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Plasmid-encoded glycosyltransferase operon is responsible for exopolysaccharide production, cell aggregation, and bile resistance in a probiotic strain, *Lactobacillus brevis* KB290

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We demonstrate here that exopolysaccharide (EPS) production, cell aggregation, and bile resistance in *Lactobacillus brevis* KB290 are conferred by three *eps* genes (*gtf27*, *gtf28*, and *orf29*) located on the 42.4-kb plasmid pKB290-1. The predicted products of *gtf27* and *gtf28* belong to the membrane-bound glycosyltransferase family whereas the *orf29* gene product showed homology with the ABC transporter. On *in silico* analysis, these genes were found to be widely distributed among lactobacilli from publicly available genomes and metagenomes, and their function is not yet elucidated. RT-PCR analysis showed that the *eps* genes were organised in an operon and their expression was markedly lower in arabinose- and xylose-containing media than in a glucose-containing medium. The three *eps* genes were cloned and expressed in homologous and heterologous strains. Considerably less EPS was produced by the plasmid-cured KB1802 strain than by the parental KB290 strain, whereas a similar amount was produced by the KB1802 strain expressing the three *eps* genes. The KB1802 strain expressing *gtf27 and gtf28* but not *orf29* did not produce EPS. Cell aggregation and bile resistance were also decreased in KB1802 strains but were complemented by *eps* genes. Moreover, the three *eps* genes conferred these phenotypes to a *Lactobacillus plantarum* strain. In conclusion, the three *eps* genes in pKB290-1 were sufficient for EPS biosynthesis with glucose and *N*-acetylglucosamine, and were responsible for cell aggregation and bile resistance. We consider these phenotypes to be at least partly responsible for KB290-specific properties.

[**Key words:** *Lactobacillus brevis*; Exopolysaccharide; Glycosyltransferase; Bile tolerance; Cell aggregation; Probiotics]

Probiotic lactobacilli (1) produce a wide variety of exopolysaccharides (EPSs), macromolecules that are considered critical for their probiotic functions, including determination of *Lactobacillus*-host interactions (2). Because large-scale genome sequencing projects illustrate a diversity of EPS-coding gene clusters among probiotic lactobacilli (3), EPSs are thought to be involved in determining strain-specific probiotic properties, such as adhesion to the intestinal epithelium, stress resistance, and involvement in modulation of host immunity (4,5). EPSs also form a layer surrounding the bacterial cell, creating a natural barrier against heat, acid, simulated gastric juice, and bile (6–9). Several types of glycosyltransferases are generally involved in the incorporation of polysaccharides into EPS in lactobacilli and bifidobacteria (4,5).

Lactobacillus brevis KB290 is a plant-derived *Lactobacillus* isolated from *Suguki*, a traditional pickle produced in Kyoto, for which the genome and plasmidome sequences were first published as whole sequences of commercial probiotic *L. brevis* species (10). This strain tolerates gastrointestinal juices (10,11), clinically stimulates immune function (12,13), and improves gut health (14–16). Scanning electron microscopy and transmission electron microscopy of KB290 show EPS layers (11). Thus, KB290 is a natural producer of EPS. KB290 also has a strong tendency to aggregate in broth medium with vigorous mixing (10,11), which may assist adhesion, colonization in the host environment, and immunomodulation of the colonic mucosa (17–19). Because of these desirable traits, KB290 has been used in fermented food products in Japan since 1993.

KB290 uniquely contains nine plasmids that range from 5.9 to 42.4 kb and house 191 protein-coding genes (10). Most of them are not previously described in *L. brevis* species. One of the plasmids harboured in KB290, pKB290-1, endows the cells with gastrointestinal tolerance and cell aggregation (10). The plasmid encodes two putative glycosyltransferase (LVISKB_P1-0027 and LVISKB_P1-0028) genes whose function and physiological relevance are unknown. Here, our hypothesis was that these genes are responsible for EPS production, and that the functional properties of KB290 are linked to EPS production. In evaluating the role of glycosyltransferase genes and the physiological properties associated with EPS, we cloned the *eps* region in pKB290-1 and expressed it in homologous and heterologous strains of lactic acid bacteria (LABs). The derived strains were then analysed for EPS production, aggregation properties, and *in vitro* resistance to bile salt-induced stress. The transcriptional level of the glycosyltransferase genes in response to different sugar sources was also analysed.

