

Using A New Mushroom Shake Flask Culture Device to Develop *Suillellus Luridus*

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Abstract—A new shake flask culture device was introduced in this paper, whose main part was comprised with mouth, body and bottom. Besides, it also included cork; pump; small bacteria filter and etc. So, the utility model had the advantages of simple structure, artful design, and it was more convenient to use. Compared with the ordinary shake flask, it solved the problem of using shaking table, occupying too much space, and avoided the low yield of mycelium. Furthermore, the culture success rate was significantly higher than that of the shaking table culture. This device can also prevent the sealing gauze from being easily polluted by medium in the fermentation process, ensuring good ventilation from fermentation flask and avoiding pollution at the same time. *Suillellus luridus* were cultured by the two kinds of fermentation devices respectively, and the highest mycelia biomass from the new shake flask culture device could achieve 2.32 g/L, while that of the normal fermentation device only could achieve 2.11 g/L.

Keywords—shake flask culture device; *Suillellu sluridus*; mycelia biomass

I. INTRODUCTION

Edible fungi are macrofungi fruiting bodies which can available for human consumption. Specifically, they are edible as mushrooms. Edible fungi are not only known as delicious and healthy food with rich content of nutrient, but also possess medicinal value to cure a variety of diseases [1-2].

Laboratorial culture of edible fungi is different from the natural living in culture conditions, because it needs to provide all kinds of superior conditions. At present, the breeding of edible fungi is usually realized by shaking table [3]. But it has shortcomings of using table, occupying too much space and having a low yield of mycelium. Beyond that, after sterilization and inoculation, the mycelium is

trained in a reciprocating shaking table, and the temperature is 37°C [4]. While the bottle is shaking, the gauze can be easily stained by medium, which could lead to the ventilation effect being greatly weakened and the tank being easily contaminated. And the opportunity of the gauze being polluted by medium can be reduced by retarding the rotation speed, but under the oxygen poor condition, the fermentation unit will be greatly reduced. At the same time, due to the different situation of the medium remained in the wall of triangular bottle, the fermentation results of mycelium even from the same batch can be very volatile. In view of the above issues, a new type of edible fungus shake flask culture device was proposed, specially.

The working principle of the shake flask culture device is that the splashed edible fungi trickled down with the inside and outside of the arc block glass after they hit the glass wall, and a small amount medium which entered into the bottleneck and the outer wall of glass tube will defluxion because of the block of annular glass. Thus, the problem of that the medium easily contaminate the sealing gauze is solved. Besides, this device combine an air inlet pipe, a connecting pipe, a pipe and an air pump to achieve ventilation.

As shown in Fig.1, number 1-13 respectively represent bottle main body, bottle mouth, bottle body, bottle bottom, cork, connecting pipe, outlet pipe, small bacteria filter, inlet pipe, pump, annular glass, arc block glass and temperature sensor.

According to the design of this device, we can conclude that the device has the following characteristics. Primarily, this device was provided with a connecting pipe, outlet pipe, inlet pipe and air pump, ensuring plentiful oxygen for fermentation in the bottle, so the phenomenon that mycelial die because of the insufficient oxygen content will not occur. Second, the upper end of the outlet pipe was installed with a small bacterial filter, to largely prevent contamination of other miscellaneous bacteria, in addition to increasing the survival rate of mycelia, but ensuring the single. And, the bottle was arranged in the middle of a circular piece of glass, and at the bottom of a piece of cambered glass, the existence of them can prevent the medium to pollute the stopper, also can stop edible fungi from sticking on the wall when bottle is oscillating. In addition, the presence of the temperature sensor can monitor the temperature in the fermentation bottle in real time, so as to ensure the good growth of the mycelium.

In particular, the connecting pipe of the device was a soft rubber tube, and the air inlet pipe and the air outlet pipe were hard glass tubes, the purpose of this design was to be more conducive to the edible fungus shake flask culture device for ventilation. It was also worth noting that the number of temperature sensor where at the outside of the bottle bottom for at least two, so that the measured temperature can be compared, that was means the temperature can be more accurate. And, the master design of the flask culture device was the arc barrier glass corresponding central angle less than 90°. According to the corresponding physical knowledge, it is clear that this design can prevent edible fungus to hanging on the bottle wall.

Suillellus luridus is a wild edible fungus; they are popular because their flesh is tender and nutrient rich. Besides, recent studies have revealed that *S. luridus* is not only low in fat but rich in polysaccharides, minerals and proteins, making it is an ideal food for preventing diabetes and cardiovascular disease [5].

In this paper, using *Suillellus luridus* as the research object, under the same conditions, through two different fermentation equipment for training, and the final yield was compared.

II. MATERIALS AND METHODS

A. Chemical and Material

All reagents and drugs used are above the analysis purity level and all the water used is the distilled water. *Suillellus luridus* was purchased from the local supermarkets (Pingwu, Mianyang, Sichuan, China).

