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NOTE

# Effect of Glucose Concentration on the Production of Erythritol by *Trichosporon* sp.

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**Abstract** The effect of glucose concentration on the production of erythritol by *Trichosporon* sp. was mainly studied. The specific growth rate and production rate of erythritol gave the highest values of 0.23 $h^{-1}$ and 4.2 g/l/h, respectively, on 100 g glucose/l of medium. The conversion yield of erythritol during the exponential phase and the stationary phase was constantly maintained at 19% and 51%, respectively, while the glucose concentration in the medium varied from 100 g/l to 400 g/l. The maximum overall erythritol conversion yield of 47% was obtained when the glucose concentration in the medium was 400 g/l. It corresponded to a 74% increase compared with the 100 g/l glucose medium. The diauxy growth of this microbe was also observed. It grew exponentially consuming glucose, then after the second lag phase, biomass slowly increased using glycerol and erythritol.

**Key words:** Erythritol, *Trichosporon* sp., osmophilic yeast, diauxy growth

Sugar alcohol erythritol is a low caloric (0.3 kcal/g), and non-cariogenic [5] sweetener [6], safe for diabetics because there is no change to blood glucose and insulin levels after the oral administration [1]. Erythritol has about 70~80% of the sweetness of sucrose and is found in fruits, mushrooms, and some fermented foods. Hajny *et al.* [2] reported that a yeast-like fungus, *Moniliella tomentosa var. pollinis* isolated from pollen produced erythritol on a medium containing 35.7% glucose giving a yield of 45.6%. Wako *et al.* [9] optimized the culture conditions for erythritol production by *Aureobasidium* sp. SN-115. According to these results, when 0.05% sodium sulfate was added to the medium, the highest yield of about 51% was obtained, and with 0.025% aluminum chloride (6 hydrates) the formation of glycerol was completely repressed. Ishizuka *et al.* [3] isolated one strain of *Aureobasidium* sp. SN-124A, which produced erythritol, and they induced a mutant using UV irradiation and NTG treatment. The mutant converted glucose to erythritol with a 47.6% yield.

The objective of this work was to study the growth pattern of *Trichosporon* sp. and to analyze erythritol production from it, particularly the effect of glucose concentration on the production yield of erythritol and the specific growth rate of *Trichosporon* sp.

## Microorganism and Cultivation

*Trichosporon* sp. was isolated from honeycombs [4]. The fermentation medium for the production of erythritol was composed of 10~50% (w/v) glucose (Doosan, Korea), 4% corn steep liquor (CSL) (Doosan, Korea), and 0.05% Tween 20 (Junsei, Japan) [4]. The glucose was separately sterilized in an autoclave and later mixed with the other parts of the culture medium. The inoculum of a selected strain of microorganism was prepared by inoculating the strain into 250-ml flasks containing 50 ml basal culture medium and cultured for 2 days with shaking at 180 rpm at 35°C (Vision Scientific, Korea).

## Analytical Methods

After adequate dilution of the fermented broth, the cell concentration was measured by absorbance at 660 nm with a spectrophotometer (Beckmann, U.S.A). Thereafter, the dry cell weight (DCW) was calculated from the relationship between absorbance and DCW. A quantitative analysis of polyol and glucose in the fermented supernatants was performed using an HPLC (Shimadzu, Japan) unit equipped with a YMC-Pack Polyamine-II column (YMC, Japan). The mobile phase was acetonitrile-water (7:3 by vol) and its flow rate was 1 ml/min. The polyol and glucose were detected with a refractive index

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(RI) detector. The amount of total nitrogen was analyzed with Kyeltec auto 1035/38 system (Tecator, Sweden).

### Calculation of the Amount of Glucose Converted into Erythritol and Cell Mass

The % weight of the produced erythritol, glycerol, and ribitol was calculated, based on the amount of glucose in the medium. When calculating the conversion yield of erythritol from glucose, it was considered that 2 M carbon dioxide were produced when 1 M glucose was converted into 1 M erythritol. This assumption was based on the erythritol biosynthetic pathway suggested by Sasaki [7].

When the conversion yield of the cell mass from glucose was estimated, it was assumed that the 8.1 g/l crude protein included in the nitrogen sources (CSL) in the medium was diverted into cell mass and the other components of the cell mass originated from the carbon source, glucose, since most of the nitrogen sources in the medium were exhausted.

