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USING *STREPTOMYCES* XYLANASE TO PRODUCE XYLOOLIGOSACHARIDE FROM CORNCOB

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ABSTRACT

Streptomyces 234P-16 and SKK1-8 are xylanase-producing bacteria. Corncob xylan were extracted using acidified method. Crude enzymes (produced by centrifuging the culture) were used to hydrolyze xylan from 2 varieties of corncob. Crude extract activity was measured by using DNS (Dinitrosalisilic Acid) method. Xylanase from strain 234P-16 has the highest activity if cultivated in 1% Hawaii xylan, whereas strain SKK1-8 on 1.5% Bisma xylan. SKK1-8 xylanase can hydrolize corncob xylan (1% Hawaii or 1.5% Bisma xylan) within 4 hours and produce xylooligosacharide with polymerization degree of 4.76 and 6.37, respectively.

Key words: Xylanase, Xylooligosacharide, *Streptomyces.*

INTRODUCTION

Besides rice, corn is the major carbohydrate sources in Indonesia especially for feed and industrial raw materials. Most of corn is used as raw material for food and non-food industries. But these applications are limited to the corn kernel, and other parts of corn plant such as corncob was not utilized yet. About 30% of the corn is corncob; the rests are corn stover and kernels. Data from Badan Pusat Statistik (Indonesian Bureau of Statistics) showed that maize production is increasing from 9.82 million ton in 2002 to 11.35 million ton in 2004. This increase is also followed by the increase of corncob as an agricultural waste.

Corncob consists of protein, fat, nitrogen free extract and also hemicellulose and cellulose (Johnson 1991). The content of xylan (major part of Hemicellulose) in corncob could go up to 40-g/100 g, the highest among all of the agricultural waste (Yang *et al.* 2005). Most xylan occurs as heteropolysaccharide, containing different substituent groups in the backbone chain and in the side chain (Beg *et al*. 2001). Due to the corncob nutritional content, it can convert into a commercial product or be used as a medium to

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cultivate microorganisms. In our previous research we have identified xylanase-producing *Streptomyces* (234P-16 and SKK1-8). Xylanase from 234P-26 has an optimum condition at pH 5 and 90 °C whereas from SKK 1-8 the optimum condition is at pH 6 and 50 °C (Meryandini 2005). In this research we will use this *Streptomyces* xylanase to produce xylooligosaccharide from corncob xylan.

MATERIALS AND METHODS

Materials

In this research we use 2 local varieties of corncobs (Bisma and Hawaii) and 2 acid-xylanases from Indonesian *Streptomyces*: *Streptomyces* 234P-16 from Padang and *Streptomyces* SKK1-8 from Sukabumi.

Delignification

Delignification of corncobs methods had been investigated previously (Widyani 2002). About 1000 g of corncob grits is immersed in 10 liters of 1% NaOCl solution for 5 hours at room temperature (28 °C). After 5 hours, the sample is decanted and rinsed by distilled water for several times and filtered, the solid part i.e. delignified samples are then dried by oven drying at 50 °C for 48 hours. The chemical composition of this sample will be examined as cellulose, hemicellulose, and lignin.

Xylan extraction & purification

The method for separation of xylan using acidified method from delignified samples has been investigated by Anggraini (2003). Delignified corncob grits are immersed in 15% NaOH solution for 24 hours at room temperature (28 °C). This step liberated the xylan into soluble fraction and neutralized by 6 N HCl solutions (pH 4.5-5.0). The supernatant is centrifuged on 4000 rpm for 30 minutes to obtain residue such as xylan.

Purification is conducted by re-dissolving the crude xylan into 4% NaOH and then filtered. Filtrate was acidified using 6 N HCl (pH 4.5-5.0), and then centrifuged on 4000 rpm for 30 minutes. Precipitates were dissolved in 95% ethanol and centrifuged, and then dehydrated using 50 °C drying oven.

Xylanase production

The *Streptomyces* isolates are cultured on YM (Yeast- Malt) agar and then on oat-spelt xylan agar medium (Yeast extract 0.2%, sucrose 10%, K_2HPO_4 1.5%, $MgSO_4 \cdot 7H_2O$ 0.025%, NaCl 0.23%, $Na_2HPO_4 \cdot 2H_2O$ 5 %, oat spelt xylan 0.5%) for 4 days at room temperature. Two cookbores of *Streptomyces* are cultured in 100 ml liquid xylan media and incubated at room temperature using a shaking incubator with an agitation speed of 240 rpm. Every day the culture should be centrifuged and the supernatans collected for enzyme assay.

Xylanase production on several substrates

Two cookbores of *Streptomyces* are cultured in 100 ml liquid xylan media (oat-spelt and several concentration of 2 local corncob xylan) in a 500 ml Erlenmeyer and incubated on a shaker for 10 to 12 days at room temperature.

