



HAL
open science

Colonization of the meat extracellular matrix proteins by O157 and non-O157 enterohemorrhagic *Escherichia coli*

Caroline Chagnot, Nelly Caccia, Estelle Loukiadis, Sarah Ganet, Alexandra Durand, Yolande Bertin, Régine Talon, Thierry Astruc, Mickaël Desvaux

► **To cite this version:**

Caroline Chagnot, Nelly Caccia, Estelle Loukiadis, Sarah Ganet, Alexandra Durand, et al.. Colonization of the meat extracellular matrix proteins by O157 and non-O157 enterohemorrhagic *Escherichia coli*. *International Journal of Food Microbiology*, 2014, 188, pp.92-98. 10.1016/j.ijfoodmicro.2014.07.016 . hal-01204410

HAL Id: hal-01204410

<https://hal.science/hal-01204410v1>

Submitted on 6 Jun 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License



Colonization of the meat extracellular matrix proteins by O157 and non-O157 enterohemorrhagic *Escherichia coli*



Caroline Chagnot^{a,b}, Nelly Caccia^a, Estelle Loukiadis^{c,d}, Sarah Ganet^{c,d}, Alexandra Durand^a, Yolande Bertin^a, Régine Talon^a, Thierry Astruc^b, Mickaël Desvaux^{a,*}

^a INRA, UR454 Microbiologie, F-63122 Saint-Genès Champanelle, France

^b INRA, UR370 Qualité des Produits Animaux, F-63122 Saint-Genès Champanelle, France

^c Université de Lyon, VetAgro Sup, LMAP Laboratory, National Reference Laboratory for *E. coli* (including STEC), F-69280 Marcy l'Etoile, France

^d Université de Lyon, UMR 5557 Ecologie Microbienne, Université Lyon 1, CNRS, VetAgro Sup, Research Group on Bacterial Opportunistic Pathogens and Environment, F-69622 Villeurbanne, France

ARTICLE INFO

Article history:

Received 21 February 2014

Received in revised form 27 May 2014

Accepted 15 July 2014

Available online 22 July 2014

Keywords:

Enterohemorrhagic *Escherichia coli*

Shiga-toxin producing *E. coli*

Bacterial adhesion

Biofilm formation

Extracellular matrix protein

Meat contamination

ABSTRACT

Enterohemorrhagic *Escherichia coli* (EHEC) are anthrozoönotic agents that range third among food-borne pathogens respective to their incidence and dangerousness in the European Union. EHEC are Shiga-toxin producing *E. coli* (STEC) responsible for foodborne poisoning mainly incriminated to the consumption of contaminated beef meat. Among the hundreds of STEC serotypes identified, EHEC mainly belong to O157:H7 but non-O157 can represent 20 to 70% of EHEC infections per year. Seven of those serogroups are especially of high-risk for human health, i.e. O26, O45, O103, O111, O121, O145 and O104. While meat can be contaminated all along the food processing chain, EHEC contamination essentially occurs at the dehiding stage of slaughtering. Investigating bacterial colonization to the skeletal-muscle extracellular matrix (ECM) proteins, it appeared that environmental factors influenced specific and non-specific bacterial adhesion of O157 and non-O157 EHEC as well as biofilm formation. Importantly, mechanical treatment (i.e. shaking, centrifugation, pipetting and vortexing) inhibited and biased the results of bacterial adhesion assay. Besides stressing the importance of the protocol to investigate bacterial adhesion to ECM proteins, this study demonstrated that the colonization abilities to ECM proteins vary among EHEC serogroups and should ultimately be taken into consideration to evaluate the risk of contamination for different types of food matrices.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The enterohemorrhagic *Escherichia coli* (EHEC) are anthrozoönotic agents responsible for foodborne poisoning incriminating contaminated animal food products, vegetables and watery drinks (Bavaro, 2012). EHEC are one of the six pathotypes of intestinal pathogenic *E. coli* (InPEC) (Kaper et al., 2004; Nataro and Kaper, 1998). Worldwide, EHEC infection is predominantly a pediatric disease frequently implicated in severe clinical illness, which is characterized by bloody diarrhea and, in most acute forms, can degenerate into hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP) (Karch et al., 2005; Tarr, 2009). While the involvement of contaminated fruits and vegetables is increasing, EHEC infections are most often linked to the consumption of beef meat and are predominantly attributed to the

E. coli O157:H7 (Berger et al., 2010). *E. coli* O157:H7 is only one of the over 300 distinct serotypes for Shiga toxin-producing *E. coli* (STEC) isolated so far (Karmali et al., 2010), i.e. the previously called verotoxin-producing *E. coli* (VTEC) (Griffin and Tauxe, 1991; Paton and Paton, 1998; Schmidt and Karch, 1996). However, only a very limited number of serotypes appear to be associated with the majority of human diseases; all EHEC are pathogenic STEC but all STEC are not systematically EHEC (or InPEC) (Karmali et al., 2003; Naylor et al., 2005). Respective to their incidence, EHEC range third in the European Union (EU) among food-borne pathogens after *Campylobacter* and *Salmonella* (EFSA and ECDC, 2013), in terms of dangerousness, however, it is the worst of the three. While these serotypes vary in frequency with the country and year, non-O157 represents between 20 and 70% of overall EHEC infections. The latest north American and EU reports, from the USDA (United States Department of Agriculture) and EFSA (European Food Safety Authority) respectively, indicated that the serogroups O26, O45, O103, O111, O121 and O145 are by far the most commonly isolated, the so-called “big six” (EFSA and ECDC, 2013; USDA, 2012). Since 2012, the USA (United States of America) legislation incorporates those non-O157 serogroups, which are considered as

* Corresponding author at: INRA Clermont-Ferrand Research Centre, UR454 Microbiology, Site of Theix, F-63122 Saint-Genès Champanelle, France. Tel.: +33 4 73 62 47 23; fax: +33 4 73 62 45 81.

E-mail address: mickael.desvaux@clermont.inra.fr (M. Desvaux).

high-risk for human health and requiring high vigilance for food safety. In EU, the serogroup O104 is watched closely since the last major outbreaks in 2011 (EFSA and ECDC, 2013).

