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Optimization of Production Xylanase from Marine Bacterium *Bacillus safensis* P20 on Sugarcane Baggase by Submerged Fermentation

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***Abstract*— Endo-1, 4-β-xylanase commonly called xylanase is an industrially important enzyme which degrades of lignocellulosic materials to sugar, alcohol and other useful product. The use of commercially xylan is too expensive for use at industrial scale production. For commercial applications, xylanases should ideally be produced from simple and inexpensive substrates. Indonesia has abundantly agro-residues such as sugar cane bagasse which is attractive to be used as carbon sources for the production of enzyme. In this study, optimization of fermentation condition extracellular xylanase from marine bacterium, *Bacillus safensis* P20 has been conducted by using sugarcane bagasse as carbon source under sub merged fermentation (SMF). Maximum xylanase production was obtained at sugar cane bagasse concentration 1.5%, pH medium 7, and temperature fermentation 20^{o}C, lactose as a carbone source and urea as a nitrogen source with activity 4.06 U/mL for 96 hours.**

***Keywords*— marine bacterium, sub merged fermentation, endo-1, 4-β-xylanase, lignocellulosic, sugar cane bagasse**

I. INTRODUCTION

Xylan is the major component of hemicelluloses which is the second most abundant renewable resources in nature. Xylaneses, in conjuction with cellulolytic enzymes, can be considered for the conversion of hemicelluloses materials. Conversions of hemicelluloses to valuable products by xylanases hold strong promise for industrial applications, such as baking and brewing industry, animal feed, bioethanol, production of xylooligosaccharides, biobleaching, kraft process, bread fluffier, baking, fruit juice and beer clarification, improving silage, bioconversion lignocellulose to fermentative products([1], [2]).

Most xylanase manufacturers produce these enzymes using submerged fermentation (SmF) techniques, in fact SmF as a producing system accounts nearly for 90% of total xylanase sells world wide ([3], [4]). Enzyme production by submerged fermentation allows better control of the conditions during fermentation [5]. For commercial applications, xylanase production by using xylan commercial is very expensive. The choice of the substrate is of great importance for the selection of the fermentation process and the successful production of xylanases. For large scale processes other alternatives have to be considered due to the cost of such substrates [4]. Indonesia has abundance biomass containing hemicellulosa such as, palm cernel cake, empty fruit bunch and sugarcane bagasse that a useful source of xylan that more attractive to be used as carbon sources for the production of xylanase. Optimization of media and fermentation conditions are the most important factors in xylanase production by using biomass as a substrat.

Xylanase has been found in a wide variety of living organisms, including marine and terrestrial bacteria. At present, there is an increasing interest in searching for new sources of xylanases. The utilization of marine bacteria to produce xylanase has not been used widely in Indonesia. *Bacillus* sp. is used more extensively than other bacteria in industrial fermentations since they secrete most of their enzymes. The purpose of this reasearch are to obtaine the optimum condition of xylanase production from marine bacterium, *Bacillus safencis* P20 using sugar cane bagasse by sub merged fermentation (SMF).

II. METHODOLOGY

A. Microorganism

Xylanase production was carried out using *Bacillus safencis* P20 from marine bacterium collection of Biocatalyst and Fermentation Laboratory, Research Center for Biotechnology, LIPI, Cibinong - Bogor.

B. *Biomass preparation*

Sugarcane bagasse, empty fruit bunch were obtained from Research Center for Biomaterial LIPI. Palm cernel cake was obtained from collection of Biocatalyst and Fermentation Laboratory, Research Center for Biotechnology, LIPI,Cibinong-Bogor.

C. *Screening of biomass as a substrate for xylanase production*

Indonesia biomass such as, sugar cane bagasse, empty fruit bunch and palm cernel cake were selected as a substrate for xylanase production by *Bacillus safencis* P20. Screening was conducted by using Congo Red methods. The biomass was selected as a substrate by xylanase production on xylan agar by observing zone of clearance.

