African Journal of Microbiology Research Vol. 6(2), pp. 379-384, 16 January, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1151
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Effect of the extracts from *Gastrodia elata BL.* on mycelial growth and polysaccharide biosynthesis by *Grifola frondosa*

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Accepted 18 November, 2011

Two groups of the extracts from different *Gastrodia elata BL*.s were separately aadded into the fermentation system of *Grifola frondosa* in submerged culture. The results showed that the two extracts could promote the biomass growth of *Grifola frondosa* significantly (*P* <0.05). However, the extracts of *Gastrodia elata BL*. in the group 2 would more contribute to *Grifola frondosa'*s biomass growth than that in the group 1, and the extracts of *Gastrodia elata BL*. in the group 2 containing 5% (v/v) markedly promoted the biomass and extracellular polysaccharide (EPS) biosynthesis of *Grifola frondosa* (*P*<0.05). Biomass and EPS production increased from 0.564±0.09 to 1.324±0.25 g/l and from 71.69±0.53 to 107.08±0.85 mg/l, which increased by 134.75 and 49.37%, respectively. However, intracellular polysaccharides (IPS) content declined from 60.38±0.87 to 45.71±0.66 mg/g, which decreased by 24.30% compared with the control's, respectively. Moreover, a fact showed that EPS-2 and IPS-2 were both the main components, and there were no new other polysaccharide components separated by DEAE-52 column in the sample. It suggested that the polysaccharide biosynthesis pathways of *Grifola frondosa* may be unchanged.

Key words: *Grifola frondosa*, *Gastrodia elata BL.*, extract, extracellular polysaccharide (EPS), intracellular polysaccharides (IPS).

INTRODUCTION

Grifola frondosa is a Basidiomycete fungus which belongs to the order *Aphyllophorales*, and family *Polyporeceae*. Its fruit bodies are called "Huishu hua" in Chinese, "Maitake" in Japanese and "Hen of the Woods" in America. It is an important medicinal fungus. Its firm and meaty texture contains a rich and woody flavor. The major anti-tumor substances, which have been obtained from Maitake's fruit body and liquid-cultured mycelium, are attributed to polysaccharides (Chienyan et al., 2008).

Nanba (1995) considered that the polysaccharide of *G. frondosa* had the strongest activity against anti-tumor in all the polysaccharide of fungus. At present, the majority of polysaccharides of fungal have been found, in addition of the polysaccharides of *G. frondosa*. The polysaccharides of *G. frondosa* have an important biological activity, and have been used to treat various types of diseases, such as anti-tumor (Suzuki et al., 1989), immunity (Gary et al., 2009; Noriko et al., 2003), HIV infections (Hiroaki et al., 2000), antioxidant and superoxide anion scavenging (En Shyh, 2011) so on. Therefore, it has become a hot topic to obtain the polysaccharides of *G. frondosa* in maximum amount, in recent years (Chienyan et al., 2006, 2008).

There are also some interesting reports that the external stimulus can affect the cell growth, polysaccharide production and biological activity by adding the traditional herbal medicine into submerged culture of the medicinal mushroom (Yanquan et al., 2006; Gao-Qiang et al., 2007; Hoon et al., 2010).

Gastrodia elata BL. (Chinese name is Tianma) belongs to Orchidaceae and it is one of the earliest and most important traditional herbal medicine in thousands of years. It has proved to have five major active components (Liu et al., 2002). Our research was supported by National Natural Science Foundation of China, and

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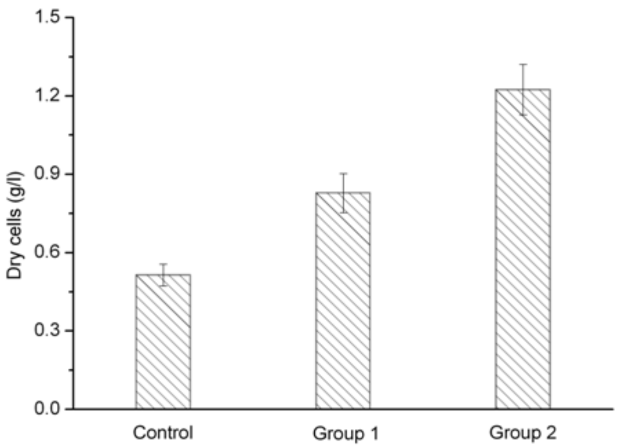

Figure 1. Effect of two extracts in a medium containing 4% (v/v) from different *Gastrodia elata BL*.s on mycelial growth of *Grifola frondosa*.

we have engaged in bio-transformation for many years, especially the bio-transformation between *G. frondosa* and *G. elata BL*. and found out some interesting experimental phenomenon in the early days. This paper focused on whether *G. elata BL*. could stimulate cell growth and polysaccharide production by *G. frondosa* submerged culture.