In any cases, EHEC/STEC contamination of food products originates from fecal contamination, the ruminants being the main natural reservoir (Chase-Topping et al., 2008; Nguyen and Sperandio, 2012). The respect of good hygienic slaughtering practices reduces the risk of contamination of carcasses but cannot guarantee the absence of EHEC/STEC from meat (Buncic et al., 2014; Rhoades et al., 2009). It has been shown that *E. coli* O157:H7 could adhere to the muscle tissue, most certainly to the extracellular matrix (ECM) (Auty et al., 2005; Cabedo et al., 1997; Chen et al., 2007; Li and McLandsborough, 1999; Medina, 2001; Rivas et al., 2006). While binding to collagen I and laminin of the cell surface of *E. coli* O157:H7 was evidenced (Medina, 2001, 2002; Medina and Fratamico, 1998), a later study could not report any significant attachment to any of the tested immobilized ECM proteins (Zulfakar et al., 2012). Using a non-toxicogenic *E. coli* O157:H7 EDL933 Δ stx strain, i.e. *E. coli* O157:H7 CM454 (for Dr Christine Martin at UR454) (Gobert et al., 2007), bacterial adhesion to muscle ECM proteins was re-investigated (Chagnot et al., 2013a,b) by taking a great care to the types of ECM proteins to ensure the highest relevance to the skeletal muscle tissue (Chagnot et al., 2012). It was evidenced that environmental factors such as the growth medium, temperature and pH had a strong influence on bacterial adhesion. Non-specific adhesion occurred when bacteria were grown in bovine small intestine content, with similar trend in DMEM (Dulbecco's modified Eagle's medium), whereas specific adhesion to some ECM fibrillar proteins occurred when bacteria were grown in ruminal content, as well as in LB (lysogenic broth) where maximal specific adhesion to collagens I and III occurred at pH 7 and 25 °C (Chagnot et al., 2013a,b). Besides the use of different environmental conditions, it was hypothesized that the discrepancies with previous investigations for the attachment of the *E. coli* O157:H7 to ECM proteins (Zulfakar et al., 2013) could be linked to bacterial-surface protein determinants potentially involved in adhesion to ECM that were damaged by mechanical shaking–centrifugation–vortexing treatment. Altogether, this prompted us to test this hypothesis using *E. coli* O157:H7 wild type (Chagnot et al., 2013a,b) as well as studying the specific and non-specific adhesion of the major non-O157 EHEC serogroups to some ECM proteins in relevant environmental conditions.

2. Material and methods

2.1. Bacterial strains and culture conditions

EHEC strains investigated in this study are listed in Table 1. One strain for each of the major EHEC serogroups (recognized at higher risk by the USDA and EFSA) was considered. Bacterial strains were cultured in different relevant nutrient media as previously determined (Chagnot et al., 2013a,b), either rich chemically defined, i.e. DMEM (Dulbecco's modified Eagle's medium, Gibco, 31885) (Dulbecco and Freeman, 1959; Eagle, 1955), or complex undefined, i.e. LB (lysogeny broth) (Bertani, 1951, 2004). From –80 °C stock culture previously

grown in the respective medium, strains were plated on the relevant agar medium and incubated overnight at 39 °C (bovine temperature) (Bertin et al., 2011; Desvaux et al., 2007). A preculture was set up from one bacterial colony grown in the respective nutrient broth medium at 39 °C in an orbital shaker till stationary phase as determined using the logarithmic growth curve of the OD_{600 nm} as previously described (Chagnot et al., 2013a,b).

2.2. Virulence profile

The presence of *stx* (encoding the Shiga-toxins), *eae* (encoding the intimin), and *ehxA* (encoding the enterohemolysin) genes and serogroup was determined by real-time PCR as previously described (Bugarel et al., 2010; Nielsen and Andersen, 2003; Perelle et al., 2004). Briefly, DNA was extracted using the InstaGene matrix kit (Biorad) from single colonies of each EHEC strain grown overnight on LB agar (Perelle et al., 2004). Pall GeneDisc Technologies' kits (GSTEHEC106006; GTOPE6C106006; and GECO104106006) and GeneDisc cyclor were used according to the manufacturer's instructions (Pall GeneDisc Technologies) (Beutin et al., 2009; Bugarel et al., 2010). Notably, the oligonucleotide primers and probes included within these discs for detection of *stx*₁, *stx*₂, *eae*, *rjbE*_{O157}, *wzx*_{O26}, *wzx*_{O103}, *wbd1*_{O111}, and *ihp1*_{O145} genes were described in the ISO/TS 13136:2012 standard (Nielsen and Andersen, 2003; Perelle et al., 2004).

2.3. Coating of microtiter plates with ECM proteins

96-well polystyrene microtiter plates (Falcon) were surface-coated with ECM proteins as previously described (Chagnot et al., 2013a,b). The ECM proteins consisted of collagen I (Millipore, 08-115), collagen III (Millipore, CC078) and a reconstituted ECM, i.e. MaxGel ECM (Sigma, E0282), including collagens, laminin, fibronectin, and elastin. BSA (bovine serum albumin (Sigma, A3803)) was used as a control for specific adhesion to muscle ECM proteins. Basically, ECM proteins were solubilized in a 0.1 M carbonate coating buffer (pH 9.6), 250 µl was dispatched at a saturating concentration of well surface (50 µg ml⁻¹) and incubated overnight at 4 °C (Chagnot et al., 2013a,b; Hennequin and Forestier, 2007). The wells were washed 3 times with Tryptone Salt (TS) at room temperature for subsequent bacterial adhesion or bio-film formation assays.

2.4. Bacterial adhesion assay

Preculture was diluted 1:100 and grown as described above. The two media LB and DMEM were adjusted with NaOH (0.1 M) to reach a pH of 7 at the time of sampling, i.e. during the exponential growth phase at an OD_{600 nm} of 0.5 (about 10⁸ CFU ml⁻¹). Chloramphenicol (90 µg ml⁻¹ final) was added to prevent de novo protein synthesis and bacterial growth during the time of the assay (Chagnot et al., 2013a,b). Prior to the crystal violet adhesion assay per se, bacterial cells were sampled and handled in two different ways, i.e. (i) a minimal mechanical

Table 1

List of O157 and non-O157 EHEC bacterial strains studied.

Strain	Somatic antigen	Flagellar antigen	Origin	Virulence profile ^a	Reference
EDL933	O157	H7	Clinical	<i>stx1</i> , <i>stx2</i> , <i>eae</i> , <i>ehxA</i>	Riley et al. (1983)
ED180	O26	H11	Clinical	<i>stx2</i> , <i>eae</i> , <i>ehxA</i>	This study
12047(Fred5)	O45	H2	Clinical	<i>stx1</i> , <i>eae</i> , <i>ehxA</i>	Voitoux et al. (2002)
CH087	O103	H2	Clinical	<i>stx1</i> , <i>eae</i> , <i>ehxA</i>	Pradel et al. (2008)
ED191	O111	H2	Clinical	<i>stx1</i> , <i>stx2</i> , <i>eae</i> , <i>ehxA</i>	This study
32316	O121	H19	Clinical	<i>stx2</i> , <i>eae</i> , <i>ehxA</i>	This study
PH27	O145	H28	Clinical	<i>stx2</i> , <i>eae</i>	Posse et al. (2008)
CB13348	O104	H4	Clinical	<i>stx2</i>	This study

^a Virulence profile of each strain was determined by real-time PCR as described in the Material and methods section.

treatment (MMT) protocol and (ii) a shaking–centrifugation–vortexing treatment (SCVT) protocol.

