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Characterizing Coaggregation among Strains of Human Gastrointestinal Bacteria

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CHARACTERIZING COAGGREGATION AMONG STRAINS OF HUMAN GASTROINTESTINAL BACTERIA

By

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INTRODUCTION

The human gastrointestinal (GI) tract harbors an ecosystem of at least 1200 documented microbial species including archaea, yeast, filamentous fungi, and both indigenous and transient bacteria (8, 18, 24). Bacteria, which range in population from 10^2 to 10^{11} cells/gram along different regions of the GI tract (21), are particularly diverse and are represented by nine phyla of which *Firmictus*, *Bacteroidetes*, and *Actinobacteria* are the most dominant (3). While transient bacteria are often enteric pathogens, the majority of GI bacteria are indigenous and have been found to aid fat storage regulation (2), improve natural and acquired host immune response, enhance the mucosal barrier, and increase protection against foreign microbes (31). Some bacteria, including members of the *Clostridium* and *Eubacterium* genera, specifically benefit host health through their production of butyrate, a short chain fatty acid resulting from the fermentation of undigested dietary fiber in the colon. Butyrate is of particular interest because of its potential role in fighting cancer. It aids in the regulation of many cell processes, but most significantly, it stimulates cell differentiation and inhibits tumor cell proliferation (6, 9, 10, 11, 23, 25, 26). Butyrate is the primary energy source of colonocytes, which in the absence of butyrate commit apoptosis. Interestingly, transformed cells experience an opposite effect and instead undergo programmed cell death in butyrate's presence (7, 10, 22). Butyrate may also be a contributor in other processes such as the reduction of inflammation and oxidative stress (10). In light of butyrate's many benefits, it is important to understand the factors modifying butyrate production. In a study conducted by Abbas (2009), it was found that growing butyrate-producing bacteria and lactic acid-

producing bacteria in a co-culture increases butyrate production levels. This increase in fermentation product may be a result of a process known as coaggregation. Coaggregation is the adherence of genetically distinct bacteria by means of specific adhesin and ligand molecules. It functions to accrete bacteria and facilitate metabolic and possibly genetic information communication between coaggregation partners (4). It is also thought to be an adhesive mechanism used in the formation of biofilms—highly structured, multi-species communities that are normally attached to a stationary substratum and that have a higher combined metabolic activity than that of the component species growing individually (27). Although coaggregation was once believed to have been a mechanism shared exclusively between plaque forming bacteria in the oral cavity, it has also been observed in bacteria isolated from freshwater drinking systems, mammalian gut, and human urogenital biofilms (27). This evidence suggests that coaggregation might occur in many different environments including the GI tract. Although Ledder *et al.* (2008) argues that coaggregation, as a mechanism for forming multi-species biofilms, is greatly limited in frequency and extent in the human gastrointestinal tract as compared to the oral cavity, co-culturing the gastrointestinal, butyrate-producing *Eubacterium plexicaudatum* ASF 492 with either the lactic acid-producing *Lactobacillus acidophilus* NCFM or *Lactobacillus johnsonii* NF-1 has resulted in considerable flocculent growth (1). The object of this study was to further examine and characterize coaggregation between these and other strains of GI microbes through coaggregation assays, some of which involved lactose inhibition and heat denaturation. It was hypothesized that gut microbes interact in a specific manner. It has been found that

the majority of GI coaggregations are mediated by protein-carbohydrate interactions (16). Therefore, it was predicted that coaggregation interactions among the GI strains in this study are mediated by protein adhesins and carbohydrate ligands.

Table 1. Microbial strains used in this study.

