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Promotion of the Hypocrellin Yield by a Co-Culture of *Shiraia bambusicola* (GDMCC 60438) with *Arthrinium* sp. AF-5 Fungus

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Abstract: Hypocrellin is a natural 3,10-xylene-4,9-anthracene derivative compound that originates from the stroma of *Shiraia bambusicola* (*S. bambusicola*) and *Hypocrella bambusae* with excellent photobiological activities. Submerged fermentation with the mycelia of *S. bambusicola* is generally regarded as an ideal technology for hypocrellin production. This study developed a co-cultivation strategy for an obvious promotion of the hypocrellin yield by incubating *S. bambusicola* (GDMCC 60438) with the endophyte fungus *Arthrinium* sp. AF-5 isolated from the bamboo tissue. The results indicated that the yield of hypocrellin A (HA) reached a 66.75 mg/g carbon source after an 84-h co-cultivation of the two strains, which was a four-time increase of that by the fermentation only with the *S. bambusicola*. The microscope observation found that the mycelia of the two strains were intertwined with each other to form the mycelium pellets during the co-cultivation. Moreover, the mycelium pellets of the co-culture showed a contracted and slightly damaged morphology. The addition of H_2O_2 in the fermentation media could further increase the HA production by 18.31%.

Keywords: hypocrellins A; *S. bambusicola*; endophyte fungi; mycelium pellets

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1. Introduction

Shiraia bambusicola Henn. and *Hypocrella bambusae* (Berk. & Broome) Sacc. (Ascomycetes) are pathogenic fungi of bamboo in east Asia [1–3]. As Chinese folk medicines, the stroma of the two fungi could be widely used to treat rheumatoid arthritis, tracheitis, and oxyhepatitis, etc. A variety of secondary metabolites, such as perylene quinones, anthraquinones, and polysaccharides, were isolated from the fungal stroma [4]. The hypocrellins belonging to perylene quinones generally include hypocrellin A (HA), hypocrellin B, hypocrellin C, and hypocrellin D [5–7] (Figure 1). The hypocrellins are photosensitizers with advanced photosensitive therapeutic activities against the tumor, virus, retinopathy, and human immunodeficiency virus (HIV) [8–12]. Unfortunately, the current supply of the hypocrellin source has failed to meet the increasing demand because of the limited productivity of the natural fungal stroma. Although the metabolic pathways' biological synthesis of the hypocrellins is still unclear, submerged fermentation with *S. bambusicola* has excellent application prospects in producing hypocrellins [13]. In recent years, various methods have been developed to increase the yield of the hypocrellins through fermentation, such as genetic modification and mutagenesis of the strains, optimization of media and cultivation conditions, and chemical and physical induction [12–15]. Through fermentation, the yield of the hypocrellins reached 90–8700 mg/L [14,15].

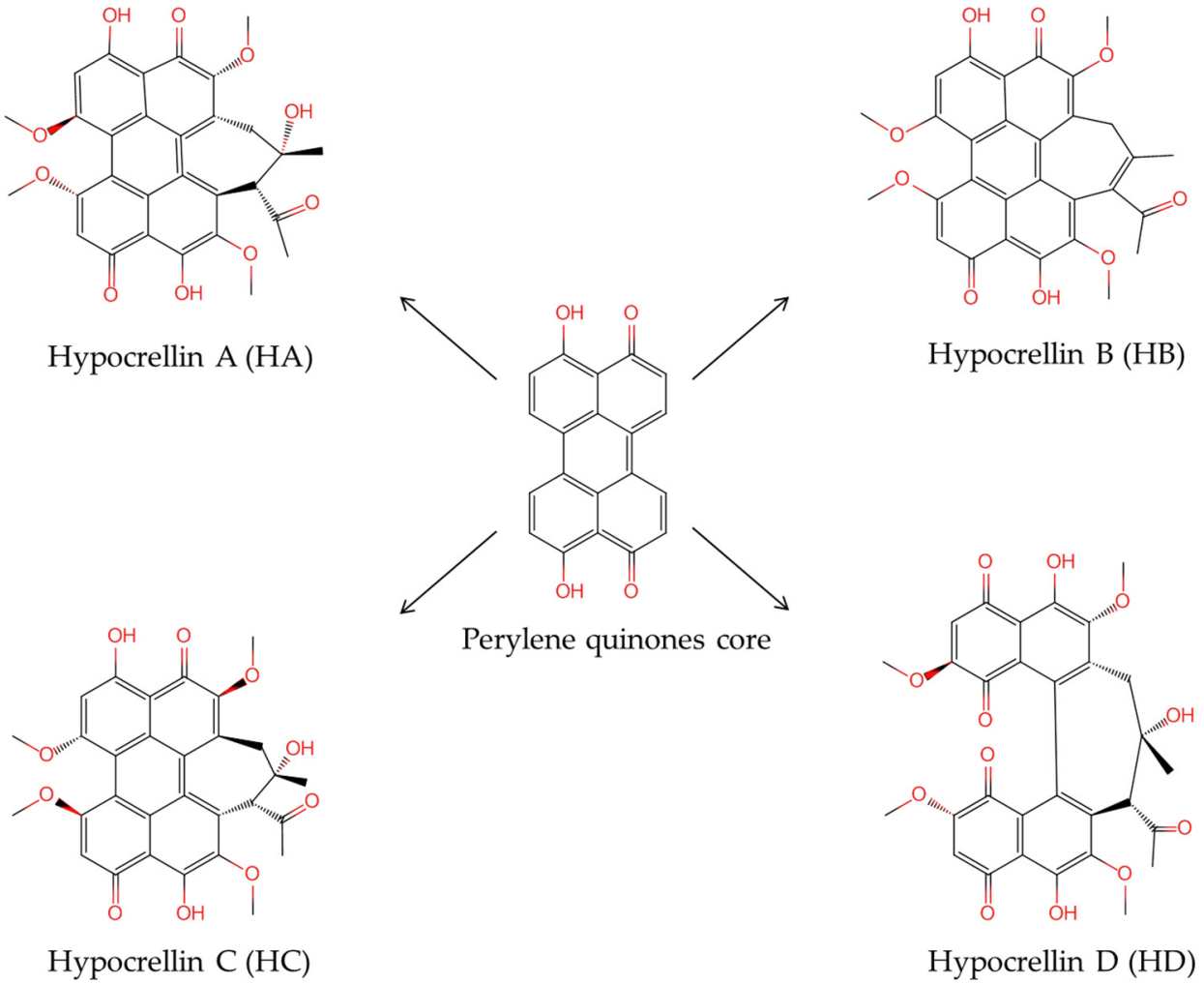

Figure 1. The chemical structure of the perylene quinones core and hypocrellins.

Fungi can elaborate the various enzymes for synthesizing secondary metabolites, most of which are critical natural sources of medicines with broad application prospects. Therefore, there has been a strong interest in scientific research and the industrialization of fungal metabolite production [16–20]. However, in the natural condition, a single strain usually failed to initiate the biological synthesis of the compounds [16,21]. The generation of secondary metabolites usually requires specific chemical and physical signal stimulation. In nature, microorganisms interact with others through competition, symbiosis, cooperation, antagonism, or parasitism [22]. The fungal strains' growth and metabolism could be influenced by the direct contact of multiple microbial cells or the communication of specific signal substances in the mixed colonies [23]. Artificial simulation of the signals encountered under natural conditions is one of the effective strategies for increasing the yield of secondary metabolites, such as the addition of elicitors and microbial co-culture [24–28]. The microbial co-culture method has become an important approach to improve the yield of products, produce new metabolites, and promote the activity of products through fermentation [29–31]. It has been widely applied in food, industry, agriculture, medicine, and pro-environment fields [32–34]. For instance, the transcription of *AFP* that codes a strong alkaline polypeptide with antifungal activity in *Aspergillus giganteus* could be triggered to start by *Fusarium oxysporum* [35]. The yield of Enniatin A1 with antibacterial activity was increased by 78 times by the co-culture of *Fusarium tricinctum* and *Bacillus subtilis* [36]. When the *Saccharomyces cerevisiae* and *Aspergillus oryzae* were simultaneously incubated with *Monascus* sp., the yield of Monascus pigments could be increased by 30 to

40 times [37]. Ma et al. firstly reported that the Pseudomonas related to the fruiting bodies of the *S. bambusicola* could stimulate the HA accumulation of *S. bambusicola*, resulting in an HA yield of 225.34 mg/L, which was about 3.25 times that of the single culture [38]. Rather than bacteria, fungi that produce distinct metabolites have great potential and should be worth studying in the co-culture with *S. bambusicola* for improving the yield of hypocrellins.

In this study, 17 endophytic fungi isolated from the bamboo branches where the stroma of *S. bambusicola* grows were submitted to the co-culture with *S. bambusicola* (GDMCC 60438) through submerged fermentation. The strain AF-5, which showed the most positive effect on promoting the HA production, was selected. The effects of the inoculation dosage and co-culture time on the HA yield were also investigated. The modifications of the morphological features of the mycelium pellets by the co-culture were also examined.

