韓 國 菌 學 會 誌 第 14 卷 第 3 號
Kor. J. Mycol. Vol. 14, No. 3, 189~194, 1986

Extracellular Wood-degradative Enzymes from *Lentinus edodes* JA01

Soon-Woo Hong, Kwang-Soo Shin, Yeup Yoon and Won-Kyu Lee*

Department of Microbiology, College of Natural Sciences, Seoul National University, Seoul 151 and National Forest Research Institute, Seoul 132, Korea*

표고 菌株 JA01에서 분비되는 細胞外 木材成分 分解酵素에 관하여

洪淳佑・申光洙・尹 燁・李元珪*

서울大學校 自然科學大學 微生物學科・國立林業試驗場*

Abstract: The aim of this study was to investigate physiological chracteristics of *Lentinus edodes* in Korea. We tried to detect properties of the several wood-degradative enzymes and in vestigate patterns of the enzyme production. A specific carbon source was used in the enzyme induction media for each enzymes, and the crude extract was used for the enzyme solution. With these enzyme solution, we investigated optimum temperature and pH conditions of their reactions. Moreover we investigated transition patterns of the enzyme production of the several wood-degradative enzmes from Complex and Saw dust media for the purpose of studying the mechanisms of the wood component degradation by this fungus. It was assumed that the order of the wood component degradation was cellulose, xylan, and then pectic substances, and that the synergistic effects of these substances also influenced the degradation of wood components.

Keywords: *Lentinus edodes*, Cellulase, Xylanase, Pectinase, Transition pattern.

Lentinus edodes is a well-known edible mushroom in Korea but the physiological chracteristics of this fungus have been poorly investigated among other fungi. So several wood-dgradative enzymes were detected and compared in this study. The mycelium of *Lentinus edodes* has been cultivated mainly on the mushroom logs of *Quercus* spp. and *Corpinus* spp. for the fruiting body development. So long as this mycelia should grow and colonize the tissue on this mushroom logs without any other nutriets, they should Save degraded the several structural components of this wood. It is also clear that this fungus produce various degradative enzymes. Oki *et al.* (1981) reported that the β-glucopyranosidase activity was increased gradually by the loss of total weight throughout the growth on wood powder of this fungus. Althougth the production of the degradative enzymes by this fungus *in vitro* is not exactly correlated with the potentiality of this fungus to produce these enzymes *in vivo*, there may be many enzymes in relation to the degradation of the wood materials, but we mainly focused on the enzymes involved in the degradation of the cellulosic and hemicellulosic substances. The most acceptable trend is to consider the cellulase system as follow according to Bisaria *et al.*

(1981) (1) endo-(1-4)-glucan glucanase(the Cx enzymes as referred to by Reese), (2) exo-(1-4)-glucanase, and (3) cellobiase, (1-4)-β glucosidase. In this report we used the name of enzymes according to their reaction substrates, as carboxymethylcellulase, avicelase and β-glucosidase. In case of hemicellulose degradative enzymes, we detected the xylanase and pectinase. According to Rombouts *et al.*(1980), pectinase has 3 types of action, that is, pectic lyase (pectin transeliminase), pectin hydrolase(polygalacturonase) and pectin esterase(pectin methyl esterase)

In addition to the comparison of these enzymes, we tried to investigate the process of the wood-components degradation by using the several media and determined the indirect order of these enzyme production.

Materials and Methods

Fungal Strain and Medium Preparation

Lentinus edodes JA01 strain was received from Forest Research Laboratories and stock cultures maintained on PDA(potato dextrose agar) slant. The inoculum was cultured at 20°C on PDA medium as well as induction medium.

