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Enhancing Effect of Egg Albumin on Ethanol Production and Its Function

Heung S. Kim, Chul S. Shin and Shaw S. Wang*
Department of Food Engineering, Yonsei University
**Department of Chemical & Biochemical Engineering*
Rutgers University, Piscataway, New Jersey 08854, USA

Egg Albumin이 알콜생산의 증진에 미치는 영향 및 기능

김 흥 선 · 신 철 수 · Shaw S. Wang*
연세대학교 식품공학과
*Rutgers대학교 화학공학과

ABSTRACT

In ethanol fermentations with *Saccharomyces sake*, phosphatidylcholine-egg albumin as a supplement in fermentation media was much more effective in enhancing ethanol production than linoleic acid-ergosterol. It came from the differences in alcohol-tolerance between egg albumin and ergosterol. The egg albumin was supposed to function as a nutrient rather than to form protective layers around the cells against ethanol.

INTRODUCTION

Keeping a high cell density in fermentation broths is important for the purpose of reducing fermentation time or increasing productivity. Cell-recycle reactor, immobilized cell system, etc., have been developed for these purposes.

Alternatively, it has been known that when unsaturated fatty acids such as linoleic acid were added with ergosterol to the fermentation media (1-3), they promoted yeast growth as well as ethanol production. They were used as a nutrient and incorporated into the membrane components of the cells. As a result, the final ethanol content to be attained at the end of fermentation could be increased owing to the improved alcohol-tolerances of the cells. Similarly, Hayashida et al.(4) and Jin et al.(5) used phosphatidylcholine and egg albumin as supplements to enhance ethanol productivity.

In this study, adding effects of phosphatidylcholine egg albumin were compared with those of linoleic acid ergosterol in terms of final ethanol content & alcohol-tolerance. In addition, considering that at least more than 1% egg albumin should be added to be effective in which its most fraction remained to be undissolved, two different mechanisms for its enhancing effects were proposed and tested based on the analyses of experimental data.

MATERIALS AND METHODS

Strain & Fermentation Medium

The microorganism used in these experiments was *Saccharomyces sake Kyokai* No. 7, a strain of *S. cerevisiae*. Fermentation medium consisted of 6 g yeast extract, 2 g KH_2PO_4, 2 g NH_4Cl, 0.2 g $MgSO_4 \cdot 7H_2O$ and 0.03 g $CaCl_2 \cdot 6H_2O$ in 1 liter tap water. But glucose content

was varied. Phosphatidylcholine, egg albumin, linoleic acid and ergosterol were added as supplements without being sterilized to the sterilized fermentation medium.

Cultivation Procedure

For a seed culture, 100ml of fermentation medium in a 300ml Erlenmeyer flask was prepared, sterilized and inoculated from a yeast slant. It was shaken at 30℃ for 24 hours. For main cultures, 20ml of the seed culture broth was transferred to a 300ml Erlenmeyer flask containing 200ml fermentation medium and cultivated at 30℃ on a rotary shaker.

Determination of Glucose & Ethanol Contents

Glucose content was measured by DNS method(6), and ethanol content was determined by using Industrial Analyzer(YSI Model 27).

Cellular Viability

Methylene-blue method was employed to measure the viabilities of cells(7).

Chemicals

Phosphatidylcholine and egg albumin were L-α-phosphatidylcholine, type Ⅱ-S, and Albumin, Egg, Crude Power Grade Ⅱ(Sigma Chemical Co.).

RESULTS AND DISCUSSION

Various supplements, such as linoleic acid-ergosterol, phosphatidylcholine-egg albumin, etc., have been used to enhance the final ethanol content in fermentation broths. From the literature (2, 8) and our lab, the optimal initial contents of these components in media were found as follows: linoleic acid, 100mg / *l* ; ergosterol, 10mg / *l* ; phosphatidylcholine, 5 g / *l* ; egg albumin, 10 g / *l* .

In our experiments, these four supplements were combined in pairs on a level with those specified above and their effects on ethanol production were tested, respectively (Fig. 1). As shown in Fig. 1, an addition of linoleic acid-ergosterol as a supplement brought about 10% increase in final ethanol concentration as compared to the control(around 90 g / *l*), and that of phosphatidylcholine-egg albumin did more than 30% (120 g / *l* or higher). Since the phosphatidylcholine used here was a phospholipid containing mostly linoleic acid, the big difference in final ethanol concentration between two kinds of supplements can be considered to be caused by that between egg albumin & ergosterol rather than that between linoleic acid & phosphatidylcholine. It has been reported that among these supplements, linoleic acid & phosphatidylcholine were usually used as a nutrient and incorporated into cellular materials(3, 9). As a result, the alcohol-tolerances of the yeast cells have been thought to be improved so that the final ethanol content in broths would be enhanced.

