Effect of specific growth rate on the extracellular expression of *Baccillus stearothermophillus* L1 lipase in recombinant *Saccharomyces cerevisiae*

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Abstract

Recombinant lipase gene (pYEGAα-lip) originated from *Bacillus stearothermophillus* L1 was overexpressed in *Saccharomyces cerevisiae*. The lipase gene expression level was compared by controlling a constant specific growth rates(μ = 0.03, 0.05, 0.07 and 0.1h^{-1}). Cell growth was successfully controlled at the desired rates by feeding rate of glucose and the formation of by-product or accumulation of the glucose was not observed. Above the growth rate of 0.1h^{-1}, the desired growth rate could not be achieved caused accumulation of by-products(ethanol). The lipase production increased as the specific growth rate decreased. The specific production rate at the lowest specific growth rate(μ =0.03) was above 2-folds than the others.

Introduction

Fed-batch fermentation is commonly used in high-density recombinant cell fermentation, as this fed-batch strategy can attain a high cell density, by avoiding the accumulation of inhibitory metabolites such as acetic acid and ethanol. The specific growth rate (μ) can be a key control parameter in the fed-batch fermentation. Nevertheless, quantitative data describing its effect on the production of recombinant protein are not available from the literature. Also, most studies have been employed for recombinant *Escherichia coli* or inducible promoter.

In this study, we investigated the effects of specific growth rate (μ) on lipase production in recombinant *S. cerevisiae* regulated the expression of lipase by the constitutive GAPDH promoter during the fed-batch fermentation.

Materials and Methods

Strain and Plasmid

S. cerevisiae 2805 (*MAT*α *pep4::HIS3 prol-*σ *can1 GAL2 his3*σ *ura3-52*) was used as the host cell in this study. Plasmid YEGAα-Lip is a shuttle vector containing the *URA3* gene, the yeast 2μ origin, and the yeast GAPDH promoter which regulated the expression of *B. stearothermophillus* L1 lipase (BSL). For the effective secretion of BSL into an extracellular medium, α-amylase signalsequence from *Aspergillus oryzae* was connected between the GAPDH promoter and the BSL gene. The plasmid was transformed into *S. cerevisiae* 2805 strain by the electroporation method.

Media and Cultivation

Minimal YNBCAD medium (2% glucose, 0.67% yeast nitrogen base without amino acids and 0.5% casamino acid) was used for the selection of transformed cells and the seed culture. The seed culture was inoculated into the 5l-jar fermenter. The fermentation was controlled at 30℃, 1 vvm air flow, and pH 5.5 (controlled with ammonia water). The batch cultivation was carried out until complete consumption of all ethanol formed by high initial concentration of glucose (20g/l) : rapid falling in OUR and CER and corresponding rise in DOT. At this point, the medium feed was controlled by using the exponential feeding method with the following equation for determining the volumetric feed rate:

$$F = \frac{\mu X_0 V_0 \exp(\mu t)}{Y_{X/S} S_F} \qquad (1)$$

where F is the volumetric medium feed rate (l/h), μ is the desired specific rate of recombinant cell growth(1/h), X_0 and V_0 are the cell concentration(g/l) and culture volume(l), respectively at the beginning of fed-batch operation, and $Y_{X/S}$ and S_F represent the biomass yield to glucose and glucose concentration (g/l) in feed, respectively.

Results and discussion

A batch fermentation preceded the feeding that began at 32h after inoculation. Fig. 1 showed a batch fermentation profiles of recombinant *S. cerevisiae* harboring pYEGA α -lip. Because of the high initial concentration of glucose (20g/l), the cells utilized the fermentative pathway for growth, resulting in buildup of ethanol. Upon complete consumption of glucose, a second growth phase was observed in which accumulated ethanol was utilized as the carbon source. Completion of the second growth phase on ethanol is the start point of glucose-complex media feeding. Glucose-complex media was fed exponentially by a PC-controlled pump during the fed-batch fermentation. To investigate effect of specific growth rate (μ) on lipase production in recombinant *S. cerevisiae* regulated the expression of lipase by the constitutive GAPDH promoter, four different exponential feedings(μ =0.03 0.05 0.07 and 0.1) were carried out. In all experiments, the specific cell growth rate was effectively well controlled and no significant accumulation of glucose and ethanol was observed during the whole period of all fed-batch fermentations.(Fig. 2(A)) The highest production of lipase was obtained in the fed-batch fermentation at the lowest specific growth rate(μ =0.03) among the four ones(Fig. 2(B)). As the specific growth rate decreased, the specific lipase production rate increased(Fig. 3). This result was compared to the one in recombinant cells regulated by the inducible promoter[2].

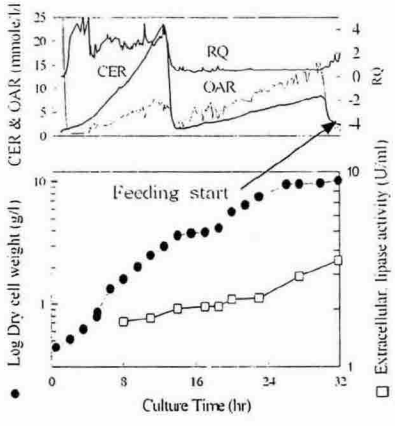

Fig. 1 Time profiles of batch fermentation with recombinant *S. cerevisiae* 2805.

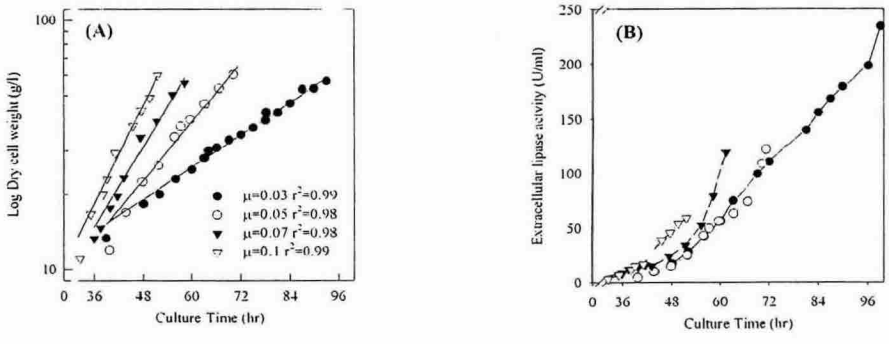

Fig. 2 Effects of growth rate on the cell density(A) and lipase prodcution(B) during fed-batch fermentation with μ=0.03(●), 0.05(○), 0.07(▼) and 0.1(▽).

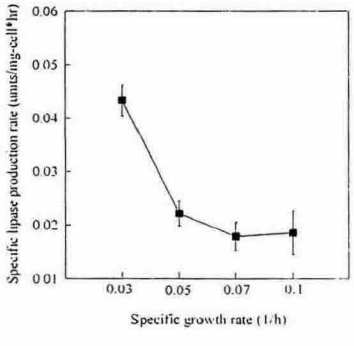

Fig. 3 Effects of specific growth rate on specific lipase production rate in recombinant *S. cerevisiae* 2805.

Reference

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