韓 國 菌 學 會 誌 第 14 卷 第 1 號
Kor. J. Mycol. Vol. 14, No. 1, 37~41, 1986

Studies on Cellulases Produced by *Pleurotus* spp. on Synthetic Medium(II)

Effects of Vitamins, Inorganic Salts and Cultural Conditions

Jai Sik Hong, Jong Bae Lee, Moo Seok Koh*,
Jeong Sook Kim**, Keug Ro Lee and Gi Tae Jung

Chonbuk National University, Chonju 520, Chonnam National University Gwangju 500, and Seokang Junior College**, Gwangju 500, Korea*

合成 培地에서 느타리屬이 生産하는 纖維素 分解酵素에 관한 硏究(제2보)

비타민類, 無機 鹽類와 培養 條件의 影響

洪載植・李種培・高武錫*・金正淑**・李克魯・鄭基泰

全北大學校・全南大學校*・光州瑞江專門大學**

Abstract: The production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017 was stimulated by folic acid and thiamine-HCl. Among the inorganic salts, optimum concentrations of KH_2PO_4 and $MgSO_4 \cdot 7H_2O$ were 0.2% (w/v) and 0.04% (w/v), respectively, but other inorganic salts were not effective for the production of the enzymes. The optimum culture temperature and pH for the production were 25°C and 5.5 for avicelase, and 30°C and 5.0 for CMCase, and 30°C and 6.5 for β-glucosidase, respectively.

Keywords: *Pleurotus sajor-caju* JAFM 1017, Avicelase, CMCase, β-Glucosidase.

In the previous paper, we reported that a *Pleurotus* spp. of potent cellulolytic enzyme productivities and rapid mycelial growth among various strains in the synthetic medium was selected, and the effects of carbon sources and nitrogen sources for the production of enzymes were investigated.

The present study was investigated the effects of inorganic salts, vitamins and cultural conditions for the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017 in synthetic medium.

Materials and Methods

The laboratory strain utilized in the present study was *Pleurotus sajor-caju* JAFM 1017. This strain was obtained from Laboratory of Mycology & Mushroom, Department of Food Science & Technology, Chonbuk National University (Chunju, Korea).

For a stock culture medium of *Pleurotus sajor-caju*, the following composition was used; malt extract 20 g, glucose 20 g, peptone 2 g, agar 15 g and distilled water 1000 ml (pH 5.5). For a preseed culture medium, the following composition

was used; glucose 10 g, peptone 2 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, thiamine-HCl 500 μg and distilled water 1000 ml (pH 5.5).

For the production of enzymes, the basal medium which was consisted of cellulose powder 1 g, urea 0.054 g, KH_2PO_4 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.02 g, thiamine-HCl 50 μg and distilled water 100 ml (pH 5.5 after autoclaving) was used. This basal medium was dispensed into 250 ml conical flasks in 50 ml amounts per flask, care being taken to shake the medium well before dispensing into each flask, and these flasks were then autoclaved for 15 min under 1.2 kg/cm^2.

To obtain the inoculum, the fungus was grown for 7 days at 25°C on the pre-seed culture medium. Cultured mycelial mats were grinded by a waring blender (15,000 rpm). Each flask was inoculated with 3 ml of mycelial suspension obtained and incubated for 10 days at 25°C in a reciprocal shaking incubator (100 rpm). After cultivation, the culture liquid was centrifuged (20 min, 6000 rpm) and the activities of enzymes in the supernatant were assayed.

To assay avicelase activity (Wakabayashi and Nisizawa, 1964), a reaction mixture consisting of 4 ml of 0.5% avicel in 0.1 M citrate-0.2 M sodium phosphate buffer (pH 4.0) and 1 ml of enzyme solution was incubated at 40°C for 120 min. To assay CMCase activity (Kim and Kim, 1982), a reaction mixture consisting of 4 ml of 0.25% Na-CMC (sodium carboxy methyl cellulose) in 0.1 M citrate-0.2 M sodium phosphate buffer (pH 4.0) and 1 ml of enzyme solution was incubated at 40°C for 60 min. To assay β-glucosidase activity (Kim and Kim, 1982), a reaction mixture consisting of 4 ml of 0.1% salicin in 0.1 M citrate-0.2 M sodium phosphate buffer (pH 4.0) and 1 ml of enzyme solution was incubated at 40°C for 60 min. The amount of reducing sugars released by the action of the enzyme was determined by the Nelson-Somogyi method using glucose as a standard. Enzyme activity was expressed as the amount (μg) of glucose that was released by 1 ml of enzyme solution under the above conditions.

