Journal of Food Protection, Vol. 75, No. 6, 2012, Pages 1090–1098
doi:10.4315/0362-028X.JFP-11-456

Effects of Bile Salt Deconjugation by Probiotic Strains on the Survival of Antibiotic-Resistant Foodborne Pathogens under Simulated Gastric Conditions

XINLONG HE,[1] YUNYUN ZOU,[1] YOUNGJAE CHO,[2] AND JUHEE AHN[1,2]*

[1]*Medical and Bio-Material Research Center and Department of Biomaterials Engineering and* [2]*Research Institute of Bioscience & Biotechnology, Kangwon National University, Chuncheon, Gangwon, Republic of Korea*

MS 11-456: Received 6 October 2011/Accepted 3 February 2012

ABSTRACT

This study was designed to evaluate the effects of bile acid deconjugation by probiotic strains on the antibiotic susceptibility of antibiotic-sensitive and multiple antibiotic–resistant *Salmonella* Typhimurium and *Staphylococcus aureus*. Eight probiotic strains, *Bifidobacterium longum* B6, *Lactobacillus acidophilus* ADH, *Lactobacillus brevis* KACC 10553, *Lactobacillus casei* KACC 12413, *Lactobacillus paracasei* ATCC 25598, *Lactobacillus rhamnosus* GG, *Leuconostoc mesenteroides* KACC 12312, and *Pediococcus acidilactici* KACC 12307, were used to examine bile acid tolerance. The ability to deconjugate bile acids was evaluated using both thin-layer chromatography and high-performance liquid chromatography. The antibiotic susceptibility testing was carried out to determine the synergistic inhibitory activity of deconjugated bile acids. *L. acidophilus, L. brevis,* and *P. acidilactici* showed the most tolerance to the conjugated bile acids. *P. acidilactici* deconjugated glycocholic acid and glycodeoxycholate from 3.18 and 3.09 mM to the detection limits, respectively. The antibiotic susceptibility of selected foodborne pathogens was increased by increasing the concentration of deconjugated bile acids. The study results are useful for understanding the relationship between bile acid deconjugation by probiotic strains and antibiotic susceptibility in the presence of deconjugated bile acids, and they may be useful for designing new probiotic-antibiotic combination therapy based on bile acid deconjugation.

Cholesterol is converted into bile acids by oxidation in the liver (neutral pathway) and hydroxylation in the extrahepatic tissue (acidic pathway), resulting in the production of cholic acid and chenodeoxycholic acid, which are stored in the gallbladder and released in the form of glycol- and tauro-conjugated bile salts into the duodenum, the jejunum, and ileum *(6, 35)*. The conjugated bile salts are detergent-like amphipathic molecules responsible for cholesterol homeostasis and emulsification, solubilization, and absorption of dietary fats and fat-soluble vitamins *(2, 15)*. Bile acids exist as conjugated forms, including mainly glycocholic acid (12.6 mM) and taurocholic acid (6.9 mM) in the duodenum, which are reduced to 1 to 3 mM and 0.4 to 1.2 mM, respectively, in the ileum *(18, 25, 36)*. Bile acids are further transformed to deconjugated bile acids such as cholic acid and deoxycholic acid *(9)*. There are two main microbial modifications of conjugated bile salts *(9, 13)*. The first step is hydrolysis of glycocholic acid, glycodeoxycholic acid, taurocholic acid, and taurodeoxycholic acid to cholic acid, which is catalyzed by bile salt hydrolase produced by intestinal bacteria such as *Enterococcus, Bifidobacterium, Lactobacillus, Clostridium, Fusobacterium, Bacteroides, Pseudomonas, Bacillus,* and *Peptostreptococcus (10, 11, 13)*. The deconjugated bile acids are further metabolized to the secondary bile salts, such as deoxycholic acid and litocholic acid, through dehydroxylation *(9)*. The bacterial deconjugation and transformation of bile acids may have an influence on the ecological changes in the gastrointestinal (GI) tract. The normal intestinal biota is exposed to bile acid stress, leading to the induction of antimicrobial activity, tolerance, and adaptation *(2)*. Therefore, it is necessary to understand the physiological interaction of pathogens with deconjugated bile salts.

Over the past few decades, the misuse and overuse of antibiotics have contributed to alterations in the intestinal biota balance and the rapid emergence of antibiotic-resistant foodborne pathogens *(43)*. Infections caused by the increasing spread of antibiotic-resistant bacteria have become a serious public health problem worldwide; these infections are primarily caused by the consumption of contaminated foods *(23, 28, 35, 42)*. Therefore, antibiotic-resistant foodborne pathogens can encounter bile stress when passing through the GI tract *(38)*. Recently, probiotic-antibiotic combination therapy has received much research attention for its improvement of intestinal biota balance and inhibition of pathogenic bacteria *(16)*. The use of probiotic strains can be a holistic approach due to their beneficial effects including intestinal biota modulation, competitive exclusion, and immune stimulation *(32, 37)*. However, there

* Author for correspondence. Tel: +82-33-250-6564; Fax: +82-33-253-6560; E-mail: juheeahn@kangwon.ac.kr.

is still a lack of information on the interaction between bile acid deconjugation and antibiotic-resistant foodborne pathogens in the GI tract in terms of the physicochemical properties of bile salts and the susceptibility of antibiotic-resistant foodborne pathogens to deconjugated bile acids. Therefore, the objectives of this study were (i) to evaluate the ability of selected probiotic strains to hydrolyze conjugated bile acids glycocholic acid, glycodeoxycholate, taurocholic acid, and taurodeoxycholic acid; (ii) to investigate the inhibitory effect of deconjugated bile acids against antibiotic-sensitive and multiple antibiotic–resistant *Salmonella* Typhimurium and *Staphylococcus aureus*; and (iii) to assess the antibiotic susceptibility patterns of selected foodborne pathogens in the presence of deconjugated bile acids cholic acid and deoxycholic acid.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strains of *Bifidobacterium longum* B6 *(1), Lactobacillus acidophilus* ADH *(44), Lactobacillus rhamnosus* GG *(17),* and *Lactobacillus paracasei* ATCC 25598 *(21)* were kindly provided by Dr. Azlin Mustapha from the University of Missouri (Columbia). *Lactobacillus brevis* KACC 10553 isolated from beer, *Lactobacillus casei* KACC 12413 isolated from cheese, *Leuconostoc mesenteroides* KACC 12312 isolated from fermenting olives, and *Pediococcus acidilactici* KACC 12307 isolated from barley were obtained from the Korean Agricultural Culture Collection (KACC; Suwon, Korea). Probiotic strains were anaerobically cultivated in de Man Rogosa Sharpe (MRS) broth (Difco, BD, Sparks, MD) supplemented with 0.05% cysteine hydrochloride at 37°C for 24 h in a GasPak anaerobic system (BBL, BD, Cockeysville, MD) with AnaeroGen (Oxoid Ltd., Hampshire, UK). Strains of *Salmonella enterica* serovar Typhimurium KCCM 40253 and *S. aureus* KACC 13236 were obtained from the Korean Culture Center of Microorganisms (KCCM; Seoul, Korea) and the KACC. *Salmonella* Typhimurium CCARM 8009 and *S. aureus* CCARM 3080 were obtained from the Culture Collection of Antibiotic Resistant Microbes (CCARM; Seoul, Korea); these are clinically isolated antibiotic-resistant strains. Pathogenic strains were cultured in Trypticase soy broth (TSB; Difco, BD) at 37°C for 20 h. The cultured cells were collected by centrifugation at 3,000 × *g* for 20 min at 4°C and washed twice with sterile 0.1% peptone water.

