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Detection of Galectin-3 Interaction with Commensal Bacteria

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A panel of commensal bacteria was screened for the ability to interact with galectin-3. Two strains of *Bifidobacterium longum* subsp. *infantis* interacted to a greater extent than did the pathogenic positive control, *Escherichia coli* NCTC 12900. Further validation of the interaction was achieved by using agglutination and solid-phase binding assays.

Galectins are a family of evolutionarily conserved proteins found across a variety of species ranging from lower invertebrates to mammals (1). These lectins are involved in a wide range of biological processes, including tumor cell adhesion and progression, inflammation, wound healing, development, and immunity (2–4). A number of natural ligands of galectins have been identified. These include galactose, lactose, polylactosamine, and *N*-acetyllactosamine (LacNAc) (1). Recently, galectins have also been shown to bind to blood group antigens expressed on the surface of bacterial cells (5).

Galectin-3 (gal-3) is a 31-kDa chimera-type lectin containing a C-terminal carbohydrate recognition domain (CRD) possessing an affinity for β-galactoside residues (6). Gal-3 is one of the most ubiquitously expressed members of the galectin family, demonstrating elevated expression levels in the epithelial cells of the digestive tract (7, 8). Within the intestinal tract, gal-3 is detected predominantly in the villus tips (9) and interacts with MUC2, a secreted mucin found in the intestine (10). This multifunctional molecule exhibits a range of functions, at times opposing one another, depending on cell localization (11). Among these functions, gal-3 is known to interact with pathogenic microorganisms. Gal-3 was first demonstrated to associate with the lipopolysaccharide (LPS) of *Klebsiella pneumoniae* through the binding of β-galactoside glycans in the outer core. In addition, interactions with *Escherichia coli* (LPS) (12), *Pseudomonas aeruginosa* (outer core of LPS) (13), *Neisseria gonorrhoeae* (lipooligosaccharides) (14), and *Helicobacter pylori* (O antigen of LPS) (15) have been described. In a recent paper, Fermino et al. (12) suggested that gal-3 may have a broader specificity than LPS. Indeed, gal-3 has been shown to bind mycolic acid, the major constituent of the *Mycobacterium tuberculosis* cell envelope (16). Additionally, *Salmonella minnesota* LPS is devoid of β-galactosides but can bind to a site within the N-terminal domain of gal-3 through its lipid A portion (17). The ability of gal-3 to bind two hydrophobic ligands, such as mycolic acid and lipid A, opens the possibility that gal-3 may also interact with lipoteichoic acid, a major constituent of the cell wall of Gram-positive bacteria (18).

Many negative effects of the gal-3–pathogen interaction have been identified. Pathogenic interaction with gal-3 may result in suppressed or exaggerated states of endotoxic shock (12, 19) or increased adhesion to host tissues (20). These studies highlight the ability of pathogens to capitalize on the presence of gal-3 to augment their capacity to colonize and survive within the host environment. Despite numerous examples of pathogen interactions with gal-3, there is very little evidence to suggest that commensal bacteria interact with and influence the activity of this protein.

In this study, we demonstrated, by using an indirect surface plasmon resonance (SPR)-based approach, the ability of commensal bacteria to interact with gal-3. The resulting interaction was further validated by using agglutination and solid-phase binding assays. Of six commensal strains examined, two strains of bifidobacteria were identified that interacted strongly with gal-3. The ability of commensal strains to interact with gal-3 may indicate an ability to exclude and displace pathogens, which has major implications for gut health.

