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Optimization of Xylanase Production by *Streptomyces costaricanus* 45I-3 Using Various Substrates through Submerged Fermentation

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Xylanase is an important hydrolytic enzymes with many application in several industries, but to obtain enzyme derived products is not easy. Thus, the optimization of efficient xylanases production is a great interest for enzyme application. This study aims to determine the type of substrate, medium composition, and optimum conditions of xylanase production by *S. costaricanus* 45I-3. Determination of substrate type was done by growing the tested bacteria on birchwood xylan, beechwood xylan, oat spelled xylan, corn cobs xylan, and tobacco xylan substrate, meanwhile the determination of medium composition and enzyme production were done by measuring xylanase activity at various substrate concentration and replacing the carbon, nitrogen, phosphate and surfactants source. The results showed that the highest enzymatic index (EI) produced from corn cob xylan substrate at 3.60 meanwhile the second highest was beechwood xylan substrate at 2.87 EI, however this substrate is purer, thus this substrate was selected and used as xylan sources for further optimization measurement. The best xylanase activity (2.29 U mL^{-1}) obtained on eighth day after inoculation on rotary incubator at 120 rpm in 28 ºC. Arabinose as the source of carbon generate the highest activity at 3.161 U mL^{-1} meanwhile the most preferred source of phosphate is Na_2HPO_4 (2.37 U mL^{-1}). Both source of nitrogen i.e. nitrogen ammonium sulphate $(NH_4)_2SO_4$ and yeast extract were able to produce xylanase at 2.57 and 2.36 U mL^{-1}. The addition of surfactant in production medium showed addition of SDS surfactant (0.146 U mL^{-1}) and Tween 80 (0.438 U mL^{-1}) showed a negative response by decreasing the activity. The conclusion showed that the xylanase activity was increased after optimization at various C, N, and P sources, and the use of nitrogen source $(NH4)_2SO_4$), become a more economical alternative to replacing a nitrogen source yeast extract so it can lower the production costs of xylanase enzyme.

Key words : fermentation, *Streptomyces costaricanus 45I-3*, substrate variation

Xilanase merupakan enzim hidrolisis penting dengan banyak aplikasi di berbagai industri, namun untuk mendapatkan produk turunan enzim tidaklah mudah. Dengan demikian, optimalisasi produksi xilanase yang efisien merupakan perhatian besar untuk aplikasi enzim. Penelitian ini bertujuan untuk mengetahui jenis substrat, komposisi medium, dan kondisi optimum produksi xilanase oleh *S. costaricanus* 45I-3. Penentuan jenis substrat dilakukan dengan cara menumbuhkan bakteri uji pada media xilan birchwood, xilan beechwood, xilan oatspelt, xilan tongkol jagung, dan xilan tembakau, sedangkan penentuan komposisi media dan produksi enzim dilakukan dengan mengukur aktivitas xilanase pada berbagai konsentrasi substrat. dan mengganti sumber karbon, sumber nitrogen, sumber fosfat dan surfaktan. Hasil penelitian menunjukkan bahwa indeks enzimatik (EI) tertinggi dihasilkan dari substrat xilan tongkol jagung sebesar 3,60 sedangkan tertinggi kedua adalah substrat xilan beechwood sebesar 2,87 EI. Substrat ini lebih murni sehingga dipilih dan digunakan sebagai sumber xilan untuk optimalisasi lebih lanjut. Aktivitas xilanase terbaik (2,29 U mL^{-1}) diperoleh pada hari kedelapan setelah inokulasi pada inkubator bergoyang 120 rpm dan suhu 28 ºC. Arabinosa sebagai sumber karbon menghasilkan aktivitas tertinggi yaitu 3,161 U mL^{-1} sedangkan sumber fosfat yang paling baik adalah Na_2HPO_4 (2,37 U mL^{-1}). Kedua sumber nitrogen yaitu amonium sulfat $(NH_4)2SO_4$ dan ekstrak khamir mampu menghasilkan xilanase pada 2,57 dan 2,36 U mL^{-1}. Penambahan surfaktan pada media produksi SDS (0,146 U mL^{-1}) dan Tween 80 (0,438 U mL^{-1}) menunjukkan respon negatif dengan penurunan aktivitas. Kesimpulan menunjukkan bahwa peningkatan aktivitas xilanase setelah dilakukan optimasi pada berbagai sumber C, N, dan P, serta penggunaan sumber nitrogen $(NH_4)2SO_4$), menjadi alternatif yang lebih ekonomis untuk menggantikan ekstrak ragi sumber nitrogen sehingga dapat menurunkan biaya produksi enzim xilanase.

Kata kunci : fermentasi, *Streptomyces costaricanus* 45I-3, variasi substrat

Xylan is the main component of hemicellulose. The main chain is composed by β-xilopironosa units with β-1,4-glycosidic bond with a subsidiary chain in

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the form of glucopyranosyl, 4-O-methyl-D-glucopyranosyl, α-L-arabinofuranosyl, acetyl, or furulil and p-coumaril (Kulkarni *et al.* 1999; Li *et al.* 2000) Endo-β-1,4-xylanase (EC 3.2.1.8) is an enzyme which is important to hydrolyze xylan perfectly thus it can produce an usable product such as xylose, and xylobiose like xylooligosaccharides (Bernier *et al.* 1983; Chakrit *et al.* 2006).