D. *Optimation culture condition*

Bacillus safensis P20 was subjected to different culture condition to obtain the optimum condition for xylanase production. Some parameter fermentation were optimised, such as substrate concentration, pH, temperature, carbon source and nitrogen source. All experiment were carried out in Erlenmeyer flask 300 mL containing 30 mL medium. Xylanase enzyme for fermentation were produced by inoculated marine bacterium *Bacillus safencis* P20 in 100 mL Flask containing 10 mL of ASW medium followed by cultivation at 30^{o}C for one days. Then, X mL of the pre-culture medium was seeded into 300 mL Flask containing 30 mL of ASW medium and incubated at 30^{o}C for six days. Inoculated were maintained in shaker at 150 rpm for 144 hours.

E. *The effect of pH media and temperature fermentation on xylanase production*

Growth and enzyme production as well as estimated at various pH (6, 7, 8, 9) and temperatures (20, 30, 40 and 50^{o}C) fermentation. Sampling were conducted every 24 hours for 144 hours culture and were centrifuged at 4^{o}C, 10.000 g for 20 min.

F. *The effect of carbon source on xylanase production*

The effect of carbon source on xylanase production were evaluated by culturing isolate in ASW medium pH 7 at 20^{o}C. Approximately 0.5% (w/v) of maltose, lactose, glucose, sucrose and xylose were separately added to the medium. Sampling were conducted every 24 hours for 144 hours culture and were centrifuged at 4^{o}C and 10.000 g for 20 min.

G. *The effect of nitrogen source on xylanase production*

The effect of nitrogen source on xylanase production were evaluated by culturing isolate in ASW medium pH 7 at 20^{o}C. Approximately 0.5% (w/v) of ammonium chloride, ammonium nitrate, ammonium sulphate, casein and urea were separately added to the medium. Sampling were conducted every 24 hours for 144 hours culture and were centrifuged at 4^{o}C and 10.000 g for 20 min.

H. *Xylanase activity assay*

Xylanase activity was measured according to Bailey *et al*. (1992). The crude xylanase preparation was obtained as the culture supernatant by centrifugation. Subsequently, the supernatant was analyzed for xylanase activity at pH 7 in sodium phosphate buffer (0.05 M) at 60^{o}C by measuring the reducing sugars using dinitrosalicylic acid (DNS) method (Miller, 1959). One unit of cellulase activity is defined as the amount of enzyme that liberates 1 µmol of D-glucose per minute under the experimental condition given.

III. Result and Discussion

We already collected of xylanolytic marine microbes from several areas in Indonesia that have ability to produce xylanolytic based on qualitative & quantitative on xylan commercial. Based on identification of our marine xilanolytic microbe using 16s rDNA analysis show that one isolates xylanolityc, P20 belong to *Bacillus safensis.*

A. *Screening of Biomass as a substrate for xylanase production*

Cost of production is one of the problems of xylanase production for industrial applications. One of alternative to decrease the cost of production was conducted xylanase production by using agricultural biomass as a substrate. The first step was selected the best substrate as a substitute of xylan commercial for xylanase production from Indonesia biomass. In this research, *Bacillus safensis* P20 was growing on some Indonesia biomass containing xylan such as, empty fruit bunch, sugar cane bagasse and palm cernel cake. Xylan commercial was used as a control. Xylanase production was detected by using Congo red solution. Fig 1 showed qualitative analysis from xylannolytic marine bacterium *Bacillus safencis* P20 on some Indonesia biomass by Congo red. This isolate have ability to form the clear zone after staining by Congo red solution on all of biomass. The diameter of clear zona around the colonies was range from 1.9 - 2.8 cm, indicated this isolate have ability to degrade these biomass to produce xylanase. Based on the data of clear zone show that the diameter clear zone on sugar cane bagasse similar with the xylan commercial, so we selected the sugar cane bagasse as a substrate for xylanase production from *Bacillus safensis* P20.