Results

Effects of Vitamins on the Enzyme Production

To elucidate the appropriate vitamin for the cellulolytic enzyme production, various vitamins (Ca-pantothenate, folic acid, inositol, riboflavin and thiamine-HCl) were added to the medium, to make a final concentrations in Table I. The results are shown in Table I.

There was no remarkable difference among the various vitamins on the enzyme production. But enzyme activities were good in vitamin amended medium than non-vitamin amended medium, and slightly stimulated by folic acid and thiamine-HCl than the others.

Effects of Various Inorganic Salts on the Enzyme Production

To study the optimum concentration of KH_2PO_4 on the production of cellulolytic enzyme, the fungus was grown in basal media that contained various amounts of KH_2PO_4. The results are shown in Fig. 1.

The enzyme production was influenced by the concentration of KH_2PO_4 in the medium. A sharp

Table I. Effects of various vitamins on the production* of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.

Vitamins	Conc. (mg/l)	Avicelase activity	CMCase activity	β-Glucosidase activity
None		103.0	438.0	318.5
Ca-pantothenate	0.3	129.7	500.0	392.0
Folic acid	0.03	160.0	584.0	490.0
Inositol	3	127.0	492.0	388.5
Riboflavin	0.3	124.5	486.5	385.0
Thiamine-HCl	0.5	157.0	571.0	481.6

* Relative activity (glucose μg/ml)

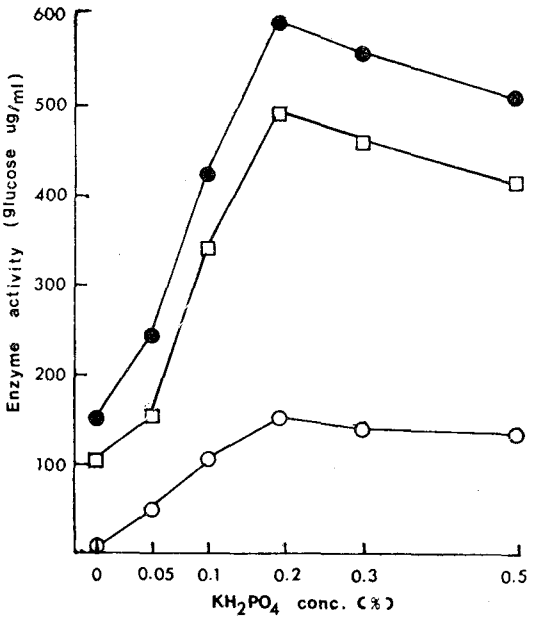

Fig. 1. Effects of potassium dihydrogen phosphate concentration on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.

○: Avicelase ●: CMCase □: β-Glucosidase

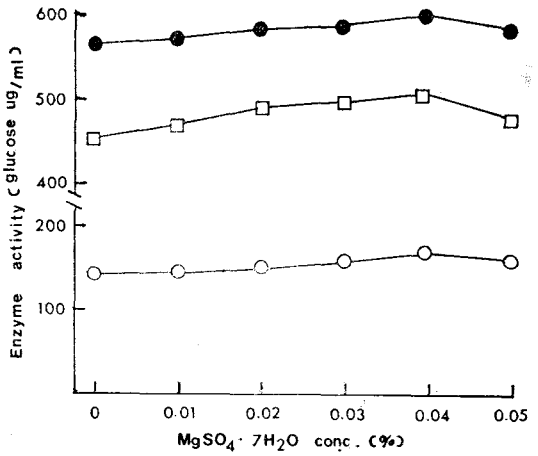

Fig. 2. Effects of magnesium sulfate concentration on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.

○: Avicelase ●: CMCase □: β-Glucosidase

increase in enzyme activities was found when the concentration of KH_2PO_4 was increased from 0 to 0.2% (w/v). The maximum activity in all three enzymes occured at 0.2%.

To investigate the optimum concentration of $MgSO_4 \cdot 7H_2O$ on the production of cellulolytic enzymes, the concentration adjusted to 0, 0.01, 0.02, 0.03, 0.04 and 0.05% (w/v). The results are shown in Fig. 2.

The effects of $MgSO_4 \cdot 7H_2O$ concentration on the enzyme production were scarcely observed. The appropriate concentration on the enzyme production was 0.04%.

To elucidate the effects of the other inorganic salts on the production of cellulolytic enzymes, $CaCl_2$, $CuSO_4$, $FeSO_4$, $MnSO_4$ and $ZnSO_4$ were added to the medium, to make final concentration in Table II. The results are shown in Table II.

