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The Use of *Rhizopus* sp. mutant for Lactic Acid Production by Solid State Fermentation

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Abstract

A fungal strain, *Rhizopus* sp. C018, is a promising filamentous fungus that produces L-(+)-lactic acid. In this study the wild strain of C018 was used for lactic acid production by solid state fermentation on cassava peel incubated at 30°C. The results showed that the strain could produce 32.4 mg/g of lactic acid at day three. Then, the wild strain of C018 was subjected to mutation by UV radiation and the mutant named UV 333 expressed the highest lactic acid production as 57.6 mg/g analyzed by Reflectometer RQflex® 10 Merck Germany at day three. For further studies, mutation by chemicals will be tested to obtain the maximum amount of lactic acid production.

Keywords : *Cassava peel, Lactic acid, Mutation, Rhizopus sp., Solid state fermentation.*

1. Introduction

Lactic acid is one of the carboxylic acid that has been used in many applications such as food, pharmaceutical, cosmetic, textile and chemical industries [3,10,13]. Recently, large amount of lactic acid has been used as a monomer to produce poly lactic acid (PLA) for bio-base in plastic production. This increases the demand of lactic acid. Lactic acid can be produced either by chemical or biosynthesis [15]. *Rhizopus* is known as a lactic acid producer and can use several cheap carbon sources for substrate such as potato pulp [8] and wheat straw [6]. This fungus has an advantage beyond bacteria in converting starch to lactic acid.

In addition, it possesses amylolytic activity and requires simple and cheap nutrients. This fungus is easy to be harvested with low expense [15].

Thailand is one of the world's largest exporters of cassava products and in cassava processing, large amounts of waste is generated and it is considered as an environmental pollution [4].Using of cassava by-products as an alternative substrate for biotechnological processes is a positive way to relieve environmental pollution [9].Therefore, it is of interest to use cassava peel as a substrate in solid state fermentation (SSF) for lactic acid production.

Solid-state fermentation (SSF) is the fermentation process in which microorga- nisms grow on solid materials without the presence of free water leading to less contamination [2]. In addition, downstream processing is easy. Thus, it is interesting to use fungus in solid state fermentation to produce lactic acid.

In the present study, wild type and a mutant strain of *Rhizopus* sp. C018 were investigated for lactic acid production using cassava peel as a substrate in solid state fermentation.

2. Materials and Methods

2.1 Microorganism and medium

Rhizopus sp. C018 (obtained from cassava waste) was cultured on potato-dextrose agar (PDA) slants at 30°C for 5 days, and then it was transferred to cassava starch agar and incubated at the same condition to obtain sporangiospores. The spores was suspended in 0.01% tween 80 solution and adjusted to the final concentration of 10^8 spores/ml.

2.2 *Rhizopus* sp. mutant

Rhizopus sp. C018 mutant was constructed using UV radiation [1]. Briefly, the spore suspension was spread on the cassava starch agar containing 0.04 g/l bromocresol green. Irradiation was performed with 30 w UV lamp at 254 nm with varying distances of 10, 20, 30 cm and the exposure time of each distance was 15, 30, 45 and 60 min. Then, the plates were kept in dark for one day for stabilization of thymine-thymine dimmers and irradiation was then repeated again. The mutant strains were collected and determined for lactic acid production.

2.3 Acid production and starch hydrolysis

Mutant strains were screened for acid production on cassava starch agar containing 0.04 g/l bromocresol green (Modified from Thalisa and Kannika [11]). The appearance of yellow color after incubation indicated the positive of acid production. For starch hydrolysis, clear zone development after overlaid cassava starch agar with iodine solution indicated positive. The mutant strains that exhibited strong acid production and starch hydrolysis were selected for further investigation.

