Properties of β -Galactosidase from *Lactobacillus zymae* GU240, an Isolate from *Kimchi*, and Its Gene Cloning

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Lactobacillus zymae GU240 was previously isolated from *Kimchi*, a Korean fermented vegetable, as a strong GABA producer. The strain showed β -galactosidase (β -Gal) activity on MRS agar plates with X-gal. When growth and β -Gal activities of GU240 were measured using MRS (glucose, 2%, w/v) and MRSL (lactose, 2%, w/v) broths, cells were found to grow slowly in MRSL, and the β -Gal activity (36 units at 4 h) was lower than that of cells grown in MRS (94 units at 16 h). The highest OD₆₀₀ value of the culture in MRS was 1.6 at 24 h at 37°C, whereas that of the culture in MRSL was 0.6 at 16 h. β -Gal activity of the culture in MRS reached the maximum (95.6 u/ml) at 16 h, decreased thereafter, and was not detected at 48 h. β -Gal activity for culture in MRSL reached its highest (36 u/ml) at 4 h and decreased gradually, but some activity (11.05 u/ml) still remained at 72 h. The structural gene encoding β -Gal in *L. zymae* GU240 was cloned as a 3.1 kb fragment, and DNA sequencing confirmed the presence of complete *lacLM* genes. *lacLM* genes from *L. zymae* GU240 showed 98–99% homologies in nucleotide sequences with other *lacLM* genes from *L. brevis*. Reverse transcription (RT)-PCR confirmed the operon structure of *lacLM*. The results indicated that *L. zymae* GU240 might be in the process of losing the ability to grow rapidly on lactose-containing medium, such as milk, due to adaptations to plant environments, including kimchi.

Keywords: *Lactobacillus zymae* GU240, β -galactosidase, *lacLM*, *Kimchi*

Introduction

β -galactosidase (β -D-galactoside galactohydrolase, EC 3.2.1.23), most commonly known as lactase, is a member of the family of glycosyl hydrolases that are known to catalyze the hydrolysis of the β -1,4-D-galactosidic linkage of lactose to its constituting monosaccharides, glucose and galactose [1].

β -galactosidase (β -Gal) is an important enzyme for lactic acid bacteria (LAB), especially for starters used for production of dairy products. One of the important prerequisites for lactic starter is the ability to utilize lactose in milk, and quickly produces acids such as lactate and

acetate, which contributes to preservation and flavor of dairy products [2]. Many studies have been done on β -Gals and the structural genes from various LAB. Two types of β -Gals, β -Gal and phospho- β -Gal, exist among LAB depending upon employed lactose transport systems [3]. In a Lac permease system, lactose enters into cell as an unmodified form and hydrolyzed by β -Gal into glucose and galactose. In a PEP (phosphoenol pyruvate)-PTS (phosphotransferase) system, lactose is phosphorylated during transport and hydrolyzed by phospho- β -Gal into galactose 6-phosphate and glucose inside cells [4]. Some β -Gals from LAB are heterodimers consisting of a large subunit (72 kDa) encoded by *lacL* and a small subunit (35 kDa) encoded by *lacM* [5, 6]. β -Gal from *Escherichia coli* is a homotetramer consisting of a 110 kDa subunit encoded by *lacZ* [7].

β -Gal gene from *E. coli* is used in molecular biological

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researches as a reporter gene [8]. β -Gal genes from various sources are used for removal of lactose from milk in food industry and production of galactooligosaccharides [1, 9]. For heterologous gene expression among LAB, a gene involved in lactose transport is used in a food-grade expression vector as a selection marker [10, 11].

β -Gal gene from LAB isolated from fermented vegetable foods are rarely studied because plants do not contain lactose in significant amount. In this report, we studied β -Gal activity of *Lactobacillus zymae* GU240, an isolate from *Kimchi*, producing GABA in large amount [12]. We also cloned the structural gene, and examined the structure of β -Gal gene. The strain and the β -Gal gene might be useful as a probiotic or a starter for foods containing lactose.

Materials and Methods

Bacterial strains and culture condition

L. zymae G9240 was grown on MRS broth (lactobacilli MRS, BD Difco, USA) and MRS agar (2%, w/v) without shaking at 37°C. When growth was tested on lactose as a carbon source, MRSL was used where all components were the same with MRS except glucose was replaced with lactose (Sigma-Aldrich, USA). *E. coli* DH5 α was grown in Luria-Bertani broth (LB) at 37°C with shaking. When cultivating cells with a plasmid, ampicillin (50 μ g/ml) was included.

Determination of β -gal activity

The enzyme activity was measured according to Miller's method [13]. *L. zymae* GU240 was grown in MRS and MRSL broth at 37°C. Aliquots were taken and cells were harvested by centrifugation (8000 $\times$ g, 10 min at 4°C). After washing twice with sterile water, cell pellet was resuspended with Z-buffer (pH 7.0, 1.61 g of $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, 0.55 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.075 g of KCl, 0.0246 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.27 ml of β -mercaptoethanol, adjust the volume to 100 ml with sterile water). Cells were disrupted by sonication using an ultrasonicator (Bandelin Electronic, Germany). Cells were sonicated for 1 min followed by pause for 1 min on ice (1 cycle), and total 5 cycles were done for each sample. Disrupted cells were centrifuged at 12,000 $\times$ g for 20 min to collect supernatant. β -gal activity was determined by using *o*-nitrophenyl- β -D-galactopyranoside (Sigma-Aldrich) as the substrate at

28°C. After yellow color appeared, 0.5 ml of 1 M sodium carbonate was added to stop the reaction. Absorbance was measured at 420 nm and 550 nm with a spectrophotometer. β -Gal activities were calculated by this following method:

$$\beta\text{-Gal Miller unit (U)} = 1000 \times (\text{OD}_{420} - 1.75\text{OD}_{550}) / (\text{T} \times \text{V} \times \text{OD}_{600}).$$

OD_{420} and OD_{550} were the values of the reaction mixture, and OD_{600} was the cell density of the washed cell suspension. T was the reaction time (min), and V (ml) was the volume of culture used in the assay.

Cloning of *lacLM* genes

L. zymae GU240 was grown in MRS broth overnight at 37°C. Cells were harvested by centrifugation (12,000 $\times$ g, 10 min, 4°C), and resuspended in 1 ml of lysis buffer (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100 mM NaCl, 2% SDS) with 40 μ l of proteinase K (Takara, Japan, 600 U/ml). After incubation for 30 min at 55°C, cell lysate was extracted with phenol and chloroform followed by ethanol precipitation. After 70% ethanol washing, DNA was dissolved in small volume of sterile water [14].

