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# Systematic Optimization of *Ganoderma lucidum* Polysaccharide Fermentation: A Guidance for Industrialization

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**Abstract:** *Ganoderma Lucidum* Polysaccharide (GLP) is one of the main active components of *G. lucidum* and a promising prebiotic for various diseases. Maximizing the production of GLP and minimizing the cost is important for the widespread use of GLP in the food/feed and pharmaceutical industries. The purpose of the present study was to optimize the fermentation condition of a single *G. lucidum* strain (isolated from the fruiting body of *G. lucidum* mushroom purchased from Linyi (Shandong, China) for GLP production under submerged-liquid fermentation, with some inexpensive substrates. The one-factor-at-a-time method was used to test the effects of inoculum culture time, inoculum size, initial pH, temperature, fermentation time, and medium components, such as carbon source, nitrogen source, $KH_2PO_4$, $MgSO_4$, and nonionic surfactant, on GLP production. Then, the response surface methodology was used to optimize the fermentation condition. According to the results, the optimal fermentation condition and medium components for GLP were as follows: 70 h inoculum culture time, 10% inoculum size, temperature of 22°C, pH of 5.56, fermentation time of 105.06 h, 14.07 g/L of glucose, 5.93 g/L of corn meal, 4 g/L of $KH_2PO_4$, 3 g/L of $MgSO_4$, 17.5 g/L of soybean meal and 0.2 mL/L of tween 80. After optimization, the production of GLP was 1.90 g/L (containing 1.00 g/L intracellular polysaccharide and 0.90 g/L exopolysaccharide) and the biomass was 15.13 g/L. The *G. lucidum* strain obtained in this study is not a good producer of GLP, while its optimized medium contains inexpensive corn meal and soybean meal and shows efficient promotion in fermentation products. These results expanded the information on strains for GLP production and provided clues for reducing the cost of industrial GLP production by using inexpensive substrates, such as corn meal and soybean meal.

**Keywords:** *Ganoderma lucidum*, Polysaccharide, Submerged Liquid Fermentation, Response Surface Methodology

## Introduction

*Ganoderma lucidum* has been used as a traditional medicine for thousands of years and its medicinal properties have been documented in ancient Chinese texts, such as "Shen Nong's herbal classic" and the "compendium of Materia Medica." *G. lucidum* contains numerous bioactive components, such as polysaccharides, triterpenoids, nucleotides, amino acids, sterols, and peptides. *G. Lucidum* Polysaccharide (GLP) is one of the primary active components found in *G. lucidum*, which includes Exopolysaccharide (EPS) and Intracellular Polysaccharide (IPS). GLP is widely present in spore powders, fruiting bodies, fermented mycelium, and broth. It has been reported to have antioxidant, immunomodulatory, anti-inflammatory, and antitumor effects *in vitro* and *in vivo* and has been regarded as a promising prebiotic for the treatment of different cancers, obesity, and other diseases (Chang *et al*., 2015; Guo *et al*., 2021; Shi *et al*., 2013). Thus, enhancing GLP production and reducing its production costs are important for the widespread use of GLP in the food/feed and pharmaceutical industries. Submerged Liquid Fermentation (SLF) is an important technology for obtaining fermentation products from *G. lucidum* (Zhang and Tang, 2008). Compared to solid substrate fermentation, SLF greatly shortens the

production cycle and increases the stability of fermentation products. Efforts have been made to establish suitable fermentation conditions to enhance GLP production by SLF. Supramani *et al*. (2019) used Response Surface Methodology (RSM) to optimize the SLF conditions, including initial pH, starting glucose concentration, and agitation rate, of *G. lucidum* strain QRS 5120 for efficient biomass (5.12 g/L), EPS (2.49 g/L) and IPS (1.52 g/L) production. Feng *et al*. (2019) optimized the concentrations of glucose, yeast powder, and $KH_2PO_4$, as well as the initial pH and inoculum size of the SLF medium by RSM, to achieve an IPS yield of up to 2.65 g/L. Supplementation of sucrose (Wei *et al*., 2016), tween 80 (Yang *et al*., 2021), $Cu^{2+}$ (Tang and Zhu, 2010), coixenolide (Zhou *et al*., 2014), selenite (Xu *et al*., 2021) and L-phenylalanine (Ma *et al*., 2019) could also increase the production of GLP and biomass. However, these optimized fermentation media were composed of high-quality carbon and nitrogen sources, such as glucose, sucrose, starch, yeast powder, yeast extract, and peptone, which are unsuitable as a reference for the industrial production of GLP. Instead, corn meal and soybean meal, as a cheap carbon source and nitrogen source, respectively, are often selected for economically viable production of polysaccharides by microorganisms. Whether they can be used to replace high-quality carbon and nitrogen sources in fermentation, however, has not been fully tested.

In the present study, a *G. lucidum* strain was isolated and evaluated for its fermentative potential. The specific aim was to optimize GLP production by the *G. lucidum* strain under SLF conditions with some inexpensive substrates. To this end, we systematically optimized the GLP production fermentation conditions (including inoculum culture time, inoculum size, initial pH, temperature and fermentation time) and medium components (carbon source, nitrogen source, $KH_2PO_4$, $MgSO_4$ and nonionic surfactant) using the One-Factor-At-A-Time (OFAAT) method, followed by RSM. We tested several inexpensive substrates, such as corn meal, corn starch, and soybean meal, for their suitability to support GLP production by the *G. lucidum* strain. Our results will guide industrial GLP production fermentation technology.

## Materials and Methods

### *Strain and Culture Condition*

The *G. lucidum* strain used in the present study was obtained from the fruiting body of *G. lucidum* that was purchased from Linyi, Shandong, China. Its internal transcribed spacer sequence is available in the GenBank database of the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/) with the accession number OR414369. This strain has a 99.67 and 99.51% similarity to the reported *G. lingzhi* strain CGMCC 5.2229 (OM721793.1) and *G. lucidum* isolates (such as MF476201.1 and GU213483.1) (Table S1). The strain was maintained on Potato Dextrose Agar (PDA) slants at 26°C for 10 days and then stored at 4°C for approximately 2 months.

The seed medium contained 20 g/L of glucose and 200 g/L of potato extract. The initial fermentation medium consisted of (g/L) glucose 20, peptone 5.0, yeast extract 5.0, $KH_2PO_4$ 3.0 and $MgSO_4$ 1.0. The agar culture from a slant was inoculated into 50 mL of seed medium in a 250 mL conical flask and incubated on a reciprocal shaker at 28°C and 150 rpm. After a 3-day culture period, 10 mL of seed culture was inoculated into 90 mL of fermentation medium and fermented at 28°C, 150 rpm for 96 h. Following fermentation, the fermentation broth was centrifuged at 10,000 g for 10 min to obtain mycelial and liquid samples (extracellular fermentation broth) for the determination of IPS, EPS, and biomass.

### *Determination of Biomass*

The mycelial samples were washed repeatedly with distilled water and dried at 60°C until the weight remained constant (Wei *et al*., 2016). The biomass of each sample was recorded. All experiments were performed in triplicate.

### *Determination of Polysaccharides*

The mycelial samples were lyophilized and ground into powder, while the liquid samples were concentrated using a rotary evaporator and then lyophilized. 10 g of mycelial powder and lyophilized liquid sample were weighed to extract the IPS and EPS, respectively. Samples were mixed with distilled water at a ratio of 1:20 (g/mL). After immersion in a boiling water bath for 2 h, the samples were centrifuged at 15,000 g for 15 min. The resulting supernatant was collected as the extract. The residue was extracted once more and the extracts pooled. Ethanol was added to the extract to achieve a final concentration of 95% (v/v) ethanol for polysaccharide extraction. After overnight extraction, the extract of crude polysaccharides was harvested by centrifugation at 15,000 g for 15 min and lyophilized. Five milligrams of each sample were weighed and diluted with distilled water to a final volume of 10 mL. The supernatant was collected to determine the polysaccharide content according to the phenol-sulfuric acid method (Nielsen, 2017).

### *Factors and Levels of Single Factor Experiment*

The OFAAT method was performed using a single-factor experiment to investigate the effects of various fermentation conditions (including inoculum culture time, inoculum size, initial pH, temperature, and fermentation time) and medium components (carbon source, nitrogen source, $KH_2PO_4$, $MgSO_4$, and nonionic surfactant) on the production of IPS and biomass. The experimental parameters are shown in Table S2. The optimal fermentation conditions and medium components were selected based on the results.

### *Plackett-Burman Design*

The Plackett-Burman Design (PBD) was used to evaluate the influence of selected variables on the production of GLP, IPS, EPS, and biomass. Each variable was tested at two levels (high and low). The experiment was conducted using the design-expert software (version 12.0.3.0; Stat-Ease, Inc., Minneapolis, MN, USA). All tests were performed in triplicate and the production of GLP, IPS, EPS, and biomass were determined. The effects of each variable on the production of GLP, IPS, EPS, and biomass were determined through Analysis of Variance (ANOVA). Significance was defined as $p \leq 0.05$ and a trend was defined as $p \leq 0.10$.

### *Steepest Ascent Design*

The Steepest Ascent Design (SAD) was performed using design-expert software (Stat-Ease, Inc.). Three variables, including the glucose-to-corn meal ratio, fermentation period and tween 80 concentration, were selected by the PBD as the most influencing and were further analyzed by SAD. The gradient direction of the experimental data was used as the climbing direction and the change step size was determined based on the effect value of each factor to approach the target area quickly and economically (Chen *et al*., 2015). Then, the IPS, EPS, GLP, and biomass production under each condition were measured according to the methods described above.

