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Efficient Production of Glucose Isomerase from *Arthrobacter* sp. L-3

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Arthrobacter sp. L-3가 생성하는 Glucose Isomerase의 최적 생성조건

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Abstract

The efficient production of glucose isomerase (G. I.) produced from *Arthrobacter* sp. L-3 was studied.
The optimum culture time of the enzyme was 40hr.
The maximum enzyme activity was found at glucose concentration 1%.
G.I. activity did not affect inoculum size.
The glucose isomerase activity was strongly influenced by the addition of glucose.

Key words: *Arthrobacter* sp. L-3, Glucose isomerase.

I. INTRODUCTION

Xylose isomerase(D-xylose ketol-isomerase, E. C. 5.3.1.5) is an intracellular enzyme that catalyzes the conversion of D-xylose to D-xylulose. Its practical significance stems from its ability to isomerase D-glucose to D-fructose. Therefore, this enzyme is often referred to as glucose isomerase and is widely used in industry for production of high fructose corn syrup [1-3]. Physicochemical properties and production of the enzyme from various sources have been extensively studied.[1,5]

Arthrobacter sp. is a good source for glucose isomerase production and it has several advantages in fermentation, but was not well studied. Generally, xylose is not demanded as an inducer for glucose isomerase production in *Arthrobacter* sp. and magnesiumion is only required for growth and in enzyme reaction[6,7]

Arthrobacter sp. is usually Gram-positive, nonsporulating and nonmotile bacteria. Morpholo-

gically and physiologically, this strain similar to Coryneform bacteria and *Actinomycetes* sp.[8]

It becomes more valuable microorganisms in industries for production of amino acids, nucleotides, enzymes, vitamins, pesticides, and single cell protein.[8]

Arthrobacter sp. was reported that putative catalytic sites and metal ion-binding amino acid residues have been predicted[9,10].

The present paper describes the efficient production of glucose isomerase from *Arthrobacter* sp. L-3.

Ⅱ. MATERIALS AND METHODS

1. Microorganism and cell culture

The microorganism for production of glucose isomerase was *Arthrobacter* sp. L-3.

The cells for seed culture were incubated at 30℃ in LB broth(tryptone 1%, yeast extract 0.5%, NaCl 0.5%, glucose 1%, pH 7.0~7.5) for 18~24hrs with shaking(rpm 100). Main cultures were grown in the fermentation medium(yeast extract 0.25%, peptone 1%, NaCl 0.5%, $MgSO_4 \cdot 7H_2O$ 0.05%, glucose 1%, pH 7.0~7.5) with jar fermentor(Marubishi MD 250~2.6L, Japan, work volume 11, rpm 200, airflow rate 1.0vvm). The inoculum size was 1%(v/v) with fresh LB grown seed cultures. After 40hr incubation, the cells were harvested and washed twice with 0.85% NaCl solution. The collected cells were preserved at 4℃ and used as an enzyme source.

2. Chemicals

Sodium dodecyl sulfate, acrylamide, dithiothretol, Sephadex G-100-50 and molecular weight standard marker protein were purchased from Sigma(U.S.A). DEAE-cellulose was obtained from Merck(Germany). Yeast extract, peptone an other medium compounds were purchased from Difco(Detroit, U.S.A).

3. Enzyme assay

The glucose isomerase activity was assayed by measuring the amounts of fructose converted from glucose by glucose isomerase.[11]

The reaction mixture was contained 1.0ml of substrate solution(1M glucose in 50mM potassium phosphate buffer pH 7.2 plus 30mM $MgSO_4 \cdot 7H_2O$) and equal volume of cells suspension (150mg of wet cells /ml).

The reaction was carried out at 60℃ for 1hr and stopped by the addition of 2ml of 0.5M perchloric acid($HClO_4$). The D-fructose produced was determined by the resorcinol method.[11] That is, to 0.5ml reactant solution were added 0.5ml of D.W. and 0.5ml of resorcinol reagent (glacial acetic acid 100ml+thiourea 0.25g+resorcinol 0.1g) and mixed throughly.

The reaction mixture was incubated at 80℃ for 10min. and cooled to room temperature. The optical density was estimated at 520nm. A linear reationship was obtained between concentration of fructose and optical density in the range 2μg to 10μg per ml(Fig. 1).

