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Ability of *Escherichia coli* to form biofilm in tomato fruits (*Solanum lycopersicum* L.)

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Abstract

Foodborne diseases are frequently caused by bacterial pathogens, many of which are able to protect themselves from unfavorable environmental conditions by forming a biofilm, allowing them to successfully colonize inert surfaces and living substrates, such as edible fruits. The objective of this study was to evaluate the formation of biofilms by *Escherichia coli* from different origins (12 strains) in order to select the best biofilm formers and investigate them on tomato fruits under greenhouse conditions. For this purpose, the microtiter technique was used, which permits measuring the adhesion of bacterial communities to surfaces. Two media were assessed (minimum essential added with glucose and Luria Bertanni broth), incubated at 37°C, and readings were taken at 24, 48 and 72 hours. The results showed that the strains differed in their biofilm production levels, with no difference between the media evaluated, reaching their maximum production level at 72 hours. The bacteria with the best production were enterohemorrhagic O157:H7, which were inoculated on tomatoes for evaluation prior to greenhouse conditions, where biofilm development was determined at two production stages (commercial and physiological maturity). The results demonstrated that *Escherichia coli* has the ability to form biofilms on tomato fruits.

Keywords: greenhouse; enteropathogen; vegetable.

Practical Application: The quantification of biofilm is essential to improve the diagnosis and prevention of ETA. To this end, an experimental model was developed that proposes creating a well in the surface of the tomato to measure the development of biofilm in fruits still on the plant, simulating development throughout the production chain of food for direct consumption and export. The importance of this study lies in the fact that biofilm-producing bacteria can reach levels of antibiotic resistance greater than free bacteria, complicating their elimination.

1 INTRODUCTION

Foodborne diseases (FBD) have increased over the past several decades due to different properties of foodborne pathogens including resistance to antimicrobial compounds and expression of bacteria components as biofilms. The increased incidence of FBD suggests that the hygienic-sanitary quality of foods may be compromised at some point prior to their ingestion. One potential type of contamination is caused by bacteria that form part of the normal intestinal microbiota of birds, pigs, and cattle but also can be human pathogens such as diarrheagenic *Escherichia coli* (Centers for Disease Control and Prevention [CDC], 2024).

Although *E. coli* participates as an intestinal commensal, some clones of the bacteria are pathogenic, causing intestinal (named diarrheagenic [DEC]) and extraintestinal (ExTEC) diseases (Sarshar et al., 2022; Wakimoto et al., 2004). DEC strains are grouped into the enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), diffuse adherent (DAEC), enteroaggregative (EAEC), and enteropathogenic (EPEC) pathotypes, each one with different factors associated with their virulence. The general mechanism of infection is by the fecal-oral route principally through the consumption of contaminated foods. Two serotypes of the bacteria (O157:H7 and O104:H4) have been associated with food outbreaks caused by the ingestion of contaminated ready-to-eat vegetables. Clinical manifestations include diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (Avila-Novoa et al., 2018; Branda et al., 2005; Castañeda-Ruelas et al., 2018; Sarti et al., 2019). Different studies have demonstrated that these bacteria can colonize and persist in vegetables because of their ability to adhere to biotic or abiotic substrates through the production of an extracellular matrix known as biofilm. This allows the bacteria to colonize a variety of surfaces (biotic, abiotic, hydrophobic, or hydrophilic), including the vegetables' epidermis. The bacteria that produce biofilms are particularly resistant to routine cleaning and disinfection procedures, and antimicrobials (Donlan, 2002; Wright et al., 2013). That is why biofilm-producing bacteria are especially relevant in food products, especially those for raw consumption.

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In Mexico, the total area of tomato (*Solanum lycopersicum* L.) cultivation is 37,056.6 hectares nationwide (Sistema de Información Agropecuaria y Pesquera [SIAP], 2021). Tomato is one of the most frequently consumed fresh local products with important economic value, representing a privileged product in production and commercialization (Sarti et al., 2019). The growing conditions of the vegetable contribute to its contamination principally by different enteropathogenic microorganisms. On the other hand, due mainly to the lack of adequate management practices during production and post-harvest, vegetables are exposed to another contamination source. Given the high susceptibility of this crop to contamination by FBD-causing bacteria as *E. coli* (Adator et al., 2018), it is fundamental to know if biofilm-producing bacteria are established in the tomato in any of its development stages.