Chemicals. Sodium salts of glycocholate, glycodeoxycholate, taurocholate, taurodeoxycholate, cholate, and deoxycholate and antibiotics used in this study (Table 1) were purchased from Sigma (St. Louis, MO). All chemicals and solvents were high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), or analytical grade, including methanol (CH_3OH; Fisher Scientific, Fair Lawn, NJ), ethylene dichloride ($C_2H_4Cl_2$; Sigma), acetic acid (CH_3COOH; Sigma), phosphomolybdic acid ($H_3[P(Mo_3O_{10})_4]$; Sigma), and phosphoric acid (H_3PO_4; Sigma).

Conjugated bile salt tolerance assay. To evaluate the tolerance to bile acids, probiotic strains (10^6 CFU/ml each) were anaerobically cultured at 37°C for 24 h in TSB containing different concentrations (0.13, 0.4, 0.08, and 0.4 mM; 0.26, 0.8, 0.16, and 0.8 mM; 0.52, 1.6, 0.32, and 1.6 mM; 1.04, 3.2, 0.64, and 3.2 mM) of taurocholic acid, glycocholic acid, taurodeoxycholic acid, and glycodeoxycholate, respectively. The viable counts were determined by pour plating serial dilutions on MRS agar. After cultivation, the supernatants were collected by centrifugation at 3,000 × *g* for 10 min at 4°C. The collected supernatants (pH 4 to 5) were adjusted to pH 7 with 1 M sodium hydroxide (NaOH) and used for deconjugation ability of probiotic strains and susceptibility testing of selected foodborne pathogens in the mixed bile salt solutions.

TABLE 1. *Description of antibiotics used in this study*

Antibiotic	Inhibitory mechanism	Abbreviation
Aztreonam	Peptidoglycan synthesis	AT
Benzylpenicillin	Peptidoglycan synthesis	BP
Chloramphenicol	Protein synthesis	CP
Cefotaxime	Peptidoglycan synthesis	CX
Ceftazidime	Cell wall synthesis	CZ
Erythromycin	Protein synthesis	EM
Kanamycin	Protein synthesis	KM
Nalidixic acid	DNA replication	NA
Novobiocin	DNA gyrase	NB
Norfloxacin	Cell division	NF
Polymyxin B	Membrane permeability	PM
Streptomycin	Protein synthesis	SM
Tetracycline	Protein synthesis	TC

HPLC analysis. Bile acids in the collected supernatants were analyzed by Waters HPLC system (M600E, M7725i/Waters, 996PDA, Waters, Milford, MA) equipped with UV detector (200 nm) using reverse-phase Zorbax Eclipse XDB-C18 column (4.6 by 250 mm, 18-μm particle size; Agilent Technologies Inc., Santa Clara, CA). The mobile phase consisted of a mixture of methanol and 0.01 M KH_2PO_4 (75:25, vol/vol), which was added with 5 N NaOH (4.2 ml/liter) and then adjusted to pH 5.35 with 85% H_3PO_4. The samples (20 ml each) were injected with a column temperature of 35°C and eluted at a flow rate of 0.7 ml/min. The conjugated bile acids (taurocholic acid, taurodeoxycholic acid, glycocholic acid, and glycodeoxycholate) were dissolved in TSB and serially diluted from 0.08 to 4.0 mM. The standard curve for each bile acid was established by plotting peak areas against dilutions. The peaks for taurocholic acid, taurodeoxycholic acid, glycocholic acid, and glycodeoxycholate were identified by retention times and corrected with library spectra obtained from standard solutions. The coefficients of determination (r^2) were 0.996 for taurocholic acid, 0.999 for taurodeoxycholic acid, 0.995 for glycocholic acid, and 0.997 for glycodeoxycholate.

TLC analysis. The deconjugation ability of probiotic strains was evaluated by TLC *(12)*. Conjugated (taurocholic acid, taurodeoxycholic acid, glycocholic acid, and glycodeoxycholate) and deconjugated (cholic acid and deoxycholic acid) bile acids were used as standard markers. TSB containing taurocholic acid, taurodeoxycholic acid, glycocholic acid, and glycodeoxycholate was used as a control. The solvent system consisted of ethylene dichloride, acetic acid, and water (10:10:1). The samples were concentrated using an evaporator at 37°C and applied on a silica gel plate (5 by 10 cm, 250-μm thickness, 17-μm particle size; Fluka, Milwaukee, WI). After chromatographic separation, the plate was dried and stained with 10% phosphomolybdic acid in alcohol and then heated for 10 min at 120°C. The stained plate was treated with ammonia water for 30 s to remove yellow background.

Time-kill studies. Time-kill studies were performed to evaluate the antimicrobial activity of the collected probiotic culture supernatants, which were previously adjusted to pH 7, against antibiotic-sensitive (*Salmonella* Typhimurium KCCM 40253 and *S.*

TABLE 2. *Growth of selected probiotic strains in TSB supplemented with different concentrations of conjugated bile acids after 24 h of cultivation*

Probiotic strain	Growth (log CFU/ml) in conjugated bile acids [TCA, GCA, TDCA, GDCA] (mM)[a]				
	[0, 0, 0, 0][b]	[0.13, 0.40, 0.08, 0.40]	[0.26, 0.80, 0.16, 0.80]	[0.52, 1.60, 0.32, 1.60]	[1.04, 3.20, 0.64, 3.20]
Bifidobacterium longum	8.84 ± 0.08 cd A[c]	8.32 ± 0.23 d A	7.36 ± 0.06 d B	6.31 ± 0.23 d C	6.27 ± 0.37 c C
Lactobacillus acidophilus	9.15 ± 0.09 ab A	9.16 ± 0.04 ab A	8.95 ± 0.10 b A	8.13 ± 0.04 c B	8.12 ± 0.11 b B
L. brevis	8.50 ± 0.05 e A	8.61 ± 0.04 cd A	8.66 ± 0.21 b A	8.74 ± 0.10 b A	8.00 ± 0.13 b B
L. casei	9.01 ± 0.04 bc A	8.83 ± 0.10 bc A	8.02 ± 0.09 c B	6.21 ± 0.13 d C	5.31 ± 0.59 d D
L. paracasei	8.96 ± 0.05 bc A	8.50 ± 0.24 cd A	6.82 ± 0.22 e B	6.06 ± 0.24 d C	5.14 ± 0.24 d D
L. rhamnosus	9.22 ± 0.23 ab A	9.11 ± 0.13 b A	7.54 ± 0.28 d B	6.33 ± 0.37 d C	4.97 ± 0.18 d D
Leuconostoc mesenteroides	8.64 ± 0.18 de A	7.77 ± 0.19 e B	5.80 ± 0.25 f C	5.07 ± 0.12 e D	3.97 ± 0.36 e E
Pediococcus acidilactici	9.37 ± 0.12 a A	9.50 ± 0.09 a A	9.64 ± 0.15 a A	9.58 ± 0.21 a A	9.33 ± 0.06 a A

[a] TCA, taurocholic acid; GCA, glycocholic acid; TDCA, taurodeoxycholic acid; GDCA, glycodeoxycholic acid.
[b] Numbers in brackets indicate the concentrations of TCA, GCA, TDCA, and GDCA, respectively.
[c] Values are means ± standard deviations. Means with different letters within a column (a through f) and a row (A through D) are significantly different at $P < 0.05$.

aureus KACC 13236) and multiple antibiotic–resistant (*Salmonella* Typhimurium CCARM 8009 and *S. aureus* CCARM 3080) strains. The probiotic culture supernatant (5 ml) was inoculated with selected foodborne pathogens (10^5 CFU/ml each) and incubated anaerobically at 37°C for 4, 8, 12, 16, 20, and 24 h. Bacterial populations were enumerated on tryptic soy agar using a plate count method.

