

Isolation, Culture and Bioactive Substances Elicitation of *Sanghuangporus Baumii*

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Abstract

Sanghuangporus baumii is a forest pathogenic fungus and also a medicine mushroom. In the wild, *S. baumii* parasitize on living host plants and there have been no reports of cultivating fruiting bodies under artificial conditions. In this study, we identified and isolated a *S. baumii* strain, and successfully cultivated the fruiting bodies on sawdust medium by optimizing culture conditions. The optimum medium, culture temperature and pH for mycelial growth of *S. baumii* were WBA and YPA, at pH 5.5–7.5 and 28°C, respectively. The contents of total flavonoids, total polysaccharides and total triterpenoids in *S. baumii* were compared with those in other two medicinal sanghuang, *S. vaninii* and *S. sanghuang*. The results showed that both the fruiting bodies and mycelia of *S. baumii* were rich in bioactive substances. The content of total flavonoids was higher in the fruiting bodies (34.91 mg/g), while the contents of total polysaccharides and total triterpenoids were higher in the mycelia (45.41 mg/g, 14.06 mg/g, respectively). In addition, the mycelia of *S. baumii* could be elicited to produce more bioactive substances. The use of sealing film in culture increased the polysaccharides content in mycelia by 51.8%, while the light increased the flavonoids content by 151.0%. The cultivation of fruiting bodies and the elicitation of bioactive substances from mycelia provide biological materials for the study and utilization of *S. baumii*.

Introduction

Sanghuang is a lethal or pathogenic fungus that damages a variety of hardwood species, causing living wood to decay and form large fruiting bodies (Kim, Hohenlohe, Kim, Seo, & Klopfenstein, 2016). However, the economic value of this pathogenic fungus outweighs the damage it causes. The fruiting bodies formed by *Sanghuangporus* spp. are well-known mushrooms that have been used as traditional medicine for thousands of years in China and other East Asian regions (Han et al., 2016). The most ancient record of sanghuang that is currently available is the “Shen Nong Materia Medica”, which was written around 102–200 A.D. Although this record was written before the advent of modern science, it was empirically found that *Sanghuangporus* spp. were indeed effective against diseases. In recent decades, *Sanghuangporus* has received widespread attention for its anti-inflammatory and anti-cancer effects (Lin, Deng, Huang, Lin, et al., 2017; Lin, Deng, Huang, Wu, Chen, et al., 2017; Lin, Deng, Huang, Wu, Lin, et al., 2017; K. Liu, Xiao, Wang, Chen, & Hu, 2017; M. M. Liu, Zeng, Li, & Shi, 2016; Xue, Sun, Zhao, Zhang, & Lai, 2011), which are due to a variety of bioactive substances produced by *Sanghuangporus* spp., such as polysaccharides (Q. Ge, Mao, Zhang, Wang, & Sun, 2013), flavonoids (Y. R. Ge et al., 2012), triterpenoids (Cai et al., 2019), and polyphenols (Zhang et al., 2019).

Currently, three species of *Sanghuangporus* have been proved to have medicinal properties, namely *S. baumii*, *S. vaninii*, and *S. sanghuang* (M. D. Wu et al., 2019; S. H. Wu et al., 2012; Zhou, Ghobad-Nejhad, Tian, Wang, & Wu, 2020). The taxonomy of *Sanghuangporus* has been a constant topic of debate due to the high levels of phenotypic plasticity. In fact, it wasn't until 2016 that the *Sanghuangporus* was identified as a new genus, rather than a clade of *Inonotus*. *Sanghuangporus sanghuang* was identified and named by Wu et al. (S. H. Wu et al., 2012) and is considered to be the only true sanghuang.

Sanghuangporus vaninii can produce fruiting bodies under artificial conditions, so it is widely sold in medicinal markets (Zhou et al., 2020). *Sanghuangporus baumii* parasitic on the trunk of *Syringa reticulata* and cannot be cultivated under artificial conditions. It also contains bioactive substances with anti-tumor and immunomodulation activity, but it is not as well-known as *S. sanghuang* and *S. vaninii*. The failure of cultivation greatly restricted the research and the industrialization of *S. baumii*. This failure is most likely due to a lack of knowledge of appropriate cultivation conditions and fruiting management. To date, it remains unknown how the medium, pH, and temperature affect the mycelial growth of *S. baumii*, and there have been very few reports on fruiting body cultivation and management.

Compared with fruiting bodies, mycelia also contain a large number of bioactive substances, and mycelia are easier to obtain through fermentation culture (Rathore, Prasad, Kapri, Tiwari, & Sharma, 2019). However, many bioactive substances are secondary metabolites whose gene clusters are silent under standard laboratory conditions. Optimization of culture conditions (medium, pH, temperature, etc.) can achieve high biomass yield, but may not increase the content of bioactive substances. Elicitation is one of the most effective techniques currently used for improving the bioactive substances production (Ramirez-Estrada et al., 2016). The elicitors are mainly carbon sources, inorganic compounds or physical factors such as oxygen and light (Tian, Dai, Song, Xu, & Ng, 2015). To the best of our knowledge, no work has been published on the elicitation bioactive substances of *S. baumii*.

In this paper, we described, isolated and cultured the medicinal mushroom *S. baumii*, and successfully cultivated the fruiting bodies. In addition, since *S. baumii*, like *S. vaninii* and *S. sanghuang*, is known for its medicinal properties, we compared the contents of total polysaccharides, flavonoids, and triterpenoids in mycelia and fruiting bodies of the three species. We also tested the contents of bioactive substances in the elicited *S. baumii* mycelia.

Materials And Methods

Strain collection, isolation and morphological study

Basidiocarps of *S. baumii*, *S. vaninii*, and *S. sanghuang* were collected in China in September 2019 (Details are shown in Table S1). The internal tissue sections of the fruiting bodies were transferred onto potato dextrose agar (PDA) and incubated at 25°C under dark conditions until the agar surface was covered with mycelium. The strains were preserved at the College of Forestry, Northeast Forestry University.

The fresh samples were photographed, and macroscopic details were described. Notably, the macro-morphological characteristics were described following the methods reported in Lodge et al. (Lodge, Ammirati, dell, & Mueller, 2004). Moreover, the Ridgeway color standards (Zimmer, 1948) were used (the corresponding keys are displayed in parentheses after the color descriptions in the Results section). Permanent sections were cut using a freezing microtome (Leica CM1520; Leica, Wetzlar, Germany), and microphotography was performed with a compound microscope (ECLIPSE Ni-U; Nikon, Tokyo, Japan).

The size of basidiospores was followed by length × width, and the length or width is in the form of (a–b–c(–d)), where a and d are the minimum and maximum values respectively, and 90% of the measured values were within the range of b–c. The samples were air-dried and deposited in the Herbarium of the College of Forestry, Northeast Forestry University, Harbin, China.