To amplify *lacLM* genes from *L. zymae* GU240, a primer pair was used: LMF (5'-CGGTGCGTCCCTACACATA-3') and LMR (5'-GACGTACTAATTTGCAATTTGCCATC-3'). Primers were designed based on the known *lacLM* sequences from National Center for Biotechnology Information (NCBI). PCR reaction mixture (50 μ l) contained 2 μ l of template DNA (20 ng/ μ l), 2 μ l of each primer (10 μ M), 5 μ l of deoxynucleoside triphosphates (0.25 mM), and 0.5 μ l of Ex *Taq* DNA polymerase (Takara). The amplification conditions were as follows: 94°C for 2 min; 30 cycles at 94°C for 40 s, 53.7°C for 40 s, 72°C for 1 min; a final extension at 72°C for 6 min. PCR product was examined by agarose gel electrophoresis using a 1% (w/v) agarose gel and stained with EcoDye™ DNA staining solution (Biofact, Korea).

Amplified fragment was ligated with pGEM T-Easy vector (Promega, USA). *E. coli* DH5 α competent cells (Enzynomics, Korea) were used for transformation. Competent cells were treated by heat-shock at 42°C for 30 s and left on ice for 2 min. Then 400 μ l of SOC medium (20 g tryptone, 5 g yeast extract, 4.8 g MgSO_4 , 3.603 g dextrose, 0.5 g NaCl, 0.186 g KCl, per liter) was added and cells were incubated for 1 h at 37°C with shaking.

Aliquots (100 μ l) were spreaded onto LB agar plates with ampicillin (100 μ g/ml) and spreaded with 100 μ l of 100 mM IPTG (isopropyl- β -D-thiogalactoside) and 20 μ l of 50 mg/ml X-Gal (5-bromo-4-chloro-3-indoxyl- β -D-galactopyranoside). Plates were incubated overnight at 37°C. Transformants were screened to identify cells harboring recombinant plasmid with *lacLM* genes. Plasmid DNA from *E. coli* DH5 α was purified using DNA-spinTM plasmid DNA purification kit (iNtRON Biotechnology, Korea). Nucleotide sequencing was done for a recombinant, and the sequences were analyzed using BLAST.

RT-PCR experiment

RNA sample was prepared from *L. zymae* GU240 grown in MRS broth for 24 h by Trizol/bead method [15]. RNA sample was treated with DNase, RQ1 RNase-free (Promega), and RT-PCR was done using one-step RT-PCR premix kit (iNtRON Biotechnology). The reaction mixture consisted of 8 μ l of premixture, 1 μ l of forward primer, 1 μ l of reverse primer, 2 μ l of RNA (1 μ g), and 8 μ l of water. The reaction was started by 30 min incubation at 45°C, followed by initial PCR denaturation at 94°C for 5 min. PCR cycles consisted of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. A total of 29 cycles were repeated, and the final extension was done at 72°C for 5 min. *lacL* was amplified by using primer A (5'-AGTCATGT GCT-TAAACG GTCAGCG-3') and B (5'-ACCCACCCTGAT ACATTGGGTATTGG-3'), and *lacM* was amplified by using primer C (5'-AAGACGGC AAGGAATGGTTGTACC-3') and D (5'-GCATTGTTTT GTGTGGTCCCTCGT-3').

Overexpression of *lacLM* in *E. coli*

lacLM gene was subcloned into pET26b(+) (Novagen, USA) after PCR product was digested with *Nco*I and *Xho*I. The recombinant (pETlacLM) was introduced into *E. coli* BL21(DE3) by electroporation. *E. coli* BL21(DE3) with pETlacLM was grown in LB until the OD₆₀₀ reached 0.6, then IPTG was added (1 mM), and the culture was grown for additional 12 h. Cells were recovered by centrifugation (12,000 $\times$ g, 10 min, 4°C), and resuspended with 4 ml of phosphate buffered saline (PBS, 10 mM PO₄³⁻, 137 mM NaCl, 2.7 mM KCl, pH 7.4). Cells were disrupted by sonication as describe above, and centrifuged. Supernatant (soluble fraction) and pellet (insoluble fraction) were obtained. Insoluble fraction was dissolved with

3 ml of PBS. Soluble and insoluble fractions were analyzed by SDS-PAGE. 10% acrylamide gel was used and SDS-PAGE was done as described previously [12].

Results and Discussion

Growth of *L. zymae* GU240 on glucose and lactose

L. zymae GU240 was grown in MRS (2% glucose, w/v) and MRSL (2% lactose, w/v) for 72 h at 37°C, and growth (OD₆₀₀) and β -Gal activities were measured, respectively (Fig. 1). *L. zymae* GU240 grew rapidly after 4 h. In MRSL, the highest OD₆₀₀ was 0.6 at 16 h whereas the highest value was 1.6 at 24 h in MRS broth. *L. zymae* GU240 grew quicker in MRS than in MRSL, and the β -Gal activities of culture grown on MRS were also higher than those of culture on MRSL. One observation was that β -Gal activity of culture on MRS increased, and the highest activity of 95.6 U was observed at 16 h. But the activity decreased rapidly after 16 h, and not detected at 48 h and thereafter. Whereas β -Gal activity of culture in MRSL increased rapidly and reached the highest point (36 U) at 4 h, and then decreased until 8 h (21 U). The activity decreased gradually after 8 h, but remained relatively stable, and significant activity (11.7 U) was still observed at 72 h. The result indicated that β -Gal production, a result of β -Gal gene expression, is apparently not tightly repressed by glucose in MRS at least until 48 h. This is contrary to the known phenomenon of glucose repression of genes involved in utilization of alternative carbon sources such as lactose [16]. It is noteworthy that although *L.*

Fig. 1. Growth and β -Gal activities of *L. zymae* GU240 in MRS and MRSL broth at 37 °C. -●-, OD₆₀₀ in MRS; -○-, β -Gal activity in MRS; -■-, OD₆₀₀ in MRSL; -□-, β -Gal activity in MRSL.

zymae GU240 grew slowly in MRS^L compared to in MRS as shown by OD₆₀₀ values, β -Gal activity of culture in MRS^L reached the highest point at 4 h, quite earlier than that of culture in MRS (16 h), and the activity remained stably until 72 h. These results indicated that β -Gal production in GU240 was induced by lactose, but not tightly repressed by glucose in MRS.

The results were contrary to those observed in *Lactococcus lactis* A2 [17] and *L. lactis* spp. *lactis* ATCC7962 [18]. In *L. lactis* A2 and 7962, maximum activity of β -Gal was observed in cells grown on lactose at 30°C, 210 U at 7 h and 325 U at 4 h, respectively. But cells grown on glucose showed very low activity, 2–3 U for A2 and 3.6 U for 7962. The reason why *L. zymae* GU240 showed higher activities on glucose is not clear. The lack of an efficient lactose transport system in *L. zymae* GU240 might be one reason for poor growth of *L. zymae* GU240 on lactose. Since lactose is not a common sugar in plant environments, *Lb. zymae* GU240 might have a problem for efficient lactose utilization. Further studies on lactose transport of *L. zymae* GU240 are necessary to answer the question.

