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Liquid Fermentation of *Ganoderma applanatum* and Antioxidant Activity of Exopolysaccharides

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Abstract: The medium composition and fermentation conditions of Ganoderma applanatum(GA) strain were optimized by the liquid shake flask fermentation, and the antioxidant activity of exopolysaccharides was investigated. The results showed that the optimal conditions of the liquid fermentation of GA strain were as follows: Carbon source was corn powder, nitrogen source was soy powder, the initial pH was 6.0, the inoculum size was 8%, the fermentation temperature was 32°C, the fermentation time was 7 d. The exopolysaccharides of GA strain could scavenge hydroxyl radicals(HR) and superoxide anion radicals(SAR), and the concentration of exopolysaccharides was positively related to the antioxidant activity.

Keywords: Antioxidant activity, GA, liquid fermentation.

1. INTRODUCTION

Ganoderma applanatum(GA) is a kind of large edible fungi, having a variety of active ingredients such as polysaccharide, triterpenoids, amino acid, polypeptide. These active ingredients have anti-tumor, anti-virus, anti-cancer, lowering blood glucose, regulating blood pressure and other pharmacological activities [1-4]. Liquid submerged fermentation technology is a modern large-scale industrial method to product fungi and their metabolites, which has the advantages of short period, simple process, low cost, high yield, suitable for industrial production etc. It was reported that culture solution produced by submerged fermentation of GA has rich nutrition and medicinal value [5-8]. Producing by liquid submerged fermentation method, GA mycelium and culture solution processed and extracted can be made of food additive and health care food. This has the positive practical significance.

The research integrated the liquid fermentation conditions of GA, which aimed to provide a theory evidence for liquid fermentation process control of GA and accumulate some fundamental and elementary investigations for large-scale industrial production. In addition, the study had further investigated the antioxidant activity of exopolysaccharides, which offered evidences to further develop functional food of GA.

2. MATERIALS AND METHODS

2.1. Materials

Bacterial strain. GA strain was provided by College of Life Science and Agronomy, Zhoukou Normal University.

Culture medium. Agarslantculture-medium(PDA medium) was composed of 20% boiled juice filtering of peeled potatos, 2% sucrose, 0.3% KH_2PO_4, 0.15% $MgSO_4$, 2% agar, natural pH. Culture medium of liquid strain was composed of 1% glucose, 2% sucrose, 1% peptone, 0.5% beef extract, 0.1% KH_2PO_4, 0.15% $MgSO_4$, natural pH.

Preparation of GA strain. GA strain, which was propagated on PDA medium for 5 d at 28℃, was grown for 4 d at 28℃ with the shaking speed of 100 r/min prior to inoculation, according to the user's manual instructions. Then, the strain had been obtained and stored at 4℃ in the refrigerator for further sutdy.

2.2. Methods

Determination of mycelial biomass. Mycelium was obtained by removing supernatant of fermentation broth centrifuged at 3000 r/min for 15 min. Mycelium which was dried at 50℃ after washing with distilled water several times was weighed.

Determination of raw exopolysaccharides. The supernatant, which was decanted after the fermentation broth centrifuged, was added 3 times volume of 95% ethanol and was set aside for 24 h at 50℃. After centrifuging at 4000 r/min for 20 min, the precipitate dried to constant weight was weighed.

Optimization of fermentation conditions. Carbon source, nitrogen source, initial pH, inoculums size, fermentation temperature, fermentation time were investigated respectively to study their effect on shake flash fermentation of GA by measuring mycelial biomass and raw exopolysaccharides.

Determination of antioxidant activity of exopolysaccharides. The scavenging effect of exopolysaccharides on HRs was measured with the reaction system of fenton [9]. The scavenging effect of exopolysaccharides on SARs was measured with the methods of pyrogallol autoxidation [10].

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3. RESULTS AND ANALYSIS

3.1. Optimization on the Liquid Fermentation Conditions of GA Strain

Effect of carbon source on shake flask fermentation of GA. Fermentation broth was prepared with 1% peptone, 0.5% beef extract, 0.1%KH_2PO_4, 0.15%$MgSO_4$ and 3% carbon source changed as glucose, maltose, sucrose, corn powder, soluble starch. 8% of GA strain were added and cultured at 28℃ for 5 d. Then, Mycelial biomass and raw exopolysaccharides were determined (Table **1**).

It was shown in Table **1** that using corn powder as the sole carbon source, mycelial biomass and raw exopolysaccharides produced by GA were significantly higher than those of the other carbon sources produced. And corn powder with relatively low cost, wide source, was suitable for use in production. Thus the optimal carbon source was corn powder.

