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Full Length Research Paper

Mycelial growth and polysaccharide content of *Polyporus umbellatus*

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Seven strains of *Polyporus umbellatus*, collected from seven provinces of China, were employed to investigate each mycelial growing rate, extra- and intracellular polysaccharide content. The results showed that Hebei strain had the highest growing rate while Heilongjiang strain did the lowest. Both Shaanxi and Heilongjiang strains produced the most dried weight of mycelium, and at the same time Shaanxi and Hubei strains exhibited the highest intra- and extracellular polysaccharide content. Correlation analysis results showed that intra- and extracellular polysaccharide content had significant and positive relationship, but extracellular content was negatively correlated with daily mycelial growing rate.

Key words: *Polyporus umbellatus*, mycelium growth, polysaccharide content.

INTRODUCTION

Polyporus umbellatus (Pers) Fries, a fungus that belongs to Polyporaceae, Basidiomycetes, is widely distributed in China, Japan, Europe and North America (Zhao, 2006). Its dried sclerotium has been used as a diuretic in Chinese medicine for centuries (National Committee of Pharmacopeia, 2005).

P. umbellatus is widely distributed in thirteen provinces of China including Shaanxi, Yunnan, Hebei, etc., among which wild medicinal materials from Shaanxi are proved to have the best quality, while Yunnan occupies the most production (Xu, 1997). Recently, sclerotium of *P. umbellatus* has been reported to exhibit various pharmacological functions such as *in vivo* antitumor-promoter, increasing immunity and antioxidant activities (Xu, 1997; Lan et al., 1999). These reports greatly stimulate the harvest of wild medicinal materials, and the harvest rate is exceedingly rapid than that of rebirth speed by species themselves. Fortunately, this situation has been found and much effort has been made to reduce the pressure of the wild through domestic cultivation.

Asexual propagation is the main pathway adopted to produce cultivated products of *P. umbellatus*, though we all know the fact that long-term asexual propagation would result in degradation of "seeds". In fact, the reason why sexual propagation cannot be applied in cultivation practices may be attributed to the key problem that the mycelium obtained from controlled experiments in the laboratory cannot form sclerotium with big shape just like those wild individuals.

In order to solve this problem, many works have been done, mainly focusing on microstructure of mycelium and sclerotium (Li et al., 2007; Zhou et al., 2008), mycelial development and formation of small sclerotium (Xu, 1997; Chen et al., 2004). However, they still cannot break through the sexual propagation "bottleneck" and this may be attributed to sole one or no more than three strains used in their studies.

The objectives of this study were:

(1) To determine mycelial growth rate of *P. umbellatus* collected from seven provinces.
(2) To investigate intra- and extracellular polysaccharide content of each strain. (3) To evaluate the relationship among daily mycelial growth rate, dried weight of

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Table 1. Seven sclerotium strains of *P. umbellatus* collected from seven provinces in China.

Strain code (#)	Origin	Provided by
1	Taibai Mountain, Shaanxi	Our laboratory
2	Shennong Mountain, Hubei	Laboratory of Fungus, Huazhong Agricultural University
3	Funiu Mountain, Henan	Research Institute of Biology, Henan Academy of Sciences
4	Changbai Mountain, Heilongjiang	Heilongjiang Institute of Edible Fungi
5	Daba Mountain, Sichuan	Mianyang Institute of Edible Fungi
6	Jizu Mountain, Yunnan	Yunnan Academy of Agricultural Sciences
7	Baishi Mountain, Hebei	Research Institute of Microbiology, Hebei Academy of Sciences

mycelium, intra- and extra-cellular polysaccharide content.

MATERIALS AND METHODS

Sclerotium materials

Seven sclerotium strains of *P. umbellatus* were collected from seven provinces out of thirteen (Table 1). They were identified and preserved at a local laboratory in each region after collection.

