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Research Article

Shigatoxigenic and Enteropathogenic *Escherichia coli* Biofilm Formation: Effects of Different Stress Conditions on Polystyrene and Stainless Steel Surfaces

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Abstract

Biofilms constitutes one of the main problems for food industry and public health due to the survival and the ability of pathogenic microorganisms to form these communities on different surfaces and culture composition. Shigatoxigenic (STEC) and Enteropathogenic (EPEC) *Escherichia coli* are some of the microorganisms that could form biofilms and cause serious diseases in humans. The aim of this study was to determine the capability to form biofilms of native STEC and atypical EPEC (aEPEC) strains on polystyrene and stainless steel and to evaluate the effects produced by different stress conditions. The expression of the fimbria curli was also studied. All strains were strong biofilm formers at 37°C on polystyrene, except STEC O145:NM and aEPEC O130:H11 which were moderated biofilm formers. No one strain formed biofilm at heat stress (54°C) or refrigeration (5°C) temperatures. Stainless steel surface reduced significantly the capability to form biofilms regardless the culture composition. Most of the STEC and aEPEC strains were curli-negative at 37°C while they showed curli-positive phenotypes at 20°C. Both curli expression and biofilm formation were significantly influenced by temperature and incubation time. STEC and aEPEC strains demonstrated to be able to form biofilm and produce fimbria curli under different conditions of medium and temperature, which is important due the risk of survival and transmission of these pathogens from reservoirs and food to humans.

Keywords: Shigatoxigenic *Escherichia coli*; atypical enteropathogenic *E. coli*; biofilms; culture; surface; curli fimbriae

Abbreviations

A/E: Adherence and Effacement; aEPEC: Atypical Enteropathogenic *E. coli*; aw: water activity; CH: Hemorrhagic Colitis; EPEC: Enteropathogenic *E. coli*; HUS: Hemolytic Uremic Syndrome; LB: Luria Bertani; MBF: Moderate-Biofilm-Forming; NBF: Non-Biofilm-Forming; OD: Optical Density; ODc: cut-off OD; SBF: Strong-Biofilm-Forming; SF: Physiological Solution; STEC: Shigatoxigenic *E. coli*; WBF: Weak-Biofilm-Forming

Introduction

Biofilms are complex communities of microorganisms that grow embedded in an extracellular matrix, adhered to biotic or abiotic surfaces. These microorganisms have an altered phenotype compared to their planktonic counterparts respect to the rate of growth and gene transcription. Biofilms could be composed of a single or several different microbial species [1]. Biofilms constitutes one of the main problems for industry and public health due to the survival and the ability of pathogenic microorganisms to form these communities on different surfaces, utensils and equipment in contact with food or on medical devices such as catheters and prostheses, among others. In the food industry, biofilms could be formed on surfaces such as plastic, glass, wood, stainless steel and on food [2]. Insufficient or inadequate disinfection of surfaces or utensils causes an increase in product contamination and the development of food poisoning [3].

Shigatoxigenic (STEC) and Enteropathogenic (EPEC) *Escherichia coli* are some of the microorganisms that could form biofilms. STEC is an emerging pathogen related to public health that causes serious diseases in humans such as Hemorrhagic Colitis (CH) and Hemolytic Uremic Syndrome (HUS) by the production of Shiga toxins and other virulence factors. Cattle are the main reservoir, being able to transmit STEC to the human through cattle derived foods and direct contact with the animals or their environment [4-6]. EPEC strains do not produce Shiga toxins but just like STEC, produce histopathological lesions of "adherence and effacement" (A/E) in the intestinal tract cells. It is transmitted via the fecal-oral route through contaminated hands, water and food. Children with diarrhea and asymptomatic children or adult carriers as well as chicken and derived food have been documented as EPEC reservoir [7,8].

