JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Resveratrol and Some Glucosyl, Glucosylacyl, and Glucuronide Derivatives Reduce *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* Scott A Adhesion to Colonic Epithelial Cell Lines

María V. Selma,*[,†] Mar Larrosa,[†] David Beltrán,[†] Ricardo Lucas,[‡] Juan C. Morales,[‡] Francisco Tomás-Barberán,[†] and Juan C. Espín[†]

[†]Research Group on Quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS-CSIC, 30100 Campus de Espinardo, Murcia, Spain

[‡]Department of Bioorganic Chemistry, Institute of Chemical Research, CSIC−University of Seville, 41092 Seville, Spain

ABSTRACT: The efficacy of resveratrol and some glucosyl, glucosylacyl, and glucuronide derivatives in inhibiting the adhesion of *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* Scott A to Caco-2 and HT-29 colonic cells was investigated. The three bacteria strains were capable of adhering to both colonic epithelial cell lines, which responded by producing the pro-inflammatory interleukin 8 (IL-8). Adhesion inhibition of *E. coli* O157:H7 and *S.* Typhimurium to colonic cells was ≥60 and ≥40%, respectively, when resveratrol and most of the resveratrol derivatives were applied. Lower adhesion inhibition was observed for the bacteria with higher adherence potential, *L. monocytogenes* (≥20%). Resveratrol-3-*O*-(6′-*O*-butanoyl)-*β*-D-glucopyranoside (BUT) (50 and 100 *μ*M) and resveratrol-3-*O*-(6′-*O*-octanoyl)-*β*-D-glucopyranoside (OCT) (50 *μ*M) reduced IL-8 secretion by 100%. These results suggest that one mechanism for the beneficial attributes of resveratrol and especially the derivatives BUT and OCT could be the ability to reduce the adhesion and consequent pro-inflammatory cytokine production in intestinal epithelial cells in response to pathogen adhesion. The potential use of these compounds in the prevention of foodborne infections, intestinal homeostasis loss, and inflammatory bowel diseases could be another step in finding coadjuvants or alternatives to antibiotic treatments.

KEYWORDS: *polyphenols, foodborne pathogens, intestinal health, HT-29 cells, interleukin 8*

INTRODUCTION

Foodborne illnesses present a significant health problem throughout the world. In the latest EFSA report on foodborne outbreaks,[1] *Salmonella* was, as in previous years, the most commonly reported cause of foodborne outbreaks in the European Union. *Salmonella* species, which cause gastroenteritis in humans, were responsible for 39.2% of all reported outbreaks in 2007. Pathogenic *Escherichia coli* strains were responsible for a lower percentage of foodborne outbreaks (1.2%). However, humans infected by this pathogen may have various symptoms from mild symptoms and diarrhea to life-threatening infections. Indeed, verotoxigenic *E. coli* of a number of different serotypes, especially O157:H7, are a well established cause of acute diarrhea, hemorrhagic colitis, and the hemolytic uremic syndrome.[2] *Listeria* infections in humans can also have a high case fatality ratio in industrialized countries. Listeriosis is fatal in up to 30% of cases. This threatening nature of listeriosis prompted the World Health Organization (WHO) to suggest that various food products must be frequently investigated for the presence of *Listeria monocytogenes* on a worldwide basis.[3] Indeed, on average, *Listeria* was the most severe pathogen associated with European outbreaks in 2006.[4] Nevertheless, *Listeria* was indicated as the causative agent in only 0.2% of all reported outbreaks in 2006 and in one reported outbreak in 2007, and all were associated with the consumption of soft cheese.

The majority of infectious diseases are initiated by the adhesion of pathogenic organisms to the tissues of the host. This is considered to be the first stage in any infectious process and is an important and critical step for colonization.[5,6] Indeed, the first direct encounter of *Salmonella* spp. with host cells is the initial recognition of and adherence to the surface of the intestinal epithelium, and this event is a prerequisite for the subsequent steps in pathogenesis that lead to mucosal infection, systemic spread, and disease.[7] *E. coli* O157:H7 also adheres intimately to and interacts with intestinal epithelial cells to cause cytoskeletal rearrangements, which result in attachment lesions and increased epithelial monolayer permeability.[8] Epidemiological evidence shows that the gastrointestinal tract is also the primary route of infection and that penetration of the intestinal epithelial cell barrier is the first step in the *L. monocytogenes* infection process.[9] *L. monocytogenes* adhesion to and invasion of intestinal epithelial cells and subsequent translocation to distant organs are critical in establishing a systemic infection in a host.[10] Therefore, the ability of *L. monocytogenes* to invade epithelial cells correlates with bacterial virulence.[9] Epithelial cell invasion by intestinal pathogens

Received: September 29, 2011
Revised: April 27, 2012
Accepted: July 4, 2012
Published: July 4, 2012

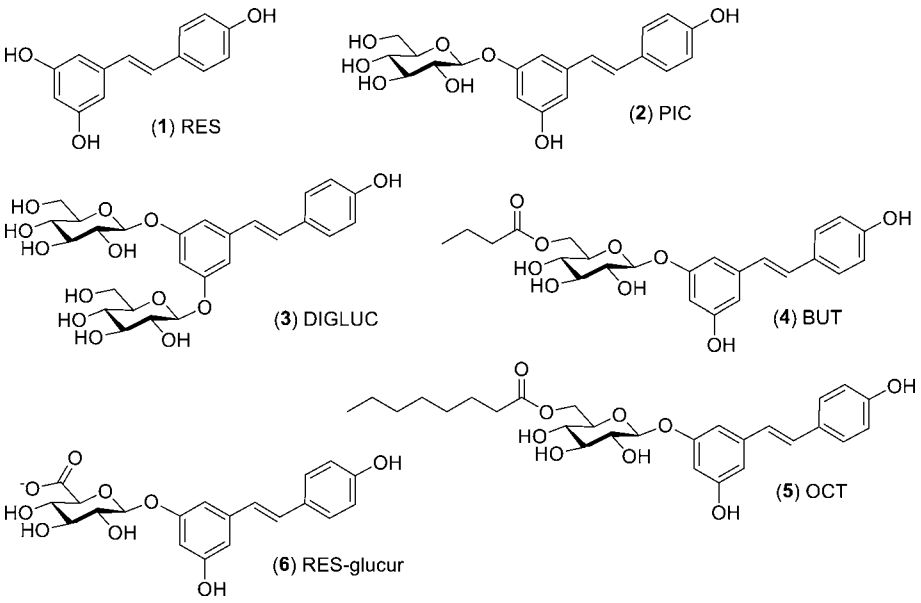

Figure 1. Resveratrol, piceid, and the different resveratrol derivatives assayed in the present study: (**1**, RES) *trans*-resveratrol; (**2**, PIC) *trans*-piceid, (**3**, DIGLUC) *trans*-resveratrol-3,5-di-*O*-*β*-D-glucopyranoside; (**4**, BUT) *trans*-resveratrol-3-*O*-(6′-*O*-butanoyl)-*β*-D-glucopyranoside; (**5**, OCT) *trans*-resveratrol-3-*O*-(6′-*O*-octanoyl)-*β*-D-glucopyranoside; (**6**, RES-glucur) *trans*-resveratrol-3-*O*-glucuronide.

