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Isolation of *Lactococcus lactis* Strain with β-Galactosidase Activity from Kimchi and Cloning of *lacZ* Gene from the Isolated Strain

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Abstract A lactic acid bacteria with β-gal activity was isolated from Kimchi, a traditional fermented vegetable food in Korea. The isolate was identified as a *Lactococcus lactis* strain and named *L. lactis* A2. The gene encoding β-gal of *L. lactis* A2 was cloned as a 5.8 kb *PstI* fragment. DNA sequencing identified the complete *lacA* (galactoside acetyltransferase)-*lacZ* (β-galactosidase) genes together with the 3' part of upstream *galT* (galactose-1-phosphate uridyltransferase), and the 5' region of downstream *galE* (UDP-galactose-4-epimerase) genes. *L. lactis* A2 had the same *gal/lac* operon structure as in *L. lactis* subsp. *lactis* 7962. Other genes of the Leloir pathway are most likely to be located in the 5' upstream of the 5.8 kb fragment on the A2 chromosome. Sequences downstream of *galE* were different from those of *L. lactis* subsp. *lactis* 7962.

Key words: Kimchi, *L. lactis* A2, β-galactosidase, *lacZ* gene, gene cloning

β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) hydrolyzes lactose into its constituting monosaccharides, glucose and galactose [15]. β-Gal and its structural gene, *lacZ*, from bacteria have been extensively studied and the topics include the distribution of β-gal activity among LAB [2], the role of β-gal in the utilization of lactose [13], and β-gal genetic study [4, 8, 9, 12]. β-Gal is a very important enzyme for many LAB, especially for those strains used as starters for dairy fermentations such as cheese and yogurt manufacturing processes [15]. β-Gal is also used for the production of lactose-free dairy products for lactose-intolerant individuals [15] and for the bioconversion of lactose in cheese whey into edible alcohol [14]. So far, studies on the β-gals and their genes have been conducted mostly for dairy LAB and few studies have been carried out for LAB from other sources. Kimchi is a traditional Korean fermented vegetable food based on natural lactic fermentation. LAB play very important roles for the ripening of Kimchi and they are responsible for the development of its characteristic flavor [5]. LAB are important for Kimchi fermentation and they include the most important genera such as *Leuconostoc*, *Lactococcus*, *Lactobacillus*, *Pediococcus*, and *Enterococcus* [7]. LAB from Kimchi are assumed to be somewhat different from those found in dairy environments in terms of their metabolic capacities, but only a few biochemical and genetic studies have been performed. In this study, the properties of the β-gal from a *Lactococcus lactis* strain isolated from Kimchi and the cloning of the *lacZ* gene are presented.

Screening and Identification of a Lac$^+$ Isolate from Kimchi

LAB were isolated from various home-made Kimchis by spreading serially diluted Kimchi supernatant on MRS plates. An isolate, designated A2, turned dark-blue on MRS plate containing X-gal, indicating the presence of β-gal activity. β-Gal assay with the crude cell extract from A2 confirmed the presence of β-gal. Biochemical properties

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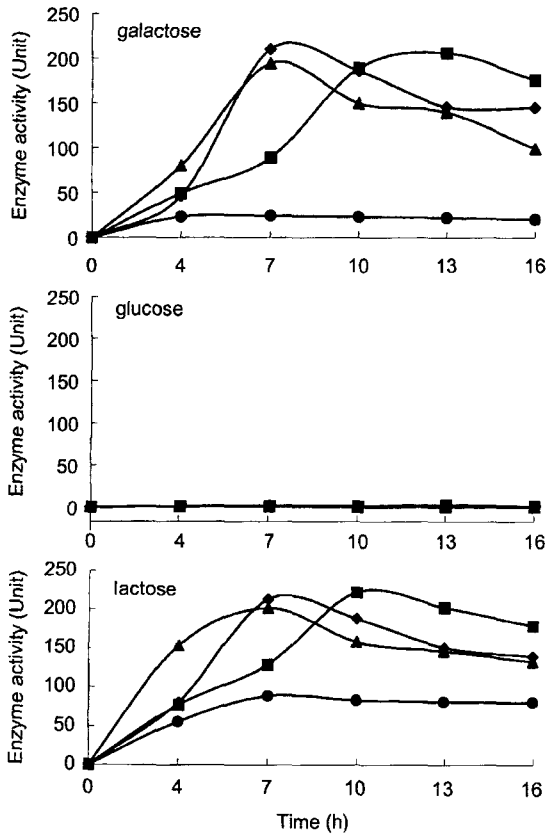

