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Purification of Glucose Isomerase from Alkalophilic *Streptomyces sp.* B-2

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호알칼리성 *Streptomyces sp.* B-2에 의한 Glucose Isomerase의 정제

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요 약

호알칼리성 방선균 *Streptomyes sp.* B-2를 Glucose Isomerase 생성을 위해 토양에서 분리했다. Glucose Isomerase(G.I.)는 high fructose glucose syrup과 fructose의 생산을 위해서 식품 공업에서 아주 중요시되고 있는 효소이다.

호알칼리성 방선균 *Streptomyces sp.* B-2가 생성하는 glucose isomerase(G. I.)를 정제하였다.

G. I.는 $(NH_4)_2SO_4$ 분획, DEAE-cellulose, Sephadex G-200 chromatography하여 순수 분리 하였다. 순수분리된 G. I.는 electrophoresis에 의해 확인을 했다.

SDS-acrylamide gel electrophoresis에 의하여 정제된 효소는 single band를 보여 주었다.

Key words: *Streptomyces sp.* B-2, glucose isomerase.

Ⅰ. Introduction

D-Xylose isomerase (E.C., 5.3.1.5) is used industrially to catalyze the isomerization of glucose to fructose, known as of glucose isomerase[1,2,3].

Glucose isomerase is an enzyme of primary industrial importance. Enzyme which was primarily derived from *Bacillus coagulans*, *Streptomyces spp.*, and *Actinoplanes spp.* was utilized in the annual conversion of 3 billion kilograms of glucose into fructose for use as high-fructose corn syrup[4].

Improvement in the enzyme process used by the sweetener industry requires higher thermal and acid stabilities of glucose isomerase, higher specific activity and overproduction of the enzyme, and a lower-cost enzyme recovery process from host cells after fermentation[5,6].

In general, sugar isomerases also require presence of at least a single divalent cation per active site. The enzyme catalyzes a fairly slow reaction, which takes about 5 molecules per second per

active site. However, triose-phosphate isomerase takes 1000-fold faster[7].

Alkalophilic *Streptomyces sp.* is a good source for glucose isomerase production.

Even though it has several advantages in fermentation, but was not well studied. Alkalophilic *Streptomyces sp.* is isolated from soil which showed an excellent glucose isomerase productivity.

The purification of glucose isomerase from Alkalophilic *Streptomyces sp.* B-2 were described in this paper.

Ⅱ. Materials and Methods

1. Microorganism

The microorganism for production of glucose isomerase was Alkalophilic *Streptomyces sp.* B-2. Alkalophilic *Streptomyces sp.* B-2 was isolated from soil.

2. Cell culture

The cells for seed culture were incubated at 30 ℃ for 50hrs with shaking(120 stroke/min), as shown in Table 1.

3. Enzyme assay

The G.I. activity was assayed by measuring the amounts of fructose converted from glucose by G.I.

D-fructose produced was determined by the resorcinol method[8].

The resorcinol method is illustrated in Fig. 1.

The reaction was carried out at 60℃ for 1hr and stopped by the additional 2m*l* of 0.5M perchloric acid.

The G.I. activity 1 unit was expressed as the 1 μmole of D-fructose produced under the conditions shown above.

Standard curve is illustrated in Fig. 2.

Table 1. Medium composition of the Glucose Isomerase by the Alkalophilic *Streptomyces sp.* B-2.

	(g/*l*)
*Glucose	4.0
*Xylose	6.0
$MgSO_4 \cdot 7H_2O$	1.0
$CoCl_2 \cdot 6H_2O$	0.1
Peptone	3.0
NaCl	3.0
Yeast extract	2.0
K_2HPO_4	5.0
*Na_2CO_3	10.0
pH	10.5

*Sterilized separately

4. Enzyme purification

The cells were disrupted by dismembrator (Fisher, Sonic dismembrator Model 300) for 30min at 4℃.

The unbroken cells and cell debris were removed by centrifugation (Hitachi, Rotor 20-2) for 20min at 15,000rpm. The supernatant treated with fine ammonium sulfate to 70% saturation, and the precipitates were harvested by centrifugation (7,000 rpm).

0.05M phosphate buffer (pH 7.0) dialized for 24hrs against the same buffer as described by Englung.

