Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

β-Galactosidase, phospho-β-galactosidase and phospho-β-glucosidase activities in lactobacilli strains isolated from human faeces

H. Honda[1], F. Kataoka[1], S. Nagaoka[2], Y. Kawai[1], H. Kitazawa[1], H. Itoh[3], K. Kimura[2], N. Taketomo[2], Y. Yamazaki[4], Y. Tateno[5] and T. Saito[1]

1 Graduate School of Agricultural Science, Tohoku University, Sendai, Japan
2 Food Science Institute, Meiji Dairies Corporation, Odawara, Japan
3 The Food Science Institute Foundation, Odawara, Japan
4 Center for Genetic Resource Information, National Institute of Genetics, Mishima, Japan
5 Center for Information Biology and DNA Data Bank of Japan, National Institute of Genetics, Mishima, Japan

Keywords
lactic acid bacteria, *Lactobacillus gasseri*, lactose, phospho-β-galactosidase, phospho-β-glucosidase.

Correspondence
Tadao Saito, Laboratory of Animal Products Chemistry, Graduate School of Agricultural Science, Tohoku University, 1-1 Tsutsumidori-Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan. E-mail: tsaito@bios.tohoku.ac.jp

2007/0092: received 21 January 2007, revised 21 March 2007 and accepted 4 April 2007

doi:10.1111/j.1472-765X.2007.02176.x

Abstract

Aims: Lactose intolerance, a serious health problem for Asians, can be solved using probiotic bacteria having high lactose hydrolysis activities. We determined the distribution of β-galactosidase (β-gal), phospho-β-galactosidase (P-βgal) and phospho-β-glucosidase (P-β-glc) activities in species of lactic acid bacteria (LAB) isolated from human faeces to select strains for potential use in fermented dairy products, e.g. yogurt.

Methods and Results: The sugar substrates, *o*-nitrophenyl-β-D- galactopyranoside 6-phosphate and *o*-nitrophenyl-β-D-glucopyranoside 6-phosphate, were synthesized and used to measure respectively P-β-gal and P-β-glc activities. Sixty-five toluene-treated strains were examined for three lactase enzyme activities. *Lactobacillus mucosae* OLL2848 showed the highest β-gal activity (107·09 U mg^{-1} of protein) among the *Lactobacillus* strains from human faeces. *Lactobacillus gasseri* OLL2836 and OLL 2948 showed the highest P-β-gal (46·58 U) and P-β-glc (50·19 U)activity, respectively, with no β-gal activity.

Conclusions: The expression of P-β-glc induced by lactose was characteristic of *Lact. gasseri*. Because this LAB is a major inhabitant of the human intestine. This enzyme is a key glycosidase involved in lactose utilization.

Significance and Impact of Study: This is the first report describing the distribution of three glycosidase activities used in lactose metabolism in LAB isolated from human faeces for possible use in functional foods.

Introduction

Lactic acid bacteria (LAB) improve the human intestinal microbiota and some strains of LAB are called probiotics because they contribute to beneficial health (Adolfsson *et al.* 2004; Parvez *et al.* 2006). The genus *Lactobacillus* is found in the human intestine (Benno *et al.* 1989; Kinoshita *et al.* 2007) and is used in various fermented milk products. Because these micro-organisms have industrial food importance, the metabolism of lactose, the principal sugar in milk, is of interest to produce foods and to prevent disease. Some strains of LAB utilize lactose and alleviate the symptoms of lactose intolerance (Alm 1982; Gilliland and Kim 1984; Marteau *et al.* 1990; De Vrese *et al.* 2001).

The symptoms of lactose intolerance are related to the presence of lactose in the colonic lumen. This is especially seen in the Asian population (Alm 1982; Fernandes *et al.* 1987). Lactose intolerance is caused when lactose cannot be digested in the intestine where lactase is not produced after weaning. The undigested lactose is used by general gut bacteria that produce several organic acids and gasses to cause the disease symptoms characterized by diarrhoea, flatulence and abdominal pain.

