GLUCOSE ISOMERASE ACTIVITY FROM *Fusarium* sp., *Streptomyces* sp. S-21 and *Streptomyces phaeochromogenes* FERM-P221

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ABSTRACT

The fast development in High Fructose Syrup Industries had led every effort to search new sources of glucose isomerase enzyme. This experiment was done to accomplish such effort.

Fifteen strains of *Fusarium* sp. and one strain of *Streptomyces* sp. have been tested for their enzyme activity. Those activities were compared with the activity of *Streptomyces phaeochromogenes* FERM-P 221. Among the fifteen strains of *Fusarium* sp. tested, one strain of the highest enzyme activity was selected.

The relative activity of *Fusarium* sp. and *Streptomyces* sp. was 70% and 93%, respectively, compared to *Streptomyces phaeochromogenes* FERM-P 221. It was also investigated that the optimum temperature of glucose isomerase enzyme from *Fusarium* sp., *Streptomyces* sp. S-21 and *Streptomyces phaeochromogenes* FERM-P 221 were 60°C, 65°C and 70°C, respectively; while, the optimum pH were 7.5, 7.0 and 8.0, respectively.

The use of Mg^{2+} activator was much better than that of the Co^{2+} activator. The concentration of Mg^{2+} and Co^{2+} activator for the optimum glucose activity from *Fusarium* sp., *Streptomyces* sp. S-21, and *S. phaeochromogenes* FERM-P 221 were 0.025 M and 0.114 M, 0.018 and 0.1 M, 0.1 M and 0.1 M, respectively.

We claimed and reported for the first time that *Fusarium* sp. showed the glucose isomerase activity.

INTRODUCTION

The increasing of sugar demand in Indonesia had lead the Government to seek an alternative sources of sugar. Indonesia as a tropical country rich in starch resources such as cassava, rice, corn, sweet potato, sago, etc.

The starch could be transformed to sugar as glucose, fructose and maltose. Fructose has long been recognized as a good alternative source of sugar due to its relatively high sweetness and other desirable physical and chemical properties. Fructose have been reported 1.5—1.7 times sweeter than sucrose and is widely used in food processing and formulation in pharmaceutical.

Recent advances in enzyme engineering stimulated industrial application of enzymes for various food and pharmaceutical process. Among various application of enzyme in food processing, production of fructose from glucose is of practical important. The production of fructose from glucose required

glucose isomerase enzyme (intracellular enzyme). A large number of micro-organisms that produce glucose isomerase have been reported (Table 1).

The purpose of this study was to search the potential glucose isomerase producing organisms originated from Indonesia.

MATERIALS AND METHODS

Materials

Fifteen strain on *Fusarium* sp., one strain of *Streptomyces* sp. S-21 and a commercial strain *Streptomyces phaeochromogene* FERM-P 221 was obtained from Biotrop, Institut Teknologi Bandung and Fermentation Research Institute, Tsukuba Japan, respectively. (Table 2).

The culture medium for propagation of *Fusarium* sp. used 2.0 g malt extract,0.5 g $(NH_4)_2SO_4$, 0.2 g KH_2PO_4.7 H_2O, 0.1 g NaCl and 0.05 g $MgSO_4$ in 100 ml of distilled water. Throughout the experiment was used medium formulated by Tsumura *et al.* (Hayashi *et al.*, 1981) namely Precultivation medium for glucose isomerase production (PGI) and Glucose isomerase production medium (GI).

Enzyme extraction was done using cationic detergents N-Cetylpyridinum chloride monohydrate (CPC) ($C_{21}H_{38}ClN.H_2O$) and N-Cetyl-N, N, N-trimethyl-ammonium bromide (CTAB) or ($C_{19}H_{42}BrN$), Merck, Darmstadt.

Methods

Method for producing crude enzyme glucose isomerase shown in Fig. 1.

Fructose was assayed by the method of Dische and Borenfreund as modified by Ryu *et al.*, 1977. The assay procedure known as "cystein-carbazole" reaction shown in Fig. 2 with minor modification and a typical standard curve shown in Fig. 3.

Glucose isomerase activity was determined by reaction of 1 ml of crude glucose isomerase in a mixture of 0.8 M glucose, 0.05 M buffer pH 7.0, 0.01 M Mg^{2+} at 60°C for 1 hour. The reaction was stopped by addition of 4.5 ml hyperchloride solution. The amount of fructose converted from glucose was then assayed for the determination of glucose isomerase activity.

For the determination of dry cell weight (DCW) a desired amount of acetone-washed cell mass was dried at 105°C for 90 minutes in an oven, the dried cells were transferred to a desiccator, cooled to room temperature and weighed. Protein determination of crude enzyme was measured by the Folin-Lowry method as described by Plummer (1979). Bovine serum albumin was used as a standard at the concentration of 50, 1000, 150, 200 and 250 ug solution. A typical standard curve for protein determination by the method of Folin-Lowry shown in Fig. 4. The spectophotometetric reading at 750 nm.

Table 1. List of Glucose Isomerase Producing Organisms.

Microorganisms	Optimum		Metal ion req.	Substrate	Inducer	References
	pH	Temp. °C				
P. hydrophila	8.5	42 - 43	Mg,Mn,As	Glu,Xil	Xil	Ryu *et al.*, 1977
A. cloacae	7.6	50	Mn,Co,Mg,As	Glu,Xil	Xil	Tsumura & Sato, 1961b
B. megaterium	7.0	35	Mg,NAD	Glu	Glu	Ryu *et al.*, 1977
A. aerogenes	6.8	39	As	Glu,Xil,Man	Xil	Ryu *et al.*, 1977
E. intermedia	7.0	40 - 50	As	Glu,Glu-6-P	Glu	Natake & Yoshimura, 1964b
P. aerogenoides	7.0 - 7.5	40	Mg,NAD	Glu,Man	Glu	Takasaki & Tanabe, 1964
L. brevis	6-7	60	Mn,Co	Glu,Xil,Rib	Xil	Takasaki, 1979
Arthrobacter sp.	8.0	60 - 65	Mg	Glu,Xil	Glu	Takasaki, 1979
B. pentosoaminoacidicum	8.3	70	Co	Glu,Xil	Xil	Ryu *et al.*, 1977
B. coagulans	7.0	60 - 70	Mg,Mn,Co	Glu,Xil,Rib	Xil	Danno, 1970c
Streptomyces sp.	7.0	80	Mg	Glu,Xil	Xil	Takasaki, 1979
Streptomyces sp. YT-5	8.0	80	Mg	Glu,Xil	Xil	Takasaki *et al.*, 1969
YT-6	7.0	85 - 90	Mg	Glu,Xil,Rib	Xil	Takasaki, 1979
S. phaeochromogenes	9.0	80	Mg,Co	Glu,Xil,Ara	Xil	Tsumura & Sato, 1965b
S. albus	7.0	70	Mg,Co	Glu,Xil,Rib, Ara,Rham,All	Xil	Sanchez & Smiley, 1975
S. griseofuscus	8.0	80	Mg,Co	Glu,Xil,Ara	Xil	Kasumi *et al.*, 1980
S. flavogriseus	7.0	70	Mg,Co	Glu,Xil	Xilan	Chen & Anderson, 1979a

