Regulation of Key Enzymes in Lentinula Edodes Mycelium Culture by Chitosan

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Research Article

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Abstract

Chitosan is a plant growth regulator. Since it has an important function, it may also play a similar role in the growth of fungi. In this study, *Lentinula edodes* was used as a model fungus, and the mycelium growth and metabolism in the cultivation bag treated with different concentrations of chitosan were investigated. The results showed when the chitosan concentration was 0.1 mg/ml, a dense mycelium was developed and the fast growth was observed. Compared with other concentrations and the control group, they had significant differences. Furthermore, the activity of CMCase, Amy, and POD were measured, which showed significant differences in the rapid growth of the mycelium compared with other concentrations. The concentrations of 0.1 mg/mL will be chosen to promote the growth of *Lentinula edodes*' mycelium in practical production.

Highlights

1. The effect of different chitosan concentration on the growth and metabolism of *Lentinus edodes* mycelium was explored
2. Chitosan could regulate the activity of key enzymes in *Lentinus edodes* mycelium by measuring the activity of several key enzymes in *Lentinus edodes*;
3. It laid a certain research foundation for searching the growth promoter of edible fungus;

Introduction

Recently, many plant growth regulators can be used to facilitate the growth of plants by adjusting a multitude of physio-biochemical processes (Khodadadi et al. 2020). Plant growth regulators are some natural substances, that can regulate the physiological processes during plant growth and development, like enhancing fruit color, floral bud differentiation, fruit ripening, etc. (Khanal and Poudel 2020). Meanwhile, it can also increase its yield, enhance cell division (Olaiya 2010), and improve the immune response of plants (Chandra et al. 2015), etc. However, the synthesis conditions and extraction technology of plant growth regulators are complicated, which limits their application in the horticultural industry (Acemi 2020). Therefore, it is of great significance to find new alternative additives in the cultivation of crops.

Chitosan is a deacetylated product of chitin, which is a naturally abundant polysaccharide (Ly et al. 2021). It has the potential to be used for many plant cultivations due to its root elongation-inhibitory effects. Besides, it also improves the yield and quality of some plants. Its effect on plants is similar to that of auxins(Arda et al. 2018). Furthermore, easy degradation characteristics in the natural environment ensure the environment is not polluted, so it is the ideal option for stimulating plant growth (Supaporn et al. 2016;Ren et al. 2021; Torres-Rodriguez et al. 2021). Similarly, it may play a similar role in the growth and development of some fungi. *Lentinula edodes* is a kind of tasty, rich in nutrients and the consumer favorite mushroom. However, the fruiting body of *Lentinula edodes* can grow normally only if it is satisfied with the supply of nutrients. In other words, the mycelium will continue to absorb nutrients from

the culture substrate to satisfy the mushroom's development. Chitosan may be an important active substance in this role. Not only that, chitosan has a certain effect on the microbial and postharvest quality of *Lentinus ed*odes (Jiang et al. 2012).

In this paper, chitosan was added to the culture substrate of *Lentinus edodes* to further investigate its effects on the growth of mycelium of *Lentinus edodes*, aiming to find a new growth promoter for edible fungi, to improve the economic benefit of *Lentinus edodes*.

Materials And Methods

Chemicals and Methods

Lentinula edodes were produced by Fuping JiaXin Planting Co.,Ltd. The chitosan (molecular weight of nearly 635000 with 85% deacetylation, and viscosity of 630 mPa s) was purchased from Sigma-Aldrich (Shanghai, China) Trading Co. Ltd. The PDA-agar medium was chosen as the cultivating medium.

Preparation of the chitosan solution

The chitosan solution was prepared and seven parallel test groups were set based on the solution concentration. The concentration was designed as follows: 0.5, 0.1, 0.05, 0.017, 0.01, 0.007, and 0.005 mg/mL.

Bag, sterilization, inoculation, and mushroom growth.

Bag and sterilization methods were used as normal.

Detection of the amount of mycelial growth

The mycelial growth amount was detected every six days when the mycelial spread to the culture medium, the mycelial growth potential, roughness, density, and color were observed, as well as the bagging time was recorded.