Phylogenetic analysis

Genomic DNA was extracted from the internal tissues of basidiocarps using the cetyltrimethylammonium bromide method (Allen, Flores-Vergara, Krasnyanski, Kumar, & Thompson, 2006). The ITS sequences were amplified by polymerase chain reaction (PCR) using the primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR conditions were as follows: 95°C for 3 min, followed by 35 cycles at 95°C for 30 s, 56°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were purified and sequenced by Boshi Biotech Co., Harbin, China. Sequence similarity searches were performed using GenBank and the basic local alignment search tool algorithm (<http://www.ncbi.nlm.nih.gov/blast>). Homologous sequences were downloaded and aligned, and a neighbor-joining tree was constructed using MEGA 5.0 software (S. H. Wu et al., 2012).

Optimization of mycelium culture conditions

Three different culture media were used to determine the optimal medium for mycelial growth. These three media consisted of PDA (20 g/L potato infusion, 20 g/L dextrose, and 15 g/L agar), wheat bran extract agar (WBA; 15 g/L wheat bran extract, 20 g/L dextrose, and 15 g/L agar), and yeast extract peptone agar (YPA; 10 g/L yeast extract, 10 g/L peptone, and 15 g/L agar), respectively. The PDA medium was used to determine the optimal pH for mycelial growth, and pH was adjusted to 5.5, 6.0, 6.5, 7.0, or 7.5, with 1 N HCl and 1 N NaOH. The PDA medium with a natural pH was used to determine the optimal temperature for mycelial growth, and the temperature was set to 18°C, 21°C, 23°C, 25°C, or 28°C. For all treatments, five replicates were used, and incubation was performed under dark conditions for 10 days. The culture temperature used to determine the optimum medium and optimum pH was 25°C.

Mycelial growth was evaluated based on the mycelial growth rate and mycelial density. The mycelial growth rate was calculated by averaging the vertical and horizontal lengths of the colony diameter. Mycelial density was classified as very scanty (+), scanty (2+), moderate (3+), somewhat abundant (4+), and abundant (Jo et al., 2009). Data analysis was conducted using SPSS version 21 (IBM, Armonk, NY) with six replicates.

Fermentation culture and cultivation

The three strains of *Sanghuangporus* were inoculated in potato dextrose broth media and maintained at 25°C and 160 rpm for 7 days before harvesting their mycelia.

The sawdust medium consisted of a mass fraction of 60% water, 34% sawdust, 5% wheat bran, 0.5% potassium dihydrogen phosphate, and 0.5% magnesium sulfate. The medium was adjusted to the optimum pH to cultivate the basidiocarps of the three strains. The mixed medium was placed in polypropylene bags and sterilized in an autoclave sterilizer at 121°C for 2 h. The strains were inoculated

on sawdust medium, and each strain was cultured in 10 bags. The inoculated bags were incubated under dark conditions and at the optimum temperature for each strain until the medium was fully colonized by the mycelia. The bags were then transferred to a mushroom chamber for subsequent fruiting management.

Elicitation of bioactive substances in *S. baumii*

Eleven different treatments (a ~ k) were evaluated to elicit the bioactive substances of *S. baumii*. The semi-solid media formulae used are shown in Table 1. All elicitation cultures were performed in glass Petri dishes. After inoculation, the Petri dishes of treatment a and treatment d-k were wrapped around the circumference twice with air-permeable Parafilm (PM-996 Parafilm® M Laboratory Film). The Petri dishes of treatment b were wrapped with air-impermeable grafting membrane (LINGS company, China). The Petri dishes of treatment c were not wrapped. Except for treatment k, all Petri dishes were incubated at 25°C under dark conditions for 12 days. The petri dishes of treatment k underwent 10 days of dark culture and 2 days of light culture with a light intensity of 200 $\mu\text{mol s}^{-1} \text{m}^{-2}$. After the elicitation culture, the colony of *S. baumii* was picked out to harvest mycelium.

Table 1
Composition of semi-solid media used in the induction.

media reagents	media composition (g/L)										
	a	b	c	d	e	f	g	h	i	j	k
Potato infusion	200	200	200	200	200	200	200	200	200	200	200
Glucose	20					20	20	20	20	20	20
Lactose				20							
Saccharose					20						
Di-potassium phosphate						1.36	40.8				
Ammonium sulfat								4			
Peptone									4		
Urea										4	
Agar	6	6	6	6	6	6	6	6	6	6	6

Assay of bioactive substances

The fruiting bodies and mycelia were sampled, dried to a constant weight at 50°C, ground into powder and sifted through a 60-mesh sieve. The total polysaccharides were determined by the phenol-sulfuric acid method (Habijanac, Berovic, Boh, Plankl, & Wraber, 2015) (Tan et al., 2018). The total flavonoids were determined by the aluminum nitrate method (Ruiz-Riaguas, Fernandez-de Cordova, & Llorent-Martinez, 2020). The total triterpenoids were determined by the method described by Ren et al. (Ren et al., 2010).

Statistical analysis

Quantitative data are presented as mean values from three independent experiments with standard deviation of the means. Statistical analysis was performed using Tukey tests with STATGRAPHICS PLUS software (STATPOINT TECHNOLOGIES, INC., Virginia). Values were considered statistically significantly different at the $p < 0.05$ level.

Results

Morphological and phylogenetic analysis

The morphological characteristics of the basidiocarps of *S. baumii* are displayed in Figure 1. The *S. baumii* basidiocarps were perennial and sessile with a cork texture when they were fresh, and a woody hard texture when they were dry. The pileus was semicircular, 7–12 cm in length, 3.5–6.0 cm in width, and up to 3.0 cm thick. The pileus surface was dark brown (7F5), glabrous, and rough, and it displayed radial cracking. There were also dense to sparse concentric annular grooves from the center to the edge of the pileus. The pore surface was light brown (6D8), and the margin was yellow ochre (5C7). There were 9–11 pores per millimeter. The setae were conical, 11–20 μm in length, and 4–8 μm in width. The basidiospores were ellipsoid, mostly sunken, and were (3.0–)3.5–4.5(–4.8) \times (2.5–)3.0–3.5(–3.8) μm in size. The generative hyphae were 2.0–3.5 μm in diameter, transparent, pale yellow, and branched. The skeletal hyphae were 2.0–3.4 μm in diameter, brown, thick-walled, and arranged closely and in parallel.

The morphological characteristics of the basidiocarps of *S. vaninii* are presented in Figure S1. The basidiocarps of *S. vaninii* were perennial and sessile, with a cork texture when they were fresh, and a hard woody texture when they were dry. The pileus was semicircular, 6–10 cm in length, 3.5–5.0 cm in width, and up to 3.5 cm thick. The pileus surface was Chinese yellow (4B7) to olive brown (4F7), and glabrous. It was characterized by dense to sparse concentric annular grooves from the center to the edge of the pileus. The pore surface was Chinese yellow (4B8), and there were 9–10 pores per millimeter. The setae were conical, 10–20 μm in length, and 4–7 μm in width. The basidiospores were ellipsoid, mostly sunken, and (2.8–)3.3–4.2(–4.3) \times (2.5–)3.0–3.5(–3.7) μm in size. The generative hyphae were 2.0–3.5 μm in diameter, transparent, pale yellow, and branched. The skeletal hyphae were 2.0–3.5 μm in diameter, golden brown, thick-walled, and closely arranged in parallel.

