KMITL Sci. Tech. J. Vol. 12 No. 1 Jan. - Jun. 2012

Ethanol Production on Jackfruit Seeds by Selected Fungi and Yeast from Loog-pang

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Abstract

Ethanol production from jackfruit seeds was carried out by using fungal strains isolated from tan-koji (loog-pang). These were *Rhizopus oryzae* MNT 006, *Aspergillus oryzae* MNT 029, *Amylomyces rouxii* MNT 037 and *Saccharomyces cerevisiae* YRK 017. Fermentation sugar production from jackfruit seeds starch by 3 fungal strains was examined. Then the fermentable sugars produced so were tested as substrates for fermentation by *S. cerevisiae* YRK 017 to produce ethanol. The results showed that *A. rouxii* was the most effective organism for producing reducing sugar. Ethanol production process was studied on separated hydrolysis and fermentation (SHF) process and simultaneous saccharification and fermentation (SSF) process. The jackfruit seeds starch fermented by SHF process produced higher yield of ethanol than that from SSF process.

Keywords: ethanol production, jackfruit seed, separated hydrolysis and fermentation, simultaneous saccharification and fermentation

1. Introduction

Bioethanol is an alternative fuel to substitute for petrol. Alcohol production by fermentation has received special attention because the world energy crisis has enhanced the interest in renewable energy sources. There is a growing interest in the utilization of starch for the production of alcohol as starch is renewable and globally available in large quantities. Simultaneous saccharification and fermentation (SSF) process and separate hydrolysis and fermentation (SHF) process have both been used for bioethanol production and other fermentation [1-3]. SSF has been shown to be more efficient than SHF in terms of overall ethanol yield [4].

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The yeast *Saccharomyces cerevisiae* is the usual choice for converting sugar to ethanol but it cannot utilize starch, for which it is necessary to add specific enzymes such as amylase, glucoamylase, pullulanase for hydrolyzing starch [2, 5-7]. High temperature hydrolysis can be more rapid but carries a cost for the heat required, so efficient low temperature hydrolysis methods are being searched for. Loog-pang, the traditional starter culture cake for Thai traditional rice wine fermentation, is composed of mixed cultures producing fungi that convert starch to sugar by enzymatic hydrolysis, then the fermentable sugars are converted to ethanol by yeast.

Jackfruit (*Artocarpus heterophyllus* Lam) is popular fruit crop that is widely grown in Thailand and other tropical areas. The ripe fruit contains well flavored yellow sweet bulbs and seeds embedded in the bulb. The edible bulbs of ripe jackfruit are consumed fresh or processed into canned products. Seeds make-up around 10 to 15% of the total fruit weight and have 30.6% of carbohydrate, 5.5% of protein and 0.2% of fat [8-9]. Seeds are normally discarded or steamed and eaten as a snack or used in some local dishes. As fresh seeds cannot be kept for a long time, seed flour can be an alternative product, which is used in some food products.

This work aims to use starch hydrolysis fungi and fermentative yeast from Loog- pang for ethanol production from jackfruit seed starch and to compare the SHF and SSF processes.

2. Materials and Methods

2.1 Organisms

Rhizopus oryzae MNT006, *Aspergillus oryzae* MNT 029, *Amylomyces rouxii* MNT 037 and *Saccharomyces cerevisiae* YRK 017 were isolated from loog-pang in Thailand [10]. These fungi were maintained on Potato Dextrose Agar (PDA) slants which were incubated at 30 °C for 7 days and then stored at 4 °C. *Saccharomyces cerevisiae* YRK 017 was used as the ethanol fermenting organism. It was cultivated on YM or malt extract-glucose-yeast extract -peptone (MGYP) (malt extract 3 gL^{-1}; glucose 10 gL^{-1}; yeast extract 3gL^{-1}, peptone 5 gL^{-1}) for one day at 30 °C and then stored at 4 °C. Agar (1.5 %) was used for preparing agar slants.

2.2 Jackfruit seeds starch hydrolysis by fungi

Jackfruit seeds were purchased from the local market, Bangkok, Thailand. They were dried in hot air oven at 40 °C for 2-3 days and milled to 1.0 mm particle size. The milled jackfruit seeds were stored in plastic bag at 4 °C. Then, 50% (w/v) jackfruit seeds starch in distilled water was supplemented with additional nutrients to give a medium composition of 0.5 gL^{-1} $MgSO_4.7H_20$, 0.5 gL^{-1} KH_2PO_4, 12.5 gL^{-1} $(NH_4)_2SO_4$. A medium was sterilized at 121 °C for 15 min. The fungi hydrolysis was carried out in 250 mL Erlenmeyer flasks containing 20 g jackfruit seed starch and 40 ml distilled water with additional nutrients. All fungi were cultivated on PDA slant and incubated at 30 °C for 7 days and spore suspension of these fungi were used for starter culture. Then, 10% aliquot of the starter culture was added separately to the medium. The culture was incubated at 30 °C for 72 h and 168 h. The amount of reducing sugar produced was determined using the Somogyi-Nelson method according to Nelson [11]. At least three samples were used in all analytical determinations and data were presented as the mean of three replications.