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MATERIALS AND METHODS

Bacterial strains and growth conditions Table 1 summarises the bacterial strains used in this study. The *Lactobacillus* strains were grown in MRS medium (Oxoid, Hampshire, UK) at 30°C whereas *Escherichia coli* was grown in LB medium (20) at 37°C. *E. coli* DH5α and the plasmid pUC19 were used for the cloning experiments. Ampicillin (100 μg/ml) (Sigma–Aldrich, St. Louis, MO, USA) was added to *E. coli* cultures and erythromycin (10 μg/ml) (Sigma–Aldrich) was added to LAB cultures as required, as was 1.0% agar.

Sequence analysis All KB290 genome sequence data have been deposited in DDBJ/GenBank/EMBL and the accession number for pKB290-1 is AP012168. We performed a similarity search for all the predicted *eps* genes against databases (non-redundant database/NCBI) using BLASTP with a cut-off E-value $\leq 10^{-10}$ and a functional classification of the genes using NCBI clusters of orthologous groups (COGs) (21) and BLASTP with a cut-off E-value $\leq e^{-5}$. Protein domains were searched against the Pfam and TIGRFAMs database (22,23) of hidden Markov models using default parameters. Synteny among genes was visualized with GenomeMatcher software (24). The putative membrane-spanning domains of *eps* genes were predicted using SOSUI (25) and TMHMM software (26).

Construction of plasmid-cured derivatives of *L. brevis* KB290 KB290 plasmid-curing was performed as described previously (10). Plasmid-cured derivatives were selected from colonies by PCR using plasmid-specific primers (10). The resulting plasmid-cured KB290 derivative, designated as *L. brevis* KB1802, was used as a host for plasmid introduction experiments.

DNA preparation and manipulation Genomic DNA of *Lactobacillus* strains was prepared from early stationary-phase cultures using standard genomic DNA affinity columns, and plasmid DNA was isolated as described (27), except that the cells were preincubated with 10 mg/ml lysozyme and 50 U/ml mutanolysin (Sigma–Aldrich for each) for 1 h at 37°C to weaken the cell wall before cell disruption. Transformation of *E. coli* and isolation of its plasmid DNA was performed using the standard method (20). Restriction endonucleases (Takara Bio, Shiga, Japan) were used as recommended by the manufacturer.

Transcription analysis of *eps* genes *Lactobacillus* RNA was extracted from early stationary-phase cultures with Isogen (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. The extracted RNA was treated with RNeasy Plus Mini (Qiagen, Tokyo, Japan) and DNase I (Takara Bio) at 37°C for 20 min. RT-PCR was performed using a OneStep RT-PCR kit (Qiagen) with the primers *orf26-gtf27*, *gtf27-gtf28*, and *gtf28-orf29* (Table S1) derived from *eps* genes to cover the intergenic spaces. For positive controls, each fragment was amplified with the same primers using total DNA from KB290 as the template; for negative controls, the reverse transcription stage was omitted.

Construction of plasmids carrying *eps* genes The three *eps* genes (*gtf27*, *gtf28*, and *orf29)* or the glycosyltransferase genes alone (*gtf27* and *gtf28*), together with promoter regions and ribosome binding sites, were amplified with primers *eps*_F and *gtf27-gtf28-orf29*_R (the 3 *eps* genes), or *eps*_F and *gtf27-gtf28*_R (the glycosyltransferase genes alone) (Table S1). The PCR products were cloned directly into *Eco*RI-*Bam*HI-digested pLES003 (28) using an In-Fusion HD cloning kit according to the manufacturer's instructions (Takara Bio), generating pLES003_*gtf27-gtf28-orf29* and pLES003_*gtf27-gtf28*. The constructs were transformed into *E. coli* DH5α cells, as was the control plasmid (pLES003; no insert). Following transformation, the purified plasmids containing the *eps* genes were sequenced to ensure that no mutations had occurred, and both plasmids were then electroporated into competent *L. brevis* KB1802 and *Lactobacillus plantarum* NC8.

Transformation of *Lactobacillus* strains Preparation and electroporation of competent cells from *Lactobacillus* strains was conducted according to described methods (29) and a personal communication with Dr. L. Axelsson (Nofima). Briefly, *Lactobacillus* strains were grown overnight in MRS broth without glucose, inoculated to an OD_{600} of 0.25 into MRS with 1% glycine (Sigma–Aldrich), and incubated at 30°C. When the OD_{600} reached 0.6, the preparation was chilled on ice and the cells were harvested, washed once with ice cold 1 mM $MgCl_2$ (Sigma–Aldrich) and once with ice cold 30% PEG-1500 (Sigma–Aldrich), and then resuspended at 1:100 in 30% PEG-1500. For transformation, 40 μl of the cell suspension and 1–2 μg of DNA were added to a 0.2-cm cuvette and pulsed at 1.5 kV, 400 Ω, and 25 μF (Gene pulser and pulse controller; Bio-Rad Laboratories, Tokyo, Japan). Immediately after the pulse, the cells were transferred to MRS broth containing 0.5 M sucrose (Sigma–Aldrich) and 0.1 M $MgCl_2$ and incubated at 30°C for 2 h, plated on MRS agar containing 10 μg/ml erythromycin, and incubated at 30°C for 36–48 h, when colonies became visible.