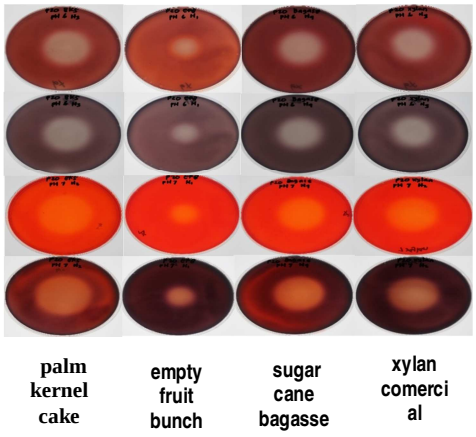

Fig 1. Data of clear zone from three biomass (palm cernel cake, empty fruit bunch and sugar cane bagasse VS xylan commercial by marine bacterium *Bacillus safensis* P20

B. Optimation culture condition

There are a series of fermentation conditions which may affect significantly to the xylanases activity, suc as substrate concentrations, pH value, temperature, carbon source and nitrogen source of the culture broth. The first step was selected sugar cane bagasse concentration to obtain the optimum xylanase activity. Effect of various substrate concentration on xylanase production was shown at Fig 2. The cell growth for a substrate concentration of 0.5%, 1.0%, and 1.5% increased from hour 0 to 72, and then decreased in 96 hours. While the growth cell on concentration of 2.0% showed a continuous increase up to 144 hours. The activity of enzyme on all substrate concentration has increased from 0 to 96 hours. while the substrate concentration 1.5% and 2.0% activity continued to increase up to 120 hours. We selected the concentration of 1.5% as the optimum conditions for the production of xylanase enzyme by *Bacillus safensis* P20 with activity 3.93 U/mL. This result was higher than was obtained by [8] produced xylanase from *Bacillus subtilis* XP10. The highest enzyme production (2.82 U/ml) was produced on xylan followed by corncob (1.8 U/ml) and wheat straw (1.7 U/ml) whereas much lower levels of xylanase activities (1.03, 1.06 U/ml) were obtained with oat and barley bran. Based on this result that sugar cane bagasse can be used as a substitute for substrate instead Birchwood xylan commercial.

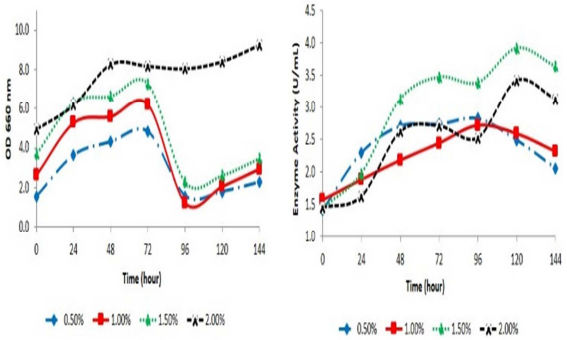

Fig 2. The growth curve (a) and the enzyme activity (b) of *Bacillus safensis* P20 at various substrate concentration

Effect of pH medium on xylanase production by *Bacillus safensis* P20 was shown at Fig 3. There is not significant diference of cell growth at various pH medium. The effect pH on xylanase activity which is in agreement with findings obtained by [9] reported that the pH did not influence xylanase production by *Bacillus circulans* D1. The value of enzyme activity at various pH is not very significant. We selected the optimum pH of the media used for the production of xylanase enzyme at pH 7. [10] in his study of xylanase enzyme producing maximum at pH 7 from *Bacillus sp*.

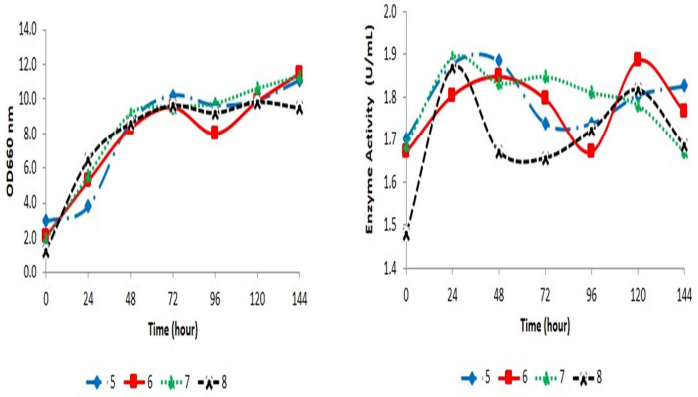

Fig 3 The Growth cell (a) and the enzyme activity (b) of *Bacillus safensis* P20 on 1.5% sugarcane bagasse at various pH medium