The morphological characteristics of the basidiocarps of *S. sanghuang* are shown in Figure S2. The *S. sanghuang* basidiocarps were perennial and sessile, with a cork texture when they were fresh, and a hard woody texture when they were dry. The pileus was irregularly semicircular, 8–15 cm in length, 5–9 cm in width, and up to 4.0 cm thick. The pileus surface was brown (6E8), glabrous, rough, and displayed irregular longitudinal cracking, as well as dense to sparse concentric annular grooves from the center to the edge of the pileus. The pore surface was yellow ochre (5C7), and the margin was buttercup yellow (4A7). There were 6–8 pores per millimeter. The setae were conical, 15–23 μm in length and 6–10 μm in width. The basidiospores were ellipsoid, mostly sunken, and (3.0–)3.4–4.2(–4.4) \times (2.6–)3.0–3.4(–3.7)

µm in size. The generative hyphae were 2.0–3.0 µm in diameter, transparent, brown, and branched. Likewise, the skeletal hyphae were 2.0–3.0 µm in diameter, but golden brown, thick-walled, and arranged closely and in parallel.

The phylogenetic tree included 20 species from the Hymenochaetaceae family and was based on the ITS sequences (Figure 2). The clustering of these sequences in the phylogenetic tree showed that the species phylogeny was divided into three clades (i.e., *Sanghuangporus*, *Tropicoporus*, and *Phellinus*). The phylogenetic analysis indicated that *S. sanghuang*, *S. vaninii*, and *S. baumii* belonged to the *Sanghuangporus* clade.

Optimal culture conditions and fruiting management

After 10 days of cultivation, the media surfaces were colonized with filamentous colonies. However, the growth status of the three strains varied between the three media (Figure S3). The mycelial growth rates and mycelial density of the three species in each culture medium are presented in Table 2.

Sanghuangporus baumii formed white filamentous colonies on the three media. The highest mycelial growth rate was observed on WBA and YPA media, while the greatest mycelial density was observed on PDA and YPA media. *Sanghuangporus vaninii* formed white colonies on YPA media and white to yellowish colonies on PDA and WBA media. The highest mycelial growth rates of *S. vaninii* were observed on PDA and YPA media, while the greatest mycelial densities were observed on WBA and YPA media. *Sanghuangporus sanghuang* formed white to yellow colonies on the three media, and the mycelium growth rate and mycelial density did not significantly differ between the media.

Table 2

Mycelial growth rates and mycelial densities of the three *Sanghuangporus* strains incubated at 25°C for 10 days on three different media.

Strains	Media	Growth rates(mm/day)	Mycelial density
<i>S. baumii</i>	PDA	3.08 ± 0.14 ^{bc}	5+
	WBA	3.62 ± 0.06 ^a	4+
	YPA	3.46 ± 0.04 ^{ab}	5+
<i>S. vaninii</i>	PDA	3.38 ± 0.24 ^{ab}	4+
	WBA	2.98 ± 0.18 ^c	5+
	YPA	3.52 ± 0.15 ^a	5+
<i>S. sanghuang</i>	PDA	2.28 ± 0.73 ^d	3+
	WBA	2.20 ± 0.16 ^d	3+
	YPA	2.15 ± 0.26 ^d	3+

Note: Values followed by the same lowercase letter are not significantly different ($p < 0.05$). PDA, potato dextrose agar; WBA, wheat bran extract agar; and YPA, yeast extract peptone agar.

All pH values between 5.5 and 7.5 were suitable for the mycelium growth of the three species (Table 3). There was no significant difference in the mycelium growth rate or mycelial density of *S. baumii* as the pH changed. As for *S. vaninii* and *S. sanghuang*, although they grew well on media of pH 5.5–7.5, the most favorable pH was 7.5, with the highest mycelium growth rates and mycelial densities, followed by pH 7.0. The mycelial growth rates of the three species increased when the temperature increased from 18°C to 28°C (Table 4). Notably, the mycelial density of *S. baumii* remained abundant across different temperatures. In contrast, the highest mycelial density was observed between 21°C and 25°C for *S. vaninii* and at 28°C for *S. sanghuang*.

Table 3

Mycelial growth rates and mycelial densities of the three *Sanghuangporus* strains incubated at 25°C for 10 days in media of differing pH.

Strains	pH	Growth rates(mm/day)	Mycelial density
<i>S. baumii</i>	5.5	3.57 ± 0.11 ^a	5+
	6	3.63 ± 0.09 ^a	5+
	6.5	3.54 ± 0.17 ^a	5+
	7	3.61 ± 0.06 ^a	5+
	7.5	3.63 ± 0.06 ^a	5+
<i>S. vaninii</i>	5.5	3.40 ± 0.17 ^b	4+
	6	3.28 ± 0.04 ^b	4+
	6.5	3.47 ± 0.16 ^b	4+
	7	3.44 ± 0.13 ^b	5+
	7.5	3.76 ± 0.04 ^a	5+
<i>S. sanghuang</i>	5.5	2.68 ± 0.03 ^d	3+
	6	3.04 ± 0.11 ^b	3+
	6.5	2.87 ± 0.08 ^c	3+
	7	3.16 ± 0.12 ^{ab}	3+
	7.5	3.20 ± 0.09 ^a	4+

Note: For each strain, values followed by the same lowercase letter are not significantly different ($p < 0.05$).

Table 4

Mycelial growth rates and mycelial densities of the three *Sanghuangporus* strains incubated on potato dextrose agar (PDA) media for 10 days at different temperatures.

Strains	Temperature (°C)	Growth rates(mm/day)	Mycelial density
<i>S. baumii</i>	18	0.96 ± 0.17 ^e	5+
	21	2.49 ± 0.10 ^d	5+
	23	2.82 ± 0.18 ^c	5+
	25	3.40 ± 0.04 ^b	5+
	28	3.73 ± 0.06 ^a	5+
<i>S. vaninii</i>	18	1.43 ± 0.12 ^c	3+
	21	2.98 ± 0.18 ^b	4+
	23	2.99 ± 0.06 ^b	4+
	25	3.14 ± 0.13 ^{ab}	4+
	28	3.29 ± 0.10 ^a	3+
<i>S. sanghuang</i>	18	1.26 ± 0.08 ^e	2+
	21	2.43 ± 0.06 ^d	3+
	23	2.69 ± 0.06 ^c	3+
	25	2.95 ± 0.04 ^b	3+
	28	3.15 ± 0.13 ^a	4+
Note: For each strain, values followed by the same letter are not significantly different (p < 0.05).			

