Available online at www.sciencedirect.com

ScienceDirect

Procedia Chemistry 18 (2016) 63 – 68

Procedia Chemistry

Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 Conference, MCLS 2015

# Scanning Electron Microscope Analysis of Rice Straw Degradation by a Treatment with α-L-arabinofuranosidase

Anita Kurniati[a], Handoko Darmokoesoemo[b], Ni Nyoman Tri Puspaningsih[a,b]*

[a]*Proteomic Study Group, Institute of Tropical Disease, Universitas Airlangga, Kampus C Mulyorejo, Surabaya 60115, Indonesia*
[b]*Departement of Chemistry, Faculty of Science and Technology,Universitas Airlangga, Kampus C Mulyorejo, Surabaya 60115, Indonesia*

**Abstract**

The purpose of this research was to analyze surface structure modification of rice straw after degradation by α-L-arabinofuranosidase. Analysis of surface structure modification was performed by scanning electron microscope. Alpha-L-arabinofuranosidase was collected from intracellular enzyme and extracellular enzyme; the ratio of enzyme mixture and incubation time were then optimized. The optimum ratio of enzyme mixture was 1:1. The optimum incubation time of rice straw was 8 hours. Rice straw, after degradation by α-L-arabinofuranosidase, was analyzed by scanning electron microscope. The rice straw, before degradation by α-L-arabinofuranosidase, was used as the control of the analysis. The result of this research indicated that enzymatic hydrolysis damaged surface structure of rice straw.


Peer-review under responsibility of the organizing committee of the Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 (MCLS 2015)

*Keywords:* Alpha-L-arabinofuranosidase; Rice straw; Scanning electron microscope

* Corresponding author. Tel.: +62-31-5922427; +62-31-5992445-6; fax: +62-31-5992445.
*E-mail address:* nyomantri@yahoo.com


Peer-review under responsibility of the organizing committee of the Molecular and Cellular Life Sciences: Infectious Diseases, Biochemistry and Structural Biology 2015 (MCLS 2015)
doi:10.1016/j.proche.2016.01.011

**Nomenclature**

| | |
|---|---|
| Abfa | Alpha-L-arabinofuranosidase |
| SEM | Scanning Electron Microscope |
| LB | Luria Bertani |
| DNS | 3,5-dinitrosalicylic acid |
| P | Intracellular enzyme |
| S | Extracellular enzyme |

## 1. Introduction

Rice straw is an abundant biomass produced from agricultural harvest. The rice straw residues were often burned at the rice field, causing environmental problems, such as air pollution and fire. Huge amount of residual rice straw is able to be potentially converted into various different value added products including biofuels, chemicals, cheap energy sources for fermentation, improved animal feeds and human nutrients[1]. Rice straw consists of three main components: cellulose (32–47%), hemicellulose (19–27%) and lignin (5–24%)[2]. By using xylanase, the hemicelluloses will degrade into its reducing sugar, which is xylose, in enzymatic hydrolysis process. Xylanases catalyze the hydrolysis of 1,4-β glycosidic bond from xylan to oligomers and xylose residues. Complete hydrolysis of xylan involves synergistic actions of endo-1,4-β-D-xylanase, 1,4-β-D-xylosidase, Exo-1,4-β-D-xylosidase, α-D-glucuronidase, α-L-arabinofuranosidase (abfa), acetyl xylan esterase, feroryl, and also p-coumaroyl esterases.

The abfas are accessory enzymes that cleave α-L-arabinofuranosidic linkages and act synergistically with other hemicellulases for complete hydrolysis of hemicelluloses[3]. These enzymes warrant substantial research efforts because they represent potential rate of limiting enzymes from agricultural residues in the degradation of lignocelluloses. The action of abfa itself or in a combination with other lignocellulose degrading enzymes represents a promising biotechnological tool as alternatives for some existing chemical technologies such as chlorination in pulp and paper industry, synthesis of oligosaccharides and pretreatment of lignocelluloses for bioethanol production. Application of abfa would further enhance the delignification of pulp as the enzyme acts to release arabinose side chain that hinder the action of other bleaching enzymes. The enzyme acted synergistically with a thermophilic xylanase in the delignification process[4].

The purpose of this research was to analyze the modification of surface structure of rice straw hydrolyzed using abfa. The surface structure modification of rice straw was determined by Scanning Electron Microscope (SEM).