Cloning of *lacLM* genes from *L. zymae* GU240

A 3.1 kb fragment was amplified (Fig. 2), and a recombinant plasmid, pGEM-TLM was obtained. Nucleotide sequence analysis confirmed that the fragment contained genes homologous to known *lacLM* genes. Nucleotide sequences and translated amino acid sequences are shown in Fig. 3. The sequences were deposited into GenBank under the accession number MN188052.

Cloned *lacLM* gene is 2,836 bp in length and the nucleotide sequences are underlined. The ORF of LacL and LacM were located by using ExpASY tool (<https://web.expasy.org/translate/>). The first codon of lacL gene is TTG, starting at 112th and ending at 1,998th, capable of encoding a protein of 628 amino acids. The first codon of *lacM* gene is ATG, starting at 1,982nd and ending at 2,947th, capable of encoding a protein of 321 amino acids. One special feature of *lacL* and *lacM* genes is that both genes overlap partially, 17 nucleotides in length. The first codon of *lacM* (ATG) locates within the ORF of *LacL*, very close to the stop codon of *lacL*. But their reading frames are different. This kind of gene overlapping is also observed for other LAB [5, 19]. The pI and molecular mass of LacL and LacM are 4.80/71,673.73 Da and

Fig. 2. PCR amplification of *lacLM* genes. M, GeneRuler 1 kb DNA Ladder (Thermo Fisher Scientific, SM0311); lane 2, PCR product.

5.26/34,569.56 Da, respectively.

lacLM showed 98–99% identities in nucleotide sequence to those of homologous genes from *L. brevis* SRCM101106 (CP021674, 2,834/2,836), *L. brevis* ATCC367 (CP000416, 2,834/2,836), *L. brevis* BSO464 (CP005977, 2,821/2,836), *L. brevis* TMW1.2111 (CP019743, 2,798/2,836), *L. brevis* NPS-QW-145 (CP015398, 2,796/2,836), and *L. brevis* KB290 (AP012167, 2,792/2,836). The high homologies were expected because *L. zymae* is very similar to *L. brevis* except few differences [12].

The deduced amino acid sequences of LacL were shown above the nucleotide sequence and the deduced amino acid sequences of LacM were shown below the nucleotide sequences. The stop codons were marked as a short line after the amino acid sequences. Amino acid sequences were aligned with those of other homologous enzymes (Figs. 4 and 5).

RT-PCR experiment

RT-PCR was done for RNA sample to confirm the operon structure of *lacLM*. The expected amplicon size was 677 bp for *lacL*, and 577 bp for *lacM*. Both amplicons with the matching sizes were obtained (Fig. 6B, lanes 2–3, and 6–7). Also, a transcript covering both *LacL* and *LacM* was detected (Fig. 6B, lanes 4 and 8), confirming the operon structure of *lacLM* genes.