Two mechanisms of lactose transport are found in LAB. In many lactobacilli species, lactose is transported using

lactose-permiase and hydrolysed by β-galactosidase (β-gal, EC 3·2·1·23). The other transport mechanism in several bacterial strains is the lactose-specific phosphoenolpyruvate-dependent phosphotransferase system (lac-PTS) where lactose is transported and phosphorylated. Then, lactose 6′-phosphate is hydrolysed by phospho-β-galactosidase (P-β-gal, LacG, EC 3·2·1·85) to galactose 6-phosphate and glucose. The structural genes coding for P-β-gal have been cloned and sequenced from *Lactobacillus casei* (Porter and Chassy 1988), *Lactococcus lactis* ssp. *lactis* (Boizet *et al.* 1988; De Vos and Gasson 1989), *Lact. acidophilus* (Kanatani and Oshimura 1994), *Staphylococcus aureus* (Breidt and Stewart 1986) and *Streptococcus mutans* (Honeyman and Curtiss 1993). Simons *et al.* (1993) found LacG-deficient strains in *L. lactis* could hydrolyse *o*-nitrophenyl-β-D-galactopyranoside 6-phosphate (ONPGal-6P) and expressed P-β-glc.

Two enzymes having P-β-gal activity were observed in the same cytosol of *Lact. gasseri* JCM 1031 (Suzuki *et al.* 1993a,b). Their respective genes were cloned and sequenced. Both enzymes showed higher homology to phospho-β-glucosidase (P-β-glc, EC 3·2·1·86) than to P-β-gal (Saito *et al.* 1998). This suggests expression of P-β-glc is induced to hydrolyse lactose 6′-phosphate in some LAB.

We previously determined the distribution of β-gal and P-β-gal activity in *Lact. acidophilus* group LAB where all test strains of *Lact. gasseri* show P-β-gal activity and little or no β-gal activity (Sasaki *et al.* 1993). Recently, the complete sequence of genomic DNA of *Lact. gasseri* ATCC33323^T was reported by Makarova *et al.* (2006). Seven different genes putatively code for P-β-gal or P-β-glc in *Lact. gasseri* where there is no putative β-gal gene found (Microbial Genome Databases of the DOE Joint Genome Institute (JGI): http://genome.jgi-psf.org/draft_microbes/lacga/lacga.home.html). Therefore, it is likely *Lact.gasseri* commonly expresses P-β-gal and/or P-β-glc to utilize lactose.

Three glycosidases, β-gal, P-β-gal and P-β-glc, take part in the fermentation of lactose. The distribution of β-gal and P-β-gal activities in various LAB have been reported (McKay *et al.* 1970; Premi *et al.* 1972) including reports from our laboratory (Sasaki *et al.* 1993). However, there are no citations for the distribution of P-β-glc activity in LAB isolated from the human faeces. The chromogenic analogs ONPGal-6P and *o*-nitrophenyl-β-D-glucopyranoside 6-phosphate (ONPGlc-6P), are used to assay for P-β-gal and P-β-glc activity, respectively, in the absence of the natural disaccharide 6′-phosphate (Hengstenberg *et al.* 1970; Witt *et al.* 1993). Here, we synthesized ONPGal-6P and ONPGlc-6P (both not commercially available) to measure P-β-gal and P-β-glc activity and investigated the characteristics of lactose metabolism in LAB. We determined the distribution of the three glycosidases in LAB from human faeces to select strains for the potential use in probiotic dairy products.

Materials and methods

Bacterial strains and growth conditions

Lactobacillus OLL strains and MEP strains isolated from human faeces were a gift from the culture collection of Meiji Dairies Corporation (Odawara, Japan). *Lactobacillus gasseri* ATCC 33323^T was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). *Lactobacillus gasseri* JCM 1031, *Lact. acidophilus* JCM 1132^T, *Lact. johnsonii* JCM 1017 and JCM 2122, and *Lact. casei* JCM 1134^T were obtained from the Japan Collection of Micro-organisms (JCM; Wako, Japan). Two LA strains were isolated from human faeces using modified LBS agar. *L. lactis* ssp. *lactis* NCFB 176 was obtained from the National Collections of Food Bacteria (NCFB; Aberdeen, UK). *L. lactis* ssp. *lactis* NIAI 527 was used as a positive control for P-β-gal activity. *Lactobacillus delbrueckii* ssp. *bulgaricus* NIAI B-6 was used as a positive control for β-gal activity. These two strains and *Lact. casei* NIAI C-9 were obtained from the National Institute of Animal Industry (NIAI; Tsukuba, Japan). All strains were stored frozen at −80°C in skim milk.