STEC and EPEC harbor virulence and adherence factors that allow adhesion and colonization to both living and inert surfaces and favor the biofilm formation, such as, the IrgA homolog adherence-conferring protein (*iha*) [9], the enterohemorrhagic *E. coli* factor of adherence (*efa*1) [10], the type 1 fimbria (*fim*) [11] and the long polar fimbria (*lpf*) [12]. They also can harbor several proteins belonging to the Autotransporter (AT) protein family such as, Antigen 43 (*agn*43), the Calcium-binding antigen 43 homolog (*cah*) [13,14] and the Enterohemorrhagic *E. coli* (EHEC) autotransporter (*ehaA*) [15], which favor the agglomeration and cell-to-cell interactions.

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The extracellular matrix in *E. coli* biofilms is formed by curli as the main component and cellulose [16]. Curli expression is regulated by different genes that code for protein subunits, such as its transcriptional regulator (*csgD*) and the indirect regulator of this operon (*crl*) [17]. Crl was proposed as a thermal sensor, which protein is more stable at low temperatures; therefore, it is considered that the maximum expression of curli occurs at less than 30°C, although it can be variable depending on the isolation, its pathogenicity and the working conditions [18].

Biofilms have been studied for decades using several *in vitro* models in an effort to understand the mechanisms of bacteria to survive and persist in the environment or into a host. Such studies reported the capability of the microorganisms to form biofilms is influenced by many factors, such as culture medium (nutrient composition, temperature, pH), hydrodynamics (continuous flow vs. batch system), substratum (roughness, chemistry), incubation times, among others [19-21]. The results obtained will depend on the combination of these factors, even using the same microorganism. For example, different abilities to form biofilms have been observed in STEC isolates from different origins according to the composition of the culture medium and the incubation times [22,23]. The effects of temperature, oxidative and nitrosative stress, pH, salt content and water activity (aw) have been the most studied factors that affect the survival and growth of STEC [24-28]. Previous studies with STEC strains isolated from cattle and food were carried out using different organic and inorganic acids, fruit and alcohol juices, and at extreme temperatures (acidic, alcoholic and thermal stress) to observe the viability of this bacterium against different stress conditions. The results showed that STEC no-O157 serotypes were more resistant to high temperatures (54°C) than O157:H7, which is the serotype routinely used to standardize the thermal inactivation protocols of these pathogens [29]. In addition, no-O157 serotypes had a better behavior to acidic pH than O157:H7 being able to survive at least 8 h at pH=2.5; suggesting the great resistance that they would have during the digestion process in the host and their potential to cause disease. In addition, the ability of these bacteria to neutralize the surrounding acidic environment has been proven, which would contribute to greater host survival [26].

In EPEC strains, biofilm formation studies were performed using different methodologies, demonstrating the ability of these bacteria to adhere to cell lines with different patterns and to form biofilm on surfaces such as polystyrene and glass [30]. The role of the expression of the BFP and EspA in the biofilm formation on polystyrene under static and continuous flow conditions has been determined in typical EPEC strains, although the quorum sensing mechanism was also necessary to form biofilm [31]. However, little is known about the influence of different stress situations or changes in temperature or pH on the biofilm formation of this *E. coli* pathotype, particularly in atypical strains.

The aim of this study was to determine the capability to form biofilms of native STEC and atypical EPEC strains on polystyrene and stainless steel and to evaluate the effects produced by different stress conditions. In addition, the expression of the fimbria curli was studied.

Materials and Methods

Bacterial Strains

A total of fifteen STEC and atypical EPEC strains (aEPEC) isolated from different sources were randomly selected from a collection of different *E. coli* pathotypes belonging of the Laboratorio de Inmunoquímica y Biotecnología (UNCPBA, Tandil, Buenos Aires, Argentina); the reference STEC strain O157:H7 EDL933 was also included. All the strains were previously serotyped and characterized in their classic virulence factors -*stx1, stx2, eae, ehxA*- [4,5,8,32,33], in other putative virulence genes present in the megaplasmid -*katP, espP, subA, stcE*- and in several adhesins and fimbriae involved in colonization and biofilm formation -*efa1, iha, fimCD, ehaA, agn43, agn43EDL933, csgA, crl*- [34,35]. Strains were grouped according the pathotype and the presence/absence of *eae*: STEC (*eae* positive/negative) and aEPEC (Table 1).

