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Production Conditions and Properties of Glucose Isomerase from *Streptomyces griseolus*

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Streptomyces griseolus 기원의 포도당 이성화효소의 생성 조건과 성질

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ABSTRACT

Cultural characteristics of *Streptomyces griseolus* isolated from the soil were investigated. This stain was disclosed to utilize D-xylose, D-glucose and D-galactose in preference order as a carbon source with the formation of glucose isomerase.

The addition of sweet potato starch also proved effective promoting the total enzyme activity measured at 29% higher than the control.

Corn cob, one of waste agricultural resources, was hydrolyzed in 2~3% H_2SO_4 solution at 100°C, 3~5 hours to produce a xylose syrup which gave rise to the recovery of 19.9% in a batch system and 28.2% in a repeated system. By the addition of both 2% of xylose syrup(Be' 28) prepared by and us 65% of corn steep liquor (total nitrogen 1.2%), enzyme induction was maximized. The enzyme activity was stimulated by the xylose and the cell growth by the C. S.L.

Also, remarkable increase of enzyme activity was noticed by the addition of protein acid hydrolysate 86.2% higher than the control.

QO_2 of the biomass cultured in 30L capacity jarfermentor recorded low oxygen requirement of 251.2 l/hr.

Maximum activity of glucose isomerase was observed noted at the 9th hour after inoculation which is 2 hours faster than the stationery phase of the biomass growth.

Glucose isomerase from the strain was activated by adding the $Co^{++}$and Mg^{++} with optimum temperature of 73°C and pH of 7.2.

Conversion ratio of 60% glucose to fructose was 42.5% after 70 hours reaction.

INTRODUCTION

Many reports have been produced of the results of experiment conducted on culturing conditions of *Streptomyces* species(Tsumura *et al.*, 1965; Takasaki, 1966; Chung *et al.*, 1976; Han *et al.*, 1978), and the characteristics of

the glucose isomerase therefrom (Takasaki, 1967; Takasaki, 1971; Schray *et al.*, 1972; Han *et al.*, 1978).

According to them, these species require D-xylose and/or xylan as the carbon source in growing and inducing the glucose isomerase activity. Also it was reported that those enzyme require divalent heavy metals such as Co^{++} and Mg^{++} etc. In this experiment, we tried to optimize the culturing conditions of the *Streptomyces griseolus* which had been isolated from the soil by the authors and to check the characteristics of glucose isomerase therefrom.

MATERIALS AND METHODS

1. Strain:

Streptomyces sp. N-3 isolated from the soil and thereafter identified as *Streptomyces griseolus* was used for this experiment.

2. Seeding line and media composition for flask scale culture:

a) Seeding line:

Stocked slant cultures were cultivated on potato dextrose agar media(30°C, 5~7 days) and activated for inoculation to the flask seed media (100m*l* in 500m*l* flask) followed by reciprocal shaking(140 strokes/minute, 30°C, 1 day) and were eventually transferred asceptically into the main culturing flask media(80m*l* in 500m*l* flask) to incubate for 2 days under the same conditions.

b) Seeding medium:

°D-xylose 1.0g, polypeptone 1.0g, Meat extract 0.5g, yeast extract 0.25g, NaCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.05g, $CoCl_2 \cdot 6H_2O$ 0.001μg and KH_2PO_4 0.01g were mixed in one liter of distilled water, sterilized and pH controlled to 7.0.

c) Main flask culture medium:

Sweet potato starch 0.75g, D-xylose 1.0g, NaCl 0.3g, $MgSO_4 \cdot 7H_2O$ 0.05g, $CoCl_2 \cdot 6H_2O$ 0.024μg, KH_2PO_4 0.05g and CSL(total nitrogen 1.2%, Be' 4~7) 40g were mixed in one liter of distilled water, sterilized and pH controlled to 7.0.

3. Culture in jar fermenter:

Two different size of jar fermenters were applied for checking the culturing time course and QO_2.

In 30L jar fermenter(Oyodenshi 30L, Japan) 15L medium was mixed and sterilized at 120 °C for 15minites, cooled down and was inoculated with 450m*l* seed culture. Culturing conditions were temperature 31±1°C, agitation speed 350rpm, internal air pressure 0.3kg/m^2, aerated 0.5v.v.m.(750m*l*/min) while controlling pH to 7.0 automatically by NH_3 injection.

