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# Promoting effect of compatible solute ectoine on the ethanol fermentation by *Zymomonas mobilis* CICC10232

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## Abstract

Supplementation effect of compatible solute ectoine on the ethanol fermentation by *Zymomonas mobilis* CICC10232 was examined in the presence of high glucose concentration. Addition of ectoine promoted the cell growth as well as volumetric ethanol productivity in the presence of 250 g/l of glucose. At the end of ethanol fermentation, cell dry weight resulted in 1.8 and 1.5 g CDW/l in the presence and absence of 1 mM ectoine, respectively. Volumetric ethanol productivity in the presence of 1 mM ectoine became 1.1 g/l/h, which resulted in 57.1% higher than that in the absence of ectoine, 0.7 g/l/h. In addition, fermentation time was shortened by 24 h when 1 mM ectoine existed in the medium. In the presence of 250 g/l of glucose together with various concentrations of ectoine, relative enzyme activities of glucokinase (GK), glucose-6-phosphate dehydrogenase (G-6-PDH), and alcohol dehydrogenase (ADH) increased by 29.9, 11.6, and 7.7% in the presence of 0.5, 0.5, and 0.25 mM ectoine compared to those without ectoine supplemented, respectively. The promoting function of ectoine on ethanol fermentation may be related to the protection of these enzyme activities under osmotic stress.
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*Keywords:* Ectoine; Compatible solute; *Zymomonas mobilis*; Ethanol fermentation; Enzyme promotion; High concentration of glucose

## 1. Introduction

When sugar-based materials are used for ethanol fermentation, Gram-negative ethanologenic bacterium *Zymomonas mobilis* possesses advantages over *Saccharomyces cerevisiae*, since the former strain has a higher sugar–ethanol conversion, faster conversion velocity and lower biomass than the latter one. In addition, *Z. mobilis* does not require the control of oxygen concentration during fermentation, and especially it has a higher tolerance for high concentrations of sugar and ethanol [1]. *Z. mobilis* accumulates sorbitol as compatible solute to counteract high external concentration of sugars [2], that is, when cells are grown in the presence of sucrose or mixture of glucose and fructose, sorbitol is synthesized by the catalysis of glucose–fructose oxidoreductase (GFOR) [3], and thus counteracts the detrimental osmotic stress [2,4]. Sorbitol was synthesized and accumulated inside the cells up to 700 mM when *Z. mobilis* was grown in the presence of 1 M sucrose [2].

Ethanol fermentation of *Z. mobilis* follows the Entner–Deudoroff (ED) pathway [5]. When the substrate is glucose, ethanol yield is near to theoretical yield [6,7]. If sucrose is used as the substrate, however, the yield becomes as low as 70% of theoretical value due to the production of levan and sorbitol as by-products [8]. Interestingly, *Z. mobilis* cannot synthesize sorbitol or any other compatible solutes when glucose is the sole source of carbon [2]. Therefore, high external glucose concentration depresses the rate of ethanol fermentation when glucose is the sole source of carbon during fermentation.

Hyperosmolarity due to high glucose concentration reduces the fermentation rates to ethanol by *Z. mobilis* [9,10]. For the strategical researches of *Z. mobilis* to overcome the hyperosmolarity resulted from high external glucose concentrations during ethanol fermentation, Loos et al. reported that compatible solute sorbitol was taken up to counteract high glucose concentrations when it was available in its habitats. Cell biomass was markedly increased when 50 mM sorbitol was added into the medium containing ≥20% of glucose (w/v). Sorbitol accumulated inside the cells increased up to 1 M when 30% glucose was present in the medium, but there was no description about sorbitol promoting ethanol fermentation [2].

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In order to enhance ethanol productivity, Kesava et al. adopted batch step-feeding of glucose to avoid the inhibition of high glucose concentration during fermentation. Glucose concentration was maintained at a lower level using the technology. However, this did not belong to high concentration glucose fermentation [11].

Therefore, it is interesting to examine whether the addition of compatible solutes has a promoting influence on ethanol fermentation against high external concentrations of glucose or starch hydrolysate. Cyclic amino acid ectoine, one of the representative of compatible solutes, is synthesized mainly by halophilic and halotolerant bacteria under high salinity [12,13]. It is similar to many compatible solutes that ectoine can protect the stabilities of proteins, nucleic acids and whole cells against external stresses of various types of environments [14–17].

In this paper we report that the addition of ectoine improved ethanol productivity by *Z. mobilis* under high external glucose concentrations. In addition, we investigated the effect of ectoine on the activities of limiting enzymes glucokinase (GK) and glucose-6-phosphate dehydrogenase (G-6-PDH) of glucose utilization in ED pathway [18] and key enzyme alcohol dehydrogenase (ADH) of ethanol fermentation [19].

## 2. Materials and methods

### 2.1. Bacterium and culture conditions

*Z. mobilis* CICC10232, which was purchased from the China Center of Industrial Culture Collection, CICC, was grown at 30 °C for 24 h in an activation medium (g/l): glucose, 100; yeast extract (Difco Laboratories, MI, USA), 5; $(NH_4)_2SO_4$, 1; $K_2HPO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.5, pH 6.6.

