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Cultural condition improvement for xylanase production by *Bacillus subtilis* GN156

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Abstract

Xylanase is important enzyme widely used in industrial process for xylose, xylitol, and xylooligosaccharides production, which is multipurpose used in food and pharmaceutical industries. To increase the production of xylanase from *Bacillus subtilis* GN156, the physical parameters were focused. By varying temperature (25, 30 and 40 °C), shaking speed of the incubator shaker (150 and 200 rmp) and pH of culture medium (4-8), the optimum condition for xylanase fermentation was found at pH 7, 30ºC and 200 rpm. The maximum xylanase was 1.86 folds higher than those from the production at the non-optimization condition at 37°C, 200 rpm and without pH control. Therefore, temperature, pH and speed of the incubator shaker were the affecting factors to xylanase production by *B. subtilis* GN156.

Keywords: xylanase, *Bacillus subtilis*, optimization

1. Introduction

Xylanase is involved in xylan degradation by hydrolyse linear polysaccharide of β-1,4-xylan backbone into oligomers and xylose (1). In commercial applications, xylanase is used as the hydrolysing agent to hydrolyse xylan and release lignin from woods in paper and pulp industry and also the bleaching agent to reduce the level of chlorine usage (2). Moreover, xylanase is required for bioconversion of lignocellulosic material and agro-wastes to fermentative products, clarification of juices, improvement in consistency of beer and the digestibility of animal feed stock (3). Xylose, xylitol, and xylooligosaccharides, multipurpose products for food and pharmaceutical industries are produced using xylanase as the key enzyme. Not only xylanase could be applied to use in many field, but the catalysis mechanism is also specific to substrate and avoid unwanted reactions occur. Therefore, an effective xylanase production is still interesting.

Among various xylanase producing bacteria (4-8), *Bacillus subtilis* GN156 has been proposed for xylanase production (9) and xylo-oligosaccharides preparation (10). Thus, to increase the xylanase activity,

the cultural condition was monitored. According to many previous studies, cultivation time, pH and temperature were identified as the major factors to xylanase production by *Bacillus* sp. (11-14). Therefore, the objective of this study was to increase the production of xylanases from *B. subtilis* GN156 by fousing on the physical parameters which were pH of culture medium, temperature and speed of the shaker.

2. Materials and Methods

1. Microorganism and inoculums preparation

B. subtilis GN156 was grown in 5 ml Nutrient Broth (NB) medium under shaking at 150 rpm for 18-20 h at 37°

2. Effect of temperature on xylanase production

One percent (v/v) of inoculums was transferred into 20 ml of NB, which contained 1.5 % (w/v) corncob powder and incubated for 18 h with shaking speed of 150 rpm at 25, 30 and 40°C. The culture broth was individually centrifuged at 4°C, 10,000g for 10 min and the cell-free supernatants were collected for assay the xylanase activity

3. Effect of shaking speed on xylanase production

Bacterial cultivation was followed the same procedure as described in (2) except incubated at optimal temperature from the previous experiment for 18 h under shaking at 150 and 200 rmp in the incubator shaker.

4. Effect of pH on xylanase production

One percent (v/v) of inoculums was added into 100 ml NB containing 1.5 % (w/v) corncobs dust. The pH was adjusted at 4, 5, 6, 7 and 8. The incubation was taken place at optimal temperature and shaking speed for 18 h. Then the experiment was followed the same method as described above.

5. Determination of enzyme activities

To determine xylanase activity, the crude enzyme was mixed with 0.5% (w/v) xylan in citrate phosphase buffer pH 5 and incubated at 50°C for 15 min. The reaction mixture was subjected to analyze reducing sugar by 3,5-Dinitrosalicylic acid (DNS) method (15). One unit of xylanase activity was defined as the amount of enzyme that could release reducing sugar equivalent to xylose 1 μmol per min

3. Results and Discussion

1. Effect of temperature on xylanase production

B. subtilis GN156 produced maximum xylanase at 30°C as illustrated in Figure 1. The xylanase activity was increased 2.4 and 1.3 times than those at 25°C and 40°C, respectively. Generally, the optimum temperature for xylanase production in submerged fermentation was around 28-32.5°C, which has been reported at 28°C for *Aspergillus niger* (14) and 30-32.5°C for *Penicillium oxalicum* ZH-30 (12). Corresponded to our result, the incubation temperatures lower than 28°C or over 40°C were not suitable for enzyme production. However, the optimized temperature at 40°C was found in solid state fermentation (SSF) by *B. pumilus*, which was recognized as thermophilic microorganism (16).