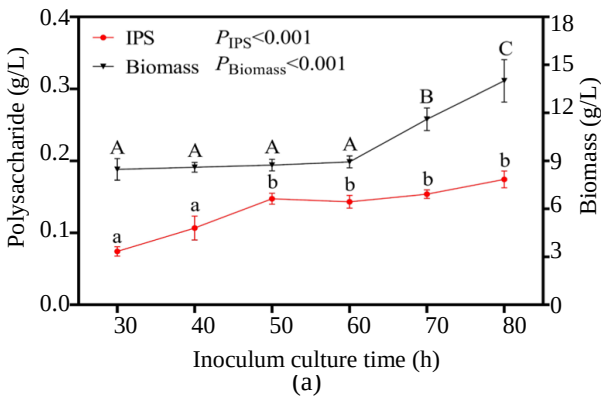
### *Central Composite Design*

The three variables, including the glucose-to-corn meal ratio, fermentation period, and tween 80 concentration, selected by PBD, were further analyzed by Central Composite Design (CCD) and RSM. Each variable was tested at three levels (-1 representing the low level, 0 representing the medium level, and +1 representing the high level). The medium-, low- and high-level values of the variables were selected according to the best, lower, and higher GLP production from the results of SAD. All data were processed using design-expert software (Stat-Ease, Inc.), to calculate the optimal values of the variables for GLP production.

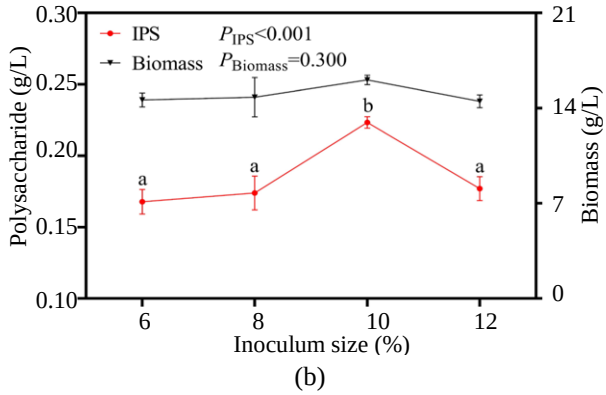
## Results and Discussion

### *Optimization of Fermentation Condition*

The important GLP production parameters during fermentation, such as inoculum culture time, inoculum size, initial pH, temperature, and fermentation time, were identified (Feng *et al*., 2016; 2019). As a part of GLP, the production of IPS showed a positive correlation with the GLP (Wei *et al*., 2016). Thus, we used IPS production to predict the efficiency of GLP production in the single-factor experiment. In the present study, the IPS and biomass production were significantly affected by the inoculum culture time and temperature ($p \leq 0.05$), whereas they were not affected by the initial pH ($p > 0.05$, Figs. 1A, C, and D). In addition, IPS production, but not biomass, was affected by inoculum size and fermentation time (Fig. 1B and E). The changes in IPS production were not consistent with the biomass production under different fermentation conditions. With an increase in inoculum culture time from 50-80 h, there was a significant increase in IPS production ($p \leq 0.05$, Fig. 1A). In contrast, the biomass showed a significant increase when the inoculum culture time was 70 and 80 h ($p \leq 0.05$, Fig. 1A). Biomass and IPS production decreased significantly as the fermentation temperature was increased from 22-25°C and a further significant decrease in biomass occurred at 31°C ($p \leq 0.05$, Fig. 1D). The IPS production increased significantly when the inoculum size was increased from 6-10%, but it significantly decreased when the inoculum size was further increased to 12% ($p \leq 0.05$, Fig. 1B). When the fermentation time was increased to 108 h, the production of IPS significantly increased ($p \leq 0.05$) but decreased thereafter (Fig. 1E). According to the results, the optimal condition for efficient IPS and biomass production was an inoculum culture time of 70-80 h, an inoculum size of 10%, a fermentation temperature of 22°C and a fermentation time of 108 h. For downstream optimization of the medium components, the fermentation condition of 70 h inoculum culture time, 10% inoculum size, unadjusted pH 5.56, 22°C and 108 h fermentation time were chosen.




(a)

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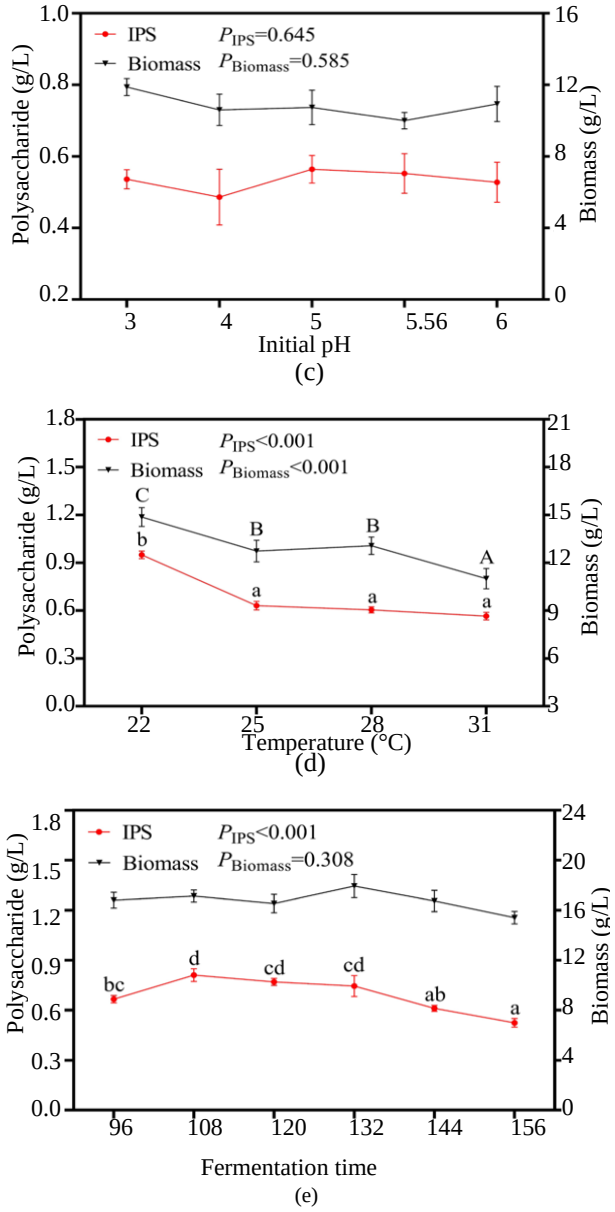
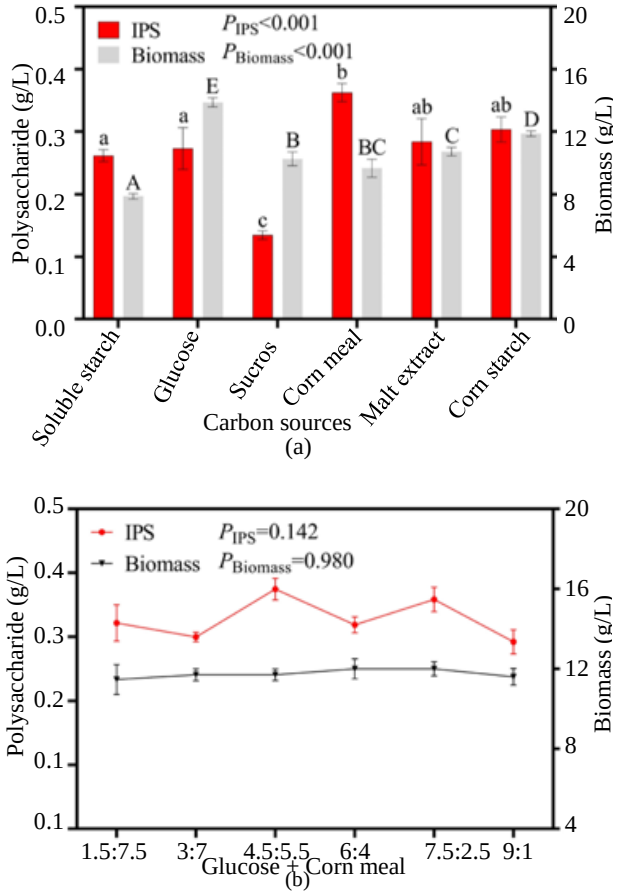



**Fig. 1:** Effect of fermentation conditions on submerged-liquid fermentation of *Ganoderma lucidum*; (A) Inoculum culture time; (B) inoculum size; (C) initial pH; (D) temperature, and (E) fermentation time. The a-c and A-C indicate p≤0.05

## *Optimization of Medium Components*

Carbon sources provide the fundamental energy for cell growth and development. In this study, the carbon source showed significant effects on the IPS and biomass production (p≤0.05, Fig. 2A). Glucose is a widely used carbon source in SLF of G. lucidum (Hsu *et al*., 2017; Supramani *et al*., 2019). Feng *et al*. (2019) reported that the optimal carbon source in SLF for IPS production by G. lucidum G0119 was glucose, followed by sucrose, sodium carboxymethyl cellulose, and starch. In contrast, Wei *et al*. (2016) identified the best substrate for IPS production in SLF of *G. lucidum* at pH 5.26 as sucrose, followed by glucose, maltose, lactose, mannose, galactose, and xylose. It is suggested that the optimal carbon source is dependent on the *G. lucidum* strain under varying fermentation conditions. Identifying the optimal carbon source for the current strain is necessary. In the present study, we investigated the effects of different types of carbohydrates, including a monosaccharide (glucose), a disaccharide (sucrose) and polysaccharides (soluble starch, corn meal, corn starch, and malt extract) (Fig. 2A). It was found that corn meal yielded the highest IPS production, whereas glucose yielded the highest biomass production (Fig. 2A). Microorganisms can utilize glucose to promote biomass without degradation (Wei *et al*., 2016). Thus, a mixed carbon source containing both glucose and corn meal was chosen to promote the production of both IPS and biomass. The optimal ratio of these two components and the optimal concentration of the mixed carbon source were investigated (Figs. 2B-C). The production of IPS and biomass were not affected by the ratio of glucose and corn meal (p>0.05, Fig. 2B), whereas they were significantly affected by the concentration of the mixed carbon source (p≤0.05, Fig. 2C). Both IPS and biomass production were highest when the mixed carbon concentration was 20 g/L (p≤0.05, Fig. 2C).



