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Biosynthetic Regulation of Inulinase from *Bacillus sphaericus* 188-1

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Bacillus sphaericus 188-1이 생성하는 Inulinase의 생합성 조절

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Abstract

Regulation of inulinase biosynthesis was studied in *Bacillus sphaericus* 188-1. Biosynthesis of inulinase was effectively induced in the presence of 0.5% inulin for 8 hrs. Fructose (0.5%) repressed the inulinase induction by inulin and as late as addition time of fructose, inulinase formation was decreased. Catabolite repression was not reduced by the addition of cAMP for 8 hrs of induction.

Key words : biosynthetic regulation, inulinase, *Bacillus sphaericus* 188-1.

INTRODUCTION

Inulinase (β-fructosidase : β-fructan hydrolase : 2,1- D-fructan-fructanohydrolase ; EC 3.2.1.7) splits β-2,1-fructanofuranosidic linkages of inulin to produce fructose[1]. The classification of inulinase was based on the occurrence of inulinase, activity on sucrose and inulin, type of reaction products (oligofructans or only fructose), secretion pattern (exo or endo) etc.

These inulinase split fructans of the inulin type either endo- or exo-wise, producing a series of oligofructans or fructose but rarely hydrolyze sucrose. Inulinase from yeasts, in particular, is able to hydrolyze inulin and levan-type fructans exo-wise and is an extracellular enzyme partially associated with the cell wall and partially excreted into the culture fluid[2]. Almost of the inulinases were glycoproteins which can be induced exclusively by inulin[1].

The inulinase has been found in several yeasts and bacteria such as *Kluyveromyces* sp.[3~5], *Debaryomyces* sp.[6], *Saccharomyces* sp.[7], *Streptococcus* sp.[8], *Clostridium* sp.[9], *Arthrobacter* sp.[10,11], *Chrysosporium* sp. [12], and in some mold such as *Aspergillus* sp. [13~15], *Penicillium* sp. [16] and *Fusarium* sp.[17]. However, only some inulinase from yeasts have mainly been used in food and pharmaceutical industries.

Illustration of biosynthetic regulation system in inulinase is required for mass production of inulinase from various sources.

Therefore, we studied the induction and repression system of intracellular inulinase in *Bacillus sphaericus* 188-1.

MATERIALS AND METHODS

1. Organism and culture condition

Bacillus sphaericus 188-1 isolated from soil was used as a source of inulinase[18]. It was cultivated in inulin broth (1.0% inulin, 0.5% Bacto-peptone, 0.8%

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$NH_4H_2PO_4$, 0.05% $MgSO_4$ and 0.05% KCl, pH 7.5).

2. Chemicals and materials

All chemicals used were of analytical grade. Inulin (chicory), glucose, dinitrosalicylic acid and cAMP were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Sucrose was from Wako Pure Chemical Ind. Ltd. (Osaka, Japan). Inulin (dahila), Bacto-tryptone, Bacto-peptone, beef extract, yeast extract and Bacto-agar were purchased from Difco Lab. (Detroit, Michigan, USA).

3. Assay of inulinase activity

Inulinase activity was measured by determining the amount of the released reducing sugar from inulin[18]. The reaction mixture containing 0.8 mL of 1% inulin in sodium phosphate buffer (pH 7.0) and 0.2 mL enzyme solution was incubated at 37℃ for 1 hr. Total reducing sugar was measured by the Somogyi-Nelson method[19]. D-Glucose was assayed using the Glucose [HK] kit. D-fructose was determined as difference between the amount of total reducing sugar and D-glucose. One unit of inulinase activity is defined as the production of 1 μM of the product per minute at 37℃ and specific activity was expressed as unit per mg protein.

4. Biosynthetic regulation of the inulinase

Induction and repression of inulinase from *Bacillus sphaericus* 188-1 was performed as followed. The incubated cells were harvested and washed twice with a biosynthesis regulation solution (50mM sodium phosphate buffer). The pellets was resuspended in the biosynthesis regulation solution containing inducer or repressor, and then incubated at 30℃ with agitation, and inulinase activity and protein content of the cell free extracts were determined.

RESULTS AND DISCUSSION

1. Induction of inulinase

Table 1 shows the effects of sugars on induction of intracellular inulinase from *Bacillus sphaericus* 188-1. Inulin served as the most effective inducer for formation of inulinase and was also induced a little by sucrose.

