Screening of Substrate Formulas for Culture of *Auricularia auricula* Mycelia with Byproducts

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Abstract [**Objectives**] This study was conducted to explore the source of substitutive substrate raw materials for cultivation of edible fungi, reduce the cutting and use of woody materials, and realize harmless treatment and resource utilization of byproducts from Chinese medicine production and bamboo processing. [**Methods**] Traditional Chinese medicine residue and bamboo shavings were used to partially replace hardwood sawdust in the conventional formula, and the growth of *Auricularia auricula* mycelia in large test tubes under different substrate formulas was studied. [**Results**] The results showed that the mycelia of *A. auricula* could grow normally on substrates with byproducts, and the mycelia grew differently with different formulas, and the performance of different strains of the same species was also different. Compared with the conventional formula, the suitable substitution amount of bamboo shavings for *A. auricula* strains was 10% - 30%, and the substitution amount of Chinese medicine residue was 5% - 15%. [**Conclusions**] This study provides reference for the efficient utilization of byproducts and the expansion of raw material sources for production of edible fungi.

Key words Byproducts; *Auricularia auricula*; Substrate formula; Mycelial culture

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Traditional Chinese medicine residue is a kind of typical biomass resource, which is the waste from the processing and preparation of traditional Chinese medicine, the production of Chinese patent medicine and other products related to traditional Chinese medicine. China, as the birthplace of traditional Chinese medicine and a big country in production and utilization, according to incomplete statistics, the annual discharge of traditional Chinese medicine residue exceeds 30 million tons. If it is not handled properly, it will not only cause a serious waste of resources, but also become a potential hazard to the ecological environment[1]. Moreover, China is rich in bamboo resources, and has the advantages of three-dimensional climate, ecological environment and germplasm resources. Bamboo cultivation techniques and bamboo industrialization level are in the leading position in the world, and the annual output of bamboo shoots, bamboo wood and bamboo shavings has reached more than one third of the world's total output[2]. Bamboo wood will produce 50% - 70% waste in the process of processing or papermaking, and a large number of bamboo shavings will be thrown into the wilderness, buried or burned, which not only causes great waste of biomass resources, but also pollutes the ecological environment[3]. How to realize the harmless comprehensive utilization of bamboo industry waste is a key problem to be solved urgently at present.

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Edible fungus industry has developed rapidly in China in recent years, becoming the sixth largest planting industry, and its total output has ranked first in the world. According to industry statistics, in 2020, the total output of edible fungi in China was 40.614 3 million yuan, and the total output value reached 346.565 billion yuan. It can be said that the edible fungus industry has become a pillar industry in many poor counties and is one of the important ways to increase farmers' income and improve agricultural efficiency. *A. auricula* is the leading variety in the development of edible fungus industry in Yinjiang County. Considering food security, the mode of "rice-*A. auricula* rotation" is adopted to develop *A. auricula* and plant rice one season a year. After harvesting the fungus, rice is planted, and after harvesting rice, *A. auricula* is cultured. The "fallow field" becomes a "treasure field", and during the period after harvesting the fungus, the rice field is not barren, achieving a "double harvest of money and grain" and generating greater value per unit area of land. Meanwhile, it has the characteristics of labor-intensive production and is closely linked with the interests of farmers, which greatly promotes the economic capacity of the main producing areas and surrounding areas. However, in the process of planting wood-rotting edible fungi, a large number of hardwood sawdust is needed as the cultivation raw material, and the contradiction between fungi and forests is becoming increasingly obvious, which fundamentally restricts the sustainable and healthy development of the industry[4]. Therefore, in this study, a large-tube mycelial culture experiment was carried out by using bamboo processing byproducts and traditional Chinese medicine residue to partially replace sawdust as the nutrient source of *A. auricula*, and suitable substrate formula combinations were screened, aiming to provide reference for the efficient utilization of byproducts and the expansion of raw material sources for production of edible fungi.

Materials and Methods

Experimental materials

Experimental strains Three *A. auricula* strains, numbered FTH2101, FTH2102 and JAUH-W-591, were provided by Brahma Fungus Industry Co., Ltd. of Guizhou Province, and are now preserved in the National Edible Fungus Germplasm Resource Bank (Guizhou) and the Microbiology Laboratory of Institute of Soil and Fertilizer, Guizhou Academy of Agricultural Sciences.

Experimental raw materials Bamboo shavings came from a bamboo processing factory in Chishui City, Guizhou Province, and Chinese medicine residue were provided by Guizhou University of Traditional Chinese Medicine. Miscellaneous sawdust, cottonseed hulls and wheat bran came from local edible fungus production enterprises.

Experimental methods

Experimental formula design As shown in Table 1, the conventional formula of *A. auricula* production was used as the control group (CK), and different gradient treatments were designed by using Chinese medicine residue and bamboo shavings to partially replace hardwood sawdust, forming five formulas in total, A to E.

