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Lactic Acid Production from Simultaneous Saccharification and Fermentation of Cassava Starch by Lactobacillus Plantarum MSUL 903

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Abstract

The objectives of this study were to select an amylolytic lactic acid bacterium for simultaneous saccharification and fermentation (SSF) of starch to lactic acid and determine the optimum conditions for SSF of cassava starch to produce lactic acid by the selected strain. Lactic acid production from SSF of cassava starch by the selected strain using a batch mode and under a non-sterile condition was also investigated. An isolate MSUL 903 was selected. It was named as *Lactobacillus plantarum* MSUL 903 according to the result of 16S rRNA gene sequences. The optimum conditions for *L. plantarum* MSUL 903 in SSF of cassava starch to produce lactic acid were determined to be at an initial pH of 6.5, 6% (w/v) of cassava starch concentration, and urea as an inexpensive nitrogen source. Lactic acid concentration at 10.34 g/L was obtained. Lactic acid concentration at 39.70 g/L was achieved from SSF of cassava starch under a non-sterile condition. The *L. plantarum* MSUL 903 has been proven to be an efficient amylolytic lactic acid bacterial strain for SSF of cassava starch to lactic acid.

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Keywords: Lactic acid production, simultaneous saccharification and fermentation, *Lactobacillus plantarum*, cassava starch

1. Introduction

Lactic acid is a compound that plays a significant role as a chemical feed stock for many industries. During the last few years, world consumption of lactic acid has increased dramatically due to their application in the

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production of polylactic acid - a biodegradable polymer. Lactic acid is mostly produced by lactic acid bacteria via fermentation of refined sugar [1]. Substrate cost is the main expense for lactic acid production. Starch could be substituted for refined sugar to minimize the production cost. Nevertheless, this is still economically unfavourable because pretreatment and enzymatic hydrolysis of starch is also costly [2]. A significant reduction in these costs could be achieved by an application of amylolytic lactic acid bacteria (ALAB) for simultaneous saccharification and fermentation (SSF) of starch to lactic acid [3]. In addition, among various starches, cassava starch is the most attractive one in terms of availability and inexpensiveness [4]. National Innovation Agency, Thailand, estimated that bioconversion of cassava root to polylactic acid could add value to cassava root by 10 times [5]. However, the study on lactic acid production from SSF of cassava starch is limited. Thus, the present research aimed at selecting an efficient amylolytic lactic acid bacterial strain and determining some optimum conditions for SSF of cassava starch to lactic acid. The performance of the selected strain in lactic acid production under a non-sterile condition was also investigated.

2. Materials and Methods

2.1. Culture Media

MRS agar (HiMedia Laboratories, India) containing 0.2% bromocresol purple (MRS-BCP) was used for isolation of lactic acid bacteria (LAB) from samples. MRS broth supplemented with 2% (w/v) cassava starch was used for inoculum preparation and screening of ALAB. A lactic acid production medium I [6] containing 2% (w/v) cassava starch as a sole carbon source was used for determination of optimum conditions for lactic acid production.

2.2. Isolation and Screening of ALAB

LAB were isolated from 6 local fermented foods and cassava pulps and activated sludges from two tapioca industries in Kalasin province, Thailand, using a spread plate technique. The plates were incubated at 35°C for 48 h. All colonies which turned the color of MRS-BCP agar to yellow were isolated and purified. They were then screened for ALAB. Each isolate was grown in MRS broth supplemented with 2% (w/v) cassava starch and incubated at 35°C for 48 h. The samples were taken at 0, 24, 48 and 72 h. An isolate producing the highest total acidity was selected. The selected ALAB strain was preserved in 15% glycerol and kept at -70°C.

2.3. Identification of the Selected ALAB

The 16S rRNA gene sequence of the selected ALAB strain was amplified [7] and then sequenced using ABI 3100 Capillary DNA Sequencer (Applied Biosystems Inc., USA). A full length of the 16S rRNA gene sequence was compared to the sequences of strains in the library of the National Center for Biotechnology Information (NCBI) using BLAST sequence analysis.

