CHAPTER 17

PHYSIOLOGY AND BIOCHEMISTRY OF LIGNOCELLULOSE UTILIZATION BY *PHOLIOTA NAMEKO*

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1. INTRODUCTION

Pholiota nemako, an edible fungus, is delicious and nutritious. It has been cultivated for 70 years in Japan and also in three provinces in Northeast China. This mushroom was cultivated with sawdust as substrate. A systemic study of the physiology and biochemistry of lignocellulose utilization by this fungus has so far been lacking. In this paper, the degradation pattern for lignin, hemicellulose and cellulose by *Pholiota nameko* have been studied.

2. MATERIALS AND METHODS

2.1. Cultivation

Pholiota nameko strain no. 2, which originated from Japan, was cultivated using the following substrate in a 500ml bottle: sawdust, 70gm; wheat bran, 17gm; $CaCO_3$, 1gm; KH_2PO_4, 0.5gm; $MgSO_4.7H_2O$, 0.5gm; dextrose, 0.5gm, water, 160ml. The substrate was autoclaved at 1kg/cm^2 for 1.5hr. After inoculation, cultures were incubated at 23-25°C, 16-20°C, 20-28°C, 14-20°C, 14-17°C and 11-14°C for 0-20, 20-72, 72-150, 150-175, 175-190 and 190-230 days, respectively.

2.2. Analysis of Components

The reduction in the amount of dry matter in the substrate, and the lignin, hemicellulose and cellulose contents were analysed according to Wang and Xu (1986).

2.3. Analysis of Enzyme Activities

Analyses of CMCase, FPase and hemicellulase were carried out according to Wang and Xu (1986). 1 unit of CMCase is equivalent to 1mg dextrose/30min/1gm dry culture; 1 unit of FPase =

1mg dextrose/60min/1gm dry culture; and 1 unit of hemicellulase = 1 mg xylose/30min/1gm dry culture.

3. RESULTS AND DISCUSSION

3.1. Quantitative Changes in Substrate Components

3.1.1. Reduction of substrate and consumption of respiration. When *Pholiota nameko* was incubated for 230 days, the dry matter in the substrate was reduced by 73.36% (Table 1). Within this figure, 60.93% was consumed by respiration of mycelium and another 12.43% was converted into fruitbody biomass (absolute biological efficiency). The production index (dry weight of fruitbody/ reduction of substrate x 100) is 16.94%. That is to say, the effective bioconversion of the substrate by *Pholiota nameko* is very low. The reason is that the fungus has a long period of vegetative growth and the nutrients in the substrate are consumed by mycelial respiration. The analysis above which is based on dry weight is less variable than when the fresh weight of fruitbody is used as is done conventionally.

It is shown also in Table 1 that the rate of lignin and hemicellulose degradation is more rapid than for cellulose during the mycelium growing stage (0-199 days), but during the fruitbody developing stage (199-230 days) the rate of cellulose degradation is far more rapid than that of lignin and hemicellulose. This increase in the amount of cellulose degraded during the fruitbody development stage was attributed to: (1). During the fruitbody development stage, the amount of cellulose in the substrate is 3-4 times greater than the hemicellulose or lignin. (2). Cellulase activity increases markedly at the fruitbody stage. (3). When the primordia appeared (199 days later), the lignin and hemicellulose that inhibit cellulase had decreased by about 80% in the substrate (Table 1). This is beneficial for the degradation of cellulose (Rajarathnam, 1979; Dekker, 1983). (4). The activity peaks of cellulase are responsible for fruitbody development. In this period, the absorption rate of nutrients from the substrate by mycelium, and the bioconversion rate, were

TABLE 1. Reduction of several main components of the substrate during the cultivation period.

stage of growth (days)*	substrate reduction (%)***	consumption-via respiration (%)***	cellulose		hemicellulose		lignin	
			content (%)	reduction (%)	content (%)	reduction (%)	content (%)	reduction (%)
0			32.26		25.62		20.09	
18	8.41	8.41	33.00	6.32	22.18	18.64	15.65	28.49
125	42.46	42.46	37.77	32.64	10.81	75.72	8.82	74.75
199	54.39	54.39	30.33	57.13	11.25	80.00	9.22	81.35
230	73.76	60.93	16.94	86.01	6.75	92.98	9.89	86.88

* 0-18 days, rapid growth stage; 18-199 d, mycelium ripening stage; 199-230 d, fruitbody development stage.
** reduction of dry substrate (%)=(A-B)/A x100
*** consumption via respiration(%) =(A-B-C)/A x100
A: dry weight of substrate at the beginning;
B: dry weight of substrate at the different stage of growth;
C: dry weight of fruitbody.

promoted. Feedback inhibition of enzyme production was lifted so that the exocellulases of the mycelium exhibited maximum hydrolytic activity. This is one of the important reasons why the cellulose degradation rate increased during fruitbody developing. (5). The pH of the substrate, 3.8-4.1, is similar to the optimum pH (4.0-4.1) of cellulase.