Medium Preparation

Induction medium was prepared according to the mineral component of medium C(Mandel *et al.*, 1962) and proper carbon sources were added to induction medium. 0.5% Avicel from Merck Chemical Company and 0.5% carboxymethyl cellulose were used for callulase induction. 1% Larch wood xylan and 1% Citrus-pectin from Sigma Chemical Company were used for xylanas and pectinase induction. Complex medium was prepared as a modulation of the natural wood composition by mixing CMC(carboxymethyl cellulose), Avicel, Ligriosulfonate-Ca salt, xylan and pectin. 0.1% saw duste were also used for the induction of several enzymes.

Cultivation and Crude Enzyme Preparation

Initial inoculum was homogenized by Omni Mixer(Du pont Instrument) and adjusted to the same inoculum size by the measurment of the fresh wet weight of mycelia. The homogenized mycelia were grown with reciprocal shaking (120 rpm) at 25°C for 12 days and the every days' culture extracts were used for the crude enzyme solution.

Enzyme Assay

1) Reducing Sugar Analysis

Carboxymethy cellulose(CMC)-saccharifying enzyme(CMCase), aviclase, xylanase and polygalacturonase(PG) activities were measured by the reducing sugar analysis according to the method of Somogyi(1952) and Nelson(1944). Each reaction mixtures was composed of 0.8 ml of the substrate(0.5% CMC, Avicel, xylan and PGA) in 0.05 M sodium acetate buffer(pH 5.0) and 0.2 ml of enzyme solution. After incubation at 40°C for 30 min., the reducing sugar was detected.

2) β-Glucosidase Activity

β-Glucosidase activity was assayed by measuring the amounts of p-nitrophenol(PNP) liberated from p-nitrophenyl-β-D-glucopyranoside (PNPG). The reaction mixture was composed of 0.8 ml of 1 mM PNPG in 0.05 M sodium acetate buffer and 0.2 ml of 1 M sodium carbonate solution was added to the mixture. The mixture was diluted with 10 ml of distilled water and the absorbance was measured(at 420 nm).

3) Pectin Transeliminase Activity

PTE(pectin transeliminase) activity was assayed by measuring the amounts of the 4,5-unsaturated galacturonate. The reaction mixture was composed of 0.1ml polygalacturonic acid (PGA) and 10^{-4}M $CaCl_2$ in 0.05 M Tris-HCl buffer(pH 8.0) and 0.1 ml of enzyme solution.

After incubation at 30°C for 1 hr, 1 ml of 1 N HCl and 0.01 M thiobarbituric acid was added and boiled for 15 min. The absorbance of the mixture was measured at 420 nm.

Results and Discussion

Effects of pH and Temperature on the Activities

The optimum pH for the enzyme activity was determined with a buffered substrate at pH values of 3, 4, 5, 6 and 7. The optimum pH of each enzymes was reprsèented in Fig. 1. Among them, β-glucosidase and xylanase activities were more affected by the pH variations. PTE activity showed somewhat higher optimum pH at 6.0.

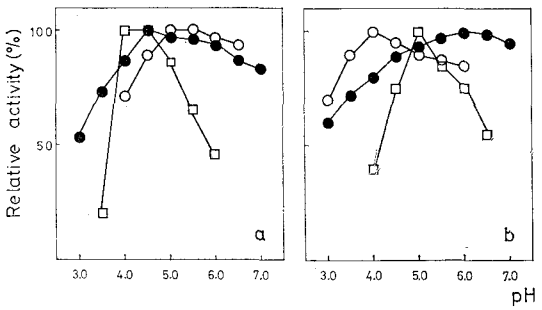

Fig. 1. Effects of pH on the extracellular wood-degradative enzyme activities. Symbols are follows;
a) Avicelase (○), CMCase (●) and β-glucosidase (□).
b) PG (○), PTE (●) and xylanase (□).