In 7k*l* jar fermenter (Miwon Co.) 3k*l* of medium was prepared and sterilized under same above conditions for further scale-up test on culturing time course. All other culturing conditions were same as above except different agitation speed of 120r.p.m. Seeding line and midia compositions are shown in table 1.

On finishing the cultivation, the broth was steam-heated at 70°C for 60 minites after adding Na_2CO_3 at concentration level of 0.3% to the broth for the purpose of heat stabilization of the glucose isomerase onto the cell matrix as well as inactivation of other enzyme systems existing in the biomass together.

4. Cell growth:

15m*l* of culture broth was taken into the test tube, centrifuged for 15 minites at 3.000 r.p.m. and its precipitation level measured directly. Dry cell weight was calculated by checking the moisture content using a Kett moisturemeter.

5. Glucose isomerase activity:

This assay was done as reported previously. (Lim *et al.*, 1982)

6. Respiratory quotient of biomass:

2m*l* of culture broth was sampled at 3, 4, 6, 8, 12, 14 hours after inoculation and was cen-

Table 1. Seeding line and media compositions for pilot plant scale culture(7k*l*)

Ingradients	Stock slant	Active slant	Flask seed (No. 1)	Flask seed (No. 2)	Jar seed (No. 1)	Jar seed (No. 2)	Main culture
Potato, sliced	30.0	30.0					
Glucose	2.0	2.0		0.50	0.50	0.50	
Xylose syrup(28Bé)			2.0	3.0	3.0		3.0
Sweet potato starch							0.5
Corn steep liquor(25Bé)				4.0	4.0	2.0	5.0
Peptone			1.0				
Yeast extract			0.25				
Meat extract			0.50				
Biomass acid hydrolysate					0.10	0.10	0.10
$MgSO_4 \cdot 7H_2O$			0.05		0.05	0.05	0.05
$CoCl_2 \cdot 6H_2O$			0.024			0.024	0.024
NaCl			0.50	0.30		0.30	0.30
KH_2PO_4				0.05	0.05	0.05	0.05
Silicone KM75					25m*l*	100m*l*	500m*l*
Agar	3.0	3.0					
pH adjusted	7.0	7.0	7.0	6.7	6.7	6.7	6.8
Container size	7'tube	7'tube	500m*l*	500m*l*	50*l*	500*l*	7k*l*
Media inleted	—	—	80m*l*	80m*l*	25*l*	250*l*	3k*l*
Culture time(hrs.)	7day	7day	24	18	15	13	24

trifuged(Kubota 220G) at 8,000 r.p.m. for 5 minutes.

Precipitation was suspended with 1.5m*l* of broth and 1m*l* was taken into the Warburg vessel and mixed with 0.8m*l* of the supernatant. Manometer level was checked directly at 4 minite intervals for measurement of the gas volume formed and respiratory quotient of biomass was calculated as follows:

$$QO_2 = \text{vessel quotient} \times \text{gas level} \times \frac{1.5}{3.5} \times 15 \times DCW^{-1} \times 10^{-3} \ (\mu l O_2/\text{hr.mg})$$

7. Preparation of xylose syrup:

For batch style hydrolysis, 100 gram of the raw material was mixed with different concentrations of acid (1,600m*l*) in 3 liter volumetric flask and autoclaved at 100~120°C for 2~8 hours. For repeated style hydrolysis, the waste cake from the above batch hydro lysis was mixed with acid for second hydrolysis and the waste cake therefrom was mixed once more with fresh acid solution for third hydrolysis.

The pH of the finished hydrolysate was adjusted to 3.3 with $CaCO_3$, filtered and passed through the ion exchange resin column consisting of SKIB(resin volume 300m*l*) and IRA 93(resin volume 600m*l*) at space velocity of 3 followed by vaccum evaporation at 65°C, 600mm Hg.

8. Preparation of protein acid hydrolysate:

220 gram of protein sources were mixed with 7% H_2SO_4 in a 3 liter round flask so as to hydrolyse at 120°C for 6 hours, cooled down and neutralized by NH_3 adjustment to 4.5 and filtered.

RESULT AND DISCUSSION

1. Effect of carbon source

As shown in table 2, glucose isomerase production was remarkably induced by the D-xylose addition(952 GIU/g-dcw) and followed by D-fructose, D-glucose and D-galactose in decreasing order. The addition of sweet potato-starch at the optimum concentration of 0.5% in the D-xylose media proved to be effective in stimulating the cell growth with an increase in the total enzyme activity.