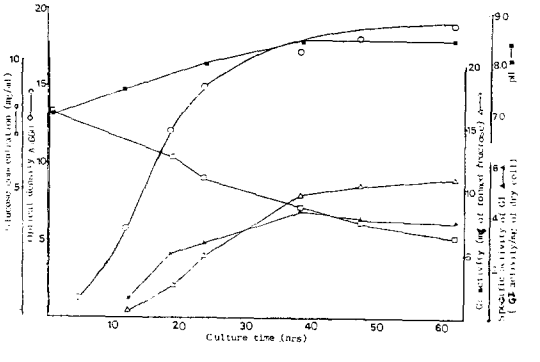

Fig. 1. The relationship of cell growth and glucose isomerase production in *Arthrobacter* sp. L-3. ○-○:cell growth, □-□:glucose concentration ■-■:pH, △-△:G.I. activity, ▲-▲:specific activity of G.I.

Ⅲ. RESULTS AND DISCUSSIONS

1. Effect of cell growth and glucose isomerase production

As shown in Fig. 1, the maximum enzyme activity was found at 40hr.

pH was varied with cell growth.

The cell growth was estimated at 660nm.

Glucose concentration was decreased by cell growth.

2. Effect of glucose concentration

The maximum enzyme activity was found at glucose concentration 1%(Fig. 2).

Cell growth was not influenced by glucose concentration.

3. Effect of inoculum size

The effect inoculum size on enzyme activity was determined at 1, 5, 10%(Fig. 3).

Inoculum size did not affect G.I. activity.

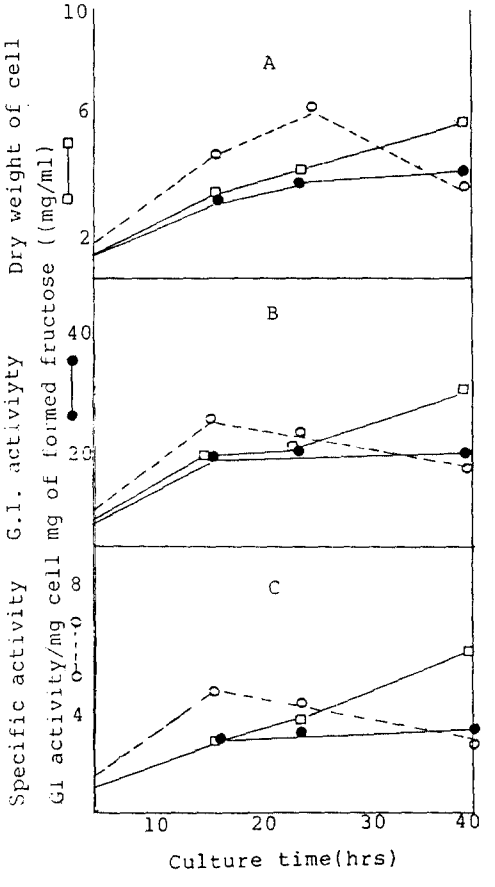

Fig. 2. Effect of glucose concentration in culture medium on cell growth and G. I. production in *Arthrobacter* sp. L-3.
A : 1%, B : 2%, C : 5%.
□-□:cell growth, ●-●:G.I. activity,
○-○:specific activity.

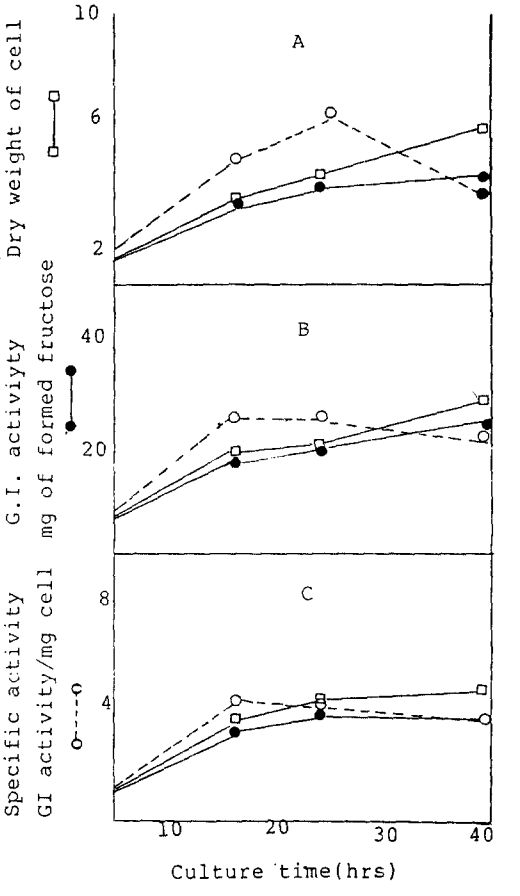

Fig. 3. Effect of inoculum size on cell growth and G. I. production in *Arthrobacter* sp. L-3.
A : 1%, B : 5%, C : 10%.
□-□:cell growth, ●-●:G.I. activity,
○-○:specific activity.