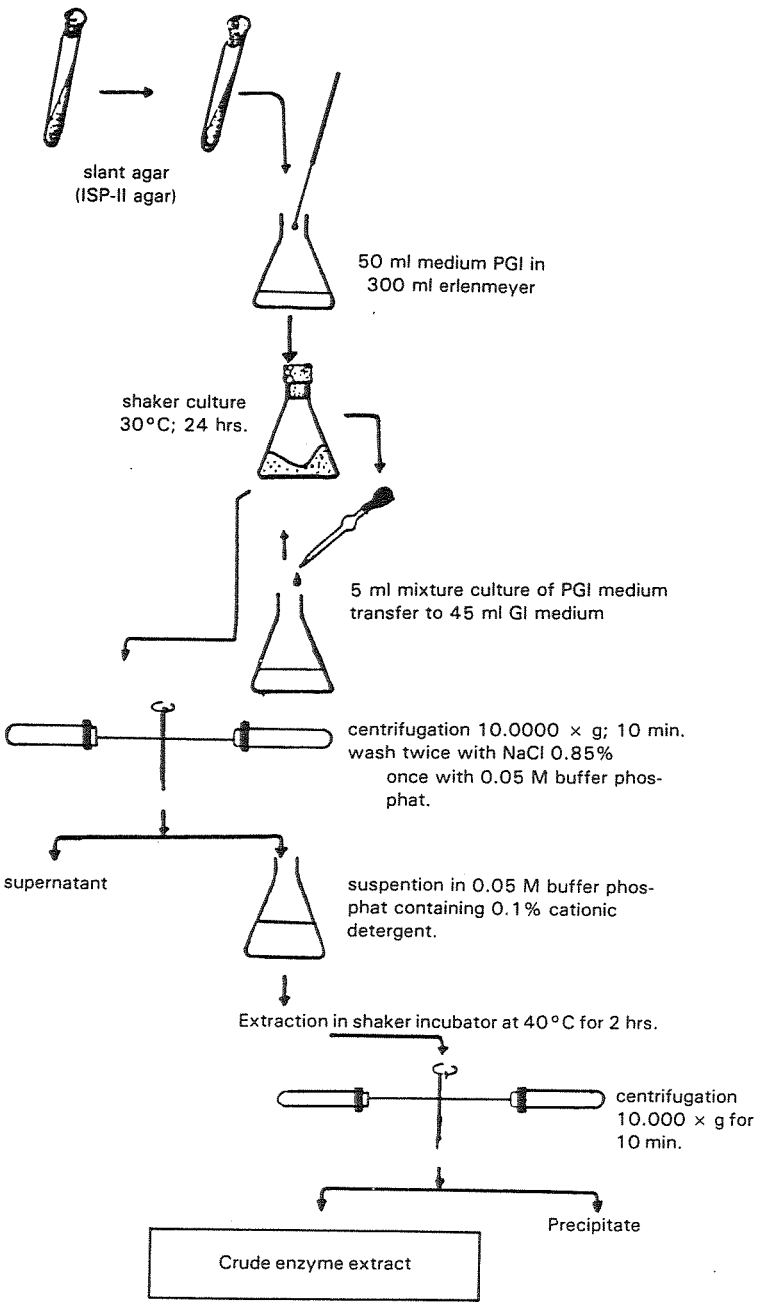

Fig. 1. Production of crude enzyme glucose isomerase.

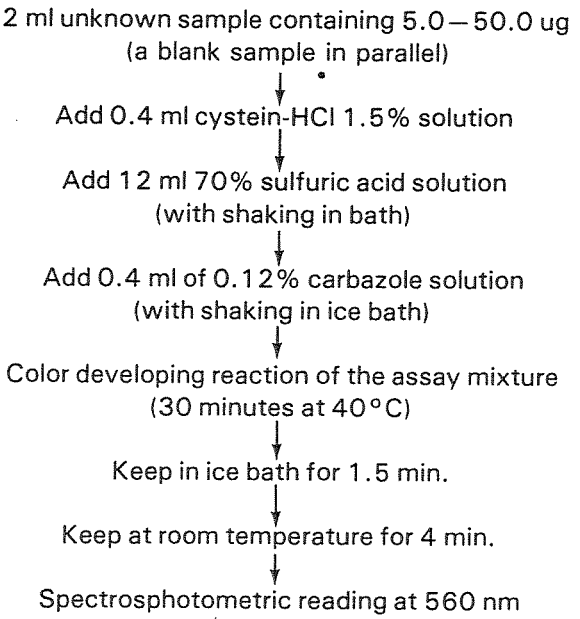

Fig. 2. The outline procedure for fructose assay (cystein-carbazole method).

Table 2. Sources of microorganisms.

No.	Microorganisms	Source	No. Strain	Obtained
1.	*Fusarium* sp.	egg plant	15	Biotrop
2.		pine seedling	21	"
3.		tomato	31	"
4.		potato tuber	115	"
5.		pepper stem	127 A	"
6.		rice stem	136	"
7.		avocado fruit	138	"
8.		papaya	140	"
9.		banana stalk	149 A	"
10.		cassava	175	"
11.		soy bean	176	"
12.		bean	178	"
13.		caladium	184	"
14.	*Fusarium* sp.	mungbean	—	BP 3
15.	*Fusarium* sp.	—	—	ITB
16.	*Streptomyces* sp.	—	S-21	ITB
17.	*S. phaeochromogenes*	soil	FERM-P 221	FRI-Japan

RESULTS AND DISCUSSION

Glucose Isomerase Activity from *Fusarium* sp.