2.4. SSF of Cassava Starch to Lactic Acid Experiment

For inoculum preparation, the selected ALAB strain was grown in MRS broth supplemented with 2% (w/v) cassava starch and incubated at 35°C for 48 h. The cells were harvested by centrifuging at 10,000*g* for 10 min and diluted with phosphate buffer (pH 7.0) to obtain cell density at 10^8 CFU/ml. To determine some optimum conditions for lactic acid production by the selected ALAB, the SSF of cassava starch was carried out in 250 mL of lactic acid production medium I containing 2% (w/v) cassava starch unless otherwise stated with various initial pH (5.0, 5.5, 6.0 and 6.5), various initial cassava starch concentrations (4%, 6% and 8% (w/v))

and various inexpensive nitrogen sources (NH_4Cl, $(NH_4)_2SO_4$ and $(NH_2)_2CO$). The 3 inexpensive nitrogen sources were only substituted for yeast extract in lactic acid production medium I containing cassava starch at an optimum concentration in order to determine a suitable inexpensive nitrogen source. An inoculum size of 3% was used for all SSF experiments. The cultures were incubated for 72 h at 45°C without agitation. The samples were taken daily to analyze for starch and lactic acid concentrations. These experiments were conducted under a sterile condition. The optimum conditions obtained were then employed for SSF of cassava starch under a non-sterile condition and at 45°C in 1.0 L of lactic acid production medium I. The concentrations of lactic acid, starch, and biomass were investigated at 0, 24, 48, 72 and 96 h.

2.5. Analytical Methods

The samples were centrifuged at 10,000*g* for 10 min. The supernatants were analyzed for total acidity [8], lactic acid concentration [9] and starch concentration [10]. For biomass concentration determination, the harvested cells were washed with the distilled water and dried at 80°C until of a constant weight. Then, the dried cells were weighed. All SSF experiments were performed in triplicate.

3. Results and Discussions

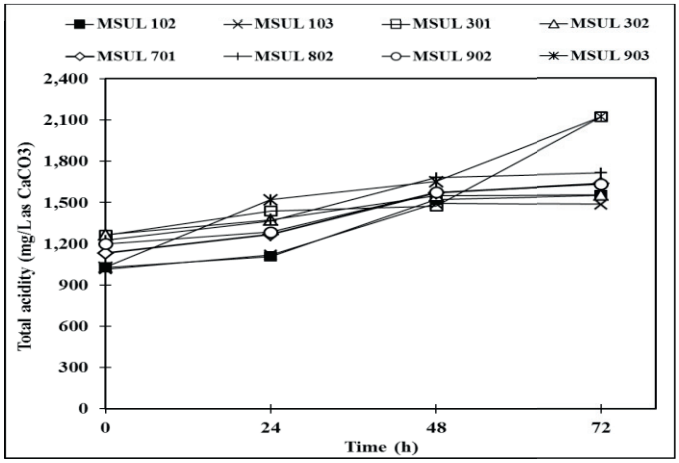

Fig. 1. Total acidity production by 8 isolates in MRS broth supplemented with 2% (w/v) cassava starch

3.1. Isolation and Identification of the Selected ALAB

A total of 19 isolates of LAB was isolated. Of these, only 8 isolates were determined for their ability to produce total acidity. The total acidity production by each isolate in MRS broth supplemented with 2% (w/v) cassava starch is showed in Fig 1. The highest total acidity at 2,122 mg/L as $CaCO_3$ was acquired from the isolate MSUL 903 which was isolated from cassava pulp. Therefore, it was selected as an efficient ALAB strain. Its sequences were similar to 16S rRNA gene sequences of *Lactobacillus plantarum* subsp. *plantarum* ATCC 14917 with 99.45% similarity. Thus, the name *Lactobacillus plantarum* MSUL 903 was proposed.

3.2. SSF of Cassava Starch to Lactic Acid

The highest lactic acid production was obtained at 72 h of incubation time which corresponded to the lowest starch concentration in all conditions studied (Fig. 2). An initial pH at 6.5 provided the highest lactic

acid production at 10.20 g/L whereas the initial pH at 5.0 and 5.5 seemed to prohibit lactic acid production. These results could be due to reduction of amylase activity to degrade starch at pH lower than 6.0 [11]. Lactic acid production by the *L. plantarum* MSUL 903 increased with increase in cassava starch concentration. However, starch concentration at 6% (w/v) instead of 8% (w/v) was selected as an optimum starch concentration due to a close proximity of lactic acid production and a cost-saving purpose. Urea was considered the best nitrogen source to promote lactic acid production and 10.34 g/L of lactic acid was obtained. These findings revealed the benefit of using *L. plantarum* MSUL 903 in which the reduction of lactic acid produced was negligible, although urea instead of yeast extract was used. The lactic acid production from SSF of cassava starch by *L. plantarum* MSUL 903 under the optimum conditions was comparable to the study of [11] and [12]. Under the optimum conditions and a non-sterile condition, the *L. plantarum* MSUL 903 still showed a high performance in lactic acid production (Fig. 3). Lactic acid concentration as high as 39.7 g/L was obtained at 96 h. Cassava starch was almost consumed within 72 h.