The bags full of mycelia were transferred to the mushroom chamber for further culture until the color of the mycelia changed from white to dark yellow, which took approximately 10 days. A sterile scalpel was then used to make incisions, which were 5–8 cm long and 0.5 cm deep, on the surface of the bags. In the mushroom chamber, the temperature was maintained at 25°C–28°C, the air humidity was of 85–95%, the light intensity was of 200–300 lx, and the chamber was ventilated twice a day. The basidiocarps of *S. baumii* and *S. vaninii* developed from the incisions (Figure 3), whereas *S. sanghuang* did not form fruiting bodies.

Comparison of bioactive substance contents in mycelia and fruiting bodies of the three species

The contents of bioactive substance of three species were significantly different. In the fruit bodies, the contents of total flavonoids and total triterpenoids of *S. vaninii* were the highest among the three species, and there was no significant difference in polysaccharides contents among the three species (Table 5). In the mycelia, the total polysaccharides content of *S. vaninii* was the highest, while the total flavonoids and triterpenoids contents of *S. sanghuang* were the highest. In addition, there were significant differences in bioactive substances between fruiting body and mycelia of the same strain. The total polysaccharides contents in the mycelia of *S. baumii*, *S. vaninii*, and *S. sanghuang* was 5.1-, 5.4-, and 4.5-fold, respectively, higher than that in their fruiting bodies, and the total triterpenoids contents in their mycelia was 2.6-, 1.1-, and 3.5-fold, respectively, higher than that in their fruiting bodies. The total flavonoids contents in the fruiting bodies of *S. baumii*, *S. vaninii*, and *S. sanghuang* was 5.4-, 19.6-, and 4.3-fold, respectively, higher than that in the mycelia.

Table 5

Content of bioactive substances in fruiting body and mycelium of the three *Sanghuangporus* strains

Strains	Part	Total Polysaccharides (mg/g)	Total Flavonoid (mg/g)	Total Triterpenoids (mg/g)
<i>S. baumii</i>	Fruiting body	8.84 ± 0.55 ^c	34.91 ± 2.14 ^b	5.44 ± 0.16 ^d
	Mycelium	45.41 ± 0.87 ^a	6.49 ± 1.12 ^{cd}	14.06 ± 1.07 ^b
<i>S. vaninii</i>	Fruiting body	8.47 ± 0.38 ^c	51.97 ± 0.97 ^a	11.74 ± 0.70 ^c
	Mycelium	46.01 ± 0.91 ^a	2.65 ± 0.46 ^d	12.89 ± 0.56 ^{bc}
<i>S. sanghuang</i>	Fruiting body	8.74 ± 0.57 ^c	39.66 ± 1.86 ^b	4.63 ± 0.28 ^d
	Mycelium	39.37 ± 0.86 ^b	9.17 ± 0.56 ^c	15.96 ± 0.42 ^a

Note: For each bioactive substance, values followed by the same letter are not significantly different (p < 0.05).

Elicitation of bioactive substances of *S. baumii*

Eleven different treatments (a ~ k) had different effects on the growth and bioactive substances contents of *S. baumii* (Figure 4 and 5). The mycelia of *S. baumii* grew poorly under the treatment g so that there were not enough mycelia to measure the bioactive substances. The mycelia of *S. baumii* could not grow under the treatment j. The treatment a used common culture conditions (PDA semi-solid media plates, sealed with Parafilm, dark culture) and was therefore used as a control. The mycelial growth rate of

treatment b was 3.61 ± 0.05 mm/day, which was the only one higher than that of the control (3.48 ± 0.11 mm/day). The biomass of the treatment c was 201.4 ± 3.26 mg, which was the only one higher than that of control (133.7 ± 8.87 mg). Other treatments inhibited the growth rate and biomass of mycelia to varying degrees. The flavonoids contents of the treatment c and k were 14.12 ± 0.38 mg/g and 26.7 ± 0.42 mg/g, respectively, which were significantly higher than that of the control (10.64 ± 0.35 mg/g). The polysaccharides contents of the treatment b (38.43 ± 1.79 mg/g), d (30.49 ± 0.52 mg/g), f (33.97 ± 0.79 mg/g), h (32.55 ± 1.30 mg/g), and k (34.90 ± 2.43 mg/g) were significantly higher than that of the control (25.31 ± 1.05 mg/g). The triterpenoids contents in all treatments were not significantly higher than that of the control.

Discussion

This study describes and cultivates a medicinal mushroom, *S. baumii*, and compares it with *S. vaninii* and *S. sanghuang*. The three species shared common characteristics of *Sanghuangporus*, such as the perennial basidiocarps with a dimitic hyphal system, a hard woody texture when they are dry, and conical setae in the hymenophore. Notably, the morphological analysis revealed that typical individuals of these three species can be distinguished by their macroscopic characteristics, despite high levels of phenotypic plasticity.

The effects of the medium, pH, and temperature on the mycelial growth of the *S. baumii* were assessed. The best growth rates and mycelium density were obtained on WBA and YPA media, which indicated that organic nitrogen (wheat bran extract and yeast extract) was beneficial to the growth of mycelia. Therefore, 5% wheat bran was added into the sawdust medium for *S. baumii* cultivation. There was no difference in the mycelial growth of *S. baumii* at pH of 5.5–7.5, while the optimum pH of *S. vaninii* and *S. sanghuang* was 7.5. Therefore, sawdust medium at pH 7.5 was used to cultivate *S. baumii*. Within the temperature range of this study, 28°C was the most suitable temperature for the mycelial growth of *S. baumii*. The optimum temperature for the mycelial growth of *S. vaninii* and *S. sanghuang* was also 28°C, indicating that the three species had the same sensitivity to temperature. In the cultivation of *S. baumii*, 28°C is the most suitable temperature for mycelial growth. When the sawdust medium is completely colonized by the mycelium, a low temperature stimulation is required to form the fruiting body. In view of the consistent temperature sensitivity of *S. vaninii* and *S. baumii*, we referred to the stimulation temperature of *S. vaninii* and successfully cultivated the fruiting body of *S. baumii* at 18°C–23°C.

There was no significant difference in the contents of the three bioactive substances in fruit bodies between *S. baumii* and *S. sanghuang*, and no significant difference in mycelia between *S. baumii* and *S. vaninii*. This means that *S. baumii* contains as much bioactive substances as *S. sanghuang* and *S. vaninii*. In addition, it was found in *S. baumii* that the content of flavonoids in fruit bodies was much higher than that in mycelia, while the contents of polysaccharides and triterpenoids in mycelia were much higher than that in fruit bodies. This indicated that the fruit bodies of *S. baumii* had advantages in the production of flavonoids, while the mycelia had advantages in the production of polysaccharides and triterpenoids.

