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Studies on Cellulolytic Enzymes Produced by *Pleurotus* spp. in Synthetic Medium(I)

Effects of Carbon and Nitrogen Sources

Jai Sik Hong, Jong Bae Lee, Moo Seok Koh*, Jeong Sook Kim**
Keug Ro Lee and Myung Kon Kim

Chonbuk National University, Chonju 520, Chonnam National University, Gwang-ju 500 and Seokang Junior College**, Gwang-ju 500, Korea*

合成培地에서 *Pleurotus*屬이 生産하는 纖維素 分解酵素에 관한 硏究(第 1 報)

炭素源과 窒素源의 影響

洪載植・李種培・高武錫*・金正淑**・李克魯・金明坤
全北大學校・全南大學校*・光州瑞江專門大學**

Abstract: Among the eight strains, *Pleurotus sajor-caju* JAFM 1017 was selected as most potent producer of cellulolytic enzymes. The avicelase and CMCase activity reached maximum levels after 10 days, and β-glucosidase activity reached a maximum level after 19 days. Among the various carbon sources, cellulose powder was most effective for the production of avicelase and β-glucosidase, and Na-CMC (sodium carboxymethyl cellulose) was good for the production of CMCase. The optimum concentration of cellulose powder was 1.0% (w/v), and glucose (1.0%) completely depressed the production of enzymes. Nitrates were effective for the production of enzymes, but nitrites did not support growth. The production of cellulolytic enzymes increased as the concentration of urea increased. The appropriate concentration of urea was 0.054% (w/v).

Keywords: Cellulase activity, *Pleurotus sajor-caju*, Avicelase, CMCase, β-Glucosidase.

Cellulose is an organic substance abundantly produced by plants and deposited every year on the earth as a main component of the plant body. It is carbohydrate abundantly available as a substrate for basidiomycetes. The production of cellulolytic enzymes may help the basidiomycetes to utilize cellulose as a source of carbon. Cellulose can be utilized as a nutrient source when it is hydrolyzed by cellulolytic enzymes to produce sugar. Therefore, the utilization of natural cellulose as an energy source, feed, food *etc.* has been paid much attention by many scientists.

Wakabayashi and Nisizawa (1964) and Wakabayashi *et al.* (1965, 1966) studied intensively the properties of cellulases from *Irpex lacteus* grown on semisynthetic media. Michalski and Beneke (1969) reported the activities of β-galactosidase, β-glucosidase and acid phosphatase in *Pleurotus ostreatus* and discussed changes in the enzyme activities during the maturing period of basidiocarp. Kawai and Abe (1972a) and Kawai (1973) presented that basidiomycetes produced

the macerating enzymes of plant tissues, amylase, cellulase and xylanase in semisynthetic media, and Hong *et al.* (1975~1981) reported the cultural conditions and properties of cellulase, hemicellulase and xylanase from *Pleurotus ostreatus*, *Lentinus edodes* and *Flammulina velutipes* grown on a rice straw medium, respectively.

In the present investigation, a *Pleurotus* spp. of potent cellulolytic enzyme productivities and rapid mycelial growth among various strains in the synthetic medium was selected, and the effect of carbon sources and nitrogen sources for production of enzymes were investigated.

Materials and Methods

The laboratory strains utilized in the present study were *Pleurotus ostreatus* JAFM 1011, 1012, 1013, 1015, 1016, 1018 *Pelurotus florida* JAFM 1014 and *Pleurotus sajor-caju* JAFM 1017. These strains were obtained from Laboratory of Mycology and Mushroom, Department of Food Science and Technology, Chonbuk National University (Chonju, Korea).

For a stock culture medium of *Pleurotus* spp., 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 pre-seed 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, peptone 0.2 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 obtained the inoculum, the fungus was grown for 7 days at 25°C on the pre-seed culture medium. Culture mycelial mass 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 liquid culture was filtrated and centrifuged (20 min, 6000 rpm) and the activities of enzymes in the supernant 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 carboxymethyl 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

Selection of Strain

In order to select a *Pleurotus* spp. of potent cellulolytic enzyme productivities and rapid mycelial growth among the various strains, all kinds of *Pleurotus* spp. were cultivated on the basal

Table I. Relative activity** of cellulolytic enzymes produced by *Pleurotus* spp.