provides early signals for the acute mucosal inflammatory response via the release of pro-inflammatory cytokines and inflammatory mediators.[11] Human colonic epithelial cell lines produce in vitro a wide range of pro-inflammatory cytokines in response to microbial pathogens such as *E. coli*, *L. monocytogenes*, *Salmonella enteritidis*, *Shigella dysenteriae*, and *Yersinia enterocolitica*.[12] However, the patterns of epithelial cytokine response vary with the site of infection and type of pathogen. *S. enteritidis*, *E. coli* O157:H7, and *L. monocytogenes* induce an instant innate immune response following their invasion, which involves the rapid expression and up-regulation of an array of pro-inflammatory cytokines, predominately interleukin 8 (IL-8).[13] Although this response is triggered to eliminate the pathogen, the persistent production of IL-8 often causes chronic inflammation that usually leads to tissue damage. Such an inflammation is characterized by high levels of IL-8 and is observed in several intestinal disorders such as ulcerative colitis, pouchitis, and Crohn's disease.[14,15] Interventions that decrease these levels have been shown to significantly alleviate the conditions.[16]

Foodborne pathogen infections such as salmonellosis and listeriosis have become more serious public health concerns due to the spread of antibiotic-resistant strains, which make their treatment difficult.[17] The extensive uses of antibiotics for the control and treatment of *Salmonella* and *Listeria* infections in farm animals and medical practices have been suggested as the predisposing factors for the evolution of multi-drug-resistant strains.[18] Therapy for *E. coli* O157:H7 infection is limited to supportive treatment, as antibiotics may increase the risk of systemic complications, such as acute renal failure associated with the hemolytic uremic syndrome, perhaps by promoting the release of preformed toxin from the periplasm.[19] For these reasons, different alternatives or coadjuvants to antibiotic treatment are being investigated.

Inhibition of pathogen adhesion to the intestinal epithelium may prevent colonization and limit opportunity for systemic infection.[20] Despite numerous studies having demonstrated the antipathogenic properties of probiotics, their effectiveness in reducing intestinal infection varies depending on which probiotic organism is used, as well as the health status of the host.[21,22] Efficient bacteriostatic agents have also been identified in foodstuffs such as wine polyphenols.[23] Foodstuffs containing inhibitors with bacteria antiadhesion agents may also be expected to emerge. These compounds do not act by killing or arresting the growth of the pathogen, as, for example, antibiotics do. Therefore, the spread of bacteria resistant to the antiadhesion agent is expected to occur at significantly lower frequencies than that of bacteria resistant to antibiotics.[5]

Resveratrol, (3,5,4′-trihydroxy-*trans*-stilbene), naturally occurring in grapes and grape-derived foodstuffs such as red wine, has been reported to exert many different health-promoting effects including antioxidant, anti-inflammatory, antitumor, antiplatelet aggregation, cardioprotective, aging-delay, antiobesity, and bactericidal properties.[24] Recently, our group demonstrated that some glucosylacyl resveratrol derivatives were much more effective than resveratrol in preventing intestinal inflammation in vivo.[25] In the present study, we explore the efficacy of resveratrol, and some glucosyl, glucosylacyl, and glucuronide resveratrol derivatives, to inhibit adhesion of *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* Scott A to Caco-2 and HT-29 colonic cells with the objective of finding potential alternatives to prevent and control human infections. Furthermore, we investigated whether resveratrol and its derivatives can alter the IL-8 production induced by foodborne pathogens.

MATERIALS AND METHODS

Chemicals. Resveratrol (*trans*-resveratrol) and different glucosyl, glucosylacyl, and glucuronide resveratrol derivatives were assayed in the present study (Figure 1), that is, piceid (PIC, *trans*-piceid, *trans*-resveratrol-3-*O*-*β*-D-glucopyranoside), resveratrol-diglucoside (DIGLUC, *trans*-resveratrol-3,5-di-*O*-*β*-D-glucopyranoside), piceid-butyrate (BUT, *trans*-resveratrol-3-*O*-(6′-*O*-butanoyl)-*β*-D-glucopyranoside), piceid-octanoate (OCT, *trans*-resveratrol-3-*O*-(6′-*O*-octanoyl)-*β*-D-glucopyranoside), and resveratrol glucuronide (RES-glucur, *trans*-resveratrol-3-*O*-glucuronide). Resveratrol derivatives were prepared as described by Larrosa et al.,[25] except RES-glucur.[26] The synthesis

