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RESEARCH PAPER

Enhanced exopolysaccharide production in submerged fermentation of *Ganoderma lucidum* by Tween 80 supplementation

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Abstract

Bioactive polysaccharides extracted from *Ganoderma lucidum* (*G. lucidum*) have been widely applied in food and medicine for their multiple functions. In this study, *G. lucidum* exopolysaccharide (EPS) production in submerged fermentation was stimulated by Tween 80. The addition of 0.25% Tween 80 on day 3 gave a maximum production of mycelial biomass and EPS, with an increase of 19.76 and 137.50%, respectively. Analysis of fermentation kinetics showed that glucose was consumed faster after adding Tween 80, while the expression of EPS biosynthesis-related genes and ATP generation were greatly improved. Moreover, Tween 80 resulted in the significant accumulation of reactive oxygen species and increased cell membrane and cell wall permeability. The EPS from Tween 80-containing medium had higher contents of carbohydrate and uronic acid, lower molecular weight, and higher antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals than those of EPS produced in the absence of Tween 80. This study provides further evidence to clarify the stimulatory effects of Tween 80 in fermentation and provides a guide for the production of bioactive *G. lucidum* EPS.

Keywords *Ganoderma lucidum* · Exopolysaccharide · Submerged fermentation · Tween 80

Introduction

Ganoderma lucidum (*G. lucidum*), commonly known as Lingzhi in China, is a traditional medicinal mushroom that has been widely used for treating and preventing various diseases in Asia [1]. Polysaccharides, the major bioactive components of *G. lucidum*, have been applied in the food and pharmaceutical industries because of their antioxidant, anti-inflammatory, antimicrobial and antitumor capabilities [2]. Polysaccharides can be isolated from fruiting bodies, cultured mycelium and cultured broth [3]. Normally, it takes months to harvest fruiting bodies by solid cultures using substrates such as wood, grain or sawdust [4]. The quality of the fruiting body is also seriously affected by cultivation management [5]. In contrast, submerged fermentation of mycelium is an effective alternative approach that can obtain more bioactive components than the harvesting of fruiting bodies with a shorter culture period, a consistent product and seasonal independence [6, 7].

Many efforts have been made to improve polysaccharide production by submerged fermentation to satisfy growing consumer demand. Controlling the pH, temperature and dissolved oxygen content or the optimization of the medium composition have been reported [4]. Recently, the addition of chemical agents has become a new strategy to improve fermentation performance. Surfactants, vegetable oil and fatty acids are low-cost materials and can be removed from fermentation mixtures by simple evaporation. Many of these materials have been used as effective stimulants to enhance the production efficiency of useful metabolites (e.g., polysaccharides [8], pigments [9], and enzymes [10]) in microorganisms.

Xiaobing Yang and Yingyin Yang are the co-first authors and have the equal contributions.

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Tween 80, namely polyoxyethylene glycol sorbitan monooleate, is one of the most important nonionic surfactants that can decrease surface and interfacial tensions as well as increase solubility and bioavailability [11]. Although Tween 80 has been widely applied to increase the production of xanthan, pullulan and curdlan [12–14], its usage to increase exopolysaccharide (EPS) production of mushrooms in submerged fermentation is not common. In particular, the effect of Tween 80 on EPS production in *G. lucidum* has not yet been studied. The mechanism underlying the increased EPS production in the presence of Tween 80 was related to the fatty acid composition of the cell membrane and the activity of polysaccharide biosynthesis enzymes [14, 15]. More information is still needed to obtain an in-depth understanding.

In this study, Tween 80 was added to the liquid medium of *G. lucidum*. The Tween 80 concentration and addition time were optimized to improve EPS yield. The mechanism by which Tween 80 enhanced *G. lucidum* EPS production was investigated. Finally, the antioxidant activity and the structural characteristics of the EPS were also evaluated primarily.

Materials and methods

Strains and culture conditions

G. lucidum YW01 was obtained from the Guangdong Institute of Microbiology (Guangzhou, China) and cultivated on potato dextrose agar (PDA; Huankai Microbial, China) plates at 28 ℃. To prepare the seed culture, fresh mycelia from PDA plates were transferred to 250 mL conical flasks containing 100 mL of modified Martin broth (20 g/L glucose, 5 g/L tryptone, 2 g/L yeast extract, 0.5 g/L $MgSO_4$ and 1 g/L KH_2PO_4), and cultured at 28 °C and 150 rpm in a shaker for 7 days. Then, the seed culture was inoculated at 10% (v/v) into 100 mL of modified Martin broth and cultured at 28 °C and 150 rpm. Tween 80 (Macklin, China) at concentrations of 0.06, 0.12, 0.25, 0.5, 0.75, 1 and 1.25% (w/v) was added to the medium on day 0 of the seven-day fermentation. Subsequently, the optimal concentration of Tween 80 was added to the medium on days 0, 1, 3, or 5 of the seven-day fermentation to optimize the addition time.