Several outbreaks of FBD associated with *E. coli* of different pathogenic groups were reported. One of them is the EHEC O157:H7, which was identified from undercooked meat, as well as some vegetables and greens. However, not much is known about the capacity of this bacterium to produce biofilm on the tomatoes' epidermis during their different growth stages. The aim of this work was to evaluate if *E. coli* of different pathotypes and origins produce biofilms using both *in vitro* and *in vivo* assays, and if the tomato (*Solanum lycopersicum* L.) development stage influences biofilm expression.

1.1 Relance of the work

Bacterial pathogens, capable of protecting themselves from unfavorable environmental conditions by means of a biofilm, are one of the main causes of foodborne diseases. Therefore, the objective of the present investigation was to evaluate the ability of enterohemorrhagic Escherichia coli strains to form biofilms on the epidermis of tomato fruits produced under greenhouse conditions, thus demonstrating the ability of these strains to colonize all stages of crop production and their relevance in countless infectious outbreaks of clinical importance.

2 MATERIALS AND METHODS

The work was carried out in different stages: 1) Evaluation of biofilm development of 12 *E. coli* strains; 2) Evaluation of biofilm development on tomatoes *in vitro*; and 3) Evaluation of biofilm development of *E. coli* under greenhouse conditions (Figure 1).

Twelve *E. coli* strains of clinical and environmental origin (Table 1) were selected from the Laboratory of Bacterial Pathogenicity at ¨Federico Gómez¨ Mexican Children's Hospital collection. The strains were stored frozen (–70°C) in preservation medium. For their recovery, the strains were transferred to 20 × 150 mm tubes with 5 mL of tryptic soy broth (OXOID®) and incubated during 24 hours at 37°C. Once growth began, the cultures were transferred to blood agar base (OXOID®) to assess their purity and MacConkey agar (BD Bioxon®) to determine their ability to ferment lactose. Biochemical (IMViC) test was performed to confirm that the cultures corresponded to *E. coli*. As a positive control, the EAEC strain OND: H10 (Eslava et al., 1998) was used, which is known to form biofilm (Jamalludeen et al., 2007). As a negative control, the *E. coli* K12 HB101 was employed, which does not form biofilm (Vanegas et al., 2009).

2.1 Biofilm formation in vitro

The biofilm expression was analyzed in an *in vitro* assay and later in an *in vivo* model using tomato fruits following the protocol established by O'Toole and Kolter (1998) with modifications (number of washes). For the *in vitro* assays, the *E. coli* cultures were adjusted to an optical density (OD) of 3×10^8 UFC/mL, corresponding to tube 1 on the McFarland scale. In a 24-well polystyrene plate (COSTAR), 50µL of the culture were mixed with 950µL of Minimal Essential Medium (MEM) with glucose (SIGMA ALDRICH); in another plate, 50µL of the culture were diluted in 950µL of Luria Bertanni broth (LBB) (BD Bioxon). Both plates were then incubated at 37°C for 24, 48 and 72h. After each of these times, the content of the wells was emptied, and each well was washed three times with a sterile saline solution. Afterward, they were stained with 300µL of crystal violet at 1% and left at room temperature for 15 min. The stain was removed, the plate was washed six times with deionized sterile water and then left to dry at room temperature for 20 min. The crystal violet was solubilized with 70% ethanol, and the absorbance of the sample was measured at 590 nm at OD in an ELISA Spectronic Genesys™ reader (Stepanović et al., 2004). According to

