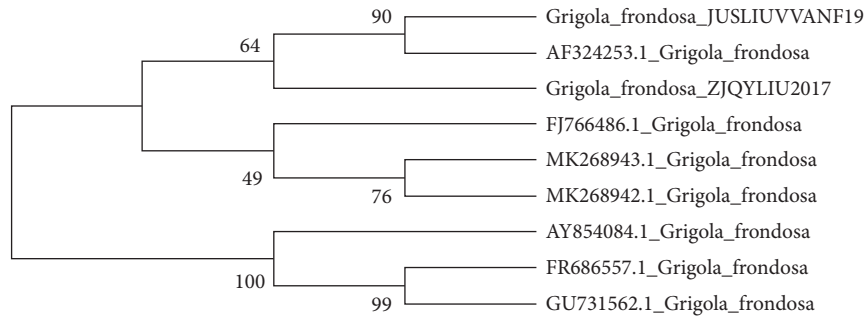


FIGURE 4: The phylogenetic tree based on gene sequence of GFA2.

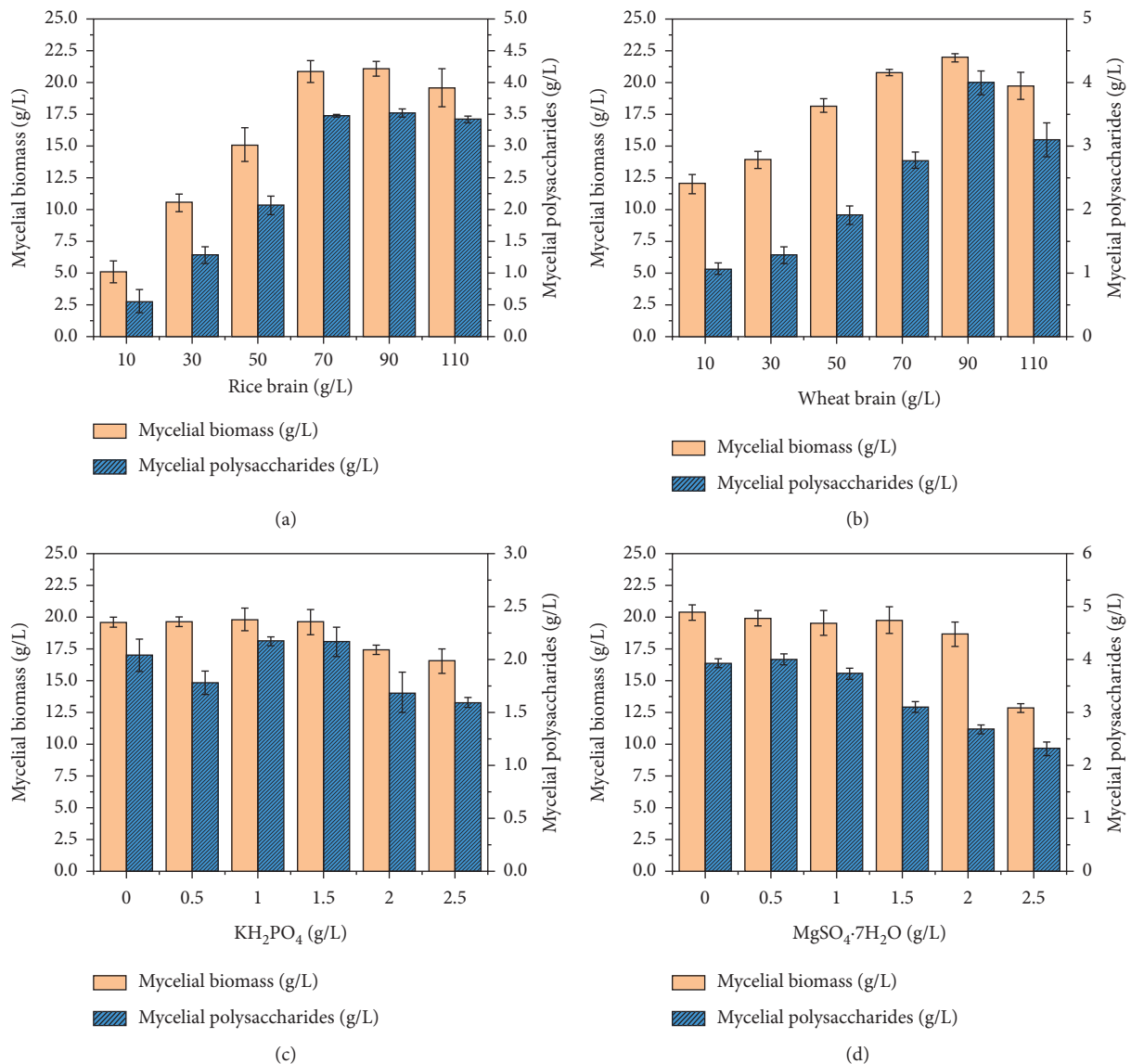


FIGURE 5: Effect of single factor on mycelial biomass and mycelial polysaccharides: effects of rice bran (a), effects of wheat bran (b), effects of KH_2PO_4 (c), and effects of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (d).