Various microbes were reported able to produce xylanase. *Bacillus* from bacteria, *Trichoderma* and *Aspergillus* from fungi, and *Streptomyces* from actinomycetes were known to be a potential microbes producing xylanase. Those microbes are known to have varied and widespread ecological niches (Collins *et al.* 2005). Bacteria from Indonesia such as *Bacillus licheniformis* strain 15 produces endo-β-1,4-xylanase which grown on banana peels substrate (Helianti *et al.* 2007*)*. Meryandini *et al.* (2007) reported that the isolate *of Streptomyces costaricanus* 45I-3 isolated from peat swamps soil in Kalimantan, Indonesia, has an optimum activity at pH 5.0 in 50 °C. However this study only used a single substrate i.e. oat spelt xylan and did not conduct the activity measurement of various carbon, phosphate and nitrogen sources also the effects of surfactants to produce an optimum conditions in producing extracellular xylanase.

The main nutrients for microorganisms growth were carbon sources, nitrogen and mineral components especially phosphate. The media formulation for the growth and fermentation *S. costaricum* is an important stage at designing an experiments in the work scale. Papagianni (2004) reported that the carbon sources, nitrogen sources and the fermentation time have a significant role in determining the enzyme production level in a culture. Therefore, it is important to optimize the conditions to produce enzyme inexpensively using the available nutrient sources abundantly. Response surface method has been used as successful statistical tools for the optimization of media composition in the fermentation process for enzyme production (Dobrev *et al.* 2007). Therefore the aim of this study was to optimize the nutritional conditions in producing xylanase using *S. costaricanus* 45I-3 by conducting a series of research stages and data analysis.

MATERIALS AND METHODS

Bacterial Strain. The bacteria used in this study was *S. costaricanus* 45I-3 isolated from peat swamp forest in Kalimantan, Indonesia, a collection of Dr. Yulin Lestari (staff of Department of Biology, Faculty of Mathematic and Natural Sciences, Bogor Agricultural University, Indonesia). These isolate was cultured on International Streptomyces Project No. 2 (ISP2), supplemented with antibiotics nalidixic acid (1 mg mL^{-1}) and cyclohexamide (5 mg mL^{-1}), and incubated for 8 days at room temperature (27 °C), and this isolate was used as stock culture for the further assays.

Morphological Characteristic of *S. costaricanus* 45I-3 in Various Growth Media. The cultivation of *S. costaricanus* 45I-3 was conducted using various solid media, which aims to see the growth and morphological characteristics in media used; nutrient agar (NA), potato dextrose agar (PDA), ISP, yeast soluble starch agar (YSA), International Streptomyces Project No. 4 (ISP4), and beechwood xylan agar medium. Each media (25 mL) were sterilized at 121 °C for 15 min. After sterilization, a loopful of *S. costaricanus* 45I- was streaked on media, then incubated at 27 °C for 8 days. The bacterial cells that showed the best growth will be used for further study.

The Measurement of β-Xylanase Activity of *S. costaricanus* 45I-3 Based on Clear Zone. A total volume of 250 mL fermentation medium (g/L) which consist of yeast extract 1.25 g, KH_2PO_4 0.25 g, 0.05 g $MgSO_4 \cdot 7H_2O$) and 0.8% (w/v) substrate (beechwood xylan) were added to 250 mL distilled water. After the medium mixed perfectly the sterilization was performed on 121 °C for 15 min. After sterilization, the medium then inoculated by 1% (v/v) bacterial culture and incubated at 27 °C for 8 days with 120 rpm agitation. Furthermore, the culture was centrifuged at 8000 rpm for 15 min to remove bacterial cells. The supernatant then was precipitated using 80% acetone solvent in accordance with Meryandini *et al.* (2007). Xylan degradation activity assay was conducted using two layers agar method with modifications based on Chen *et al.* (2004). The base layer of agar medium consists of 50 mM acetate buffer (sodium acetate + acetic acid) pH 6.0 and 1.7% agar. Meanwhile the upper layer consists of 0.8% (w/v) xylan substrate (beechwood, birchwood, oat spelled, corn cobs, and tobacco) and 1.7% (w/v) agar in 50 mM acetate buffer pH 5.0. A total of 3, 5, 7, and 10 mL precipitated xylanase were dripped on each media with different substrates and dried subsequently at 27 °C for 15 min, then incubated at 37 °C for 4 days. After the incubation process the media were stained using 1% congo red for 15 min and rinsed using 1 mM NaCl. The hydrolysis of β-xylanase indicated by the clear zone (halo) formed with an orange margin around (Carder, 1986). This

halo was measured for subsequent calculation of the enzymatic index (EI) using the equation.

$$EI = \frac{\text{Diameter of hydrolysis zone}}{\text{Diameter of colony}}$$

The Effect of Xylanase Production using Various Concentration of Xylan. In this study a beechwood xylan was used as a carbon source. To determine the minimum concentration with the best activity in producing β-xylanase, a varying concentration of xylan were used in fermentation media. Beechwood xylan was added to each fermentation medium with concentrations 0.2, 0.4, 0.6, 0.8, and 1.0% (w/v) respectively, meanwhile the 0% (w/v) medium (without the addition of beechwood xylan) used as a control.