### Typical Fermentation Pattern of Erythritol Production

Results from a typical batch run with an initial glucose concentration of 200 g/l are illustrated in Fig. 1. The glucose concentration in the medium declined at a constant rate, 3.0 g/l/h, after the lag phase of cell growth. Cells grew exponentially until most of the nitrogen sources were exhausted, and then the biomass slightly increased, actively producing erythritol during the stationary phase. Finally, the cells grew up to 54 g/l and its growth rate became slower than in the first exponential phase. In this second growth phase, it consumed the produced glycerol and erythritol as carbon sources without change of total nitrogen concentration. This result shows a typical diauxy growth of *Trichosporon* sp. In the culture broth, the pH changed from 3.7 to 3.1 after 2 days and maintained until the end of the fermentation (data not shown).

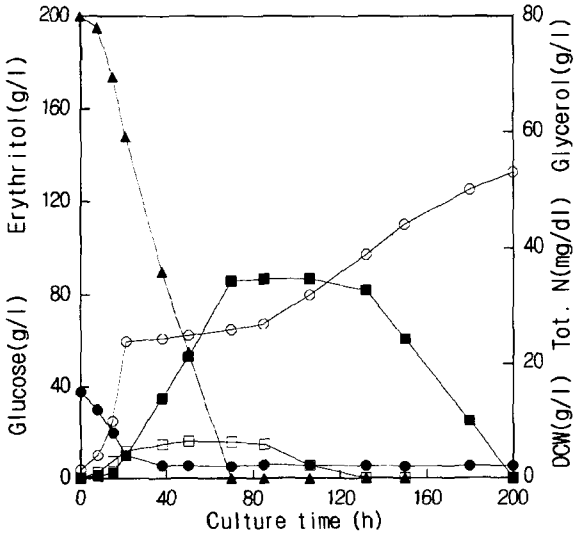


**Fig. 1.** Time course of cultivation in 20% glucose medium.

Cultivation was done in flasks containing 50 ml of medium. The medium consisted of 20% glucose, 4% CSL, and 0.05% Tween 20. Concentration of residual glucose (▲), concentration of erythritol (■), dry cell weight (○), concentration of glycerol (□), and concentration of total nitrogen (●) are indicated.

**Table 1.** Conversion ratio of glucose into cell mass and other compounds.

| | Cell mass (%) | Erythritol (%) | Glycerol (%) | Ribitol (%) |
|---|---|---|---|---|
| Total growth phase | 8 | 65 | 3 | 5 |
| Exponential phase (from 0 to 21 h) | 30 | 28 | 9 | 12 |
| Stationary phase (from 21 h to 70 h) | 1 | 77 | 1 | 2 |

Cells were cultivated on 20% glucose, 4% CSL and 0.05% Tween 20 medium at 35°C in a shaking incubator.

Erythritol was produced at a constant rate of 1.6 g/l/h from the late exponential phase of cell growth, when the nitrogen sources were not limited and until the glucose was wholly depleted. The glycerol conversion increased in parallel with the cell mass during the exponential phase of cell growth and slowly declined after the glucose was exhausted. Thereafter, erythritol was used as the last carbon source (Fig. 1).

On a rough basis, glucose appeared to be converted into cell mass (8% (w/w)), erythritol (65%), glycerol (3%), and ribitol (5%). In detail, glucose was diverted into cell mass (30%), erythritol (28%), glycerol (9%), and ribitol (12%) during the exponential phase (from 0 to 21 h), and cell mass (1%), erythritol (77%), glycerol (1%), and ribitol (2%) during the stationary phase (from 21 to 70 h) (Table 1).

### The Effect of Glucose Concentration on Erythritol Production

Initial concentration of glucose in the medium had a significant effect on erythritol production and cell growth. For example, a 50% decrease to 100 g/l of glucose in the medium gave the results shown in Fig. 2 and Table 2. Although similar trends to those shown in Fig. 1 were indicated, these represented an increase of ca. 35% and 160% in the specific growth rate and production rate of erythritol, respectively, and represented a decrease of ca. 37% in the conversion yield of erythritol. While 100 g/l of glucose in the medium is optimum for the production of erythritol and cell growth, the total conversion yield (27%) of erythritol was lower than that in the 200 g/l glucose medium (43%). The was because the period of stationary phase in the 200 g/l glucose medium, in which