Enzyme activity was measurably induced by the joint addition of 2% xylose syrup (Be' 28) and 65% corn steep liquor(total nitrogen 1.2 %) where it seems the former is effective for inducing the enzyme activity and the latter is

Table 2. Effect of carbon sources on the growth of *Streptomyces griseolus*

Carbon source (1%, dry base)	Enz. activity (GIU/g-DCW)	Relative activity(%)	Cell volume* (10m*l*)
none	140	1.47	0.38
D-xylose	952	100	0.87
D-glucose	712	74.8	1.01
D-sucrose	168	1.76	0.38
D-fructose	828	87.0	0.91
L-arabinose	332	34.7	0.57
D-galactose	68	70.2	0.74
Cellulose	368	3.87	0.34
Sweet potato starch	342	34.6	1.18

1. Basal medium(%): NaCl 0.3, $MgSO_4 \cdot 7H_2O$ 0.05, $CoCl_2 \cdot 6H_2O$ 0.024, corn steep liquor (Bé4~7, TN 1.2 %) 40. 2. Seed culture : cell volume 1.5, pH 5.2, 95 CP.
*cell volume precipetated 10m*l* test tube after centrifugation at 3000 rpm for 15 minutes.

Table 3. Effect of xylose syrup in combination with corn steep liquor on *Streptomyces griseolus*.

xylose syrup(%)		Corn steep substances(%)			final	DCW	Enz. activity	Total activity
Xylup+	Nagase++	Miwon*	Nagase**	Sanwa***	pH	(g/*l*)	(GIU/g-DCW)	(GIU×10^{-4}/1)
2	0	35	0	0	7.1	12.5	1356	1.695
2	0	65	0	0	6.6	15.0	1560	2.340
2	0	0	4	0	6.4	14.8	1450	2.146
2	0	0	0	2	6.1	8.0	948	0.758
0	2	35	0	0	7.3	9.5	1540	1.463
0	2	65	0	0	6.8	15.0	1622	2.433
0	2	0	4	4	6.9	10.3	1532	1.578
0	2	0	0	2	7.1	8.6	1370	1.178
2	0	0	0	0	6.2	7.5	1440	1.080
0	0	35	0	0	6.4	9.5	1200	1.140
0	0	65	0	0	6.9	11.5	971	1.117

\+ : Concentrated xylose syrup made by the author (Bé28).
++ : Xylose syrup made by Nagase Industrial Co.(Bé 32).
* : CSL made by Miwon Co. (Bé 3.0, total N 1.2%, pH4.1).
** : CSL made by Nagase Industrial Co. (Bé 27, pH4.5).
*** : corn steep powder made by Sanwa Starch Co. (Japan)

Table 4. Effect of solid vs. liquid ratio on xylose syrup produced by "batch hydrolysis"

Volume of 3% H_2SO_4 solution	Vol. of acid soln. recovered after hydrolyis	Total pentose (%)	Yield (%)
800m*l*	540m*l*	2.55	13.8
1000m*l*	870m*l*	2.03	17.7
1200m*l*	1050m*l*	1.66	18.3
1400m*l*	1250m*l*	1.48	18.5
1600m*l*	1440m*l*	1.38	1.99

Hydrolyzed at 100°C for 2 hours.

effective for growth of biomass (table 3).

When the corn cob, which had been identified as the effective raw material for xylose source, was hydrolyzed with 2~3% H_2SO_4 at 100°C for 3~5 hours xylose showed a 42.9% higher recovery by the repeated system than the batch system while the respective recovery figures for repeated and batch were 28.2% and 19.9% (table 4, 5),

2. Effect of nitrogen source

Four kinds of corn steep liquor had been tested as shown in table 6, which shows increasing trend of cell growth by the corn steep liquor whereas decreasing trend of the enzyme activity per unit cell weight. Maximum total enzyme activity was recorded by adding 40~50% of CSL(Be' 3.0, total nitrogen 0.41%) of which result is similar to that of Lee *et al.*, (1977) who had reported maximum enzyme induction at 36% CSL level(total nitrogen 0.5%) and that of Takasaki(1967) and Chung *et al.*(1976).

