Effect of Different Fruiting Treatments on the Enzymic Activity of *Tricholoma giganteum* Mycelia

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Abstract In order to achieve the transformation from the conventional soil-covering cultivation of *Tricholoma giganteum* with bags to the soilless cultivation with bottles and understand the mechanism of primordium formation of *Tricholoma giganteum*, with *Tricholoma giganteum* mycelia as experimental materials, this paper studied the effect of different fruiting treatments on the activity of three enzymes in different time. The results showed that from the mycelial recovery to primardial formation and budding under three treatment groups which could form primordium, the tyrosinase activity was relatively stable, and under two treatment groups which could not form primordium, the tyrosinase activity dropped after the rise and reached a maximum on the 9th day, significantly higher than under the other three groups, indicating that too high tyrosinase inhibited primardial formation; the prolease and amylase activity was effectively activated before primardial formation, and the enzymic activity was significantly higher than under the two treatment groups which could not form primordium.

Key words *Tricholoma giganteum*, Mycelium, Primordial formation, Enzymic activity

1 Introduction

Tricholoma giganteum, belongs to Tricholomataceae of Tricholoma, is also well-known as the rare large medicinal mushroom which can be used as food and medicine[1]. Over the years, the covering-based fruiting has been the production mode of *Tricholoma giganteum*[3]. Based on the cultivation method of *Pleurotus eryngii* and other edible mushrooms[4], the bottle is used for bacterination.

In the actual production process, there are some problems in the soilless cultivation of *Tricholoma giganteum* with bottles, such as irregular fruiting and long fruiting time. The formation of edible mushroom primordium is the basis of fruiting, and the primordium formation is also closely related to the activity of related enzymes in addition to the basic nutrition and appropriate environmental conditions[5].

These enzymes are mostly induced enzymes, and the expression order and synergistic effect of their activity is an important part of endogenous biochemical metabolism of edible fungi. Some substances that facilitate the synthesis or expression of these enzymes are added to the matrix so as to promote induction, accelerate metabolism and increase the level of expression of the enzymic activity, or some inhibitors are added to the matrix so as to reduce the expression of the enzymic activity, control fruiting body and increase yield. The growth of fruiting body is studied from the perspective of growth and metabolism[6-8].

Therefore, through different methods of fruiting, we studied the mycelium enzymic activity of *Tricholoma giganteum* from mycelium overgrowth to fruiting body formation and found the enzyme related to the formation of primordium, which provided a reference for understanding the nutrition needs of *Tricholoma giganteum* in soil-covering cultivation and soilless cultivation and determining the law of use.

Received: July 4, 2017 Accepted: September 18, 2017

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2 Materials and methods

2.1 Test materials and instruments Mushroom: *Tricholoma giganteum* for test. Reagents: potassium dihydrogen phosphate, dipotassium hydrogen phosphate, L-tyrosine, trichloroacetic acid, casein, Folin reagent, hydrochloric acid, 3, 5-dinitrosalicylic acid, starch and maltose.

The main instruments: biochemical incubator, high-speed refrigerated centrifuge, UV-visible spectrophotometer, constant temperature water bath, micro-pipette.

2.2 Test methods

2.2.1 Mycelium culture and fruiting treatment. After the bag was covered with mycelium and there was physiologically yellow water, the bag was opened and covered with a layer of 3 cm garden soil[9].

At the same time, after the bottle was filled with mycelium and there was physiologically yellow water, the mycelium stimulation was conducted, water was injected into the bottle, and then the bottle was inverted and put straight.

Subsequently, several different fruiting treatment methods were managed in the same way every day, and the mushroom room temperature was maintained to be 28 ℃ - 30 ℃ and humidity 85% - 95%[10].

2.2.2 Determination methods of tyrosine enzymic activity. It was based on the method of Wei Yaowu[11] and modified. 1 g of fresh mycelium culture medium was placed in a mortar, and 5 mL of pre-cooling 0.05 mol/L phosphate buffer with pH of 6.8 was added and homogenized. After 20 min of 5000 r/min centrifugation at 4 ℃, the supernatant was as enzyme extract. In the cuvette,

3.95 mL of 1 mmol/L L-tyrosine phosphate buffer (pH 6.8) was added, and 0.05 mL of enzyme solution was rapidly mixed.