Inhibition kinetics. Time-kill curves were fitted to the modified Gompertz model *(24),* and the lag-phase duration (LPD) was estimated as follows:

$$\log N = A + C \cdot \exp\left[-\exp\left(\frac{-B}{(t-M)}\right)\right] \quad (1)$$

$$\mathrm{LPD} = M - \left(\frac{1+e^{1-e^{BM}}}{B}\right) \quad (2)$$

where N is the viable bacterial number (CFU per milliliter) at time t; A is the initial bacterial number at time zero (CFU per milliliter); B is the growth rate at the inflection point (1/h), C is the number of log cycles of bacterial growth (CFU per milliliter), and M is the time needed to reach maximum growth rate (hours).

Antibiotic susceptibility assay. The antibiotic susceptibility of selected foodborne pathogens was determined in Mueller-Hinton broth containing different concentrations of cholic acid and deoxycholic acid (1:1; 0, 0.03, 0.1, and 0.5 mM) according to the Clinical and Laboratory Standards Institute procedure *(8)*. The antibiotics were dissolved in Mueller-Hinton broth and then serially diluted (1:2) to concentrations ranging from 0.25 to 256 μg/ml in 96-well plates. Strains of *Salmonella* Typhimurium KCCM 40253, *S. aureus* KACC 13236, *Salmonella* Typhimurium CCARM 8009, or *S. aureus* CCARM 3080 were inoculated at approximately 10^6 CFU/ml in the diluted antibiotic solutions and then incubated anaerobically for 18 h at 37°C to evaluate the susceptibility by determining MICs. Absorbance was measured using a microplate reader (ELx800, BioTek, Seoul, Korea) at 600 nm.

Statistical analysis. Time-kill curves were analyzed using a nonlinear curve fitting function of MicroCal Origin version 7.5 (Microcal Software Inc., Northampton, MA). Data were analyzed using the General Linear Model and the least significant difference procedures of SAS (Statistical Analysis Systems Institute, Cary, NC). Significant mean differences among treatments were compared by Fisher's least significant difference at $P < 0.05$.

RESULTS AND DISCUSSION

Bile acid tolerance of selected probiotic strains. The inhibitory effect of conjugated bile acids on the growth of probiotic strains was evaluated in the mixtures with varying concentrations of taurocholic acid, glycocholic acid, taurodeoxycholic acid, and glycodeoxycholate (Table 2). All probiotic strains grew well up to 8 to 9 log CFU/ml in the absence of conjugated bile acids after 24 h of incubation and in TSB supplemented with 0.13, 0.40, 0.08, and 0.40 mM of taurocholic acid, glycocholic acid, taurodeoxycholic acid, and glycodeoxycholate, respectively, with the exception of *L. mesenteroides*. However, the growth of *B. longum, L. casei, L. paracasei, L. rhamnosus,* and *L. mesenteroides* was significantly inhibited by increasing the concentration of taurocholic acid, glycocholic acid, taurodeoxycholic acid, and glycodeoxycholate, which were further increased up to 1.04, 3.20, 0.64, and 2.20 mM, respectively. The strain most tolerant to bile acids was *P. acidilactici,* followed by *L. acidophilus* and *L. brevis,* showing more than 8 log CFU/ml at 1.04, 3.20, 0.64, and 2.20 mM of taurocholic acid, glycocholic acid, taurodeoxycholic acid, and glycodeoxycholate, respectively (Table 2). The strains of *L. rhamnosus* and *L. mesenteroides* were susceptible to bile acids compared with other probiotic strains. The deconjugated forms have more antimicrobial activity against many GI bacteria than do conjugated forms *(11, 15, 33)*. Therefore, the probiotic deconjugation can be considered as a probiotic selection criterion *(29)*. Probiotic strains are well known to produce potential health benefits, including the competitive exclusion and inhibition of intestinal pathogens and the improvement of intestinal biota balance *(19, 30)*. Among the probiotic strains used in this study, *L. acidophilus, L. brevis,* and *P. acidilactici* were highly tolerant to the bile acids, while other strains did not grow at the high concentrations of bile acids (Table 2). The results confirm previous reports that bile salt tolerance and susceptibility varied from strain to strain, depending on membrane protein profiles and cell surface properties *(5, 7, 14, 26, 34)*. Bile salt adaptation can lead to cross protection against different stresses such as low pH, cold, osmotic pressure, and other bile acids *(22, 26, 31)*.