Determination of biomass, polysaccharide production and residual sugar

Mycelia were separated from the fermentation broth by vacuum filtration. The mycelia were washed with distilled water and dried at 50 ℃ to a constant weight for biomass determination. The dry mycelia were then extracted with boiling water for 2 h. The extract and mycelium-free fermentation broth were added with four volumes of absolute ethanol, respectively, and allowed to stand overnight at 4 ℃. The precipitated intracellular polysaccharide (IPS) from mycelia and EPS from the fermentation broth were collected by centrifugation. The contents were assayed by the phenol sulfuric acid method [16]. The amount of residual sugar in the fermentation broth was measured by the 3,5-dinitrosalicylic acid method [17].

Compositional analysis of EPS

The carbohydrate content of the EPS was measured by the phenol sulfuric acid method. The protein content was measured by Bradford's method [18]. The uronic acid content was estimated by the meta-hydroxydiphenyl method [19]. The residual Tween 80 content was determined by the cobalt thiocyanate ammonium color-developing method [20].

The molecular weight (Mw) of the EPS was determined using a high-performance gel permeation chromatography system (Shimadzu, Japan) equipped with a TSK-G5000PWXL column (7.5 × 300 mm) and a refractive index detector. The mobile phase was ultrapure water with a flow rate of 1 mL/min, and the column temperature was maintained at 30 ℃. The Mw of the EPS was calculated using the calibration curve of dextran standards.

Real-time quantitative PCR

Fresh mycelia were collected from the fermentation broth and ground into a powder with liquid nitrogen. Total RNA was extracted from 100 mg of mycelium powder using a Plant RNA Extraction Kit (Tiangen Biotech, China) according to the manufacturer's instructions. The PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Japan) was used for cDNA synthesis, and TB Green Premix Ex Taq (Takara) was used for real-time quantitative PCR. PCR was performed on an Applied Biosystems QuantStudio 6 (Thermo Scientific, USA). The *18S rRNA* gene was used as an internal gene for normalization, and the relative gene expression was analyzed according to the $2^{-\Delta\Delta C_T}$ method [21]. The primers used are shown in Supplemental Table S1.

ATP detection assay

Fresh mycelia were ground into a powder with liquid nitrogen. Then, 20 mg of mycelium powder was resuspended in the lysis reagent of an ATP Assay Kit (Beyotime, China) and centrifuged at 4 ℃ and 12,000 rpm for 5 min. The supernatant was used for ATP detection according to the protocol of the ATP Assay Kit, which is based on the luciferase-luciferin reaction.

Assessment of cell membrane permeability

Fresh 5-day-fermented mycelia (300 mg) were treated with 1 mL of 1.5% (w/v) lywallzyme (Guangdong Institute of Microbiology) containing 0.6 M mannitol at 30 ℃ for 2 h. The cells were washed twice and stained with propidium iodide (PI; Sigma, USA) for 30 min at room temperature in the dark. The fluorescence intensity was measured using a flow cytometer (BD Bioscience, USA).

Quantification of β-1,3-glucan and chitin

Five-day-fermented mycelia were ground into a fine powder with liquid nitrogen. The aniline blue assay was used to determine the content of β-1,3-glucan [22]. Briefly, 20 mg mycelium powder was extracted in 1 mL of 1 M NaOH at 52 ℃ for 30 min. Then, 50 µL of the extract was mixed with 185 µL of aniline blue solution (0.067% aniline blue, 0.35 N HCl and 0.98 M glycine–NaOH; pH 9.5). The mixture was incubated at 52 ℃ for 30 min and then cooled to room temperature. The absorbance was measured using a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek, USA) with excitation and emission wavelengths of 405 nm and 460 nm, respectively.

A chitin detection assay was performed as follows [22]: first, 5 mg of mycelium powder was treated with 3 mL of saturated KOH at 130 ℃ for 1 h. After cooling to room temperature, the sample was mixed with 8 mL of ice-cold 75% (v/v) ethanol and placed in an ice bath for 15 min. The mixture was combined with 300 µL of 13.3% (w/v) Celtite 545 and centrifuged at 4 ℃ and 5,000 rpm for 5 min. The precipitate was washed with ice-cold 40% (v/v) ethanol and ice-cold water and resuspended in 0.5 mL of distilled water. The resuspended solution, standard solution (10 µg/mL glucosamine) and blank control (distilled water) were combined with 0.5 mL of 5% (w/v) $NaNO_2$ and 0.5 mL of 5% (w/v) $KHSO_4$ and centrifuged at 4 ℃ at 10,000 rpm for 5 min. Then, 150 µL of supernatant was mixed with 450 µL of water and 200 µL of 12.5% (w/v) NH_4 sulfamate. The mixture was combined with 0.5 mL of 5 mg/mL 3-methylbenzthiazolinone-2-hydrazone and boiled for 3 min. After cooling to room temperature, the solution was incubated with 200 µL of 0.83% (w/v) $FeCl_3$ for 30 min. The absorbance at 650 nm was read on a visible light spectrophotometer (Thermo Scientific). The concentration of glucosamine decomposed by chitin was calculated as follows: [(A_{650} unknown − A_{650} blank) × 10 µg/mL] / (A_{650} standard − A_{650} blank).