## 2. Methods

### *2.1. Microorganism*

A xylanolytic gene cluster from a thermophilic bacterium *Geobacillus thermoleovorans* IT-08 isolated from Gunung Pancar hotspring (Bogor, West Java, Indonesia) was successfully cloned into the plasmid pTP510 in *Escherichia coli* DH5α. This gene cluster encodes exo-xylanase (GenBank accession number DQ387047), β-xylosidase (DQ345777), and abfa (DQ387046)[5]. Abfa was successfully sub-cloned into *Escherichia coli* BL21[6].

### *2.2. Media culture*

This *Escherichia coli* BL21 was used further in this research and grown in Luria Bertani (LB) medium. LB medium contains tryptone 1%, yeast extract 0.5%, and sodium chloride 1%. The LB medium was sterilized by autoclaving for 15 minutes at 121°C before it was used[7].

### *2.3. Enzymatic assay*

Abfa activity was routinely assayed by measuring the amount of reducing sugars released from 1% (w/v) oat spelt xylan, using the 3,5-dinitrosalicylic acid (DNS) method, with arabinose as the standard of the method. One percent (w/v) oat spelt xylan was preliminarily suspended homogeneously in a 100 mM phosphate citrate buffer (pH

6.0). The reaction mixture containing 100 µl enzyme solutions and 100 µl oat spelt xylan was incubated at 70°C for 60 minutes. After incubation, 600 µl of dinitrosalicylic acid reagent was added to the reaction mixture and boiled for 15 minutes. Absorbance was measured at 550 nm using a UV– Vis spectrophotometer (UV 1800, Shimadzu)[8]. The absorbance was converted into moles of reducing sugar which is produced by using a standard curve generated by arabinose. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 µmol of arabinose per minute at 70°C[9].

### *2.4. Sample preparation*

Rice straw was collected from Mojokerto, East Java, Indonesia. They were chopped by a cutter into small pieces of about 3 to 4 cm in length for SEM analysis. Then, the small pieces of rice straw were washed and dried at 40°C[10].

### *2.5. Optimization of Intracellular enzyme (P) and Extracellular enzyme (S) mixture ratio*

The optimum mixture ratio of P and S was determined using rice straw as the substrate. The reaction mixture contains enzyme solution and substrate. The mixture was incubated at 70°C for 60 minutes. After incubation, 600 µl of DNS reagent was added into 200 µl of reaction mixture and was boiled for 15 minutes. Absorbance was measured at 550 nm using a spectrophotometer (UV-1800, Shimadzu)[10].

### *2.6. Optimization of hydrolysis incubation time*

The optimum hydrolysis incubation time was determined using rice straw as the substrate. The reaction mixture contains enzyme solution and substrate. The mixture was incubated at 70°C for 1, 2, 4, 8, 12 and 24 h. After incubation, 600 µl of DNS reagent was added to 200 µl of reaction mixture and boiled for 15 min. Absorbance was measured at 550 nm by using a spectrophotometer (UV-1800, Shimadzu)[10].

### *2.7. SEM analysis*

After the abfa was hydrolyzed at optimum condition, the modification of surface structure of rice straw was analyzed by SEM (Phenom). Sample was coated with gold before being measured by SEM[11]. In this method, rice straw which was not treated with abfa was used as the control in this analysis.

## 3. Results and discussion

### *3.1. Enzymatic assay*

The result of abfa activity assay is summarized in Table 1.

Table 1. The abfa activity at different P and S ratio

| Ratio P : S | Activity (Unit/mL) |
|---|---|
| 1 : 0 | $1.70 \times 10^{-1}$ |
| 1 : 10 | $2.97 \times 10^{-2}$ |
| 1 : 20 | $1.61 \times 10^{-2}$ |
| 0 : 1 | $8.30 \times 10^{-3}$ |

### *3.2. Optimization of P and S mixture ratio*

The optimization result of P and S mixture ratio is summarized in Table 2. The optimum ratio of P and S mixture for rice straw hydrolysis was 1:1.

Table 2. The optimization of P and S mixture ratio for rice straw hydrolysis

| Ratio P : S | Reducing sugar concentration (μg/mL) |
|---|---|
| 1 : 10 | $2.88 \times 10^2$ |
| 1 : 8 | $3.06 \times 10^2$ |
| 1 : 4 | $3.17 \times 10^2$ |
| 1 : 2 | $3.56 \times 10^2$ |
| 1 : 1 | $3.79 \times 10^2$ |

### *3.3. Optimization of hydrolysis incubation time*

The optimization result of incubation time in rice straw hydrolysis is summarized in Table 3. The highest reducing sugar concentration was detected at 8h. After 8 h of incubation time, the abfa activity decreased.

