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Enhanced active extracellular polysaccharide production from *Ganoderma formosanum* using computational modeling

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ABSTRACT

Extracellular polysaccharide (EPS) is one of the major bioactive ingredients contributing to the health benefits of *Ganoderma* spp. In this study, response surface methodology was applied to determine the optimal culture conditions for EPS production of *Ganoderma formosanum*. The optimum medium composition was found to be at initial pH 5.3, 49.2 g/L of glucose, and 4.9 g/L of yeast extract by implementing a three-factor–three-level Box–Behnken design. Under this condition, the predicted yield of EPS was up to 830.2 mg/L, which was 1.4-fold higher than the one from basic medium (604.5 mg/L). Furthermore, validating the experimental value of EPS production depicted a high correlation (100.4%) with the computational prediction response model. In addition, the percentage of β-glucan, a well-recognized bioactive polysaccharide, in EPS was 53 ± 5.5%, which was higher than that from *Ganoderma lucidum* in a previous study. Moreover, results of monosaccharide composition analysis indicated that glucose was the major component of *G. formosanum* EPS, supporting a high β-glucan percentage in EPS. Taken together, this is the first study to investigate the influence of medium composition for *G. formosanum* EPS production as well as its β-glucan composition.

1. Introduction

Lingzhi (*Ganoderma* spp.), generally recognized as a safe medical mushroom, has been used for centuries as a nutraceutical to promote health and longevity [1]. Nowadays, the global *Ganoderma* market has an estimated size of US$2.5 billion [2]. Polysaccharide is one of the major components contributing to the health benefits of *Ganoderma* spp., including immune-modulation, anti-inflammation, and

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obesity management [3]. For commercialization of dietary supplement, however, it takes several months to cultivate the fruit bodies of *Ganoderma*, and it is difficult to control their quality when they come from different batches [4]. As a result, submerged fermentation has received considerable attention by dint of its time-saving and economic properties for industrial production.

Ganoderma formosanum is an endemic species of *Ganoderma* in Taiwan [5]. Currently, owing to its novelty, few studies have demonstrated its pharmacological potential, among which the investigation of bioactive extracellular polysaccharide (EPS) is the most understood [6–9]. PS-F2, an EPS fraction from the submerged cultivation of *G. formosanum*, was reported to stimulate macrophage activation in mice via multiple pattern-recognition receptors including TLR4, CR3, and Dectin-1 [7,8]. Moreover, PS-F2 exerted antitumor effects toward melanoma, adenocarcinoma, and sarcoma in mice without adverse effects [9]. Nevertheless, optimization for the production of *G. formosanum* EPS has not yet been explored.

Optimizing medium composition is a key step to ameliorate the production of bioactive components. The effects of initial pH, carbon source, nitrogen source, inoculation density, and temperature have been investigated to ameliorate polysaccharide and biomass production in *Ganoderma* spp. [10–13]. A previous study indicated that a maximum level in the biomass of *Ganoderma lucidum* was obtained at high initial pH (6.5), whereas low initial pH (3.5) was favorable for EPS production [10]. Another study reported that an increase in glucose concentration (from 20 g/L to 50 g/L) gradually led to a higher biomass and endopolysaccharide production of *G. lucidum*. However, the production of polysaccharide was inhibited at a higher concentration of glucose (65 g/L) because of the high osmotic pressure [14]. Therefore, it was noteworthy that the fermentation response was not in proportion to the cultivation parameter. In view of this, a more reliable statistical strategy is needed to optimize fermentation variables.

Traditionally, one-factor-at-a-time (OFAT) approach has been carried out by analyzing the effect of a single factor on experimental response during fermentation [15]. Although this technique provides a simple way to monitor the influence of the variables studied, some drawbacks still exist, including time consumption, laboriousness, and diseconomy [16]. Furthermore, the major downside of OFAT is that it does not depict the interaction between different factors. Therefore, a collection of statistical and mathematical approaches is needed to investigate the multiple variables and their interaction during cultivation. Response surface methodology (RSM) is a statistical approach based on the fit of a polynomial regression model, which can be applied to validate the value of independent variables considering the interaction among them.

