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Molecular cloning of phospho-β-galactosidase gene of *Lactobacillus casei* in *Escherichia coli*

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*Lactobacillus casei*의 phospho-β-galactosidase 유전자의 대장균내 분자클로닝

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ABSTRACT : Gene for lactose catabolism in *Lactobacillus casei* SW-M1 was encoded by a 60Kb metabolic plasmid. A derivative of only 10kb, pPLac 15 of recombinant plasmid, was constructed by introducing into pBR322 and was cloned into *E. coli* using restriction endonuclease Pst I. A 10kb insert DNA in plasmid pBR322 was identified as a gene encoded phospho-β-galactosidase by the determination of enz yme activity. Phospho-β-galactosidase was apparently expressed in *E. coli*. The enzyme activities of cell-free extract from transformant *E. coli* HB101 carrying pPLac 15 DNA were not different from that of *L. casei* as a donor strain on the basis of enzyme properties. However, specific activity of phospho-β-galactosidase in the cloned strain with Lac Y^- phenotype of *E. coli* HB101 was lower than that in *L. casei* strain.

KEY WORD □ Phospho-β-galactosidase gene, molecular cloning, *Lactobacillus casei*

In lactose utilization of prokaryotes, several strains of *Lactobacillus casei* of different origines contained a β-galactosidase, others a phospho-β-galactosidase and others with both enzymes (Jimeno *et al.*, 1984). The regulation of expression of lactose metabolism genes in procaryotes appears quite complex. Gram-positive bacteria have been shown to transport and phosphorylate many sugars by the phosphoenolpyruvate (PEP)-dependent phos photransferase system(PTS).The genetic result of biochemical studies of lac-PTS in *Streptococcus lactis* by Park and McKay (1982) was proved in favor of a seperate gal-PTS. Chassy & Thompson (1983) reported similar conclusions from on analysis of PTS activities in *Lactobacillus casei*. The lactose metabolism genes are encoded by extrachromosomal elements, *L. casei* 64H contains a 35kb plasmid, pLZ64, that determines both the lactose-PTS and phospho-β-galactosidase activities of the strain (Lee *et al.*, 1982). A shotgun clone bank of chimeric plasmids containing restriction enzyme digest fragments of pLZ64 was constructed in *E. coli* K-12. One clone contained the gene encoding for phospho-β-galactosidase on 7.9kb Pst I fragment cloned into the vector pBR322 in *E. coli* strain (Lee *et al.*, 1982). We cloned the phospho-β-galactosidase gene of *Lactobacillus casei* SW-MI into *E. coli* as the regulation of lactose utilization. In *L. casei* strain SW-MI, pPLac plasmid(60kb), has been identified as encoding genes for lactose catabolism. Here we describe the cloning of this region into *E. coli* and the expression of the lactobacillus gene for phospho-β-galactosidase.

MATERIALS AND METHODS

Bacterial strains and plasmids : *Escherichia coli* HB101 was used as recipient in the cloning experiment. The cloning vehicle included the plasmid pBR322. The *Lactobacillus casei* SW-MI included pPLac plasmid. All strains used in this are listed in Table 1.

Table 1. *Bacterial strains and plasmids*

Strains and plasmids	Relevant genotype or phenotype	Source and reference
Lactobacillus casei SW-M1	Chromosomal *Lac'* ; contains lactose utilization plasmid pPLac	This work
Escherichia coli HB101	F-*hsd* s20 *rec*AB *ara*-14 *pro*A2 *lac*Y1 *gal*K2 *rps*L20(str) *xyl*-5 *al*., 1986 *mtl*-1 *sup*E44 λ	Frederick *et al*., 1986
Plasmids		
pPLac	*Lac'* ; 60kb(39Md)	This work
pBR322	Tc^rAp^r ; 4.3kb	Lee *et al*., 1982

Media and reagents : *L. casei* cultures were grown in TCM (Tomochika medium) by Bae *et al*. (198 5). Materials were obtained from the following sources : o-nitrophenyl-β-D-galactopyranoside 6-phosphate (ONPG-6-P) from Sigma Chemical Co. ; Calf intestinal alkaline phosphatase (CIP), restriction endonucleases and T_4 DNA ligase from Bethesda Research Laboratories, Inc., and Korea Steel Chemical Co.