Duration of xylan hydrolysis by crude xylanase

Streptomyces xylanase produced in 1% of corncob xylan was used to hydrolyze 1% of corncob xylan for 4 hours at optimum pH and temperature condition. Degree of hydrolysis and degree of polymerization will be monitored every hour and the reducing sugars analyzed by Dinitroasalicilycacid Method, and total sugar by Phenol-H_2SO_4 method.

Assay of xylanase activity

The culture was centrifuged 5 minutes at 10.000 x g to obtain the xylanase crude extract. Xylanase activity was measured with oat-spelt xylan as a substrate. Enzyme solution (100 µl) was added to 1 ml substrate solution which contain 0.5% xylan and the mixture was incubated at optimum temperature for each enzyme (90 °C for 234P-26 and 50 °C for SKK 1-8) for 30 minutes. Crude extract activity was measured by using DNS (Dinitrosalisilic Acid) method by Miller (1959) with xylosa as the standard.

The reducing sugar of the references samples (substrate solution incubated without enzyme and diluted enzyme solution in buffer) were deduced from the values of the test samples. The reducing-sugar was detected by spectrophotometer (λ = 540 nm). One unit xylanase activity was defined as the amount of enzyme which produces 1 µmol xylosa per minute.

RESULTS AND DISCUSSIONS

Xylan extraction

The chemical composition of corncob is described in Table 1, and the delignification effect of corncobs and recovery of cellulose fraction to the fiber components are shown in Table 2.

Table 1. Chemical composition of corncobs

Constituent	Bisma Variety	Hawaii Variety
Ash (% db)	1.62	1.67
Lipid (% db)	3.02	4.68
Crude Protein (%db)	2.41	4.82
Crude Fiber (% db)	38.07	40.65
Carbohydrate (% db, by difference)	51.93	44.14

Table 2. Composition of fibers before and after delignification.

Constituent	Before delignification (%)	After delignification (%)
Bisma Variety		
Cellulose	65.96	44.36
Hemicelulose	10.82	30.38
Lignin	23.74	19.21
Hawaii Variety		
Cellulose	60.04	41.88
Hemicelulose	18.11	30.18
Lignin	16.14	15.12

Elimination of lignin should be conducted since lignin will reduce the effectiveness of the utilization of xylan as carbon source for microbial growth and will influence the production of enzyme (Agustine 2005). Post-delignification, the product composition was analyzed and small amount of lignin liberated. The initial lignin of Bisma Variety (23.74%) and Hawaii Variety (16.14%) were reduced to 19.27% (Bisma Variety) and 15.2% (Hawaii Variety), respectively. Delignification cannot eliminate lignin from lignocellulosic materials completely. Cellulose microfibril was integrated in hydrophobic matrix covered by lignin, and the lignin linked with cellulose and hemicellulose in covalent linkages (Agustine 2005).

According to Fengel and Wegener (1995), lignin, cellulose and hemicellulose cannot separate completely even by special separation and purification. Lignin and cellulose can be detected in purified cellulose and lignin. Delignification was conducted using 1% NaOCl as strong oxidant (Agustine 2005). Anggraini (2003) and Widyani (2002) stated that hypochlorite ion from NaOCl can cleave the carbon linkage on lignin structure, and delignification caused the opening of the linkages between lignin and other polysaccharides and influenced the increasing use of xylan by bacteria.

Xylan extraction was conducted by submerging the delignified materials into 15% NaOH. Anggraini (2003) and Widyani (2002) concluded that hemicellulose can be dissolved in alkaline solution, such as 15% NaOH can produce brighter and clear powder, relatively clean from impurities, and easily dissolved in water and produced high yield. Acid solution 6 N HCl was added to neutralize up to pH 4.5-5.0 to precipitate again the xylan.

The yield of xylan recovery was 9.26% from Bisma Variety, and 9.94% from Hawaii Variety. Based on research results of Widyani (2002) and Anggraini (2003) 7.64-12.94 % and 7.31-11.45 %, respectively, of xylan can be produced from corncob using the same acidified methods.

Production of xylanase

The daily production curve of xylanase *Streptomyces* 234P-16 and SKK 1-8 assayed at pH 7.2 and 37 ^{o}C is shown in Figure 1. The highest xylanase production was reached on day-10 with the activity of 0.625 Unit/ml for SKK1-8 and on day-5 with the activity of 0.27 U/ml for 234P-16. The optimum time of xylanase production was then used as the standard harvest time for the next xylanase production.