2.4 Solid state fermentation preparation

The production medium consisting of cassava starch 20 g, $(NH4)_2SO_4$ 2 g, KH_2PO_4 0.6 g, $MgSO_4.7H_2O$ 0.25 g, $ZnSO_4.7H_2O$ 0.04 g, calcium carbonate 1 g, cassava peel was added to obtain 100 g in thermal resistant plastic bag. The 70% initial moisture content and various pH (6, 7, 8, 9 and 10) were adjusted with 1 N NaOH and distilled water. After autoclaved at 121°C for 15 min, spore suspension (~1×10^8 spores/ml) of either wild type or mutant strains was inoculated separately into the production medium and mixed then spread on Tray (sterilized with UV radiation) covered with cheesecloth (Figure 1) incubated at 30°C. Fermentation studies were conducted in duplicate. Each day of incubation, 5 g of sample was taken and mixed with 45 ml of sterile distilled water. Samples were taken randomly at different area on tray. Then, it was left 1-2 h before filtration. The filtrated obtained was then analyzed.

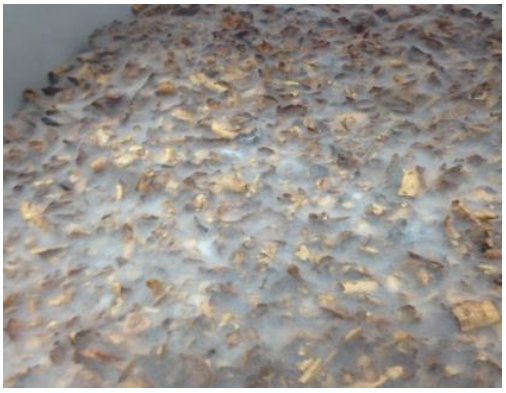
(A)  **(B)** 

Figure 1. (A) Tray fermenting cover with cheese cloth, (B) Growth of fungus on tray fermenting.

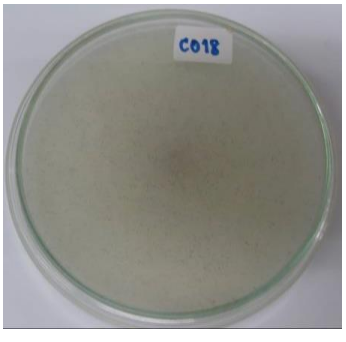
2.5 Analytical methods

pH, reducing sugar and lactic acid (mg /g) of the filtrated were analyzed by pH meter (Sartorius), DNS methods [7] and Reflectometer RQflex® 10 (Merck Germa- ny), respectively.

The survival rate of *Rhizopus* sp. C018 after treatment with UV radiation revealed that the highest survival rate of the mutants was detected at 30 cm. distances with 30 minute exposure time. (Figure 2)

3. Results and discussion

3.1 Mutagenesis by UV radiation and determination of survival rate

(A) 

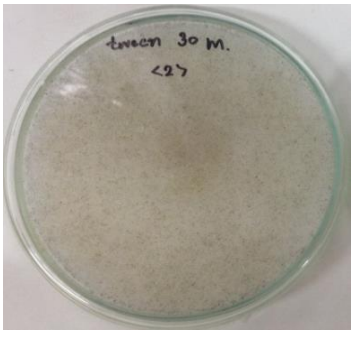
(B) 

Figure 2. (A) Growth of *Rhizopus* sp. C018 wild strain, (B) Growth of *Rhizopus* sp. C018 mutant strain.

3.2 Acid production and starch hydrolysis

Ten mutant strains (UV331. UV332, UV333, UV334, UV335, UV336, UV337, UV338, UV339 and UV3310) were selected and investigated for acid production and starch hydrolysis. After 3 days of incubation, the mutant strain UV333 exhibited strong acid production and starch hydrolysis when compared with the wild strain (Figure 3A and 3B). This mutant strain was selected for solid state fermentation.

