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Studies on the Microbial Glucose Isomerase

Part 2. Culture Conditions of *Streptomyces* sp. K-14 in Producing Glucose Isomerase

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微生物의 葡萄糖 異性化酵素에 關한 硏究

(第 2 報) *Streptomyces* sp. K-14 菌株의 培養特性에 關하여

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Abstract

Cultural characteristics of a strain of *Streptomyces* sp. K-14 (KFCC 35051) producing glucose isomerase were demonstrated. The glucose isomerase was produced when the strain was grown in the medium containing pure xylan or xylan-containing materials such as wheat bran or corn cob. The optimum condition was attained in a culture medium composed of 3% wheat bran or corn cob, 2% corn steep liquor, 0.1% $MgSO_4 \cdot 7H_2O$ and 0.012% $CoSO_4 \cdot 7H_2O$ for the production of the glucose isomerase. The production of the enzyme reached to a maximum level when the strain was cultured for 40 hrs 30°C and pH 7.0.

Introduction

Experimental culture conditions for the production of glucose isomerase from a strain of *Streptomyces* sp. have been studied by Takasaki et al. [1] They reported that glucose isomerase of *Streptomyces* sp. was generally induced by xylose and by xylan, and also discovered that glucose isomerase could be produced by *Streptomyces* sp. in culture medium composed of constituents containing xylan such as wheat bran and corn cob.

In the previous article, [2] we reported a newly isolated strain of *Streptomyces* sp. K-14, which is different from those species previousely reported by others and which showed the highest yield of glucose isomerase among other isolated strains. In this study, we carried out a series of experiments for culture condition of *Streptomyces* sp. K-14 to optimize production of glucose isomerase.

Materials and Methods

Experimental Strain : The microbial strain used

in the present experiment is *Streptomyces* sp. K-14 (KFCC 35051) isolated from a soil sample. Microbial characteristics of this strain has been determined[2].

Procedures of Microbial Culture The strain of *Streptomyces* sp. K-14 was cultured in 250m*l* shaking flasks containing 50m*l* of the basic culture medium (Table 1) composed of xylan as a carbon source for the production of glucose isomerase. The culture flasks were incubated at 30°C for 30~35 hrs. after inoculation of the microbes from an agar slant of a stock culture. This primary culture was used as a seed for a larger volume of liquid culture for the enzyme production. The secondary fermentation process was carried out in 5 *l* jar fermentors (Model MJ 5, Marubishi) with 2 *l* culture media containing either pure xylan or raw material such as wheat bran (xylan content about 21%) and corn cob (xylan content about 35%) (Table 2). The culture media were sterilized at 121°C for 30 min followed by inoculation of 100 m*l* of the seed culture after cooling the media to room temperature. The inoculated fermentors were placed in a water bath and incubated at 30°C for 40 hrs with stirring speed 200 rpm and aeration rate 1.5-2-1/min before recovery of myce lium for analysis of the enzyme activity.

Table 1. Medium Used for Seed Culture.

Xylan	10g
Polypepton	3g
K_2HPO_4	3g
$MgSO_4 \cdot 7H_2O$	1g
Distilled Water	1,000m*l*

pH 7.0±0.2

Table 2. Medium Used for Enzyme Production.

Xylan	10g
Corn steep liquor	20g
$MgSO_4 \cdot 7H_2O$	1g
$CoSO_4 \cdot 7H_2O$	0.1g
Distilled Water	1,000m*l*

pH 7.0±0.2

Determination of Cell Growth : Cell growth was determined by measuring turbidity at 660nm after dilution of the original culture broth to 20 times with distilled water. Direct measurement of protein content in the culture media was also applied for the estimation of cell mass.

Determination of Xylan Content Xylan contents in wheat bran and corn cob were determined by the method of pentosan determination[3]. Pure xylan was analyzed by determining reducing sugar according to the Somogy's method after hydrolysis of samples with 4% H_2SO_4 for 2.5 hrs. Other procedures were same as described in the previous paper[2].

