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Degradation Pattern of CMC, Xylan, Lignin Components of Rice Straw by *Bacillus subtilis* DO4

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Bacillus subtilis DO4에 의한 볏짚의 CMC, Xylan 및 Lignin 성분의 분해양상에 관하여

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ABSTRACT

To investigate the biodegradation pattern of rice straw, mainly composed of cellulose, hemicellulose and lignin components, by the isolate stran *Bacillus subtilis* DO4, the change of cell population was observed on CMC (carboxymethyl cellulose), larch wood xylan and lignosulfonate as a carbon source respectively. Also, the transition pattern of enzyme activities of cellulase and xylanase and lignin contents was measured on rice straw and mixed substrate according to growth.

The results in these experiments revealed that xylanase activity was first appeared and cellulase activity in the next, while lignin component was almost not changed through the culture period.

INTRODUCTION

Rice straw, like as other plant residues, is mainly composed of cellulose, hemicellulose and lignin, which is widely used as an animal feed, especially for ruminants (Han et al., 1978a and 1978b; Morrison, 1979). Unfortunately, its low digestibility and low protein content presently prevent its use in feed-lots.

Various methods have been advocated for enhancing the digestibility and the nutritive value of straw, for example, by treating the straw with sodium hydroxide or applying microbial fermentation to it (Grant *et al.*, 1978; Han *et al.*, 1975 and 1976).

Different from these investigations, this paper reports the change of each components according to growth and biodegradation pattern of them on rice straw or mixed substrates of cellulose, xylan and lignosulfonate.

MATERIALS AND METHODS

1. Isolation of bacterial strains.

The *Bacillus subtilis* DO4 strain used in these investigations was isolated from manure and wheat bran for animal feed. 1 g of sample was suspended in 100ml distilled water, mixed with 120 rpm rotary shaker for 1 hour at 30°C, and allowed to stand for 30min at room temperature. Then the suspension was plated on minimal salt medium (Table 1) containing rice straw as a carbon source.

The isolate DO4 used in this study was selected on the basis of its rapid growth rate on

Table 1. Minimal salt medium

KH_2PO_4	0.5gm
K_2HPO_4	0.5gm
NaCl	6.0gm
$(NH_4)_2SO_4$	1.0gm
$MgSO_4.7H_2O$	0.1gm
$CaCl_2.2H_2O$	0.1gm
Distilled water	1,000ml
Agar, if necessary	18~20gm

rice straw powder containing medium.

2. Substrates.

Annual rice straw, sun-dried and ground to pass a 0.149mm pore size sieve, was used as the substrate. Lignosulfonate (NH_4-salt) was obtained from Dr. Youn W. Han, Department of Agriculture, Oregon state University,Oregon, U.S.A. Substrates such as sodium carboxymethyl cellulose (CMC) and larch wood xylan were commercial products from Wako Pure Chemical Industries, Ltd. and Sigma Chemical Co., respectively.

3. Growth conditions.

Stock culture for the isolate DO4 strain were maintained on nutrient agar slants, stored in the refrigerater and transferred biweekly. For liquid culture, Erlenmeyer flasks (500ml capacity) containing 50ml media (0.5% substrate concentration) were used. Seed culture was prepared as follows: one loopful of precultured strains were inoculated into the 500ml flask containing 50ml nutrient broth and incubated at 30°C for 14-16 hours with shaking.

One ml aliquot of inoculum were used to inoculate each culture flask, which then was incubated 9days at 30°C on a rotary shaker of 120rpm. Each other day's sampling was carried out to establish growth kinetics for the isolate DO4.

4. Measurement of growth.

The growth was determined by counting viable cells, using the spread-plate technique.

5. Analytical procedures.

All the enzyme activities were expressed in terms of a change of optical density (O.D.). Cellulase and xylanase activities were measured in the reaction mixture of 0.25ml of 1% CMC and xylan(xylan was dissolved in a small amount of 0.5M NaOH and neutralized with 0.5N acetic acid) respectively, 0.5ml of 0.1M acetate buffer (pH 4.5) and 0.25ml of enzyme solution. After the reaction mixture was incubated at 42°C for 2 hours, total reducing sugars produced in the mixture were estimated from the optical density at 660nm measured by the method of Somogyi-Nelson.