EPS isolation and detection EPSs were isolated as described (30). Briefly, cell-bound EPS was extracted from the bacterial cells using 0.05 M EDTA (Dojindo, Kumamoto, Japan), precipitated with ethanol, and dialyzed against water (6- to 8-kDa dialysis membrane [Spectra/Por, VWR International, PA, USA]). The crude EPSs were treated with 2 μg/ml DNase and 2 μg/ml RNase (Sigma–Aldrich for each) at 37°C for 6 h. The protein components were then digested with 20 μg/ml proteinase K (Takara Bio) at 37°C for 16 h (the reaction was stopped by heating at 95°C for 10 min), and the crude EPSs were precipitated with ethanol. The total amount of EPS isolated was estimated using the phenol-sulfuric acid method (31). The amount of EPS was expressed as glucose equivalents per 10^9 cells.

For monosaccharide analysis, the isolated EPSs were hydrolysed with 4 M trifluoroacetic acid (TFA) (J-Oil Mills, Tokyo, Japan) at 100°C for 3 h. The carbohydrate composition of the TFA-hydrolysed EPS was analysed by labelling the sample using an ABEE labelling kit (J-Oil Mills), followed by high-performance liquid chromatography analysis using a Honenpak C_{18} column (4.6 mm I.D. × 75 mm) (J-Oil Mills) (32).

Evaluation of cell survival in the presence of bile For the survival study, washed cells were suspended in 50 mM sodium phosphate buffer (pH 7.0), and bile salt (Sigma–Aldrich) solution was added to a final concentration of 0.2% (w/v) (10). The mixtures were then incubated in a water bath at 37°C. Samples were taken at 0 and 3 h and plated on MRS agar (33). Colonies were counted after an overnight incubation at 30°C.

Measurement of cell aggregation The aggregation index (defined below) was calculated as described (34) with slight modification. Briefly, early stationary-phase *Lactobacillus* cultures were vigorously mixed until fibrous-like aggregates became visible. After 5 min at room temperature, most of the aggregates precipitated to the bottom of the tubes. The OD_{600} of the supernatant was represented as OD_{NA} (i.e., OD_{600} of non-aggregated cells). The aggregation index (AI) was defined as follows:

$$\mathrm{AI}\ (\%) = [(\mathrm{OD}_{\mathrm{total}} - \mathrm{OD}_{\mathrm{NA}})/\mathrm{OD}_{\mathrm{total}}] \times 100 \qquad (1)$$

Analysis of the glycosyltransferase gene transcription level in response to different sugars *L. brevis* strains were cultured in MRS, or MRS containing 2% arabinose or 2% xylose instead of 2% glucose (Wako Tokyo, Japan for each). Cultures were grown to early stationary-phase, and RNA was isolated as described above. The transcription levels of *gtf27* were quantified using real-time RT-PCR with primers *gtf27*_F and *gtf27*_R (Table S1) using an iScript one-step RT-PCR kit with SYBR green on a Chromo4 real-time instrument (Bio-Rad Laboratories for each). The threshold cycles, as calculated using a Chromo4 Opticon Monitor 3 (Bio-Rad Laboratories), were used to determine the relative changes between samples. After amplification, template specificity was ensured by melting-curve analysis. The expression analysis results were normalized for each experiment using the phosphoketolase gene as a housekeeping gene with primers keto_V and keto_R (35) (Table S1). For positive controls, each fragment was amplified with the same primer using total DNA from each culture as a template, and the same was done for negative controls but without the reverse transcription stage.

TABLE 1. Bacterial strains and plasmids.

Strain/plasmid[a]	Relevant characteristics[b]	Reference/source
Strain		
E. coli DH5α		Takara Bio
L. brevis KB290 (JCM 17312)	Wild-type producer of EPS, containing nine plasmids, pKB290-1– pKB290-9	10
L. brevis KB1802	Plasmid-cured strain of KB290, containing pKB290-5, −7, −8, and −9	This study
L. plantarum NC8	Silage isolate, plasmid-free	29
Plasmid		
pUC19	Ap^r	Takara bio
pLES003	*Lactobacillus-E. coli* shuttle vector; Em^r	28
pLES003_*gtf27-gtf28*	pLES003 containing *gtf27-gtf28* locus	This study
pLES003_*gtf27-gtf28-orf29*	pLES003 containing *gtf27-gtf28-orf29* locus	This study

[a] JCM, Japan Collection of Microorganisms.