Measurement of reactive oxygen species (ROS)

The level of intracellular ROS was detected by the 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma) assay. Five-day-fermented mycelia were stained with 10 µM DCFH-DA in the dark for 20 min and washed twice with phosphate-buffered saline (PBS; Sangon, China). The fluorescence was detected using a Cytation 5 Cell Imaging Multi-Mode Reader with excitation and emission wavelengths of 488 and 525 nm, respectively.

Antioxidant activity assay

The antioxidant activity of the EPS was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma) radical scavenging method [23]. Briefly, 1 mL of EPS solution at a series of concentrations was mixed with 1 mL of 0.1 mM DPPH. Each mixture was incubated in the dark at room temperature for 30 min, and then their absorbances were measured at 517 nm.

The DPPH radical scavenging activity of each sample was calculated according to the following equation:

$$\text{Scavenging activity } (\%) = \left[A_0 - \left(A_x - A_i\right)\right] / A_0 \times 100\%,$$

where A_0 is the absorbance of the control group without EPS, A_x is the absorbance of the EPS sample, and A_i is the absorbance for the background without DPPH.

Statistical analysis

Unless otherwise stated, all data are presented as the mean ± standard deviation of three replicates. Statistical analysis was performed using the Student's *t* test. A *p* value of < 0.05 was considered statistically significant.

Results and discussion

Mycelial growth and EPS production under different Tween 80 concentrations and addition times

The *G. lucidum* mycelial biomass and EPS yield achieved by supplementing 0.06–1.25% Tween 80 on day 0 are shown in Fig. 1a. Tween 80 at concentrations of 0.06–0.25% had no significant effect on mycelial biomass accumulation, while at a concentration above 0.25%, Tween 80 inhibited mycelial growth. EPS production reached a maximum yield of 1.02 g/L when the added Tween 80 concentration increased to 0.25% but then decreased to 0.71 g/L when the Tween 80 concentration increased to 1.25%. The optimal concentration of Tween 80 for mycelial growth and EPS production of *G. lucidum* was found to be 0.25%. A high concentration of Tween 80 resulted in the formation of excessive foam, which caused an adverse effect not only on the sterile environment but also on mass and heat transfer during

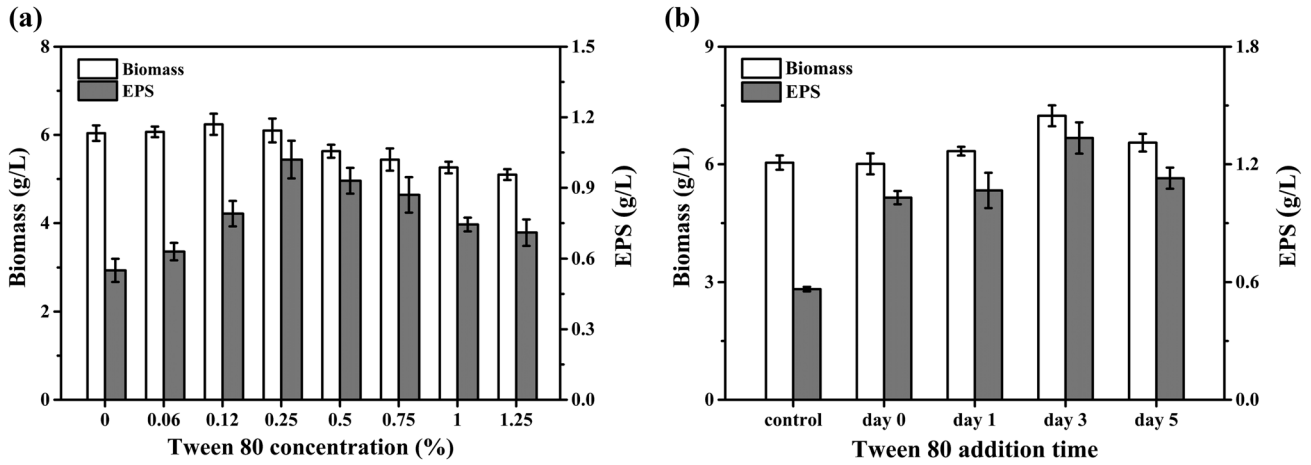

Fig. 1 Influences of Tween 80 **a** concentration and **b** addition time on *G. lucidum* mycelial biomass and EPS production during 7 days of fermentation

submerged fermentation [8]. This might explain the reduction in *G. lucidum* biomass and EPS yield when the Tween 80 concentration was higher than 0.25%. Previous studies reported that the optimal concentrations of Tween 80 used for EPS production of *Aureobasidium pullulans* and *Agrobacterium* sp. were 0.5 and 1.6%, respectively [12, 14]. This discrepancy indicated that different species responded differently to this additive.

