Adherence to abiotic surface induces SOS response in *Escherichia coli* K-12 strains under aerobic and anaerobic conditions

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During the colonization of surfaces, *Escherichia coli* bacteria often encounter DNA-damaging agents and these agents can induce several defence mechanisms. Base excision repair (BER) is dedicated to the repair of oxidative DNA damage caused by reactive oxygen species (ROS) generated by chemical and physical agents or by metabolism. In this work, we have evaluated whether the interaction with an abiotic surface by mutants derived from *E. coli* K-12 deficient in some enzymes that are part of BER causes DNA damage and associated filamentation. Moreover, we studied the role of endonuclease V (*nfi* gene; 1506 mutant strain) in biofilm formation. Endonuclease V is an enzyme that is involved in DNA repair of nitrosative lesions. We verified that endonuclease V is involved in biofilm formation. Our results showed more filamentation in the *xthA* mutant (BW9091) and triple *xthA nfo nth* mutant (BW535) than in the wild-type strain (AB1157). By contrast, the mutant *nfi* did not present filamentation in biofilm, although its wild-type strain (1466) showed rare filaments in biofilm. The filamentation of bacterial cells attaching to a surface was a consequence of SOS induction measured by the SOS chromotest. However, biofilm formation depended on the ability of the bacteria to induce the SOS response since the mutant *lexA Ind* did not induce the SOS response and did not form any biofilm. Oxygen tension was an important factor for the interaction of the BER mutants, since these mutants exhibited decreased quantitative adherence under anaerobic conditions. However, our results showed that the presence or absence of oxygen did not affect the viability of BW9091 and BW535 strains. The *nfi* mutant and its wild-type did not exhibit decreased biofilm formation under anaerobic conditions. Scanning electron microscopy was also performed on the *E. coli* K-12 strains that had adhered to the glass, and we observed the presence of a structure similar to an extracellular matrix that depended on the oxygen tension. In conclusion, it was proven that bacterial interaction with abiotic surfaces can lead to SOS induction and associated filamentation. Moreover, we verified that endonuclease V is involved in biofilm formation.

**INTRODUCTION**

*Escherichia coli* is a predominant species among facultatively anaerobic bacteria of the gastrointestinal tract. Both its frequent community lifestyle and the availability of a wide array of genetic tools contributed to establish *E. coli* as a relevant model organism for the study of surface colonization (Beloin et al., 2008).

Bacterial adherence is a key step in bacterial physiology and pathogenesis and this adherence to biotic and abiotic surfaces is a complex process that, in many cases, involves the participation of several distinct adhesins, all of which may act at the same time or at different stages during colonization (Ofek et al., 2003; Rendón et al., 2007). During colonization, the bacteria often encounter DNA-damaging agents and these agents can induce various defence mechanisms.

The SOS system in *E. coli* is regulated by LexA–RecA repressor-activator proteins, respectively. In the *E. coli* SOS
response, the expression of approximately 40 unlinked genes is induced after the cell is exposed to DNA-damaging agents (Fernández de Henestrosa et al., 2000; Friedberg et al., 2006). Many of these gene products are involved in chromosome recombination, replication, repair, and segregation during cell division (Friedberg et al., 2006; Gotoh et al., 2010). Filamentation is a consequence of the SOS response. During SOS, premature cell division is prevented, and genes for DNA damage repair are induced. SulA, the SOS gene product, inhibits cell division by binding to FtsZ to block septum formation until the DNA damage has been repaired (Higashitani et al., 1995; Gotoh et al., 2010). Although the filamentation protects daughter cells from receiving damaged copies of the bacterial chromosome, the filamentous phenotype can also involve other programmes that are designed to promote bacterial survival (Justice et al., 2008).

Recently, it has been shown that the activation of the SOS response is involved in the adherence to abiotic surfaces by E. coli (Hoffman et al., 2005; Linares et al., 2006; Gotoh et al., 2010), Vibrio cholerae (Hung et al., 2006; Gotoh et al., 2010) and Mycobacterium avium (Geier et al., 2008; Gotoh et al., 2010).

Aerobically grown cells are exposed to endogenous reactive oxygen species (ROS), including singlet oxygen, superoxide, hydrogen peroxide and the hydroxyl radical. The ROS can damage intracellular components such as lipids, proteins and DNA (Inlay, 2003; Sakai et al., 2006). The mutagenesis of DNA may be generated through spontaneous mutations. However, most organisms possess mechanisms to detoxify the ROS, removing the oxidative DNA damage (Sakai et al., 2006).

Base excision repair (BER) is dedicated to the repair of the oxidative DNA damage caused by ROS generated by chemical and physical agents or by metabolism. BER involves two classes of enzymes, bifunctional glycosylases such as endonuclease III encoded by nth gene, and apurinic/apyrimidinic endonucleases, such as endonuclease IV and exonuclease III, encoded by genes nfo and xth, respectively (Wallace, 1997). E. coli possesses a homologue of every BER enzyme identified (Eisen & Hanawalt, 1999). The redundancy in BER may provide evidence for its biological significance to E. coli strains.