[b] Ap^r, ampicillin resistance; Em^r, erythromycin resistance.

RESULTS

Characteristics of the *eps* genes in pKB290-1 The alignment between pKB290-1 and similar *Lactobacillus* plasmids, pWCFS103 (36) and plca36 (37), revealed several genes unique to pKB290-1 as shown in Fig. 1. The region contained two putative glycosyltransferase genes (LVISKB_P1-0027 and LVISKB_P10028), with LVISKB_P1-0029 adjacent to them downstream (Fig. 2A). Although these genes were widely distributed among lactobacilli from publicly available genomes and metagenomes, the function of this 3-gene set has not yet been elucidated.

LVISKB_P1-0027 was similar to WcaA glycosyltransferase involved in cell wall biosynthesis (COG0463) and GtrA-like protein (PF04138) (Table 2). LVISKB_P1-0028 was similar to BcsA glycosyltransferase, the catalytic subunit of cellulose synthase, and poly-beta-1,6-*N*-acetylglucosamine synthase (COG1215), CelA: cellulose synthase catalytic subunit (TIGR03030), Glycos_transf_2: glycosyltransferase, group (PF00535), and PilZ: Type IV pilus assembly protein PilZ (PF07238) (Table 2). We assigned LVISKB_P1-0027 to *gtf27* and LVISKB_P1-0028 to *gtf28*. Both protein products were predicted to be localised in the cell membrane (Table 2). The predicted domain architecture of the *gtf28*-encoded protein was similar to that of the known cellulose synthases of *Gluconacetobacter xylinus* (38,39), *Thermosynechoccus vulcanus* (34), and *Agrobacterium tumefaciens* (40) (Fig. 2B). They all harbour a highly conserved D, D, D, QXXRW motif essential for their catalytic activity (41,42). LVISKB_P1-0029 showed 68% homology with the ABC transporter permease from *Lactobacillus* sp. CBA3606 (WP_105449149.1) encoded by *orf29*. To confirm whether *gtf27*, *gtf28*, and *orf29* were expressed as a single transcript, RNA was extracted from an early stationary-phase culture and analysed by RT-PCR. Three amplifications covered all the intergenic *eps* regions except the region between *orf26* and *gtf27* (data not shown).

Characteristics of the *eps* gene products The 5-kb *eps* genetic locus (*gtf27*, *gtf28*, and *orf29*) in pKB290-1 (Fig. 2A) was cloned and expressed in homologous and heterologous LAB

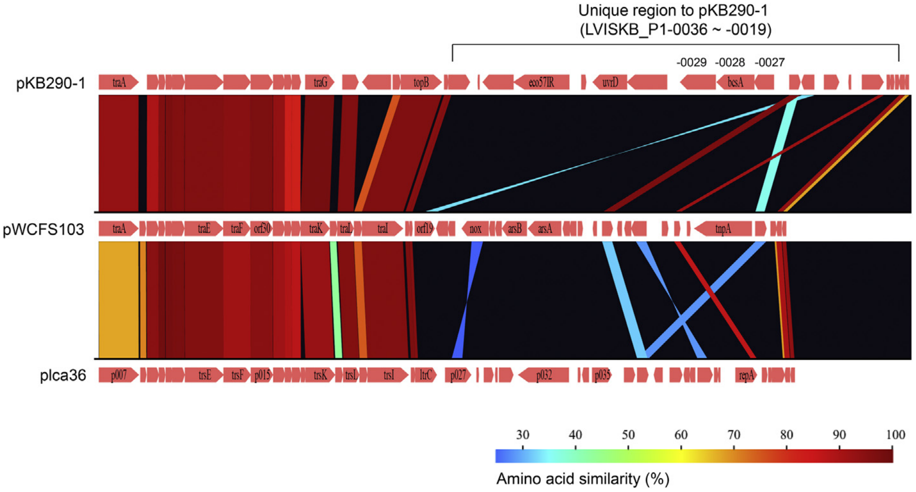

FIG. 1. Sequence similarity among pKB290-1 and other *Lactobacillus* plasmids. Genes and their orientations are depicted with arrows.