Briefly, RSM is composed of three steps: (1) executing a set of designed experiment; (2) evaluating the coefficients of polynomial model; and (3) predicting the response model and obtaining the optimum value. Box–Behnken design (BBD) and central composite design (CCD) are the most popular RSM alternatives; however, BBD is preferable to CCD when three factors are used as it decreases the number of experiments [17,18].

To date, there is still no consensus about the optimal cultivation conditions for EPS production of *Ganoderma* spp. Therefore, the aim of this study was to investigate the optimal initial pH, glucose, and yeast extract concentration of the medium for *G. formosanum* ESP production using the RSM technique, while revealing the relationship between morphology and EPS production. The percentage of β-glucan in EPS was also studied. The insights gleaned from this study enable us to increase EPS production for further application.

2. Materials and methods

2.1. Fungal strain and fermentation

G. formosanum (ATCC76537) was obtained from the American Type Culture Collection (Rockville, MD, USA). The mycelia were cultured on potato dextrose agar (PDA) plates at 25°C for 10 days. For preparation of seed inoculums, an 8-cm^2 mycelium from dish culture was inoculated to an Erlenmeyer flask (250 mL) containing 100 mL potato dextrose broth and incubated at 25°C on a rotary shaker (120 rpm) for 7 days. For the fermentation stage, a 10% (v/v) inoculum was poured to the basic medium that was obtained from a previous study with modification [19], including 35 g glucose, 7.5 g yeast extract, 0.88 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, and 0.05 g vitamin B_1 per liter of deionized water, with 120 rpm shaking at 25°C for 9 days. To optimize the culture condition for EPS, the medium was supplemented with various levels of glucose, yeast extract, and initial pH in a total of 13 different conditions (Tables 1 and 2).

2.2. Determination of biomass and exopolysaccharide (EPS)

To determine the mycelial biomass of *G. formosanum*, mycelia were separated from culture broth with mesh filter and washed with sterile water, then dried to a constant weight in the lyophilizer (T10; HCS, New Taipei City, Taiwan). For EPS preparation, the culture broth without mycelia was added with 95% ethanol by four volume times to precipitate EPS at 4°C overnight. After isolation of EPS by centrifugation at 7,200 × *g* for 15 minutes, EPS was resuspended with 95% ethanol and centrifuged again. The insoluble component was dissolved with 1N NaOH at 60°C for 1 hour, and the amount of EPS was measured using the phenol–sulfuric acid method [19].

2.3. Effects of carbon, nitrogen, and pH levels (experimental design)

A three-level–three-factor Box–Behnken design response surface methodology (BBD-RSM) was applied to optimize

Table 1 – Levels of factors chosen for the Box–Behnken design.

Factors	Symbols	Coded levels		
		−1	0	1
Initial pH	X_1	3.5	5	6.5
Glucose (g/L)	X_2	25	45	65
Yeast extract (g/L)	X_3	0	3.75	7.5

Table 2 – Box–Behnken design matrix and experimental results of EPS production, biomass accumulation, and final pH.

Run order	Factors			EPS (mg/L)	Biomass (g/L)	Final pH
	X_1	X_2	X_3			
1	5	45	3.75	855.07	9.07	3.24
2	5	45	3.75	817.73	10.74	3.38
3	5	45	3.75	840.07	10.12	3.32
4	3.5	25	3.75	246.07	7.22	3.11
5	5	45	3.75	754.73	10.51	3.19
6	6.5	65	3.75	709.40	16.71	3.79
7	3.5	65	3.75	201.73	13.94	2.99
8	5	25	7.5	642.06	12.36	3.70
9	3.5	45	0	179.79	3.89	3.52
10	5	45	3.75	686.06	10.34	3.21
11	5	25	0	153.40	3.61	3.50
12	3.5	45	7.5	298.37	15.47	3.14
13	5	65	0	199.93	4.46	3.53
14	6.5	25	3.75	299.73	9.04	3.42
15	5	65	7.5	613.73	18.30	3.78
16	6.5	45	7.5	427.73	14.34	3.71
17	6.5	45	0	304.20	5.43	3.63