In order to investigate the reason why egg albumin were remarkably more effective in enhancing the final ethanol content than ergosterol, the alcohol-tolerances of the cells

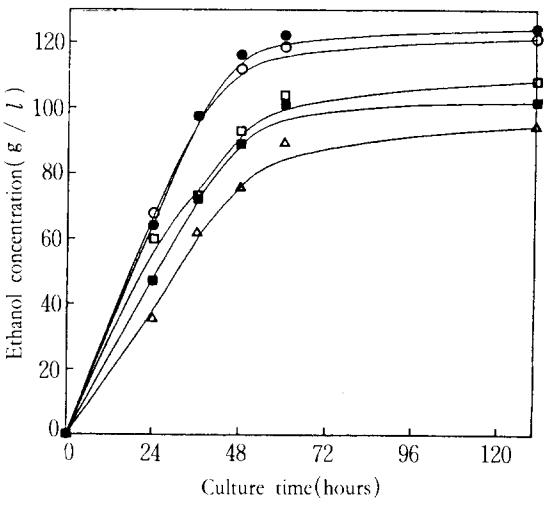

Fig. 1. Ethanol production under the various combinations of different supplements.
*Each supplement was added to the media prior to the beginning of fermentation and their concentrations indicate the final contents in media.
△ : no supplement
■ : 10mg / *l* ergosterol & 100mg / *l* linoleic acid
○ : 10 g / *l* egg albumin & 5 g / *l* phosphatidylcholine □ : 10mg / *l* ergosterol & 5 g / *l* phosphatidylcholine
● : 10 g / *l* egg albumin & 100mg / *l* linoleic acid

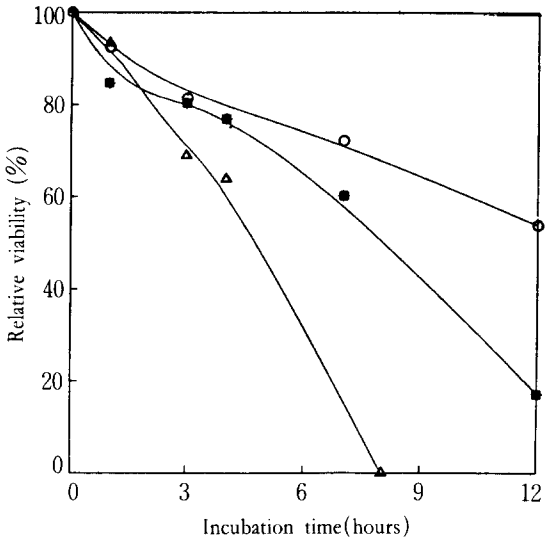

Fig. 2. Comparisons in alcohol-tolerance between the cells grown in the presence of different supplements.
*Each supplement was added to the media prior to the beginning of fermentation and their concentrations indicate the final contents in media.
△: no supplement
■: 100mg / l linoleic acid & 10mg / l ergosterol ○: 10g / l egg albumin & 5g / l phosphatidylcholine

grown in a medium containing the supplements were determined via cellular viability test. The cells grown in each supplemented medium were dipped in an ethanol solution (13%, w / v) and their viabilities were estimated at certain intervals, respectively. Fig. 2 shows that they were the lowest for the cells grown in the medium containing no supplement(control), middle for linoleic acid-ergosterol and the highest for phosphatidylcholine-egg albumin. These results indicate that the final ethanol content to be attained at the end of fermentation was proportionally related to the alcohol-tolerances of the cells.

On the other hand, since only a small fraction of the added albumin could be dissolved, two hypotheses for its function were made below. Firstly, the soluble fraction of the albumin was to be incorporated as a nutrient into cells so that their alcohol-tolerances would be changed. Or, secondly, its insoluble fraction formed a film layer around the cells so that it could protect the cells against alcohol. In order to test them, two different experiments were carried out. After the albumin was boiled or encapsulated in a dialysis membrane sac, it was added to the fermentation media. And, then, their effects on ethanol production were observed(Fig. 3). In both cases, the final ethanol content was increased more than 30% as compared to the control and there was little difference between them.

