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Inhibition of Pathogenic Bacterial Adhesion by Acidic Polysaccharide from Green Tea (*Camellia sinensis*)

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An acidic polysaccharide CS-F2 from *Camellia sinensis* was examined to characterize its anti-adhesive effects against pathogenic bacteria, most notably *Helicobacter pylori*, *Propionibacterium acnes*, and *Staphylococcus aureus*. CS-F2 showed marked inhibitory activity against the pathogen-mediated hemagglutination with a minimum inhibitory concentration (MIC) between 0.01 and 0.1 mg/mL, which is lower than the previously reported MIC values for *Panax ginseng* and *Artemisia capillaris*. The inhibitory effects of CS-F2 on the adhesion of *H. pylori* to AGS adenocarcinoma gastric epithelial cells, or *P. acnes* and *S. aureus* to NIH 3T3 fibroblast cells, were further assessed resulting in MIC values between 0.063 and 0.13 mg/mL. Importantly, CS-F2 showed no inhibitory effects against *Lactobacillus acidophilus*, *Escherichia coli*, or *Staphylococcus epidermidis*. Our results suggest that CS-F2, which is a pectin-type polysaccharide with a molecular weight of approximately 8.0×10^4 Da, may exert a selective anti-adhesive effect against certain pathogenic bacteria, while exerting no effects against beneficial and commensal bacteria.

KEYWORDS: Anti-adhesive; green tea acidic polysaccharide; *Helicobacter pylori*; *Propionibacterium acnes*; *Staphylococcus aureus*

INTRODUCTION

Helicobacter pylori is a microaerophilic Gram-negative bacterium that exclusively colonizes humans and primates. This bacterium resides persistently in the human gastric mucosa of over half of the world's population and has been identified as a principal etiological agent of chronic active or type B gastritis, duodenal ulcer, gastric carcinoma, and mucosa-associated lymphoid tumors (*1*). *Propionibacterium acnes* is an anaerobic Gram-positive bacterium and a primary inhabitant of adult human skin (*2*). This bacterium has been implicated primarily in acne, the most frequently encountered disease of the skin (*3*). *Staphylococcus aureus* is an aerobic Gram-positive bacterium, which is harbored principally on mucosal surfaces. This bacterium has been recognized as largely responsible for the known complication of atopic dermatitis (*4*) and also as the primary causative agent of life-threatening hospital-acquired infections (*5*). All of these bacteria require iron to facilitate basic cellular processes, including respiration and DNA synthesis. The hemagglutinate erythrocytes bind to receptors on different tissue culture cells and efficiently employ heme and hemoglobin for their growth (*6*).

The attachment of bacteria to human epithelial cells is a key step in the initiation of infection, which leads to the development of pathogenic diseases (*7*). It has now been well-established that cell surface carbohydrates mediate cell−cell recognition, via single or multiple interactions. The specificities of bacteria−host interactions have been the subject of a host of recent studies, from which a number of putative host receptors for *H. pylori*, *P. acnes*, *S. aureus*, and *Porphyromonas gingivalis* have been discovered (*8*−*13*). Nevertheless, the ability of these bacteria to bind to host cell carbohydrates has yet to be thoroughly elucidated.

Green tea made from *Camellia sinensis* is a popular beverage throughout Asia and has been recognized as an herbal remedy. It provides a dietary source of biologically active compounds considered to be beneficial to human health (*14*). Green tea extract contains polyphenols and caffeine (*15*). Green tea polysaccharides have been reported to possess immunological, anti-radiation, anti-blood coagulation, anti-cancer, anti-HIV, anti-oxidant, and hypoglycemic qualities (*16*, *17*). In previous studies, acidic polysaccharides isolated from *Panax ginseng* C.A. Meyer (Araliaceae) and *Artemisia capillaris* (Asteracae) were determined to exert marked inhibitory effects against the adherence of *H. pylori* to human gastric epithelial cells and erythrocytes

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(*18*−*20*). The objective of the present study was to evaluate the anti-adhesive role of an acidic polysaccharide from *C. sinensis*, which was tested against *H. pylori*, *P. gingivalis*, *Actinobacillus actinomycetemcomitans*, *P. acnes*, and *S. aureus* as well as *Lactobacillus acidophilus*, *Escherichia coli*, and *Staphylococcus epidermidis* via hemagglutination, urea phenol red, and colony counting assays. Our results were then compared with those obtained using other polysaccharides.