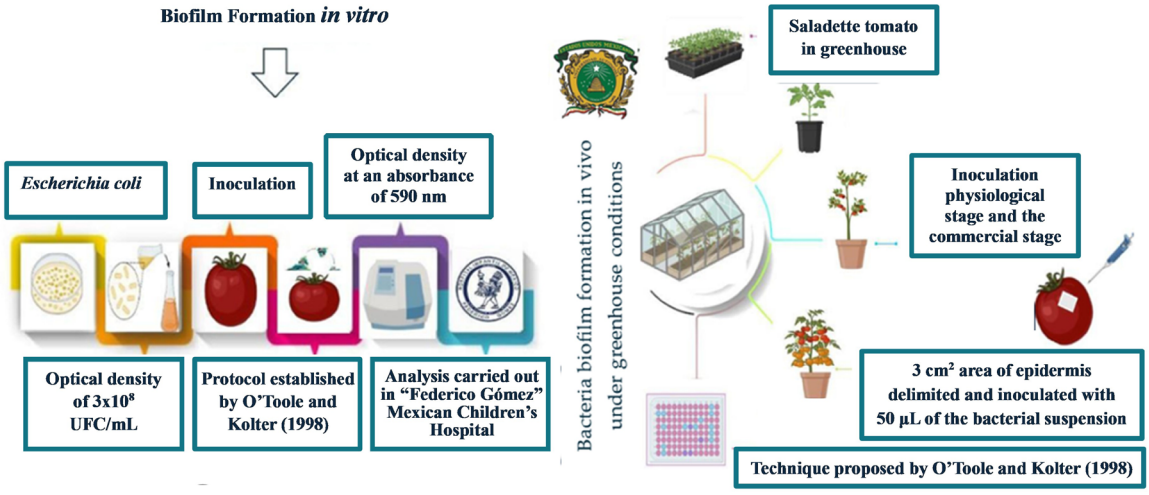


Figure 1. Biofilm formation.

Table 1. *Escherichia coli* strains used in the biofilm formation assays.

Group	Pathotype	Strain code	Serotype	Isolation source	Reference
Diarrheagenic	DAEC	E66438		Human	Tamayo-Legorreta et al., 2020
	EAEC[1]	49766	OND:H10	Human	Eslava et al., 1998
	EHEC		O157:H7	Human	Park et al., 2006
	EHEC	DL933	O157:H7	Human	Mobley et al., 1990
Extra-intestinal	UPEC	CFT073	O6:H10	Human	
Environmental[2]		SPEC DC+		Bovine	López-Islas, 2004
		3FCL+2		Bovine	
		IFL+6		Bovine	
		LPL+.		Bovine	
		3PL+C		Bovine	
		6PBL+.		Bovine	
		3FCL+2		Bovine	
Non-pathogenic[3]		HB101		Human	Tamayo-Legorreta et al., 2020

[1]Strain used as positive control due to known biofilm formation; [2]*Escherichia coli* isolated from the surfaces of bovine carcasses; [3]Non-pathogenic strain of *E. coli* generated in the laboratory from *E. coli* K12; DAEC: diffusely adherent *E. coli*; EAEC: enteroaggregative *E. coli*; EHEC: enterohemorrhagic *E. coli*; UPEC: uropathogenic *E. coli*.

the OD obtained considering a cutoff point (OD.c) (defined as three standard deviations above the mean OD of the negative control), the biofilms formed by the tested strains were classified as: non-producers [OD ≤ OD.c]; weak [OD.c < OD ≤ (2 × OD.c)]; moderate [(2 × OD.c) < OD. ≤ (4 × OD.c)] and strong producers [(4 × OD.c) < OD] (Stepanović et al., 2004). All tests were performed in duplicate in three independent assays, using as reference (blank) an assay with no bacterial inoculation.

2.2 Bacteria biofilm formation in vitro

The strains identified as strong biofilm producers in the *in vitro* assay and the negative control (HB101) were inoculated into the tomatoes' epidermis that were in two different ripening stages: physiological ripening (PR), when there is a defined green color in more than 90% of the tomato epidermis, and commercial ripening (CR), when there is a red color in more than 90% of the fruit epidermis. A 1 cm^2 well of single-component silicone with acetic curing (Dow Corning 732) was placed in each fruit. This well allowed an adequate incubation of the study bacteria and the correct handling of the technique even on the epidermis of the fruit. Once the study area was defined, the fruits were placed in plastic domes (hinged containers) lined with cotton previously moistened with sterile water and sterilized with ultraviolet light for 20 min. Then, using a micropipette, 50µL of the bacterial suspension (3×10^8 UFC/ml) was added to the surface of each fruit inside the 1 cm^2 area delimited with silicone. The fruits were incubated inside the plastic domes at 37°C for 24, 48, and 72h. Biofilm formation was quantified in accordance with the protocol of O'Toole and Kolter (1998). The assays were done in duplicate in three independent assays.

