Korean J. Food & Nutr. Vol. 15. No. 2, 126~130 (2002)

THE KOREAN JOURNAL OF FOOD AND NUTRITION
한국식품영양학회

Biosynthetic Regulation of Invertase from Thermophilic and Alkalophilic *Bacillus* sp. TA-11

Na-Mi Kim*, Dong-Woo Kim** and Jae-Ho Kim†

Dept. of Genetic Engineering, Paichai University, Daejeon 302-735, Korea
**Division of Product Development, Korea Tabacco & Ginseng Company Central Institute, Daejeon 305-345, Korea*
***Division of Food Science and Technology, Woosong Technical College, Daejeon 300-832, Korea*

고온성이며 호알칼리성인 *Bacillus* sp. TA-110이 생성하는 Invertase의 생합성 조절

김나미* · 김동우** · 김재호†

배재대학교 유전공학과, *한국담배인삼공사 중앙연구원 제품개발부,
**우송공업대학 식품과학계열

Abstract

Regulation of invertase biosynthesis was studied in thermophilic and alkalophilic *Bacillus* sp. TA-11. Biosynthesis of the invertase was effectively induced in the presence of 10 mM sucrose for 180 min. Glucose repressed the invertase induction by sucrose and as late as addition time of glucose, the invertase formation was increased, indicating that glucose repression was occurred by inducer exclusion. Catabolite repression was reduced a little by the addition of cAMP for 180 min of induction.

Key words : biosynthetic regulation, invertase, *Bacillus* sp. TA-11.

Introduction

Invertase (β -fructofuranosidase; β -D-fructofuranoside fructohydrolase; EC 3.2.1.26) hydrolyzes sucrose to glucose and fructose, and also catalyzes transfructosylation reaction with sucrose as substrate, resulting in the formation of various isomers of kestose type trisaccharides (fructosyl sucrose)[1~4]. It is widely used in food and medical industries for the production of fructose syrup and fructooligosaccharides which are low-calory sweetener, bifidus growth factor and anti-caries effector[1,3].

Although invertase has been found in various plants[4,5~10] and microorganisms[11~23], studies on the enzyme have been carried out exclusively in yeast[16~23] and some plants[4,6,10]. It was known that two forms of invertase exist in *Saccharomyces* sp. : a large, secreted mannoprotein of 270 KDa molecular mass containing 50% mannose and 3% glucosamine by weight, and a smaller, intracellular glycan enzyme with a molecular mass of 135 KDa [23]. In addition, the molecular mass of microbial invertase were 58 KDa from *Zymomonas mobilis*[14], 180 KDa and 270 KDa from *Saccharomyces* sp.[17], 340 KDa from *Aspergillus niger*[13], 48 KDa from *Streptococcus mutans*[12], 52 KDa from *Arthrobacter* sp. K-1[15] and 25 KDa from *Bacillus* sp. TA-11[24].

There is little information on the biosynthetic and

† Corresponding author : Jae-Ho Kim

molecular biological studies of intracellular invertase from microorganisms and plant. Only a single gene encoding both the intracellular and extracellular forms of yeast invertase and sucrase gene from *Bacillus* subtilis[25] have been cloned and sequenced[26].

To elucidate the regulation mechanism of invertase biosynthesis, induction and repression system of intracellular invertase were investigated in themophilic and alkalophilic *Bacillus* sp. TA-11.

Materials and Methods

1. Strains and Culture Condition

Alkalophilic and thermophilic *Bacillus* sp. TA-11 which was initially isolated from soil was used as a source of invertase[24]. The strain was cultivated in a fermentor (KFM-7, Korea Fermentor Co.) at 50℃ for 36 hrs in SY broth containing 1% (w/v) sucrose, 0.6% (w/v) yeast extracts and 0.1% (w/v) each of KH_2PO_4 and K_2HPO_4 with 0.5 vvm aeration and pH was maintained to 9.5 by adding 1.0 M Na_2CO_3[24].

MY medium (1.0% maltose, 0.6% yeast extract, 0.1% K_2HPO_4 and KH_2PO_4) was used for regulation study.