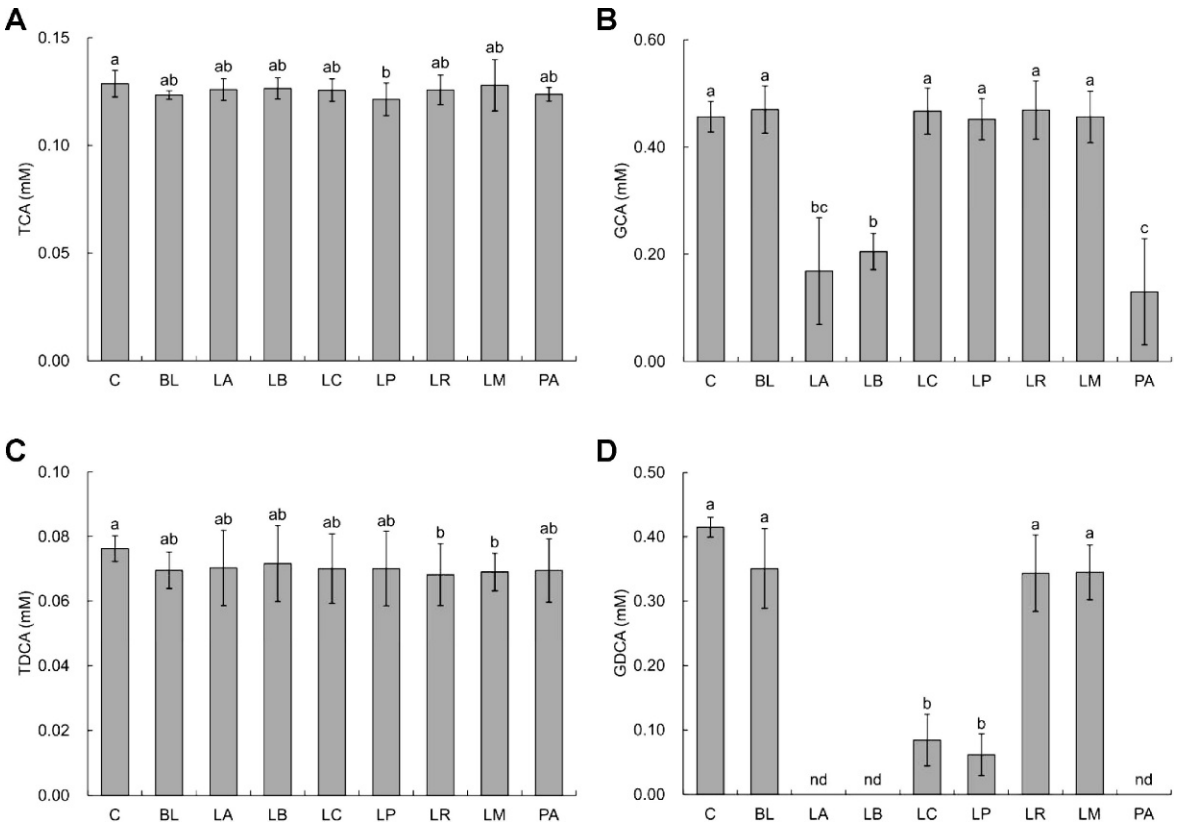

FIGURE 1. *Deconjugation of (A) taurocholic acid (TCA), (B) glycocholic acid (GCA), (C) taurodeoxycholic acid (TDCA), and (D) glycodeoxycholic acid (GDCA) in TSB supplemented with 0.13 mM TCA, 0.40 mM GCA, 0.08 mM TDCA, and 0.40 mM GDCA after 24 h of cultivation. Different letters on the bars indicate significantly different at* $P < 0.05$. *ND, not detected; C, control; LA,* L. acidophilus*; LB,* L. brevis*; LC,* L. casei*; LM,* L. mesenteroides*; LP,* L. paracasei*; LR,* L. rhamnosus*; BL,* B. longum*; PA,* P. acidilactici.

Deconjugation of bile acids by selected probiotic strains. The remaining bile acid concentrations were measured to evaluate the deconjugation ability of probiotic strains grown at the different concentrations of bile acids for 24 h (Figs. 1 and 2). The recovery rates ranged from 95 to 114% at 0.13, 0.40, 0.08, and 0.40 mM of taurocholic acid, glycocholic acid, taurodeoxycholic acid, and glycodeoxycholate, respectively, and 94 to 99% at 1.04, 3.20, 0.64, and 2.20 mM of taurocholic acid, glycocholic acid, taurodeoxycholic acid, and glycodeoxycholate, respectively. No significant reduction in the amount of taurocholic acid was observed in any probiotic culture broth, except for *L. paracasei* grown at 0.13, 0.40, 0.08, and 0.40 mM of taurocholic acid, glycocholic acid, taurodeoxycholic acid, and glycodeoxycholate, respectively, compared with the control (Fig. 1A). The highest amount of taurocholic acid was 0.129 mM in the control, while the least amount was 0.121 mM in *L. paracasei* culture broth. The amounts of glycocholic acid in *L. acidophilus, L. brevis,* and *P. acidilactici* cultures were significantly decreased to 0.169 mM (63% reduction), 0.205 mM (55% reduction), and 0.130 mM (72% reduction) after 24 h of cultivation compared with the control (Fig. 1B). Similar to taurocholic acid, there were no significant differences in the amounts of taurodeoxycholic acid among all probiotic culture broths (Fig. 1C). After 24 h of cultivation, glycodeoxycholate was not detected in *L. acidophilus, L. brevis,* and *P. acidilactici* culture broths (Fig. 1D). The amounts of glycodeoxycholate were reduced to 0.084 mM (80% reduction) and 0.062 mM (85% reduction), respectively, in *L. casei* and *L. paracasei* culture broths.

The amounts of taurocholic acid were significantly reduced to 0.919, 0.923, and 0.924 mM, respectively, in *B. longum, L. acidophilus,* and *P. acidilactici* cultured in media containing 1.04, 3.20, 0.64, and 2.20 mM of taurocholic acid, glycocholic acid, taurodeoxycholic acid, and glycodeoxycholate, respectively, when compared with the control (0.983 mM) (Fig. 2A). The amounts of glycocholic acid in *L. acidophilus* and *L. brevis* culture broths were significantly reduced by 91% (0.291 mM) and 75% (0.795 mM), respectively, as compared with the control (3.184 mM) (Fig. 2B). No glycocholic acid was detected in *P. acidilactici* culture. The least amount of taurodeoxycholic acid was 0.492 mM in *L. acidophilus* culture broth, followed by *L. paracasei, B. longum,* and *L. brevis*. As shown in Figure 2D, glycodeoxycholate was not detected in *L. brevis,* and *P. acidilactici* cultures and the amount of glycodeoxycholate was significantly reduced by 98% (0.067 mM).

The deconjugation of bile acids by probiotic strains was examined using TLC (Fig. 3). The conjugated acids (glycocholic acid and glycodeoxycholate) and deconjugated bile acids (cholic acid and deoxycholic acid) were well separated on TLC plates, while the taurine-conjugated stereoisomers (taurocholic acid and taurodeoxycholic acid) were poorly resolved (Fig. 3). The spots of conjugated bile acids (taurocholic acid or taurodeoxycholic acid,