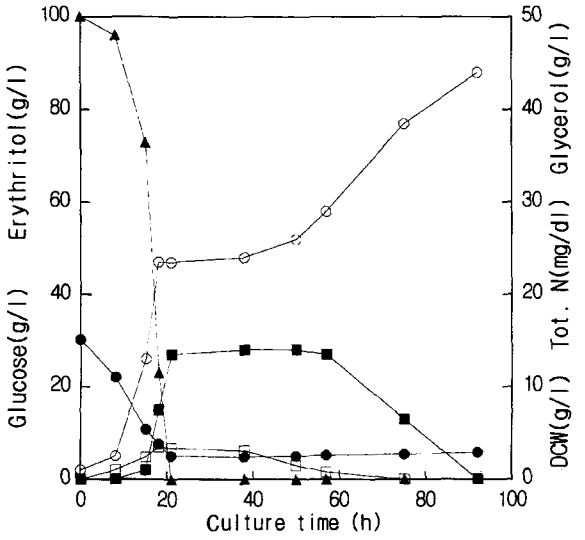


**Fig. 2.** Time course of cultivation in 10% glucose medium.

Cultivation was done in flasks containing 50 ml of medium. The medium consisted of 100% glucose, 4% CSL, and 0.05% Tween 20. Concentration of residual glucose (▲), concentration of erythritol (■), dry cell weight (○), concentration of glycerol (□), and concentration of total nitrogen (●) are indicated.

**Table 2.** Effect of glucose concentration.

| Concentration of glucose (g/l) | μm ($h^{-1}$) | $\frac{dP}{dt}$[a] (g/l/h) | $Y_{p/se}$[b] (%) | $Y_{p/ss}$[c] (%) | $Y_{p/st}$[d] (%) | Productivity[e] (g/l/h) |
|---|---|---|---|---|---|---|
| 100 | 0.23 | 4.2 | 19(28)[f] | 52(78)[f] | 27 | 1.29 |
| 200 | 0.17 | 1.6 | 19(28) | 51(77) | 43 | 1.23 |
| 400 | 0.10 | 1.4 | 20(30) | 51(77) | 47 | 1.18 |

Cells were cultivated in different concentrations of glucose, 4% CSL and 0.05% Tween 20 medium in 35°C in a shaking incubator. [a]Production rate of erythritol during staionary phase. [b]$Y_{p/se}$ is the conversion yield of erythritol from glucose up to the exponential phase. [c]$Y_{p/ss}$ is the conversion yield of erythritol from glucose during stationary phase. [d]$Y_{p/st}$ is the conversion yield of erythritol from glucose up to the stationary phase. [e]Volumetric productivity during whole period of cultivation. [f]Conversion ratio of glucose into erythritol and carbon dioxide.

the conversion yield of erythritol remains at a higher level, was shorter (3 h) than that in the 200 g/l glucose medium (49 h) (Fig. 1, Fig. 2).

A two-fold increase to 400 g/l glucose in the medium gave the results shown in Fig. 2. Compared with the results in the 200 g/l glucose medium, the *Trichosporon* sp. showed a decreased specific growth rate, 0.10 $h^{-1}$, and lower production rate of erythritol, 1.4 g/l/h, but it gave the highest overall erythritol conversion yield of about 47%. This result may be due to the fact that the stationary phase was maintained longer (128 h) than in the other cases (Fig. 3).

With 500 g/l glucose in the medium, specific growth rate, conversion yield of erythritol, and cell mass were all markedly declined, probably due to osmotic pressure from the high concentration of glucsoe (data not shown).

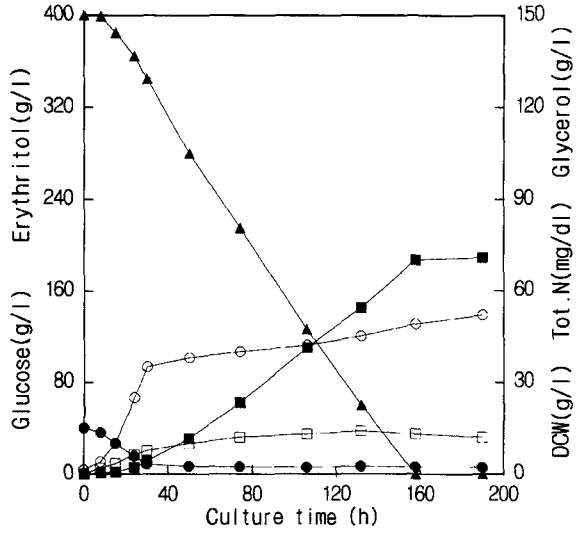


**Fig. 3.** Time course of cultivation in 40% glucose medium.

Cultivation was done in flasks containing 50 ml of medium. The medium consisted of 40% glucose, 4% CSL, and 0.05% Tween no. 20. Concentration of residual glucose (▲), concentration of erythritol (■), dry cell weight (○), concentration of glycerol (□), and concentration of total nitrogen (●) are indicated.