All strains were successively propagated three times in lactobacilli MRS broth (Difco Laboratories, Detroit, MI, USA); then twice in MRSL broth using incubation for 24 h at 30°C for *L. lactis* and *Lact. casei* NIAI C-9 and 37°C for all other strains. MRSL broth is a modified MRS broth where lactose (2%) is substituted for glucose. One hundred microlitres of the cultures were inoculated into 10 ml of MRSL broth and incubated for 18 h at the appropriate optimum temperature. The bacterial cells were harvested by centrifugation at 3000 *g* for 15 min and washed with 0·05 mol l^{-1} phosphate buffer (Na_2HPO_4–KH_2PO_4, pH 6·8). The cells were resuspended in distilled water and lyophilized.

Synthesis of sugar substrates

ONPGal-6P and ONPGlc-6P were respectively synthesized from *o*-nitrophenyl-β-D- alactopyranoside (ONPGal, Sigma-Aldrich, St Louis, MO, USA) and *o*-nitrophenyl- β-D-glucopyranoside (ONPGlc, Sigma-Aldrich) using the method of Hengstenberg and Morse (1969).

Assay of enzymes

β-gal, P-β-gal and P-β-glc activities were measured using the methods of Citti *et al.* (1965) and Fisher *et al.* (1985) with some modifications. One millilitre of cell suspension

(0·5 mg of dry weight ml^{-1}) in 0·05 mol l^{-1} phosphate buffer was vigorously mixed with 50 μl of toluene-acetone (1/9, v/v) solution for three min at room temperature. A 25 μl volume of this cell suspension was incubated with 100 μl of 5 mmol l^{-1} of each substrate in the phosphate buffer for 15 min at 37°C. The β-gal, P-β-gal or P-β-glc activity was assayed using ONPGal, ONPGal-6P, or ONPGlc-6P, respectively. Adding 125 μl of 0·5 mol l^{-1} Na_2CO_3 stopped the reaction. Cells were removed by centrifugation at 2000 *g* for 15 min and the absorbance of the supernatant was measured at 405 nm. One unit of enzyme activity was defined as one micromole of *o*-nitrophenol liberated from each substrate per mg protein per minute. The amount of protein in the cell suspension was determined using the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA).

Results

The three lactase activities in 65 LAB strains including the two control LAB strains, *Lact. delbrueckii* ssp. *bulgaricus* NIAI B-6 and *L. lactis* ssp. *lactis* NIAI 527, were determined. The highest β-gal activity (844·47 U) was found in the control strain, *Lact. delbrueckii* ssp. *bulgaricus* NIAI B-6 (Table 1). Among the tested strains from human faeces, *Lact. mucosae* OLL 2848 had the highest β-gal activity (107·09 U); however, the activity was only one-eighth as high as the control strain, NIAI B-6. The P-β-gal activity of *Lact. gasseri* OLL 2836 was the highest (46·58 U) of the tested lactobacilli strains from human faeces and was as high as the control strain, NIAI 527 (49·89 U). *Lactobacillus gasseri* OLL 2948 showed higher P-β-glc activity (50·19 U) than any of the other examined strains.

Most *Lactobacillus* strains (except *Lact. gasseri*) have higher β-gal activities than P-β-gal and P-β-glc. Among the strains, generally *Lact. mucosae* showed high β-gal activity. In contrast, *Lact. gasseri* strains showed little or no β-gal activity and commonly expressed P-β-gal and/or P-β-glc activities in lactose medium.