Biofilm Formation Assays

The detection and quantification of biofilms were performed in 96-well polystyrene microtiter plates and stainless steel coupons under previously described laboratory conditions [23], with modifications. Briefly, the experiments were performed in two sections, according to different stress conditions and surfaces. In section 1 (polystyrene surface), biofilm formation assay was performed under different temperatures of incubation: standard (37°C), heat stress (54°C) and refrigeration temperature (5°C) and different culture conditions: standard, acidic or alcoholic at 37°C. Acidic and alcoholic conditions were achieved adding acetic acid (0,05 M, pH=5,5) or ethyl alcohol (6%) to Luria Bertani (LB) medium (with and without glucose 0,5%) respectively. In section 2 (stainless steel surface), biofilm formation was performed under different culture conditions: standard, acidic and alcoholic as described above. In this section, 24-well polystyrene plates were used as support for stainless steel coupons (1x1cm, finishing number 304#4) which were previously cleaned and sterilized according to Joseph, et al. [36].

In each section, the surfaces were washed, fixed and stained with crystal violet (0.1%) according to the protocol described previously [23]. The dye adhered to the biofilms was eluted with 200 µl of ethylic alcohol 96% and the OD_{570} was read in a Labsystem Multiscan EX microplates reader (I.C.T, S.L. Instrumentación Científica Técnica, S.L.). In section 2, only the stainless steel coupons were staining to obtain the OD corresponding to the biofilm on this surface.

Three consecutive wells were used for each strain. The OD of each well was averaged and corrected by a cut-off OD (average OD of three wells with sterile medium and 3 times its standard deviation). According to the corrected OD, the strains were classified into four categories: Non-Biofilm-Forming (NBF), Weak-Forming (WBF), Moderate-Forming (MBF) and Strong Biofilm-Forming (SBF) [37]. Three independent experiments were made and data obtained were statistically analyzed by ANOVA InfoStat 2015E (National University of Cordoba, Argentina).

Curli Expression Assays

The curli phenotype of STEC and aEPEC strains was studied by the Congo red assay at optimum growth temperature (37°C) and at room temperature (≈20°C) and then, the biofilm formation from colonies incubated at both temperatures was evaluated. Briefly, one

Table 1: STEC and aEPEC serotypes used in this study.

E. coli group	Serotype	Virulence profiles
eae-positive STEC	O103:H2	*vt1, vt2, ehxA, eae, espP, subA, efa1, fimCD, ehaA, agn4, csgA, crl*
	O111:NM	*vt2, ehxA, eae, katP, espP, efa1, iha, fimCD, ehaA, agn43$_{EDL933}$, agn43, csgA, crl*
	O145:NM	*vt2, ehxA, eae, katP, espP, efa1, iha, fimCD, ehaA, agn43$_{EDL933}$, agn43, csgA, crl*
	O26:H11	*vt1, vt2, ehxA, eae, katP, espP, efa1, iha, fimCD, ehaA, agn43$_{EDL933}$, agn43, csgA, crl*
	O157:H7	*vt2, ehxA, eae, katP, espP, stcE, efa1, iha, fimCD, ehaA, agn43$_{EDL933}$, agn43, csgA, crl*
eae-negative STEC	O91:H21	*vt2, ehxA, saa, espP, iha, fimCD, ehaA, agn43, csgA, crl*
	O113:H21	*vt2, ehxA, saa, espP, subA, iha, fimCD, ehaA, agn43, csgA, crl*
	O130:H11	*vt1, vt2, ehxA, saa, espP, subA, iha, fimCD, ehaA, agn43, csgA, crl*
	O178:H19	*vt1, vt2, ehxA, saa, espP, subA, iha, fimCD, ehaA, agn43, csgA, crl*
	O8:H19	*vt2, ehxA, espP, iha, fimCD, ehaA, agn43, csgA, crl*
aEPEC	O19:H11	*eae, fimCD, agn43, csgA, crl*
	O130:H11	*eae, fimCD, agn43, csgA, crl*
	O40:NM	*eae, fimCD, agn43, csgA, crl*
	O40:H10	*eae, fimCD, agn43, csgA, crl*
	O108:H9	*eae, fimCD, csgA, crl*
EDL933†	O157:H7	*vt1, vt2, ehxA, eae, katP, espP, stcE, efa1, iha, fimCD, ehaA, agn43$_{EDL933}$, agn43, csgA, crl*

