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Formation of D-Glucose Isomerase by *Streptomyces* sp.

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Streptomyces sp.에 의한 포도糖 異性化酵素의 生成

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Abstract

A source of D-xylose was required for the enhanced production of D-glucose isomerase of *Streptomyces* sp. strain K-17. D-glucose supported the luxuriant growth of the organism as well as D-xylose, but D-glucose isomerase activity was hardly detected in the D-glucose-grown cells. When the D-glucose-grown cells were incubated aerobically for a few hours in 0.5% xylose solution in 0.05 M phosphate buffer, pH 7.0, it was found that inductive formation of D-glucose isomerase occurred in the cells without multiplication. In the non-growth phase of cells the inductive formation of D-glucose isomerase occurred because a source of nitrogen for the synthesis of enzymes was obtained from turnover of protein accumulated in cells. D-ribose, L-arabinose, D-glucose, D-mannose, citrate, succinate and tartrate could not induce the formation of D-glucose isomerase, but D-xylose could induce. Inductinn of D-glucose isomerase was repressed by D-glucose and its catabolites; glycerol, succinate and citrate. Inductive formation of the enzymes in the non-growth phase was stimulated by Ba^{2+}, Mg^{2+} and Co^{2+}, and inhibited by Cu^{2+}, Cd^{2+}, Ag^{+} and Hg^{2+}. The synthesis of enzymes in the induction system composed of 0.5% xylose solution was disrupted by actinomycin D, streptomycin, chloramphenicol, kanamycin, tetracycline, p-chloromercuribenzo ate, arsenate and 2,4-dinitrophenol, but not disrupted by mitomycin C and penicillin G.

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Introduction

Almost Streptomyces isomerases were an inducible enzymes requiring the presence of D-xylose as an inducer in the culture medium for the production [1~5]. According to Magasanik[6], the production of some inductive enzymes were repressed by their catabolites. The relations between induction and repression for the formation of L-arabinose isomerase in *Pediococcus pentosaceus* and *Lactobacillus plantarum* were studied by Dobrogosz and De Moss[7] and Chakravorty[8], respectively. Bhattacharya and Chakravorty[9] were studied that the catabolite repression of L-arabinose isomerase in *Salmonella typhimurium* LT2 was reversed by cyclic 3,5-adenosine monophosphate (AMP) and cyclic 3,5-AMP had no effect on the induction in normal cells, but the cyclic nucleotide enhanced synthesis of the enzyme

in ethylenediamine tetraacetic acid (EDTA)-treated cells.

There were a few reports about the induction and repression of L-arabinose isomerase[7~9] but few reports on the D-glucose isomerases. The induction and repression of D-glucose isomerase in *Streptomyces sp.* K-17 was initially reported in the previous paper[10]. When the intact cells grown in the D-glucose containing medium without D-xylose as an inducer, which has very low activity of D-glucose isomerase, were suspended and incubated in the D-xylose solution, the isomerases were synthesized inductively. The inductive formation of D-glucose isomerase in *Bacillus coagulans* and *Streptomyces sp.* were studied by Danno[11] and Takasaki[12]. The bacteria without enzyme activity wasincubated for the inductive formation of the isomerase on the medium containing pepton and D-xylose as energy source and inducer by Danno[11] and Takasaki[12] whereas Rhee and Seu[10] induced the enzyme formation on the special condition without nitrogen source.

In the present investigation the effect of the induction on the above condition without nitrogen source, catabolite repression and antibiotics on the process of the enzyme synthesis are discussed.

Materials and Methods

Microorganism

The microorganism used in this study was *Streptomyces sp.* strain K-17 as a potent producer of D-glucose isomerase [2,13,14], which was similar to *Streptomyces antibioticus*[15]. The microorganism was maintained on the agar slant previously described[15].

Cultures

One loop of the microorganism was inoculated to 100m*l* of the media in a 500 m*l* shaking flask. The medium was composed of 1.0% peptone, 0.05 % K_2HPO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.1% NaCl and 1.0% D-glucose. The microorganisms inoculated were incubated at 30°C for 26 hr on the shaker.

Assay of cell mass

Five ml of the intact cell mixture was filtered through a Toyo No. 2 filter paper. The retained solid on the filter was washed three times with distilled water, and dried at 90°C until the constant weight. The cell mass was calibrated from the standard curve by the determination of the soluble protein concentration in the dried cells prepared by the method of Schmidell[16].