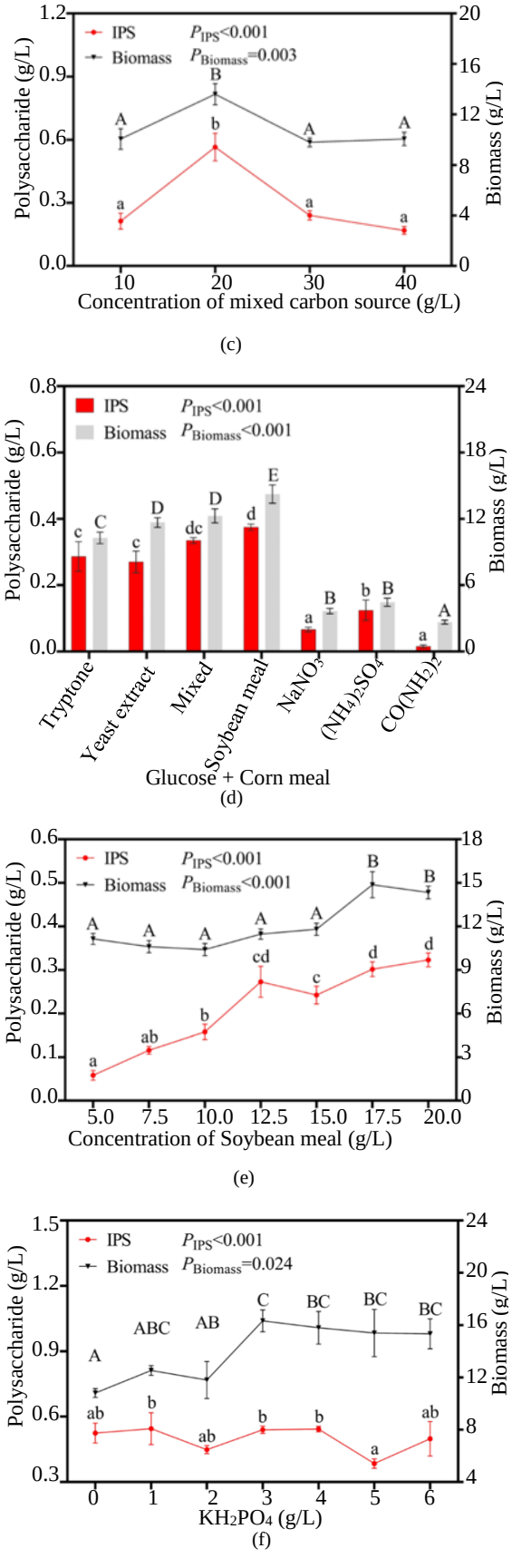



**Fig. 2:** Effect of medium components on submerged-liquid fermentation of *Ganoderma lucidum*; (A) Different carbon sources; (B) ratio of mixed carbon sources; (C) concentration of mixed carbon source; (D) different nitrogen sources; (E) concentration of nitrogen source, and (F) concentrations of $KH_2PO_4$; (G) $MgSO_4$; (H) tween 20 and (I) tween 80. The a-e and A-E indicate $p \leq 0.05$. [1]Mixed = Mixed nitrogen source containing tryptone and yeast extract with a ratio of 1:1

Nitrogen sources provide the primary raw materials for the biosynthesis of enzymes, proteins, and nucleic acids during the growth and development of microorganisms. In total, we estimated seven different kinds of nitrogen sources, including four organic nitrogen sources (tryptone, yeast extract, soybean meal, and urea), two inorganic nitrogen sources ($NaNO_3$ and $(NH_4)_2SO_4$), and one mixed organic nitrogen source (containing

tryptone and yeast extract in a 1:1 ratio). We identified that these nitrogen sources had significant effects on both IPS and biomass production ($p \leq 0.05$, Fig. 2D). Compared to the inorganic nitrogen sources and urea, the tryptone, yeast extract, and soybean meal yielded higher IPS and biomass production ($p \leq 0.05$), with soybean meal having the highest production ($p \leq 0.05$). Figure 2E further shows that with the increasing concentration of soybean meal, the production of IPS and biomass was significantly increased ($p \leq 0.05$). The highest IPS and biomass production were obtained when the concentration of soybean meal was 17.5 and 20.0 g/L (Fig. 2E). Considering the goal of minimizing the cost of the medium, soybean meal with a concentration of 17.5 g/L was selected for further analysis.

The supplementation of phosphate and magnesium can promote fungal growth and increase the production of IPS, EPS, and biomass (Feng *et al*., 2019; Yuan *et al*., 2012). Among the different kinds of phosphates, $KH_2PO_4$ was the most effective for increasing IPS production, followed by $K_2HPO_4$, $Na_2HPO_4$, and $NaH_2PO_4$ (Feng *et al*., 2019). In the present study, we examined the effects of different concentrations of both $KH_2PO_4$ and $MgSO_4$ (Figs. 2F-G). The IPS and biomass production were significantly affected by the concentration of $KH_2PO_4$ and $MgSO_4$ ($p \leq 0.05$, Figs. 2F-G). Concentrations of 3 g/L $KH_2PO_4$ and 2 g/L of $MgSO_4$ yielded the highest IPS and biomass production. Thus, those concentrations were chosen for downstream analysis.

Tween 20 and 80 are common nonionic surfactants that can decrease surface and interfacial tensions, increase solubility and bioavailability, and promote enzyme efficiency (Kaar and Holtzapple, 1998; Singh *et al*., 2007). In the present study, although the concentrations of both tween 20 and 80 had significant effects on the IPS and biomass production (Figs. 2H-I), tween 20 did not promote but instead significantly inhibited the production of both IPS and biomass when supplemented at 0.02 and 0.10 mL/L ($p \leq 0.05$, Fig. 2H). Compared to 0 mL/L of tween 80, the addition of 0.02 mL/L of tween 80 resulted in a numerical increase in IPS and biomass production. When the concentration of tween 80 exceeded 0.02 mL/L, it exerted an inhibitory effect (Fig. 2I), which was consistent with the reported effects of tween 80 on EPS by Yang *et al*. (2021). Thus, we chose 0.02 mL/L of tween 80 instead of tween 20.

## *Important Variables for the Production of Polysaccharides and Biomass*

Based on the results of a single-factor experiment, nine variables that affected the production of IPS and biomass were chosen for PBD, including inoculum culture time, inoculum size, temperature, fermentation time, glucose-to-corn meal ratio and the concentrations of soybean meal, $KH_2PO_4$, $MgSO_4$ and tween 80 (Table 1), to filter the most important variables. To achieve optimal GLP production, 12 levels were designed for nine variables, and GLP, IPS, EPS, and biomass production were taken into consideration in further optimization (Table 2). Among the 12 levels, the production of GLP, IPS, EPS, and biomass ranged from 0.90-1.62, 0.46-0.93, 0.34-0.95, and 15.40-18.40 g/L, respectively (Table 2). These results suggested the necessity for additional optimization of the fermentation conditions and medium components. Regarding the production of GLP and EPS, the significant factors were the glucose-to-corn meal ratio, tween 80, and fermentation time ($p \leq 0.05$, Table 3 and S3). For IPS production, the significant terms were the inoculum size, $MgSO_4$, and fermentation time ($p \leq 0.05$, Table S4). For biomass production, the significant terms were the inoculum culture time, inoculum size, $KH_2PO_4$, and fermentation time ($p \leq 0.05$, Table S5).

## *Optimization of Fermentation Condition and Medium Components by RSM*

The glucose-to-corn meal ratio, tween 80 concentration, and fermentation time were selected for RSM based on the findings of PBD. In total, six levels of the three variables were performed in SAD (Table 4). Among the six levels, GLP and IPS had the highest production at 1.99 and 1.18 g/L, respectively, when the glucose-to-corn meal ratio was 3:7, the tween 80 concentration was 0.2 mL/L and the fermentation time was 104 h (Table 4). Incongruent with the production of GLP and IPS, the maximum production of EPS (0.99 g/L) was observed when the glucose-to-corn meal ratio was 4:6, the tween 80 concentration was 0.15 mL/L and the fermentation time was 92 h. Biomass production reached a maximum of 15.00 g/L at a glucose-to-corn meal ratio of 9:1, 0.30 mL/L of tween 80, and a fermentation time of 128 h (Table 4).