Moreover, we had documented the fact that there was a significant difference in the two groups of extracts from *G. etata BL.*, and the second group of extract could promote the mycelia growth and EPS production of *Grifola frondosa* more extremely. Therefore, this study not only provided a reference that the external stimulus could affect the mycelia growth and polysaccharide production of the medicinal mushroom, but also suggested a potential and novel method to bring the traditional herbal medicine into modern development by submerged culture.

MATERIALS AND METHODS

Gastrodia elata BL. and its extractions

G. etata BL. was purchased in county of Dejiang, Guizhou (People's Republic of China). Both two groups of *G. elata BL*. were both *G. elsta* f. *flavida.* Firstly, wash *G. etata BL.*, and then cook and dry at 55°C. Finally, smash it and pound it to powder (40 mesh), then store. However, the first group of *G. etata BL.* was smoked by sulfur in the process, and without smoke was the second group.

For the preparation of the extracts, 10 g of *G. elata BL*. were extracted in 100 ml of 75% ethanol for 48 h before the obtained extracts filtered, and the ethanol was removed under reduced pressure to obtain dry extracts, and then couple with 100 ml water to re-dissolve and filter the obtained. Here, the prepared alcohol extracts were to cultivate *G. frondosa*.

Strain and medium

The strain of *G. frondosa* (51616) was purchased from China Agricultural Culture Collection Center (Beijing, People's Republic of China). The stock culture was maintained on potato dextrose agar (PDA). For seed culture, the medium composition was (g/l): glucose, 20; peptone, 2; KH_2PO_4, 2; $MgSO_4$, 1; and the initial pH was natural. For fermentation, the medium composition was (g/l): glucose, 30; peptone, 5.5; KH_2PO_4, 1.5; $MgSO_4$, 0.75; and the initial pH was natural.

Culture conditions

The slants were inoculated with mycelium and incubated at 25°C for 12 d. The seed culture was grown in a 250 ml shake flask with 100 ml of liquid medium and incubated at 25°C for 8 d with 150 rpm/min. The fermentation cultivation was inoculated at 15% (v/v) of the above seed culture medium and kept at 25°C and with 150 rpm/min for 7 d.

Determination of biomass, EPS and IPS

Biomass was obtained by centrifuging a sample at 4,000 rpm for 15 min, washing the precipitated cells three times with distilled water, and drying at 60°C for a sufficient time to constant weight.

For the determination of EPS, after removing the mycelia by centrifugation, the crude EPS was precipitated with 95% (v/v) ethanol by four times of volume at 4°C refrigerator for 24 h from the centrifuged liquid, and then separated by centrifugation at 4,000 rpm for 15 min. After that, the obtained was washed with 80% (v/v) ethanol one time, and then dried to remove residual ethanol at 60°C. Finally, the EPS content was determined by phenol-sulphuric acid assay (Dubois et al., 1956).

For the analysis of intracellular polysaccharides (IPS), the dried mycelia (ca.100 mg) were grinded and extracted with 10 ml of 1M NaOH at 60°C (1 h). The extracts solution from dried mycelia was precipitated with 95% (v/v) ethanol by four times of volume at 4°C refrigerator for 24 h, and then the subsequent processes and the determination of IPS were just as those of EPS.

Polysaccharide component analysis

Two groups of 1 ml polysaccharide, that is, EPS 49 mg/l and IPS 38 mg/l, respectively from the control and the sample containing 5% (v/v) extract from *G. etata BL.* were collected by DEAE-52 cellulose column separation (1.2 cm ×7 cm). Then they were eluted with H_2O and 1 M NaCl at a flow rate of 1.5 ml/min. Finally, 3 ml was taken from each fraction to determine and analyze the polysaccharide component by the phenol-sulphuric acid.

Statistical analysis

All analysis was performed in triplet by SPSS 17.0 version. The data were expressed as the mean ± SD. The significance of the mean difference between the control and sample groups was determined by t-test. A level of difference of P <0.05 was considered significantly.

RESULTS AND DISCUSSION

Effect of two extracts from *Gastrodia elata BL*.s on the cell growth of *Grifola frondosa*

Figure 1 show that the two extracts in a medium containing

Table 1. Effect of the extracts concentration in the group 2 on the cell growth, EPS and IPS production in the submerged culture of *Grifola frondosa*.