Endonuclease V plays the initiating role in base recognition and strand nicking. Because endonuclease V nicks at the 3’ side of the lesion and the base damage is not removed from DNA, other enzymes have to act to remove nicks at the 3’ side of the lesion. Several proposals have been put forward to explain the removal of the damaged base by 3’ exonuclease or endonuclease action (Cao, 2013).

Defence mechanisms invoked against the oxidative stress can be involved in various bacterial responses such as antibiotic resistance (Kohanski et al., 2007), increase in survival within macrophages (Suvarnapunya et al., 2003), and the production of diversity and adaptability in biofilm communities (Boles & Singh, 2008).

The essential role of oxygen in biofilm formation is controversial since Colón-González et al. (2004) reported that E. coli K-12 strains are unable to form biofilms under anaerobic conditions and Bjergbaek et al. (2007) reported that E. coli K-12 strains had the ability to form biofilm in the absence of oxygen.

In this study, we evaluated whether the adherence to abiotic surfaces would be able to generate genotoxic effects in mutants deficient in BER that were derived from E. coli K-12 with induction of the SOS system and associated filamentation. The current study showed that the filamentation observed was a consequence of SOS induction, triggered by attachment to the glass surface. In part, the filamentation and SOS induction observed in these experiments were related to ROS production, since the decrease in filamentous growth and SOS response occurred even when the assays of adherence were performed under anaerobic conditions.

Moreover, we verified the participation of nitrous agents in biofilm formation under anaerobic and aerobic conditions using the mutant deficient in endonuclease V (nfi mutant), since this enzyme is responsible for recognizing the DNA damage caused by these agents.

**METHODS**

**Bacterial strains.** The bacterial strains derived from E. coli K-12 used in this study are listed in Table 1. All strains are isogenic, except for the particular mutation of interest. The prototype EAEC 042 strain (AAF/II), originally isolated from an infant with diarrhoea in Lima, Peru (Nataro et al., 1987), was used as a positive control. E. coli K-12 DH5α strain was used as a negative control in all assays. Microorganisms were stored at −70 °C in Luria broth (LB; Merck) with 20% glycerol.

**Bacterial culture conditions.** Biofilm formation assays were done with bacterial strains grown in LB for 18 h at 37 °C, under aerobic and anaerobic conditions. The anaerobic conditions were generated by an AnaeroGen kit (Oxoid).

**Biofilm methods.** To assess qualitative biofilm formation, 1 ml Dulbecco’s modified Eagle’s medium (DMEM; Sigma) containing 0.45% glucose was added to each well of 24-well tissue culture plates with circular glass coverslips (Nunc International). Bacterial cultures (10 μl, approx. 10⁷ bacterial cells) grown overnight in LB, under aerobic and anaerobic conditions, were then incubated with the DMEM 18 h at 37 °C, under aerobic and anaerobic conditions. At the end of the incubation time, the culture medium was aspirated, and the substratum was washed three times with Dulbecco’s PBS (PBS-D), fixed with methanol, and stained with 0.5% crystal violet (CV) for 5 min. The stained coverslips were mounted on glass slides and examined by light microscopy (Sheikh et al., 2001).

To assess semiquantitative biofilm formation, 200 μl DMEM containing 0.45% glucose in a 96-well flat-bottom polystyrene microtitre plate (TPK) was inoculated with 5 μl (approx. 10⁶ bacterial cells) of a bacterial culture in LB grown overnight at 37 °C, under aerobic and anaerobic conditions. The plate was incubated for 18 h at 37 °C, under aerobic and anaerobic conditions. Planktonic cells were then removed by rinsing three times with water, and the substratum was stained with 0.5% CV for 5 min. After washing with water and blotting with paper towels, the biofilm formation was quantified spectrophotometrically by the addition of 200 μl 93% ethanol to each
measurements of the SOS Chromotest (Quillardet & Hofnung, 1985; Asad et al., 2010). The induction of the SOS system was determined as described in biofilm methods with some modifications. After an 18 h incubation period at 37 °C, under aerobic or anaerobic conditions, the substratum was washed with water and stained by placing 200 μl staining solution (1 μl SYTO 9 stain and 1 μl propidium iodide stain in 1 ml filter-sterilized water) onto the biofilm sample for 30 min, at room temperature, protected from light. After washing with water, the stained coverslips were transferred to a new microtitre plate, and the absorbance was determined with an ELISA plate reader at 570 nm (Mohamed et al., 2007).