2.3 Bacteria biofilm formation in vivo *under greenhouse conditions*

A crop of 200 plants of saladette-type *Peter* variety tomato seedlings in germination trays in a greenhouse at the Faculty of Agricultural Studies (Facultad de Ciencias Agrícolas) of the Autonomous University of Mexico State (Universidad Autónoma del Estado de México; UAEMex). The seedlings were then transplanted into 35 × 35 cm pots in a chemically sterilized substrate composed of 40% field soil, 40% vermicompost, and 20% pumice. The plants were watered three times per week with well water and kept under greenhouse conditions for the whole production cycle.

Prior to the assay, a microbiological assay of the seedlings was performed, the substrate mixture, and the irrigation water (with periodic evaluation throughout the crop cycle) to rule out the presence of thermotolerant coliform bacteria. Ninety-six days after transplanting, PR and CR tomato fruits were haphazardly selected from the greenhouse plants. On each fruit, we delimited a 3 cm^2 area of epidermis with silicone and inoculated it with 50µL of the bacterial suspension 3×10^8 UFC/mL, as described above. In this assay, only the two strongest biofilm-forming strains identified during the *in vitro* assay were tested, as well as the positive and negative control strains (OND: H10 and HB101, respectively). Sampling was done after 24, 48, 72, and 96 h, evaluating the fruits obtained according to the previously described method.

2.4 Experimental design and statistical analyses

We used three separate three-way analysis of variance (ANOVA; $p < .05$) to determine the effect of *E. coli* strain and culture medium, and to determine the different strains, incubation times, and their interaction on the biofilm formation in the two *in vivo* assays (on tomato fruit epidermis in the laboratory and in the greenhouse). Upon finding significant effects in the ANOVAs, we applied the minimum significant difference (MSD) test at 5% to determine pairwise differences among groups. Statistical tests were performed with the Statistical Analysis Software program (SAS/STAT User's Guide. Software version 9.0. Cary: N.C., EE. UU., 2002).

3 RESULTS AND DISCUSSION

3.1 Colonial morphology of the studied strains

The characterization of the *E. coli* strains analyzed included colonial morphology on agar where colonies between 2–4 mm in diameter of white color, shiny surface, opaque density, and

convex elevation were observed. In the blood agar, some strains showed hemolysis around the colonies. In MacConkey agar, all the strains except negative control (HB101) were lactose positive and the biochemical test (Koneman et al., 2008) showed an IMViC (+,+,-,-) for all strains.

3.2 In vitro *biofilm formation*

The biofilm formation on polystyrene plates was positive for all *E. coli* strains except for the negative control (HB101) at the three incubation times. Except for strain E66438, which was a weak biofilm former, the rest of the DAEC strains were classified as strong biofilm formers. The extraintestinal uropathogenic *E. coli* (UPEC) strain CFT073 was classified as moderate biofilm former and the environmental (bovine-derived) strain, as weak biofilm former (Table 2).

In this study, no statistically significant differences in biofilm formation were observed between the two-growth media evaluated (glucose-enriched MEM and LBB). Studies by Cáceres et al. (2019) reported an increase in the formation of biofilms by *E. coli* and other enterobacteria in cell culture medium glucose enriched. They proposed that glucose is useful as a substrate for the exopolysaccharide matrix formation and, therefore, increases the total biomass. The potential effect of glucose on biofilm development was confirmed in two of the EHEC O157:H7 strains (Table 2). On the other hand, Mauad et al. (2023) related a stronger biofilm formation in minimal media as the LBB used in the present study. Some authors mentioned that bacterial adherence and the biofilm formation are stimulated under conditions of scarce nutrients in the medium (Reisner et al., 2006; Skyberg et al*.,* 2007; Wang et al*.,* 2013). Similarly, Pratt and Kolter (1998) reported an increase in biofilm formation by *E. coli* strains grown in LBB and low production when using a minimal broth supplemented with a carbon source such as glucose or glycerol. When comparing the nature of both media, it can be concluded that the characteristics of the culture medium and the expression of the exopolysaccharide depend, to a large extent, on the microorganism and the capacity for biofilm formation in any external condition (Cáceres et al*.,* 2019), which gives them an excellent capacity for adaptation and survival.

