0273-2289/94/4546—0655$03.00

Cellulase and Xylanase Production by *Aspergillus sp.* G-393

Scientific Note

S. L. WANG,*[,1] L. G. CHEN,[2]
C. S. CHEN,[2] AND L. F. CHEN[2]

[1]*Department of Food Engineering, Da-Yeh Institute of Technology, Chan-Hwa, Taiwan 51505, ROC; and* [2]*Department of Food Science, Purdue University, West Lafayette, IN*

Index Entries: Cellulase; xylanase; *Aspergillus*; production; agricultural wastes.

INTRODUCTION

Cellulosic material is the most abundant organic matter on the earth and is produced approximately at the rate of 100 billion tons each year through photosynthesis. However, utilization of cellulosic materials is limited by high processing costs to degrade cellulose and hemicellulose. Degradation of cellulosic materials using enzymes avoids many problems experienced with acid hydrolysis, such as inhibition of fermentation by undesirable byproducts. For this reason, enzymatic hydrolysis of cellulosic materials for fuel production has been an area of active research (*1–5*).

The production of inexpensive cellulolytic enzymes is an important element in the utilization of cellulosic materials. In this study, the agricultural waste was used to screen for an organism that has the ability to produce cellulolytic enzymes.

*Author to whom all correspondence and reprint requests should be addressed.

MATERIALS AND METHODS

Isolation and Screening of Cellulase Producing Fungi

First Screening

Microorganisms isolated from soils collected at five different locations in Taiwan were screened on agar plates containing 0.02% pulp (wood pulp), 0.1% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% yeast extract, and 1.5% agar. The pH was about 7, and wood pulp was used as the only carbon source at 37°C for 3–7 d. Those colonies that grew well under such environment, or those cultures that showed a clear zone around the colonies were isolated and retained for second screening. Two hundred and fifty fungal candidates were obtained from the first screening.

Second Screening

Those isolated organisms from the first screening were cultured in liquid media in flasks on a rotary shaker (37°C and 180 rpm). After incubation for 6 d, culture broth (medium containing different combinations of rice bran, wheat bran, and bagasse, as shown in Table 1) was centrifuged, and the supernatants were collected for measuring enzyme activities. Three out of 250 isolates from first screening showed a high enzyme activity in the culture broth. Among the three, the strain G-393, which was isolated from soils collected from northern Taiwan, showed the highest capability in producing both cellulase and xylanase. This strain was identified as a specie of *Aspergillus*, and it was used in this entire experiment for the production of cellulolytic enzymes. After studying the morphological, cultural, biochemical, and physiological characteristics of the microorganism, identification was carried out according to Raper and Fennell (*6*) and Klich and Pitt (*7*).

Preparation of the Enzymes

Fungal cell was removed from a slant and inoculated into a 250-mL Erlenmeyer flask containing 100 mL of culture medium. The culture was incubated in a rotary shaker (180 rpm) at 37°C. Culture samples were taken every 24 h. The samples were centrifuged at 10,000 × *g* in a refrigerated centrifuge to remove the cells. The supernatants were then taken for measuring enzyme activities. The experiment was carried out in duplicates, and average values were reported.

Assay of Cellulase Activity

Cellulase activity was measured with CMC as substrate. Enzyme solution (0.1 mL) was mixed with 0.4 mL of 1.25% (w/v) CMC in 1.25 m*M* acetate buffer (0.05*M*, pH 5.0). After the mixture was incubated at 37°C for 30 min, the amount of reducing sugar was determined by method of

Table 1
Effect of Percentage of Rice Bran, Wheat Bran, and Bagasse Added for the CM-Cellulases and β-Xylanase Production of *Aspergillus sp.* G-393

Percentage added to the medium			CM-cellulose activity, U/mL broth	β-xylanase activity, U/mL broth
Rice	Wheat	Bagasse		
5	0	0	0.228	3.892
0	5	0	0.213	1.993
0	0	5	0.149	1.898
2.5	2.5	0	0.235	3.181
0	2.5	2.5	0.277	3.086
2.5	0	2.5	0.320	4.746
1.67	1.67	1.67	0.277	4.224

Miller (*8*) with glucose as the reference compound. One unit of cellulase activity was defined as the amount of enzyme catalyzing the release of 1 μmol of reducing sugar/min, at 37°C and a pH of 5.

Assay of Xylanase Activity

Xylanase activity was measured with larch wood xylan as substrate. Enzyme solution (0.1 mL) was added to 0.4 mL of substrate solution, which contained 0.5% xylan in an acetate buffer solution (0.125*M*, pH 5.0), and the mixture was incubated at 37°C for 10 min. The mixture was centrifuged, and the amount of reducing sugar produced in the supernatant was determined according to Miller (*8*), with xylose as the reference compound. One unit of xylanase activity was defined as the release of 1 μmol of reducing sugar/min at 37°C and a pH of 5.