MATERIALS AND METHODS

Materials. The dried green tea leaves used in this study were purchased from a local market in Seoul in May 2005. The voucher specimen (CS-F2) was deposited at the Laboratory of Structural Bioinformatics, Department of Biotechnology, at Korea University in Chungnam. *H. pylori* (ATCC 43504), *P. gingivalis* (ATCC 33277), *A. actinomycetemcomitans* (ATCC 29522), *P. acnes* (ATCC 6919), and *L. acidophilus* (ATCC 4356) cells were acquired from the Korean Culture Center of Microorganisms (KCCM) (Seoul, Korea). *E. coli* strains BL21(DE3) and BL21(DE3)pLysS were purchased from Novagen (Madison, WI). *S. aureus*, *S. epidermidis*, and human erythrocytes were obtained from the Korea University Hospital (Ansan, Korea). Trypsin (EC 3.4.21.4), low molecular weight heparin (LMWH), citrus pectin, ribonuclease A from bovine pancreas (RNase), deoxyribonuclease I from bovine pancreas (DNase), bovine serum albumin (BSA), 2,6-di-*o*-methyl-β-cyclodextrin (CD), and dimethyl sulfoxide (DMSO) were from Sigma (St. Louis, MO). Sucrose octasulfate (SOS) was acquired from Toronto Research Chemicals (North York, Canada). Carbazole, phenol, and H_2SO_4 were purchased from Junsei Chemicals (Tokyo, Japan). GC internal standards, *myo*-inositol, and mannoic acids lactone, arabinose, fucose, galactose, glucose, rhamnose, mannose, and xylose were also purchased from Sigma (St. Louis, MO).

Bacterial Cell Growth. The *H. pylori* cells were grown in Brucella broth (Difco) containing 10% (v/v) fetal bovine serum (FBS), 0.2% (w/v) 2,6-di-*o*-methyl-β-cyclodextrin (CD), and antibiotics (cefsulodin, vancomycin, trimethoprim, and amphotericin B). The cells were incubated for 43 h in a 10% CO_2 atmosphere at 37 °C. The *H. pylori* cells were quickly thawed and grown in Brucella broth containing 10% (v/v) FBS, CD, and antibiotics at 37 °C for 43 h in a shaker in a microaerobic environment, which was achieved using BBL Campypak-Plus envelopes (Cockeyville, MD). The cultured bacteria were then identified via urease and catalase reactions, and these identifications were verified via polymerase chain reaction based on *VagA*, *Cag1*, *IceA1*, *IceA2*, *BabA*, and *UreA* primers (data not shown) (*20*). *P. acnes* was grown for 2 days in trypticase soy broth (Difco) at 37 °C under an atmosphere of 80% N_2, 10% H_2, and 10% CO_2. *S. aureus* and *S. epidermidis* were grown aerobically in trypticase soy broth at 37 °C. *E. coli* was cultured aerobically on Luria−Bertani (LB) agar plates. The colonies were inoculated and grown for 6 h in LB broth without antibiotics at 37 °C. *P. gingivalis* and *A. actinomycetemcomitans* were grown for 2 days in trypticase soy broth (Difco) supplemented with 0.6% yeast extract, hemin (10 mL/L), and vitamin K3 (0.2 mL/L) (for *P. gingivalis*) at 37 °C in an atmosphere of 80% N_2, 10% H_2, and 10% CO_2. All bacterial cell stocks were maintained with 20% (v/v) glycerol in a liquid nitrogen tank until required. *L. acidophilus* was used directly from the stocks obtained from KCCM, without further growth.

Extraction and Isolation of Polysaccharide CS-F2. The dried green tea leaves (20 g) were homogenized and extracted with hot distilled water (300 mL) for 3 h. After centrifugation for the removal of any insoluble materials, the supernatant was precipitated using absolute ethanol (final 70% concentration) at 4 °C. The precipitate was then dissolved in PBS (100 mM sodium phosphate, pH 7.3, and 150 mM NaCl) containing 10 mM $MgCl_2$ and 1 mM $CaCl_2$ and then incubated for 3 h with RNase and DNase at 37 °C. The enzyme reactions were stopped by 5 min of heating at 100 °C. After centrifugation, cold ethanol was added to the supernatant (final 70% concentration). The precipitate was then dialyzed against 20 mM Tris-HCl (pH 8.0), which was applied to a Q-Sepharose fast flow column (2.5 cm × 5 cm), which had been equilibrated with the same buffer. After extensive washing, the polysaccharide fractions were eluted with a linear gradient of increasing NaCl concentrations (0−0.4 M). The fractions were detected at 226 nm and analyzed via phenol−sulfuric acid and carbazole assays at 490 and 525 nm, respectively. The polysaccharide fractions eluting at 0.30−0.32 M NaCl exhibiting anti-adhesive activity were pooled, dialyzed against distilled water, and lyophilized. This solution was then dissolved in a minimum volume of distilled water and applied to a Sephacryl S200 gel-filtration column (1.5 cm × 70 cm), which generated a major peak of polysaccharide CS-F2.