The CCD was performed on the three-factor response surface analysis experiment, with the glucose-to-corn meal ratio (A), the tween 80 concentration (B), and the fermentation time (C) used as independent variables (Table 5). To achieve optimal GLP production, the GLP production was used as the response variable. According to the SAD results, the condition of a 7:3 ratio of glucose to corn meal, 0.2 mL/L tween 80 and 104 h fermentation time was chosen as level 0 of CCD. The experimental design and results are shown in Table 6. The quadratic multiple regression equation for GLP was as follows:

$$Y = 1.95 + 0.0084A + 0.0055B + 0.0689C - 0.0227AB + 0.0085AC - 0.0394BC - 0.2861A^2 - 0.2155B^2 - 0.2497C^2 \quad (1)$$

where:
*A* represents the glucose-to-corn meal ratio
*B* represents the tween 80 concentration
*C* represents the fermentation time

**Table 1:** Factors of Plackett-Burman design

| | | Level | |
|---|---|---|---|
| Factor | Coding | -1 | 1 |
| Inoculum culture time (h) | $X_1$ | 65 | 75 |
| Inoculum size (%) | $X_2$ | 8 | 12 |
| Glucose-to-corn meal ratio | $X_3$ | 6:4 | 9:1 |
| Soybean meal (g/L) | $X_4$ | 15 | 20 |
| $KH_2PO_4$ (g/L) | $X_5$ | 3 | 5 |
| $MgSO_4$ (g/L) | $X_6$ | 2 | 4 |
| Temperature (°C) | $X_7$ | 20 | 24 |
| Tween 80 (mL/L) | $X_8$ | 0.1 | 0.3 |
| Fermentation time (h) | $X_9$ | 84 | 108 |

**Table 2:** Experimental design and the response results of Plackett-Burman design

| No. | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | $X_6$ | $X_7$ | $X_8$ | $X_9$ | GLP[1] (g/L) | IPS[2] (g/L) | EPS[3] (g/L) | Biomass (g/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 75 | 12 | 9:1 | 15 | 3 | 2 | 24 | 0.3 | 84 | 1.21 | 0.68 | 0.53 | 15.80 |
| 2 | 65 | 12 | 9:1 | 20 | 3 | 2 | 20 | 0.1 | 108 | 1.43 | 0.83 | 0.60 | 17.70 |
| 3 | 75 | 12 | 6:4 | 20 | 5 | 4 | 20 | 0.1 | 84 | 0.90 | 0.56 | 0.34 | 15.40 |
| 4 | 75 | 8 | 6:4 | 15 | 5 | 2 | 24 | 0.1 | 108 | 1.24 | 0.77 | 0.47 | 18.20 |
| 5 | 75 | 12 | 6:4 | 15 | 3 | 4 | 20 | 0.3 | 108 | 1.20 | 0.68 | 0.52 | 16.60 |
| 6 | 65 | 8 | 6:4 | 20 | 3 | 4 | 24 | 0.3 | 84 | 1.00 | 0.46 | 0.54 | 17.40 |
| 7 | 65 | 8 | 9:1 | 15 | 5 | 4 | 20 | 0.3 | 108 | 1.61 | 0.66 | 0.95 | 17.00 |
| 8 | 65 | 12 | 9:1 | 15 | 5 | 4 | 24 | 0.1 | 84 | 1.11 | 0.61 | 0.50 | 15.80 |
| 9 | 75 | 8 | 9:1 | 20 | 3 | 4 | 24 | 0.1 | 108 | 1.35 | 0.65 | 0.70 | 17.40 |
| 10 | 75 | 8 | 9:1 | 20 | 5 | 2 | 20 | 0.3 | 84 | 1.41 | 0.67 | 0.74 | 16.00 |
| 11 | 65 | 8 | 6:4 | 15 | 3 | 2 | 20 | 0.1 | 84 | 0.90 | 0.55 | 0.35 | 18.20 |
| 12 | 65 | 12 | 6:4 | 20 | 5 | 2 | 24 | 0.3 | 108 | 1.62 | 0.93 | 0.69 | 16.90 |

**Table 3:** Effect evaluations of each factor under Plackett-Burman design based on *Ganoderma lucidum* polysaccharide production

| Factors | Effect | Standard error | F-value | p-value | Significance |
|---|---|---|---|---|---|
| Inoculum culture time (h) | -0.030 | 0.018 | 2.890 | 0.231 | |
| Inoculum size (%) | -0.004 | 0.018 | 0.044 | 0.854 | |
| Glucose-to-corn meal ratio | 0.105 | 0.018 | 36.030 | 0.027 | * |
| Soybean meal (g/L) | 0.037 | 0.018 | 4.360 | 0.172 | |
| $KH_2PO_4$ (g/L) | 0.068 | 0.018 | 15.080 | 0.060 | |
| $MgSO_4$ (g/L) | -0.053 | 0.018 | 9.200 | 0.094 | |
| Temperature (°C) | 0.006 | 0.018 | 0.132 | 0.752 | |
| Tween 80 (mL/L) | 0.094 | 0.018 | 28.890 | 0.033 | * |
| Fermentation time (h) | 0.160 | 0.018 | 83.160 | 0.012 | * |

**Table 4:** Experimental design and results of the steepest ascent experiment

| No. | Glucose: Corn meal | Tween 80 (mL/L) | Fermentation period (h) | GLP[1] (g/L) | IPS[2] (g/L) | EPS[3] (g/L) | Biomass (g/L) |
|---|---|---|---|---|---|---|---|
| 1 | 5:5 | 0.10 | 80 | 1.27 | 0.83 | 0.45 | 9.40 |
| 2 | 6:4 | 0.15 | 92 | 1.79 | 0.89 | 0.99 | 12.20 |
| 3 | 7:3 | 0.20 | 104 | 1.99 | 1.18 | 0.86 | 12.40 |
| 4 | 8:2 | 0.25 | 116 | 1.71 | 1.12 | 0.84 | 13.00 |
| 5 | 9:1 | 0.30 | 128 | 1.13 | 0.97 | 0.36 | 15.00 |
| 6 | 10:0 | 0.35 | 140 | 1.07 | 0.92 | 0.33 | 14.80 |

[1]GLP = *Ganoderma lucidum* polysaccharide, [2]IPS = intracellular polysaccharide, [3]EPS = exopolysaccharide

**Table 5:** Factors and levels of central composite design

| | | Level | | |
|---|---|---|---|---|
| Factor | Coding | -1 | 0 | 1 |
| Glucose: Corn meal | A | 5:5 | 7:3 | 9:1 |
| Fermentation period (h) | B | 80 | 104 | 128 |
| Tween 80 (mL/L) | C | 0.1 | 0.2 | 0.3 |

**Table 6:** Experimental design and results of central composite design

| No. | A | B | C | GLP[1] (g/L) | IPS[2] (g/L) | EPS[3] (g/L) | Biomass (g/L) |
|---|---|---|---|---|---|---|---|
| 1 | 7:3 | 0.2 | 128 | 1.60±0.08 | 1.06±0.21 | 0.54±0.25 | 16.80±0.35 |
| 2 | 5:5 | 0.3 | 80 | 1.17±0.03 | 0.73±0.02 | 0.44±0.02 | 14.20±0.87 |
| 3 | 5:5 | 0.1 | 128 | 1.29±0.19 | 0.92±0.19 | 0.37±0.17 | 15.73±0.61 |
| 4 | 7:3 | 0.2 | 104 | 2.00±0.24 | 1.38±0.08 | 0.62±0.32 | 16.00±0.35 |
| 5 | 9:1 | 0.1 | 128 | 1.40±0.12 | 1.18±0.11 | 0.22±0.01 | 13.67±0.58 |
| 6 | 7:3 | 0.2 | 104 | 1.89±0.20 | 1.55±0.11 | 0.34±0.25 | 18.80±1.04 |
| 7 | 7:3 | 0.2 | 104 | 2.07±0.06 | 1.61±0.04 | 0.46±0.07 | 18.00±1.00 |
| 8 | 5:5 | 0.2 | 104 | 1.59±0.25 | 1.07±0.12 | 0.52±0.25 | 14.07±0.81 |
| 9 | 5:5 | 0.1 | 80 | 1.07±0.16 | 0.67±0.06 | 0.40±0.18 | 13.00±0.50 |
| 10 | 9:1 | 0.1 | 80 | 1.11±0.10 | 0.81±0.05 | 0.30±0.08 | 12.80±0.20 |
| 11 | 7:3 | 0.2 | 104 | 2.00±0.11 | 1.55±0.01 | 0.45±0.11 | 15.00±0.53 |
| 12 | 5:5 | 0.3 | 128 | 1.27±0.06 | 0.92±0.08 | 0.35±0.14 | 15.77±0.35 |
| 13 | 7:3 | 0.1 | 104 | 1.61±0.22 | 1.34±0.21 | 0.27±0.12 | 16.13±1.70 |
| 14 | 9:1 | 0.3 | 128 | 1.25±0.03 | 1.06±0.05 | 0.19±0.06 | 16.87±0.87 |
| 15 | 7:3 | 0.3 | 104 | 1.68±0.09 | 1.40±0.23 | 0.28±0.14 | 17.60±3.22 |
| 16 | 9:1 | 0.2 | 104 | 1.55±0.07 | 1.11±0.09 | 0.44±0.15 | 17.07±0.12 |
| 17 | 9:1 | 0.3 | 80 | 1.16±0.01 | 0.86±0.11 | 0.30±0.12 | 11.40±0.53 |
| 18 | 7:3 | 0.2 | 104 | 2.05±0.09 | 1.49±0.06 | 0.56±0.12 | 17.40±0.53 |
| 19 | 7:3 | 0.2 | 104 | 2.00±0.14 | 1.52±0.03 | 0.48±0.14 | 18.07±0.49 |
| 20 | 7:3 | 0.2 | 80 | 1.62±0.08 | 1.05±0.09 | 0.57±0.11 | 13.67±1.15 |