All fifteen strain of *Fusarium* sp. were tested their glucose isomerase activity under acids pH 4.6 and base pH 7.9 condition for 24 hours reactions.

Most of the *Fusarium* sp. exhibit glucose isomerase activity under acid condition while seven strain exhibit glucose isomerase activity under base condition namely strain No: 2, 3, 8, 12, 13, 14, and 15. (Table 2).

Some considerations have been made and select strain No 14 as a representative among the fifteen strain of *Fusarium* sp. The characteristics of the 15 strain of *Fusarium* sp. shown in Table 3. Glucose Isomerase Extraction.

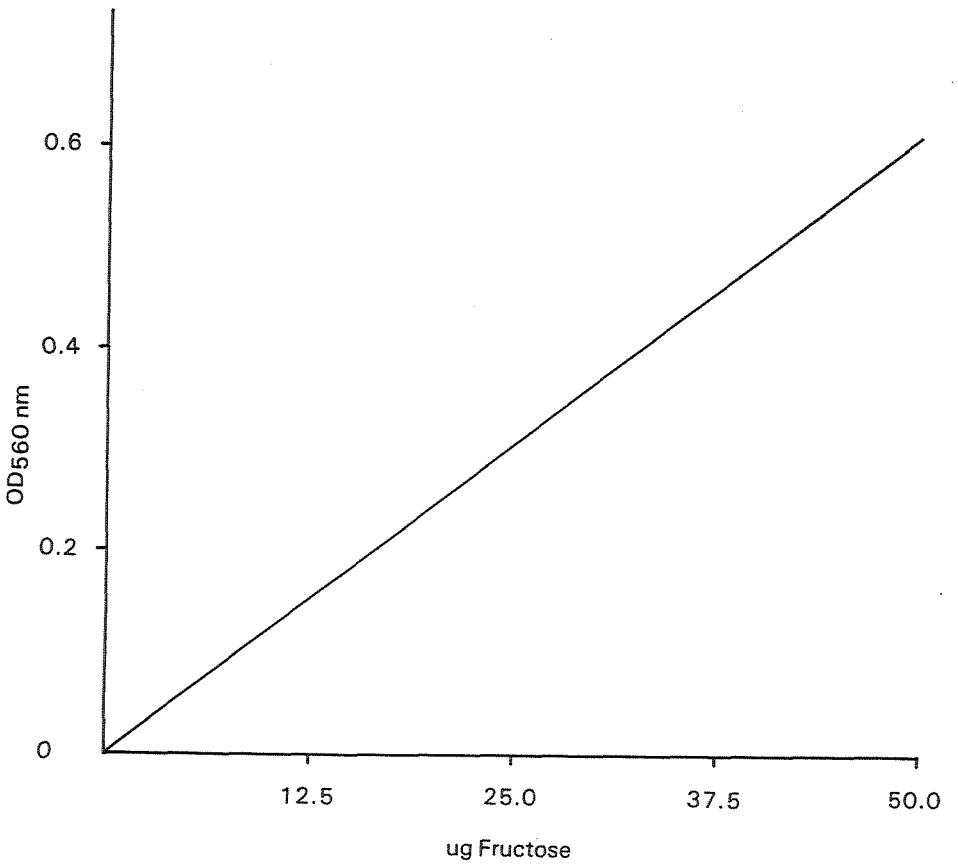

Fig. 3. Standard curve of Fructose (Cystein-carbazole - method).

Method of cell breakage in order to extract the enzyme was done chemically using cationic detergent as described by Takasaki *et al.,* 1969 and Chen *et al.,* 1979, they used cationic detergents N- Cetyl Pyridinum Chloride monohydrate (CPC) and N- Cetyl-N, N, N- Trymethyl Ammonium Bromide (CTAB).

Experimental results in Table 4 shows statistically no significant difference between extraction using CTAB and CPC.