The optimal concentration of Tween 80 (0.25%) was then added on days 0, 1, 3, and 5 within the fermentation period. These time points represented the beginning, early exponential phase, middle exponential phase and late exponential phase of fermentation, respectively, based on the strain culturing features. The results revealed that the maximal mycelial biomass (7.24 g/L) and EPS yield (1.33 g/L) were achieved by adding Tween 80 at the middle exponential phase (on day 3) and were significantly increased by 19.76 and 137.50% compared to the control, respectively (Fig. 1b). A previous study found that Tween 80 as an oxygen vector increased oxygen transfer efficiency and promoted the production of metabolites [24]. The oxygen demand was remarkably increased as cell growth and metabolic activity increased, leading to the removal of dissolved oxygen in the middle and late exponential phase [25]. The addition of Tween 80 at these phases might efficiently alleviate oxygen limitation, resulting in improved mycelial growth and EPS production. It is noteworthy that the addition of Tween 80 at the late exponential phase caused lower mycelial biomass and EPS yield than those achieved with the addition of Tween 80 at the middle exponential phase. This was possibly due to the decreased cell viability in the late exponential phase. Thus, 0.25% Tween 80 added at day 3 was selected for subsequent studies.

Effects of Tween 80 on fermentation kinetics of *G. lucidum*

Comparison of the fermentation performance of *G. lucidum* with and without 0.25% Tween 80 is shown in Fig. 2. The results revealed that the mycelial growth rate of the Tween 80 group was faster than that of the control, and the biomass increased to 7.39 g/L on day 8 versus 6.08 g/L in the control (Fig. 2a). IPS production was also significantly promoted by Tween 80 (Fig. 2b). The IPS yield of the Tween 80 group reached the maximum level of 0.51 g/L on day 8 and then decreased until day 10. The EPS yield of the Tween 80 group increased faster than that of the control and reached 1.45 g/L on day 10 (Fig. 2c). Tween 80 increased EPS production primarily by stimulating mycelial growth and IPS production. Furthermore, the glucose in the Tween 80-containing medium was consumed more rapidly than that in the absence of Tween 80 (Fig. 2d). At the end of the fermentation, the glucose content in the Tween 80-containing medium (2.76 g/L) was lower than that in the control group (4.61 g/L). These results implied that the nutrient absorption efficiency was enhanced in Tween 80-treated *G. lucidum*, and it subsequently increased the mycelial biomass and EPS yield.

Culture pH is one of the important environmental factors affecting the cell growth and polysaccharide production of *G. lucidum* [26]. Time-course changes in pH value with and without the presence of 0.25% Tween 80 are shown in Fig. S1. During the first 6 days of fermentation, the consumption of substrate and dissolved oxygen could cause a decrease in the pH value [26]. The minimum pH value of the Tween 80 group was 3.61 on day 6 compared to 3.70 in the control, which was in accordance with the higher glucose consumption rate of the Tween 80 group. Thereafter, the pH of the