Strain	Classification	Main Fermentation Product (s)	Plated Media	Liquid Media	Growth Requirements
Bacteroides fragilis ATCC 25285	Gram-negative	Succinate, propionate, acetate	Blood Agar	RCM	Obligate anaerobe
Bacteroides thetaiotamicron ATCC 29148	Gram-negative	Succinate, propionate, acetate	Blood Agar	RCM	Obligate anaerobe
Bifidobacterium adolescentis ATCC 15703	Gram-positive	Lactic acid	MRS + NaN_3	MRS	Obligate anaerobe
Bifidobacterium catenalatum ATCC 27539	Gram-positive	Lactic acid	MRS + NaN_3	MRS	Obligate anaerobe
Bifidobacterium longum ATCC 15707	Gram-positive	Lactic acid	Brucella Agar	Brucella Broth	Obligate anaerobe
Candida albicans SC5314	Yeast	Ethanol	TSA	TSB	Facultative anaerobe
Clostridium sp. ASF 500	Gram-positive	Butyrate	Brucella Agar	Brucella Broth	Obligate anaerobe
Enterococcus faecalis OG1S	Gram-positive	Lactic acid	Brucella Agar	Brucella Broth	Facultative anaerobe
Eubacterium plexicaudatum ASF 492	Gram-positive	Butyrate, acetate, formic acid, lactic acid	Brucella Agar	Brucella Broth	Obligate anaerobe
Lactobacillus acidophilus NCFM	Gram-positive	Lactic acid	Brucella Agar	Brucella Broth	Facultative anaerobe
Lactobacillus casei ATCC 393	Gram-positive	Lactic acid	MRS + NaN_3	MRS	Facultative anaerobe
Lactobacillus johnsonii NF-1	Gram-positive	Lactic acid	Brucella Agar	Brucella Broth	Facultative anaerobe
Lactobacillus murinus ASF 361	Gram-positive	Lactic acid	MRS + NaN_3	MRS	Facultative anaerobe
Lactobacillus paracasei ATCC 27092	Gram-positive	Lactic acid	MRS + NaN_3	MRS	Facultative anaerobe
Lactobacillus plantarum ATCC39542	Gram-positive	Lactic acid	MRS	MRS	Facultative anaerobe
Lactobacillus rhamnosus ATCC 53103	Gram-positive	Lactic acid	MRS + NaN_3	MRS	Facultative anaerobe

MATERIALS AND METHODS

Microbe strains and culture conditions

Microbial strains used in this study are listed in Table 1. Strains were first streaked on plated media, incubated for 24-48 hours, transferred to liquid media at 37°C as indicated by Table 1, and incubated for 24-48 hours. Solid media included: Blood Agar TSA II 5% Sheep Blood (Blood Agar; BD, Sparks, MD), Brucella Agar (Acumedia, Lansing, MI), Lactobacilli MRS Agar (MRS; EMD Chemicals Inc., Darmstadt, Germany), and Tryptic Soy Agar (TSA; Acumedia, Lansing, MI). MRS+NaN_3 Agar was prepared by supplementing MRS agar with 0.01% sodium azide. Liquid media included: Brucella Broth (Acumedia, Lansing, MI), Lactobacilli MRS Media (MRS; EMD Chemicals Inc., Darmstadt, Germany), Reinforced Clostridia Media (BD, Sparks, MD), and Tryptic Soy Broth (TSB; Acumedia, Lansing, MI). All strains, excluding *Candida albicans* SC5314, were grown under anaerobic conditions using the GasPak system (BD, Sparks, MD). Isolates were characterized and identified morphologically by gram staining after transfer to liquid broth as well as before centrifugation. Cells were harvested via centrifugation at 10,000 x g (4°C for 10 min) and were washed three times and suspended in a coaggregation buffer (5) comprised of 0.1mM $MgCl_2$, 0.1mM $CaCl_2$, 150mM NaCl, 0.02% NaN_3, and 1mM Tris adjusted to a pH of 8.0.