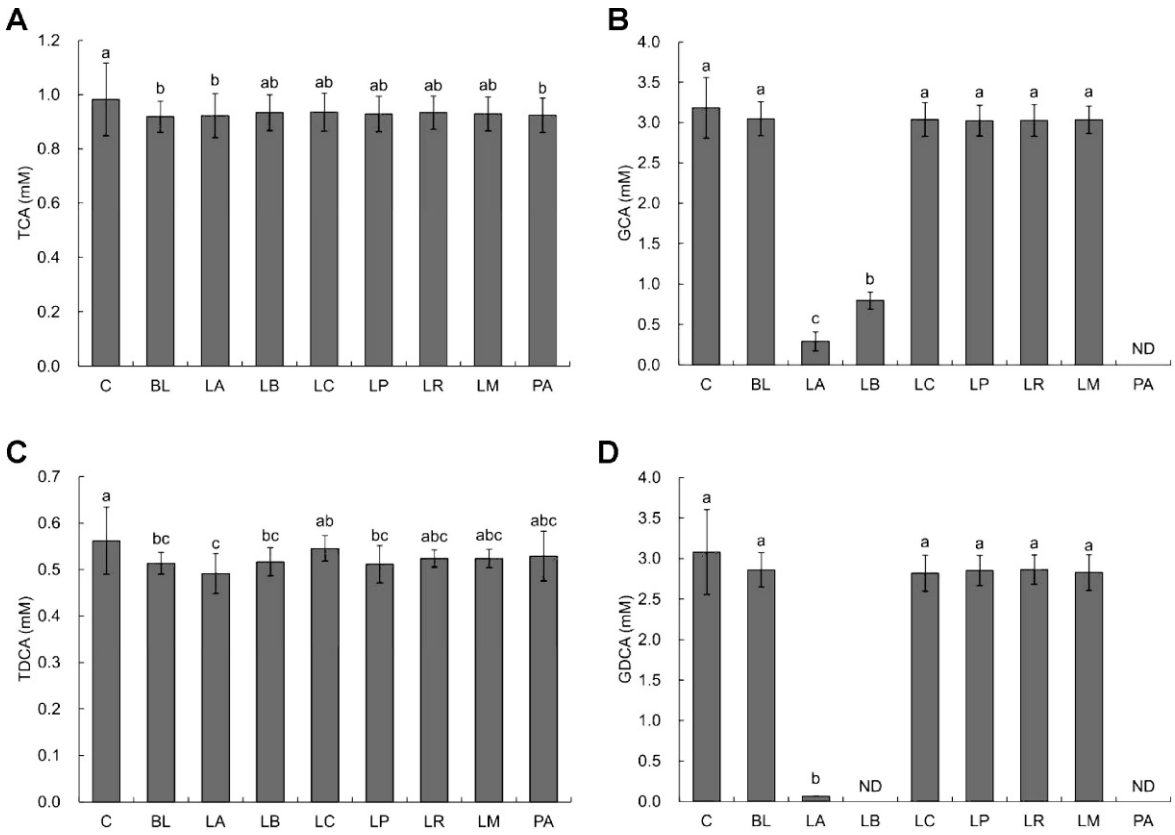

FIGURE 2. *Deconjugation of (A) taurocholic acid (TCA), (B) glycocholic acid (GCA), (C) taurodeoxycholic acid (TDCA), and (D) glycodeoxycholic acid (GDCA) in TSB supplemented with 1.04 mM TCA, 3.20 mM GCA, 0.64 mM TDCA, and 3.20 mM GDCA after 24 h of cultivation. Different letters on the bars indicate significantly different at* $P < 0.05$. *ND, not detected; C, control; LA,* L. acidophilus*; LB,* L. brevis*; LC,* L. casei*; LM,* L. mesenteroides*; LP,* L. paracasei*; LR,* L. rhamnosus*; BL,* B. longum*; PA,* P. acidilactici.

glycocholic acid, and glycodeoxycholate) were observed in *L. casei, L. mesenteroides, L. paracasei, L. rhamnosus,* and *B. longum*. No glycocholic acid and glycodeoxycholate spots were observed in *L. acidophilus, L. brevis,* and *P. acidilactici*. The conjugated bile acids (glycocholic acid and glycodeoxycholate) were deconjugated by *L. acidophilus, L. brevis,* and *P. acidilactici,* showing apparent spots of cholic acid and deoxycholic acid (Fig. 3). The conjugated bile acids, glycocholic acid and glycodeoxycholate, were noticeably deconjugated by *L. acidophilus, L. brevis,* and *P. acidilactici,* while none of the probiotic strains had significant ability to deconjugate taurocholic acid and taurodeoxycholic acid (Figs. 1 and 2). The observation suggests that *L. acidophilus, L. brevis,* and *P. acidilactici* might prefer to use glycocholic acid and glycodeoxycholate rather than taurocholic acid and taurodeoxycholic acid, which is in good agreement with the previous reports that glyco-conjugated bile acids were more efficiently hydrolyzed by probiotic strains than were tauro-conjugated bile acids *(3, 20, 39, 40)*.

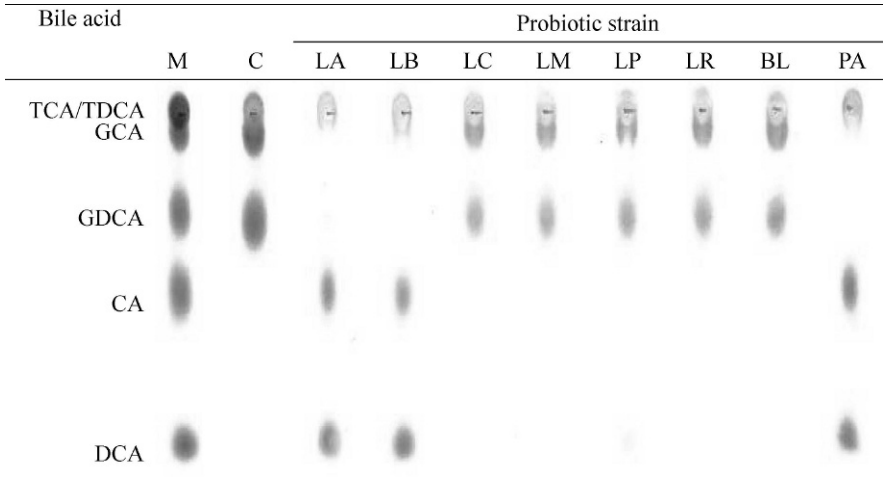

FIGURE 3. *Typical TLC chromatogram of taurocholic acid (TCA), glycocholic acid (GCA), taurodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA), cholic acid (CA), and deoxycholic acid (DCA) in TSB supplemented with 1.04 mM TCA, 3.20 mM GCA, 0.64 mM TDCA, and 3.20 mM GDCA after 24 h of cultivation. M, bile acid marker; C, control; LA,* L. acidophilus*; LB,* L. brevis*; LC,* L. casei*; LM,* L. mesenteroides*; LP,* L. paracasei*; LR,* L. rhamnosus*; BL,* B. longum*; PA,* P. acidilactici.

TABLE 3. *Lag-phase duration of selected foodborne pathogens grown in different probiotic culture supernatants without bile acids or with low or high bile acids during 24 h of cultivation*[a]