2. Materials and Methods

2.1. Preparation of the Endophytic Fungi Inculumn for Co-Culture

The 17 endophytic fungi strains (AF-1, AF-2, AF-3, AF-4, AF-5, AF-6, AF-7, BF-1, BF-2, BF-3, BF-4, BF-5, BF-6, BF-7, BF-8, BF-9, BF-10) isolated from *Pleioblastus amarus* (Keng) Keng (Hangzhou, Zhejiang, China) were cultivated at 28 °C on potato dextrose agar (PDA, Guangdong Huankai Microbial Technology Co., Ltd., Guangzhou, China) medium for 7 days. The mycelia with the PDA medium were cut into 1 × 1 cm blocks and then transferred into a 250-mL flask containing 50 mL potato dextrose broth (PDB, Guangdong Huankai Microbial Technology Co., Ltd., Guangzhou, China) media. After the cultivation performed at 28 °C for 48–60 h, the broth was centrifuged at 9000 rpm for 10 min to collect the fresh fungal mycelium (FFM) and fungus suspension (FS) of the endophytic fungi. After the collected FFM was freeze-dried for 10 h, fungal crude polysaccharide (FCP) of endophytic fungi were extracted by hot-water extraction. The proteins were removed from the FFM by the Sevag method [39].

2.2. HA Fermentation by Co-Culture of S. bambusicola and Endophytic Fungi

The *S. bambusicola* (GDMCC 60438) was cultivated on PDA and at 28 °C for 5 days. The plate was washed with 8 mL of sterile H_2O containing 2% (*w*/*w*) Tween 80 (Damao chemical reagent factory, Tianjin, China). The suspension was incubated at 28 °C at 150 rpm in a 250-mL flask containing 50 mL PDB media. After 48–60 h, the prepared seed inoculum was adjusted to 0.6 g/mL and submitted into a fermentation medium containing 10 g/L glycerol (Macklin Inc., Shanghai, China), 4 g/L potato extract (Yuanye Biotechnology Co., Ltd., Shanghai, China), and 12 g/L beef extract (Guangdong Huankai Microbial Technology Co., Ltd., Guangzhou, China) with a dosage of 10% (*v*/*v*). The fermentation for the hypocrellins only with *S. bambusicola* inoculum was conducted for 72 h at 28 °C and 150 rpm. After a 24-h fermentation with the *S. bambusicola* inoculum in a 250-mL flask containing 50 mL fermentation media, the FFM, FS, and FCP of the endophytic fungi were separately added into the broth at the dosages of 0.06 g fresh weight (FW)/mL, 10% and 0.06 g/mL to start the co-cultivation, respectively. The co-cultivation with different inoculation substrates was conducted for 72 h. Fermentation of the HA only with the *S. bambusicola* (GDMCC 60438) was set as the control.

2.3. Strain Identification

The extraction of the DNA from the AF-5 strain was conducted by the CTAB method [40]. The mycelium of the strain was mixed with 500 µL CTAB buffer and ground with a plastic rod. After incubated at 65 °C for 1 h, the mixture was centrifuged at 12,000 rpm for 10 min. The collected supernatant was extracted with saturated phenol and chloroform. The DNA was precipitated with absolute ethanol. The DNA was air-dried and dissolved in 100 µL TE buffer (Sangon Biotech, Shanghai, China) before use. The internal transcribed spacer (ITS) sequences were amplified using the ITS5 (GGAAGTAAAAGTCGTAACAAGG) and ITS4 (TCCTCCGCTTATTGATATGC) primers [41]. The PCR products were detected by

1% gel electrophoresis and then sequenced by Sangon Biotech Co., Ltd. (Shanghai, China). The BLAST was used to compare the similarity of the ITS sequence of the AF-5 strain with reported sequences in the Genbank (https://www.ncbi.nlm.nih.gov/, the link was last accessed on 1 June 2020). The Clustalx 1.81 was used to compare multiple sequences, and the phylogenetic tree was constructed by the MEGA-X.

2.4. The Effect of Cultivition Conditions on the HA Yields with the S. bambusicola (GDMCC 60438) and the AF-5 Strain

The effect of inoculum time, dosage of the endophytic fungi, and co-culture time on the HA yields were studied. The experimental levels of inoculum time were 0, 12, 24, 36, and 48 h after the start of the fermentation. The inoculum volumes of 0.02, 0.06, 0.1, and 0.14 g FW/mL and the co-cultivation times of 24, 36, 48, 60, 72, 84, 96, and 108 h were applied. The activity of the H_2O_2 (30%, *w/w*, Sinopharm Co., Ltd., Beijing, China) on the promotion of HA yield through co-cultivation was explored; H_2O_2 solution was added into the fermentation broth along with inoculation of the endophytic fungi. The concentrations of H_2O_2 in the broth were 0, 0.5, 1, 5, and 10 mM.

2.5. Morphological Observation of the Mycellium Pellets from the Co-Culture

Morphology of mycelium pellets and mycelium were observed by stereomicroscope and scanning electron microscope (SEM), respectively. The mycelium after fermentation for 12, 24, 36, 48, and 60 h were washed with 0.1 M phosphate buffer saline solution (PBS). The mycelium pellets were observed by a CX41 stereomicroscope (Olympus Corporation, Tokyo, Japan) after suspending in the cell culture dish containing 0.1M PBS. The Image-Pro Plus 6.0 (Media Cybernetics, Inc., Silver Spring, MD, USA) was used to measure mycelium pellets' diameter. The washed mycelium was mixed with 4% glutaraldehyde solution at 4 °C for 4 h. After centrifugated at 8000 rpm for 5 min, the mycelium was collected and washed using 0.1M PBS. The mycelium was dried and coated with gold by an EM SCD500 high vacuum sputter coater (Leica microsystems, Wetzlar, Germany). The morphology of the mycelium was observed by a ZEISS EVO SEM (Merlin, Germany) with a 5.00 KV scanning voltage [42].

2.6. Analytical Assays

The fungal mycelium pellets were collected by filtration using the qualitative filter paper (diameter 7 cm, Jiaojie, Fushun, China) at the end of the fermentation. The collected solids were freeze-dried for 12 h after being washed twice by distilled water. The mycelium dry weight (DW) was obtained using the BS224S analytical balance (Sartorius, Germany). A 0.05 g portion of dried mycelium was mixed into a 10 mL centrifuge tube containing an equal amount of quartz sand (Acmec Biochemical Co., Ltd., Shanghai, China) and 5 mL of dichloromethane (Zhiyuan Reagent Co., Ltd., Tianjin, China). The tube was sealed and the hypocrellinses were extracted under ultrasonic irradiation by an AS20500ATH (Tianjin Automatic Science Instrument, China) at 40 KHz for 30 min. Before use, the extraction solution was filtered through a filter membrane (ø = 0.22 μm, Jinteng Experimental Equipment Co., Ltd., Tianjin, China). An e2695 HPLC system (Waters Corporation, Milford, MA, USA) equipped with a Inertsil ODS-3 column (5 μm, 4.6 mm × 250 mm, Shimadzu Corporation, Japan) and a PDA detector (2988, Waters Corporation, Milford, MA, USA) was used for the determination of HA content in the extraction solution. The HPLC operating conditions were as follows: mobile phase (methanol/acetic acid, 90/10, *v*/*v*); flow rate, 1 mL/min; column temperature, 30 °C; detector wavelength, 467 nm; injection volume, 10 μL; sample running time, 12 min. Calculation of the HA content in mycelia, HA conversion yield, and HA yield were according to Equations (1)–(3), respectively.

$$\frac{\text{HA content in mycelia}}{(\text{mg g biomass})} = \frac{\text{HA content in the extract } (\text{mg/mL}) \times \text{extraction volume } (5\ \text{mL})}{\text{dry weight of mycelia } 0.05\ \text{g}}, \quad (1)$$

$$\text{HA conversion yield (mg/g carbon source)} = \frac{\text{HA content in mycelia (mg/g biomass)} \times \text{mycelium biomass (g DW / L)}}{\text{dosage of carbon source (10 g)}}, \quad (2)$$

$$\text{HA yield (mg L)} = \frac{\text{HA content in mycelia (mg/g biomass)} \times \text{mycelium biomass (g DW/L)}}{\text{volume of fermentation broth (0.05 L)}} \quad (3)$$

2.7. Statistical Analysis

All results were reported as mean value ± standard deviation (n = 3). Kruskal–Wallis method was used for nonparametric one-way analysis of variance. The analysis was performed using an SPSS Statistics 20 (IBM Corporation, Armonk, NY, USA) program with a significance level of 0.05 and 0.01.

3. Results and Discussion

3.1. The HA Yields by Co-Culture of S. bambusicola (GDMCC 60438) with Different Endophytic Fungi

As shown in Table 1, the endophytic fungi had a distinct effect on the growth of the mycelium biomass in the co-culture system. Among all the strains, the AF-4, AF-5, AF-7, BF-1, BF-3, BF-5~BF-9 showed a significant positive effect on both the mycelium biomass ($p < 0.05$) and the HA yield ($p < 0.01$). After the co-culture of the AF-5 strain and the *S. bambusicola* (GDMCC 60438), the HA content in the mycelia and HA yield reached 40.5 mg/g and 332.92 mg/L, which was about 2.1 times and 2.8 times the control, respectively. In addition, the AF-2 strain showed a strong inhibition effect on the HA synthesis and growth of the mycelia. The biomass and HA yield had a decline of 26.02% and 68.62%, respectively. Although the AF-4 or BF-5 strains had no remarkable effect on the mycelium growth of the *S. bambusicola* (GDMCC 60438), the HA contents in the mycelium were increased by 66.71% and 54.18%, respectively.