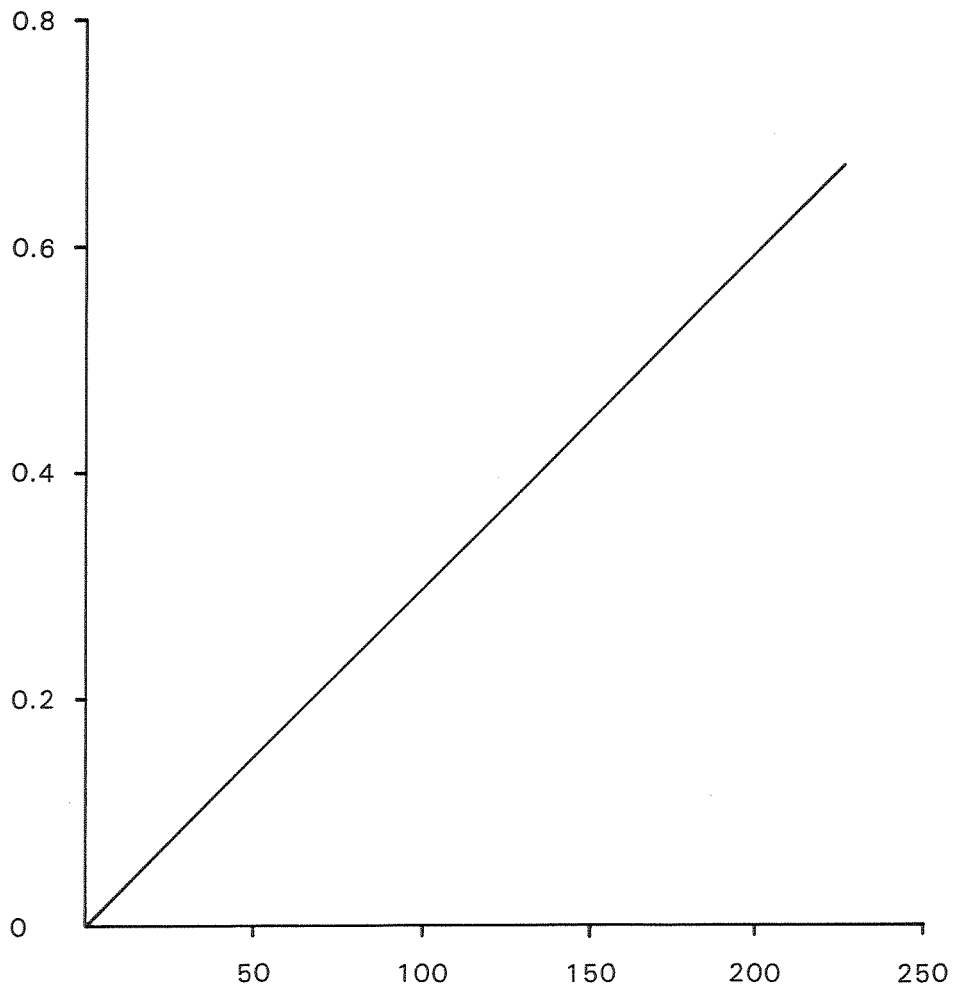

Fig. 4. Standard curve of protein using Bovin Serum Albumin (Folin-Lowry method).

Table 3. Characteristics of 15 strain *Fusarium* sp.

No. Strain	Cell Weight (mg Wet Cell/ml broth)	End pH	Activity at 60°C; 24 hours (mg Fructose/g Wet Cell Weight pH) 4.6	7.9
1.	90.89	2.7	0.06	0
2.	40.53	2.7	0.06	0.02
3.	27.86	2.4	0.78	0.02
4.	37.98	2.7	0	0
5.	36.62	2.5	0.06	0
6.	56.69	2.8	0.12	0
7.	21.65	2.5	0.26	0
8.	26.65	2.5	0.47	0.05
9.	10.73	2.4	0.53	0
10.	44.30	2.9	0.27	0
11.	42.38	2.9	0.11	0
12.	51.22	2.9	0.10	0.20
13.	36.67	3.1	0.14	0.30
14.	47.66	2.9	0.18	0.40
15.	37.22	3.1	0.13	0.13

No. Strain			Activity at pH 7.0 (mg Fru/g wet cell) 40 C	60 C
14.	20.08		0.147	0.208
16.	20.00		0.231	0.792

Strain	(mg DCW/ml broth)		mg Fru/g DCW/hr. (60 C) pH 4.5	pH 7.5
14.	9.53	5.2	0.7	3.7
16.	1.32	5.8	0.5	6.4
FERM-P 221	2.42	5.8	1.5	8.0

Table 4. The effect of cationic detergent CPC and CTAB on the glucose isomerase activity.

Microorganisms/ cationic detergent		Activity (mg Fructose/mg protein)
Fusarium sp.	CTAB	6.234
	CPC	6.576
Streptomyces sp.	CTAB	9.138
	CPC	9.708
Streptomyces phaeochromogenes FERM P-221	CTAB	9.846
	CPC	10.082

Takasaki *et al.,* 1969 reported enzyme extraction using cationic detergent give the excellent result compare to extraction physically using ultrasonication. It could be understood in this study the used of cationic detergent to extract the enzyme. In addition Bucke (1983) reported that cell breakage chemically due to the lipoprotein react with detergent agent caused membrane solubilization resulting intracellular enzyme released.

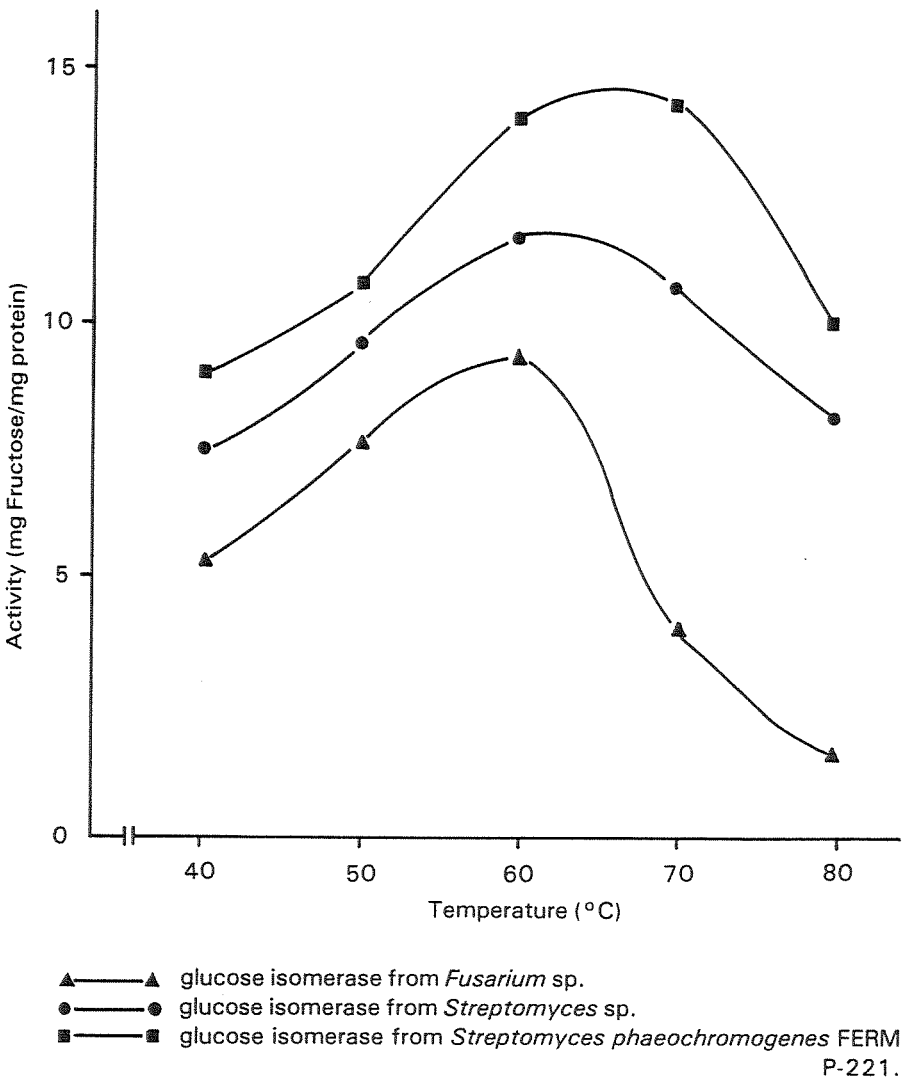

Fig. 5. The effect of temperature on the glucose isomerase activity.

