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Effect of Flavan-3-ols on the Adhesion of Potential Probiotic Lactobacilli to Intestinal Cells

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ABSTRACT: The effect of dietary flavan-3-ols on the adhesion of potential probiotic lactobacilli strains to intestinal cells was unraveled. The inhibitory activity of these compounds on intestinal cells was highlighted. The cytotoxic effect was shown to depend on both the compound's chemical structure (galloylation and polymerization) and degree of differentiation of intestinal cells. The effect of flavan-3-ols on bacteria adhesion differed greatly between compounds, strains, and intestinal cells. All flavan-3-ols inhibited significantly *Lactobacillus acidophilus* LA-5 and *Lactobacillus plantarum* IFPL379 adhesion except epigallocatechin gallate, which enhanced *L. acidophilus* LA-5 adhesion to Caco-2. Procyanidins B1 and B2 increased remarkably the adhesion of *Lactobacillus casei* LC115 to HT-29 cells, whereas epigallocatechin increased *L. casei* LC115 adhesion to Caco-2. These data showed the potential of flavan-3-ols to alter gut microecology by modifying adhesion of lactobacilli strains to intestinal cells.

KEYWORDS: *polyphenols, flavan-3-ols, lactobacilli, adhesion*

INTRODUCTION

Flavan-3-ols, also known as flavanols, are the main constituents of polyphenol intake in the diet, being present in a wide range of foods and beverages as both monomers and oligomeric procyanidins. The richest sources of flavan-3-ols include cocoa, red wine, green tea, red grapes, berries, and apples. These dietary polyphenols have received increased attention due to their claimed health benefits as they may act as antioxidant, anticarcinogenic, cardiopreventive, antimicrobial, antiviral, and neuroprotective agents.[1,2] It is clear that their resulting in vivo bioactivity is dependent on bioavailability, absorption, and metabolism in the body after ingestion.

There is an emerging consensus that the gut microbiota may play a crucial role in the potential health benefits of polyphenols.[3,4] The microbiota present in the intestinal tract could metabolize dietary polyphenols to more bioactive compounds with different physiological significance.[5] Parallel to this microbial metabolism, polyphenols could also modify the intestinal bacteria population composition and/or activity. Thus, dietary phenolic compounds are often transformed by gut microbiota, and the gut microbial population is modulated by dietary polyphenols in a two-way phenolic−microbiota interaction.[6]

The intestinal microbiota constitutes a dynamic and high-density ecosystem that comprises around 500−1000 microbial species (typically 10^{11}−10^{12} microbes/mL of luminal colon content). This microbial ecosystem serves numerous important functions for human host health, including the maintenance of intestinal homeostasis.[7,8] The healthy microbiota may be considered a good source of future probiotics.

Some specific lactic acid bacteria (LAB) are considered to be probiotics, which have been defined as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host".[9] Among LAB, the genus most commonly used as probiotics for oral delivery in human consumption is *Lactobacillus*. Probiotics are believed to temporarily colonize the intestine by adhering to intestinal surfaces. Therefore, adhesion to the intestinal mucosa is considered one of the main criteria for the selection of potential probiotics, as it may increase their persistence in the intestine and thus allow the probiotic to exert its effects.[10]

Due to the difficulty of assaying bacterial adherence in vivo, this feature has been studied using intestinal cell lines of human origin in culture as in vitro models for the intestinal epithelium.[11] These cultured, polarized intestinal cell lines express in different ways the specific characteristics of several of the cell phenotypes that line the intestinal epithelium.[12] The Caco-2 cell line, originally isolated from a human colon adenocarcinoma, spontaneously differentiates under standard culture conditions, and the differentiated cells express characteristics of mature enterocytes.[13]

In addition to host−bacteria and bacteria−bacteria relationships, diet has a very strong influence in establishing a balanced intestinal microbiota on the extent to which different intestinal bacteria colonize the intestine. Therefore, it is important to examine whether intake of dietary components may influence the colonization of intestinal microorganisms. In an in vitro study, berry juice polyphenol fractions were shown to inhibit the adhesion of *Neisseria meningiditis* to human epithelial cells.[14]

This study aims to investigate the effect of flavan-3-ols on the adherence of potential probiotic *Lactobacillus* strains to human intestinal cell lines. The antimicrobial activity of these compounds against the strains assayed was also examined. Taking into account the interaction that could be established between diet components, host, and bacteria, the possible

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growth-inhibitory activities of these dietary components on the intestinal cell lines was also highlighted.