Table 1. Effect of different endophyte co-culture with *S. bambusicola* (GDMCC 60438) on the biomass and HA productivity.

Strain Number	* Mycelium Biomass (g DW/L)	** HA Content in Mycelium (mg/g Biomass)	** HA Conversion Yield (mg/g Carbon Source)	** HA Production (mg/L)
Control	6.24 ± 0.19	18.99 ± 1.36	11.84 ± 0.65	118.39 ± 6.5
AF-1	5.39 ± 1.31	19.79 ± 1.34	10.54 ± 1.85	114.60 ± 13.46
AF-2	4.58 ± 0.34	8.12 ± 1.07	3.72 ± 0.54	37.15 ± 5.38
AF-3	6.75 ± 1.67	11.85 ± 0.6	7.93 ± 1.57	79.34 ± 15.74
AF-4	6.38 ± 0.57	31.69 ± 4.18	20.10 ± 0.88	201.02 ± 8.76
AF-5	8.23 ± 0.61	40.5 ± 1.4	33.19 ± 1.31	332.92 ± 13.09
AF-6	8.91 ± 0.49	9.6 ± 0.53	8.54 ± 0.04	85.4 ± 0.36
AF-7	10.53 ± 0.52	22.92 ± 1.35	24.10 ± 0.23	240.87 ± 2.26
BF-1	6.22 ± 0.91	33.17 ± 2.73	20.51 ± 1.3	205.07 ± 13.04
BF-2	9.88 ± 1.74	10.72 ± 0.67	10.51 ± 1.25	105.14 ± 12.46
BF-3	8.07 ± 0.69	31.29 ± 1.55	25.2 ± 0.92	251.95 ± 9.17
BF-4	8.71 ± 1.12	11.71 ± 0.4	10.88 ± 0.36	108.79 ± 3.58
BF-5	5.93 ± 0.13	29.25 ± 1.19	17.33 ± 0.33	173.35 ± 3.34
BF-6	6.95 ± 0.33	28.11 ± 0.69	19.52 ± 0.91	195.19 ± 9.14
BF-7	9.09 ± 0.04	23.45 ± 0.25	21.31 ± 0.32	213.13 ± 3.23
BF-8	8.08 ± 0.32	25.43 ± 0.06	20.67 ± 1.06	206.72 ± 10.65
BF-9	7.29 ± 0.47	24.11 ± 2.577	17.52 ± 0.75	175.20 ± 7.48
BF-10	6.51 ± 0.27	12.89 ± 0.62	8.4 ± 0.73	85.96 ± 7.27

Values shown represent the averages of triplicate samples (mean value ± standard deviation). Levels for significance of differences * $p < 0.05$, ** $p < 0.01$.

3.2. Molecular Identification and the Culture Features of the Endophytic AF-5 Strain with the S. bambusicola (GDMCC 60438)

A 609-bp ITS DNA fragment of the AF-5 strain was obtained. Blast analysis showed that the sequence was most homologous with *Arthrinium arundinis* (MK461045.1) and *Arthrinium arundinis* (MK460910.1). The AF-5 strain should belong to the *Arthrinium* species due to their close evolutionary distance in the phylogenetic tree (Figure 2). The AF-5 strain was recognized as *Arthinium* sp. AF-5.

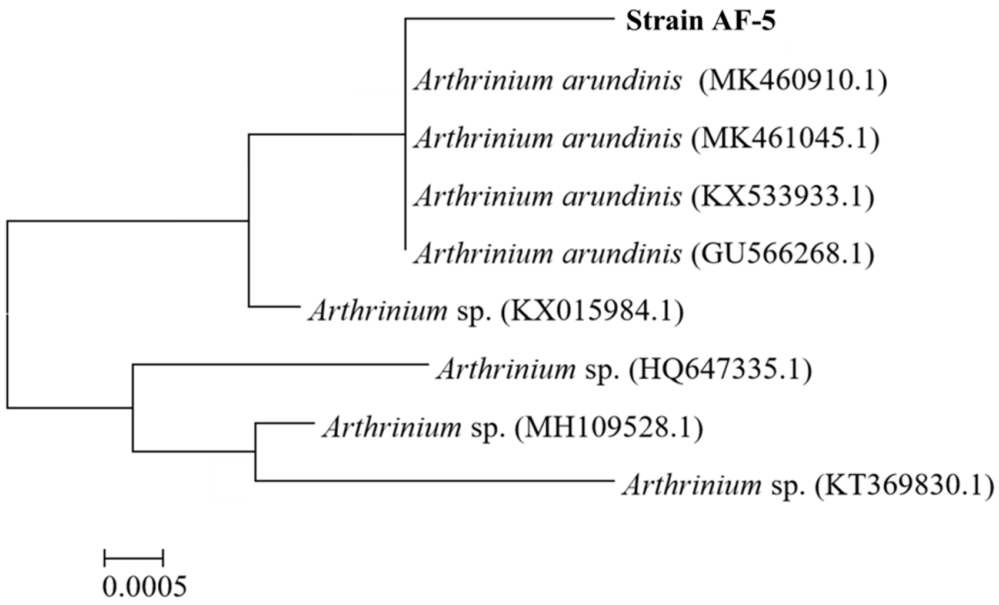

Figure 2. Phylogenetic tree of the strain AF-5 based on the ITS sequences.

On the PDA medium, the *S. bambusicola* (GDMCC 60438) colony appeared bright red in the middle, but a concentric annular yellow–brown circle appeared on the reverse side of the medium (Figure 3(a1,a2)). When *Arthinium* sp. AF-5 was inoculated on the PDA medium at 28 °C, the fungal colony could grow to 40 mm in diameter and cover the whole medium on days 7–10 with white filaments (Figure 3(b1,b2)). When the *S. bambusicola* (GDMCC 60438) and AF-5 strains were co-cultured together on the PDA medium for 7 days, the contacting edge of the two fungal colonies was in dark red from the middle (Figure 3(c1,c2)). It was revealed that the AF-5 strain could coexist well with *S. bambusicola* (GDMCC 60438) and promote its HA production. Moreover, the growth rate of the AF-5 was higher than that of the *S. bambusicola* (GDMCC 60438), with a larger AF-5 colony on the PDA medium. To avoid the competition at the early stage, it was necessary to conduct the separated cultivation of the two stains before the co-cultivation during the submerged fermentation.

After the independent cultivation of the *S. bambusicola* (GDMCC 60438) (Figure 4(a1)) and the *Arthinium* sp. AF-5 in the PDB medium for 60 h (Figure 4(b1)), the mycelia of the *Arthinium* sp. AF-5, which grew more rapidly, adhered and aggregated into about 10 mm blocks, while the *S. bambusicola* (GDMCC 60438) formed a relatively tiny mycelium pellet with a diameter of about 1 mm. There was no noticeable color change in the mycelia of either of the strains. Figure 4(a2,b2) shows the morphology of the two strains after being cultured in the fermentation medium for 72 h. The results show that the color of the fermentation broth had a slight change when the strain AF-5 was cultured alone, indicating that no pigment was produced. After inoculating the *S. bambusicola* (GDMCC 60438) in the fermentation substrate and cultivating for 24 h, the mycelia of the *Arthinium* sp. AF-5 were added to start the co-culture for another 48h. Due to the intensive accumulation of HA in the mycelium, the pellet of the co-culture turned dark red and even black (Figure 4c), while the pellet of the *S. bambusicola* (GDMCC 60438) culture without AF-5 turned to bright red (Figure 4(a2)).