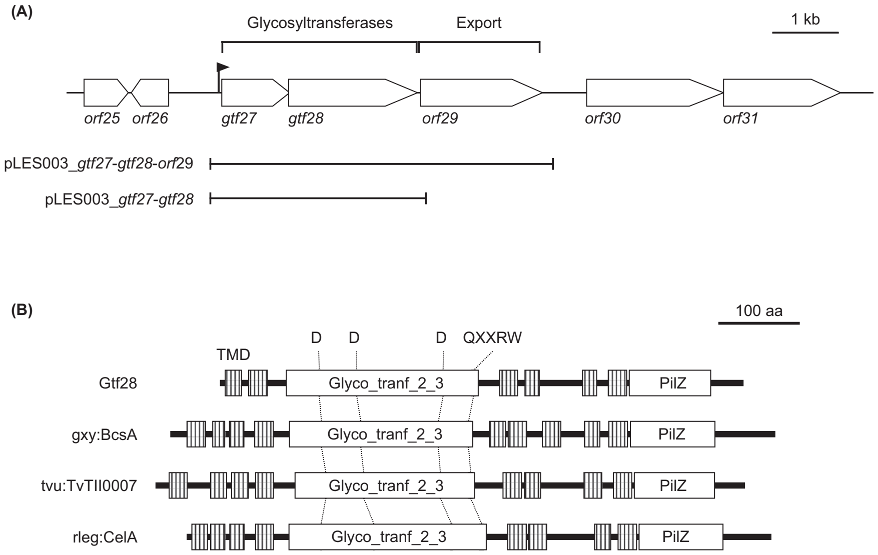

FIG. 2. Schematic overview of the *eps* gene loci. (A) The *eps* gene cluster of plasmid pKB290-1. Genes and their orientation are depicted with arrows, and gene designations are indicated under the arrows. The flag indicates the putative promoter. The plasmids listed were used for heterologous and homologous expression of *eps* genes. (B) Domain architecture of Gtf28 and known cellulose synthases. gxy, *Gluconacetobacter xylinus*; tvu, *Thermosynechococcus vulcanus*; rleg, *Rhizobium leguminosarum*; TMD, transmembrane domain; The D, D, D, QXXRW motif is shown by a dotted line.

TABLE 2. Components of the *eps* locus in pKB290-1.

Gene name (locus tag[a])	No. of amino acids	No. of transmembrane domains	Predicted encoded function	COG functional category, Pfam and TIGRFAM domains
gtf27 (LVISKB_P1-0027)	344	4	Glycosyltransferase	WcaA glycosyltransferase involved in cell wall biosynthesis (COG0463), GtrA-like protein (PF04138)
gtf28 (LVISKB_P1-0028)	654	6	Glycosyltransferase	BcsA glycosyltransferase, catalytic subunit of cellulose synthase and poly-beta-1,6-*N*-acetylglucosamine synthase (COG1215), CelA: cellulose synthase catalytic subunit (TIGR03030), Glycos_transf_2: glycosyltransferase, group (PF00535), PilZ: Type IV pilus assembly protein PilZ (PF07238)
orf29 (LVISKB_P1-0029)	617	12	ABC transporter permease	No hit

[a] Locus tags from published sequence AP012168.

strains. The DNA fragment containing the genes was inserted into pLES003 and introduced into the plasmid-cured KB1802, designated as *L. brevis* KB1802_*gtf27-gtf28-orf29*. The genes were also inserted into *L. plantarum* NC8, designated as *L. plantarum* NC8_*gtf27-gtf28-orf29*. We confirmed that *gtf27* was expressed in the recombinants by RT-PCR (data not shown).

Considerably less EPS was detected in the plasmid-cured KB1802 strain expressing pLES003 (0.37 ± 0.07 μg) than in the parental KB290 strain (13.01 ± 3.13 μg), whereas a similar amount was detected in the KB1802 strain expressing pLES003_*gtf27-gtf28-orf29* (11.63 ± 1.87 μg). The KB1802 strain expressing pLES003_*gtf27-gtf28* but not *orf29* did not increase EPS (0.94 ± 0.11 μg). Moreover, increased amounts of EPS were detected in *L. plantarum* NC8 expressing pLES003_*gtf27-gtf28-orf29* (8.29 ± 0.18 μg) than in *L. plantarum* NC8 expressing pLES003 (1.20 ± 0.07 μg).

The chromatogram of acid-hydrolysed EPS from KB290 revealed distinct monosaccharide peaks (Fig. 3A), corresponding to glucose and *N*-acetylglucosamine as previously reported (11). Considerably less monosaccharide peaks were detected in the KB1802 strain expressing pLES003 (Fig. 3B) than in the parental KB290 strain, whereas similar monosaccharide peaks were detected in the KB1802 strain expressing the three *eps* genes (Fig. 3C). Moreover, the three *eps* genes conferred similar monosaccharide peaks in *L. plantarum* NC8 (Fig. 3D, E).