Coaggregation assays

Coaggregation assays and treatments were performed according to the procedure used by Kolenbrander and London (1992). Cell suspensions were adjusted to an absorbency of $A_{600}\approx1$. A visual assay to test coaggregation between strains was

performed by mixing equal amounts of turbid solutions of each partner, vortexing the mixture for 5 seconds, rocking the mixture 100 times, and visualizing the mixture under the lens of a manual colony counter (American Optical Corp.). A coaggregation score was assigned according to a qualitative, 0-4 scaled system which is described in Table 2. In this study, a score of 1 is a weak interaction, scores of 2 or 3 are considered moderate, and a score of 4 is considered strong. Occasionally, (+) and (-) symbols were attached to numerical scores for coaggregation interactions that did not completely satisfy the description of a score outlined in Table 2, but rather had an intermediate value. Lactose inhibition assays were performed by adding lactose to pair-wise mixtures at a final concentration of 300mM and repeating the visual assay. The effects of heat were determined for each pair-wise mixture by heating both strains at 85°C for 30 minutes and repeating the visual assay procedure for the following combinations of microbial strains: heat-treated (HT) strain 1 x untreated (UT) strain 2, strain 1 (HT) x strain 2 (HT), and strain 1 (UT) x strain 2 (HT). All assays were repeated three times before a final coaggregation score was assigned. All assays for each microbial strain were preformed using cell suspensions prepared from the same broth culture.

Table 2. Description of coaggregation scores (adapted from Kolenbrander and London, 1992).

Coaggregation	Description
0	Even, turbid suspension of bacteria
1	Finely dispersed clumps in turbid background (weak)
2	Definite clumps of bacteria are easily seen but do not settle immediately and remain n turbid background (moderate)
3	Clumps settle immediately with a slightly turbid background (moderate)
4	Clumps settle immediately and supernatant is completely clear (strong)

RESULTS

Coaggregation assays

Of the 16 strains tested, 13 exhibited coaggregation with at least one other strain (Table 3). *Lactobacillus rhamnosus* ATCC 53103 was the most promiscuous coaggregator, displaying positive assay scores with 10 other microbial strains. *L. rhamnosus* ATCC 53103 demonstrated coaggregation with *Bacteroides spp.*, *Bifidobacterium spp.*, *Eubacterium spp.*, *Clostridium sp.* ASF 500, and *Lactobacillus spp.* *L. acidophilus* NCFM had weak coaggregation interactions with *C. albicans* SC 5314 and *L. rhamnosus* ATCC 53103, but displayed coaggregation scores of 3 with both *Clostridium sp.* ASF 500 and *E. plexicaudatum* ASF 492. *L. plantarum* ATCC 39542 displayed moderate coaggregation interactions with *C. albicans* SC5314, *Clostridium sp.* ASF 500, and *E. plexicaudatum* ASF 492.

There was no complete reversal of coaggregation for any of the assays when lactose was added. All but four assays experienced no change in coaggregation score upon lactose addition. The scores for *L. acidophilus* NCFM with *Clostridium sp.* ASF 500 and *L. acidophilus* NCFM with *E. plexicaudatum* ASF 492 were reduced from 3 to 2+ when lactose was added, the score for *L. plantarum* ATCC 39542 with *C. albicans* SC5314 was reduced from 2 to 1-, and the score for *L. plantarum* ATCC 39542 with *Clostridium sp.* ASF 500 was reduced from 3 to 3-.

Heat assays were performed for each pair of strains that had a positive coaggregation score. Nearly all heat assays that included *L. rhamnosus* ATCC 53103 displayed unexpected results (Table 4). When heat-treated *L. rhamnosus* ATCC 53103

was mixed with all but one of its untreated coaggregation partners, the coaggregation score remained within ±1 point of the original value (both strains untreated). When both *L. rhamnosus* ATCC 53103 and its coaggregation partner were heat-treated, every score increased except for *L. rhamnosus* ATCC 53103(HT) with *L. murinus* ASF 361(HT), which remained the same. Most strikingly, all coaggregation scores increased when untreated *L. rhamnosus* ATCC 53103 was mixed with one of its heat-treated partners. The largest increase in coaggregation score was observed for the mixture of *L. rhamnosus* ATCC 53103 (UT) with *Bacteroides fragilis* ATCC 25285 (HT). When only *B. fragilis* ATCC 25285 was heated, the coaggregation score rose from an original score of 1 to a higher score of 3+.