X_1 = initial pH; X_2 = glucose; X_3 = yeast extract.

initial pH (X_1), glucose (X_2), and yeast extract (X_3), for the production of EPS (Y). The coded and uncoded (actual) levels of the independent variables are listed in Table 1. As shown in Table 2, a total of 17 run experiments were performed to verify the optimization conditions for EPS production, and results were analyzed using the Minitab software (version 16; Minitab Inc., State College, PA, USA). A full quadratic equation for BBD-RSM model is presented below:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=1}^{3} \beta_{ij} X_i X_j, \quad (1)$$

where Y is the response variable, β_0 is a model constant, and β_i, β_{ii}, and β_{ij} are coefficients for linear, quadratic, and interaction effects of the model estimated by multiple regression analysis, respectively. X_i and X_j are the independent variables.

2.4. Measurement of morphology

Images of the mycelial morphology of samples were taken via a 12-megapixel iSlight camera (A1687; Apple Inc., Cupertino, CA, USA) with 1.22 μ pixels. The camera captured images of 4032 × 3024 pixels, each with ISO-100, 4-mm focal distance, *f*/2.2 aperture and 1/4-second exposure time.

2.5. Analysis of β-glucan

Aniline blue is a chromophoric compound that specifically binds to the helical structure of β-glucan, which forms a fluorescent complex leading to fluorescence [20]. The amount of β-glucan in EPS was determined through aniline blue staining according to previous studies with slight modifications [20,21]. After precipitation and washing, EPS from various samples was dissolved in 4 mL 0.5N NaOH (containing 0.5M NaCl) and stirred at room temperature for at least 2 hours. After this, the pH was adjusted to 11.50 ± 0.1 and made constant volume to 10 mL with Na_2HPO_4-NaOH (containing 0.5M NaCl). A 2-mL sample was reacted with 0.2 mL aniline blue (Ferak, Berlin, Germany) for 2 hours at room temperature. The absorbance was measured at an excitation wavelength of 395 nm and an emission wavelength of 495 nm using a florescence spectrophotometer (F4500, Hitachi Inc., Tokyo, Japan).

2.6. Monosaccharide composition

The monosaccharide composition of EPS was analyzed by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as previously described with slight modifications [9]. Briefly, dehydrated EPS (1 mg) was reacted with 2M HCl in absolute methanol at 80°C for 12 hours. After removal of the reagent, EPS was hydrolyzed with 2M trifluoroacetic acid at 100°C for 1 hour and further evaporated to remove the trifluoroacetic acid. Next, EPS was dissolved in high performance liquid chromatography-grade distilled water and determined by HPAEC-PAD with a Bioscan 817 system (Metrohm, Herisau, Switzerland) and an electrochemical detector (E1 = +0.05 V, 480 milliseconds; E2 = +0.80 V, 180 milliseconds; E1 = −0.3 V, 360 milliseconds). For separation of monosaccharide, the CarboPac PA1 column (Dionex Inc., Sunnyvale, CA, USA) with guard column (4 mm × 50 mm) was used (eluent = 19mM NaOH, flow rate = 1 mL/min). Finally, Metrodata IC Net 2.1 software (Metrohm) was applied to perform data analysis.

2.7. Statistical analysis

Results of EPS production were analyzed using analysis of variance, and $p < 0.05$ was regarded as statistically significant. The fitness of the polynomial model is validated by the coefficient of determination (R^2). Three-dimensional and contour plots were obtained from regression models. All statistical analyses were performed with Minitab Pro 16 (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Response model fitting and adequacy checking

The medium composition certainly affects the yield of *G. formosanum* EPS. However, there is still no agreement about the optimal EPS production conditions for *Ganoderma* spp. [22]. For this reason, several carbon (fructose, glucose, lactose and sucrose) and nitrogen (ammonium acetate, peptone, malt extract, and yeast extract,) sources were screened for optimizing *G. formosanum* EPS production. The result showed that glucose and yeast extract were favorable nutrients for the yield of *G. formosanum* EPS (Table 3).