2. Assay of Invertase Activity

Invertase activity was assayed by measuring the amount of reducing sugar released from sucrose as the substrate [24]. The assay mixture contained 0.1 M sucrose, 0.1 M phosphate buffer (pH 6.5), and 0.2 mL of an enzyme solution in total volume of 1 mL. After incubation for 2 hrs at 37℃, reducing sugar formed in the reaction mixture was measured by the methods of dinitrosalisylic acid method. One unit of invertase activity was defined as the amount of enzyme which require to produce reducing sugar equivalent to 1 μM of glucose per min.

3. Biosynthetic Regulation of the Invertase

Induction and repression test of the invertase from *Bacillus* sp. TA-11 was performed as follows. After harvested the cells, washed twice with a biosynthesis regulation solution (MY medium without maltose). The pellets were resuspended in the biosynthesis regulation solution containing inducer or repressor and then incubated at 50℃ with agitation, and invertase activity and protein content of cell free extract were determined.

4. Chemicals

All chemicals used in this study were of analytical grade which obtained from Sigma Chemical Co.(St. Louis, Mo. USA).

Results and Discussion

1. Induction of the Invertase *Bacillus* sp. TA-11

Table 1 shows the effects of sugars on induction of invertase from thermophilic and alkalophilic of *Bacillus* sp. TA-11. Sucrose served as the most effective inducer for formation of invertase and the invertase was also induced a little by raffinose.

To investigate the effects of sucrose concentration and culture time on the induction of invertase, sucrose ranging from 1 mM to 50 mM was added in the biosynthesis regulation medium and then induced for 5 hrs at 50℃ (Fig. 1).

Induction of the invertase was increased with increasing sucrose concentration up to 10 mM and the induction was maximized at 10 mM sucrose. However, the induction was not changed at over 10 mM of sucrose. However, induction of the invertase by raffinose was maximized at 20 mM concentration(data not shown).

Fig. 2 shows the effect of culture time on the invertase

Table. 1. Effects of various sugars on induction of invertase from *Bacillus* sp. TA-11

Sugar (30mM)	Specific activity (Unit/mg protein)
Glucose	3.8
Fructose	3.5
Glycerol	5.2
Maltose	4.9
Raffinose	9.0
Ribose	5.5
Xylose	6.9
Sucrose	23.7
Control(no sugar)	4.9

* After cultivation of *Bacillus* sp. TA-11 in 0.6% yeast extract containing enzyme regulation medium at 50℃ for 18 hrs, the cell was harvested, suspended in enzyme regulation medium (without yeast extract) and sugars were added to a final concentration of 30 mM for further cultivation at 50℃ for 24 hrs.

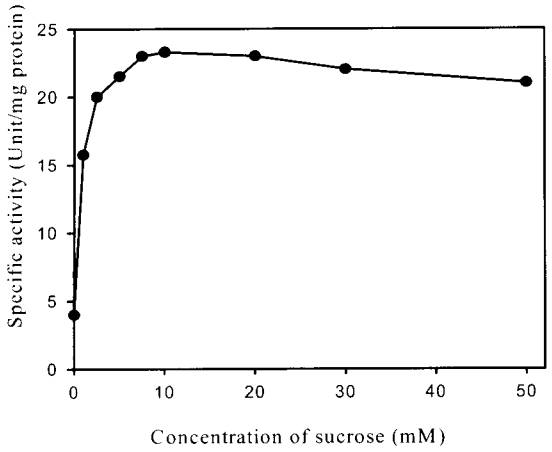

Fig. 1. Effect of sucrose concentration on induction of invertase from *Bacillus* sp. TA-11. After cell harvested from culture, suspended in biosynthesis regulation medium containing 0~50 mM of sucrose and then incubated at 50℃ for 5 hrs.