Immediately, the UV spectrophotometer was used to measure the change in optical absorbance values at 317 nm for 10 min. The initial linear segment slope with the increase in absorbance was used to calculate the enzyme activity. Enzyme activity unit (U) was defined as 0.1 – 1 unit of enzyme activity causing OD change per minute under the assay conditions (20 ℃).

2.2.3 Determination methods of protease activity. 1 g of fresh mycelium culture medium was placed in a mortar and mixed with 5 mL of 0.02 mol/L phosphate buffer with pH of 7.4 and sufficiently ground, followed by 10 min of 10000 r/min centrifugation at 4 ℃. The supernatant was just the crude enzyme solution.

Protease activity was measured by the method of Huang Zhuolie[12]. Under certain reaction conditions, 1 μg of tyrosine enzyme generated by the casein hydrolysis per minute was 1 unit of enzyme activity.

2.2.4 Determination methods of amylase activity. 1 g of fresh mycelium culture medium was placed in a mortar and 5 mL of 0.05 mol/L phosphate buffer (pH 6.8) was added and thoroughly ground, followed by 10 min of 10000 r/min centrifugation at 4 ℃. The supernatant was the crude enzyme solution of mycelium.

Amylase activity was measured by the method of Huang Zhuolie[12]. Under certain reaction conditions, the amount of 1 μmol maltose enzyme from through the starch hydrolysis and catalysis of amylase was defined as 1 unit of enzyme activity.

2.3 Data analysis DPSv 9.50 mathematical statistical software was used for statistical analysis.

3 Results and analysis

3.1 Effect of different fruiting treatments on the tyrosinase within *Tricholoma giganteum* mycelia As shown in Fig. 1, the enzymic activity of tyrosine in mycelia under 5 different treatments first increased and then decreased.

After casing and bottle mycelium stimulation, under treatment (putting-straight 1 and upside-down 1), the tyrosine enzymic activity was relatively low in the whole process; after bottle mycelium stimulation, under treatment (putting-straight 2 and upside-down 2), the tyrosine enzymic activity drastically increased after the 6th d, and peaked to 55.6633 U/g and 88.2803 U/g on the 9th d, respectively, but then declined. After the 18th d, the tyrosine enzymic activity of mycelia under 5 different treatments was similar.

3.2 Effect of different fruiting treatments on the protease in *Tricholoma giganteum* mycelia As shown in Fig. 2, after casing and bottle mycelium stimulation, under treatment (putting-straight 1 and upside-down 1), the protease activity continued to increase, and peaked to 582.075 U/g, 664.6975 U/g and 677.5793 U/g on the 21st d (budding stage), respectively, significantly higher than the protease activity under treatment (putting-straight 2 and upside-down 2).

Under treatment (putting-straight 2 and upside-down 2), the protease activity was not effectively activated and the protease activity did not change significantly in the whole process, always at a low level.

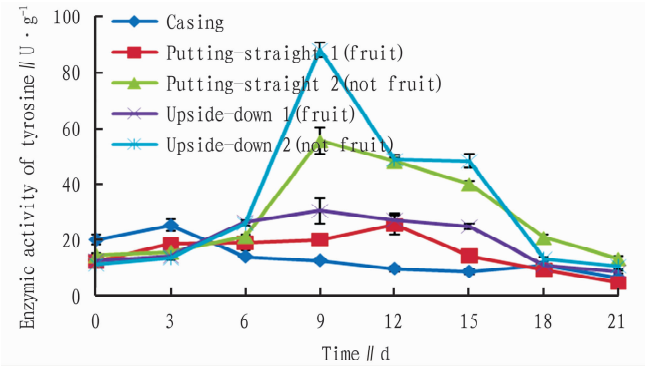

Fig. 1 Effect of different fruiting treatments on the enzymic activity of tyrosine in *Tricholoma giganteum* mycelia

