Characteristics of Cellulose Produced by Cellvibrio Mixtus UV4 Isolated from Sugarcane Leaf

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Abstract

This study aims to characterize the crude extract of cellulase that produced by Cellvibrio mixtus UV4. This is an experimental research. Cellvibrio mixtus UV is obtained from the sugarcane leaf. Stages of this research were include (1) Sterilization of tools and materials; (2) production of cellulase by aerobic fermentation using liquid CMC 1% for 6 days; (3) isolation of crude extract cellulase; (4) determination of protein standard curve using Bovine Serum Albumin (BSA); (5) determination of the cellulase crude extract protein by the Bradford method; (6) determining the specific activity of crude extract cellulase; (8) analysis of the molecular weight of crude extract cellulase by SDS-PAGE. Analysis of the crude extract cellulase by SDS-PAGE produce proteins with molecular weights ranging from 127 kDa, 116 kDa, 98 kDa, 90 kDa, 70 kDa, 54 kDa and 46 kDa. Characteristics of the crude extract cellulase produced by C. mixtus UV4 resulting cellulase with activity of 0,011 U/mL, protein content 0.872 µg/mL, and the specific activity of 12.62 U/mg.

Keywords: crude extract of cellulose, Cellvibrio mixtus UV4, specific activity of cellulose, sugarcane leaf

I. INTRODUCTION

The leaves of sugarcane is agricultural waste from sugar cane production in Indonesia. The high production of sugarcane and meningkat need for sugar in Indonesia of course, coupled with the increasing waste sugar cane leaves. Sugarcane leaf composition consisting of water (10.37%), cellulose (38.30%), hemicellulose (30.06%), lignin insoluble (8.88%) and ash (3.98%) (Yahya and Scallop, 2008). One component of sugar cane leaves that are difficult to decompose cellulose. Cellulose is a linear polysaccharide of glucose residues with bond-1,4-glycosidic β. Cellulose can be degraded by microbes cellulolitic with the aid of cellulase.

Cellulolytic microbes using cellulose as a source of energy and carbon by producing cellulase enzymes that can degrade cellulose component overaull and a long and its derivatives to glucose (Hardjo et al., 1989). Cellulolytic microbes that have great ability to produce cellulase enzymes need to be revealed and known characteristics of the enzyme. It aims to determine the conditions of how these enzymes work optimally. Among the characters of cellulase enzymes include enzyme activity, specific activity of the enzyme, protein content and molecular weight proteins.

II. MATERIALS and METHODS

This research was conducted at the Laboratory of Microbiology and Molecular Genetics Laboratory, Faculty of Science and Technology, Airlangga University in June 2012 to February 2013.

The tools used in this study were Autoclave, Laminar Air Flow, Spectrophotometer Spectronic, micro cuvette, incubators, ovens, clear bottles vol 100 mL, 250 mL and 500 mL Erlenmeyer flask, shaker, test tube, vortex, pH meter, thermometer, needle ose, petri dish, pipette volume of 2 mL, 5 mL and 10 mL, micropipette 100-1000 µL, Eppendorf tubes, blender, UV-Vis spectrophotometer and refrigerator.

The materials used in this study include Cellvibrio mixtus UV4 (Firmani, 2013), distilled water, liquid CMC 1%, (NH₄)₂SO₄, 0.05M and 0.02M phosphate buffer pH 7, Bovine Serum Albumin (BSA), anhydrous glucose reagent Bradford, 0.1N HCl, BaCl₂, and a cellophane bag. Material for separating gel 15% among others 2500 mL acrilamid-bis 30%, 1250 mL of 1.5 M Tris HCl pH 8.8, 1175 akuabides mL, 50 mL of 10% SDS, TEMED 6 mL and 25 mL of 10% APS. Materials maker 4% stacking gel,
among others, 325 mL acrylamid-bis 30%, 625 mL of 0.5 M Tris HCl pH 6.8, 1525 akuabides mL, 25 mL of 10% SDS, 2.5 mL TEMED, 12.5 mL APS 10% and running buffer.

III. METHODS

The procedures in this study include (1) Sterilization of tools and materials; (2) production of cellulase by aerobic fermentation using liquid CMC 1% for 6 days; (3) isolation of crude extract cellulase; (4) determination of protein standard curve using Bovine Serum Albumin (BSA); (5) determination of the cellulase crude extract protein by the Bradford method; (6) determining the specific activity of crude extract cellulase; (8) analysis of the molecular weight of crude extract cellulase by SDS-PAGE