The filter paper and the retained cells were added to 10m*l* of N-NaOH, and were heated in a water bath at the boiling temperature for 30 min. After the filtration the soluble protein concentration was measured by the Lowry's method[17] using a Bausch and Lomb Spectronic 20 spectrophotometer at 660 nm. The cell mass represented as mg of cell per ml of the medium.

Enzyme activity

To determine D-glucose isomerase activity the dried cells prepared as described in the previous paper [18] were used as a enzyme source. Five to twenty five mg of the dried cells were added to the reaction mixture which was composed of 5m*l* of 0.2M glucose solution containing 2×10^{-3} M $MgSO_4$ and 5m*l* of 0.2M phosphate buffer, pH 7.2. After the incubation at 70°C for 60min, amounts of D-fructose converted from D-glucose were determined by the method of cysteine carbazole sulfuric acid[19] as described in the previous paper[13]. One unit of the activity was defined as the enzyme quantity which converted to 1mg fructose per m*l* reaction mixture per min.

Enzyme induction

The cells obtained from the liquid medium containing 1% D-glucose instead of D-xylose as an inducer were aseptically washed twice with 0.9% saline and once again with the distilled water. The washed cells contained about 75% moisture. After 1.5g of the washed cells were suspended in 15m*l* of 0.1M phosphate buffer, pH 7.0, which was sterilized previously, 15m*l* of the suspension were added to 15m*l* of 1.0% D-xylose in a 500m*l* shaking flask which were boiled for 15min (the intact cell mixture). The intact cell mixture in 0.5% D-xylose was incubated at 30°C on the shaker for the enzyme induction.

Concentration of D-xylose

The liquid obtained after the filtration of the

intact cell mixture, carried out in order to determine the cell concentration, was used to measure D-xylose content consumed during the induction by the method of Somogyi and Nelson[20].

Total RNA

The total RNA in the cells was fractioned by the method of Schmidt-Thanhauser and Schneider [21]. The content of the fractionated RNA was determined by the absorbance at 260nm in Schimatzu double beam spectrophotometer model UV-200.

Reagent

Chloramphenicol from Kukje pharm. Co., mitomycin C from Takeda pharm. Co. in Japan, oxytetracycline crystal HCl from Kupung pharm. Co., streptomycin from Donga pharm. Co. and actinomycin D from Makor Chemicals Ltd. in Israel were obtained.

Results

Induction pattern

For the enzyme induction the cells grown in the medium containing 1.0% glucose without an inducer were incubated in 0.5% D-xylose in 0.05% phosphate buffer, pH 7.0, at 30°C with constant shaking. The induction pattern represents that the enzyme formation arose to be propotional to the induction time. Also the concentration of D-xylose in the intact cell mixture and total RNA in the cells were decreased to be propotional to the induction time. The cell mass estimated by the Lowry's method was not changed during the enzyme induction. So the cell growth was not shown because any nitrogen sources were not added to the induction system. The total RNA content in the cell seemed to be decreased because the degradation of RNA by RNase occurred much more than the formation of m-RNA for D-glucose isomerase on the non-growth phase without multiplication, even though m-RNA for the enzyme was synthesized (Fig. 1). The induction was carried out on the non-growth phase without the multiplication unless otherwise stated. The washed cells grown in the liquid medium containing 1.0% glucose, 0.5% xylose or 1.0% xylose were induced in 0.5% xylose solution. If the amout of the enzyme formed was presumed 100 after the induction for 11hr, the level of the enzyme activity before the induction was 4%, 53% and 73% of that activity on the

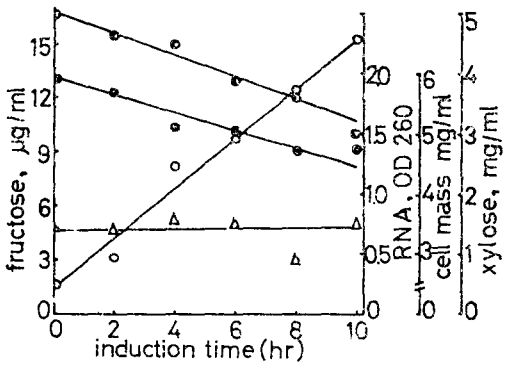

Fig. 1. Induction pattern of D-glucose isomerase in *Streptomyces* sp. strain K-17. Cells grown in the medium containing 1.0% glucose were induced in 0.5% xylose solution. ○—○, enzyme activity; △—△, cell mass; ●—●, total RNA; ◐—◐, content of xylose.