MATERIALS AND METHODS

Bacterial Strains and Media. Four *Lactobacillus* strains from different sources were used in this study. *Lactobacillus plantarum* IFPL935 and IFPL379 isolated from raw milk cheeses[15] (IFPL Collection, CSIC, Madrid, Spain) and *Lactobacillus casei* LC115 isolated from a commercial probiotic product and kindly supplied by Dr. A. Martínez (Instituto de Investigación en Ciencias de la Alimentación, CSIC, Spain) were grown at 37 °C in MRS. *Lactobacillus acidophilus* LA-5 (Simbiotic drink, Priégola, Madrid, Spain) was grown in MRS supplemented with maltose (0.5%) at 37 °C under an atmosphere of 5% CO_2 in a CB 210 incubator (Binder GmbH, Tuttlingen, Germany). Growth of bacterial cultures was routinely monitored by measuring the optical density at 600 nm (OD_{600}).

Cell Culture. Two human epithelial intestinal cell lines, colorectal adenocarcinoma (Caco-2) and colon carcinoma (HT-29), were used to assess the cytotoxicity of the different flavan-3-ols and the adhesion abilities of the four LAB. The culture and maintenance of the cell lines were carried out following standard procedures; thus, cells were grown in a 75 cm^2 flask (Sarstedt, Nümbrecht, Germany) using Dulbecco's modified Eagle's medium (DMEM) for Caco-2 and McCoy's medium for HT-29 cells. Both media were supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin, and cell cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. The medium was changed every 2 days, and the cells were subcultured at 80% confluence every week. Media and reagents were purchased from Sigma (Poole, Dorset, UK) and Invitrogen (Paisley, UK).

Cell Viability Assay. Gallic acid, monomeric flavan-3-ols [(+)-catechin (C), (−)-epicatechin (EC), (−) epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG)], and procyanidin dimers A2 (PA2), B1 (PB1), and B2 (PB2) were assayed on Caco-2 and HT-29 cell lines. Flavan-3-ols were purchased from Extrasynthese (Genay, France), and stock solutions were dissolved in DMEM or McCoy's medium plus 1% dimethyl sulfoxide (DMSO) to improve solubility. Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were plated at a density of 1×10^4 cells/well in 96-well plates, cultured overnight, and then treated with various concentrations of the polyphenolic compounds (5−250 μM); the final concentration of the solvent in the wells was 0.1%. DMEM or McCoy's medium plus 0.1% of solvent was added to the control wells. At the end of the treatment period (24 h), 50 μL of MTT solution (0.5 mg/mL) was added to each well, and plates were incubated for 3 h at 37 °C. The formazan crystals formed were solubilized with 100 μL per well of DMSO and quantified by reading the absorbance at 570 nm on a Varioskan Flash microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). Cell viability was calculated as a percentage of untreated cells (cell growth measured in the control wells without the addition of the tested compound). The effect on cell viability was expressed as IC_{50} value (concentration inducing 50% inhibition of cell growth when compared to the control) of the flavan-3-ols assayed, and the data were stated as the mean ± standard error (SE) of three independent experiments carried out in triplicate. IC_{50} was determined using a nonlinear regression model based on the equation for a sigmoid dose−response curve (GraphPadPrism v.4.01, GraphPad, La Jolla, CA, USA). Treatment concentrations were logarithmically transformed, and the analysis was performed with log (inhibitor) versus response, variable slope fit. IC_{50} values are reported with 95% confidence intervals.

Adhesion Assays. Caco-2 and HT-29 cells were seeded (1×10^4 cells/mL) in 24-well plates and incubated to obtain confluence (1×10^7 cells/mL) for 14 ± 1 days at 37 °C in a humidified atmosphere containing 5% CO_2, prior to the adhesion assays.