Table 5 displays the effects of heat treatment on coaggregation assays between *L. acidophilus* NCFM and four other microbial strains. When *L. acidophilus* NCFM was mixed with *C. albicans* SC5314, *Clostridium sp.* ASF 500, or *E. plexicaudatum* ASF 492, heat treatment of either both partners or *L. acidophilus* NCFM alone produced no coaggregation. When untreated *L. acidophilus* NCFM was mixed with latter two of these heat-treated strains, coaggregation scores remained the same as the original scores. However, as already discussed, when heat-treatment effects on coaggregation assays were assessed for the mixture of *L. acidophilus* NCFM with *L. rhamnosus* ATCC 53101, it was found that heat treatment enhanced some coaggregation scores. Additionally, in the mixture of *L. acidophilus* NCFM with *C. albicans* SC5314, heat treatment of *C. albicans* SC5314 alone caused the coaggregation score to increase from 1 to 3.

Table 3. Coaggregation scores of GI strains. Superscripts represent coaggregation scores after the addition of lactose. Intermediate scores are indicated by (+) and (-). Scores were determined after 3 trials of each assay.

	B. fragilis ATCC 25285	*B. thetaiotamicron* ATCC 29148	*B. adolescentis* ATCC 15703	*B. catenalatum* ATCC 27539	*B. longum* ATCC 15707	*C. albicans* SC5314	*Clostridium sp.* ASF 500	*E. faecalis* OG1S	*E. plexicaudatum* ASF 492	*L. acidophilus* NCFM	*L. casei* ATCC 393	*L. johnsonii* NF-1	*L. murinus* ASF 361	*L. paracasei* ATCC 27092	*L. plantarum* ATCC 39542	*L. rhamnosus* ATCC 53103
***B. fragilis* ATCC25285**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{1}
B. thetaiotamicron* ATCC *29148		0	0	0	0	0	0	0	0	0	0	0	0	0	0	1^{1}
***B. adolescentis* ATCC 15703**			0	0	0	0	0	0	0	0	0	0	0	0	0	0
***B. catenalatum* ATCC 27539**				0	0	0	0	0	0	0	0	0	0	0	0	2^{-2}
***Bifidobacterium longum* ATCC 15707**					0	0	0	0	0	0	0	0	0	0	0	2^{-2}
***C. albicans* SC5314**						0	0	0	0	1^{1}	0	0	0	0	2^{1}	0
***Clostridium sp.* ASF 500**							0	0	0	3^{2+}	0	0	0	0	3^{3-}	$2+^{2+}$
***E. faecalis* OG1S**								0	0	0	0	0	0	0	0	2^{-2}
***E. plexicaudatum* ASF 492**									0	3^{2+}	0	0	0	0	3^{3}	2^{-2}
***L. acidophilus* NCFM**										0	0	0	0	0	0	1^{1}
***L. casei ATCC* 393**											0	0	0	0	0	0
***L. johnsonii* NF-1**												0	0	0	0	1^{1}
***L. murinus* ASF 361**													0	0	0	3^{3}
***L. paracasei* ATCC 27092**														0	0	0
***L. plantarum* ATCC 39542**															0	0
***L. rhamnosus* ATCC 53103**																0

Heat-treatment assays of *L. plantarum* ATCC 39542 mixed with either *C. albicans* SC5314, *Clostridium sp.* ASF 500, or *E. plexicaudatum* ASF 492 (Table 6) showed no coaggregation when either *L. plantarum* ATCC 39542 was heated alone or when both strains were treated. When heat-treated *C. albicans* SC5314, *Clostridium sp.* ASF 500, or *E. plexicaudatum* ASF 492 were mixed with untreated *L. plantarum* ATCC 39542, coaggregation scores remained within ±1 of the original score.