General Methods. Total carbohydrates, uronic acid, and protein contents were determined via the phenol−sulfuric acid, carbazole (*21*), and Bradford methods (*22*), respectively, using glucose, galacturonic acid, and bovine serum albumin as the respective standards. For gas chromatography (GC), the carbohydrate compositions of the polysaccharides were analyzed as alditol acetates (*23*, *24*). The sample (2 mg) or a carbohydrate standard (0.5 mg) was hydrolyzed for 2 h with 3 M trifluoroacetic acid (1 mL) at 120 °C in a screw-capped vial. The hydrolyzate was then evaporated to dryness at reduced pressure with repeated additions of methanol and reduced with 10 mg of $NaBH_4$ in 1 M NH_4OH solution. The reduced monosaccharides, alditol or aldonic acid, which were separated using Q sepharose fast flow ion-exchange resin, were acetylated with acetic anhydride and pyridine (1:1), resulting in the formation of alditol acetate derivatives. The resultant alditol acetates produced from the aldoses and from uronic acids were then analyzed separately. After the reagents had been completely removed via vacuum evaporation, the dried residuals were extracted with ethyl acetate/water (1:1) and vortexed, and the ethyl acetate fractions were evaporated. This extraction procedure was repeated three times. After the final ethyl acetate had been evaporated to a minimum volume (20 μL) and centrifuged, GC analyses were conducted on a HP5890 series II instrument, which was equipped with a flame ionization detector and a DB-225 column (0.25 mm i.d. × 30 m). Chromatography was then conducted isothermally at 220 °C. The peaks were identified via comparison with the monosaccharide standard peak, and the weights of each of the monosacchrides in the sample were quantified with internal standards, using *myo*-inositol and mannonic acid lactone as references from the integrated peak areas. The mol % was calculated for each of the samples (*23*).

Hemagglutination Assays. Human erythrocytes suspended in 10 mL of PBS were incubated with trypsin (2 mg) at 37 °C for 3 h and washed three times in PBS. The trypsinized erythrocytes were suspended at 2% (w/v) in the same buffer and used for the hemagglutination assays (*25*), the results of which were evaluated via microscopic inspection. Erythrocytes in the bacterial suspension in the absence of anti-adhesive agents were used as positive controls, and erythrocytes not in the bacterial suspension were employed as negative controls.

The CS-F2 was then evaluated (final concentrations 0.01−2.0 mg/mL) with regard to its hemagglutination inhibitory activities. The inhibitory effects of pectin, LMWH, and SOS were determined at final concentrations of 0.01−1.0 mg/mL. Aspartic and glutamic acids were tested as nonspecific reactions and exhibited no inhibitory activities. Equal volumes of 2-fold dilutions of the bacterial suspensions and the purified inhibitor or carbohydrates were then mixed and incubated at room temperature for 30 min. Each of the mixtures was then added to O-type blood samples in U-shaped 96 microwell plates. The hemagglutination inhibition assay was visualized via microscopic inspection.

Anti-adhesion Assay via Urea Phenol Red Method. AGS cells (ATCC CRL 1739, a human gastric adenocarcinoma epithelial cell line) were grown to confluence in tissue culture flasks (75 cm^2) in RPMI 1640 medium supplemented with 10% (v/v) FBS and 1% (v/v) antibiotics−antimycotic in a 5% CO_2 incubator at 37 °C for 3 days. For replating, the monolayers were detached for 5 min using trypsin−EDTA, then washed twice with RPMI medium supplemented with 10% (v/ v) FBS and 1% (v/v) antibiotics−antimycotic.

For the adhesion assay, the cells were seeded in flat-bottomed 96-microwell plates (Corning, NY) in 100 μL of complete culture medium at a concentration of approximately 5×10^4 cells/mL. After achieving confluence in 1.5−2 days, the cultures were evaluated for anti-adhesive activity via urea phenol red assays (*26*). The confluent monolayers on the 96-well microtiter plates were washed three times with PBS. Nonspecific binding was blocked by 1 h of incubation at 37 °C with 0.5% BSA, prior to two rinses in PBS. Equal volumes of 2-fold dilutions

of *H. pylori* suspensions (OD_{600} = 1.0; 1 × 10^8 bacterial cells/mL) and purified inhibitor at various concentrations were incubated at room temperature for 30 min. The bacteria−carbohydrate mixture (100 μL) was added to the AGS cells in each well of the 96-well microtiter plates and incubated at 37 °C for 1 h. Control experiments were conducted in the absence of carbohydrate inhibitors. The 96-well microtiter plates were washed twice with PBS and then finally with 0.03% phenol red solution in PBS (pH 5.2). After the removal of the washing buffer, urea phenol red solution (0.03% phenol red and 2% urea in PBS, pH 5.2) was added to each well, and absorbance was measured at 560 nm with a microplate reader after 15 min of incubation. The percentage of attached *H. pylori* cells was calculated as follows: attached % = 100 − [($OD_{experimental}$ − $OD_{negative}$)/($OD_{positive}$ − $OD_{negative}$) × 100]. The negative controls contained only epithelial monolayers with no bacteria. The positive controls contained bacteria and monolayers with no added inhibitor and were used to establish 100% attachment. Each experiment was conducted in triplicate on different days.