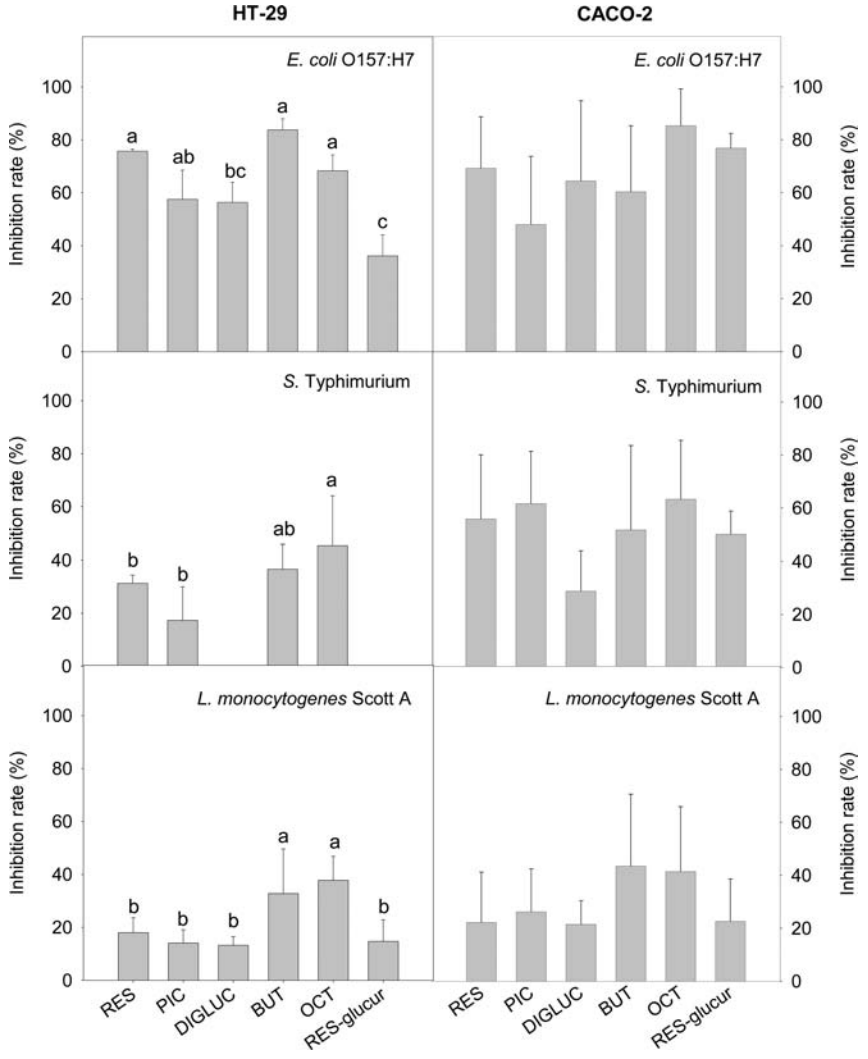

Figure 2. Effect of resveratrol and derivatives on adhesion of foodborne pathogens to Caco-2 (right) and HT-29 (left) cells. The results represent the mean values of relative adhesion compared to control in the absence of resveratrol and the standard error of the means for three different experiments. Different letters above columns indicate that the values of inhibition are significantly different ($P \leq 0.05$). (RES) *trans*-resveratrol; (PIC) *trans*-piceid, (DIGLUC) *trans*-resveratrol-3,5-di-*O*-β-D-glucopyranoside; (BUT) *trans*-resveratrol-3-*O*-(6′-*O*-butanoyl)-β-D-glucopyranoside; (OCT) *trans*-resveratrol-3-*O*-(6′-*O*-octanoyl)-β-D-glucopyranoside; (RES-glucur) *trans*-resveratrol-3-*O*-glucuronide.

process of glucosylated and acylglucosyl resveratrol derivatives, and their use as anti-inflammatory compounds is patented (PCT/ES2010/070826).

Caco-2 and HT-29 Cell Cultures. Human colonic epithelial cell lines HT-29 and Caco-2 were obtained from the European Collection of Cell Cultures (ECACC, Porton Down, Salisbury, UK) and grown to confluence in 24-well plates (Costar, High Wycombe, UK). HT-29 cells were cultured in DMEM containing 10% fetal bovine serum (FBS), 2 mM glutamine, 100 units/mL of penicillin, and 100 μg/mL of streptomycin and maintained at 37 °C and 5% CO_2. Caco-2 cells were grown in Eagle's Minimal Essential Medium (EMEM) containing 2 mM glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, 100 mM sodium piruvate, and nonessential amino acids and supplemented with 10% FBS. Cells were maintained at 37 °C in a 5% CO_2 humidified atmosphere. All cell culture reagents were from Gibco (Invitrogen, Cergy-Pontoise, France).

Bacterial Cultures. Strains of *S. enteritidis* serovar Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* Scott A were used in this study. *S.* Typhimurium (NCTC 12023), which contained plasmid pFVP25.1 carrying gfpmut3A under the control of constitutive promoter for fluorescence visualization, was kindly provided by Dr. Beuzón.[27] *E. coli* O157:H7 (CECT 5947) was obtained from the Spanish type culture collection (Valencia, Spain), and *L. monocytogenes* Scott A (LIS 1) isolated from cooked pita meat was obtained from the Laboratory of Food Microbiology and Food Preservation (LFMFP, Gent University, Belgium). *S.* Typhimurium and *E. coli* O157:H7 were grown in nutrient broth (Oxoid, Basingtoke, UK), whereas *L. monocytogenes* Scott A was grown in TSB (Oxoid) with 1% glucose. The 24 h cultures of bacteria were washed three times by centrifugation (4000*g*/15 min) with 0.05 M sterile phosphate buffer (PBS), pH 7, and the final pellets were resuspended in 10 mL of serum free DMEM or EMEM as the inocula (10^9 cfu/mL) for adhesion assays in HT-29 and Caco-2 cultures, respectively and also for cytokine production assay.

Adherence Assay. HT-29 and Caco-2 cells were grown in 24-well microtiter plates. The cells were seeded at 2×10^4 cells/well and incubated until they reached confluence (2×10^5 and 6×10^5 for HT-29 and Caco-2 cells, respectively). One day prior to the assay the cells were washed twice with PBS and incubated in antibiotic- and serum-free DMEM or EMEM, respectively. To determine the effect of resveratrol and its glucosylacyl derivatives on adhesion of *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* Scott A to HT-29 and Caco-2 cell layers, 1 mL of DMEM or EMEM medium containing 5 μL of the assayed compound (25 μM final concentration) and 5 μL of bacterial inocula (10^7 cfu) were maintained for 1 h at room temperature. Following this preincubation, three wells of HT-29 and Caco-2 cell layers, respectively, were filled with 300 μL of these

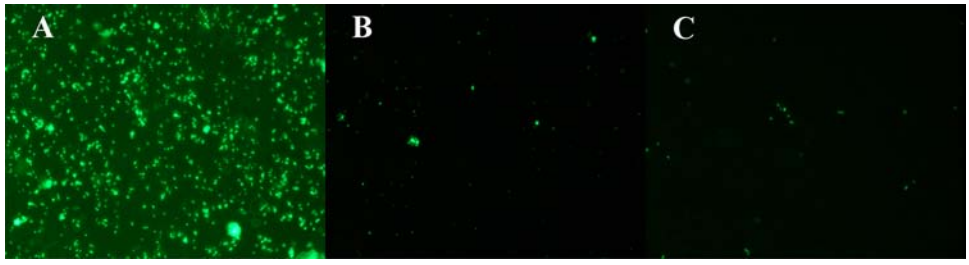

Figure 3. Visualization of *Salmonella* Typhimurium adhesion on Caco-2 cells using a Nikon Diaphot-TMD microscope equipped with fluorescence: (A) fluorescence microscopy of bacterial adhesion on Caco-2 cells in the absence of resveratrol; (B, C) fluorescence images of bacterial adhesion on Caco-2 in the presence of (B) resveratrol and (C) *trans*-resveratrol-3-*O*-(6′-*O*-octanoyl)-*β*-D-glucopyranoside (OCT). *S.* Typhimurium expressed GFP constitutively.

suspensions, and microtiter plates were incubated for 2 h at 37 °C in a 5% CO_2 humidified atmosphere. HT-29 multilayers and Caco-2 cell monolayers were washed three times with 1 mL of PBS and resuspended in 100 μL of PBS. Cells were lysed with 1 mL of distilled water with 20% glycerol and frozen at −70 °C for bacteria counting. Adhered *S.* Typhimurium and *E. coli* O157:H7 were determined by serial dilution and cultured on plates of nutrient agar, whereas adhered *L. monocytogenes* were counted in BHI agar. Results were expressed as the percentage of bacteria adhered relative to an adherence control (ARC).