DNA isolation : Plasmids were isolated by the modification of the method of Maniatis(1982) and then further purified by using the procedure developed by Maniatis (1982) and Klaenhammer(198 4).

Cloning of the phospho-β-galactosidase gene : *L. casei* SW-MI plasmid DNA was digested with restriction endonucleases and ligated to similarly cleaved, calf-intestinal alkaline phosphatase-treated pBR322 plasmid DNA under the restriction conditions recommended by the enzyme suppliers. Preperation of competent *E. coli* HB101 cells and transformations were done as described by Maniatis (1982) and Jagusztyn(1970). The transformation of recombinant plasmids into *E. coli* strains with selection for acquisition of antibiotic resistances.

Preparation of cell-free extract : Phospho-β-galactosidase assay with *E. coli* were carried out with cells grown overnight in 20ml of L. broth supplemented with lactose. Cells were harvested and suspended in 0.1M sodium phosphate buffer, pH 6.8 and washed by centrifugation at 6000 rpm for 10min. The washed cells were resuspended in 1ml of the same buffer and broken by 3 min of sonic disruption with a microtip sonifier probe. Cell debris and insoluble matter were removed by centrifugation at 12000rpm for 30min and the supernatant was used for the assay of phospho-β-galactosidase and protein.

Enzyme assay : Cell-free extract, 0.1ml was added to 50μl of 100mM sodium phosphate buffer(pH 6.8)-10mM ONPG-6-P. The mixture were incubated at 37℃. Cold 1M sodium carbonate, 850μl, was added to stop the reaction. The amount of o-nitrophenol (ONP) released was then determined spectrophotometrically (Hengstenberg *et al*., 197 0). Specific activity was recorded as micromoles of ONP released per milligram of protein per minute. *L. casei* SW-MI phospho-β-galactosidase assays were carried out by the same procedure described in previous paper (Moon *et al*., unpublished).

Protein determination : Protein concentration was

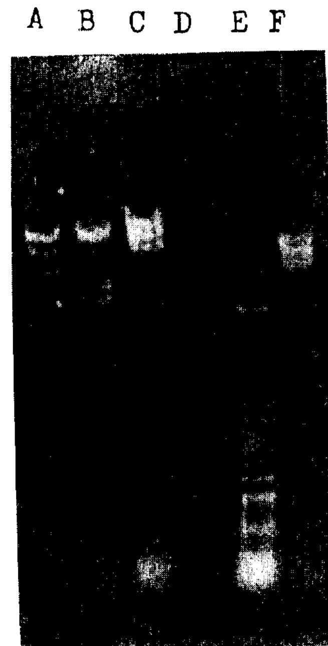

Fig. 1. *Restriction pattern of pPLac by restriction endonucleases* Lane A ; Size marker λ/Hind Ⅲ(*)
Lane B ; Size marker λ/Hind Ⅲ-EcoR Ⅰ
Lane C ; pPLac/Pst Ⅰ
Lane D ; pPLac/BamH Ⅰ
Lane E ; pPLac/Hinf Ⅰ
Lane F ; pPLac/EcoR Ⅰ

determined by the procedure of Lowry *et al.* (1951).

RESULTS

Restriction pattern of pPLac plasmid : As previous paper (Moon *et al.*, 1989), We isolated plasmid pPLac from *Lactobacillus casei* SW-Ml. It was too large to make a restriction map for the cloning of phospho-β-galactosidase gene. On the view point of cloning experiment, it was examined the restriction pattern of pPLac plamid using Pst I, BamH I, EcoR I, Hinf I. The result of restriction pattern of the plasmid was shown in Figure 1 and Table 2. pPLac plasmid was divided into 6 fragments by Pst I, 5 fragments by BamH I, 10 fragments by EcoR I, and 19fragments by Hinf I. On the other hand, pBR322 plasmid as vector DNA was also cut by Pst I, BamH I, EcoR I single site on it to be cloned with foreign DNA fragments.