Anti-adhesion Assay via Colony Counting Method. NIH 3T3 cells (ATCC CRL-1658, a mouse fibroblast epithelial cell line) were grown to confluence in tissue culture flasks (75 cm^2) in DMEM medium supplemented with 10% (v/v) FBS and 1% (v/v) antibiotics−antimycotic in a 5% CO_2 incubator at 37 °C for 3 days. For replating, the monolayers were detached for 5 min using trypsin−EDTA, then washed twice with DMEM medium supplemented with 10% (v/v) FBS and 1% (v/ v) antibiotics−antimycotic.

NIH 3T3 cells were seeded at 1 × 10^4 cells/mL of DMEM containing 10% fetal bovine serum and antibiotics in 96-well plates. After achieving confluence in 1.5−2 days, the confluent monolayers on the 96-well microtiter plates were washed three times with PBS and replaced with fresh medium devoid of antibiotics. Various concentrations of carbohydrates were dissolved in the antibiotics-free culture medium and incubated with 1 × 10^7 cfu/mL of bacteria (*P. acnes* or *S. aureus*) at 30 min. A 100 μL aliquot of each mixture containing the bacteria in the presence or the absence of carbohydrates was added to the NIH 3T3 cells, incubated at 37 °C for 30−60 min, and washed three times with PBS to remove the unbound bacteria from the cell surface. After treatment of the cells with PBS containing 0.1% Triton X-100, the recovered cell extracts were diluted with PBS and plated on trypticase soy agar (TSA) or Brucella agar. The activity to inhibit bacteria from binding to NIH 3T3 cells was estimated as follows: attached (%) = (cfu of bound bacteria in the presence of polysaccharide/cfu of bound bacteria in the absence of polysaccharide) × 100 (*27*).

RESULTS

Purification of Acidic Polysaccharide CS-F2 from Green Tea. The purification of the acidic polysaccharide CS-F2 from *C. sinensis* followed the previously established procedure (*20*), with some minor modifications, including the addition of DNase and RNase treatment and a second ethanol precipitation. The precipitate was then dialyzed against 20 mM Tris-HCl and purified via using Q Sepharose anion exchange chromatography. The bound fractions with high uronic acid content were eluted at a 0.3 M NaCl concentration, which suggests that CS-F2 may be primarily comprised of acidic polysaccharides (**Figure 1**). These fractions were determined to exhibit profound anti-adhesive activity against *H. pylori* and were further purified into a single peak via Sephacryl S200 gel-filtration chromatography (**Figure 2**). No proteins were detectable in this CS-F2 peak, which was found to exhibit the most potent anti-adhesive activity against *H. pylori* among the fractions using the hemagglutination assay.

The acidic polysaccharide CS-F2 was estimated to be approximately 80 kDa using dextran molecular weight standards (25−150 kDa) (data not shown), suggesting that it may comprise approximately 400−450 sugar units, calculated from the average molecular weight of 180 Da per sugar unit. GC analysis revealed that CS-F2 is composed mainly of galacturonic acid (49%) and glucuronic acid (26%), as well as small quantities of rhamnose, arabinose, fucose, galactose, mannose, and xylose (**Table 1**). Thus, CS-F2 may be an arabinogalactan-type polysaccharide, which is mainly composed of galacturonic and glucuronic acids, much like the pectin-type polysaccharide.

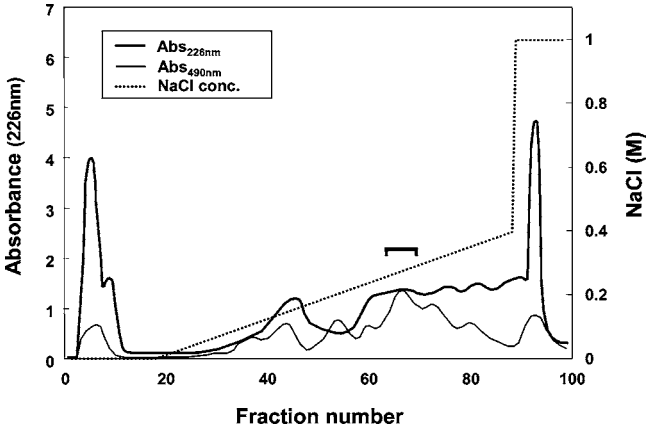

Figure 1. Elution profile of polysaccharide fractions from *C. sinensis* on ion-exchange chromatography. Q Sepharose fast flow column (2.5 cm × 5 cm) was used at a flow rate of 1 mL/min. The thick solid line represents the absorbance at 226 nm, the thin solid line represents the absorbance at 490 nm from the phenol–sulfuric acid assay, and the dotted line represents the NaCl concentration gradient. The pooled fractions were indicated by the bar above the peak.