†Reference STEC strain O157:H7 EDL933 was incorporated to the eae-positive STEC group in order to a better analysis of the results.

aliquot of each strain stored at -70°C was reactivated in 500µL of LB for 2 h, at 37°C with moderate agitation. Then, they were seeded in Congo Red Agar plates (CRA) and incubated for 24 h at 37°C, after that they were incubated an additional 24 h at room temperature (≈20°C). The phenotypes expressed at each temperature were observed and colonies were classified as *rdar* ("red, dry and rough"; curli and cellulose production), *sar* ("red and smooth"; only curli production) or *saw* ("white and smooth"; not curli or cellulose production).

One individual colony from CRA incubated at 37°C and then at 20°C was taken for each strain. Colonies were separately homogenized in 1 ml of physiological solution (SF). From that homogenate, 50µl was seeded in 24-well polystyrene plates (three consecutive wells were used for each strain and six wells for culture medium controls). The plates were incubated at 37°C and 20°C statically for 48 h following the same protocol used above. Biofilm formation was estimated by the violet crystal technique as previously described and the OD obtained was related to the curli expression phenotypes.

Results and Discussion

Biofilm Formation

The biofilm formation for STEC and aEPEC strains was variable according the culture conditions on polystyrene. Indeed, under different temperatures of incubation all strains were SBF at 37°C, except STEC O145:NM and aEPEC O130:H11 that resulted MBF. No one strain formed biofilm at thermal stress (54°C) or refrigeration (5°C) temperatures (Figure 1). When the average of biofilm formation was compared by *E. coli* group (*eae*-positive STEC, *eae*-negative STEC, and aEPEC strains), it was found that *eae*-negative STEC were SBF at 37°C than aEPEC and *eae*-positive STEC strains (Figure 1).

Under different culture conditions -standard, acidic or alcoholic- all strains were MBF or SBF; aEPEC group resulted more biofilm-forming than the STEC groups in all conditions (Table 2, Figure 2).

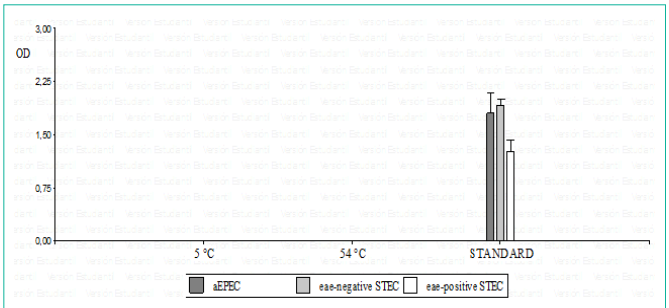

Figure 1: Comparison of the biofilm formation under different temperatures of grows.

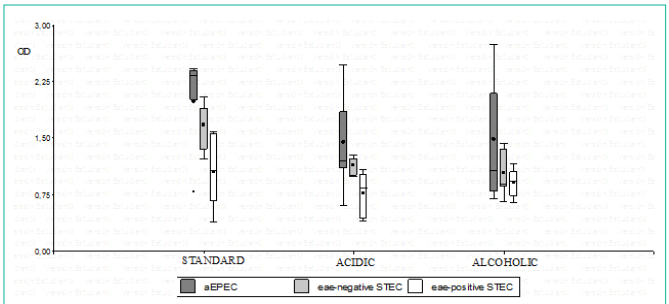

Figure 2: Comparison of biofilm formation between groups of *E. coli* strains under different culture medium on polyestyrene.

In the standard condition the strongest biofilm formers were the strains belonging to the serotypes O40:H10, O91:H21 and O157:H7 from aEPEC, *eae*-negative STEC and *eae*-positive STEC, respectively. Under acidic conditions, the aEPEC serotypes O108:H9, O19:H11 and O40:NM were SBF; while O157:H7 and O113:H21 were the best biofilm formers from the *eae*-positive and *eae*-negative STEC, respectively. Under alcoholic conditions, the aEPEC strains O108:H9 and O19:H11, *eae*-negative STEC O130:H11 and *eae*-positive STEC

Table 2: The estimated biofilm formation (OD_{570}) under different culture conditions according to the surfaces used.

