KOREAN J. FOOD & NUTR.
Vol. 10, No. 1, 117~121(1997)

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

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Arthrobacter sp. L-3로부터 Glucose Isomerase의 생산

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요 약

Arthrobacter sp. L-3로부터 glucose isomerase의 생산성을 검토하였다. 배지의 탄소원으로는 glucose와 xylose를 혼합해서 공급한 것이 효소 생산성이 가장 높았다. 질소원으로는 yeast extract가 가장 높은 효소 생산성을 나타내었다. Glucose isomerase의 생산성은 배양시간이 40시간일 때 가장 높게 나타났다.

주요어 : *Arthrobacter* sp. L 3, glucose isomerase, productivity

INTRODUCTION

Xylose isomerase (D-xylose ketol isomerase, E.C. 5.3.1.5), which reversibly catalyzes the isomerization reaction between D-xylose and D-xylulose, can also convert D-glucose into D-fructose ; hence, the enzyme is often referred to glucose isomerase[1].

Glucose isomerase is an enzyme of primary industrial importance. Glucose isomerase (G.I. : D-xylose-ketol-isomerase, E.C. 5.3.1.5) is one of the valuable enzymes in food industries which is used in the production of high fructose syrup (HFS)[2-6].

Arthrobacter sp. is a good source for glucose isomerase production and it has several advantages in fermentation, but was not well studied. *Arthrobacter* sp. was isolated from soil which showed an excellent glucose isomerase productivity and glucose isomerase productivity of the strain was improved by mutagenesis.

The productivity of glucose isomerase of *Arthrobacter* sp. L-3 were described in this paper.

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MATERIALS AND METHODS

1. Microorganism and cell culture

The microorganism used for the 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 (100rpm). 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 250ml-2.6L, Japan, working volume 1L, 200rpm, air flow 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. Enzyme assay

The glucose isomerase activity was assayed by

measuring the amounts of fructose converted from glucose by glucose isomerase[7-12]. 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 cell suspension(150mg of wet cells/ml). The reaction was carried out at 60℃ for 1hr and stopped by addition of 2mL of 0.5M perchloric acid ($HClO_4$). The D-fructose produced was determined by the resorcinol method(Fig. 1)[8]. 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 0.25 g + resorcinol 0.1g) and mixed thoroughly. The reaction mixture was incubated at 80℃ for 10min and cooled to room temperature. The optical density was measured at 520nm. A linear relationship was obtained between concentration of fructose and optical density in the range of 2μg to 10μg per mL(Fig. 2).

RESULTS AND DISCUSSION

1. Effect of xylose and xylan on glucose isomerase activity in *Arthrobacter* sp. L-3

The concentration of carbon sources which were added to fermentation medium was 10mg /ml. The effect of carbon sources on glucose