Crude enzyme preparation

All the mycelia in every group were cultured for 12 days, and a crude enzyme solution was prepared every six days. All the samples were put in the refrigerator at 4℃ to detect the activity.

Enzymatic activity measurements

The activities of carboxymethyl cellulase (CMCase), amylase (Amy), and guaiacol peroxidase (POD) were measured with reference to the previous literature (Wang et al. 2019; Thongsaiklaing et al. 2014; Golan et al. 2019).

Isoenzyme electrophoresis

Peroxidase isoenzyme has used SDS-PAGE for enzymatic protein analysis. The stacking gel T=2.5%, the separating gel T=7%. The designed amount of sample was 50 μL, and the initial electrophoresis current was 20 mA. The current was set at 40 mA when the front indicator reached the interface of the stacking gel and separation gel.

The electrophoresis should be paused once the front indicator was 1 cm away from the bottom, and then dyed after degumming.

Results

Effect of chitosan on the diffusion rate of the mycelium

The effect of different concentrations of chitosan on the diffusion rate of the mycelium is given in Table 1. There was an obvious effect on the *Lentinula edodes* mycelium growth of the different chitosan solutions. When the solution concentrations were 0.1 mg/ml and 0.5 mg/ml, the mycelium grew fast and dense, which was significantly different from the control group. Among them, it performed better at the 0.1 mg/ml concentrations. However, the mycelium growth rate of the two groups was not significantly different. When the solution concentrations were 0.017 mg/ml, 0.007 mg/ml, and 0.005 mg/ml, the mycelium growth rate was not significantly different from the control group.

Table 1
Effect of different concentrations of chitosan on mycelium diffusion rate of the *Lentinula edodes* on the substratum(mm/d)

Chitosan concentration (mg/ml)	Average growth rate of mycelium(mm/d)	Significant Difference		Mycelium growth
		α = 0.05	α = 0.01	
Control	3.005	c	B	+
0.005	4.018	bc	AB	+
0.007	4.385	bc	AB	++
0.01	4.678	b	AB	++
0.017	4.683	bc	AB	+
0.05	4.703	b	AB	+++
0.1	6.360	a	A	++++
0.5	6.265	ab	A	++++
Notations: “++++” means strong growth of mycelium;“+++”general growth;“++”and “+” poor growth.				

Chitosan is a natural cationic polymer that could stimulate the development of the roots and stems and enhance the resistance of the plant. Meanwhile, it can also improve the quality and quantity of the plants

(Olaiya 2010).The mechanism of the chitosan to stimulate the *Lentinula edodes* mycelium growth could be the stimulation of the mycelium cell to divide and grow. It may also promote the physiological activity of *Lentinula edodes* mycelium cells, thereby activating and improving the related enzyme activity. Afterward, enhance the absorption and utilization of the nutrients, resulting in the acceleration of the mycelium growth.

Effect of chitosan on mycelial CMCase activity

The effect of chitosan supplementation on CMCase activity of mycelial cells is shown in Fig. 1.The mycelial CMC enzyme activity of all groups treated with chitosan was higher than the control group when the chitosan concentration was 0.1 mg/ml, and its mycelial CMCase enzyme activity was the highest; Compared with the control group, when the chitosan concentration was 0.05 mg/ml, the mycelial CMCase enzyme activity was increased in the first 12 days, and the mycelial enzyme activity of CMCase was obviously decreased from the 18th day. When the chitosan concentrations were 0.017 mg/ml, 0.01 mg/ml, and 0.007 mg/ml, the mycelial CMCase enzyme activity was the same as that of the control group. The trend of the mycelial CMCase enzyme activity was different with time, its activity increased from day-0 to day-18, and then decreased, there was a second activity peak for 0.1 mg/ml, 0.5 mg/ml, 0.007 mg/mL and the control, but it was smaller than the first peak, and then the mycelial CMCase enzyme activity decreased.