Table 3. The optimization of hydrolysis incubation time

| Time (hour) | Reducing sugar concentration (μg/mL) |
|---|---|
| 1 | $2.23 \times 10^2$ |
| 2 | $2.16 \times 10^2$ |
| 4 | $2.63 \times 10^2$ |
| 8 | $3.25 \times 10^2$ |
| 12 | $2.86 \times 10^2$ |
| 24 | $2.44 \times 10^2$ |

### *3.4. SEM analysis*

The result of SEM analysis showed that there was a modification in rice straw's surface structure, following the abfa treatment. The modification of surface structure can be seen in fig. 1.

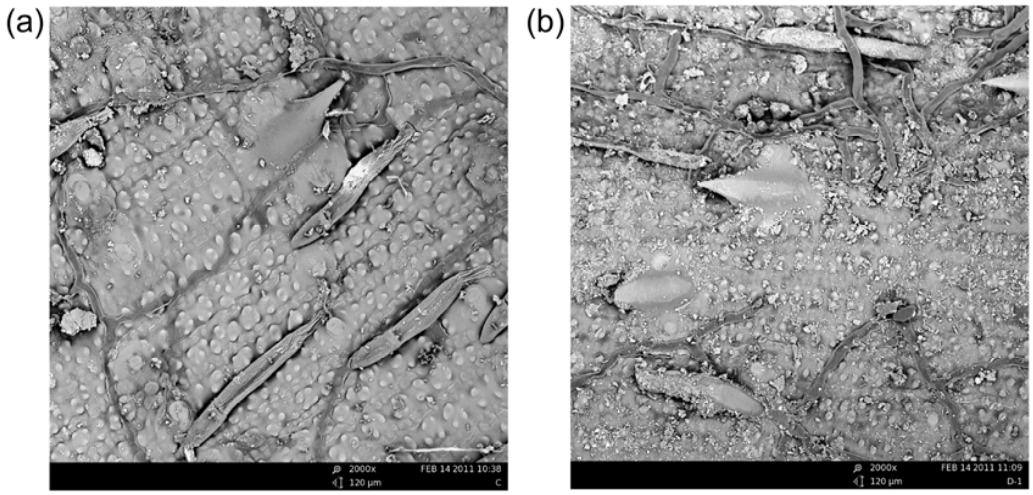


Fig. 1. The SEM analysis result of rice straw's surface structure (a) before treatment; (b) after treatment in 2000x resolution

Abfa from *Escherichia coli* BL21 was both intracellular and extracellular enzyme; however, the extracellular one had a low activity[6]. In this research, we used the mixture of intracellular and extracellular enzyme to get a low cost for its application in industry. Firstly, we optimized ratio of the enzyme mixture. Table 1 showed that the mixture of P and S in 0:1 ratio had the lowest activity, while 1:0 ratio of mixture P and S had the highest activity. Based on the first experiment, we conducted second optimization experiment with the mixture of P and S in 1:0 up to 1:10. DNS method was used to determine the best mixture ratio of P and S in rice straw hydrolysis process. The hydrolysis process of rice straw was determined by measuring the amount of reducing sugar. Table 2 showed that reducing sugar concentration increased from ratio 1:10 to 1:1 of P and S mixture, used in hydrolysis process. The optimum hydrolysis process was obtained at mixture ratio of P and S = 1: 1. Based on the second experiment, we performed the third experiment, i.e. the optimization of incubation time. Table 3 showed that the activity of abfa in rice straw hydrolysis increased from 1 to 8 h, and decreased after 8 h incubation. The optimum incubation time was 8 h. Kurniati *et al.*[10] did a hydrolysis of water hyacinth with the same enzyme (abfa). The result of this experiment was as the same as that of this research. The optimum incubation of water hyacinth hydrolysis was 8 h. These results regarding thermostability properties of abfa were consistent to the previous study[6]. According to the research, the abfa had a stable activity during 5 to 10 h incubation and after 10 to 24 h of incubation, the abfa activity decreased.