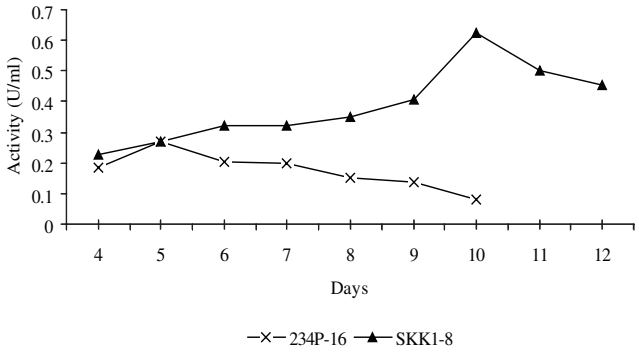


Figure 1. Production curve of *Streptomyces*. 234P-16 and SKK 1-8 xylanase assayed on 37 °C and pH 7.2.

Xylanase activity on several concentrations of corncob xylan

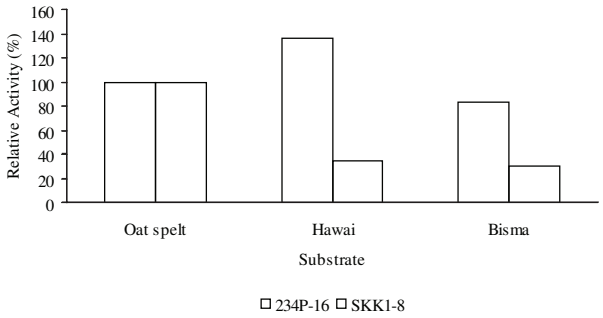


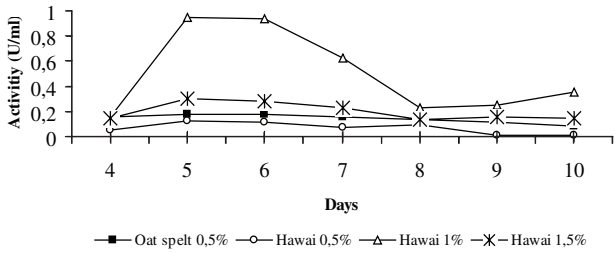
Figure 2. Relative activity of *Streptomyces* xylanases on 0.5% xylan from oat spelt, Bisma dan Hawaii corncob

Figure 2 shows differences in xylanase activity on several 0.5% substrates. Xylanase from strain 234P-16 showed the highest activity on Hawaii xylan, while xylanase from SKK1-8 on oat spelt. Xylanase 234P-16 showed the lowest activity on Bisma xylan, whereas xylanase 45I-3 on Hawaii xylan. Hendarwin (2005) also reported activity differences in various substrates.

Xylanase activity in several concentrations of corn xylan and 0.5% oat spelt xylan

Streptomyces was inoculated in 0.5%, 1% and 1.5% Bisma or Hawaii xylan medium and compared with 0.5% oat spelt xylan. Figure 3 shows the *Streptomyces* 234P-16 xylanase activity on several concentrations of Hawaii xylan (Figure 3A) and several concentrations of Bisma xylan (Figure 3B). Xylanase from strain 234P-16 has the highest activity if cultivated in 1 % Bisma xylan (0.57 U/ml) or in 1% Hawaii xylan (0.947 U/ml)

A.



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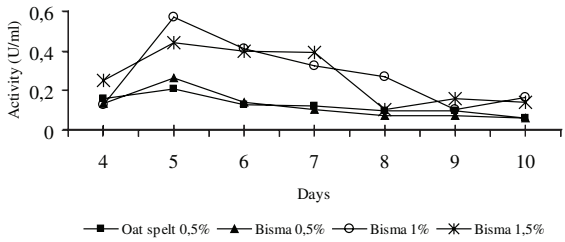
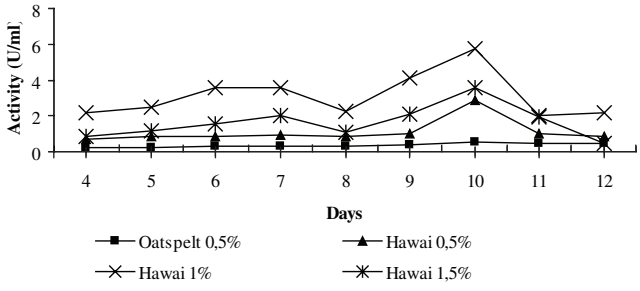


Figure 3. Xylanase activities from *Streptomyces* 234P-16 on (A) Hawaii corncob ylan and (B) Bisma corncob xylan compared with oat spelt xylan

Figure 4 shows the *Streptomyces* SKK1-8 xylanase activity on several concentrations of Hawaii xylan (Figure 4 A) and several concentrations of Bisma xylan (Figure 4B). Xylanase from strain SKK1-8 has the highest activity if cultivated in 1.5 % Bisma xylan (49 U/ml) or in 1% Hawaii xylan (5.75 U/ml).

A.