Effect of temperature fermentation on xylanase activity was shown at Fig 4. The cell growth at temperature of 50°C is lower than others. While for a temperature of 20°C, 30°C, and 40°C has similar cell growth. Xylanase activity reaches a maximum at 20°C at 96 hours with activity 3.70 U/mL. This result similar with [11] who produce xylanase from *B. subtilis*, where highest enzyme activity was achieved at low temperature (25°C).

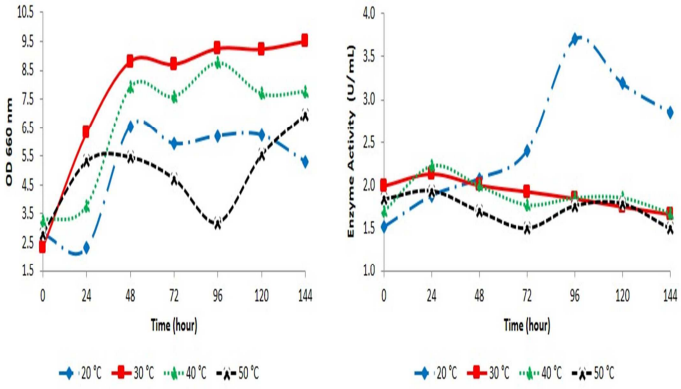

Fig 4. The growth cell (a) and the enzyme activity (b) from *Bacillus safensis* P20 at various temperature fermentation

Effect of various carbon sources on xylanase production was studied for enhanced production of xylanase. Effect of various carbon sources on xylanase production was shown in Fig 5. Based on the growth curve obtained peak cell growth with maltose carbon source occurred at 120 hours, whereas lactose, glucose, and sucrose occurred at 96 hours, and xylose have lower cell growth compared to the others. The effect of carbone source not to significant for xylanase activity. The enzyme activity of xylanase produced using lactose has a value greater activity than others (2.65 U/mL). This result contrasts with the result was obtained by [12] for *Cellulosimicrobium sp* where sucrose was found to be the second best of the carbon sources used after xylan (96.33 U/mL), producing 91.10 U/ml of xylanase. Xylan in combination with sucrose combination with sucrose found stimulatory for increased production of xylanase.

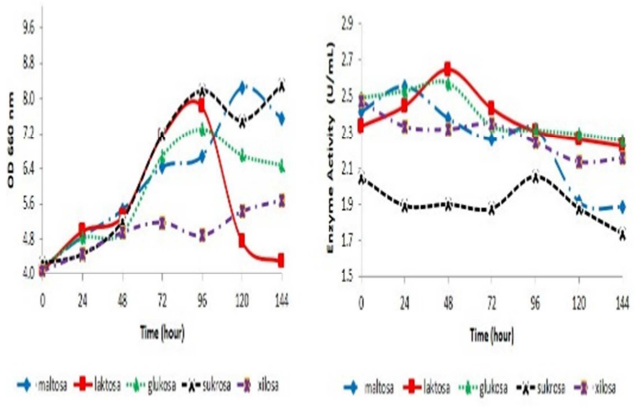

Fig 5. The effect of carbone source on growth curve (a) and the enzyme activity (b) of *Bacillus safensis* by using sugarcane bagasse as substrate

Effect of nitrogen source on xylanase activity was shown at Fig 6. The results showed that the cell growth on all of nitrogen source reach optimal at 96 hours fermentation, but casein give a greater cell growth than others. All nitrogen source not give the diferrent activity until 72 hours, but urea give higher activity than the others (4,06 U/mL) at 96 hours fermentation. The results show that additional nitrogen sources in the media significantly can be increasing the activity of xylanase.