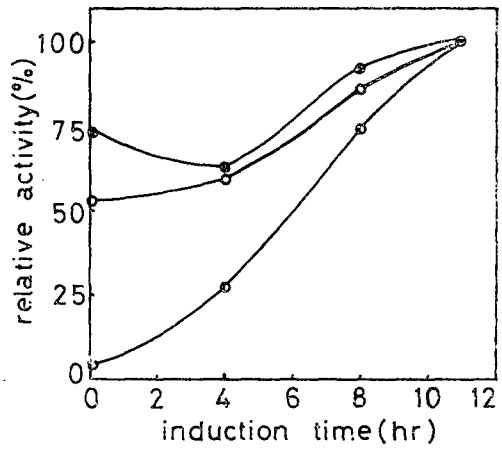

Fig. 2. Induction of D-glucose isomerase in *Streptomyces* sp. strain K-17. Cells grown in the medium containing 1.0% glucose (◐—◐). 0.5% xylose (○—○), or 1.0% xylose (●—●) as a carbon source were induced in 0.5% xylose solution.

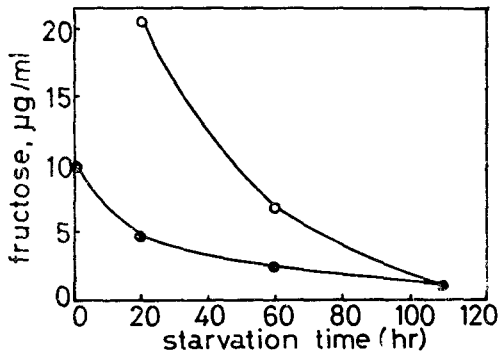

Fig. 3. Induction of D-glucose isomerase in the starved cells. Cells were induced in 0.5% xylose solution, after starvation at 30°C. ●—●, enzyme activity of no induced cells; ○—○, enzyme activity of induced cells after starvation.

cell grown in the liquid medium containing 1.0% glucose, 0.5% xylose or 1.0% xylose, respectively (Fig. 2).

Effect of starvation on the enzyme induction

The cell protein seemed to be turnover to the enzyme protein during the induction because the enzyme was produced on the non growth state without the addition of nitrogen sources. If the reservoir of the cell protein was consumed, enzyme production would be stopped in the above condition. To exhaust the reserved protein in the cells, the cells were starved in the M/15 phosphate buffer, pH 7.0, at 30°C on the shaker. After the starvation, the cells were induced by the usual method. The enzyme formation was induced weakly after the starvation for 60 hr and was not induced in the cells depleted of protein for the enzyme synthesis after 110 hr (Fig. 3).

Induction and repression of the D-glucose isomerase in *Streptomyces sp.*

The effect of carbon compounds as an inducer on the enzyme formation in the non-growth phase was investigated. After each 0.5% of D-glucose, D-xylose, D-ribose, D-arabinose, D-mannose, citrate, succinate and tartrate was added to the induction system, cell mixture was incubated at 30°C for 10 hr.

D-xylose among the carbon compounds was only an inducer (Table 1). During the induction with 0.5% D-xylose, 0.5% catabolite which was easily assimilated was added to the induction system. The enzyme induction was not affected by D-fructose and D-sorbitol, but the enzyme formation was we-

Table 1. Effect of carbon compounds as an inducer on the enzyme formation in the non-growth phase.

Inducer (0.5%)	Relative activity (%)
D-xylose	100*
D-ribose	0
L-arabinose	0
D-glucose	0
D-mannose	0
citrate	0
succinate	4
tartrate	0

*The activity obtaining with 0.5% D-xylose as an inducer was set as 100%.

Table 2. Effect of sugars and their catabolite as a repressor for the enzyme induction. Cells were induced in 0.5% D-xylose solution containing 0.5% concentration of each compound.

Carbon compounds (0.5%)	Relative activity (%)
D-glucose	25
D-fructose	105
D-mannose	66
D-galactose	63
D-sorbitol	133
D-maltose	82
L-arabinose	86
glycerol	89
citrate	22
succinate	55
none	100*

*The activity obtained with 0.5% D-xylose only as an inducer was set as 100%.

akly repressed by the addition of D-mannose, D-maltose, D-galactose, L-arabinose, glycerol and succinate, and strongly repressed by the addition of D-glucose and citrate to the induction system (Table 2).