AAGTATAAATCTAAACGGGACGGGGCAGTCAATTTACTTTGATGGTTGTTGCGATAACCCGGATTCCGGTATAATGGTCAGTGAAGCGTT	90
<u>M K A D L T W L D D P Q T F R I N Q L P A H S</u>	23
TTATAAAAGGAGGAAGTAGCTTGAAGCAGATCTTACTTGGTAGATGATCCGCAGACATTTCCGATCAATCAACTACCAGCGCACAGT	180
<u>D H R G Y A S V E E A T A Q H S S L V Q S L D G T W Q F A F</u>	53
GATCACCGTGGGTATGCTTCAGTTGAGGAAGCCACCGCGCAGCAGTAGCTGGTTCAAAGTCTCGACGGGACCTGGCAATTTGCCCTTT	270
<u>A P D P V H R F E G F Y Q P D Y D R S A F D R L T V P G H I</u>	83
GGCCAGACCCAGTGCATCGATTGAAGGCTTCTACCAACCTGATTATGACCGTTACGCCCTTGGATCGGCTGACGGTGCCAGGTCAATTT	360
<u>E L A G Y G Q I Q Y I N T A Y P W E G H H Y R R P A Y S M G</u>	113
GAGCTAGCTGTTATGGTCAGATTGAGTATATCAATACCGCTATCCGTGGGAGGGGATCATTATCGACGACCGGCTTACAGTATGGGC	450
<u>A D Q P E E G M F S T D P Q N T V G A Y V K H F T L N P A L</u>	143
GCTGATCAGCCAGGAAGAAGGGATGTTTAGTACGGATCCCAAAATACAGTTGGGGCTTACGTCAAGCACTTTACCTTGAACCCAGCGCTA	540
<u>A N Q R V S I E F D G V E Q A M F L W L N G Q F V G Y A E D</u>	173
GCCAATCAACGGGTAGCATTTGAATTTGACGGTGTGGAACAGGCCATGTTTCTCTGGCTCAACGGCCAGTTTGTGGGCTACGCCGAAGAT	630
<u>S F S R S E F D L T P Y L Q A G Q N L L A V E V F K H S T A</u>	203
AGCTTTAGCGCTTGAATTTGATCTGACGCCCTTATTACAGGCCGGGCAAAATTTATTGGCGTGGGAAGTCTTTAAACATAGTACGGCC	720
<u>A F L E D Q D M F R F S G I F R S V R L V A K P E L H V E D</u>	233
GCTTTTTTGAAGACCAAGATATGTTCCGGTTTTCCGGGTATCTTCCGGAGTGTTCGGTTGGTTGCCAAGCCGAGCTGCATGTAGAAGAT	810
<u>L T I R A G L D D A F Q T G D L K V R L Q L T A A S Q L S G</u>	263
TTAACCATTCGAGCTGGTTTAGTACGCGCTTTCAGACAGGTGATTTAAAGTGGCTTGCAACTGACGGCTGCTAGCCAACTTTCCGGG	900
<u>T A T A Q L L T A D G Q E V W A T E Q P A A S T L D L A A A</u>	293
ACTGCGACGGCGCAACTACTAACGGCCGATGGTCAAGAAGCTGGGCAACTGAGCAACCGCGCGTAGTACGTTAGACTTGGCGGCCGCG	990
<u>I D H V H L W D H H D P Y L Y Q L R I T L K D V A G Q V V E</u>	323
ATTGATCAGCTCCACCTGTGGGATCACCACGATCCTTACCTTTACCAATTCGCGATTACTTTAAAGATGTTGCCGGTCAAGTGGTGGAA	1080
<u>V V P Y P V G F R R I E L K D K V M C L N G Q R L I L N A V</u>	353
GTGCTCCCTTATCCAGTGGGCTTCCGGCGCATTAAGTCAAGATAAAGTATGCTGCTTAAACGGTCAAGCGGTAAATTTTAAATGGTGTG	1170
<u>N R H E W D A H R G R A V T M A D M T Q D L Q T F H D N H I</u>	383
AATCGCCACGAATGGGATGCGCATCGTGGCGGGCCGTGACTATGGCGGACATGACACAGGATCTCCAACTTTTCAGGACAACCATATC	1260
<u>N A D R A K S N Y E M F K N H P A V L F W S L G N E S Y A G D</u>	413
AATGCGGTGCGGACGTGCCACTATCCGACCAAGATGCTGGTATTACTTATGTGATCAGCAAGGTATTACATGATGGCTGAAAATAAC	1350
<u>L E T H G T W Q K M G A V E P S Y N V P G S L P Q W Q L A V</u>	443
TTGGAGACGCGCAAGTATGGCAAAAAATGGGGGCTGTGGAGCCGTCTTACAATGTGCCAGGAAGCTTGCCCAATGGCAGTTAGCCGTG	1440
<u>L D R A K S N Y E M F K N H P A V L F W S L G N E S Y A G D</u>	473
CTTGATCGGGCAAGAGTAAGTATGAGATGTTTAAAGATCATCAGCCGTTTATTCTGGTCACTGGGGAACGAATCCTATGCTGGCGAT	1530
<u>N I A A M D A F Y H H A D P T R L T H Y E G V C R N R V Y E</u>	503
AACATCGCGCAATGGATGCTTCTACCAACCGCCGATCCGACGCGCTTAACTCATTATGAAGGGGTGTGCGGTAAATCGGCTATGAA	1620
<u>D R I S D M E S M M Y D P P R A I E D Y L K N D P Q K P F V</u>	533
GATCGCATCTCGGATATGGAAGTATGATGATGATCGCCACGTGGGATCGAAGATTACCTTAAAAACGATCCCGAGAAACCCCTTTGTC	1710
<u>N C E Y M H D M G N S L G M A S Y D A L I D Q Y P M Y Q G</u>	563
AATTGCGAGTACATGACATGGGCAACTCACTGGCGGGATGGCCAGTTATGATGCGTTGATGACCAATACCCAATGTATGACGGT	1800
<u>G F I W D F I D Q A L W V K D E V T G Q P V L R Y G G D F D</u>	593
GGGTTTATTGGGACTTCATTGACCAAGCCTTGTGGGTTAAAGATGAGGTTACGGGACAGCCAGTACTGCGGTATGGCGGTGACTTTGAC	1890
<u>D R H S D Y E F S G D G L L F A D R T P K P A L Q E V D Y T</u>	623
GATCGCCATTCTGATTATGAATTTCCGGTGACGGGCTGTTATTTGCTGACCGGACACCAAGCCAGCTTTACAGGAGGTGGACTATTAC	1980
<u>Y G Q H D - 628</u>	
TATGGACAACACGACTAAGTTAAACGTGATTTTGGTGACGTGACGTTAGGGGTCAGCGGACAGGTTTTTCATTACATTTTTCGCTATGA	2070
<u>M D N T T K L N V I F G D V T L G V S G P G F H Y I F A Y D</u>	30
TCGCGCGCGCTTGGAAATCCTTGGTGCAAGACGGCAAGGAATGGTTGTACCGGACGCCGATGCCAGCGTTGTGGCGAGCCAGCAGGATAA	2160
<u>R G G L E S L V Q D G K E W L Y R T P M P A L W R A T T D N</u>	60
TGACCGTGGCAATGGATTTTCGACTAAGTCCGCCCAAGGGCTAGGGGCAGATCTCTTTAGCAGCTGCGATCATATTAGCGTGGCAATGTA	2250
<u>D R G N G F S T K S A Q R L G A D L F S S C D H I S V A I D</u>	90
CGGCCAATCAATTCGTTACCGATTGCCCTGAAAAATACCGCTATTCCGATCAGCAAAACGGCTACAACGTGGCTGTGACGTTTACCTA	2340
<u>G Q S I P L P I A P E N N R Y S D H E T A T T V A V T F T Y</u>	120
TACCACCAACGACGCGACGCAACCATTCGGGTGACCTACACGGTAGCTGCTAGTGGGCGATGACGGTTGCGGTACACTATGCAGG	2430
<u>T T P T T P A T T I A V T Y T V A A S G A M T V A V H Y A G</u>	150
CAAGGCGGACTTGCCGGAATGCCAGCGCTGGGGTTGCGATTAGTCATGCCGACGCCAGCTTAGGATTTGCTTACCAGGGACTTTCCGG	2520
<u>K A D L P E L F A L G L R L V M P T P A L G F A Y Q G D Y S</u>	180
GGAAACGATTCGGATCGCAAGCCGGTGGCCGTAAAGGGATTACCAAGGTAGCTGGCTGCCGTAACCCCTTATCTGGTGCCGCAAGA	2610
<u>E T Y P D R K A G G R K G I H Q V A G L P V T P Y L V P Q E</u>	210
GTGCGGTATGCAGCTCGCAACAGTGGGTGACGTTGACACGAGGACACACAAACAATGCCGATGCTGACCAAGATGCTTTAGCCT	2700
<u>C G M H V D N Q W V T V T R G T T Q N N A D A D H D A F S L</u>	240
AAAAGTCCGGCAACGACGACCACTTTGCGTTTAGTTGCTTACCTATACGCCAACGGAATTAGAAAATGCGACGCAACCAAGAGGAGTT	2790
<u>K V R Q T Q H H F A F S C L P Y T P T E L E N A T H Q E E L</u>	270
GCCGGTGGCCGCGCGACAGTTTACGCGCGCTTCCGGGAGTCCGGGGAATCGATAGTTGGGGCTCCGATGCGGAAGCTCC	2880
<u>P V P R R T V L T I Y G A V R G V G G I D S W G S D A E A P</u>	300
TTACCACATTGCTGGTGACAGCGACCACTTTAGCTTTGAAATTCGCCGGCCAAACCGCGTTTAAACGATTAAAAAAGAGTCAGGG	2970
<u>Y H I A G D S D H D F S F I A G P T P V - 321</u>	
ATCAATTCGCGGCTCTTTTGTGCGGCAATATCAATCAAGTGAACCTTTTATGGTAAGATTGACTAAAAGACAAGCAATAGTTAA	3060
<u>T</u>	3061

Fig. 3. Nucleotide sequence of *lacLM*. Nucleotide sequences of ORFs are underlined. Translated amino acids are shown above the nucleotides (LacL) or below the nucleotides (LacM). The first codon of *lacL* gene is TTG, starting at 112th and the first codon of *lacM* gene is ATG, starting at 1,982nd.