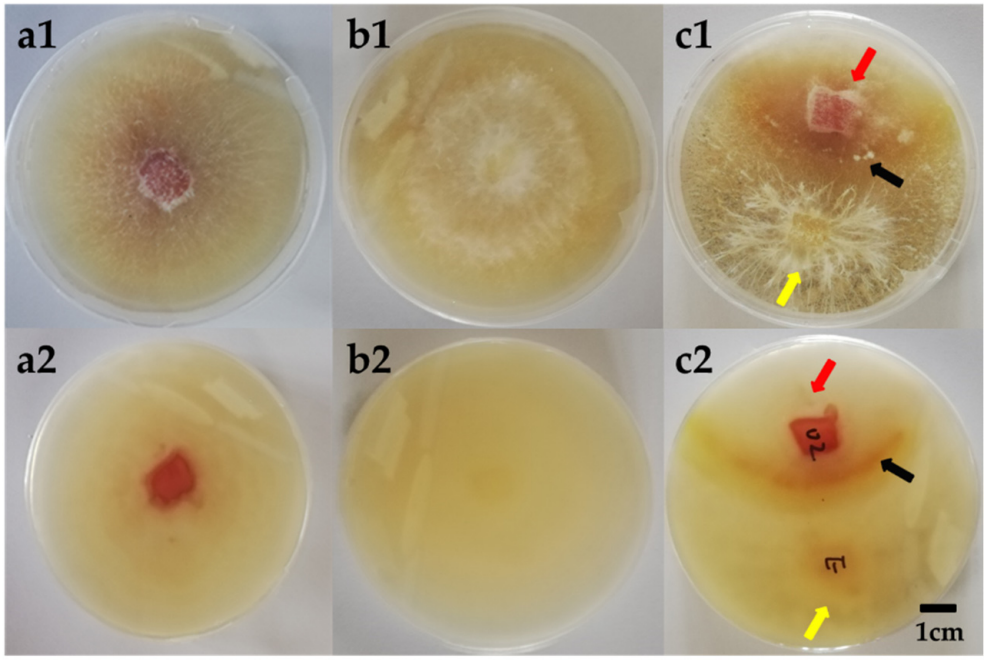

Figure 3. Growth of the mycelia of the *S. bambusicola* (GDMCC 60438) and the *Arthinium* sp. AF-5. (**a1**). Top side of *S. bambusicola* (GDMCC 60438) plate; (**a2**). Reverse side of *S. bambusicola* (GDMCC 60438) plate; (**b1**). Top side of strain AF-5 plate; (**b2**). Reverse side of strain AF-5 plate; (**c1**). Top side of fungus–fungus resistance plate; (**c2**). Reverse side of fungus–fungus resistance plate. The red arrow points to *S. bambusicola* (GDMCC 60438), the yellow arrow points to strain AF-5, and the black arrow points to the junction of the two species.

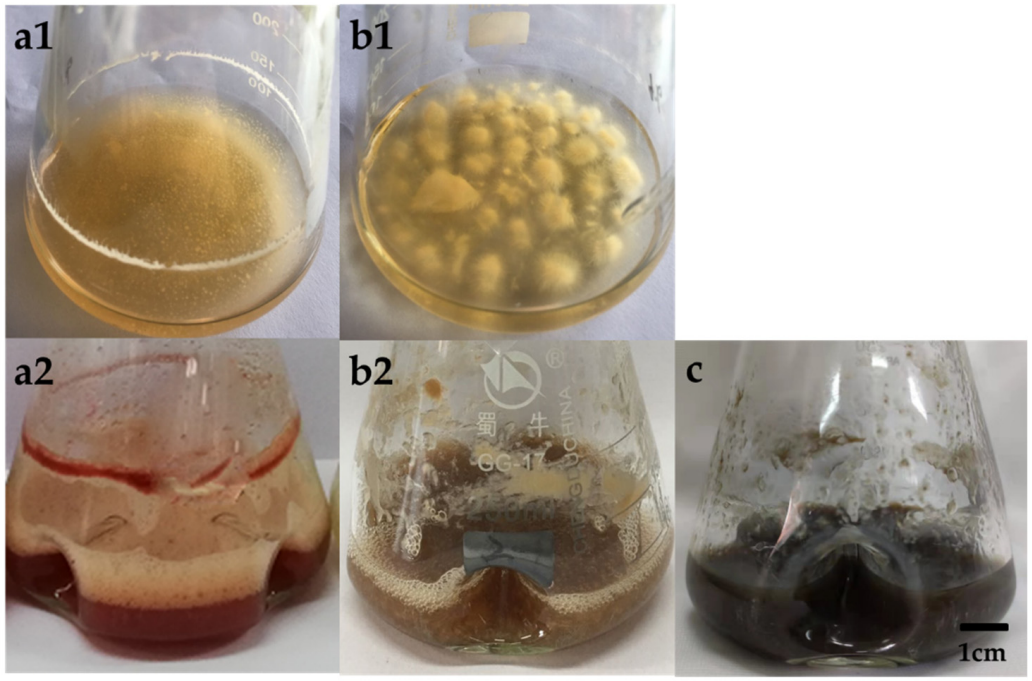

Figure 4. The culture of *S. bambusicola* (GDMCC 60438) and the *Arthinium* sp. AF-5. (**a1**). Seed of the *S. bambusicola* (GDMCC 60438); (**a2**). Broth of the *S. bambusicola* (GDMCC 60438) after a 72-h fermentation; (**b1**). Seed of the *Arthinium* sp. AF-5; (**b2**). Broth of the *Arthinium* sp. AF-5 after a 72-h fermentation; (**c**). broth of the co-culture of *S. bambusicola* (GDMCC 60438) and the *Arthinium* sp. AF-5 after a 60-h fermentation (the *Arthinium* sp. AF-5 was added at the 12 h mark).

3.3. Effect of Different Types of the AF-5 Inoculums on the HA Yields

To determine the effect of the different inoculums on the HA yields, the FP, FS, and FFM of *Arthrinium* sp. AF-5 were prepared and submitted to the co-cultivation with the *S. bambusicola* (GDMCC 60438). After the HA fermentation, the mycelium biomass and the HA content of the culture with the FFM were significantly increased compared to those with the FS and FP (Figure 5a). The addition of FFM could effectively stimulate the biosynthesis of HA by the *S. bambusicola* (GDMCC 60438). Compared with the regular fermentation by the *S. bambusicola* (GDMCC 60438), the HA conversion yield increased from 14.23 mg/g carbon source to 24.77 mg/g carbon source with an increased rate of 74.07% (Figure 5b).

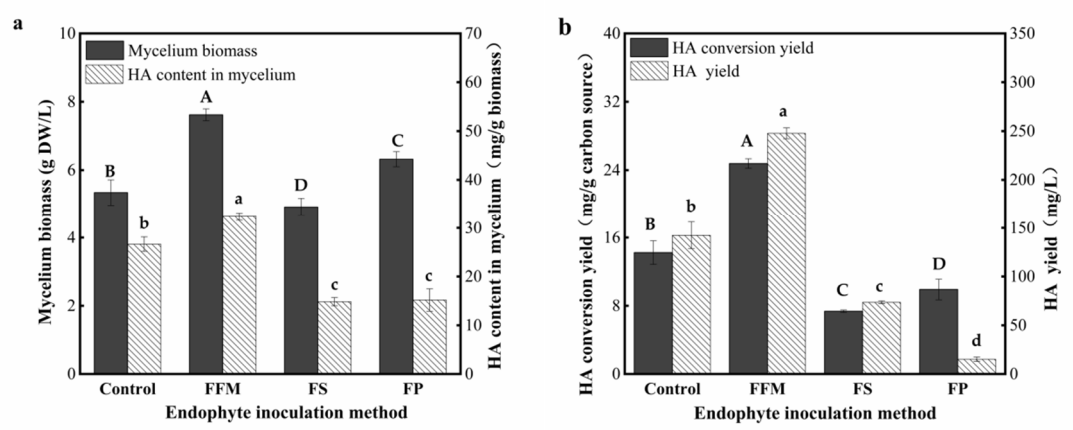

Figure 5. Effects of *Arthrinium* sp. AF-5 inoculation methods on (**a**). mycelium biomass and the HA content in mycelium; (**b**). HA conversion yield and HA yield. Different letters indicated that data were significantly different ($p < 0.05$), and comparisons were made between columns of the same color, the significance between a set of data is marked uniformly in uppercase letters or uniformly in lowercase letters.

It was considered that the polysaccharides, peptides, and glycoproteins derived from microorganisms and plant cells were the most commonly used elicitors for promoting the production of fungal secondary metabolites [43,44]. For example, the addition of protein elicitor PB90 isolated from *Pasteurella pestis* during the liquid fermentation of *S. bambusicola* could promote the synthesis of hypocrellins and increase the accumulation of phenolic compounds [45]. The induction of elicitors to producing strains is affected by the type, addition dosage, and additional time of elicitors [46]. Du et al. found that the mixed culture of *Phoma* sp. BZJ6 and *S. bambusicola* could increase the laccase yield by up to 9.18 times that of the pure culture and enhance the synthesis of total phenols and flavonoids, but it led to a decrease in the biomass [47]. In another work, they introduced bacteria to the *S. bambusicola* culture and found that they had no significant effect on the growth of the biomass but could increase the hypocrellin yield. The effects on the improvement of the hypocrellin yield were different depending on the media [48]. In this study, the addition of FP could only increase the mycelium biomass rather than the HA yields. It was similar to the report that crude polysaccharides from bacteria had no significant effect on the accumulation of hypocrellins by the *Shiraia* sp. S9 [45]. It was interesting that the filtrate of the *Arthinium* sp. AF-5 broth inhibited the growth of *S. bambusicola*. The inhibition may likely be caused by the defense response against the metabolism molecules in the aqueous phase [43,49].

3.4. Effect of Introducing Time of the AF-5 Strain on the HA Yield

The results showed that the biomass, HA content in the mycelium, HA conversion yield, and HA yield were significantly improved when the AF-5 strain was introduced at 12, 24, and 36 h of the fermentation (Figure 6). Adding the *Arthinium* sp. AF-5 at the early or late stage of the fermentation led to no remarkable increase in the HA yield. It was indicated that the promotion effect of AF-5 on the HA yield could depend on the

metabolism status of S. bambusicola (GDMCC 60438). When adding the *Arthinium* sp. AF-5 at 12 h, the biomass and HA conversion yield of S. bambusicola (GDMCC 60438) reached 7.28 g DW/L and 32.8 mg/g carbon source, which increased by 16.6% and 177% compared with the control, respectively.