3.3 Biofilm expression in vitro *on tomato epidermis*

In this assay, it was observed that the serotypes O157:H7 (EHEC) and OND: H10 (EAEC) were strong biofilm-forming bacteria on tomato epidermis. It is important to point out that the strain OND: H10 of the EAEC group was isolated from the autopsy of a child (Eslava et al., 1994) and is used as a positive control in biofilm assays on abiotic surfaces (polyethylene plates). The biofilm formation on the tomato epidermis of OND: H10 began at 24h (Figure 2A) and reached its maximum expression at 72h (Figure 2B).

Regarding the time of expression, the specific behaviors of each of the strains evaluated showed statistically significant differences. In this respect, it was observed that at 24h the reference strain DL933 (O157:H7) showed 66% higher production (considering the highest peak as 100% OD) compared to the positive control strain (OND:H10). This indicates that the EAEC strains are excellent biofilm formers on biotic surfaces under natural conditions, making the aggregative pathotype of *E. coli* especially relevant as potential triggers of epidemic outbreaks. In about the year 2011, there was an outbreak in Germany related to an *E. coli* strain O104:H4, which carried genes both of EAEC and EHEC (Wang et al., 2013). This fact lends greater impact to the present work given that this is the first time that the ability of EAEC strains to colonize raw consumption vegetables was reported.

Our results coincide with previous studies that mentioned that the flagella, pili, outer membrane proteins, and biofilm production, allow the bacteria to initially interact with the surfaces and then adhere in a specific manner through cell receptors (Ryu et al*.,* 2004). Bacteria can communicate using chemical signals to detect cellular density and coordinate gene expression (Hughes & Sperandio, 2008), a process known as quorum sensing (QS). *E. coli* O157:H7 has been shown to utilize QS signals to communicate with plants and to regulate the expression of virulence and flagella genes (Carey et al*.,* 2009). Cell-cell signals between the bacteria and their hosts are regulated by acil-homoserin lactones (Hughes & Sperandio, 2008). Carey et al. (2009) pointed out that these factors are involved in the formation and

Table 2. Formation of biofilm *in vitro* by strains of *Escherichia coli* at three different incubation times in two culture media.

Group	Pathotype	Strain code	MEM ($\overline{X} \pm SD$)			LBB ($\overline{X} \pm SD$)			CF
			24h	48h	72h	24h	48h	72h	
Diarrheagenic	DAEC	E66438	0.215 ± 0.003^{a}	0.208 ± 0.002^{a}	0.264 ± 0.049^{a}	0.233 ± 0.011^{a}	0.235 ± 0.010^{a}	0.317 ± 0.030^{a}	WBF
	EAEC[1]	49766	5.49 ± 0.378^{c}	6.055 ± 0.680^{c}	6.36 ± 0.177^{c}	5.524 ± 0.423^{c}	5.949 ± 0.554^{c}	6.367 ± 0.179^{c}	SBF
	EHEC	9330	3.223 ± 0.763^{b}	4.208 ± 0.071^{b}	4.363 ± 0.173^{b}	3.385 ± 0.571^{b}	4.211 ± 0.087^{b}	4.388 ± 0.198^{b}	SBF
	EHEC	DL933	2.42 ± 0.019^{ab}	2.85 ± 0.101^{ab}	3.60 ± 0.398^{ab}	0.257 ± 0.006^{ab}	2.963 ± 0.226^{ab}	3.72 ± 0.277^{ab}	SBF
Extraintestinal	UPEC	CFT073	1.219 ± 0.138^{a}	1.850 ± 0.087^{a}	1.906 ± 0.007^{a}	1.209 ± 0.099^{a}	1.880 ± 0.044^{a}	1.719 ± 0.395^{a}	MBF
Environmental[2]		SPEC DC+	0.140 ± 0.049^{a}	0.166 ± 0.015^{a}	0.185 ± 0.013^{a}	0.150 ± 0.046^{a}	0.169 ± 0.017^{a}	0.197 ± 0.026^{a}	WBF
		3FCL+2	0.176 ± 0.017^{a}	0.231 ± 0.103^{a}	0.300 ± 0.029^{a}	0.191 ± 0.022^{a}	0.216 ± 0.080^{a}	0.332 ± 0.033^{a}	WBF
		IFL+6	0.445 ± 0.048^{a}	0.680 ± 0.046^{a}	0.720 ± 0.045^{a}	0.452 ± 0.052^{a}	0.645 ± 0.055^{a}	0.792 ± 0.019^{a}	WBF
		LPL+.	0.265 ± 0.040^{a}	0.317 ± 0.029^{a}	0.328 ± 0.028^{a}	0.345 ± 0.036^{a}	0.375 ± 0.030^{a}	0.390 ± 0.007^{a}	WBF
		3PL+C	0.294 ± 0.052^{a}	0.351 ± 0.036^{a}	0.363 ± 0.031^{a}	0.299 ± 0.053^{a}	0.371 ± 0.020^{a}	0.395 ± 0.002^{a}	WBF
		6PBL+.	0.496 ± 0.104^{a}	0.547 ± 0.038^{a}	0.613 ± 0.015^{a}	0.506 ± 0.016^{a}	0.560 ± 0.035^{a}	0.612 ± 0.051^{a}	WBF
Non-pathogenic[3]		HB101	0.169 ± 0.043^{a}	0.186 ± 0.031^{a}	0.282 ± 0.025^{a}	0.170 ± 0.039^{a}	0.196 ± 0.031^{a}	0.289 ± 0.023^{a}	NBF