In this study, a more reliable statistical approach, RSM, was used to optimize medium composition instead of using OFAT. The impact of three variables—initial pH (X_1), glucose (X_2), and yeast extract (X_3)—on EPS production were investigated by BBD-RSM (Table 1). The results of BBD are summarized in Table 2 and analyzed by multiple regression analysis. Furthermore, the following second-order polynomial equation was obtained with Minitab software:

Table 3 – Carbon and nitrogen sources selection for EPS production of *Ganoderma formosanum*.

Carbon source[a]	Fructose	Glucose	Lactose	Sucrose
EPS (mg/L)[b,c]	139.3 ± 3.2	339.9 ± 18.4	141.3 ± 7.6	179.0 ± 12.3
Biomass (g/L)[b,c]	14.0 ± 1.4	10.45 ± 0.85	13.32 ± 1.4	13.32 ± 1.6
Nitrogen source[d]	**AA**	**Malt extract**	**Peptone**	**Yeast extract**
EPS (mg/L)[b,c]	49.0 ± 4.1	292.5 ± 16.7	195.97 ± 5	378.5 ± 48
Biomass (g/L)[b,c]	1.21 ± 0.3	7.7 ± 0.2	11.74 ± 0.7	11.9 ± 0.5

AA = ammonium acetate; EPS = extracellular polysaccharide.
[a] At 35 g/L carbon source and 7.5 g/L yeast extract.
[b] Cultivation time (7 days).
[c] The results are presented as the means of three replicates.
[d] At 7.5 g/L nitrogen source and 35 g/L glucose.

$$Y_{EPS} = 790.732 + 101.895X_1 + 47.941X_2 + 143.079X_3 - 263.136{X_1}^2 - 163.363{X_2}^2 - 225.088{X_3}^2 + 113.502X_1X_2 + 1.223X_1X_3 - 18.715X_2X_3 \quad (2)$$

The analysis of variance for the model is shown in Table 4, and the fitness of it was examined using the determination coefficient ($R^2 = 0.9087$), which suggests that the sample variation of 90.87% for EPS production was associated with the variable factors. In addition, the lack of fit for the model was insignificant ($p > 0.05$), verifying the accuracy fit of the second-order model (Equation 2) to the true response of EPS production. Moreover, the F value of 7.74 and p value < 0.05 for the regression, supporting the second-order model, adequately approximated the response surface (Table 4). As a result, canonical analysis demonstrated that the predicted maximum of EPS production was 830.2 mg/L at initial pH 5.3, 49.2 g/L glucose, and 4.9 g/L yeast extract. The results clearly indicated that all these variables influenced EPS yield.

3.2. *Effects of factors on EPS production*

Three-dimensional surface and contour plots (Figure 1) were generated to reveal the interaction among the three independent variables studied and to depict the combined effects of these variables on EPS yield. As the influence of two variables on response surface was plotted, the other variable was kept at its zero level. As shown in Figure 1, EPS production gradually increased with the increase of initial pH, glucose, and yeast extract. However, it was noted that the yield of EPS will decrease while these three factors continuously increased. These results demonstrated that our response surface generated from a quadratic model was defined as maximum surface [16], supporting this experimental design to achieve the optimum point of these factors for EPS production. As described above, RSM provides a more robust and efficient way to evaluate fermentation parameters for response and avoid false-positive results. For example, if OFAT had been used to investigate the optimization value of yeast extract for response in this study, it would have shown that 45 g/L of glucose was preferable to 65 g/L. However, the predicted value of glucose was 49.2 g/L with RSM modeling. Furthermore, BBD surpasses OFAT in reducing large amounts of experimental replicates. When three factors are designed in the experiment, 15–18 total runs are required in BBD-RSM (12 runs for three factors with three levels; 3–6 center points), whereas OFAT has 27 total runs (3 factors × 3 levels × 3 replicates). As a whole, BBD-RSM not only reduced the replicate and cost of experiment, but

Table 4 – Analysis of variance (ANOVA) for response surface quadratic model.