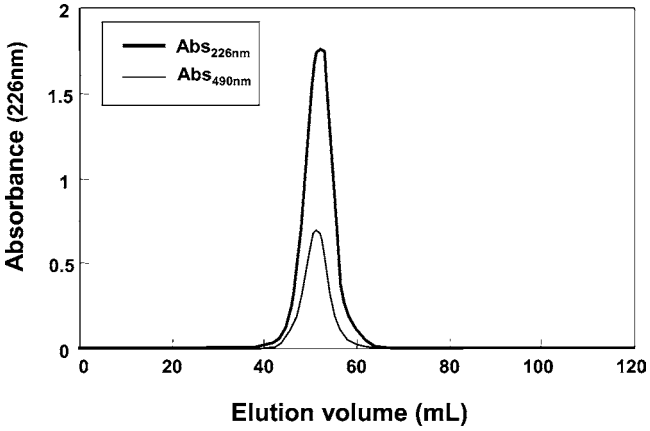

Figure 2. Elution profile of CS-F2 on gel-filtration chromatography. A Sepharcyl S200 column (1.5 cm × 70 cm) was used at a flow rate of 0.5 mL/min. The thin line represents the absorbance at 490 nm from the phenol–sulfuric acid assay.

Table 1. Characteristics of Polysaccharide Purified from *C. sinensis*

sugar composition (mol %)[a]	CS-F2
rhamnose	6.28
fucose	5.52
arabinose	6.10
xylose	0.28
mannose	2.82
galactose	3.44
galacturonic acid	49.25
glucuronic acid	26.32

[a] Mol % of total carbohydrate content.

CS-F2 Inhibits *H. pylori* Adhesion to AGS Gastric Cell. Previously, the adherence of *H. pylori* to AGS gastric cells was evaluated via scanning electron microscopy (SEM) by counting *H. pylori* microcolonies attached to the surfaces of gastric cells (*20*), and the acidic polysaccharide from *Panax ginseng* inhibited

Table 2. Inhibition of Bacteria-Induced Hemagglutination by Acidic Polysaccharide from *C. sinensis* and by a Range of Carbohydrates

		minimum inhibitory concentration (mg/mL)[a]							
inhibitors	MW (Da)	*H. p*[b]	*P. g*	*A. a*	*P. a*	*S. a*	*S. e*	*E. c*	*L. a*
CS-F2[c]	80000	0.01	–	–	0.05	0.1	–	–	–
PG-F2[c]	12000	0.1	0.1	0.25	0.5	0.5	–	–	–
pectin[d]	20000	–	0.0001	–	0.01	0.01	–	–	–
LMWH[d]	3000	–	0.02	–	0.1	1.0	–	–	0.1
SOS[d]	1159	–	0.01	–	1.0	1.0	–	–	–
sialyllactose[e]	655.5	0.7							

[a] All the values are MIC in mg/mL, corresponding to the average in triplicates. Dash represents no inhibition at high concentrations above 2.0 mg/mL. Aspartic and glutamic acids were used as non-carbohydrate acidic compounds, and bacterial binding did not cause nonspecific reactions (data not shown). [b] *H. p*: *Helicobacter pylori*, *P. g*: *Porphyromonas gingivalis*, *A. a*: *Actinobacillus actinomycetemcomitans*, *P. a*: *Propionicbacterium acnes*, *S. a*: *Staphylococcus aureus, S. e*: *Staphylococcus epidermidis*, *E. c*: *Escherichia coli*, and *L. a*: *Lactobacillus acidophilus.* [c] CS-F2 and PG-F2 (*29*) are polysaccharides from *C. sinensis* and *P. ginseng*, respectively. [d] Pectin showed the highest inhibition against *P. gingivalis* with an MIC of ca. 0.0001 mg/mL but did not show inhibitory activity above 0.1 mg/mL (*28*). LMWH and SOS represent low molecular weight heparin and sucrose octasulfate, respectively. [e] Sialyllactose showed an inhibitory effect of about 41% in 0.7 mg/mL by scanning electron microscopy analysis (*20*).