Overnight cultures of the four lactobacilli strains, grown under standard conditions, were harvested by centrifugation, washed twice in Dubelcco's PBS solution, and resuspended in DMEM or McCoy's medium without antibiotics at a concentration of about 10^8 colony-forming units (cfu/mL). Meanwhile, cell line monolayers were also washed twice in Dubelcco's PBS to remove antibiotics before the bacterial suspension was added (at a ratio of bacteria/eukaryotic cell of 10:1). Then, plates were incubated for 1 h at 37 °C under 5% CO_2. After the incubation period, supernatants were removed and wells were softly washed three times with Dulbecco's PBS buffer to remove nonattached bacteria. Finally, Caco-2 and HT-29 monolayers were trypsinized with 0.25% trypsin−EDTA solution (Sigma), and bacterial counts were carried out in the appropriate agar media. Adhesion data were expressed as the percentage of bacteria adhered compared to the total of bacteria added (cfu bacteria adhered/cfu bacteria added). Two independent experiments, in which each of the tested LAB strains was analyzed in triplicate, were carried out.

The effect of flavan-3-ols on bacteria adhesion was assayed as described above. In this case, stock solutions of the polyphenols in DMEM or McCoy's medium plus 1% DMSO were diluted and added to Caco-2 and HT-29 monolayers cells for a final concentration of 50 μM along with the bacterial suspensions at a ratio 10:1 (bacteria/eukaryotic cell). For control wells bacterial suspensions were incubated in the same media without addition of the polyphenolic compounds. Then, the plates were incubated for 1 h at 37 °C under 5% CO_2. Nonadherent bacteria were removed, and attached bacteria were determined by plate counting as mentioned before. Percentage change in the number of adherent bacteria was determined as follows: (cfu_{sample} /$cfu_{control}$ × 100), where cfu_{sample} is the amount of adhered bacteria in the presence of the polyphenol and $cfu_{control}$ is the amount of adhered bacteria in the control wells.

Antibacterial Activity Assay. Antimicrobial activity of flavan-3-ols against the LAB studied was performed as previously described[14] with some modifications. Thus, 450 μL of overnight cultures grown in MRS media of the LAB tested were added to 24-well plates in the same amount as in the adhesion-inhibition assay (about 10^8 cfu/mL) along with 50 μL of the polyphenol compounds assayed (0 - 200 μM) in DMEM media plus 0.1% DMSO. For control wells bacterial suspensions were incubated in the same media without addition of the polyphenolic compounds. The mixtures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 1 h. The antibacterial activity of the polyphenols against the LAB studied was determined by plating the incubation mixtures on its appropriate MRS agar plates. Experiments were run in triplicate, and the colony forming units were determined.

Statistical Analysis. Statistical study of the results was performed using one-way analysis of variance (software package Statgraphics Centurion XVI.I., Warranton, VA, USA) to determine significant differences ($P < 0.05$) in percentage of adhesion.

RESULTS AND DISCUSSION

Flavan-3-ols: Effect on Cell Viability. The growth-inhibitory activity of monomeric flavan-3-ols and procyanidin dimers were assayed on two intestinal cell lines showing different degrees of differentiation, Caco-2 and HT-29 cells. The highly enterocyte-like differentiated Caco-2 cell line is used as an in vitro model that resembles the human small intestinal epithelium, whereas the colorectal HT-29 cell line is a moderately differentiated primary cancer cell.[16] The effect of gallic acid that has been reported as a metabolite of the microbial conversion of the galloylated flavan-3-ol monomers[17] was also examined. Caco-2 and HT-29 cells were treated with a range of concentrations (5−250 μM) of the compounds tested for 24 h. All of the polyphenolic compounds studied inhibited the viability of both intestinal cell lines in a dose-dependent manner. The IC_{50} values (Table 1) showed that the cytotoxic effect varies depending both on the compound assayed and also on the cell line examined. For most of the compounds, Caco-2 cells displayed lower growth inhibition sensitivity (IC_{50} ranging

Table 1. IC_{50} Values (Concentration Inducing 50% Inhibition of Cell Growth) of the Polyphenolic Compounds Assayed[a]

	Caco-2	HT-29
catechin (C)	211.5 (±6.2)	215.1 (±14.4)
epicatechin (EC)	214.4 (±9.3)	192.0 (±18.0)
epicatechin gallate (ECG)	198.7 (±6.7)	64.0 (±3.4)
epigallocatechin (EGC)	160.1 (±5.7)	233.4 (±16.1)
epigallocatechin gallate (EGCG)	199.2 (±6.5)	12.15 (±2.2)
procyanidin A2 (PA2)	192.6 (±9.5)	136.7 (±8.3)
procyanidin B1 (PB1)	211.7 (±5.9)	88.5 (±7.5)
procyanidin B2 (PB2)	170.9 (±3.1)	36.06 (±3.2)
gallic acid	206.6 (±7.3)	26.62 (±2.8)