Source	Coefficient	Degree of freedom	Sum of squares	F	p
Constant	790.732	9			0.000
X_1	101.895	1			0.047
X_2	47.941	1			0.296
X_3	143.079	1			0.012
X_1^2	−263.136	1			0.003
X_2^2	−163.363	1			0.027
X_3^2	−225.088	1			0.006
X_1X_2	113.502	1			0.101
X_1X_3	1.223	1			0.984
X_2X_3	−18.715	1			0.764
Regression		9	1,004,428	7.74	0.007
Linear		3	265,220	6.13	0.023
Square		3	686,270	15.86	0.002
Interaction		3	52,938	1.22	0.370
Residual		7	100,935		
Lack of fit		3	81,380	5.55	0.066
Pure error		4	19,555		
Total		16	1,105,363		

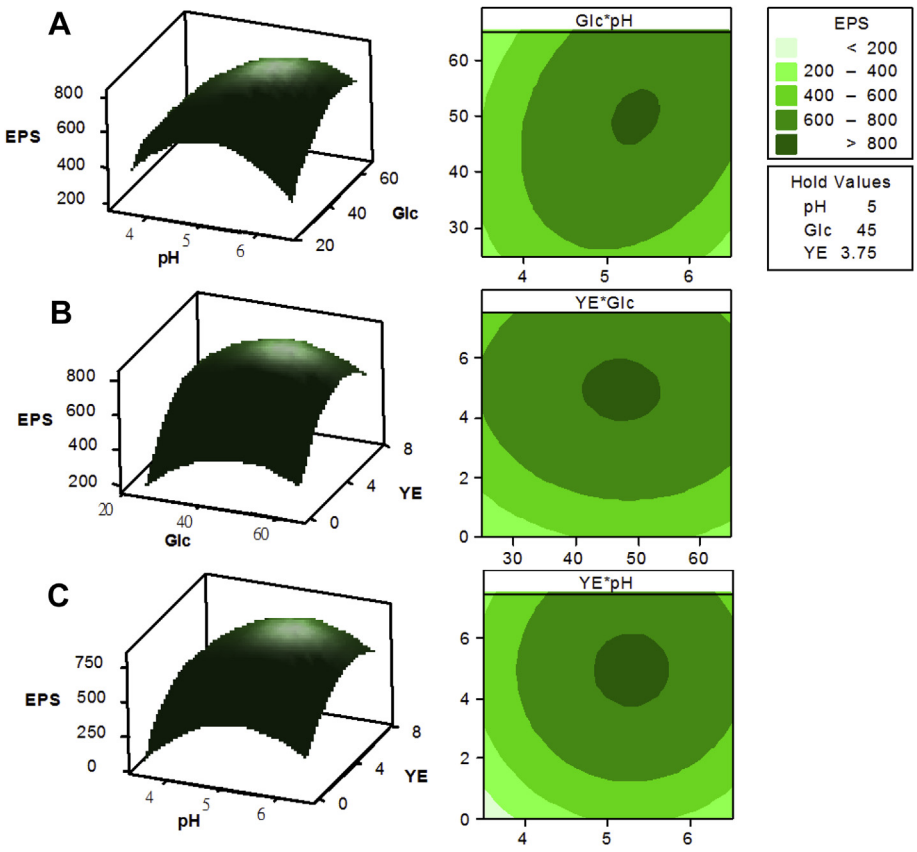

Figure 1 – Three-dimensional surface and contour plots of three factors on EPS production. The effect of (A) initial pH and glucose, (B) initial pH and yeast extract, and (C) glucose and yeast extract on EPS yield. EPS = extracellular polysaccharide; Glc = glucose; YE = yeast extract.

also verified the optimal independent variables for experimental response.