The CMCase is an important cellulose enzyme, which could hydrolyze and generate certain glucose with the help of the CMCase, promoting the *Lentinula edodes* mycelium cell growth. Moreover, the mycelial growth rate reached its maximum from the 12th to the 18th day. The interesting thing was that the mycelium entered the physiological maturity period from the twenty-fourth to thirty-sixth day, and the CMCase enzyme activity was decreased. The reason was most probably because the CMCase enzyme was an inducible enzyme, as the exocellular enzyme of the mycelium cell, which was secreted little during the early growth period of the microorganism. Research also discovered the enzymatic secretion increased following the growth of the mycelium and was related to the age of mycelium.

Effect of chitosan on the mycelium amylase activity

The effect of chitosan solutions on the mycelium Amy activity is represented in Fig. 2. When the chitosan concentration was 0.1 mg/ml the Amy activity of *Lentinula edodes mycelium* was the highest, which was significantly higher than the control group.

At the chitosan concentration of 0.05 mg/ml, the Amy activity was significantly higher than that of the control after 12 days of continuous culture. Surprisingly, the activity of Amy was reversed and decreased as in the control group after 18 days., and then it stayed normal. Overall, the amylase activity showed an earlier increase and later decrease trend, and the amylase activity reached a maximum on the 12th day.

The starch in the medium can be hydrolyzed into glucose under the effect of the Amy, and the glucose is an important carbon source for the *Lentinula edode* growth. Amy of *Lentinula edode* exocellular had high

catalytic activity, but its activity was reduced and then always kept at low level when the mycelium came into the mature periods. The starch was the rapid carbon source that was used by the *Lentinula edode* in the early growth period, and the amylase activity decreased with the growth of the mycelium, corelating to the mycelium growth rate. The chitosan could be a growth regulator for the *Lentinula edode*, playing a role in improving the Amy activity, and providing the carbon sources and energy for the mycelium growth.

Effect of chitosan on the POD activity of mycelium

The effect on the mycelium POD enzyme activity in the cultivation of different chitosan is shown in Fig. 3. The mycelium POD enzyme activity of the control group changed little. In the first 12 days, the activity of each concentration had changed to some degree. The mycelium POD enzyme activity of other groups was higher than that of the control group until 18 days of cultivation, but at other times the activity was almost lower than that of the control. POD is a lignin enzyme, that could accelerate the degradation and use of the lignin aromatic compounds, lignin, cellulose, and hemicellulose are the main components of the plant skeleton (Sakurai 1998). Also, the enzymatic activity of POD reached its highest peak in the mycelium's rapid growth periods (from 12th to 20th day), and then the mycelium entered the physiology maturity periods (from 24th to 36th day). When the chitosan concentration was 0.1 mg/ml, the POD enzyme activity was significantly higher than the control group, which showed not only the mycelium of the *Lentinula edode* mycelium POD enzyme activity, but also the degradation of the lignin. At the same time, the degradation of the cellulose was increased and the mycelium grew better after being treated with chitosan.

Effect of chitosan on the mycelium peroxidase isozyme

The effect on the mycelium peroxidase isozyme in the cultivation of the different chitosan is shown in Fig. 4. The *Lentinula edodes* peroxidase isozyme has 3 enzyme belts. The strength of the first and second belt was different when compared to the control. The third belt of all the groups was strengthened compared to the control when the chitosan concentration was 0.5 mg/ml and 0.1 mg/ml, the enzyme belt was the widest, colored the fastest and dyed the deepest, and at this concentration, there was the most obvious enhancement effect. When the chitosan concentrations were 0.017 mg/ml and 0.05 mg/ml, the enzyme belt was wider, colored faster, and dyed deeper, and the enhancement effect was better, but the effect of enhancement was not obvious.

Discussion

The market demand for *Lentinus edodes* is increasing day by day, so exploring the important role of the growth regulator in this fungus has provided an important way for the high yield technology cultivation of *Lentinus edodes*. The results showed that chitosan, as a fungal growth regulator, could regulate the growth of *Lentinus edodes* obviously, and it might have a certain influence on the yield, which was verified in our experiment. Compared with the control group, adding a certain concentration of chitosan can significantly change the activities of a variety of biological enzymes and affect the growth rate of mycelia. In the future research process, we will deepen the study of its molecular mechanism.