The Effect of Various Carbon, Nitrogen, Phosphate, and Surfactant Sources towards *β*-Xylanase Produced by *S. costaricanus* 45I-3. Observation of various nutrients addition i.e. carbon, nitrogen, phosphate and surfactant sources, towards β-xylanase production was done by replacing the resource of various nutrients with a variety of other sources. The production of β-xylanase with the addition of major carbon source in the form of beechwood xylan 0.8% (w/v) with various other carbon sources, such as glucose, galactose, maltose, xylose, fructose, sucrose, arabinose and corn cob xylan with 0.3% (w/v) concentration, respectively. Meanwhile, the nitrogen source used were malt extract, peptone, tryptone, yeast extract, ammonium sulfate $(NH_4)2\ SO_4$, and ammonium persulfate $(NH_4)2\ S_2O_8$ respectively 0.3% (w/v). The effect of various phosphate sources addition was done by replacing the main source of phosphate KH_2PO_4 with other phosphate sources, i.e. Na_2HPO_4, NaH_2PO_4, K_2HPO_4 and $AlPO_4$ respectively 0.03% (w/v). Measurement of xylanase activity with 0.03% (w/v) surfactants addition was performed by adding Tween 80, SDS (sodium dodecyl sulfate), and DMSO (dimethyl sulfoxide) respectively.

Xylanase Activity Assay. The released reducing sugars were measured using DNS method (Miller, 1959). The DNS method was conducted by reacting 0.5 mL of 0.8% beechwood xylan substrate with 0.5 mL xylanase in acetate buffer (0.05 M, pH 5.0) and incubated at room temperature for 30 min. The reaction stopped by adding 1.5 mL of 3.5 dinitrosalicylic acid and heated for 15 min at 100 ºC. The solution then allowed to cool down and the absorbance value was measured using a spectrophotometer at 540 nm. Xylanase activity was calculated by using xylose standard curve. One unit of enzyme activity is defined as the amount of enzyme required to produce 1 mol xylose sugar per minute in standard conditions.

RESULTS

Morphological Characteristic of *S. costaricanus* 45I-3. *S. costaricanus* 45I-3 bacterial culture showed several morphological characteristics when grown on a various growing medium as shown in Figure 1. The *S. costaricanus* 45I-3 colony was round-shaped hyphae, undulate margin with mealy surface, the substrate mycelia are yellow, and the aerial mycelia are yellowish white with a bright yellow pigmentation on beechwood xylan medium, meanwhile on the other media (YMA, YSA, NA, PDA and ISP4) the aerial mycelia are brown with a yellowish white to brownish gray substrate mycelium (Table 1). Bacterial strains isolated from peat swamp forest in Kalimantan have *flexous* hyphae but has a spiral type on ISP4 medium.

The spores have a smooth morphological characteristic. *S. costaricanus* 45I-3 was able to grow well in six different media as shown in Figure 1, but the most rapid and abundant growth were in beechwood xylan and YMA medium, the growth on those media was started on 2nd day after incubation, the growth begins with substrate hyphae formation and followed

Table 1 Morphological characteristic of *S. costaricanus* 45I-3 on various media

Isolate Code	Growth Media	Color		Pigmentation on Media	Hyphae Type	Spore Morphology
		Aerial Mycelia	Substrate Mycelia			
45I-3	YMA	Grey	Brownish grey	None	Flexous	Smooth
	YSA	Grey	Brownish grey	None	Flexous	Smooth
	Beechwood xylan	Yellowish white	Yellow	Bright yellow	Flexous	Smooth
	NA	White	Yellowish white	None	Flexous	Smooth
	PDA	Grey	Yellowish grey	None	Flexous	Smooth
	ISP4	Grey	Greyish white	None	Spiral	Smooth

with aerial hyphae covering the majority of growth media which followed by sporulation process. On the other hand, the four other media showed a slower growth and the aerial hyphae which formed was thinner and did not cover the overall surface of growth media.

Xylanase Activity on Various Xylan Sources. This measurement aimed to determine which xylan source preferred by tested bacteria that could be seen its EI value. The highest EI value (3.6) was obtained in the solid culture which was added with corn cob xylan, while the lowest in tobacco xylan-enriched medium with a mere of 2.08 EI value. In addition, three other substrates i.e. birchwood xylan, beechwood xylan and oat spelt xylan produces an adjacent EI values respectively at 2.80, 2.87 and 2.77 (Fig. 2).

