Highlights

Resveratrol and some derivatives inhibit the adhesion of foodborne pathogens (*S.*Typhimurium, *E. coli* O157:H7 and *L. monocytogenes*) to colonic cells. >The glucosyl-acyl derivatives resveratrol-3-*O*-(6'-*O*-butanoyl)-β-D-glucopyranoside and resveratrol-3-*O*-(6'-*O*-octanoyl)-β-D-glucopyranoside reduce IL-8 expression by colonic cells infected with *L. monocytogenes*.> The potential use of these compounds in the prevention of food-borne infections, intestinal homeostasis loss and inflammatory bowel diseases could be another step in finding coadjuvants or alternatives to antibiotic treatments.

Resveratrol and some glucosyl-, glucosyl-acyl- and glucuronide derivatives reduce *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes* Scott A adhesion to colonic epithelial cell lines

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ABSTRACT

The efficacy of resveratrol, naturally occurring in grapes and wine, and some glucosyl-, glucosyl-acyl and glucuronide derivatives, in inhibiting adhesion of *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A to Caco-2 and HT-29 colonic cells was investigated. *E. coli* O157:H7, *S.* Typhimurium and *L. monocytogenes* Scott A were capable of adhering to the human colonic epithelial cell types tested, which responded producing the pro-inflammatory interleukin 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%). The presence of resveratrol and most of the derivatives tested did not alter interleukin 8 expression by HT-29 infected with *L. monocytogenes* with the exception of resveratrol-3-*O*-(6'-*O*-butanoyl)-β-D-glucopyranoside (BUT) and resveratrol-3-*O*-(6'-*O*-octanoyl)-β-D-glucopyranoside (OCT). BUT concentrations of 50 and 100 μM reduced IL-8 secretion by 44 and 74%, respectively while OCT concentration of 50 μM reduced IL-8 secretion by 100%. The results of the present study 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 food-borne infections, intestinal homeostasis loss and inflammatory bowel diseases could be another step in finding coadjuvants or alternatives to antibiotic treatments.

***Keywords*: Polyphenols, food-borne pathogens, intestinal health, Caco-2 cells, HT-29 cells, interleukin 8.**

1. Introduction

Food-borne illnesses present a significant health problem throughout the world. In the latest EFSA report on foodborne outbreaks (EFSA, 2009), *Salmonella* was, as in previous years, the most commonly reported cause of food-borne outbreaks in the European Union. *Salmonella* which cause gastroenteritis in humans were responsible for 39.2% of all reported outbreaks in 2007. Pathogenic *Escherichia coli* were responsible for a lower percentage of foodborne outbreaks (1.2%). However, humans infected by this pathogen may have varying 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 (Blaser, 2004). *Listeria* infections in humans can also have a high case fatality ratio in industrialised 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 *L. monocytogenes* on a worldwide basis (WHO, 1990). Indeed, on average, *Listeria* was the most severe pathogen associated with European outbreaks in 2006 (EFSA, 2007). Nevertheless, *Listeria* was only indicated as the causative agent in 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 the first stage in any infectious process and is an important and critical step for colonization (Ofek et al., 2003; Jankowska et al., 2008). 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 (Hohmann et al., 1978). *E. coli* O157:H7 also adheres intimately and interacts with intestinal epithelial cells to cause cytoskeletal rearrangements, which result in attachment lesions and increased epithelial monolayer permeability (Ceponis et al., 2005). 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 (Moroni et al., 2006). *L. monocytogenes* adhesion to and invasion of intestinal epithelial cells and the subsequent translocation to distant organs are critical in establishing a systemic infection in a host (Vásquez-Boland et al., 2001). Therefore, the ability of *L. monocytogenes* to invade epithelial cells correlates with bacterial virulence (Moroni et al., 2006). Epithelial cell invasion by intestinal pathogens provide early signals for the acute mucosal inflammatory response via the release of proinflammatory cytokines and inflammatory mediators (Li et al., 1998). Human colonic epithelial cell lines produce *in vitro* a wide range of proinflammatory cytokines in response to microbial pathogens such as *E. coli, L. monocytogenes*, *Salmonella enteritidis, Shigella dysenteriae*, and *Yersinia enterocolitica* (Jung et al., 1995). 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, predominantly interleukin 8 (IL-8) (Zhou et al., 2003). 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 characterised by high levels of IL-8 and is observed in several intestinal disorders like ulcerative colitis, pouchitis, and Crohn's disease (Hecht and Savkovic, 1997; Hata et al., 2001). Interventions that decrease these levels have been shown to significantly alleviate the conditions (Casellas et al., 1998).

