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



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ABSTRACT

Enterohemorrhagic *Escherichia coli* (EHEC) are anthropozoonotic 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 0157:H7 but non-0157 can represent 20 to 70% of EHEC infections per year. Seven of those serogroups are especially of high-risk for human health, i.e. 026, 045, 0103, 0111, 0121, 0145 and 0104. 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 0157 and non-0157 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.

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1. Introduction

The enterohemorrhagic *Escherichia coli* (EHEC) are anthropozoonotic 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

* 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. 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 verotoxinproducing 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 (Unites States of America) legislation incorporates those non-O157 serogroups, which are considered as

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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-toxigenic E. coli O157:H7 EDL933 ∆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 reinvestigated (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 nonspecific 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

Table 1				
List of O157	and non-O157	EHEC bacterial	strains	studied.

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 $OD_{600 \text{ nm}}$ 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; GTOP6EC106006; and GEC0104106006) and GeneDisc cycler 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*, *rfbE*₀₁₅₇, *wzx*₀₂₆, *wzx*₀₁₀₃, *wbd1*₀₁₁₁, and *ihp1*₀₁₄₅ 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 $OD_{600 \text{ nm}}$ of 0.5 (about 10^8 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

Strain	Somatic antigen	Flagellar antigen	Origin	Virulence profile ^a	Reference
EDL933	0157	H7	Clinical	stx1, stx2, eae, ehxA	Riley et al. (1983)
ED180	026	H11	Clinical	stx2, eae, ehxA	This study
12047(Fred5)	045	H2	Clinical	stx1, eae, ehxA	Voitoux et al. (2002)
CH087	0103	H2	Clinical	stx1, eae, ehxA	Pradel et al. (2008)
ED191	0111	H2	Clinical	stx1, stx2, eae, ehxA	This study
32316	0121	H19	Clinical	stx2, eae, ehxA	This study
PH27	0145	H28	Clinical	stx2, eae	Posse et al. (2008)
CB13348	0104	H4	Clinical	stx2	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 supramolecular 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 proteincoated 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 centrifugated (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 µl 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 µl 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 microtitre

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 < 0.001, ***).

3. Results

3.1. Mechanical treatment greatly impacts bacterial adhesion assay of *E. coli* 0157: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



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.

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. 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 026, 045, 0103, 0111, 0121 and 0145 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-toxigenic isogenic mutant strain (Chagnot et al., 2013a,b), E. coli O157:H7 EDL933 ∆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 0157 and non-0157 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 0157: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 nonspecific 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 surpramolecular 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 0157: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.

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