Discussion

P-β-gal activity is usually detected using chemically synthesized ONPGal-6P. The sugar substrate is prepared by phosphorylation of ONPGal at the primary-OH group of the galactose residue with phosphorus oxychloride. However, as described by Thompson *et al.* (2002), this method cannot be used for the synthesis of lactose 6′-phosphate from lactose as a starting materials, because the presence of two primary-OH groups in lactose yields a mixture of lactose 6-phospate, lactose 6′-phosphate and lactose 6,6′-diphosphate. Therefore, it is difficult to detect each free

Table 1 β-Gal, P-β-gal and P-β-glc activities in toluene-treated cell suspensions using various lactic acid bacteria

Strains	β-gal	P-β-gal	P-β-glc
Lactobacillus acidophilus			
JCM1132^T	12·87	0·40	1·33
LA117	10·89	0·10	0·82
Lact. amylovorus			
MEP181R45	6·47	0·18	48·02
MEP181R50	7·29	0·25	4·80
MEP181R51	6·35	0·02	1·81
Lact. casei			
JCM1134^T	12·66	3·45	1·45
MEP181R52	0·00	2·55	0·42
MEP181R53	0·00	0·14	0·29
MEP181R54	0·00	0·11	0·26
NIAI C-9	5·18	2·98	0·43
Lact. crispatus			
MEP181R55	5·03	0·04	1·12
MEP181R56	4·42	0·12	0·76
Lact. fermentum			
MEP181R57	8·30	0·03	0·08
MEP181R58	1·06	0·03	0·05
Lact. gasseri			
ATCC33323^T	0·00	4·18	5·72
JCM1031	0·00	1·38	5·36
LA2	0·00	3·49	6·45
OLL2836	0·00	46·58	2·20
OLL2948	0·00	1·81	50·19
MEP181R34	0·00	18·73	3·41
MEP181R35	0·00	9·63	3·37
MEP181R36	0·00	7·61	3·05
MEP181R40	0·00	15·78	2·53
MEP181R41	0·05	9·91	40·37
MEP181R42	0·00	1·27	31·96
MEP181R43	0·06	7·31	28·56
MEP181R44	0·00	0·93	19·66
MEP181R46	0·00	30·63	50·02
MEP181R47	0·06	1·20	22·55
MEP181R48	0·00	8·32	22·07
MEP181R49	0·00	35·57	19·84
MEP181R65	0·10	2·46	2·92
Lact. johnsonii			
JCM1017	2·94	0·00	1·21
JCM2122	3·32	0·13	2·78
Lact. mucosae			
OLL2848	107·09	0·60	4·85
MEP181R12	84·26	0·00	0·00
MEP181R66	32·61	0·00	0·00
MEP181R67	24·79	0·09	0·04
MEP181R68	29·02	0·03	0·11
MEP181R69	44·45	0·02	0·00
MEP181R70	43·91	0·07	0·10
MEP181R71	38·30	0·11	0·00
MEP181R72	37·61	0·06	0·04
MEP181R73	26·98	0·00	0·00
MEP181R74	34·39	1·75	2·57
MEP181R75	33·13	0·00	0·05
MEP181R76	41·06	0·00	0·00

Table 1 (*Continued*)

Strains	β-gal	P-β-gal	P-β-glc
MEP181R77	11·62	0·00	0·00
MEP181R78	8·09	0·00	0·04
MEP181R79	25·87	0·00	0·00
MEP181R80	38·98	0·00	0·03
MEP181R81	34·66	0·05	0·10
MEP181R82	28·26	0·00	0·00
Lact. oris			
MEP181R83	11·32	0·00	0·00
MEP181R84	7·49	0·06	0·13
MEP181R85	7·25	0·00	0·00
Lact. plantarum			
MEP181R86	5·40	0·17	1·81
MEP181R87	4·64	0·15	1·51
Lact. reuteri			
MEP181R88	8·58	0·00	0·00
Lact. salivalius			
MEP181R89	1·62	0·00	0·00
MEP181R90	2·96	0·00	0·00
Lact. vaginalis			
MEP181R91	6·13	0·23	2·19
Lact. delbrueckii ssp. *bulgaricus*			
NIAI B-6	844·47	0·00	0·00
L. lactis ssp. *lactis*			
NCFB 176	0·00	9·38	2·71
NIAI 527	0·00	49·89	0·84

Cells were grown in MRS broth containing 2% lactose.
A superscript T after strain number shows the strain is the type strain of the species.
Numbers in the table indicate the units of enzyme activity.

sugar with high sensitivity after hydrolysis of lactose 6′-phosphate. Therefore, we made synthetic substrates to measure the three glycosidase activities.