3.1.2. Degradation of lignocellulosics. When the cultivation ended, the cellulose, hemicellulose and lignin in the substrate were reduced by 86.01%, 92.98% and 86.88%, respectively (Table 1). This showed that *Pholiota nameko* is a wood-rotting fungus that has a very strong ability to degrade sawdust.

Through analysis of the rates listed in Table 2, the nutritional characteristics of *P. nameko* at different growth stages are as follows: (1). In the stage of rapid mycelial growth (0-18 days), the amount of lignocellulose degraded was more than the reduction of substrate. At the same time, the amount of reducing sugar in the substrate also decreased rapidly and about 32% of degraded lignocellulose was converted into mycelial biomass. (2). In the stage of mycelial ripening, the amount of lignocellulose degraded was slightly lower than the reduction in the weight of the substrate. In this stage (18-199 days), the total amount of non-lignocellulosics in the substrate did not increase. Non-lignocellulosics were consumed to a small extent, equivalent to 6.96% of the reduced weight of substrate. (3). The nutrients consumed by fruitbody (used for respiration and constructing the fruitbody) originate from the degraded cellulose (49.13%), hemicellulose (17.54%), lignin (5.85%), and mycelium biomass or bioconverted product (27.48%), respectively. That is to say, cellulose is the main carbon source for fruitbody development, and the amount of cellulose degraded at this stage markedly affected the yield of the fruitbody. It is of great physiological significance that the lignin and hemicellulose are degraded largely during the stage of vegetative growth. This is beneficial to the degradation of cellulose in the substrate at the stage of fruitbody development. (4). In the stage of fruitbody development, the amount of hemicellulose degraded is not enough. This phenomenon does not mean that the hemicellulase activity was weak. In fact, degradation due to hemicellulase was reduced markedly before the stage of fruitbody development. It is necessary to study the regulation of the physiology of mycelial ripening (it could be delayed by high temperatures or promoted by low temperatures). This regulation affects the fruitbody development and the yield of fruitbody.

3.2. Cellulase Activity of *Pholiota nameko*

As shown in Figure 1, peaks of CMCase, FPase and hemicellulase activity appeared during the

TABLE 2. Relationship between the amount of degraded lignocellulose and the reduction of substrate at different stages of growth.

stages of growth	LR(g)	HR(g)	CR(g)	LHCR(g)	SR(g)	LR/SR x100	HR/SR x100	CR/SR x100	LHCR/SR x100
0-18	4.49	3.71	1.60	9.80	6.60	68.03	56.21	24.24	148.48
18-199	8.33	12.37	12.86	33.56	36.07	23.09	34.29	35.65	93.04
199-230	0.87	2.61	7.31	10.79	14.88	5.85	17.54	49.13	72.51

"LR"-reduction in lignin; "HR"-reduction in hemicellulose; "CR"-reduction in cellulose.
"LHCR"-reduction in lignocellulosics=LR+HR+CR; "SR"-reduction in substrate.

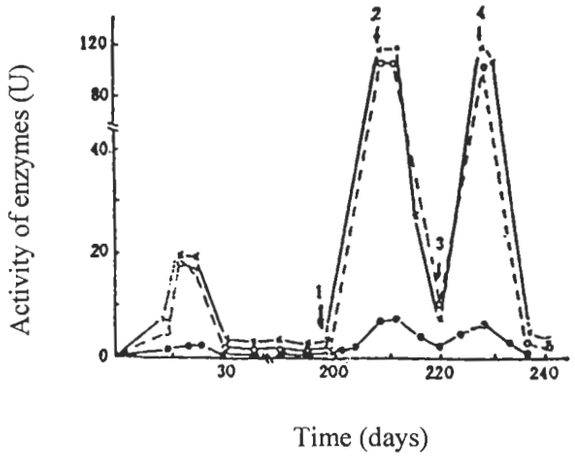

FIGURE 1. Activity of lignocellulases of *Pholiota nameko* in substrate. o—o CMCase cellulase; ●—● FP cellulase; x—— x hemicellulase; 1, 2, 3,4 show the primordia stage and the ripening stage of the first and second flushes.

stage of mycelium growth and the stage of fruitbody development. However, the highest peaks corresponded to the later stage. This is very beneficial to fruitbody development. The increase in enzyme activity was related to fruitbody development because the enzyme activity increased rapidly during the first and second flush stages and decreased rapidly between the two flushes (212-220 days). Additional experimental results are shown in Figs. 2, 3, 4 and 5. When cultivated at 25°C, or if the primordia were cut off to stop the fruitbody development, the peak of cellulase disappeared (only 10-18 units). This fact indicates that the low temperature is an essential condition for induction of fruitbody development.