Table 1 Formula design of bamboo shaving substrates for *A. auricula* culture in large test tubes %

Formula	Chinese medicine residue	Bamboo shavings	Miscellaneous sawdust	Wheat bran	Lime	Gypsum
CK		–	78	20	1	1
A	5	10	63	20	1	1
B	10	20	48	20	1	1
C	15	30	33	20	1	1
D	20	40	18	20	1	1
E	28	50	–	20	1	1

Experimental methods Raw materials such as Chinese medicine residue, bamboo shavings, miscellaneous sawdust, wheat bran and cottonseed hull were weighed according to the formula proportion, mixed evenly and blended with appropriate amount of water to keep the water content at 55% – 60%. Large glass test tubes with specifications of 25 mm × 200 mm were used for accommodating the substrates, and each tube was filled to 4/5 volume. The test tubes filled with substrates were sterilized at 121 ℃ for 1 h, cooled to normal temperature, and inoculated with strain cakes with a diameter of 6 mm. The strains were cultured in a biochemical incubator at 25 ℃, and the germination time, growth rate, appearance and growth vigor of *A. auricula* mycelia were observed and determined. Each strain was set with 10 parallel control tubes, in three replicates.

Statistical analysis of data SPSS and EXCEL were used to analyze the recorded data.

Results and Analysis

Mycelial growth of *A. auricula* FTH2101 with different byproduct substrate formulas

As shown in Table 2, the mycelial germination time of *A. auricula* FTH2101 cultured in large test tubes was in the range of 2 – 4 d, and the order was formula E > CK = formula A = formula D > formula B = formula C. In terms of mycelial growth rate, formula B was the fastest and better than the conventional formula, reaching (5.5 ±0.4) mm/d, followed by formula C and formula A, which were similar to the CK, and formula E was the slowest, with a growth rate at (1.1 ±0.4) mm/d. Except for formula E and formula D with mycelia sparse in appearance, yellow or white in color and weak in growth, other treatments showed white and dense mycelia, and formula B had the most vigorous growth, followed by the CK, formula A and formula C with similar growth.

Table 2 Mycelial growth of FTH2101 cultured in large test tubes with byproduct substrates

Formula	Germination time//d	Growth rate//mm/d	Appearance	Mycelial growth
A	3	4.6 ±0.2	White, relatively dense	+ + +
B	2	5.5 ±0.4	White, dense	+ + + +
C	2	4.8 ±0.5	White, dense	+ + +
D	3	3.3 ±0.3	Relatively white, relatively sparse	+ +
E	4	1.1 ±0.4	Relatively yellow, sparse	+
CK	3	4.8 ±0.3	White, dense	+ + +

Mycelial growth of *A. auricula* FTH2102 with different byproduct substrate formulas

It can be seen from Table 3 that the mycelial germination time of *A. auricula* FTH2102 with formula B and formula C was 2 d, followed by the CK and formula A, which were all better than formula D and formula E with a germination time of 4 d. The mycelial growth rates of various formulas were between 2.2 and 6.7 mm/d, and the order was formula C > formula B > CK > formula A > formula D > formula E, in which the rate of formula C was (6.7 ± 0.5) mm/d, which was better than that of the conventional formula (5.7 ±0.4) mm/d and 3.05 times that of the slowest formula E. On the other hand, in terms of appearance traits, formula B and formula C showed white and dense mycelia, which were similar to those of the conventional formula and superior to other treatments. The mycelia of formula E were yellow, aged and relatively sparse.

Table 3 Mycelial growth of FTH2102 cultured in large test tubes with byproduct substrates

Formula	Germination time//d	Growth rate//mm/d	Appearance	Mycelial growth
A	4	4.9 ±0.6	White, relatively dense	+ +
B	3	6.0 ±0.3	White, relatively dense	+ + +
C	2	6.7 ±0.5	White, dense	+ + + +
D	2	4.7 ±0.4	Relatively white, relatively sparse	+ +
E	3	2.2 ±0.5	Relatively yellow, sparse	+
CK	4	5.7 ±0.4	White, dense	+ + +

Mycelial growth of *A. auricula* JAUH-W-591 with different byproduct substrate formulas

The results showed that the mycelial germination rates of *A. auricula* JAUH-W-591 with formula A and formula B were the fastest, at 2 d, while that of treatment E was the slowest, at 5 d. Treatments C and D and the CK were the same. On the whole, treatment A and the CK showed similar mycelial growth, which was superior to that of other treatments, and their mycelial growth rates reached (4.8 ±0.2) and (5.1 ±0.3) mm/d, respectively, and the mycelia exhibited white and dense appearance and vigorous growth potential. However, with the increase of the contents of residue and bamboo shavings in byproducts and the decrease of the content of sawdust, the mycelial growth rate, density and growth potential decreased. Among them, treatment E showed slowest mycelial growth, which was (3.6 ±0.5) mm/d, and the mycelia exhibited white and sparse appearance and weakest growth potential.