Table 4. Coaggregation scores of GI strains after heat treatment (indicated by*). (L) represents *L. rhamnosus* ATCC 53103 and (S) represents its partner strain. Intermediate scores are indicated by (+) and (-). Scores were determined after 3 trials of each assay.

Lactobacillus rhamnosus ATCC 53103 (L) and treatment	Coaggregation score with strain(S):									
	Bacteroides fragilis ATCC25285	*Bacteroides thetaiotamicron* ATCC 29148	*Bifidobacterium catenalatum* ATCC 27539	*Bifidobacterium longum* ATCC 15707	*Clostridium sp.* ASF 500	*Enterococcus faecalis* OG1S	*Eubacterium plexicaudatum* ASF 492	*Lactobacillus acidophilus* NCFM	*Lactobacillus johnsonii* NF-1	*Lactobacillus murinus* ASF 361
LS	1	1	2-	2-	2+	2-	2-	1	1	3
L*S	1	1+	2-	2-	2	2-	1	1	1	1
L*S*	2+	2+	2-	2	2+	2-	2	3	3	3
LS*	3+	3	2	3-	3	2	3-	3	3	4

Table 5. Coaggregation scores of GI strains after heat treatment (indicated by*). (L) represents *L. acidophilus* NCFM and (S) represents its partner strain. Scores were determined after 3 trials of each assay.

Lactobacillus acidophilus NCFM (L) and treatment	Coaggregation score with strain(S):			
	Candida albicans SC5314	*Clostridium sp.* ASF 500	*E. plexicaudatum* ASF 492	*L. rhamnosus* ATCC 53103
LS	1	3	3	1
L*S	0	0	0	3
L*S*	0	0	0	3
LS*	3	3	3	1

Table 6. Coaggregation scores of GI strain after heat treatment (indicated by*). (L) represents *L. plantarum* ATCC 39542 and (S) represents its partner strain. Scores were determined after 3 trials of each assay.

Lactobacillus plantarum ATCC 39542 (L) and treatment	Coaggregation score with strain(S):		
	Candida albicans SC5314	*Clostridium sp.* ASF 500	*Eubacterium plexicaudatum* ASF 492
LS	2	3	3
L*S	0	0	0
L*S*	0	0	0
LS*	3	4	3

DISCUSSION

Coaggregation is likely an integral part of biofilm formation in the GI tract and may facilitate increased metabolic function of microbial components, including greater production of the anti-cancer agent butyrate. The possible health benefits of a GI biofilm create a need for characterization of the coaggregation mechanisms that facilitate its formation. This study investigated the coaggregation mechanisms of multiple GI strains, focusing specifically on interactions between butyrate and lactic acid-producing bacteria.

This study uncovered 16 positive coaggregation interactions between 12 strains of GI bacteria and the single eukaryote, *C. albicans* SC5314. The most promiscuous GI coaggregators were all from the genus *Lactobacillus* and included *L. rhamnosus* ATCC 53103, *L. acidophilus* NCFM, and *L. plantarum* ATCC 39542. These findings are not surprising considering lactobacilli display surface proteins that may be involved in intestinal wall adherence (20, 30). Having the ability to adhere to both the intestinal wall and other microbes would make lactobacilli bacteria a key contributor to biofilm formation.

