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Growth Conditions of *Termitomyces albuminosus*

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Abstract. In order to solve the problem of difficult isolation of *Termitomyces albuminosus*, a strain of wild *T. albuminosus* collected from Jiajiang County, Leshan City, Sichuan Provence,China, was isolated and purified by tissue separation method. The mycelium growth rate was used as an indicator to explore the optimal growth carbon source, nitrogen source, KH_2PO_4 concentration, $MgSO_4$ concentration, temperature and pH. The results showed that the optimum carbon source for vegetative mycelial growth was corn flour; the optimum nitrogen source was ammonium nitrate. The concentration of KH_2PO_4 with faster growth of *T. albuminosus* was between 0.10% to 0.20%, the optimum concentration was 0.15%. The concentration of MgSO4 with faster growth of *T. albuminosus* was between 0.025% to 0.075%, the optimum concentration was 0.050%. The growth temperature ranges from 22 to 30°C, the optimum temperature was 26 °C. The pH range was 4.5 to 8.0, and the optimum pH was 5.0.

INTRODUCTION

Termitomyces albuminosus (Berk. Heim) belongs to the genus *Termitemyces*, Lyophyllanceae, Agaricales, Agaricomycetidae, Basidiomycetes [1], also known as Umbrella Mushroom, Three Mushrooms, Chicken Mushroom, Termite Mushroom, Triadella, etc. [2], is a very rare large-scale fungus for food and medicine. Termitemyces are mainly distributed in southern Africa, South Asia and subtropical regions. There are 24 species of Termitemyces genus known in China, mainly distributed in Yunnan, Sichuan, Guizhou and other places, including 20 species in Yunnan, 9 species in Sichuan, 8 species in Guizhou, and 4 species in Guangdong, and only 1 to 2 species in other provinces [3-4]. *Termitomyces albuminosus* not only has the characteristics of fleshy fat, fine silky white, crisp and refreshing, fresh and delicious, and rich in nutrients [5], but also has clearing the spirit, enhancing human immunity, curing phlegm, preventing intestinal cancer, nourishing blood, moistening, and Spleen and stomach and other effects [6-7]. Studies have shown that domestication and cultivation of *T. albuminosus* had been achieved, but so far there has been no artificial cultivation and reported production. It can be seen that artificial cultivation of *T. albuminosus* is still difficult [8]. Many scholars have studied the relationship between *T. albuminosus* and termites [9], the classification and identification of *T. albuminosus*, the isolation and purification of *T. albuminosus* [10-12], the deep submerged fermentation of *Termitomyces albuminosus* and the enrichment of *Termitomyces albuminosus*, etc. [13-14].

In this study, the mycelium was isolated from a wild *T. albuminosus* from Jiajiang County, Leshan City, Sichuan Provence, China, by tissue separation method. The growth conditions were studied to provide a reference for the artificial cultivation of *T. albuminosus* in the Leshan area.

MATERIALS AND METHODS

Strains Used in Experiment

The test strain was isolated from a fruiting body with a morphology similart to that of *T. albuminosus* in Jiajiang County, Leshan City, on May 15, 2018.

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Culture Medium

The basal medium was a PDA comprehensive medium, and its formulation was 200 g potato, 20 g glucose, 1 g KH2PO4, 0.5 g MgSO4, 0.2 g NaCl, 0.2 g MnSO4, 0.15 g vitamin B6, 2.1 g peptone in percent 1000 mL water, pH natural. Bevel culture medium of *T. albuminosus* was additionally increases 0.075 g Vitamin B1.

Separation and Purification of *Termitomyces albuminosus*

Remove the base impurities, wash the surface with water, then disinfect the surface with 75% ethanol solution, and then use the sterilized knife to cut the fruit body from it. Take a soybean-sized tissue block at the intersection of the cap and the stipe. Transfer to the center of the bevel of the PDA medium in the test tube. Place the test tube into the tissue block and incubate in the dark at 23 °C until the white hyphae grow. Pick a little mycelium and purify it on the PDA medium. After the mycelium grows over the slope, transfer it to 4 °C refrigerator preservation reserve.