Strains	Mycelium growth (NE*)	Avicelase activity	CMCase activity	β-Glucosidase activity
Pleurotus ostreatus JAFM 1011	++	120.0	469.1	324.0
Pleurotus ostreatus JAFM 1012	++	74.0	287.8	200.0
Pleurotus ostreatus JAFM 1013	+++	87.7	328.9	251.0
Pleurotus ostreatus JAFM 1015	+	106.8	414.5	287.0
Pleurotus ostreatus JAFM 1016	+++	67.8	263.1	182.0
Pleurotus ostreatus JAFM 1018	+++	78.8	305.9	212.0
Pleurotus florida JAFM 1014	++	115.0	447.4	310.0
Pleurotus sajor-caju JAFM 1017	+++	127.0	495.0	370.0

*NE: Naked eye observation, +: Growth present, ++: Moderate growth, +++: Heavy growth
**Glucose μg/ml

medium. After cultivation, the enzyme activities and mycelial growth were investigated. The results are shown in Table I. As shown in Table I, the strain for the potent enzyme productivities and rapid mycelial growth was *Pleurotus sajor-caju* JAFM 1017. Therefore, *Pleurotus sajor-caju* JAFM 1017 was performed in futher expriments.

Effect of Cultural Period on the Enzyme Production

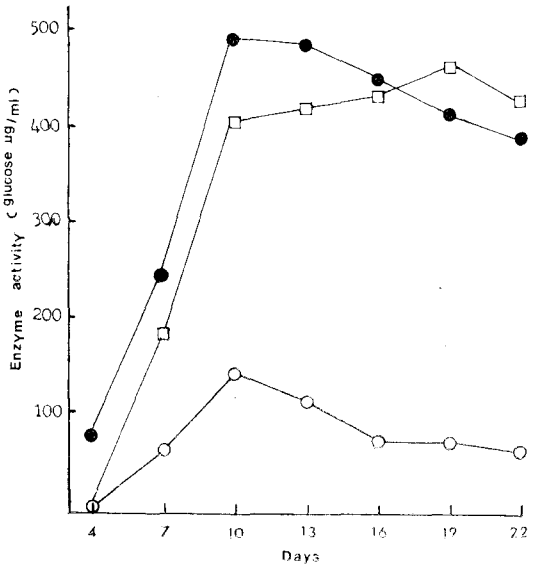

Fig. 1. Effect of cultural period on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.
○: Avicelase ●: CMCase □: β-Glucosidase

Fig. 1 illustrates the production of enzymes between the cultural period of 22 days at 25°C. The avicelase and CMCase activities reached maximum levels after 10 days, and β-glucosidase activity reached a maximum level after 19 days, respectively.

Effect of Carbon Sources on the Enzyme Production

To find carbon sources effective for induction of cellulolytic enzymes, the effect of the various carbon sources was investigated. The results are shown in Table Ⅱ. Among the various carbon sources tested cellulose powder was effective to induce the production of avicelase and β-glucosidase, and Na-CMC was most effective for the production of CMCase, but the addition of 1% glucose completely depressed the production of

Table II. Effect of carbon sources on the production* of cellulolytic enzymes by *Pleurtus sajor-caju* JAFM 1017.

Carbon sources 1% (w/v)	Avicelase activity	CMCase activity	β-Glucosidase activity
Avicelase	99.0	414.3	0
Cellulose powder	127.0	495.0	370.0
Na-CMC	86.8	643.5	115.0
Salicin	0	375.0	206.5
Glucose	0	0	0

*Relative activity (glucose μg/ml)

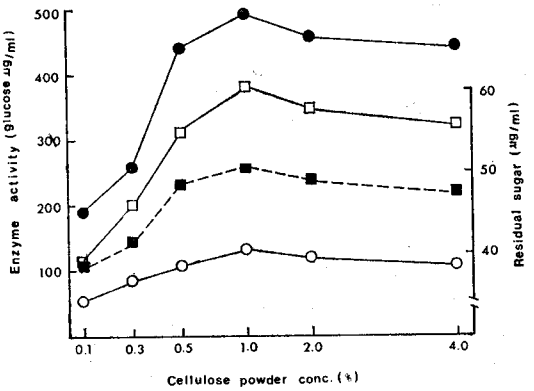

Fig. 2. Effect of cellulose powder concentration on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.
○: Avicelase ●: CMCase □: β-Glucosidase
■: Residual sugar

enzymes. Avicelase and β-glucosidase activities were not observed in salicin and avicel media, respectively.

To investigate the optimum concentration of cellulose powder which was relatively effective for the production of cellulolytic enzymes, the fungus was grown in basal media that contained various amounts of cellulose powder. The results were shown in Fig. 2. An increase of enzyme activities was observed when concentration of the cellulose powder was increased from 0.1 to 0.5% (w/v). For all three enzyme activities, the maximum induction was found in the culture containing 1.0% cellulose powder.

To study the effect of glucose on the production of cellulolytic enzymes, glucose was added to the medium containing cellulose powder. The total weight of glucose and cellulose powder was maintained at 1.0 g/100 ml but the ratio of cellulose powder to glucose was varied. The results were shown in Fig. 3. As shown in Fig 3, the enzyme production was influenced by the concentration of glucose in the media. The enzyme production was not observed in the medium containing 1.0% (w/v) glucose alone.