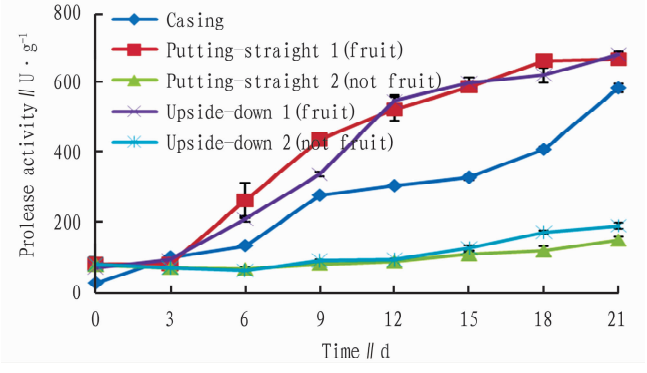

Fig. 2 Effect of different fruiting treatments on the prolease activity of *Tricholoma giganteum* mycelia

3.3 Effect of different fruiting treatments on the amylase activity of *Tricholoma giganteum* mycelia As shown in Fig. 3, from 0 to the 9th d, there was no significant difference in the amylase activity under different treatments, all at a low level.

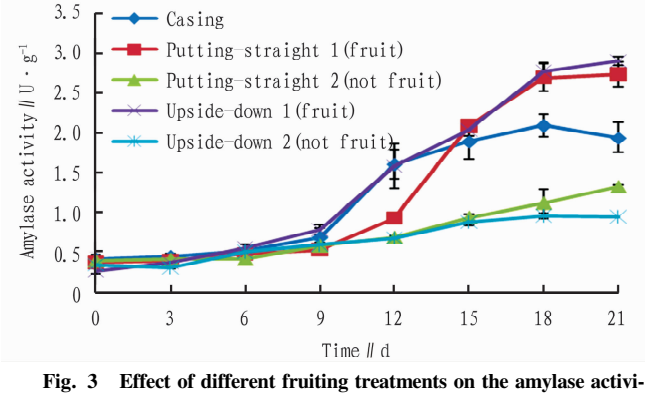

Fig. 3 Effect of different fruiting treatments on the amylase activity of *Tricholoma giganteum* mycelia

From the 9th d, after casing, under treatment (putting-straight 1 and upside-down 1), the amylase activity increased rapidly, significantly higher than under the two other treatments. Under treatment (putting-straight 2 and upside-down 2), the amylase activity increased at the late stage, but the change was not significant.

4 Conclusions and discussions

4.1 Conclusions This paper studied the changes of enzymic activity in mycelia under different treatments after casing and bottle mycelium stimulation of *Tricholoma giganteum*, and found that there were significant differences in activity of three kinds of enzymes at different stages.

After casing and bottle mycelium stimulation, under treatment (putting-straight 1 and upside-down 1), the primordium could be formed on the 18th d, and small buds appeared on the 21st d; after bottle mycelium stimulation, under treatment (putting-straight 2 and upside-down 2), the primordium was not formed in the whole process of determination.

Therefore, it was concluded that from the mycelial recovery period to primardial formation and budding period of *Tricholoma giganteum*, there was no need of high tyrosine enzymic activity, and high tyrosinase activity inhibited mycelial kink to form primordium; the prolease and amylase needed to be effectively activated before primardial formation, and the enzymic activity was significantly higher than under the treatment groups which could not form primordium.

4.2 Discussions Tyrosinase is a widespread class of Cu-containing desmoenzyme in the plant body, participating in the redox reactions at the breathing end[13]. It was found in this study that under the treatment groups which could form primordium, the tyrosinase activity increased on the 6th d and then leveled off; compared with the treatment groups which could not form primordium, it was at a low level, possibly because an effective inhibitor of tyrosinase was generated in the mycelia[14].

After bottle mycelium stimulation, under treatment (putting-straight 2 and upside-down 2), the tyrosinase activity dropped after an initial increase, and reached a peak on the 9th d, significantly higher than under the other three treatments. The possible reason was that the mycelium respiration was blocked, stimulating the activity of tyrosinase, the enzymic activity was high, and the mycelial growth did not come to end, unable to form primordium by kink and have the internal conditions of fruiting.