Optimum Carbon and Nitrogen Sources for Mycelial Growth

The purified hyphae of *T. albuminosus* were inoculated on the basal medium with glucose, sucrose, maltose, soluble starch, corn flour, lactose and ethanol as carbon sources, and the basal medium with bovine powder, peptone, soy flour, acid hydrolyzed casein, urea, ammonium nitrate and yeast extract as nitrogen sources. Group was set in 3 parallel. All treatments were cultivated at 25 °C and natural pH conditions. The growth rate of the hyphae were recorded regularly every day.

Optimum Concentration of Inorganic Salt for Mycelial Growth

The purified hyphae of *T. albuminosus* were inoculated into KH_2PO_4 basal medium at concentrations of 0.05%, 0.10%, 0.15%, 0.20%, 0.25%, and 0.30%, respectively. And $MgSO_4$ basal medium with concentrations of 0.025%, 0.050%, 0.075%, 0.100%, 0.125%, and 0.150%, respectively. Group was set in 3 parallel. All treatments were cultivated at 25 °C and natural pH conditions. The growth rate of the hyphae were recorded regularly every day.

Cultivate Environmental Conditions for Mycelial Growth

The purified hyphae of *T. albuminosus* were inoculated onto the plate medium at 22, 23, 24, 25, 26, 27, 28 °C, pH natural conditions and 23 °C, pH 4.5,. 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 gradient medium plates. All treatments were cultivated. The growth rate of the hyphae were recorded regularly every day.

Representation Method of Mycelial Growth Rate

The growth rate of the hyphae ($mm \cdot d^{-1}$) was ratio of net growth of mycelium to growing days.

RESULTS

Screening of Solid Medium Carbon Source

T. albuminosus can use different types of carbon sources, and different types of carbon sources had different effects on mycelial growth (Table 1). When corn flour was used as the carbon source, the hyphae grow to a maximum of 1.41 $mm \cdot d^{-1}$, followed by glucose, sucrose and maltose. Because corn flour contains rich growth factors and other nutrients in addition to the basic carbon source, the mycelial growth value was the highest when corn flour was used as the carbon source. Therefore, corn flour was used as the solid medium carbon source of chicken bacillus.

TABLE 1. Effect of different carbon souce on the mycedium growth rate

Carbon Source	Mycelium growth rate(mm•d^{-1})				
	1	2	3		average value
Glucose	1.04	1.15	0.99	1.06	bB
Sucrose	1.09	1.16	1.14	1.13	bB
Maltose	0.95	0.99	1.15	1.03	bBC
Llactose	0.48	0.61	0.5	0.53	dE
Ethanol	0.79	0.82	0.81	0.81	cD
Corn flour	1.43	1.42	1.39	1.41	aA
Soluble starch	0.92	0.89	0.88	0.90	cCD

Screening of Nitrogen Source in Solid Medium

T. albuminosus can used different types of nitrogen sources, and different types of nitrogen sources had a greater impact on mycelial growth (Table 2). The three nitrogen sources with faster mycelial growth rate were yeast extract paste, acid hydrolysis casein and ammonium nitrate. When the yeast extract paste was nitrogen source, the mycelial growth rate was 1.49 mm•d^{-1}. When ammonium nitrate and When ammonium nitrate was used as the nitrogen source, the mycelial growth rate was 1.22 mm•d^{-1}. Due to the high price of acid hydrolyzed casein and yeast leaching cream, considering the feasibility and economic conditions of the test, ammonium nitrate was selected as the nitrogen source.