Many of the *Lact. mucosae* strains show higher β-gal activity than the other strains from human faeces. The data suggest intestinal strains such as *Lact. mucosae* have an important role for continuous lactose utilization in adult human intestine although the highest β-gal activity of OLL 2848 was much lower than the dairy *Lact. delbrueckii* ssp. *bulgaricus* NIAI B-6. Most LAB strains having neither P-β-glc activity nor P-β-gal activity have high β-gal activity.

Lactobacillus mucosae was first isolated from pig intestine (Roos *et al.* 2000) and later detected in humans (Decroos *et al.* 2005; Kinoshita *et al.* 2007). Our data shows *Lact. mucosae* displays the highest β-gal activity among the lactobacilli from human faeces. Further, *Lact. mucosae* have a homolog of the mucus-binding protein (Roos *et al.* 2000). These factors likely contribute to its ability to colonize, survive and adapt in the human intestine having a potential role as a probiotics.

The enzyme activities shown by each strain differ with species. However, some strains of *Lact. casei* show higher β-gal activity and other strains show higher P-β-gal activity. This is in agreement with Jimeno *et al.* (1984).

Recently, seven structural genes of *Lact. gasseri* ATCC33323^T were found to be putatively coded as phospho-β-glycosidase. We found *Lact. gasseri* MEP181R46 and MEP181R49 had both high P-β-gal and P-β-glc activities. The data suggests the number of expressed enzymes may be strain-specific in *Lact. gasseri*. *Lactobacillus gasseri* is known to be the predominant lactobacilli occurring in Japanese people (Benno *et al.* 1989). Also, *Lact. gasseri* is used for many fermented milk products in Japan. In spite of this, little is known about the metabolic utilization of lactose by *Lact. gasseri*.

Although it is difficult to distinguish among the six species of the *Lact. acidophilus* group LAB [*Lact. acidophilus* (A_1 subgroup), *Lact. crispatus* (A_2), *Lact. amylovorus* (A_3), *Lact. gallinarum* (A_4), *Lact. gasseri* (B_1), and *Lact. johnsonii* (B_2)] using several biochemical characteristics, lactose metabolism using P-β-gal and/or P-β-glc and no β-gal was shown in our data as characteristic of *Lact. gasseri*. Therefore, *Lact. gasseri* can be easily identified using this difference among the *Lact. acidophilus* group LAB. Because *Lact. gasseri* is known to be the predominant lactobacilli in the Japanese people's intestinal content(Benno *et al.* 1989) and, the number of patients having lactose intolerance is very high in adults in Japan, our data suggests using selected strains of *Lact. gasseri* may reduce and improve the symptoms of lactose intolerance caused by lactose in dairy foods.

As described by He *et al.* (2006), lactose fermentation in the colon plays a central role in lactose intolerance. The colonic adaptation to lactose is achieved in people with lactose intolerance using microbiota therapy (Hertzler and Savaiano 1996). An altered microbiota is demonstrated in some Japanese adults who cannot produce lactase to be able to consume lactose in the dairy products without experiencing lactose intolerance disease. This suggests lactose utilization by the LAB microbiota is an important factor in mitigating lactose intolerance.

Experiments are now in progress in our laboratory to clarify the characteristics of the glycosidases in three selected strains: *Lact. mucosae* OLL 2848 and *Lact. gasseri* (OLL 2836 and OLL 2948) for potential use in functional dairy products..

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research (B) (no. 16380179) from the Japan Society for the Promotion of Science (JSPS) and the Food Science Institute Foundation to T. Saito. We thank Meiji Dairies Corporation for the gift of the OLL strains and MEP strains.

References

Adolfsson, O., Meydani, S.N. and Russel, R.M. (2004) Yogurt and gut function. *Am J Clin Nutr* **80**, 245–256.

