J. Dairy Sci. 94:5811–5820
doi:10.3168/jds.2011-4479

β-Galactosidase with transgalactosylation activity from *Lactobacillus fermentum* K4

G. X. Liu,* J. Kong,† W. W. Lu,† W. T. Kong,† H. Tian,* X. Y. Tian,* and G. C. Huo*[1]
*State Key Laboratory of Dairy Science, Northeast Agricultural University, Ministry of Education, No. 59 Mucai Street, Xiangfang District, Harbin, Heilongjiang Province, 150030, China
†State Key Laboratory of Microbial Technology, Shandong University, No. 27 Shanda South Road, Jinan, Shandong Province, 250100, China

ABSTRACT

The LacLM β-galactosidase of *Lactobacillus fermentum* K4 is encoded by 2 consecutive genes, *lacL* (large subunit) and *lacM* (small subunit), that share 17 overlapping nucleotides. Phylogenetic analysis revealed that this enzyme was closely related to other *Lactobacillus* β-galactosidases and provided significant insight into its common and distinct characteristics. We cloned both the *lacL* and *lacM* genes of *L. fermentum* K4 and heterologously expressed each in *Escherichia coli*, although the recombinant enzyme was only functional when both were expressed on the same plasmid. We evaluated the enzymatic properties of this species-specific LacLM β-galactosidase and discovered that it acts as both a hydrolase, bioconverting lactose into glucose and galactose, and a transgalactosylase, generating prebiotic galacto-oligosaccharides (GOS). The recombinant β-galactosidase showed a broad pH optimum and stability around neutral pH. The optimal temperature and Michaelis constant (K_m) for the substrates *o*-nitrophenyl-β-D-galactopyranoside and lactose were, respectively, 40°C and 45 to 50°C and 1.31 m*M* and 27 m*M*. The enzyme activity was stimulated by some cations such as Na^+, K^+, and Mg^{2+}. In addition, activity was also enhanced by ethanol (15%, wt/vol). The transgalactosylation activity of *L. fermentum* K4 β-galactosidase effectively and rapidly generated GOS, up to 37% of the total sugars from the reaction. Collectively, our results suggested that the β-galactosidase from *L. fermentum* K4 could be exploited for the formation of GOS.

Key words: β-galactosidase, *Lactobacillus fermentum*, lactose, galacto-oligosaccharides

INTRODUCTION

The carbohydrate-active enzymes (CAZymes) are divided among 5 functional classes: glycoside hydrolases (**GH**), glycosyltransferases, polysaccharide lyases, carbohydrate esterases, and carbohydrate-binding modules (Cantarel et al., 2009). The β-galactosidases (**β-gal**, EC 3.2.1.23) belong to 4 different GH families (GH1, GH2, GH35, and GH42; http://www.cazy.org/) and catalyze the hydrolysis and the transgalactosylation of β-D-galactopyranoside substrates such as lactose. β-Galactosidases are widely distributed throughout nature and have been characterized in animals, plants, and microorganisms, including bacteria, fungi, and yeast. The β-gal from *Escherichia coli* has been particularly well described because of the universal application of the lactose operon as a molecular tool. Furthermore, the transgalactosylation activity of β-galactosidases has gained considerable attention for its ability to produce galacto-oligosaccharide (**GOS**) prebiotics (Otieno, 2010; Park and Oh, 2010).

Galacto-oligosaccharides are enymatically produced upon lactose conversion, and they vary in saccharide chain length (between 2 and 8 monomeric units) and the types of linkages between the units. Recently, however, certain invariable characteristics were described. The saccharide chain is composed of a single terminal glucose, galactose monosaccharides, and disaccharides comprising 2 galactose units (Tzortzis and Vulevic, 2009). Industrial processes aimed at producing low-lactose or lactose-free items are concerned with undesirable GOS byproducts, for fear of unknown side effects that may stimulate symptoms of lactose intolerance. However, GOS have demonstrated beneficial effects that are distinct from lactose. The GOS can increase the numbers of *Bifidobacterium* strains and other probiotics (Onishi and Tanaka, 1995; Rabiu et al., 2001; Rastall and Maitin, 2002; Macfarlane et al., 2008) and contribute to metabolic activity of colon microbiota (Knol et al., 2005). As such, GOS have been proposed as an emerging special class of prebiotics and have gained popularity as supplemental components to

Received April 24, 2011.
Accepted September 5, 2011.
[1]Corresponding author: gchuo58@126.com