GU240	MKADLTWLDDPQTFRINQLPAHSDHGRGYASVEEATAQHSSLVQSLDGTWQFAFAPDPVHR	60
SRCM101106	MKADLTWLDDPQTFRINQLPAHSDHGRGYASVEEATAQHSSLVQSLDGTWQFAFAPDPVHR	
ATCC 367	MKADLTWLDDPQTFRINQLPAHSDHGRGYASVEEATAQHSSLVQSLDGTWQFAFAPDPVHR	
BSO 464	MKADLTWLDDPQTFRINQLPAHSDHGRGYASVEEATAQHSSLVQSLDGTWQFAFAPDPVHR	
TMW 1.2111	MKADLTWLDDPQTFRINQLPAHSDHGRGYASVEEATAQHSSLVQSLDGTWQFAFAPDPVHR	
NPS-QW-145	MKADLTWLDDPQTFRINQLPAHSDHGRGYASVEEATAQHSSLVQSLDGTWQFAFAPDPVHR	
KB290	MKADLTWLDDPQTFRINQLPAHSDHGRGYASVEEATAQHSSLVQSLDGTWQFAFAPDPVHR	
	*	*
GU240	FEGFYQPDYDRSAFDRLTVPGHIELAGYGQIQYINTAYPWEHGHYRRPAYSMGADQPEEG	120
SRCM101106	FEGFYQPDYDRSAFDRLTVPGHIELAGYGQIQYINTAYPWEHGHYRRPAYSMGADQPEEG	
ATCC 367	FEGFYQPDYDRSAFDRLTVPGHIELAGYGQIQYINTAYPWEHGHYRRPAYSMGADQPEEG	
BSO 464	FEGFYQPDYDRSAFDRLTVPGHIELAGYGQIQYINTAYPWEHGHYRRPAYSMGADQPEEG	
TMW 1.2111	FEGFYQPDYDRSAFDRLTVPGHIELAGYGQIQYINTAYPWEHGHYRRPAYSMGADQPEEG	
NPS-QW-145	FEGFYQPDYDRSAFDRLTVPGHIELAGYGQIQYINTAYPWEHGHYRRPAYSMGADQPEEG	
KB290	FEGFYQPDYDRSAFDRLTVPGHIELAGYGQIQYINTAYPWEHGHYRRPAYSMGADQPEEG	
	*	*
GU240	MFSTDPQNTVGAYVKHFTLNPALANQRVSI EFDGVEQAMFLWLNQGFVGYAEDSFSRSEF	180
SRCM101106	MFSTDPQNTVGAYVKHFTLNPALANQRVSI EFDGVEQAMFLWLNQGFVGYAEDSFSRSEF	
ATCC 367	MFSTDPQNTVGAYVKHFTLNPALANQRVSI EFDGVEQAMFLWLNQGFVGYAEDSFSRSEF	
BSO 464	MFSTDPQNTVGAYVKHFTLNPALANQRVSI EFDGVEQAMFLWLNQGFVGYAEDSFSRSEF	
TMW 1.2111	MFSTDPQNTVGAYVKHFTLNPALANQRVSI EFDGVEQAMFLWLNQGFVGYAEDSFSRSEF	
NPS-QW-145	MFSTDPQNTVGAYVKHFTLNPALANQRVSI EFDGVEQAMFLWLNQGFVGYAEDSFSRSEF	
KB290	MFSTDPQNTVGAYVKHFTLNPALANQRVSI EFDGVEQAMFLWLNQGFVGYAEDSFSRSEF	
	*	*
GU240	DLTPYLQAGQNLLAVEVFKHSTAFLLEDQDMFRFSGIFRSVRLVAKPELHVEDLTIRAGL	240
SRCM101106	DLTPYLQAGQNLLAVEVFKHSTAFLLEDQDMFRFSGIFRSVRLVAKPELHVEDLTIRAGL	
ATCC 367	DLTPYLQAGQNLLAVEVFKHSTAFLLEDQDMFRFSGIFRSVRLVAKPELHVEDLTIRAGL	
BSO 464	DLTPYLQAGQNLLAVEVFKHSTAFLLEDQDMFRFSGIFRSVRLVAKPELHVEDLTIRAGL	
TMW 1.2111	DLTPYLQAGQNLLAVEVFKHSTAFLLEDQDMFRFSGIFRSVRLVAKPELHVEDLTIRAGL	
NPS-QW-145	DLTPYLQAGQNLLAVEVFKHSTAFLLEDQDMFRFSGIFRSVRLVAKPELHVEDLTIRAGL	
KB290	DLTPYLQAGQNLLAVEVFKHSTAFLLEDQDMFRFSGIFRSVRLVAKPELHVEDLTIRAGL	
	*	*
GU240	DDAFQTGDLKVRQLTAASQLSGTATAQLLTADGQEVWATEQPAASTLDLAAAIDHVHLW	300
SRCM101106	DDAFQTGDLKVRQLTAASQLSGTATAQLLTADGQEVWATEQPAASTLDLAAAIDHVHLW	
ATCC 367	DDAFQTGDLKVRQLTAASQLSGTATAQLLTADGQEVWATEQPAASTLDLAAAIDHVHLW	
BSO 464	DDAFQTGDLKVRQLTAASQLSGTATAQLLTADGQEVWATEQPAASTLDLAAAIDHVHLW	
TMW 1.2111	DDAFQTGDLKVRQLTAASQLSGTATAQLLTADGQEVWATEQPAASTLDLAAAIDHVHLW	
NPS-QW-145	DDAFQTGDLKVRQLTAASQLSGTATAQLLTADGQEVWATEQPAASTLDLAAAIDHVHLW	
KB290	DDAFQTGDLKVRQLTAASQLSGTATAQLLTADGQEVWATEQPAASTLDLAAAIDHVHLW	
	*	*
GU240	DHHDPLYQLRITLKDVGAGQVVEVPYPVGFRRIELKDKVMCLNGQRLILNGVNRHEWDA	360
SRCM101106	DHHDPLYQLRITLKDVGAGQVVEVPYPVGFRRIELKDKVMCLNGQRLILNGVNRHEWDA	