Activation culture of sclerotium

The seven original sclerotium strains were activated on slope of PDA (potato dextrase agar) medium in tube at 25℃ within 7 days and then were transferred to petri dish with PDA medium at 25℃. When mycelium covered all dish plane, aseptical hole puncher (diameter 0.5 cm) was used to obtain five random mycelium samples along the edge of each mycelium colony. The five mycelium samples of each strain were cultivated in five new petri dishes with PDA medium, respectively. From the third day, the diameter of mycelium colony in each dish was measured by vernier caliper at every 48 h. Three directions were randomly selected to obtain the mean value of diameters. When the mycelium colony covered dish again, dynamic growth curve of mycelium would be drawn along with recording growth speed and color of each mycelium colony.

Liquid culture

The activated mycelium were transplanted on PDA medium again and cultivated at 25℃ under dark condition. After mycelium was full of dish plane, aseptical hole puncher (diameter 0.5 cm) was used again to obtain five random mycelium samples along the edge of cultivated mycelium. The five mycelium samples were cultivated in 100 ml liquid medium (corn flour 30 g/L, glucose 20 g/L, peptone 1.0 g/L, yeast extract 5 g/L, KH_2PO_4 1.0 g/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, pH 6.8) contained in 250-ml conical flask with the rotation speed 150 rpm at 25℃ for 10 days (Guo et al., 2002).

Mycelium observation

Fifty microlitre cultural liquid was drawn by aseptical glass sucker and uniformly laid on PDA medium. The mixed medium was then cultivated at 25℃ in dark for 15 days after inserting microscope cover glass with 45° angle. The diameter of mycelium was measured by microscope after when, over glass was put on microscope slide and then recorded (Zhou et al., 2008).

Intracelluar polysaccharide content

The rest cultural liquid of each strain was extracted by vacuum and then mycelium and filtrate would be separate. The harvest mycelium was washed three times by aseptical distilled water and dried to constant weight at 60℃. The dried mycelium was ground to powder and then followed the steps provided by Su et al. (2002) to obtain intracellular polysaccharide. The content was determined by our past work (Tian et al., 2007).

Extracellular polysaccharides content

Twenty millilitre filtrate liquid of each strain was measured and put into circular flask. 80 ml absolute alcohol was added in flask and other steps were followed by Su et al. (2002) and Tian et al. (2007).

Correlation analysis

The raw data was compiled by taking the means of all strains taken for each index and replication for different strains in the experiment. Mean values, standard deviation, minimum and maximum were calculated for each index in each strain. A fixed-effects model ANOVA was performed to check index variations between and within strains using SPSS 11.5. For each index, when the null hypothesis related to the ANOVA was rejected, multiple comparisons using the least significant difference (LSD) test were carried out to determine differences between strains. All LSD tests were carried out at $\alpha = 0.05$ significance level. Correlation analysis was performed to determine the relationships between all traits by using correlate program in SPSS 11.5.

RESULTS

Mycelium growth and mycelium colony characteristics

The mycelial growth rate among different strains was not obvious except that 7# was slightly faster. The diameter of mycelium colony and mean daily growth rate were shown in Table 2 and Figure 1.

Mycelium colony characteristics were observed after 45 days' culture in PDA medium at 25℃ (Figure 2). All mycelium colonies produced circular secretion except 7# and the secretion of six strains were all proved to be

Table 2. Mycelial growth rate and mycelium diameter of *P. umbellatus* strains from seven provinces in China.

Strain [a] (#)	Mycelium colony diameter (mm) after 13 days' growth	Mean daily growth rate (mm)	Colony growth curve of the proposed synthesis [b]	Correlation index (r)
1	64.988	4.999	Y = 0.4623X + 0.3982	0.9996
2	64.688	4.976	Y = 0.4331X + 0.8024	0.9988
3	70.136	5.395	Y = 0.4502X + 1.1081	0.9978
4	63.504	4.885	Y = 0.4740X + 0.1753	0.9990
5	74.290	5.715	Y = 0.5314X + 0.4493	0.9986
6	65.375	5.029	Y = 0.4454X + 0.4503	0.9899
7	79.686	6.130	Y = 0.5514X + 1.3469	0.9779

[a] The strain information was listed in Table 1; [b] Y represented diameter of mycelium colony; X represented number of days.