In addition to the corn steep liquor, several kinds of protein acid hydrolysate were tested to find out the most effective in promoting the enzyme activity especially under the presence of CSL by additions of the biomass hydrolysate(table 7). It was supposed that high nitrogen content seems a major activity induction factor.

3. Effect of defoaming agent

To defoam the sweet potato starch media several antifoaming agents were tested; the most effective were NOPCO (USA), sillicone and adecanol in order.

4. Respiration quotient of biomass

Maximum QO_2 of 251.1 1/hr/mg-d.c.w. was recorded at the 4th hour after inoculation cultured in 30 liter jar fermenter and thereafter decreased rapidly down to 50% at 14th hour

Table 5. Xylose formed from corn cobby "repeated hydrolysis."

Flask no.	First filtrate		Second filtrate		Third filtrate	
	pentose(%)	pentose(g)	pentose(%)	pentose(g)	pentose(%)	pentose(g)
1	0.31	4.9	1.25	19.0	3.2	37.1
2	0.21	3.0	0.86	11.5	4.1	46.1
3	0.27	3.8	0.92	12.4	4.0	42.1
4	0.56	5.4	1.36	13.8	4.8	39.0
5	0.25	3.6	0.70	8.8	3.7	38.0
6	0.22	3.3	0.97	13.0	3.8	45.1
7	0.43	6.3	1.23	15.3	4.8	49.2
X	0.32	4.3	1.04	13.4	4.1	42.3

Solid vs. liquid 100 : 1500 Hydrolyzed with 3% H_2SO_4 at 100°C for 3 hours

Table 6. Effect of corn steep substances on *Streptomyces griseolus*

Corn steep substance (%)		Final pH	Viscosity(CP)	DCW(g/l)	Enz. activity (GIU/g-DCW)	Total activity (GIU×10⁻⁴/l)
Miwon(L)*1	5	6.7	210	4.63	981	0.454
	10	6.5	229	6.13	720	0.441
	15	6.8	285	9.88	2040	2.016
	20	7.1	250	10.00	1881	1.881
	30	6.9	230	15.21	1281	1.948
	40	6.5	222	14.91	1956	2.916
	50	7.0	223	15.90	1642	2.611
	60	6.2	273	13.74	1245	1.711
Miwon(H)*2	2	7.0	230	10.33	1926	1.990
	3	6.5	222	11.82	1890	2.234
	4	7.2	223	7.00	1845	1.292
	5	6.7	273	7.25	1807	1.310
Nagase(H)*3	2	7.5	225	9.38	1404	1.351
Sanwa(P)*4	2	6.8	285	14.00	1260	1.764

*1 : Made by Miwon Co., low Bé 3.0 (total nitrogen 0.41%)
*2 : Made by Miwon Co., high Bé 28 (total nitrogen 3.19%)
*3 : Made by Nagase Industrial Co., Bé 32 (total nitrogen 3.94%)
*4 : Made by Sanwa Starch Co., (moisture8.0%)

Table 7. Effect of protein acid hydrolysate on *Streptomyces griseolus* cultured in media with and without corn steep liquor

PHA(5%)	Final pH		DCW		Enz.activity(GIU/g-DCW)		Total activity(GIU ×10⁻⁶/1)	
	with	without	with	without	with	without	with	without
BM*	7.2	—	12.1	—	1715		2.075	—
BM+CSL(H)4%	—	7.3	—	14.7	—	1610	—	2.367
BM+SAH	7.0	6.7	14.7	12.5	2222	2275	3.226	2.844
BM+AAH	7.0	5.7	13.2	123	238	2530	3.139	3.112
BM+BAH	7.2	6.2	12.4	12.5	3115	2907	3.863	3.634
BM+GAH	7.4	5.5	13.0	9.7	2400	2603	3.120	2.525
BM+SAH	6.9	4.9	10.8	7.6	1788	1520	1.931	1.115

*BM(basal media) : xylose syrup 2.5, NaCl 0.3, sweet potato starch 0.5, $KH_2PO_4 \cdot 7H_2O$ 0.05, $CoCl_2 \cdot 2H_2O$ 0.024%.
PAH : Protein acid hydrolysate
SAH : Soybran acid hydrolysate
AAH : Amino acid hydrolysate
BAH : biomass acid hydrolysate
GAH : gluten(corn) acid hydrolysate
SAH : Silkworm acid hydrolysate

Total oxygen demand was 0.002v.v.m. at 8th hour culture (table 8), which is a very low aerobic state.

