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호알칼리성, 고온성 *Bacillus* sp. TA-11의 β-galactosidase의 생합성 조절

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Regulation of β-Galactosidase Biosynthesis in Alkalophilic, Thermophilic *Bacillus* sp. TA-11

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자연계에서 분리, 동정한 호알칼리성, 고온성 *Bacillus sp.* TA-11의 β-galactosidase 생합성 조절 기작을 조사 하였다. 시험균주의 β-galactosidase 생합성은 isoprophyl-β-Dthiogalactopyranoside (IPTG) 보다 lactose에 의해 더욱 효과적으로 유도 되었고 glucose는 lactose의 세포내 유입을 방해하면서 β-galactosidase의 생합성을 억제 하였다. 또한 이러한 glucose의 효소합성 억제효과는 cAMP에 의해 완화되지 못했다.

Regulation of β-galactosidase biosynthesis was studied with alkalophilic, thermophilic *Bacillus* sp.TA-11 . Biosynthesis of the enzyme was effectively induced by lactose and some low level by isoprophyl-β-D-thiogalactopyranoside(IPTG) . When 30mM glucose was added at the different intervals to the culture that had been in contact with lactose, the different levels of the enzyme synthesis were observed. So, this suggests that glucose interfered with the entry of the lactose into the cells.The glucose inhibitory effect was not relieved by adding cAMP to the culture.

Keywords ; Regulation, β-galactosidase, biosynthesis, *Bacillus* sp. TA-11.

Introduction

In previous publication[1] we have been studied on the isolation of alkalophilic, thermophilic *Bacillus* sp. TA-11 and production of β-galactosidase. β-Galactosidase production was maximized when it was incubated in synthetic medium containing 1.5% lactose, 0.4% peptone, 0.4% yeast ext., 0.2% $MgSO_4$, 0.05% NH_4Cl, and 0.2% NaCl (initial pH 10.0) at 50°C for 2 days.

Regulation of β-galactosidase biosynthesis in bacteria have been extensively studied.[1~12] β-Galactosidase synthesis is constitutive or induced by inducer[1~3,5~9] and it is affected in several ways by the carbon source or chemical inducer and another chemicals.[3,7,8,11] Furthermore, β-galactosidase biosynthesis is repressed by accumulation of glucose and the other metabolite even though inducer existed much in cell,[4] and glycerol show the transient repression of β-galactosidase collaboration with glucose.[11,12] cAMP overcome inhibition of glucose to β-galactosidase biosynthesis and this effect is caused by stimulation for transcription of inducible catabolic operon.[5]

The present work discussed on the induction and repression system in β-galactosidase of alkalophilic, thermophilic *Bacillus* sp. TA-11 which was isolated from natural source.

Material and methods

1. Reagents

Isopropyl-β-D-thiogalactopyranoside (IPTG), adenosin-3'.5'-cyclic monophosphate (cAMP) were purchased from Sigma Chemical Company and peptone, yeast ext., from Difco Laboratories and other chemicals were used commercial special grade or first grade products.

2. Culture condition

Bacillus sp. TA-11 described previously[1] incubated in YP medium (yeast ext. 0.4% ; polypeptone 0.4% ; K_2HPO_4 and KH_2PO_4, each 0.1% ; $MgSO_4$, 0.2% ; NH_4Cl, 0.05% ; NaCl, 0.2% ; pH 9.5 with 20% Na_2CO_3) at 50°C for 24 hrs in reciprocal shaker.

3. Induction and repression of the β-galactosidase

After harvested cells from the cultures, washed and inoculated in enzyme biosynthesis control medium (YP medium without yeast ext.) containing 30mM lactose and then incubated at 50°C for 2 hrs.[7]

4. Assay of β-galactosidase activity

β-Galactosidase activity was estimated as reported previously.[1]

Results and discussion

1. Induction system of β-galactosidase biosynthesis in *Bacillus* sp. TA-11

1) Effect of sugars

Effect of various sugars on the β-galactosidase biosynthesis were investigated by adding 30mM glucose, galactose, fructose, lactose, sucrose, maltose, ribose, xylose, and glycerol in enzyme - producing medium.[1]

Enzyme formation by lactose was 83.5 Unit/A_{660}, remarkably effective to induce the biosynthesis of β-galactosidase and were also induced relatively high by ribose and glycerol but low that by glucose and xylose (Table 1).

This results indicate lactose was good inducer in the β-galactosidase formation

of alkalophilic, thermophilic *Bacillus* sp. TA-11.

