RESEARCH NOTE

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Characterization of Xylanase of *Cladosporium cladosporioides* H1 Isolated from Janggyeong Panjeon in Haeinsa Temple

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***Cladosporium cladosporioides* H1 was found to be the most abundant microbe in Janggyeong Panjeon. *C. cladosporioides* H1 produced a 20 kDa xylanase, which was generally stable below 60°C and had specialized activity in an acidic condition. Our results may lead to the development of a strategy for preservation of organic cultural heritage environments.**

KEYWORDS : *Cladosporium cladosporioides*, Janggyeong Panjeon, Xylan, Xylanase Activity

The Janggyeong Panjeon in Haeinsa temple, a United Nations Educational, Scientific and Cultural Organization (UNESCO) world heritage site, contains the Tripitaka Koreana (Palman Daejanggyeong), which was carved into 81,350 wooden printing blocks in the 13th century. The Tripitaka Koreana has been well-preserved in an impeccable condition over the last 7 centuries, a testimony to the outstanding achievements of medieval Koreans in science and technology with religious significances. However, the Tripitaka Koreana has recently become contaminated by harmful elements, especially fungi, due to changes in the environment. Deterioration of wooden round columns and square posts of Janggyeong Panjeon have been described [1, 2].

Hemicellulose is among the constituents of the wooden culture heritages such as the Janggyeong Panjeon and Tripitaka Koreana. Hemicellulose is a polysaccharide composed of various compounds of saccharides, which plays an important structural role in combination with cellulose and lignin. It is first invaded or degraded by microorganisms, prior to the decomposition of cellulose [3]. Thus, preventing microbial-mediated hemicellulose destruction may be a means of preserving the entire wooden structure. The major component of hemicellulose is xylan. Xylanase which degrade xylan, is an extracellular enzyme produced by microorganisms [4].

There have been few studies on the characteristics of microorganisms damaging organic cultural heritages. Also, the enzymatic characteristics of microorganisms in Janggyeong Panjeon have not been reported.

We described the purification and characteristics of xylanase produced by *Cladosporium cladosporioides* H1 isolated from Janggyeong Panjeon.

We isolated *Cladosporium cladosporioides* H1 from the atmosphere of Janggyeong Panjeon. *C. cladosporioides* H1 was the most abundant microbe in Janggyeong Panjeon. The organism had been previously demonstrated to be capable of degrading carboxymethyl cellulose, xylan, and lignin [5]. *C. cladosporioides* H1 was presently identified by sequencing using internal transcribed spacer primers and BLAST search.

C. cladosporioides H1 was maintained on solid potato dextrose agar medium. The spore suspension (3×10^6 spores) of *C. cladosporioides* H1 was added to a 250 mL Erlenmeyer flask containing 50 mL of medium composed of 0.5% oat spelt xylan, 0.1% yeast extract, 0.07% K_2HPO_4, 0.02% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, and 0.11% $MgSO_4 \cdot 7H_2O$ (pH 7.0). The strain was cultured on a rotary shaking incubator at 150 rpm and 28°C. The supernatant was collected as the crude enzyme containing fraction for the assay of xylanase activity. The thermostability of the crude enzyme was determined by incubation at 55, 60 and 65°C in pHs of 5, 7 and 9 from 1 to 3 hr [6]. Xylanase activities were assayed by using 1% oat spelt xylan as substrate in a 100 mM sodium phosphate buffer of pH 7.0. The enzyme was incubated for 30 min at 50°C. Reducing sugars produced as the result of these assays were determined using the dinitrosalicylic acid method [7]. Xylose was used as the standard. A unit enzyme activity was defined as the amount of enzyme that produces

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1 μmol of reducing sugar per min at 550 nm using a model UV-2550 spectrophotometer (Shimadzu, Tokyo, Japan) [8]. The control was *Aspergillus niger* KACC 43547, the official test fungus used in fumigation of cultural heritages, and which secretes a highly active xylanase.

Crude protein of supernatant was precipitated with ammonium sulfate between 30% to 80% saturation. The precipitate was dissolved in 10 mM acetate buffer (pH 5.5) and desalted by using Vivaspin 2 (Sartorius Stedim Biotech, Goettingen, Germany). The precipitated protein was added to a glass column of CM-Sepharose fast flow (GE Healthcare, Buckinghamshire, UK) equilibrated with the same buffer. The adsorbed xylanase was eluted with a stepwise gradient of 0~0.4 M NaCl. The xylanase activities in all fractions were measured. The molecular weight of eluted xylanase was confirmed by 10% SDS-PAGE and matrix-assisted laser desorption/ionization-time of flight spectrometry (MALDI-TOF) using an Ultraflex TOF/TOF apparatus (Bruker Daltonics Inc., Bremen, Germany). The protein concentration was determined by a bicinchoninic acid protein assay kit (Thermo Scientific, Pittsburgh, PA, USA), using bovine serum albumin as the standard.

