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SHORT COMMUNICATION

Characterization of Xylanase from *Streptomyces* spp. Strain C1-3

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Xylan is the major constituent of hemi cellulose. Several enzymes are needed to hydrolyse xylan completely, including xylanase. Currently, there is an increasing use of this enzyme. This study was carried out to characterize the xylanase from *Streptomyces* spp. strain C1-3. Results showed that the xylanase displayed its highest activity at pH 3 and 90 °C and was stable up to 10 hours at this conditions. Its activity increased after the addition of Cu^{2+}, Fe^{2+}, and Co^{2+} under concentration of 1 and 5 mM, respectively. The activity however, decreased after the addition of Mg^{2+}, Ca^{2+} at 1 mM and Zn^{2+} at 5 mM. After a test with five kinds of xylan (i.e. from Birchwood, Beechwood, Arabinoxylan, Oat spelt and CMC), the xylanase of *Streptomyces* spp. C1-3 showed its preferences to Birchwood- and Arabino-xylan. The results showed that the xylanase of *Streptomyces* spp. C1-3 was characterized as a thermostable acid xylanase.

Key words: xylanase, *Streptomyces*, stability, CMCase

Hemicellulose is the second most abundant heteropolysaccharides after cellulose and xylan is the major constituent of it. Xylan has a backbone chain of 1,4 linked β-D xylopiranose units. The backbone consists of O-acetyl, α-L-arabinocyl, 4-metilglucuronic acid. An enzyme system is needed to hydrolyze xylan completely. This enzyme system consist of endo-β-1,4 xylanase, β-xilosidase, galactosidase, α-arabinofuranosidase, α-D-glucuronidase, and acetyl xylan esterase (Sunna *et al.* 1997; Subramaniyan & Prema 2002).

Currently, xylanase application showed highly increase due to its necessity of the products produced from dissolving pulp such as rayon, cellophane, and chemicals. Xylanase is also used in bioconversion of lignocellulotic material, agro-waste products, clarifying juice and feedstock. As xylan hydrolysis is a main factor in all processes above (Beg *et al.* 2001; Subramaniyan & Prema 2002), hence the use of xylanase for all those purposes should be characterized.

Xylanase is produced by fungi (Lin *et al*. 1999; Saha 2002; Ryan *et al*. 2003), bacteria (Beg *et al*. 2001), and protozoa (Devillard *et al*. 2003). One of the potential bacteria group that produced xylanase is Actinomycetes, especially *Streptomyces* (Ruiz-arribas *et al*. 1995; Georis *et al*. 2000; Kaneko *et al*. 2000; Wang *et al*. 2003; Kansoh & Nagieb 2004). The aim of this research was to characterize *Streptomyces* spp. C1-3 xylanase isolated from Cicurug Sukabumi soil sample. It was one of several *Streptomyces* collections we had in Dept. of Biology.

The *Streptomyces* spp. C1-3 isolate was rejuvenated in YM agar-agar media (0.4% yeast extract, 1% malt extract, 1.5% glucose, and 1.5% agar-agar). The isolate was then grown in a xylan media (1% yeast extract, 10.3% sucrose, 0.5% Birchwood Xylan, 1.5% agar-agar) and was incubated at 30 °C for seven days. Two cockbors (diameter = 2 cm) of the isolate grown in the xylan media was subsequenthly inoculated to 100 ml xylan media in 500 ml Erlenmeyer. They were incubated in 140 rpm agitation at 30 °C for 10 days. The culture was centrifuged every day (5 minutes) at 10,000 x g to obtain the xylanase crude extract. The extract activity was measured by using DNS (Dinitrosalisilic Acid) method of Miller (1959) with xylosa as the standard. The reducing sugar of the reference samples (substrate solutions incubated without enzyme and a diluted enzyme solution in a buffer) was deduced from the values of the test samples. The yielded reducing-sugar substance was assessed by spectrophotometer (λ = 540 nm). One unit xylanase activity was defined as the amount of enzyme producing 1 μmol xylosa per minute. Protein concentration (mg/ml) was defined by using Bradford method (1976) and *Bovine serum albumin* (BSA) was used as the standard.