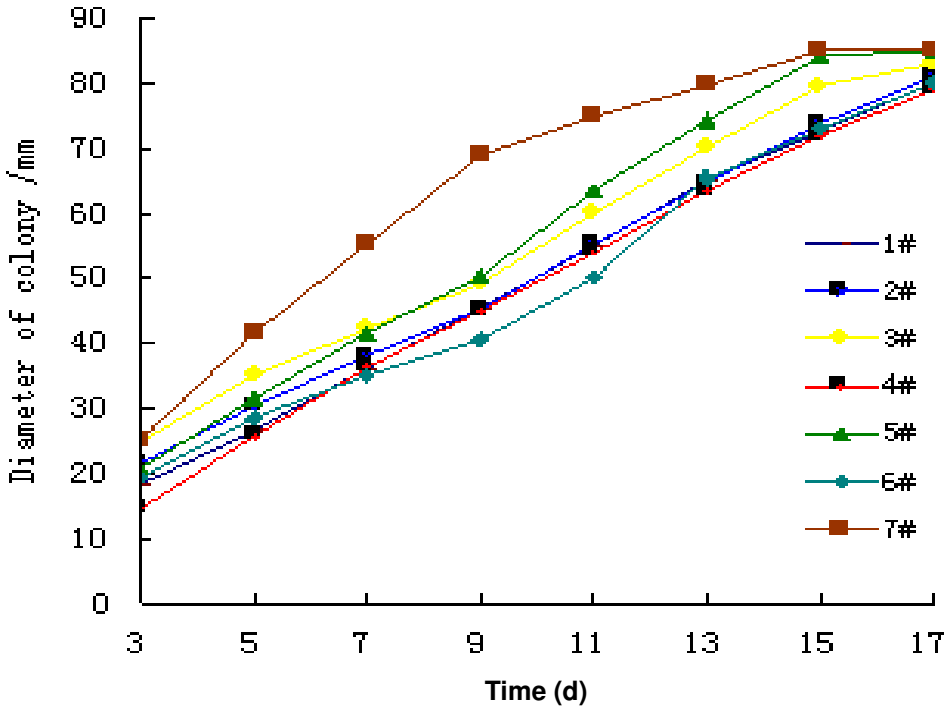

Figure 1. Mycelium growth dynamics of seven strains of *P. umbellatus* collected from seven provinces in China when cultivated in PDA medium with different days.

polysaccharide crystals. The secretion color was found varied accompanying with mycelium growth. The color of both 1# and 4# varied from yellow to coffee and to final black while the others (2#, 3#, 5# and 6#) displayed from white to yellow.

Mycelium characteristics

The mycelium diameter ranged from 1.5 to 4.0 μm (Table 3). Asexual spores and calcium oxalate crystals were observed in all strains (Figure 3; Table 3). The dried weight of mycelium was also found different, among which 1# exhibited the most followed by 4# (Table 4).

Polysaccharide content

The results showed that intracellular polysaccharide was obviously higher than that of extracellular content (Table 5). Correlation analysis exhibited that intracellular polysaccharide was significantly correlated with extracellular content ($r = 0.802$, $P < 0.05$). However, extracellular polysaccharide content was found negatively and significantly correlated with daily mycelial growth rate ($r = -0.788$, $P < 0.05$) (Table 6).