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 (Helms et al., 2002). 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 multidrug-resistant strains (White et al., 2001). 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 haemolytic uremic syndrome, perhaps by promoting the

113 release of pre-formed toxin from the periplasm (Wong et al., 2000). For these reasons, different
114 alternatives or coadjuvants to antibiotic treatment are being investigated.

115 Inhibition of pathogen adhesion to the intestinal epithelium may prevent colonization and limit
116 opportunity for systemic infection (Finlay, 1997; Burkholder et al., 2009). Despite numerous studies
117 having demonstrated the antipathogenic properties of probiotics, their effectiveness in reducing intestinal
118 infection varies depending on which probiotic organism is used, as well as the health status of the host
119 (Patterson and Burkholder, 2003; Eutamene and Bueno, 2007). Efficient bacteriostatic agents have also
120 been identified in foodstuffs such as wine polyphenols (Requena et al., 2010). Foodstuffs containing
121 inhibitors with bacteria anti-adhesion agents may also be expected to emerge. These compounds do not
122 act by killing or arresting growth of the pathogen, as, for example, antibiotics do. Therefore, the spread of
123 bacteria resistant to the anti-adhesion agent is expected to occur at significantly lower frequencies than
124 that of bacteria resistant to antibiotics (Ofek et al., 2003).

125 Resveratrol, (3,5,4´-trihydroxy-*trans*-stilbene), naturally occurring in grapes and grape-derived
126 foodstuffs such as red wine, has been reported to exert many different health-promoting effects including
127 antioxidant, anti-inflammatory, antitumor, anti-platelet aggregation, cardioprotective, aging-delay, anti-
128 obesity and bactericidal properties (Vang et al., 2011). Recently, our group demonstrated that some
129 glucosyl-acyl resveratrol derivatives were much more effective than resveratrol to prevent intestinal
130 inflammation *in vivo* (Larrosa et al., 2010). In the present study, we explore the efficacy of resveratrol,
131 and some glucosyl-, glucosyl-acyl- and glucuronide resveratrol derivatives, to inhibit adhesion of
132 *Salmonella* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* Scott A to Caco-2 and HT-29 colonic
133 cells with the objective of finding potential alternatives to prevent and control human infections.
134 Furthermore, we investigated whether resveratrol and its derivatives can alter the IL-8 production induced
135 by food-borne pathogens.

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2. Materials and methods

2.1. Resveratrol and derivatives

Resveratrol (*trans*-resveratrol), and different glucosyl-, glucosyl-acyl- and glucuronide resveratrol derivatives, were assayed in the present study (**Figure 1**), i.e. 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. (2010), except RES-glucur (Lucas et al. 2009). The synthesis process of glucosylated and acyl-glucosyl-resveratrol derivatives and their use as anti-inflammatory compounds is patented (PCT/ES2010/070826).

2.2. 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, United Kingdom) and grown to confluence in 24-well plates (Costar, High Wycombe, United Kingdom). 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).

2.3. Bacterial cultures

Strains of *S. enteritidis* serovar Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* Scott A were used in this study. *S.* Typhimurium (NCTC 12023) which contained a plasmid pFVP25.1,

carrying gfpmut3A under the control of constitutive promoter for fluorescence visualization was kindly provided by Dr. Beuzón (Beuzón et al., 2002). *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, United Kingdom) while *L. monocytogenes* Scott A was grown in TSB (Oxoid, Basingtoke, United Kingdom) 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.

2.4. 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 x 10^5 and 6 x 10^5, HT-29 and Caco-2 cells respectively). One day prior to the assay the cells were washed twice with phosphate buffered saline (PBS) and incubated in antibiotic and serum free DMEM or EMEM medium, respectively. To determine the effect of resveratrol and its glucosyl-acyl derivatives on adhesion of *S.* Typhimurium*, E. coli* O157:H7 *and L. monocytogenes* Scott A to HT-29 and Caco-2 cell monolayers, 1 mL DMEM or EMEM medium containing 5 µL of the assayed compound (25 µM final concentration) and 5 µL bacterial inocula (10^7 cfu) were maintained for 1 h at room temperature. Following this pre-incubation, three wells of HT-29 and Caco-2 cell monolayers, respectively were filled with 300 µL of these suspensions and microtiter plates were incubated for 2 h at 37 °C in a 5% CO_2 humidified atmosphere. HT-29 and Caco-2 cell monolayers were washed three times with 1 mL PBS and resuspended in 100 µL of PBS. Cells were lysed with 1 mL of distilled water with 20 % glycerol and cells were frozen at -70 ºC for bacteria counting. Adhered *S.* Typhimurium and *E. coli* O157:H7 were determined by serial dilution and cultured on plates of nutrient

agar while adhered *L. monocytogenes* were counted in BHI agar. Results were expressed as the percentage of bacteria adhered relative to an adherence control (ARC).