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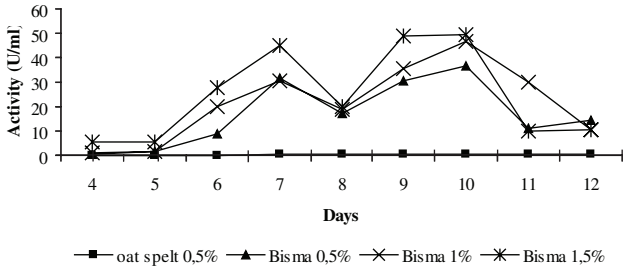

Figure 4. Xylanase activities from *Streptomyces* SKK1-8 on (A) Hawaii corncob xylan and (B) Bisma corncob xylan compared with oat spelt xylan

The production of xylanase in 0.5% oat spelt xylan was compared with the production in several concentrations of corncob xylan. Xylanase from strain 234P-16 has the highest activity if cultivated in 1% Hawaii xylan, whereas strain SKK1-8 on 1.5% Bisma xylan (Figure 3 and 4).

The production of xylanase is usually induced in medium containing pure xylan or xylan-rich residues. Several reports showed that xylanase can be induced with lignocellulose material such as wheat bran, rice straw, corncob and sugarcane bagase (Beg *et al.* 2001).

Corncob xylan is a rich carbon source with several kinds of carbon content as according to Fengel and Wegener (1995) lignin, cellulose and hemicellulose cannot be separated perfectly although the usage of a special separation and purification methods and this content gives an effect on xylanase production. All carbon sources will give an effect on enzym production as reported by Ambarawati (2005) that medium containing mannan can induce xylanase from strain 45I-3 and vice versa.

The optimum concentration to induce xylanase was 1% for Hawaii xylan and for Bisma xylan. This greater result on Hawaii xylan could be due to the higher concentration of Hemicellulose in Hawaii xylan.

Hydrolysis of xylan

Sugar composition was monitored by the increasing of reducing sugar and the decreasing degree of polymerization and the result is shown in Table 3.

Table 3 shows the decreasing of DP during hydrolysis, while the reducing sugar continued to increase. Smaller number of DP shows that polysaccharide was depolymerized into short-chains compounds.

Li *et al.* (2000) also reported that each microorganism will liberate specific xylanase, and showed different activity on the same substrate. High diversity of xylanase may be caused by many different structures of xylan in the nature. Isolates SKK1-8, but not 234P-16, can be used for XOS production using corncob xylan within 4 hours hydrolysis time.

Chen *et al.* (1997) stated that xylo-oligosaccharide (XOS) composed of 2-5 units xylose. Vazque *et al.* (2000) reported that XOS for food application should be 2-4 unit monomers, especially DP 2 xylobiose.

Table 3. Reducing sugar, total sugar and the degree of polymerization

Isolate	Substrate	Time (hours)	Sugar production (mg/ml)	Total sugar (mg/ml)	Degree of Polymerization
234P-16	1 % Hawaii xylan	0	2.05	184.79	90.14
		1	2.91	184.79	63.50
		2	3.4	184.79	54.35
		3	4.3	184.79	43.18
		4	4.8	184.79	38.50
	1% Bisma xylan	0	1.53	151.47	99
		1	2.06	151.47	73.53
		2	2.39	151.47	63.38
		3	3.13	151.47	48.39
		4	3.39	151.47	44.68
SKK1-8	1 % Hawaii xylan	0	12.24	184.56	15.08
		1	27.43	184.56	6.73
		2	36.29	184.56	5.09
		3	34.84	184.56	5.30
		4	38.70	184.56	4.76
	1.5% Bisma xylan	0	1.36	182.61	134.27
		1	2.45	182.61	74.53
		2	26.84	182.61	6.80
		3	24.72	182.61	7.39
		4	28.65	182.61	6.37

CONCLUSIONS

Xylanase from strain 234P-16 has the highest activity if cultivated in 1% Hawaii xylan, whereas strain SKK1-8 on 1.5% Bisma xylan. Using 1.5% of Bisma xylan, xylanase from SKK1-8 can produce xylooligosacharide with 6.37 degree of polymerization whereas using 1 % of Hawaii xylan with 4.76 degree of polymerization within 4 hours of hydrolysis time. Xylanase from strain 234P-16 is not suitable for producing xylooligosacharides. Xylanase from strain 234P-16 has the highest activity if cultivated in 1% Hawaii xylan, whereas strain SKK1-8 on 1.5% Bisma xylan. Using 1.5% of Bisma xylan, xylanase from SKK1-8 can produce xylooligosacharide with 6.37 degree of polymerization whereas using 1 % of Hawaii xylan with 4.76 degree of polymerization within 4 hours of hydrolysis time. Xylanase from strain 234P-16 is not suitable for producing xylooligosacharides.

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