Production, characterization and hydrolysation products of xylanase from *Bacillus subtilis* GN156

Presented in the 3rd International Conference for Value Added Agricultural Products (3rd FerVAAP Conference)

Jakkapan Pratumteep[1]
Jirakarn Sansernsuk[1]
Sunee Nitisinprasert[2]
Jirawan Apiraksakorn[1,3*]

Abstract

To improve the production of xylanase from *Bacillus subtilis* GN156, xylan and corncob powder were tested separately as inducers. By varying the concentrations of both inducers from 0-2 % (w/v), the xylanase production from xylan containing medium was higher than those from corncob powder containing medium in all treatments. The highest xylanase activity of 1.47 U/ml was obtained from the production medium supplement with xylan 1.5 % (w/v). The effects of pH and temperature on the enzyme activity and stability were also studied. Xylanase activity was effective at wide range of pH 4-7 with relative activity more than 75%, while its optimum pH and optimum temperature were shown at 5 and 40°C, respectively. Moreover, this enzyme was stable to the temperature up to 50°C for 30 min with the remaining activity more than 50 %. The hydrolysation products from the action of xylanase were investigated by using xylan as a substrate and determined by the technique of Thin Layer Chromatography (TLC). Xylose and 3 different kinds of xylo-oligosaccharides were detected as the products.

Keywords: xylanase, *Bacillus subtilis*, xylo-oligosaccharides, production

[1]*Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand*
[2]*Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand*
[3]*Fermentation Research Center for Value-Added Agricultural Products, Khon Kaen University, Khon Kaen 40002, Thailand*
**Corresponding author, e-mail: jirapi@kku.ac.th*

Introduction

Xylanase is a biological catalyst for xylan hydrolysis. Due to the xylanase properties which are very specific to substrate and active under mild condition without undesired byproducts contamination (Tan et al., 2008), xylanases have been used in industrial process for bleaching in pulp and paper industry (Dhillon et al., 2000; Sá-Pereira, et al., 2002), textile refinery and bioconversion of lignocellulosic materials to xylose and xylo-oligosaccharides (Vázquez et al., 2006). Xylose is needed as a substrate for xylitol and ethanol production, while xylo-oligosaccharides have been reported for various applications in food and pharmaceutical industries such as drug-released carriers, raw materials for degradable plastic, non-degradable fiber in functional food and prebiotics. According to the potential of xylo-oligosaccharides to support the health promoting bacteria and prevent cancer in digestive system (Nabarlatzet al.,2007; Wang and Zhang, 2006), xylanases and its products have been monitored.

Xylanases from various bacteria have been isolated and characterized. Bacterial xylanases range from acidic to alkaline and from moderate to extremely thermostable depending on the strain (Dey et al., 1992; Park et al., 1992; Blanco et al., 1995 Bataillon et al., 2000; Molares et al., 1993; Breccia et al., 1998; Dhillone et al., 2000). *Bacillus subtilis* GN156 has been reported for cellulolytic enzymes producting bacteria (Apiraksakorn et al., 2006). Among several cellulases and hemicellulases, xylanase is proposed to a high potential enzyme in applications. Therefore, the aims of this study were improve of xylanase production and characterization of xylanase from *Bacillus subtilis* GN156. Furthermore, the products from xylan hydrolysis were also studied.

Materials and Methods

1. Enzyme production

B. subtilis GN156 was grown in 5 ml Nutrient Broth (NB) medium under shaking at 150 rpm for 18-20 h at 37°C. One percent (v/v) of inoculum was transferred into 100 ml of NB, which contained 1.0 % (w/v) xylan, or corncob powder. After 18 h of incubation, the culture was centrifuged at 4°C, 10,000g for 10 min and the cell-free supernatant was stored at -20°C for further studies.

2. Determination of enzyme activities

Xylanase activity was determined by the modified method of Okeke and Obi (1995) by performing the reaction mixture of 0.1 ml of cell-free supernatant and 0.1 ml of 1% (w/v) xylan (Sigma) in 50 mM citrate phosphate buffer pH 5.5 at 50°C for 20 min. The amount of reducing sugar released was determined by Dinitrosalicylic acid (DNS) method (Miller, 1959). One unit of enzyme was defined as the amount of enzyme that released xylose 1 μmol per min.

3. The effect of pH on xylanase activity

The optimum pH was determined from the reactions of the enzyme and xylan in various pH of the buffers (citrate phosphate buffer pH 3-6, phosphate buffer pH 7-8) at 50°C for 20 min.

4. The effect of temperatrue on xylanase activity

The optimum temperature was determined from the enzyme activities at various temperatures of 20-70°C for 20 min under the optimum pH and buffer.