Table 1. Effect of carbon source on shake flask fermentation of GA.

Carbon source	Mycelial biomass (g /100 mL)	The content of raw exopolysaccharides (g /100 mL)
Glucose	0.181±0.012d	0.166±0.005b
Maltose	0.345±0.008b	0.074±0.005d
Sucrose	0.237±0.013c	0.149±0.002c
Corn powder	0.435±0.001a	0.420±0.007a
Soluble starch	0.085±0.019e	0.066±0.008d

Effect of nitrogen source on shake flask fermentation of GA strain. Fermentation broth was prepared with 3% corn powder, 0.1%KH_2PO_4, 0.15%$MgSO_4$ and 1.5% nitrogen source changed as peptone, yeast extract, beef extract, bran, soy powder.. 8% of GA strain were added and cultured at 28℃ for 5 d. Then, Mycelial biomass and raw exopolysaccharides were determined. The results were shown at Table **2**.

Table 2. Effect of nitrogen source on shake flask fermentation of GA.

Nitrogen Source	Mycelial biomass (g /100 mL)	The content of raw exopolysaccharides (g /100 mL)
Peptone	0.181±0.008c	0.064±0.002d
Yeast extract	0.356±0.006b	0.150±0.003c
Beef extract	0.204±0.008c	0.167±0.001c
Bran	0.338±0.021b	0.478±0.001a
Soy powder	0.485±0.009a	0.356±0.021b

Table **2** showed that the effect of bran and soy powder utilized by GA was significantly higher than that of yeast extract, beef extract and peptone, while the difference between the soy powder and bran was significant. Both soy powder and bran are easily available raw materials. They could be selected according to desired product fermented by GA strain in practical production. If the desired product was mycelium, soy powder was the best nitrogen source; If the desired product was exopolysaccharides, bran was the best nitrogen source.

Effect of initial pH on shake flask fermentation of GA strain. Fermentation broth was prepared with 3% corn powder, 1.5% soy powder 0.1%KH_2PO_4, 0.15%$MgSO_4$ and initial pH changed as 4.0、5.0、6.0、7.0、8.0. 8%of GA strain were added and cultured at 28℃ for 5 d. Then, Mycelial biomass and raw exopolysaccharides were determined (Table **3**).

Table 3. Effect of initial PH on shake flask fermentation of GA.

PH value	Mycelial biomass (g /100 mL)	The content of raw exopolysaccharides (g /100 mL)
4.0	0.383±0.003c	0.277±0.017e
5.0	0.449±0.016b	0.442±0.015c
6.0	0.541±0.014a	0.511±0.001a
7.0	0.365±0.031c	0.480±0.004b
8.0	0.324±0.009c	0.316±0.007d

It was shown in Table **3** that, with the rise of pH value, the mycelial biomass and raw exopolysaccharides increased gradually. At pH 6.0, both mycelial biomass and raw exopolysaccharides reached to the peak, and then decreased correspondingly with pH value rise. Therefore, GA strain is suitable to grow in acidic environment. The most suitable pH value for GA was 6.0.

Effect of inoculum size on shake flask fermentation of GA strain. Fermentation broth was prepared with 3% corn powder, 1.5% soy powder 0.1%KH_2PO_4, 0.15%$MgSO_4$ and initial pH6.0. Inoculum size of GA strain changed as 4%, 6%, 8%, 10%and 12%were added and cultured at 28℃ for 5 d. Then, Mycelial biomass and raw exopolysaccharides were determined (Table **4**).

Table **4** showed that when the inoculums size was 8% or 10%, mycelial biomass fermented by GA strain was significantly higher than the other. While the difference between 8% and 10% was not significant. When the inoculum size was 6% or 8%, the content of raw exopolysaccharides fermented by GA strain was significantly higher than the other. While the difference between 6% and 8% was not significant. All things considered, the suitable inoculum size was 8%.

Effect of fermentation time on shake flask fermentation of GA strain. Fermentation broth was prepared with 3% corn powder, 1.5% soy powder 0.1%KH_2PO_4, 0.15%$MgSO_4$ and initial pH6.0. 8% of GA strain were added and cultured at 28℃, and fermentation time was changed as 1 d, 3 d, 5 d, 7 d and 9 d. Then, Mycelial biomass and raw exopolysaccharides were determined. *The results were shown at Table* ***5****.*

Table 4. Effect of inoculum size on shake flash fermentation of GA.