Table 1. Effect of various sugars on the ß-galactosidase formation by *Bacillus* sp. TA-11

Carbon source (30mM)	Cell yield (A_{660})	Specific activity (Units/A_{660})
Fructose	1.10	26.3
Galactose	1.09	50.5
Glucose	1.50	33.8
Glycerol	1.07	59.0
Lactose	1.36	83.5
Maltose	1.32	21.8
Ribose	1.05	60.6
Sucrose	1.00	50.0
Xylose	1.32	27.8

The cell were grown in the YP medium described in material and method. Cultivation was done at 50℃ with shaking for 24 hours.

2) Effect of lactose and IPTG

To compare the effect of lactose (natural inducer) and IPTG (chemical inducer) on the induction of ß-galactosidase biosynthesis of *Bacillus* sp. TA-11, 30mM lactose and 5mM IPTG were added in enzyme biosynthesis control medium described above, respectively and then induced the ß-galactosidase biosynthesis for 2 hrs at 50°C (Fig. 1).

As seen in Fig. 1 lactose was more effective in induction of the enzyme than IPTG. This results indicate inducer of ß-galactosidase of *Bacillus* sp. TA-11 was lactose or allolactose, and it was similar with that of Jobe and Bourgeois[9] which lactose was diverted in allolactose by ß-galactosidase in *E. coli* and it reacted as natural inducer of lactose operon, but was different in that of *Lactobacillus sporogenes*[7] which synthesis of the enzyme was effectively induced by IPTG or galactose, and to much lower level by lactose.

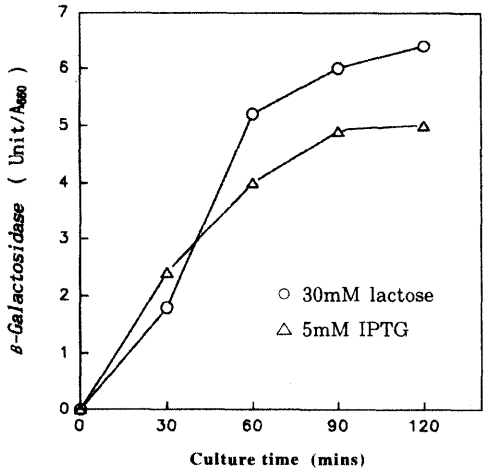

Fig. 1. Effect of lactose and IPTG on the ß-galactosidase induction of *Bacillus* sp. TA-11.
The cells were grown in the enzyme biosynthesis control medium described in material and method.

3) Effect of carbon source on the ß-galactosidase induction by lactose

Effect of carbon sources on the ß-galactosidase induction by lactose were investigated by adding 30mM of various sugars in lactose-containing enzyme biosynthesis control medium and then culturing for 2 hrs at 50°C (Table 2).

Table 2. Effect of carbon source on the biosynthesis of ß-galactosidase induced by lactose

Carbon source (30mM)	Specific activity (Units/A_{660})
Fructose	6.23
Galactose	3.88
Glucose	3.39
Glycerol	6.64
Maltose	5.74
Sucrose	5.27
Xylose	8.21
Ribose	6.00

The cells were grown in the enzyme biosynthesis control medium described in material and method. At the start of the experiment lactose was added to a final concentration of 30mM. Cultivation was done at 50℃ with shaking for 2 hours.

Specific activity of glucose and galactose were 3.39 and 3.38Unit/A_{660}, respectively and they inhibited for β-galactosidase induction by lactose remarkably.

2. Repression system of β-galactosidase biosynthesis in *Bacillus* sp. TA-11

1) Effect of glucose

We discussed on the inhibition of glucose in induced synthesis of β-galactosidase of *Bacillus* sp. TA-11.

For the investigation the inhibition mechanism of glucose, 30mM glucose was added at the different interval to the lactose containing-enzyme biosynthesis control medium and its β-galactosidase formation was then measured (Fig. 2).

As late as addition time of glucose, β-galactosidase formation was increased and at 15 min. after addition of lactose, its specific activity show 3.5Unit/A_{660}. We guessed glucose inhibition was caused by interference of glucose to entry of lactose into cell(that is. lactose exclusion) alike in *E. coli.*[5]

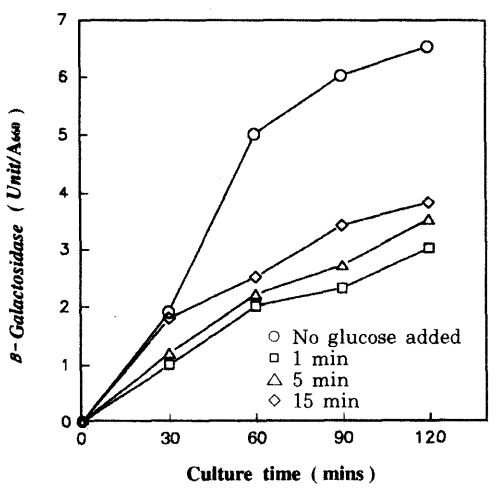

Fig. 2. Effect of glucose on the synthesis of β-galactosidase induced by 30mM lactose in *Bacillus* sp. TA-11 .
The cells were grown in the enzyme biosynthesis control medium. Glucose was added to a final concentration of 30mM at various culture time after the start of induction by 30mM lactose.