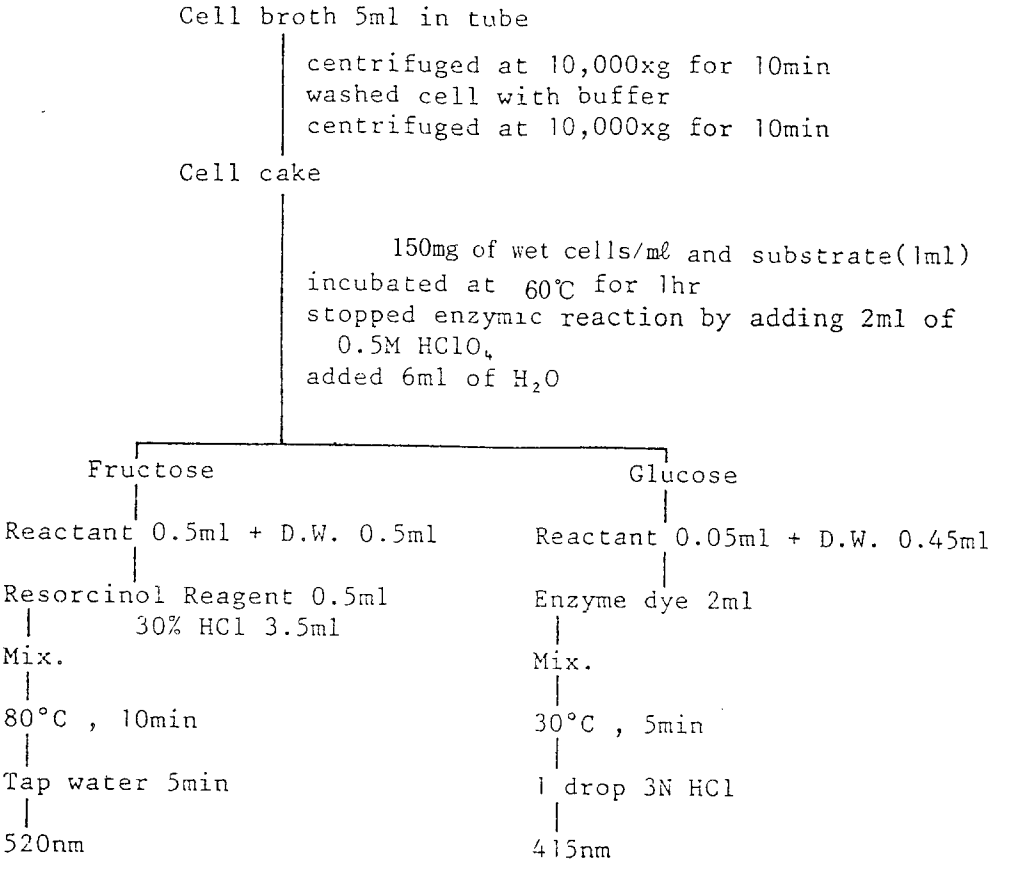

Fig. 1. Procedure for determination of glucose isomerase activity by resorcinol method.
Substrate : 50mM phosphate buffer(pH 7.2), 1M glucose, 30mM $MgSO_4 \cdot 7H_2O$
Buffer : 0.05M phosphate buffer(pH 7.2)
Resorcinol reagent : glacial acetic acid 100ml, thiourea 0.25g, resorcinol 0.1g
Enzyme dye : o-diansidine 17.5mg, 0.1M acetate buffer (pH 5.0) 100ml

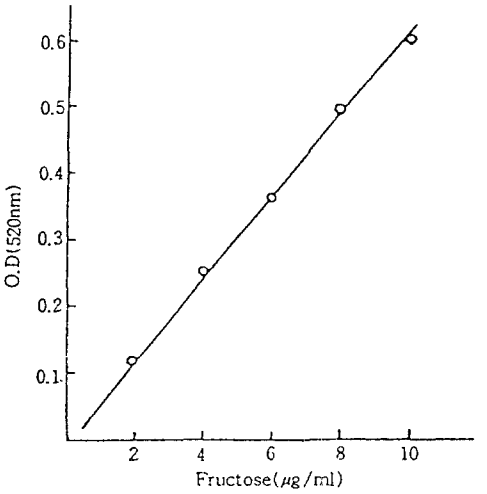

Fig. 2. Standard curve for determination of fructose by resorcinol method.

isomerase productivity of *Arthrobacter* sp. L-3 is described in Table 1.

D-glucose isomerase is used to catalyze the isomerization of glucose to fructose *in vivo*. A very important factor for glucose isomerase production is carbon source of medium. Glucose isomerase productivity is influenced by the addition of xylose[14-16]. In general , glucose isomerase requires xylose as an inducer. Addition of glucose and xylose as a carbon source showed the maximum productivity of glucose isomerase.

2. Effect of nitrogen sources on glucose isomerase productivity in *Arthrobacter* sp. L-3

The final concentration of added nitrogen sources was 50mg /ml in the culture medium. The effect of various nitrogen sources on glucose isomerase productivity was investigated. Addition of yeast extract as a nitrogen source showed higher productivity of glucose isomerase than any other nitrogen sources.

