The improvement of glucoamylase production

by Emma Moko

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The improvement of glucoamylase production by UV irradiated strains of *Aspergillus awamori* KT-11

I Nuryana[1], U Perwitasari[1], J Mawey[2], J Ngangi[2], E Moko[2], R Melliawati[1], and Yopi[3]

[1]Research Center for Biotechnology, Indonesian Institute of Sciences, Indonesia
[2]State University of Manado, North Sulawesi, Indonesia
[3]Center for Research and Human Resources Development, National Agency of Standardization, Indonesia

E-mail: isa.nuryana@lipi.go.id

Abstract. In many food industries, glucoamylase is extensively applied because of its ability to convert oligosaccharides into glucose. Recently, mutagenic treatments of *Aspergillus awamori* have been conducted to improve and enhance glucoamylase production. This research was focused on the study of glucoamylase production by strains of *A. awamori* after being exposed to ultra-violet (UV) irradiation. The parental strain was cultivated on the PDA plates and treated by exposing under UV lamp from 0 to 90 minutes with the time intervals of 30 min. Solid state fermentation was carried out to produce glucoamylase using cassava peel as a substrate. As compared to all strains, the mutant strain of *A. awamori* after being treated by 30 min of UV exposure time gave the highest activity of glucoamylase at 5.9 U/ml. The activity was two fold higher than the parental strain. The same mutant strain also reached its maximum value of total protein content at 97.31 μg/ml and 23% higher than parental strain. Glucoamylase production can be achieved and improved by the strain of *A. awamori* after UV mutagenic treatment.

1. Introduction

Glucoamylase (GA) is the key and essential enzyme in starch processing because of its role for the conversion of starch and oligosaccharides into glucose [1]. It performs a hydrolysis of 1,4 glycosidic bonds from the non-reducing ends of starch and releases L-D-glucose through catalytic process [2]. The enzyme can be produced using microbial processes in liquid, submerged, or solid state fermentation [3] [4]. The end products such as glucose and fructose syrup are useful in food industries [5].

Some fungi are reported for its ability to produce GA such as *Aspergillus niger, A. awamori, A. oryzae*, *Rhizopus* sp, *Penicillium* sp, and yeast [6] [7]. In addition, GAs from microbial sources are acceptable in food and industrial application because they are considered as safe status [5] [8]. Due to the high demands of the enzyme, some experiments have been conducted to optimize the cultural conditions in order to obtain the efficient processes for increasing GA production. In recent years, UV irradiation treatments are found to be cost-efficient and effective way to improve the strain performance for enhancing GA production [9].

In this work, the utilization of cassava peel is an effort to obtain value added from agricultural waste as it contains the amount of starch and other oligosaccharides. Furthermore, the previous research showed that cassava peel can be used as a good substrate for *A. awamori* KT-11 to produce

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GA and provide its productivity in the conversion of cassava starch into glucose [10] [11]. Based on these facts, this research was focused on the study of GA production using cassava peel as a low-cost substrate of solid-state fermentation by fungal strain of *A. awamori* KT-11 after being exposed to ultraviolet (UV) irradiation within different interval time. The research helps to find an efficient process to obtain the better GA production.

2. Materials and Methods

2.1. Microorganism

The strain of *A. awamori* KT-11 was a culture collection from Laboratory of Biocatalyst and Fermentation, Research Center for Biotechnology, Indonesian Institute of Sciences. The fungal strain was then refreshed and propagated on the slant Potato Dextrosa Agar (PDA) media and incubated for 7 days at room temperature. The fungal strains were then used as parental strains for UV treatments.

2.2. Medium

Cassava peel was used as a medium of solid state fermentation for GA production. It was washed and placed into a jar then added distilled water with a ratio of medium to water of 5:1 (w/v). In addition, the medium was supplemented by adding 5% $(NH_4)_2SO_4$, 0,5% KH_2PO_4, and 0,5% K2HPO4 (w/w) and covered using paper before being sterilized at 121°C, 1 atm for 15 minutes.