Probiotic culture	LPD (h)			
	S. aureus KACC 13236	*S. aureus* CCARM 3080	*Salmonella* Typhimurium KCCM 40523	*Salmonella* Typhimurium CCARM 8009
NBA				
Bifidobacterium longum	2.98 ± 0.66 b A[b]	2.92 ± 0.62 b A	2.08 ± 0.92 c A	1.55 ± 0.64 bcd A
Lactobacillus acidophilus	4.33 ± 0.85 a A	4.07 ± 0.26 a A	2.63 ± 0.36 bc B	1.65 ± 0.63 bcd B
L. brevis	3.55 ± 0.04 ab A	2.83 ± 0.49 b B	3.07 ± 0.18 ab AB	1.62 ± 0.18 bcd C
L. casei	3.38 ± 0.79 ab A	2.47 ± 0.79 b AB	2.56 ± 0.20 bc AB	1.05 ± 0.03 cd B
L. paracasei	2.30 ± 0.82 b B	2.55 ± 0.20 b AB	3.78 ± 0.07 a A	2.01 ± 0.54 abc B
L. rhamnosus	2.94 ± 0.61 b A	2.70 ± 0.58 b A	2.95 ± 0.44 abc A	2.87 ± 0.43 a A
Leuconostoc mesenteroides	3.50 ± 0.56 ab AB	2.08 ± 0.30 b B	3.93 ± 0.35 a A	0.76 ± 0.10 d C
Pediococcus acidilactici	3.47 ± 0.20 ab A	3.13 ± 0.31 ab AB	3.08 ± 0.28 ab AB	2.12 ± 0.72 ab B
LBA				
B. longum	6.58 ± 0.30 cd A	3.58 ± 1.36 bc B	3.54 ± 0.55 a B	3.74 ± 0.08 a B
L. acidophilus	11.25 ± 0.17 a A	5.92 ± 0.33 a B	3.50 ± 0.50 a C	3.41 ± 0.58 ab C
L. brevis	10.49 ± 1.03 ab A	3.99 ± 0.18 bc B	2.98 ± 0.99 a B	2.36 ± 1.63 ab B
L. casei	5.68 ± 0.25 cd A	3.47 ± 0.12 bc B	3.25 ± 0.26 a B	2.59 ± 1.51 ab B
L. paracasei	5.60 ± 0.18 cd A	3.12 ± 0.07 c B	3.27 ± 0.97 a B	3.63 ± 0.04 a AB
L. rhamnosus	5.91 ± 0.28 cd A	4.79 ± 1.66 ab AB	3.46 ± 0.73 a B	2.74 ± 0.11 ab C
L. mesenteroides	4.29 ± 0.63 d A	3.62 ± 0.36 bc A	3.09 ± 1.06 a A	2.81 ± 0.96 ab A
P. acidilactici	8.32 ± 2.58 abc A	3.74 ± 0.33 bc B	3.48 ± 0.59 a B	3.52 ± 0.39 a B
HBA				
B. longum	6.59 ± 0.30 ab A	6.54 ± 1.38 b A	5.96 ± 0.04 a A	5.32 ± 0.49 ab A
L. acidophilus	NC[c]	NC	6.25 ± 1.10 a A	5.80 ± 0.27 a A
L. brevis	NC	NC	6.17 ± 0.04 a A	5.40 ± 0.06 ab A
L. casei	5.68 ± 0.22 bc A	3.96 ± 0.50 c B	5.76 ± 0.46 a A	4.96 ± 0.03 ab AB
L. paracasei	5.30 ± 0.62 c A	3.11 ± 0.14 c B	5.58 ± 1.12 a A	3.62 ± 0.48 c B
L. rhamnosus	6.83 ± 0.28 a AB	8.73 ± 0.95 a A	5.63 ± 1.21 a B	4.51 ± 1.13 bc B
L. mesenteroides	3.98 ± 0.61 d A	3.93 ± 0.05 c A	3.89 ± 0.86 b A	3.49 ± 0.34 c A
P. acidilactici	NC	NC	6.45 ± 0.19 a A	5.81 ± 0.74 a A

[a] LPD, lag-phase duration; NBA, no bile acids; LBA, low bile acids; HBA, high bile acids. LBA: 0.13 mM TCA, 0.40 mM GCA, 0.08 mM TDCA, and 0.40 mM GDCA; HBA: 1.04 mM TCA, 3.20 mM GCA, 0.64 mM TDCA, and 3.20 mM GDCA.

[b] Values are means ± standard deviations. Means with different letters within a column (a through d) and a row (A through C) are significantly different at $P < 0.05$.

[c] NC, not calculated because bacterial growth inhibition was observed.

Inhibitory activity of deconjugated bile acids against antibiotic-resistant foodborne pathogens. Lag times of antibiotic-sensitive and multiple antibiotic–resistant *Salmonella* Typhimurium and *S. aureus* strains were estimated from the modified Gompertz equation to evaluate the inhibitory effect of deconjugated bile acids. Table 3 shows the inhibitory effect of culture supernatants in which probiotic strains were cultured at different concentrations of bile acids: no bile acid, low bile acid concentration (0.13 mM taurocholic acid, 0.40 mM glycocholic acid, 0.08 mM taurodeoxycholic acid, and 0.40 mM glycodeoxycholate), and high bile acid concentration (1.04 mM taurocholic acid, 3.20 mM glycocholic acid, 0.64 mM taurodeoxycholic acid, and 3.20 mM glycodeoxycholate). The LPDs increased with increasing bile acid concentrations. More significant differences in LPDs as a function of bile concentration were observed in antibiotic-sensitive *S. aureus* KACC 13236 when compared with other pathogenic indicators. At low bile acid concentration, the LPDs of antibiotic-sensitive *S. aureus* KACC 13236 were increased to 11.25, 10.49, and 8.32 h in the culture supernatants of *L. acidophilus, L. brevis,* and *P. acidilactici,* respectively. The antibiotic-sensitive *S. aureus* KACC 13236 was more sensitive to deconjugated bile acids than was the antibiotic-resistant *S. aureus* CCARM 3080. At high bile acid concentration, the culture supernatants of *L. acidophilus, L. brevis,* and *P. acidilactici* had bactericidal effects against both antibiotic-sensitive *S. aureus* KACC 13236 and antibiotic-resistant *S. aureus* CCARM 3080 (Table 3). The LPD values increased with increasing probiotic growth and deconjugation activity, showing that the culture supernatants of *L. acidophilus, L. brevis,* and *P. acidilactici* effectively delayed the growth of antibiotic-sensitive and multiple antibiotic–resistant *Salmonella* Typhimurium and *S. aureus* (Table 3). These observations suggest that the presence of deconjugated bile acids (cholic acid and deoxycholic acid) can effectively inhibit the growth of foodborne pathogens, specifically antibiotic-sensitive and -resistant *S. aureus* in this study. The deconjugated bile acids directly contribute to the disruption of cell membrane permeability, leading to enhanced antimicrobial activity *(4, 11, 33, 41, 45).*

The antibiotic susceptibility patterns of selected foodborne pathogens in the presence of deconjugated bile acids

TABLE 4. *Susceptibility of selected foodborne pathogens to antibiotics in the simulated deconjugated bile acids*[a]

Strain	CA+DCA (mM)	MIC (μg/ml)											
		AT	BP	CP	CX	CZ	EM	KM	NA	NB	NF	PM	SM	TC
Staphylococcus aureus KACC 13236	0.00 + 0.00	>256	<0.25	2	2	8	<0.25	8	16	<0.25	2	64	8	0.5
	0.03 + 0.03	>256	<0.25	2	2	8	<0.25	8	16	<0.25	2	64	8	0.5
	0.10 + 0.10	>256	<0.25	2	1	8	<0.25	8	16	<0.25	1	<0.25	4	<0.25
	0.50 + 0.50	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
S. aureus CCARM 3080	0.00 + 0.00	>256	32	8	>256	128	>256	>256	256	0.5	256	64	8	128
	0.03 + 0.03	>256	16	8	>256	128	>256	>256	256	<0.25	128	64	8	128
	0.10 + 0.10	>256	16	8	>256	128	>256	>256	256	<0.25	128	4	8	128
	0.50 + 0.50	>256	8	4	256	128	>256	64	128	<0.25	128	1	4	64
Salmonella Typhimurium KCCM 40523	0.00 + 0.00	<0.25	16	8	<0.25	0.5	64	16	8	>256	<0.25	4	16	1
	0.03 + 0.03	<0.25	16	8	<0.25	0.5	64	8	8	>256	<0.25	2	16	1
	0.10 + 0.10	<0.25	16	8	<0.25	0.5	64	8	8	>256	<0.25	<0.25	16	1
	0.50 + 0.50	<0.25	16	4	<0.25	0.5	32	8	8	>256	<0.25	<0.25	16	1
Salmonella Typhimurium CCARM 8009	0.00 + 0.00	<0.25	>256	4	<0.25	0.5	64	>256	16	>256	<0.25	8	>256	256
	0.03 + 0.03	<0.25	>256	4	<0.25	0.5	64	>256	8	>256	<0.25	8	256	256
	0.10 + 0.10	<0.25	>256	4	<0.25	0.5	64	>256	8	>256	<0.25	4	256	128
	0.50 + 0.50	<0.25	>256	4	<0.25	0.5	32	>256	4	>256	<0.25	0.5	256	128

[a] CA, cholic acid; DCA, deoxycholic acid; AT, aztreonam; BP, benzylpenicillin; CP, chloramphenicol; CX, cefotaxime; CZ, cefatazidime; EM, erythromycin; KM, kanamycin; NA, nalidixic acid; NB, novobiocin; NF, norfloxacin; PM, polymyxin B; SM, streptomycin; TC, tetracycline.