Characterization of the crude extract enzyme were carried out to determine the optimum temperature and pH, enzyme stability, and the influence of bivalent cations. All data in this study were from duplo trials. The assessment of pH was carried out within a pH range of 3.0-9.0 with 0.5 interval. The determination of optimum temperature was performed from 30 up to 90 °C with 10 °C interval. The stability of xylanase crude extract was tested by incubating the extract without substrate in two different temperatures the first at its optimum temperature and the second at 3 °C of storage temperature. To observe the influence of cations on the enzyme activity, six cations (i.e. Ca^{2+}, Zn^{2+}, Cu^{2+}, Mg^{2+}, Fe^{2+}, and Co^{2+}) derived from $CaCl_2$, $ZnCl_2$, $CuCl_2$, $MgCl_2$, $FeSO_4 \cdot 7H_2O$, and $CoCl_2$, were added separately to a final concentration of 1 and 5 mM. Determining the enzyme activity in several kinds of xylan substrates was defined by assessing the extract activity in Birchwood Xylan, Oatspelt Xylan, Wheat Arabinoxylan, Beechwood Xylan, and Carboxy Methyl Cellulose (CMC).

The daily production of xylanase was tested at pH 7.2 and a temperature of 37 °C. The highest enzyme production was reached on day-8 with the activity of 0.65 Unit/ml (Figure 1). This optimum time of xylanase production was used as the standard harvest time in the next xylanase production.

The influence of pH on the specific activity was measured at 37 °C (Figure 2). Xylanase had its highest activity at pH 3, but also demonstrated quite high activity at pH 4.5- 6.0 and 7.5. The effect of temperature on specific activity was tested at pH 3.0 Xylanase *Streptomyces* spp. C1-3 displayed its optimum temperature at 90 °C (Figure 3). Furthermore, xylanase showed its stability in both 90 and at 3 °C conditions, (Figures 4 & 5). After ten hours incubation at 90 °C the xylanase still showed its 90% activity, and after ten hours incubation at 3 °C the xylanase still had 91% activity. The influence of cations (1 and 5 mM in concentration) on the activity of xylanase was tested at 90 °C and pH 3 (Figure 6). Generally, the cations increased the activity in both concentration. However, Mg^{2+}, and Ca^{2+} at the concentration of 1 mM and Zn^{2+} at the concentration 5 mM, reduced 11, 5, and 32%, of the enzyme activity, respectively.

Xylanase demonstrated its highest activity in Birchwood- and Arabino-xylan with an activity of 2.4 and 2.55 U/ml, respectively (Figure 7). Beg *et al.* (2001) reported that xylanase were usually secreted in media containing pure xylan or xylan-rich residues. The decreasing activity after the optimum time might be due to the accumulation of xylose in media (Mountfort & Asher 1989; Beck *et al*. 2001).

Enzymes show various activity range in different pH and are categorized as acidophil, alkaliphil, and neutral. The

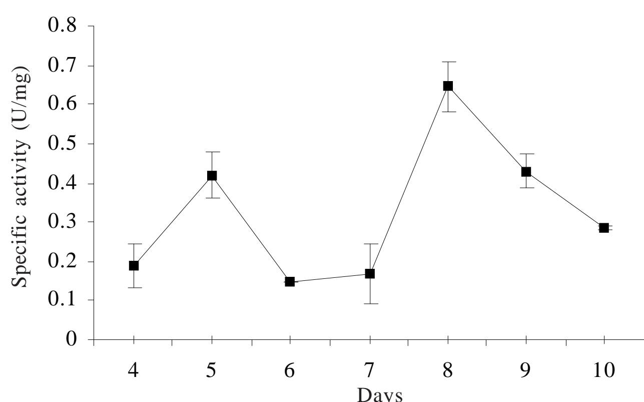

Figure 1. *Streptomyces* spp. strain C1-3. Specific activity of xylanase (U/mg) measured at 37 °C and pH 7.2.

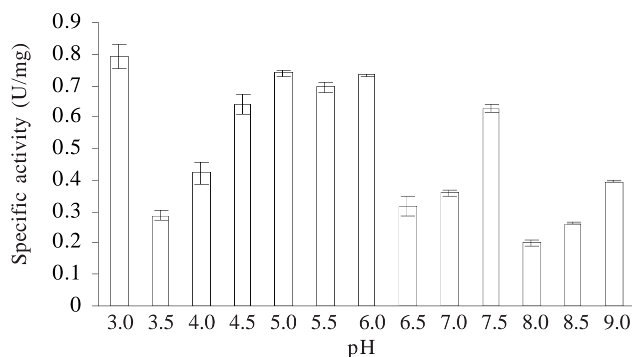

Figure 2. *Streptomyces* spp. C1-3. Effect of pH on xylanase activity measured at 37 °C.