[a]Caco-2 and HT-29 cells were treated with various concentrations of polyphenolic compounds (5−250 μM) for 24 h, and the effect on cell cytotoxicity was assayed by the MTT method. IC_{50} (μM) was determined on the basis of the dose−response curve. Data are represented by means ± SE from triplicate determinations.

from 160.1 to 214.4 μM) than HT-29 cells (IC_{50} between 12.15 and 233.4 μM); nevertheless, for C, EC, and EGC the effectiveness in inhibiting cell growth was similar in both cell lines. The stronger inhibitory activity on cell viability was measured when HT-29 cells were treated with the galloyl monomers (ECG and EGCG), gallic acid, and PB2 (Table 1).

These results point out the impact of the flavanol chemical structure on the cytotoxic effect. In this regard, ECG and EGCG differ from EC and EGC by the presence of a gallic acid molecule on the B-ring, although the two former were shown to be more biologically active as a cytotoxic agent than the non-galloyl molecules. Other studies have also shown that galloylation of catechins could be an important chemical characteristic for their activity;[18,19] thus, EGCG was proven to be the most potent flavanol of the catechin type for inhibiting cell proliferation of prostate cell lines.[20] The galloylation of flavanols is not the only structural modification that can influence their growth-inhibitory potential. Procyanidins, oligomerized flavanols, have also been reported to be more cytotoxic than monomer flavanols in a variety of human cell lines;[21] accordingly, when HT-29 cells were treated with a natural polyphenolic extract, it was clearly shown that both polymerization and galloylation enhance the cytotoxic effect of polyphenolic fractions.[22] Additionally, the molecule of gallic acid has been reported to have a significant inhibition of cell viability in a series of cancer cell lines in a dose- and time-dependent manner.[23] This effect could be due to the fact that the galloyl moiety increases the hydrophobicity of the flavan-3-ol molecule, resulting in a higher affinity for lipid cell membranes.[24] Other studies also suggested that the higher toxicity of gallic acid on cell lines may be partly due to the generation of reactive oxygen species (ROS) in media.[25]

In addition, the galloyl monomers along with gallic acid and the procyanidins studied displayed a differential growth inhibitory effect on the two cell lines examined, inducing a decrease in cell viability in HT-29 cells but not in Caco-2 cells at physiological concentrations. A different sensitivity to growth inhibition after incubation with propolis extracts has been reported for HCT116, SW480, and HT-29 colon cancer cells compared to Caco-2 cells; thus, a great decrease in growth was measured in those cell lines with a high proliferation rate, whereas the propolis extract showed only a marginal growth inhibition in human normal colonic epithelial cells.[26] Highly proliferating cells were also shown to be critical to growth inhibition by the extract of bilberry extracts.[27] The effect of dietary flavanols on apoptosis/proliferation in Caco-2 and SW480 colon cancer cell lines also suggested a different cytotoxicity of these compounds depending on their different activity and the degree of differentiation of the colon cancer cell line.[28]

Adhesion to the Cell Lines. Adhesion to intestinal mucosa is regarded as a prerequisite for colonization and is an important characteristic related to the ability of probiotic strains to confer a health benefit. Adhesion of the four selected lactobacilli strains was evaluated using the in vitro Caco-2 and HT-29 cell line models (Figure 1).