Materials Xylose and xylan were purchased from Sigma Chemical Company. Wheat bran and corn cob were obtained from a local market. Corn steep liquor was a gift from Cheoneel Goksan Company and dried in a spray drier at KIST. Other chemicals used were of reagent grade.

Results

Effect of Carbon Source The productivity of glucose isomerase is dependent of carbon source applied in the culture medium. The effect of various carbon sources on the enzyme production of *Streptomyces* sp. K-14 are summarized in Table 3. It is of interest to note that glucose isomerase is

Table 3. Effect of Carbon Source in Culture Medium. The basic medium contains corn steep liquor 2%, $MgSO_4 \cdot 7H_2O$ 0.1%, $CoCl_2 \cdot 7H_2O$ 0.01% and carbohydrate 1%, The enzyme activity was determined after 45 hrs culture at 30°C.

Carbon Source (1%)	Enzyme Activity (unit/m*l* broth)	Relative Activity (%)
None	0.27	1.0
Xylose	28.12	100
Xylan	16.56	58.9
Glucose	0.86	3.1
Fructose	0.68	2.4
Sorbitol	1.44	5.1
Mannose	1.28	4.6
Sucrose	1.10	3.9
Mannitol	1.84	6.5
Raffinose	0.75	2.7
Maltose	0.45	1.6

induced by the presence of xylose or by xylan whose effect is one half of that xylose. Other sugars did not induce glucose isomerase activity (or less than 10%) although cells were grown normally.

Table 4 shows the effect of xylan concentrations on the enzyme activity. It is noted that the enzyme activity gradually increases as xylan concentration increases up to 5%. The optimum concentration of xylan is about 1~2%.

Table 4. Effect of Concentration of Xylan in Culture Medium. Culture condition is same as shown in Table 3 except xylan concentration.

Xylan (%)	Enzyme Activity (unit/m*l* broth)	Relative Activity (%)
0	0.15	0.9
0.2	1.29	7.9
0.5	4.96	30.5
1	10.25	63.1
2	14.80	91.1
5	16.25	100
10	16.20	99.7

Effect of Nitrogen Source Effect of organic as well as inorganic nitrogen sources were examined (Table 5). It was found that only corn steep liquor appeared best for the production of glucose isomerase from *Streptomyces* sp. K-14, whereas other organic and inorganic nitrogen sources were not effective.

Teble 5. Effect of Nitrogen Source in Culture Medium. The basic medium contains $MgSO_4 \cdot 7H_2O$ 0.1%, $CoSO_4 \cdot 7H_2O$ 0.01% and Xylan 1%. Enzyme activity was determined after 45 hrs culture at 30°C.

Nitrogen Source (1%)	Enzyme Activity (unit/m*l* broth)	Relative Activty (%)
None	0.28	1.8
Corn steep liquor	15.30	100
Yeast extract	2.40	15.7
Beef extract	1.40	9.2
Pepton	1.20	7.8
Soy protein*	1.04	6.8
Ammonium sulfate	2.75	11.4
Ammonium chloride	1.88	12.3
Potassium nitrate	2.25	14.7
Sodium nitrate	1.75	11.4
Urea	1.00	6.5

*Acid hydrolyzed soy protein

The optimum concentration of corn steep liquor is about 1~2% and the maximum enzyme activity was produced at the concentration of 2% followed by decrease in the enzyme productivity (Table 6).

Table 6. Effect of Concentration of Corn Steep Liquor. The culture condition is same as indicated in Table 5 except concentrations of corn steep liquor.

Corn Steep Liquor (%)	Enzyme Activity (unit/m*l* broth)	Relative Activity
None	1.68	10.8
0.5	12.31	79.3
1	15.06	97.0
2	15.52	100
3	11.34	73.1
5	11.43	73.6

Effect of Metal Ions Table 7 shows effect of various inogranic ions on the productivity of glucose isomerase by *Streptomyces* sp. K-14 cultured in the basic medium containing xylan and corn steep liquor. It was observed that only Mg^{++} and Co^{++} were effective on the enzyme production, whereas other inorganic ions showed no effect. The optimum final concentrations of Mg^{++} and Co^{++} were 4.0mM and 1-2mM, respectively as seen in Table 8 and 9.