Bacterial interaction with gal-3 was assessed indirectly by assaying various commensal strains for their respective abilities to inhibit the well-defined interaction between gal-3 and asialofetuin (ASF) by using an SPR-based biosensor system as outlined by Maljaars et al. (21) (Biacore X100 system, solutions, and reagents; GE Healthcare UK Ltd., Buckinghamshire, United Kingdom). The experimental design is summarized in Fig. 1. SPR is a microfluidics-based methodology used to investigate the real-time interaction between two or more unlabeled molecules under flow conditions. Interactions between two molecules are measured as a change in mass on the chip surface, detected as a change in the reflected pathway of a laser beam directed at the gold chip surface. An indirect approach was used because of the functional nature of gal-3. As oligomerization is often required for full activity, immobilization would prevent a representative biological response. A carboxymethyl dextran-coated (CM5) chip was prepared in accordance with the manufacturer's instructions. Briefly, flow cell 1 was prepared by the injection of a 1:1 mixture of 0.05 M *N*-hydroxysuccinimide (NHS) and 0.2 M 1-ethyl-3-(3-dimethylpropyl)-carbodiimide (EDC) at a flow rate of 10 μl/min, and ASF was immobilized (10 μl, 0.2 mg/ml in 10 mM sodium acetate buffer, pH 4.5; flow rate, 5 μl/min) to a level of ~11,000 resonance units (RU). The remaining NHS esters were blocked by the injection of a 1 M ethanolamine hydrochloride solution (70 μl, pH 8.5; flow rate, 10 μl/min). Flow cell 2 was activated with NHS-EDC and then blocked with ethanolamine to create the reference flow cell to control for nonspecific binding to the carboxymethyl dextran-coated surface. A stock concentration of 0.22 mg/ml of human recombinant gal-3 in HBS-EP buffer (0.01 M HEPES [pH 7.4],

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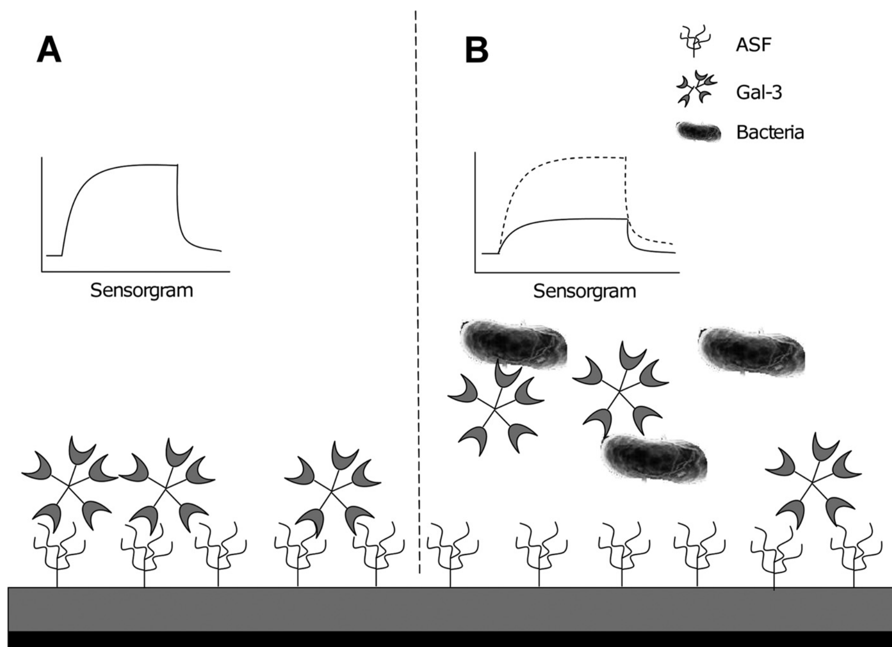

FIG 1 Experimental overview of SPR screening study. (A) Gal-3 interacts with ASF on the chip surface. (B) Preincubation of gal-3 with bacteria decreases the gal-3 interaction with ASF, as indicated by a reduced resonance response.

0.15 M NaCl, 3 mM EDTA, 0.005% [vol/vol] Surfactant P20) was used for all injections following preliminary optimization experiments. Gal-3 stock solution (10 μl) was incubated with 30 μl (optical density at 600 nm of 1.0) of a bacterial suspension in HBS-EP buffer for 3 min prior to each injection. The mixture was injected over the sensor chip surface for 2 min. Results were calculated on the basis of the difference between the number of RU immediately before injection and that following the postinjection washing. Five measurements were taken, commencing with completion of the postinjection washing and every 20 s thereafter for 80 s. The measurements were averaged to calculate the response value for each injection. The surfaces were then regenerated with 0.1 M lactose (a well-characterized ligand of gal-3) in HBS-EP (10 μl) for 2 min. All measurements were performed at a flow rate of 5 μl/min. Each injection was repeated in triplicate for each bacterial strain tested, and the average of the three results is reported. Mean responses were compared against those obtained with the positive control, *E. coli* NCTC 12900, by using Student's *t* test, and significance was defined as $P < 0.05$.