As previously mentioned (Chagnot et al., 2013a,b), the MMT protocol was aimed at preserving the cell surface proteins especially supra-molecular structures, such as pili and flagella. Thus, no vigorous shaking, vortexing or centrifugation was ever applied in the MMT protocol and large-orifice pipet tips were used to handle bacterial cell suspension at all stages. Briefly, bacteria were grown as described above but under low orbital shaking (70 rpm). Chloramphenicol was added and mixed gently (tube inverted three times) and the bacterial suspension (200 μ l) was directly inoculated in the relevant protein-coated wells of the microtiter plates.

The SCVT protocol is usually described in different forms in the literature but always involves shaking, centrifugation, and vortexing treatment steps. Basically, the bacterial cells were here grown under vigorous orbital shaking (150 rpm). The cells were then centrifuged (3210 g, 5 min, 4 °C) and washed twice in sterile growth medium. Each time, the cells were resuspended by pipetting using classical tips and vortexing to achieve homogeneous resuspension. After the last resuspension, the bacterial suspension was deposited (200 μ l) in the wells of the microtiter plates using classical tips.

Prior to the crystal violet bacterial adhesion assay, the microtiter plates with cells subjected to MMT or SCVT protocol were incubated statically at 25 °C for 3 h. As a control, some wells were filled with sterile growth medium. After incubation, bacterial suspension was removed by pipetting for the crystal violet bacterial adhesion assay. To remove loosely attached cells, wells were washed with TS. Adherent bacteria were fixed with 200 μ l absolute ethanol during 20 min. Wells were then emptied by pipetting and dried for 30 min prior to staining with 200 μ l of an aqueous solution of crystal violet (0.1% w/v) during 15 min. Then, wells were washed a second time with TS to remove the excess of unbound crystal violet dye, and dried for 30 min. For 1 min, under orbital shaking, the bound dye was solubilized from stained cells using 200 μ l of an aqueous solution of acetic acid (33% v/v). Finally, contents of each well (150 μ l) were transferred to a clean microtiter plate and a microtiter plate reader was used to measure absorbance at 595 nm. The readings were corrected by subtracting the average absorbance from control wells.

2.5. Biofilm formation assay

This assay was based on a previously described protocol (Chagnot et al., 2013a,b). Preculture was diluted 1:100, 200 μ l of bacterial cell suspension was dispatched in protein-coated wells of the microtiter

plate and further incubated at 25 °C statically. Wells filled with sterile growth medium were used as a control. To estimate the attached biomass after 24 h of sessile development, wells were subjected to the same sequential procedure as described for the bacterial adhesion assay, namely, washing with TS, fixation with absolute ethanol, air drying, staining with an aqueous crystal violet solution, washing with TS, air drying, dye recovery with an aqueous acetic acid solution and reading of the absorbance at 595 nm.

2.6. Statistical analysis

Statistical analysis was performed from Excel using XLSTAT v2009.3.02. Results are presented as mean \pm standard deviation (SD) and correspond to the mean values of at least three independent experiments, i.e. 3 biological replicates. For each independent experiment, the values result from the average of repetitions performed in triplicate at fewest. The mean values from the biological replicates were compared to the mean values obtained with BSA used as a control of specificity. Data were statistically analyzed following the Student's t-test with differences considered significant ($p < 0.05$, *), very significant ($p < 0.01$, **), highly significant ($p < 0.001$, ***) or very highly significant ($p < 0.0001$, ****).

3. Results

3.1. Mechanical treatment greatly impacts bacterial adhesion assay of *E. coli* O157:H7 EDL933 to immobilized ECM proteins

To test the hypothesis that SCVT (shaking–centrifugation–vortexing treatment) could affect the results of bacterial adhesion to ECM proteins, it was compared with the MMT (minimal mechanical treatment) protocol previously described (Chagnot et al., 2013a,b). Using the reference strain *E. coli* O157:H7 EDL933, two conditions were tested, i.e. using bacterial cells grown (i) in LB, which induce bacterial specific adhesion to collagens I and III, and (ii) in DMEM inducing non-specific adhesion to ECM proteins. Specific bacterial adhesion of *E. coli* O157:H7 EDL933 to the tested collagens occurred when MMT was applied (Fig. 1A). However, the decrease of the specific bacterial adhesion was very highly statistically significant upon SCVT. With DMEM, bacterial cells adhered similarly to collagens I and III and to BSA, indicating that bacterial adhesion was non-specific (Fig. 1B). Again, bacterial adhesion was statistically very much lower upon SCVT than with the MMT protocol. This study clearly evidenced that mechanical treatment can

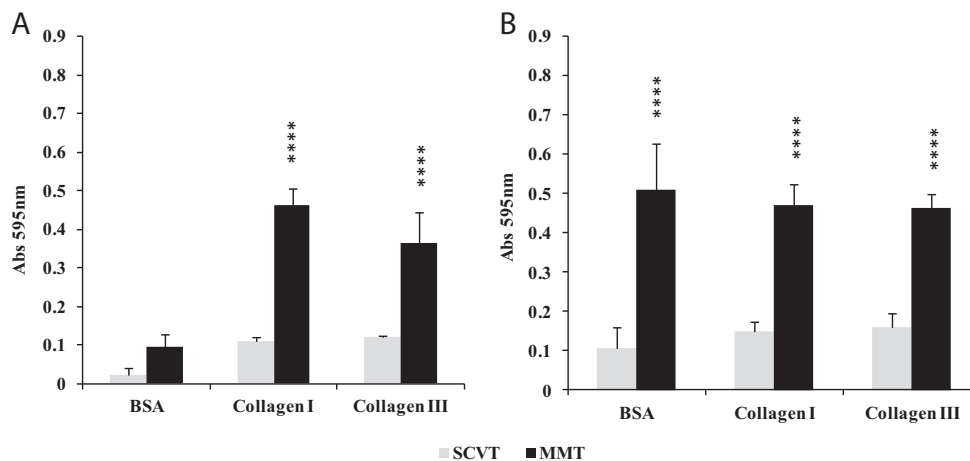


Fig. 1. Effect of mechanical treatment on the adhesion of *E. coli* O157:H7 EDL933 wt to immobilized ECM proteins. Specific adhesion and non-specific bacterial adhesion to relevant collagens I and III were tested in LB (A) and in DMEM (B) respectively to compare the effect of SCVT (shaking–vortexing–centrifugation treatment) and MMT (minimal mechanical treatment) protocols. BSA was used as a negative control for specific adhesion to ECM proteins. Bacterial adhesion assay was performed using the crystal violet staining method.

influence and bias the results of bacterial adhesion assay when investigating the interaction of *E. coli* O157:H7 EDL933 with ECM proteins.

3.2. Bacterial adhesion of the major non-O157 EHEC to immobilized ECM proteins

The attachment ability of strains belonging to the 7 major non-O157 EHEC serogroups (Table 1) was tested using previously reported conditions inducing the most exacerbated specific and non-specific adhesion of *E. coli* O157:H7 EDL933 against collagens I and III or reconstituted ECM respectively (Chagnot et al., 2013a,b), i.e. at 25 °C pH 7 using LB or DMEM as growth medium and applying the MMT (minimal mechanical treatment) protocol.