Optimal Incubation Time in Producing Xylanase. The maximum activity of xylanase was obtained in 8th day after incubation. The optimum activity of extracellular enzyme was generated on 8th day i.e. at 2.29 U mL^{-1} (Fig. 3).

The Effect of Various Concentration of Substrate/Beechwood Xylan for Xylase Production. Every microorganism requires a certain concentration of carbon source for their growth. The results in Figure 4 shows that the addition of a carbon source ranging from 0-1% (w/v) beechwood xylan give a different effect on xylanase production. In general, it can be concluded that the addition of higher concentrations produce a greater xylanase. However, the provision of 0.8% (w/v) and 1.0% (w/v) carbon source at 7th and 8th day after incubation showed not significant result with a nearly same amount of xylanase production i.e. 2.29 U mL^{-1}.

The Effect of Various Carbon Sources towards Xylanase Production. Figure 5 shows the effect of various carbon sources usage for xylanase produced by *S. costaricanus* 45I-3 isolates, then different treatments were done by adding various carbon sources (0.3%) i.e. glucose, galactose, maltose, xylose, fructose, sucrose, arabinose, and corn cob xylan. The addition of 0.3% arabinose and fructose as additional carbon sources produced the highest (3.16 U mL^{-1}) and lowest xylanase (0.45 U mL^{-1}), respectively.

The Effect of Various Nitrogen Sources towards Xylanase Activity. Ammonium sulphate $(NH_4)_2SO_4$ and yeast extract are the best nitrogen sources for xylanase production i.e. 2.52 and 2.36 U mL^{-1} individually compared to the other organic and inorganic nitrogen sources which used, such as malt extract, peptone, tryptone and ammonium persulfate (Fig. 6).

The Effect of Various Phosphate Sources towards Xylanase Activity. The results obtained indicate that the best phosphate source for xylanase production was Na_2HPO_4. The addition of Na_2HPO_4 can induce xylanase production up to 2.37 U mL^{-1}.

The Effect of Various Surfactants towards Xylanase Activity. The effect of surfactant on xylanase production was tested using 3 different surfactants sources including Tween 80, SDS, and DMSO. The results showed that both of 0.03% Tween 80 and SDS had a significant effect to decline of xylanase production. The addition of Tween 80 decreased xylanase production to 0.44 U mL^{-1} and SDS addition also decreased xylanase production to 0.15 U mL^{-1}. The addition of DMSO (dimethyl sulfoxide) did not affect xylanase production which was proven with the xylanase production that still reached 2.46 U mL^{-1}.

DISCUSSION

Xylan is one of the carbon sources used by microorganisms, particularly bacteria, to be reformed into a simpler molecular structure which can then be used as an energy source. Several carbon sources used in this study were birchwood xylan, beech wood xylan, oat spelled xylan, corncob xylan and tobacco xylan. This may occur because xylan from corn cobs, birchwood, beechwood and oats contains many growth factors needed, vitamins and proteins which can provide source of carbon and nitrogen for the test bacteria (Sasmitaloka *et al.* 2019; Revanker and Lele 2006), low lignin and silica (Battan *et al*. 2006).

Based on the results, xylanase production peaked at the eighth dayof incubation, accounted at 2.29 U mL^{-1}. Several other research results showed that the optimal incubation time to produce xylanase depends on the type of bacteria used regarding with the time of bacterial growth. Gram-negative and gram-positive bacteria, which are non-actinomycetes, tend to have a shorter incubation time compared to actinomycetes group. Several studies reported that *Bacillus subtilis* cho40 merely produced xylanase during 4 days of incubation time (Khandeparker *et al*. 2011). Another study revealed the optimal extracellular xylanase production time, carried out by Li *et al*. (2010), of actinomycetes isolate, *Streptomyces rameus* L2001, was on the 7th day. Kavya and Padmavathi (2009) reported that the xylanase produced by *Aspergillus niger* was at 6.11 U mL^{-1} on the 6th day after incubation. As can be observed from curve, bacterial cells started to decrease on the 8th day which was the peak of exponential period. In this phase, bacterial cells grow dramatically and require