Most of the elicitors used in this study had adverse effects on the mycelial growth and bioactive substances contents of *S. baumii*. Among them, different sealing methods and light obtained satisfactory effects. In biological laboratories, agar culture plates are often wrapped to avoid dehydration and contamination, but this sealing also severely limits the rate of gas flow in and out of the culture container, thus affecting the growth, development and gene expression of organisms (Xu, Li, Shabala, Jian, & Zhang, 2019). Sealing with Parafilm reduces the gas exchange rate, while the sealing with grafting membrane prevents gas exchange. The biomass and flavonoids content of mycelia in unwrapped treatment c increased significantly, while that in air-impermeable grafting membrane wrapped treatment b decreased significantly. This indicated that the different sealing methods or gas exchange rates affected both primary metabolites and secondary metabolites of *S. baumii*. Interestingly, these effects were not simply inhibiting or promoting, because although the mycelia biomass of treatment b was decreased, the growth rate and polysaccharides content of mycelia were both increased. The effect of gas exchange rates on mycelia metabolism was then analyzed in detail by transcriptome sequencing (data not shown). Light significantly increased the contents of flavonoids and polysaccharides in the mycelia. Studies have shown that light exposure, especially blue light, promotes the accumulation of flavonoids by up-regulating the genes of flavonoids synthesis in *S. vaninii* (Ma et al., 2021).

Conclusion

Sanghuangporus baumii is a kind of sanghuang with similar morphological characteristics to *S. vaninii* and *S. sanghuang*. The fruiting bodies and mycelia of *S. baumii* are rich in bioactive substances. The fruiting bodies of *S. baumii* can be successfully cultured under appropriate culture and management conditions. The mycelia of *S. baumii* can be elicited to produce more bioactive substances, among which gas exchange rate and light are effective elicitors.

Declarations

Author contributions Shixin Wang, Ruipeng Liu and Li Zou contributed to the study conception and design. Material preparation, data collection and analysis were performed by Xutong Wang, Yawei Li and Zengcai Liu. The first draft of the manuscript was written by Shixin Wang and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Conflict of interest The authors have no conflict of interest to declare.

Ethical approval This article does not contain any studies with human participants or animal experiments.

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Tables

Table 1. Composition of semi-solid media used in the induction.

media reagents	media composition (g/L)										
	a	b	c	d	e	f	g	h	i	j	k
Potato infusion	200	200	200	200	200	200	200	200	200	200	200
Glucose	20					20	20	20	20	20	20
Lactose			20								
Saccharose				20							
Di-potassium phosphate					1.36	40.8					
Ammonium sulfate							4				
Peptone								4			
Urea										4	
Agar	6	6	6	6	6	6	6	6	6	6	6

Table 2. Mycelial growth rates and mycelial densities of the three *Sanghuangporus* strains incubated at 25°C for 10 days on three different media.

Strains	Media	Growth rates(mm/day)	Mycelial density
<i>S. baumii</i>	PDA	3.08 ± 0.14 ^{bc}	5+
	WBA	3.62 ± 0.06 ^a	4+
	YPA	3.46 ± 0.04 ^{ab}	5+
<i>S. vaninii</i>	PDA	3.38 ± 0.24 ^{ab}	4+
	WBA	2.98 ± 0.18 ^c	5+
	YPA	3.52 ± 0.15 ^a	5+
<i>S. sanghuang</i>	PDA	2.28 ± 0.73 ^d	3+
	WBA	2.20 ± 0.16 ^d	3+
	YPA	2.15 ± 0.26 ^d	3+

Note: Values followed by the same lowercase letter are not significantly different ($p < 0.05$). PDA, potato dextrose agar; WBA, wheat bran extract agar; and YPA, yeast extract

peptone agar.

Table 3. Mycelial growth rates and mycelial densities of the three *Sanghuangporus* strains incubated at 25°C for 10 days in media of differing pH.

Strains	pH	Growth rates(mm/day)	Mycelial density
<i>S. baumii</i>	5.5	3.57 ± 0.11 ^a	5+
	6	3.63 ± 0.09 ^a	5+
	6.5	3.54 ± 0.17 ^a	5+
	7	3.61 ± 0.06 ^a	5+
	7.5	3.63 ± 0.06 ^a	5+
<i>S. vaninii</i>	5.5	3.40 ± 0.17 ^b	4+
	6	3.28 ± 0.04 ^b	4+
	6.5	3.47 ± 0.16 ^b	4+
	7	3.44 ± 0.13 ^b	5+
	7.5	3.76 ± 0.04 ^a	5+
<i>S. sanghuang</i>	5.5	2.68 ± 0.03 ^d	3+
	6	3.04 ± 0.11 ^b	3+
	6.5	2.87 ± 0.08 ^c	3+
	7	3.16 ± 0.12 ^{ab}	3+
	7.5	3.20 ± 0.09 ^a	4+

Note: For each strain, values followed by the same lowercase letter are not significantly different ($p < 0.05$).

Table 4. Mycelial growth rates and mycelial densities of the three *Sanghuangporus* strains incubated on potato dextrose agar (PDA) media for 10 days at different temperatures.

Strains	Temperature (°C)	Growth rates(mm/day)	Mycelial density
<i>S. baumii</i>	18	0.96 ± 0.17 ^e	5+
	21	2.49 ± 0.10 ^d	5+
	23	2.82 ± 0.18 ^c	5+
	25	3.40 ± 0.04 ^b	5+
	28	3.73 ± 0.06 ^a	5+
<i>S. vaninii</i>	18	1.43 ± 0.12 ^c	3+
	21	2.98 ± 0.18 ^b	4+
	23	2.99 ± 0.06 ^b	4+
	25	3.14 ± 0.13 ^{ab}	4+
	28	3.29 ± 0.10 ^a	3+
<i>S. sanghuang</i>	18	1.26 ± 0.08 ^e	2+
	21	2.43 ± 0.06 ^d	3+
	23	2.69 ± 0.06 ^c	3+
	25	2.95 ± 0.04 ^b	3+
	28	3.15 ± 0.13 ^a	4+

Note: For each strain, values followed by the same letter are not significantly different ($p < 0.05$).

Table 5. Content of bioactive substances in fruiting body and mycelium of the three *Sanghuangporus* strains

Strains	Part	Total Polysaccharides (mg/g)	Total Flavonoid (mg/g)	Total Triterpenoids (mg/g)
<i>S. baumii</i>	Fruiting body	8.84 ± 0.55 ^c	34.91 ± 2.14 ^b	5.44 ± 0.16 ^d
	Mycelium	45.41 ± 0.87 ^a	6.49 ± 1.12 ^{cd}	14.06 ± 1.07 ^b
<i>S. vaninii</i>	Fruiting body	8.47 ± 0.38 ^c	51.97 ± 0.97 ^a	11.74 ± 0.70 ^c
	Mycelium	46.01 ± 0.91 ^a	2.65 ± 0.46 ^d	12.89 ± 0.56 ^{bc}
<i>S. sanghuang</i>	Fruiting body	8.74 ± 0.57 ^c	39.66 ± 1.86 ^b	4.63 ± 0.28 ^d
	Mycelium	39.37 ± 0.86 ^b	9.17 ± 0.56 ^c	15.96 ± 0.42 ^a

Note: For each bioactive substance, values followed by the same letter are not significantly different ($p < 0.05$).