TABLE 2. Effect of different nitrogen souce on the mycedium growth

Nitrogen Source	Mycelium growth rate(mm•d^{-1})				
	1	2	3	average value	
Urea	0.82	0.78	0.84	0.81	dD
Ammonium nitrate	1.22	1.25	1.2	1.22	bB
Acid hydrolyzed casein	1.23	1.22	1.21	1.22	bB
Soy flour	0.82	0.78	0.81	0.80	dD
Beef extract	1.05	1.04	0.98	1.02	cC
Yeast extract	1.45	1.52	1.51	1.49	aA
Peptone	1.02	1.03	1.06	1.04	cC

Screening of the Concentration of KH_2PO_4 and $MgSO_4$ in Solid Medium

Mycelium of *T. albuminosus* can grow on different concentrations of KH_2PO_4, and different concentrations of KH_2PO_4 have a greater effect on mycelial growth (Figure 1). The concentration of KH_2PO_4 added to the culture base is good for growth from 0.10% to 0.20%, but it is very slow to grow below 0.10% and above 0.20%. When the concentration of KH_2PO_4 was 0.05%, the growth rate of mycelium reached 0.84 mm•d-1; the concentration of KH_2PO_4 was 0.25%, and the growth rate of mycelium reached 0.82 mm•d-1. When the concentration of KH_2PO_4 was between 0.05% and 0.15%, the growth rate of hyphae increased with the increase of KH_2PO_4 concentration. When the concentration of KH_2PO_4 was 0.15%, the growth rate was the fastest, and the growth rate of mycelium reached 1.10 mm•d-1. The growth rate of mycelium was also better. When the concentration rose to 0.20%, the growth rate of mycelium decreased. Therefore, the optimum growth concentration of the mycelium of the chicken gizzard is 0.10% to 0.20%.

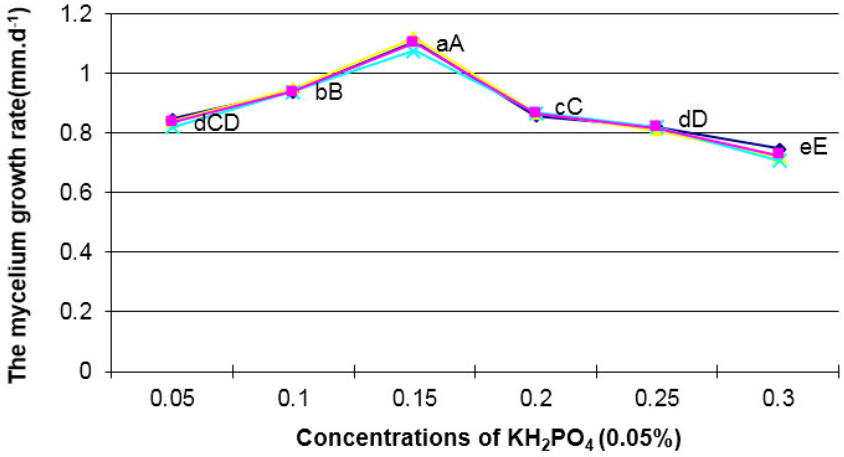

FIGURE 1. Effect of different Concentrations of KH_2PO_4 on the mycelium growth rate

When the concentration of $MgSO_4$ was 0.100%, the growth rate of mycelium reached 0.80 mm•d^{-1}; when the concentration of $MgSO_4$ was 0.125%, the growth rate of mycelium reached 0.77 mm•d^{-1}. When the concentration of $MgSO_4$ was between 0.025% and 0.050%, the growth rate of hyphae increased gradually with the increase of $MgSO_4$ concentration. The growth rate of $MgSO_4$ was the fastest at 0.050%, and the mycelial growth rate reached 0.95 mm•d^{-1}, the mycelium has the best growth, the hyphae are thick, white and dense; when the concentration was increased to 0.075%, the mycelial growth rate was reduced. Therefore, the optimum growth concentration of the chicken sputum was 0.025% to 0.075%.