It was demonstrated by the results presented in Fig. 4 that D-glucose isomerase formation in this organism was specially sensitive to the repression by the high concentration of D-glucose and citrate. This phenomenon initially described by Magasanik [6] seemed to be catabolite repression. When 0.5% glucose was added to the induction system in the middle of the induction period, enzyme formation was repressed in the same way as D-glucose was added to the system at the beginning of the induction (Fig. 5). The phenomenon of catabolite repression by D-glucose was investigated on the growth phase which the bacteria were allowed to grow in the medium containing 0.5% D-xylose as an inducer. The enzyme formation was not repressed by less than 0.2% of D-glucose, but considerably repressed by more than 0.4% of D-glucose (Fig. 6).

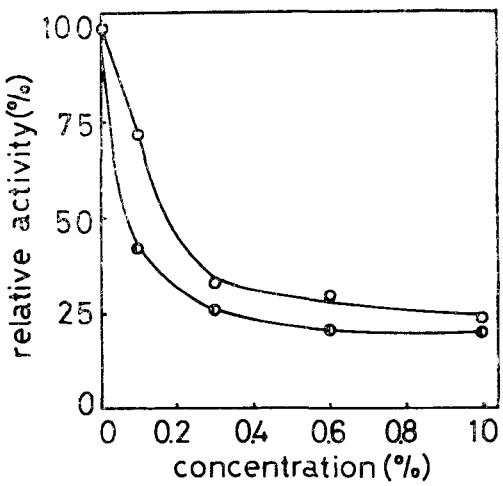

Fig. 4. **Effect of D-glucose and citrate on the repression of enzyme formation in the non-growth phase. Cells were induced in 0.5% xylose solution containing various concentration of D-glucose (○—○) and citrate (◐—◐). The isomerase activity was same as Table 2.**

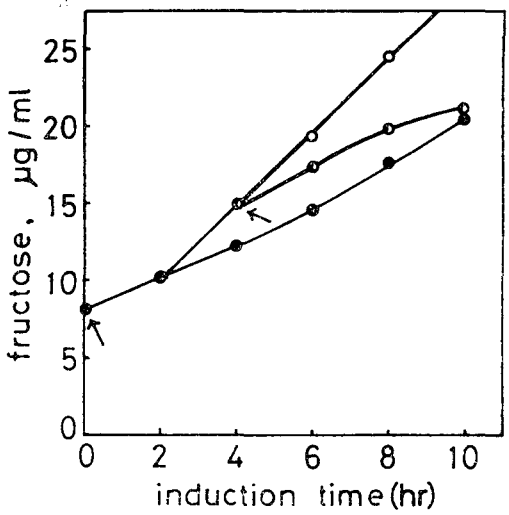

Fig. 5. **Glucose repression of D-glucose isomerase formation in non-growth phase. Cells were induced in 0.5% xylose solution (○—○). 0.5% glucose was added to induction system at 0 (●—●) and 4hr (◐—◐). Arrows repersented the addition time of glucose.**

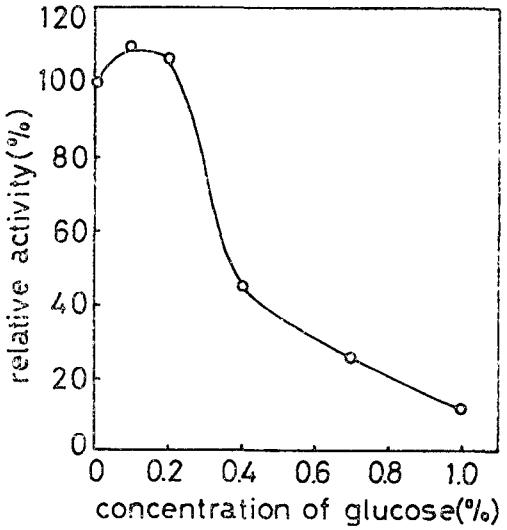

Fig. 6. **Effect of D-glucose on the repression of enzyme formation in the growth phase. Cells were grown with addition of various concentration of D-glucose to the medium containing 0.5% xylose. The activity was same as table 2.**

Effect of metal ions on the enzyme induction

To investigate the effect of metal ions on the

enzyme induction, 10^{-3}M metal ions were added to the induction system. The enzyme formation was inhibited by Cu^{2+}, Cd^{2+} and Ag^{+}, was activated by Ba^{2+}, Mg^{2+} and Co^{2+}, and was not affected by Ca^{2+}, Mn^{2+} and Zn^{2+} (Table 3). The induced formation of the enzyme was remarkably activated by the addition of 10^{-3}M Ba^{2+}, 10^{-3}M Co^{2+} and 5×10^{-3}M Mg^{2+} to the induction system (Fig. 7).