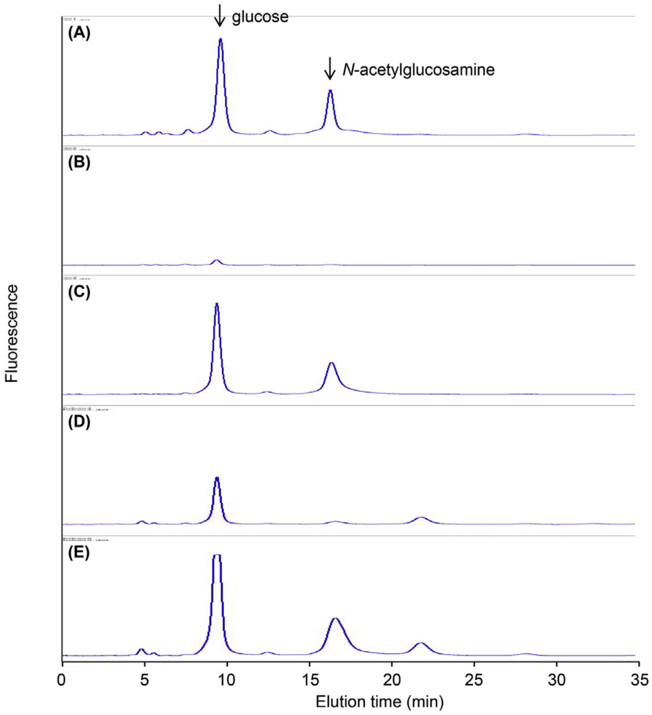

FIG. 3. HPLC profiles of isolated acid-hydrolysed surface EPS. (A) KB290, (B) KB1802 expressing pLES003, (C) KB1802 expressing pLES003_*gtf27-gtf28-orf29*, (D) *L. plantarum* NC8, and (E) *L. plantarum* NC8 expressing pLES003_*gtf27-gtf28-orf29*. The arrows indicate the peaks corresponding to glucose and *N*-acetylglucosamine.

***L. brevis* KB290 EPS is linked to cell aggregation and bile resistance** Cell aggregation was decreased in KB1802 relative to that in the parental strain but was complemented by the three *eps* genes (Fig. 4). The KB1802 cells expressing glycosyltransferase genes alone did not aggregate at all. Moreover, the 3 *eps* genes conferred aggregation properties to *L. plantarum* NC8.

Bile resistance was also decreased in KB1802 and was complemented by *eps* genes (Fig. 5). The KB1802 cells expressing only glycosyltransferase genes were not bile resistant. Moreover, the three *eps* genes conferred bile resistance in *L. plantarum* NC8.

Effect of sugar source on glycosyltransferases gene expression *L. brevis* KB290 cultured in glucose, arabinose, or xylose showed comparable growth (data not shown), but *gtf27* expression was markedly lower in arabinose- and xylose-containing media than in glucose-containing medium (Fig. 6A). The same was true for the aggregation level (Fig. 6B). Glycosyltransferase gene (LVISKB_P1-0027) expression was correlated with cell aggregation.

DISCUSSION

In this study of the properties conferred by genes on the *L. brevis* KB290 plasmid pKB290-1, we elucidated the function of a 3-gene set contributing to the unique characteristic features of KB290, suggesting that this region is responsible for the probiotic properties specific to KB290.

The GtrA-like protein (PF04138) encoded by *gtf27* is involved in the synthesis of cell surface polysaccharides, including O-antigen modification, biosynthesis of the serotype-specific polysaccharide antigen, and teichoic acid glycosylation (43–45). The *gtf27* gene product is predicted to act as the priming glycosyltransferase that adds the first sugar monomer to the carrier. The glycosyltransferase-like family 2 domain (PF00535) of *gtf28* is found in a diverse family of glycosyltransferases that transfer the sugar moiety from UDP-glucose, UDP-*N*-acetyl-galactosamine, GDP-mannose, or CDP-abequose to a range of substrates including cellulose, dolichol phosphate, and teichoic acids (46). The predicted domain architecture of *gtf28* was similar to that of the known cellulose synthases with highly conserved motifs. The PilZ domain (PF07238) of *gtf28* is known to bind to 3′,5′-cyclic diguanylic acid (c-di-GMP), an intracellular second messenger (47,48), and to activate cellulose synthase (49,50). This suggests that *gtf27* and *gtf28* add some component to the EPS of KB290. On the other hand, as *orf29* showed homology with the ABC transporter permease, its function is likely to involve export of EPS across the cell membrane. Consistent with these roles, the proteins were predicted to be located in the cytoplasmic membrane.