B. Liquid Culture

S. luridus was primitively cultivated on the synthetic potato dextrose agar (PDA) medium (2% glucose, 1% peptone, 0.2% KH₂PO₄, 0.1% MgSO₄·7H₂O) at 25°C for 4 d. Thus, the mycelium of *S. luridus* was obtained. Subsequently, to further development, the resulting liquid culture (2.5 mL) was transformed to a 250 mL flask fermentation culture medium and incubated for 5, 6, 7 d at 25°C on a shaking table at a speed of 120 rpm [5-8]. We set up three groups parallel to avoid the occurrence of contingency and named them group 1, group 2, group 3. Repeating the above operation, the mycelium was inoculated into the shake flask culture device and then cultured under the same conditions and called it group 0.

C. Determination of The Biomass in Fermentation Liquid

The mycelia biomass was considered as the index to the growth of edible fungi, so the mycelia were obtained by filtration of the culture broths. The fermentation liquid in the culture flasks and shake flask culture device were centrifuged at 5000rpm for 20 min to separate the biomass from the liquid medium. The precipitated biomass was washed three times with distilled water and freeze-dried to give the biomass dry mass. Finally, the biomass dry mass of mycelium was measured [9-10].

III. RESULTS AND DISCUSSION

The mycelia biomass of shaking table culture and shake flask culture device are shown in Table I. When the fermentation time was 7 days, the biomass of mycelium in shake flask culture device was 2.32 g/L, was the highest in all experimental group. And the biomass from the ordinary fermentation device only can reach 2.11 g/L, 0.21 g/L fewer than in the shake flask culture device. The following was 2.25 g/L, which train condition was the culture time was 6 days, the fermentation device was the shake flask culture device. And, the biomass from shake flask culture device was still higher than culture flasks when fermentation time was 5 days. On the whole, when the culture time is same, the mycelia biomass from shake flask cultivation device is

higher than the normal fermentation device, which fully demonstrates that using the shake flask cultivation device can improve the yield of mycelium, and this conclusion provides a new way of fermentation to commercialization for edible fungus culture.

Table I also shows that the biomass of the culture time was 5 days, and the fermentation device was the shake flask culture device was 2.03 g/L, while the biomass of the culture time was 6 days; the fermentation device was 2.05 g/L. And the biomass of both were roughly same, but the shake flask culture device used fewer time relatively. The reasons for this result may be there was sufficient oxygen in the shake flask device and the culture temperature is constant, so the environment had been in a good state. Or, there may be no other bacteria exist, all the nutrients were only used by *S. luridus*.

Compared with the solid cultivation, liquid fermentation has more advantages. For instance, the training time is effectively shorter, and the production of liquid fermentation is greatly higher than that of solid culture [11]. In addition, according to the results of our research, we can conclude that using the new shake flask culture device can be better than solid cultivation and shaking table to commercially culture edible fungi. At the same time, some studies have shown that the fat content of mycelial polysaccharides is low, but the content of protein is high, they also contain a variety of trace elements. Besides, it is also proved that mycelial polysaccharides have antioxidant activity and hypoglycemic activity, and their nutritional value is very high. [12-14]. But the mycelial polysaccharides are difficult to gain. So, extracting polysaccharides will consume a large amount of mycelium. Thus, there's no doubt that the new shake flask culture device will play a great role, because it can increase mycelial biomass and decrease the fermentation time.

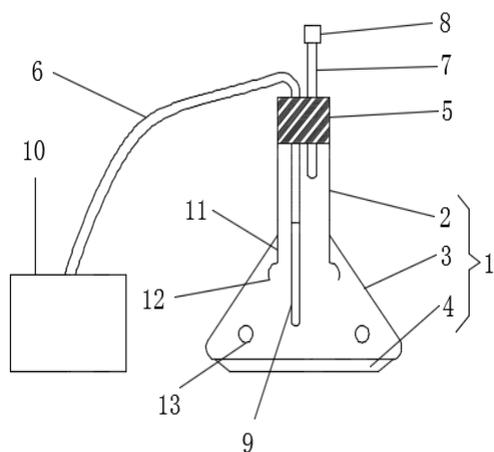


Figure 1. The new shake flask culture device

TABLE I. THE MYCELIA BIOMASS OF SHAKE FLASK CULTURE DEVICE AND SHAKING TABLE CULTURE

Culture	Dry mycelia weight (g/L)				AVG ^a
	Group 0	Group 1	Group 2	Group 3	
Day 5	2.03	1.84	1.79	1.86	1.83
Day 6	2.25	2.06	2.01	2.09	2.05
Day 7	2.32	2.13	2.11	2.08	2.11

Group 0: the group using shake flask culture device; group 1-3: the groups using shaking table; a. the average mycelia biomass of the groups using shaking table.

IV. CONCLUSIONS

The consequence of this study demonstrates that the shake flask culture device can increase the mycelial biomass of *Suillellus luridus* and reduce the fermentation time, which can be suggested to as new mycelia fermentation device whatever used in laboratory or commerce. Therefore, the advantages of this device can be shown more, which includes the design is clever, the structure is simple, and the another feather is that it is convenient to use. Furthermore, the shake flask culture device is equipped with connecting pipe, outlet pipe, inlet pipe and pump, to ensure the oxygen concentration; Small bacteria filter and temperature sensor is also advantageous to the mycelium fermentation, in other words, they can shorten fermentation time and increase the yield of mycelium.

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