Glucose Isomerase Activity

Throughout the experiment the term of enzyme activity mean the amount of fructose (mg) converted from glucose per mg protein in one hour reaction, and the relative activity was calculated from the average glucose activity compare to the reference of *Streptomyces phaeochromogenes* FERM-P 221 under condition as described in the above method.

The relative activity of glucose isomerase from *Fusarium* sp. was 70% while *Streptomyces* sp. S-21 was 93%. (Table 5).

Table 5. Relative activity of crude enzyme.

Microorganisms	Relative activity (%)
Fusarium sp.	70
Streptomyces sp. S-21	93
Streptomyces phaeochromogenes FERM P-221	100

Both strain *Fusarium* sp. and *Streptomyces* sp. S-21 are potentially showed glucose isomerase activity. Probably the enzyme activity could be increased by another research on the medium culture, inducer, mutagenicity and other factor related.

Factors Affecting the Glucose Isomerase activity

1. *Temperature*

The optimum temperature reaction of glucose isomerase shown in Table 6.

Table 6. The optimum temperature of glucose isomerase.

Microorganisms	Temperature (°C)
Fusarium sp.	55 - 60
Streptomyces sp.	60 - 65
Streptomyces phaeochromogenes FERM P-221	65 - 70

The experimental result on the optimum temperature shows lower than reported in Table 1., probably due to the crude enzyme extract is not as resistance as crude enzyme of the whole cells, secondly the mineral act as an activator also affecting the optimum temperature. Tsumura and Sato (1965) reported that addition of Co mineral will increased the optimum temperature from 80°C to 90°C. Fig. 5 shows the effects of temperature on the enzyme activity and at the temperature of 80°C all enzyme activity decreased.

2. *pH*

The optimum pH reaction of glucose isomerase enzyme shows higher than pH 7.0, its range from pH 7.0—8.0, the results agree with some other report (Table 1).

Table 7 shows the effects of different pH reactions on the glucose isomerase activity.

Table 7. The effect of different pH reactions on the glucose isomerase activity.

pH	Activity (mg Fructose/mg protein)		
	Fusarium sp.	*Streptomyces* sp.	*S. phaeochromogenes*
4.0	0.252	2.226	1.028
4.5	1.008	3.574	1.350
5.0	1.230	4.344	1.588
5.5	2.418	4.538	1.977
6.0	5.718	6.932	7.428
6.5	6.376	9.368	8.138
7.0	11.296	18.494	10.836
7.5	12.918	17.422	17.194
7.5	14.490	16.246	18.686
8.0	9.856	8.288	18.704
8.5	6.000	4.998	12.812
9.0	5.272	3.604	7.734
9.0	2.778	4.796	2.366
9.5	0.806	0.536	1.650
10.0	0.177	0.170	0.994

The preliminary study on the selection of *Fusarium* sp. showed that at pH 4.6 (acid) most of the strain exhibit glucose isomerase activity, we are not going for further observation on this matter. One might take the advantages of seraching the glucose isomerase enzyme which active at acid condition, however the acid effects on sugar have to be considered.

3. *Substrate concentration*

The effect of substrate concentration on the glucose isomerase activity shown in Fig. 6, Fig. 7 and Fig. 8, originally from *Fusarium* sp., *Streptomyces* sp. and *Streptomyces phaeochromogenes* FERM P-221 respectively.

All three curves shows the rectangular hyperbola pattern as its obey the Michaelis-Menten kinetics.

The values of K_m and V_s shown in Table 8., the result still in the range of K_m glucose isomerase ever reported.

The K_m value of glucose isomerase from *Fusarium* sp. nearly of *L. Brevis* (0.92), while K_m value of glucose isomerase from *Streptomyces* sp. almost

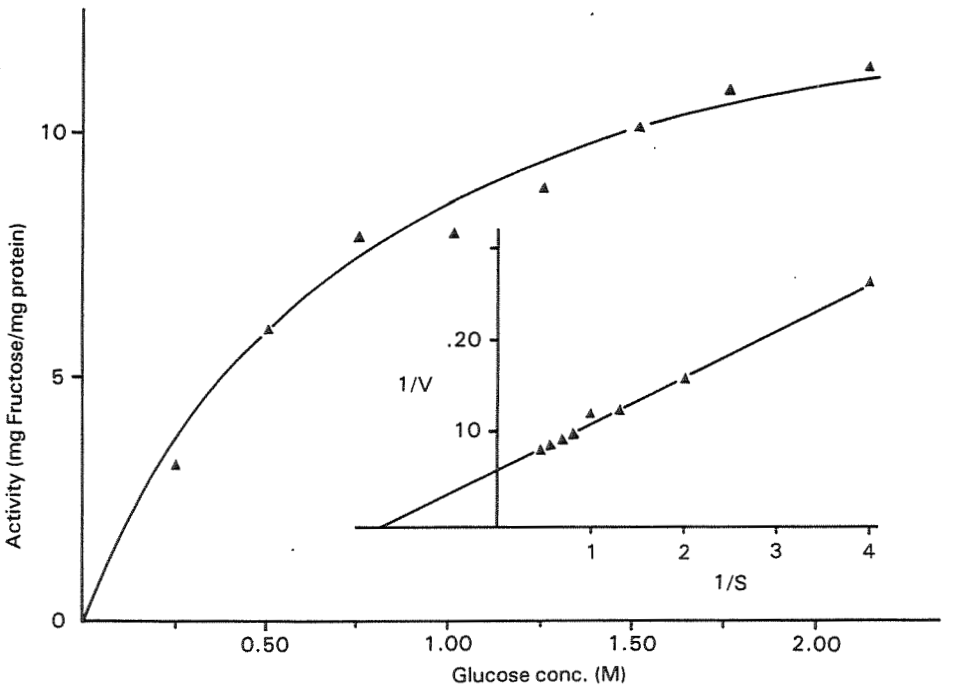

Fig. 6. The effect of glucose concentration on the glucose isomerase activity from *Fusarium* sp.