The facts that the albumin heated or encapsulated in a dialysis membrane sac could not form protective layers around the cells and the added albumin was gradually consumed as the fermentation proceeded suggest that it would be used as a nutrient for cells. In order to reassure the hypothesis, albumin-hydrolysate was introduced because it consists of low molecular components which can func-

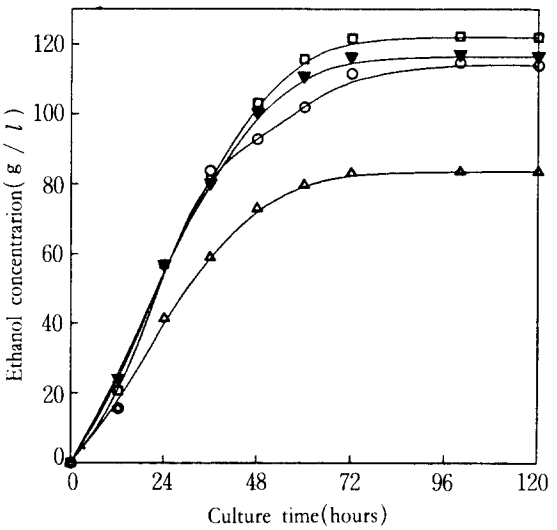

Fig. 3. Effect of various types of egg albumin on ethanol production.
*In all cases, 10g / l egg albumin & 5g / l phosphatidylcholine as a final concentration in media were added prior to the fermentation.
△: no supplement
□: egg albumin
▼: heat-denaturated egg albumin
○: egg albumin held in dialysis membrane sac

tion as a nutrient. As shown in Fig. 4, the final ethanol content in fermentation broths was gradually enhanced by increasing the albumin-hydrolysate concentration and approached the level similar to that with 0.3% albumin.

Finally, it can be concluded that the enhancing effect of the albumin on the final ethanol content came chiefly from the improved alcohol-tolerances due to the composi tional changes of the cells rather than the formation of protective layers around them.

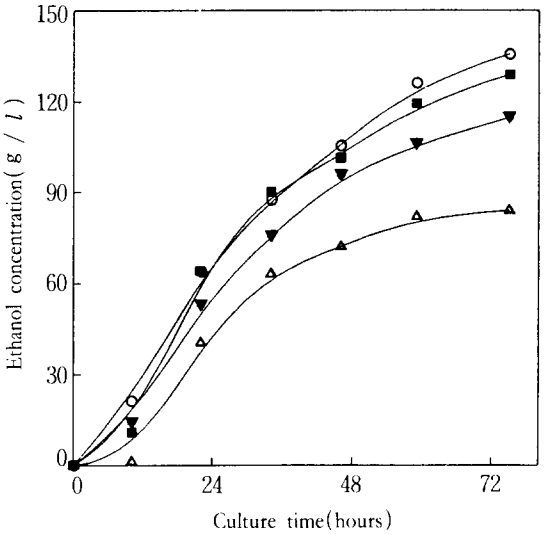

Fig. 4. Ethanol production at different concentrations of albumin-hydrolysate.
△: no supplement added
▼: 0.1% albumin-hydrolysate & 0.5% phosphatidylcholine added
■: 0.2% albumin-hydrolysate & 0.5% phosphatidylcholine added
○: 0.3% albumin-hydrolysate & 0.5% phosphatidylcholine added

요 약

*Saccharomyces sake*를 이용한 알콜발효에서 배지의 첨가물로 egg albumin및 phosphatidylcholine이 최종 알콜농도에 미치는 영향과 그의 기작에 대하여 분석하였다.

Phosphatidylcholine-egg albumin이 첨가될 때 최종 알콜농도는 120 g / l 정도 얻어졌으며, 첨가물없는 경우에 비해 대략 30% 정도, 첨가물 linoleic acid-ergosterol에 비하여 20% 정도 증가되었다. 최종 알콜농도가 높을수록 균체의 알콜내성도 비례적으로 증가하였으며, 두 첨가물 사이의 차이는 지방산보다는 albumin과 ergosterol의 영향으로부터 주로 비롯되었다.

열변성 혹은 가수분해된 albumin을 첨가한 결과로부터 albumin은 균체외부에 보호막을 형성하기보다는 영양분으로 균체내로 흡수되어 균체성질을 변화시켜 알콜내성을 증진시키며, 이로부터 궁극적으로 최종 알콜농도를 증가시키는 것으로 판단되었다.

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