2.3 Ethanol fermentation by separated hydrolysis and fermentation (SHF)

The fermentation was undertaken at the same condition as for starch hydrolysis and carried out in 250 mL Erlenmeyer flasks under nonaerated conditions at 30 °C. Then, 10% aliquot of spore suspension of selected fungi were used in the saccharification process of jackfruit seed medium.

After 72 h of saccharification, inocula of yeast was prepared from an overnight pre-culture on PDA medium. Fermentation was carried out by inoculation of 10% of cell suspension (10^6 cells/mL of yeast) into the medium and incubated for 96 h. The amount of reducing sugar and ethanol produced in the medium were then determined.

2.4 Ethanol fermentation by simultaneous saccharification and fermentation (SSF)

SSF experiments were undertaken at the same condition for starch hydrolysis and carried out in 250 mL Erlenmeyer flasks under nonaerated conditions at 30 °C, selected fungi and *S. cerevisiae* YRK017 were simultaneously inoculated in the medium. Fermentation was allowed for 168 h. The amount of reducing sugar and ethanol produced in the medium were then determined.

2.5 Analytical methods

Reducing sugar was determined by the Somogyi-Nelson method according to Nelson [11]. Ethanol was determined by gas chromatography (GC-17A Chromatograph, Shimadzu), flame ionization detector (FID, Shimadzu), DB-WAX (30 m × 0.53 mm × 1 μm) capillarycolumn (Agilent J&W GC Column). The condition employed was split mode (split ratio,1:10). The oven temperature was 60 °C. The injector and detector temperatures were maintained at 150 °C. Helium was used as a carrier gas at a flow rate of 45 cm/s and 2-propanol concentration (10 % v/v) was used as an internal standard. A mixture containing 0.2 g of NaCl, 200 μL of 2-propanol and 200 μL of a sample supernatant was heated at 70 °C for 6 minutes and 0.5 ml of the gas head space of the mixture was injected to the Gas Chromatography instrument.

3. Results and Discussion

3.1 Reducing sugar liberated from starch hydrolysis

From Table 1 it was found that *Amylomyces rouxii* MNT 037 gave the highest reducing sugar yield about 2.37 gL^{-1} after 72 h followed by *Rhizopus oryzae* MNT 006 and *Aspergillus oryzae* MNT 029, respectively.

3.2 SHF process

In order to bring a sufficient amount of reducing sugar liberated before the fermentation, 72 h - saccharification was carried out on jackfruit seed starch medium by mono-culture of *Amylomyces rouxii* MNT 037 and co-culture of *A. rouxii* MNT 037 and *R. oryzae* MNT 006 as primary inoculum. After saccharification periods, the fermentation process began after the addition of the secondary inoculum of *S. cerevisiae* YRK 017 for 96 h. This study process was applied from the procedure of traditional alcoholic beverage that made from Loog- pang. The results showed that ethanol production by SHF process with mono-culture of *A. rouxii* MNT 037 (18.98 gL^{-1}) was higher than that of other treatments (Table 2).

Table 1 Yield of reducing sugar from hydrolysis of jackfruit seed starch by 3 fungal strains

Microorganisms	Reducing sugar (gL^{-1})*	
	72 h	168 h
- *Rhizopus oryzae* MNT 006	0.25^{b}	1.76^{a}
- *Aspergillus oryzae* MNT 029	0.09^{c}	0.03^{b}
- *Amylomyces rouxii* MNT 037	2.37^{a}	1.98^{a}

* Means (from 3 replicates) in column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

Table 2 Comparison of ethanol production in culture with mono-culture of *A. rouxii* MNT 037 and co-culture of *A. rouxii* MNT 037 and *R. oryzae* MNT006 followed by *S. cerevisiae* YRK 017 on SHF and SSF process

Process	Reducing sugar (gL^{-1})*	Ethanol (gL^{-1})*
SHF		
- *A. rouxii* and *S. cerevisiae*	0.55^{b}	18.98^{a}
- *A. rouxii*, *R. oryzae* and *S. cerevisiae*	0.63^{b}	15.76^{b}
SSF		
- *A. rouxii* and *S. cerevisiae*	0.89^{a}	14.58^{b}
- *A. rouxii*, *R. oryzae* and *S. cerevisiae*	0.90^{a}	14.24^{b}

* Means of 3 replications in column followed by the same letter are not significantly different at the 5% level by Duncan's multiple range test.

3.3 SSF process

For SSF process, jackfruit seed starch medium was added with mono-culture of *A. rouxii* MNT 037 compared with co-culture of *A. rouxii* MNT 037 and *R. oryzae* MNT 006, and *S. cerevisiae* YRK 017 was simultaneously added and fermented for 168 h. SSF was performed at 30 °C. Each experiment was carried out in triplicate. Jackfruit seed starch was fermented to ethanol and ethanol yields were given in Table 2. The results showed that ethanol yields of 14.58 gL^{-1} was slightly lower than that in the SHF process.

