Simultaneous Saccharification and Fermentation Using Environmental-adapted Yeast by Preculture

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Ethanol production from lignocellulosic biomass has attracted attention for utilization as an alternative fuel for internal combustion. Pretreatment is the first step in the conversion of lignocellulosic biomass to ethanol. Fermentation inhibitors generated by hydrothermal pre-treatment have an inhibitory influence on yeast growth and fermentation.

Simultaneous saccharification and fermentation (SSF) is a promising, cost-effective method demonstrated in this study. We showed that yeast acquired thermotolerance in preculture at 35 °C and the thermotolerant yeast developed tolerance to 5-HMF by adding 5-HMF in preculture. Thus, we showed that Saccharomyces cerevisiae has the ability to tolerate stress caused by multiple factors including heat and inhibitors.

Key Word

SSF, Cellulose, Preculture

1. Introduction

Environmental issues are a growing worldwide concern. Most important is the progressive decrease in renewable energy sources due to the depletion of fossil fuel along with increase in global warming. Biomass is being recognized as a promising alternative. Lignocellulosic biomass is a widely available resource and remains largely underutilized in Japan. Therefore, adequate use of lignocellulosic biomass is a key factor to overcoming the problem of renewable energy and its environmental impact.

Ethanol production from lignocellulosic biomass has drawn attention as an alternative fuel for internal combustion. In general, lignocellulosic biomass undergoes 3 processes during ethanol production: pre-treatment, enzymatic hydrolysis, and ethanol fermentation. These steps need to be simplified to increase efficiency of ethanol production.

Simultaneous saccharification and fermentation (SSF) has been widely investigated for efficient ethanol production. In this method, the use of thermotolerant yeast for fermentation is recommended, as optimum temperature of enzymatic conversion is approximately 50 °C. Thermotolerant yeast, Kluyveromyces marxianus, which is different from Saccharomyces cerevisiae has been used in SSF. Ethanol production by K. marxianus is higher than that by Saccharomyces cerevisiae, at high temperature. However, high ethanol yield and productivity by K. marxianus depends
on experimental condition including temperature, feedstock and enzyme. Therefore, we used *Saccharomyces cerevisiae* as this system could be adapted to different types of biomass.

*S. cerevisiae* adaptation has been widely investigated previously, for effective ethanol production, including temperature profiling using iterative dynamic programing and thermotolerance achieved by long-term adaptation. These results suggest that thermotolerant yeast can be generated by pre-treatment involving high-temperature incubation.

In addition to the issue of high temperature, production of fermentation inhibitors during the process of ethanol production from lignocellulosic biomass is a serious problem as these inhibitors affect the efficiency of fermentation. Among these inhibitors, 5-hydroxymethyl furfural (5-HMF) inhibits yeast growth and ethanol fermentation even at low concentrations. Therefore, we investigated the effect of preculture with 5-HMF on yeast fermentation, as pre-treatment with high temperature yielded thermotolerant yeast. In this study as well, yeast developed thermotolerance in a preculture at 35°C. Additionally, by adding 5-HMF in pre-culture, thermotolerant yeast developed tolerance to 5-HMF, which otherwise affected yeast fermentation.

Thus, we demonstrated that *S. cerevisiae* has the ability tolerate stress from multiple factors including heat and inhibitors.

### 2. Material and methods

Before preculture experiments, yeast was prepared using *S. cerevisiae* type 2 (Sigma-Aldrich), cultured in yeast extract dextrose medium (YPD; yeast extract, peptone, dextrose; Difco) at 30°C. The 100 μl of overnight cultures added into 5 ml of YPD. The temperature conditions changed from 30°C to 35°C as needed. This preculture experiment performed using test tube with shaking. 1 mL of overnight preculture cells were used for further analysis.

SSF performed using elrenmeyer flask and consisted of 50 mL 0.1 M acetic buffer (pH5), while 77.52 U/g cellulase (cellulase from *Trichoderma reesei* ATCC 26921, Sigma-Aldrich) and 111.25 U/g β-glucosidase (Novozymes) were used for enzymatic hydrolysis. As feedstock, 5 g of cellulose (Sigmacell 20 μm, Sigma-Aldrich) was added to the flask. For ethanol fermentation, preculture yeast were added into the flask. Incubation temperature ranged from 30°C to 35°C with shaking. Inhibitory experiments were performed by addition of 5 mM 5-HMF (Sigma-Aldrich) both in preculture and SSF. The quantity of ethanol was measured by high-performance liquid chromatography (HPLC, Shimadzu) using a SUGAR KS-802 (Shodex) column operated at 60°C with water as the eluent at 0.8 cm³/min.

### 3. Results and discussions

Normally, optimum growth temperature for *S. cerevisiae* is 28–30°C. At higher temperature (approximately 37°C), *S. cerevisiae* exhibits a protective mechanism. In order to induce adaptation during preculture, the environmental conditions must be set such that they exert mild stress on the yeast cells so that they survive. In this study, rate of cell growth during preculture at higher temperature was lower than that of cells incubated at optimal temperature (data not shown). Additionally, we investigated effect of preculture temperature on ethanol production (Fig. 1). Ethanol production by normal-grown cells was higher than that of heat-stressed cells. This result was caused by the difference in the number of the cells after preculture.

In previous study, *S. cerevisiae* has the ability to adapt to high temperature. In this study, slightly increased temperature (35°C) in SSF increased ethanol production compared with normal temperature (Fig. 2). Interestingly, ethanol production by cells precultured at higher temperature was more efficient than normal temperature (Fig. 2). This suggested that yeast adapted to high temperature by preculture at 35°C.

Production of fermentation inhibitors during pre-treatment is a serious problem during the process of ethanol production. Therefore, we investigated the effect of 5-HMF on ethanol production at constant temperature. Fig. 3 shows the effect of fermentation inhibitor on SSF, where 5 mM 5-HMF was added during preculture and SSF. As a result, ethanol production at a higher temperature was higher than that at normal temperature (Fig. 3).
did not use yeast extract, peptone etc. as added nutrition for yeast growth in this study. The issue of cost-effectiveness still remains a challenge. Moreover, the growth of yeast cells has to be avoided during SSF, by addition of nutrients. Additionally, raw material that can be used for energy production differs from species to species. This means that the composition differs among various sources of biomass. Therefore, we focused on cellulose utilization in minimum medium. Thus, we directly investigated the effects of temperature and presence of inhibitor on yeast cells. As a result, increase in temperature during SSF causes an increase in ethanol production by pre-stressed cells. We suggested that \textit{S. cerevisiae} has adaptation ability by multi-stressed preculture.

4. Conclusions

In this study, \textit{S. cerevisiae} had the ability to adapt to high temperature by preculture at 35 °C. Also, we demonstrated that yeast can acquire tolerance to 5-HMF and heat by pre-culture. As a result, \textit{S. cerevisiae} has an adaptability to environment by preculture.

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References