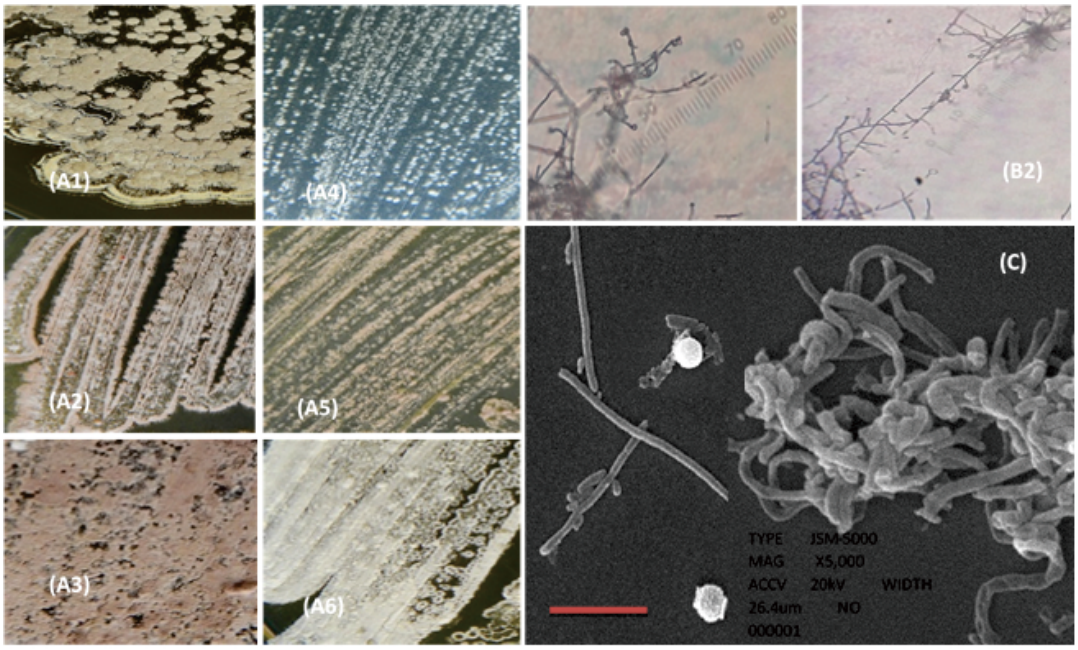

Fig 1 Morphological characteristic of *Streptomyces costaricanus* 45I-3 on various substrates. A1 (beechwood xylan), A2 (yeast solube startch), A3 (YMA), A4 (ISP4), A5 (PDA) and A6 (NA). B1 (morphology of aerial hyphae), B2 (crossed hyphae). C (morphological spore and hyphae) figure taken using *Scanning electron microscope* (SEM).

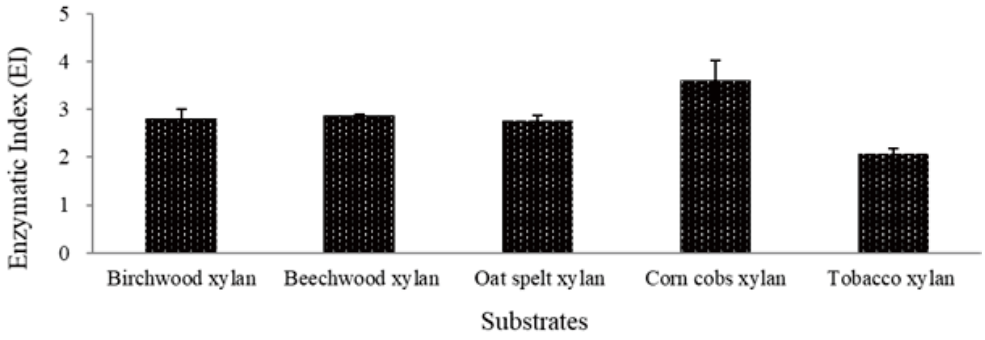

Fig 2 The effect of different substrates on xylanase production by *Streptomyces costaricanus* 45I-3 after 4 days incubation at 28 °C.

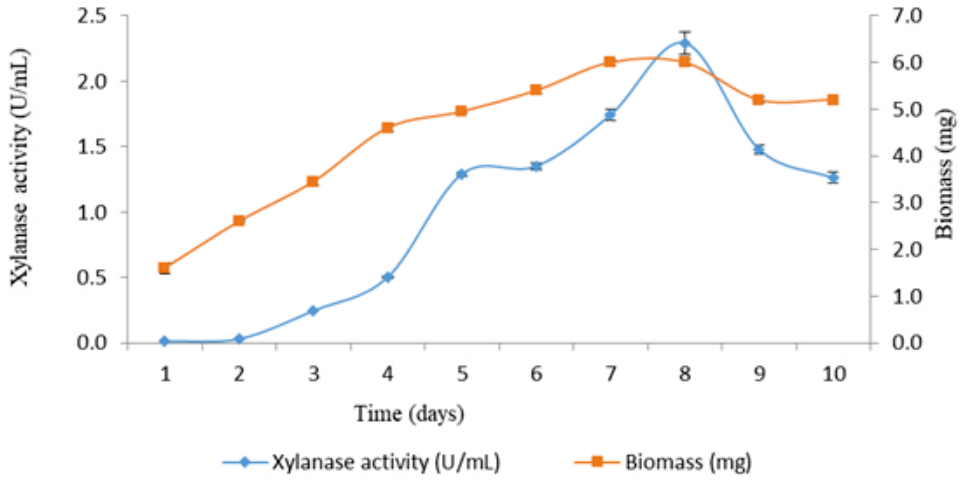

Fig 3 Cell biomass of *S. costaricanus* 45I-3 (mg mL^{-1}) (—■—) and *S. costaricanus* 45I-3 xylanase activity (—●—) on 0.8% beechwood xylan medium at 120 rpm in room temperature (27 °C).