The results showed that these inorganic salts were not effective for the production of enzymes.

Effects of Cultural Conditions on the Enzyme Production

To elucidate the effect of cultural temperature on the production of cellulolytic enzymes, the fungus was cultured at 20, 25, 30 and 35°C. The results are shown in Fig. 3.

The optimal temperature for the production of avicelase was at 25°C, and those for CMCase and β-glucosidase were at 30°C.

To investigate the effect of pH on the production of cellulolytic enzymes, the initial pH (after autoclaving) of media described above was adjusted to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0

Table II. Effects of various minerals on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.

Inorganic salts	Conc. (mg/l)	Avicelase activity	CMCase activity	β-Glucosidase activity
None		177.0	594.6	505.9
$CaCl_2 \cdot 2H_2O$	0.3	184.0	652.7	516.8
$CuSO_4 \cdot 5H_2O$	0.1	173.4	581.4	509.8
$FeSO_4 \cdot 7H_2O$	0.3	139.7	494.0	453.9
$MnSO_4 \cdot 5H_2O$	0.1	170.0	584.4	503.0
$ZnSO_4 \cdot 7H_2O$	0.1	184.3	590.0	506.3

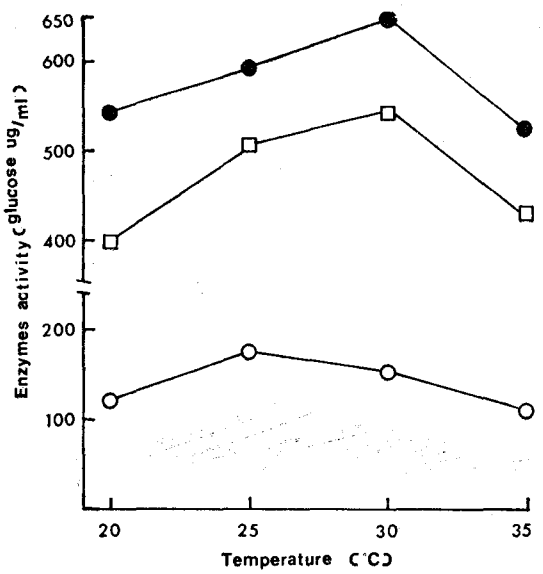

Fig. 3. Effects of cultural temperature on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.
○: Avicelase ●: CMCase □: β-Glucosidase

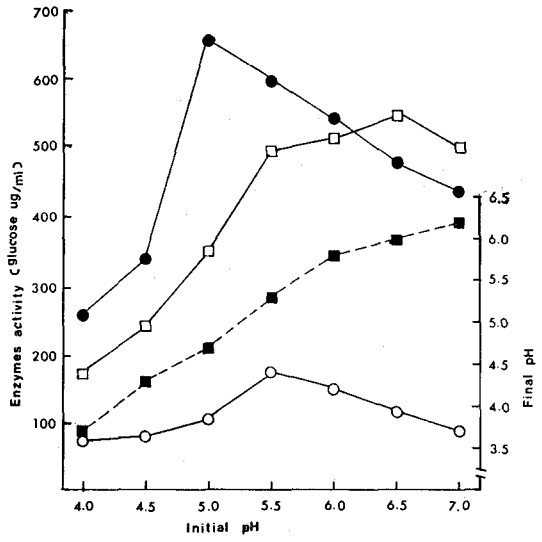

Fig. 4. Effects of initial pH on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.
○: Avicelase ●: CMCase
□: β-Glucosidase ■: Final pH

by adding HCl and NaOH. The results are shown in Fig. 4.

The results showed that the optimum initial pH for the production of avicelase, CMCase and β-glucosidase were 5.5, 5.0 and 6.5, respectively.

Discussion

The enzyme production and mycelial growth (data not shown) were stimulated by folic acid and thiamine-HCl. These results were similar to the investigations of Hong *et al.* (1978, 1981 and 1983), while not agreed with the findings of Sugimori *et al.* (1971) that mycelial growth of basidiomycetes was good in Ca-pantothenic acid amended medium.

The enzyme production was quite stimulated by KH_2PO_4. In this respect, *Pleurotus sajor-caju* resembles *Asp. niger* (Lee *et al.*, 1976) and *Trichoderma* spp. (Kim *et al.*, 1981). The enzyme production was good in 0.04% $MgSO_4$ amended medium. The result of this investigation was contrary to the finding of Kim *et al.* (1974) who showed that optimum concentration of $MgSO_4$ was 0.015%. Other inorganic salts were not effective for the production of enzymes. Similar observations of other fungi have also been made (Murao *et al.*, 1979).