Table 7. Effect of Metal Ions in Culture Medium. The basic medium contains xylan 1% and corn steep liquor 2%. The enzyme activity was determined after 35 hrs culture at 30°C

Salts used (1×10^{-3}M)	Enzyme Activity (unit/m*l* broth)	Relative Activity (%)
None	0.20	1.80
$MgSO_4$	10.18	91.5
$MgCl_2$	11.12	100
$ZnSO_4$	5.22	46.9
$MnCl_2$	4.32	38.8

$NiCl_2$	4.50	40.5
KCl	3.82	34.4
$CoSO_4$	10.54	94.8
$CoCl_2$	9.98	89.7
$FeCl_2$	3.86	34.7
Li_2SO_4	5.41	48.7
$CaCl_2$	3.33	29.9

Table 8. Effect of Mg^{++} Concentration in Culture Medium. The basic medium contains xylan 1%, corn steep liquor 2% and 1mM of $CoSO_4 \cdot 7H_2O$. The enzyme activity was determined after 45 hrs culture at 30°C.

$MgSO_4 \cdot 7H_2O$ (mM)	Enzyme Activity (unit/*ml* broth)	Relative Activity (%)
None	9.82	81.8
2.0	11.80	98.3
4.0	12.00	100
8.0	8.52	71.0
12.0	9.12	76.0
20.0	9.10	75.8

Table 9. Effect of Co^{++} Concentration in Culture Medium. The basic medium contains xylan 1%, corn steep liquor 2% and 5mM of $MgSO_4 \cdot 7H_2O$. The enzyme activity was determined after 40 hrs culture at 30°C.

$CoSO_4 \cdot 7H_2O$ (mM)	Enzyme Activity (unit/ml broth)	Relative Activity (%)
None	10.2	51.5
1	19.8	100
2	19.3	97.5
3	6.8	34.3
4	5.0	25.3
5	4.8	24.2

Effect of pH Fig 1. demonstrates a pH profile of the enzyme production by *Streptomyces* sp. K-14 in the basic medium containing pure xylan as a carbon source. It is noted that the pH profile is a bell shape and the maximum peak appears at pH 7.0. The range of optimum pH for production of glucose isomerase appear between pH 7.0~7.5.

Time Course of Enzyme Production From the experimental results described above, optimum

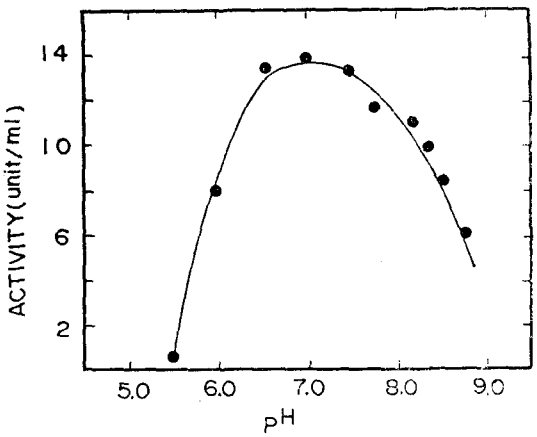

Fig. 1. Effect of pH on the Production of Glucose Isomerase. The composition of culture medium is described in Table 2. The enzyme activity was determined after 40 hrs culture at 30°C.

composition of culture medium for the production of glucose isomerase of *Streptomyces* sp. K-14 can be summarized as shown in Table 10. In order to study fermentation kinetics, we examined time dependent changes of the enzyme production, growth of cell mass, pH of the medium, and xylan consumption by culturing the microbial strains in 300 m*l* of shaking flasks contained the optimized culture media with pure xylan (Fig. 2). After inoculation of 5% seed culture, the culture flasks were incubated at 30°C until subjected to experimental tests.

Table 10. Optimum Composition of Culture Medium for the Enzyme Production by *Streptomyces* sp. K-14.