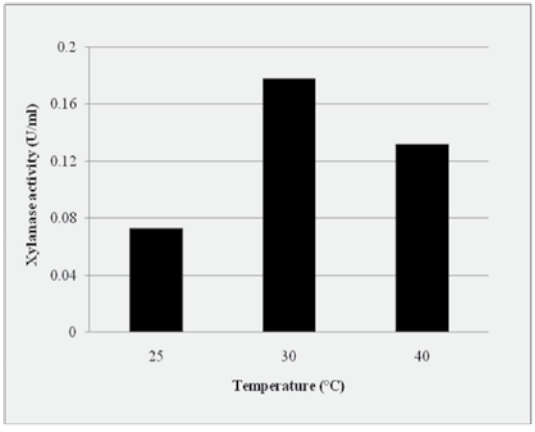

Figure 1. Effect of temperature on xylanase production by *B. subtilis* GN156

2. Effect of shaking speed on xylanase production by *B. subtilis* GN156

For fermentation improvement, aeration is one of the affecting factors to aerobic fermentation. To optimize the aerobic condition in shake flask cultivation, the experiments were carried out under the variation of shaking speed at 150 and 200 rpm. As shown in Figure 2, the highest enzyme production was obtained at 200 rpm, which was the same optimum agitation rate for xylanase production by *B. subtilis* ASH (13). Therefore, xylanase production depended on speed of incubator shaking, since higher speed provided more efficient for uniform distribution of nutrient and supplied oxygen for the cultivation (13).

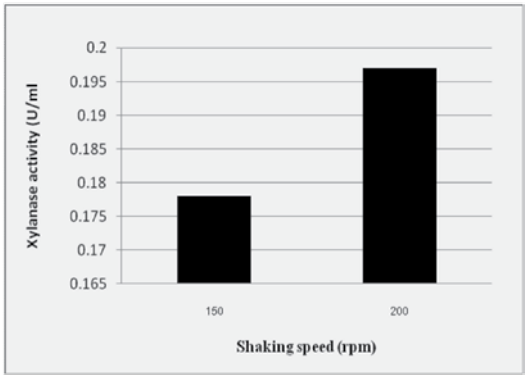

Figure 2. Effect of shaking speed on xylanase production by *B. subtilis* GN156

3. Effect of pH on xylanase production by *B. subtilis* GN156

The optimal pH for xylanase production by *B. subtilis* GN156 was shown in the range of 6-7 (Figure 3) which was same pH as reported previously for xylanases fome *P. oxalicum* ZH-30 (12), *B. subtilis* ASH (13), *B. subtilis* (17) and *Bacillus* sp.AQ-1 (18). Normally, the optimum pH for enzyme reaction corresponds to the optimum pH for enzyme production condition, but the optimum pH of the reaction and production of *B. subtilis* GN156 xylanase were slightly different which shifted from pH 5 to 6-7. However, it still was grouped into acid xylanase according to the optimum pH of the reaction (10) and suitable for the bioconversion of lignocellulosic materials (19).

Unlike some alkaline tolerant xylanases which were produced under optimum pH condition at pH 9 (20; 11; 21) and these kinds of xylanase were aimed to use in the pulp and paper industry to decrease the consumption of chlorine bleaching.

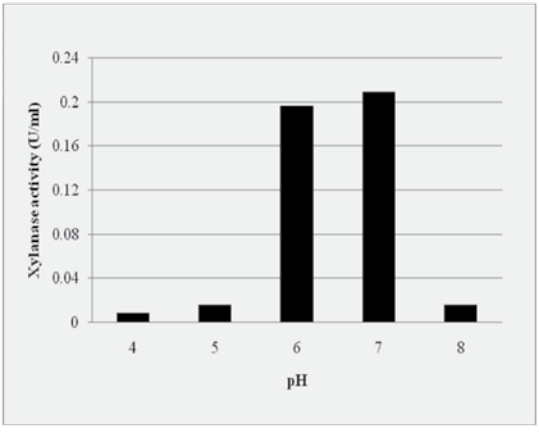

Figure 3. Effect of pH on xylanase production by *B. subtilis* GN156

4. Optimal condition for xylanase production by *B. subtilis* GN156

Under the optimized condition at 30ºC, pH 7 and 200 rpm, xylanase was produced 1.86 folds higher

than that from the control condition at 37°C, 150 rpm and without pH control. The xylanase production enhancement could be provided by optimizing the physical factors, which was confirmed by several studies as summaried in Table 1. It was noticed that the optimized conditions were similar even though different strains were used and resulted in 2-11 folds increase in xylanase production as compared to un-optimized condition (13; 12; 14). Therefore, temperature, pH and shaking speed affected to xylanase production by *B. subtilis* GN156. Furthermore, to obtain the maximum xylanase activity, not only the physical factors but chemical factors especially medium composition should be concerned.

Table 1. Comparison of xylanase production enhancement under the optimized condition by various microorganisms

Microorganisms	Cultural condition	Enhancement (fold)	Reference
B. subtilis GN156	30ºC, pH 7 and 200 rpm	1.86	This study
B. subtilis ASH	pH 7 and 200 rpm*	2.51	(13)
P. oxalicum ZH-30	31.1ºC and pH 7.38	10.6	(12)
A. niger	28ºC and 180 rpm	-	(14)

*By using the optimized media

3. Conclusion

Xylanase could be produced by *B. subtilis* GN156. To enhance its activity, physical parameters revealing with fermentation were monitored. The highest xylanase activity was obtained from cultivation temperature at 30°C. The optimal shaking speed was shown at 200 rpm. The pH of culture medium also effected on xylanase fermentation and the optimal pH for xylanase production was around normal at pH 6-7. Then, xylanase production under the optimized and non-optimization condition was compared; higher activity of 1.86 folds was achieved under the improved condition.