5. Time course in 7kl jar fermenter

As shown in fig. 1, the logarithmic phase was quickened to 8~16 hours after seed inoculation from 20~30 hours in flask culture. glucose isomerase activity has been peaked at 9th hour, 2 hour earlier than the stationary phase of cell growth. This phenomena mean the quite opposite to both reports of Chung *et al.* (1976) who had expressed maximum activity level recorded at 15~20th hour later than the peak point of cell growth by the former

Table 8. Oxygen demand of *Streptomyces griseolus*

Time (hrs.)	DCW (g/l)	QO_2 (μl/hr/mg)	TotalQO_2 (1/hr)	Air volume required(1/hr)
3	5.54	212.6	1.178	5.64
4	5.76	251.1	1.446	6.92
6	7.57	210.9	1.597	7.64
8	10.12	159.1	1.711	8.19
10	12.26	134.9	1.654	7.91
12	13.24	116.2	1.538	7.36
14	13.39	111.6	1.486	7.11

Culture conditions in 301 jar fermenter: media volume 151, 420 rpm, 0.5kg/cm, 0.5v.v.m.

and simultaneous maximizing the levels in both activity and cell growth by the latter.

pH profiles showed decrease down to 5.4 at 15th hour and turned rapidly upward thereafter.

6. Major characteristic properties of glucose isomerase from *Streptomyces griseolus* were investigated.

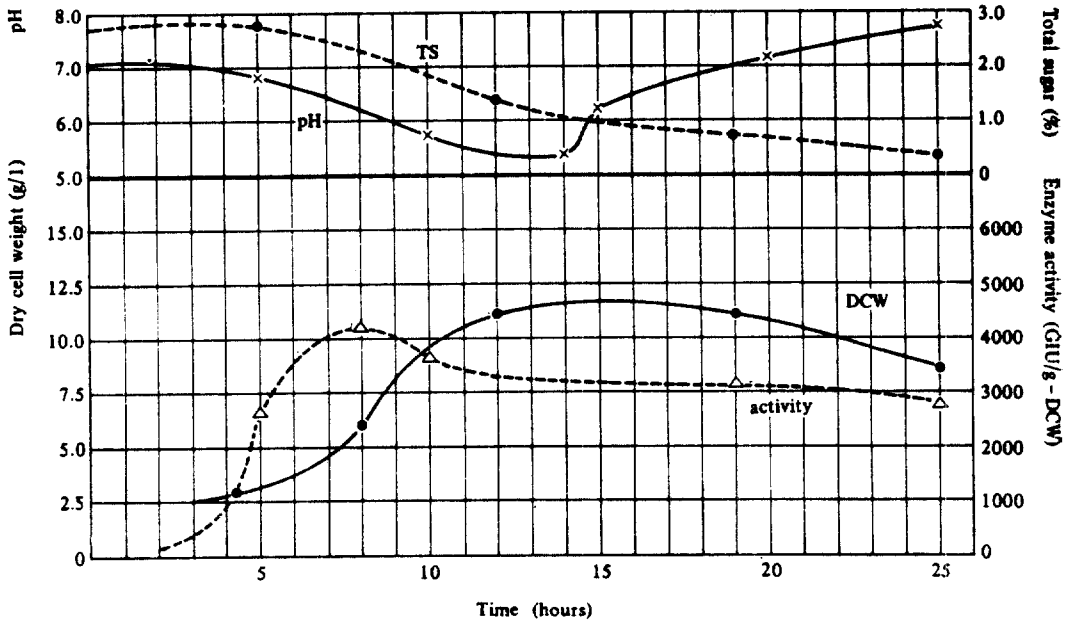
Considering this enzyme requires divalent metals, four typical kinds were examined. Co^{++} and Mg^{++} ions proved to be effective while partly inactivated by Mn^{++} and Al^{++}. Optimum concentration of Co^{++} and Mg^{++} was 0.006% and 0.24% respectively when added together (table 9).

These trends are similar to the results of Han *et al.* (1978) and of Kasumi *et al.* (1982) but opposite to Chou et al. (1976) who had reported Co^{++} exhibition of strong inhibition to the enzyme induction especially during the early stage of mycelium propagation.