Bifidobacterium longum subsp. *infantis* ATCC 15702 and ATCC 15697 were found to be capable of significantly inhibiting the interaction between gal-3 and ASF (Fig. 2). Indeed, these strains inhibited the interaction to a greater extent than did the positive control, *E. coli* O157:H7 NCTC 12900. *B. longum* subsp. *infantis* ATCC 15702 and ATCC 15697 demonstrated 93.1% ($P < 0.0001$) and 73.1% ($P < 0.0001$) inhibition of the ASF–gal-3 interaction, respectively. The remaining commensal strains displayed intermediate inhibition of the gal-3 interaction with ASF, ranging between 36 and 44%. The positive pathogen control used in the study exhibited 54% inhibition (Fig. 2). To rule out the possibility of bacterial interference with the chip surface, the SPR experiments were replicated with the two strains of bifidobacteria, as performed previously, followed by a centrifugation step (8,000 × *g* for 5 min) to remove bacteria prior to injection. The results indicate that the bacteria did not actively or passively interfere with the gal-3–ASF interaction at the chip surface (Fig. 3).

The direct interaction between *B. longum* subsp. *infantis* ATCC 15702 and gal-3 was further assessed by employing an agglutination assay as outlined by Hynes et al. (22). Gal-3 effectively agglutinated the bacterial suspension of *B. longum* subsp. *infantis* ATCC 15702, as demonstrated by a carpet of aggregated cells on the bottoms of the wells (Fig. 4, column A). Alternatively, in the absence

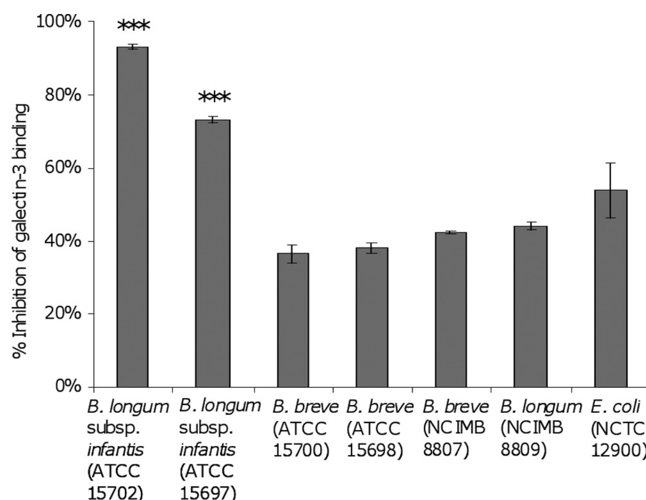

FIG 2 Inhibition of gal-3 interaction with immobilized ASF by a panel of commensal bacteria by an SPR biosensor approach. *E. coli* NCTC 12900 was included as a positive control. Inhibition is defined as the ability of bacteria to interact with gal-3, thereby reducing its ability to generate a surface plasmon response through interaction with the ASF chip surface. Gal-3 injected over the ASF surface in the absence of bacteria was used to define the 0% inhibition SPR response. Experiments were carried out in triplicate ($n = 3$), and the results are presented as mean inhibition ± the standard deviation. ***, $P < 0.0001$.

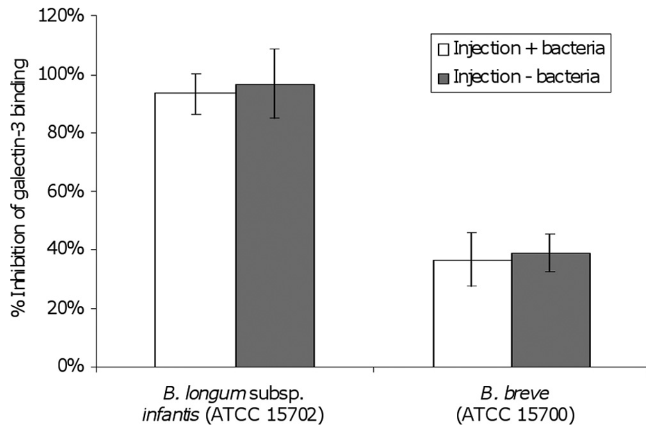

FIG 3 SPR analysis of bacterium-free injections of gal-3 over an immobilized ASF surface. Inhibition is defined as the ability of bacteria to interact with gal-3, thereby reducing its ability to generate a surface plasmon response through interaction with the ASF chip surface. Gal-3 injected over the ASF surface in the absence of bacteria was used to define the 0% inhibition SPR response. The effects of injections with and without bacteria are compared, and data are presented as mean inhibition ± the standard deviation. Experiments were carried out in triplicate ($n = 3$).