IL-8 Production. Bacterial inflammatory effect was assessed by measuring levels of IL-8 cytokine secretion in the culture supernatants of HT-29 cells infected with *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* Scott A, respectively. HT-29 cells were grown to confluence in 96-well microtiter plates (3×10^4 cells/well). One day prior to the assay the cells were washed twice with PBS and incubated in antibiotic- and serum-free DMEM. HT-29 cell multilayers were used to determine the effect of resveratrol and its glucosylacyl derivatives on bacteria-induced production of IL-8. Twelve hundred microliters of DMEM containing 6 μL of the assayed compound (25 μM final concentration) and 6 μL of bacterial inocula (10^7 cfu) were maintained for 1 h at room temperature. Following this preincubation, five wells of HT-29 cell multilayers, respectively, were filled with 200 μL of these suspensions. TNF-*α* (0.4 ng/well) was used as control. Six hours after infection with bacteria, the culture supernatants of the plate were collected, centrifuged at 10000*g* for 10 min, and stored at −80 °C. IL-8 was measured by enzyme-linked immunoabsorbent assay using the Human IL-8 kit (Diaclone, Cedex, France) according to the manufacturer's instructions, on an Infinite 200 plate reader (Tecan, Grodig, Austria). The lowest sensitivity limit of the assay was 8 pg/mL. Any test wells with optical density values above this sensitivity were considered to be positive for IL-8.

Statistical Analysis. Each adhesion and cytokine expression assay was repeated on three separate experiments. The mean value was determined, and the standard error of the mean from triplicate experiments was calculated. Analysis of variance (ANOVA), followed by Tukey's method with a significant level of $P \leq 0.05$, was carried out on these data using SPSS (Windows 2000, Statistical Analysis).

RESULTS

Adherence of Foodborne Pathogenic Bacteria to Caco-2 and HT-29 Cell Cultures. The efficiencies of adhesion of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* Scott A to HT-29 and Caco-2 cells were evaluated. The level of adhesion of these bacteria to Caco-2 and HT-29 cells ranged from 1.1 ± 0.1 to 8.8 ± 1.1%. The level of adhesion varied depending on the bacteria. Thus, *L. monocytogenes* was the most efficient bacteria in terms of adhesion to epithelial cells, and the values were 8.8 ± 1.1 and 5.4 ± 1.6% with Caco-2 and HT-29 cells, respectively. Levels of adhesion of *E. coli* O157:H7 and *S.* Typhimurium ranged from 1.1 ± 0.1 to 1.4 ± 0.5% with both human intestinal cell lines.

Effect of Resveratrol and Derivatives on the Adherence of Foodborne Pathogenic Bacteria to Caco-2 and HT-29 Cell Cultures. We sought to determine the influence of resveratrol and some glucosylated, glucosylacyl, and glucuronide derivatives on the adhesion of foodborne pathogens to intestinal cell lines by exposing bacteria to test compounds for 1 h prior to intestinal cell infection. A significant inhibition of adhesion ($P \leq 0.01$) to HT-29 and Caco-2 cells of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* Scott A pre-exposed to resveratrol and derivatives was observed (Figure 2). The degree of inhibition depended on both the bacterial species and the resveratrol derivative applied. Adhesion inhibition of *E. coli* O157:H7 was ≥60 ± 9% for most of the resveratrol derivatives applied. Lower adhesion inhibition was observed in the case of *S.* Typhimurium, with which most of the resveratrol derivatives achieved an inhibition rate of ≥40 ± 4%. The lowest adhesion inhibition was observed for *L. monocytogenes* Scott A (≥20 ± 5% for most of the resveratrol derivatives) (Figure 2).

Figure 3 shows *S.* Typhimurium adhesion on Caco-2 cells in the presence and absence of resveratrol and OCT. In Caco-2 cells, the efficacies of the different resveratrol derivatives for inhibiting each bacteria adhesion were similar. Only a slightly higher inhibition of *L. monocytogenes* adhesion with no statistical significance was observed when BUT and OCT were applied (Figure 2). In contrast, significant differences in *L. monocytogenes* adhesion were observed when different resveratrol derivatives were applied in HT-29 cells ($P \leq 0.01$). BUT and OCT were especially effective in the inhibition of *L. monocytogenes* adhesion to HT-29 cells. Higher efficacy of BUT and OCT with respect to other resveratrol derivatives was also observed for inhibiting *E. coli* O157:H7 and *S.* Typhimurium adhesion to HT-29 cells (Figure 2).

Effect of Foodborne Pathogen Infection, Resveratrol, and Derivatives on Cytokine Expression by HT-29 Cells. IL-8 secretion by HT-29 cells was examined for *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* at different incubation times. Infection of HT-29 cells with these pathogenic bacteria resulted in secretion of IL-8 into the medium (Figure 4). IL-8 secretion by HT-29 cells was significantly higher in the presence of *L. monocytogenes* than with *E. coli* O157:H7 and *S.* Typhimurium. After 16 h of infection with *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes*, IL-8 concentrations were 433 ± 7, 572 ± 78, and 1673 ± 246 pg/mL, and all induced IL-8 secretion compared to control ($P \leq 0.01$) (Figure 4). Visually, cytotoxicity was also observed earlier during infection with *L. monocytogenes* than during infection with *E. coli* O157:H7 and *S.* Typhimurium. Thus, after 8 h, alteration of HT-29 cells was observed in HT-29 cells infected with *L. monocytogenes*, whereas

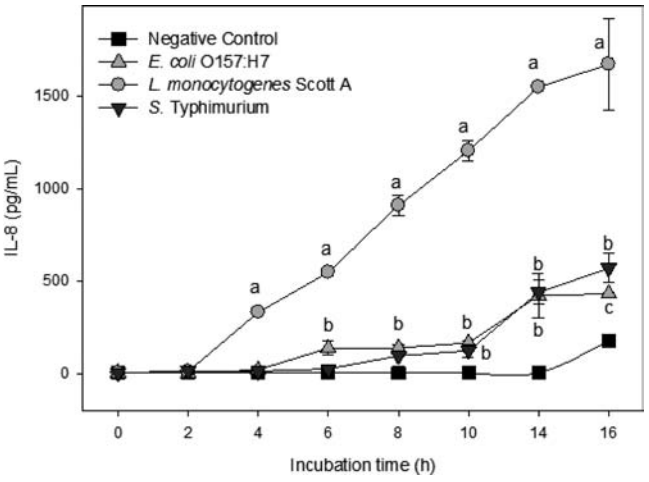

Figure 4. Time course induction of interleukin 8 (IL-8) synthesis by *Escherichia coli* O157:H7, *Listeria monocytogenes* Scott A, and *Salmonella* Typhimurium in HT-29 cells. Symbols with different letters indicate significant differences ($P \leq 0.05$) between the IL-8 levels of cells exposed to foodborne pathogens.