[1]Strain used as positive control; [2]*Escherichia coli* isolated from the surfaces of bovine carcasses; [3]Non-pathogenic strain of *E. coli* generated in the laboratory from *E. coli* K12; SD: standard deviation; MEM: minimum essential medium; LBB: Luria Bertani broth; CF: biofilm-forming capacity; NBF: non-biofilm former; WBF: weak biofilm former; MBF: moderate biofilm former; SBF: strong biofilm former; Different letters represent statistically significant differences.

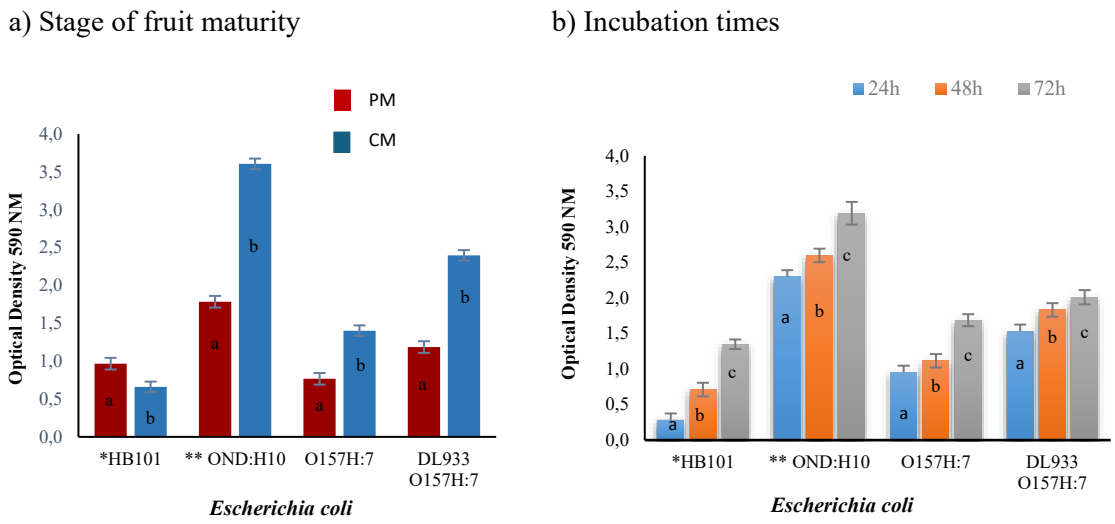
mobility of biofilms, resulting in the colonization of different horticultural products, which demonstrates the results of this study. (Adator et al., 2018; Lindsay & von Holy, 2006; Ma et al., 2019; Park et al., 2006; Reid, 2004; Skyberg et al., 2007).