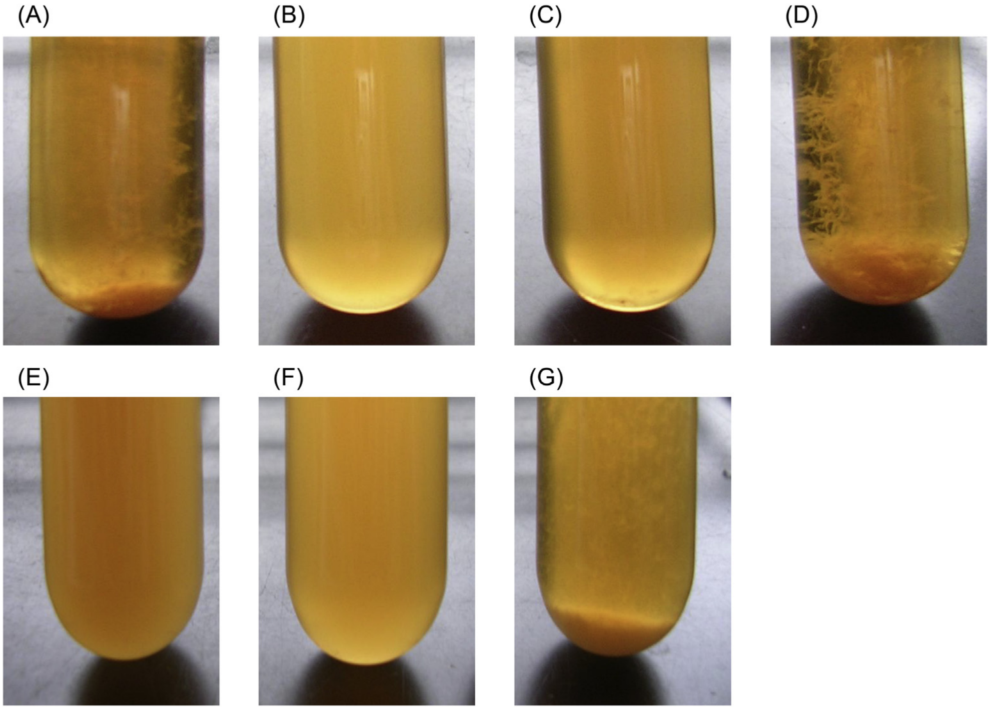

FIG. 4. Cell aggregation of KB290 and *eps* gene derivatives. (A) KB290, (B) KB1802 expressing pLES003, (C) KB1802 expressing pLES003_*gtf27-gtf28*, (D) KB1802 expressing pLES003_*gtf27-gtf28-orf29*, (E) *L. plantarum* NC8 expressing pLES003, (F) *L. plantarum* NC8 expressing pLES003_*gtf27-gtf28*, and (G) *L. plantarum* NC8 expressing pLES003_*gtf27-gtf28-orf29*.

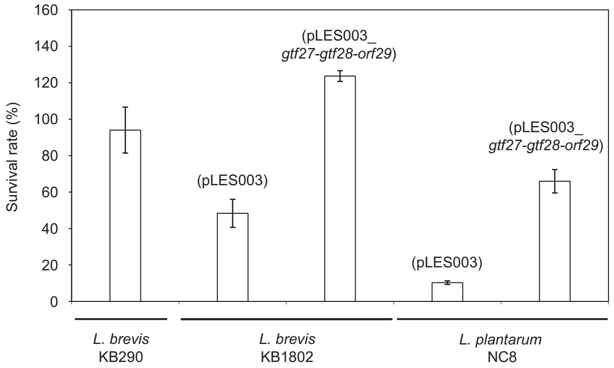

FIG. 5. Survival rates of KB290 and *eps* gene derivatives in bile salt-induced stress.

Our findings that *gtf27*, *gtf28*, and *orf29* comprise an operon suggest that transcription is initiated at *gtf27*.