ATCC 367	DHHDPLYQLRITLKDVGAGQVVEVPYPVGFRRIELKDKVMCLNGQRLILNGVNRHEWDA	
BSO 464	DHHDPLYQLRITLKDVGAGQVVEVPYPVGFRRIELKDKVMCLNGQRLILNGVNRHEWDA	
TMW 1.2111	DHHDPLYQLRITLKDVGAGQVVEVPYPVGFRRIELKDKVMCLNGQRLILNGVNRHEWDA	
NPS-QW-145	DHHDPLYQLRITLKDVGAGQVVEVPYPVGFRRIELKDKVMCLNGQRLILNGVNRHEWDA	
KB290	DHHDPLYQLRITLKDVGAGQVVEVPYPVGFRRIELKDKVMCLNGQRLILNGVNRHEWDA	
	*	*
GU240	HRGRAVTMADMTQDLQTFHDNHINAVRTCHYPDQDAWYYLCDQGGIYMAAENNLETHGTW	420
SRCM101106	HRGRAVTMADMTQDLQTFHDNHINAVRTCHYPDQDAWYYLCDQGGIYMAAENNLETHGTW	
ATCC 367	HRGRAVTMADMTQDLQTFHDNHINAVRTCHYPDQDAWYYLCDQGGIYMAAENNLETHGTW	
BSO 464	HRGRAVTMADMTQDLQTFHDNHINAVRTCHYPDQDAWYYLCDQGGIYMAAENNLETHGTW	
TMW 1.2111	HRGRAVTMADMTQDLQTFHDNHINAVRTCHYPDQDAWYYLCDQGGIYMAAENNLETHGTW	
NPS-QW-145	HRGRAVTMADMTQDLQTFHDNHINAVRTCHYPDQDAWYYLCDQGGIYMAAENNLETHGTW	
KB290	HRGRAVTMADMTQDLQTFHDNHINAVRTCHYPDQDAWYYLCDQGGIYMAAENNLETHGTW	
	*	*
GU240	QKMGAVEPSYNVPGSLPQWQLAVLDRAKSNYEMFKNHPAVLFWSLGNESYAGDNI AAMDA	480
SRCM101106	QKMGAVEPSYNVPGSLPQWQLAVLDRAKSNYEMFKNHPAVLFWSLGNESYAGDNI AAMDA	
ATCC 367	QKMGAVEPSYNVPGSLPQWQLAVLDRAKSNYEMFKNHPAVLFWSLGNESYAGDNI AAMDA	
BSO 464	QKMGAVEPSYNVPGSLPQWQLAVLDRAKSNYEMFKNHPAVLFWSLGNESYAGDNI AAMDA	
TMW 1.2111	QKMGAVEPSYNVPGSLPQWQLAVLDRAKSNYEMFKNHPAVLFWSLGNESYAGDNI AAMDA	
NPS-QW-145	QKMGAVEPSYNVPGSLPQWQLAVLDRAKSNYEMFKNHPAVLFWSLGNESYAGDNI AAMDA	
KB290	QKMGAVEPSYNVPGSLPQWQLAVLDRAKSNYEMFKNHPAVLFWSLGNESYAGDNI AAMDA	
	*	*
GU240	FYHHADPTRLTHYEGVCRNRVYEDRISDMESMMYDPPRAIEDYLKNDPQKPFVNCEYMH	540
SRCM101106	FYHHADPTRLTHYEGVCRNRVYEDRISDMESMMYDPPRAIEDYLKNDPQKPFVNCEYMH	
ATCC 367	FYHHADPTRLTHYEGVCRNRVYEDRISDMESMMYDPPRAIEDYLKNDPQKPFVNCEYMH	
BSO 464	FYHHADPTRLTHYEGVCRNRVYEDRISDMESMMYDPPRAIEDYLKNDPQKPFVNCEYMH	
TMW 1.2111	FYHHADPTRLTHYEGVCRNRVYEDRISDMESMMYDPPRAIEDYLKNDPQKPFVNCEYMH	
NPS-QW-145	FYHHADPTRLTHYEGVCRNRVYEDRISDMESMMYDPPRAIEDYLKNDPQKPFVNCEYMH	
KB290	FYHHADPTRLTHYEGVCRNRVYEDRISDMESMMYDPPRAIEDYLKNDPQKPFVNCEYMH	
	*	*
GU240	MGNSLGGMASYDALIDQYPMYQGGFIWDFIDQALWVKDEVTGQPVLYRYGGDFDDRHS	600
SRCM101106	MGNSLGGMASYDALIDQYPMYQGGFIWDFIDQALWVKDEVTGQPVLYRYGGDFDDRHS	
ATCC 367	MGNSLGGMASYDALIDQYPMYQGGFIWDFIDQALWVKDEVTGQPVLYRYGGDFDDRHS	
BSO 464	MGNSLGGMASYDALIDQYPMYQGGFIWDFIDQALWVKDEVTGQPVLYRYGGDFDDRHS	
TMW 1.2111	MGNSLGGMASYDALIDQYPMYQGGFIWDFIDQALWVKDEVTGQPVLYRYGGDFDDRHS	
NPS-QW-145	MGNSLGGMASYDALIDQYPMYQGGFIWDFIDQALWVKDEVTGQPVLYRYGGDFDDRHS	
KB290	MGNSLGGMASYDALIDQYPMYQGGFIWDFIDQALWVKDEVTGQPVLYRYGGDFDDRHS	
	*	*
GU240	FSGDGLLFADRTPKPALQEVDDYGGQHD-	628
SRCM101106	FSGDGLLFADRTPKPALQEVDDYGGQHD-	
ATCC 367	FSGDGLLFADRTPKPALQEVDDYGGQHD-	
BSO 464	FSGDGLLFADRTPKPALQEVDDYGGQHD-	
TMW 1.2111	FSGDGLLFADRTPKPALQEVDDYGGQHD-	
NPS-QW-145	FSGDGLLFADRTPKPALQEVDDYGGQHD-	
KB290	FSGDGLLFADRTPKPALQEVDDYGGQHD-	

