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Studies on the Microbial Glucose Isomerase

Part 1. Isolation and Characterization of *Streptomyces* species 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1報) 葡萄糖 異性化酵素 生産菌株의 分離 및 性質에 關하여

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Abstract

Five strains of *Streptomyces* spp. with high productivity of glucose isomerase (15-30 units/m*l*) were obtained among 280 microbial strains isolated from 150 soil samples. These strains produced glucose isomerase with xylose as an inducer. These 5 strains were also identified to be different strains of *Streptomyces* spp. : *streptomyces* sp. K-14, K-53, K-71, K-77 and K-733. It was found that *Streptomyces* sp. K-14 produced the highest enzyme activity. The spore chains of these strains were rectiflexible and spore surface was smooth except *Steptomyces* sp. K-77 and K-733, with spiny surface.

Introduction

Microbial glucose isomerase was initially found by Marshall and Kooi[1] from *Pseudomonas hydrophyla* cultured in a xylose medium. Since they demonstrated that glucose could be converted to fructose by the catalysis of glucose isomerase, much work has been done to put this scientific finding into practical use. In fact, recent development of enzyme technology in the production of microbial glucose isomerase and the immobilized enzyme made application of this particular enzyme successful for the industrial production of high fructose corn syrup, a sugar substitute.

Glucose isomerase was found in various microorganisms such as *Aerobactor cloacae*[2], *A. aerogenes*[3], *Escherichia intermedia*[4]. *Lactobacillus brevis*[5]. *Bacillus megaterium*[6]. and *B. coagulans*[7], in addition to a number strains of the genus *Streptomyces*. *Streptomyces*. spp. producing glucose isomerase was initially reported by Tsumura

and Sato[8]. Subsequent studies involving purification, crystalization and kinetic characterization, demonstrated that the enzyme from this genus converted D-xylose as well as D-glucose to their respective ketoses[9]. This enzyme was then identified as D-xylose keto isomerase (EC 5.3.1.5).

Takasaki[10] succeeded in isolating a strains of *Streptomyces* sp. with the best yield of glucose isomerase as cultured in medium containing xylose. This strain was identified later to be *Streptomyces cinereus* series. He also successfully isolated a strain of *Streptomyces* sp. utilizing xylan (D-xylose polymer) instead of xylose and produced glucose isomerase economically by applying wheat bran or corn cob in the culture media[11]. Seu[12] reported that a strain of the genus *Actinomyces*, producing glucose isomerase, isolated from soil samples did not require xylose as an enzyme inducer.

In the course of studies on microbial glucose isomerase for the practical application of the enzyme to the industrial production of high fructose corn syrup, we have attempted to isolate strains of *Streptomyces* spp. with relatively high productivity of glucose isomerase from soil samples of various part of the country. These strains were registered in KFCC (Korean Federation of Culture Collection); *Streptomyces* sp. K-14 (KFCC 35051) *Streptomyces* sp. K-53 (KFCC 35052), *Streptomyces* sp. K-71 (KFCC 35053), *Streptomyces* sp. K-77 (KFCC 35054), and *Streptomyces* sp. K-733 (KFCC 35056). Among these strains we were able to obtain *Streptomyces* sp. K-14 as the best strain for the production of glucose isomerase in the culture media containing xylose or xylan. In the first report, authers should like to discuss isolation procedures and microbial characteristics of these strains producing glucose isomerase.

Materials and Methods

Soil Samples About 150 soil samples were used for the isolation of the microbes. The soil samples were collected from various parts of the country, particularly from the wet spots of forests, farm yards, and rice fields of the North and South Kyongsang Province and the Kyonggee Province, and from the residential and suburban swamp area of Seoul City, between March and May, 1974.

Isolation of Microbial Strains

One gram of collected soil samples were suspended in 100m*l* of physiological saline solution and 1m*l* of supernatant of the soil suspension was used for the initial isolation of microbial strains. The supernatant was applied on agar plates containing isolation media (Table 1 and 2) sterilized at 121°C for 20 min and incubated at 30 °C for 5~6 days spotting colonies which appear to be *Streptomyces*

Table 1. Medium Used for Microbial Isolation (1)

Soluble Starch	10 g
NH_4Cl_2	0.5g
K_2HPO_4	0.5g
Agar	20 g
Distilled water	1,000 ml

pH 7.0±0.2

Table 2. Medium Used for Microbial Isolation (2)

Glucose	10 g
K_2HPO_4	1 g
$NaNO_3$	2 g
$MgSO_4 \cdot 7H_2O$	0.5g
KCl	0.5g
$FeSO_4$	0.5g
Agar	20 g
Distilled water	1,000 ml

pH 7.0±0.2

like strains. Microbial colonies which are non-motile aerial spores produced and not borne on verticillate sporosphores were selected and transferred for the pure culture collections.