The resulted solution was applied to DEAE-cellulose column which was equilibrated with 0.05M phosphate buffer (pH 7.0). After washing the column with the same buffer elution was carried out by two stage NaCl gradient from 0.5M to 2.0M. The eluate was tested for G.I. activity and determined protein concentration by spectrophotometer at 280nm. Resulted enzyme fractions were collected, dialized against distilled water and lyophilized.

Ⅲ. Results and Discussions

1. Purification of G.I. by DEAE-cellulose column chromatography

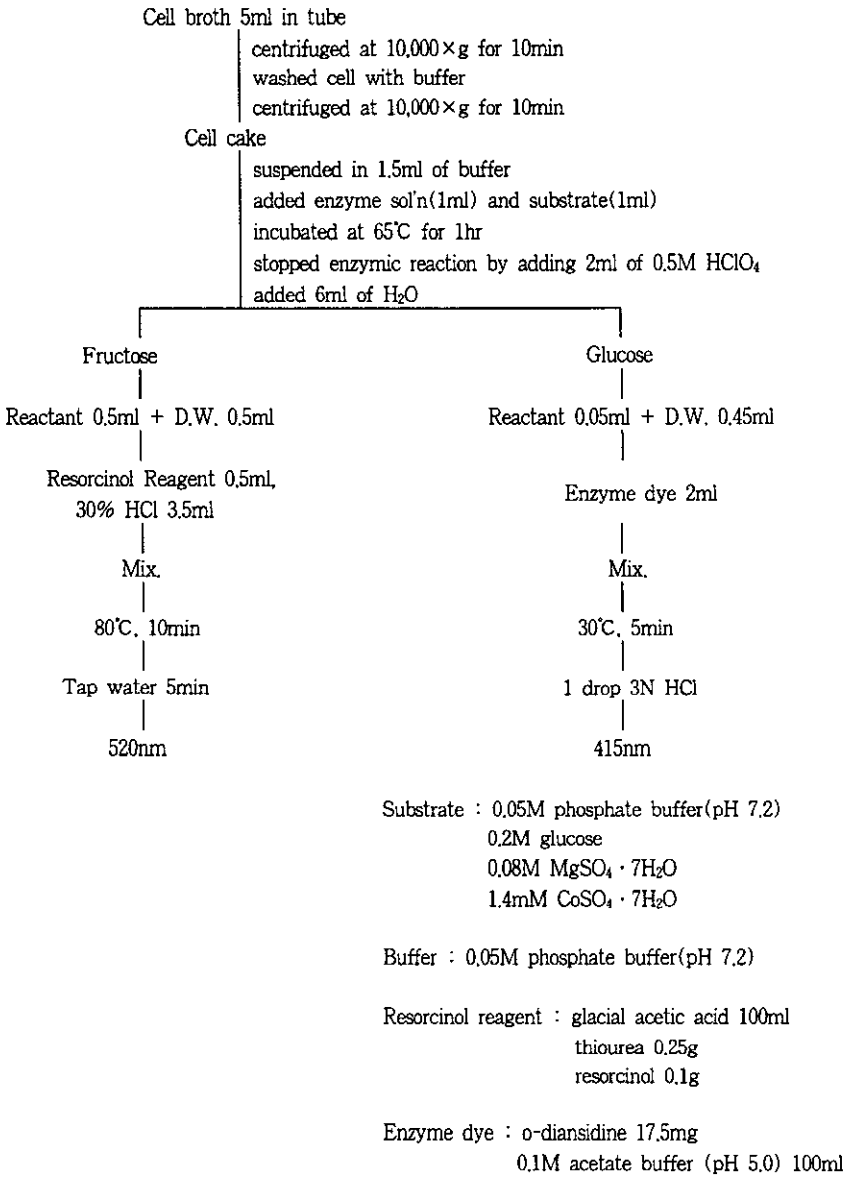

Fig. 1. Procedure for determination of glucose isomerase activity by resorcinol method.

The crude enzyme solution was disrupted by sonication, precipitated by 70% ammonium sulfate and dialyzed with 50mM phosphate buffer solution (pH 7.2). Then, it was applied to DEAE-cellulose column (35×450mm), which was equilibrated with the same buffer solution. Elution was carried out by NaCl gradient elution(Fig. 3). Each fractional volume was 7.5ml, and all fraction was tested G.I. activity. Quantity of protein was also determined by spectrophotometer (Shimazu, Japan) at 280nm. With the two-stage of NaCl gradient elution, the enzyme was purified in one step.