Table 1. Effects of various sugars on inulinase induction by *Bacillus sphaericus* 188-1

Sugars (0.5%)	Specific activity (Unit/mg protein)
Maltose	15.4
Lactose	9.8
Galactose	9.3
Xylose	13.7
Glycerol	9.2
Glucose	9.2
Fructose	9.3
Soluble starch	9.3
Sucrose	37.7
Raffinose	28.6
Inulin	288.8
Control(no sugar)	28.0

* After cultivation of *Bacillus sphaericus* 188-1 in 0.4% peptone containing enzyme regulation medium at 30℃ for 10 hrs, the cell was harvested, suspended in 50 mM sodium phosphate buffer and sugars were added to a final concentration of 0.5 % for further cultivation at 30℃ for 18 hrs.

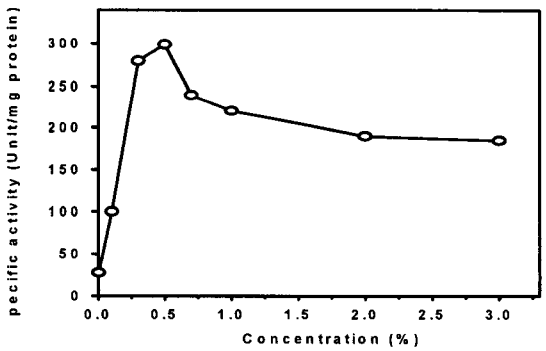

Fig. 1. Effects of inulin concentration on inulinase induction by *Bacillus sphaericus* 188-1.

To investigate the effects of inulin concentration on induction of the inulinase, inulin ranging from 0 to 5% was added in biosynthesis regulation solution and then induced for 10 hrs at 30℃(Fig. 1). Induction of the inulinase by inulin was maximized at 0.5% and as increasing as the concentration of inulin, the induction was decreased gradually by increasing inulin concentration over 3.0%.

Fig. 2 shows the effect of culture time on the inulinase induction by inulin. 8 hrs of cultivation was

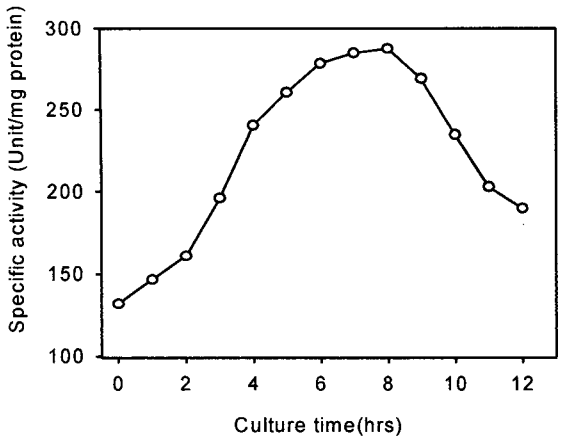

Fig. 2. Effects of culture time on inulinase induction by *Bacillus sphaericus* 188-1.

Table 2. Effect of sugars on the repression of inulinase in *Bacillus sphaericus* 188-1

Sugars (0.5%)	Specific activity (Unit/mg protein)
Glucose	105.7
Fructose	80.3
Galactose	292.8
Xylose	108.9
Glycerol	249.0
Maltose	287.0
Lactose	273.9
Soluble starch	214.4
Sucrose	145.5
Raffinose	207.6
Inulin	304.2

* After cultivation of *Bacillus sphaericus* 188-1 in 0.4% peptone containing enzyme regulation medium at 30℃ for 10 hrs, the cell was harvested, suspended in 50 mM sodium phosphate buffer, and then sugars were added to a final concentration of 0.5% for further cultivation at 30℃ for 8 hrs.

effective in the inulinase induction(Fig. 2).

2. Repression of inulinase

The effect of sugars on repression of the inulinase were investigated by addition of 0.5% sugars in inulin-containing biosynthesis regulation solution and further incubating for 8 hrs at 30℃.

As shown in Table 2, almost of sugars examined inhibited induction of the inulinase by inulin, and especially fructose inhibited significantly the inulinase induction by inulin (Table 2).