Enzyme Stability

In the pH stability test, the broth containing the enzymes was preincubated at 37°C and various pHs (buffers used were in a concentration of 25 m*M*) for 10 min. The enzyme activities were then determined after the addition of substrate solution buffered at pH 5 with 100 m*M* sodium acetate. In the testing of thermal stability, the enzyme solutions (culture broth) were incubated at various temperatures in buffered solution at a pH of 7.8 for 10 min. The buffered enzyme solutions were then chilled quickly in ice, and the remaining CM-cellulase and β-xylanase activities were assayed.

pH and Temperature Profiles of the Enzymes

The pHs of the enzyme activities were studied by mixing the enzyme solution (culture broth) with 50 m*M* buffer solution of various pHs and

Table 2
Optimum Culture Conditions for CM-Cellulase and β-Xylanase Production by *Aspergillus sp.* G-393

Medium (100 mL)	Rice bran	2.5%
	Bagasse	2.5%
	Pulp	0.3%
	K_2HPO_4	0.1%
	$MgSO_4 \cdot 7H_2O$	0.05%
Condition	Initial pH	7
	Temperature	37°C
	Cultivation time	6 d
	Oscillatory speed	180 rpm

were incubated at 37°C for 30 min for the assay of CM-cellulase activity and 10 min for xylanase activity. For temperature profile, the enzyme activities were studied at pH 5, and incubated at various temperatures for 30 min for cellulase and 10 min for xylanase.

RESULTS AND DISCUSSION

Effect of Different Carbon Sources on Enzyme Production

Three types of agricultural residues (rice bran, wheat bran, and sugar cane bagasse) were used as substrate for enzyme production by strain G-393. The results are shown in Table 1. The strain G-393 produced the highest enzyme activities in the culture broth containing 2.5% rice bran and 2.5% bagasse. Table 2 lists the optimal culture conditions for the production of CM-cellulase and xylanase.

Time-Course of Cultivation

During the process of incubation, CM-cellulase, β-xylanase, and pH in broth were measured every 24 h. The results are shown in Fig. 1. The pH increased steadily to a value of about 8.5. The CM-cellulase activity increased to a maximum of 0.32 U/mL within 6 d and remained constant thereafter. The activity of xylanase reached a maximum of about 4.7 U/mL by the fifth day, and the activity dropped slowly to a value of 2.4 U/mL by the 12th day.

pH and Thermal Stability of Enzymes

Figure 2 shows the pH stability of the crude enzymes. The enzyme CM-cellulase, produced by the strain G-393, retained its 90% activity within the pH range between 2 and 9. Xylanase was stable within the pH range between 4 and 10.5.

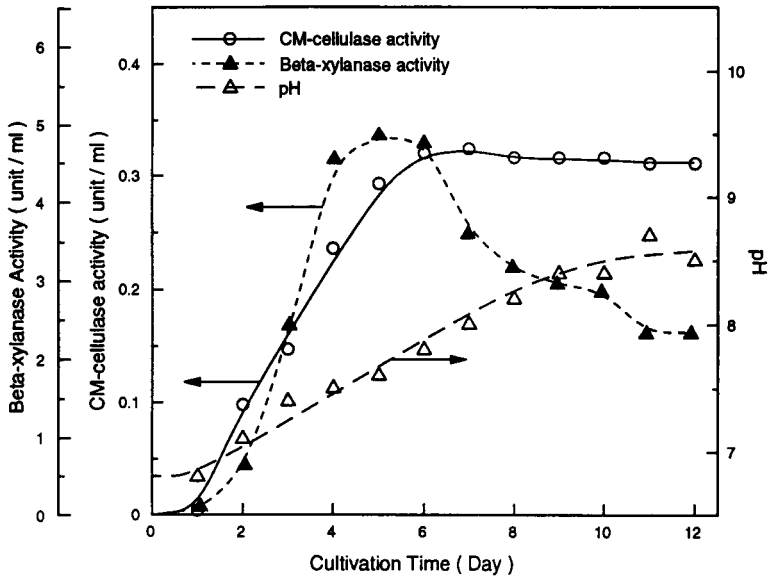

Fig. 1. Time-course of enzyme productions.