The lignin content of culture medium was determined with the absorbancy at 280nm, spectrophotometrically.

RESULTS AND DISCUSSION

1. Characteristics of the isolate DO4.

The isolate DO4 was identified according to "Bergy's Manual of Determinative Bacteriology (8th ed.)" and "Manual for the identification of medical bacteria (S.T. Cowan)". The morphological and physiological characteristics of the isolate DO4 were described in Table 2. Thus, it was identified *Bacillus subtilis* DO4, comparing with *Bacillus subtilis* ATCC 6633 as a standard strain.

Table 2. Major taxonomical characteristics of the strain DO4

Test.	Reaction
Gram staining	+
Cell shape	Rod
Spore	+
Motility	+
Requirement of oxygen	Aerobic
Catalase	+
Acid from glucose	+
No_3-NO_2	+
7%NaCl	+
VP-test	+
Starch hydrolysis	+
Casein hydrolysis	+

2. Growth of *B. subtilis* DO4 on CMC, xylan and lignosulfonate.

B. subtilis DO4 was cultured on CMC, xylan and lignosulfonate (0.5%) as a sole carbon source and the growth phase was investigated by the plate spreading technique. The results are shown in Fig. 1.

The number of microbial cells increased gradually on CMC as a carbon source. On the other hand, it reached a plateau during 1-3 days' culture period and decreased after 3 days' culture with xylan and lignosulfonate. However, in case of lignosulfonate, rapid growth in early phase seemed to be due to the lignin impurities (i. e. carbohydrates), which was confirmed by quantifying the lignin contents as shown in Fig. 1. Also, the viable cell number decreased after 3 days of incubation, probably due to cell death after exhaustion of one or more limiting nutrients or growth factors. At any rate, Fig.1 shows that *B. subtilis* DO4 can utilize xylan faster than CMC during the early culture period.

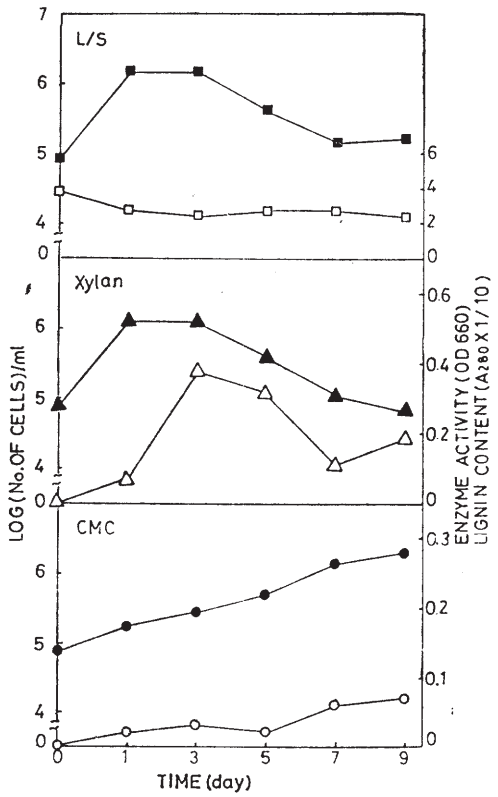

Fig. 1. Growth and enzymatic activities of *B. subtilis* DO4 on lignosulfonate, xylan and CMC as carbon sources. lignosulfonate (L/S) (■—■), absorbancy at 280nm (□—□). xylan (▲—▲), xylanase (△—△), CMC (●—●) and CMCase (○—○).

3. The transition pattern of each components according to growth.

Enzymatic activities and lignin contents was measured to investigate the transition pattern of each components according to growth on rice straw as a substrate (Fig. 2). shown in Fig. 2, the xylanase activity reached the maximum value in 3 day culture and decreased thereafter, while cellulase activity was delayed and detected after 3 day culture. Lignin contents did not change almost through the culture period. Therefore, it was presumed that the lignin component may not be degraded by the isolate strain DO4.