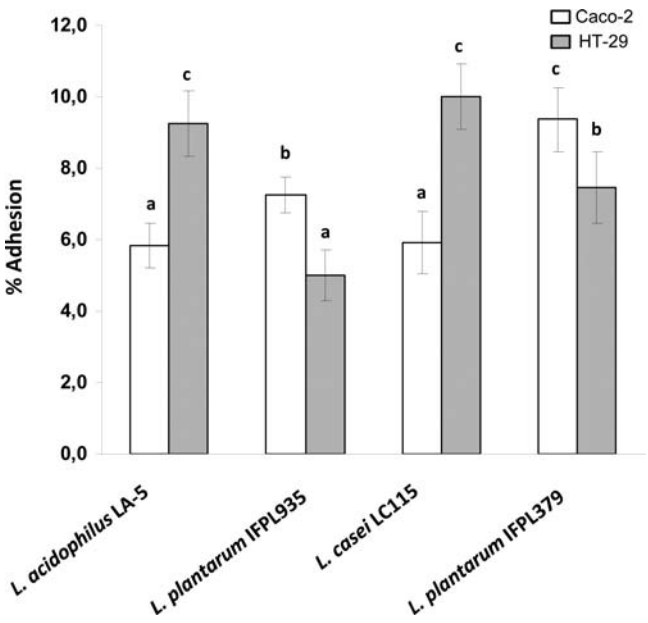

Figure 1. Percentage of adhesion (bacteria adhered with respect to the amount of bacteria added) of four lactobacilli strains to the intestinal Caco-2 (white bars) and HT-29 (gray bars) cell lines. Each adhesion assay was conducted in triplicate with cells from three successive passages. Adhesion assays were monitored after 1 h of incubation. Mean and SE are shown. For each cell line, columns that do not share the same letter are significantly different ($P < 0.05$).

Overall, the adhesion levels of the strains studied varied significantly ($P < 0.05$) depending on the in vitro model assayed; thus, *L. acidophilus* LA-5 and *L. casei* LC115 were shown to exhibit poorer adherence properties to Caco-2 cells, but exhibited better adhesion abilities when HT-29 cell monolayers were used (Figure 1). The adhesion abilities of the lactobacilli studied also demonstrated variability among strains except for *L. acidophilus* LA-5 and *L. casei* LC115, for which no significant differences ($P < 0.05$) between them, in both cell lines, were found. In general, the lactobacilli strains selected for this study (and mainly *L. plantarum* IFPL379) adhered quite well to Caco-2 cells compared to *L. acidophilus* LA-5, which was previously stated to possess significant adhesion abilities.[29] Moreover, these strains exhibited better adhesion percentages to both cell lines than that reported for the highly adhesive strain *Lactobacillus rhamnosus* GG,[11] under the same assay conditions.[30]

The variability in bacterial adhesion found among strains and in vitro models used is in accordance with previous studies.[31] Overall, these differences could be attributed to differences in