Wheat bran or corn cob	30g
Corn steep liquor	20g
$MgSO_4 \cdot 7H_2O$	1g
$CoSO_4 \cdot 7H_2O$	0.12g
Distilled Water	1,000m*l*

pH 7.0±0.2

As shown in Fig 2, the productivity of the enzyme increased drastically after 20 hrs and reached

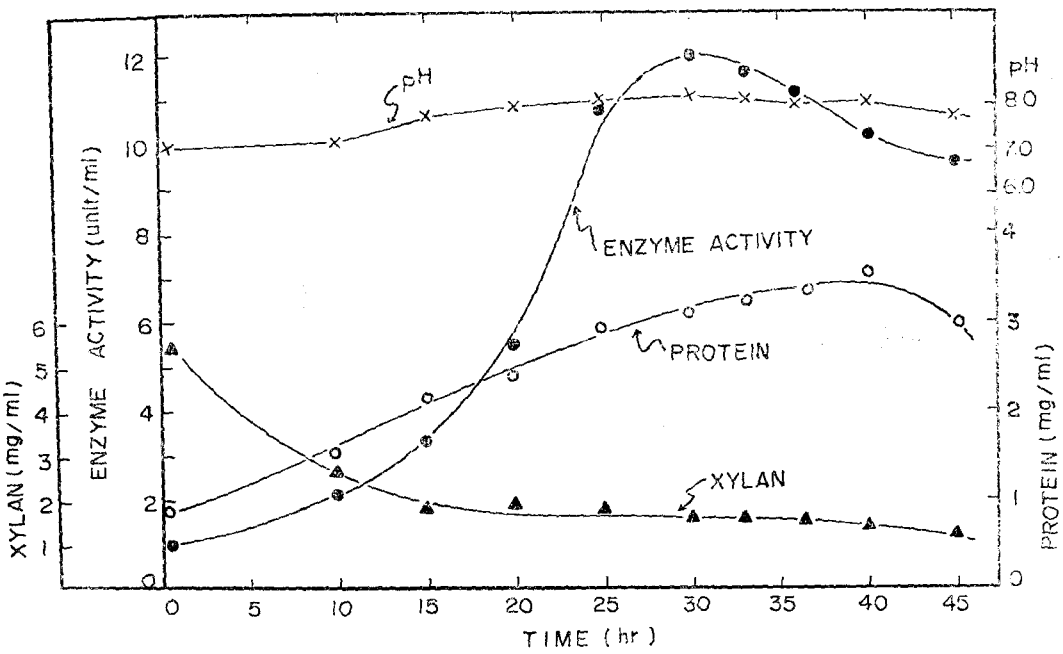

Fig. 2. Time Dependent Changes in the Enzyme Activity, Protein Concentration, Xylan Consumption, and pH of the Culture Medium. The composition of the medium (xylan medium) is described in Table 2 and the fermentation process was carried out under the following conditions: air flow, 2*l*/min; temperature, 30°C.

to a maximum level (about 12 units/m*l* of broth) at 30 hrs after the incubation was started. There was, however, an initial lag phase for about 10 hrs before emerging to the production phase of the enzyme. Cell growth took place rapidly for 15~20 hrs reaching constant level followed by a slight decrease. Degree of xylan consumption was proportionally related to the cell growth and turbidity due to insoluble macromolecular xylan was completely cleared after 30 hrs incubation. The clearance of turbidity appears to be due to the hydrolysis of xylan producing small molecular units such as xylose. The pH of the medium raised up to 8.0 from the initial pH 7.0.

Time dependent changes in the enzyme production were also observed in a jar fermentor with a medium of wheat bran or corn cob which contains a considerable amount of xylan instead of pure xylan (Fig. 3). The kinetic pattern of the enzyme production with such as crude medium is similar to that of a medium containing pure xylan.

The growth of cell mass reached to maximum level and maintained steady state after 20 hrs, while the productivity of the enzyme gradually increased reaching a peak at 43 hrs followed by gradual decrease in the enzyme activity. Thus, it is concluded that the optimum culture time for the production of glucose isomerase by *Streptomyces* sp. K-14 is about 40 hrs when wheat bran or corn cob is used as a carbon source.