The effect of temperature on enzyme stability was measured the remaining activity after incubating the enzyme in optimum pH buffer at

various temperatures (20-7°) for 30 min. Reaction of the enzyme was performed min under the optimum pH and temperature for 20 min.

5. Thin layer chromatography (TLC) analysis

The action pattern of xylanase on xylan hydrolysis was determined by the modified method of Apiraksakorn et al. (2008) for the thin layer chromatography (TLC) with xylose, glucose and cellobiose (Sigma) as standards. Two µl of the hydrolysation products containing 2 µg/µl reducing sugar were applied on TLC plate (Kieselgel 60, Merck) and developed in mixture solvent of butanol : isopropanol : ethanol : deionized water in the ratio of 2 : 3 : 3 : 2, respectively. The TLC plate was dipped in 0.2 % (w/v) orcinol in 10 % (v/v) sulfuric acid in ethanol and further placing in 100°C for 15 min.

Results and Discussion

The effect of carbon sources on xylanase production

B. subtilis GN156 is a cellulosic enzyme producing bacteria. It can produce many enzymes revealed with cellulosic material hydrolysis such as β-1,3-1,4-glucanase, carboxymethylcellulase and xylanase (Apiraksakorn et al., 2006). However, xylanase activity of 0.56 U/ml was produced by using 1% (w/v) carboxymethylcellulose (CMC) as a carbon source (Apiraksakorn et al., 2006). To improve the production of xylanase from *Bacillus subtilis* GN156, xylan and corncob powder were tested separately as carbon sources. Considering corncob component, it composes of xylan 35-40 % which is the highest amount of xylan found from agricultural residues (Tan et al., 2008, Yang et al., 2005, Yang et al., 2006). Therefore, corncob powder was chosen as a natural xylan source for comparing with commercial xylan (Sigma). By varying the carbon source concentrations from 0-2 % (w/v), the xylanase production from xylan containing medium was higher than those from corncob powder containing medium in all treatments (Figure 1).

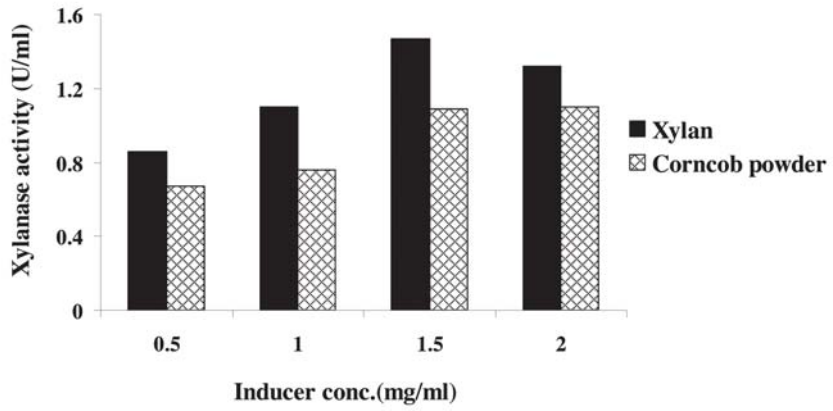

Figure 1. Effect of xylan and corncob powder on xylanase production.

Moreover, xylanase was significantly higher produced in all treatments than those from the control with no carbon source added. This would be described by the concept of gene expression that xylanolytic enzymes is regulated by the carbon source in the medium and the induction of xylanase take place in the presence of an inducer molecule (Parachin et al., 2009). The result from this study confirmed that xylanase is an inducible enzyme, its activity could be enhanced along with an increase of the appropriate inducer concentrations. The highest xylanase activity of 1.47 U/ml was obtained from the production medium supplement with xylan 1.5 % (w/v), which was 2.6 fold higher than those of previously reported by using 1% (w/v) CMC as the inducer (Apiraksakorn et al., 2006). Consider to same concentration of the inducer at 1 % (w/v), the xylanase production from xylan, corncob powder and CMC were 1.10, 0.76 and 0.56 U/ml, respectively. Therefore, xylan was the most suitable carbon source for xylanase induction.

The effect of pH on xylanase activity

The optimum pH of xylanase was determined at 50°C of various pH values from 3-8. The highest activity was shown at pH 5, while xylanase activity was effective at wide range of pH 4-7 with relative activity more than 75% (Figure 2). Generally, xylanases from *Bacillus* sp. show optimum pH around neutral at pH 6-7, those have been proposed from *Bacillus* sp. NCIM 59 (Dey et al., 1992), *Bacillus sp.* YC335 (Park et al., 1992), *Bacillus* sp. SPS-0 (Batailon et al., 2000), *Bacillus amyloliquefaciens* (Breccia et al., 1998) and *Bacillus circulans* AB16 (Dhillon, et al., 2000). In contrast, xylanase $X_{34}E$ from *Bacillus polymyxa* (Molares et al., 1993) and xylanase from *Bacillus* sp. BP-23 (Blanco et al., 1995) show optimum pH in acidic range of 4-6 and 5.5, respectively.