3.4. Optimization of the GFA2 Fermentation Medium. Rice and wheat brans were the main carbon and nitrogen sources in the submerged fermentation media for the GFA2 strain. Figure 5(a) shows the influence of rice bran on the

production of mycelial biomass and polysaccharide concentration. Biomass reached a maximum of 21.08 g/L when rice bran increased to 90 g/L but decreased to 19.58 g/L at higher bran concentrations. Mycelial polysaccharide

TABLE 1: Uniform design of $U_{11}(11^3)$ parameters and results for GFA2 fermentation optimization. Each data point is the mean of three replicate samples.

Runs	Factors			Biomass Y_1 (g/L)	Mycelial polysaccharides Y_2 (g/L)
	1x_1	2x_2	3x_3		
1	2.2727	2	2.25	21.61 ± 0.57	3.38 ± 0.17
2	9.909	0.25	2	18.33 ± 0.44	2.39 ± 0.13
3	1.1818	0.5	0.5	18.38 ± 1.16	2.42 ± 0.09
4	6.6363	1.25	2.5	20.76 ± 0.35	2.15 ± 0.12
5	5.5454	0	1	17.15 ± 1.38	1.72 ± 0.20
6	8.8181	1	0	19.40 ± 1.11	1.98 ± 0.06
7	0.0909	1.5	1.25	17.71 ± 2.12	2.44 ± 0.13
8	11	1.75	0.75	22.76 ± 1.18	3.15 ± 0.15
9	3.3636	0.75	1.75	21.10 ± 1.42	2.25 ± 0.22
10	7.7272	2.5	1.5	18.58 ± 0.74	1.80 ± 0.13
11	4.4545	2.25	0.25	19.27 ± 0.91	2.09 ± 0.06

1x_1 : the ratio of rice bran to wheat bran (total amount of rice and wheat brans was 120 g/L). 2x_2 : the concentration of KH_2PO_4 . 3x_3 : the concentration of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.

concentration rose from 0.55 ± 0.02 g/L (rice bran concentration 10 g/L) to a maximum of 3.51 ± 0.07 g/L (rice bran 90 g/L). Wheat bran from 10 to 70 g/L gave a relatively consistent yield of mycelial biomass (Figure 5(b)), which then peaked at 21.96 g/L with 90 g/L wheat bran (while polysaccharide concentration peaked at 4.00 ± 0.17 g/L). Low bran concentrations provided insufficient substrate, leading to a low yield of mycelia.

Appropriate concentrations of macromineral elements are essential for substrate utilization by fungi [39]. Concentrations of mycelial biomass and polysaccharides peaked at 19.83 ± 0.88 g/L and 2.18 ± 0.03 g/L, respectively, when KH_2PO_4 was 1.0 g/L (Figure 5(c)). Notably, as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ increased, biomass concentrations fell, in contrast to mycelial polysaccharide concentrations (Figure 5(d)) which reached 4.01 ± 0.10 g/L when $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was 0.5 g/L. K^+ and Mg^{2+} regulate cellular osmotic pressure and influence the metabolic processes of mycelial growth. Moreover, Mg^{2+} , as an enzyme cofactor, affects the activities of many intracellular and extracellular enzymes, thus impacting the synthesis of polysaccharides in mycelia and their ability to degrade substrates. In agreement with the current study, Mg^{2+} has been reported to increase the mycelial growth of *Ganoderma lucidum* cau5501 [40]. It is notable that high concentrations of KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ suppressed mycelial growth and polysaccharide accumulation. Yuan et al. [41] also reported that when KH_2PO_4 exceeded its optimum concentration of 5.0 g/L, cell growth slowed, and inulinase activity was inhibited.

TABLE 2: ANOVA data of mycelial polysaccharides concentration in rice and wheat brans fermentation media for GFA2.