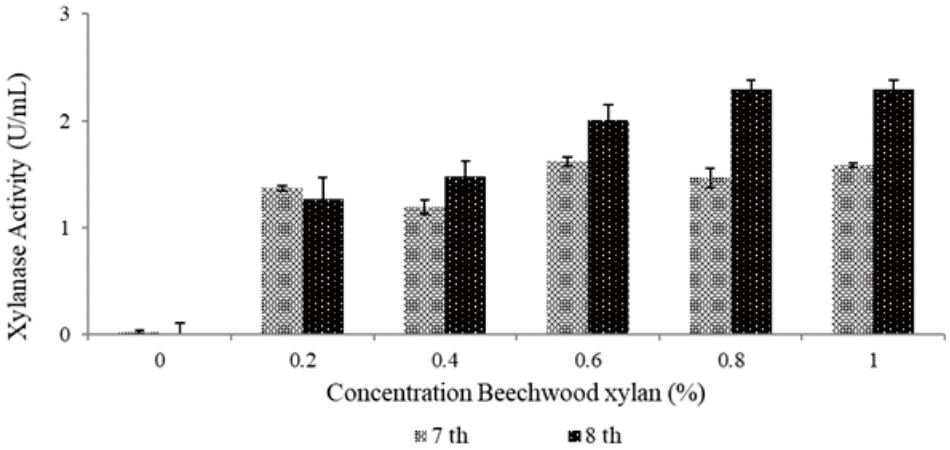

Fig 4 Screening of ISB64 isolate-producing cellulase thermophilic bacteria from the Ie Seuum hot spring, Aceh Besar. (a) Clear zone, (b) Bacterial colony, and (c) CMC media flooded with 1% congo red.

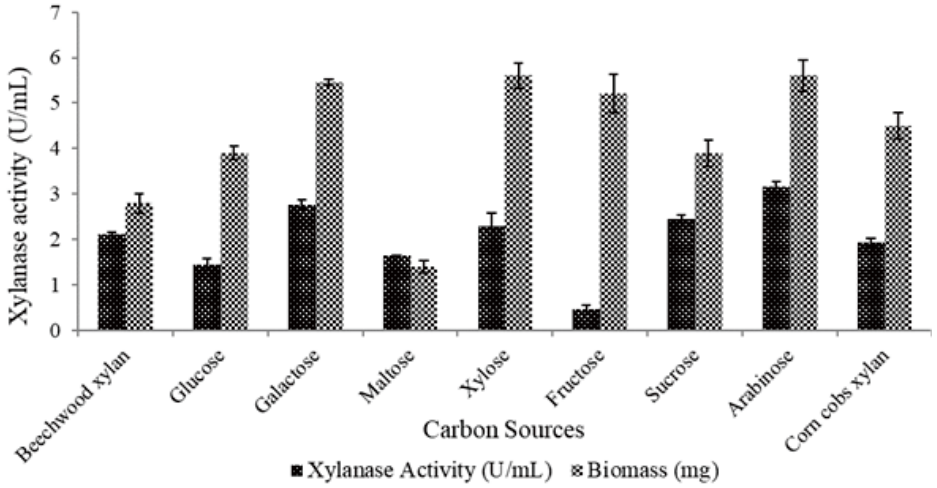

Fig 5 The effect of various carbon sources towards xylanase produced by *S. costaricanus* 45I-3 after incubated for 8 days.

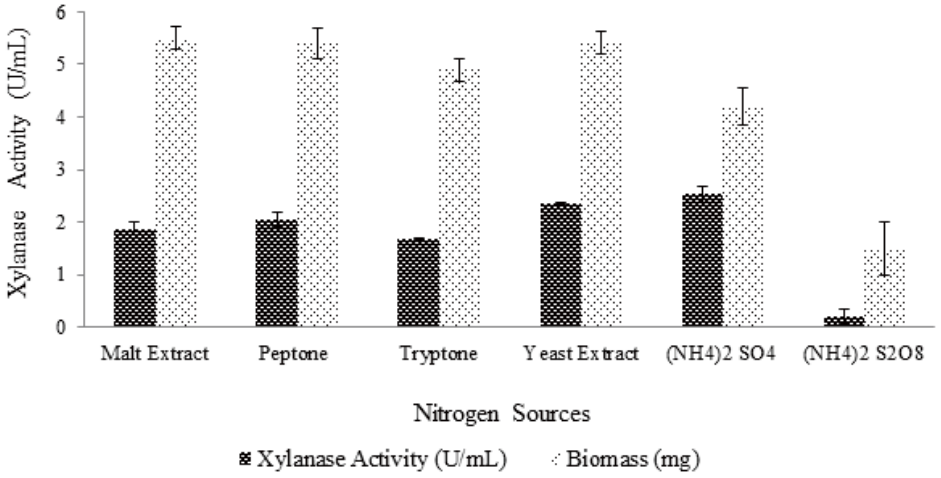

Fig 6 The effect of various phosphate sources towards xylanase produces by *S. costaricanus* 45I-3 after incubated for 8 days.