The concentration of extracts in the second group (% v/v)	Biomass (g/l)	EPS production (mg/l)	IPS content (mg/g DW)
0	0.564±0.09	71.69±0.53	60.38±0.87
1	0.716±0.15	80.92±0.48	50.26±0.76
3	1.076±0.17	91.31±0.62	51.03±0.80
5	1.324±0.25	107.08±0.85	45.71±0.66
7	1.182±0.21	98.62±1.02	38.18±0.71
9	0.948±0.19	89.77±0.97	40.87±0.54

4% (v/v) from different *G. elata BL*.s both could promote biomass growth of *G. frondosa* significantly (*P* <0.05). However, the extracts of *G. elata BL*. in the group 2 would more contribute to *G. frondosa's* mycelial growth than that of *G. elata BL.* in the group 1. Furthermore, *G. elata BL.* in the group 1 was smoked by sulfur. Therefore, *G. elata BL.* in the group 2 was chosen finally.

Effect of the extract concentrations in the group 2 on the cell growth, EPS and IPS production

The results from Table 1 indicate that the cell growth and EPS production both rose with an increase of the extract concentration from *G. elata BL*. in the group 2 within the range of 1–9%. However, IPS content decreased. The maximum biomass and EPS production were obtained in a medium containing 5% (v/v) the extracts of *G. elata BL.*, which reached 1.324 ± 0.25 g/l and 107.08 ± 0.85 mg/l, and increased by 134.75 and 49.37% compared with the control, respectively. IPS content decreased by 24.30% compared with the control. The extracts concentration in the group 2 at 5% (v/v) could markedly promote the biomass and extracellular polysaccharide (EPS) biosynthesis of *G. frondosa*, however, inhibited the IPS content.

Effect of the extracts in the group 2 on fermentation kinetics

Figure 2 shows the effect of the extracts in the group 2 on fermentation kinetics through a comparison between the second and control group, when the 5% (v/v) extract containing in the second group. Figure 2A and C suggested that the biomass and EPS production gradually and steadily increase with the fermentation time in two groups, and the second group showed a significant improvement after the second day. Figure 2B indicated that the pH fell slightly in both two groups on the first day, but came to rise after then, which reached the maximum on the fifth day and the sixth day. However, there was a sharp decline respectively, just in the exponential and stationary phase. Maybe the glucose metabolism was greatly vigorous, and it would produce some organic acid, as led into this sharp decline. Figure 2D meant that the IPS content also gradually and steadily increased over time, however, the IPS content in group 2 was lower than that of the control, indicating the extracts of *G. elata BL*. might inhibited the IPS biosynthesis.

Effect of the extracts solution of *Gastrodia elata BL*. in the group 2 on the polysaccharide component diversity

Figure 3 showed that EPS of *G. frondosa* had five main components, and they were named as EPS-1, EPS-2, EPS-3, EPS-4 and EPS-5, respectively. The EPS-2 was the main component from the control and sample group. Figure 4 indicated the IPS of *G. frondosa* had four main components, and they were named as IPS-1, IPS-2, IPS-3 and IPS-4, respectively. The IPS-2 was the main component from the control and sample group. The EPS and IPS from each fraction were shown in Table 2: the results indicated that the each fraction in the control was different from that of the group 2 containing the extract of 5% (v/v). Although there was no new polysaccharide component in the second group separated by DEAE-52 column compared with the control, the amount of polysaccharides had been redistributed in the group 2.

That the submerged fermentation of the medicinal mushroom would accelerate cell growth and metabolite production has been a hot topic, and studies about effect of environmental conditions, oxygen supply (Tang and Zhong, 2003) and fed-batch fermentation (Tang and Zhong, 2002), etc. on fermentation have been explored. In addition, some inducers to increase the cell growth and polysaccharide production have been reported, including NaCl (Xiang et al., 2006), oil (Changhua et al., 2009; Chienyan et al., 2008; Hung-Chang et al., 2009), ethanol (Hai et al., 2004) and organic acids (Hua et al., 2010). However, it is the first time to report the effect of the extracts from *G. elata BL*. on the cell growth, extracellular polysaccharide (EPS) and intracellular polysaccharide (IPS) biosynthesis by *G. frondosa* in the submerged