of gal-3, control wells exhibited a tight bacterial pellet, indicating a lack of agglutination (Fig. 4, column B).

In order to characterize the interaction between *B. longum* subsp. *infantis* ATCC 15702 and gal-3, a solid-phase binding platform was used. The two *B. longum* subsp. *infantis* strains and *E. coli* NCTC 12900 were stained with TAMRA (carboxytetramethylrhodamine; Invitrogen) (intracellular localization) as outlined by Alemka et al. (23) and exposed to gal-3-coated wells (InnoCyte ECM cell adhesion assay kit; Calbiochem [Merck, Darmstadt, Germany]) under anaerobic conditions (Anaerocult A gas pack; Merck, Darmstadt, Germany) for 1 h at 37°C. The wells were washed three times with phosphate-buffered saline (PBS), and the fluorescence in each well was recorded at an excitation wavelength of 530 nm with detection at 590 nm with a Synergy HT plate reader (BIO-TEK, Bedfordshire, United Kingdom; sensitivity setting of 45). *B. longum* subsp. *infantis* ATCC 15702 displayed a significantly higher level of interaction with immobilized gal-3 than did the other two bacterial strains assayed (Fig. 5A). While *B. longum* subsp. *infantis* ATCC 15702 and ATCC 15697 both dem-

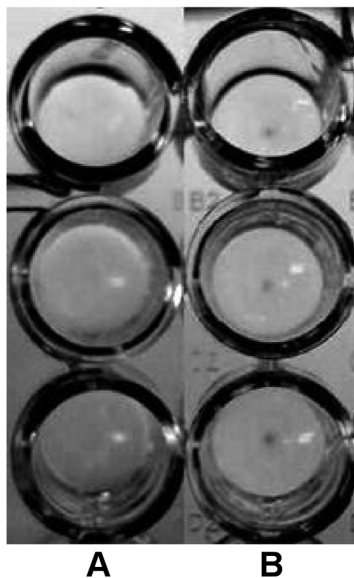

FIG 4 Agglutination assay for direct visualization of bacterium–gal-3 interaction. Gal-3 or PBS alone (negative control) was incubated with *B. longum* subsp. *infantis* ATCC 15702. Gal-3 exposure resulted in agglutination of the bacteria, as evidence by the absence of a dense pellet of bacteria in the bottom of the well (A). PBS alone did not agglutinate the bacteria, resulting in pellet formation at the bottom of the well (B).