2.5. IL-8 production

Bacterial inflammatory effect was assessed 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 x 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 monolayers were used to determine the effect of resveratrol and its glucosyl-acyl derivatives on bacteria-induced production of IL-8. 1200 µL of DMEM medium containing 6 µL of the assayed compound (25 µM final concentration) and 6 µL bacterial inocula (10^7 cfu) were maintained for 1 h at room temperature. Following this pre-incubation, five wells of HT-29 cell monolayers, 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 10,000 *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 positive for IL-8.

2.6. Statistical analysis

Each adhesion and cytokine expression assay was repeated on three separated 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).

3. Results

3.1. Adherence of food-borne pathogenic bacteria to Caco-2 and HT-29 cell cultures

The efficiencies of adhesion of *Escherichia 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% to 8.8%. 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% and 5.4% with Caco-2 and HT-29 cells, respectively. Levels of adhesion of *E. coli* O157:H7 and *S.* Typhimurium ranged from 1.1% to 1.4% with both human intestinal cell lines.

3.2. Effect of resveratrol and derivatives on the adherence of food-borne pathogenic bacteria to Caco-2 and HT-29 cell cultures

We sought to determine the influence of resveratrol and some derivatives some glucosylated, glucosyl-acyl and glucuronide derivatives on adhesion of food-borne 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 specie and the resveratrol derivative applied. Adhesion inhibition of *E. coli* O157:H7 was ≥60% for most of the resveratrol derivatives applied. Lower adhesion inhibition was observed in the case of *S.* Typhimurium where most of resveratrol derivatives achieved an inhibition rate ≥40%. The lowest adhesion inhibition was observed for *L. monocytogenes* Scott A (≥20% for most of 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 efficacy of the different resveratrol derivatives for inhibiting each bacteria adhesion was 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**).

3.3. 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, *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 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 concentration was 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, citotoxicity 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 14 h were needed for observing 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.

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 HT-29 model, was exposed to a low concentration of resveratrol and some glucosylated, glucosyl-acyl and glucuronide derivatives derivatives (25 µM) for 1 h prior to HT-29 cell infection. After 6 h of infection with *L. monocytogenes*, IL-8 concentration was 418±20 pg/mL. The presence of resveratrol and most of derivatives tested did not alter IL-8 expression by HT-29 infected with *L. monocytogenes* (**Table 1**). However, a significant decrease of IL-8 production ($P \leq 0.05$) was observed when the HT-29 cells were infected with *L. monocytogenes* pre-treated 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**). BUT concentrations of 50 and 100 µM reduced IL-8 secretion by 44 and 74%, respectively. 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**).

4. Discussion

A wide range of intestine and food-borne bacteria interactions can ultimately lead to disease. However, the adhesion of bacterial cells to the intestinal epithelium is generally considered to be the first step in pathogenesis preceding invasion (Ofek et al., 2003; Jankowska et al., 2008). 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 (Moroni et al. 2006), differences between adhesion of some bacteria such as *L. monocytogenes* on Caco-2 cells and adhesion on HT-29 cells was noted. *L. monocytogenes* was the best performing specie 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 (2007) showed that *L. monocytogenes* 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. (2006) 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 where the level of adhesion ranged from 0.01 to 9.45% (Moroni et al. 2006). This difference in adhesion capacity as well as in invasion ability may explain the difference in virulence among *L. monocytogenes* strains.

Plant materials possessing anti-adhesion activities are attractive candidates for antibacterial agents. There is, however, a relative paucity of information regarding the anti-adhesive properties of most plant

294 materials. In the present study, we examined the abilities of the polyphenol resveratrol and some
295 derivatives to block the adherence of three food-borne pathogens with different adhesion potentials to
296 human colonic cells. Our data clearly indicated that resveratrol and most of the derivatives tested inhibit
297 the adherence of *S.* Typhimurium, *E. coli* O157:H7 and *L. monocytogenes* to human colonic cells. We
298 observed higher adhesion inhibition (≥60% and ≥40%) in the case of the bacteria with lower adherence
299 potential (*E. coli* O157:H7 and *S.* Typhimurium, respectively) than adhesion inhibition level (≥20%) of the
300 bacteria with higher adherence potential (*L. monocytogenes*). Other phenolic compounds, especially
301 those obtained from cranberry (*Vaccinium macrocarpon*) have been analyzed with respect to their anti-
302 adhesion activities (Ofek et al., 2003). Extracts of cranberries containing proanthocyanidins in their
303 condensed form inhibited adhesion of P fimbriated *E. coli* to erythrocytes (Foo et al., 2000). Tea and hop
304 bract polyphenols have also been identified as inhibitors of buccal epithelial adhesion (Ooshima et al.,
305 1993; Tagashira et al., 1997). Pectin oligosaccharides extracted from orange albedo have recently been
306 shown to be invasion inhibitors of *Campylobacter jejuni* to Caco-2 cells but to have no significant effect
307 on the adhesion of bacteria to colonic cells (Ganan et al., 2010).