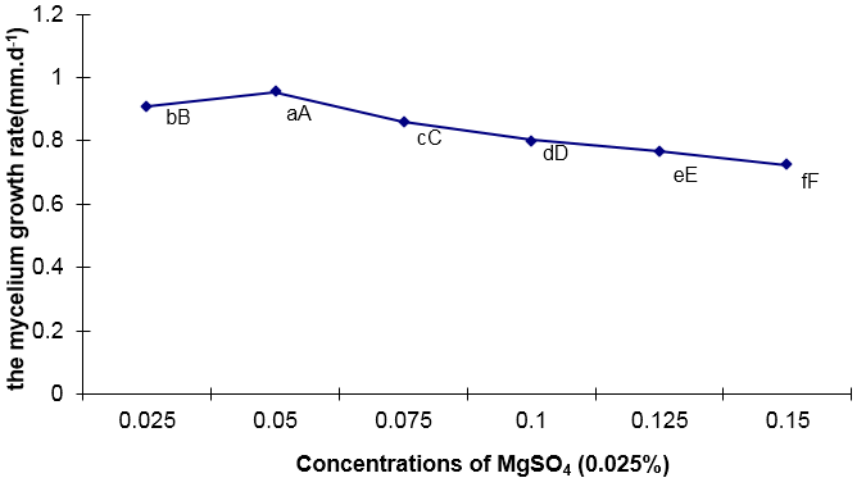

FIGURE 2. Effect of different Concentrations of $MgSO_4$ on the mycelium growth rate

Screening of Culture Temperature

The effect of different temperature on mycelial growth rate was significant differences. The mycelial growth rate basically stabilized with the increase of temperature. When the temperature was 26 °C, the mycelial growth reached a maximum of 1.21 mm•d^{-1}, followed by 27 °C and 28 °C. Therefore, the optimum temperature for the fungus of the fungus is 26 to 28 °C.

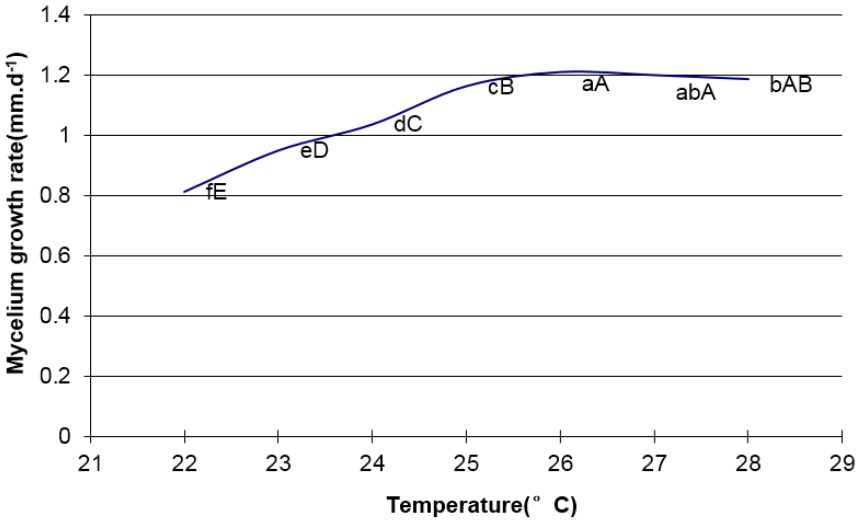

FIGURE 3. Effect of different temperature (°C) on the mycelium growth rate

Screening of pH in Solid Medium

The mycelium of *T. albuminosus* can grew on different pH in solid medium. The effect of different pH on mycelial growth was significant differences. The effect degree is: pH5.0>pH 5.5>pH6.0>pH4.5>pH6.5>pH7.0>pH7.5>pH8.0, in which the mycelial growth rate reached the maximum (1.20 mm•d^{-1}) and the growth was best on the medium with pH 5.0. The mycelial growth rate was the slowest (0.81 mm•d^{-1}) on a medium with a pH of 8.0. Therefore, the optimum pH of the mycelium of chicken bacterium was 5.0-6.0.