E. coli Group	Serotype	Polystyrene*			Stainless Steel‡		
		Standard	Acidic	Alcoholic	Standard	Acidic	Alcoholic
eae-positive STEC	O157:H7	1,590	1,083	1,157	0	0	0
	O26:H11	1,567	0,425	0,645	0,0632	0	0
	O103:H2	0,986	1,021	1,054	0	0	0
	O111:NM	0,662	0,779	0,731	0,1257	0	0
	O145:NM	0,387	0,395	0,989	0,1527	0,1606	0,0153
	EDL933	1,175	0,896	0,874	0,0158	0	0
eae-negative STEC	O91:H21	2,053	1,227	1,357	0	0,0511	0,1609
	O113:H21	1,905	1,220	0,853	0,0898	0,1130	0,2890
	O130:H11	1,889	0,988	1,425	0,0920	0,1146	0,2549
	O178:H19	1,221	1,273	0,898	0,2102	0,1287	0,2426
	O8:H19	1,347	1,004	0,656	0,3170	0,1433	0,2848
aEPEC	O40:H10	2,425	1,101	0,789	0,4529	0	0
	O19:H11	2,399	1,853	2,101	0,6016	0,3563	0,0930
	O108:H9	2,336	2,479	2,744	0,1000	0,1129	0
	O40:NM	2,008	1,195	1,077	0,5673	0	0,0700
	O130:H11	0,789	0,611	0,702	0,0964	0,0531	0

References:

*Strains were considered as biofilm formers when their OD was greater than the established cut-off OD (ODc=0,192) on polystyrene. Strains whose values were included to 2ODc (0,385) were considered WBF; values between 2ODc and 4ODc (0,770) were considered MBF, and more than 4ODc were classified as SBF.

‡Strains were biofilm former when their OD were greater than ODc=0,541 on stainless steel. Strains whose values were included to 2ODc (1,082) were considered WBF. No one strain could be classified as MBF or SBF since no value was greater than 2ODc on this surface.

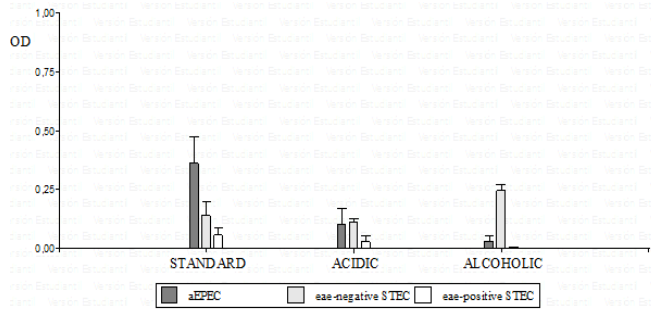

Figure 3: Biofilm formation under different culture conditions on stainless steel surface. It is notably that the OD's average obtained were never greater than 0.50 in the three groups.

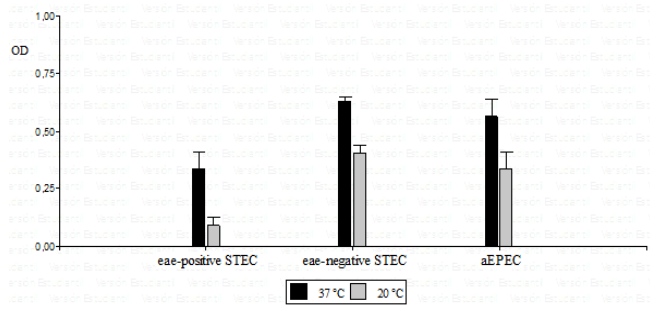

Figure 4: Differences in the biofilm formation between *E. coli* groups according with the temperature of incubation.

O157:H7 were SBF.