C. cladosporioides H1 produced xylanase at a rapid rate. As with other studies, the level of xylanase production was related to the growth of the cells [6, 9]. To determine the viability of *C. cladosporioides* H1, we assessed the level of xylanase between *C. cladosporioides* H1 and *A. niger* KACC 43547 for 21 days. *C. cladosporioides* H1 showed the highest activity of xylanase at day 3 and activity declined after 5 days. The xylanase activity of *A. niger* exhibited a peak at 5 days and displayed a significant decrease after 9 days. Xylanase activity of several cellulolytic strains of fungi isdependent on pH. Xylanases of *C. cladosporioides* H1 and *A. niger* showed maximum activity at pH 5.0 (3.8 unit and 5.3 unit, respectively). Xylanase activities decreased gradually as the pH increased. This data is consistent with the previous report that the optimal activity of most xylanases occurs in acid conditions [10].

Next, we tested the thermostability for xylanase. Xylanase was incubated at indicated temperatures for 1, 2, and 3 hr prior to measuring its activity. As shown in Fig. 1A, the relative activities of xylanase from *C. cladosporioides* H1 were 66%, 46% and 26% at pH 5.0, 11, 15, respectively, and 8% at pH 7.0 after being incubated for 1 hr at 55, 60 and 65°C, respectively. On the other hand, the relative activities of xylanase from *A. niger* were 89%, 87% and 66% in samples incubated for 1 hr at pH 7.0 at 55, 60 and 65°C, respectively (Fig. 1B) and showed a significant decrease above 60°C at pH 5.0 (Fig. 1B). Taken together, these data indicate that the activity of xylanase from *C. cladosporioides* H1 varies depending on the pH and temperature, and suggests that its stability is lower than that of *A. niger*, especially at pH 7.0.

The purification steps of xylanase from *C. cladosporioides* H1 are summarized in Table 1. The total and specific activities of crude xylanase were 605.6 unit and 4.7 unit/mg, respectively (Table 1). The specific activity of xylanase precipitated by ammonium sulfate was 49.0 unit/mg, reflective of the 10.4-fold purification. Fig. 2 depicts the extraction of xylanase from the column at concentrations of 0.05 M NaCl. Xylanase purified using ion exchange chromatography on CM-Sepharose resulted in a major peak representing xylanase activity (Fig. 2A). CM-Sepharose

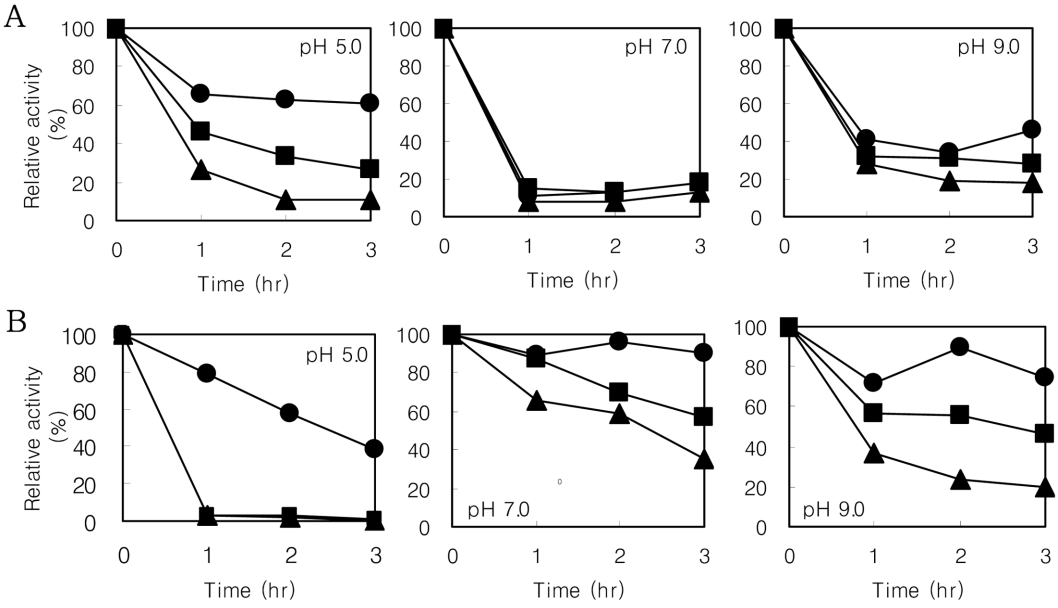

Fig. 1. Thermal stabilities (●, 55°C; ■, 60°C; ▲, 65°C) of xylanase from *Cladosporium cladosporioides* H1 (A) and *Aspergillus niger* KACC 43547 (B) at pH 5.0, 7.0 and 9.0.