With reference to fig. 2 the pH profile showed optimum activation of enzyme at pH 7.2 and unstable in Co^{++} excluded solution while the optimum temperature was established as 70~72°C.

Experimental result on effect of D-glucose concentration as a substrate showed 42.5% isomerization degree at 70 hour when 60% concentration was added and major conversion was carried out before 45 hour reaction(fig. 3).

Fig. 1. Time course of Streptomyces griseolus cultured in 7kl jar fermentor



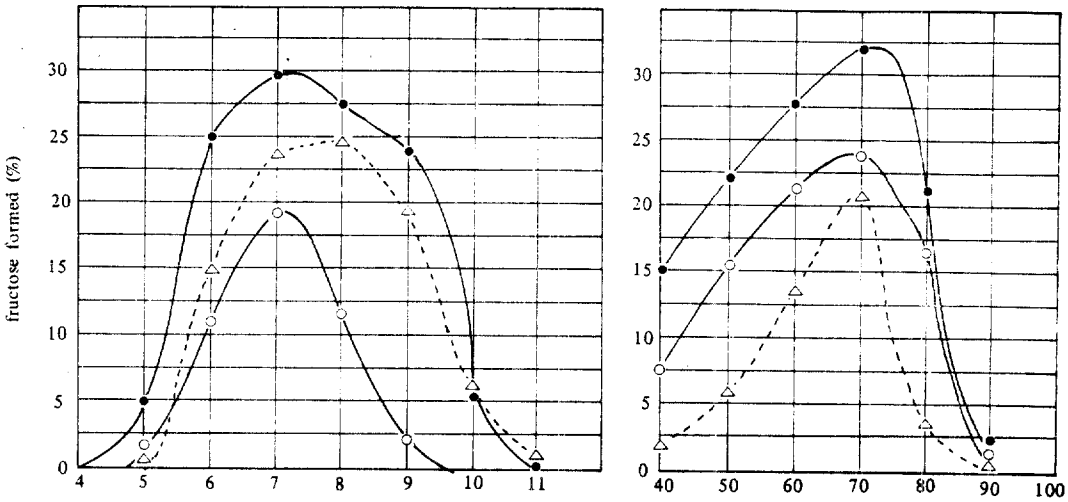


Fig. 2. Effect of pH and temperature on glucose isomerase activity

Table 9. Effect of Co^{++} and Mg^{++} on glucose isomerase activity (unit : %).

Concentration		Fructose produced			Relative
$CoCl_2 \cdot 6H_2O$	$MgSO_4 \cdot 7H_2O$	18hrs.	42hrs.	96hrs.	activity (66hrs.)
0	0	8.2	14.9	18.7	10
0	0.024	8.8	12.5	19.9	16
0	0.060	10.5	13.7	21.0	112
0	0.120	11.1	15.7	22.6	121
0	0.240	8.2	12.0	20.8	111
0.0024	0	11.8	17.2	21.4	114
0.0024		9.5	14.9	19.9	106
0002.4	0.060	12.2	17.6	21.8	117
0.0024	0.120	10.5	16.8	21.4	114
0.0024	0.240	11.8	16.8	20.3	109
0.006	0	8.8	17.9	22.9	122
0.006	0.024	9.3	19.3	24.7	132
0.006	0.060	12.5	20.7	24.7	132
0.006	0.120	10.7	20.7	24.3	130
0.006	0.240	12.3	21.1	26.4	141
0.012	0	9.3	17.6	23.3	125
0.012	0.024	10.7	20.4	23.6	126
0.012	0.060	10.9	19.7	24.7	132
0.012	0.120	11.9	21.8	26.1	140
0.012	0.240	10.2	20.4	24.3	130
0.024	0	9.7	11.6	24.7	132
0.024	0.027	10.5	19.3	23.3	125
0.024	0.060	13.7	20.7	23.6	126
0.024	0.120	11.9	20.7	24.7	132
0.024	0.240	11.2	20.4	23.6	126