Figures

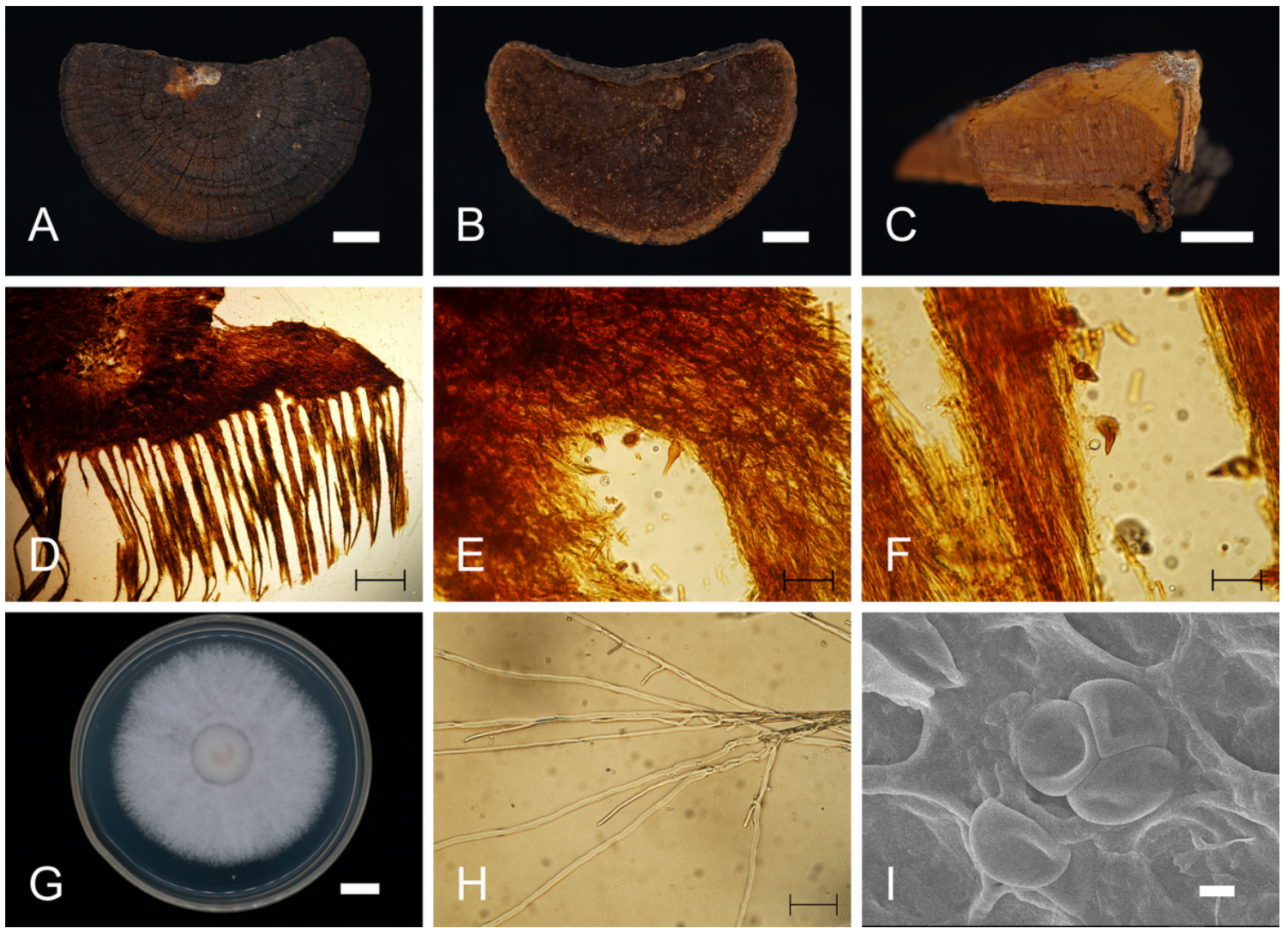


Figure 1

Morphology of *Sanghuangporus baumii*. (A, B) Mature basidiocarps. (C) The longitudinal section of the basidiocarps. (D–F) The hymenophore. (G) Mycelia cultured at 25°C on a potato dextrose agar (PDA) medium for 10 days. (H) Branched vegetative hyphae. (I) Basidiospores under a scanning electron microscope. Scale bars: 1 cm (A–C, G); 0.5 mm (D); 33.3 μm (E, H); 20 μm (F); and 1 μm (I).