Cloning fragments of plasmid pPLac into E. coli: Previous work (Moon *et al.*, 1989) had indicated that the restriction endonucleases BamH I, EcoR I, Pst I, and Hinf I would be useful in constructing a gene bank of plasmid pPLac in *E. coli*; the enzymes cut the plasmid into a limited number of fragments, some of which were fairly large. We chose the plasmid pBR322 to use as vector, since they had useful single restriction endonuclease sites in antibiotic resistance genes to permit indirect selection of cloned DNA fragment (Chang *et al.*, 1978). The vector DNAs were treated with Calf Intestinal Phosphatase (CIP) for dephospholylation. Appropriate restricted DNAs were ligated and transformed into HB101 (Figure 2) : transformants were screened for insertional inactivation as shown in Table 2. By the shotgun experiment, 57 clones were obtained. All of these were screened by agarose gel electrophoresis to find the size of their chimeric plasmids; we determinded that for restriction endonuclease Pst I, we had cloned all of the possible

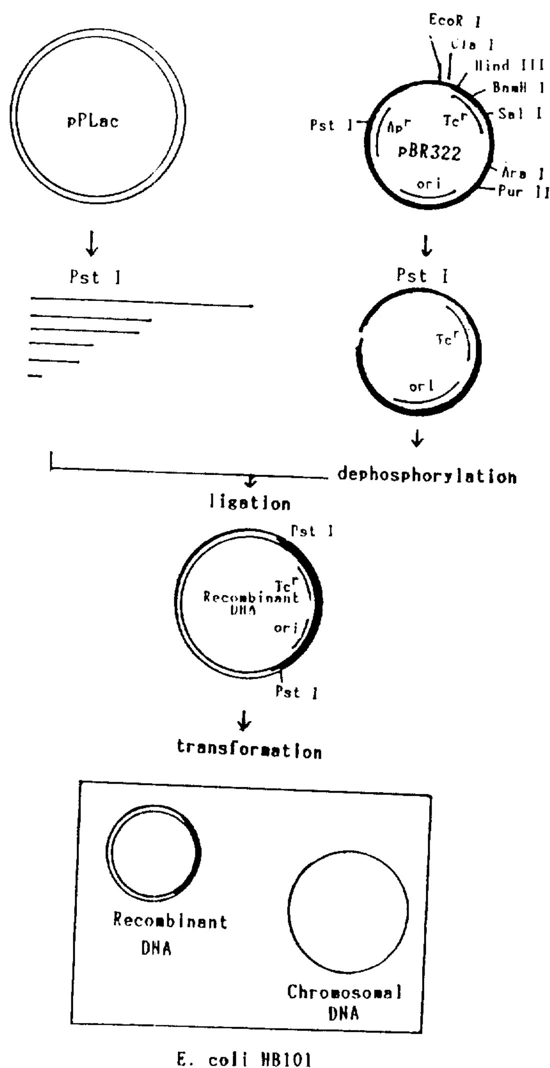

Fig. 2. *Construction of recombinant plasmid DNA.*

Table 2. *Cloning of pPLac restriction fragments into E. coli*

Digestion fragment of pPLac			*E. coli* clone with pPLac fragment		
Restriction endonucleases	No. of fragment	Size range(kb)	Vector plasmid	Phenotype selection*	
				Direct	Indirect
Pst I	6	25 − 0.9	pBR322	Tc^r	Ap^s
BamH I	5	23 − 2	pBR322	Tc^r	Ap^r
Hinf I	19	4.3 −			
EcoR I	10	15 − 1			

*Phenotypes were screened on antibiotic media for senssensitive or resistant to tetracycline(Tc) and ampicillin(Ap).

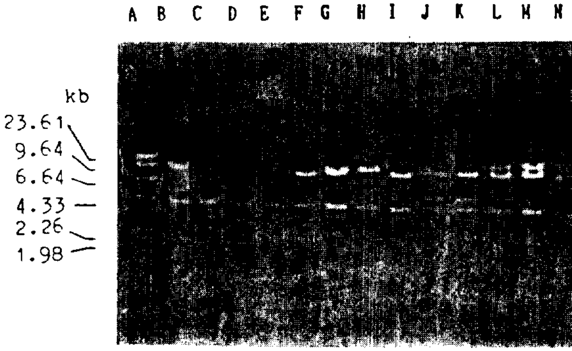

Fig. 3. *Restriction pattern of transformant clones by Pst I.*
Lane A : Size marker λ-Hind III
Lane B : pPLac-Pst I fragments
Lane C : pBR322-Pst I DNA
Land D-N : Transforments with pPLac 6, 36, 15, 21, 51, 1, 22, 20, 40 and 39