2.3. Ultra-violet (UV) Mutagenic Treatment

The parental strains of *A. awamori* KT-11 were cultivated on the PDA plates for 5 days. Strains with large colonies were selected and treated by exposing under UV lamp (Philips TUV 30W/G30 T8) from 0 to 90 min with the time intervals of 30 min (0, 30, 60, and 90 min). After UV treatment, each strain was cultivated on the PDA slant and plate. Fungal strain was incubated at room temperatur for 5 days, then used for inoculum preparation. Fungi with UV treatments were named as mutant strains while other without treatment was used as control or parental strain.

2.4. Inoculum Preparation

The fungal spores on the slant PDA media were dissolved by adding 5 ml of sterile distilled water into the tubes. The spore suspension of *A. awamori* KT-11 was ready used as inoculum of solid state fermentation for GA production.

2.5. Solid State Fermentation for GA Production

A spore suspension of *A. awamori* KT-11 was inoculated into the sterile solid substrate in the jar with a final consentration of 5% (w/v). The culture was then properly mixed with solid medium. Fermentation was carried out for 7 days at room temperature. After the fermentation process was done, solid cultures were dried using oven at 55°C for 2 days and then crushed into fine powder.

2.6. Analysis of Scanning Electron Microscope

Fungal strains were observed using SEM to examine the morphology of fungal filaments after being exposed under UV lamp.

2.7. Crude Enzyme Extraction

Crude Enzyme was extracted by diluting 1 g of fine powder into 9 ml of 0.2 M citrate-phosphate buffer (pH 4.8). Extraction was carried out by shaking at temperature of 22°C, 150 rpm for 20 min. All samples were centrifuged at 4°C, 10,000 g for 20 min [12]. The supernatants were collected as crude enzyme extracts which contain Gas and used for analysis of GA activity.

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2.8. *Glucoamylase Assay*

A 1% soluble starch in 0,1 M sodium acetate buffer (pH 5.0) was used as a subtrate solution to measure GA activity in crude enzyme extract. The assay was started by transferring 0.5 ml of crude enzyme extract into the tube followed by the addition of 0.5 ml of 1% substrate solution. The mixture was mixed properly using vortex and incubated at 55ºC for 30 min. The released glucose was measured using 3,5-Dinitrosalicylic acid (DNS) reagent and spectrofotometer at 540 nm according to Miller method [13]. The GA activity was then calculated using a calibration curve prepared with D-glucose as standard. A negative control used was the solid medium without *A. awamori* KT-11. One unit of GA activity was defined as the amount of enzyme that released 1 μmol glucose equivalent per min from soluble starch under the assay condition at 55ºC and pH 5.0.

2.9. *Protein Assay*

Protein was measured by adding 0.1 ml crude enzyme extract into 0.9 ml of distilled water and mixed using vortex. A volume of 0.5 ml was transferred into a new tube followed by the addition of 0.5 ml Bradfrod reagent and then properly mixed. The optical density was measured using spectrofotometer at 595 nm.

3. Results and Discussion

3.1. *The morphology of the colonies*

The strains of *A. awamori* KT-11 characteristically have and present coarsely roughened conidia ornamentation with the cylindrical shape and black colour of conidia. The fungal cell itself contains GA gene hence it can provide the ability of GA synthesis to convert starch into disacharide and glucose. As depicted in Figure 1, it can be seen the growth of *A. awamori* KT-11 on PDA plates after UV irradiation treatment followed by the incubation at room temperature for 5 days. The wild type was a strain without UV treatment, namely parental strain. It could be distinguished to mutant UV irradiated strains because the morphological colony was completely circular with normal colony diameter (35-49 mm) [14].