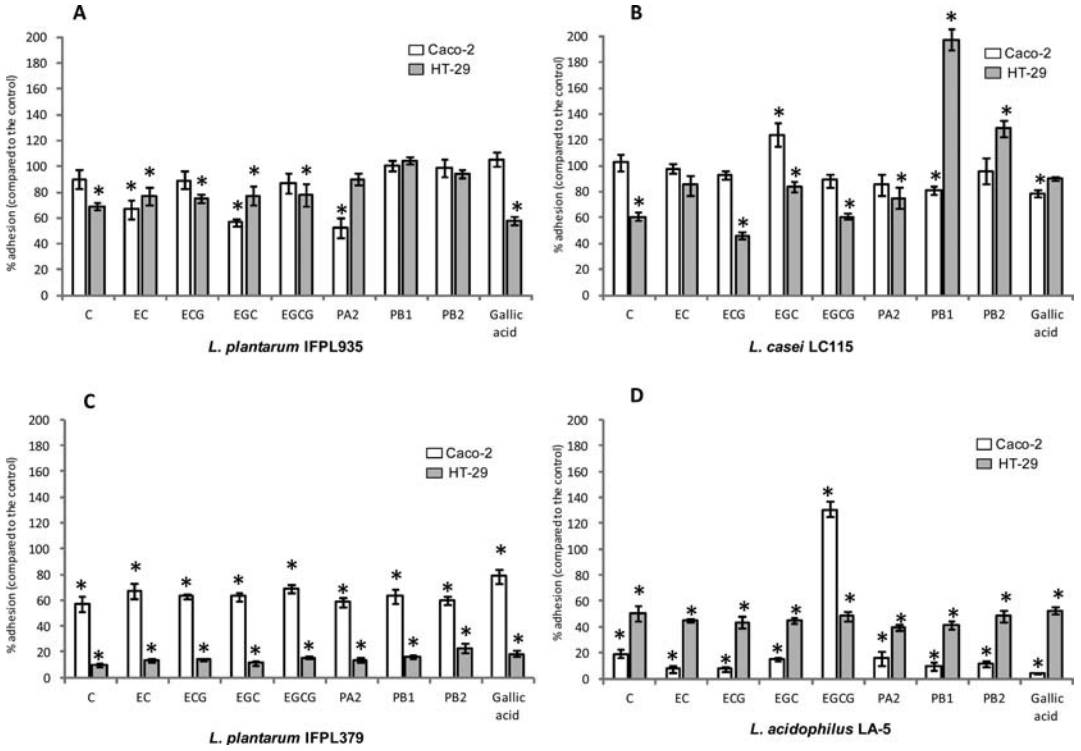

Figure 2. Adhesion of *L. plantarum* IFPL935 (A), *L. casei* LC115 (B), *L. plantarum* IFPL379 (C), and *L. acidophilus* LA-5 (D) to the intestinal Caco-2 (white bars) and HT-29 (gray bars) cell lines after polyphenol treatment. Values are expressed as percentage of bacterial adhesion, after 1 h of treatment with 50 μM concentrations of the flavan-3-ol compounds, compared to the control. Values are the mean ± SE of three independent assays. For each of the cell lines and bacteria studied, an asterisk (∗) indicates significance difference ($P < 0.05$) of the values when compared to the control.

the mechanisms involved as well as the strain aggregation patterns.[32] Although the mechanisms of adhesion are not fully understood, components of proteinaceous nature have been pointed out as key components involved in bacterial adhesion to the intestinal mucin types and/or epithelial cells.[33,34] Thus, mucus-binding proteins and mannose-specific adhesins are examples of important effector molecules involved the adhesion of lactobacilli to the host.[35,36] Novel proteins secreted by *L. plantarum*, which might play an important role in the cellular adhesion, were identified.[37] Recently, the heterologous expression of the collagen-binding protein Cnb in *L. casei* ATCC393 was shown to increase its adhesion abilities to Caco-2 cells but also resulted in higher competition ability against *Escherichia coli* O157:H7 and *Listeria monocytogenes* adhesion to Caco-2 cells than the parental strain.[38] Furthermore, other cell surface molecules such as S-layer proteins, lipoteichoic acid, and exopolysaccharides also contribute to specific and/or non-specific adhesion to host epithelial cells.[39] A possible relationship between the hydrophobicity of the bacteria and their ability to adhere to intestinal mucosa was also suggested.[40]

It is important to bear in mind that in vitro results of bacteria adhesion are difficult to extrapolate to in vivo conditions, as different factors present in the dynamic environment of the gastrointestinal tract (i.e., host responses, cell-to-cell communication molecules, resident microbiota, peristaltic flow) are likely to modify the bacteria adhesion.[39] However, in vitro experiments are essential to understand the mechanisms of adhesion and provide important information regarding strain differences.

Influence of Flavan-3-ols on Bacteria Adhesion. The daily mean intake of total flavan-3-ols throughout the European Prospective Investigation into Cancer and Nutrition (EPIC) calibration cohort countries was estimated to range from 125 to 450 mg. If the colon volume is about 2 L, it a flavan-3-ol colon concentration of about 60 to 225 mg/L can be estimated, depending on the daily intake. On the basis of these figures and taking into account other factors such as the percentage that can reach the colon, a concentration of 50 μM of the flavan-3-ols was used to investigate their effects on bacteria adhesion; at these doses, it was shown that the flavan-3-ol compounds were not cytotoxic to the enterocyte-like differentiated Caco-2 cells, as the cells were found to be ≥85% viable at up to 125 μM for the most toxic compounds tested. To understand the effect of these compounds on bacterial adhesion to intestinal cells, it was important to study their antibacterial activity. At the conditions assayed, none of the flavan-3-ols, in the range of concentrations tested (0−200 μM), affected the viability of the lactobacilli strains to any extent (data not shown).