Glucose had a positive effect on EPS production of *Ganoderma*, and a similar result was also reported in a previous study [23]. Furthermore, glucose was not only involved in glycolysis pathway to produce energy but also converted to glucose-1-phosphate, which is a precursor for sugar nucleotides in polysaccharide synthesis [24]. However, excess glucose had an inhibitory effect in the yield of EPS that might be attributable to unfavorable osmotic pressure (Figure 1 and Table 2). The sluggish growth of microorganisms was often observed at high osmotic pressures, while initial sugar concentration exceeded a certain level [25]. By contrast, both initial pH and yeast extract (X_1 and X_3) had a significant positive effect on EPS yield ($p < 0.05$), when yeast extract concentration had the largest coefficient in Equation 2 (Table 4).

Organic nitrogen source was widely used in the cultivation of *Ganoderma*, suggesting that essential amino acid could be synthesized from organic nitrogen sources instead of inorganic sources. The preliminary results of this study also indicated that an organic nitrogen source (yeast extract) was more favorable for EPS and biomass production in *G. formosanum* than an inorganic nitrogen source (ammonium acetate) (Table 3). The yield of EPS increased with the elevation of yeast extract concentration, but high concentrations of it resulted in a countereffect (Figure 1). It was supposed that certain by-products existing at high concentrations of yeast extract would lead to abatement in EPS yield, such as NH_4+ and L-pyroglutamate [26,27].

In addition, initial pH was associated with the yield of EPS (Figure 1). EPS production at the initial pH of 3.5 was lower than that at pH 6.5, whereas the concentrations of glucose and yeast extract were at the same level (Table 2). We inferred that initial pH 3.5 was unfavorable for the yield of EPS. Moreover, the final pH of every run ranged from 2.99 to 3.71, showing that the pH at the end of fermentation was less than 3.7. This reflects the fact that initial pH 3.5 is unsuitable for the yield of EPS. However, a previous study suggested that low initial pH (3.5) enhanced the EPS production of *G. lucidum* [10]. This conflicting result arose from the different strains of *Ganoderma* used.

Even though initial pH, glucose, and yeast extract exerted a positive effect on biomass accumulation of *G. formosanum*, there is no obvious correlation between biomass and EPS production. For instance, the biomass of run 15 was 2-fold higher than that of run 1 (Table 2), but the EPS yield of run 15 was lower than that of run 1.

Therefore, the amount of biomass did not show a significant influence on EPS accumulation [28].

3.3. *Relationship between morphology and EPS yield*

The mycelial morphology of *Ganoderma* is associated with metabolism, eventually leading to variable productivity of the

target products. In addition, the morphology of pellet form was influenced by pH, sugar concentration, and inoculum density [4]. The yield of *G. lucidum* endopolysaccharide, for instance, is lower in larger pellets because of nutrient and oxygen limitation inside the pellet center. In contrast, larger pellets result in higher ganoderic acid production of *G. lucidum* owing to certain limited oxygen tension [13]. For the yield of EPS, dispersed pellets was reported to have a favorable morphology for EPS production in the submerged fermentation of *G. lucidum*. By contrast, feather-like pellets were regarded as an unfavorable form for EPS production [29]. Therefore, it is necessary to elucidate the underlying mechanism of EPS synthesis and mycelial morphology.

As depicted in Table 2, the favorable initial pH for the yield of *G. formosanum* EPS was at 5, and not 3.5 or 6.5. The unsuitability of initial pH 3.5 is probably attributable to the larger pellet form (Figure 2). It was inferred that the lower surface area and oxygen limitation inside the pellet core led to lower EPS production. By contrast, the compact pellet induced greater yield of EPS than the larger pellet as a result of high surface area and better oxygen diffusion (Figure 2 and Table 2). Taken together, the yield of polysaccharide was inversely proportional to pellet size owing to the influence of cellular respiration [14].