Table 1. Effects of xylose and xylan on glucose isomerase a productivity of *Arthrobacter* sp. L-3

Carbon source	Glucose	Xylose	Xylan	Glucose xylose	Glucose xylan
GI activity, mg of formed fructose	10	7.0	2.6	10.7	5.2
Relative value %	100	70	26	107	52

3. Effect of NaCl on glucose isomerase productivity of *Arthrobacter* sp. L-3

The NaCl concentration was determined by the 25~0.60%. The optimum NaCl concentration for the production of glucose isomerase was 0.5%. Table 3 shows the effect of NaCl concentration on the glucose isomerase productivity.

4. The relationship of cell growth and glucose isomerase productivity of *Arthrobacter* sp. L-3

The optimum culture time was determined by varying the culture time. Fig. 3 showed the relationship of cell growth and glucose isomerase

Table 2. Effect of nitrogen sources on glucose isomerase productivity of *Arthrobacter* sp. L-3

N-sources	GI activity (mg of formed fructose)	Relative value (%)
Yeast ext.(Difco)	10.5	100
Beef ext.	5.7	54
Peptone	5.8	55
Tryptone	4.8	45
Malt ext.	2.5	23
Casein	2.7	25
c.s.l.	2.2	20
Casein hydrolysate	2.4	22
Soybean-meal	3.1	30
Peanut-meal	3.0	28

Table 3. Effect of NaCl concentration on glucose isomerase productivity of *Arthrobacter* sp. L-3

NaCl(%)	GI activity (mg of formed fructose)
0.25	5.6
0.30	7.3
0.40	9.5
0.45	10.1
0.50	10.6
0.55	9.4
0.60	8.8

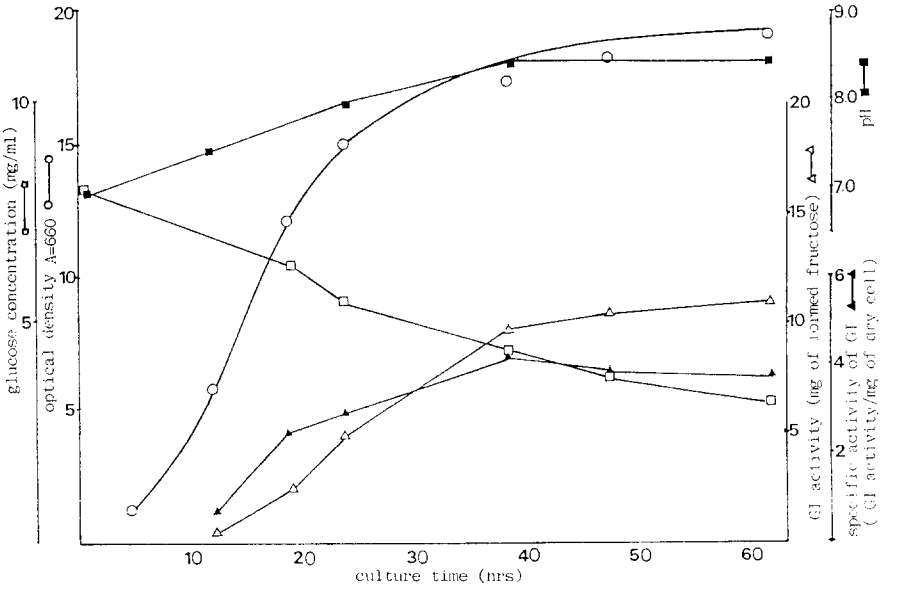

Fig. 3. The relationship of cell growth and glucose isomerase productivity of *Arthrobacter* sp. L-3.
○----○ : cell growth, □----□ : glucose concentration,
■----■ : pH, △----△ : glucose isomerase activity,
▲----▲ : specific activity of glucose isomerase.

productivity of *Arthrobacter* sp. L-3. The optimum culture time for the production of glucose isomerase was 40hrs.

ABSTRACT

The glucose isomerase productivity of *Arthrobacter* sp. L-3 was studied. Glucose plus xylose showed higher productivity of glucose isomerase than any other carbon sources. Yeast extract showed higher productivity of glucose isomerase than any other nitrogen sources. The optimum culture time for the production of glucose isomerase was 40hrs.

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

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(1997년 3월 14일 접수)