Table 8. K_m and V_s values of glucose isomerase.

Microorganisms		Vs mg fru/mg protein/hr
Fusarium sp.	8.5×10^{-1}	16.69
Streptomyces sp.	5.8×10^{-1}	16.45
S. phaeochromogenes	7.5×10^{-1}	25.77

similar to the K_m value of *B. pentosoaminoacidicum* (0.6) (Kasumi *et al.*, 1982).

The K_m value of glucose isomerase from *Streptomyces phaeochromogenes* FERM P-221 higher than the value reported by Tsumura and Sato, 1979. The different result in this study due to the used of crude enzyme, while Tsumura and Sato used the Immobilized enzyme. The high K_m value indicate that enzyme-substrate affinity is low. The effect of substrate concentration on the glucose isomerase activity shown in Table 9.

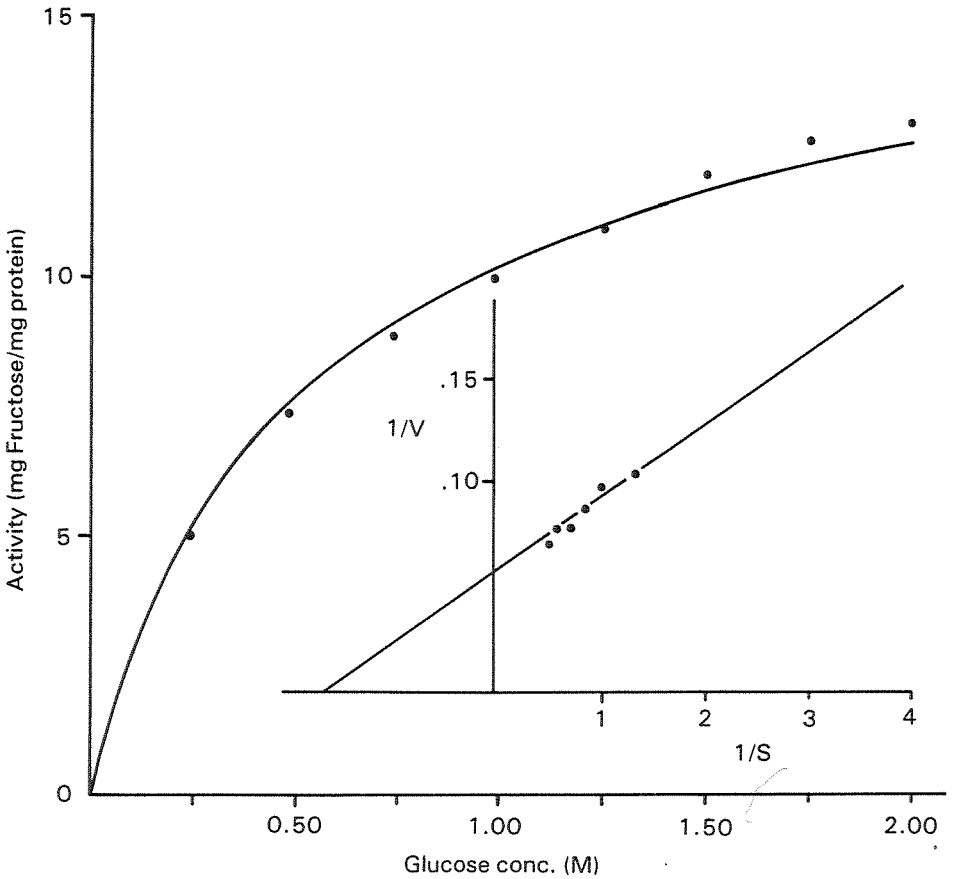

Fig. 7. The effect of glucose concentration on the glucose isomerase activity from *Streptomyces* sp.

Table 9. The effect of substrate concentration on the glucose isomerase activity.

Substrate Conc. (M)	Activity (mg Fructose/mg protein)		
	Fusarium sp.	*Streptomyces* sp.	*S. phaeochromogenes*
0.25	3.786	5.059	6.341
0.50	6.240	7.467	8.918
0.75	8.230	8.906	11.594
1.00	8.259	10.051	15.073
1.25	9.929	11.079	16.272
1.50	10.621	12.062	16.519
1.75	11.461	12.734	17.445
2.00	11.949	13.707	18.015