4. Effect of acidic condition

The effect of acidic condition on the stability of *Arthrobacter* sp. L-3 glucose isomerase was shown in Fig. 4.

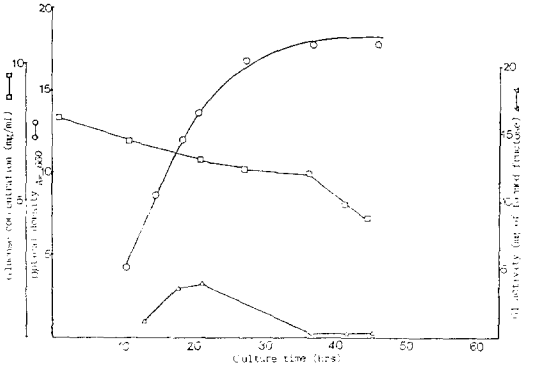

Fig. 4. Cell growth and glucose isomerase production of *Arthrobacter* sp. L-3 cultured in acidic condition.
□-□:glucose concentration, △-△:G.I. activity, ○-○:cell growth.
The pH was adjusted to 5.0 by 5N HCl with pH controller.

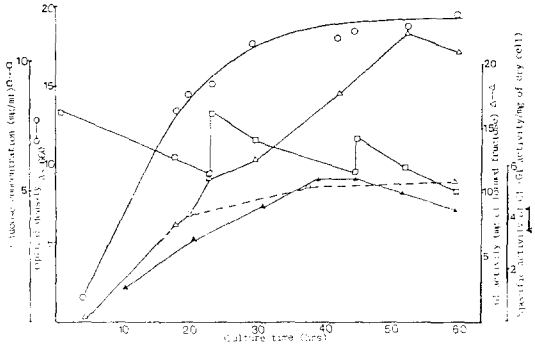

Fig. 5. Effect of intermittent feeding of nutrient on cell growth and glucose isomerase activity in *Arthrobacter* sp. L-3.
○-○:cell, growth,
□-□:glucose concentration,
△-△:G.I. activity,
▲-▲:specific activity of G.I.,
△-△:control G.I. activity shown in Fig. 1.

5. Effect of intermittent feeding

The addition of glucose was determined at culture time 20hr and 45hr(Fig. 5).

The glucose isomerase activity was strongly influenced by the addition of glucose.

The addition of glucose gave the highest glucose isomerase.

With intermittent feeding, the glucose isomerase production in *Arthrobacter* sp. L-3 was about four times higher than that.

6. Effect of continuous feeding

Fig. 6 shows the effect of continuous feeding on the G.I. activity.

The activity of glucose isomerase and cell growth was increased at continuous feeding.

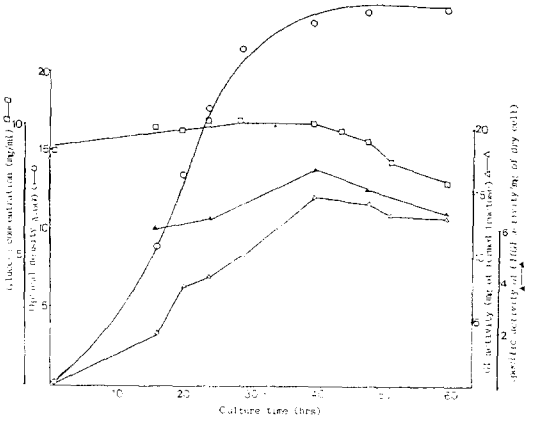

Fig. 6. Effect of continuous feeding of nutrient on cell growth and glucose isomerase production in *Arthrobacter* sp. L-3.
□-□:glucose concentration,
○-○:cell growth,
△-△:G.I. activity,
▲-▲:specific activity of G.I.

Ⅳ. 요 약

Arthrobacter sp. L-3가 생성하는 glucose isomer-

ase(G.I.)의 최적생성 조건을 검토하였다.

Cell growth에 따라 pH는 상승하였으며, cell growth는 40hr에 최대치에 도달하였고, glucose concentration은 cell growth가 증가함에 따라 감소하였으며, glucose concentration이 1%일때 G.I. activity가 가장 높았다.

Inoculum size가 증가해도 G.I. activity는 증가하지 않았으며, acidic condition에서는 G.I. activity가 반으로 줄었다.

Intermittent feeding으로 G.I. activity는 2.2~3.6배, specific activity는 1.3배 증가하였으며, continuous feeding으로 G.I. activity는 1.3배, specific activity는 2배 증가하였다.

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