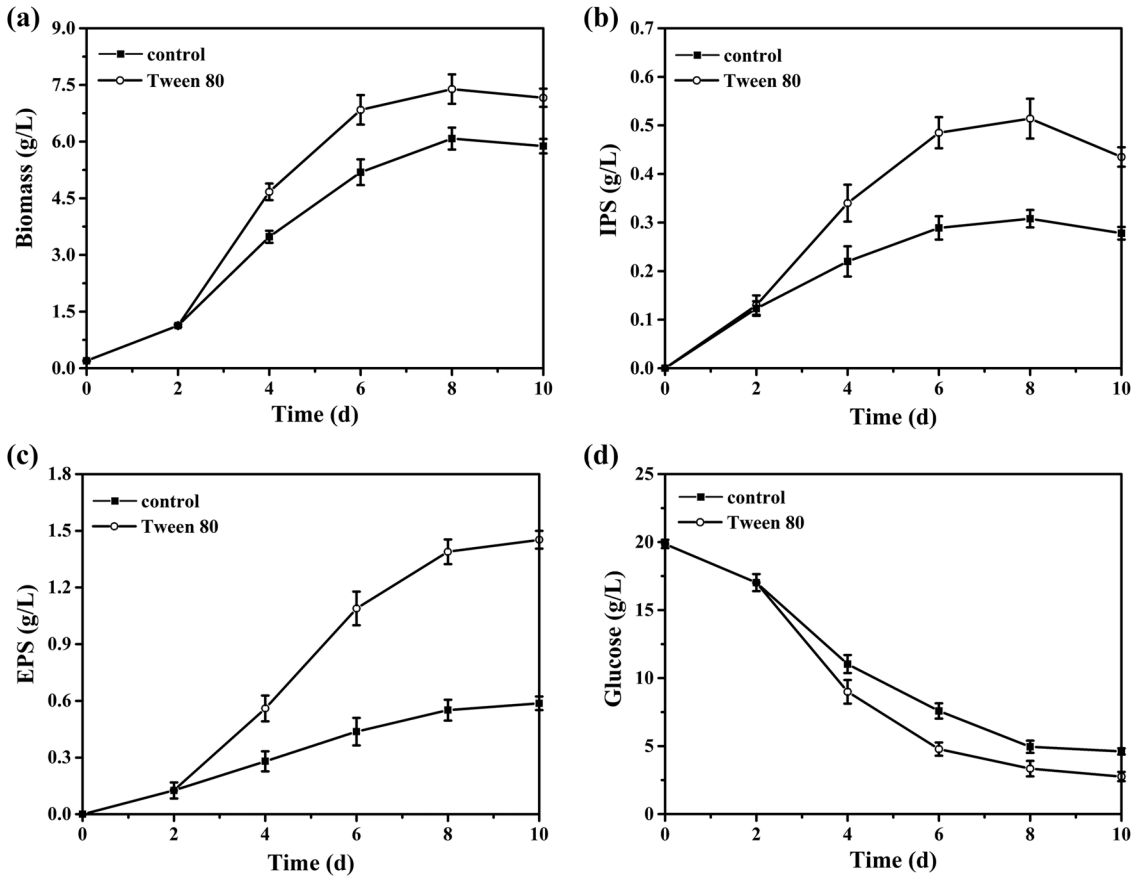

Fig. 2 Fermentation profiles of *G. lucidum* with or without the addition of Tween 80. **a** Mycelial biomass, **b** IPS production, **c** EPS production, and **d** glucose consumption. Tween 80 (0.25%) was added on the third day of the ten-day fermentation. Values are the mean ± standard deviation of three replicates

control group marginally increased to 3.83, while that in the Tween 80 group remained relatively constant between 3.61 and 3.67. It was reported that the intracellular components would be released due to the lysis of cells at the end of fermentation, thereby increasing the pH value of the culture broth [27]. The lower final pH value of the Tween 80-containing medium implied that Tween 80 could prevent disintegration due to the shearing forces, improving EPS yield [15].

Expression of EPS biosynthesis-related genes with Tween 80 addition

The polysaccharide biosynthetic pathway of *G. lucidum* has been partially elucidated. Phosphoglucomutase (PGM) and uridine diphosphate glucose pyrophosphorylase (UGP) are key enzymes for the production of precursors of polysaccharides [28]. The expression levels of the genes *pgm* and *ugp* in Tween 80-treated *G. lucidum* are presented in Fig. 3. The results showed that the expression levels of *pgm* and *ugp* were greatly stimulated by the presence of Tween 80. The maximum transcription levels of these two genes were achieved on day 8, and they were 3.14- and 2.98-fold greater than those observed in the control, respectively. These findings corresponded to the EPS production pattern under Tween 80 treatment (Fig. 2c). Tween 80 also induced transcription of the related polysaccharide biosynthesis genes in *Aureobasidium pullulans* and *Agrobacterium* sp. for pullulan and curdlan production, respectively [12, 24]. Therefore, it can be proposed that Tween 80 regulated EPS biosynthesis by affecting the expression level of related genes in *G. lucidum*.

Intracellular ATP levels under Tween 80 conditions

Since ATP directly provides energy for various cellular processes, such as precursor generation and EPS secretion in the biosynthesis of EPS [28], the ATP levels were also measured during fermentation. A large amount of energy was needed to support the rapid growth of mycelia and EPS synthesis during the first 6 days of fermentation, which led to a fast increase in ATP generation (Fig. 4). Thereafter, ATP

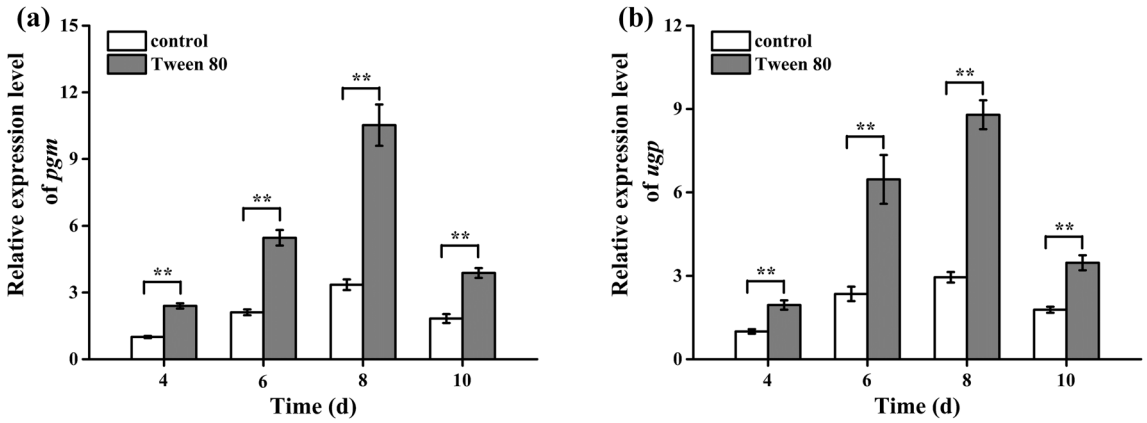

Fig. 3 Transcription levels of the **a** *pgm* and **b** *ugp* genes in Tween 80-treated *G. lucidum*. Data are presented as the mean ± standard deviation of three replicates. $^{**}P < 0.01$