To confirm this phenomena, the isolate strain DO4 was cultured on the mixed substrate, which is composed of CMC, xylan and lignosulfonate in a suitable proportion.The results from

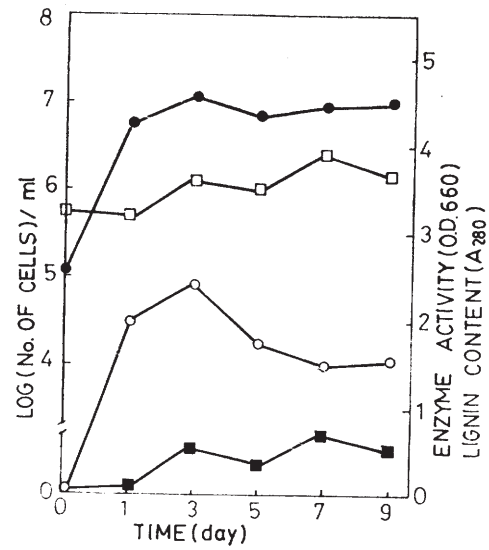

Fig. 2. The change of enzymatic activities of CMCase, xylanase and lignin contents according to growth. growth on rice straw (●—●), xylanase (○—○) and CMCase activities (■—■), absorbancy at 280nm (□—□).

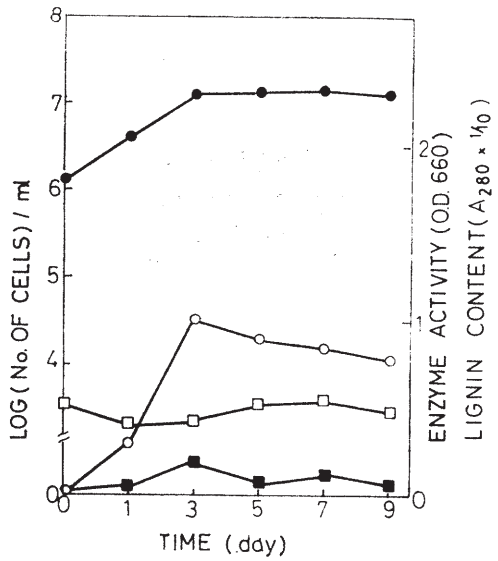

Fig. 3. The change of enzymmatic activities and lignin contents according to growth. (The ratio of substrate composition is CMC: xylan: L/S=5 : 5 : 1) All abbreviations are the same as Fig. 2.

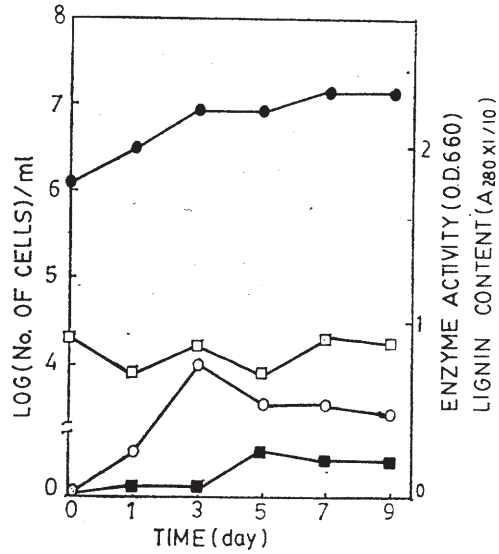

Fig. 4. The change of enzymmatic activities and lignin contents according to growth. (The ratio of substrate composition is CMC:xylan: L/S=2 : 2 : 1) All abbreviations are the same as Fig. 2.

this experiment are shown in Fig. 3. and 4. Similar transition pattern was observed as in case of rice straw. That is, xylanase activity was first appeared and cellulase activity in the next place. Lignin contents was nearly changeless through the culture period. These results suggest that the hemicellulose component of rice straw was first attacked by this strain.