Identification of Microbial Strains Identification procedures of the isolated microbial strains were carried out according to the methods of Shirling and Gottlieb[13] and Nonomura[14]. Morphological characteristics of aerial mycelium were observed with an optical microscope after either staining or slide culture according to the general procedure [15]. Morphology of Spores was observed with an

electron microscope (Hitachi Model HU-125 C, 1968) at 20,000-time enlargement after staining spore chains with 1 % uranyl acetate[16] on a carbon grid.

Selection of Active Strains Microbial strains that produce glucose isomerase were selected out of 280 strains of *Streptomyces* spp. to determine the enzyme activity in the mycelium. The mycelia were cultured in the presence of xylan as an enzyme inducer in shaking culture flasks containing 50 ml of basal medium composed of xylose or xylan as a sole carbon source (Table 3 and 4) at 30°C for 30~50 hours (125 strokes/min). The resulted mycelia were collected by centrifugation and used for the enzyme preparation.

Table 3. Medium Used for Enzyme Production(1)

Xylan or Xylose	10g
Polypepton	3g
K_2HPO_4	3g
$MgSO_4 \cdot 7H_2O$	1g
Distilled water	1,000ml

pH 7.0±0.2

Table 4. Medium Used for Enzyme Production(3)

Xylan	10g
Corn steep liquor*	20g
$MgSO_4 \cdot 7H_2O$	1g
$CoCl_2 \cdot 7H_2O$	0.1g
Distilled water	1,000ml

pH 7.0±0.2

*Spray dried corn steep liquor

Determination of Glucose Isomerase Activity Glucose isomerase activity was determined according to the method of Takasaki[10]. The enzyme reaction was initiated by adding 0.2 ml of enzyme preparation to 2 ml of reaction mixture containing final concentration of 0.05 M phosphate buffer (pH 7.2), 0.1 M D-glucose, and 0.05 M $MgSO_4 \cdot 7H_2O$. After incubating the reaction mixture at 70 °C for 1 hr, the enzyme reaction was stopped by adding 2 ml of 0.5 M perchloric acid and D-fructose produced was determined by the cystein-carbazol method[17]. Whole cell recovered from the culture medium was used as an enzyme source. Or else otherwise, the cell suspension was sonified with a sonicator (Blackstone Model BP 5) at a frequency of 20 KC for 10 min with 1 min interval followed by centrifugation at 10,000 rpm for 10min. The resulting supernatant was used for the determination of enzyme activity. It was noted that most of glucose isomerase, an intracellular enzyme, was released in the supernatant. In this study, one unit of the glucose isomerase activity is defined as the amount of enzyme that produces one mg of D-fructose under the assay conditions shown above.

Materials Xylose and xylan were purchased from Sigma Chemical Company. Corn steep liquor was a gift from Cheoneel Goksan Co. and dried in a spraydrier at KIST. Other chemicals used were of reagent grade.

Results and Discussion

Isolation of Strains Producing Glucose Isomerase In this experiment, we were able to obtain 37 strains which demonstrated glucose isomerase activity out of total 280 strains isolated from 150 soil samples. Among these 37 strains, most of them showed low enzyme activity (lower than 5 units/ml of culture broth) and only 5 strains produced the enzyme more than 15 units/ml of broth (Table 5). These 5 strains were designated to be K-14, K-53, K-61, K-77 and K-733 and subjected to further characterization of the microorganisms.

Table 5. Glucose Isomerase Activity of 37 Microbial Strains Isolated

Enzyme Activity (units/ml broth)	Number of Strains
0~5	27
6~10	4
11~15	1
16~20	1
21~25	3
26~30	1

Glucose isomerase from these strains was induced by xylose in the culture medium. Among these

Table 6. Morphological and Cultural Characteristics of the Glucose Isomerase Producing Strains

	Strains of *Streptomyces* spp.				
	K-14	K-53	K-71	K-77	K-733
Morphological Characteristics					
Colony form Mycelium (Spore-chain)	Velvety RF*	Velvety RF	Velvety RF	Velvety RF	Velvety RF
Spore surface	smooth	smooth	smooth	spiny	spiny
Size (μ)	0.6-0.8x 1.0-1.5	0.6-0.8x 0.9-1.3	0.7-1.1x 1.4-1.6	0.7-0.9x 1.0-1.2	1.0-1.2x 1.4-1.5
Aerial mass	Gray	Redgray	Whitegray	Whitegray	Whitegray
Melanoid pigment	+	+	+	−	+
Reverse color Other soluble	Brown	Red	Yellow	Violet	Gray
color to pH change	−	+	+	−	+
Flagella	−	−	−	−	−
Gram staining	+	+	+	+	+
Motility	−	−	−	−	−
Cultural Characteristics					
Oatmeal agar	Gray	Redgray	Grayyellow	Whitegray	Gray
Glycerol asparagin agar	Gray	Whitegray	Gray	Gray	Gray
Tyrosin agar	Whitegray	Whitegray	Whitegray	Violet	Gray
Emerson agar	Gray	Reddish gray	Gray	Gray	Gray
Czapek broth	Pellicle, White	Pellicle, Yellow	Pellicle, Gray	Pellicle, Yellow	Pellicle, White

