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

LIU Zhong-hua*, HOU Xiao-ge, Zhao Jin-hui and HE Le

College of Life Science and Agronomy, Zhoukou Normal University, Zhoukou, Henan, 466000, China

**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 potatoes, 2% sucrose, 0.3% KH$_2$PO$_4$, 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$_2$PO$_4$, 0.15% MgSO$_4$, natural pH.

**Preparation of GA strain.** GA strain, which was propagated on PDA medium for 5 d at 28°C, was grown for 4 d at 28°C 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°C in the refrigerator for further study.

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°C 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°C. 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].
3. RESULTS AND ANALYSIS

3.1. Optimization on the Liquid Fermentation Conditions of GA Strain

Effect of carbon source on shake flash fermentation of GA. Fermentation broth was prepared with 1% peptone, 0.5% beef extract, 0.1%KH₂PO₄, 0.15%MgSO₄ and 3% carbon source changed as glucose, maltose, sucrose, corn powder, soluble starch. 8% of GA strain were added and cultured at 28°C 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 flash 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 flash fermentation of GA strain. Fermentation broth was prepared with 3% corn powder, 0.1%KH₂PO₄, 0.15%MgSO₄ 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°C 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 flash 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 flash fermentation of GA strain. Fermentation broth was prepared with 3% corn powder, 1.5% soy powder 0.1%KH₂PO₄, 0.15%MgSO₄ 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°C for 5 d. Then, Mycelial biomass and raw exopolysaccharides were determined (Table 3).

Table 3. Effect of initial PH on shake flash 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±0.017c                                  |
| 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 flash fermentation of GA strain. Fermentation broth was prepared with 3% corn powder, 1.5% soy powder 0.1%KH₂PO₄, 0.15%MgSO₄ and initial pH6.0. Inoculum size of GA strain changed as 4%, 6%, 8%, 10% and 12%. The results were shown at Table 4. It 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 flash fermentation of GA strain. Fermentation broth was prepared with 3% corn powder, 1.5% soy powder 0.1%KH₂PO₄, 0.15%MgSO₄ and initial pH6.0. 8% of GA strain were added and cultured at 28°C, 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.
and cultured for 7 d, and fermentation temperature was significantly with the increase of fermentation temperature. The content increased to a peak value at 7 d, but then lowered down significantly with the increase of fermentation time. The content 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% KH₂PO₄, 0.15% MgSO₄, and initial pH 6.0. 8% of GA strain were added and cultured for 7 d, and fermentation temperature was 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 temperature. The content increased to a peak value at 32°C, 28°C, 25°C, 22°C, and 22°C. Then, Mycelial biomass and raw exopolysaccharides were determined (Table 4).

It was shown in Table 5 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.

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

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                     |
CONFLICT OF INTEREST

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

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