Table 3. Effect of metal ions on the enzyme induction.

Metal ion	Relative activity (%)
10^{-3}M Ca^{2+}	81
Mg^{2+}	121
Ba^{2+}	148
Mn^{2+}	95
Co^{2+}	180
Cu^{2+}	43
Cd^{2+}	36
Ag^{+}	38
Hg^{2+}	62
Zn^{2+}	90
none	100*

*The activity obtained without metal ions was set as 100%.

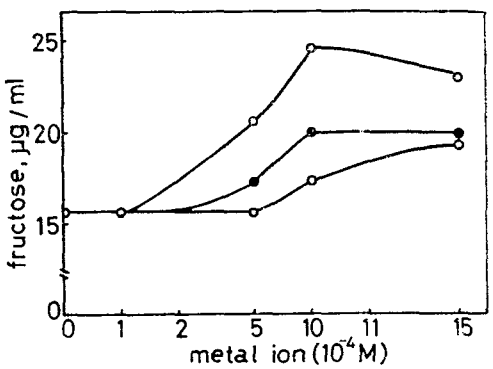

Fig. 7. Enzyme induction as effected by the concentration of metal ions. ○—○, Co^{2+}; ●—●, Ba^{2+}; ◐—◐, Mg^{2+}.

Effect of antibiotics and inhibitors on the enzyme induction

The effects of antibiotics on the enzyme induction were investigated to clarify whether the enzyme synthesis initiate from DNA synthesis, transcription level or translation level on the non-growth phase. The D-glucose isomerase activity obtaining without the addition of antibiotics was represented as 100 %. The enzyme formation was inhibited by actinomycin D, chloramphenicol, kanamycin, streptomycin and tetracycline, but not inhibited by mitomycin C and penicillin G (Table 4). Also the induced formation of the enzymes was inhibited by 10^{-3}M arsenate, 5×10^{-3} M 2, 4-dinitrophenol and 5×10^{-3} M p-chloromercuribenzoic acid (p-CMB) as well as antibiotics (Table 5).

Table 4. Effect of antibiotics on the enzyme inductien in the non-growth phase.

Antibiotics (μg/ml)		Relative activity (%)
none		100*
mitomycin C	5	100
actinomycin D	5	9
chloramphenicol	50	21
kanamycin	10	32
streptomycin	50	17
tetracycline	50	35
penicillin G	100	100

*The activity obtaining without antibiotics was set as 100%.

Table 5. Effect of inhibitor on the enzyme induction in the non-growth phase.

Inhibitor		Relative activity (%)
none		100*
Na-arsenate	10^{-3}M	52
	10^{-4}M	72
p-CMB**	5×10^{-4}M	33
	10^{-4}M	93
2·4-DNP***	5×10^{-3}M	33
	5×10^{-4}M	104

*The activity obtained without inhibitor was set as 100%.

**p-chloromercuribenzoate

***2·4-dinitrophenol

Discussion

Almost *Streptomyces* isomerases were inducible enzymes requiring the presence of D-xylose in the cultured medium for the production [1~5]. Takasaki [3] reported *Streptomyces* sp. capable of using xylans, thereby producing the inducer in the medium from xylans. *Streptomyces* sp. strain K-17 also required D-xylose as an inducer for the isomerase production on the non-growth phase as well as the growth phase[2] (Table 1).

In this experiment the induction of D-glucose isomerase was carried out on the non-growth phase without the addition of any nitrogen sources. If *Streptomyces* sp. strain K-17 was grown in the medium eliminated D-xylose as an inducer, little amounts of D-glucose isomerases were produced in the cells. On the non-growth state without the addition of nitrogen source, the enzyme proteins were synthesized. So the nutrients in the cells were exhausted by the starvation in M/15 phosphate buffer, pH 7.0 at 30°C. The enzyme formation was decreased as a result of the absence of the protein for the enzyme synthesis in the cells by the starvation (Fig. 3). The enzyme proteins seemed to be synthesized by the turnover of the cell proteins in the presence of the inducer on the non-growth state. But Danno[11] and Takasaki[12] carried out the induction of D-glucose isomerase on the growth phase to which D-xylose as an inducer and polypeptone as a nitrogen source were added.