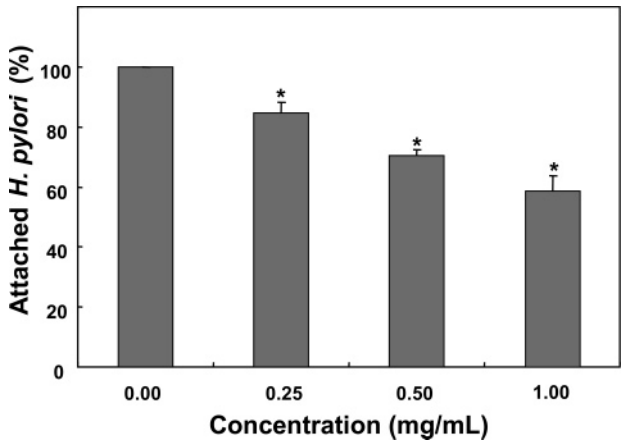

Figure 3. Anti-adhesive activity of CS-F2 on the adhesion of *H. pylori* to AGS cells. Anti-adhesive activity was determined via the urea phenol red method. The positive control (0 mg/mL) without inhibitor was used to establish 100% attachment. The results are the means ± SE for three independent experiments. Asterisk indicates significant difference from control ($p < 0.05$ by paired *t*-test).

the adhesion of *H. pylori* on AGS gastric cells. In this study, CS-F2 was determined to inhibit hemagglutination fairly effectively, showing high anti-adhesive activity against *H. pylori* at a concentration of 0.01 mg/mL (**Table 2**). When CS-F2 was evaluated with regard to its ability to inhibit the adhesion of *H. pylori* to human AGS gastric epithelial cells via a urea phenol red assay, a significant inhibition of *H. pylori* attachment was observed with a reduction of ca. 20−40% in a range of concentrations between 0.25 and 1.0 mg/mL (**Figure 3**).

Anti-adhesive Activity of CS-F2 against Skin and Oral Bacteria. CS-F2 was further assessed with regard to its anti-adhesive effects against *P. gingivalis*, *A. actinomycetemcomitans*, *P. acnes*, and *S. aureus* via hemagglutination assays. CS-F2 was determined to exhibit marked anti-adhesive activity against *P. acnes* with a minimum inhibitory concentration (MIC) of 0.05 mg/mL and against *S. aureus* with an MIC of 0.1 mg/mL (**Table 2** and **Figure 4**). However, it displayed only minimal inhibitory activity against the oral pathogens *P. gingivalis* and *A. actinomycetemcomitans*. Importantly, when CS-F2 was evaluated for its effects against the beneficial or commensal bacteria, *L. acidophilus*, *E. coli*, and *S. epidermidis*, it exerted no detectable effects against them, even at concentrations of above 2.0 mg/mL (**Table 2**).

It was previously reported that the acidic polysaccharide from *P. ginseng* inhibited the attachment of *A. actinomycetemcomitans*, *P. acnes*, and *S. aureus*, as well as *H. pylori* and *P. gingivalis* in a concentration range of 0.1−0.5 mg/mL (**Table 2**) (*28*, *29*). Notably, pectin exhibited the most potent anti-adhesive effects against *P. gingivalis* and significant effects against *P. acnes* and *S. aureus*, albeit only within a limited range of concentration. However, the effects disappeared at concentrations above 0.1 mg/mL. LMWH and SOS were found to be highly active only against *P. gingivalis*. Interestingly, sialyl-3′-lactose, an oligosaccharide that occurs naturally in both human and bovine milk, and one of the most active oligosaccharides, showed inhibitory effects at a concentration of 0.7 mg/mL (ca. 1.1 mM). The inhibitory activity of sialyl-3′-lactose against *H. pylori*-mediated hemagglutination is much lower than that of CS-F2 and the acidic polysaccharide from *P. ginseng* (**Table 2**).

Inhibition of Skin Bacterial Adhesion to NIH 3T3 Fibroblast Cells. CS-F2 was also evaluated with regard to its ability to inhibit the adhesion of *P. acnes* and *S. aureus* to NIH 3T3 cells via colony counting assays. A significant inhibition of the bacterial attachment was observed with a reduction of ca. 30% in a range of concentrations between 0.06 and 1.0 mg/mL (**Figure 5**), reaching more than 50% at 2.0 mg /mL. In light of the results that were obtained via hemagglutination assays, CS-F2 also demonstrated profound inhibition against the adhesion of *P. acnes* and *S. aureus* to the NIH 3T3 cell.

DISCUSSION

The human body hosts a variety of microbial flora, composed primarily of bacteria. This microbial flora comprises a total number of cells greater than those constituting the rest of the human body. The acquisition of normal microflora in the respiratory and gastrointestinal tracts, as well as on the skin, provides crucial stimuli for the development of innate immunity. These microflora also generate metabolites that function as essential nutrients for human cells and exert protective effects against more pathogenic bacteria. An increasing amount of evidence suggests that the interaction between human and commensal flora may play an important role in human health (*30*).