Table 1. Purification of xylanase from *Cladosporium cladosporioides* H1

Step	Total protein (mg)	Total activity (unit)	Specific activity (unit/mg)	Purification (fold)	Yield (%)
Culture supernatant	127.5	605.6	4.7	1.0	100
Ammonium sulfate precipitation	8.5	417.8	49.0	10.4	69.0
CM-Sepharose chromatography	0.31	194.8	637.2	135.6	46.6

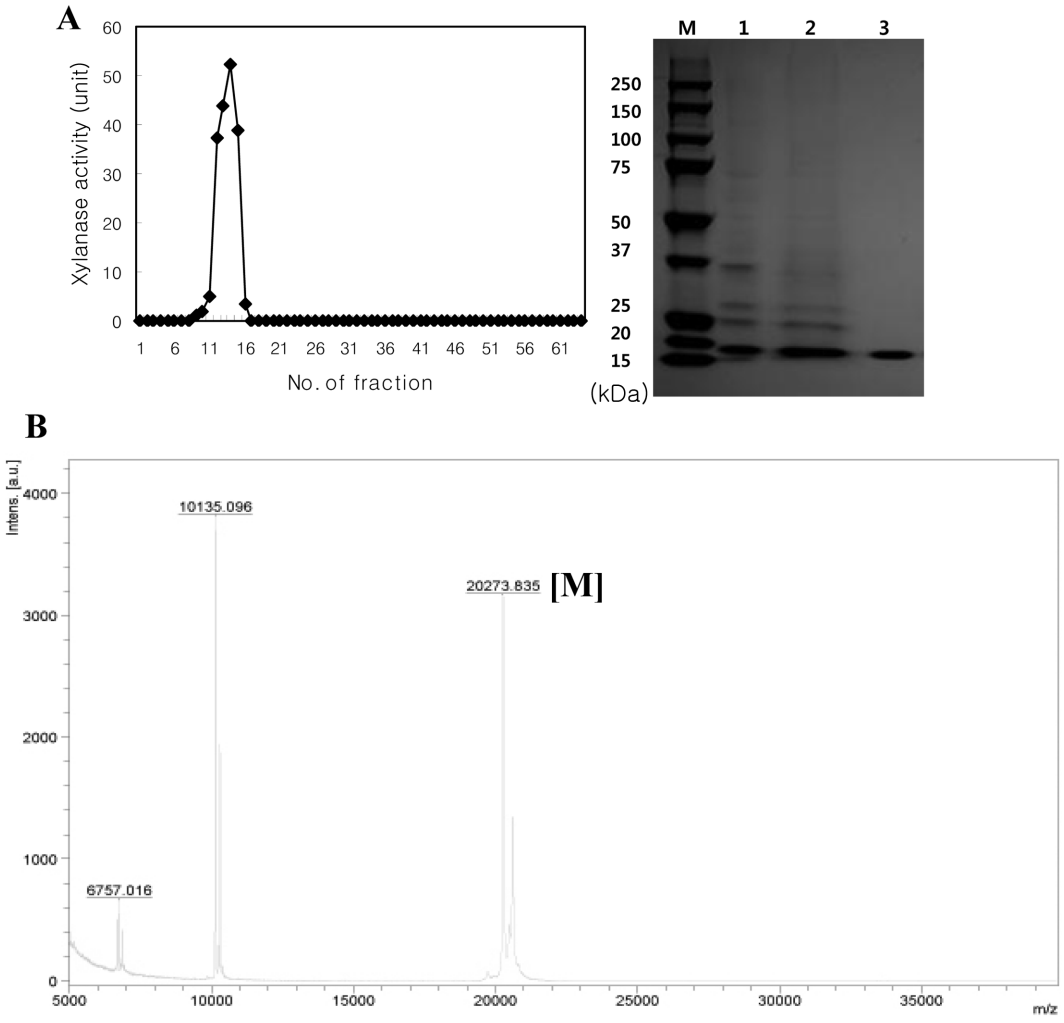

Fig. 2. Analyses of the crude and purified xylanase extract. A, Ion exchange chromatography of crude extract (left) and SDS-PAGE analysis of extracellular protein and purified xylanase from *Cladosporium cladosporioides* H1 (right). Lane M, standard protein (kDa); lane 1, culture supernatant; lane 2, 80% ammonium sulfate precipitate; lane 3, fraction after choromatography CM-Sepharose from *C. cladosporioides* H1 on CM-Sepharose; B, Matrix-assisted laser desorption/ ionization-time of flight spectrometry analysis of purified 20 kDa xylanase from *C. cladosporioides* H1.

chromatography showed that specific activities of xylanase in the fraction increased considerably from 49.0 to 637.2 unit/mg. The increased specific activities indicated a 135.6-fold purification.

To determine the product form of xylanase, the supernatant and precipitate from the culture were subjected to SDS-PAGE analysis. SDS-PAGE of the supernatant showed several major polypeptides. A single protein band with a molecular weight of approximately 20 kDa was detectable in the fraction eluted at 0.05 M NaCl (Fig. 2A, lane 3). We confirmed that the molecular weight of xylanase eluted at 0.05 M NaCl was 20 kDa through MALDI-TOF analysis (Fig. 2B).

In conclusion, this study shows that *C. cladosporioides* H1 in the air of Janggyeong Panjeon in Haeinsa Temple has a short phase of growth. *C. cladosporioides* H1 produces a 20 kDa xylanase. Although this xylanase is stable at various temperatures, it is especially active in

acidic conditions. In Korea, organic cultural heritages, including Janggyeong Panjeon, mostly consist of wood that is easily contaminated by fungi. Therefore, our results may lead to the development of a strategy for preservation of organic cultural heritages.

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