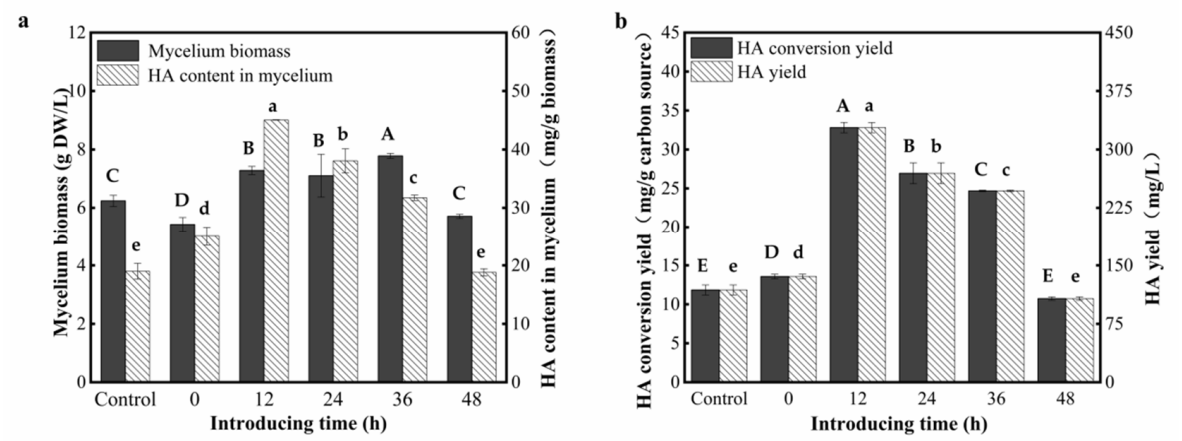

Figure 6. Effects of introducing time of *Arthrinium* sp. AF-5 on (**a**). the mycelium biomass and HA content in mycelium; (**b**). HA conversion yield and HA yield. Different letters indicated that data were significantly different ($p < 0.05$), and comparisons were made between columns of the same color, the significance between a set of data is marked uniformly in uppercase letters or uniformly in lowercase letters.

3.5. Effect of Dosage of the AF-5 Strain on the HA Yield

To determine the optimal content of the *Arthrinium* sp. AF-5 for HA production in the co-culture system, dosages of 1 g (0.02 g FW/mL), 3 g (0.06 g FW/mL), 5 g (0.1 g FW/mL), and 7 g (0.14 g FW/mL) of the *Arthrinium* sp. AF-5 were added into the pure culture of the *S. bambusicola* at 12 h. Pure culture of the *S. bambusicola* without the *Arthrinium* sp. AF-5 was set as the control. As shown in Figure 7a,b, the increasing dosage of AF-5 gradually promoted the growth of the co-cultured biomass. When the content of the *Arthrinium* sp. AF-5 reached 0.14 g FW/mL, the highest co-cultured biomass of 10.48 g DW/L could be achieved, which was 67.9% higher than the control. It was indicated that the accumulation of the HA in the mycelia reached the highest level of 55.43 mg/g when the dosage of the *Arthrinium* sp. AF-5 was 0.06 g FW/mL. An over-inoculation of the *Arthrinium* sp. AF-5 could have a significantly negative effect on both the HA conversion yield and the production of HA. The highest HA conversion yield of 44.74 mg/g carbon source could be achieved when the initial content of the *Arthrinium* sp. AF-5 was 0.06 g FW/mL.

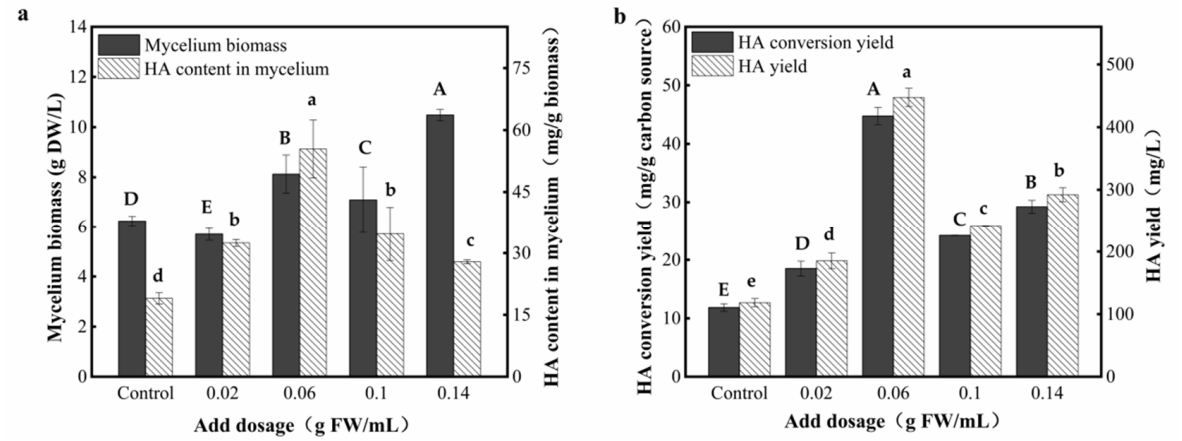

Figure 7. Effects of *Arthrinium* sp. AF-5 addition dosage on (**a**). the mycelium biomass and HA content in mycelium; (**b**). HA conversion yield and HA yield. Different letters indicated that data were significantly different ($p < 0.05$), and comparisons were made between columns of the same color, the significance between a set of data is marked uniformly in uppercase letters or uniformly in lowercase letters.

3.6. Effect of the Co-Culture Time on the HA Yield

The effect of the co-culture time on the HA yield was studied with 0.06 g FW/mL of the *Arthrinium* sp. AF-5 co-cultured in the broth of the pure culture of the *S. bambusicola*. Along with the increase of the co-culture time from 24 h to 96 h, the mycelium biomass gradually increased from 3.83 g DW/L to 8.41 g DW/L (Figure 8a). After an 84-h co-cultivation, the mycelium biomass reached 8.28 g DW/L, 50% higher than that of the control group. The HA content in the mycelium, the HA conversion yield, and the HA yield reached 80.68 mg/g, 66.75 mg/g carbon source, and 667.47 mg/L, respectively (Figure 8b–d).

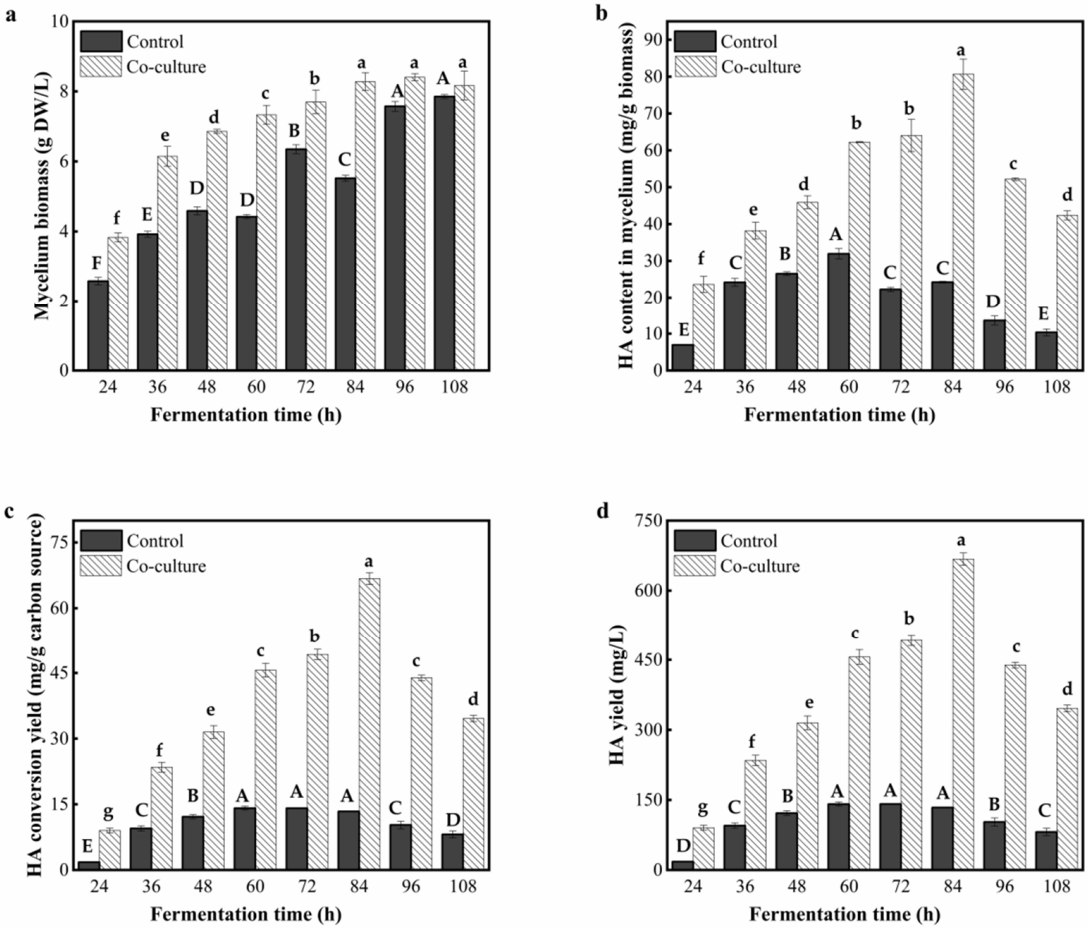

Figure 8. The variation tendency of (**a**). mycelium biomass, (**b**). HA content in mycelium, (**c**). HA conversion yield, and (**d**). HA yield in the co-culture system. Different letters indicated that data were significantly different ($p < 0.05$), and comparisons were made between columns of the same color, the significance between a set of data is marked uniformly in uppercase letters or uniformly in lowercase letters.