Alm, L. (1982) Effect of fermentation on lactose, glucose and galactose content in milk and suitability of fermented milk products for lactose intolerant individuals. *J Dairy Sci* **65**, 346–352.

Benno, Y., Endo, K., Mizutani, T., Namba, Y., Komori, T. and Mitsuoka, T. (1989) Comparison of fecal microflora of elderly persons in rural and urban areas of Japan. *Appl Environ Microbiol* **55**, 1100–1105.

Boizet, E., Villeval, D., Slos, P., Novel, M., Novel, G. and Mercenier, A. (1988) Isolation and structural analysis of the phospho-β-galactosidase gene from *Streptococcus lactis* Z268. *Gene* **62**, 249–261.

Breidt, F. Jr and Stewart, G.C. (1986) Cloning and expression of the phospho-β-galactosidase gene of *Staphylococcus aureus* in *Escherichia coli*. *J Bacteriol* **166**, 1061–1066.

Citti, J.E., Sandine, W.E. and Elliker, P.R. (1965) β-galactosidase of *Streptococcus lactis*. *J Bacteriol* **89**, 937–942.

De Vos, W.M. and Gasson, M.J. (1989) Structure and expression of the *Lactococcus lactis* gene for phospho-β-galactosidase (LacG) in *Escherichia coli* and *L. lactis*. *J Gen Microbiol* **135**, 1833–1846.

De Vrese, M., Stegelmann, A., Richter, B., Fenselau, S., Laue, C. and Schrezenmeir, J. (2001) Probiotics-compensation for lactase insufficiency. *Am J Clin Nutr* **73**, 421–429.

Decroos, K., Vanhemmens, S., Cattoir, S., Boon, N. and Verstracte, W. (2005) Isolation and characterization of an equal-producing mixed microbial culture from a human fecal sample and its activity under gastrointestinal conditions. *Arch Microbiol* **183**, 45–55.

Fernandes, C.F., Shahani, K.M. and Amer, M.A. (1987) Therapeutic role of dietary lactobacilli and lactobacillic fermented dairy products. *FEMS Microbiol Rev* **46**, 343–356.

Fisher, K., Johnson, M.C. and Ray, B. (1985) Lactose hydrolyzing enzymes in *Lactobacillus acidophilus* strains. *Food Microbiol* **2**, 23–29.

Gilliland, S.E. and Kim, H.S. (1984) Effect of viable starter culture bacteria in yogurt on lactose utilization in humans. *J Dairy Sci* **67**, 1–6.

He, T., Prieve, M.G., Harmsen, H.J.M., Stellaard, F., Sun, X., Welling, G.W. and Vonk, R.J. (2006) Colonic fermentation may play a role in lactose intolerance in humans. *J Nutr* **136**, 58–63.

Hengstenberg, W. and Morse, M.L. (1969) An improved method of synthesis of *o*-nitrophenyl β-D-galactopyranoside 6-phosphate. *Carbohydr Res* **10**, 463–465.

Hengstenberg, W., Penberthy, W.K. and Morse, M.L. (1970) Purification of the Staphylococcal 6-phospho-β-D-galactosidase. *Eur J Biochem* **14**, 27–32.

Hertzler, S.R. and Savaiano, D.A. (1996) Colonic adaptation to daily lctose feeding in lactose maldigesters reduces lactose inlolerance. *Am J Clin Nutr* **64**, 232–236.

Honeyman, A.L. and Curtiss, R. III (1993) Isolation, characterization and nucleotide sequence of the *Streptococcus mutans* lactose-specific enzyme II (*lacE*) gene of the PTS and the phospho-β-galactosidase (*lacG*) gene. *J Gen Microbiol* **139**, 2685–2694.

Jimeno, J., Casey, M. and Hofer, F. (1984) The occurrence of β-galactosidase and β-phosphogalactosidase in *Lactobacillus casei* strains. *FEMS Microbiol Lett* **25**, 275–278.

Kanatani, K. and Oshimura, M. (1994) Isolation and structural analysis of the phospho-β-galactosidase gene from *Lactobacillus acidophilus*. *J Biosci Bioeng* **78**, 123–129.