Discussion

In recent year microbial glucose isomerase became

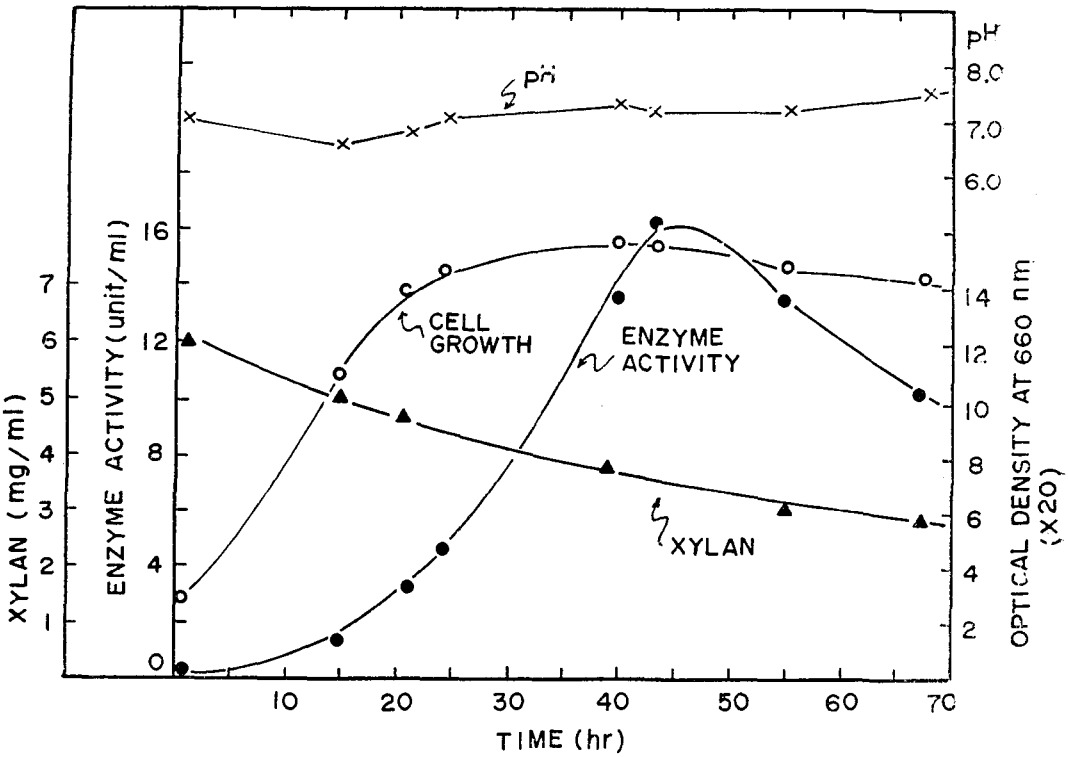

Fig. 3. Fermentation Kinetics of Glucose Isomerase Production. The composition of the medium (wheat bran medium) is described in Table 10. The fermentation conditions are same as described in Fig. 2.

one of the popular industrial enzymes for the prod uction of high fructose corn syrup. There are many reports and patents dealing with production of glucose isomerase from various microorganisms including several species of the genus *Streptomyces*. Takasaki[4] reported 23 strains of *Streptomyces* species. Among those reported the followings are well known strains that produce glucose isomerase; *Streptomyces flavovirens St. achromogenes, St. echinatus, St. albus, St. phaeochromogenes* and *St. olivochromogenes*. Particularly the enzyme from *Streptomyces cinerues* series, *Streptomyces* sp. ATCC 21175 and 21176 are actually being used for the industrial production of high fructose corn syrup. In the previous paper, it was reported that *Streptomyces* sp. K-14 isolated in our laboratory was taxonomically different from those reported by others [4,5]. Particularly, the strains of ATCC 211 75 and ATCC 21176 have spiny spore, whereas *Streptomyces* sp. K-14 has smooth spore surface.