While *E. coli* O157:H7 EDL933 exhibited specific adhesion ability towards collagen I or III in LB, EHEC O26:H11 ED180 could not adhere to those ECM proteins in tested conditions (Fig. 2A). In contrast, EHEC O45:H2 12047, O103:H2 CH087 and O111:H2 ED191 and O104:H4 CB13348 adhered similarly to collagens I and III as well as to BSA, indicating that bacterial adhesion was non-specific. However, the levels of non-specific bacterial adhesion for EHEC O103:H2 CH087 and O111:H2 ED191 were statistically and significantly much lower than those observed for O45:H2 12047 and O104:H4 CB13348. Besides *E. coli* O157:H7 EDL933, it appears that EHEC O145:H28 PH27 adhered specifically to both collagens I and III whereas EHEC O121:H19 32316 adhered specifically to collagen I only.

Bacterial adhesion was further tested in DMEM (Fig. 2B). It first appeared that O103:H2 CH087 and O121:H19 32316 did not adhere in the tested conditions. As for *E. coli* O157:H7 EDL933, all the remaining

non-O157 EHEC tested strains adhered similarly to a reconstituted ECM and BSA, indicating that non-specific bacterial adhesion occurred. However, the levels of non-specific bacterial adhesion were statistically and significantly higher for EHEC O45:H2 12047 and especially for O104:H4 CB13348. In the conditions tested and together with EHEC O111:H2 ED191, those strains exhibited non-specific adhesion ability both in LB and DMEM (Fig. 2).

3.3. Biofilm formation of the major non-O157 EHEC on immobilized ECM proteins

Besides bacterial adhesion, biofilm formation of strains belonging to the 7 major non-O157 EHEC serogroups (Table 1) was investigated at 24 h using previously reported conditions promoting sessile development of *E. coli* O157:H7 EDL933 (Chagnot et al., 2013a,b), i.e. at 25 °C using LB with immobilized collagens I and III or DMEM with reconstituted ECM.

The presence of immobilized collagen I or III chiefly increased the amount of EHEC O157:H7 EDL933 sessile biomass at 24 h in LB compared to a surface coated with BSA (Fig. 3A). EHEC O145:H28 PH27 formed biofilm specifically on collagen I or III, whereas O111:H2

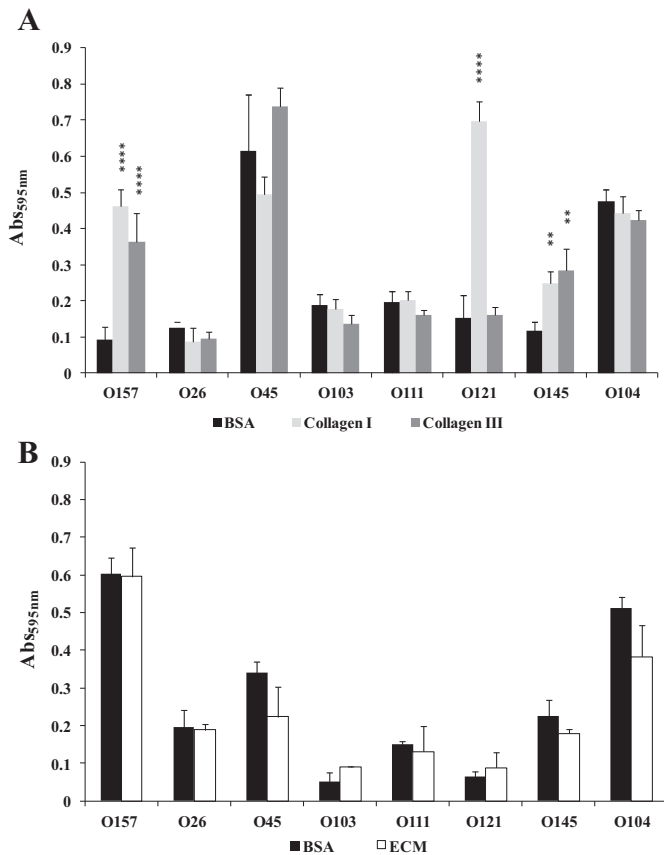


Fig. 2. Adhesion of the major non-O157 EHEC to immobilized ECM proteins. Specific adhesion and non-specific bacterial adhesion to collagens I and III or a reconstituted ECM were tested in LB (A) and in DMEM (B) respectively applying the MMT (minimal mechanical treatment) protocol. BSA was used as a negative control for specific adhesion to ECM proteins. Bacterial adhesion assay was performed using the crystal violet staining method. Tested bacterial strains are listed in Table 1.

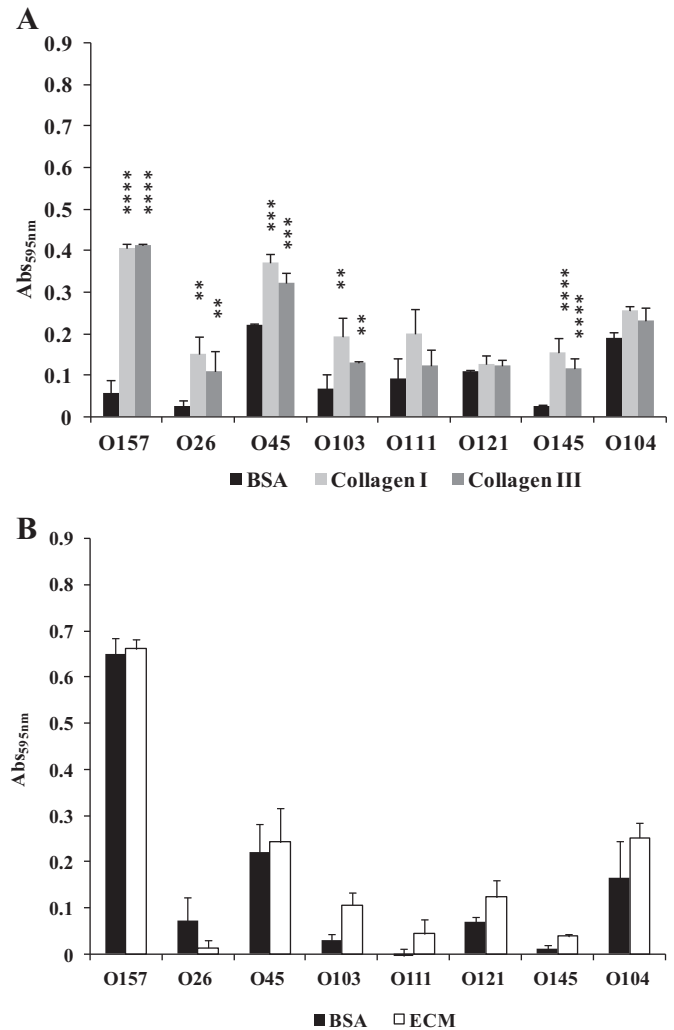


Fig. 3. Colonization of immobilized ECM proteins by *E. coli* O157:H7 EDL933 and the major non-O157 EHEC. Biofilm formation on immobilized collagen I or a reconstituted ECM was performed in LB (A) and in DMEM (B) respectively. BSA was used as a negative control for specific adhesion to ECM proteins. The development of the sessile biomass was assayed at 24 h using the crystal violet staining method. Tested bacterial strains are listed in Table 1.

