RESEARCH NOTE

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

Jin-Young Hong*, Young-Hee Kim, Mi-Hwa Jung, Chang-Wook Jo and Jung-Eun Choi

National Research Institute of Cultural Heritages, Daejeon 305-380, Korea

(Received October 7, 2011. Revised October 24, 2011. Accepted October 25, 2011)

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 × 10⁶ 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% K₂HPO₄, 0.02% KH₂PO₄, 0.1% (NH₄)₂SO₄, and 0.11% MgSO₄·7H₂O (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...
Characterization of Airborne Microbe in Janggyeong Panjeon 307

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 is dependent 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.](image-url)
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 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 chromatography 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.
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.

Acknowledgements

The purposes of this study, which was performed as a part of the project, have been achieved with the support of the Conservation Restoration Technology Research and Development project, which has been organized by the National Research Institute of Cultural Heritage of Cultural Heritage Administration.

References

1. Kim YS, Kim KH, Kim YS. Science of wood conservation. Gwangju: Jeonnam National University Press; 2004. p. 29-32.
2. Kim YS, Yoon JH, Kang HY, Park SJ. Deterioration and preservation technique of wooden cultural properties (part 1): biodeterioration of wooden columns, Janggeongpanjeon. Mokchae Konghak 2007;35:64-72.
3. Kim YS, Han SM. Deterioration and preservation technique of wooden cultural properties (part 2): biodeterioration of square post to support wooden printing blocks shelves, Janggeongpanjeon. Mokchae Konghak 2007;35:64-72.
4. Dekker RF. Biodegradation of the hemicellulose. In: Higuchi T, editor. Biosynthesis and biodegradation of wood components. Tokyo: Academic Press; 1985. p. 505-33.
5. Hong JY, Kim YH, Jung MH, Jo CW, Choi JE. Antifungal activities on organic heritage fungi and antioxidative effect of Phellodendron amurense extractives. J Korea TAPPI 2010; 42:54-61.
6. Ko CH, Lin ZP, Tu J, Tsai CH, Liu CC, Chen HT, Wang TP. Xylanase production by Paenibacillus campinasensis BL11 and its pretreatment of hardwood kraft pulp bleaching. Int Biodeterior Biodegradation 2010;64:13-9.
7. Martin C, Manzanares P. A study of the decolourization of straw soda-pulping effluents by Trametes versicolor. Bioresour Technol 1994;47:209-14.
8. Khasin A, Alchanati I, Shoham Y. Purification and characterization of a thermostable xylanase from Bacillus stearothermophilus T-6. Appl Environ Microbiol 1993;59: 1725-30.
9. Dhillon A, Khanna S. Production of a thermostable alkali-tolerant xylanase from Bacillus circulans AB 16 grown on wheat straw. World J Microbiol Biotechnol 2000;16:325-7.
10. Wong KK, Tan LU, Saddler JN. Multiplicity of β-1,4-xylanase in microorganisms: functions and applications. Microbiol Rev 1988;52:305-17.