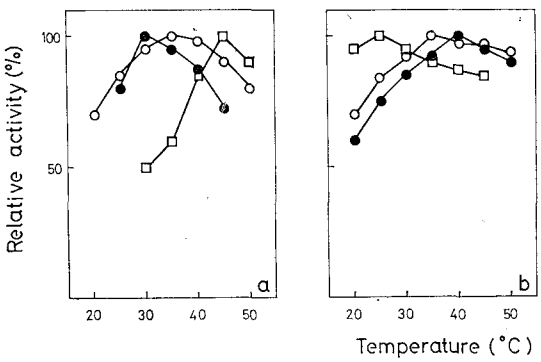

Fig. 2. Effects of temperature on enzyme activities. Symbols are the same as Fig. 1.

Optimum temperature of each enzyme activities was shown in Fig. 2. In case of β-glucosidase, the maximum activity was detected at 45°C and 30°C in CMCase, 35°C in avicelase, 35°C in PG, 40°C in PTE and 25°C in xylanase. As compared with other enzymes, xylanase was slightly psychrophilic and stable on temperature change. Cellulase was stable on temperature variation in this organism.

Enzyme Activities in Induction Media

1) Cellulase Activity

CMCase activity in the culture medium was increased over 4 days and this enzyme activity was much decreased after 6 days. Although avicelase activity showed slight differences in compared to CMCase, both CMCase and avicelase activity seemed to be released earlier than β-glucosidase (Fig. 3).

2) Xylanase and Pectinase Activity

Xylanase activity appeared at first and its activity lasted for a while. PG and PTE activity appeared between 4 and 5 days of its cultivation and their activities were not lost rapidly. PTE

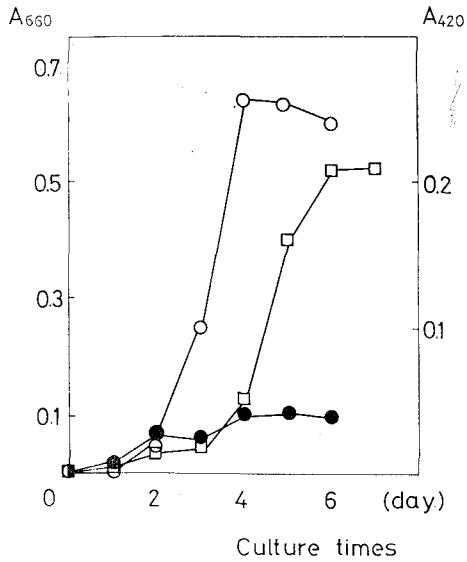

Fig. 3. Transition pattern of cellulases in single induction medium. Symbols are follows; Avi celase (○), CMCase (●) and β-glucosidase (□).

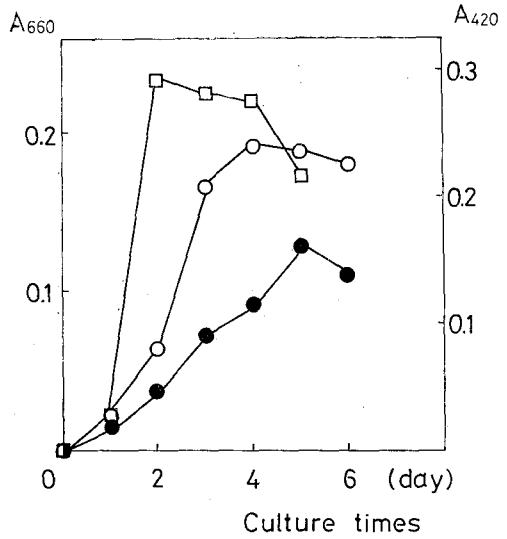

Fig. 4. Transition pattern of hemicellulases activities in single induction medium. Symbols are follows; PG (○), PTE (●) and xylanase(□).

activity was too low to detect in this fungus (Fig. 4).

3) Transition Patterns of the Enzyme Activities

CMCase activity from saw dust medium appeared early within 2 or 3 days and its activity was higher than that from complex medium. Avicelase also showed higher enzyme activity from saw dust medium than from complex and single induction medium. In case of β-glucosidase, the enzyme activity was much higher from saw dust medium than from complex and single induction medium. This results also included some possibilities of the involvement of some inducer materials or the ruptures of the enzymes from cell lysis by the toxic materials that might be produced during the degradation of lignin substances.