The attachment of pathogenic bacteria to human cells is a pivotal step in the initiation of infections, which lead to the development of a variety of diseases (*7*, *31*). *H. pylori* shows a broad spectrum of different specificities in adhesion to the gastric epithelial cells, indicating a multifactorial adherence. A number of cell surface carbohydrates that appear to mediate cell adhesion have been previously discovered (*8*, *9*). In particular, the Lewis b blood group antigen has been demonstrated to mediate the adherence of *H. pylori* to human gastric mucosa (*32*), and this antigen is typically expressed on the surfaces of human gastric epithelial cells including AGS adenocarcinoma cells. The acidic polysaccharides obtained from *P. ginseng* roots and *A. capillaris* leaves were previously determined to have remarkable inhibitory properties against the adherence of *H. pylori* to human erythrocytes, with MICs of 0.1 and 0.6 mg/mL, respectively (*19*, *20*). *P. ginseng* polysaccharide also demonstrated a comparable inhibitory effect against *P. gingivalis* (*28*). In this study, CS-F2 was shown to inhibit the *H. pylori*-mediated hemagglutination with an MIC of 0.01 mg/mL (0.12

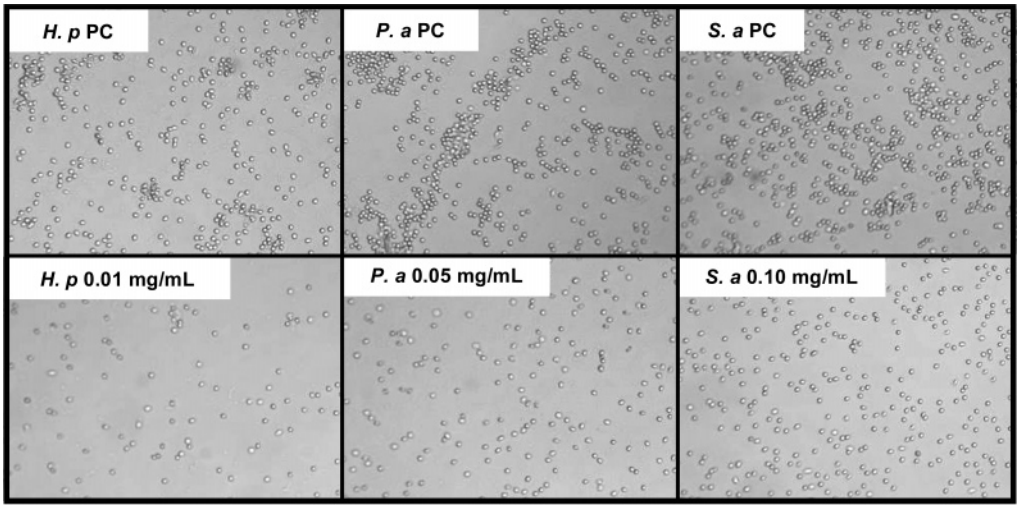

Figure 4. Micrograph images of hemagglutination inhibition assays with *H. pylori*, *P. acnes*, and *S. aureus* (magnification ×100). Hemagglutination was inhibited by the active polysaccharide CS-F2 from *C. sinensis*, CS-F2, against *H. pylori*, *P. acnes*, and *S. aureus*, and the minimum inhibitory concentrations were 0.01, 0.05, and 0.1 mg/mL, respectively. A positive control without inhibitor (PC) is shown along with each pathogenic bacterium.

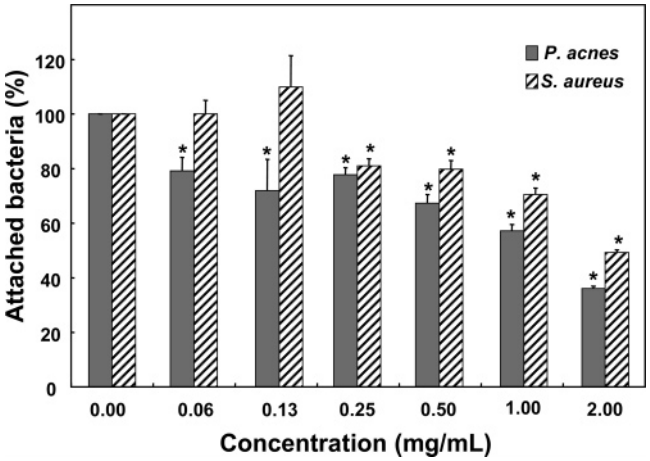

Figure 5. Anti-adhesive activity of CS-F2 on the adhesion of *P. acnes* and *S. aureus* to NIH 3T3 cells. Anti-adhesive activity was determined via colony counting method. The results were expressed as the relative percentage of attached bacteria, as compared with the positive control as 100%. The results are the means ± SE for three independent experiments. Asterisk indicates significant difference from control ($p < 0.05$ by paired *t*-test).