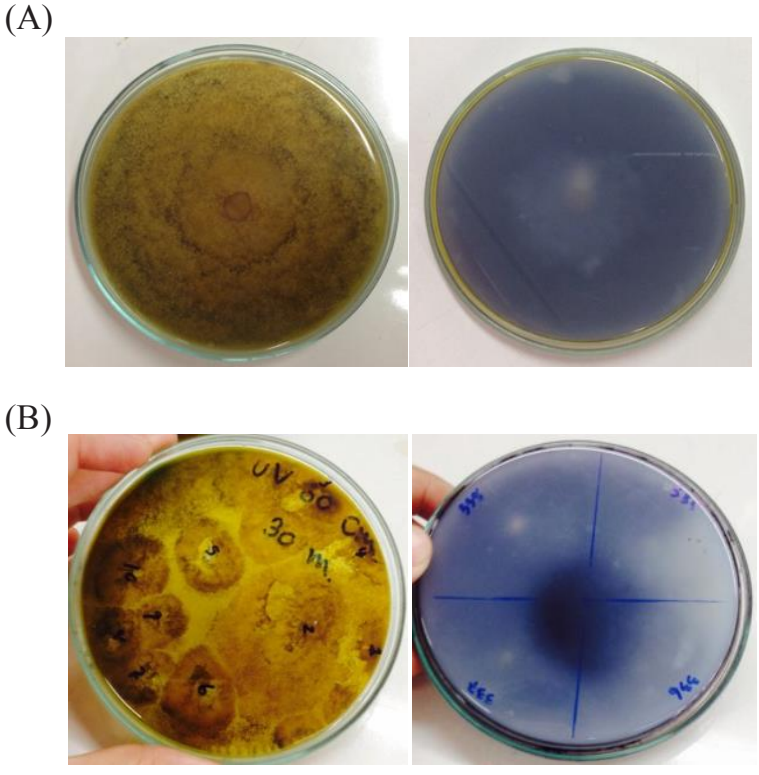
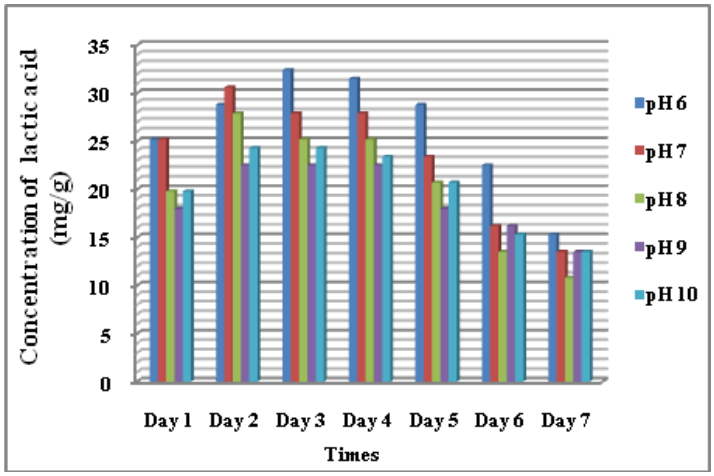


Figure 3. (A) Acid production and starch hydrolysis of wild strain, (B) Acid production and starch hydrolysis of mutant strain UV333.

3.3 Solid state fermentation

Comparison of lactic acid production between *Rhizopus* sp. C018 wild strain and the mutant (UV333) strain was evaluated at various pH (pH 6-10). The results indicated that there was significant difference in lactic acid production among the wild strains and mutant strain. Lactic acid was produced and more accumulated at day 2 and higher at day 3 (Figure 4). At pH 6, the wild and mutant strains produced 32.4 mg/g and 57.6 mg/g lactic acid, respectively at day 3 analyzed by Reflectometer RQflex® 10 Merck Germany. This correlates to the previous work which demonstrated that the optimal pH for lactic acid production was between 6-7 [12] and Huang [5] reported that the best pH for *Rhizopus* sp. fermentation would be in the range of 5.0-6.0. Yin [14] also proved the production of lactic acid by UV radiation and the result found that mutant strain *Rhizopus oryzae* LA-UN-1 produced concentration of lactic acid reached 59.5 g/l on batch fermentation. During fermentation the fungus can utilize the reducing sugar to produced lactic acid. The results on Figure 5 showed the profiled of reducing sugar that derived from starch hydrolysis. After 6 days of incubation, at pH 6 the concentration of mutant strains was consumed after entire experiment and nearly exhausted. (Figure 5A and 5B).