摘 要

볏짚의 주 구성성분이 되는 cellulose, hemicellulose 그리고 lignin이 분리된 균주 *Bacillus subtilis* DO4에 의한 분해양상을 알기 위해서 각 성분에 해당하는 CMC (carboxymethyl cellulose), xylan 및 lignosulfonate를 각각 탄소원으로 주었을 때의 cell population 변화를 관찰하였으며 볏짚과 CMC, xylan 및 lignin을 혼합한 것(혼합비율 2 : 2 : 1 및 5 : 5 : 1)을 기질로 배양했을 때의 성장에 따른 CMC, xylan 분해 효소의 활성도와 lignin량의 변화를 측정하였다. 그결과 xylanase activity가 먼저나타나고 cellulase activity가 뒤이어 서서히 나타남을 확인할 수 있었으며 lignin량은 배양중에 큰 변화가 없었다.

REFERENCES

Bernier, R., Jr., M. Desrochers, L. Jurasek, and M. G. Paice, 1983. Isolation and characterization of a xylanase from Bacillus subtilis. *Appl. Environ. Microbiol.* 46(2), 511-514.

Eriksson, O., and B.O. Lindgren, 1977. About the linkage between lignin and hemicelluloses in wood. Svensk Papperstidn. 80, 59-63.

Grant, G.A., Y.W. Han, and A.W. Anderson, 1978. Pilot-scale semisolid fermentation of straw. *Appl. Environ. Microbiol.* 35(3), 549-553.

Han, Y.W., P.R. Cheeke, A.W. Anderson, and C. Lekprayoon, 1976. Growth of Aureobasidium pullulans on straw hydrolysate. *Appl. Environ. Microbiol.* 32(6), 799-802.

Han, Y.W., and Anderson, 1975. Semisolid fermentation of ryegrass straw. *Appl. Microbiol.* 30(6), 930-934.

Han, Y.W., P.L. Yu, and S.K. Smith, 1978. "Alkali treatment and fermentation of straw for animal feed. *Biotech. Bioeng.* 22, 1015-1026.

Han, Y.W., 1978. Microbial utilization of straw. in Advancesin Applied Microbiology (Eds. D. Perlman). p. 119-153. Academic, N.Y.

Hiroi, T., and K.E. Eriksson, 1976. Influence of cellulose on the degradation of lignins by the white-rot fungus Pleurotus ostreatus.

Kanda, T., and K. Nisizawa. 1976. Xylanase activity of an endocellulase type from Irpex lacteus (Polyporus tulipiferae). *J. Biochem.* 79, 989-995.

Lindenfelser, L.A., R.W. Detroy, J.M. Ramstack, and K.A. Worden, 1978. Biological modification of the lignin and cellulose components of wheat straw by Pleurotus ostreatus. *Dev. Ind. Microbiol.* 21, 799-802.

Morrison, I.M., 1979. The degradation and utilization of straw in the rumen. in Straw Decay and its Effect on Disposal and Utilization (Eds. Dr. E. Grossbard) p. 237-245. John Wiley and Sons, N.Y.

Nisizawa, T., H. Suzuki, and K. Nisizawa, 1971. "De novo" synthesis of cellulase induced by sophorose in Trichoderma viride cells. *J. Biochem.* 07, 387-393.

Plasse, F., M.L. Fardeau, and J.P Belaich, 1980. Microcalonimetric evaluation of the efficiency of the alkaline treatment on the fermentation of straw. *Biotechnol. Lett.* 2(1), 11-16.

Roncero, M.I.G., 1983. Genes controlling xylan utilization by Bacillus subtilis. *J. Bacteriol.* 156(1), 257-263.

Saddler, J.N., E.K.C. Yu, M. Mes-Hartree, N. Levitin, and H.H. Brownell, 1983. Utilization of enzymatically hydrolyzed wood hemicelluloses by microorganisms for production of liquid fuels. *Appl. Environ. Microbiol.* 45(1), 153-160.

Shaw, T.V., and H. Oujesky, 1978. Characterization of the growth and cellulolytic activities of a Trichoderma sp. soil isolate. *Dev. Ind. Microbiol.* 21, 553-562.