14 h was needed to observe damage in HT-29 cells infected with *E. coli* O157:H7 or *S.* Typhimurium (Figure 5). After 6 h of infection with *L. monocytogenes*, cell integrity was not affected.

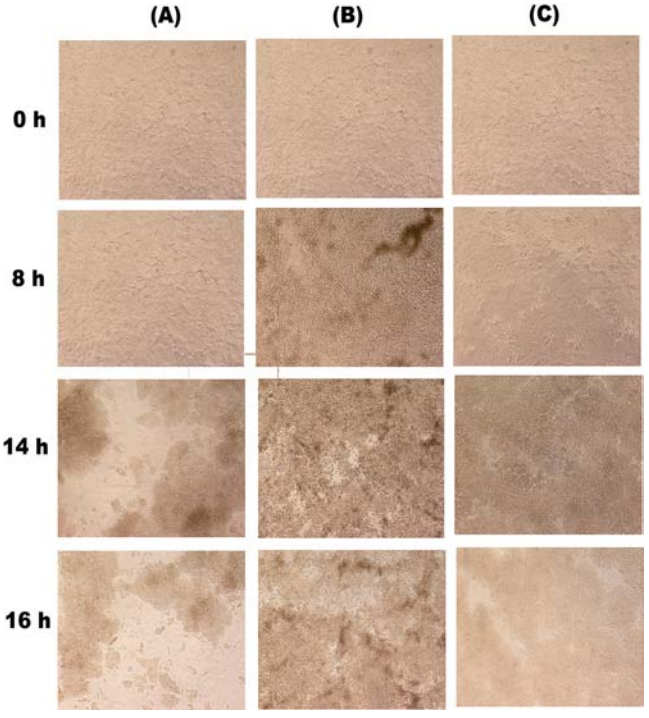

Figure 5. HT-29 culture during incubation with different foodborne pathogens: (A) *Escherichia coli* O157:H7; (B) *Listeria monocytogenes* Scott A; (C) *Salmonella* Typhimurium.

To investigate the effect of resveratrol and derivatives on IL-8 secretion, *L. monocytogenes*, the highest IL-8 elicitor among the three strains tested in the HT-29 model, was exposed to a low concentration of resveratrol and some glucosylated, glucosylacyl, and glucuronide derivatives (25 μM) for 1 h prior to HT-29 cell infection. After 6 h of infection with *L. monocytogenes*, the IL-8 concentration was 418 ± 20 pg/mL. The presence of resveratrol and most of the derivatives tested did not alter IL-8 expression by HT-29 infected with *L. monocytogenes* (Table 1).

Table 1. Interleukin 8 Secretion in HT-29 Cells Exposed to *Listeria monocytogenes* Scott A in the Presence of Resveratrol and Derivatives

elicitor	treatment[a] (25 μm)	IL-8 secretion (pg/mL)
none		29 ± 7[b]
Listeria monocytogenes		418 ± 20
Listeria monocytogenes	resveratrol	442 ± 2
Listeria monocytogenes	PIC	419 ± 35
Listeria monocytogenes	DIGLUC	360 ± 58
Listeria monocytogenes	BUT	315 ± 68[b]
Listeria monocytogenes	OCT	289 ± 77[b]
Listeria monocytogenes	RES-glucur	409 ± 5
TNF-α (2 ng/mL)		1535 ± 1.2[b]

[a]PIC, piceid; DIGLUC, resveratrol diglucoside; BUT, piceid butyrate; OCT, piceid octanoate; RES-glucur, resveratrol glucuronide. [b]Significantly different ($P \leq 0.05$) from *L. monocytogenes* Scott A exposed cells in the absence of resveratrol compounds.

However, a significant decrease of IL-8 production ($P \leq 0.05$) was observed when the HT-29 cells were infected with *L. monocytogenes* pretreated with BUT and OCT. Higher concentrations of BUT and OCT (50 and 100 μM) significantly reduced IL-8 secretion ($P \leq 0.01$) (Figure 6).

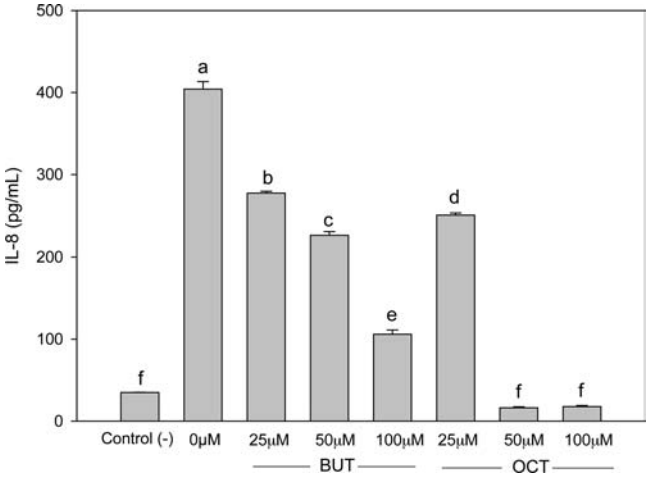

Figure 6. Interleukin 8 (IL-8) secretion in HT-29 cells exposed to *Listeria monocytogenes* Scott A in the presence of different concentrations of *trans*-resveratrol-3-*O*-(6′-*O*-octanoyl)-β-D-glucopyranoside (OCT) and *trans*-resveratrol-3-*O*-(6′-*O*-butanoyl)-β-D-glucopyranoside (BUT). Bars with different letters indicate significant differences ($P \leq 0.01$).

BUT concentrations of 50 and 100 μM reduced IL-8 secretion by 44 and 74%, respectively. An OCT concentration of 50 μM reduced IL-8 secretion by 100%, obtaining IL-8 levels under the limit of detection (8 pg/mL), which are lower than those obtained in HT-29 cells not infected with *L. monocytogenes* (17 ± 0.3 pg/mL) (Figure 6).