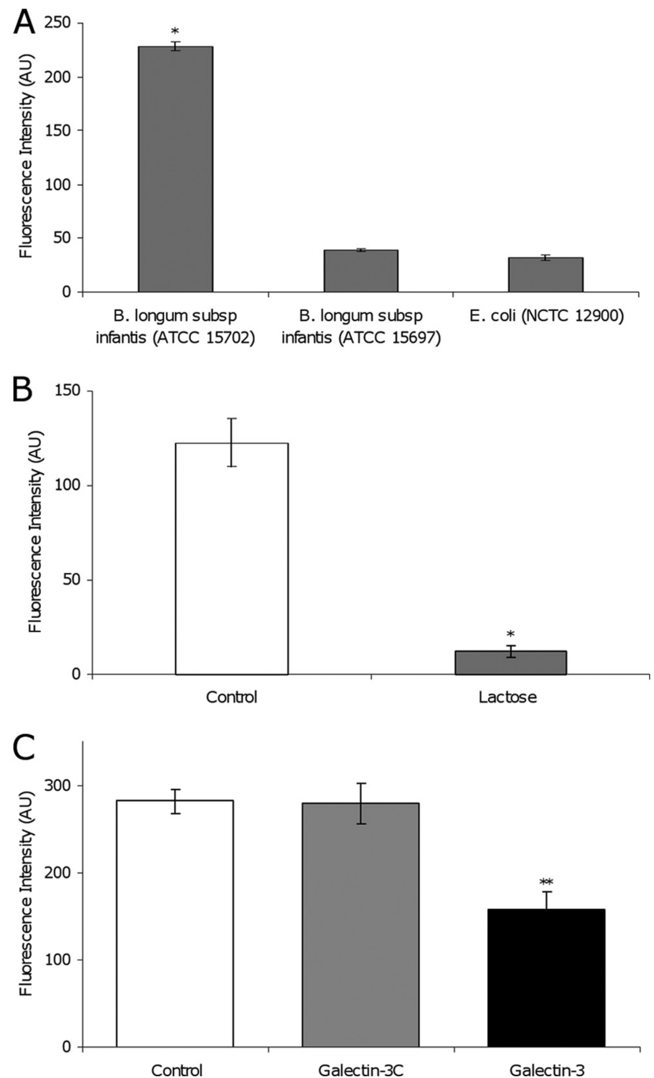

FIG 5 (A) Binding of TAMRA-labeled bacteria to recombinant gal-3 in a solid-phase binding assay. *B. longum* subsp. *infantis* ATCC 15702 and ATCC 15697 and *E. coli* O157:H7 NCTC 12900 were incubated in gal-3-coated wells for 1 h. (B) Binding of TAMRA-labeled *B. longum* subsp. *infantis* ATCC 15702 to recombinant gal-3 in a solid-phase binding assay. TAMRA-labeled bacteria were incubated in gal-3-coated wells (white bars) or in gal-3-coated wells in the presence of lactose (gray bars). (C) Binding of TAMRA-labeled *B. longum* subsp. *infantis* ATCC 15702 to recombinant gal-3 in a solid-phase binding assay. Bacteria were incubated in gal-3-coated wells (white) or in gal-3-coated wells in the presence of the soluble gal-3 CRD (gal-3C; gray) or full-length soluble gal-3 (black). All experiments were carried out in triplicate ($n = 3$). The data are presented as means ± the standard deviations. *, $P < 0.05$; **, $P < 0.01$; AU, arbitrary units.

onstrate a comparatively high level of gal-3 inhibition in the SPR experiments, ATCC 15697 does not interact as strongly under the solid-phase experimental conditions used, likely because of the difference in incubation times with gal-3 (1 h versus 3 min), as well as the differing means of detection. Resuspension of *B. longum* subsp. *infantis* ATCC 15702 in PBS containing 50 mM lactose (a known ligand for gal-3) prior to interaction with immobilized gal-3 resulted in a 90% reduction of bacterial adherence ($P < 0.05$) (Fig. 5B), indicating the involvement of a carbohydrate ligand in the bacterium–gal-3 interaction. To investigate the interaction further, studies were carried out with full-length soluble gal-3 protein and the gal-3 CRD. Resuspension of *B. longum* subsp. *infantis* ATCC 15702 in PBS containing a 20-fold molar excess (3.85 μM, in relation to that immobilized on the well surface) of full-length gal-3 prior to well exposure resulted in a 44% reduction of bacterial binding ($P < 0.01$; Fig. 5C), while the presence of the gal-3 CRD (3.85 μM) did not result in a significant level of inhibition. These results highlight the importance of the full-length gal-3 protein for maximal binding. Negative-control experiments with bacteria that are known not to interact with gal-3 were difficult to design. Such controls are not available, given that galactose and other moieties recognized by gal-3 are commonly found on the surface of most bacteria (12, 17). Further work is required to fully characterize the interaction between gal-3 and *B. longum* subsp. *infantis* ATCC 15702 or ATCC 15697 and to determine its implications for host health and the integrity of the intestinal microflora.

In conclusion, the results presented here, to the best of our knowledge, demonstrate for the first time an interaction between gal-3 and commensal intestinal bacteria. The results identify a higher affinity of gal-3 for two specific strains of bifidobacteria, with gal-3 requiring the full-length protein for enhanced activity, similar to findings reported by Fermino et al. (12). This novel interaction may potentially play a role in colonization resistance and host infection by limiting the availability of gal-3 to pathogens.

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