Table 4 Mycelial growth of JAUH-W-591 cultured in large test tubes with byproduct substrates

Formula	Germination time//d	Growth rate//mm/d	Appearance	Mycelial growth
A	2	4.8 ±0.2	White, dense	+ + + +
B	2	4.6 ±0.4	White, dense	+ + +
C	3	3.9 ±0.3	White, relatively dense	+ + +
D	3	3.6 ±0.5	White, relatively sparse	+ +
E	5	3.2 ±0.4	Relatively white, relatively sparse	+ +
CK	3	5.1 ±0.3	White, relatively dense	+ + + +

Conclusions and Discussion

The results showed that there were some differences in the culture of *A. auricula* mycelia in large test tubes using substrate formulas with different contents of Chinese medicine residue and bamboo shavings, and the performance of different strains of the same species was also different. In comparison, the optimum substrate formula for *A. auricula* strain FTH2101 included sawdust 48%, wheat bran 20%, bamboo shavings 20%, Chinese medicine residue 10%, lime 1% and gypsum 1%. Under this formula, the mycelial germination time was 2 d, and the mycelia grew at a rate of (5.5 ±0.4) mm/d, and was white and dense in appearance, showing strong growth vigor. The optimum substrate formula for *A. auricula* strain FTH2102 was miscellaneous sawdust 33%, bamboo shavings 30%, wheat bran 20%, traditional Chinese medicine residue 15%, lime 1%, and gypsum 1%. Under this formula, the mycelial germination time was 2 d, and the mycelia grew at a rate of (6.7 ±0.5) mm/d, and was white and dense in appearance, showing relatively strong growth vigor. The optimum substrate formula of *A. auricula* strain JAUH-W-591 was miscellaneous sawdust 63%, wheat bran 20%, bamboo shavings 10%, Chinese medicine residue 5%, lime 1%, and gypsum 1%, with which the growth rate was (4.8 ±0.2) mm/d, and the mycelia were white and dense in appearance, showing strong growth vigor. The comprehensive performance was superior to that of the conventional formula.

Chinese medicine residue not only contains nutrients such as carbon source, nitrogen source, growth factor and inorganic salt, but also very little harmful heavy metal elements such as mercury, lead, cadmium and arsenic. It meets the national green food production control standards and is an excellent resource for cultivating edible fungi. Traditional Chinese medicine residue contains a large number of nutrients suitable for the growth of edible fungi, such as cellulose and lignin. After processing, cellulose has loose tissue structure and can be decomposed and utilized by enzymes in edible fungi[5]. Moreover, the fiber porosity of Chinese medicine residue is high, which facilitates the growth of mycelia, so it is an excellent culture medium for fungi and suitable for the cultivation of edible fungi. Using traditional Chinese medicine residue as a substitutive culture medium for edible fungi can improve the yield of fruiting bodies and enrich the active ingredients in them. Chen *et al.* [6] cultivated *Pleurotus ostreatus* with the non-medicinal parts of *Magnolia officinalis*. When the amount of non-medicinal parts of *M. officinalis* was 67%, the yield increased by 18.3% and the protein content of fruiting body increased by 15.6%. It was found that the cultivation of *A. auricula* with traditional Chinese medicine waste can promote the antioxidant capacity of its fruiting body including scavenging DPPH free radicals, hydroxyl free radicals, peroxides and reducing capacity[7]. China is rich in bamboo resources, and bamboo products show a rapid growth trend, resulting in a large number of bamboo byproducts, which contain nutrients

(such as cellulose, lignin and ash) needed for the cultivation of edible fungi. Zhong *et al.* [8] carried out the cultivation experiment of *P. ostreatus* with the mixture of bamboo shavings, sawdust and straw as the main raw materials. The results showed that the formula with half of bamboo shavings and half of sawdust and straw achieved the highest yield and the biological efficiency reached 42%. However, there are few studies on the safety of cultivating edible fungi with bamboo shavings, such as excessive heavy metal content, pesticide residues, chemical reagents and other quality and safety issues. In this study, the suitable addition of Chinese medicine residue and bamboo shavings to partially replace sawdust for mycelial culture of *A. auricula* in large test tubes was discussed, providing reference for the preparation of original strain and mushroom production in the next step. Meanwhile, the breeding of strains adaptive to byproducts, the optimization of substrate formulas and how to improve the yield and quality of fruiting bodies need further study.