Coaggregation interactions of *Lactobacillus rhamnosus* ATCC 53103

L. rhamnosus ATCC 53103 had the most coaggregation partners out of any of the GI strains, and many of its pairings demonstrated an unexpected reaction to heating. *L. rhamnosus* ATCC 53103 had moderate, positive interactions with butyrate-producing bacteria— *Clostridium sp.* ASF 500 and *E. plexicaudatum* ASF 492—and weak or moderate interactions with *B. fragilis* ATCC 25285, *Bacteroides thetaiotamicron* ATCC 29148, *Bifidobacterium catenalatum* ATCC 27539, *Bifidobacterium longum* ATCC

15707, *Enterococcus faecalis* OG1S, *L. acidophilus* NCFM, *L. johnsonii* NF-1, and *Lactobacillus murinus* ASF 361. Lactose was added to coaggregation solutions to determine whether a ligand of an adhesin-ligand mediated interaction was lactose. A reversal or reduction in coaggregation score would indicate that the free lactose was acting by competitive inhibition against a ligand by blocking the binding site on the adhesin. Upon performing lactose-inhibition assays, it was found that lactose addition did not reverse or even reduce any *L. rhamnosus* ATCC 53103 coaggregation scores, indicating that, even if these coaggregation interactions are mediated by lectin-carbohydrate interactions, the carbohydrate receptors are not lactose.

Heat treatment assays are used to indicate if one or both coaggregation partners have a protein adhesin. Normally heat treatment will denature adhesin proteins, resulting in a reversal or reduction in coaggregation score. However, sometimes, as when N-acetylsuccinimide treated *A. naeslundii* W752 is mixed with untreated *S. sanguis* M5, gram-positive bacteria show a stronger coaggregation score when one partner is treated than when both partners are untreated (5). This phenomenon is more commonly seen in coaggregation involving gram-negative bacteria. Kolenbrander *et al.* (1989) found that all investigated intergeneric pairings of gram-negative bacteria showed enhanced coaggregation with heat treatment of one partner by the activation of a putative heat-sensitive site. In this study, when heat-treated *L. rhamnosus* ATCC 53103 was mixed with an untreated partner, the coaggregation score was close to the original score, with the exception *L. rhamnosus* ATCC 53103 with *Lactobacillus murinus* ASF 361, which dropped from an original score of 3 to a score 1. These results indicate that it is unlikely

that *L. rhamnosus* ATCC 53103 has the primary protein adhesin responsible for its coaggregation interactions. When both *L. rhamnosus* ATCC 53103 and its partner were treated, coaggregation scores were either the same as the original or slightly enhanced. The most dramatic increase in coaggregation score for all *L. rhamnosus* ATCC 53103 mixtures occurred when untreated *L. rhamnosus* ATCC 53103 was paired with a treated partner. In the mixing of untreated *L. rhamnosus* ATCC 53103 with the heat-treated, gram-negative *B. fragilis* ATCC 25285, the coaggregation score increased from an original value of 1 to a score of 3+, which is consistent with the observation that there is often an increase in coaggregation score after the heat-treatment of a gram-negative bacterium (12). Surprisingly, there are also substantial increases for the *Eubacterium* and *Lactobacillus* partners. These findings suggest that there may be a heat-activated site on strains mixed with *L. rhamnosus* ATCC 53103 that increases coaggregation interactions. These stronger coaggregation interactions, however, would likely never exist *in vivo* as a result of heat denaturation, because the body temperature of a living human is approximately 37°C. It is possible that these sites may be exposed under different conditions, such as conformation changes brought on by the binding of other substrates or the presence of a different reduction potential in the environment.

Coaggregation interactions of *Lactobacillus acidophilus* NCFM

Lactobacillus acidophilus NCFM had weak coaggregation scores with *C. albicans* SC5314 and *L. rhamnosus* ATCC 53103, but showed moderate coaggregation interactions with the butyrate-producing bacteria *E. plexicaudatum* ASF 492 and *Clostridium sp.* ASF 500. Of the four mixtures, those with *Clostridium sp.* ASF 500 and

E. plexicaudatum ASF 492 showed only a slight decrease in score after the addition of lactose from 3 to 2+. Lactose does not appear to be the ligand moiety in *L. acidophilus* NCFM coaggregation interactions.