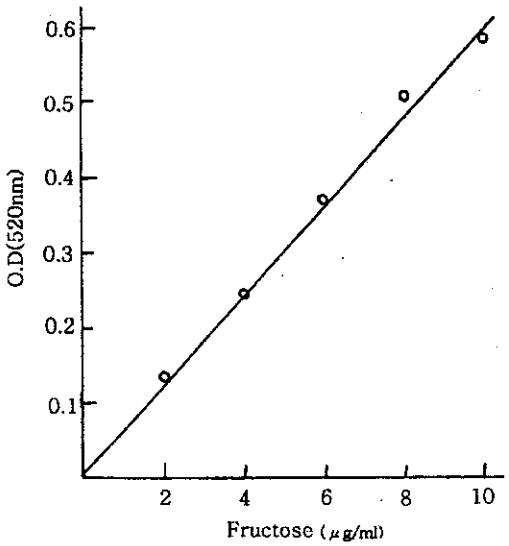

Fig. 2. Standard curve for determination fructose by resorcinol method.

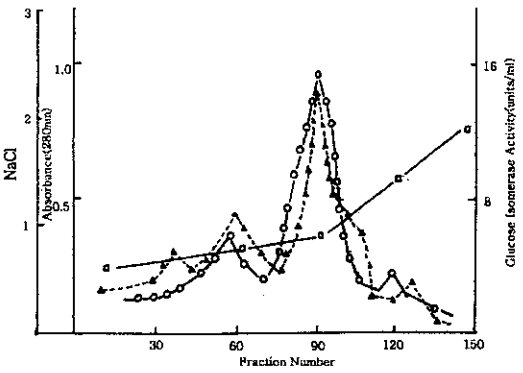

Fig. 3. DEAE-cellulose column chromatography pattern of glucose isomerase.
Column size: 3.4×45cm, One fraction : 7.5ml
○ : glucose isomerase activity
△ : protein
□ : NaCl concentration

2. Sephadex G-200 rechromatography

Sephadex G-200 rechromatography carried out from G.I. active fraction by DEAE-cellulose ion exchange chromatography.

Sephadex G-200 column (5.0~6.0cm), which

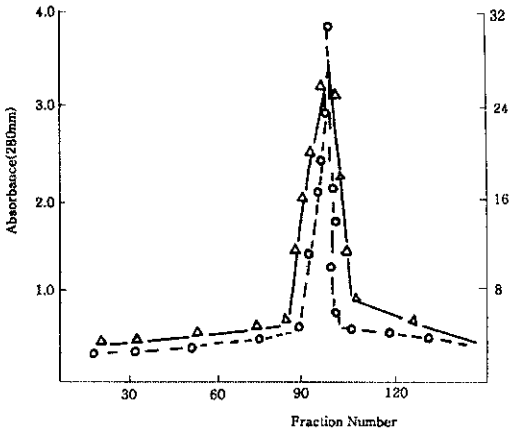

Fig. 4. Gel filtration chromatography of glucose isomerase produced by alkalophilic *Streptomyces sp.* B-2 on Sephadex G-200.

Fig. 5. Disc gel electrophoresis of the glucose isomerase produced by alkalophilic *Streptomyces sp.* B-2.

was equilibrated with 0.05M phosphate buffer(pH 7.0) containing 0.1M NaCl.

After the column was successively washed with the same buffer as above, the enzyme was eluted with a linear gradient increase in NaCl at flow rate of 12ml/hr.

7.5ml fractions were collected, and each fraction was assayed.

Column development carried out at 4℃.

The typical elution profile is shown in Fig. 4.

The enzyme activity was assayed by measuring the amount of D-fructose resulted from D-glucose(Fig. 2).

3. SDS-acrylamide gel electrophoresis

Sodium dodecyl sulfate (SDS) gel electrophoresis were carried out according to the Yamanaka, K[9,10,15].

The purified enzyme preparation showed a single band on SDS-acrylamide gel electrophoresis as shown in Fig. 5. This means that the enzyme purification was completely performed.

Ⅳ. Abstract

The purification of glucose isomerase produced by Alkalophilic *Streptomyces sp.* B-2 were studied.

Numerous studies on glucose isomerase by acidophiles or neutrophiles have been reported by several workers[11~14].

Glucose isomerase was purified from the culture filtrate of the organism through chromatography on DEAE-cellulose and Sephadex G-200.

The purified glucose isomerase was found homogenous in polyacrylamide gel electrophoresis.

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