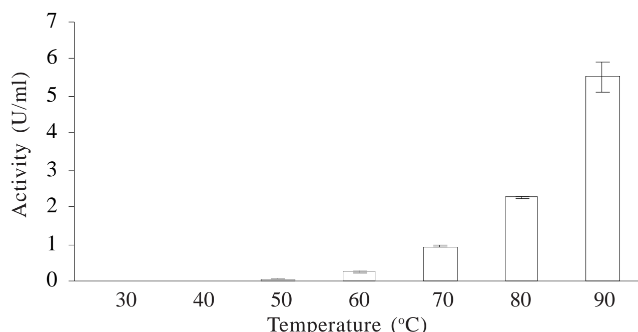

Figure 3. *Streptomyces* spp. C1-3. Temperature effect on xylanase specific activity (U/mg) measured at pH 3.

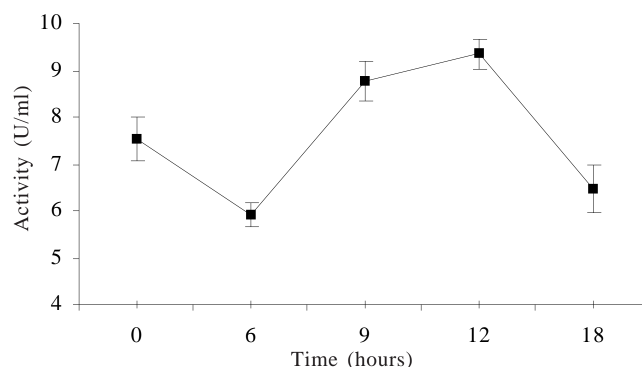

Figure 4. *Streptomyces* spp. C1-3. Stability curve of xylanase at 90 °C.

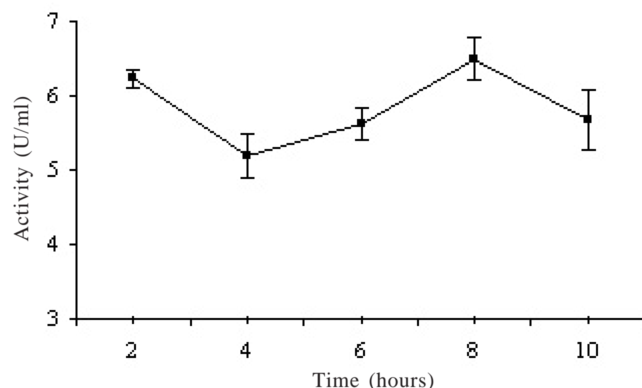

Figure 5. *Streptomyces* spp. C1-3. Stability curve of xylanase at 3 °C.

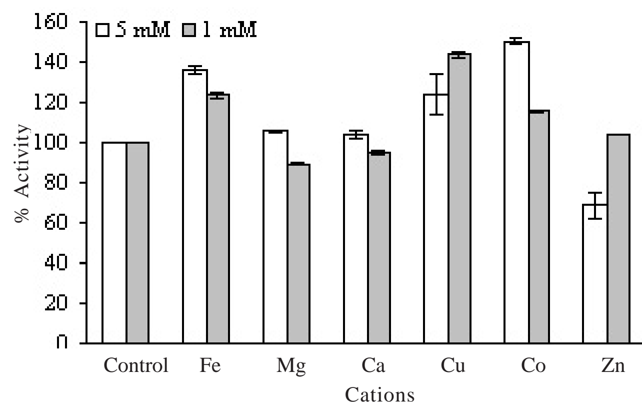

Figure 6. *Streptomyces* spp. C1-3. Cation effects on xylanase measured at 90 °C and pH 3.