[1]GLP = *Ganoderma lucidum* polysaccharide; [2]IPS = intracellular polysaccharide; [3]EPS = exopolysaccharide

**Table 7:** The regression results of the central composite design

| Factors | df | Sum of squares | Mean squares | F-value | p-value | Significance |
|---|---|---|---|---|---|---|
| Model | 9 | 2.140 | 0.238 | 22.330 | < 0.001 | ** |
| A | 1 | 0.001 | 0.001 | 0.067 | 0.801 | |
| B | 1 | 0.000 | 0.000 | 0.028 | 0.870 | |
| C | 1 | 0.048 | 0.048 | 4.460 | 0.061 | |
| AB | 1 | 0.004 | 0.004 | 0.387 | 0.548 | |
| AC | 1 | 0.001 | 0.001 | 0.054 | 0.820 | |
| BC | 1 | 0.012 | 0.012 | 1.170 | 0.306 | |
| $A^2$ | 1 | 0.225 | 0.225 | 21.130 | 0.001 | * |
| $B^2$ | 1 | 0.128 | 0.128 | 11.990 | 0.006 | * |
| $C^2$ | 1 | 0.172 | 0.172 | 16.100 | 0.003 | * |
| Residual | 10 | 0.107 | 0.011 | | | |
| Lack of fit | 5 | 0.088 | 0.018 | 4.800 | 0.055 | Not significant |
| Pure error | 5 | 0.018 | 0.004 | | | |
| Cor total | 19 | 2.250 | | 4.800 | 0.051 | |

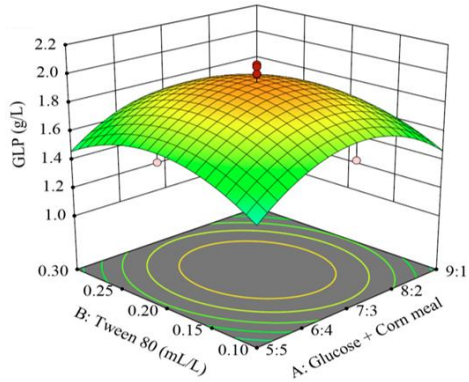
$R^2$ = 0.9526; C.V. = 6.57%; Adj-$R^2$ = 0.9099; Pred-$R^2$=0.7882; * and ** represented significant difference at $p<0.05$ and $p<0.01$, respectively

According to the results of the ANOVA, the regression model was found to be reliable and effectively fit the experimental data ($p\leq0.05$, Table 7). The $R^2$ of the fitted model indicated that the quadratic equation model could explain and predict 95.26% of the variable responses (Table 7). The correction determination coefficient (Adj-$R^2$) implied the significance of the fitted model and aligned with the predicted $R^2$. Thus, the regression model could be used to predict GLP production. In the regression model, the quadratic terms of $A^2$, $B^2$, and $C^2$ showed significant effects on the GLP production ($p\leq0.05$), whereas no significant effect was found for the other terms (Table 7).

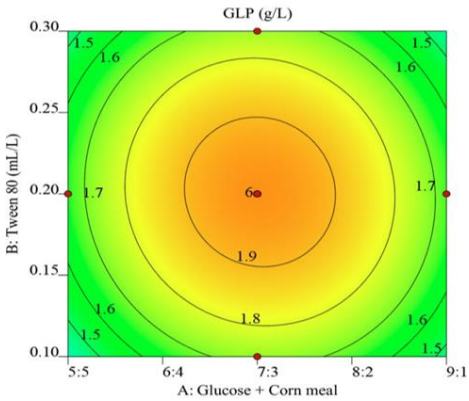
The combined effects of the glucose-to-corn meal ratio, tween 80 concentration, and fermentation time are depicted in three-dimensional plots in Fig. 3. The maximum GLP production was obtained at a glucose-to-corn meal ratio of 7.03:2.97, 0.20 mL/L of tween 80 and a fermentation time of 105.06 h. Under that condition, the predicted maximum theoretical GLP production was 1.95 g/L.

### *Verification of the Optimized Conditions*

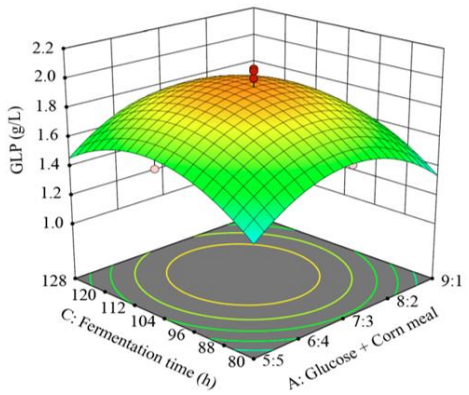
The production of IPS, EPS, GLP and biomass was determined under the optimized condition. After optimization, the production of GLP was significantly increased by 2.39-fold, from 0.56-1.90 g/L, IPS production by 1.94-fold, from 0.34-1.00 g/L, EPS production by 3.09-fold, from 0.22-0.90 g/L and the biomass production by 0.23-fold, from 12.27-15.13 g/L (Fig. 4).



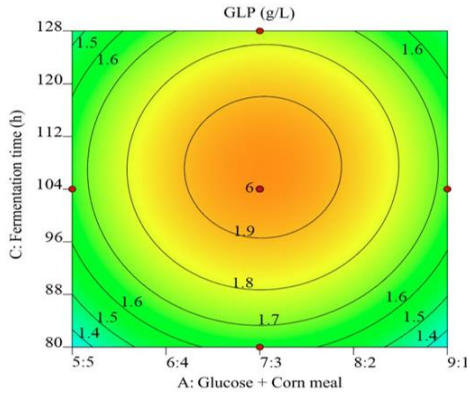

(a)



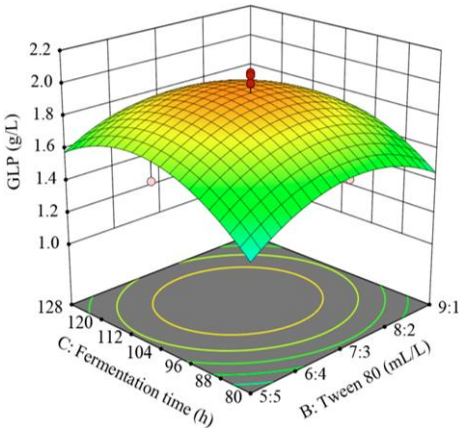

(b)



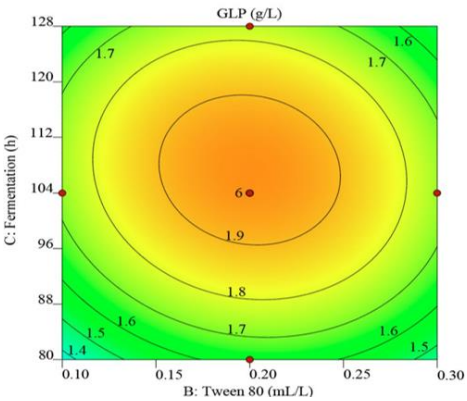

(c)




(d)




(e)




(f)

**Fig. 3:** Response surface curve of polysaccharide production from *Ganoderma lucidum* showing the interactions among glucose-to-corn meal ratio, tween 80 concentration, and fermentation time. The three-dimensional (3D) surface plot; (A) and contour plot; (B) showing the interaction between the glucose-to-corn meal ratio and tween 80 concentration. The 3D surface plot; (C) and contour plot; (D) showing the interaction between the glucose-to-corn meal ratio and fermentation time. The 3D surface plot (C) and contour plot; (D) showing the interaction between the tween 80 concentration and fermentation time

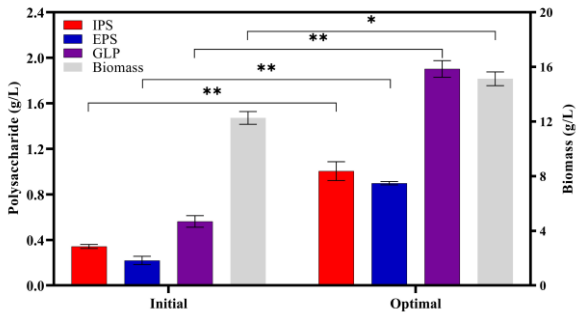



**Fig. 4:** The production of *Ganoderma Lucidum* Polysaccharide (GLP), Intracellular Polysaccharide (IPS), Exopolysaccharide (EPS), and biomass before and after optimization. * represents p≤0.05, and ** represents p≤0.01

Compared to the reported *G. lucidum* strains, such as QRS 5120 (Supramani *et al*., 2019), the Chinese strain (Asadi *et al*., 2021), G0119 (Feng *et al*., 2019), G0041, G0045, and G0059 (Wang *et al*., 2016), the *G. lucidum* strain used in the present study was not a high-producing GLP strain. The RSM was an efficient method for optimizing the fermentation conditions and medium and could increase GLP production by more than 2-fold (Zhang *et al*., 2016). Consistently, the RSM also increased the production of GLP, IPS, and EPS by about 2-3 times. Although the GLP production was not the highest, it could basically meet the requirement for industrial production. The present strain demonstrated a good ability to produce GLP using an inexpensive carbon source (corn meal) and nitrogen source (soybean meal), which is beneficial for minimizing the cost of industrial-scale production. Further study is needed to investigate the mechanism of efficient utilization of corn meal and soybean meal by the *G. lucidum* strain investigated in this study in order to find clues to minimize the cost during industrial production.