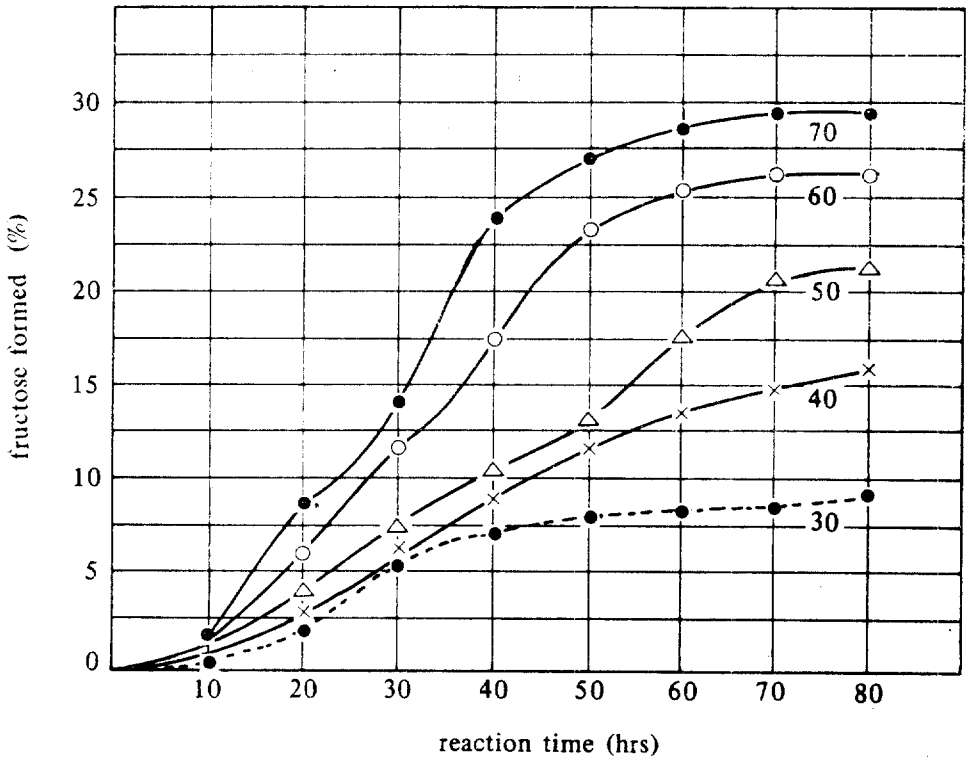


Fig. 3. Effect of glucose concentration on glucose isomerase activity 100ml of reaction solution containing $MgSO_4 \cdot 5H_2O$(0.12%) and $CoCl_2 \cdot 6H_2O$(0.012%) was incubated at 67°C. Reaction solution includes 5% phosphate buffer (pH 7.2%) consisted of 1/15 M KH_2PO_4 (4 parts). 8 unit of enzyme was added per one gram of glucose.

적 요

토양에서 분리한 *Streptomyces griseolus*의 배양 특성에 관하여 검토하였다. 본 균은 포도당 이성화 효소를 생성함에 있어서 탄소원으로서 D-xylose, D-glucose 및 D-galactose를 순서대로 자화하였다.

고구마전분의 첨가에 의하여서도 효소의 총 역가는 무첨가시에 비하여 29%가 증가하였다.

농산폐자원의 일종인 옥수수 심(蕊)을 2~3% H_2SO_4 용액으로 100°C에서 3~5시간 가수 분해하였을 때에 xylose syrup의 대원료 생성수율이 회분식에서 19.9%, 반복식으로 28.2%를 나타내었다. xylose syrup(Be' 28) 2%와 corn steep liquor(총질소 1.2%) 65%의 첨가에 의하여 효소의 생성은 극대화되었다. 효소활성은 xylose에 의하여 증진되는 반면, 균의 성장은 corn steep liquor에 의하여 촉진되었다. 또한 효소 활성은 단백질산가수 분해물의 첨가에 의하여 86.2%로 현저히 증가하였다.

30*l* 발효조에서 배양시 균의 QO_2는 251.2*l*/hr.로 낮은 산소요구량을 나타내었고, 7k*l* 발효조를 이용한 배양실험결과 포도당이성화 효소의 최대 활성은 접종 후 9시간으로서 이는 균성장의 stationery phase보다 2시간이 빠른 것이다. 본 균이 분비한 포도당 이성화 효소는 Co^{++} 및 Mg^{++}에 의하여 활성화되었고, 최적온도는 73°C, 최적 pH는 7.2이었다.

60% 포도당 용액을 기질로 한 이성화실험에서 70시간 후에 포도당의 42.5%가 과당으로 전환되었다.

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