308 Resveratrol has been reported to exert a number of health benefits (Vang et al., 2011). However, like
309 other phenolics, resveratrol is rapidly absorbed and conjugated by Phase II enzymes to yield mostly
310 sulphate and glucuronate derivatives, which reduces resveratrol delivery to the distal parts of the gut and
311 decreases its topical effectiveness in the mucosa. In this context, our group synthesized a number of
312 resveratrol derivatives which were much more effective than resveratrol in the prevention of intestinal
313 inflammation (Larrosa et al., 2010). Bearing this in mind, we hypothesized that these compounds,
314 especially those with glucosyl-acyl-residues could improve the resveratrol efficacy including the
315 antimicrobial properties in distal part of the intestine. In addition, we also explored the ability of the
316 compound resveratrol 3-*O*- glucuronide (RES-glucur) since this metabolite is very relevant in the lumen of
317 the intestine after the intake of resveratrol (Azorín-Ortuño et al., 2011).

318 In the present study, we show for the first time the potential of resveratrol, and especially some
319 glucosyl-acyl derivatives, to reduce the adherence of food-borne pathogens to intestinal cells. Recently,

320 our research group demonstrated that resveratrol is able to increase the level of lactobacilli and
321 bifidobacteria in intestinal bowel disease murine models (Larrosa et al., 2009; Larrosa et al., 2010).
322 Competition of commensal and probiotic bacteria including lactobacilli and bifidobacteria species with
323 pathogens for adhesion and colonization is one of the important protective mechanisms of the
324 gastrointestinal tract (Zareie et al., 2006; Eutamene and Bueno, 2007). Probiotics are able to prevent
325 infections by pathogens when sufficient numbers are present in the intestinal flora (Ingrassia et al., 2005;
326 Moroni et al., 2006; Johnson-Henry et al., 2007; Burkholder and Bhunia, 2009). Taking into account the
327 potential of several species of lactobacilli and bifidobacteria to reduce adherence and invasion of
328 pathogens to the intestinal cells, the action mechanism of resveratrol against pathogen infection could be
329 directly inhibiting adhesion and also indirectly promoting lactobacilli and bifidobacteria colonic population
330 with pathogen antiadhesion properties. Some resveratrol derivatives synthetized in our laboratory such
331 as BUT and OCT, exerted higher efficacy with respect to other resveratrol derivatives, for inhibiting *L.*
332 *monocytogenes*, *E. coli* O157:H7 and *S.* Typhimurium adhesion to HT-29 cells.

333 The production of host cytokines like IL-8 in the infected epithelial tissue can be determined as a
334 measure for virulence since secretion of such as IL-8 as a response against food-borne pathogens can
335 affect intestinal homeostasis and causes diarrhea and chronic inflammation (Oliveira et al., 2011). Our
336 data in this study clearly indicate that the better adherence ability of *L. monocytogenes* Scott A compared
337 to *E. coli* O157:H7 and *S.* Typhimurium was associated with a higher IL-8 production by HT-29 cells.
338 Furthermore, the higher efficacy of BUT and OCT respect to other resveratrol derivatives for inhibiting *L.*
339 *monocytogenes* adhesion also was correlated to a higher efficacy of BUT and OCT inhibiting IL-8
340 secretion by HT-29. This anti-inflammatory effect is in agreement with our previous study in which, mice
341 feeding with a very low dose (equivalent to 10 mg for a 70 kg-person) of BUT or OCT drastically
342 prevented colitis symptoms and improved 6-fold the disease activity index compared to resveratrol in a
343 murine colitis model (Larrosa et al., 2010). The results of the present study suggest that one mechanism
344 for the beneficial attributes of resveratrol and especially BUT and OCT could be the ability to inhibit the
345 adhesion and consequently cytokine production in intestinal epithelial cells as a response to food-borne

pathogen adhesion. Its potential use in the prevention of food-borne infections, intestinal homeostasis loss and inflammatory bowel diseases could be another step in finding coadjuvants or alternatives to antibiotic treatments.