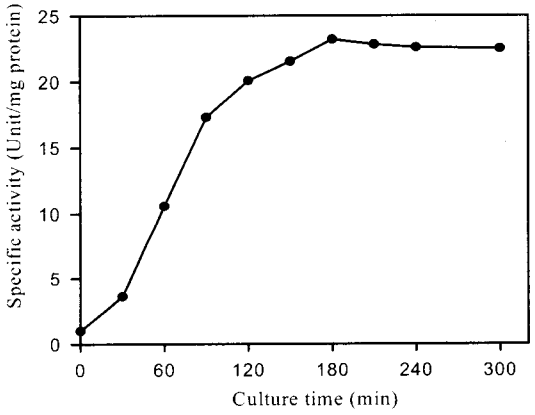

Fig. 2. Effects of culture time on induction of invertase from *Bacillus* sp. TA-11. After cell harvested from culture medium, suspended in biosynthesis regulation medium containing 10 mM sucrose and incubated at 50℃ for 30~300 min.

induction by sucrose. The invertase was induced effectively for 3 hrs of incubation.

2. Repression of the Invertase

The effects of sugars on repression of the invertase were investigated by addition of 30 mM of sugars in sucrose-containing biosynthesis regulation medium and further incubating for 3 hrs at 50℃. As shown in Table 2, glucose inhibited significantly the invertase induction by sucrose.

Table 2. Effects of sugars on repression of invertase from *Bacillus* sp. TA-11

Sugar (30mM)	Specific activity (Unit/mg protein)
Glucose	11.4
Fructose	26.1
Glycerol	20.3
Maltose	24.0
Raffinose	27.1
Ribose	35.0
Xylose	39.4
Control (10mM sucrose)	24.6

* After harvested cell from cultures, suspended in enzyme regulation medium containing 10 mM of sucrose, and then various sugars were added to a final concentration of 30 mM for further incubation at 50℃ for 3 hrs.

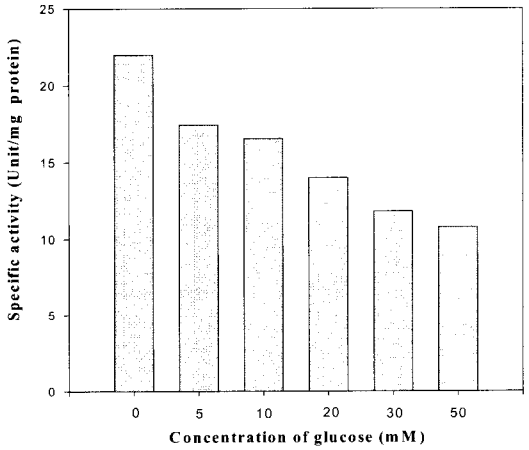

Fig. 3. Effects of glucose concentration on induction of invertase by sucrose in *Bacillus* sp. TA-11. After cell harvested, suspended in biosynthesis regulation medium containing 10 mM sucrose, various concentration of glucose were added and incubated for 3 hrs at 50℃.

Fig. 3 shows the effect of glucose concentration on the induction of invertase by sucrose. The invertase induction was markedly inhibited at 30 mM of glucose.

To investigate the inhibition mechanism of glucose, 30 mM of glucose was added at the different intervals to the sucrose-containing biosynthesis regulation medium and its invertase formation was measured (Fig. 4). As late as addition time of glucose, the invertase formation was increased, indicating that glucose inhibition was occurred by inducer exclusion, that is, interference of glucose to

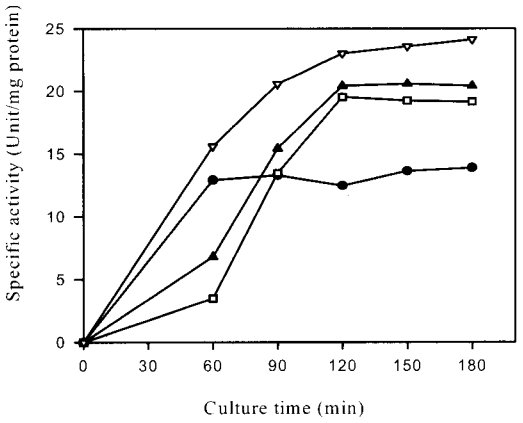

Fig. 4. Effects of addition time of glucose on inductions of invertase by 10mM sucrose in *Bacillus* sp. TA-11. After cell harvested, suspended in biosynthesis regulation medium containing 10 mM of sucrose, glucose was added to a final concentration of 30 mM at various culture time after the start of induction by 10mM sucrose. ▽: Control-sucrose only, ●: Added after 0 min, □: Added after 5 min, ▲: Added after 15 min.

entry of sucrose into cell.