The temperature requirements for enzyme production observed in the present study agreed with the investigations of Hong and Kim (1981), and this investigation was similar that the optimum temperature for mycelial growth of *Flavorus arcularius* was 25~30°C (Kitamoto and Kasai, 1968). The pH requirements for enzyme production and mycelial growth (data not shown) were similar to the findings of Hong *et al.* (1978, 1981, 1981 and 1983).

The enzymatic products of cellulose were mostly glucose when the culture filtrate of *Pleurotus sajor-caju* was assayed. These facts might be useful for the production of edible glucose from

cellulosic substances.

적 요

Pleurotus spp.중 纖維素 分解酵素 生産力이 가장 강한 *Pleurotus sajor-caju* JAFM 1017을 合成培地 상에서 vitamin類, 無機鹽類와 培養條件의 영향을 檢討한 結果는 다음과 같다.

纖維素 分解酵素 生産은 folic acid와 thiamine-HCl에 의해 촉진되었고 KH_2PO_4와 $MgSO_4$의 最適濃度는 각각 0.2%, 0.04%(w/v)이었으며 그 밖의 無機鹽類는 效果가 없었다.

酵素生産에 最適인 培養溫度와 培地 pH는 avicelase가 25°C, 5.5이었고, CMCase는 30°C, 5.0이었으며, β-glucosidase는 30°C, 6.5이었다.

References

Hong, J.S. (1978): Studies on the physico-chemical properties and the cultivation of oyster mushroom (*Pleurotus ostreatus*). *J. Kor. Agri. Chem. Sci.* **21**: 150~184.

Hong, J.S. and Kim, D.H. (1981): Studies on the enzymes produced by basidiomycetes. I. The production of crude enzymes. *J. Kor. Agri. Chem. Soc.* **24**: 7~14.

Hong, J.S., Lee, K.S. and Choi, D.S. (1981): Studies on basidiomycetes. I. On the mycelial growth of *Agaricus bitorquis* and *Pleurotus ostreatus*. *Kor. J. Mycol.* **9**: 19~24.

Hong, J. S., Kwon, Y. J. and Jung, G. T.(1983): Studies on basidiomycetes. II. Production of mushroom mycelium (*Pleurotus ostreatus* and *Auricularia auricula-judae*) in shaking culture. *Kor. J. Mycol.* **11**: 1~7.

Kim, K.C., Kim, C.K. and Kim, C.H. (1981): Studies on the microbial decomposition of cellulosic materials. I. Isolation of cellulase producing microorganisms and characterization of enzymes activities. *J. Kor. Agri. Chem. Soc.* **24**: 85~93.

Kim, S.H. and Kim, W.S. (1982): Studies on the isolation, purification and characterization of a c_x-enzyme produced by *Pyricularia oryzae*, C-7^{++}. *Kor. J. Mycol.* **10**: 67~73.

Kim, Y.B., Yi, P.K. and Choi, S.H. (1974): Studies on the cellulase of *Penicillium* sp. isolated from soils. III. Culture conditions of *Penicillium* sp. C 13-13 strain. *Kor. J. Mycol.* **2**: 25~29.

Kitamoto, Y. and Kasai, Z. (1968): Fruit-body formation of *Flavorus arcularius* on a synthetic medium. *Agri. Chem. Soc. Japan* **42**: 255~259.

Lee, K.H., Koh, J.S. and Park, S.O. (1976): Studies on the production of fermented feeds from agricultural waste product. III. On the production of cellulase by *Aspergillus niger* and *Trichoderma viride*. *J. Kor. Agri. Chem. Soc.* **19**: 130~137.

Murao, S., Kanamoto, J. and Arai, M.(1979): Isolation and identification of the cellulolytic enzymes producing microorganism. *J. Ferment. Technol.* **57**: 151~156.

Somogyi, N. (1952): Notes on sugar determination. *J. Biol. Chem.* **195**: 19~23.

Sugimori, T., Oyama, Y. and Ominichi, T. (1971): Studies on basidiomycetes. II. Productions of mycelium and fruiting body from noncarbohydrate organic substances. *J. Ferment. Technol.* **49**: 435~446.

Wakabayashi, K. and Nisizawa, K. (1964): On the cellulase system of *Irpex lacteus*. *J. Ferment. Technol.* **42**: 347~355.

〈Received October 7, 1985;
Accepted November 21, 1985〉