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467

468 **FIGURE CAPTIONS**

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

473 **Figure 2.** Effect of resveratrol and derivatives on adhesion of food-borne pathogens to Caco-2 (A) and
474 HT-29 (B) cells. The results represent the mean values of relative adhesion compared to control in
475 absence of resveratrol, and the standard error of the means for three different experiments. Different
476 letters above columns indicate that the values of inhibition are significantly different ($P \leq 0.05$). (**1**, RES)
477 *trans*-resveratrol; (**2**, PIC) *trans*-piceid, (**3**, DIGLUC) *trans*-resveratrol-3,5-di-*O*-β-D-glucopyranoside; (**4**,
478 BUT) *trans*-resveratrol-3-*O*-(6'-*O*-butanoyl)-β-D-glucopyranoside; (**5**, OCT) *trans*-resveratrol-3-*O*-(6'-*O*-
479 octanoyl)-β-D-glucopyranoside; (**6**, RES-glucur) *trans*-resveratrol-3-*O*-glucuronide.

480 **Figure 3.** Visualization of *Salmonella* Typhimurium adhesion on Caco-2 cells using a Nikon Diaphot-TMD
481 microscope equipped with fluorescence. (A) Phase contrast image (magnification X 200) of bacteria
482 inoculated on Caco-2 cells. (B) Fluorescence microscopy of bacterial adhesion on Caco-2 cells in the
483 absence of resveratrol; Fluorescence images of bacterial adhesion on Caco-2 in the presence of
484 resveratrol (C), and *trans*-resveratrol-3-*O*-(6'-*O*-octanoyl)-β-D-glucopyranoside, OCT (D). *S.* Typhimurium
485 expressed GFP constitutively.

486 **Figure 4.** Time-course induction of interleukin 8 (IL-8) synthesis by *Escherichia coli* O157:H7, *Listeria*
487 *monocytogenes* Scott A and *Salmonella* Typhimurium in HT-29 cells. Significant differences ($P \leq 0.05$)
488 between the IL-8 levels of cells exposed to food-borne pathogens and control cells are indicated by
489 letters.

490 **Figure 5.** HT-29 culture during incubation with different food-borne pathogens. (A) *Escherichia coli*
491 O157:H7, (B*) Listeria monocytogenes* Scott A and (C) *Salmonella* Typhimurium.

492

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).

519 **Table 1.** Interleukin 8 secretion in HT-29 cells exposed to *Listeria monocytogenes* Scott A in presence of
520 resveratrol and derivatives.
521
522

ELICITOR	TREATMENT (25 µM)	IL-8 SECRETION (pg/mL)
None	-	29 ± 7
Listeria monocytogenes	-	418 ± 20
Listeria monocytogenes	Resveratrol	442 ± 2
Listeria monocytogenes	PIC	419 ± 35
Listeria monocytogenes	DIGLUC	360 ± 58
Listeria monocytogenes	BUT	315 ± 68 *
Listeria monocytogenes	OCT	289 ± 77 *
Listeria monocytogenes	RES-glucur	409 ± 5
TNF-α (2 ng/mL)		1535 ± 1.2

523
524 PIC, piceid; DIGLUC, resveratrol-diglucoside; BUT, piceid butyrate; OCT, piceid octanoate; RES-glucur,
525 resveratrol glucuronide. $P \leq 0.05$. *Significantly different from *Listeria monocytogenes* Scott A exposed
526 cells in absence of resveratrol compounds.
527

Figure 1

Fig. 1.