## Conclusion

In summary, the optimal SLF condition and medium components for obtaining GLP from the *G. lucidum* strain in this study were as follows: A 70 h inoculum culture time, a 10% inoculum size, a temperature of 22°C, a pH of 5.56, a fermentation time of 105.06 h, 14.07 g/L of glucose, 5.93 g/L of corn meal, 4 g/L of $KH_2PO_4$, 3 g/L of $MgSO_4$, 17.5 g/L of soybean meal and 0.2 mL/L of tween 80. After optimization, the production of GLP, IPS, EPS, and biomass were 1.90, 1.00, 0.90 and 15.13 g/L, respectively. These values basically meet the requirements for the industrial production of GLP. The optimized medium contains inexpensive corn meal and soybean meal and it has shown efficient promotion in fermentation products. This provides clues for minimizing costs during industrial production. The *G. lucidum* strain used in this study is not a good producer of GLP, but its mechanism of utilization of corn meal and soybean meal deserves further study. Moreover, further study should focus on identifying the most efficient GLP producers and investigating the potential use of corn meal and soybean meal to enhance GLP production.

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## Author's Contributions

**Bin Yang:** Contributed to material preparation, data collection, and analysis, drafted the initial preparation, and provided comments.

**Junhu Kai:** Contributed to material preparation, data collection, and analysis.

**Dehui Dai, Guicai Chen and Weilian Hu:** Conceived and designed the study, and provided comments on previous versions of the manuscript.

## Ethics

All authors have read and approved the manuscript and no ethical issues are involved.

## References

Asadi, F., Barshan-Tashnizi, M., Hatamian-Zarmi, A., Davoodi-Dehaghani, F., & Ebrahimi-Hosseinzadeh, B. (2021). Enhancement of exopolysaccharide production from *Ganoderma lucidum* using a novel submerged volatile co-culture system. *Fungal Biology*, *125*(1), 25-31. https://doi.org/10.1016/j.funbio.2020.09.010

Chang, C. J., Lin, C. S., Lu, C. C., Martel, J., Ko, Y. F., Ojcius, D. M., ... & Lai, H. C. (2015). *Ganoderma lucidum* reduces obesity in mice by modulating the composition of the gut microbiota. *Nature Communications*, *6*(1), 1-19. https://doi.org/10.1038/ncomms8489

Chen, H., Niu, J., Qin, T., Ma, Q., & Wang, L. (2015). Optimization of the medium for lactobacillus acidophilus by Plackett-Burman and steepest ascent experiment. *ACTA Scientiarum Polonorum Technologia Alimentaria*, *14*(3), 227-232. https://doi.org/10.17306/J.AFS.2015.3.24

Feng, J., Feng, N., Tang, Q. J., Liu, Y. F., Yang, Y., Liu, F., ... & Lin, C. C. (2019). Optimization of *Ganoderma lucidum* polysaccharides fermentation process for large-scale production. *Applied Biochemistry and Biotechnology*, *189*, 972-986. https://doi.org/10.1007/s12010-019-02968-5

Feng, J., Feng, N., Zhang, J. S., Yang, Y., Jia, W., & Lin, C. C. (2016). A new temperature control shifting strategy for enhanced triterpene production by *Ganoderma lucidum* G0119 based on submerged liquid fermentation. *Applied Biochemistry and Biotechnology*, *180*(4), 740-752. https://doi.org/10.1007/s12010-016-2129-1

Guo, C., Guo, D., Fang, L., Sang, T., Wu, J., Guo, C., ... & Wang, X. (2021). *Ganoderma lucidum* polysaccharide modulates gut microbiota and immune cell function to inhibit inflammation and tumorigenesis in colon. *Carbohydrate Polymers*, *267*, 118231. https://doi.org/10.1016/j.carbpol.2021.118231

Hsu, K. D., Wu, S. P., Lin, S. P., Lum, C. C., & Cheng, K. C. (2017). Enhanced active extracellular polysaccharide production from ganoderma formosanum using computational modeling. *Journal of Food and Drug Analysis*, *25*(4), 804-811. https://doi.org/10.1016/j.jfda.2016.12.006

Kaar, W. E., & Holtzapple, M. T. (1998). Benefits from tween during enzymic hydrolysis of corn stover. *Biotechnology and Bioengineering*, *59*(4), 419-427. https://doi.org/10.1002/(SICI)1097-0290(19980820)59:4<419::AID-BIT4>3.0.CO;2-J

Ma, Z., Xu, M., Wang, Q., Wang, F., Zheng, H., Gu, Z., ... & Ding, Z. (2019). Development of an efficient strategy to improve extracellular polysaccharide production of *Ganoderma lucidum* using L-phenylalanine as an enhancer. *Frontiers in Microbiology*, *10*, 2306. https://doi.org/10.3389/fmicb.2019.02306

Nielsen, S. S. (2017). Total carbohydrate by phenol-sulfuric acid method. *Food Analysis Laboratory Manual*, 137-141. https://doi.org/10.1007/978-3-319-44127-6_14

Shi, M., Zhang, Z., & Yang, Y. (2013). Antioxidant and immunoregulatory activity of *Ganoderma lucidum* Polysaccharide (GLP). *Carbohydrate Polymers*, *95*(1), 200-206. https://doi.org/10.1016/j.carbpol.2013.02.081

Singh, A., Van Hamme, J. D., & Ward, O. P. (2007). Surfactants in microbiology and biotechnology: Part 2. Application aspects. *Biotechnology Advances*, *25*(1), 99-121. https://doi.org/10.1016/j.biotechadv.2006.10.004

Supramani, S., Ahmad, R., Ilham, Z., Annuar, M. S. M., Klaus, A., & Wan, W. A. A. Q. I. (2019). Optimisation of biomass, exopolysaccharide and intracellular polysaccharide production from the mycelium of an identified *Ganoderma lucidum* strain QRS 5120 using response surface methodology. *AIMS Microbiology*, *5*(1), 19. https://doi.org/10.3934/microbiol.2019.1.19

Tang, Y. J., & Zhu, L. W. (2010). Improvement of ganoderic acid and *Ganoderma* polysaccharide biosynthesis by *Ganoderma lucidum* fermentation under the inducement of Cu2+. *Biotechnology Progress*, *26*(2), 417-423. https://doi.org/10.1002/btpr.352

Wang, G., Feng, J., Feng, N., Tang, C., & Zhang, J. (2016). Screening and kinetic study of high-yield intracellular polysaccharide strains of *Ganoderma lucidum* in liquid submerged fermentation. *Acta Agriculturae Shanghai*, *32*(6), 10-17. https://www.cabdirect.org/cabdirect/abstract/20173103466

Wei, Z. H., Liu, L., Guo, X. F., Li, Y. J., Hou, B. C., Fan, Q. L., ... & Zhong, J. J. (2016). Sucrose fed-batch strategy enhanced biomass, polysaccharide and ganoderic acids production in fermentation of *Ganoderma lucidum* 5.26. *Bioprocess and Biosystems Engineering*, *39*, 37-44. https://doi.org/10.1007/s00449-015-1480-x

Xu, M., Zhu, S., Wang, L., Wei, Z., Zhao, L., Shi, G., & Ding, Z. (2021). Influence of selenium biofortification on the growth and bioactive metabolites of *Ganoderma lucidum*. *Foods*, *10*(8), 1860. https://doi.org/10.3390/foods10081860

Yang, X., Yang, Y., Zhang, Y., He, J., & Xie, Y. (2021). Enhanced exopolysaccharide production in submerged fermentation of *Ganoderma lucidum* by tween 80 supplementation. *Bioprocess and Biosystems Engineering*, *44*, 47-56. https://doi.org/10.1007/s00449-020-02418-1

Yuan, B., Chi, X., & Zhang, R. (2012). Optimization of exopolysaccharides production from a novel strain of *Ganoderma lucidum* CAU5501 in submerged culture. *Brazilian Journal of Microbiology*, *43*, 490-497. https://doi.org/10.1590/S1517-83822012000200009

Zhang, D., Wang, X., & Liu, G. (2016). Medium optimization for β-glucan production from *ganoderma lingzhi* in submerged fermentation. *Mycosystema*, *35*(6), 750-759. https://www.cabdirect.org/cabdirect/abstract/2016328349

Zhang, W., & Tang, Y. J. (2008). A novel three-stage light irradiation strategy in the submerged fermentation of medicinal mushroom *Ganoderma lucidum* for the efficient production of ganoderic acid and *Ganoderma* polysaccharides. *Biotechnology Progress*, *24*(6), 1249-1261. https://doi.org/10.1002/btpr.36