According to the optimum pH xylanase from *B. subtilis* GN156 can be grouped into acid xylanase. Even alkaline xylanases are more familiar due to their application in the pulp and paper industry, but a few xylanases in acid group could be a key enzyme to improve the bioconversion of lignocellulosic materials (Parachin et al., 2009).

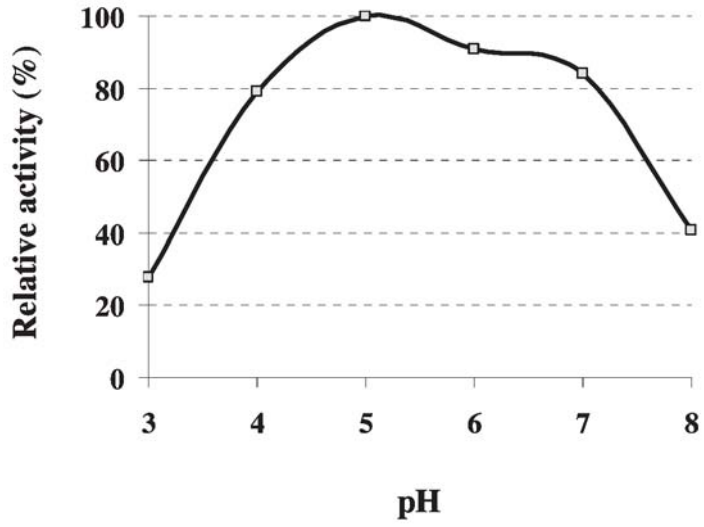

Figure 2. Effect of pH on xylanase activity from *B. subtilis* GN156 was carried out at 50°C for 20 min.

The effect of temperature on xylanase activity

The optimum temperature for xylanase was observed at various temperatures from 20-70°C. The highest xylanase activity was at 40°C, while it could be effective in the range of from 30-60°C with the relative activity more than 70 % as shown in Figure 3. Considering stability of xylanase on temperatures, it was stable at 20-50°C for 30 min with remaining activity around 55 % (Figure 4).

Xylanases from *Bacillus* sp. usually effective at the temperature around 50-60°C (Dey et al., 1992; Park et al., 1992; Blanco et al., 1995), except those from *Bacillus* sp. SPS-0 (Bataillon et al., 2000) which showed optimum and temperature stability at 75°C and 70°C, respectively and it could be classified as thermostable xylanase.

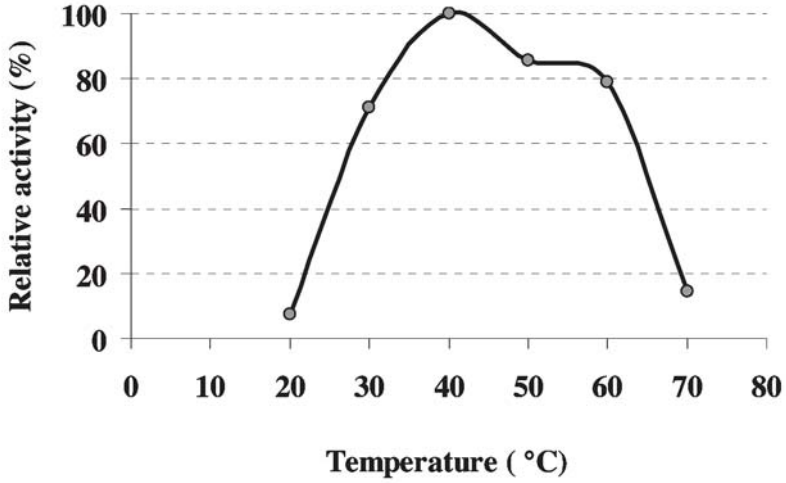

Figure 3. Effect of temperature on xylanase activity from *B. subtilis* GN156 was carried out in citrate phosphate buffer pH 5 for 20 min.