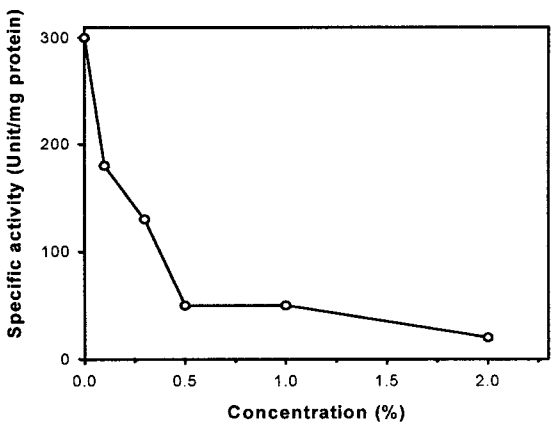

Fig. 3. Effects of fructose concentration on induction of the inulinase by 0.5% inulin in *Bacillus sphaericus* 188-1.

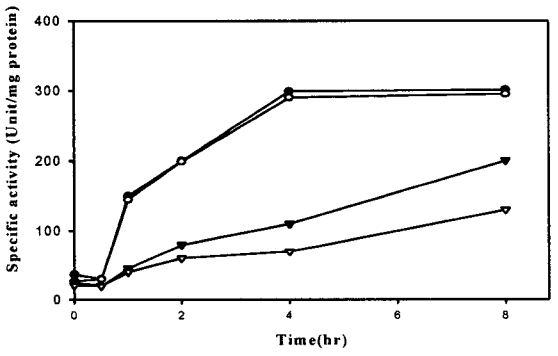

Fig. 4. Effects of addition time of fructose on the inulinase induction by 0.5% inulin in *Bacillus sphaericus* 188-1. ● : No fructose, ○ : Added after 0 min, ▼ : Added after 5 min, ▽ : Added after 15min.

Fig. 3 shows the effect of fructose concentration on induction of the inulinase by inulin. The inulinase induction was markedly inhibited at 0.5% of fructose.

To investigate the inhibition mechanism of fructose, 0.5% of fructose was added at the different intervals to the inulin-containing biosynthesis regulation solution and its inulinase induction was measured (Fig. 4). As late as addition time of fructose, inulinase induction was decreased. This results were quite different with those of invertase[20] and β-galactosidase[21] in *Bacillus* sp. TA-11 which glucose inhibition of invertase and galactose inhibi-

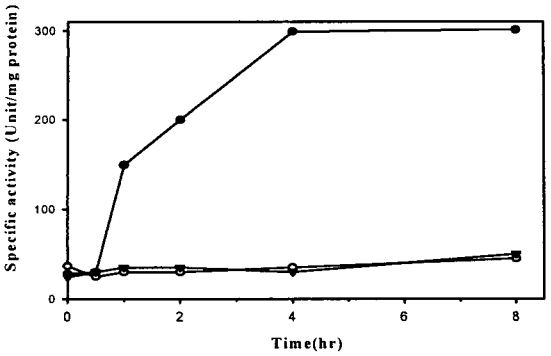

Fig. 5. Effects of cAMP on the catabolite repression of inulinase synthesis in *Bacillus sphaericus* 188-1. After cell harvested, suspended in biosynthesis regulation medium containing 0.5% inulin, fructose (0.5%) and cAMP (0.5mM) were added separately or together and then incubated at 30℃ for 10 hrs. ● : Inulin only, ▼ : cAMP, ○ : Fructose+cAMP.

tion of β-galactosidase were occurred by inducer exclusion.

To investigate effect of cAMP on catabolic repression of the inulinase, mixture of 0.5 mM cAMP and 0.5% fructose was added in 0.5% inulin-containing biosynthesis regulation solution, and then inulinase induction was measured. Catabolic repression was not reduced by addition of cAMP(Fig. 5).

요 약

Inulinase의 생합성 조절 기작을 규명하여 이들의 대량생산을 위한 자료로 활용하고자 *Bacillus sphaericus* 188-1이 생성하는 inulinase의 생합성 조절에 관하여 조사하였다. Inulinase의 생합성은 0.5% inulin을 함유한 생합성 조절용액에서 8시간에 효율적으로 유도되었고 0.5% fructose의 첨가는 inulin에 의한 inulinase의 생합성 유도를 억제시켰으며 fructose를 늦게 첨가할수록 inulinase 생합성 유도가 낮아졌다. cAMP의 첨가는 catabolite repression을 감소시키지 못하였다.

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