(cholic acid and deoxycholic acid) are shown in Table 4. The antibiotic sensitivities were increased in the presence of deconjugated bile acids against *S. aureus* KACC 13236, *S. aureus* CCARM 3080, *Salmonella* Typhimurium KCCM 40523, and *Salmonella* Typhimurium CCARM 8009, showing that the MICs decreased with increasing concentrations of deconjugated bile acids. The MICs of all antibiotics were less than 0.25 μg/ml in the presence of 0.5 mM cholic acid and 0.5 mM deoxycholic acid. The MICs of aztreonam against antibiotic-sensitive *S. aureus* KACC 13236 were dramatically decreased from >256 μg/ml in the absence of deconjugated bile acids to <0.25 μg/ml in the presence of 0.5 mM cholic acid and 0.5 mM deoxycholic acid. Antibiotic-resistant foodborne pathogens were more resistant to antibiotics tested than antibiotic-sensitive foodborne pathogens. The antibiotic-resistant *S. aureus* CCARM 3080 was highly resistant to aztreonam, cefotaxime, and erythromycin, showing MICs of >256 μg/ml, and the antibiotic-resistant *Salmonella* Typhimurium CCARM 8009 was highly resistant to kanamycin, novobiocin, and streptomycin regardless of the concentration of deconjugated bile acids. The antibiotic susceptibility of antibiotic-sensitive and multiple antibiotic–resistant *Salmonella* Typhimurium and *S. aureus* was increased in the presence of deconjugated bile acid (cholic acid and deoxycholic acid) (Table 4). With increased concentration of cholic acid and deoxycholic acid, *S. aureus* KACC 13236 showed increased susceptibility to aztreonam, chloramphenicol, cefotaxime, cefatazidime, kanamycin, nalidixic acid, norfloxacin, polymyxin B, streptomycin, and tetracycline; *S. aureus* CCARM 3080 showed increased susceptibility to benzylpenicillin, chloramphenicol, cefotaxime, kanamycin, nalidixic acid, novobiocin, norfloxacin, polymyxin B, streptomycin, and tetracycline; *Salmonella* Typhimurium KCCM 40523 showed increased susceptibility to chloramphenicol, erythromycin, kanamycin, and polymyxin B; and *Salmonella* Typhimurium CCARM 8009 showed increased susceptibility to erythromycin, nalidixic acid, polymyxin B, streptomycin, and tetracycline. These results are in good agreement with previous reports, in which antibiotic susceptibility was significantly increased in the presence of deconjugated bile acids due to the destruction of membrane permeability *(6, 27)*. The synergistic inhibitory effects of antibiotics and deconjugated bile acids were more obvious against gram-positive bacteria than gram-negative bacteria.

In conclusion, the deconjugation ability varied with strain, corresponding to the endogenous conjugated bile acid tolerance. *L. acidophilus, L. brevis,* and *P. acidilactici* were more likely to deconjugate the glyco-conjugated bile acids as substrates than tauro-conjugated bile acids, which induced antimicrobial activity against selected foodborne pathogens. Thus, deconjugation ability could be an important selection criterion for probiotic strains. In this study, deconjugation by probiotic strains enhanced the antibiotic susceptibility of selected antibiotic-resistant *Salmonella* Typhimurium and *S. aureus* exposed to deconjugated bile acids (cholic acid and deoxycholic acid). These results may shed light on the susceptibility of antibiotic-resistant pathogens in the presence

of deconjugated bile acids; they also provide guidance in designing effective antibiotic therapy. However, further studies are needed to clearly delineate the antimicrobial mechanisms of deconjugated bile acids in the complex GI system.

ACKNOWLEDGMENTS

This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0026113) and partially supported by the Grant of the Korea Ministry of Education, Science and Technology (The Regional Core Research Program/Medical & Bio-Material Research Center). We also thank Ms. Mi-Suk Yang in the Central Laboratory of Kangwon National University for her technical assistant in the analytical field using HPLC.