DISCUSSION

A wide range of intestine and foodborne bacteria interactions can ultimately lead to disease. However, the adhesion of bacterial cells to the intestinal epithelium is generally

dx.doi.org/10.1021/jf203967u | J. Agric. Food Chem. 2012, 60, 7367−7374

considered to be the first step in pathogenesis preceding invasion.[5,6] In the present study, *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* Scott A were capable of adhering to the human colonic epithelial cell types tested, HT-29 and Caco-2. In accordance with a previous study,[9] differences between adhesion of some bacteria such as *L. monocytogenes* on Caco-2 cells and adhesion on HT-29 cells were noted. *L. monocytogenes* was the best performing species among the tested species in this study in adhering to the colonic epithelial cells (8.8 and 5.4% with Caco-2 and HT-29 cells, respectively). Kim and Wei[28] showed that *L. monocytogenes* samples obtained from both humans and retail meat products were able to better invade Caco-2 cells than *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* or *Salmonella* isolates including *S.* Typhimurium, *Salmonella* Agona, and *Salmonella* Heidelberg. On the other hand, Moroni et al.[9] showed the abilities of *L. monocytogenes* to adhere to HT-29 or Caco-2 cells vary widely depending on the strain tested. *L. monocytogenes* Scott A strain tested in the present study showed a high adherence ability compared to 14 *L. monocytogenes* strains tested in a previous study in which the level of adhesion ranged from 0.01 to 9.45%.[9] This difference in adhesion capacity as well as in invasion ability may explain the difference in virulence among *L. monocytogenes* strains.

Plant materials possessing antiadhesion activities are attractive candidates for antibacterial agents. There is, however, a relative paucity of information regarding the antiadhesive properties of most plant materials. In the present study, we examined the abilities of the polyphenol resveratrol and some derivatives to block the adherence of three foodborne pathogens with different adhesion potentials to human colonic cells. Our data clearly indicated that resveratrol and most of the derivatives tested inhibit the adherence of *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* to human colonic cells. We observed higher adhesion inhibition (≥60 and ≥40%) in the case of the bacteria with lower adherence potential (*E. coli* O157:H7 and *S.* Typhimurium, respectively) than adhesion inhibition level (≥20%) of the bacteria with higher adherence potential (*L. monocytogenes*). Other phenolic compounds, especially those obtained from cranberry (*Vaccinium macrocarpon*), have been analyzed with respect to their antiadhesion activities.[5] Extracts of cranberries containing proanthocyanidins in their condensed form inhibited adhesion of P-fimbriated *E. coli* to erythrocytes.[29] Tea and hop bract polyphenols and red wine proanthocyanidins have also been identified as inhibitors of buccal epithelial adhesion.[30−32] Pectic oligosaccharides extracted from orange albedo have recently been shown to be invasion inhibitors of *Campylobacter jejuni* to Caco-2 cells but to have no significant effect on the adhesion of bacteria to colonic cells.[33]

Resveratrol has been reported to exert a number of health benefits.[24] However, like other phenolics, resveratrol is rapidly absorbed and conjugated by phase II enzymes to yield mostly sulfate and glucuronate derivatives, which reduces resveratrol delivery to the distal parts of the gut and decreases its topical effectiveness in the mucosa. In this context, our group synthesized a number of resveratrol derivatives, which were much more effective than resveratrol in the prevention of intestinal inflammation.[25] Bearing this in mind, we hypothesized that these compounds, especially those with glucosylacyl residues, could improve the resveratrol efficacy including the antimicrobial properties in distal parts of the intestine. In addition, we also explored the ability of the compound resveratrol 3-*O*-glucuronide (RES-glucur) because this metabolite is very relevant in the lumen of the intestine after the intake of resveratrol. [34]

In the present study, we show for the first time the potential of resveratrol, and especially some glucosylacyl derivatives, to reduce the adherence of foodborne pathogens to intestinal cells. However, further research should be done to determine the mechanistic pathway of these compounds to reduce foodborne pathogen adherence to intestinal cells and corroborate it by means of in vivo studies in which the indigenous microbiota is present. Recently, our research group demonstrated that resveratrol is able to increase the level of lactobacilli and bifidobacteria in intestinal bowel disease murine models.[35] Competition of commensal and probiotic bacteria including lactobacilli and bifidobacteria species with pathogens for adhesion and colonization is one of the important protective mechanisms of the gastrointestinal tract.[21,36] Probiotics are able to prevent infections by pathogens when sufficient numbers are present in the intestinal flora.[9,37−39] Taking into account the potential of several species of lactobacilli and bifidobacteria to reduce adherence to and invasion of pathogens of the intestinal cells, the action mechanism of resveratrol against pathogen infection could be direct inhibition of adhesion and also indirect promotion of lactobacilli and bifidobacteria colonic population with pathogen antiadhesion properties. Some resveratrol derivatives synthesized in our laboratory, such as BUT and OCT, exerted higher efficacy with respect to other resveratrol derivatives for inhibiting *L. monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium adhesion to HT-29 cells.

The production of host cytokines such as IL-8 in the infected epithelial tissue can be determined as a measure for virulence because secretion of, for example, IL-8 as a response against foodborne pathogens can affect intestinal homeostasis and cause diarrhea and chronic inflammation.[40] Our data in this study clearly indicate that the better adherence ability of *L. monocytogenes* Scott A compared to *E. coli* O157:H7 and *S.* Typhimurium was associated with a higher IL-8 production by HT-29 cells. Furthermore, the higher efficacy of BUT and OCT (25 μM) with respect to other resveratrol derivatives (25 μM) for inhibiting *L. monocytogenes* adhesion also was correlated to a higher efficacy of BUT and OCT inhibiting IL-8 secretion by HT-29. These results suggest that *L. monocytogenes*-induced IL-8 secretion is not the consequence of a soluble secreted bacterial factor and needs direct contact between bacteria and cells as occurred with other pathogens such as *Helicobacter pullorum*.[41] This anti-inflammatory effect is in agreement with our previous study in which the feeding of mice with a very low dose (equivalent to 10 mg for a 70 kg person) of BUT or OCT drastically prevented colitis symptoms and improved 6-fold the disease activity index compared to resveratrol in a murine colitis model.[25] In the present study, to confirm the higher efficacy of BUT and OCT with respect to resveratrol and other resveratrol derivatives for inhibiting IL-8 secretion by HT-29 even at 25 μM, higher concentrations of BUT and OCT were assayed. Higher concentrations of BUT and OCT (50 and 100 μM) reduced further IL-8 secretion with respect to BUT and OCT concentrations of 25 μM. We did not observe any sign of cytotoxicity as floating or detached cells at the assayed concentrations of resveratrol and derivatives even after exposure to 50 and 100 μM OCT and BUT. Furthermore, previous studies in HT-29 cells have found that the IC_{50} for a 72 h treatment was 80 μM resveratrol[42] and that BUT and OCT have less cytotoxicity than their parent compound,

resveratrol.[25] Therefore, OCT and BUT concentrations of 50 and 100 μM, although high, are effective in reducing IL-8 secretion.