3.4. *Verification of optimization*

To validate the predicted *G. formosanum* EPS production, a verification fermentation with the predicted optimal value of variables (initial pH 5.3, 49.2 g/L glucose, and 4.9 g/L yeast extract) was implemented. As shown in Table 5, a high correlation between predicted and experimental yield of EPS was

Table 5 – Comparison of basic and optimum medium on EPS production.

Factors	Basic medium	Optimum medium
Initial pH	Nature (5.8)	5.3
Glucose (g/L)	35	49.2
Yeast extract (g/L)	7.5	4.9
EPS yield (mg/L)[a]	604.5 ± 52.9	833.9 ± 70.8
β-Glucan (mg/L)[a]	321.6 ± 62.4	442.9 ± 56.8
β-Glucan/EPS (%)	52.9 ± 6	53.2 ± 5.5
Biomass (g/L)[a]	17.5 ± 0.6	15.5 ± 1.3

EPS = extracellular polysaccharide.
[a] The results are presented as the means of three replicates.

conformity to the design response model. The EPS production was 833.9 ± 70 mg/L after the 9-day fermentation, which was approximately 1.4 times higher than the one from basic medium (Table 5). In addition, it was recorded as the highest EPS production of *G. formosanum* ever reported. EPS production of *G. formosanum* from a previous study was only 500 mg/L [19], and it can be inferred that the difference in EPS yield from this study was attributable to the different types of sugar used (lactose and glucose). In our preliminary test, glucose was more favored in the production of EPS than lactose (Table 3), supporting this inference.

3.5. *Content of β-glucan and monosaccharide composition in EPS*

Beta-glucan (beta-1,3-glucan) is one of the major components found in fungal cell wall for development of fungi, which is well recognized as a bioactive polysaccharide for immune-

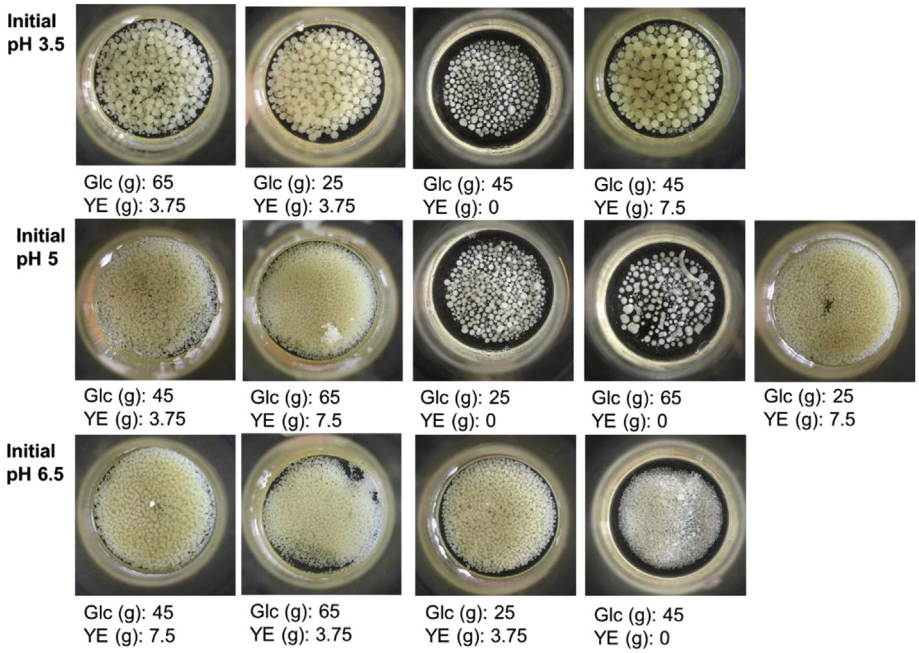

Figure 2 – Effect of initial pH and medium composition on the morphology of mycelial pellets. Glc = glucose; YE = yeast extract.