In addition to the plasmid, the chromosome of KB290 contains a cell wall-associated polysaccharide biosynthesis gene cluster composed of 17 *eps* genes reaching up to 19 kb in length (10), whose organization is similar to that of other sequenced LABs, including *L. plantarum* NC8, which was used as the recombinant host strain in this study. These genes are predicted to be genetic elements required for EPS production by encoding factors responsible for regulation, chain-length determination, repeat-unit assembly, polymerization and export (10). Although the precise scope and nature of plasmid-chromosome cross-regulation are not yet fully understood, the EPS phenotypes in *L. brevis* and *L. plantarum* used in this study were clearly affected by the presence of the three *eps* genes of pKB290-1. Thus, further studies are required to determine

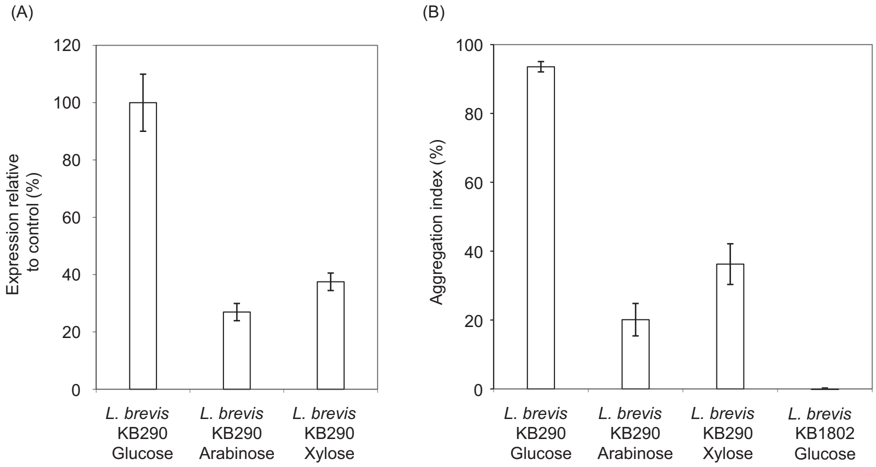

FIG. 6. Effect of sugar source on glycosyltransferase gene expression and cell aggregation. (A) Expression ratios of the KB290 *gtf27* gene in MRS broth containing glucose, arabinose, or xylose. (B) Aggregation index of KB290 in MRS broth containing glucose, arabinose, or xylose and of KB1802 in MRS broth containing glucose.

the precise coordination between the plasmid and chromosome for EPS production in KB290.

Our analysis of *eps* gene derivatives showed the same monosaccharide peaks, indicating a secreted heteropolysaccharide consisting of glucose and *N*-acetylglucosamine as previously reported (11) and these findings are consistent with transmission electron microscopy results showing that KB290 produces an outer cell surface layer presumed to be an EPS capsule. Our findings suggest that the EPS surface layer produced by KB290 has a specific composition and structure defined by the 3-gene set. Since the presence of genes encoding specific glycosyltransferases has a direct impact on the repeating units of polymers (4,5), further studies are needed to determine the structure of the repeat unit conferred by these glycosyltransferases.

While we found that the *eps* genes were responsible for cell aggregation, the mechanism by which EPS induces aggregation is unknown. However, it is possible that EPS mediates cell–cell attachment. Aggregation of LAB or their co-aggregation with other commensals or pathogens could be important factors in inhibiting the adherence of pathogenic bacteria to intestinal mucosa as well as the colonization and immunomodulation of colonic mucosa (17,18). Our future experiments will focus on EPS interactions with human epithelial cells, co-aggregation of KB290 or purified EPS with various pathogenic bacteria, and the role of EPSs in adhesion and probiotic potential.

We also found that the *eps* genes in KB290 were responsible for bile resistance, which is consistent with the findings of a positive correlation between EPS production and resistance to bile salts in *Bifidobacterium* species (6–9). Interestingly, both the technological and gastrointestinal durability of *Lactobacillus* species were significantly improved by equipping the strain with an inherent ability to produce EPS (51–53). Heterologous expression of the genes in *Lactobacillus paracasei* increased its heat tolerance 60-fold, its acid-tolerance 20-fold, its ability to survive in simulated gastric juice 15-fold, and its ability to survive in bile 5.5-fold (53). In KB290, the EPS capsule might protect the cells during delivery to the gastrointestinal tract.

In KB290, *gtf27* expression varied with the sugar substrate and correlated with cell aggregation. This is consistent with previous reports that sugar availability may modulate EPS production (54–56).

In summary, the *eps* genes (*gtf27*, *gtf28*, and *orf29*) in pKB290-1 were shown to be sufficient for EPS biosynthesis with glucose and *N*-acetylglucosamine. The functions of these genes might include biosynthesis of the EPS repeating unit (*gft27* and *gtf28*) and export of EPS (*orf29*). These genes are also responsible for cell aggregation and bile resistance. However, the precise molecular mechanism by which EPS induces these phenotypes remains unknown.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2019.04.008.

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