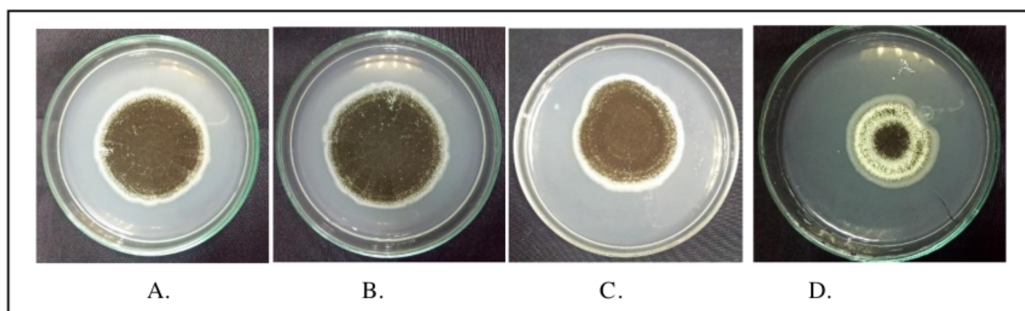

Figure 1. Photographs of the colonies of *A. awamori* KT-11 strains on PDA media: A. without treatment, B. after 30 min of UV irradiated treatment, C. after 60 min of UV irradiated treatment, and D. after 90 min of UV irradiated treatment.

On the other hand, the colony diameter of mutant after 30 min of UV irradiation showed the biggest diameter (42 mm) compared to other strains. The smallest colony diameter reached at 26 mm and could be found in strain with a 60 min of exposure time (Table 1). Ultra-violet irradiation could induce gene mutation. It might cause doubling of replication rate. The previous research reveled that the higher and longer irradiation dose and time, the lower percentage of survival rate of *A. awamori* [15]. In this study, the optimum and most effective exposure time could be obtained in 30 min. Strains

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with UV exposure time more than 30 min suffered the spore and cell damages as depicted in Figure 2, consequently the growth of cell was inhibited.

Table 1. Diameter of the colonies after UV treatments

No	Exposure time (min)	Diameter of the colonies (mm)
1	0	37
2	30	42
3	60	32
4	90	26

3.2. The morphology of the fungal filaments and spores using SEM

The morphology of *A. awamori* filaments and its spores have been observed using Scanning Electron Microscope (SEM) to study more details and deeper regarding the cell damage after being treated using UV irradiation. Without treatment, it could be seen that parental strain showed ellipsoid conidia and mycellium in normal shape and size. These were shown by mutant strain with 30 min treatment as well. In contrast, the other mutants beared a burden of conidia damage. It might cause the delayed germination and inhibit fungal cell to survive.

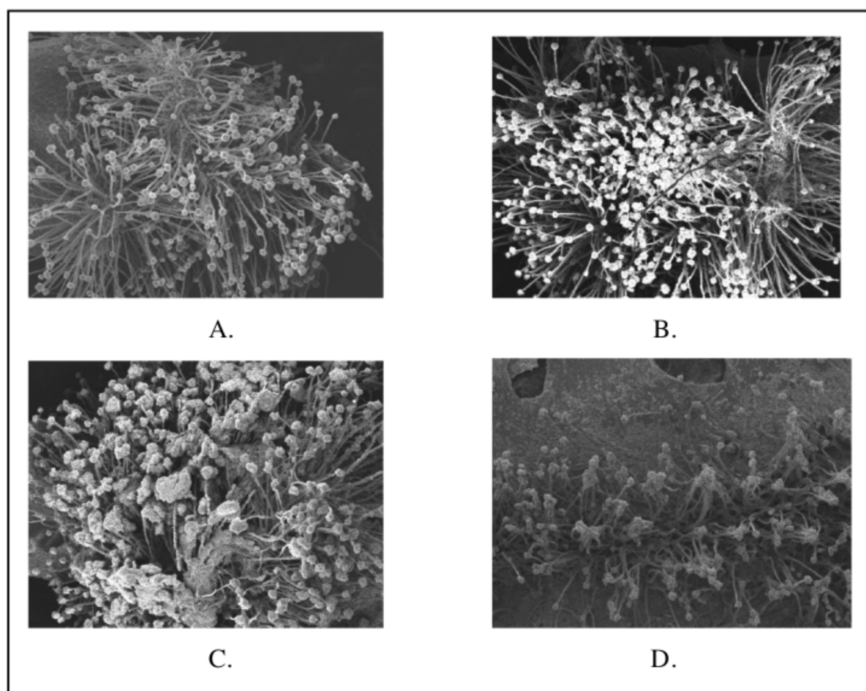

Figure 4. The morphology of the filaments of *A. awamori* KT-11 observed using SEM: A. without treatment, B. after 30 min of UV irradiated treatment, C. after 60 min of UV irradiated treatment, and D. after 90 min of UV irradiated treatments.