ED191 and O104:H4 CB13348 formed biofilm similarly on collagen I or III as well as BSA, indicating that sessile development was not specific to ECM proteins. As expected, O45:H2 12047 and O103:H2 CH087 could specifically colonize collagens I and III and O121:H19 32316 formed non-specific biofilm from previous adhesion results. Unexpectedly from previous adhesion results, O26:H11 ED180 formed specific biofilm to ECM proteins tested.

Investigating biofilm formation in DMEM (Fig. 3B), EHEC O45:H2 12047 and O104:H4 CB13348 formed a biofilm as well as EHEC O157:H7 EDL933 but with statistically and significantly lower level of biomass adhered. The biofilm was formed at 24 h similarly on BSA and reconstituted ECM indicating that colonization was not specific. Unexpectedly and while they could adhere non-specifically to reconstituted ECM and BSA, the remaining non-O157 EHEC tested strains did not form a biofilm in the tested conditions.

4. Discussion

Worldwide, many foodborne outbreaks linked to the consumption of meat product contaminated by EHEC strains are regularly identified. EHEC infection principally affects young children where it generally leads to very serious complications, even death. Despite the existence of strict hygiene rules and efficient techniques for STEC detection, contaminated meat is still responsible for human infections. The serogroup most commonly isolated from human infection is O157, however, non-O157 EHEC strains represent also a major risk to human health and include O26, O45, O103, O111, O121 and O145 serogroups as well as O104, a serogroup responsible for the main EHEC outbreak in 2011 (EFSA and ECDC, 2013; USDA, 2012). Primary bacterial contamination of meat occurred on slaughtering, especially when bacteria can be transferred from the hide to the carcass (Rhoades et al., 2009). Due to the large number of human cases and the mortality rate in the consumption of meat contaminated by EHEC, understanding bacterial molecular attachment properties of the major serogroups to specific structures of meat is a prerequisite to improve safety.

As previously observed with a non-toxicogenic isogenic mutant strain (Chagnot et al., 2013a,b), *E. coli* O157:H7 EDL933 Δ stx (recently renamed *E. coli* O157:H7 EDL933 CM454) adhered and formed biofilm specifically to collagens I and III in LB but non-specifically to ECM proteins in DMEM. While validating the use of *E. coli* O157:H7 CM454 for such investigation, it further evidenced the importance of handling the bacterial cells in a correct way for bacterial adhesion assay. Shaking, centrifugation as well as washing procedures, involving repeated pipetting and vortexing to resuspend bacterial cells, were early suggested to damage cell-surface molecular determinants potentially involved in bacterial adhesion abilities (An and Friedman, 1997; Fletcher and Floodgate, 1973). Although it could hinder the true ability of the bacterial cells to adhere to ECM proteins, this crucial aspect has not been taken seriously into consideration and was actually completely overlooked until very recently (Dourou et al., 2011; Zulfakar et al., 2012). Here, the importance of applying the MMT protocol prior to the crystal violet adhesion assay was undeniably evidenced, indicating that it must be carefully and thoughtfully considered by future investigations for rigorous assessment of bacterial adhesion ability.

As such, we evidenced that there are significant differences in bacterial adhesion abilities to the tested ECM proteins between O157 and non-O157 *E. coli* strains, which clearly contrast with the conclusions from a previous investigation (Zulfakar et al., 2012) but corroborate the findings that *E. coli* O157:H7 bacterial cells bind collagen (Medina, 2001). The present study focused on experimental conditions where *E. coli* O157:H7 showed specific adhesion and non-specific adhesion to ECM proteins, which were carefully chosen to correspond to the ECM components of the skeletal muscle tissue. However, it was shown that the type of ECM proteins, the growth medium, the temperature and the pH greatly influence the adhesion ability of *E. coli* O157:H7 (Chagnot et al., 2013a,b). Of course, further in-depth investigations

would be required to characterize the influence of those key parameters on the adhesion and biofilm formation abilities of non-O157 EHEC to skeletal muscle ECM components.

The bacterial adhesion and biofilm formation results showed disparity between EHEC O157 and non-O157 serogroups. This implies that different surface determinants involved in adhesion are differentially expressed. Furthermore, differential transcriptional regulation between DMEM and LB has been reported for EHEC O157:H7 respective to the autoinducer activity (Sperandio et al., 1999) and bicarbonate was also identified as an influential nutrient for gene expression, especially related to bacterial colonization (Abe et al., 2002). It cannot be excluded, though, that additional nutritional factors are involved in the regulation, which the network linked to a wide variety of molecular determinants involved in the adhesion and biofilm formation processes is far from being completely understood in EHEC. As recently reviewed (Chagnot et al., 2012, 2013b), various cell-surface proteinaceous determinants could be responsible for bacterial adhesion to ECM proteins, i.e. the protein MSCRAMM (Microbial Surface Components Recognizing Adhesive Matrix Molecules). In diderm-LPS bacteria like EHEC, it can be either single proteins or supramolecular protein structures, (i) anchored to the outer membrane by β -barrel mainly secreted by the Type V secretion system (T5SS) such as autotransporters (T5aSS), or (ii) secreted and assembled by few secretion systems into supramolecular structures, such as flagella (T3bSS) and different types of pili, e.g. Type 4 pili (T2cSS), pili T (T4aSS), pili F (T4bSS), injectisome (T3aSS), Type 1 pili (T7SS) or curli (T8SS) (Chagnot et al., 2013a,b). Regarding non-specific bacterial adhesion, it was shown to be associated with autoaggregation in *E. coli* O157:H7 EDL933 (Chagnot et al., 2013a,b). The Ag43 (antigen 43) is a surface adhesin expressed by many strains of *E. coli*, including pathogenic strains. Its involvement in the phenomenon of autoaggregation in *E. coli* has been demonstrated several times (Torres et al., 2002). In *E. coli* K12, the expression of the Ag43 is submitted to phase variation (Owen et al., 1996) but its regulation is not fully understood as yet (Chauhan et al., 2013) and has not been thoroughly investigated in pathogenic *E. coli*, except for one study in *E. coli* O157:H7 (Torres et al., 2002). In addition to Ag43, other supramolecular surface protein structures could play a role in EHEC autoaggregation such as the curli (Tree et al., 2007) and type 4 hemorrhagic coli pili (HCP) (Xicohtencatl-Cortes et al., 2009). Besides Ag43, several other autotransporters could be involved in specific adhesion to ECM, e.g. EhaA (EHEC autotransporter A) (Wells et al., 2008), EhaB (Wells et al., 2009), EhaG (Totsika et al., 2012), EhaJ (Easton et al., 2011), EspP (Dziva et al., 2007), Sab (Herold et al., 2009) or AIDA (Yin et al., 2009). In addition to curli and HCP, a wealth of pili could be further responsible for ECM colonization, namely the injectisome (T3aSS) (Shaw et al., 2008), Lpf (long polar fimbriae) (Torres et al., 2007), ECP/Mat (*E. coli* common pilus/meningitis-associated and temperature-regulated fimbriae) (Lehti et al., 2013), SFP (Sorbitol-fermenting EHEC fimbriae) (Brunder et al., 2001), F9 (Low et al., 2006), F18 (Bardiau et al., 2010) or ELF (*E. coli* YcbQ laminin-binding fimbriae) (Samadder et al., 2009). The respective contribution and regulation of those different determinants are extremely complex and are far from being understood at a global scale even in the most investigated EHEC O157:H7. Further studies to investigate the protein determinants implicated in specific and non-specific adhesion as well as biofilm formation on immobilized ECM proteins and muscle tissues are undoubtedly required to better understand the behavior of O157 and non-O157 strains to contaminate meat but also other food matrices.