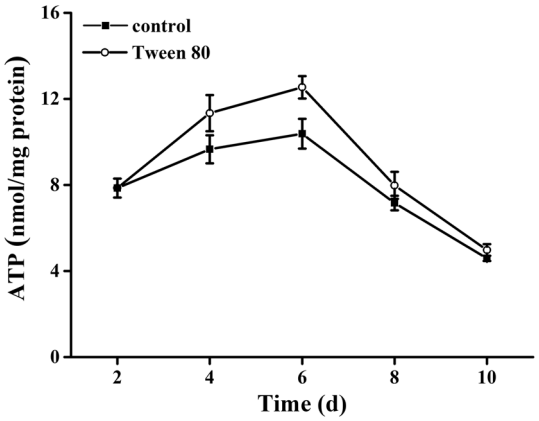

Fig. 4 Comparison of intracellular ATP levels between control *G. lucidum* and Tween 80-treated *G. lucidum*

generation gradually decreased as cell viability decreased. It is noteworthy that the ATP levels of the Tween 80 group were greater than those of the control during the whole fermentation process. A previous study also showed that Tween 80-treated *Agrobacterium* sp. produced more ATP and curdlan than the untreated strain [12]. ATP generation mainly depends on the oxidative phosphorylation of the respiratory chain [29]. The increase in oxygen transfer efficiency induced by Tween 80 could be conducive to oxidative phosphorylation, which provided more ATP for EPS synthesis and secretion [12, 24].

Changes in cell membrane permeability and cell wall components by Tween 80

PI is a red-fluorescent nuclear dye and is normally blocked by the cell membrane. This dye will only bind to nucleic acids of cells where the plasma membrane has been permeabilized [30]. Therefore, the effect of Tween 80 on cell membrane permeability was studied by measuring the fluorescence intensity of PI-stained cells. As seen in Fig. 5, the fluorescence intensity of Tween 80-treated *G. lucidum* cells was 1.79-fold higher than that of control cells, indicating that Tween 80 significantly increased the cell membrane permeability. The increased cell membrane permeability might be attributed to changes in the composition and contents of unsaturated fatty acids in the cell membrane, which could facilitate the uptake of nutrients as well as secretion of macromolecule EPS [14, 15].

The contents of β-1,3-glucan and chitin in the cell wall of *G. lucidum* were also measured. As shown in Fig. 6a, the contents of β-1,3-glucan and chitin of the cell wall in the Tween 80 treatment group were significantly decreased by 17.66 and 23.19% compared with those in the control, respectively. Moreover, the transcription levels of putative glucan synthase genes (GL20535, GL24465 and GL24554) and chitin synthase genes (GL15273, GL18134, GL25613, GL27969, GL28060, GL30737, GL30799 and GL31550) [31] were determined. The transcription levels of these genes were significantly downregulated in Tween 80-treated *G. lucidum* (Fig. 6b, c). As the main components of the fungal cell wall, β-1,3-glucan and chitin play an important role in cell wall structure [32]. The decrease of β-1,3-glucan and chitin had a positive impact on cell wall permeability, which was beneficial for EPS secretion [22].

Previous studies confirmed that ROS accumulation resulted in the increased cell membrane and cell wall permeability [33, 34]. The increased ROS level in Tween 80-treated *G. lucidum* (Fig. S2) here might have also contributed to the elevation of the cell membrane and cell wall permeability. The new evidence we provided is the first to show that Tween 80 modulates fungal cell membrane and cell wall permeability. A high concentration of Tween 80 inhibited mycelial growth and EPS production (Fig. 1a) because it severely affected the cell membrane and cell wall