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5. References

(1) Bastawde KB. Review: Xylan structure, microbial xylanases, and their mode of action. Would J. Microbiol. Biotech. 1992; 8: 353-68.

(2) Shoham Y, Schwartz Z, Khasin A, Gat O, Zosim Z, Rosenberg E. Delignification of wood pulp by a thermostable xylanase from *Bacillus stearothermophilus* strain T-6. Biodegradation. 1992; 3: 207-18.

(3) Wong KKY, Tan LUL, Saddler JN. Multiplicity of β-1,4-xylanase in microorganisms: Functions and applications. Microbiol. Rev. 1988; 52(3):

305-17.

(4) Breccia JD, Siñeriz F, Baigori MD, Castro GR, Kaul RH. Purification and characterization of a thermostable xylanase from *Bacillus amyloliquefaciens*. Enzyme Microb. Technol. 1998; 22(1): 42-9.

(5) Dey D, Hinge J, Shendye A, Rao M. Purification and properties of extracellular endoxylanases from alkalophilic thermophilic *Bacillus* sp. Can. J. Microbiol. 1992; 38: 436-42.

(6) Duarte MCT, Portugal EP, Ponezi AN, Bim MA, Tagliari CV, Franco TT. Production and purification of alkaline xylanases. Bioresource Technol. 1999; 68(1): 49- 53.

(7) Morales P, Madarro A, González JAP, Sendra, JM, Piñaga F, Flors A. Purification and characterization of alkaline xylanases from *Bacillus polymyxa*. Appl. Environ. Microbiol. 1993; 59(5): 1376-82.

(8) Ragauskas A, Poll KM, Cesternino AJ. Effect of xylanase pretreatment procedures on nonchlorine bleaching of kraft pulps. Enzyme Microb. Technol. 1994; 16: 492-5.

(9) Apiraksakorn J, Buwjoom T, Nitisinprasert S. Characterization of grass degrading bacteria active on ß-1,3-1,4-D-glucans from *Bacillus subtilis* GN156 potential use for grass silage-making. Kasetsart J. (Nat. Sci.). 2006; 40: 136-47.

(10) Pratumteep J, Sansernsuk J, Nitisinprasert S, Apiraksakorn J. Production, characterization and hydrolysation products of xylanase from *Bacillus subtilis* GN156. KKU Res. J. 2010; 15(5): 343-50.

(11) Bocchini DA, Alves-Prado HF, Baida LC, Roberto IC, Gomes E, Da Silva R. Optimization of xylanase production by *Bacillus circulans* D1 in submerged fermentation using response surface methodology. Process Biochem. 2002; 38(5): 727-31.

(12) Li Y, Liu Z, Zhao H, Xu Y, Cui F. Statistic optimization of xylanase production from ner isolated Penicillium oxalicum ZH-30 in submerged fermentation. Biochem. Eng. J. 2007; 34: 82-6.

(13) Naga S, Carg N, Sanghi A, Kuhad RC, Gupta VK. Enhancement of xylanase production by *Bacillus subtilis* ASH in submerged fermentation using response surface methodology. Int. J. Microbes Environ. Management. 2010; 1(1): 1-9.

(14) Yuan Q, Wang J, Zhang H, Qian Z. Effect of temperature shift on production of xylanase by *Aspergillus niger*. Process Biochem. 2005; 40(10): 3255-7.

(15) Miller GL, Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959; 31(3): 426–8.

(16) Kapilan R, Arasaratnam V. A novel *Bacillus pumilus* strain for alkaline xylanase production at and above 40°C. Cey. J. Sci. 2010; 39(1): 61-70.

(17) Sá-Pereura P, Mesquita A, Duarte JC, Barros MRA, Costa-Ferreira M. Rapid production of thermostable cellulose-free xylanase by a strain of *Bacillus subtilis* and its properties. Enzyme Microb. Technol. 2002; 30(7): 924-33.

(18) Wahyuntari B, Mubarak NR, Setyahadi IS. Effect of pH, temperature and medium composition on xylanase production by *Bacillus* sp. AQ-1 and partial characterization of the crude enzyme. Microbiol. Indones. 2009; 3(1): 17-22.

(19) Parachin NS, Siqueira S, de Faria FP, Torres FAG, de Moraes LMP. Xylanase from *Cryptococcus flavus* isolate I-11: Enzymatic profile, isolation and heterologous expression of *CfXYN1* in *Saccharomyces cerevisiae*. J. Molecular Catalysis

B: Enzymatic. 2009; 59(1): 52-7.

(20) Annamalai N, Thavasi R, Jayalakshmi S, Balasubramanian T. Thermostable and alkaline tolerant xylanase production by *Bacillus subtilis* isolated from marine environment. Indian J. Biotechnol. 2009; 8(3): 291-7.

(21) Kapilan R, Arasaratnam V. Paddy husk as support for solid state fermentation to produce xylanase from *Bacillus pumilus*. Rice Sci. 2011; 18(1): 36-45.