The results of the present study suggest that one mechanism for the beneficial attributes of resveratrol and especially BUT and OCT could be the ability to inhibit the adhesion and consequently cytokine production in intestinal epithelial cells as a response to foodborne pathogen adhesion. Its potential use in the prevention of foodborne infections, intestinal homeostasis loss, and inflammatory bowel diseases could be another step in finding coadjuvants or alternatives to antibiotic treatments. These results reinforce the concept of resveratrol as a beneficial dietary compound to prevent intestinal infections at doses possibly attainable with resveratrol-enriched nutraceuticals.

AUTHOR INFORMATION

Corresponding Author

*E-mail: mvselma@cebas.csic.es. Phone: +34 968 396102. Fax: +34 968 396213.

Funding

This work has been supported by Projects 200670F0131 (CSIC) and CICYT-BFU2007-60576 and CSD2007-00063 (Fun-C-Food; Consolider Ingenio 2010). M.L. and R.L. are holders of a JAE-DOC grant from CSIC, and M.V.S holds a Ramón y Cajal contract from MCINN (Spain).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) EFSA. The Community Summary Report on Food-borne Outbreaks in the European Union in 2007. *EFSA J.* **2009**, *271*, 3−128.

(2) Blaser, M. J. Bacteria and diseases of unknown cause: hemolytic-uremic syndrome. *J. Infect. Dis.* **2004**, *189*, 552−555.

(3) WHO. Foodborne listeriosis. *Bull. WHO* **1990**, *66*, 421−428.

(4) EFSA. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union in 2006. *EFSA J.* **2007**, *130*, 1−2.

(5) Ofek, I.; Hasty, D. L.; Sharon, N. Anti-adhesion therapy of bacterial diseases: prospects and problems. *FEMS Immunol. Med. Microbiol.* **2003**, *38*, 181−191.

(6) Jankowska, A.; Laubitz, D.; Antushevich, H.; Zabielski, R.; Grzesiuk, E. Competition of *Lactobacillus paracasei* with *Salmonella enterica* for adhesion to Caco-2 cells. *J. Biomed. Biotechnol.* **2008**, 1−6.

(7) Hohmann, A. W.; Schmidt, G.; Rowley, D. Intestinal colonization and virulence of *Salmonella* in mice. *Infect. Immun.* **1978**, *22*, 763−770.

(8) Ceponis, P. J.; Riff, J. D.; Sherman, P. M. Epithelial cell signaling responses to enterohemorrhagic *Escherichia coli* infection. *Mem. Inst. Oswaldo Cruz* **2005**, *100*, 199−203.

(9) Moroni, O.; Kheadr, E.; Boutin, Y.; Lacroix, C.; Fliss, I. Inactivation of adhesion and invasion of food-borne *Listeria monocytogenes* by bacteriocin-producing *Bifidobacterium* strains of human origin. *Appl. Environ. Microbiol.* **2006**, *72*, 6894−6901.

(10) Vásquez-Boland, J. A.; Kuhn, M.; Berche, P.; Chakraborty, T.; Domínguez-Bernal, G.; Goebel, W.; González-Zorn, B.; Wehland, J.; Kreft, J. *Listeria* pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev.* **2001**, *14*, 584−640.

(11) Li, C. K. F.; Seth, R.; Gray, T.; Bayston, R.; Mahida, Y. R.; Wakelin, D. Production of proinflammatory cytokines and inflammatory mediators in human intestinal epithelial cells after invasion by *Trichinella spiralis*. *Infect. Immun.* **1998**, *66*, 2200−2206.

(12) Jung, H. C.; Eckmann, L.; Yang, S. K.; Panja, A.; Fierer, J.; Morzycka-Wroblewska, E.; Kagnoff, M. F. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. *J. Clin. Invest.* **1995**, *95*, 55−65.

(13) Zhou, X.; Giron, J. A.; Torres, A. G.; Crawford, J. A.; Negrete, E.; Vogel, S. N.; Kaper, J. B. Flagellin of enteropathogenic *Escherichia coli* stimulates interleukin 8 production in T84 cells. *Infect. Immun.* **2003**, *71*, 2120−2129.

(14) Hata, K.; Andoh, A.; Sato, H.; Araki, Y.; Tanaka, M.; Tsujikawa, T.; Fujiyama, Y.; Bamba, T. Sequential changes in luminal microflora and mucosal cytokine expression during developing of colitis in HLA-B27/beta2- microglobulin transgenic rats. *Scand. J. Gastroenterol.* **2001**, *36*, 1185−1192.

(15) Hecht, G.; Savkovic, S. D. Effect or role of epithelia in inflammation: interaction with bacteria. *Aliment. Pharm. Ther.* **1997**, *3*, 64−69.

(16) Casellas, F.; Borruel, N.; Papo, M.; Guarner, F.; Antolin, M.; Videla, S.; Malagelada, J. R. Antiinflammatory effects of enterically coated amoxicillin clavulanic acid in active ulcerative colitis. *Inflamm. Bowel Dis.* **1998**, *4*, 1−5.

(17) Helms, M.; Vastrup, P.; Gerner-Smidt, P.; Molbak, K. Excess mortality associated with antimicrobial drug-resistant *Salmonella* Typhimurium. *Emerg. Infect. Dis.* **2002**, *8*, 490−495.

(18) White, D. G.; Zhao, S.; Sudler, R.; Ayers, S.; Friedman, S.; Chen, S.; McDermott, P. F.; McDermott, S.; Wagner, D. D.; Meng, J. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *New Engl. J. Med.* **2001**, *345*, 1147−1154.

(19) Wong, C.; Lelacic, S.; Habeeb, R. L.; Watkins, S. L.; Tarr, P. I. The risk of the hemolytic−uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infection. *New Engl. J. Med.* **2000**, *342*, 1930−1936.

(20) Burkholder, K. M.; Kim, K. P.; Mishra, K. K.; Medina, S.; Hahm, B. K.; Kim, H.; Bhunia, A. K. Expression of LAP, an SecA2-dependent secretory protein, is induced under anaerobic environment. *Microbes Infect.* **2009**, *11*, 859−867.

(21) Eutamene, H.; Bueno, L. Role of probiotics in correcting abnormalities of colonic flora induced by stress. *Gut* **2007**, *56*, 1495−1497.

(22) Patterson, J. A.; Burkholder, K. M. Application of prebiotics and probiotics in poultry production. *Poult. Sci.* **2003**, *82*, 627−631.