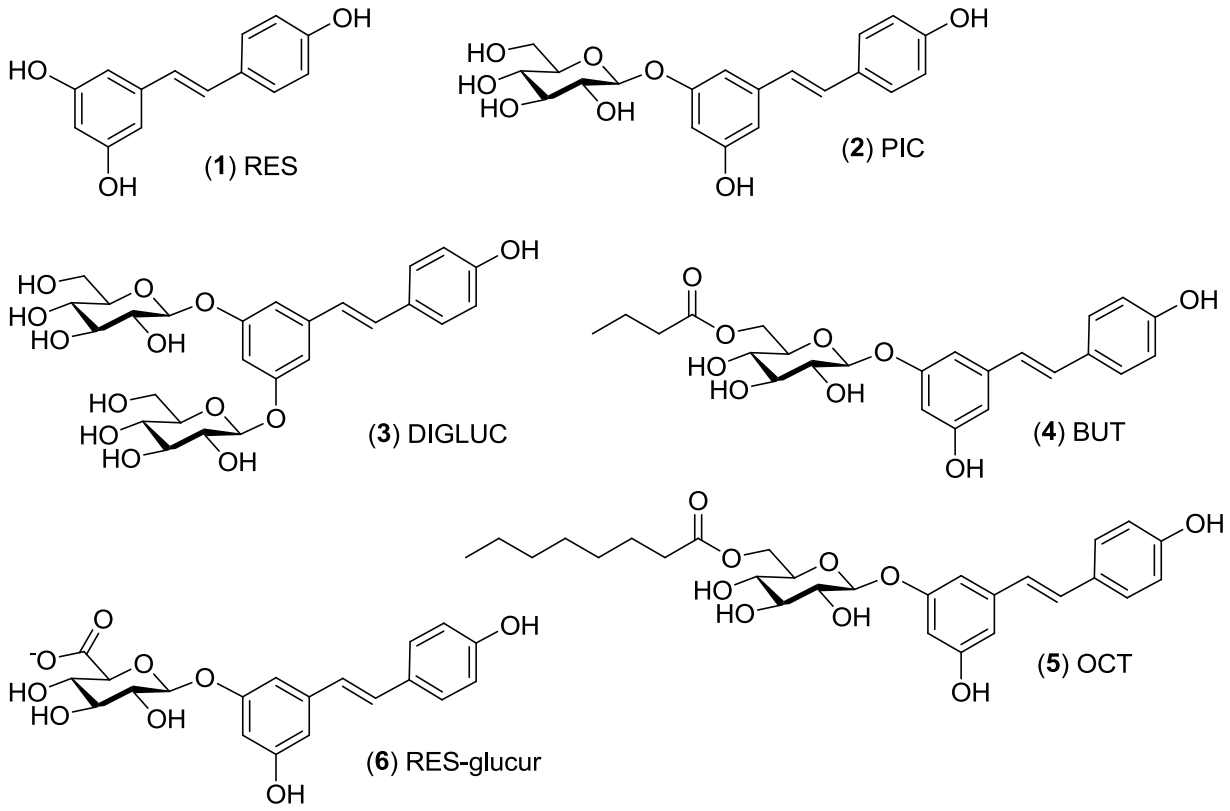

Figure 2

Fig. 2.