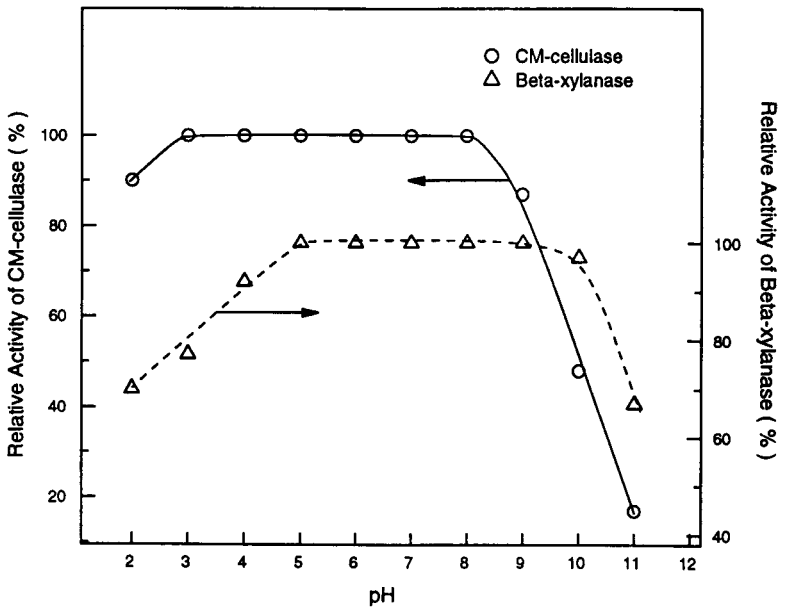

Fig. 2. pH Stability of the enzymes.

For thermal stability, xylanase was stable up to a temperature of 50°C, whereas CM-cellulase retained its activity (>90%) up to a temperature of 65°C (Fig. 3). These observations indicated that these enzymes can be used in cellulolytic reaction around 60°C; however, a long incubation time should be examined.

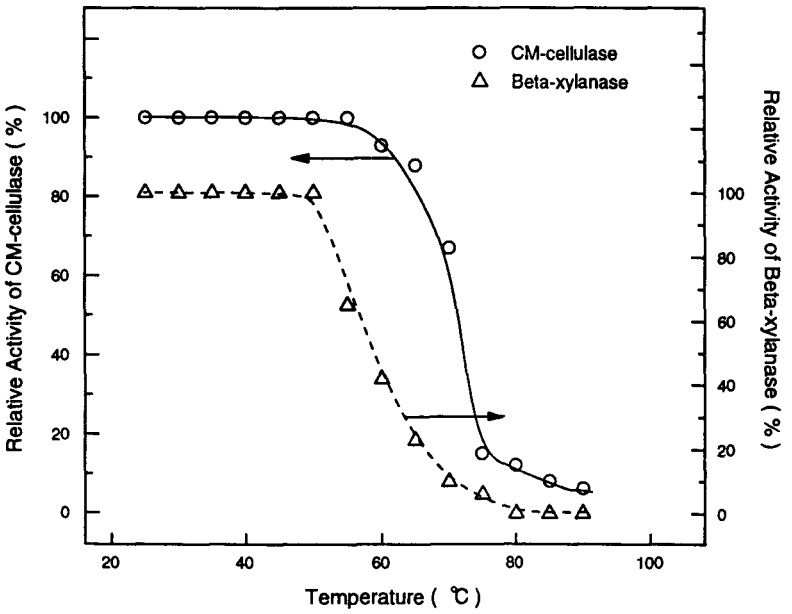

Fig. 3. Thermal stability of the enzymes.

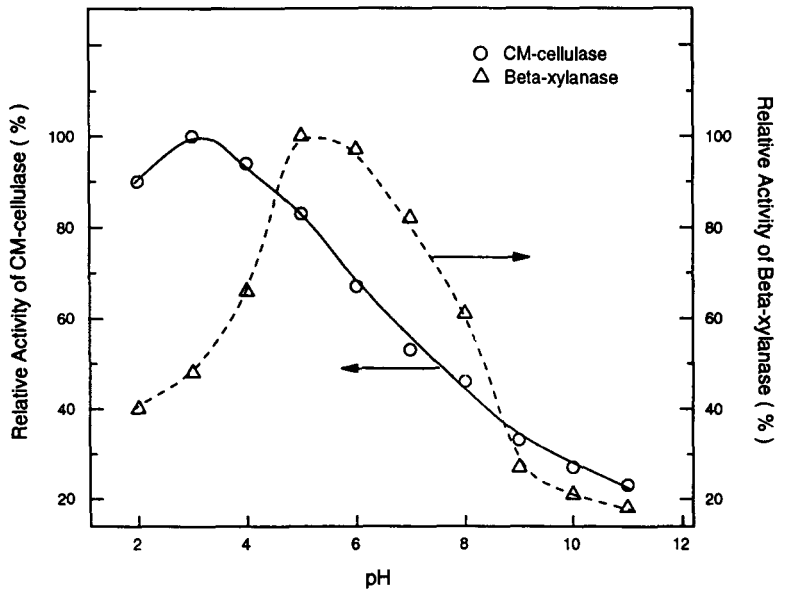

Fig. 4. Optimum pH of the enzymes.