Fig. 1. β-Gal activity of *L. lactis* A2 cells.
L. lactis A2 cells were grown on M17 broth containing lactose, galactose, or glucose (1% concentration). One ml aliquots were taken at intervals and crude cell extracts were prepared for enzyme measurements. -■-, 25°C grown cells; -◆-, 30°C grown cells; -▲-, 37°C grown cells; -●-, 42°C grown cells.

such as sugar utilization, growth temperature range, and NaCl tolerance and 16S rDNA sequencing results identified A2 as a *Lactococcus lactis* strain. A ca. 300 bp fragment was amplified using primer sets (Leu1; 5'-GCGGCGTG-CCTAATACATGCAAGTCG-3' and Leu2; 5'-GACCCG-

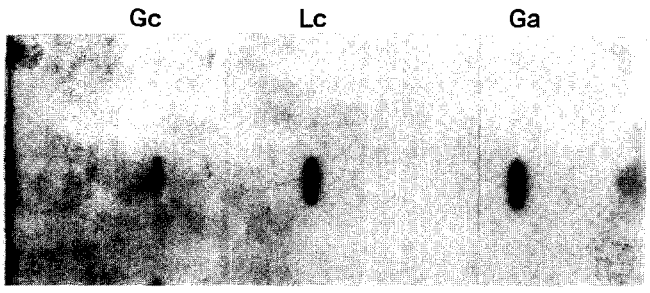

Fig. 2. Slot blot for detecting β-gal transcripts of *L. lactis* A2.
A2 cells were grown on M17 broth containing lactose, galactose, or glucose (1% concentration). Total RNAs were prepared by using the RNeasy kit (Qiagen, Valencia, CA, U.S.A.). Ten μg of RNA was applied to each slot. Gc, Lc, and Ga represent glucose, lactose, and galactose, respectively.

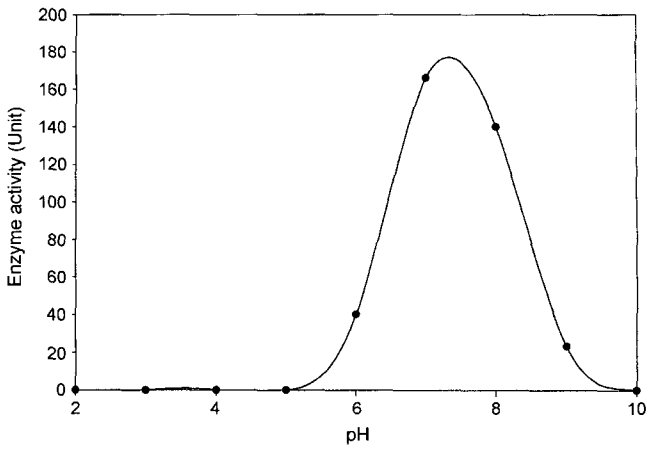

Fig. 3. The effect of pH on the β-gal activity of *L. lactis* A2 cells.

GGAACGTATTCACCGCGGC-3'), hybridizing to the internal region of 16 rRNA gene. When the sequence of the PCR fragment was compared with other genes in the data library, the sequence showed 98% homology with 16S rRNA genes of *Lactococcus lactis* strains (X64887, AJ271851). *L. lactis* A2 grew at both 10°C and 40°C, and also at NaCl concentration of up to 3%. *L. lactis* subsp. *lactis* grew at 40°C and in 4% NaCl. In contrast, *L. lactis* subsp. *cremoris* did not [3]. To confirm the subspecies, PCR amplification for *acmA* was performed, as described by Garde *et al.* [3], however, the results did not clearly indicate the subspecies status (results not shown). A2 was therefore, designated *L. lactis* A2.

β-Gal Activity

Expression of the *lacZ* gene in *L. lactis* A2 is under catabolite repression, as is the *lacZ* gene of *L. lactis* 7962

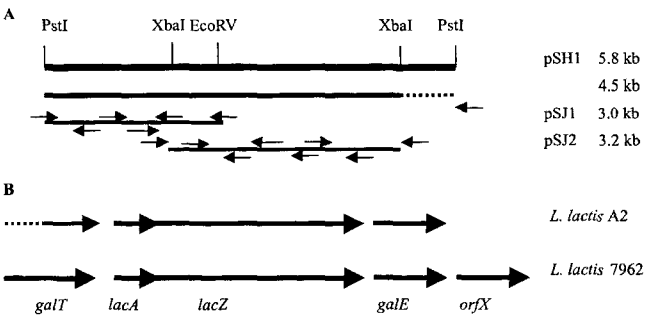

Fig. 4. The restriction map of 5.8 kb *PstI* insert in pSH1.
A: The sequenced 4.5 kb region is shown below the restriction map as a thick line together with an undetermined 1.3 kb region (dotted line). The fragments subcloned into pUC19 generating pSJ1 and pSJ2, respectively, are also shown. The positions of primers used for sequencing are shown as short arrows. B: The order of *gal/lac* operon genes in *L. lactis* A2 is shown. The genes constituting the *gal/lac* operon of *L. lactis* 7962 are also shown for comparison.