Effect of Nitrogen Sources on the Enzyme Production

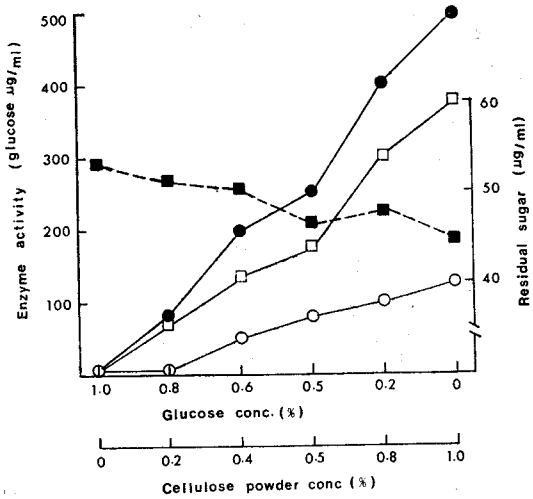

Fig. 3. Combined effect of glucose together with cellulose powder on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.
○: Avicelase ●: CMCase □: β-Glucosidase
■: Residual sugar

In order to determine suitable nitrogen sources for the production of cellulolytic enzymes, several kinds of nitrogen sources (0.026% as nitrogen) were added to the medium. The results were shown in Table Ⅲ. In the nitrogen sources

Table III. Effect of nitrogen sources on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.

Nitrogen sources 0.026% (w/v)*	Avicelase activity	CMCase activity	β-Glucosidase activity
None	70.0	187.5	80.0
Peptone	127.0	495.0	370.0
Urea	157.0	571.0	481.6
$(NH_4)_2HPO_4$	139.8	446.5	307.9
$(NH_4)_2SO_4$	120.0	420.0	305.0
NH_4NO_3	50.0	152.5	72.5
KNO_3	47.5	125.0	65.0
$NaNO_3$	45.0	102.5	62.5
KNO_2	0	0	0
$NaNO_2$	0	0	0

*As nitrogen

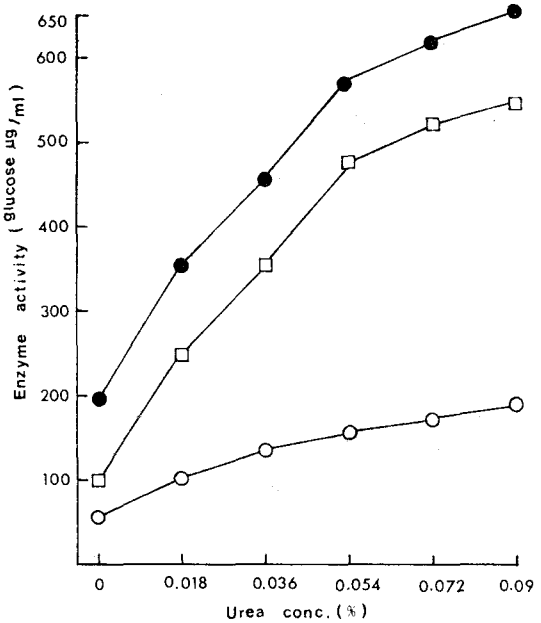

Fig. 4. Effect of urea concentration on the production of cellulolytic enzymes by *Pleurotus sajor-caju* JAFM 1017.
○: Avicelase ●: CMCase □: β-Glucosidae

tested, urea was most effective for the enzyme production. Nitrates were effective for enzyme production, but nitrites did not support the mycelial growth at all.

To investigate the optimum concentration of urea for the production of cellulolytic enzymes, the fungus was grown in the basal medium that contained 1.0% (w/v) cellulose powder and various amounts of urea. The results were shown in Fig. 4. Increase in the concentration of urea was effective for the increase of the cellulolytic enzyme activities. A sharp increase of CMCase and β-glucosidase activities was observed when the concentration of urea was increased from 0 to 0.054%, while higher the concentration of urea gradually increased the production of avicelase.

Discussion

Among the eight strains of *Pleurotus* spp., the strains of potent enzyme productivities were *Pleurotus sajor-caju* JAFM 1017, *Pleurotus ostreatus* JAFM 1011 *Pleurotus florida* JAFM 1014, *Pleurotus ostreatus* 1015, and strains of rapid mycelial growth were *Pleurotus ostreatus* JAFM 1013, 1016, 1018, and *Pleurotus sajor-caju* JAFM 1017, respectively. There seemed to be little or no relationship between the enzyme production and the mycelial growth. This is in agreement with the finding of Hong and Kim (1981a).

The ability of *Pleurotus sajor-caju* to produce cellulolytic enzymes and to degrade insoluble or native forms of cellulose was shown by their assimilation of avicel and cellulose powder. The effective cellulose powder concentration for the enzyme production observed in the present study did not agree with those of their fungi (Chung, 1971; Gunasekaran, 1980; Sakamoto *et al.*, 1982). This may mean that the optimum amount of carbon source is different depending on the fungal strains used.