3.7. Morphological Observation of the Mycelium Pellets during the Co-Culture

The development of the mycelial pellets during the co-culture was observed under the microscope. As shown in Figure 9, both the mycelial pellets of the pure culture of *S. bambusicola* and the co-culture system gradually could turn to a dark color with the extension of the cultivation time. However, the pellets could maintain the intact globosity structure but shrink obviously at the end of fermentation. In the beginning, the average diameters of the mycelial pellets of the pure culture and the co-culture gradually reduced to 3.31 (±0.05) mm and 2.50 (±0.07) mm, respectively. After 36 h, the change in the size of the mycelial pellet was almost stable in the control group, but the development of the pellet size continued in the co-culture system. In the late stage of the fermentation, the size of the mycelium pellets in the pure culture and the co-culture systems decreased by 26.44% and 44.44%, respectively. It was revealed that the time period between 24 h and 48 h could be the key stage for mycelium growth and HA accumulation. The change in the

mycelial pellets might be related to the HA synthesis, which was similar to the result of the co-culture of *Shiraia* sp. S9 with bacteria [50].

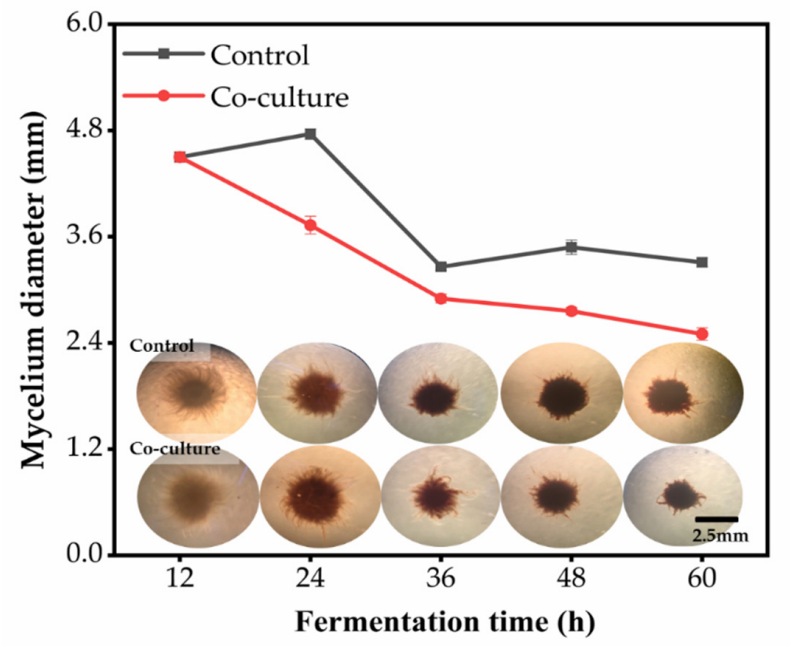

Figure 9. Change in the morphology and size of the mycelium pellets during the fermentation.

SEM observation showed that the *Arthrinium* sp. AF-5 had flat, horizontal segmentation mycelia with a diameter of 2.20 (±0.13) μm (Figure 10(c1,c2)). The diameter of the *S. bambusicola* (GDMCC 60438) mycelia was about 1.82 (±0.11) μm (Figure 10(a1,a2)) with a branched-tubular structure. The mycelia of the *Arthrinium* sp. AF-5 and *S. bambusicola* (GDMCC 60438) contracted and intertwined with each other with an interwoven pattern in the co-culture system (Figure 10(b1,b2)).

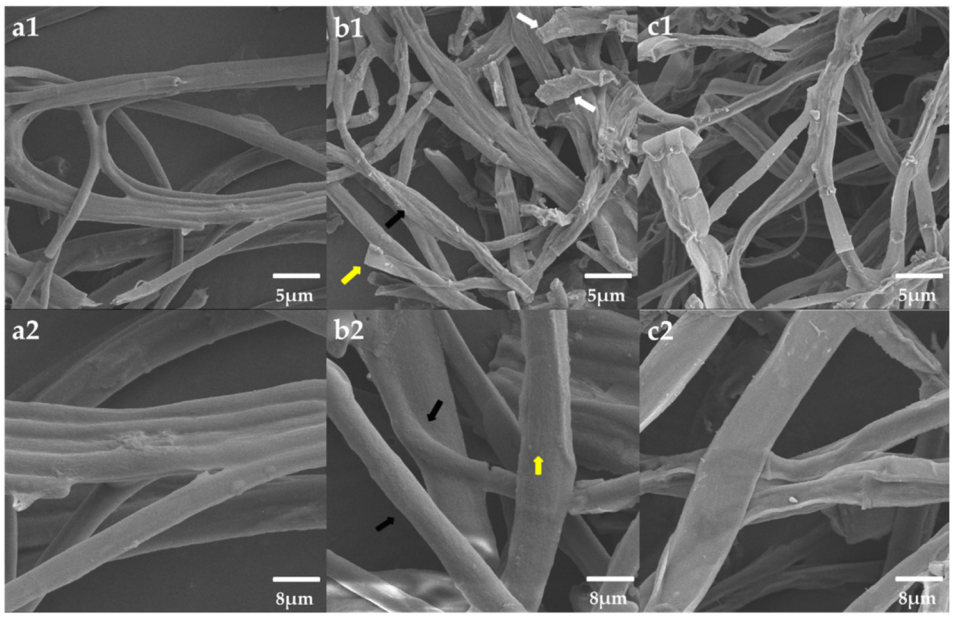

Figure 10. SEM image of mycelium, *S. bambusicola* (GDMCC 60438) (**a1**,**a2**), co-culture (**b1**,**b2**), and *Arthrinium* sp. AF-5 (**c1**,**c2**). The black arrow represents the mycelia of the *S. bambusicola* (GDMCC 60438), the yellow arrow represents the mycelia of the *Arthrinium* sp. AF-5, and the white arrow represents the damaged structure of the *S. bambusicola* (GDMCC 60438) mycelia.

In the co-culture system, the increased HA yield was generally contributed by the accelerated HA accumulation in the mycelia rather than the stimulation of the growth of the mycelium biomass. RNA sequencing and the transcriptomic analysis confirmed that the expression of the polyketide synthase and ATP-binding cassette of *S. bambusicola* was up-regulated during the co-culture of *S. bambusicola* with bacteria, suggesting the endophytic fungi may promote the metabolism of polyketone compounds, the precursor of HA [24]. It was speculated that the regulatory mechanisms of the *Arthrinium* sp. AF-5 promoting the HA accumulation in *S. bambusicola* (GDMCC 60438) could rely on regulating the metabolism enzyme system through interaction communication [37].

3.8. Effect of the H_2O_2 Addtives on the HA Yield during the Co-Culture

Considering that *S. bambusicola* (GDMCC 60438) and the *Arthrinium* sp. AF-5 were oxygen-consuming fungi, the increase in the inoculation dosage in the co-culture system will accelerate the increase in the oxygen demand. Except for providing oxygen, it was reported that H_2O_2 may mediate the signaling pathways for HA accumulation [51]. In this study, the H_2O_2 was introduced in the co-culture system, and its effect on the HA yield was investigated.

The oxygen metabolism can be balanced by adding an exogenous oxidant or reductant in the fermentation broth [52]. Adding H_2O_2 in the co-culture system could promote the growth of the mycelium biomass and the accumulation of the HA in the mycelia. For example, when adding 0.5 mM H_2O_2, the HA yield increased slightly to 51.32 mg/g carbon source, 18.31% higher than that of the control (Figure 11). The adding of H_2O_2 could be helpful in further promoting the HA yields, but the dosage should be controlled at a proper level.