To investigate the effect of cAMP on catabolic repression of the invertase, mixture of 5 mM cAMP and 30 mM glucose was added in 10 mM sucrose- containing biosynthesis regulation medium and then the invertase formation was measured (Fig. 5). Catabolic repression

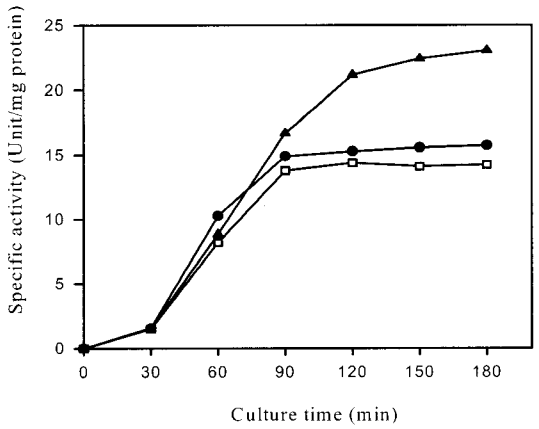

Fig. 5. Effects of cAMP on the catabolite repression of invertase in *Bacillus* sp. TA-11. After cell harvested, suspended in biosynthesis regulation medium containing 10 mM of sucrose, glucose (30 mM) and cAMP (5 mM) were added separately or together and then incubated at 50℃ for 3hrs. ▲: Control-sucrose only, ●: Glucose+cAMP, □: Glucose.

was reduced a little by addition of cAMP for 3 hrs of incubation.

요 약

고온성이며 호알칼리성인 *Bacillus* sp. TA-11이 생성하는 Invertase의 생합성 조절 기작을 규명하고자 먼저 이들의 유도와 억제에 관하여 검토하였다. Invertase는 10mM sucrose을 함유한 생합성 조절배지에서 3시간에 효율적으로 유도되었고 glucose는 sucrose에 의한 invertase 유도를 inducer exclusion 방식으로 억제시켰다. cAMP의 첨가로 glucose에 의한 catabolic repression이 다소 줄어들었다.

References

1. Mybrack, K. : Invertase. The enzyme, P. D. Boyer, H. Lardy, and K. Mybrack (ed.), Academic Press. Inc., New York., Vol. 4, p. 379~396(1960).
2. Straathof, A., Kieboom, A. P. G. and Bekkum, H. : Invertase catalyzed fructosyl transfer in concentrated solutions of sucrose, *Carbohydr. Res.*, **146**, 154~159(1986).
3. Fitzgerald, R. J., Spinell, D. M. and Stoudt, T. H. : Enzymatic removal of artificial plague, *Arch. Oral. Biol.*, **13**, 125~128(1968).
4. Obenland, D., Simmen, U., Boller, T. and Wiemken, A. : Purification and characterization of three soluble invertase from barley leaves, *Plant Physiol.*, **101**, 1331~1339(1993).
5. Pressey, R. : Separation and properties of potato invertase and invertase inhibitor, *Arch. Biochem. Biophys.*, **113**, 667~674(1966).
6. Porntaveewat, W., Takayanagi, T. and Yokotsuka, K. : Purification and properties of invertase from Muscat Bailey A grapes, *J. Ferment. Bioeng.*, **78**, 288~292(1994).
7. Nakagawa, H., Kawasaki, Y., Ogura, N. and Takehata, H. : Purification and properties two types of β-fructofuranosidase from tomato fruit, *Agric. Biol. Chem.*, **36**, 18~26(1971).
8. Krishnan, H. B., Blanchette, J. T. and Okita, T. W. : Wheat invertase, *Plant Physiol.*, **78**, 241~245(1985).
9. Unger, C., Hofsteenge, J. and Sturm, A. : Purification and characterization of a soluble β-fructofuranosidase from *Daucus carota*, *Eur. J. Biochem.*, **204**, 915~921(1992).
10. Kim, B. M. : Studies on invertase from Korean ginseng, *Panax ginseng* C. A. Meyer. *Korean J. Food. Sci. Technol.*, **12**, 1~5(1980).
11. Park, J. B. and Choi, Y. J. : Purification and characterization of inulin fructotransferase from *Arthrobacter* sp.