3.4 In vivo *biofilm expression under greenhouse conditions*

To evaluate biofilm formation under greenhouse conditions, tomato fruits at two stages of maturity and different incubation times were inoculated with different *E. coli* strains. The results showed biofilm formation obtaining 95% confidence intervals for each of the attained means with statistically significant differences. The formation of biofilm for both strains of *E. coli* (O157:H7 and OND: H10) occurred in the PM and CM stages (Figure 3A).

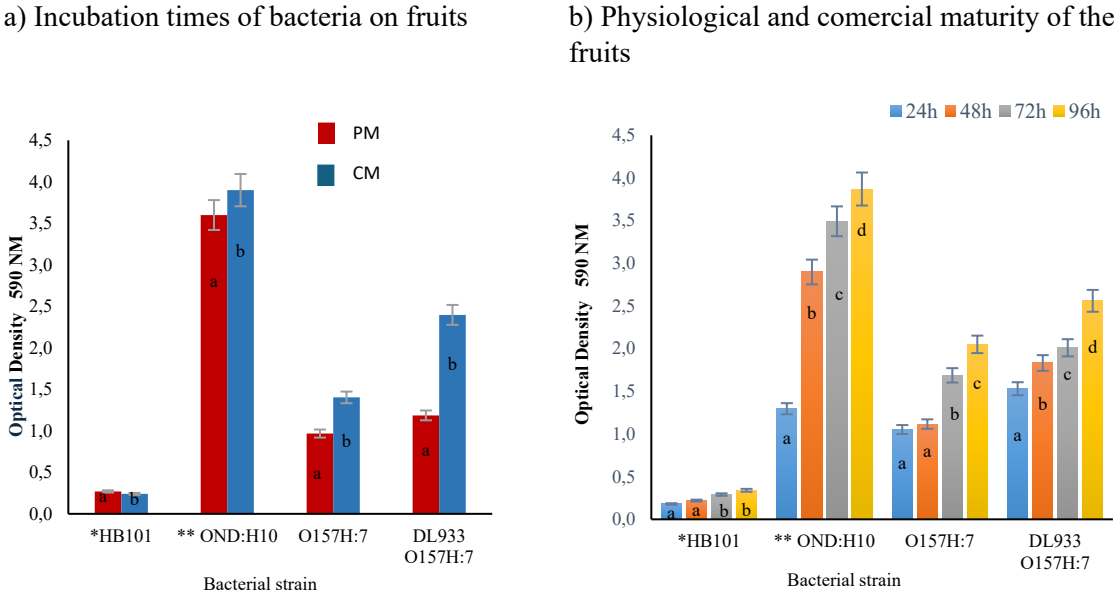
One important observation during the CM stage of the fruits was the fact that they presented internal changes in the mesocarp that increased during maturation. The development of biofilm in the greenhouse tomatoes began at 24h with a notable increase and the highest peak at 96h (Figure 3B).

Wang et al. (2013) reported differences among *E. coli* strains under controlled growth conditions, pointing out that the ability to form biofilms was not restricted to a particular serotype. This could be due to the participation of the different elements in the adhesion of the bacteria, such as fimbriae, *curli,* cellulose, exopolysaccharide, and autotransporter proteins. Other investigators suggested that virulence genes constitute a key element for the formation of biofilm (Lajhar et al., 2018; Ogasawara et al., 2010; Uhlich et al., 2013). This allows us to conclude that



*Non-pathogenic strain of *Escherichia coli* generated in the laboratory from *E. coli* K12; **Strain used as the positive control due to known biofilm formation; PM: physiological maturity; CM: commercial maturity; Different letters represent statistically significant differences.

Figure 2. Formation of biofilm on the epidermis of tomato fruits under greenhouse conditions.



*Non-pathogenic strain of *Escherichia coli* generated in the laboratory from *E. coli* K12; **Strain used as the positive control due to known biofilm formation; PM: physiological maturity; CM: commercial maturity; Different letters represent statistically significant differences.