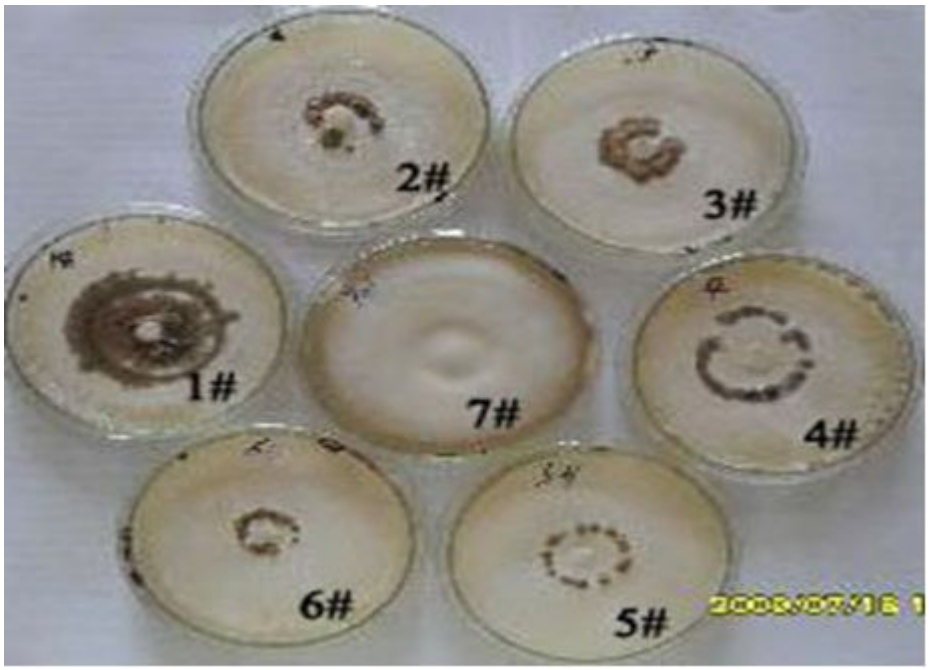

Figure 2. Mycelium morphology of seven *P. umbellatus* strains after 45 days' culture in PDA medium at 25℃. The circular secretions were all proved as polysaccharide.

Table 3. Calcium oxalate crystals and asexual spores observed in mycelium colonies of seven strains of *P. umbellatus* after 15 days' culture in PDA medium.

Strain (#)	Mycelium diameter (μm)	Number of calcium oxalate crystals *	The number of asexual spores *
1	2.0 - 3.0	+++	+++
2	1.5 - 3.0	++	++
3	1.5 - 3.0	++	++
4	2.0 - 4.0	++++	++
5	1.5 - 3.0	++	+
6	1.5 - 3.5	+++	+
7	2.5 - 3.5	+	++

Note: * The number of plus sign did not mean the accurate but the relative number. Both calcium oxalate crystals and asexual spores were observed in 40 × 10 multiple.

DISCUSSION

Based on the results of mycelial growth and mycelium diameter, no obvious difference has been found among seven strains of *P. umbellatus* collected from different provinces. This may be attributed to the fact that they belonged to one species. Therefore, they have genetic similarity and the difference possibly caused by environmental heterogeneity.

The results also showed that both 1# and 4# had the highest dried weight of mycelium, and 1# and 2# produced the most extra- and intracellular polysaccharide. Therefore, 1# could be considered as the best strain, which is in accordance with the better medicinal effect of Shaanxi materials reported by Xu (1997). In addition, 1# was found to produce the most asexual spores. So, the relationship between the number of asexual spores and polysaccharide content need further investigation.

Correlation analysis results displayed the positive relationship between intra- and extracellular polysaccharide content, which indicated that alternative content could be selected when detecting polysaccharide. Extracellular content was found negatively correlated with daily mycelial growth rate; this provided a clue to researchers and farmers that over-quick mycelial growth did not facilitate the accumulation of polysaccharide.