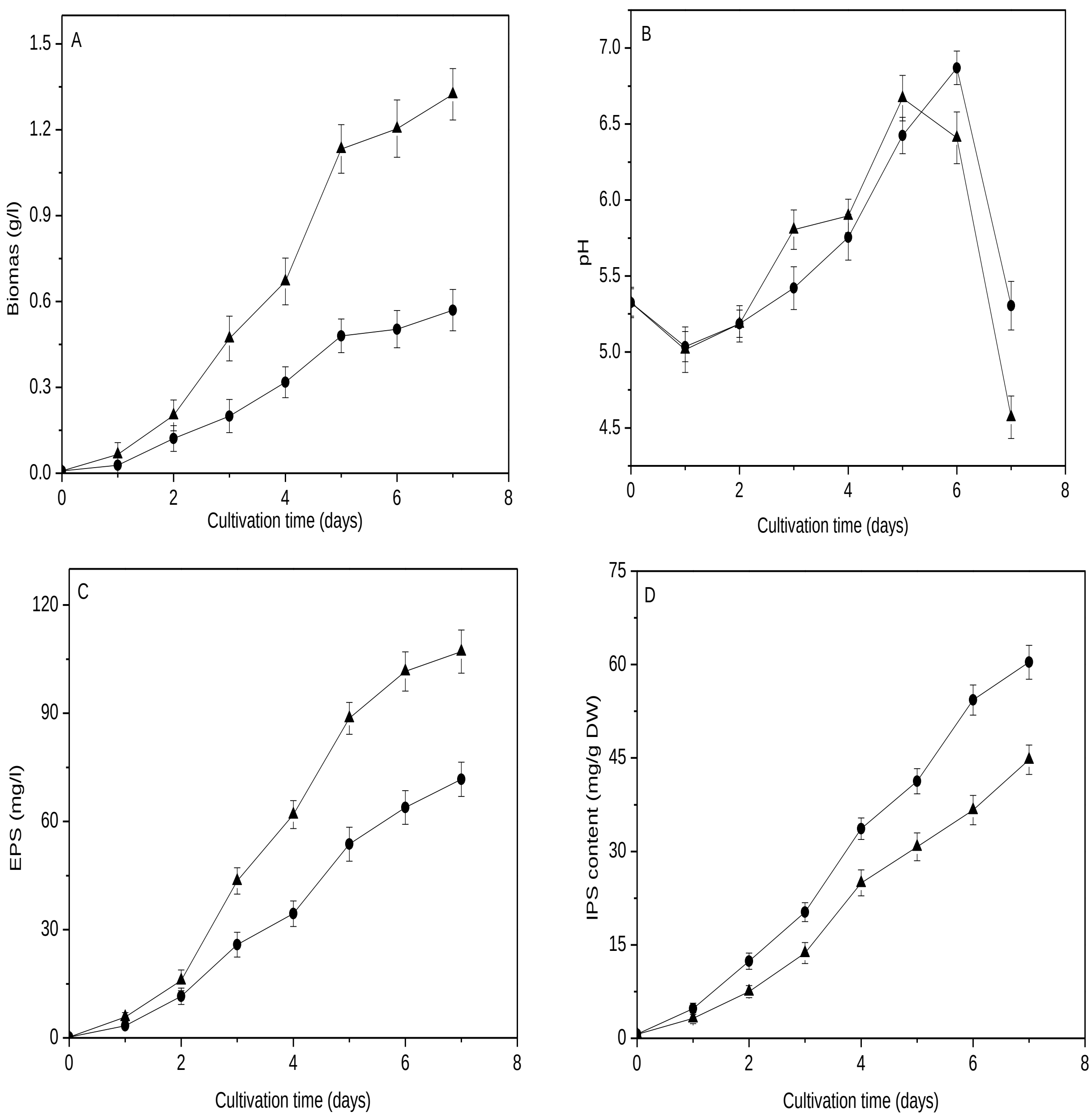

Figure 2. A comparison of the cell growth (A), pH (B), EPS production (C) and IPS content (D) between the second group (▲) and the control (●) when the 5% (v/v) extract containing in the second group. The error bar ranges denote the standard deviations of three trials.

culture. So it will give us a potential and novel way to spread the application of the traditional herbal medicine.

Conclusion

We confirmed that the extract of *G. elata BL*. could significantly increase the cell growth and polysaccharide biosynthesis by *G. frondosa* in the submerged culture, and it was some extracts from *G. elata BL*. that might have altered some enzyme activity in the polysaccharide biosynthesis pathways. However, the ingredients of the extracts from *G. elata BL*. responsible for enhancement of cell growth and EPS production are unclear currently. Therefore, it is necessary to further study to confirm the key components promoting the cell growth and EPS production from the extracts of *G. elata BL*.