modulation and antitumor activities [28]. β-Glucan is also one type of conserved microbial structures in *Ganoderma*, serving as pathogen-associated molecular patterns to be recognized by pattern recognition receptors including several Toll-like receptors (TLR-4, TLR-2/6) [30,31]. In addition, it is recognized by immune receptors, such as complement receptor 3 and Dectin-1, to active macrophages, and dendritic and natural killer cells [31,32]. However, the percentage of β-glucan in EPS production has not been explored extensively, and the optimum culture conditions for β-glucan remain unclear in previous studies. In view of the bioactive quality concern for polysaccharides, the yield of β-glucan should be analyzed in the fermentation process. As shown in Table 5, the yield of β-glucan from basic and optimum medium was 321.6 ± 62.4 mg/L and 442.9 ± 56.8 mg/L, respectively, suggesting that not only EPS production, but also β-glucan yield, was increased after medium optimization. Moreover, it was noteworthy that the β-glucan percentage in EPS was 53.2 ± 5.5%, which was higher than that reported in *G. lucidum* EPS (4.9–20.3%) in a previous study [22]. This is the first study to suggest that the percentage of β-glucan in EPS produced by *G. formosanum* is probably higher than that from *G. lucidum*.

In view of the structural differences of polysaccharides from various *Ganoderma* strains [7], the monosaccharide composition of *G. formosanum* EPS from optimum medium was determined through HPAEC-PAD. As presented in Table 5, EPS comprised 59.29% glucose, 22.69% mannose, 13.7% galactose, and 4.3% fucose, indicating that glucose was the major component in *G. formosanum* EPS. Furthermore, monosaccharide composition of EPS from the optimum medium exhibited a similar composition to that from the basic medium. Accordingly, the high percentage of β-glucan in EPS (Table 5) was associated with the dominant glucose composition in EPS (Table 6). It was reported that the percentage of (1,3; 1,6)-β-glucan in the polysaccharide of *G. lucidum* under static cultivation (33.9–48.4%) was higher than that from shaking cultivation (1.1–27.8%) [28]. However, the inherent drawback of static cultivation is that it requires more time compared with shaking cultivation. It was found that the structure and composition of polysaccharides may change in response to medium composition in shaking culture [22]. The composition of the optimum medium in the present study probably contributed to the high β-glucan percentage in *G. formosanum* EPS. Therefore, medium optimization provides a reinforced approach to produce polysaccharide in shaking cultivation considering productivity and bioactivity.

Table 6 – Monosaccharide composition of EPS from basic and optimum medium.

Sugar components	Molar percentage (%)	
	Basic medium	Optimum medium
Fucose	2.9 ± 0.95	3.48 ± 1.18
Galactose	17.69 ± 0.26	13.3 ± 0.58
Glucose	61.42 ± 2.83	60.98 ± 2.39
Mannose	21.85 ± 3.72	22.24 ± 0.63
Xylose	ND	ND

EPS = extracellular polysaccharide; ND = not detected.

4. Conclusion

The medium composition has significant influence on the EPS production of *G. formosanum* by submerged fermentation. Our preliminary study indicated that glucose and yeast extract were favorable carbon and nitrogen sources for the yield of *G. formosanum* EPS. Furthermore, the optimum value of independent variables for EPS production was evaluated and predicted by BBD-RSM. As a result, initial pH 5.3, 49.2 g/L glucose, and 4.9 g/L yeast extract of the experimental design favored the formation of EPS. The result demonstrated that EPS production was 833.9 mg/L at RSM-optimized medium, which was 100.4% of the software-predicted value. The consistent correlation between predicted and experimental values validated the response model. It is noteworthy that low initial pH (3.5) led to the larger pellet size of mycelium and lower EPS production, suggesting that the mycelial morphology of *G. formosanum* was associated with the yield of EPS and influenced by initial pH. In addition, a high β-glucan percentage in EPS (53.2 ± 5.5%) suggested that the EPS produced by *G. formosanum* possesses notable immunomodulation potential.

In conclusion, this is the first study to elucidate the effect of medium composition on *G. formosanum* EPS maximum production, and it provides a new insight into the large-scale production and industrial application of *G. formosanum* EPS in the future.

Conflicts of interest

All authors declare no conflicts of interest.

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