In the present study, the cellulolytic enzyme production was repressed in the presence of 1% glucose. Other investigations indicated that glucose repressed the CMCase production in the other fungi grown in the presence of CMC (Gunasekaran, 1980), and sucrose depressed the macerating enzyme production by basidiomycetes (Kawai and Abe, 1972).

The nitrogen source requirements for the enzyme production observed in the present study were generally similar to those of the other fungi (Chung, 1971; Kim *et al.*, 1981). The production of cellulolytic enzymes increased as initial urea supplements increased. Similar observations have been also made in other fungi (Sakamoto *et al.*, 1982).

摘 要

8개의 *Pleurotus* spp.를 合成培地에 培養하여

纖維素分解酵素 生産力이 제일 강하고 菌絲生育이 良好한 菌絲를 選別하여 培養期間 및 炭素源, 窒素源의 影響을 檢討한 結果는 다음과 같다.

纖維素分解酵素 生産力이 강한 菌株로 *Pleurotus sajor-caju* JAFM 1017을 選別하였다. Avicelase와 CMCase活性은 10日 培養하였을때 最大値를 보였으며 β-glucosidase 活性은 19日 培養하였을 때가 가장 높았다. 炭素源중에서 avicelase와 β-glucosidase 生産은 cellulose powder를, CMCase 生産은 Na-CMC를 添加하였을 때가 가장 좋았다. 酵素生産은 cellulose powder 1.0%에서 가장 良好하였으며 glucose는 濃度의 增加에 따라 현저하게 減少되었다. 酵素生産에 良好한 窒素源은 urea였고 亞窒酸性 窒素는 酵素生産을 抑制하였으며 酵素生産에 適當한 urea의 濃度는 0.054%였다.

References

Chung, D.H. (1971): Studies on the cellulase produced by *Myriococcum albomyces*. *J. Kor. Agri. Chem. Soc.* **14**: 59~95.

Gunasekaran, M. (1980): Physiological studies on *Phymatotrichum omnivorum*. XI. Cellulolytic enzymes. *Mycologia* **72**: 759~765.

Hong, J.S. and Namgung, H. (1975): Studies on the enzymes produced by *Pleurotus ostreatus*. I. Properties of crude cellulase. *Bul. Agri. Col. Jeonbug N. Univ.* **6**:101~105.

Hong, J.S. (1976): Studies on hemicellulolytic enzymes produced by *Pleurotus ostreatus*. I. Properties of crude hemicellulolytic enzymes. *Bul. Agri. Col. Jeonbug N. Univ.* **7**: 89~92.

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

Hong, J.S. (1978b): Studies on the enzymes produced by *Flammulina velutipes*. I. Properties of crude cellulase. *Bul. Agri. Col. Jeonbug N. Univ.* **9**: 97~100.

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

Hong, J.S. and Kwon, Y.J. (1981b): Studies on the enzymes produced by basidiomycetes. II. Properties of cellulase and xylanase. *J. Kor. Agri. Chem. Soc.* **24**: 260~266.

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

Kawai, M. and Abe, S. (1972a): Maceration of plant tissues by basidiomycetes. I. Screening tests of basidiomycetes on the production of macerating enzymes. *J. Ferment. Technol.* **50**: 685~690.

Kawai, M. (1972b): Maceration of plant tissues by basidiomycetes. II. On some sorts of polysaccharide decomposing activities of crude enzyme preparations. *J. Ferment. Technol.* **50**: 691~697.

Kawai, M. (1973): Productivity of amylolytic, cellulolytic and xylolytic enzymes among the basidiomycetes. *J. Agri. Chem. Soc. Japan* **47**: 529~534.

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 Cx-enzyme produced by *Pyricularia oryzae*, C-7^{++}. *Kor. J. Mycol.* **10**:67~73.

Michalski, C.J. and Beneke, E.S. (1969): Enzymatic activities during basidiocarp development of *Pleurotus ostreatus*. *Mycologia* **61**: 1041~1047.

Sakamoto, R., Shooro, I., Arai, M. and Murao, S. (1982): Effect of culture conditions on cellulase production by *Aspergillus aculeatus*. *J. Ferment. Technol.* **60**:327~332.

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

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

Wakabayashi, K., Kanda, T. and Nisizawa, K.

(1965): On the fractionation and some properties of cellulase *Irpex lacteus*. *J. Ferment. Technol.* **43**: 739~749.

Wakabayashi, K., Kanda, T. and Nishizawa, K. (1966): Separation of two cellulase components from a culture filtrate of *Irpex lacteus* and some of their properties. *J. Ferment. Technol.* **44**: 669~681.

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