Zhou, H., Bi, P., Wu, X., Huang, F., & Yang, H. (2014). Improved polysaccharide production in submerged culture of *Ganoderma lucidum* by the addition of coixenolide. *Applied Biochemistry and Biotechnology*, *172*, 1497-1505. https://doi.org/10.1007/s12010-013-0623-2

**Table S1:** Similarity of the *Ganoderma lucidum* strain used in the present study to other reported strains

| Description | Scientific Name | Max score | Total Score | Query cover (%) | E-value | Per. ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| *Ganoderma lingzhi* strain CGMCC5.2229 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1109 | 1109 | 99 | 0 | 99.67 | 627 | OM721793.1 |
| *Ganoderma lingzhi* isolate AL-R5 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1105 | 1105 | 99 | 0 | 99.51 | 638 | MH160076.1 |
| *Ganoderma lingzhi* isolate AL-R1 small subunit ribosomal RNA gene, partial sequence; internal transcribed | *Ganoderma lingzhi* | 1105 | 1105 | 99 | 0 | 99.51 | 749 | MH160073.1 |

**Table S1:** Continue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | | | | | | | | |
| *Ganoderma lucidum* isolate 39 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1105 | 1105 | 99 | 0 | 99.51 | 925 | MF476201.1 |
| *Ganoderma lucidum* isolate 49 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1105 | 1105 | 99 | 0 | 99.51 | 917 | MF476200.1 |
| *Ganoderma lucidum* isolate 32 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1105 | 1105 | 99 | 0 | 99.51 | 824 | MF476197.1 |
| *Ganoderma lucidum* isolate YS 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1105 | 1105 | 99 | 0 | 99.51 | 765 | KX589250.1 |
| *Ganoderma lingzhi* voucher Dai 12573 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1105 | 1105 | 99 | 0 | 99.51 | 822 | JQ781855.1 |
| *Ganoderma lucidum* strain Gl-22 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1105 | 1105 | 99 | 0 | 99.51 | 765 | GU213483.1 |
| *Ganoderma lucidum* strain Gl-20 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1105 | 1105 | 99 | 0 | 99.51 | 766 | GU213481.1 |
| *Ganoderma lucidum* strain Gl-10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1105 | 1105 | 99 | 0 | 99.51 | 773 | GU213479.1 |
| *Ganoderma* sp. strain DMS2021-8-2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma* sp. | 1103 | 1103 | 99 | 0 | 99.51 | 641 | OK643763.1 |
| *Ganoderma lingzhi* voucher Li245 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1103 | 1103 | 99 | 0 | 99.51 | 664 | JQ781863.1 |
| *Ganoderma lucidum* isolate 46 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1101 | 1101 | 99 | 0 | 99.34 | 914 | MF476199.1 |
| *Ganoderma lucidum* voucher Cui 9164 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1101 | 1101 | 98 | 0 | 99.5 | 625 | JN048774.1 |
| *Ganoderma lucidum* strain GLVN02 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 98 | 0 | 99.5 | 637 | MN636776.1 |
| *Ganoderma lucidum* isolate 61 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 909 | MF476198.1 |
| *Ganoderma lingzhi* isolate SFC20150918-07 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1099 | 1099 | 98 | 0 | 99.5 | 624 | KY364248.1 |
| *Ganoderma lingzhi* isolate SFC20150812-48 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1099 | 1099 | 98 | 0 | 99.5 | 630 | KY364247.1 |
| *Ganoderma lingzhi* isolate SFC20120721-08 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1099 | 1099 | 98 | 0 | 99.5 | 629 | KY364244.1 |
| *Ganoderma lucidum* isolate TK internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 736 | KX589249.1 |
| *Ganoderma lucidum* isolate RB internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 737 | KX589248.1 |
| *Ganoderma lucidum* isolate JQ internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 751 | KX589247.1 |
| *Ganoderma lucidum* isolate HZ 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 762 | KX589246.1 |
| *Ganoderma lucidum* isolate DB 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 770 | KX589245.1 |
| *Ganoderma lucidum* isolate 203 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 770 | KX589244.1 |
| *Ganoderma lingzhi* isolate G32 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1099 | 1099 | 99 | 0 | 99.34 | 786 | KR093032.1 |
| *Ganoderma lucidum* isolate Iran76 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 728 | ON076052.1 |
| *Ganoderma lucidum* isolate Univ.M1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 98 | 0 | 99.5 | 651 | ON256156.1 |
| *Ganoderma lucidum* strain 1904 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 98 | 0 | 99.5 | 636 | MW748295.1 |
| *Ganoderma lucidum* strain FCL194 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 98% | 0 | 99.5 | 636 | JN008870.1 |
| *Ganoderma lucidum* strain CSAAS0801 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 98 | 0 | 99.5 | 651 | FJ940919.1 |
| *Ganoderma lucidum* strain Gl-23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 782 | GU213484.1 |
| *Ganoderma lucidum* strain Gl-14 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 768 | GU213480.1 |
| *Ganoderma lucidum* strain Gl-8 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 99 | 0 | 99.34 | 784 | GU213478.1 |
| *Ganoderma lucidum* strain Gl-7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1099 | 1099 | 98 | 0 | 99.5 | 643 | FJ379262.1 |
| Ganoderma sichuanense isolate B1_1406706 clone 1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | Ganodermasichuanense | 1098 | 1098 | 98% | 0 | 99.34 | 662 | KT693254.1 |
| *Ganoderma lingzhi* clone lynk2103 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1098 | 1098 | 98 | 0 | 99.67 | 612 | OP784796.1 |
| *Ganoderma lingzhi* isolate TQN4R internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1098 | 1098 | 98 | 0 | 99.5 | 604 | MZ497287.1 |
| *Ganoderma lingzhi* isolate LCN8T internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1098 | 1098 | 98 | 0 | 99.5 | 605 | MZ470735.1 |
| *Ganoderma lingzhi* strain CGMCC5.2231 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1098 | 1098 | 99 | 0 | 99.34 | 629 | OM721795.1 |

**Table S1:** Continue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Ganoderma lingzhi* strain CGMCC5.2230 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1098 | 1098 | 99 | 0 | 99.34 | 628 | OM721794.1 |
| *Ganoderma lingzhi* strain CGMCC5.2228 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1098 | 1098 | 99 | 0 | 99.34 | 624 | OM721792.1 |
| Ganoderma sp. strain TS2021-8-12 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma* sp. | 1098 | 1098 | 99 | 0 | 99.34 | 659 | OL998882.1 |
| *Ganoderma lingzhi* isolate AL-R2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1096 | 1096 | 99 | 0 | 99.18 | 636 | MH160074.1 |
| *Ganoderma lingzhi* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: FB-0001 | *Ganoderma lingzhi* | 1096 | 1096 | 98 | 0 | 99.34 | 702 | LC090753.1 |
| *Ganoderma lingzhi* isolate TQN3R internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1096 | 1096 | 97 | 0 | 99.67 | 606 | MZ497286.1 |
| *Ganoderma* sp. isolate LCN10T internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | Ganoderma sp. | 1096 | 1096 | 97% | 0 | 99.67 | 606 | MZ489261.1 |
| *Ganoderma lucidum* voucher S32_3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1096 | 1096 | 98% | 0 | 99.5 | 605 | MW520976.1 |
| *Ganoderma lucidum* strain Gl-16 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1096 | 1096 | 98% | 0 | 99.34 | 644 | FJ379263.1 |
| *Ganoderma lucidum* strain WD-565 voucher TFM-F 15131 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1096 | 1096 | 98 | 0 | 99.34 | 636 | EU021455.1 |
| *Ganoderma lingzhi* isolate TG2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1094 | 1094 | 97 | 0 | 99.67 | 602 | MN372059.1 |
| *Ganoderma lingzhi* isolate AL-R13 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1094 | 1094 | 99 | 0 | 99.18 | 755 | MH160082.1 |
| *Ganoderma lingzhi* isolate SFC20160420-01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1094 | 1094 | 98 | 0 | 99.34 | 625 | KY364250.1 |
| *Ganoderma lucidum* voucher TS5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 627 | ON196269.1 |
| *Ganoderma lucidum* voucher TS45 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 624 | ON196266.1 |
| *Ganoderma lucidum* voucher 522635MF42 (HGASMF01-4994) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 97 | 0 | 99.67 | 608 | MZ057681.1 |
| *Ganoderma lucidum* isolate XH002 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 636 | MW947476.1 |
| Ganoderma sp. ASI 7038 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma* sp. ASI 7038 | 1094 | 1094 | 98% | 0 | 99.34 | 636 | JQ520210.1 |
| *Ganoderma lucidum* strain IUM 4303 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 636 | JQ520182.1 |
| *Ganoderma lucidum* cultivar Yeongji-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 636 | JQ520169.1 |
| *Ganoderma lucidum* strain IUM 4002 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 636 | JQ520178.1 |
| *Ganoderma lucidum* strain IUM 0047 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 636 | JQ520174.1 |
| Ganoderma sp. isolate G8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma* sp. | 1094 | 1094 | 98 | 0 | 99.34 | 613 | MN579547.1 |
| Ganoderma sp. isolate G7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma* sp. | 1094 | 1094 | 98 | 0 | 99.34 | 613 | MN579546.1 |
| Ganoderma sp. isolate G2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma* sp. | 1094 | 1094 | 98 | 0 | 99.34 | 614 | MN579544.1 |
| *Ganoderma* sp. Jfl1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma* sp. Jfl1 | 1094 | 1094 | 98 | 0 | 99.34 | 636 | HQ689696.1 |
| *Ganoderma lucidum* strain FCL188 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 636 | JN008869.1 |
| *Ganoderma lucidum* strain XZ-G-B 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 660 | HQ235632.1 |
| *Ganoderma lucidum* strain XZ-G-A1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 660 | HQ235630.1 |
| *Ganoderma lucidum* strain Gt-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 99 | 0 | 99.18 | 785 | GU213487.1 |
| *Ganoderma lucidum* strain Gl-3B 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 99 | 0 | 99.18 | 786 | GU213477.1 |
| *Ganoderma lucidum* strain Gl-3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 99 | 0 | 99.18 | 768 | GU213476.1 |
| *Ganoderma lucidum* strain WD-2038 voucher TFM-F 18922 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1094 | 1094 | 98 | 0 | 99.34 | 637 | EU021456.1 |
| *Ganoderma* sp. strain 16SHD01-01 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma* sp. | 1092 | 1092 | 98 | 0 | 99.17 | 647 | MN911335.1 |
| *Ganoderma* sp. strain 16JL01-01 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma* sp. | 1092 | 1092 | 98 | 0 | 99.18 | 628 | MN911333.1 |
| *Ganoderma lingzhi* isolate HN internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1092 | 1092 | 98 | 0 | 99.34 | 615 | MN372067.1 |
| *Ganoderma lucidum* strain H1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1092 | 1092 | 99 | 0 | 99.18 | 607 | KX262899.1 |
| *Ganoderma lucidum* strain P2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1092 | 1092 | 99 | 0 | 99.17 | 611 | KX262898.1 |
| *Ganoderma lucidum* strain P internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1092 | 1092 | 98 | 0 | 99.34 | 613 | KX262896.1 |
| Ganoderma sichuanense isolate B1_1406706 clone 2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | Ganoderma sichuanense | 1092 | 1092 | 97% | 0 | 99.83 | 646 | KT693255.1 |
| *Ganoderma lucidum* voucher TS55 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1092 | 1092 | 98 | 0 | 99.34 | 624 | ON196273.1 |
| *Ganoderma lucidum* voucher TS38 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1092 | 1092 | 98 | 0 | 99.34 | 634 | ON196261.1 |