Fig. 4. Alignment of amino acid sequence of LacL with homologous enzymes from *L. brevis* strains. GU240 (*L. zymae* GU240, MN188052), SRCM101106 (*L. brevis* SRCM101106, CP021674), ATCC367 (*L. brevis* ATCC367, CP000416), BSO464 (*L. brevis* BSO464, CP005977), TMW 1.2111 (*L. brevis* TMW1.2111, CP019743), NPS-QW-145 (*L. brevis* NPS-QW-145, CP015398), and KB290 (*L. brevis* KB290, AP012167). Amino acids different from other enzymes are marked as asterisks above the amino acids

GU240	MDNTTKLNVIFGDVTLGVSGPGFHYIFAYDRGGLESVQDGKEWLYRTPMPALWRATTDN	60
SRCM101106	MDNTTKLNVIFGDVTLGVSGPGFHYIFAYDRGGLESVQDGKEWLYRTPMPALWRATTDN	
ATCC 367	MDNTTKLNVIFGDVTLGVSGPGFHYIFAYDRGGLESVQDGKEWLYRTPMPALWRATTDN	
BSO 464	MDNTTKLNVIFGDVTLGVSGPGFHYIFAYDRGGLESVQDGKEWLYRTPMPALWRATTDN	
TMW 1. 2111	MDNTTKLNVIFGDVTLGVSGPGFHYIFAYDRGGLESVQDGKEWLYRTPMPALWRATTDN	
NPS-QW-145	MDNTTKLNVIFGDVTLGVSGPGFHYIFAYDRGGLESVQDGKEWLYRTPMPALWRATTDN	
KB290	MDNTTKLNVIFGDVTLGVSGPGFHYIFAYDRGGLESVQDGKEWLYRTPMPALWRATTDN	
	* * *	
GU240	DRNGFSTKSAQRLGADLFSSCDHISVAIDGQSIPLPIAPENNRYSDEHTATTVAVTFTY	120
SRCM101106	DRNGFSTKSAQWLGADLFSSCDHISVAIDGQSIPLPIAPENNRYSDEHTATTVAVTFTY	
ATCC 367	DRNGFSTKSAQWLGADLFSSCDHISVAIDGQSIPLPIAPENNRYSDEHTATTVAVTFTY	
BSO 464	DRNGFSTKSAQWLGADLFSSCDHISVAIDGQSIPLPIAPENNRYSDEHKATTVAVTFTY	
TMW 1. 2111	DRNGFSTKSAQWLGADLFSSCDHISVAIDGQSIPLPIAPENNRYSDEHTATTVAVTFTY	
NPS-QW-145	DRNGFSTKSAQWLGADLFSSCDHISVAIDGQSIPLPIAPENNRYSDEHTATTVAVTFTY	
KB290	DRNGFSTKSAQWLGADLFSSCDHISVAIDGQSIPLPIAPENNRYSDEHTATTVAVTFTY	
	*	
GU240	TTPTTPATTIAVYTVAAASGAMTVAVHYAGKADLPELPALELRLVMPALGFAYQGLSG	180
SRCM101106	TTPTTPATTIAVYTVAAASGAMTVAVHYAGKADLPELPALELRLVMPALGFAYQGLSG	
ATCC 367	TTPTTPATTIAVYTVAAASGAMTVAVHYAGKADLPELPALELRLVMPALGFAYQGLSG	
BSO 464	TTPTTPATTIAVYTVATSGAMTVAVHYAGKADLPELPALELRLVMPALGFAYQGLSG	
TMW 1. 2111	TTPTTPATTIAVYTVAAASGAMTVAVHYAGKADLPELPALELRLVMPALGFAYQGLSG	
NPS-QW-145	TTPTTPATTIAVYTVAAASGAMTVAVHYAGKADLPELPALELRLVMPALGFAYQGLSG	
KB290	TTPTTPATTIAVYTVAAASGAMTVAVHYAGKADLPELPALELRLVMPALGFAYQGLSG	
	*	
GU240	ETYPDRKAGGRKG IHQVAGLPVTPYLPVQECGMHVDNQWVTVTRGTTQNNADADHDAFSL	240
SRCM101106	ETYPDRKAGGRKG IHQVAGLPVTPYLPVQECGMHVDNQWVTVTRGTTQNNADADHDAFSL	
ATCC 367	ETYPDRKAGGRKG IHQVAGLPVTPYLPVQECGMHVDNQWVTVTRGTTQNNADADHDAFSL	
BSO 464	ETYPDRKAGGRKG IHQVAGLPVTPYLPVQECGMHVDNQWVTVTRGTTQNNADADHDAFSL	
TMW 1. 2111	ETYPDRKAGGRKG IHQVAGLPVTPYLPVQECGMHVDNQWVTVTRGTTQNNADADHDAFSL	
NPS-QW-145	ETYPDRKAGGRKG IHQVAGLPVTPYLPVQECGMHVDNQWVTVTRGTTQNNADADHDAFSL	
KB290	ETYPDRKAGGRKG IHQVAGLPVTPYLPVQECGMHVDNQWVTVTRGTTQNNADADHDAFSL	
	*	
GU240	KVRQTQHHAFAFSLPYTPTELENATHQEELPVPRRTVLT IYGAVRGVGGIDSWGSDAEAP	300
SRCM101106	KVRQTQHHAFAFSLPYTPTELENATHQEELPVPRRTVLT IYGAVRGVGGIDSWGSDAEAP	
ATCC 367	KVRQTQHHAFAFSLPYTPTELENATHQEELPVPRRTVLT IYGAVRGVGGIDSWGSDAEAP	
BSO 464	KVRQTQHHAFAFSLPYTPTELENATHQEELPVPRRTVLT IYGAVRGVGGIDSWGSDAEAP	
TMW 1. 2111	KVRQTQHHAFAFSLPYTPTELENATHQEELPVPRRTVLT IYGAVRGVGGIDSWGSDAEAP	
NPS-QW-145	KVRQTQHHAFAFSLPYTPTELENATHQEELPVPRRTVLT IYGAVRGVGGIDSWGSDAEAP	
KB290	KVRQTQHHAFAFSLPYTPTELENATHQEELPVPRRTVLT IYGAVRGVGGIDSWGSDAEAP	
	*	
GU240	YH IAGDSHDHDFSFEIAGPTPV-	321
SRCM101106	YH IAGDSHDHDFSFEIAGPTPV-	
ATCC 367	YH IAGDSHDHDFSFEIAGPTPV-	
BSO 464	YH IAGDSHDHDFSFEIAGPTPV-	
TMW 1. 2111	YH IAGDSHDHDFSFEIAGPTPV-	
NPS-QW-145	YH IAGDSHDHDFSFEIAGPTPV-	
KB290	YH IAGDSHDHDFSFEIAGPTPV-	

Fig. 5. Alignment of amino acid sequence of LacM with homologous enzymes from *Lactobacillus* strains. GU240 (*L. zymae* GU240, MN188052), SRCM101106 (*L. brevis* SRCM101106, CP021674), ATCC367 (*L. brevis* ATCC367, CP000416), BSO464 (*L. brevis* BSO464, CP005977), TMW 1.2111 (*L. brevis* TMW1.2111, CP019743), NPS-QW-145 (*L. brevis* NPS-QW-145, CP015398), and KB290 (*L. brevis* KB290, AP012167). Amino acids different from other enzymes are marked as asterisks above the amino acids

Overexpression of *lacLM* in *E. coli*

SDS-PAGE results showed that LacM was overproduced in *E. coli* BL21(DE3) as shown in Fig. 7. A thick 35 kDa band was observed from insoluble fraction from *E. coli*

BL21(DE3) harboring pETlacLM (Fig. 7, lane 6), which matched well with the expected size of LacM (34,569.56 Da). LacL production was not significant compared with LacM, and the reason is not known. LacM was produced

Fig. 6. The operon structure of *lacL* and *lacM*. (A) Operon structure of *lacL* and *lacM* genes in *L. zymae* GU240. The arrows indicate the binding sites for each primer used for RT-PCR. (B) Agarose gel electrophoresis result. Lane M, iVDye 1kb DNA ladder (Gendepot, USA); 1-4, RNA preps; 5-8, RNA preps treated with DNase I. Lanes 1 and 5, RT-PCR using universal primers (27F, 1492R) for 16S rRNA genes; 2 and 6, RT-PCR using primers A and B; 3 and 7, RT-PCR using primers C and D; 4 and 8, RT-PCR using primers A and D.