Table 3. *Specific activity of phospho-β-galactosidase in transformants*

Transformants	*Sp. activity of p-β-galactosidase (μmole/min/mg protein)×10^{-1}
E. coli HB101	<
pPLac 15/*E. coli* HB101	2.98
pPLac 3/*E. coli* HB101	<
pPLac 21/*E. coli* HB101	<
pPLac 36/*E. coli* HB101	<
pPLac/*L. casei* SW-M1	7.40

* Expressed as micromoles of 0-nitrophenol released per minute per miligram of protein.
Abbr. : Sp ; Specific.

restriction fragments of pPLac except the largest (Table 2).

Identification of phospho-β-galactosidase clone : As shown in Figure 3, lane B showed pPLac-Pst I fragment and PBR322-Pst I DNA in lane C. Lane D to lane N were transformants with pPLac fragments. All Pst I clones were screened for evidence of phospho-β-galactosidase activity. Only on clone produced a strong yellow color from the ONPG-6-phosphate substrate ; the recombinant plasmid in this clone was named pPLac 15. This plasmid contained the 10kb Pst I C fragment (third largest one) of pPLac cloned into the pBR322 β-lactamase gene (Figure 3).

Specific activities of phospho-β-galactosidase : The presence of phospho-β-galactosidase activities in transformants, which were cloned with derivatives of plasmid pPLac, were tested using cell-free extracts. Enzyme activity was detected only for plasmid pPLac 15 in *E. coli* HB101, and this recombinant DNA was defined a region between Pst I site as encoding the gene for phospho-β-galactosidase. Specific activities were calculated for *E. coli* & *L.casei* cell-free extracts of the positive strains. As shown in Table 3, enzyme activity of transformant

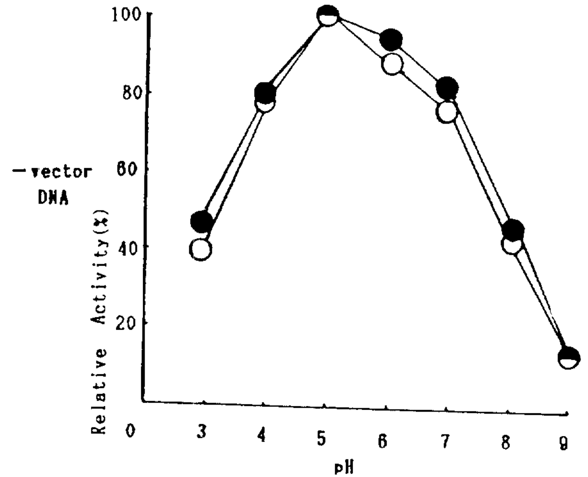

Fig. 4. *Effect of pH on phospho-β-galactosidase activities in the cell-free extract from Lactobacillus casei SW-M1(●) and pPLac 15/E. coli HB101(○).*

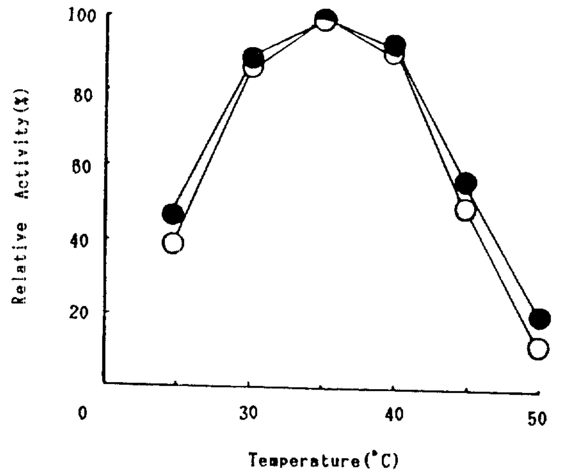

Fig. 5. *Effect of temperature on phospho-β- galactosidase activities in the cell-free extract from Lactobacillus casei SW-M1(●) and pPLac 15/E. coli HB101(○).*