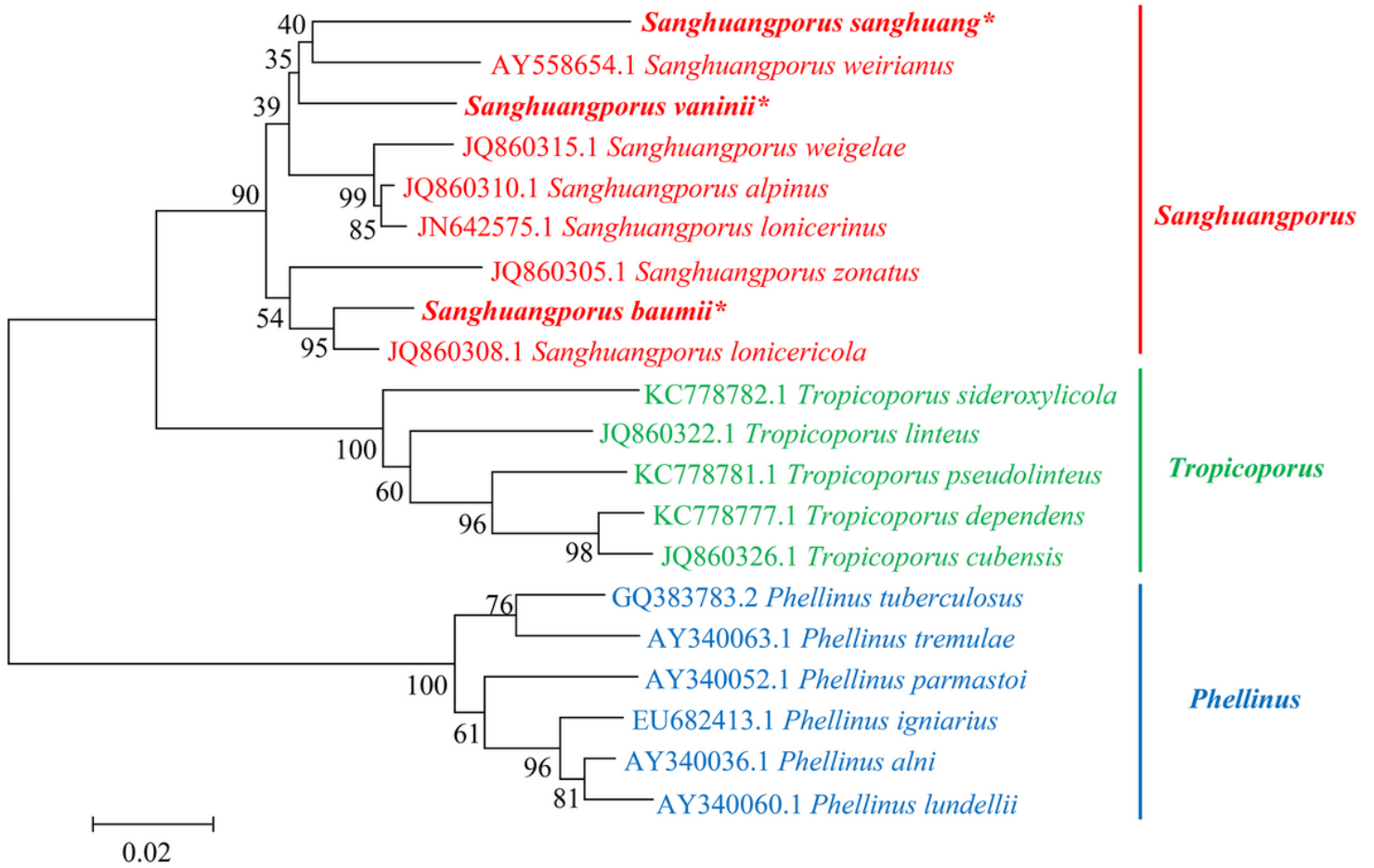


Figure 2

Phylogenetic tree obtained using the neighbor-joining method from internal transcribed sequence (ITS) datasets.

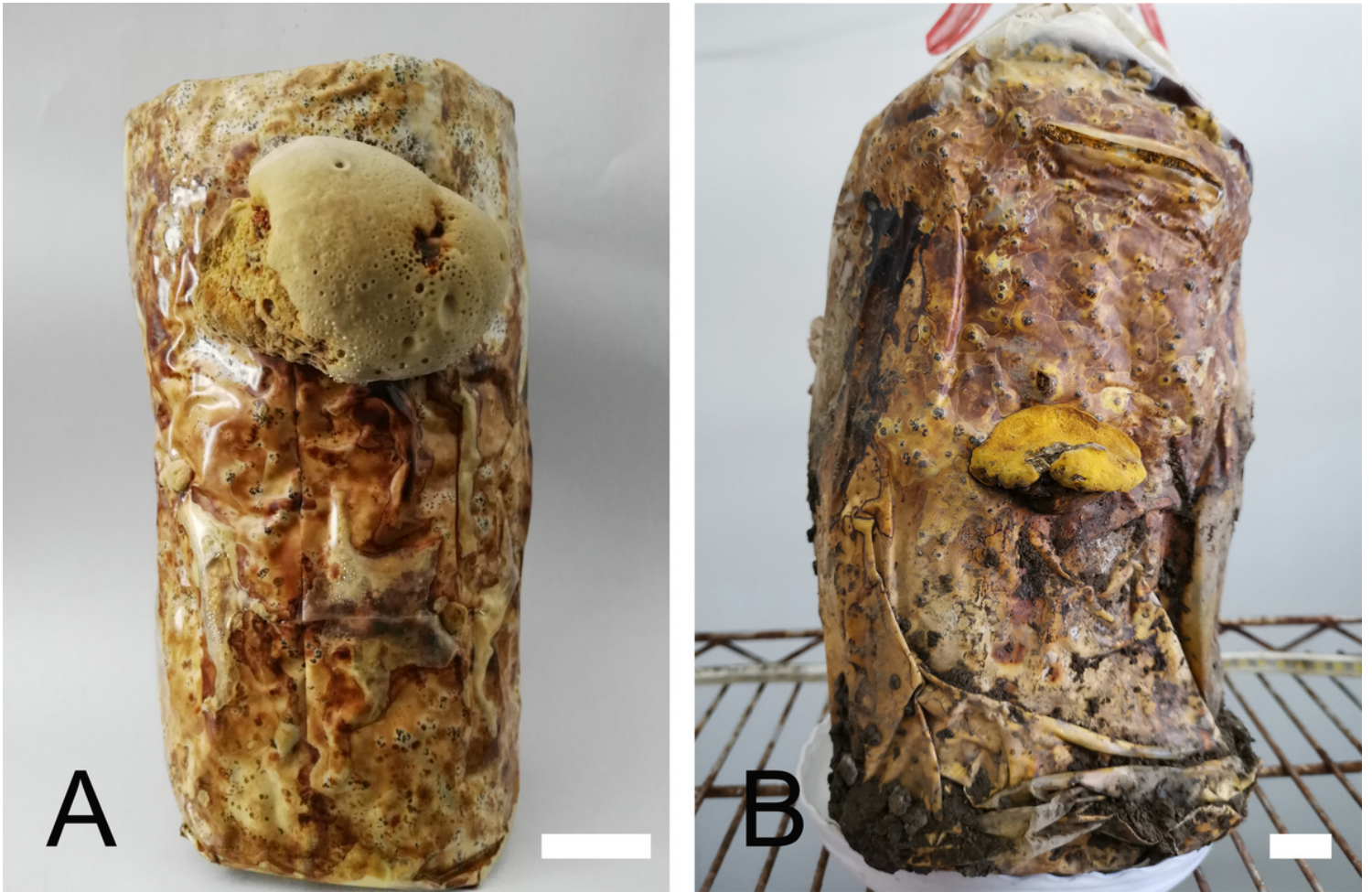


Figure 3

Cultivated fruiting bodies of *Sanghuangporus baumii* and *Sanghuangporus vaninii*. (A) *S. baumii*. (B) *S. vaninii*. Scale bars: 1 cm.