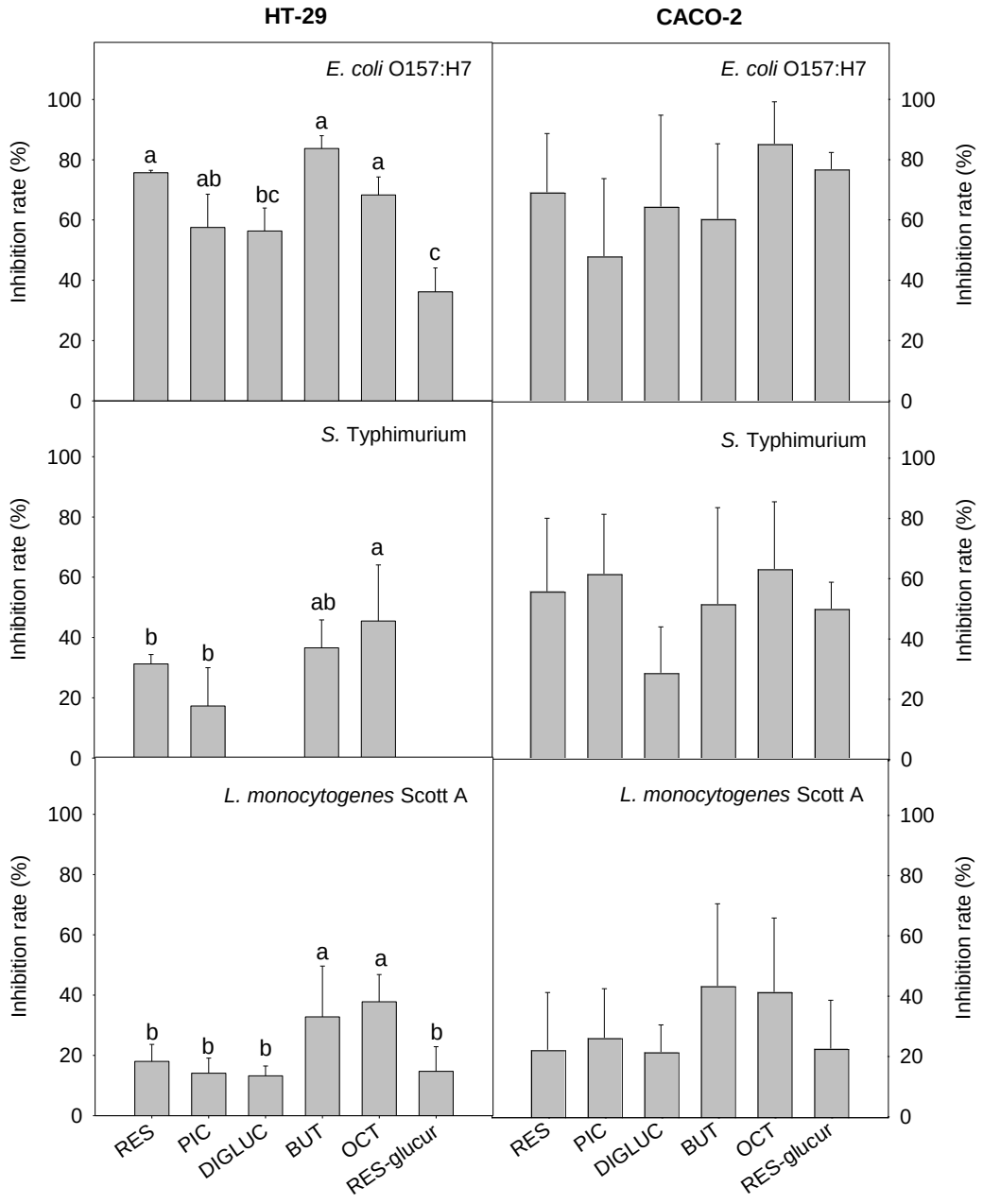

Figure 3

Fig. 3.

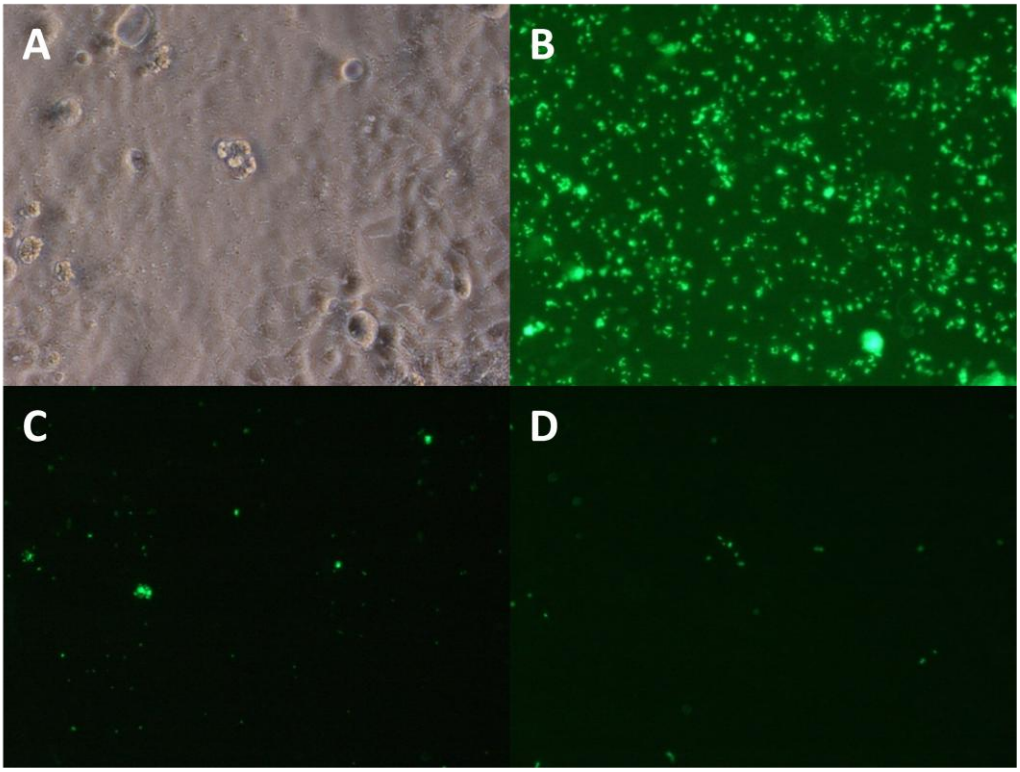

Figure 4

Fig. 4.