2) Effect of glycerol on the inhibition of glucose

The effect of glycerol on the glucose inhibition in β-galactosidase biosynthesis of *Bacillus* sp. TA-11 was investigated by addition of lactose(as inducer) in enzyme biosynthesis control medium containing glucose alone, glycerol alone and mixture of glycerol and glucose (Fig. 3).

Cells placed in the medium containing glucose only produced β-galactosidase at a different rate approximately one-half that observed in the cell growing in the medium containing glycerol. The cells placed in the medium containing glucose as well as glycerol produced the enzyme at low rate, approximately one-third that of glycerol only. This results indicate transient repression of the β-galac-

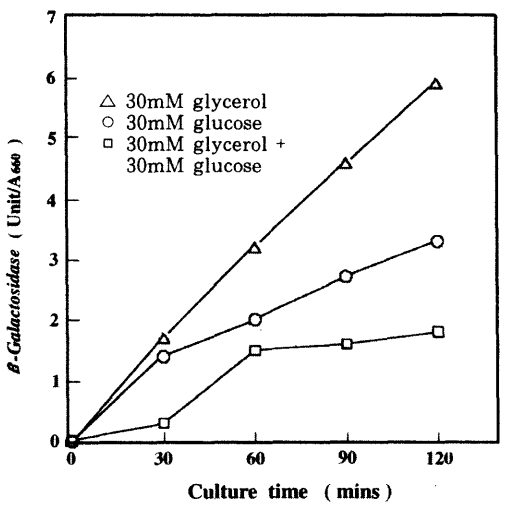

Fig. 3. Catabolite repression of β-galactosidase by glucose in *Bacillus* sp. TA-11.
The cells were grown in the enzyme biosynthesis control medium containing 30mM lactose.

tosidase occured when glucose together with the glycerol was added to a culture growing in a inducer containing medium.

3) Effect of cAMP on the inhibition of glucose

Fig. 4 presented the results of the effect of cAMP on the glucose inhibition in the *ß*-galactosidase biosynthesis. *ß*-Galactosidase biosynthesis inhibition with glucose was not reduced by addition of 5mM cAMP in the enzyme biosynthesis control medium containing glucose. It was similar to those of other *Bacillus* sp.[5] and *L. sporogenes*,[7] not in *E. coli*.[5]

From this all of data, we concluded that lactose was effective inducer in *ß*-galactosidase biosynthesis of *Bacillus* sp.

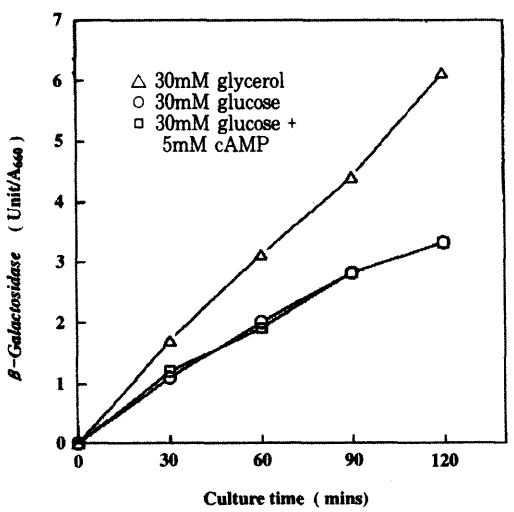

Fig. 4. Effect of cAMP on the catabolite repression of *ß*-galactosidase synthesis in *Bacillus* sp. TA-11.
The cells were grown in the enzyme biosynthesis control medium containing 30mM lactose.

TA-11 and glucose inhibited the enzyme biosynthesis by inducer exclusion and its inhibitory effect was not reduced by addition of cAMP.

We think determination of cAMP content and adenylate cyclase activity of the cell and other following experiments are necessary to elucidate more exactly the mechanism of induction and repression system in *ß*-galactosidase biosynthesis of alkalophilic, thermopilic *Bacillus* sp. TA-11.

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