REFERENCES

1. Adhikari, K., A. Mustapha, I. U. Gruen, and L. Fernando. 2000. Viability of microencapsulated *Bifidobacteria* in set yogurt during refrigerated storage. *J. Dairy Sci.* 83:1946–1951.
2. Begley, M., C. G. M. Gahan, and C. Hill. 2005. The interaction between bacteria and bile. *FEMS Microbiol. Rev.* 29:625–651.
3. Begley, M., C. Hill, and C. G. M. Gahan. 2006. Bile salt hydrolase activity in probiotics. *Appl. Environ. Microbiol.* 72:1729–1738.
4. Bernstein, C., H. Bernstein, C. M. Payne, S. E. Beard, and J. Schneider. 1999. Bile salt activation of stress response promoters in *Escherichia coli*. *Curr. Microbiol.* 39:68–72.
5. Charteris, W. P., P. M. Kelly, L. Morelli, and J. K. Collins. 1998. Antibiotic susceptibility of potentially probiotic *Bifidobacterium* isolates from the human gastrointestinal tract. *Lett. Appl. Microbiol.* 26:333–337.
6. Charteris, W. P., P. M. Kelly, L. Morelli, and J. K. Collins. 2000. Effect of conjugated bile salts on antibiotic susceptibility of bile salt–tolerant *Lactobacillus* and *Bifidobacterium* isolates. *J. Food Prot.* 63: 1369–1376.
7. Chateau, N., A. M. Deschamps, and A. H. Sassi. 1994. Heterogeneity of bile salts resistance in the *Lactobacillus* isolates of a probiotic consortium. *Lett. Appl. Microbiol.* 18:42–44.
8. Clinical and Laboratory Standards Institute. 2009. M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 8th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
9. De Boever, P., and W. Verstraete. 1999. Bile salt deconjugation by *Lactobacillus plantarum* 80 and its implication for bacterial toxicity. *J. Appl. Microbiol.* 87:345–352.
10. Floch, M. H. 2002. Bile salts, intestinal microflora and enterohepatic circulation. *Digest. Liver Dis.* 34:S54–S57.
11. Floch, M. H., H. J. Binder, B. Filburn, and W. Gershengoren. 1972. The effect of bile acids on intestinal microflora. *Am. J. Clin. Nutr.* 25: 1418–1426.
12. Gregg, J. A. 1966. New solvent systems for thin-layer chromatography of bile acids. *J. Lipid Res.* 7:579–581.
13. Grill, J. P., C. Manginot-Dürr, F. Schneider, and J. Ballongue. 1995. Bifidobacteria and probiotic effects: action of *Bifidobacterium* species on conjugated bile salts. *Curr. Microbiol.* 31:23–27.
14. Gueimonde, M., L. Noriega, A. Margolles, C. G. de los Reyes-Gavilan, and S. Salminen. 2005. Ability of *Bifidobacterium* strains with acquired resistance to bile to adhere to human intestinal mucus. *Int. J. Food Microbiol.* 101:341–346.
15. Gunn, J. S. 2000. Mechanisms of bacterial resistance and response to bile. *Microb. Infect.* 2:907–913.
16. Hammad, A. M., and T. Shimamoto. 2010. Towards a compatible probiotic-antibiotic combination therapy: assessment of antimicrobial resistance in the Japanese probiotics. *J. Appl. Microbiol.* 109:1349–1360.
17. He, X., Y. Zou, W.-B. Yoon, S.-J. Park, D.-S. Park, and J. Ahn. 2011. Effects of probiotic fermentation on the enhancement of biological and pharmacological activities of Codonopsis lanceolata extracted by high pressure treatment. *J. Biosci. Bioeng.* 112:188–193.
18. Hofmann, A. F., G. Molino, M. Milanese, and G. Belforte. 1983. Description and simulation of a physiological pharmacokinetic model for the metabolism and enterohepatic circulation of bile acids in man. Cholic acid in healthy man. *J. Clin. Invest.* 71:1003–1022.
19. Kheadr, E., N. Dabour, C. Le Lay, C. Lacroix, and I. Fliss. 2007. Antibiotic susceptibility profile of *Bifidobacteria* as affected by oxgall, acid, and hydrogen peroxide stress. *Antimicrob. Agents Chemother.* 51:169–174.
20. Kim, G. B., S. H. Yi, and B. H. Lee. 2004. Purification and characterization of three different types of bile salt hydrolases from *Bifidobacterium* strains. *J. Dairy Sci.* 87:258–266.
21. Lee, H. Y., H. Xu, H. J. Lee, T. I. Lim, Y. B. Choi, J. R. Ko, J. Ahn, and A. Mustapha. 2008. Prophylactic uses of probiotics as a potential alternative to antimicrobials in food animals. *Food Sci. Biotechnol.* 17:191–194.
22. Leverrier, P., D. Dimova, V. Pichereau, Y. Auffray, P. Boyaval, and G. Jan. 2003. Susceptibility and adaptive response to bile salts in *Propionibacterium freudenreichii*: physiological and proteomic analysis. *Appl. Environ. Microbiol.* 69:3809–3818.
23. Liebowitz, L. D. 2009. MRSA burden and interventions. *Int. J. Antimicrob. Agents* 34:S11–S13.
24. Linton, R. H., W. H. Carter, M. D. Pierson, and C. R. Hackney. 1995. Use of a modified Gompertz equation to model nonlinear survival curves for *Listeria monocytogenes* Scott A. *J. Food Prot.* 58:946–954.
25. Mallory, A., F. Kern, J. Smith, and D. Savage. 1973. Patterns of bile acids and microflora in the human small intestine. I. Bile acids. *Gastroenterology* 64:26–33.
26. Margolles, A., L. García, B. Sánchez, M. Gueimonde, and C. G. de los Reyes-Gavilán. 2003. Characterisation of a *Bifidobacterium* strain with acquired resistance to cholate—a preliminary study. *Int. J. Food Microbiol.* 82:191–198.
27. Marrie, T. J., E. V. Haldane, C. A. Swantee, and E. A. Kerr. 1981. Susceptibility of anaerobic bacteria to nine antimicrobial agents and demonstration of decreased susceptibility of *Clostridium perfringens* to penicillin. *Antimicrob. Agents Chemother.* 19:51–55.
28. Mayrhofer, S., P. Paulsen, F. J. M. Smulders, and F. Hilbert. 2004. Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. *Int. J. Food Microbiol.* 97:23–29.
29. Neville, B. A., and P. W. O'Toole. 2010. Probiotic properties of *Lactobacillus salivarius* and closely related *Lactobacillus* species. *Future Microbiol.* 5:759–774.
30. Noriega, L., C. G. de los Reyes-Gavilán, and A. Margolles. 2005. Acquisition of bile salt resistance promotes antibiotic susceptibility changes in *Bifidobacterium*. *J. Food Prot.* 68:1916–1919.
31. Noriega, L., M. Gueimonde, B. Sánchez, A. Margolles, and C. G. de los Reyes-Gavilán. 2004. Effect of the adaptation to high bile salts concentrations on glycosidic activity, survival at low pH and cross-resistance to bile salts in *Bifidobacterium*. *Int. J. Food Microbiol.* 94: 79–86.
32. Nurmi, E., L. Nuotio, and C. Schneitz. 1992. The competitive exclusion concept: development and future. *Int. J. Food Microbiol.* 15:237–240.
33. Rees, E. N., and S. J. Elliott. 1998. The influence of bile on antimicrobial activity in vitro. *J. Antimicrob. Chemother.* 41:659–660.
34. Sánchez, B., L. Noriega, P. Ruas-Madiedo, C. G. de los Reyes-Gavilán, and A. Margolles. 2004. Acquired resistance to bile increases fructose-6-phosphate phosphoketolase activity in *Bifidobacterium*. *FEMS Microbiol. Lett.* 235:35–41.
35. Sánchez García, M. 2009. Early antibiotic treatment failure. *Int. J. Antimicrob. Agents* 34:S14–S19.
36. Sandine, W. 1979. Roles of *Lactobacillus* in the intestinal tract. *J. Food Prot.* 42:259–262.

37. Servin, A. L. 2004. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiol. Rev.* 28:405–440.
38. Silbergeld, E. K., J. Graham, and L. B. Price. 2008. Industrial food animal production, antimicrobial resistance, and human health. *Ann. Rev. Pub. Health* 29:151–169.
39. Tanaka, H., H. Hashiba, J. Kok, and I. Mierau. 2000. Bile salt hydrolase of *Bifidobacterium longum*—biochemical and genetic characterization. *Appl. Environ. Microbiol.* 66:2502–2512.
40. Taranto, M. P., F. Sesma, and G. Font de Valdez. 1999. Localization and primary characterization of bile salt hydrolase from *Lactobacillus reuteri*. *Biotechnol. Lett.* 21:935–938.
41. Thanassi, D., L. Cheng, and H. Nikaido. 1997. Active efflux of bile salts by *Escherichia coli*. *J. Bacteriol.* 179:2512–2518.
42. Van, T. T. H., G. Moutafis, L. T. Tran, and P. J. Coloe. 2007. Antibiotic resistance in food-borne bacterial contaminants in Vietnam. *Appl. Environ. Microbiol.* 73:7906–7911.
43. Walsh, F. M., and S. G. Amyes. 2004. Microbiology and drug resistance mechanisms of fully resistant pathogens. *Curr. Opin. Microbiol.* 7:439–444.
44. Xu, H., J. Gou, G. P. Choi, H. Y. Lee, and J. Ahn. 2009. Functional properties of squid by-products fermented by probiotic bacteria. *Food Sci. Biotechnol.* 18:761–765.
45. Zarate, G., A. P. Chaia, S. Gonzalez, and G. Oliver. 2000. Viability and β-galactosidase activity of dairy propionibacteria subjected to digestion by artificial gastric and intestinal fluids *J. Food Prot.* 63: 1214–1221.