The results of this study showed that under the treatment groups which could form primordium, the protease activity showed a rising trend, consistent with the previous description[15]. The increase in protease activity accumulated nitrogen sources for the synthesis of new substances, and when it was accumulated to a certain extent, the primordium was induced. Under the treatment groups which could not form primordium, there was no significant change in protease activity, always at a low level, indicating that the treatment lacked the conditions for the induction of protease response, lacked material conversion and accumulation, and mycelium grew excessively and was maintained at the vegetative growth stage, so it was difficult to form primordium, which was consistent with the findings of Zhang Le[16].

Many studies have shown that the amylase is high at the mycelial growth stage of edible fungus but low at the fruiting stage[17], indicating that in the early cultivation period, most of starch substances are decomposed and used, while in the late cultivation period, the substances are seldom used. It was found in this study that under 5 treatments, the amylase activity was low at the early stage, and from the 9th d, under the treatment group which could form primordium, the amylase activity rapidly rose.

This might be because after the small molecules in culture material, which did not use amylase for decomposition, were digested at the mycelial recovery and vegetative growth stage, there was a need to further decompose starch macromolecules to meet the nutrition needs of mycelia to continuously grow, kink and form primordium. The studies of Zhao Yadong on ectoenzyme of Pleurotus geesteranus indicated that extracellular amylase activity level was irrelevant with the budding period of Pleurotus geesteranus[18].

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production efficiency of growing-finishing pigs. Theoretically, fine division of feeding stages will have better feeding effect, but too fine division will be limited by operability in the production practice. Therefore, some European countries such as Denmark, Germany, and Britain applied liquid feed system. Using this method, two kinds of liquid daily ration with different nutrition levels were prepared. According to varieties of pigs and daily ration, 2 kinds of daily ration were mixed to prepare the daily ration with nutrition level, so make the daily nutrition supply get closer to nutrition demands of pigs[8]. Such new feeding technology has gone beyond the scope of multi-stage feeding, posing enormous challenges to traditional feeding method, to realize fine feeding with popularization and extension of necessary equipment and feed products. At present, in the swine production of China, three-stage feeding is widely applied due to too few stage division and simple operation, but such feeding method can not really obtain optimum production efficiency, and will lead to waste of protein and deteriorate the pollution[9]. In order to explore efficient feeding methods for growing-finishing pigs, many scholars studied feeding effect of four-stage and five-stage feeding on the basis of three-stage feeding[8-11]. According to the study of Qiao Jianguo *et al.* [8], the four-stage feeding had better production performance, lower feeding cost, and higher economic benefits than three-stage feeding; according to the study of Liu Jing *et al.* [10] five-stage feeding had higher production performance, lower feeding cost, and higher economic benefit than three-stage feeding. According to results of this experiment, five-stage and four-stage feeding increased the body weight by 4.18% and 2.41% than three-stage feeding, and average economic income increased by 5.47% and 3.59% respectively. Fine division of feeding can significantly raise production performance and economic benefits of growing-finishing pigs. Previous researches have shown that, without influencing the production performance, if the crude protein of growing-finishing pigs drops 1%, it can reduce about 8% – 10% total nitrogen excretion[12], proving that multi-stage feeding of growing-finishing pigs has environmental protection[13]. In this experiment, the crude protein consumption from average body weight increase of growing-finishing pig was 463.44 g/kg and 466.31 g/kg for five-stage feeding and four-stage feeding, which was 2.28% and 1.68% lower than that of three-stage feeding, indicating that fine division of feeding stages for growing-finishing pigs has advantage of environmental protection. Compared with the three-stage feeding, four-feeding and five-stage feeding can significantly improve the production performance of growing-finishing pigs, increase the feed utilization efficiency, reduce the feed cost, increase the economic benefits, and significantly reduce the crude protein consumption from average body weight increase. Compared with the four-stage deeding, the five-stage feeding has better feeding effect.

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