Factor	Coefficient estimate	Standard error	t value	p value
x_1	0.299	0.080	3.739	0.013
x_2	17.134	3.240	5.288	0.003
x_3	11570	3.157	3.665	0.015
x_1x_1	-0.023	0.008	-2.976	0.031
x_2x_2	-62.756	13.668	-4.591	0.006
x_3x_3	-44.624	13.226	-3.374	0.020

The results of uniform experiments on biomass and mycelial polysaccharides are shown in Table 1. SPSS was used to analyze the linear regression of rice: wheat bran ratio (x_1), KH_2PO_4 (x_2), and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (x_3) against mycelial biomass (Y_1) and mycelial polysaccharides (Y_2):

$$Y_1 = 0.299x_1 + 17.134x_2 + 11.570x_3 - 0.023x_1^2 - 62.756x_2^2 - 44.624x_3^2. \quad (2)$$

The previous equation was the mycelial biomass model equation with the best degree of fit and strongest significance of regression equation and coefficient. The correlation coefficient ($R^2 = 0.990$) and high adjusted determination coefficient ($R^2_{\text{adj}} = 0.978$) show that this model is highly reliable (Table 2). Both coefficients are highly significant ($p < 0.01$). Equation (2) yields maximum mycelial biomass of 28.91 g/L when wheat bran is 16.0 g/L, rice bran is 104.0 g/L, KH_2PO_4 is 1.4 g/L, and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ is 1.3 g/L:

$$Y_2 = 602.174 - 131.163x_1 - 5394.101x_2 - 203.406x_3 + 10.571x_1^2 + 310.670x_1x_2 - 3556.507x_1x_3 + 19567.853x_2^2 - 13258.589x_2x_3 + 18948.745x_3^2. \quad (3)$$

The previous equation was the mycelial polysaccharide model equation with the best degree of fit and strongest significance of regression equation and coefficient. The

correlation coefficient ($R^2 = 1.000$) and high adjusted determination coefficient ($R^2_{\text{adj}} = 1.000$) show that this model is extremely reliable ($p < 0.05$) and fits the experimental data

TABLE 3: ANOVA data of mycelial biomass in rice and wheat brans fermentation media for GFA2.

Factor	Coefficient estimate	Standard error	<i>t</i> value	<i>p</i> value
a	602.174	5.000	120.444	0.005
x_1	-131.163	1.279	-102.580	0.006
x_2	-5394.101	65.488	-82.368	0.008
x_3	-203.406	26.490	-7.679	0.082
x_1x_1	10.571	0.092	114.596	0.006
x_1x_2	310.670	3.964	78.382	0.008
x_1x_3	-356.507	3.769	-94.600	0.007
x_2x_2	19567.853	230.209	85.000	0.007
x_2x_3	-13258.589	171.039	-77.518	0.008
x_3x_3	18948.745	210.091	90.193	0.007

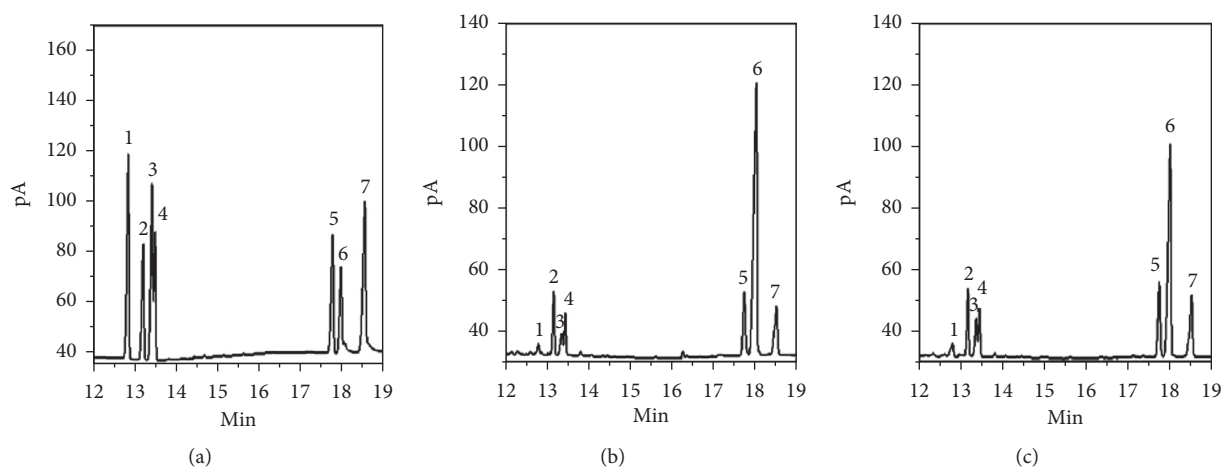


FIGURE 6: GC profiles of nitrile acetates of (a) monosaccharide standards, (b) the parent strain GF0 polysaccharides, (c) the mutant strain GFA2 polysaccharides. Peaks (Rt: min): 1, rhamnose; 2, arabinose; 3, fucose; 4, xylose; 5, mannose; 6, glucose; and 7, galactose.

very well (Table 3). Equation (3) yields a maximum mycelial polysaccharide concentration of 5.90 g/L when wheat bran is 110 g/L, rice bran is 10 g/L, and KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ are zero. KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ had no significant effect on mycelial polysaccharide concentration, so the addition of KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was optional within the experimental value range.