References

[1] FU Y, QUAN J, YANG TJ, *et al.* Study on safety evaluation and utilization potential of six kinds of traditional Chinese medicine residues[J]. Lishizhen Medicine and Materia Medica Research, 2022, 33(2): 459 – 463. (in Chinese).

[2] XIAO JH. Bamboo forest management in China[M]. Beijing: Science Press, 2010. (in Chinese).

[3] ZENG FQ, YING GH, JIANG J, *et al.* Experiment of cultivating *Lentinula edodes* with *Phyllostachys edulis* sawdust[J]. Edible Fungi of China, 2022, 41(2): 30 – 33, 42. (in Chinese).

[4] HUANG Y, ZHENG YD, ZHANG CB, *et al.* The contradiction between mushroom and forest has become a bottleneck restricting the development of mushroom and *Auricularia auricula* industry[J]. Edible and Medicinal Mushrooms, 2018, 26(6): 333 – 337. (in Chinese).

[5] LI Y, ZENG X, CHEN J, *et al.* Advances in utilization of Chinese medicine wastes by edible fungi[J]. Acta Edulis Fungi, 2021, 28(2): 123 – 129. (in Chinese).

[6] CHEN XQ, LUO X, YU MY, *et al.* Cultivation of *Pleurotus ostreatus* using non-medicinal parts of *Magnolia officinalis*[J]. Acta Edulis Fungi, 2009, 6(4): 31 – 34. (in Chinese).

[7] GAO JJ, ZHUANG XH, TAN XS, *et al.* Cultivation of *Pleurotus ostreatus* using non-medicinal parts of *Magnolia officinalis*[J]. Chinese Traditional Patent Medicine, 2024, 46(2): 536 – 541. (in Chinese).

[8] ZHONG FY, ZENG FQ, JIANG J, *et al.* Experiment of *Stropharia rugosoannulata* cultivated in bamboo sawdust[J]. Heilongjiang Agricultural Sciences, 2021(3): 95 – 98. (in Chinese).

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU

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[16] SAFRAN M, DALAH I, ALEXANDER J, *et al.* GeneCards Version 3: The human gene integrator[J]. Database(Oxford), 2010: baq020.

[17] SZKLARCZYK D, GABLE AL, LYON D, *et al.* STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets[J]. Nucleic Acids Res, 2019, 47(D1): D607 – D613.

[18] SHERMAN BT, HAO M, QIU J, *et al.* DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update)[J]. Nucleic Acids Res, 2022,50(W1): W216 – W221.

[19] GOODSELL DS, ZARDECKI C, DI COSTANZO L, *et al.* RCSB protein data bank: Enabling biomedical research and drug discovery[J]. Protein Sci, 2020, 29(1): 52 – 65.

[20] CHEN XN, WANG RQ, SHAN HY, *et al.* Quercetin alleviates H_2O_2-induced oxidative stress damage to human endometrial stromal cells by inhibiting the p38 MAPK/NOX4 signaling pathway[J]. Journal of Sichuan University: Medical Science Edition, 2024, 55(3): 552 – 558. (in Chinese).

[21] ISLAM A, ISLAM MS, RAHMAN MK, *et al.* The pharmacological and biological roles of eriodictyol[J]. Arch Pharm Res, 2020, 43(6): 582 – 592.

[22] LIU YN, KANG JW, ZHANG Y, *et al.* Vanillin prevents the growth of endometriotic lesions through anti-inflammatory and antioxidant pathways in a mouse model[J]. Food Funct, 2023, 14(14): 6730 – 6744.

[23] HINZ N, JÜCKER M. Distinct functions of AKT isoforms in breast cancer: A comprehensive review[J]. Cell Commun Signal, 2019, 17(1): 154.

[24] FRESNO VJA, CASADO E, DE CASTRO J, *et al.* PI3K/Akt signalling pathway and cancer[J]. Cancer Treat Rev, 2004, 30(2): 193 – 204.

[25] TIWARI A, TASHIRO K, DIXIT A, *et al.* Loss of HIF1A from pancreatic cancer cells increases expression of PPP1R1B and degradation of p53 to promote invasion and metastasis[J]. Gastroenterology, 2020, 159(5): 1882 – 1897. e5.

[26] HUA G, GEORGE JW, CLARK KL, *et al.* Hypo-glycosylated hFSH drives ovarian follicular development more efficiently than fully-glycosylated hFSH: enhanced transcription and PI3K and MAPK signaling[J]. Hum Reprod, 2021, 36(7): 1891 – 1906.

[27] CHANDLER RL, DAMRAUER JS, RAAB JR, *et al.* Coexistent ARID1A-PIK3CA mutations promote ovarian clear-cell tumorigenesis through pro-tumorigenic inflammatory cytokine signalling[J]. Nat Commun, 2015(6): 6118.

Editor: Yingzhi GUANG Proofreader: Xinxiu ZHU