Most of industrial strains of *Streptomyces* produces glucose isomerase induced by xylose as well as xylan. Glucose isomerase of *Streptomyces* sp. K-1 4 is also induced by xylose and xylan. Since use of pure xylan is not practical for the scale-up pur pose, one can replace it with plant material containing xylan. Xylan is one of the major constituents of hemicellulose in plant material such as rice straw, wheat bran, corn cob, and woods. It is likely that xylan in the culture medium is hydrolyzed in to xylose by the action of xylanase, an extracellular enzyme produced by *Streptomyces* sp. [6]. Subsequ ently the resulted xylose can be utilized by the microorganisms as an energy source and an enzyme inducer[7]. The cell growth occured rapidly in the initial phase of the culture which corresponds to the initial lag phase for the production of glucose isomerase. This lag phase appears to be due to the

induction period of the enzyme.

It is of interest to note that only corn steep liquor is effective in increasing the enzyme productivity, whereas other nitrogen sources were not. It is not yet understood as to which components of corn steep liquor are associated with the promotion of the enzyme productivity. There is, however, a report that sulfide compounds can stimulate the enzyme productivity of *Streptomyces*[5].

The fact that productivity of glucose isomerase is very sensitive to metal ions such as Co^{++} and Mg^{++} can be related to the increased stability of the enzyme by these metal ions. The metal ions can also act as enzyme activators. It is noted that the effect of Co^{++} is biphasic in such a way that it gradually activates the enzyme activity up to the concentration level of 1~2 mM and it becomes inhibitory at higher concentration.

Although taxonomical characteristics of *Streptomyces* sp. K-14 isolated in our laboratory is quite different from those originally reported by Takasaki[7], the cultural characteristics of the strain for the production of glucose isomerase appear to be later. It is likely that inductivity of xylose and requirement of metal ions such as Co^{++} and Mg^{++} are common properties of glucose isomerase produced by the genus *Streptomyces*.

In any event, *Streptomyces* sp. K-14 (KFCC 3 5051) in a new microbial strain with the best pro ductivity of glucose isomerase isolated so far and with cultural characteristics which are practically suitable for industrial production of the enzyme. Further studies on enzymatic properties will be reported in the subsequent paper.

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요 약

토양에서 분리한 glucose isomerase 생산균주 *Streptomyces* sp. K-14 (KFCC 35051)를 시험균주로 선정하여 몇가지 glucose isomerase 생산의 최적조건을 규명하여 다음의 결과를 얻었다.

1. 본 균주는 탄소원으로 xylose 대신 xylan에서도 glucose isomerase가 유도되며, xylan의 최적농도는 1~2%였다.

2. 유기태 질소원으로 corn steep liquor를 사용할때에 glucose isomerase 생산이 가장 양호하였고 무기태 질소는 거의 영향이 없었다. corn steep liquor의 최적농도는 1~2%였다.

3. 본균주의 효소생산에 무기이온으로 Mg^{++}과 Co^{++}만이 영향이 있었고 기타의 무기이온은 영향이 없었으며, 그 최적농도는 $MgSO_4 \cdot 7H_2O$로서 4.0mM이고 $CoSO_4 \cdot 7H_2O$로서 1~2mM이였다.

4. 본 균주의 효소생산 최적pH는 7.0~7.5였다.

5. 밀기울 및 옥수수혜축을 사용한 효소생산 최적배지조성은 밀기울 및 옥수수혜축 3%, 분말 corn steep liquor 2%, $MgSO_4 \cdot 7H_2O$ 0.1% 및 $CoSO_4 \cdot 7H_2O$ 0.012%이며, 최적 배양조건은 배지의 pH 7.0, 통기량 1.5~2 *l*/min, 회전수 200rpm, 배양온도 30°C에서 약 40시간 배양하여 최대 효소활성을 나타내였다.

이상의 결과를 검토한바 *Streptomyces* sp. K-14 (KFCC 35051) 균주는 glucose isomerase 생산을실용화하는데 적합한 균주라고 생각된다.

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