Heat treatment assays between *L. acidophilus* NCFM and *E. plexicaudatum* ASF 492, *Clostridium sp.* ASF 500, and *C. albicans* SC5314 all had coaggregation scores of zero when 1) only *L. acidophilus* NCFM was treated and 2) when both partners were treated. This indicates that L. *acidophilus* NCFM contains the major protein adhesin responsible for its coaggregation interactions. Interestingly, when untreated *L. acidophilus* NCFM was mixed with treated *C. albicans* SC5314, the coaggregation score increased from 1 to 3, suggesting that *C. albicans* SC5314 may have a heat-activated site that increases coaggregation interactions.

As mentioned previously, the interaction between *L. acidophilus* NCFM and *L. rhamnosus* ATCC 53103 does not appear to have the same coaggregation mechanism as the rest of *L. acidophilus* NCFM's mixtures. In this case, it is *L. acidophilus* NCFM that likely has the heat-activated site that enhances coaggregation.

Coaggregation interactions of *L. plantarum* ATCC 39542

L. plantarum ATCC 39542 had coaggregation interactions with *C. albicans* SC5314, and the butyrate-producing *Clostridium sp.* ASF 500 and *E. plexicaudatum* ASF 492. The mixtures of *L. plantarum* ATCC 39542 with *C. albicans* SC5314 and *Clostridium sp.* ASF 500 showed slight reductions in coaggregation scores with the addition of lactose from 2 to 1- and from 3 to 3-, respectively. It is likely that lactose is not the ligand responsible for coaggregation between these strains. In all cases, reversal

of coaggregation when *L. plantarum* ATCC 39542 was heated reveals that *L. plantarum* ATCC 39542 contains the primary protein adhesin responsible for its positive coaggregation interactions.

Summary

To the author's knowledge, many of the coaggregation mixtures made in this study have not been previously tested. Therefore, the most significant findings of this study are the 16 positive coaggregation interactions displayed in Table 3. These positive interactions qualify the 13 microbial strains that were involved— especially *L. rhamnosus* ATCC 53103, *L. acidophilus* NCFM, and *L. plantarum* ATCC 39542 who all had 3 or more coaggregation partners—as candidates for the use in future research on GI biofilm formation, and particularly in the development of *in vitro* biofilms.

This study highlights several important qualities of the observed coaggregation interactions, but their full characterization remains incomplete. It has been found that coaggregation among oral strains and between oral and GI strains is most often mediated by protein-carbohydrate interactions (16). These findings influenced the design of the present experiment. Inhibition tests were performed under the assumption that for a single interaction one partner would have a protein adhesin and the other would have a carbohydrate ligand, with the possibility that the coaggregation interaction may be a result of two or more specific adhesin-ligand interactions. Although most oral strain coaggregation interactions are inhibited by the addition of lactose (12, 13, 17, 28, 29), lactose did not inhibit any of the coaggregation interactions here—indicating that ligand components of the tested GI strains do not have a structure similar to lactose.

The heat inhibition assays showed that *L. acidophilus* NCFM contains the protein adhesins in all of its interactions, excluding its interaction with *L. rhamnosus* ATCC 53103. *L. plantarum* ATCC 39542 contains the protein components for all of its interactions. *L. rhamnosus* ATCC 53103, on the other hand, does not appear to have any protein adhesins, except perhaps in its binding to *L. murinus* ASF 361. This leaves open the possibility that the majority of *L. rhamnosus* ATCC 53103's partner strains contain lectin-like proteins. The activation of heat-sensitive sites on partner strains, however, makes this impossible to confirm using this method. In addition, it is possible that the interactions involving *L. rhamnosus* ATCC 53103 do not have a protein component at all and work by some other mechanism. Therefore, although *L. rhamnosus* ATCC 53103 displayed the most positive coaggregation interactions, these interactions remain uncharacterized.