Table 1. Bacterial strains and plasmids included in this study

Strain or plasmid	Characteristic[1]	Source
Strain		
Lactobacillus fermentum K4	Isolated from Chinese traditional dairy products	This work
Escherichia coli Origami B (DE3)	F′ *ompT hsdS*$_B$(r_B′ m_B′) *gal dcm lacY1 ahpC*(DE3) *gor522*:: Tn *10* *trxB* (Kan^r, Tet^r); derived from a *LacZY* mutant of DE3 and carries *trxB/gor* mutations for cytoplasmic disulfide bond formation	Novagen, Germany
Plasmid		
pET-22b(+)	Amp^r, 5.5 kb, C-terminal His-Tag, T7 promoter/lac operator, *pelB* leader	Novagen, Germany
pET Duet-1	Amp^r, 5.4 kb, T7 promoter/lac operator, *ColE1* replicon, two MCS, His-Tag, S-Tag	Novagen, Germany
p22bLM	Amp^r, 8.3 kb, pET 22b(+) derivative with *lacLM* genes inserted before His-Tag	This work
pDuetL	Amp^r, 7.3 kb, pET Duet-1 derivative with *lacL* gene inserted after His-Tag	This work
pDuetLM	Amp^r, 8.2 kb, pET Duet-1 derivative with *lacL* gene inserted after His-Tag and *lacM* gene inserted before S-Tag	This work

[1]Amp^r = ampicillin resistant; Kan^r = kanamycin resistant; Tet^r = tetracycline resistant; MCS = multiple clone site.

infant formula powder, wherein they replicate the oligosaccharide effect of human milk (Torres et al., 2010). It is now believed that combining prebiotic GOS with probiotics in food sources will strongly benefit overall human health.

Lactic acid bacteria (**LAB**) are an established and crucial component of modern dairy processing and the food industry. The most common species applied are from the genera *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, and *Streptococcus*. *Lactobacillus fermentum* is a heterofermentative LAB that acts within a broad range of environmental niches, including dairy, meat, cereal, and vegetable fermentations, and even in the human gastrointestinal tract (Walter, 2008). The probiotic properties of some *L. fermentum* strains have been described, such as that of the ME-3 strain, which is also considered to elicit a prebiotic effect (Calderon Santoyo et al., 2003; Songisepp et al., 2004, 2005; Mikelsaar and Zilmer, 2009).

In recent years, whole-genome sequencing studies of LAB model strains have provided significant insights into the molecular mechanisms by which these bacteria affect biological processes. The principal objective of this study was to investigate the transgalactosylation properties of β-gal from *L. fermentum* K4. To this end, the LacLM β-gal was heterologously expressed and the recombinant protein purified. The amino acid sequences of LacLM and putative active sites were analyzed, and homology with other GH2 β-gal from various LAB strains was investigated. Our results indicated that the β-gal from *L. fermentum* K4 could be used to yield GOS.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Lactobacillus fermentum strain K4 (16S rDNA GenBank accession no. EU621851; Table 1) was grown anaerobically at 37°C in standard Lactobacillus de Man, Rogosa, and Sharpe broth (Difco, Detroit, MI) containing 2% lactose (wt/vol). *Escherichia coli* Origami B (DE3) (Table 1) was grown at 37°C under aeration in Luria-Bertani broth, supplemented with 100 μg/mL ampicillin and 30 μg/mL kanamycin for plasmid maintenance.

Gene Cloning and Vector Construction

Chromosomal DNA was extracted from *L. fermentum* K4 using the TIANamp bacteria genomic DNA extraction kit (Tiangen, Beijing, China). Amplification primers for the *lacL* and *lacM* genes encoding β-gal were designed according to the complete genome sequences of *L. fermentum* IFO 3956 (GenBank accession no. AP008937) and *L. fermentum* CECT 5716 (GenBank accession no. CP002033; Table 2). Amplification of the *lacLM* genes using Lf22b-F and Lf22b-R primers resulted in introduction of (5′) *Nco*I and (3′) *Xho*I restriction enzyme recognition sites, respectively. Likewise, amplification of the large subunit (*lacL*) gene using LfDuetL-F and LfDuetL-R primers introduced (5′) *Bam*HI and (3′) *Pst*I sites, and amplification of the small subunit (*lacM*) gene using LfDuetM-F and LfDuetM-R primers introduced (5′) *Nde*I and (3′) *Bgl*II sites.

Expression vectors pETDuet-1 and pET-22b(+) (Novagen, Darmstadt, Germany) were restructured with digested PCR products of *lacL* and *lacLM* genes, respectively, to generate pDuetL and p22bLM. Subsequently, pDuetL was used to construct the pDuetLM plasmid containing the complete *lacLM* genes. The restructured plasmids (Table 1) were confirmed by restriction enzyme digestion and sequencing.

Expression and Purification

The recombinant plasmids p22bLM, pDuetL, and pDuetLM were transformed into *E. coli* Origami B