The effect of the different flavan-3-ol compounds on bacteria adhesion differs depending on both the lactobacilli strain and the cell line tested (Figure 2). On the one hand, all of the compounds inhibited significantly *L. acidophilus* LA-5 and *L. plantarum* IFPL379 adherence to the intestinal cell lines except EGCG, which increased the adhesion of *L. acidophilus* LA-5 to Caco-2 cells (131%). Percentages of adhesion, with respect to the control, to Caco-2 cells were 57−78% for *L. plantarum* IFPL379 and 4−19% for *L. acidophilus* LA-5, whereas these adhesion percentages to HT-29 cells ranged between 10 and 19% and between 40 and 52% of control values for both strains, respectively. On the other hand, and focusing on Caco-2 cells, only EC, EGC, and PA2 significantly lowered *L. plantarum* IFPL935 adhesion to 52−66% of control values, whereas just PB1 and gallic acid demonstrated significant inhibition on *L. casei* LC115 adhesion. Interestingly, for this latter strain,

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pretreatment with EGC increased the adhesion. Although most of the polyphenols tested demonstrated an inhibitory effect on the lactobacilli adhesion to HT-29 cells, addition of either PB2 or PB2 increased remarkably the adhesion of *L. casei* LC115 to HT-29 cell line (Figure 2). For EGCG and gallic acid, the cytotoxicity of these compounds on HT-29 cells at the concentration assayed could be partially responsible for the inhibitory effect on adhesion. Nevertheless, addition of PB2, which also markedly diminished HT-29 cell viability when used at 50 μM, resulted in different effects on bacteria adhesion depending on the lactobacilli strain assayed (Figure 2). On the basis of these data, a relationship between the chemical structure of the flavan-3-ols and their effect on bacterial adhesion to the cell lines assayed cannot be determined. These results are in agreement with previous findings[41] showing that of all of the polyphenols assayed only phloridzin and rutin enhanced the adherence of *L. rhamnosus*.

The mechanisms by which polyphenols could influence the bacterial adhesion are not fully investigated. Polyphenols are able to interact with microbial membrane proteins, enzymes, and lipids, thereby altering cell permeability and permitting the loss of protons, ions, and macromolecules. There is also evidence that polyphenols could interfere with biofilm formation, the first step of which is bacterial adhesion, and quorum-sensing.[42] These bioactive molecules could have an influence of pathogen adhesion to host tissues, as adhesion is a requirement for the initiation of the majority of infectious diseases. Plant polyphenols have been reported to inhibit glucosyltransferases that mediate the adherence of *Streptococcus mutans* and other oral bacterial species to tooth surfaces, contributing to the formation of dental plaque biofilms. Moreover, polyphenolic compounds may also inhibit bacterial adherence by reducing the hydrophobicity of mutans streptococci.[43] Related to other pathogens and host tissues, current evidence favors that the cranberry polyphenols' ability to antagonize uropathogenic *Escherichia coli* relies on its antiadhesion properties; cranberry juices diminished the adhesive forces in P fimbriated bacteria through a change in surface macromolecules.[44] Berry juice polyphenolic fractions also are able to inhibit the binding of *N. meningiditis* pili to inmobilized human epithelial cells,[45] and apple peel polyphenol-rich extracts were shown to display an in vitro inhibitory effect against *Helicobacter pylori*.[46] Recently, resveratrol and some derivatives were reported to inhibit *E. coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* Scott A adhesion to colonic epithelial cell lines and consequent cytokine production as a response to foodborne pathogen adhesion.[47]

Adhesion to the intestinal mucosa is thought to be crucial for colonization in the intestine and is thought to stimulate the host immune system, and these actions have been related to certain beneficial effects of probiotics.[48] Probiotics help to maintain gut mucosal defenses by modification of the environmental conditions, competition for nutrients and adhesion sites, production of antimicrobial metabolites, and modulation of the immune and nonimmune defense mechanisms of the host. Thus, increasing the adhesion of probiotic bacteria could help to restore the balance of intestinal microbiota in the host and play important roles in health.

A dynamic relationship exists among the gastrointestinal microbiota, the intake and metabolism of dietary bioactive food components such as polyphenols, and the intestinal mucosal cells. Both the numbers and types of microbes and dietary factors can influence the host health status. In this work, several common dietary flavan-3-ols were shown to have potential to alter gut microecology by modifying adhesion of probiotic *Lactobacillus* strains to intestinal cells. Thus, the consumption of a diet rich in these bioactive molecules could influence the intestinal microbiota and improve microbiota imbalances caused by different factors, thereby favoring the host's overall well-being. Further studies on the effect of these flavan-3-ols on the viability of other bacteria representative of the gut ecosystem, such as pathogens, and their adhesion to enterocytes will help to gain a better insight into the interaction of these polyphenols on the complex gut microbiota.

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Notes

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