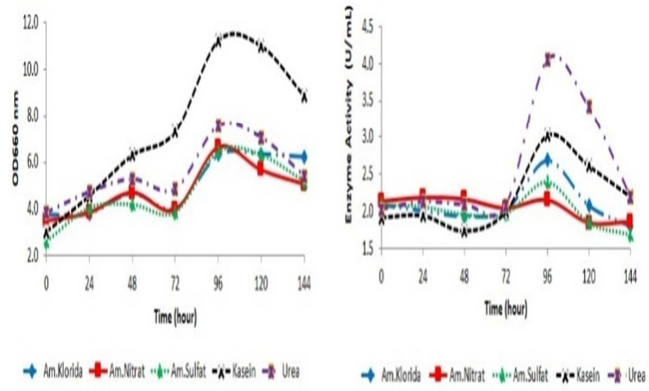

Fig 6. The effect of nitrogen source on growth curve (a) and xylnase activity (b) of *Bacillus safensis* by using sugarcane bagasse as substrate

IV. Conclusion

The optimal condition used sugarcane bagasse as a substrate for xylanase production by *Bacillus safensis* P20 on sub merged fermentation were substrate concentration 1.5%, pH medium 7, temperature 20°C, lactose as carbon source, and urea as nitrogen source with activity 4.1 U/mL at 96 hours fermentation. The additional of carbon source not influent to xylanase activity, but additional of nitrogen source significantly increasing of xylanase activity from 3.7 U/mL to 4.1 U/mL. Based on this result that xylanase synthesis on xylan or xylan containing substrates suggests that xylan is necessary for the effective induction of xylanase by *Bacillus* strains. This data may be explained not only because xylan is the main carbon source, but probably also because its hydrolysis products act as inducers [8].

Acknowledgment

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References

[1] Annie Deborah Harris and C. Ramalingam. 2010. Xylanases and its Application in Food Industry: A Review. Journal of Experimental Sciences Vol. 1, Issue 7, Pages 01-11.

[2] Sharma, M and Anil Kumar. 2013. Xylanases: An Overview. British Biotechnology Journal 3(1): 1-28

[3] Polizeli M.L., Rizzatti A.C., Monti R., Terenzi H.F., Jorge J.A., Amorim D.S. 2005. Xylanases from fungi: properties and industrial applications. Appl Microbiol Biotechnol, 67: 577-591.

[4] Octavio Loera Corral and Francisco Villaseñor-Ortega Xylanases. Research Signpost Advances in Agricultural and Food Biotechnology, 2006: 305-322 ISBN: 81-7736-269-0

[5] Frost G M, Moss D M. Production of enzymes by fermentation, in: Rehm H J, Reed G (eds.,) Biotechnology; 1987.

[6] Bailey MJ, Peter Biely and Kaisa Poutanen. 1992. Interlaboratory testing of methods for assay of xylanase activity. Journal of Biotechnology, 23 (1992) 257-270 257

[7] Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. J Anal Chem. 31(3):426-428.

[8] Tork, S and Magda M. Aly , Saleha Y. Alakilli and Madeha N. Al-Seeni. 2013. Production and characterization of thermostable xylanase from Bacillus subtilis XP10 isolated from marine water. African Journal of Biotechnology Vol. 12(8), pp. 780-790.

[9] Bocchini DA, Alves-Prado HF, Baida LC, Roberto IC, Gomes E, Da-Silva R (2002). Optimization of xylanase production by Bacillus circulans D1 in submerged fermentation using response surface methodology. Process Biochem. 38: 727–731.

[10] Kuhad RC, Chopra P, Battan B, Kapoor M, Kuhad S. 2006. Production, partial purification and characterization of a thermo-alkali stable xylanase from Bacillus sp.RPP-1. Indian Journal of Microbiology. 46: 13-23.

[11] Heck JX, Soares LH, Ayub MAZ (2005). Optimization of xylanase and mannanase production by Bacillus circulans strain BL53 on solid-state cultivation. Enzyme Microb. Technol. 37(4): 417-423

[12] Rajashri D. Kamble, Anandrao R. Jadhav. 2012. Production, purification and characterisation of alkali stable xylanase from Cellulosimicrobium sp. MTCC 10645. Asian Pacific Journal of Tropical Biomedicine S1790-S1797.