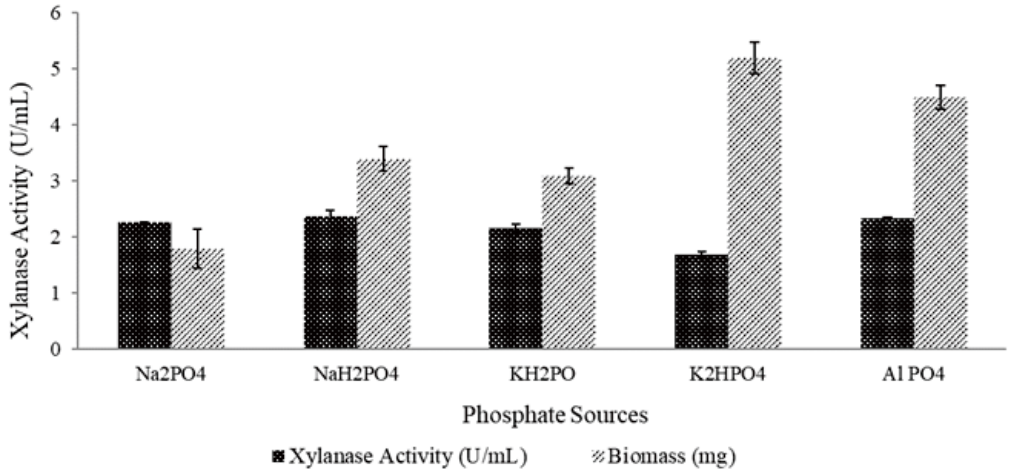

Fig 7 The effect of various phosphate sources towards xylanase produced by *S. costaricanus* 45I-3 after incubated for 8 days.

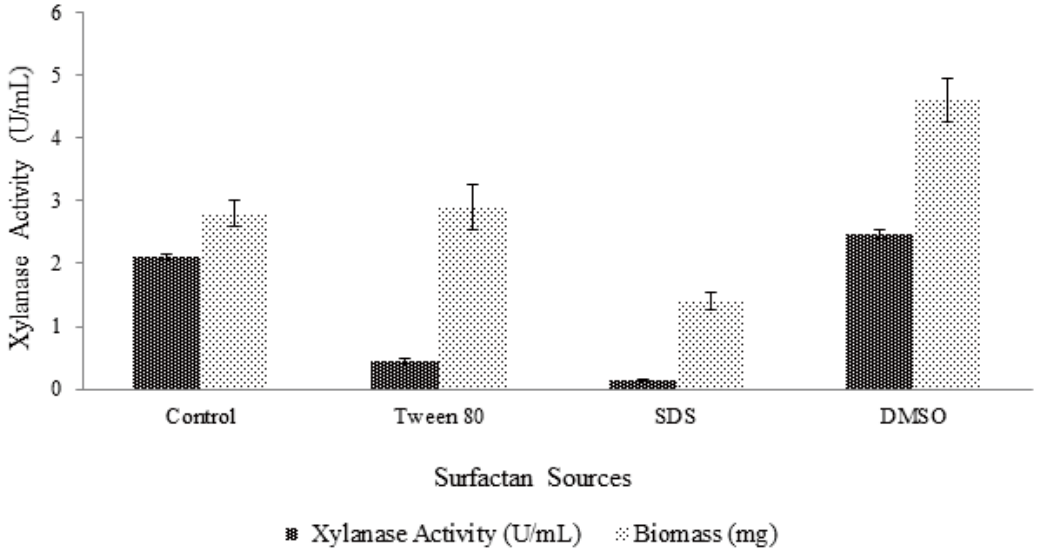

Fig 8 The effect of various surfactant towards xylanase produced by *S. costaricanus* 45I-3 after incubated for 8 days.

more energy source for survival than in other periods. Therefore, production of xylanase was excreted abundantly in order to gain more energy in terms of degrading media-contained xylan.

Moreover, xylan concentration strongly related to enzyme production and for this reason, this study also monitored the effect of several concentrations of xylan to xylanase activity. Based on the results presented in Figure 4, the concentration used in the next optimization test was 0.8% (w / v). This result is in accordance with Guha *et al.* (2013) which states that the best concentration to produce xylanase is to provide a carbon source (xylan) from 0.25 to 1.0% (w / v). Lawrence *et al*. (2015) reported that the xylan concentration variation of 0.5-1% (w / v) added to the fermentation medium did not have a significant effect on xylanase production at concentration more than 1%. The administration of 0.5-3.0% molasses as a supplement for *B. subtilis* and *B. megaterium* can reduce xylanase production in line with the increase in the concentration of molasses given. This probably due to the nutrients contained in molasses produce catabolite suppressants in xylanase production (Irfan *et al*. 2016).