IV. RESULT AND DISCUSSION

Samples 1, 2 and 3 is a protein that is derived from crude extract cellulase enzymes isolated from cultures of C. mixtus UV4 CMC in liquid media. From Figure 1 can be concluded that there are 7 protein bands in the crude extract of cellulase enzyme with a molecular weight ranging from 127 kDa, 116 kDa, 98 kDa, 90 kDa, 70 kDa, 54 kDa and 46 kDa. However, the protein bands that show cellulase enzyme is not yet known because it was mixed with other proteins. In order to determine the protein bands of cellulase enzymes, needed further analysis by zimogram. Analysis of protein with zimogram can be used to determine protein bands were active against cellulase enzyme inducers substrate, in this study CMC Cellulase enzymes there are 3 types of β endo-1,4 glucanase or endoglucanase, β exo-1,4 glucanase or selobiohidrolase and β-glucosidase. The third of these enzymes work synergistically to break the ties β-glycosidic that make up cellulose. In this study cannot be known types of cellulase enzymes produced by Cellvibrio mixtus UV4 because it does not test substrate specificity. Test substrate specificity can be used to determine the type of enzyme because it uses a special substrate which can only induce the enzyme according to its kind, namely the endoglucanase, eksoglukanase or β-glucosidase. Ayuningtyas research results (2008) indicate that the molecular mass of the enzyme cellulase cellulolytic bacteria from cow rumen is at 40.7 kDa. Research results Celestino et al. (2006) states that the enzyme β-molecular weight of 33.7 kDa endoglucanase. Based on the findings of Lee et al. (2011) mentions that the molecular weight or eksoglukanase selobiohidrolase enzyme is 60 kDa. According to Chang et al. (2010), the molecular weight of β-glucosidase enzyme was 52 kDa. Endoglucanase and selobiohidrolase synergism work together and break down the cellulose into cellobiose and short chain oligosaccharides, which is then converted by β-glucosidase into glucose (Tomme et al., 1996 and Zhang et al., 2006). Selobiohidrolase is a type of cellulase enzymes work in breaking the crystalline regions of cellulose and produce cellobiose, while endoglucanase randomly break up the central regions of the cellulose chain is amorphous regions of cellulose (Parkkinen et al., 2008 and Teeri et al., 1998).

Fig. 1: Profile protein of crude extract cellulase produced by C. mixtus UV4
Table - 1

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Crude extract of cellulase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>100</td>
</tr>
<tr>
<td>Cellulase activity (U/mL)</td>
<td>1.1 $\times 10^2$</td>
</tr>
<tr>
<td>Totally activity (U)</td>
<td>1.1</td>
</tr>
<tr>
<td>Amount of cellulase protein (µg/mL)</td>
<td>0.872</td>
</tr>
<tr>
<td>Total amount of cellulase protein (mg)</td>
<td>8.72$\times 10^2$</td>
</tr>
<tr>
<td>Specific activity (U/mg)</td>
<td>12.62</td>
</tr>
</tbody>
</table>

From Table 1 it is known that the activity of the crude extract produced by cellulolytic bacteria from sugarcane leaf C. mixtus UV4, was 0.11 U / mL, meaning that to produce 1 mol product per minute needed enzyme hydrolysis of 0.11 units. The volume of the crude extract cellulase is too less. The specific activity of C. mixtus UV4 amounted to 12.62 U / mg. Activity and specific activity of cellulase crude extract produced by C. mixtus UV4 is higher than that produced by cellulolytic bacteria in other studies. The research result Rani (2009) states Acidithermus cellulolitycus bacteria that grown on media CMC produce activity of cellulase crude extract 2,64$\times 10^{-3}$ U / mL and specific activity 6.07$\times 10^{-3}$ U / mg.

Results of research conducted by Purwadaria, et al (2003), which isolate bacteria and fungi from termites showed the highest specific activity of the enzyme cellulase Bacillus pumilus PU 4-2 of 1 U / mg of protein (48 hours incubation), whereas the highest specific activity of Aspergillus flavus S-13 of 2.1 U / mg protein with an incubation period of 5 days. Purwadaria research results, et al (2003) showed that the specific activity of cellulase enzyme is lower than the specific activity of the enzyme cellulase produced by isolates of C. mixtus UV4 in this research that is equal to 12.62 U / mg protein with a 6-day incubation period. Crude extract of cellulase protein content greater than cellulase protein content that has been deposited. Cellulase enzyme crude extract still contains many non-enzyme proteins that increase the amount of protein content. The addition of ammonium sulfate and dialysis resulted in decreased levels of protein because protein having a molecular weight less than 20 kDa are not filtered in a cellophane membrane.

**V. CONCLUSION**

SDS-PAGE analysis of crude extract of cellulase produce 7 protein band with a molecular weight ranging from 127 kDa, 116 kDa, 98 kDa, 90 kDa, 70 kDa, 54 kDa and 46 kDa. Characteristics of the crude extract of cellulase of C. mixtus UV4 that the cellulase activity of 0.011 U / mL, the protein content of 0.872 µg / mL and specific activity of 12.62 U / mg.

**REFERENCES**