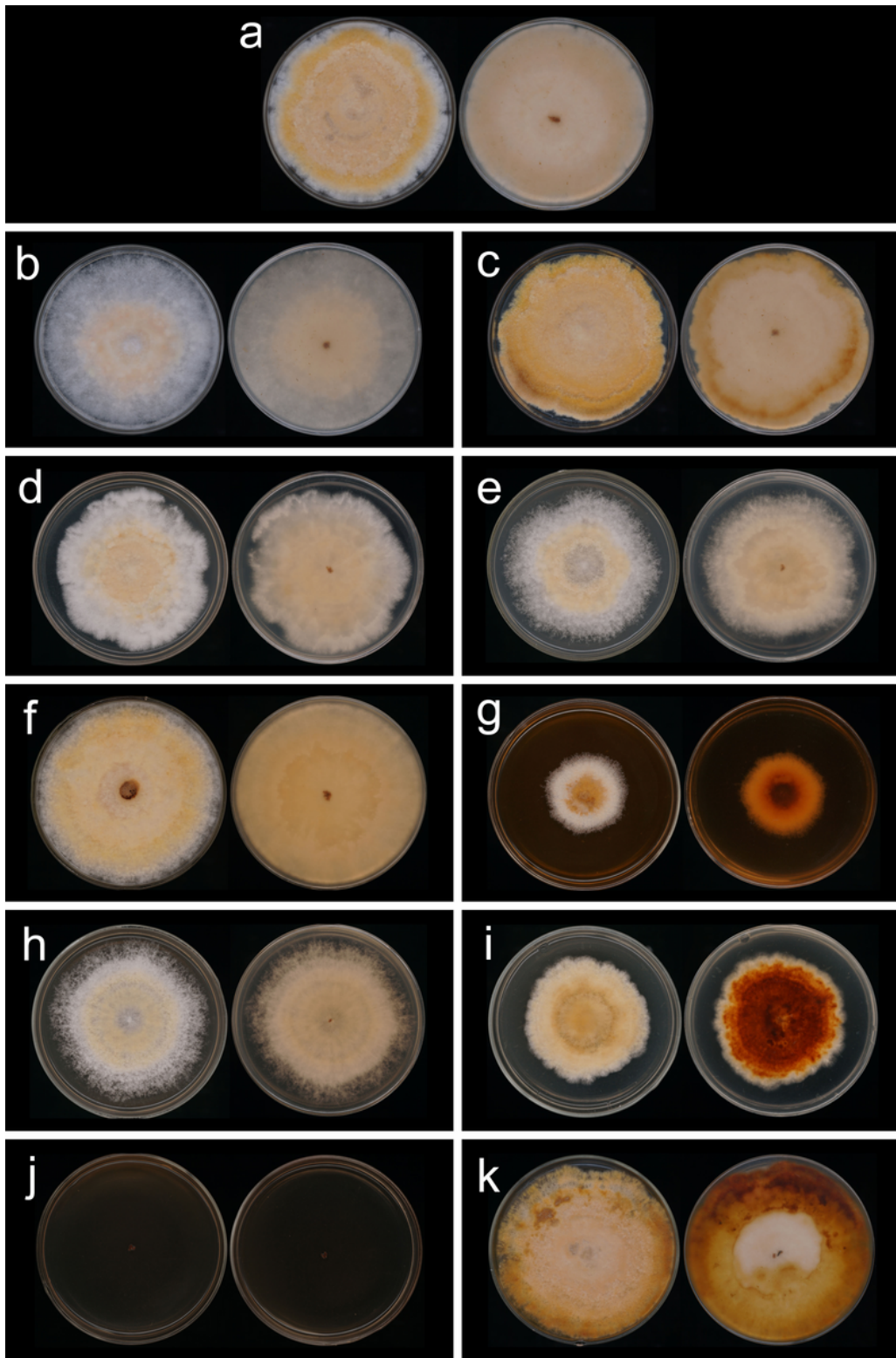
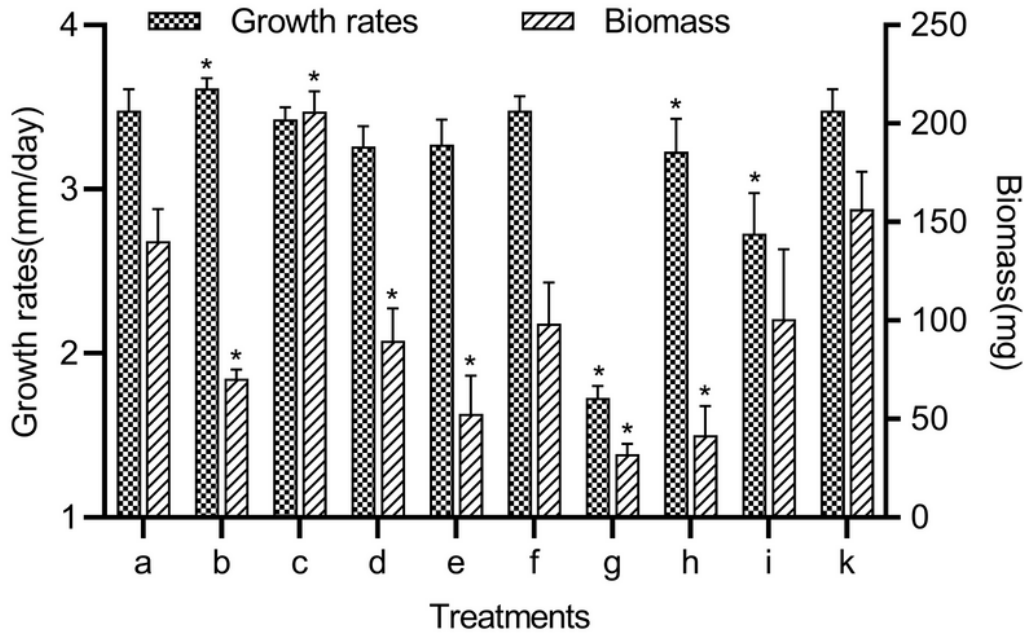
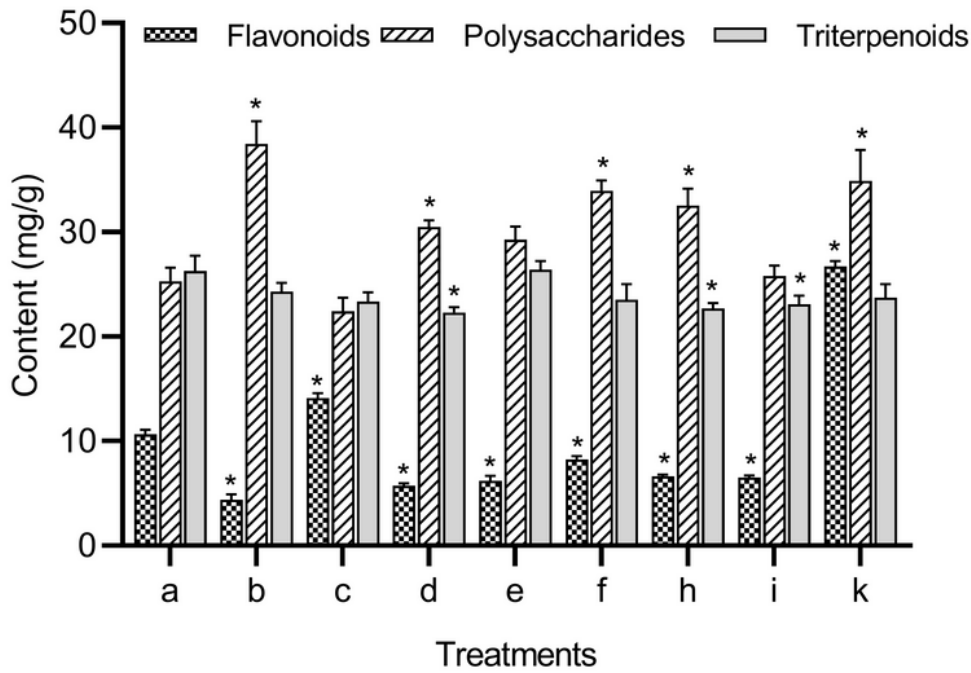


Figure 4

The front and back sides of the *Sanghuangporus baumii* mycelia after elicitation. Treatment a to k were: a control; b grafting membrane; c Parafilm; d lactose; e saccharose; f phosphate \square ; g phosphate \square ; h ammonium sulfate; i peptone; j urea; k light.

A**B****Figure 5**

The growth and bioactive substances contents of *S. baumii* mycelia after elicitation. (A) The growth rates and biomass. (B) The contents of total flavonoids, polysaccharides and triterpenoids.

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