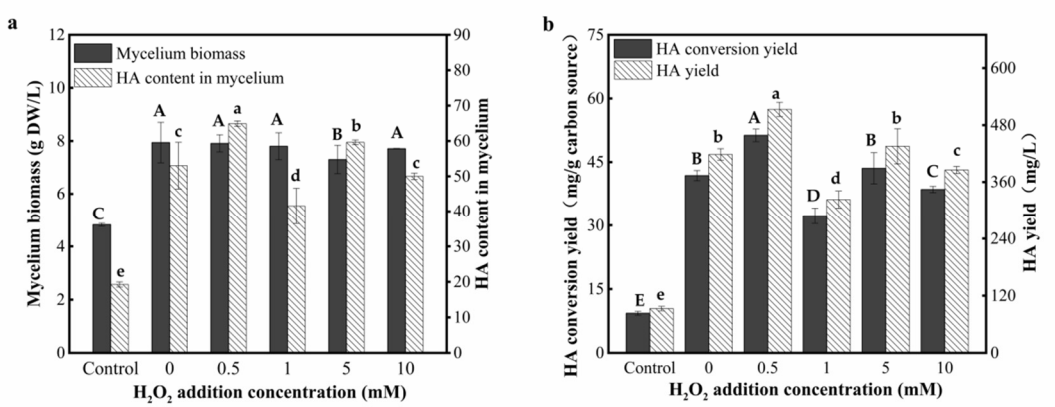

Figure 11. Effect of exogenous H_2O_2 on mycelium biomass and HA yield in the co-culture system. (**a**). mycelium biomass and HA content in mycelium; (**b**). HA conversion yield and HA yield. Different letters indicated that data were significantly different ($p < 0.05$), and comparisons were made between columns of the same color, the significance between a set of data is marked uniformly in uppercase letters or uniformly in lowercase letters.

The co-culture of *S. bambusicola* (GDMCC 60438) with the *Arthrinium* sp. AF-5 was an effective strategy to promote the HA yields through the submerged fermentation. With an in-depth understanding of the possible role and mechanism of the endophytic fungi in the regulation of the HA production, more techniques, e.g., feeding of precursor, nutrition, or even signal molecules, could be developed and applied to the biosynthesis of the HA in the future. Additionally, the biomass of the co-culture was higher than the total of the two fungal biomasses obtained from separate cultivation. However, it was difficult to determine the biomass of *S. bambusicola* (GDMCC 60438) alone as the mycelia of the two strains entangled together in the co-culture system. The effect of the co-culture on the individual fungal biomass growth could be further investigated through the analysis of the anatomical structure of the pellets using the fluorescent staining method.

4. Conclusions

An endophytic fungi *Arthrinium* sp. AF-5 was isolated to enhance the HA production of strain *S. bambusicola* (GDMCC 60438). The optimal form of inoculation (FFM), the optimal inoculation time (12 h), the optimal addition amount (0.06 g FW/mL), and the optimal fermentation time (84 h) were determined for the co-culture. The co-cultivation of the *S. bambusicola* (GDMCC 60438) with the endophytic *Arthrinium* sp. AF-5 strain could significantly promote the HA yield, with the highest HA production of 667.47 mg/L and the highest HA conversion yield of 66.75 mg/g. During the co-cultivation, the mycelium biomass was increased by 11.01%, and the accumulation of the HA in the mycelia was promoted by 3.84 times, reaching 52.19 mg/g. The enhanced HA production could be related to the contact communication between the two strains by intertwined hyphae in the mycelial pellets. In addition, proper amounts of H_2O_2 may supplement oxygen in the co-culture system for further increasing HA production. However, an additional investigation would be necessary to understand the molecular mechanisms by which the co-culture works according to evidence.

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References

1. Ali, S.M.; Olivo, M. Efficacy of Hypocrellins Pharmacokinetics in Phototherapy. *Int. J. Oncol.* **2003**, *21*, 1229–1237. [CrossRef]
2. Cheng, T.F.; Jia, X.M.; Ma, X.H.; Lin, H.P.; Zhao, Y.H. Phylogenetic Study on *Shiraia Bambusicola* by Rdna Sequence Analyses. *J. Basic Microbiol.* **2010**, *44*, 339–350. [CrossRef] [PubMed]
3. Doungporn, M.; Hiroko, K.; Tatsuji, S. Molecular Diversity of Bamboo-Associated Fungi Isolated from Japan. *FEMS Microbiol. Lett.* **2010**, *1*, 10–19.
4. Jia, X.M.; Xu, X.H.; Zhuang, B.C.; Lin, H.P. The Progress of Biological Research of Medicinal Fungus *Shiraia Bambusicola*. *Microbiology* **2006**, *33*, 147–150.
5. Ca, I.Y.; Ding, Y.; Tao, G.; Liao, X. Production of 1,5-Dihydroxy-3-Methoxy-7-Methylanthracene-9,10-Dione by Submerged Culture of *Shiraia Bambusicola*. *J. Microbiol. Biotechnol.* **2008**, *18*, 322.
6. Kishi, T.; Tahara, S.; Taniguchi, N.; Tsuda, M.; Takahashi, S. New Perylenequinones from *Shiraia Bambusicola*. *Planta Med.* **1991**, *57*, 376–379. [CrossRef]
7. Cai, Y.; Wei, Z.; Liao, X.; Ding, Y.; Sun, J. Characterization of Three Extracellular Polysaccharides from *Shiraia* sp. Super-H168 under Submerged Fermentation. *Carbohydr. Polym.* **2010**, *82*, 34–38. [CrossRef]
8. Ali, S.M.; Chee, S.K.; Yik, G. Hypericin and Hypocrellins Induced Apoptosis in Human Mucosal Carcinoma Cells. *J. Photochem. Photobiol. B Biol.* **2001**, *65*, 59–73. [CrossRef]
9. Wang, N.; Zhang, Z. Relationship between Photosensitizing Activities and Chemical Structure of Hypocrellins a and B. *J. Photochem. Photobiol. B* **1992**, *14*, 207–217.
10. Chaloupka, R.; Sureau, F.; Kocisova, E.; Petrich, J.W. Hypocrellins a Photosensitization Involves an Intracellular Ph Decrease in 3t3 Cells. *Photochem. Photobiol.* **2010**, *68*, 44–50. [CrossRef]
11. Hudson, J.; Zhou, J.; Chen, J.; Harris, L.; Yip, L.; Towers, G. Hypocrellins, from Hypocrella Bambuase, Is Phototoxic to Human Immunodeficiency Virus. *Photochem. Photobiol.* **2010**, *60*, 253–255. [CrossRef] [PubMed]
12. Zhenjun, D.; Lown, J.W. Hypocrellins and Their Use in Photosensitization. *Photochem. Photobiol.* **1990**, *52*, 609–616. [CrossRef] [PubMed]
13. Yang, H.; Xiao, C.; Ma, W.; He, G. The Production of Hypocrellins Colorants by Submerged Cultivation of the Medicinal Fungus *Shiraia Bambusicola*. *Dye. Pigment.* **2009**, *82*, 142–146. [CrossRef]