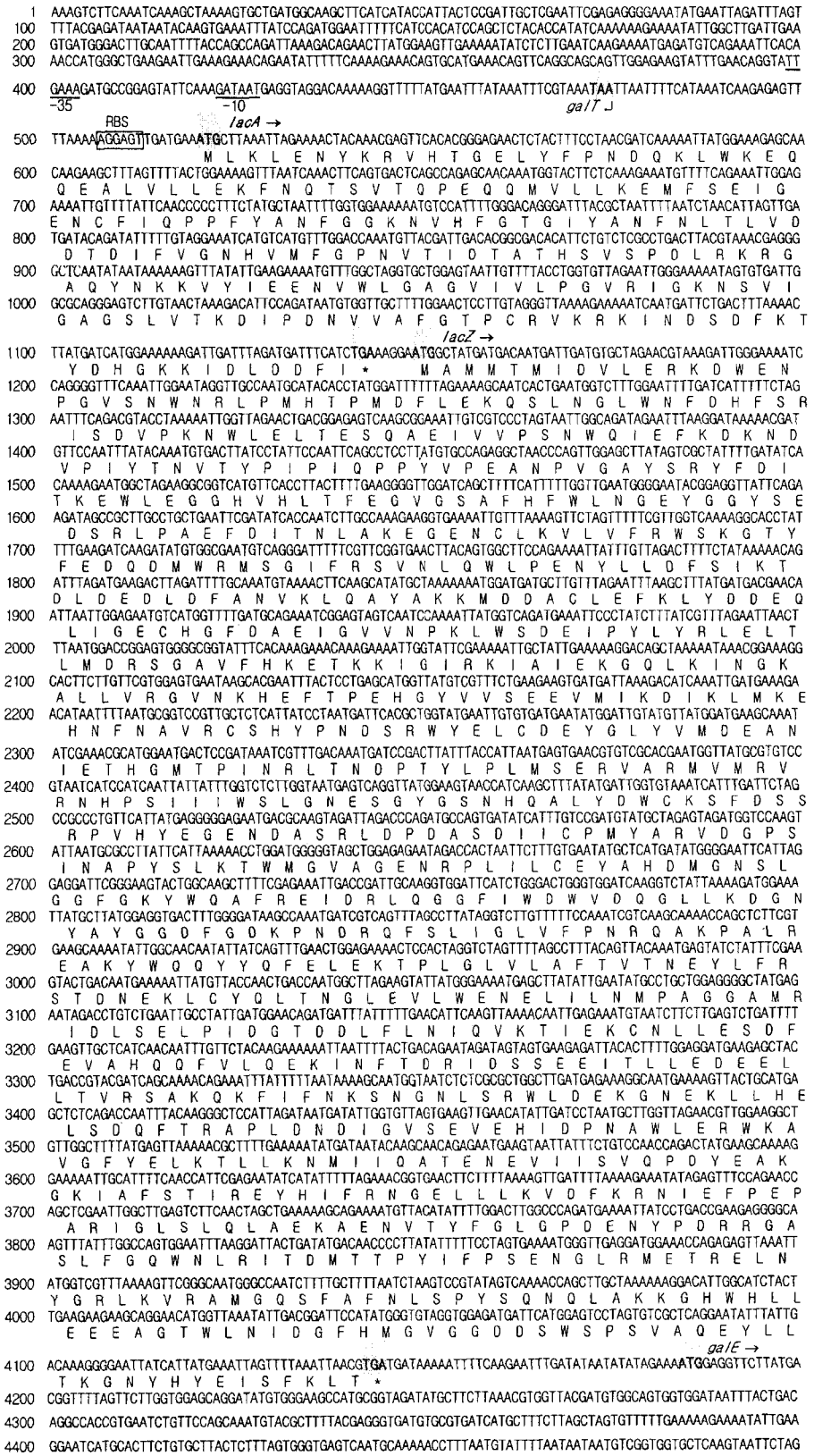

Fig. 5. Nucleotide sequences of the *lacA* and *lacZ* genes of *L. lactis* A2.
The putative-35 and -10 promoter sequences in front of *lacA* are underlined. The ribosome binding sites for *lacA* and *lacZ* are boxed. The bold and shaded letters correspond to the translation start and stop codons of *lacA* and *lacZ*. The sequences of the 3' part of *galT* and the 5' region of *galE* are also shown.