Fig. 7. Overexpression of *lacLM* in *E. coli*. M, DokDo-Marker (EBM-1034, Elpisbiotech, Korea); lanes 1-3, culture supernatant (1), soluble fraction (2), insoluble fraction (3) of *E. coli* BL21(DE3) [pET-26b(+)]]; lanes 4-6, culture supernatant (4), soluble fraction (5), insoluble fraction (6) of *E. coli* BL21(DE3) [pETlacLM], no IPTG added; lanes 7-9, culture supernatant (7), soluble fraction (8), insoluble fraction (9) of *E. coli* BL21(DE3) [pETlacLM], IPTG (1 mM) added.

as inclusion body, and this is a common phenomenon when a foreign gene is overproduced in *E. coli* using an efficient expression system such as a pET vector and *E. coli* BL21(DE3). Renaturation steps are necessary if overproduced β -Gal in *E. coli* is to be utilized.

L. zymae GU240 possesses intact *lacLM* genes but the strain grows poorly in lactose containing medium. The result indicates a possibility that *L. zymae* GU240 might

be in the process of adaptation to plant environments including *Kimchi* where lactose is not a major nutrient.

There is no need for a host cell to maintain an ability to grow rapidly in lactose as long as the environments contain no lactose since maintaining the ability is expensive for LAB which get less energy through metabolisms. Although lactose utilization is not a major energy generating pathway in *L. zymae*, still *lacLM* from *L. zymae* GU240 might be useful as reporter genes or a selection marker for hosts such as *Lactococcus lactis* and other LAB actively growing on milk and other dairy products. Also the gene might be useful for the production of galactooligosaccharides, a prebiotic with industrial importance [20].

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

- Juers DH, Matthews BW, Huber RE. 2012. LacZ β -galactosidase: structure and function of an enzyme of historical and molecular biological importance. *Protein Sci.* **21**: 1792-1807.
- De Vos WM, Vaughan EE. 1994. Genetics of lactose utilization in lactic acid bacteria. *FEMS Microbiol. Rev.* **15**: 217-237.
- De Vos WM, Simons G. 1988. Molecular cloning of lactose genes in dairy lactic streptococci: the phospho- β -galactosidase and β -galactosidase genes and their expression products. *Biochimie* **70**: 461-473.
- Vaughan EE, Pridmore RD, Mollet B. 1998. Transcriptional regulation and evolution of lactose genes in the galactose-lactose operon of *Lactococcus lactis* NCDO2054. *J. Bacteriol.* **180**: 4893-4902.
- Nguyen TH, Splechna B, Yamabhai M, Haltrich D, Peterbauer C. 2007. Cloning and expression of the β -galactosidase genes from *Lactobacillus reuteri* in *Escherichia coli*. *J. Biotechnol.* **129**: 581-591.
- Obst M, Meding ER, Vogel RF, Hammes WP. 1995. Two genes encoding the β -galactosidase of *Lactobacillus sake*. *Microbiology* **141**: 3059-3066.

7. Adams RM, Yoast S, Mainzer SE, Moon K, Palombella AL, Estell DA. *et al.* 1994. Characterization of two cold-sensitive mutants of the β-galactosidase from *Lactobacillus delbruckii* subsp. *bulgaricus*. *J. Biol. Chem.* **269**: 5666-5672.
8. Franco D, de Boer PA, de Gier-de Vries C, Lamers WH, Mooman AF. 2001. Methods on in situ hybridization, immunohistochemistry and beta-galactosidase reporter gene detection. *Eur. J. Morphol.* **39**: 3-25.
9. Oliveira C, Guimaraes PMR, Domingues L. 2011. Recombinant microbial systems for improved β-galactosidase production and biochemical applications. *Biotechnol. Adv.* **29**: 600-609.
10. Platteeuw C, van Allen-Boerrigter I, van Schalkwijk S, de Vos EM. 1996. Food-grade cloning and expression system for *Lactococcus lactis*. *Appl. Environ. Microbiol.* **62**: 1008-1013.
11. Landete JM. 2017. A review of food-grade vectors in lactic acid bacteria: from the laboratory to their application. *Crit. Rev. Biotechnol.* **37**: 296-308.
12. Park JY, Jeong SJ, Kim JH. 2014. Characterization of a glutamate decarboxylase (GAD) gene from *Lactobacillus zymae*. *Biotechnol. Lett.* **36**: 1791-1799.
13. Miller JH. 1972. Experiments in molecular genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
14. Heo K, Cho KM, Lee CK, Kim GM, Shin JH, Kim JS, *et al.* 2013. Characterization of a fibrinolytic enzyme secreted by *Bacillus amylo-liquefaciens* CB1 and its gene cloning. *J. Microbiol. Biotechnol.* **23**: 974-983.
15. Sa HD, Park JY, Jeong SJ, Lee KW, Kim JH. 2015. Characterization of glutamate decarboxylase (GAD) from *Lactobacillus sakei* A156 isolated from *Jeot-gal*. *J. Microbiol. Biotechnol.* **25**: 696-703.
16. Saier MH, Chauvaux S, Cook GM, Deutscher J, Paulsen IT, Reizer J, *et al.* 1996. Catabolite repression and inducer control in Gram-positive bacteria. *Microbiology* **142**: 217-223.
17. Park RJ, Lee KW, Kim SJ, Park JY, Nam SJ, Park JY, *et al.* 2002. Isolation of *Lactococcus lactis* strain with β-galactosidase activity from *Kimchi* and cloning of *lacZ* gene from the isolated strain. *J. Microbiol. Biotechnol.* **12**: 157-161.
18. Kim TY, Lee JM, Chang HC, Chung DK, Lee JH, Kim JH, *et al.* 1999. Effect of temperature and a carbon source on the expression of β-galactosidase gene of *Lactococcus lactis* spp. *lactis* ATCC7962. *J. Microbiol. Biotechnol.* **9**: 201-205.
19. David S, Stevens H, van Riel M, Sinons G, de Vos W. 1992. *Leuconostoc lactis* β-galactosidase is encoded by two overlapping genes. *J. Bacteriol.* **174**: 4475-4481.
20. Chanalia P, Gandhi D, Attri P, Dhanda S. 2018. Purification and characterization of β-galactosidase from probiotic *Pediococcus acidilactici* and its use in milk lactose hydrolysis and galactooligosaccharide synthesis. *Bioorg. Chem.* **77**: 176-189.