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3.3. Glucoamylase activity and total protein content

This study highlighted to the improvement of GA production. UV irradiation with an optimum exposure time was an effective way that has proven to increase and enhance GA activity and total protein content, according to previous study [9]. As it is shown in Figure 3, among the all mutant and parental strains, the strain with 30 min of UV treatment had the highest GA activity and reached at 5.9 ± 0.974 U/ml. As the control, the GA activity was achieved by the parental strain at 2.93 ± 0.228 U/ml, while the other mutants gave the lower levels of GA activities. They were 2.68 ± 0.190 and 2.39 ± 0.271 U/ml with a 60 and 90 min of UV treatments, respectively.

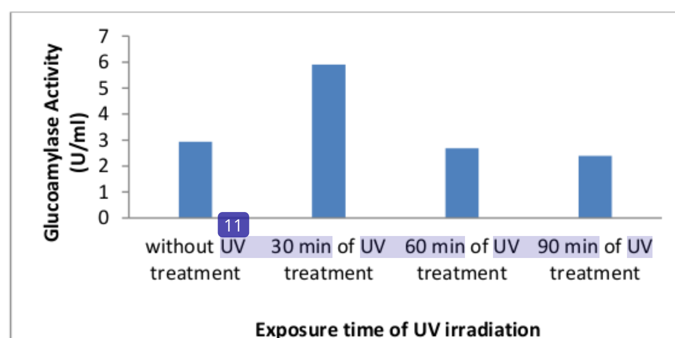

Figure 3. Glucoamylase activity of *A. awamori* KT-11 in different exposure time of UV irradiation

The enhancement of GA production might occure due to the increase of mutation rate and copy number of gene [16]. As consequent, the gene expression improved and affected to the hyper production of the enzyme. The activity of GA enzyme was two fold increased by mutant after 30 min UV treatment, compare to other strains. In this case, UV mutation could be an inducer to modify the structure of pyrimidine which cause the formation of thyamine dimmer and alter the DNA structure. Hence, it could disrupt the replication process and might lead some organisms gain the better adaptation in their environment with improved biocatalytic performance [17].

The highest content of total protein was achieved by mutant strain with 30 min of UV treatment as well, as shown in Table 2. It is directly proporsional to the enhancement of GA production. The increase of the gene number significantly contributed the enhance of the others enzyme and protein expression.

Table 2. Total protein content of *A. awamori* KT-11 after UV treatments

No	Exposure time (min)	Protein content (μg/ml)
1	0	75.88 ± 1.499
2	30	97.31 ± 0.654
3	60	62.85 ± 0.284
4	90	46.46 ± 2.348

The basic principle of strain improvement in this study is random mutagenesis. This approach is widely used in the food and industrial needs for the classical strain development and generally aiming to enhance the enzyme production at low production costs [18]. The mechanism of random mutagenesis is perfomed by introducting random mutations into the genom of interest, categorizing of a large number of variants, screening of desired mutant strains with high quality, and characterizing of huge number of survivors for further use [19] [20] [21].

4. Conclusion

Glucoamylase production can be improved by mutant strains of *A.awamori* KT-11 after UV irradiation treatment. There are positive and better results for the enzyme activity and total protein content in parental strains after 30 minutes of being exposured to UV irradiation. Our study may effectively contribute to the enhancement of glucoamylase production by UV irradiated strains of *A. awamori* KT-11 in solid state fermentation using cassava peel as a substrate.

5. Acknowledgments

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