Xylanase activity showed little difference between saw dust medium and single induction medium and little lower from complex medium, but this enzyme might rapidly degrade the substance. PG and PTE activities from single induction medium were higher than those from

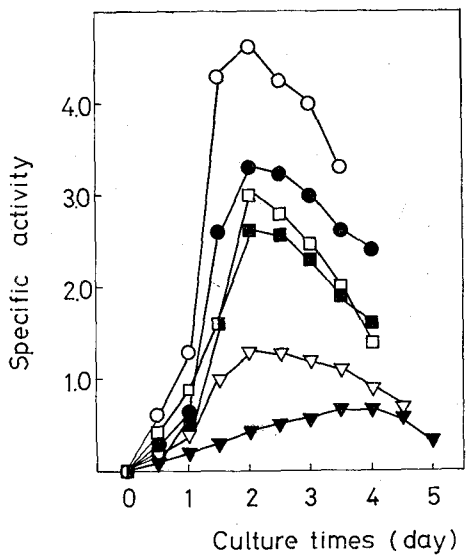

Fig. 5. Transition pattern of extracellular wood-degradative enzyme activities in complex medium. Symbols are follows; Avicelase (●), CM Case (□), β-glucosidase (▼), PG (▽), PTE (■) and Xylanase (○).

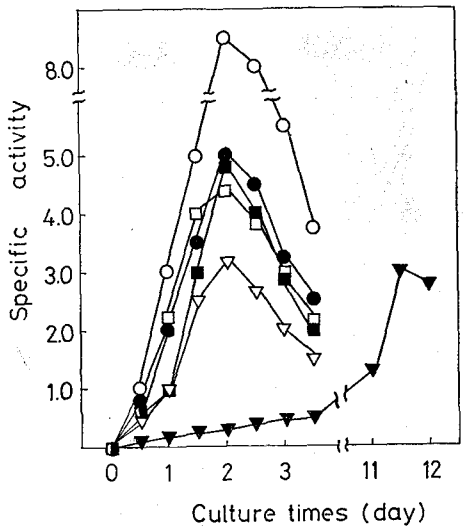

Fig. 6. Transition pattern of extracellular enzyme activities in saw dust medium. Symbols are the same as Fig. 5.

any other media (Fig. 5, 6). As a result, the sequences of the wood-component degradation in the artificial complex medium were as follows; cellulose, xylan, pectin and lignin at last (unpublished data). But in case of saw dust

medium, xylanase, cellulase and pectinase appeared earlier at once and also showed higher activities than from complex medium. Although this study does not exactly reflect the production of the enzymes *in vivo*, we could assume that this fungus utilize the cellulosic and hemicellulosic materials and the synergistic effects of this two substances contribute to the degradation process.

적 요

한국산 표고菌의 생리적 조사의 일원으로서 이 菌株가 목재에서 생장시 필요로하는 목재분해 효소들의 특징 및 생성경향을 조사하였다.

각 효소들의 유도를 위한 배지로서는 각각 단일의 특정탄소원을 첨가한 배지를 사용하였고 이때 추출한 조효소를 효소용액으로 사용하였으며 이들 효소에 대한 최적온도 및 최적 pH등의 조건을 조사하였다. 또한 이 균주가 목재구성성분을 분해하는 기작을 간접적으로 추정하기 위하여 복합배지 및 톱밥배지 내에서의 각 효소생성 양상을 측정하였다. 이 균주가 목재성분을 분해할 때에는 cellulose와 hemicellulose의 하나인 xylan을 초기에 분해하여 이용하고, 조금지나서 pectin질을 분해함을 알수있었다. 또한 이들기질들은 서로 상호작용에 의해 분해가 좀더 원활하게 이루어지게 하는데 효과가 있었다.