A better understanding of the molecular and cellular mechanisms involved in adhesion of major EHEC to meat is necessary to limit the risk of food outbreak, causing serious diseases (SHU and PTT) especially for young children (Hermos et al., 2011). The discrepancies in the colonization abilities of EHEC to ECM proteins could ultimately be taken into consideration to evaluate the risk of contamination of different types of food products (meat, milk and vegetable products) and eventually lead to the development of more efficient preventing strategy of carcass contamination by bacterial pathogens in the meat industry.

Acknowledgments

This work was supported in part by INRA (French National Institute for Agronomical Research) with the MICEL project funded by the divisions CEPIA (Science and Process Engineering of Agricultural Products) and MICA (Microbiology and Food Chain) AIP (“Action Incitative Programmée”) (grant no. 504910/505566), COST (European Cooperation in Science and Technology) Action BacFoodNet (A European Network For Mitigating Bacterial Colonization and Persistence On Foods and Food Processing Environments) (grant no. FA1202), and “CPER (Contrat de Plan État-Région) ASTERisk (Auvergne STEC Risque)” (grant no. 23000566). The authors are grateful to Patrick Fach and Frédéric Auvray (ANSES, Maisons-Alfort, France) for kindly providing *E. coli* O45:H2 12047 (Fred5) and *E. coli* O145:H28 PH27 respectively, Patricia Mariani-Kurkdjian (Hôpital Robert Debré, Paris, France) for *E. coli* O121:H19 32316, and Lothar Beutin (Nationales Referenzlabor für *Escherichia coli*, Berlin, Germany) for *E. coli* O104:H4 CB13348, *E. coli* O26:H11 ED180 and *E. coli* O111:H2 ED191. Dr. Caroline Chagnot was a PhD research fellow granted by the “Région Auvergne – FEDER (Fonds Européen de Développement Régional) (grant no. 23000410).