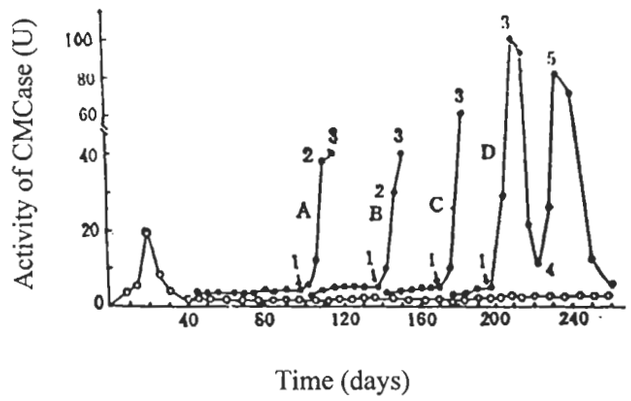

FIGURE 2. Effects of incubation temperature on *Pholiota nameko* fruitbody formation and activity of CMCase in the substrate. o—o incubated at 23-25°C for 0-260 days; ● — ● incubated at 9-11°C; A, B, C, D incubated at 9-11°C after 40, 102, 142 and 178 days, respectively; 1, 2, 3 stages at primordia formation, pileus opening and spore dissemination of first flush; 4, 5 stages at primordia formation and spore dissemination of second flush, respectively.

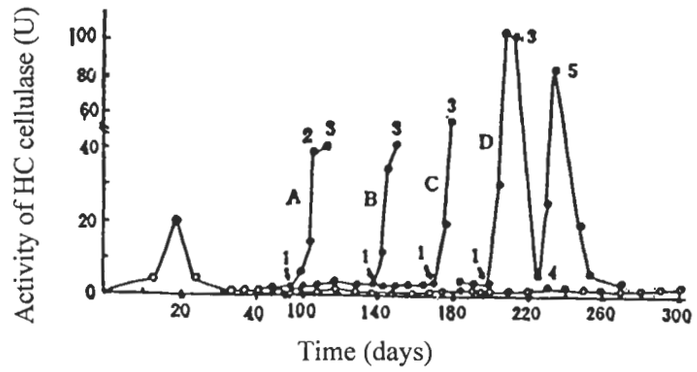

FIGURE 3. Effects of incubation temperature on *Pholiota nameko* fruitbody formation and activity of hemicellulase in the substrate (Legend as for FIGURE 2).

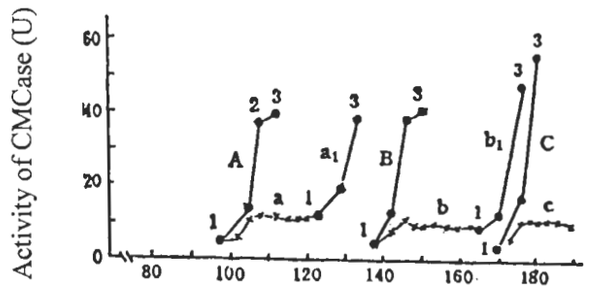

FIGURE 4. Cutting off of primordia to inhibit the activity of CMCase. o —— o enzyme activity when fruiting; A, B, C treatment at 9-10°C after different incubation times; x—— x enzyme activity after cutting off primordia; a, b, c treatment at 9-10°C after different incubation times; 1, 2, 3 different stages of fruitbody development; a1, b1 when fruiting again, the enzyme peak appears in substrate.

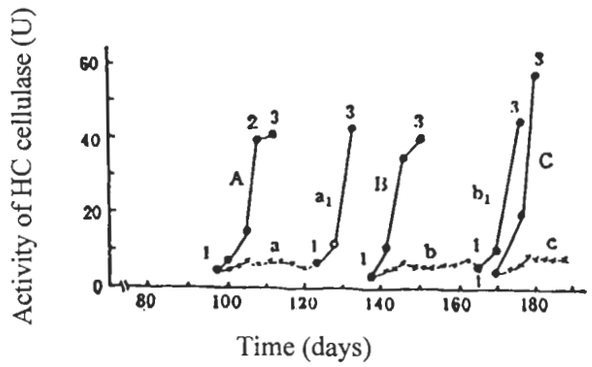

FIGURE 5. Cutting off of primordia to inhibit the activity of hemicellulase (Legend as for FIGURE 4).

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