### 2.2. Ethanol fermentation

100 ml of fermentation medium was added to 300 ml of Erlenmeyer flask. Each bottle was fitted with a fermentation bung. The bungs were partially filled with concentrated sulfuric acid, permitting only $CO_2$ to evolve from the culture. By inoculation of 5% preculture, fermentation was started without agitation at 30 °C. Ethanol fermentation was carried out in a fermentation medium (g/l) containing glucose, 150–300 (as stated in text); yeast extract, 5; $(NH_4)_2SO_4$, 1; $K_2HPO_4$, 1; $MgSO_4 \cdot 7H_2O$, 0.5, pH 6.6.

### 2.3. Determination of $CO_2$ yield, ethanol and glucose concentrations

The fermentation was followed by designated intervals $CO_2$ evolution, which was monitored by the decrease in weight of the whole culture [20,21]. The weight loss was defined as $CO_2$ yield. The distillation of ethanol was terminated after the volume was reduced to 70% of the initial volume, and water was added up to the same volume as the original. The specific gravity of liquid was measured based on the density bottle method [22]. Ethanol concentration was determined according to the standard table based on the relationship between the specific gravity and density. For the determination of glucose concentration, glucose kit (Wako Pure Chemical Industries, Osaka, Japan) was used.

### 2.4. Determination of enzyme activities

*Z. mobilis* CICC10232 was cultivated at 30 °C for 24 h in an activation medium, and then the culture was centrifuged for 15 min at 12,000 rpm. The pellets were washed by sterile physiological saline solution. The sample was pretreated according to the method by Algar and Scopes [23]. Activities of GK,

G-6-PDH, and ADH were measured with the methods by Algar and Scopes [23], and Miyata and Yonehara [24].

## 3. Results

### 3.1. Effect of glucose concentration on the ethanol fermentation

An attempt was made to examine the ethanol fermentation process by *Z. mobilis* CICC10232 in the presence of different concentrations of glucose, 150–300 g/l, where $CO_2$ production rates (the percentage after the weight loss of $CO_2$ divides the weight of initial glucose) were determined. As shown in Fig. 1, $CO_2$ production rates were reduced with the increase of glucose concentrations. When glucose concentration was increased from 150 to 300 g/l, $CO_2$ production rates decreased from 41.3 to 23.5% at the corresponding time of fermentation termination. In the presence of more than 150 g/l of glucose the lag periods of $CO_2$ production rates was recognized, in which they tended to extend in parallel with the increase of glucose concentration, that is, lag periods resulted in 12, 24, and 36 h in the presence of 200, 250, and 300 g/l of glucose, respectively.

For a precise comparison of kinetic parameters under different glucose concentrations, ethanol and glucose concen-
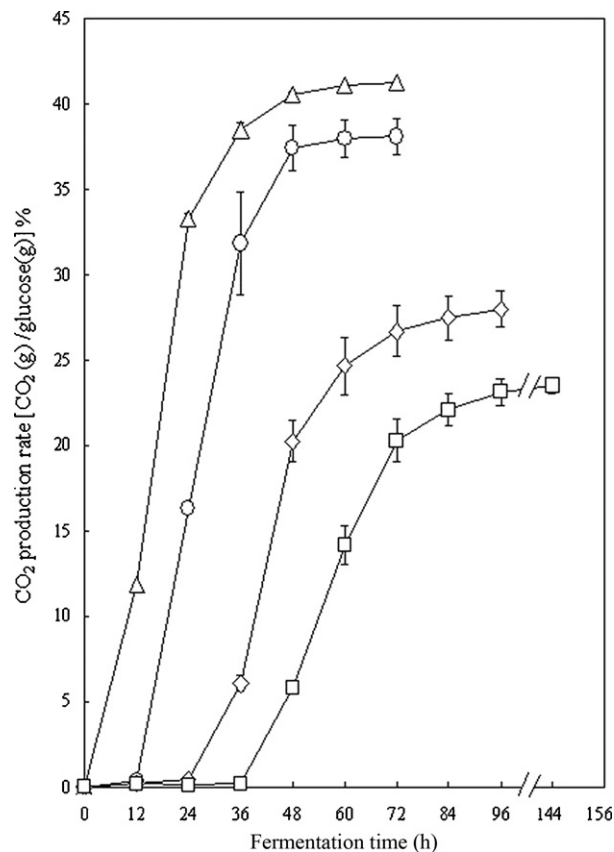


Fig. 1. Effect of initial concentration of glucose on $CO_2$ production rate during ethanol fermentation by *Z. mohilis* CICC10232. Strain CICC10232 was anaerobically grown in the fermentation medium containing 150 (△), 200 (○), 250 (◇) and 300 g/l (□) of glucose at 30 °C. At designed intervals, $CO_2$ production rate was monitored according to the method described in Section 2. The values are the averages ± S.D. from three independent experiments.