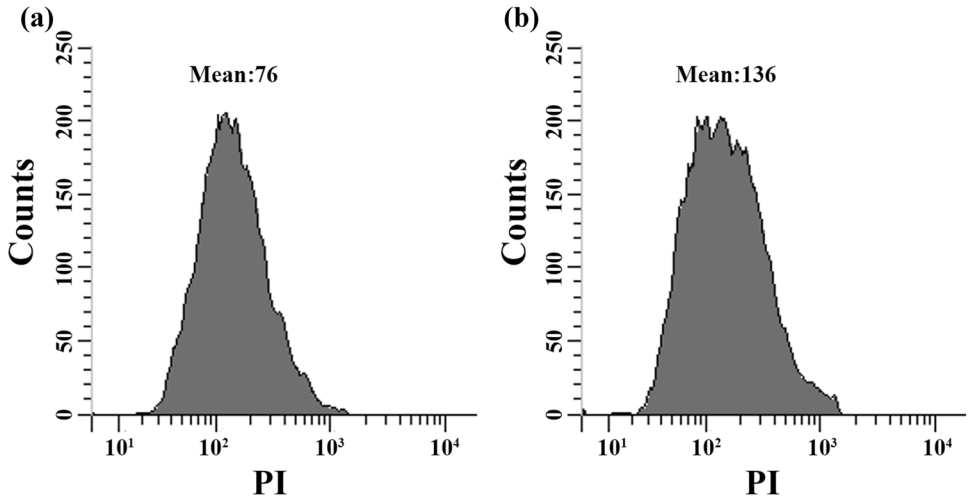

Fig. 5 Effect of Tween 80 on the cell membrane permeability of *G. lucidum*. The cells of **a** control *G. lucidum* and **b** Tween 80-treated *G. lucidum* were stained with PI. The fluorescence intensity was determined by flow cytometry

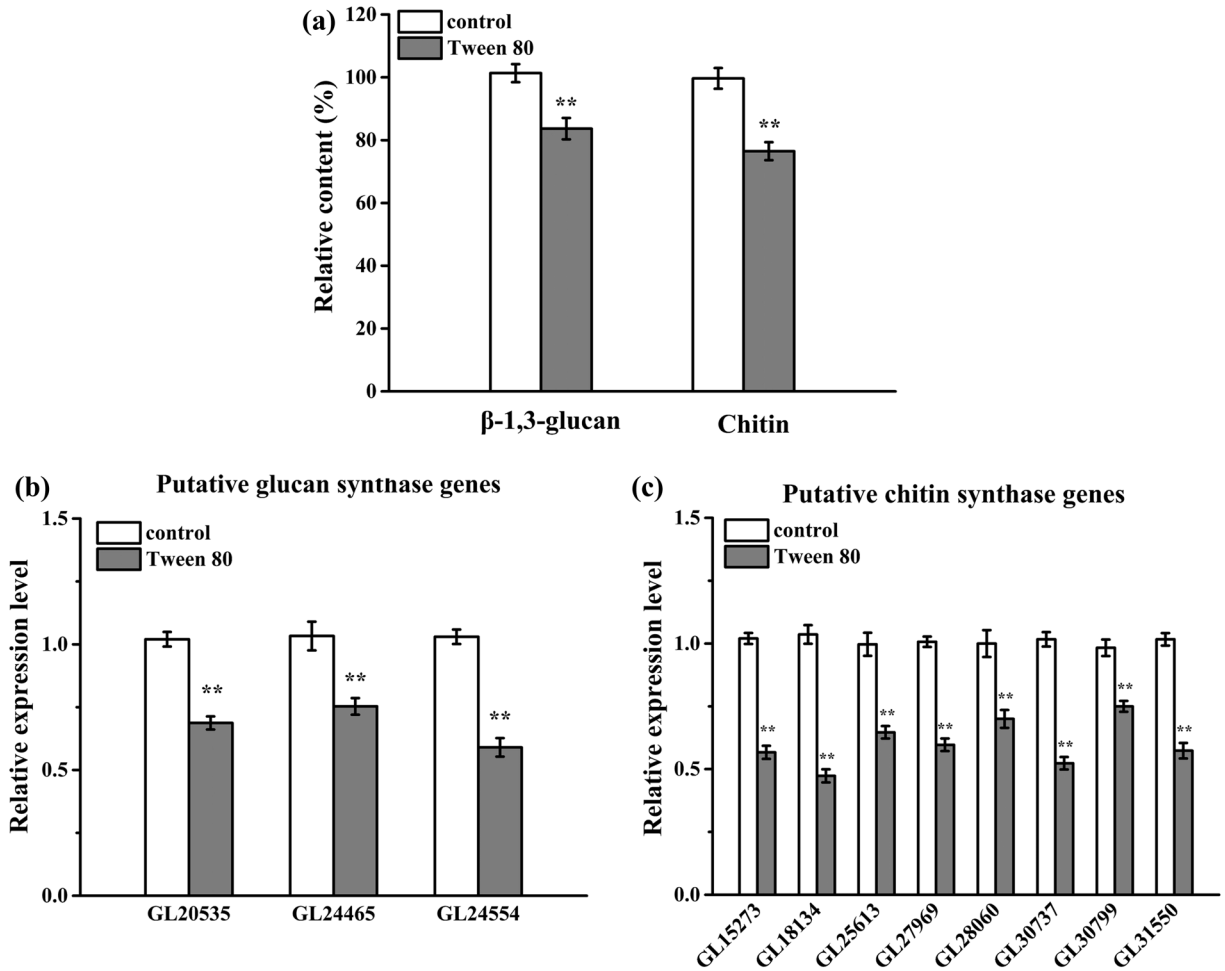

Fig. 6 The cell wall components were altered in Tween 80-treated *G. lucidum*. **a** The contents of β-1,3-glucan and chitin. **b** The expression levels of putative glucan synthase genes. **c** The expression levels of putative chitin synthase genes. Values are the mean ± standard deviation of three replicates. $^{**}P < 0.01$

integrity, which might be harmful to the cells. The addition of Tween 80 at the early exponential phase might cause irreversible injury to the immature cell membrane and cell wall, leading to lower biomass and EPS yield outcomes (Fig. 1b).