Danno[11] suggested that de novo synthesis of protein was required for the induced formation of D-glucose isomerase. The induced formation of D-glucose isomerase by *Streptomyces sp.* strain K-17 on the non-growth state was inhibited by actinomycin D as an inhibitor of RNA synthesis, and streptomycin, chloramphenicol, kanamycin and tetracycline as the inhibitor of protein synthesis, but not inhibited by mitomycin C as an inhibitor of DNA synthesis and penicillin G as an inhibitor of cell wall synthesis. So the enzyme formation on the non-growth state seemed to require the de novo synthesis started from the transcription level (Table 4).

Since the inductive formation of D-glucose isomerase on the same condition was inhibited by the uncoupler for the phosphorylation; arsenate and 2,4-DNP, the participation of ATP seemed to be attendant on the enzyme formation. The enzyme induction by D-xylose was activated by Ba^{2+}, Co^{2+} and Mg^{2+}. Mg^{2+} as an activator and Co^{2+} as a protector for heat denaturation were applied for the activity of D-glucose isomerase in *Streptomyces* sp. strain K-17[2]. It can be deduced that the enzyme formation was activated by Mg^{2+} and the inactivation of the enzyme was reduced by Co^{2+} (Table 3).

The synthesis of the inducing enzyme was limited due to catabolite repression by D-glucose and its related metabolites[6,22,23]. Tahara[22] reported the induction of polygalacuronase by *Aspergillus niger* was repressed by some catabolites derived from glycolysis while Nisizawa[23] reported the induction of cellulase by *Trichoderma viride* was repressed by the compounds of glycolysis and the intermediates of TCA cycle. The phenomenon of catabolite repression for D-glucose isomerase formation by *Streptomyces sp.* strain K-17 was the same as the latter (Table 2).

Since the catabolite repression in the sugar metabolism was excluded in this experimental condition, the special state, added only D-xylose as an inducer and energy source without the nitrogen source, would be prepared for the best condition of the enzyme synthesis.

要 約

Streptomyces sp. strain K-17의 포도당 異性化酵素의 강력한 분비를 위해서는 inducer로서 D-xylose를 필요로 하고 있다. 그런데 D-xylose를 가하지 않고 1.0% glucose를 가한 培地에서 培養한 異性化酵素 力價가 낮은 균체를 모아서 이것을 다시 0.05M인 산 완충액(pH 7.0)에 현탁시켜 0.5% xylose를 가하여 好氣的으로 해주었을 때 酵素의 induction pattern을 調査한 結果 酵素活性이 10시간까지는 처리시간에 따라 직선상으로 增加하고 이에 비례해서 D-xylose의 양이 減少했으나 cell mass에 있어서는 거의 變動이 없었다. 이때 酵素蛋白의 合成이 일어나고 있지만, 전 RNA 함량에 있어서는 오히려 減少하였다. 이와같이 窒素源을

가하지 않는 non-growth phase에서도 酵素蛋白의 合成이 일어나는 것은 細胞內에 축적되어 있는 蛋白質의 turn-over에 의한다는 것을 starvation實驗에서 알 수 있었다. D-xylose 이외에 D-ribose, L-arabinose, D-glucose, D-mannose, citrate, succinate 및 tartrate는 inducer로서의 효과가 없었다. 酵素의 induction時, D-glucose를 가했을 경우 catabolite repression이 일어났으며 succinate나 citrate에 의해서도 강하게 酵素生成이 억제되었다. 이와 같은 현상은 growth phase에서도 마찬가지 결과를 나타내었다. Induction時, Ba^{2+}, Mg^{2+} 및 Co^{2+}가 酵素生成을 促進시켰으며, Cu^{2+}, Cd^{2+}, Ag^{+} 및 Hg^{2+}와 같은 重金屬이 酵素生成을 沮害하였고, mitomycin C 및 penicillin G는 酵素生成에 영향을 주지 못하였으나, actinomycin D, streptomycin, chloramphenicol 및 tetra cycline등에 의해 강하게 沮害되었다. 또 p-CMB 및 uncoupler인 arsenate와 2·4-DNP에 의해서도 酵素生成이 沮害되었다.

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