μM), a figure 10-fold higher than that determined with *P. ginseng*. When the CS-F2-mediated inhibition of the adhesion of *H. pylori* to AGS gastric cells was determined via a urea phenol red assay, *H. pylori* attachment was shown to be inhibited by ca. 30% at a concentration of 0.5 mg/mL (**Figure 3**). In the case of sialyl-3′-lactose, at least 0.7 mg/mL (ca. 1.1 mM) was required for anti-adhesive activity, a concentration far higher than that of CS-F2 (**Table 2**). Thus, CS-F2 exerts the most potent anti-adhesive effects against *H. pylori* among any of the carbohydrates evaluated in this study. In the case-control study, the infection rate of *H. pylori* was lower in the group that consumed a larger quantity of green tea (*33*).

CS-F2 was additionally assessed with regard to its anti-adhesive effects against *P. acnes* and *S. aureus* and demonstrated much more profound activities (0.05−0.1 mg/mL in MICs) than were seen with *P. ginseng* (ca. 0.5 mg/mL). Furthermore, the anti-adhesive activity of CS-F2 was evalutated via colony counting method using NIH 3T3 cells. *P. acnes* or *S aureus* attachment was shown to be inhibited by ca. 30% at a concentration of 0.5 mg/mL (**Figure 5**), which was much higher than that determined with *P. ginseng* (unpublished data). At 2.0 mg/mL, CS-F2 inhibited more than 60% of *P. acnes* attachment to the cells. *P. acnes* and *S. aureus* are involved in the pathogenesis of inflammatory acne and atopic dermatitis, respectively. Strong evidence for the association between *P. acnes* and inflammatory acne was found in a study in which the failure of erythromycin treatment in acne patients was associated with the development of erythromycin resistance in *P. acnes* (*34*). The evolution of methicillin-resistant *S. aureus* (MRSA) is an emerging problem, and this strain of *S. aureus* has become a significant community pathogen. The formation of biofilm from the fibrin fibers and glycocalyx is an important first step in the adhesion of *S. aureus* to the skin, as well as in the development of resistance to antimicrobial agents. The ever increasing number of antibiotic-resistant bacterial strains, as well as the reduced efficacy of antibiotic treatments, requires an alternative approach to antibiotics therapy.

Acidic polysaccharides have recently been reported to show a variety of biological activities, including immunostimulatory, antioxidant, antitumor, and antiviral activities (*35*). It is interesting to note that the acidic polysaccharides in this study have in common a high uronic acid content. Like heparan sulfate, the negatively charged groups may perform a crucial function in host−bacterial adhesion. High levels of uronic acids have been detected in both the CS-F2 from *C. sinensis* and the acidic polysaccharide from *P. ginseng* (76 and 94% in total carbohydrates, respectively) (*29*), and the digestion of these polysaccharides with pectinase resulted in an abrogation of inhibitory activity (data not shown). An acidic polysaccharide from *A. capillaris* was also demonstrated to exert a profound inhibitory effect on the adhesion of *H. pylori* to erythrocytes (*19*), comparable to that observed with *P. ginseng*. Although the carbohydrate composition of CS-F2 is similar to that of pectin, which is composed primarily of glucuronic and galacturonic acids, pectin alone did not reveal any significant activity in a concentration-dependent manner, thereby implying that some carbohydrate components of CS-F2 other than uronic acid may play a role in the observed inhibition of host−bacterial adhesion (*36*). Recently, xylitol and farnesol have been shown to have some potential with regard to the modulation of the skin−microfloral balance, with selective effects and the inhibition of biofilm formation (*12*).

In conclusion, our results suggest that the acidic polysaccharide found in green tea shows a selective anti-adhesive activity against some pathogenic bacteria and a marked degree of activity against gastric and skin pathogenic bacteria. The design of synthetic glycomimetics, predicated on the structure of carbohydrate epitopes, may eventually be utilized in the development of carbohydrate-based anti-adhesive drugs. Anti-adhesive agents that govern susceptibility to bacterial adherence and colonization are currently under development, and this cell surface adhesion-based strategy promises to facilitate further investigations into carbohydrate-mediated cell−cell interactions.

ABBREVIATIONS USED

CS-F2, purified acidic polysaccharide from *Camellia sinensis*; EDTA, ethylenediamine tetraacetic acid; PBS, phosphate buffered saline; LMWH, low molecular weight heparin; SOS, sucrose octasulfate.

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