The biofilm formation on stainless steel was significantly lower than that obtained on polystyrene ($p<0.0001$). The highest OD values were observed in aEPEC strains which ranged from 0,4 to 0,6 in standard culture conditions (Table 2). The 81% of the strains were considered WBF under standard culture and the rest were NBF (19%). Under acidic and alcoholic conditions, the strains decreased their biofilm formation (WBF in a 56 and 50%, respectively) and increased the percentage of NBF (44 and 50%, respectively). The aEPEC group formed more biofilm under standard culture medium, while *eae*-negative STEC were better in acidic and alcoholic mediums. The *eae*-positive STEC group were the lowest biofilm former in all condition studied (Figure 3).

Curli Expression

In this assay, data showed that temperature significantly influenced both biofilm formation and curli expression (Table 3). The biofilm formation was greater at 37°C than at 20°C ($p=0.001$) and both *eae*-negative STEC and aEPEC strains were better biofilm-forming than *eae*-positive STEC at both temperatures ($p=0.003$) (Figure 4). The curli phenotype was affected by temperature inversely to biofilm formation, so more positive curli colonies ("sar" and "rdar") were observed at 20°C than at 37°C ($p=0.0007$). A direct association between the curli phenotype and the biofilm-forming capacity could not be established ($p=0.059$).

The ability of bacteria to produce biofilms depends on many factors inherent to the microorganism, the environment and the surface where it can adhere. The studies of biofilm by STEC and EPEC have been based especially on the risk that these bacteria represent for

Table 3: The estimated biofilm formation (OD_{570}) and fimbria curli expression under different temperatures of incubation.

E. Coli group	Serotype	Incubation temperatures					
		37°C			≈20°C		
		Curli expression	Biofilm formation	Classification	Curli expression	Biofilm formation	Classification
eae-positive STEC	O103:H2	saw-sar	0,610	MBF	sar	0,267	WBF
	O111:NM	saw	0,171	WBF	saw	0,050	WBF
	O145:NM	saw	0,331	WBF	saw	0,038	WBF
	O26:H11	saw-sar	0,480	MBF	sar	0,085	WBF
	O157:H7	saw	0,150	WBF	saw	0,046	WBF
	EDL933	saw	0,288	WBF	sar	0,062	WBF
eae-negative STEC	O91:H21	saw	0,663	MBF	sar	0,446	MBF
	O113:H21	saw	0,631	MBF	sar	0,419	MBF
	O130:H11	saw	0,562	MBF	saw	0,263	WBF
	O178:H19	saw	0,609	MBF	sar	0,479	MBF
	O8:H19	saw	0,676	SBF	sar	0,400	MBF
aEPEC	O19:H11	saw	0,415	MBF	sar	0,508	MBF
	O130:H11	saw-sar	0,779	SBF	sar	0,128	WBF
	O40:NM	saw-sar	0,677	SBF	sar	0,461	MBF
	O40:H10	sar	0,387	MBF	sar	0,395	MBF
	O108:H9	saw-sar	0,564	MBF	rdar	0,198	WBF

public health worldwide. Most STEC biofilm studies have focused on serotype O157:H7 [38-42] and few reports have been published on STEC no-O157 or EPEC [30,43,44]. In these studies, variables such as the culture medium, different surfaces, the expression of several adherence factors such as curli and cellulose, have been studied to find out how these pathogens form biofilm.

This study was aimed to investigate the ability to form biofilms of a set of native STEC and aEPEC strains under different conditions of culture media, surfaces and temperatures in order to know whether these conditions affect their biofilm formation. The temperature was the variable that more affected the biofilm formation, since at extreme temperatures (heat and cold) the strains did not form biofilms. Previous studies have evaluated the biofilm formation at refrigeration temperature (4 and 15°C) but they used longer incubation times (up to 16 days) finding a greater biofilm formation at 15°C more than at 4°C, regardless of the surface and substrate [20,42]. Other authors have used temperatures that favored the production of fimbria curli and cellulose, and greater adhesion and biofilm formation were obtained at 28°C, 30°C or 37°C on semi-solid and solid surfaces such us, stainless steel, teflon and glass [41, 44]. In agreement with our results, these findings suggest that temperature have a great influence on the ability to form biofilms, since STEC and aEPEC strains were favored by temperatures near those found in their natural environment (animal host or human) and not by temperatures that would constitute a stress condition.