It must be pointed out that equations (2) and (3), which model the relationships between variables and criteria, were selected from many equations according to the principles of multiple regression modeling. This selection may differ between researchers, leading to differing interpretations of the data.

3.5. Monosaccharide Composition. After hydrolysis and derivatization, monosaccharide composition and molar ratios of the polysaccharides from GF0 and mutant GFA2 were determined by gas chromatography (Figure 6). The strains had the same monosaccharide composition (rhamnose, arabinose, fucose, xylose, mannose, glucose, and galactose) but in different molar ratios: 1.14:7.81:3.60:5.04:10.85:61.45:10.09 for GF0 polysaccharides and 1.65:9.73:6.24:6.75:13.71:48.04:13.89 for GFA2 polysaccharides.

G. frondosa mycelial polysaccharides obtained by fermentation were heteropolysaccharides and glucose was the main monosaccharide.

3.6. FT-IR Analysis. The bonds and functional groups of the mutant and parent strain polysaccharides were analyzed by FT-IR spectroscopy (Figure 7). Stretching vibration of O-H caused a wide, strong band around 3397 cm^{-1} , while the weak band near 2927 cm^{-1} was ascribed to C-H stretching vibrations [42]. Absorption around 1654 cm^{-1} was associated with water [43]. The band in the polysaccharide fingerprint region $1400\text{--}1000\text{ cm}^{-1}$ was attributed to tensile vibrations of C-C, C=O, C-O-H, and C-O-C groups. The existence of these characteristic peaks confirmed this component as a polysaccharide. The peaks at approximately 1079 cm^{-1} and 1025 cm^{-1} were due to C-O-C glycosidic bond vibrations, which indicated the presence of pyranose monomers [44]. The small absorption peaks at 910 cm^{-1} and 844 cm^{-1} were assigned to β -glycosidic and α -glycosidic bonds in the polysaccharides [45]. The main absorption bands were similar in GF0 polysaccharides and GFA2 polysaccharides, indicating the same basic structures, with only a few differing peaks.

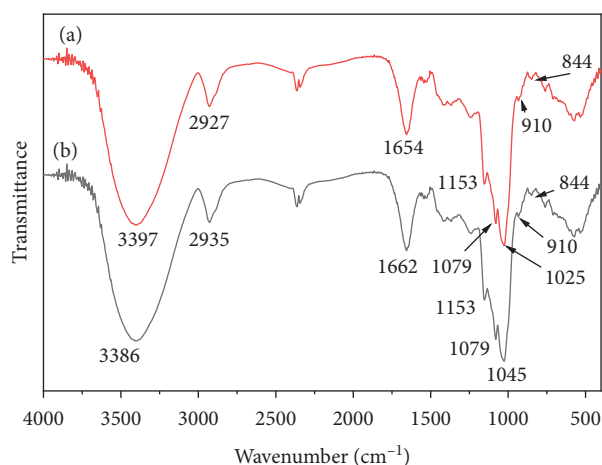


FIGURE 7: FT-IR spectra of (a) the parent strain GF0 polysaccharides and (b) the mutant strain GFA2 polysaccharides.

4. Conclusions

A mutant strain of *G. frondosa* (GFA2) exhibiting stable growth and fermentation performance was induced by ARTP and showed improved mycelial growth rate (6.0 mm/d) and polysaccharide production (2.65 g/L). Uniform design experiments optimized the culture conditions and maximized the mycelial biomass (28.91 g/L) and polysaccharide production (5.90 g/L). The mycelial polysaccharides from the parent and mutated strains had the same monosaccharide composition (rhamnose, arabinose, fucose, xylose, mannose, glucose, and galactose) at different molar ratios. Ongoing experiments are elucidating the detailed mechanisms of mycelial biomass and polysaccharide synthesis in *G. frondosa* by analyzing their related transcriptional reactions. The effects and mechanisms of *G. frondosa* polysaccharides in the prevention of chronic diseases are worthy of further study.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of Interest

All authors declare that they have no conflicts of interest.

Authors' Contributions

W. M. Liu conceived the study, participated in its design and coordination, and reviewed the manuscript. W. W. Yang analyzed results and drafted the manuscript. Y. Cheng and J. Wu revised the manuscript. T. Wang performed experiments and analyzed results. Z. C. Wei, H. L. Ma, L. Q. Yang, and F. J. Cui were responsible for the project. F. J. Cui, X. M.

Yang, and J. K. Yan revised the manuscript. K. A. Ampofo and, Hao Zhang performed partial experiments. All authors read and approved the manuscript.

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