Acknowledgments

The authors wish to thank Dr. Jae-Myung Cho for supplying the fungal strain.

References

Ahlgrem, E., Eriksson, K.E. and Vesterberg, O. (1967): Chracterization of cellulase and related enzymes by isoelectric focusing, gel filtration and zone electrophoresis. *Acta Chem. Scand.* **21**:937-944.

Barker, K.R. and Walker, J.C. (1962): Relationship of pectolytic and cellulolytic enzyme production by strains of *Pellicularia filamentosa* to their pathogenicity. *Phytopathol.* **52**: 1119-1125.

Bateman, D.F. (1966): Hydrolytic and trans-eliminative degradation of pectic substances by extracell ular enzymes of *Fusarium solani* f. *phasoeli. Phytopathol.* **56**: 238-244.

Bisaria, V.S. and Ghose, T.K. (1981): Biodegradation of cellulosic materials: substrates, microorganisms, enzymes and products. *Enz. Microb. Technol.* **3**: 90-103.

Choe, Y.T. and Kim, K.J. (1984): Degradation pattern of CMC, xylan, lignin components of rice straw by *Bacillus subtilis* DO_4. *Korean J. Microbiology* **22**: 97-101.

Hildebrand, D.C. (1971): Pectate and pectin gels for differentiation of *Pseudomonas* sp. and other bacterial plant pathogens. *Phytopathol.* **61**: 1430-1436.

Mandels, M., Parrish, F.W. and Reese, E.T.(1962): Sophorose as an inducer of cellulase in *Trichoderma viride. J. Bacteriol.* **83**: 400-408.

Mandels, M. and Wever, J. (1969): The production of cellulases. *Advan. Chem. Ser.* **95**: 391-414.

Millar, R.L. (1965): Polygalactronase trans-eliminase production by *Fusarium* spp. isolated from alfalfa roots. *Phytopathol.* **55**: 131.

Mount, M.S., Bateman, D.F. and Basham, H.G. (1969): Induction of electrolyte loss, tissue maceration, and cellular death of potato tissue by an endopolygalaturonate trans-eliminase. *Phytopathol.* **60**: 925-931.

Nelson, N. (1944): A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* **153**: 375-380.

Nisizawa, J., Suzuki, H. and Nisizawa, K. (1972): Catabolite repression of cellulase formation in *Trichoderma viride. J. Biochem.* **71**: 999-1007.

Oki, T., Watanabe, H. and Ishikawa, H. (1981): The biodegradation of lignin by Shiitake *Lentinus edodes* (Berk.) Sing. *Mokuzai Gakkaishi* **27**: 696-702.

Oki, T., Senba, Y. and Ishikawa, H. (1982): The biodegradation of softwood lignin and guaiacylglycerol-β-guaiacyl ether by extracellular enzyme in Shiitake *Lentinus edodes* (Berk.) Sing. *Mokuzai*

Gakkaishi **28**: 800-808.

Rombouts, F.M. and Pilnik, W. (1980): Microbial enzymes and bioconversions, *Economic Microbiology* Vol. 5 Academic Press, New York, pp. 227.

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

Tomita, Y., Suzuki, H. and Nisizawa, K. (1974): Further purification and properties of "Avicelase", a cellulase component of less random type from *Trichoderma viride*. *J. Ferment. Technol.* **52**: 233-246.

Woodward, J. and Wiseman, A. (1982): Fungal and other β-glucosidase their properties and applications. *Enz. Microb. Technol.* **4**:73-79.

Zucker, M. and Hankin, L. (1970): Regulation of pectate lyase synthesis in *Pseudomonas fluorescens* and *Erwinia carotovora*. *J. Bacteriol.* **104**: 13-18.

〈Received March 21, 1986;
Accepted April 25, 1986〉