*RF: Rectus Flexiblilis

strains only the K-14 strain demonstrated the highest yield of glucose isomerase. more than 25 units /ml of broth. The rest of 4 strains, K-53, K-71, K-77, and K-733 were in the range of 10~25 units/ml.

Characterization of Microbial Strains In order to indentify the 5 isolated strains that produced glucose isomerase *Streptomyces* spp. various microbiological characteristics of these strains were examined. Table 6 summarizes the morphological and cultural characteristics of these 5 selected strains. Fig. 1 demonstrates electron microscopic pictures of spore chains of these strains. It was noted that most of the strains formed white-gray and velvety colonies on agar plates, except K-53 strain which showed light reddish colonies. Under the light microscope, it was observed that morphology of the aerial mycelium of all 5 strains was rectiflexible and showed no spiral structure. Shapes of spores were round or elipsoidal with either smooth surface (K-14, K-53, and K-71) or spiny surface (K-77 and K-733). The color of reverse side of colonies appeard differently among strains examined (Table 6). All of these strains were grown excellently in liquid culture and formed whitish-gray pellicles.

The physiological and biochemical characteristics of the strains selected above are summarized in Table 7 and 8. Growth of the strains were aerobic and mesophilic. Optimum temperature for the growth of the strains were 30 °C, but no growth was observed at 50 °C. Comparing the results discussed above, it is concluded that these strains are different species of the genus *Streptomyces*, which can be identified best by color formation of colony, morphology of aerial mycelium and conidia forming linear spore chains at the end of the mycelial structure[18].

Among these strains, K-71 can be tentatively identified as *Streptomyces antibioticus* according to

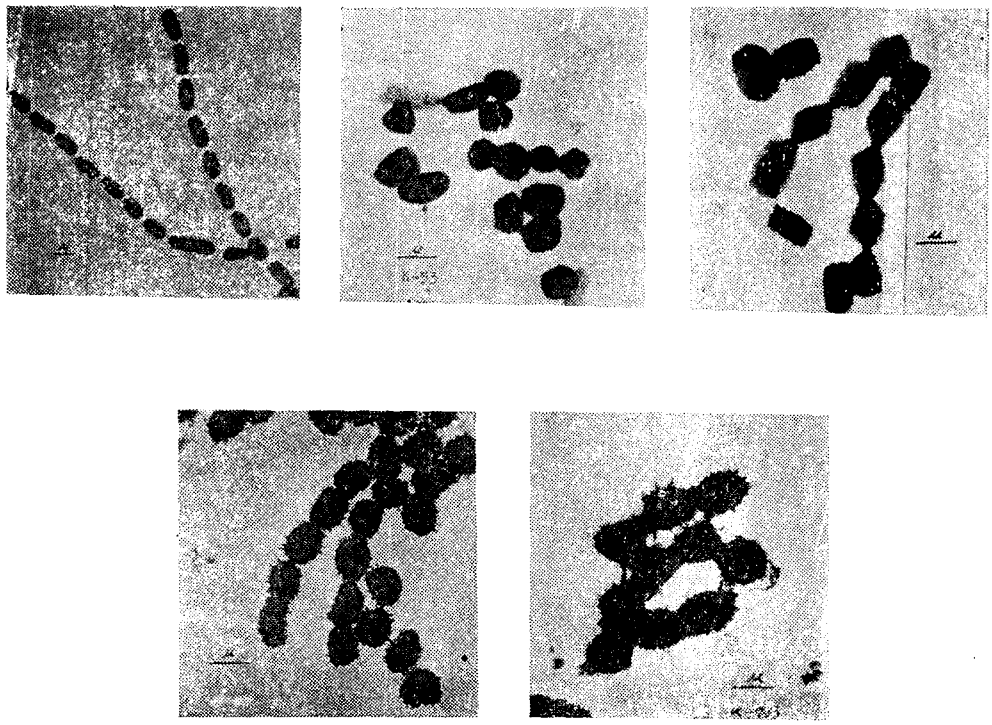

Fig 1. Eletron Photomicrographs of Spore Chains of *Streptomyces* spp. K-14, K-53, K-71, K-77 and K-733.