Effects of Tween 80 on antioxidant activity and structural composition of EPS

DPPH is a stable radical used to assess the free radical scavenging ability of antioxidants in vitro. Multiple hydroxyls in polysaccharides contribute to their antioxidant activity, as they act as donors of hydrogen atoms or electrons in the transformation of DPPH radicals into their reduced form [35]. EPS of the control group (EPS-control) and Tween 80 group (EPS-Tween 80) scavenged DPPH radicals in a dose-dependent manner (Fig. 7). The DPPH radical scavenging activity of EPS-Tween 80 was significantly higher than that of EPS-control, and a maximum of 56.13% at 2 mg/mL was observed. The enhanced production of EPS-Tween 80 with higher antioxidant activity might respond to the ROS accumulation stimulated by Tween 80 (Fig. S2). When ROS accumulation exceeded a certain extent, it could induce oxidative stress and cell damage [34]. In this case, the fungus produced more EPS with higher antioxidant activity to protect cells under stress by maintaining cell morphology and eliminating ROS [34, 36, 37].

The bioactivity of polysaccharides is strongly affected by their structural characteristics, and the chemical composition and molecular weight of EPS were investigated. As shown in Table 1, there was no Tween 80 left in EPS-Tween 80 and it could be confirmed that there was no disturbance in the antioxidant activity assay. The carbohydrate and uronic acid contents of EPS-Tween 80 were significantly higher than those of EPS-control. Moreover, there was no significant difference in protein content between the two groups (Table 1). It was found that the polysaccharides in some

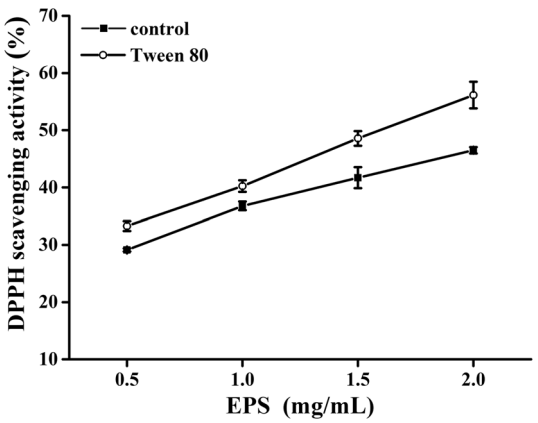

Fig. 7 Influence of Tween 80 on the DPPH radical scavenging activity of *G. lucidum* EPS

Table 1 Chemical content and molecular weight of EPS with and without the addition of Tween 80

	Control	Tween 80
Carbohydrate content (wt%)	74.54 ± 2.05 a	80.13 ± 1.62 b
Protein content (wt%)	12.91 ± 0.29 a	12.64 ± 0.35 a
Uronic acid content (wt%)	2.48 ± 0.08 a	3.72 ± 0.12 b
Tween 80 content (wt%)	None	None
Molecular weight (Da × 10^5)	4.88 ± 0.09 a	4.61 ± 0.07 b

wt% is expressed as the weight percentage. Mean ± standard deviation values ($n = 3$) in each row followed by different letters are significantly different ($P < 0.05$)

plants and fungi with relatively high carbohydrate and uronic acid contents exhibited strong antioxidant activity due to the enhanced electron- or hydrogen atom-donating capacity [18, 38, 39]. We assumed that the higher antioxidant activity of EPS-Tween 80 than that of EPS-control was related to its higher carbohydrate and uronic acid contents. Furthermore, polysaccharides with relatively low Mw seemed to have more reductive hydroxyl groups for the elimination of free radicals than those with high Mw [38]. Thus, the lower Mw of EPS-Tween 80 than that of EPS-control might contribute to a higher antioxidant activity (Table 1). Overall, Tween 80 improved the antioxidant activity of EPS by altering its chemical composition and molecular weight. It should be noted that the antioxidant activity of EPS-Tween 80 might be affected by other structural characteristics, such as monosaccharide composition and the glycosidic bond content, which need to be further investigated.

Conclusions

In summary, the addition of Tween 80 at the middle exponential phase of *G. lucidum* under submerged fermentation could significantly increase mycelial growth and EPS production. ROS accumulation stimulated by Tween 80 might result in elevation of the cell membrane and cell wall permeability, thereby facilitating nutrient absorption and EPS biosynthesis. The expression of EPS biosynthesis-related genes and ATP generation were also stimulated by Tween 80. Moreover, Tween 80 increased the antioxidant activity of EPS by altering its structural composition. Due to the low cost of Tween 80, this is an economically feasible application to enhance bioactive *G. lucidum* EPS production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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