Our result was in agreement with Marques *et al.* [12], who studied conversion of recycled paper sludge to ethanol by SHF and SSF using *Pichia stipitis.* Their results showed that a slightly higher conversion yield was attained on SHF, but fermentation time was longer. Ochaikul *et al.* [13] studied production of ethanol from jackfruit seed starch by enzymatic hydrolysis and subsequent fermentation with *S. cerevisiae* YRK 017. The results showed that the maximum ethanol yield of 16.42 gL^{-1} was achieved after 72 h of SSF which was higher than SHF process. Ooshima *et al*. [14] showed that SSF process was effective for accelerating ethanol production (as judged by a higher ethanol volumetric production rate) when compared with the SHF process.

The results of ethanol production in mono-culture and co-culture are shown in Table 2. More ethanol was produced by the mono-culture of *A. rouxii* MNT 037 than co-culture *of A. rouxii* MNT 037 and *R. oryzae* MNT 006 from both SHF and SSF processes.

4. Conclusions

Jackfruit seeds could be used as an alternative raw material for production of ethanol. *Amylomyces rouxii* MNT 037 was effective fungal strain to hydrolyze jackfruit seed starch. The maximum ethanol yield was achieved by SHF process.

References

[1] Wingren, A., Galbe, M. and Zacchi, G., **2003**. Techno-economic evaluation of producing ethanol from softwood a comparison of SSF and SHF and identification of bottlenecks. *Biotechnology Progress*, 19, 1109-1117.

[2] Montesinos, T. and Navarro, J. M., **2000**. Production of alcohol from raw wheat flour by amyloglucosidase and *Saccharomyces cevevisiae*. *Enzyme and Microbial Technology*, 27, 362-372.

[3] John, R. P., Anisha, G. S., Nampoothiri, K. M. and Pandey, A., **2009**. Direct lactic acid fermentation focus on simultaneous saccharification and lactic acid production. *Biotechnology Advance*, 27, 145-152

[4] Soderstrom, J., Galbe, M. and Zacchi, G., **2005**. Separate versus simultaneous saccharification and fermentation of two-step steam pretreated softwood for ethanol production. *Journal of Wood Chemistry and Technology*, 25,187-202

[5] Bandaru, V. V. R., Somalanka, S. R., Mendu, D. R., Madicherla, N. R. and Chityala, A., **2006**. Optimization of fermentation conditions for the production of ethanol from sago starch by co-immobilized amyloglucosidase and cells of *Zymomonas mobilis* using response surface methodology. *Enzyme and Microbial Technology*, 38, 209-214.

[6] Jamai, L., Ettayebi, K., Yamani, J. E. L. and Ettayebi, M., **2007**. Production of ethanol from starch by free and immobilized *Candida tropicalis* in the presence of amylase. *Bioresource Technology*, 98, 2765-2770.

[7] Ebrahimi, F., Khanahmadi, M., Roodpeyma, S. and Taherzadeh, M. J., **2008**. Ethanol production from bread residues. *Biomass Bioenergy*, 32, 333-337.

[8] Bobbio, F. O., El-Dash, A. A., Bobbio, P. A. and Rodrigues, L. R., **1978**. Isolation and characterization of the physicochemical properties of the starch of jackfruit seeds *(Artocarpus heterorphyllus). Cereal Chemistry*, 55, 505-511.

[9] Kumar, S., Singh, A. B., Abidi, A. B., Upadhyay, R. G. and Singh, A., **1988**. Proximate composition of jack fruit seeds. *Journal of Food Science and Technology*. 25, 308-309.

[10] Rattanapreedakul, W., **2006**. *Study on fungi and yeast isolated from Look-Pang in comparison with pure culture for sato production*. Master Degree. King Mongkut's Institute of Technology Ladkrabang.

[11] Nelson, N. A., **1944**. Photometric adaptation of somogyi method for the determination of glucose. *Journal of Biological Chemistry*. 15, 375-380.

[12] Marques, S., Alves, L., Roseiro, J. C., Girio, F.M., **2008**. Conversion of recycled paper sludge to ethanol by SHF and SSF using *Pichia stipitis*. *Biomass and Bioenergy*, 32, 400-406.

[13] Ochaikul, D., Poungmalee, B., Yimyai, T. and Keawkaew, S., **2010**. Production of ethanol from jackfruit seeds starch by enzymatic hydrolyzation and *Saccharomyces cerevisiae* fermentation. **In:** *Proceeedings NZMS and NZSBMB Joint Meeting*, 30 November -3 December, The University of Auckland, New Zealand, 65-66.

[14] Ooshima, H., Ishitani,Y. and Harano, Y., **1985**. Simultaneous saccharification and fermentation of cellulose-effect of ethanol on enzymatic saccharification of cellulose. *Biotechnology and Bioengineering*, 27,389-397.