14. Lei, X.Y.; Zhang, M.Y.; Ma, Y.J.; Wang, J.W. Transcriptomic Responses Involved in Enhanced Production of Hypocrellins a by Addition of Triton X-100 in Submerged Cultures of *Shiraia Bambusicola*. *J. Ind. Microbiol. Biotechnol.* **2017**, *44*, 1425–1429. [CrossRef] [PubMed]
15. Deng, H.; Liang, W.; Fan, T.P.; Zheng, X.; Cai, Y. Modular Engineering of *Shiraia Bambusicola* for Hypocrellins Production through an Efficient Crispr System. *Int. J. Biol. Macromol.* **2020**, *165*, 796–803. [CrossRef]
16. Bertrand, S.; Bohni, N.; Schnee, S.; Schumpp, O.; Gindro, K.; Wolfender, J.L. Metabolite Induction Via Microorganism Co-Culture: A Potential Way to Enhance Chemical Diversity for Drug Discovery. *Biotechnol. Adv.* **2014**, *32*, 1180–1204. [CrossRef]
17. Bérdy, J. Thoughts and Facts About Antibiotics: Where We Are Now and Where We Are Heading. *J. Antibiot.* **2012**, *65*, 385–395. [CrossRef]
18. Brakhage, A.A. Regulation of Fungal Secondary Metabolism. *Nat. Rev. Microbiol.* **2013**, *11*, 21–32. [CrossRef]
19. Keller, N.P.; Turner, G.; Bennett, J.W. Fungal Secondary Metabolism-from Biochemistry to Genomics. *Nat. Rev. Microbiol.* **2005**, *3*, 937–947. [CrossRef]
20. Boruta, T.; Milczarek, I.; Bizukojc, M. Evaluating the Outcomes of Submerged Co-Cultivation: Production of Lovastatin and Other Secondary Metabolites by *Aspergillus Terreus* in Fungal Co-Cultures. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 5593–5605. [CrossRef]
21. Bader, J.; Mast-Gerlach, E.; Popovi, M.K.; Bajpai, R.; Stahl, U. Relevance of Microbial Coculture Fermentations in Biotechnology. *J. Appl. Microbiol.* **2010**, *109*, 371–387. [CrossRef] [PubMed]
22. Smid, E.; Lacroix, C. Microbe–Microbe Interactions in Mixed Culture Food Fermentations. *Curr. Opin. Biotechnol.* **2013**, *24*, 148–154. [CrossRef] [PubMed]
23. Scherlach, K.; Hertweck, C. Triggering Cryptic Natural Product Biosynthesis in Microorganisms. *Org. Biomol. Chem.* **2009**, *7*, 1753–1760. [CrossRef] [PubMed]
24. Chagas, F.O.; Dias, L.G.; Pupo, M.T. A Mixed Culture of Endophytic Fungi Increases Production of Antifungal Polyketides. *J. Chem. Ecol.* **2013**, *39*, 1335–1342. [CrossRef]
25. Marmann, A.; Aly, A.; Lin, W.; Wang, B.; Proksch, P. Co-Cultivation—a Powerful Emerging Tool for Enhancing the Chemical Diversity of Microorganisms. *Mar. Drugs* **2014**, *12*, 1043–1065. [CrossRef]
26. Brakhage, A.A.; Schroeckh, V. Fungal Secondary Metabolites—Strategies to Activate Silent Gene Clusters. *Fungal Genet. Biol. FG B* **2011**, *48*, 15–22. [CrossRef]
27. Chávez, R.; Fierro, F.; García-Rico, R.O.; Vaca, I. Filamentous Fungi from Extreme Environments as a Promising Source of Novel Bioactive Secondary Metabolites. *Front. Microbiol.* **2015**, *6*, 903. [CrossRef]
28. Netzker, T.; Fischer, J.; Weber, J.; Mattern, D.J.; König, C.C.; Valiante, V.; Schroeckh, V.; Brakhage, A.A. Microbial Communication Leading to the Activation of Silent Fungal Secondary Metabolite Gene Clusters. *Front. Microbiol.* **2015**, *6*, 299. [CrossRef]
29. Brenner, K.; You, L.; Arnold, F.H. Engineering Microbial Consortia: A New Frontier in Synthetic Biology. *Trends Biotechnol.* **2008**, *26*, 483–489. [CrossRef]
30. Zuck, K.M.; Shipley, S.; Newman, D.J. Induced Production of N-Formyl Alkaloids from *Aspergillus Fumigatus* by Co-Culture with *Streptomyces Peucetius*. *J. Nat. Prod.* **2011**, *74*, 1653. [CrossRef]
31. Wang, L.; Wei, W.; Tian, X.; Shi, K.; Wu, Z. Improving Bioactivities of Polyphenol Extracts from *Psidium Guajava* L. Leaves through Co-Fermentation of *Monascus Anka* Gim 3.592 and *Saccharomyces Cerevisiae* Gim 2.139. *Ind. Crop. Prod.* **2016**, *94*, 206–215. [CrossRef]
32. Yan, P.-M.; Xue, W.-T.; Tan, S.-S.; Zhang, H.; Chang, X.-H. Effect of Inoculating Lactic Acid Bacteria Starter Cultures on the Nitrite Concentration of Fermenting Chinese Paocai. *Food Control* **2008**, *19*, 50–55. [CrossRef]
33. Szambelan, K.; Nowak, J.; Czarnecki, Z. Use of Zymomonas Mobilis and Saccharomyces Cerevisiae Mixed with Kluyveromyces Fragilis for Improved Ethanol Production from Jerusalem Artichoke Tubers. *Biotechnol. Lett.* **2004**, *26*, 845–848. [CrossRef]
34. Xie, X.H.; Fan, F.X.; Yuan, X.W.; Liu, N.; Liu, J.S. Isolation of High Performance Bacterial Consortium Ff and Its Decolorization Effects on Reactive Black 5. *J. Donghua Univ. (Nat. Sci.)* **2013**, *39*, 802–813.
35. Meyer, V.; Stahl, U. The Influence of Co-Cultivation on Expression of the Antifungal Protein in *Aspergillus Giganteus*. *J. Basic Microbiol.* **2003**, *43*, 68–74. [CrossRef]
36. Ola, A.; Thomy, D.; Lai, D.; Br Tz-Oesterhelt, H.; Proksch, P. Inducing Secondary Metabolite Production by the Endophytic Fungus *Fusarium Tricinctum* through Coculture with *Bacillus Subtilis*. *J. Nat. Prod.* **2013**, *76*, 2094–2099. [CrossRef] [PubMed]
37. Shin, C.S.; Kim, H.J.; Kim, M.J.; Ju, J.Y. Morphological Change and Enhanced Pigment Production of *Monascus* When Cocultured with *Saccharomyces Cerevisiae* or *Aspergillus Oryzae*. *Biotechnol. Bioeng.* **2015**, *59*, 576–581. [CrossRef]
38. Ma, Y.J.; Zheng, L.P.; Wang, J.W. Bacteria Associated with *Shiraia* Fruiting Bodies Influence Fungal Production of Hypocrellins A. *Front. Microbiol.* **2019**, *10*, 2023. [CrossRef]
39. Jian-Feng, L.I.; Chen, L.I.; Liao, L.M. Study on Extraction of Honeysuckle Polysaccharide by Internal Boiling Method. *Food Mach.* **2016**, *19*, 155–159.
40. Cubero, O.F.; Crespo, A.; Fatehi, J.; Bridge, P.D. DNA Extraction and Pcr Amplification Method Suitable for Fresh, Herbarium-Stored, Lichenized, Other Fungi. *Plant Syst. Evol.* **1999**, *216*, 243–249. [CrossRef]
41. Liu, X.; Chen, Y.; Wu, L.; Wu, X.; Huang, Y. Optimization of Polysaccharides Extraction from *Dictyophora Indusiata* and Determination of Its Antioxidant Activity-Sciencedirect. *Int. J. Biol. Macromol.* **2017**, *103*, 175–181. [CrossRef]
42. Hu, M.; Cai, Y.; Liao, X.; Hao, Z.; Liu, J. Development of an Hplc Method to Analyze and Prepare Elsinochrome C and Hypocrellins a in the Submerged Fermentation Broth of *Shiria* sp. Super-H168. *Biomed. Chromatogr.* **2012**, *26*, 737–742. [CrossRef] [PubMed]

43. Wen, W.J.; Wu, J.Y. Effective Elicitors and Process Strategies for Enhancement of Secondary Metabolite Production in Hairy Root Cultures. *Adv. Biochem. Eng. Biotechnol.* **2013**, *134*, 55–89.
44. Zhu, L.W.; Zhong, J.J.; Tang, Y.J. Significance of Fungal Elicitors on the Production of Ganoderic Acid and Ganoderma Polysaccharides by the Submerged Culture of Medicinal Mushroom *Ganoderma Lucidum*. *Process Biochem.* **2008**, *43*, 1359–1370. [CrossRef]
45. Ishihara, A.; Nakao, T.; Mashimo, Y.; Murai, M.; Ichimaru, N.; Tanaka, C.; Nakajima, H.; Wakasa, K.; Miyagawa, H. Probing the Role of Tryptophan-Derived Secondary Metabolism in Defense Responses against *Bipolaris Oryzae* Infection in Rice Leaves by a Suicide Substrate of Tryptophan Decarboxylase. *Phytochemistry* **2011**, *72*, 7–13. [CrossRef] [PubMed]
46. Simic, S.G.; Tusevski, O.; Maury, S.; Hano, C.; Delaunay, A.; Chabbert, B.; Lamblin, F.; Laine, E.; Joseph, C.; Hagege, D. Fungal Elicitor-Mediated Enhancement in Phenylpropanoid and Naphtodianthrone Contents of *Hypericum Perforatuml*. Cell Cultures. *Plant Cell Tissue Organ. Cult.* **2015**, *122*, 213–226. [CrossRef]
47. Du, W.; Liang, Z.; Zou, X.; Han, Y.; Liang, J.; Yu, J.; Chen, W.; Wang, Y.; Sun, C. Effects of Microbial Elicitor on Production of Hypocrellins by *Shiraia Bambusicola*. *Folia Microbiol.* **2013**, *58*, 283–289. [CrossRef]
48. Wang, J.W.; Zhang, Z.; Tan, R.X. Stimulation of Arteisinin Production in *Artemisia Annua* Hairy Roots by the Elicitor from the Endohytic *Colletotrichum* sp. *Biotechnol. Lett.* **2001**, *23*, 857–860. [CrossRef]
49. Wakefield, J.; Hassan, H.M.; Jaspars, M.; Ebel, R.; Rateb, M.E. Dual Induction of New Microbial Secondary Metabolites by Fungal Bacterial Co-Cultivation. *Front. Microbiol.* **2017**, *8*, 1284. [CrossRef]
50. Ma, Y.J.; Zheng, L.P.; Wang, J.W. Inducing Perylenequinone Production from a Bambusicolous Fungus *Shiraia* sp. S9 through Co-Culture with a Fruiting Body-Associated Bacterium *Pseudomonas Fulva* Sb1. *Microb. Cell Factories* **2019**, *18*, 121. [CrossRef]
51. Du, W.; Sun, C.; Wang, J.; Xie, W.; Wang, B.; Liu, X.; Zhang, Y.; Fan, Y. Conditions and Regulation of Mixed Culture to Promote *Shiraia Bambusicola* and *Phoma* Sp. Bzj6 for Laccase Production. *Sci. Rep.* **2017**, *7*, 17801. [CrossRef] [PubMed]
52. González-Siso, M.I.; García-Leiro, A.; Tarrío, N.; Cerdán, M.E. Sugar Metabolism, Redox Balance and Oxidative Stress Response in the Respiratory Yeast *Kluyveromyces Lactis*. *Microb. Cell Factories* **2009**, *8*, 46. [CrossRef] [PubMed]