The result of SEM analysis (Fig. 1) showed that there was some modification in the surface structure of rice straw after abfa hydrolysis. The surface of rice straw before enzymatic treatment was regular and compact (Fig. 1). After the abfa treatment, the surface of rice straw got hollower, chapped and cracked (Fig. 1). This modification was caused by abfa activity. Abfas are enzymes that catalyze the hydrolysis of arabinofuranosidic bonds in hemicelluloses, such as arabinoxylan, arabinan and other arabinose containing polysaccharides[12]. The L-arabinofuranoside substitutions on xylan strongly inhibit the action of xylan-degrading enzymes; thus it prevents the complete degradation of the polymer to its basic xylose units. The Abfa acts synergistically with other hemicellulases and pectinases for the complete degradation of hemicelluloses and pectins. Abfas (EC 3.2.1.55) are among key enzymes of the hemicellulase system which is tremendously useful in biobleaching of paper pulp, bioconversion of lignocelluloses material until fermentative products for subsequent production of fuel alcohol[13] as well as the improvement of animal feed-stock digestibility. Thus, in this case, abfa enhanced the delignification process of rice straw by releasing arabinose side chains linkaged to lignin. The Abfa acted synergistically with a thermophilic xylanase in the delignification process, releasing 19.2% of lignin[4].

## 4. Conclusion

The research concluded that an abfa activity could damage the surface structure of rice straw.

## Acknowledgements

This research was supported by *Hibah Tim Pascasarjana DP2M DIKTI*, Ministry of Education and Culture, Republic of Indonesia.

## References

1. Howard RL, Abotsi E, Jansen van Rensburg EL, Howard S. Lignocellulose biotechnology: issues of bioconversion and enzyme production. *African Journal of Biotechnology* 2003;Vol. 2 (12), pp. 602-619.
2. Parameswaran Binod, Raveendran Sindhu, Reeta Rani Singhania, Surender Vikram, Lalitha Devi, Satya Nagalakshmi,y Noble Kurien, Rajeev K, Sukumaran, Ashok Pandey. Bioethanol production from rice straw: An overview. *Bioresource Technology* 2010;101:4767–4774.
3. Birgisson H, Fridjonsson O, Mougeot FKB, Hreggvidsson GO, Kristjansson JK, Mattiasson B. A new thermostable α-L-arabinofuranosidase from a novel thermophilic bacterium. *Biotechnology Letters* 2004;26: 1347–1351.
4. Numan MT, Bhosle NB. Alpha-L-arabinofuranosidase: The Potential Applications in Biotechnology. *J Ind Microbial Biotechnol* 2006;33:247-260.
5. Puspaningsih NNT. *Charachterization of Xylanolytic Enzyme and Cloning Xylosidase Gene from Geobacillus thermoleovorans IT-08*. PhD Disertation. Bogor Agricultural Univesity, Indonesia. 2004.
6. Puspaningsih NNT, Baktir A, Rachmania N, Suryani, Djisktra BW, Dijkhuizen L. *Structure Function Study of β-xylosidase and α-L-arabinofuranosidase from Bacillus thermoleovorans IT-08 Expressed in Escherichia coli DH5α.* Research Report of KNAW mobility Program (04-MP-19), Report of Join Research, Airlangga University (Indonesia), University of Groningen, KNAW (Netherland). 2005.

7. Basar B, Shamzi MM, Rosfarizan M, Puspaningsih NNT, Ariff AB. Enhanced Production of Thermophilic Xylanase by Recombinant Escherichia coli DH5α through Optimation of Medium and Dissolved Oxygen Level. *Int. J. Agric Biotech* 2010;12: 321-328.
8. Kim DY, Han MK, Lee JS, Oh HW, Park DS, Shin DH, Bae KS, Son KH, Park HY. Isolation and characterization of a cellulase-free endo-β-1,4-xylanase produced by an invertebrate-symbiotic bacterium, Cellulosimicrobium sp. HY-13. *Process Biochemistry* 2009;44 : 1055–1059.
9. Lee JW, Park JY, Kwon M, Choi IG. Purification and Characterization of a Thermostable Xylanase from the Brown-Rot Fungus Laetiporus sulpureus. *J. Biosci. Bioeng* 2009;107(1): 33-37.
10. Kurniati A, Darmoekoesoemo H, Puspaningsih NNT. Modification of surface structure and crystallinity of water hyacinth (Eichhornia crassipes) following recombinant α-L-arabinofuranosidase (abfa) treatment. *Journal of Agricultural Biotechnology and Sustainable Development* 2011;Vol. 3(9), pp. 182 -188.
11. Pongjanta J, Utaipatanacheep A, Naivikul O, Piyachomkwan K. Enzymes-resistant Starch (RS III) from Pullulanase-Debranched High Amylose Rice Starch. *Kasetsart J (Nat. Sci.)* 2008;42: 198-205.
12. Subramaniyan S, Prema P. Biotechnology of Microbial Xylanases : Enzymology, Molecular Biology and Application. *Critical Rev Biotechnol* 2002;22:33-64.
13. Saha BC. Hemicellulose Bioconversion. *J Ind Microbial Biotechnol* 2003;30:279-291.