(A)



(B)

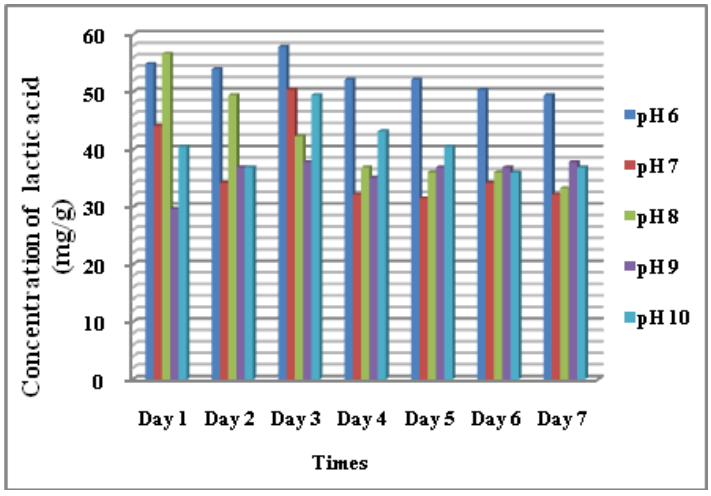


Figure 4. Lactic acid concentration (mg/g) of wild strain (A), And mutant strain UV333 (B).

The result indicated that starch was quickly hydrolyzed to glucose and utilized to produce acid. Therefore, glucose residue was decreased at pH 6.

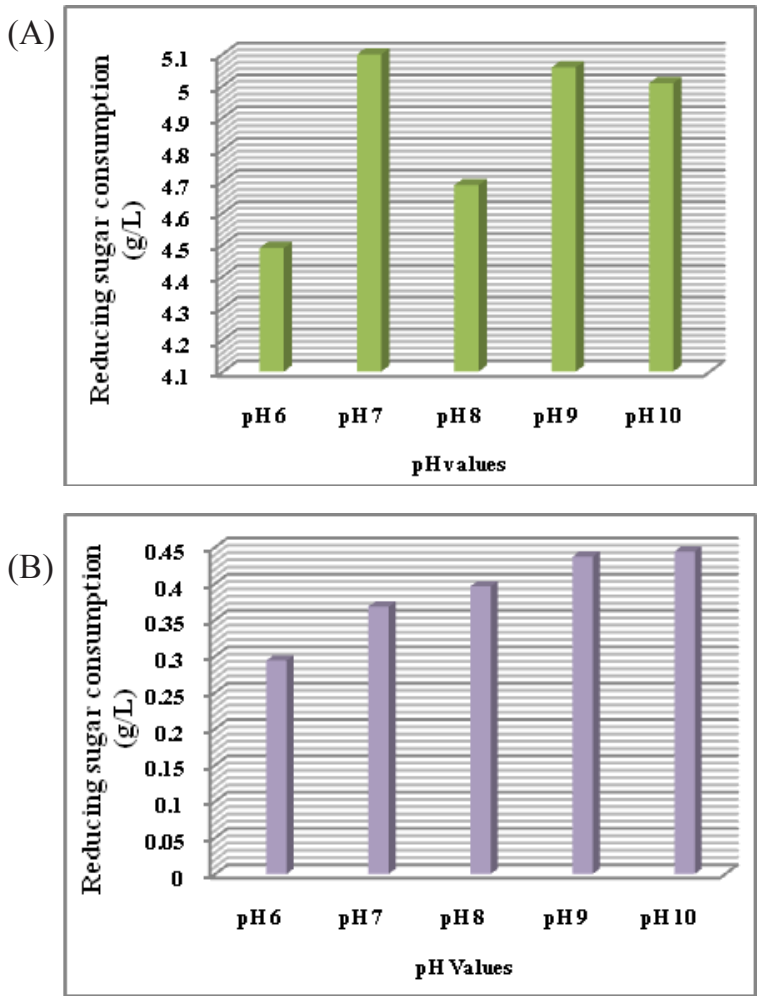


Figure 5. Reducing sugar consumption (g/l) of wild strain (A), And mutant strain UV333 (B).

There were reported that UV was effective mutagenic agents of improvement Rhizopus sp. and improved strains can increase productivity and reduce the cost of processing [1].

4. Conclusion

In this study, high level of lactic acid production of Rhizopus sp. C018 was detected in the mutant strain UV333 obtained from UV radiation. It was constructed with 30 w UV lamps at 254 nm with 30 cm distances and 30 min exposure time. The wild and mutant strains produced 32.4 mg/g and 57.6 mg/g lactic acid, respectively on day three at pH 6 analyzed by Reflectometer RQflex® 10 Merck Germany.

5. Acknowledgment

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