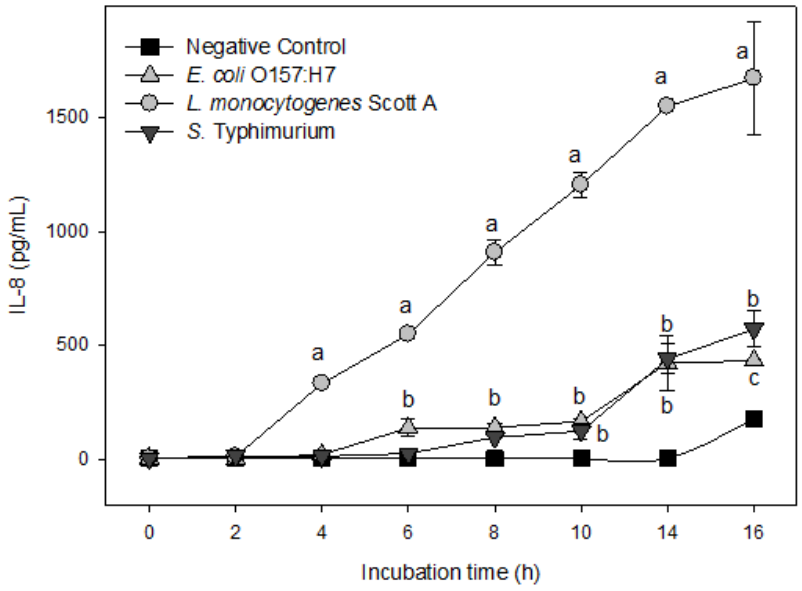

Figure 5

Fig. 5.

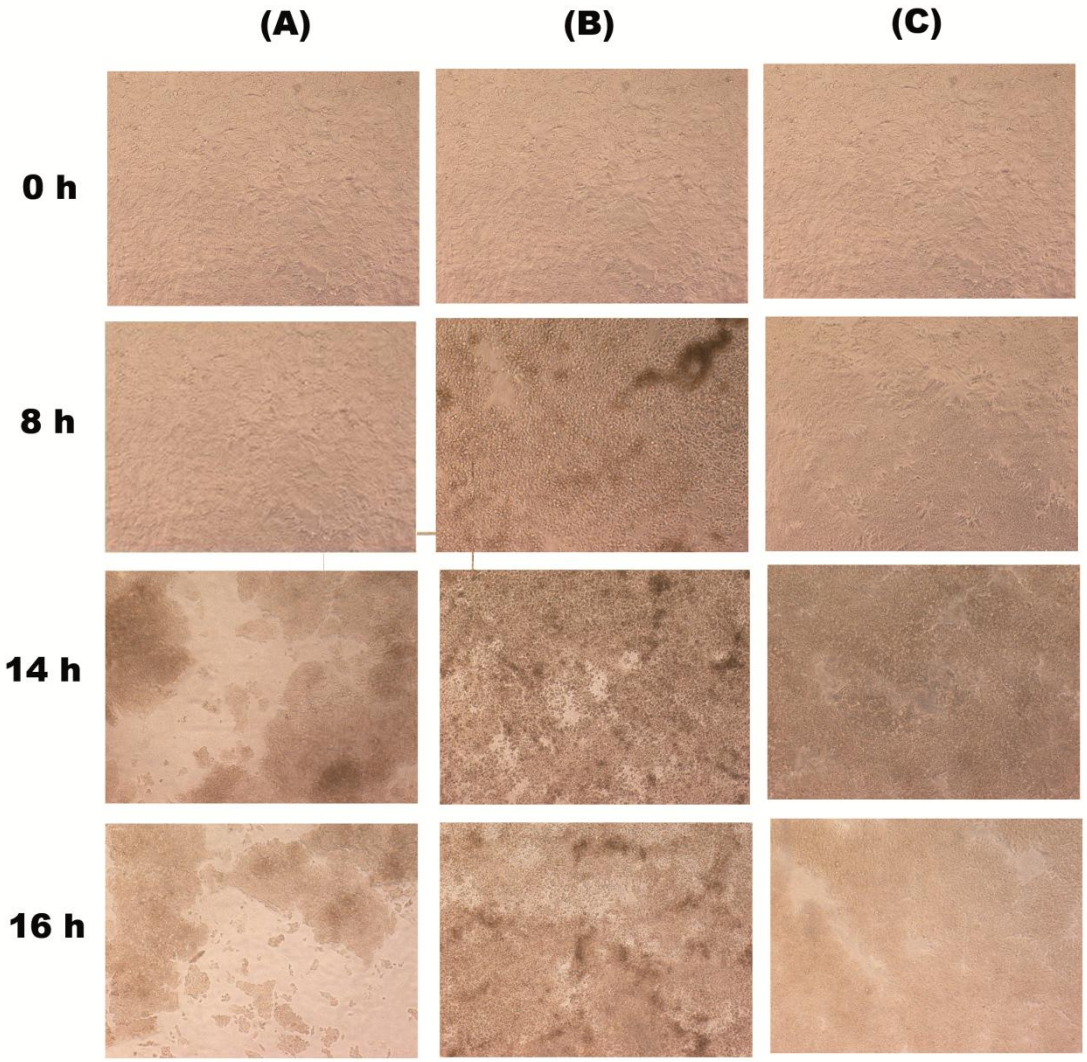

Figure 6

Fig. 6.