Table 7. Physiological and Biochemical Characteristics of the Glucose Isomerase Producing Strains

	Strains of *Streptomyces*				
	K-14	K-53	K-71	K-77	K-733
Starch hydrolysis	+	+	+	+	+
Catalase Production	+	+	+	+	+
Gelatine liquefaction	+	+	−	+	+
Protease production	+	−	−	+	−
Citrate utilization	+	−	−	−	−
Oxygen relation	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Optimum temperature	30 °C	30 °C	30 °C	30 °C	30 °C
Range of pH	5.0-9.0	5.0-9.0	5.0-9.0	5.0-9.0	5.0-9.0
Optimum pH	7.0	7.0	7.0	7.0	7.0

the description in the Bergey's Manual[19]. Other strains, however, were not attempted to specify the species, which appeared not having been described as known *Streptomyces* spp.

There are a number of *Streptomyces* spp. that produce glucose isomerase[20], such as *St. flavorirens*, *St. achromogenes*, *St. echinatus*, *St. ealbus*, *St. phaechromogenus*, *St. olivochromogenus*, and *Streptomyces* sp. ATCC 21175 [18]. Interestingly those reported *Streptomyces* spp. appear differently from the newly isolated *Streptomyces* sp. K-14 (KFCC 35051) in our laboratory (Table 9). Thus

Table 8. Utilization of Carbon Sources by the Glucose Isomerase Producing Strains

	Strains of *Streptomyces* spp. K-14	K-53	K-71	K-77	K-733
No carbon	−	−	−	−	−
Glucose	+	+	+	+	+
Fructose	+	+	+	+	+
Soluble Starch	+	+	+	+	++
Xylose	+	+	+	+	+−
Maltose	+	++	++	++	+−
Rhamnose	+	+	+	+	+
Raffinose	+	−	−	−	−
Lactose	+	+	+	+	+
Mannitol	++	+	+	+	++
Sucrose	++	−	−	−	−
Salicin	±	−	−	±	+
Inositol	+	+	+	+	+

we conclude that *Streptomyces* sp. K-14 is a new strain with good productivity of glucose isomerase.

요 약

Xylose를 효소 유도물질로한 배지에서 glucose isomerase를 생산하는 균주를 분리하기 위하여 전국각지의 토양시료 150점에서 약 280주의 *Streptomyces* 속 균주를 분리하여 그 중에서 효소 활성능이 높은 5주를 분리하였다. 이들 균주의 성질을 조사한 결과, 서로 다른 균종임을 확인하고 분류가 완성될때까지 *Streptomyces*(*st*) spp. K-14, K-53, K-71, K-77 및 K-733 이라 부르기로 하였다 특히 K-77과 K-733 균주는 포자 표면이 침상형임으로 다른 균종과 완전히 구별되었다. 이들 균주 중에서 K-14 균주는 효소 활성능이 가장 양호하였다.

Table 9. Summary of Characteristics of *Streptomyces* spp. Producing Glucose Isomerase

Species name	Aerial mass color (a)	Melanoid pigment (b)	Reverse side pigment (c)	Soluble pigment (b)	Spore chain (d)	Spore surface (e)	Arabinose	Xylose	Inositol	Mannitol	Fructose	Rhamnose	Sucrose	Raffinose	References
St. cinerues series	Gy	1	1	v	RF	sm	+	+	+	+	+	+	−	−	(10)
St. flavovirens	Gy	0	1	0	RF	sm	+	+	−	+	−	+	−	−	(20)
St. achromogenes	Gy	1	0	0	RF	sm	+	−	±	+	+	±	−	−	(20)
St. echinatus	Gy	1	1	0	SRA	sp	+	+	+	+	+	+	−	+	(20)
St. albus	W(Y)	0	0	0	S	sm	±	+	−	+	+	−	−	±	(20)
St. Phaeochromogenus	R	1	0	0	RF	sm	+	+	+	+	+	+	+	+	(20)
St. olivochromogenus	X	1	0	v	S	sm	+	+	+	+	+	+	+	−	(20)
St. sp. (ATCC 21175)	Br	0	v	0	S	sp	+	+	+	+	+	+	−	−	(18)
St. sp. (KFCC 35051)	Gy	1	1	0	RF	sm	+	+	+	+	+	+	+	+	Present study

a) GY: Gray, W(Y); White(Yellow); R; Red, Br; Brown, X; not determined
b) 1: produced, O; not produced, V; variable
c) 1: distinctive, O: not distinctive or none
d) RF: Rectiflexibiles, RA: Retinaculiaperti, S: Spirales,
e) sm: smooth, sp; spiny

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