NISR Research GRANT

2005 Young Investigator Research Grant

Development of Target-adhesive Bacteria and Its Application to Fermentation Process

Kazuaki NINOMIYA

Department of Biotechnology, Graduate School of Engineering, Osaka University

Research aims

Recently, the use of bioethanol as alternative transportation fuel has rise considerable interest due to the public conscious of environmental problem and petroleum price. One of the most common methods for bioethanol production is through fermentation of renewable biomass such as starch using yeast, *Saccharomyces cerevisiae*. Since the yeast lacks α-amylase and glucoamylase, we have developed some recombinant yeast that displays these amylolytic enzymes on the surface of the yeast (arming yeasts) for direct ethanol fermentation from starch. Moreover, we have also constructed arming yeasts system using flocculent yeast as a host (flocculent arming yeast), since the flocculent yeast can be recovered without centrifugation, contributing to saving of equipment cost especially for onsite bioethanol production[1,2].

In general enzyme reactions, an enzyme and its substrate diffuse and associate together to initiate the reaction. On the other hand, in the direct fermentation from starch using the arming yeast, α-amylase is immobilized on the yeast and the starch granule as substrate is huge molecule with a very low diffusion rate. In the case of flocculent arming yeast, to make things worse, it is considered that the starch granules cannot approach to α-amylase molecules existed inside of the floc, where the apparent specific activity of α-amylase might decrease drastically. These decrease in an apparent activity of α-amylase would be critical for a specific ethanol production rate, since the rate limiting step in the direct ethanol fermentation using the arming yeasts was the conversion step from starch to oligosaccharide catalyzed by α-amylase, as described previously[1].

Therefore in the present study, to verify the effect of yeast flocculency on the performance in the direct ethanol fermentation as predicted above, we firstly conducted the fermentation using flocculent and non-flocculent arming yeast. Moreover, the microscopic observation was carried out to examine the surface contact between yeast cells and starch granules during the fermentation using the arming yeasts. Finally, to promote the surface contact between the arming yeast and starch granules, we demonstrated the direct fermentation under the packed culture condition, where the yeast cells and starch granule precipitated together.

Methods

We used flocculent arming yeast (YF207/pGA11/pUFLA) and non-flocculent (YF237/pGA11/pUFSA) constructed previously[1,2]. One gram of corn starch (Wako Pure Chemical Industries, Ltd, Tokyo) and 0.5 ml of the yeast suspension (400 g wet cell$\cdot$l^{-1}) were taken in a test tube and filled up to 10 ml with YP medium (yeast extract 10 g$\cdot$l^{-1}, polypeptone 20 g$\cdot$l^{-1}, pH 5.0). The tube was capped with a backflow valve and shaked vigorously at 30°C in the suspended condition. On the other hand, in the packed condition, the test tube was centrifuged at 1600×g for 5 minutes and 8 ml of the supernatant was kept in another bottle, then the remainder containing the cells and starch granules was mixed by a vortex and finally the kept supernatant was overlaid carefully on the mixture. The tube was capped with the backflow valve and incubated statically at 30°C. With a time interval, the sedimented cells and starch granules were resuspended and centrifuged to prepare the sample for the ethanol quantification. The ethanol concentration of the supernatant was analyzed by a gas chromatography.

Results

To investigate the effect of yeast flocculency on the performance in ethanol fermentation from starch, we conducted the fermentation using flocculent and non-flocculent arming yeast. It was found that the specific ethanol production rate (ρ) for the flocculent arming yeast was 0.04 g$\cdot$g dry cell$^{-1}\cdot$h^{-1}, which was almost comparable to the ρ value for non-flocculent arming yeast (0.06 g$\cdot$g dry cell$^{-1}\cdot$h^{-1}). Moreover, the microscopic observation proved that many starch granules were captured inside the yeast flocs during the fermentation using the flocculent arming yeast. Therefore, it could be said that the captured starch granules contributed to increase in the apparent α-amylase activity, because of an increase in the ratio of α-amylase molecule contacting with starch granule. In other words, the surface contact between yeast cells and starch granules seems to be an important factor that influences the performance of direct ethanol fermentation of raw starch by the arming yeasts.

Therefore, to promote the surface contact between the arming yeast and starch granules, we conducted the direct fermentation under the packed culture condition. It was found that the larger ρ value was obtained from the packed culture condition, compared with the usual suspended culture condition. The ρ values were 0.09 g$\cdot$g dry cell$^{-1}\cdot$h^{-1} and 0.07 g$\cdot$g dry cell$^{-1}\cdot$h^{-1} under the packed and suspended culture condition, respectively. It was considered that these results were due to more surface contact between

starch granule and yeast under packed condition, which leads to the increase in apparent α-amylase activity toward starch. On the other hand, it could be said that the yeast cells did not always contact with a starch granule in the suspended condition, even though the amylolytic enzymes displayed on the cell surface have starch binding domains.

Conclusion

In conclusion, we have demonstrated that the specific ethanol production rate in the direct ethanol fermentation could be enhanced by increasing the surface contact between the starch granules and the flocculent arming yeasts. These results provides important information to develop a more feasible process for direct ethanol fermentation using the arming yeasts, such as solid state fermentation where the arming yeasts and starch existed closely. The advantages of solid state fermentation over conventional liquid fermentation are; less osmotic pressure caused by the high concentration of sugars at the initial stage of fermentation, cost saving for equipment (as the solid state fermentation requires smaller volume of fermenter tank than the conventional liquid fermentation) and less power consumption for distillation of ethanol. Our researches for development of solid state fermentation process are in progress.

References

1) Khaw T. S., Katakura Y., Koh J., Kondo A., Ueda M., and Shioya S. Evaluation of performance of different surface-engineered yeast strains for direct ethanol production from raw starch. *Appl Microbiol Biotechnol*. **70**, 573–579 (2005).
2) Khaw T. S., Katakura Y., Ninomiya K., Bito Y., Katahira S., Kondo A., Ueda M., and Shioya S. Effect of flocculation on performance of arming yeast in direct ethanol fermentation. *Appl Microbiol Biotechnol*. **73**, 60–66 (2006).
3) Khaw T. S., Katakura Y., Ninomiya K., Kondo A., Ueda M., and Shioya S. Enhancement of ethanol production by promoting surface contact between starch granules and arming yeast in direct ethanol fermentation. *J. Biosci. Bioeng*. (2007) in press.