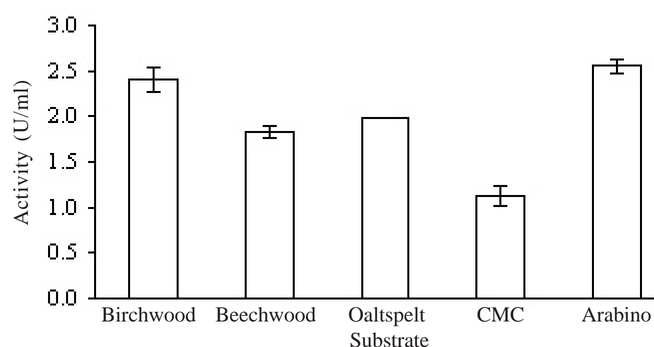

Figure 7. Activity of *Streptomyces* spp. C1-3 xylanase on several substrat measured at pH 3 and 90 °C.

xylanase of *Streptomyces* spp. C1-3 had optimum condition at pH 3. Hence, it can be categorized as an acidophilic enzyme (Madigan & Martinko 2006). *Streptomyces acromogenes* ISP5028 was also reported had its highest activity was on pH 4.5-8.5 (Belfaquih *et al.* 2002). Other xylanase that have been reported as acidophil was from *Penicillium capsulatum* with its optimum pH at 3.8 (Ryan *et al.* 2003).

Streptomyces spp. C1-3 xylanase optimum temperature of 90 °C and was had the categorized as extremozyme (Madigan & Martinko 2006). Several xylanases were also reported as extremozyme such as from *Bacillus thermantarcticus* which has optimum temperature at 80 °C (Lama *et al.* 2004), *Microtetraspora flexuosa* S11X at 85 °C or *Streptomyces achromonas* ISP5028 at 70 °C (Belfaquih *et al.* 2002).

Streptomyces spp. C1-3 xylanase can also be categorized as thermostable enzyme as shown in the stability curve. *Thermotoga maritima* xylanase that has its optimum temperature at 90 °C is the most stable enzyme because it retained 82% its activity after two hours incubation (Xue & Shao 2004). *Bacillus thermantarcticus* xylanase have half-life time of 50 minute at 80 °C, however, it was stable for 24 hours at 60 °C (Lama *et al.* 2004), while *Bacillus* sp. strain SPS-O xylanase lost its activity in 15 minute at 80 °C (Bataillon *et al.* 2000).

The activity of *Streptomyces* spp. C1-3 xylanase was hindered slightly by cation Mg^{2+} and Ca^{2+} in concentration of 1 mM and Zn^{2+} in concentration of 5 mM. Wang *et al.* (2003) reported that *Streptomyces actuosin* A-151 xylanase increased its activity by 30 mM Mn^{2+}, while *Bacillus thermoantarthicum* xylanase was hindered by cation Cu^{2+} and Fe^{2+} (Lama *et al.* 2004). *Penicillium capsulatum* xylanase was hindered by Zn^{2+}, Cu^{2+}, Ca^{2+}, and Mn^{2+} (Ryan *et al.* 2003).

Streptomyces spp. C1-3 xylanase demonstrated its highest activity in Birchwood- and Arabino-xylan. In Birchwood-xylan, about 94% of carbohydrate consists of xylose, which makes it a suitable substrate for standardization of various xylanases activity. The difference in xylose chain length and the number of the side-chains at xylan substrate affects the xylanase performance in hydrolyzing the xylan structure. The shorter is the xylan chain, the slower is the xylanase hydrolytic activity. This is due to the decrease of xylanase's bounding site over its substrates (Li *et al*. 2000). *Trichoderma longibrachiatum* xylanase demonstrated its highest activity in oat spelt xylan (Chen *et al.* 1997), whereas *Sulfolobus solfataricus* xylanase performed high in beechwood and oat spelt and lower in birchwood (Cannio *et al.* 2004). The highest activity of *Streptomyces malaysiensis* xylanase was in larchwood-, oatsplet-, and birchwood xylan (do Nascimento *et al.* 2003). *Streptomyces* spp. C1-3 xylanase demonstrated its activity in the substrate of carboxy methyl cellulose (CMC). Xylanase examination in CMC substrate needs to be conducted because there are several xylanase that is able to hydrolyze not only xylan but also cellulose (Tjusibo *et al*. 1992). Overall, on the basis of this research, xylanase *Streptomyces* spp. C1-3 has an optimum condition at pH 3, 90 °C, and have a CMCase activity.

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