Another variable that can influence the development of biofilms is the culture medium. Several studies evaluated STEC biofilms in mediums with different nutrient availability, from minimal medium to food products fluids such as milk, minced meat, meat juice etc., and demonstrated that there was a greater biofilm formation in nutrient medium than in minimal or diluted medium [38,42]. Other studies have evaluated the bacteriostatic effects of organic acid solutions on pathogenic strains such as *Escherichia coli* and *Salmonella spp*. on cattle carcasses surfaces, and they have found that lactic acid was more effective than acetic acid [45,46]. The ability to survive in solutions with low concentrations of acid and alcohol allows the biofilm development on surfaces commonly used in industry and the domestic environment, constituting a risk to underestimate the presence of STEC or aEPEC in foods. Biofilm studies in EPEC strains are limited to use minimal medium such as DMEM or nutritive such as LB, since the study of biofilms in this pathotype, especially atypical EPEC, is quite recent [30,31,47-49].

In this study, STEC and aEPEC strains were subjected to different medium with pH and chemical composition (low pH, presence of acetic acid and alcohol). No significant differences were found in biofilm formation under standard culture medium condition and its acid and alcoholic variants, although STEC has been able to grow and survive for more than 24 h at the same acetic acid and ethyl alcohol concentrations [27]. The behavior of the strains was very variable within each *E. coli* group. It was observed that certain serotypes such as O108:H9 (aEPEC), O91:H21 and O157:H7 (*eae*-negative and *eae*-positive STEC, respectively) were the best biofilm formers on polystyrene surface. This is in agreement with results obtained by Wang, et al. [50], who showed that *eae*-negative O113 and O91 serogroups exhibited a greatest potential to form biofilms and by Molina, et al [27] who found that STEC O91:H21 was the most resistant serotype against acidity conditions. STEC O145:NM formed more biofilm under standard, acidic and alcoholic conditions on stainless steel than the other strains. Among the *eae*-negative STEC, O8:H19 was the serotype that formed the greater biofilm in the three conditions studied on stainless steel, however this serotype was one of the lowest biofilm-forming on polystyrene. STEC O157:H7 is the most frequently serotype used as a reference for the development of

control and thermal inactivation protocols of STEC in food [51,52]. In this study, this serotype showed a differently behavior according to its origin: the bovine strain was more biofilm former than the reference strain EDL933 O157:H7 isolated from human.

During the adhesion to the surface and maturation of the biofilm, the fimbria curli plays an important role in favoring cell aggregation and interaction with cellulose to create hydrophobic networks in the extracellular matrix [53]. Curli has been studied in the biofilm formation of *E. coli*, especially in STEC O157:H7, on surfaces commonly used in the food industry such as stainless steel, polystyrene, glass and rubber [54], finding that the production of this fimbria is more favored by temperatures below 30°C, varying not only according to the serotype, but within the same serotype [39,55]. Curli expression and biofilm formation at different temperatures from colonies incubated in CRA at 37°C and 20°C were compared. The results showed that most STEC and aEPEC strains were curli-negative at 37°C, but 12 of 16 strains (mostly *eae*-STEC and aEPEC) were moderate or strong biofilm. However, at 20°C, most of the strains were curli-positive ("sar" or "rdar") but they were weak biofilm-forming. These results differ with those found by other authors where a relationship between curli expression and biofilm formation could be established [41,56,57]. However, other authors did not find a clear association between curli production and an increased biofilm formation [22,47]. In agreement with others studies, we suggest that curli could need more time of incubation to play an important role in the adhesion and maturation of biofilm under certain laboratory conditions [58]. The production of curli, cellulose, or both, may not be an indispensable factor to form biofilm, however they were suggested as important contributing factors [39,59].

Conclusion

Native STEC and aEPEC strains demonstrated to be able to form biofilm and produce fimbria curli under different conditions of culture medium and temperature. This is important because of the risk of transmission and survival of these pathogens from reservoirs and food to humans. The recreation of food industry environment into the laboratory result a useful tool to understand the behavior of these foodborne pathogens and to find new strategies for their prevention and control.

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