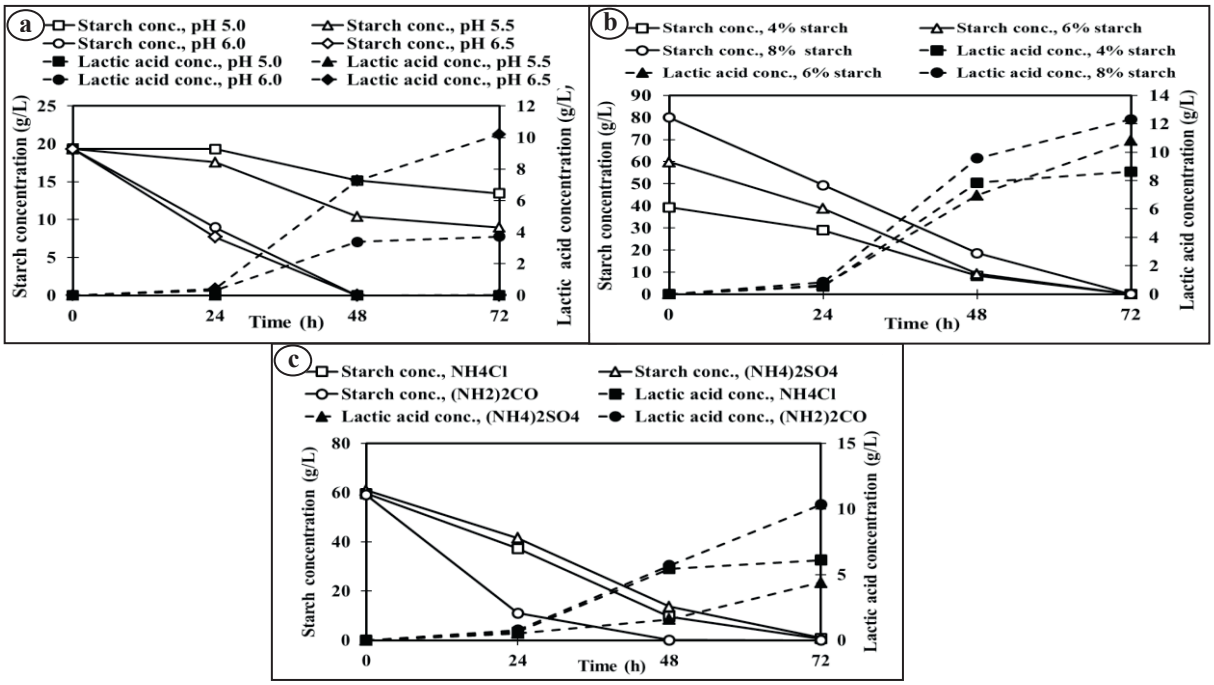

Fig. 2. Effects of (a) initial pH; (b) initial cassava starch concentration; and (c) nitrogen source on lactic acid production by L. plantarum MSUL 903 in SSF of cassava starch

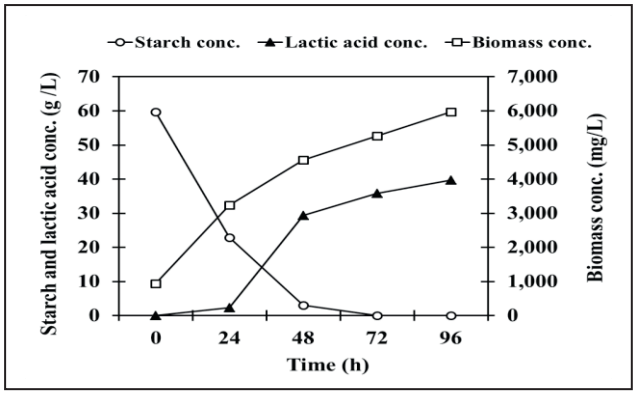

Fig. 3. SSF of cassava starch to lactic acid by L. plantarum MSUL 903 under a non-sterile condition

4. Conclusion

The *L. plantarum* MSUL 903 was selected as an efficient ALAB strain for SSF of cassava starch to lactic acid. It showed a high potential for production of lactic acid from the cheap carbon and nitrogen sources.

Acknowledgements

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