A-6, *Korean J. Microbiol. Biotech.*, **6**, 402~406(1996).
12. Tanzer, J. M., Brown, A. T. and McInerney, M. F. : Identification, preliminary characterization, and evidence for regulation of invertase in *Streptococcus mutans*, *J. Bacteriol.*, **116**, 192~202(1973).
13. Hirayama, M., Sumi, N. and Hidaka, H. : Purification and properties of a fructooligosaccharides-producing β-fructofuranosidase from *Aspergillus niger* ATCC 20611, *Agric. Biol. Chem.*, **53**, 667~673(1989). .
14. Yanase, H., Fukushi, H., Ueda, N., Maeda, Y., Toyoda, A. and Tonomura, K. : Cloning, sequencing and characterization of the intracellular invertase gene from *Zymomonas mobilis*, *Agric. Biol. Chem.*, **55**, 1383~1390(1991).
15. Fujita, K., Hara, K., Hashimoto, H., and Kitahata, S. : Purification and some properties of β-fructofuranosidase I from *Arhrobacter* sp. K-1, *Agric. Biol. Chem.*, **54**, 913~919 (1990).
16. Dodyk, F. and Rothstein, A. : Factors influencing the appearance of invertase in *Saccharomyces cerevisiae*, *Arch. Biochem. Biophys.*, **104**, 478~475(1964).
17. Neumann, N. and Lampen, J. O. : Purification and properties of yeast invertase, *Biochem.* **6**, 468~475(1967).
18. Kidby, D. K. and Davies, R. : Inverase and disulphide bridge in the yeast wall, *J. General Microbiol.*, **61**, 327~333(1970).
19. Ottolenghi, P. : A comparison of five genetically distinct invertase from *Saccharomyces*, *Eur. J. Biochem.*, **18**, 554~552(1971).
20. Arnold, W. N. : Location of acid phosphatase and β-fructofuranosidase within yeast cell envelopes, *J. Bacteriol.*, **112**, 1346~1352(1972).
21. Babczinski, P. : Partial purification, characterization and localization of the membrane-associated invertase of yeast, *Biochim. Biophys. Acta.*, **614**, 121~133(1980).
22. Montenecourt, B. S., Kuo, S. C. and Lampen, J. O. : *Saccharomyces mutans* with invertase formation resistant to repression by hexoses, *J. Bacteriol.*, **114**, 233~238 (1973).
23. Abrams, B. B., Hckel, R., Mizunaga, T. and Lampen, J. O. : Relationship of large and small invertase in *Saccharomyces* mutant selectively deficient in small invertase, *J. Bacteriol.*, **135**, 809~817(1994).
24. Choi, Y. J. and Lee, J. S. : Production of β-fructofuranosidase from alkalophilic, thermophilic *Bacillus* sp. TA-11, *Korean J. Appl. Microbiol. Biotechnol.*, **23**, 197~202(1995).
25. Martin, I., Debarbouille, M., Ferrari, E., Klier, A. and Rapoport, G. : Characterization of the levanase gene of *Bacillus* subtilis which shows homology to yeast invertase, *Mol. Gen Genet.*, **208**(1-2), 177~184(1987).
26. Fouet, A., Klier, A. and Rapoport, G. : Nucleotide sequence of the sucrase gene of *Bacillus subtilis*, *Gene.*, **45**(2), 221~225(1986).

(2002년 3월 9일 접수)