**Table S1:** Continue

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Ganoderma lucidum* voucher TS36 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1092 | 1092 | 98 | 0 | 99.34 | 624 | ON196259.1 |
| *Ganoderma lucidum* voucher TS28 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1092 | 1092 | 98 | 0 | 99.34 | 626 | ON196252.1 |
| *Ganoderma lucidum* voucher NH1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1092 | 1092 | 98 | 0 | 99.34 | 639 | ON196224.1 |
| *Ganoderma lucidum* strain Han G internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1092 | 1092 | 99 | 0 | 99.18 | 640 | JX162764.1 |
| *Ganoderma lucidum* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: NBRC 31863 | *Ganoderma lucidum* | 1092 | 1092 | 98 | 0 | 99.17 | 636 | AB733122.1 |
| *Ganoderma lingzhi* isolate SCIM 1006 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lingzhi* | 1092 | 1092 | 97 | 0 | 99.83 | 621 | MT741782.1 |
| *Ganoderma lucidum* voucher TS9 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 623 | ON196281.1 |
| *Ganoderma lucidum* voucher TS7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 633 | ON196279.1 |
| *Ganoderma lucidum* voucher TS58 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 624 | ON196276.1 |
| *Ganoderma lucidum* voucher TS37 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 623 | ON196260.1 |
| *Ganoderma lucidum* voucher TS2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 620 | ON196245.1 |
| *Ganoderma lucidum* voucher TS13 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 618 | ON196239.1 |
| *Ganoderma lucidum* voucher NH8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 623 | ON196234.1 |
| *Ganoderma lucidum* voucher NH7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 623 | ON196233.1 |
| *Ganoderma lucidum* voucher NH6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 623 | ON196232.1 |
| *Ganoderma lucidum* voucher NH3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.34 | 623 | ON196229.1 |
| *Ganoderma lucidum* strain FCL195 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence | *Ganoderma lucidum* | 1090 | 1090 | 98 | 0 | 99.17 | 636 | JN008871.1 |

**Table S2:** Factors and levels of the single-factor experiment

| | Fermentation conditions | | | | | Medium components | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Levels | Inoculum culture time (h) | Inoculum size (%) | Initial pH | Temperature (°C) | Fermentation time (h) | Carbon source | Ratio of glucose and corn meal | Mixed carbon (g/L) | Nitrogen source | Soybean meal (g/L) | $KH_2PO_4$ (g/L) | $MgSO_4$ (g/L) | Tween 20 (mL/L) | Tween 80 (mL/L) |
| 1 | 30 | 6 | 3 | 22 | 96 | Soluble starch | 1.5:7.5 | 10 | Tryptone | 5.0 | 0 | 0.0 | 0.0 | 0.0 |
| 2 | 40 | 8 | 4 | 25 | 108 | Glucose | 3:7 | 20 | Yeast extract | 7.5 | 1 | 1.0 | 0.2 | 0.2 |
| 3 | 50 | 10 | 5 | 28 | 120 | Sucrose | 4.5:5.5 | 30 | tryptone +10 yeast extract (1:1) | 2.0 | 2 | 0.4 | 0.4 | |
| 4 | 60 | 12 | 5.56 (natural) | 31 | 132 | Corn meal | 6:4 | 40 | Soymeal | 12.5 | 3 | 3.0 | 0.6 | 0.6 |
| 5 | 70 | | 6 | | 144 | Malt extract | 7.5:2.5 | | $NaNO_3$ | 15.0 | 4 | 4.0 | 0.8 | 0.8 |
| 6 | 80 | | 7 | | 156 | Corn starch | 9:1 | | $(NH4)_2SO_4$ | 17.5 | 5 | 5.0 | 1.0 | 1.0 |
| 7 | | | | | | | | | $CO(NH_2)_2$ | 20.0 | | | | |

**Table S3:** Effect evaluations of each factor under Plackett-Burman design based on exopolysaccharide production

| Factors | Effect | Standard error | F-value | p-value | Significance |
|---|---|---|---|---|---|
| Inoculum culture time (h) | -0.028 | 0.011 | 5.920 | 0.135 | |
| Inoculum size (%) | -0.049 | 0.011 | 18.370 | 0.050 | |
| Glucose: Corn meal | 0.093 | 0.011 | 67.070 | 0.015 | * |
| Soybean meal (g/L) | 0.025 | 0.011 | 4.690 | 0.163 | |
| $KH_2PO_4$ (g/L) | 0.040 | 0.011 | 12.330 | 0.072 | |
| $MgSO_4$ (g/L) | 0.015 | 0.011 | 1.670 | 0.325 | |
| Temperature (°C) | -0.006 | 0.011 | 0.287 | 0.646 | |
| Tween 80 (mL/L) | 0.084 | 0.011 | 54.610 | 0.018 | * |
| Fermentation time (h) | 0.077 | 0.011 | 46.330 | 0.021 | * |

**Table S4:** Effect evaluations of each factor under Plackett-Burman design based on intracellular polysaccharide production

| Factors | Effect | Standard error | F-value | p-value | Significance |
|---|---|---|---|---|---|
| Inoculum culture time (h) | -0.002 | 0.009 | 0.063 | 0.826 | |
| Inoculum size (%) | 0.045 | 0.009 | 25.860 | 0.037 | * |
| Glucose: corn meal | 0.012 | 0.009 | 1.970 | 0.296 | |
| Soybean meal (g/L) | 0.012 | 0.009 | 1.870 | 0.305 | |
| $KH_2PO_4$ (g/L) | 0.028 | 0.009 | 10.230 | 0.085 | |
| $MgSO_4$ (g/L) | -0.068 | 0.009 | 58.970 | 0.017 | * |
| Temperature (°C) | 0.012 | 0.009 | 1.980 | 0.295 | |
| Tween 80 (mL/L) | 0.010 | 0.009 | 1.400 | 0.358 | |
| Fermentation time (h) | 0.083 | 0.009 | 87.640 | 0.011 | * |

**Table S5:** Effect evaluations of each factor under Plackett-Burman design based on biomass

| Factors | Effect | Standard error | F-value | p-value | Significance |
|---|---|---|---|---|---|
| Inoculum culture time (h) | -0.015 | 0.003 | 19.060 | 0.049 | * |
| Inoculum size (%) | -0.025 | 0.003 | 52.940 | 0.018 | * |
| Glucose: Corn meal | -0.013 | 0.003 | 13.240 | 0.068 | |
| Soybean meal (g/L) | -0.003 | 0.003 | 0.941 | 0.434 | |
| $KH_2PO_4$ (g/L) | -0.016 | 0.003 | 21.240 | 0.044 | * |
| $MgSO_4$ (g/L) | -0.013 | 0.003 | 15.060 | 0.060 | |
| Temperature (°C) | 0.003 | 0.003 | 0.529 | 0.543 | |
| Tween 80 (mL/L) | -0.013 | 0.003 | 13.240 | 0.068 | |
| Fermentation time (h) | 0.022 | 0.003 | 39.760 | 0.024 | * |