[6, 11]. As shown in Fig. 1, β-gal activities was much higher in cells grown on lactose (230 unit) or galactose (220 unit) than those grown on glucose (2–3 unit). Slot blot results (Fig. 2) showed that more transcripts were synthesized in cells grown on lactose or galactose than cells grown on glucose, indicating the operation of catabolite repression.

Interestingly, β-gal activities of cells grown at 25°C or 30°C were relatively higher than cells grown at 37°C, although cells grew faster at 37°C (results not shown). The reason for the higher enzyme activities at lower temperatures is not clear; however, the same phenomena were also reported for β-gals from *L. lactis* 7962 and *Lactobacillus acidophilus* CRL639 [6, 10]. β-Gal of *L. lactis* A2 was most active at pH 6.5–8.0 and completely inactive below pH 5.5 (Fig. 3).

Cloning of the *lacZ* Gene from *L. lactis* A2

Five blue colonies on LB plates containing tetracycline and X-gal were identified out of 5,000 *E. coli* transformants. All transformants contained the same plasmid harboring a 5.8 kb insert. Restriction mapping (Fig. 4) showed that all the inserts were the same. The recombinant plasmid was named pSH1. Southern blot was performed using the 5.8 kb *Pst*I insert as a probe and the result confirmed that the 5.8 kb fragment was originated from *L. lactis* A2 chromosome (results not shown). Subcloning of a 3.0 kb *Pst*I-*Eco*RV fragment and a 3.2 kb internal *Xba*I fragment into pUC19 produced pSJ1 and pSJ2, respectively (Fig. 4).

Sequence Analysis

The 4.5-kb region starting from the 5' end of the 5.8-kb insert was sequenced by the primer walking method as shown in the Fig. 4 and the sequences (accession # AY030302) are shown in Fig. 5. Blast analyses of the sequences confirmed the presence of the 3' part of *galT*, complete *lacA*, *lacZ*, and the 5' part of *galE* genes in the 4.5-kb region, and the genes apparently were part of an operon (Fig. 5). A truncated *galT* gene encoding the C-terminus 158 amino acids of *galT* is the first ORF. At 40 bp downstream from the stop codon of *galT*, an ORF encoding *lacA* starts. *lacA* is 624 bp in size and thus encodes *lacA* of 207 amino acids. At 5 bp downstream from the stop codon of *lacA*, *lacZ* starts. *LacZ* is 2,997 bp in size and encodes a protein of 998 amino acids. Figure 5 shows the nucleotide sequences of *lacA* and *lacZ* genes. *LacZ* of *L. lactis* A2 was almost identical with those of *L. lactis* 7962 (U60828, 97% identity), a *L. lactis* strain (X80037, 97% identity), and *L. lactis* IL1403 (AE006428, 96% identity). Amino acid sequence comparison of *lacZ* from A2 with that from 7962 showed 31 different amino acids (3.1%), whereas *lacA* proteins were different at 8 amino acids (3.9%). *GalE* starts at 39 bp downstream from the stop codon of *lacZ* and the 5' region corresponding to the N-terminus 104 amino acids of *galE* was sequenced. The 1.3 kb downstream region was not sequenced; however, the partially determined sequences near the 3' end of the 5.8 kb insert did not indicate any homology with other genes in the data library, including the *orfX* of *L. lactis* 7962 (U60828). Considering the size of *galE*, the 1.3 kb downstream region contained only the 3' part of *galE*. *OrfX*, a putative gene in the immediate downstream of *galE* in *L. lactis* 7962, was not present in *L. lactis* A2. These results strongly indicate that *L. lactis* A2 most likely has the same *gal/lac* operon structure as observed in *L. lactis* 7962, where the *lacA* and *lacZ* genes are inserted between *galT* and *galE* [8, 16]. The same operon structure indicates that *L. lactis* 7962, a wild-type strain and slow lactose-fermenter [1], is similar to *L. lactis* A2 in terms of sugar utilization. Since the *gal/lac* operon structure of *L. lactis* A2 is highly likely the same as that of *L. lactis* 7962, the transcriptional control mechanism employing *ccpA* [11] is also expected to operate in *L. lactis* A2.

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