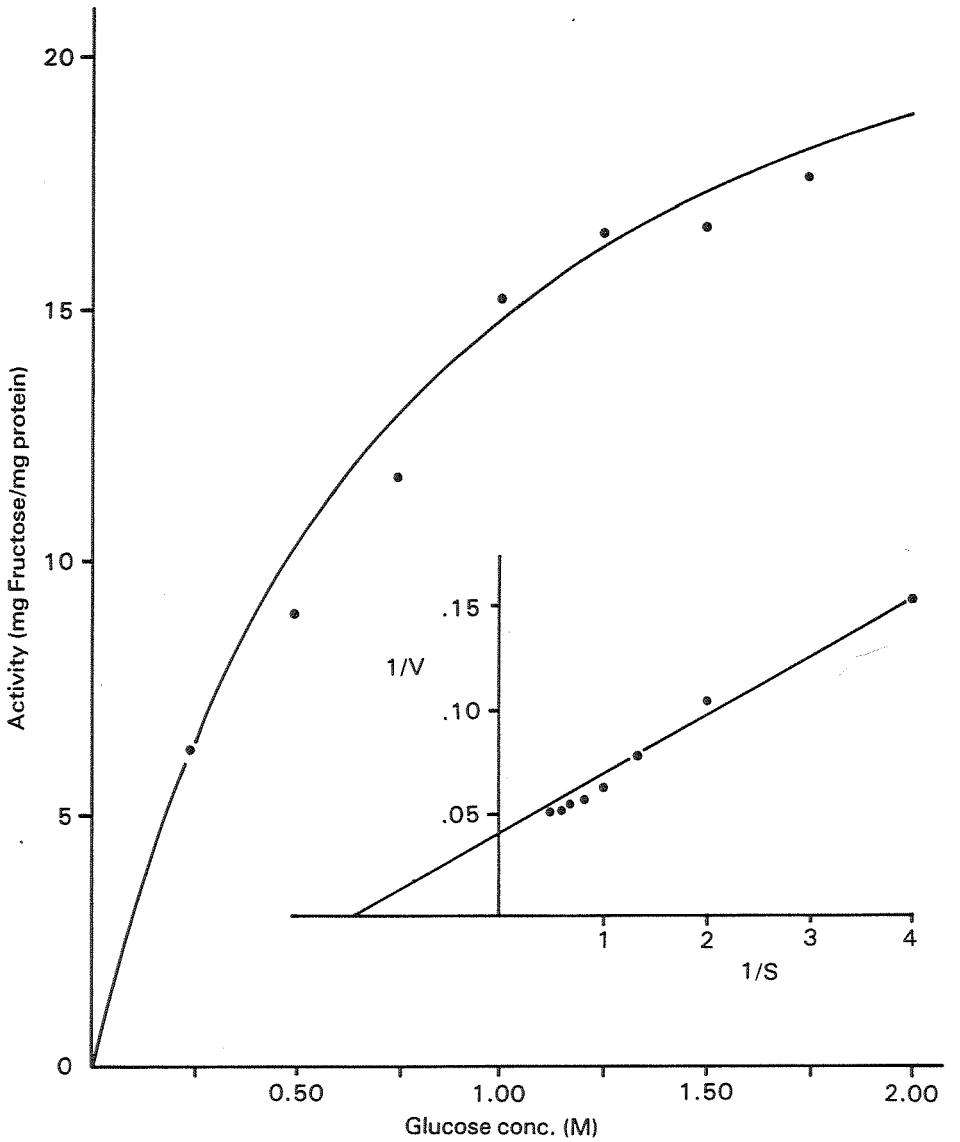

Fig. 8. The effect of glucose concentration on the glucose isomerase activity from *Streptomyces* sp.

4. *Mg^{2+} and Co^{2+} concentration*

The effect of different metal ion on the glucose isomerase activity shown in Fig. 9, Fig. 10, and Fig. 11.

The experimental result shows that generally Mg^{2+} much better than Co^{2+} as an activator, this agreed with other report (Tsumura and Sato, 1965).

The Mg^{2+} required for the optimum glucose activity lesser than Co^{2+}. Kasumi *et al.* (1980) found that Co^{2+} gave only 56% reaction activity compared to Mg^{2+}.

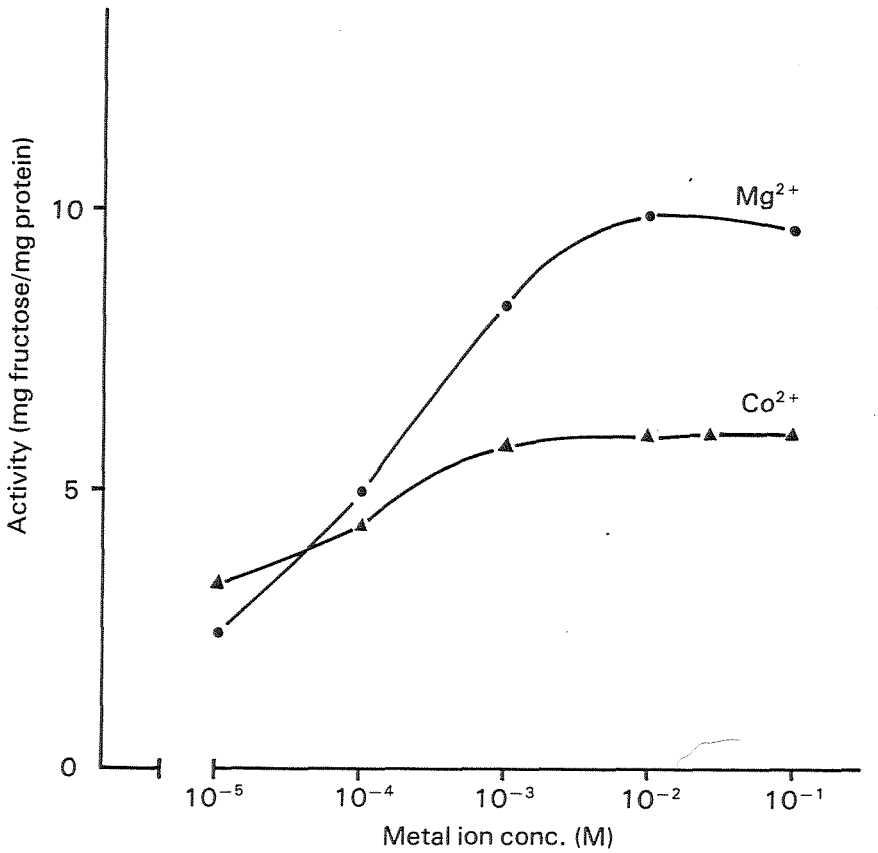

Fig. 9. The effect of different metal ion concentration on the glucose isomerase activity from *Fusarium* sp.