Declarations

Data Availability

The data used to support the findings of this study are included within the article.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Author Contributions

Dr. Xunyou Yan conceived and wrote this paper; Dr. Sizhu Ren commented and revised the manuscript. All authors contributed to the manuscript preparation and find a great deal of relevant literatures according to the professional experience.

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Figures

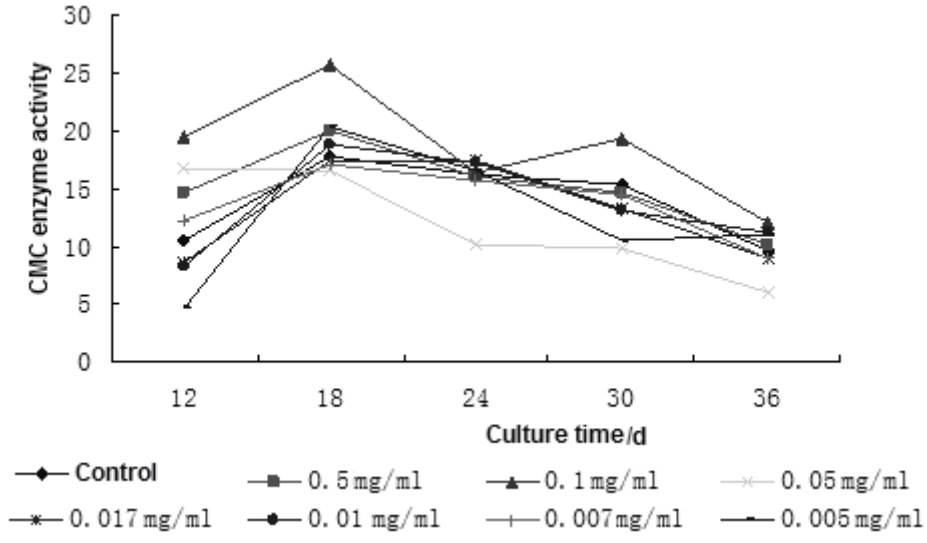

Figure 1

Effect of different concentrations of chitosan on CMCase activities of the *Lentinula edodes* on the substratum

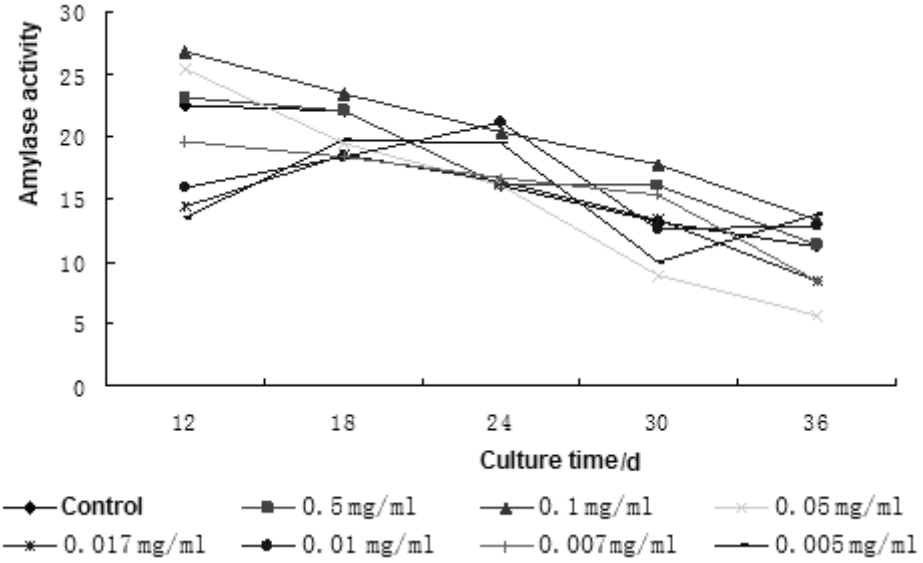

Figure 2

Effect of different concentrations of chitosan on amylase activity of the *Lentinula edodes* on the substratum