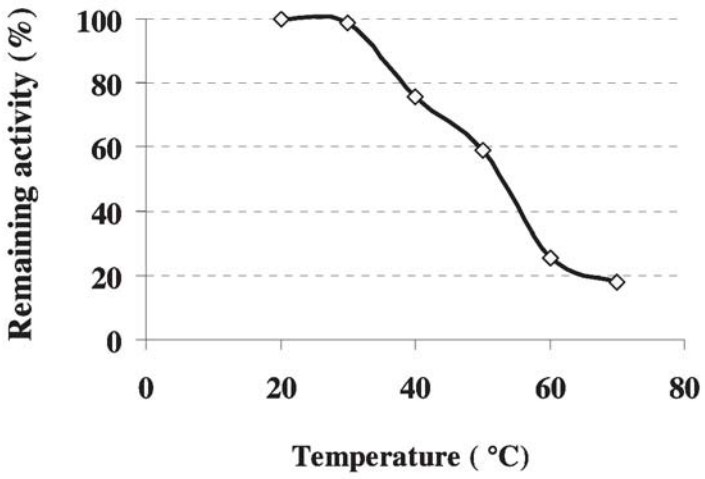

Figure 4. Temperature stability of xylanase was treated at various temperatures for 30 min.

The hydrolysation product

The degradation products from the action of xylanase on xylan were analyzed by thin layer chromatography and the result was shown as Figure 5. Considering the product mobilities compared to those of the standard; xylose, glucose and cellobiose, the results shows that the xylan degradation products from xylanase activity were xylose and 3 different kinds of xylo-oligosaccharides. From the action pattern, it indicates that xylanase from *B. subtilis* GN156 was endo-xylanase.

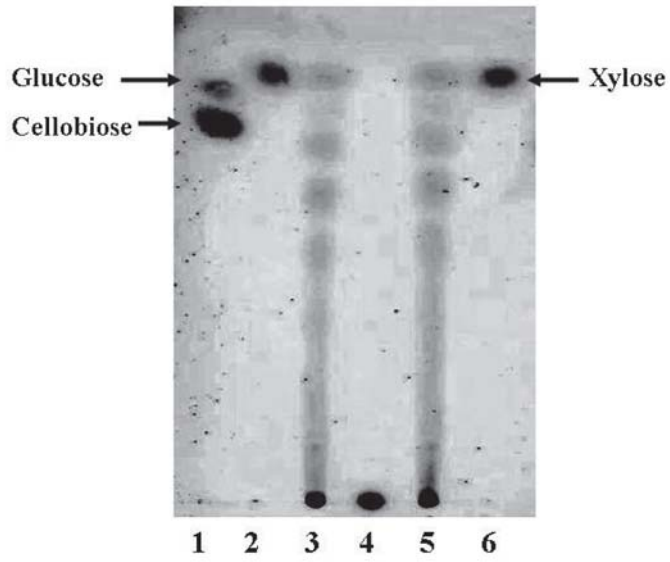

Figure 5. Thin layer chromatography of xylan hydrolysation products by xylanase from *B. subtilis* GN156. 1:glucose and cellobiose; 2, 6: xylose; 3, 5: hydrolysation products; 4: xylam

Xylo-oligosaccharides are the mixture of oligosaccharides derived from break down the ß-1,4-linkages of xylan-containing substrate with endo-xylanase. Normally, the major products are xylobiose and xylotriose (Yang et al., 2006). The hydrolysis patterns of xylanases from *Bacillus* sp. NCIM 59 demonstrated that xylanases were endoxylanase. They yielded mainly xylobiose, xylotriose, and higher xylooligosaccharides, with traces of xylose from xylan. (Dey et al., 1992). Purified xylanase from *Bacillus* sp. strain SPS-0 showed the products of bichwood xylan hydrolysis were xylose, xylobiose, xylotriose and xylotetraose, while the major products of wheat bran arabinoxylan and oat spelt xylan hydrolysate were xylode, xylobiose and arabinoxylan (Bataillon et al., 2000). From several previous reports, it is concluded that bacterial xylanases release various products depend on strain of organisms and substrates used.

Conclusion

Xylanase from *Bacillus subtilis* GN156 was an inducible enzyme, its activity could be enhanced along with an increase of xylan and corncob powder. The highest xylanase activity of 1.47 U/ml was obtained from the production medium supplement with xylan 1.5 % (w/v). Xylanase showed optimum pH and optimum temperature at 5 and 40°C, respectively. It was stable at 20-50°C for 30 min with remaining activity around 55 %. The hydrolysation products from the action of xylanase were xylose and 3 different kinds of xylo-oligosaccharides. From the action pattern, it indicated that xylanase from *B. subtilis* GN156 was an endo-xylanase.

Acknowledgements

Appreciation is expressed to Yongsuwat Agritrade Co. Ltd. for supporting corncob powder through all of this research. Special thanks to Assoc. Prof. Dr. Rasana Wongratanacheewin, Assoc. Prof. Dr. Pattana Laopaiboon, Dr. Korakoch Hamsupo and Dr. Prekamol Kranrit for their assistances.

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