ACKNOWLEDGEMENT

This work was supported by the Natural Science

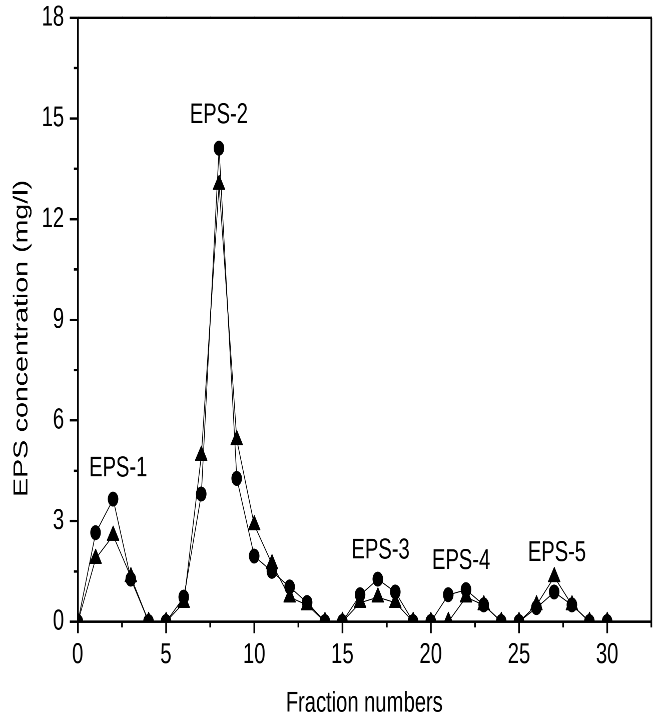

Figure 3. DEAE-52 elution profile of the EPS, the column was eluted stepwise with H_2O, 1M NaCl solutions. ▲, the sample containing 5% (v/v) the extract in the group 2 ; ●, the control.

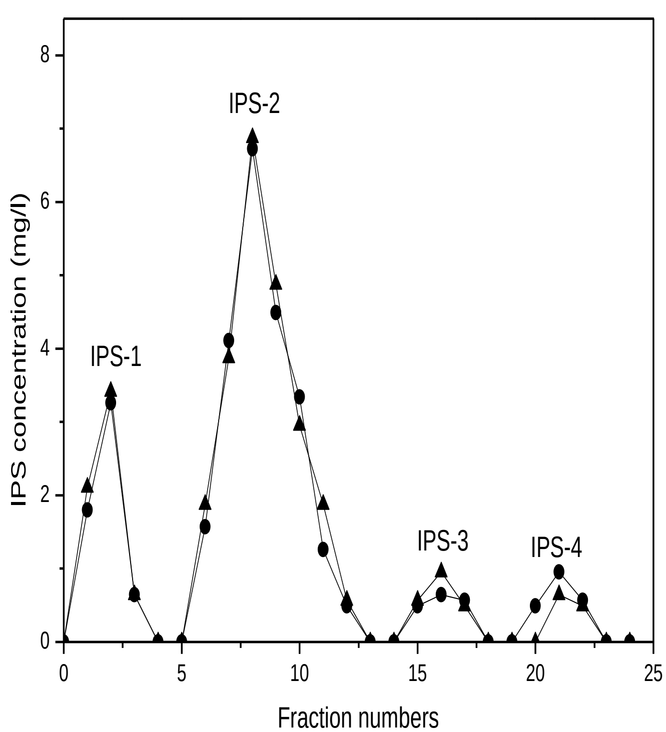

Figure 4. DEAE-52 elution profile of the IPS, the column was eluted stepwise with H_2O, 1M NaCl solutions. ▲, the sample containing 5% (v/v) the extract in medium ; ●, the control.

Table 2. A comparism of each fraction of EPS and IPS from the control and the sample containing 5% (v/v) the extract in medium.

	Control (mg/l)	containing 5% (v/v) the extracts in medium (mg/l)
EPS fraction		
EPS-1	7.55	5.78
EPS-2	27.94	29.78
EPS-3	2.94	1.86
EPS-4	2.25	1.22
EPS-5	1.78	2.32
IPS fraction		
IPS-1	5.71	6.17
IPS-2	21.98	22.91
IPS-3	1.71	2.02
IPS-4	2.02	1.14

Foundation of China (No.31060272) and also supported by Guizhou Province Natural Science Foundation of China (No.2009106).

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