Optimum pH and Temperature of the Enzyme Activities

Figure 4 shows that the optimum pH for CM-cellulase was 3 and xylanase was 5. At pH 2, the CM-cellulase activity retained 90% of its maximum. Such a low optimal pH of the CM-cellulase is desirable in saccharification of cellulose, because a low pH environment is more resistant to contamin-

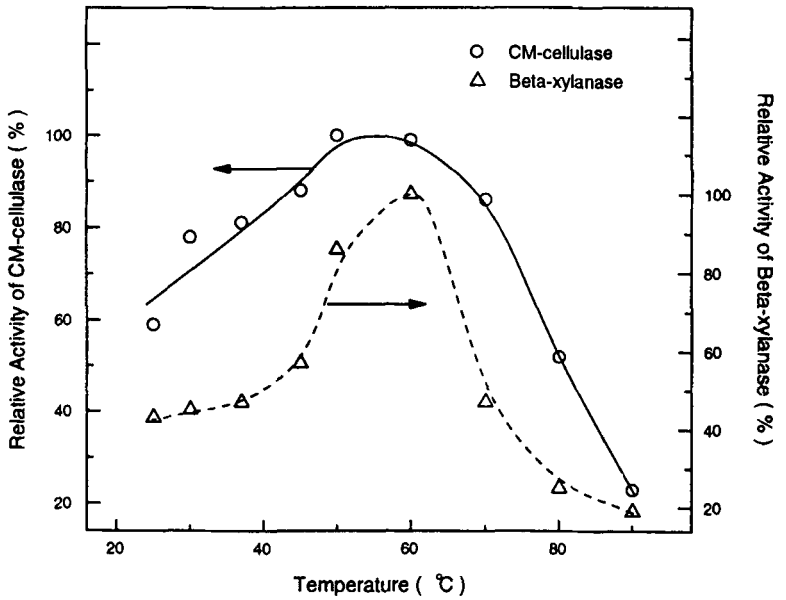

Fig. 5. Optimum temperature of the enzymes.

ations. However, this characteristic is not desirable, if a simultaneous saccharification and fermentation (SSF) type of process is desired in the case of ethanol production. The optimal temperature for CM-cellulase was 55°C, and for xylanase was 60°C (Fig. 5).

Comparison with Enzymes from Other Microorganisms

Cellulase produced by the strain G-393 is stable up to a temperature of 65°C, whereas different cellulases produced by other *Aspergillus* species are stable in a range from 30 to 50°C (*9*). The optimal pH of the cellulase produced by G-393 is 3, whereas that for cellulasese produced by *Aspergillus aculeatus* and *Trichoderma viride* is pH 5 (*9*).

Xylanase from G-393 is stable over a pH range from 4 to 10. The pH range of xylanase from *Aspergillus aculeatus* is 4 to 7 and from *Trichoderma viride* is 2 to 7 (*9*). The optimum pH of xylanse from the strain G-393 is the same as that produced by other *Aspergillus* species.

CONCLUSION

The production of cellulase and xylanase can be achieved by *Aspergillus sp.* G-393 using agricultural wastes. Enzymes produced by the strain G-393 were stable over wide range of pHs, and had an optimal temperature of about 60°C. These conditions are suitable for carrying out cellulolytic activities of biomass utilization. The results presented in this article represent only a shake-flask level of work, which needs to be qualified for real applications.

REFERENCES

1. Chahal, P. S., Chahal, D. S., and Andre, G. (1992), *J. Ferm. Bioeng.* **74(2),** 126–128.
2. Qu, Y., Zhao, X., Gao, P., and Wang, Z. (1991), *Appl. Biochem. Biotechnol.* **28/29,** 363–368.
3. Myburgh, J., Prior, B. A., and Kilian, S. G. (1991), *J. Ferm. Bioeng.* **72(2),** 135–137.
4. Mohagheghi, A., Grohmann, K., and Wyman, C. E. (1988), *Appl. Biochem. Biotechnol.* **17/18,** 263–277.
5. Rapp, P. and Wagner, F. (1986), *Appl. Environ. Microbiol.* **51(4),** 746–752.
6. Raper, K. B. and Fennell, D. I. (1965), *The Genera Aspergillus,* Williams & Wilkins, Baltimore, p. 686.
7. Klich, M. A. and Pitt, J. I. (1988), *A Laboratory Guide to Common* Aspergillus *Species and Their Teleomorphs,* CSIRO Division of Food Processing, Noth Rydo, N.S.W., p. 116.
8. Miller, G. L. (1959), *Anal. Chem.* **31,** 426–428.
9. Murao, S., Arai, M., and Sakamoto, R. (1987), *Cellulases,* Koudanshya, Japan, p. 112.