of pPLac 15/*E. coli* HB101 was specially expressed apparently to be 2.98 units $x10^{-1}$ per minute per mg protein, while those of phospho-β-galactosidase of other transformants ; pPLac 3/*E. coli* HB101, pPLac 21/*E. coli* HB101 and pPLac 36/*E. coli* HB1 01 were not expressed like those of recipient strain, *E. coli* HB101. Therefore, it was found that pPLac 15/*E. coli* HB101 was cloned the gene of phospho-β-galactosidase of *L. casei* SW-Ml. However, specific activity of phospho-β-galactosidase in pPLac 15/*E. coli* HB101 was lower than those of enzyme in *L. casei* SW-Ml strain. This phenomenon will be explained at the discussion.

Properties of phospho-β-galactosidase : To compared with phospho-β-galactosidase of *E. coli* HB1 01 carrying pPLac 15 & *L. casei* SW-Ml contained pPLac plasmid, enzyme properties were examined on the basic study. Optimum pH and temperature were tested with the cell-free extracts of *E. coli* HB1 01 strain and *L. casei* SW-M1 carrying gene for phospho-β-galactosidase strain. The result of this experiment was shown in Figure 4 and 5. Optimum pH of enzyme from the extracts for two strains was to be 5.0 and optimum temperature of phospho-β-galactosidase of those of two strains was 35℃. It has been suggested that these two results showed the evidence of same gene for phospho-β-galactosidase.
ase, was found to depend upon the presence of a region of plasmid pPLac. Enzyme activity was

DISCUSSION

Activity of the key lactose splitting enzyme in *Lactobacillus casei* SW-M1, phospho-β-galactosid- correlated with the presence of a 60kb plasmid that was identified after gene expression of DNA cloned in *E. coli*. This enzyme protein is in good agreement with 56KD phospho-β-galactosidase from *L. casei* that has been purified, and also cloned into *E. coli* (Lee *et al.*,1982). Even if species name was the same *L. casei*, one plasmid of *L. casei* SW-Ml ; pPLac was different size compared with that of *L. casei* 64H. Therefore, they cloned 7.9kb Pst I fragment of pLZ64 into *E. coli* x1849, but our cloning experiment was fragments pattern of pPLac by restriction endonuclease was apparently different from that of pLZ64

As has been found for other lactic streptococci (McKay *et al.*, 1970), the phospho-β-galactosidase enzyme activity of *S. lactis* 712 was induced by growth in presence of lactose or galactose. When the gene was clone into *E. coli* only about 4% of the fully induced level of activity was found, inducibility could not be demonstrated. They assumed that one possible reason for lack of induction was the Lac Y^- phenotype of *E. coli* HB101.

In our result, the enzyme activity of the clone with pPLac 15DNA fragment was lower in *E. coli* than that in *L. casei* strain. It was probably because the correct inducer molecule is not produced in that host or because a control component(s) has been deleted during cloning of the phospho-β-galactosidase.

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적 요

Lactobacillus casei SW-Ml의 pPLac plasmid DNA에 유당 분해 효소인 phospho-β-galactosidase gene이 존재함을 보고한 바 있다(Moon *et al.*, 1989). pPLac plasmid 의 restriction endonuclease pattern을 비교 고찰한 결과, Pst I에 의해서는 6개, BamH I에 의해서는 5개, EcoR I에 의해서는 10개 등의 절편으로 절단됨을 확인하였다. pPLac plasmid의 Pst I 절편들을 pBR322 vector의 Pst I site에 insertion하여 shot-gun experiment에 의하여 *E. coli* HB101내에서 phospho-β-galactosidase gene의 expression을 확인 할 수 있었다. Transformants 중 pPLac 15의 경우는 10kb가 insertion된 recombinant plasmid로 확인되었으며, 이들 cloned strain의 효소활성을 측정한 결과 높은 활성을 보여주었지만 모균주 보다는 낮은 활성을 나타내었다. 이것은 *E. coli*내에서 높은 phospho-β-galactosidase gene이 손상을 입었던가 또는 promoter나 insert된 size 관계로 나타난 것이 아닌가 사료된다. *L. casei*와 *E. coli* transformant 의 phospho-β-galactosidase활성은 동일한 최적 pH와 온도를 나타내었다.

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