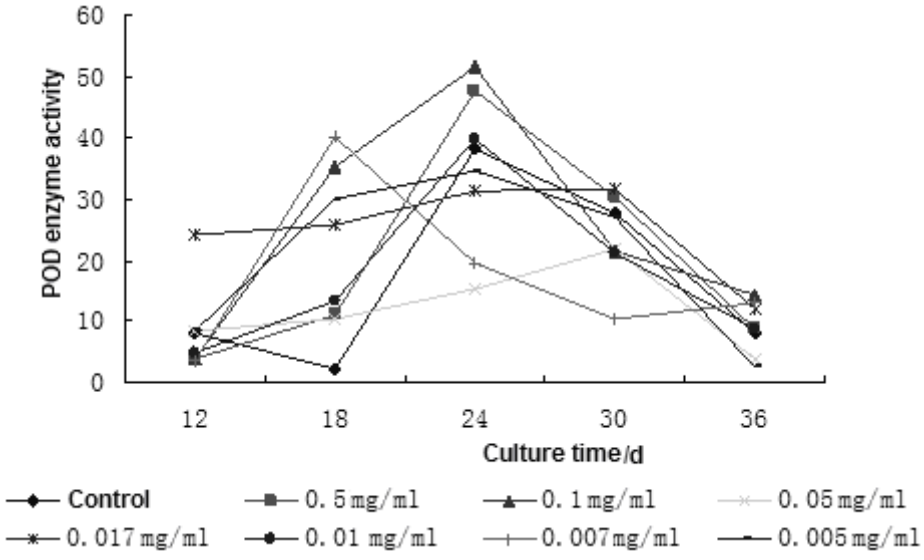

Figure 3

Effect of different concentrations of chitosan on POD of the *Lentinula edodes* on the substratum

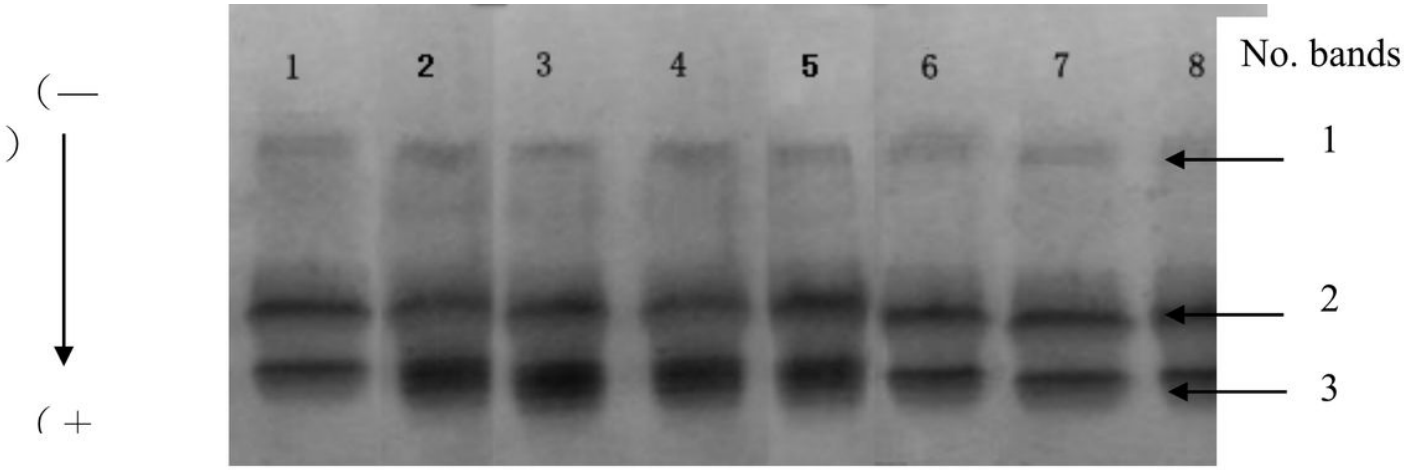

Figure 4

Effect of different concentrations of chitosan on peroxidase isozyme of the *Lentinula edodes* on the substratum

1、control、2、0.5 mg/ml、3、0.1 mg/ml、4、0.05 mg/ml、5、0.017 mg/ml、6、0.01 mg/ml、7、0.007 mg/ml、8、0.005 mg/ml

Supplementary Files

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