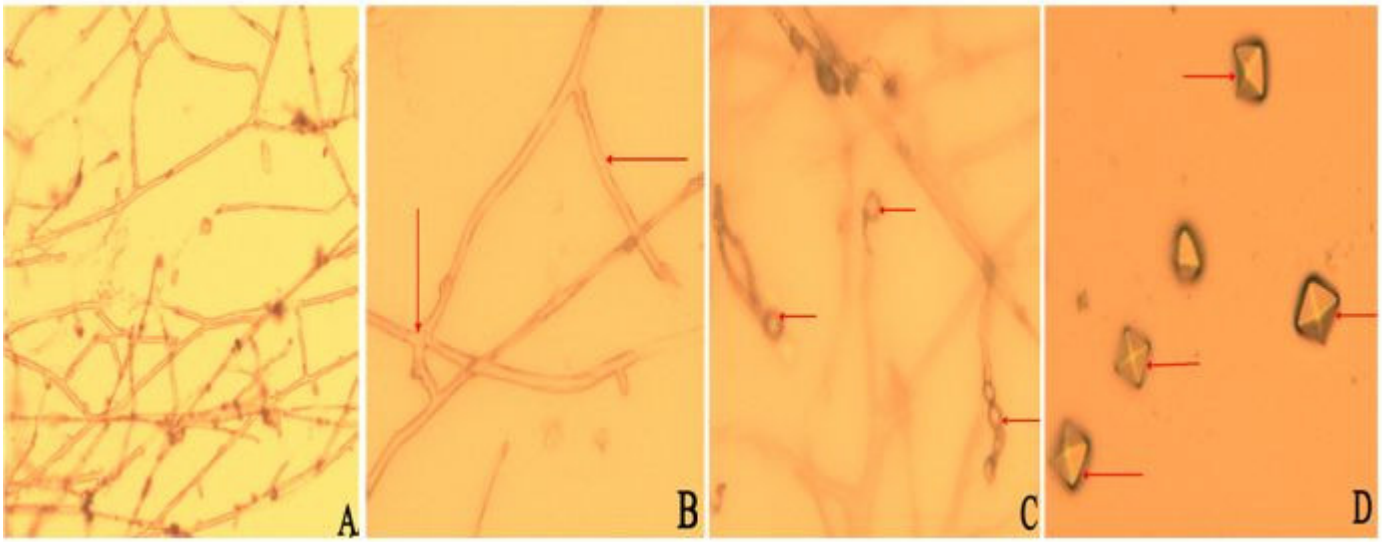

Figure 3. Mycelial morphology, asexual spores and calcium oxalate crystals of *P. umbellatus* after fifteen-day culture in PDA medium at 25℃. A is 2# mycelium (20 × 10 multiple); B is 1# mycelium in which clamp connection and H-type can be found (red arrow); C exhibits asexual spores observed in 1# mycelium (red arrow); D are calcium oxalate crystals found in 4# mycelium (red arrow). B, C and D are observed in 40 × 10 multiple.

Table 4. The dry weight of seven mycelium strains of *P. umbellatus* after ten-day liquid culture at 25℃.

Strain (#)	Dry weight of mycelium (g/L)
1	7.640
2	5.395
3	6.050
4	7.330
5	6.645
6	4.500
7	5.966

Table 5. Intra- and extracellular polysaccharide content of seven *P. umbellatus* strains detected by spectrophotometer.

Strain (#)	Absorbance A	Intracellular polysaccharide content (mg/g)	Absorbance A	Extracellular polysaccharide content (mg/ml)
1	0.568	64.496	0.572	2.598
2	0.531	60.289	0.542	2.462
3	0.222	25.153	0.560	1.590
4	0.339	38.457	0.481	2.184
5	0.227	25.721	0.525	1.192
6	0.250	28.337	0.505	2.293
7	0.208	23.561	0.362	1.643

Table 6. Correlation analysis on four indices about mycelium growth and polysaccharide content of *P. umbellatus.*

	Mean daily growth rate (mm/d)	Dry weight of mycelium (g/l)	Intracellular polysaccharide content (mg/g)	Extracellular polysaccharide content (mg/ml)
Mean daily growth rate (mm/d)	1			
Dry weight of mycelium (g/l)	-0.067	1		
Intracellular polysaccharide content (mg/g)	-0.637	0.324	1	
Extracellular polysaccharide content (mg/ml)	-0.788 *	-0.004	0.802 *	1

* represented significance ($P < 0.05$).

Conclusion

The 7# strain of *P. umbellatus* collected from Hebei had the highest mycelium growth rate. Both 1# (Shaanxi) and 4# (Jilin) produced the most dried weight of mycelium, and 1# and 2# (Hubei) did the highest intra- and extracellular polysaccharide content. Correlation analysis exhibited that intra- and extracellular polysaccharide content had significant and positive relationship, but extracellular content was negatively correlated with daily mycelial growth rate.

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