Application of several additional carbon sources also shows an influence on xylanase activity. Figure 5 shows the addition of fructose to the beech wood xylan production media significantly decreased the xylanase production, it was because of the xylanase produced in beech wood xylan media is 2.11 U / mL without the addition of fructose. Additional fructose can inhibit xylanase production, since fructose as a simple sugar will be used first as a carbon source by *S. costaricanus* 45I-3 isolates or bacterial cell growth. As supposed by Guan *et al.* (2016), simple sugars, including fructose,

have no remarkable effect with xylanase production since, perhaps, those sugars could prevent synthesis of enzymes. This is also supported by study conducted by Ajijolakewu *et al.* (2016) who suggested that there were repressive effects produced by simple carbohydrate molecules in producing xylanase. Indeed, those were only utilized for growth. On the other hand, there is breakthrough discovering that *S. thermocoprophilus* TC12W was able to produce 1204.8 U/g of xylanase by using alkaline pretreated empty fruit bunch (APEFB) as a carbon source, which was the first report utilizing that (Sinjaroonsak *et al.* 2019).

Giving different nitrogen sources turned out to have various effects on xylanase activity, indicating that yeast extract had the effect of being the best nitrogen source on xylanase activity. Bhardwaj et al. (2019) stated in their review that nitrogen is one of the most important elements for metabolisms, including enzymatic activity. A study by Zuhri *et al.* (2013) showed that ammonium sulfate is the best nitrogen source for the cultivation of *Bacillus* sp. M123 for the production of alkaline proteases. Addition of 1% casein played role in both the highest xylanase activity and xylanse production, 1.78 U g^{-1} and 3.69 U mgG^{-1}, respectively (Tai *et al.* 2019). Sudan and Bajaj (2007) reported that adding 0.3% ammonium sulfate to the production medium was able to make *Aspergillus niveus* RS2 produce 15 U/mL xylanase after 5 days of incubation which was the second best after yeast extract. The role of nitrogen, especially ammonium sulfate, from xylanase excretion was found from *Anoxybacillus kamchatkensis* strain NASTPD13 (Yadav *et al.,* 2018). Ravindran *et al.* (2019) reported the highest xylanase (6495.6 IU/g of dry SCW) produced by *A. niger* was successfully resulted using media supplemented with 0.2g/g of yeast extract as nitrogen source. All nitrogen sources added to the production media showed that all of them were able to promote cell biomass growth and xylanase production except for ammonium persulfate $(NH_4)2S_2O_8$ which inhibited cell biomass growth and xylanase production. This is presumably because $(NH_4)2\ S_2O_8$ is toxic so that it inhibits the growth of *S. costaricanus* 45I-3 cells and also inhibits xylanase production.

The effect of different inorganic phosphate resources towards xylanase production has been tested and presented in Figure 7, shows that *S. costaricanus* 45I-3 isolates was able utilizing other phosphate sources i.e. NaH_2PO_4, KH_2PO_4, and $AlPO_4$ to produce xylanase, it is seen from the xylanase production which were relatively stable at 2.17 to 2.34 U mL^{-1}. A slightly different results in the addition of phosphate sources was K_2HPO_4 which only capable inducing xylanase production at 1.69 U mL^{-1}. A similar result were reported by Mandal (2015) which stated that Na_2HPO_4 is the best phosphate sources to *Bacillus cereus* BSA1 for xylanase production, with the production of xylanase at 5.53 U mL^{-1}.

The phosphate salts with a certain concentration can encourage organism growth and stimulates the synthesis of extracellular enzymes in production medium Chellapandi and Jani (2008). Microbes using phosphate source as an ingredient synthesis of nucleic acids and phospholipids on the cell wall.The addition of other biosurfactant such as 0.03% sodium dodecyl sulfate (SDS) decrease xylanase activity by 13 fold (0.15 U mL^{-1}) compared with the production media without surfactant addition i.e. 2.11 U mL^{-1} (Figure 8). Silva *et al*. (2015) reported that the addition of SDS on production media decreasing xylanase activity by 7.75 fold (12.9 U mL^{-1}) which is lower than controls (100 U mL^{-1}). SDS is a strong denaturant, the usage of SDS at certain concentrations can interfere enzyme activity function and lipid solubility (Wamack *et al*. 1983)

As conclusion, from the results obtained it can be concluded that *S. costraricanus* 45I-3 was able to grow on a variety of media, but the best growth medium was medium containing xylan (beechwood xylan) which was produced a yellow aerial hyphae, smooth-shaped spores and produces bright yellow pigmentation. The usage of 0.8% beechwood xylan substrate on 8th day after incubation was able to produce the highest xylanase production. The best fermentation conditions were on the addition of arabinose carbon source, and nitrogen source i.e. ammonium sulfate $(NH_4)2\ SO_4$ and yeast extract. The most preferred source of phosphate was Na_2HPO_4. The addition of surfactants such as SDS and Tween-80 gave negative effects because it decreased the level of xylanase production while the addition of DMSO did not decrease the activity.

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