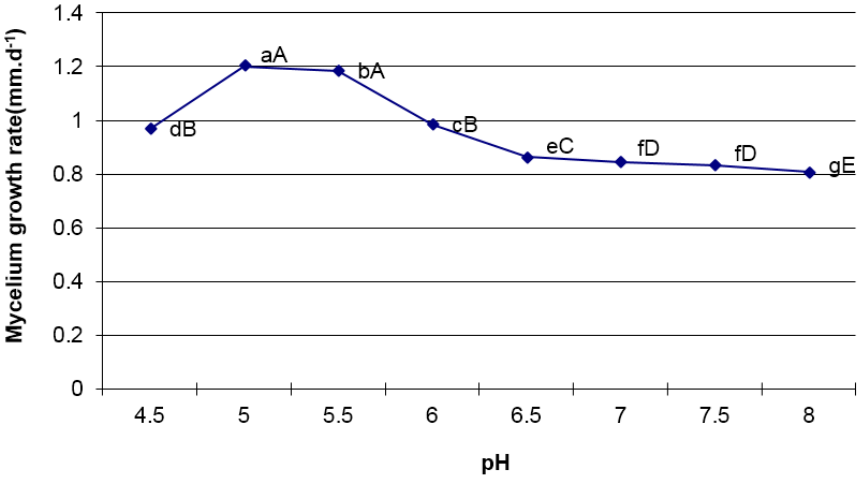

FIGURE 4. Effect of different pH on the mycelium growth rate

DISCUSSIONS AND CONCLUSION

The mycelium of *T. albuminosus* was cultured by different temperature, pH, carbon source, nitrogen source, KH_2PO_4 concentration and $MgSO_4$ concentration, and the mycelial growth of the strain was measured. It was found that the growth rate of the mycelium of the mycelium of *T. albuminosus* was significantly different on each medium. It indicated that temperature, pH and carbon source, nitrogen source, KH_2PO_4 concentration and $MgSO_4$ concentration

had certain effects on the growth of hyphae. The most suitable carbon source for chicken hyphae was corn flour, and its mycelial growth rate was 1.41 mm•d^{-1}. The most suitable nitrogen source was ammonium nitrate, and its mycelial growth rate was 1.49 mm•d^{-1}. Suitable KH_2PO_4 concentration was between 10% to 0.20%, in which mycelial growth rate was 0.87 ~ 1.10 mm•d^{-1}. MgSO4 optimal concentration was between 0.025% to 0.075%, the mycelial growth rate was 0.86 to 0.95 mm•d^{-1}. The optimum growth temperature was between 26-28 °C, and the mycelial growth rate was 1.19 ~ 1.21 mm•d^{-1}. The optimum growth pH was between 5.0 ~ 6.0, and the mycelial growth rate was 0.98 ~ 1.20 mm•d^{-1}. In addition, the test also exhibit different culture groups different growth stages Termitomyces hyphal impact speed is not a sample, which shows the entire process is not a constant speed mycelial growth, and may be a variable growth curve.

ACKNOWLEDGMENTS

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REFERENCES

1. X.L. Cai, L.F. Yu and W. He. Journal of Dali University **9**, 61 (2010).
2. Y.L. Li, B.W. Den, M.M. Huang, X.C. Xie and H. Peng, Heilongjiang Agricultural Sciences **10**, 87 (2017).
3. Q.X. Hu, Acta Edulis Fungi **8**, 54 (2001).
4. X.H. Yao,Y.X. Xu and S.C. Xu, Acta Edulis Fungi **2001**, 8, 59 (2001).
5. S.Q. Hu, T.G. Liu and X.B. Li, Journal of Food Science and Biotechnology **27**, 67 (2008).
6. S.L. Wang, K.Y. Wang, Y.L. Peng. Chinese Veterinary Science, **41**, 1276 (2011).
7. N. Baruah and N. Baruwati, International Journal of Plant Sciences, **11**, 144 (2016).
8. Y.J. Zhang and H.C. Guo, Acta Microbiologica Sinica **50**, 1288 (2010).
9. D. Wilson, Microbiology **73**, 274 (1995).
10. F. Fang, B. Xu, J.J. Li and Z.X. Huang, Acta Microbiologica Sinica, **52**, 466 (2012).
11. Z. Mu, Acta Botanica Yunnanica **3**, 367 (1981).
12. L.K. Zou and X. Pan, Northern Horticulture **6**, 217 (2009).
13. P. Mitra, N.C. Mandal, K. Acharya and J. Verbr, Lebensm **11**, 25 (2016).
14. R. Splivallo, S. Ottonello and A. Mello, New Phytologist **189**, 688 (2011).