Kinoshita, H., Uchida, H., Kawai, Y., Kitazawa, H., Miura, K., Shiiba, K., Horii, A. and Saito, T. (2007) Quantitative evaluation of adhesion of lactobacilli isolated from human intestinal tissues to human colonic mucin using surface plasmon resonance (BIACORE assay). *J Appl Microbiol* **102**, 116–123.

Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., Pavlov, A., Pavlova, N. *et al.* (2006) Comparative genomics of the lactic acid bacteria. *Proc Natl Acad Sci USA* **103**, 15611–15616.

Marteau, P., Flourie, B., Pochart, F., Chastang, C., Desjeux, J.F. and Rambaud, J.C. (1990) Effect of the microbial lactase (EC 3·2·1·23) activity in yogurt on the intestinal absorption of lactose: an in vivo study in lactase-deficient humans. *Br J Nutr* **64**, 71–79.

McKay, L., Miller, A. III, Sandine, W.E. and Elliker, P.R. (1970) Mechanisms of lactose utilization by lactic acid streptococci: enzymatic and genetic analyses. *J Bacteriol* **102**, 804–809.

Parvez, S., Malik, K.A., Ah Kang, S. and Kim, H.-Y. (2006) Probiotics and their fermented food products are beneficial for health. *J Appl Microbiol* **100**, 1171–1185.

Porter, E.V. and Chassy, B.M. (1988) Nucleotide sequence of the β-D-phosphogalactoside galactohydrolase gene of *Lactobacillus casei*: comparison to analogous *pbg* genes of other Gram-positive organisms. *Gene* **62**, 263–276.

Premi, L., Sandine, W.E. and Elliker, P.R. (1972) Lactose-hydrolyzing enzymes of *Lactobacillus* species. *Appl Microbiol* **24**, 51–57.

Roos, S., Karner, F., Axelsson, L. and Jonsson, H. (2000) *Lactobacillus mucosae* sp. nov., a new species with in vitro mucus-binding activity isolated from pig intestine. *Int J Syst Evol Microbiol* **50**, 251–258.

Saito, T., Suzuki, M., Konno, K., Kitazawa, H., Kawai, Y., Itoh, T. and Kamio, Y. (1998) Molecular cloning and sequencing of two phospho-β-galactosidase I and II genes of *Lactobacillus gasseri* JCM 1031 isolated from human intestine. *Biosci Biotechnol Biochem* **62**, 2318–2327.

Sasaki, K., Samant, S.K., Suzuki, M., Toba, T. and Itoh, T. (1993) β-Galactosidase and phospho-β-galactosidase activities in strains of *Lactobacillus acidophilus* complex. *Lett Appl Microbiol* **16**, 97–100.

Simons, G., Nijhuis, M. and de Vos, W.M. (1993) Integration and gene replacement in the *Lactococcus lactis lac* operon:

induction of a cryptic phospho-β-glucosidase in LacG-deficient strains. *J Bacteriol* **175**, 5168–5175.

Suzuki, M., Saito, T. and Itoh, T. (1993a) Purification and characterization of 6-phospho-β-galactosidase in the cytosol of *Lactobacillus gasseri* JCM1031. *Biosci Biotech Biochem* **60**, 139–141.

Suzuki, M., Saito, T. and Itoh, T. (1993b) Coexistence of two kinds of 6-phospho-β-galactosidase in the cytosol of *Lactobacillus gasseri* JCM1031 – purification and characterization of 6-phospho-β-galactosidase II. *Biosci Biotech Biochem* **60**, 708–710.

Thompson, J., Lichtenthaler, F.W., Peters, S. and Pikis, A. (2002) β-Glucoside kinase (BglK) from *Klebsiella pneumoniae*. *J Biol Chem* **277**, 34310–34321.

Witt, E., Frank, R. and Hengstenberg, W. (1993) 6-Phospho-β-galactosidases of Gram-positive and 6-phospho-β-glucosidase B of Gram-negative bacteria: comparison of structure and function by kinetic and immunological methods and mutagenesis of the *lacG* gene of *Staphylococcus aureus*. *Protein Eng* **6**, 913–920.