Figure 3. Formation of biofilm on the epidermis of tomato fruits inoculated under greenhouse conditions.

the strains evaluated in the study, due to their ability to develop biofilms and adhere to tomato fruits, make them a potential threat of infectious intestinal diseases.

The formation of biofilms by *E. coli* strains reveals the survival capabilities of the pathogen. Therefore, the contact of the microorganism with the fruit is not favorable for the consumer.

Under controlled conditions, the formation of biofilm exhibited higher development at 96h after inoculation, presenting readings with a mean value of 1.86 OD. Although the inoculation was controlled in the study, uncontrolled inoculation in the field during cultivation or at various points during the fruits' processing and commercialization could occur through vehicles such as water, substrate, and inadequate crop management. Thus, it is feasible that in the lapse of a few days, bacteria inoculated in this manner could colonize and form biofilm on tomato fruits meant for consumption. Erickson (2012) suggested that pathogenic bacteria like *E. coli* O157:H7 can survive on the surface of the plant, penetrate the epicarp and eventually establish in and colonize the mesocarp. This, in conjunction with the biofilm formation on the epidermis, makes it difficult to eliminate bacteria by traditional methods (Xicohtencatl-Cortes et al., 2009). When a vegetable or fruit like tomato is exposed to bacteria, the bacteria tend to attach to its epidermis; firm adhesion generally takes up to a few hours. At that point, the adhesion becomes strong enough to resist conventional washing, making bacteria removal more difficult. The situation can become more serious with persistent humidity, which allows the synthesis of polymers, and therefore, the formation of biofilms (Avila-Novoa et al., 2018).

The strains analyzed in this study showed the capacity to produce biofilm on inert and live surfaces (tomato) within 24h of incubation. Furthermore, it has been reported that O157:H7 strains of *E. coli* can penetrate natural openings in the plant, such as the sub-stomatal cavities of the leaves (Brandl, 2008; Erickson, 2012; Kroupitski et al., 2009). Once the bacterial cells are found inside the plant or protected by an exopolysaccharide matrix, they are protected from most superficial disinfectants (Gomes et al*.,* 2009). Therefore, if the pathogen possesses the ability to form biofilm and can adhere to plant tissues in a crop, there will be a latent risk to human health from the ingestion of the contaminated product (Deering et al., 2012; Warriner et al*.,* 2003). Importantly, *E. coli* O157:H7 established on fruits and vegetables maintains its virulence to humans (Mukhopadhyay et al., 2014), such that the presence of *E. coli* O157:H7 in food practically guarantees a disease outbreak, which could have serious consequences (Figueroa-Arredondo, 2011; Lajhar et al., 2018; Torres Armendáriz et al., 2016).

4 CONCLUSION

E. coli in general, and some members of the diarrheagenic group, are of great clinical and epidemiological importance. These bacteria have different virulence factors, including the ability to express biofilms.

The strains subjected to the biofilm formation study differed in their biofilm production levels with ranges of NBF, WBF, MBF, and SBF. Biofilm formation increased with incubation time, appearing at 24h and maximizing at 72h. The strains of *E. coli* O157:H7 subjected to the first study of fruits in the laboratory gave rise to the analysis of fruits under greenhouse conditions where our findings showed that regardless of the state of maturity or the incubation time of the strains, 100% of the fruits managed to produce a biofilm. This is relevant because the physiology of the tomato fruit allows the producer to harvest the fruits without reaching commercial maturity, and in the marketing period, the fruit continues the ripening process. This is of great significance since it was demonstrated that microorganisms can colonize the fruit while it is still green and develop the protective barrier at the same rate of fruit maturity, reaching its final consumer with a high risk of generating a disease. For this reason, they emphasize the importance of constant preventive practices because actions designed to kill or inactivate pathogens have, to date, been relatively ineffective. Considering that the formation of biofilms protects bacteria from routine surface cleaning, strategies that seek to prevent contamination in the first place and detect it when it occurs may be an especially effective way to protect food safety, making it one of the most successful outcomes. The impact was the ability to adapt a technique designed for an *in vitro* analysis carried out on polystyrene plates to a living surface, with the help of the well, thus being able to measure the adaptation capacity of the strains to different environments. This technique strengthens and energizes the arsenal of applicability of future research, developments, and innovations.

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