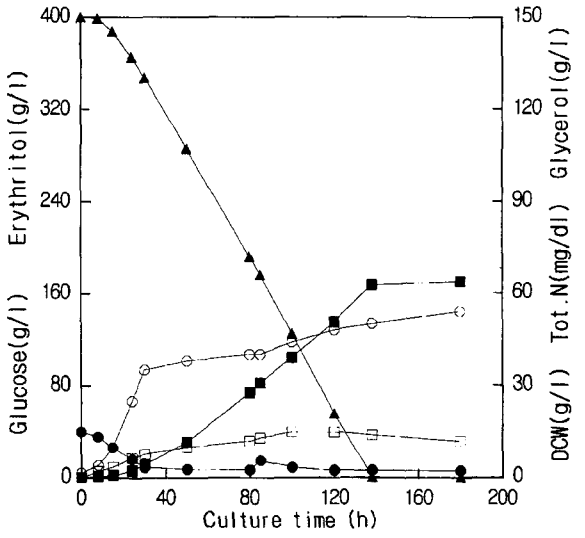


**Fig. 4.** Time course of cultivation in 40% glucose medium to which 1% CSL was added at the stationary phase.

Cultivation was done in flasks containing 50 ml of medium. The medium consisted of 40% glucose, 4% CSL, and 0.05% Tween 20. Concentration of residual glucose (▲), concentration of erythritol (■), dry cell weight (○), concentration of glycerol (□), and concentration of total nitrogen (●) are indicated.

### The Effect of Nitrogen Source on Erythritol Production

To find the effect of the nitrogen source on the production rate and conversion yield of erythritol, CSL was added to 1% concentration at the stationary phase of growth (80 h). As shown in Fig. 4, the production rate of erythritol increased from 1.4 g/l/h to 1.6 g/l/h, but the

**Table 3.** Content of total nitrogen in dry cell mass.

| Fermentation time (h) | Content of total nitrogen (%) |
|---|---|
| 18 | 4.64 |
| 48 | 4.82 |
| 70 | 3.50 |
| 95 | 3.20 |

Strains were cultivated on 10% glucose, 4% CSL and 0.05% Tween 20 medium at 35°C in a shaking incubator.

conversion yield of erythritol, $Y_{p/ss}$, was lowered from 51% to 44% after the addition of CSL to the culture broth. Cell mass and glycerol increased slightly.

### Strategies for High Erythritol Production Yield

*Trichosporon* sp. shows a typical diauxy growth. It grows exponentially consuming glucose at first, then after the second lag phase, cell mass slowly increases using glycerol and erythritol. In particular, the cell consumes glucose and nitrogen in the first stage, but only uses the produced glycerol and erythritol with no decrease in total nitrogen concentration in the second stage. The content of total nitrogen in dry cell mass was lowered from 4.82% to 3.20% during the second growth stage (Table 3, Fig. 2), indicating that carbon-to-nitrogen ratio of biomass increased. The nature of such accumulated materials is not known and is beyond the scope of this investigation. A similar phenomenon was observed in the case of *Aureobasidium pullulans* [8], which produces exopolysaccharide, pullulan. Its growth started with a decrease in both carbon and nitrogen sources and continued after the complete exhaustion of the nitrogen source.

It was reported that many microorganisms synthesized the erythritol to protect the high osmotic pressure from salts or sugars [10]. In *Trichosporon* sp., the erythritol seems to be produced not only as a osmoregulator but also as a storage carbohydrate. The produced erythritol was used as carbon source in the second growth phase, when the other carbon sources are used up.

Erythritol production was begun at the late exponential phase of cell growth before the nitrogen sources were wholly exhausted. When nutrient (CSL) was added during the stationary phase in which erythritol was being actively produced, the production rate of erythritol was slightly increased, although the conversion yield of erythritol decreased (Fig. 4). It is assumed that the nutrients, including nitrogen sources, stimulate the metabolic rate of glucose without inhibiting the erythritol production.

As the glucose concentration in the medium rose above 200 g/l, the specific growth rate and production rate of erythritol declined, while the conversion yield of cell mass and erythritol was maintained at the same level up to 400 g/l glucose in the medium (Table 2). These results suggest that the osmotic pressure from up to 400 g/l glucose in the medium has no effect on the conversion yield of cell mass and erythritol.

In summary, when the glucose concentration in the medium reached 400 g/l, the maximum conversion yield of erythritol of about 47% was obtained, since the period of stationary phase, in which the conversion yield of erythritol remains at a higher level, was maintained longer than in the other cases. The conversion yield of 47% in the 40% glucose medium was corresponded to 74% increase compared with that in the 10% glucose medium.

While the 47% conversion yield of erythritol was the highest in the 40% glucose medium, the volumetric productivity of erythritol, 1.18 g/l/h, was the lowest due to the low specific growth rate and erythritol production rate (Table 2). To increase the volumetric productivity of erythritol as well as the conversion ratio of erythritol, we are studying the fed-batch culture methods. Erythritol will be rapidly and effectively produced with constant feeding of glucose, after the fast growth of microorganisms in the 10% glucose medium.

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