(23) Requena, T.; Monagas, M.; Pozo-Bayón, M. A.; Martín-Álvarez, P. J.; Bartolomé, B.; del Campo, R.; Ávila, M.; Martínez-Cuesta, M. C.; Peláez, C.; Moreno-Arribas, M. V. Perspectives of the potential implications of wine polyphenols on human oral and gut microbiota. *Trends Food Sci. Technol.* **2010**, *21*, 332−344.

(24) Vang, O.; Ahmad, N.; Baile, C. A.; Baur, J. A.; Brown, K.; Csiszar, A.; Das, D. K.; Delmas, D.; Gottfried, C.; Lin, H. Y.; Ma, Q. Y.; Mukhopadhyay, P.; Nalini, N.; Pezzuto, J. M.; Richard, T.; Shukla, Y.; Surh, Y. J.; Szekeres, T.; Szkudelski, T.; Walle, T.; Wu, J. M. What is new for an old molecule? Systematic review and recommendations on the use of resveratrol. *PLoS One* **2011**, *6*, e19881.

(25) Larrosa, M.; Tomé-Carneiro, J.; Yáñez-Gascón, M. J.; Alcántara, D.; Selma, M. V.; Beltrán, D.; García-Conesa, M.; Urban, C.; Lucas, R.; Tomás-Barberán, F. A.; Morales, J. C.; Espín, J. C. Preventive oral treatment with resveratrol pro-prodrugs drastically reduce colon inflammation in rodents. *J. Med. Chem.* **2010**, *53*, 7365−7376.

(26) Lucas, R.; Alcántara, D.; Morales, J. C. A concise synthesis of glucuronide metabolites of urolithin-B, resveratrol, and hydroxytyrosol. *Carbohydr. Res.* **2009**, *344*, 1340−1346.

(27) Beuzón, C. R.; Salcedo, S. P.; Holden, D. W. Growth and killing of a *Salmonella enterica* serovar Typhimurium sifA mutant strain in the cytosol of different host cell lines. *Microbiology* **2002**, *148*, 2705−2715.

(28) Kim, S. H.; Wei, C. I. Invasiveness and intracellular growth of multidrug-resistant *Salmonella* and other pathogens in Caco-2 cells. *J. Food Sci* **2007**, *72*, M72−M78.

(29) Foo, L. Y.; Lu, Y.; Howell, A. B.; Vorsa, N. A-type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated *Escherichia coli*. *J. Nat. Prod.* **2000**, *63*, 1225−1228.

(30) Daglia, M.; Stauder, M.; Papetti, A.; Signoretto, C.; Giusto, G.; Canepari, P.; Pruzzo, C.; Gazzani, G. Isolation of red wine components with anti-adhesion and anti-biofilm activity against *Streptococcus mutans*. *Food Chem.* **2010**, *119*, 1182−1188.

(31) Ooshima, T.; Minami, T.; Aono, W.; Izumitani, A.; Sobue, S.; Fujiwara, T.; Kawabata, S.; Hamada, S. Oolong tea polyphenols inhibit experimental dental caries in SPF rats infected with mutans streptococci. *Caries Res.* **1993**, *27*, 124−129.
(32) Tagashira, M.; Uchiyama, K.; Yoshimura, T.; Shirota, M.; Uemitsu, N. Inhibition by hop bract polyphenols of cellular adherence and water-insoluble glucan synthesis of mutans streptococci. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 332−335.
(33) Ganan, M.; Collins, M.; Rastall, R.; Hotchkiss, A. T.; Chau, H. K.; Carrascosa, A. V.; Martinez-Rodriguez, A. J. Inhibition by pectic oligosaccharides of the invasion of undifferentiated and differentiated Caco-2 cells by *Campylobacter jejuni*. *Int. J. Food Microbiol.* **2010**, *137*, 181−185.
(34) Azorín-Ortuño, M.; Yáñez-Gascón, M. J.; Vallejo, F.; Pallarés, F. J.; Larrosa, M.; Lucas, R.; Morales, J. C.; Tomás-Barberán, F. A.; García-Conesa, M. T.; Espín, J. C. Metabolites and tissue distribution of resveratrol in the pig. *Mol. Nutr. Food Res.* **2011**, *55*, 1154−1168.
(35) Larrosa, M.; Yañez-Gascón, M. J.; Selma, M. V.; González-Sarrías, A.; Toti, S.; Cerón, J. J.; Tomás-Barberán, F. A.; Dolara, P.; Espín, J. C. Effect of a low dose of dietary resveratrol on colon microbiota, inflammation and tissue damage in a DSS-induced colitis rat model. *J. Agric. Food Chem.* **2009**, *57*, 2211−2220.
(36) Zareie, M.; Johnson-Henry, K.; Jury, J.; Yang, P. C.; Ngan, B. Y.; McKay, D. M.; Soderholm, J. D.; Perdue, M. H.; Sherman, P. M. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut* **2006**, *55*, 1553−1560.
(37) Burkholder, K. M.; Bhunia, A. K. *Salmonella enterica* serovar Typhimurium adhesion and cytotoxicity during epithelial cell stress is reduced by *Lactobacillus rhamnosus* GG. *Gut Pathog.* **2009**, *1*, 14.
(38) Ingrassia, I.; Leplingard, A.; Darfeuille-Michaud, A. *Lactobacillus casei* DN-114 001 inhibits the ability of adherent-invasive *Escherichia coli* isolated from Crohn's disease patients to adhere to and to invade intestinal epithelial cells. *Appl. Environ. Microbiol.* **2005**, *71*, 2880−2887.
(39) Johnson-Henry, K. C.; Hagen, K. E.; Gordonpour, M.; Tompkins, T. A.; Sherman, P. M. Surface-layer protein extracts from *Lactobacillus helveticus* inhibit enterohaemorrhagic *Escherichia coli* O157:H7 adhesion to epithelial cells. *Cell. Microbiol.* **2007**, *9*, 356−367.
(40) Oliveira, M.; Wijnands, L.; Abadias, M.; Aarts, H.; Franz, E. Pathogenic potential of *Salmonella* Typhimurium DT104 following sequential passage through soil, packaged fresh-cut lettuce and a model gastrointestinal tract. *Int. J. Food Microbiol.* **2011**, *148*, 149−155.
(41) Varon, C.; Duriez, A.; Lehours, P.; Ménard, A.; Layé, S.; Zerbib, F.; Mégraud, F.; Laharie, D. Study of *Helicobacter pullorum* proinflammatory properties on human epithelial cells *in vitro*. *Gut* **2009**, *58*, 629−635.
(42) Juan, M. E.; Wenzel, U.; Daniel, H.; Planas, J. M. Resveratrol induces apoptosis through ROS-dependent mitochondria pathway in HT-29 human colorectal carcinoma cells. *J. Agric. Food Chem.* **2008**, *56*, 4813−4818.