Inoculum size(%)	Mycelial biomass (g /100 mL)	The content of raw exopolysaccharides (g /100 mL)
4	0.269±0.009d	0.377±0.002c
6	0.392±0.005c	0.552±0.008a
8	0.546±0.004a	0.541±0.003a
10	0.556±0.005a	0.517±0.007b
12	0.442±0.014b	0.388±0.011c

Table 5. Effect of fermentation time on shake flash fermentation of GA.

Fermentation time(d)	Mycelial biomass (g /100 mL)	The content of raw exopolysaccharides (g /100 mL)
1	0.179±0.002c	0.169±0.009e
3	0.307±0.003b	0.334±0.018d
5	0.560±0.001a	0.427±0.015c
7	0.556±0.006a	0.588±0.010a
9	0.554±0.001a	0.537±0.008b

Table **5** showed that the longer the fermentation time, the higher was the mycelial biomass. The mycelial biomass increased to a peak value at 5 d, but then did not changed with the extension of time, which meant the growth curve of GA strain reached the stationary phase after fermenting for 5 d; The content of raw exopolysaccharides increased significantly with the increase of fermentation time. The content increased to a peak value at 7 d, but then lowered down significantly with the extension of time. The time taken to reach the top amount of mycelial biomass was not consistent with the time taken to reach the top amount of exopolysaccharides. Therefore, the growth of mycelia and secretion of exopolysaccharides was not synchronized, and the secretion of exopolysaccharides lagged some time. All things considered, the suitable fermentation time was 7 d.

Effect of fermentation temperature on shake flash fermentation of GA strain. Fermentation broth was prepared with 3% corn powder, 1.5% soy powder 0.1%KH_2PO_4, 0.15%$MgSO_4$ and initial pH6.0. 8% of GA strain were added and cultured for 7 d, and fermentation temperature was changed as 22℃, 25℃, 28℃, 32℃ and 35℃. Then, Mycelial biomass and raw exopolysaccharides were determined (Table **6**).

It was shown in Table **6** that, effect of fermentation temperature on shake flash fermentation of GA was significant. Mycelial biomass and raw exopolysaccharides increased significantly with the increase of fermentation temperature. The content increased to a peak value at 32℃, but then lowered down with the rise of temperature. Its reason might be that high temperature caused the strain premature aging, and metabolites can also be reduced accordingly. Therefore, the optimum fermentation temperature was 32℃.

Table 6. Effect of fermentation temperature on shake flash fermentation of GA.

Fermentation temperature (d)	Mycelial biomass (g /100 mL)	The content of raw exopolysaccharides (g /100 mL)
22	0.341±0.015e	0.357±0.017e
25	0.391±0.009d	0.431±0.023d
28	0.547±0.007b	0.581±0.002b
32	0.659±0.009a	0.631±0.011a
35	0.431±0.021c	0.490±0.004c

3.2. Antioxidant Activity of Exopolysaccharides

It was shown in Table **7** that, the exopolysaccharides could scavenge HRs and SARs, and the more radicals were scavenged with the increase of concentration of raw exopolysaccharides. It indicated that its scavenging activity might be related to exopolysaccharides. When the concentration of exopolysaccharides was 1.2 mg/mL, the ratio of HRs scavenged had exceeded 50%; When the concentration of exopolysaccharides was 1.8 mg/mL, the ratio of SARs scavenged had reached 50%, too.

Table 7. The scavenging effect of exopolysaccharides on HRs and SARs.

The concentration of exopolysaccharides (mg/mL)	The ratio of HRs scavenged(%)	The ratio of SARs scavenged(%)
0.5	33.2±0.9e	22.0±0.5e
0.8	46.6±0.4d	31.7±0.4d
1.2	54.0±0.1c	41.1±0.3c
1.8	61.1±0.3b	50.1±0.1b
2.5	63.9±0.1a	58.8±0.1a

CONCLUSION

The medium composition and fermentation conditions for the production of GA were studied. The optimal liquid fermentation parameters of GA were as follows: Carbon source was corn powder, nitrogen source was soy powder, the initial pH was 6.0,the inoculums size was 8%, the fermentation temperature was 32℃, the fermentation time was 7 d. In addition, the antioxidant activity of exopolysaccharides which were produced by the fermentation of GA was also studied.The result showed that the exopolysaccharides had the ability to scavenge HRs and SARs, and the concentration of exopolysaccharides was positively related to the antioxidant activity.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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