The multiple findings of this study were compiled into a diagrammatical depiction (Figure 1) of the tested GI strains' specific coaggregation interactions. It has been found in previous studies that *B. longum* B6 and *L. rhamnosus* GG have a high hydrophobicity and display strong *in vitro* adhesion to Caco-2 cells, both of which indicate likely *in vivo* adhesion to the GI wall (32). For this reason, Figure 1 depicts *L. rhamnosus* ATCC 53103 and *B. longum* ATCC 15707 as the possible biofilm components that adhere to the GI wall through an unknown mechanism.

Future directions of this research are extensive. It has been found that manipulating the solvent of bacterial suspensions can affect coaggregation scores (19). In order to create the most accurate model of GI coaggregation, a buffer must be used that

best mimics the environment of the GI tract. In addition, all ligands and some adhesins in this study remain uncharacterized. Testing a more extensive selection of carbohydrates and using protease treatments to further identify partners with protein adhesins would more completely characterize these GI microbe interactions. The 16 strains studied here represent a very small fraction of the diverse GI environment. Increasing the number of tested microbial strains would give a more complete understanding of GI biofilm formation.

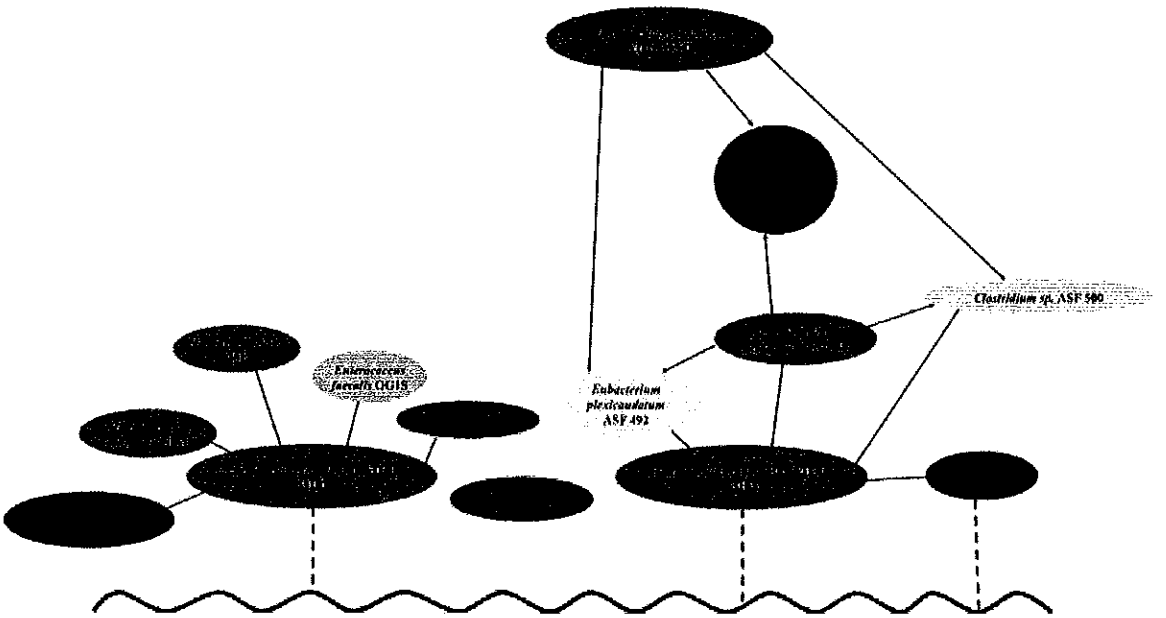

Figure 1. Diagrammatic representation of coaggregation interactions among GI strains. The dashed lines represent uncharacterized adhesion components that connect strains to the GI wall. The solid lines represent uncharacterized coaggregation components that are probably lectin-like proteins belonging to the respective partners of *L. rhamnosus* ATCC 53103. The arrows represent heat-inactivated components that are probably lectin-like proteins belonging to the attached *L. plantarum* ATCC 39542 or *L. acidophilus* NCFM. Organisms of the same genus are represented with the same color.

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