References

- Abe, H., Tatsuno, I., Tobe, T., Okutani, A., Sasakawa, C., 2002. Bicarbonate ion stimulates the expression of locus of enterocyte effacement-encoded genes in enterohemorrhagic *Escherichia coli* O157:H7. *Infect. Immun.* 70, 3500–3509.
- An, Y.H., Friedman, R.J., 1997. Laboratory methods for studies of bacterial adhesion. *J. Microbiol. Methods* 30, 141–152.
- Auty, M., Duffy, G., O’Beirne, D., McGovern, A., Gleeson, E., Jordan, K., 2005. In situ localization of *Escherichia coli* O157:H7 in food by confocal scanning laser microscopy. *J. Food Prot.* 68, 482–486.
- Bardiau, M., Szalo, M., Mainil, J.G., 2010. Initial adherence of EPEC, EHEC and VTEC to host cells. *Vet. Res.* 41, 57.
- Bavaro, M.F., 2012. *Escherichia coli* O157:H7 and other toxigenic strains: the curse of global food distribution. *Curr. Gastroenterol. Rep.* 14, 317–323.
- Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., Frankel, G., 2010. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ. Microbiol.* 12, 2385–2397.
- Bertani, G., 1951. Studies on lysogeny. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J. Bacteriol.* 62, 293–300.
- Bertani, G., 2004. Lysogeny at mid-twentieth century: P1, P2, and other experimental systems. *J. Bacteriol.* 186, 595–600.
- Bertin, Y., Girardeau, J.P., Chaucheyras-Durand, F., Lyan, B., Pujos-Guillot, E., Harel, J., Martin, C., 2011. Enterohaemorrhagic *Escherichia coli* gains a competitive advantage by using ethanolamine as a nitrogen source in the bovine intestinal content. *Environ. Microbiol.* 13, 365–377.
- Beutin, L., Jahn, S., Fach, P., 2009. Evaluation of the ‘GeneDisc’ real-time PCR system for detection of enterohaemorrhagic *Escherichia coli* (EHEC) O26, O103, O111, O145 and O157 strains according to their virulence markers and their O- and H-antigen-associated genes. *J. Appl. Microbiol.* 106, 1122–1132.
- Brunder, W., Khan, A.S., Hacker, J., Karch, H., 2001. Novel type of fimbriae encoded by the large plasmid of sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:H(-). *Infect. Immun.* 69, 4447–4457.
- Bugarel, M., Beutin, L., Fach, P., 2010. Low-density microarray targeting non-locus of enterocyte effacement effectors (*nle* genes) and major virulence factors of Shiga toxin-producing *Escherichia coli* (STEC): a new approach for molecular risk assessment of STEC isolates. *Appl. Environ. Microbiol.* 76, 203–211.
- Buncic, S., Nychas, G.J., Lee, M.R., Koutsoumanis, K., Hebraud, M., Desvaux, M., Chorianopoulos, N., Bolton, D., Blagojevic, B., Antic, D., 2014. Microbial pathogen control in the beef chain: recent research advances. *Meat Sci* 97, 288–297.
- Cabedo, L., Sofos, J.N., Schmidt, G.R., Smith, G.C., 1997. Attachment of *Escherichia coli* O157:H7 and other bacterial cells grown in two media to beef adipose and muscle tissues. *J. Food Prot.* 60, 102–106.
- Chagnot, C., Listrat, A., Astruc, T., Desvaux, M., 2012. Bacterial adhesion to animal tissues: protein determinants for recognition of extracellular matrix components. *Cell. Microbiol.* 14, 1687–1696.
- Chagnot, C., Agus, A., Renier, S., Peyrin, F., Talon, R., Astruc, T., Desvaux, M., 2013a. *In vitro* colonization of the muscle extracellular matrix components by *Escherichia coli* O157:H7: the influence of growth medium, temperature and pH on initial adhesion and induction of biofilm formation by collagens I and III. *PLoS One* 8, e59386.
- Chagnot, C., Zorgani, M.A., Astruc, T., Desvaux, M., 2013b. Proteinaceous determinants of surface colonization in bacteria: bacterial adhesion and biofilm formation from a protein secretion perspective. *Front. Microbiol.* 4, 303.
- Chase-Topping, M., Gally, D., Low, C., Matthews, L., Woolhouse, M., 2008. Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nat. Rev. Microbiol.* 6, 904–912.
- Chauhan, A., Sakamoto, C., Ghigo, J.M., Beloin, C., 2013. Did I pick the right colony? Pitfalls in the study of regulation of the phase variable antigen 43 adhesin. *PLoS One* 8, e73568.
- Chen, J.R., Rossman, M.L., Pawar, D.M., 2007. Attachment of enterohemorrhagic *Escherichia coli* to the surface of beef and a culture medium. *LWT – Food Sci. Technol.* 40, 249–254.
- Desvaux, M., Scott-Tucker, A., Turner, S.M., Cooper, L.M., Huber, D., Nataro, J.P., Henderson, I. R., 2007. A conserved extended signal peptide region directs posttranslational protein translocation via a novel mechanism. *Microbiology* 153, 59–70.
- Dourou, D., Beauchamp, C.S., Yoon, Y., Geornaras, I., Belk, K.E., Smith, G.C., Nychas, G.J., Sofos, J.N., 2011. Attachment and biofilm formation by *Escherichia coli* O157:H7 at different temperatures, on various food-contact surfaces encountered in beef processing. *Int. J. Food Microbiol.* 149, 262–268.
- Dulbecco, R., Freeman, G., 1959. Plaque production by the polyoma virus. *Virology* 8, 396–397.
- Dziva, F., Mahajan, A., Cameron, P., Currie, C., McKendrick, I.J., Wallis, T.S., Smith, D.G., Stevens, M.P., 2007. EspP, a Type V-secreted serine protease of enterohaemorrhagic *Escherichia coli* O157:H7, influences intestinal colonization of calves and adherence to bovine primary intestinal epithelial cells. *FEMS Microbiol. Lett.* 271, 258–264.
- Eagle, H., 1955. Nutrition needs of mammalian cells in tissue culture. *Science* 122, 501–514.
- Easton, D.M., Totsika, M., Allsopp, L.P., Phan, M.D., Idris, A., Wurlup, D.J., Sherlock, O., Zhang, B., Venturini, C., Beatson, S.A., Mahony, T.J., Cobbold, R.N., Schembri, M.A., 2011. Characterization of Ehaj, a new autotransporter protein from enterohemorrhagic and enteropathogenic *Escherichia coli*. *Front. Microbiol.* 2, 120.
- EFSA, ECDC, 2013. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. *EFSA J.* 11, 3129.
- Fletcher, M., Floodgate, G.D., 1973. An electron-microscopic demonstration of an acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. *J. Gen. Microbiol.* 74, 325–333.
- Gobert, A.P., Vareille, M., Glasser, A.L., Hindre, T., de Sablet, T., Martin, C., 2007. Shiga toxin produced by enterohaemorrhagic *Escherichia coli* inhibits PI3K/NF-kappaB signaling pathway in globotriaosylceramide-3-negative human intestinal epithelial cells. *J. Immunol.* 178, 8168–8174.
- Griffin, P.M., Tauxe, R.V., 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *Escherichia coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* 13, 60–98.
- Hennequin, C., Forestier, C., 2007. Influence of capsule and extended-spectrum β -lactamases encoding plasmids upon *Klebsiella pneumoniae* adhesion. *Res. Microbiol.* 158, 339–347.
- Hermos, C.R., Janineh, M., Han, L.L., McAdam, A.J., 2011. Shiga toxin-producing *Escherichia coli* in children: diagnosis and clinical manifestations of O157:H7 and non-O157:H7 infection. *J. Clin. Microbiol.* 49, 955–959.
- Herold, S., Paton, J.C., Paton, A.W., 2009. Sab, a novel autotransporter of locus of enterocyte effacement-negative Shiga-toxinogenic *Escherichia coli* O113:H21, contributes to adherence and biofilm formation. *Infect. Immun.* 77, 3234–3243.
- Kaper, J.B., Nataro, J.P., Mobley, H.L., 2004. Pathogenic *Escherichia coli*. *Nat. Rev. Microbiol.* 2, 123–140.
- Karch, H., Tarr, P.I., Bielaszewska, M., 2005. Enterohaemorrhagic *Escherichia coli* in human medicine. *Int. J. Med. Microbiol.* 295, 405–418.
- Karmali, M.A., Mascarenhas, M., Shen, S., Ziebell, K., Johnson, S., Reid-Smith, R., Isaac-Renton, J., Clark, C., Rahn, K., Kaper, J.B., 2003. Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. *J. Clin. Microbiol.* 41, 4930–4940.
- Karmali, M.A., Gannon, V., Sargeant, J.M., 2010. Verocytotoxin-producing *Escherichia coli* (VTEC). *Vet. Microbiol.* 140, 360–370.
- Lehti, T.A., Bauchart, P., Kukkonen, M., Dobrindt, U., Korhonen, T.K., Westerlund-Wikstrom, B., 2013. Phylogenetic group-associated differences in regulation of the common colonization factor Mat fimbria in *Escherichia coli*. *Mol. Microbiol.* 87, 1200–1222.
- Li, J., McLandsborough, L.A., 1999. The effects of the surface charge and hydrophobicity of *Escherichia coli* on its adhesion to beef muscle. *Int. J. Food Microbiol.* 53, 185–193.
- Low, A.S., Dziva, F., Torres, A.G., Martinez, J.L., Rosser, T., Naylor, S., Spears, K., Holden, N., Mahajan, A., Findlay, J., Sales, J., Smith, D.G., Low, J.C., Stevens, M.P., Gally, D.L., 2006. Cloning, expression, and characterization of fimbrial operon F9 from enterohemorrhagic *Escherichia coli* O157:H7. *Infect. Immun.* 74, 2233–2244.
- Medina, M.B., 2001. Binding of collagen I to *Escherichia coli* O157:H7 and inhibition by carrageenans. *Int. J. Food Microbiol.* 69, 199–208.
- Medina, M.B., 2002. Biosensor studies of the binding of extracellular matrix components with immobilized *Escherichia coli* O157:H7 and inhibition by polysulfated polysaccharides. *Biotechnol. Lett.* 24, 77–84.
- Medina, M.B., Fratamico, P.M., 1998. Binding interactions of collagen I, laminin and fibronectin with immobilized *Escherichia coli* O157:H7 using a surface plasmon resonance biosensor. *Biotechnol. Tech.* 12, 235–240.
- Nataro, J.P., Kaper, J.B., 1998. Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* 11, 142–201.
- Naylor, S.W., Gally, D.L., Low, J.C., 2005. Enterohaemorrhagic *Escherichia coli* in veterinary medicine. *Int. J. Med. Microbiol.* 295, 419–441.
- Nguyen, Y., Sperandio, V., 2012. Enterohemorrhagic *Escherichia coli* (EHEC) pathogenesis. *Front. Cell Infect. Microbiol.* 2, 90.
- Nielsen, E.M., Andersen, M.T., 2003. Detection and characterization of verocytotoxin-producing *Escherichia coli* by automated 5’ nuclease PCR assay. *J. Clin. Microbiol.* 41, 2884–2893.
- Owen, P., Meehan, M., de Loughry-Doherty, H., Henderson, I.R., 1996. Phase-variable outer membrane proteins in *Escherichia coli*. *FEMS Immunol. Med. Microbiol.* 16, 63–76.
- Paton, J.C., Paton, A.W., 1998. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clin. Microbiol. Rev.* 11, 450–479.
- Perelle, S., Dilasser, F., Grout, J., Fach, P., 2004. Detection by 5’-nuclease PCR of Shiga-toxin producing *Escherichia coli* O26, O55, O91, O103, O111, O113, O145 and O157:H7, associated with the world’s most frequent clinical cases. *Mol. Cell. Probes* 18, 185–192.
- Posse, B., De Zutter, L., Heyndrickx, M., Herman, L., 2008. Quantitative isolation efficiency of O26, O103, O111, O145 and O157 STEC serotypes from artificially contaminated food