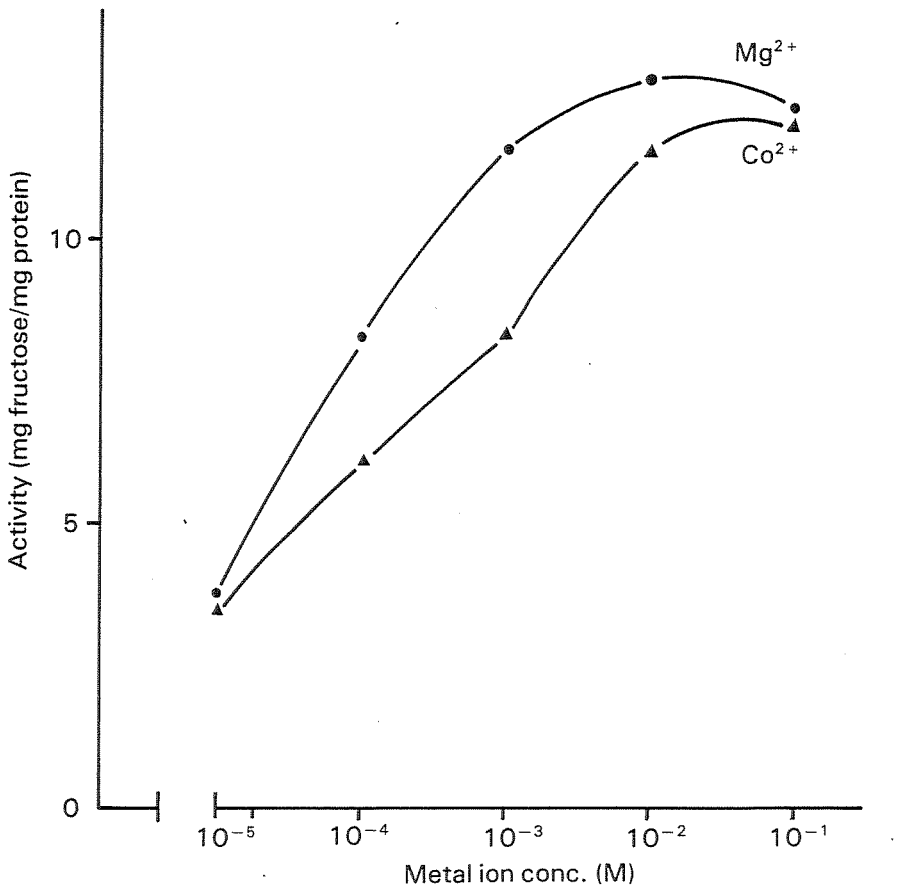

Fig. 10. The effect of different metal ion concentration on the glucose isomerase activity from *Streptomyces* sp.

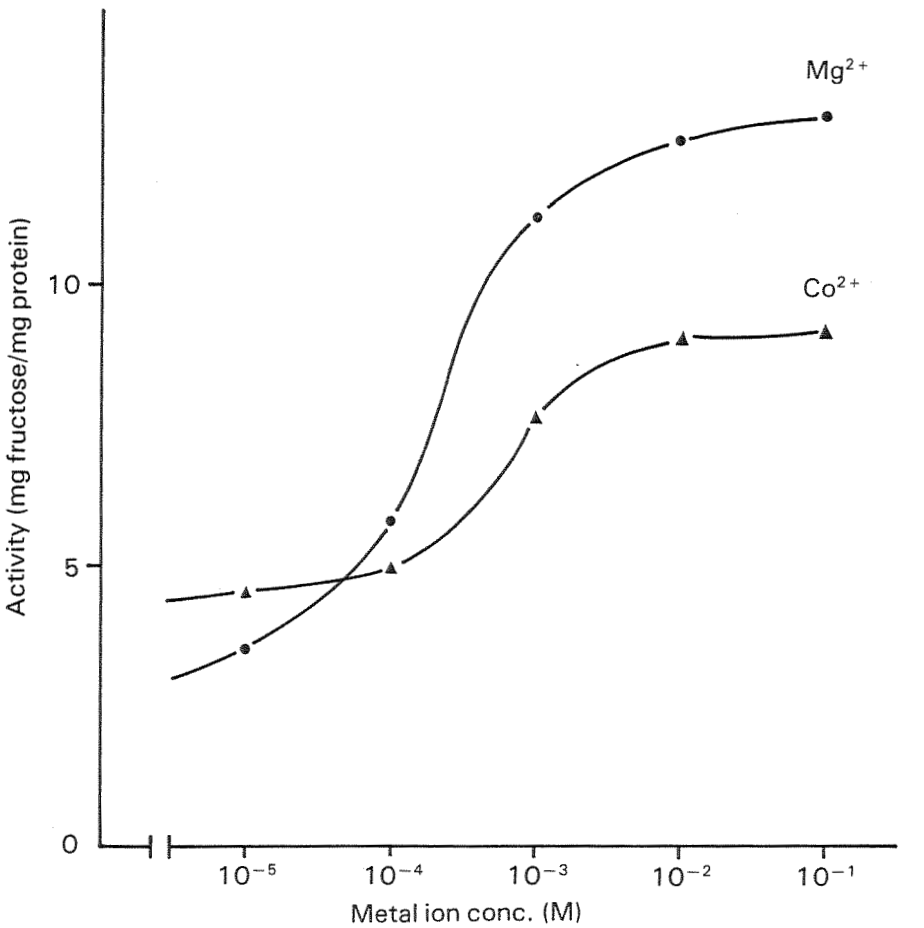

Fig. 11. The effect of different metal ion concentration on the glucose isomerase activity from *Streptomyces phaeochromogene* FERM P-221.

REFERENCES

BUCKE, C. 1983. The Biotechnology of Enzyme. Isolation and Purification. In Principles of Biotechnology. A. Wiseman, editor. Surrey University Press, London, New York.

CHEN, W.P., A.W. ANDERSON AND Y.W. HAN. 1979. Production of Glucose Isomerase by *Streptomyces flavogriseus.* Appl. Environ. Microbiol. 37: 324.

HAYASHI, K., A. SEINO, T. KASUMI, N. KUBO AND N. TSUMURA. 1981. Taxonomical studies on a Strain of Streptomyces Producing Glucose Isomerase. J. Ferment. Technol. 59: 83.

KASUMI, T., K. HAYASHI AND N. TSUMURA. 1980. Purification and Enzymatic Properties of Glucose Isomerase from *Streptomyces griseofuscus,* S-41. Agric. Biol. Chem. 45: 1087.

PLUMMER, D.T. 1979. An Introduction to Practical Biochemistry, second edition. Tata Mc Graw-Hill, New Delhi.

RYU, D.Y., S.H. CHUNG AND K. KATOH. 1977. Performance of the Continuous Glucose Isomerase Reactor Systems for the Production of Fructose Syrup. Biotech. Bioeng. 19: 159.

TAKASAKI, Y., Y. KOSUGI AND A. KANBAYASHI. 1969. Studies on Sugar-Isomerizing Enzyme. Purification, Crystallization and some Properties of Glucose Isomerase from *Streptomyces* sp. Agric. Biol. Chem. 33: 1527.

TSUMURA, N. AND T. SATO. 1965. Enzymatic Conversion of D-glucose to D-Fructose. Part V and VI. Agric. Biol. Chem. 29: 1123-1129.