- and cattle faeces samples using a new isolation protocol. *J. Appl. Microbiol.* 105, 227–235.
- Pradel, N., Bertin, Y., Martin, C., Livrelli, V., 2008. Molecular analysis of Shiga toxin-producing *Escherichia coli* strains isolated from hemolytic-uremic syndrome patients and dairy samples in France. *Appl. Environ. Microbiol.* 74, 2118–2128.
- Rhoades, J.R., Duffy, G., Koutsoumanis, K., 2009. Prevalence and concentration of verocytotoxigenic *Escherichia coli*, *Salmonella enterica* and *Listeria monocytogenes* in the beef production chain: a review. *Food Microbiol.* 26, 357–376.
- Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A., Cohen, M.L., 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* 308, 681–685.
- Rivas, L., Dykes, G.A., Fegan, N., 2006. Attachment of Shiga toxin-producing *Escherichia coli* to beef muscle and adipose tissue. *J. Food Prot.* 69, 999–1006.
- Samadder, P., Xicohtencatl-Cortes, J., Saldana, Z., Jordan, D., Tarr, P.I., Kaper, J.B., Giron, J.A., 2009. The *Escherichia coli* *ycbQRST* operon encodes fimbriae with laminin-binding and epithelial cell adherence properties in Shiga-toxigenic *E. coli* O157:H7. *Environ. Microbiol.* 11, 1815–1826.
- Schmidt, H., Karch, H., 1996. Enterohemolytic phenotypes and genotypes of Shiga toxin-producing *Escherichia coli* O111 strains from patients with diarrhea and hemolytic-uremic syndrome. *J. Clin. Microbiol.* 34, 2364–2367.
- Shaw, R.K., Berger, C.N., Feys, B., Knutton, S., Pallen, M.J., Frankel, G., 2008. Enterohemorrhagic *Escherichia coli* exploits EspA filaments for attachment to salad leaves. *Appl. Environ. Microbiol.* 74, 2908–2914.
- Sperandio, V., Mellies, J.L., Nguyen, W., Shin, S., Kaper, J.B., 1999. Quorum sensing controls expression of the Type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15196–15201.
- Tarr, P.I., 2009. Shiga toxin-associated hemolytic uremic syndrome and thrombotic thrombocytopenic purpura: distinct mechanisms of pathogenesis. *Kidney Int. Suppl.* S29–32.
- Torres, A.G., Perna, N.T., Burland, V., Ruknudin, A., Blattner, F.R., Kaper, J.B., 2002. Characterization of Cah, a calcium-binding and heat-extractable autotransporter protein of enterohaemorrhagic *Escherichia coli*. *Mol. Microbiol.* 45, 951–966.
- Torres, A.G., Milflores-Flores, L., Garcia-Gallegos, J.G., Patel, S.D., Best, A., La Ragione, R.M., Martinez-Laguna, Y., Woodward, M.J., 2007. Environmental regulation and colonization attributes of the long polar fimbriae (LPF) of *Escherichia coli* O157:H7. *Int. J. Med. Microbiol.* 297, 177–185.
- Totsika, M., Wells, T.J., Beloin, C., Valle, J., Allsopp, L.P., King, N.P., Ghigo, J.M., Schembri, M.A., 2012. Molecular characterization of the EhaG and UpaG trimeric autotransporter proteins from pathogenic *Escherichia coli*. *Appl. Environ. Microbiol.* 78, 2179–2189.
- Tree, J.J., Ulett, G.C., Hobman, J.L., Constantinidou, C., Brown, N.L., Kershaw, C., Schembri, M.A., Jennings, M.P., McEwan, A.G., 2007. The multicopper oxidase (CueO) and cell aggregation in *Escherichia coli*. *Environ. Microbiol.* 9, 2110–2116.
- USDA, 2012. Rules and regulations: Shiga toxin-producing *Escherichia coli* in certain raw beef products. *Fed. Regist.* 77, 31975–31981.
- Voitoux, E., Lafarge, V., Collette, C., Lombard, B., 2002. Applicability of the draft standard method for the detection of *Escherichia coli* O157 in dairy products. *Int. J. Food Microbiol.* 77, 213–221.
- Wells, T.J., Sherlock, O., Rivas, L., Mahajan, A., Beatson, S.A., Torpdahl, M., Webb, R.L., Allsopp, L.P., Gobius, K.S., Gally, D.L., Schembri, M.A., 2008. EhaA is a novel autotransporter protein of enterohemorrhagic *Escherichia coli* O157:H7 that contributes to adhesion and biofilm formation. *Environ. Microbiol.* 10, 589–604.
- Wells, T.J., McNeilly, T.N., Totsika, M., Mahajan, A., Gally, D.L., Schembri, M.A., 2009. The *Escherichia coli* O157:H7 EhaB autotransporter protein binds to laminin and collagen I and induces a serum IgA response in O157:H7 challenged cattle. *Environ. Microbiol.* 11, 1803–1814.
- Xicohtencatl-Cortes, J., Monteiro-Neto, V., Saldana, Z., Ledesma, M.A., Puente, J.L., Giron, J.A., 2009. The type 4 pili of enterohemorrhagic *Escherichia coli* O157:H7 are multipurpose structures with pathogenic attributes. *J. Bacteriol.* 191, 411–421.
- Yin, X., Chambers, J.R., Wheatcroft, R., Johnson, R.P., Zhu, J., Liu, B., Gyles, C.L., 2009. Adherence of *Escherichia coli* O157:H7 mutants in vitro and in ligated pig intestines. *Appl. Environ. Microbiol.* 75, 4975–4983.
- Zulfakar, S.S., White, J.D., Ross, T., Tamplin, M.L., 2012. Bacterial attachment to immobilized extracellular matrix proteins in vitro. *Int. J. Food Microbiol.* 157, 210–217.
- Zulfakar, S.S., White, J.D., Ross, T., Tamplin, M., 2013. Effect of pH, salt and chemical rinses on bacterial attachment to extracellular matrix proteins. *Food Microbiol.* 34, 369–375.