**Biofilm viability.** Biofilm viability was evaluated by a FilmTracer live/dead biofilm viability kit (Invitrogen). The qualitative biofilm formation was determined as described in biofilm methods with some modifications. After an 18 h incubation period at 37 °C, under aerobic or anaerobic conditions, the substratum was washed with PBS-D and overlaid with 1 ml SYTO 9 stain and 1 μl propidium iodide stain in 1 ml filter-sterilized water) onto the biofilm sample for 30 min, at room temperature, protected from light. After washing with water, the stained coverslips were mounted on glass slides and examined by confocal microscopy.

**SOS induction.** The bacterial strain PQ35 was cultured in 5 ml LB containing 50 μg ampicillin ml⁻¹ for 18 h at 37 °C, under aerobic and anaerobic conditions. Cells were then centrifuged, resuspended in 1 ml PBS-D, and 10 μl (approx. 10⁶ bacterial cells) of the bacterial suspension was added to 24-well tissue culture plates with glass slides, and DMEM containing 0.45 % glucose was added. After an 18 h incubation period, under aerobic or anaerobic conditions, the substratum was washed twice with PBS-D and overlaid with 1 ml 1 % Triton X-100 in PBS-D for 30 min. Samples of bacterial cells were removed from the biofilm, centrifuged and resuspended in a saline buffer (Alves et al., 2010). The induction of the SOS system was measured in E. coli PQ35 (adherence to glass and planktonic cells) by means of an sfi::lacZ operon fusion according to the principle of the SOS Chromotest (Quillardet & Hofnung, 1985; Asad et al., 1997). The measurements of β-galactosidase and alkaline phosphatase activities were performed as described by Quillardet & Hofnung (1985) and Asad et al. (1997). The results were calculated according to the ratio, R, of β-galactosidase units (B) to alkaline phosphatase units (P): R=A_B×P/ incubation time (min). Results were considered statistically significant when P<0.05.

**Scanning electron microscopy.** For scanning electron microscopy (SEM), biofilm specimens (18 h) obtained from glass slides were fixed in 2.5 % glutaraldehyde, post-fixed in 1 % osmium tetroxide and dehydrated in a graded series of ethanol. Subsequently, the samples were subjected to critical-point drying with carbon dioxide, covered with gold-palladium to a 10 nm layer and examined with a JEOL JSM 5310 scanning electron microscope.

**Statistical analysis.** All assays were performed three times under each condition in duplicate. The means from each experiment were analysed by Student’s t-test using the GraphPad Prism statistical software. Results were considered statistically significant when P≤0.05.

### RESULTS

**Expression of filamentous phenotype during adherence of bacterial strains to abiotic surfaces**

We showed previously that adherence to HEp-2 cells is accompanied by filamentous growth and SOS induction in a wild-type strain (AB1157), and especially in BER mutants (BW9091 and BW535) (Costa et al., 2012). Therefore, we wondered whether the attachment to glass could lead to DNA damage in the same BER mutants, resulting in a filamentation response.

Microscopic inspection of bacterial adherence to glass after 18 h of incubation revealed that all strains of E. coli K-12 tested were able to adhere to glass (Fig. 1, Table 2).

Moreover, this adherence to glass was accompanied by filamentous bacterial growth in the xthA mutant (BW9091) (Fig. 1b) and especially in the triple xthA nfo nth mutant (BW535) (Fig. 1c). This result is similar to that observed in the adherence to HEp-2 cells where BER mutants (BW9091 and BW535) also revealed higher filamentation (Costa et al., 2012). Nevertheless, the wild-type strain (AB1157) presented rare filaments (Fig. 1a) and the strain deficient in SOS induction (lexAInd⁻, DM49) did not exhibit bacterial cell filamentation (Fig. 1d). The nfi mutant (1506) also did not show filaments, in contrast to its isogenic wild-type strain (1466), which showed rare filaments (Table 2).

**Influence of oxygen tension on filamentous growth during the adherence to glass of E. coli K-12 strains**

To examine the involvement of ROS in the filamentation of strains adhering to glass, we compared the filamentation of E. coli cells grown and attached to glass under anaerobic or aerobic conditions. The results displayed in Fig. 2 and Table 2 show that all strains of E. coli K-12 tested were able to

### Table 1. Bacterial strains derived from E. coli K-12 used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB1157</td>
<td>Wild-type</td>
<td>J. D. Hall and P. Howard-Flanders*</td>
</tr>
<tr>
<td>BW9091</td>
<td>xthA</td>
<td>B. Weiss†</td>
</tr>
<tr>
<td>BW535</td>
<td>nfo-1::kan nth-1::kanΔ(xth-pncA)90</td>
<td>B. Weiss†</td>
</tr>
<tr>
<td>DM49</td>
<td>lexA3</td>
<td>J. D. Hall and P. Howard-Flanders*</td>
</tr>
<tr>
<td>PQ35</td>
<td>sfiA::Mud(Ap lac) ets lacU169</td>
<td>P. Quillard and M. Hofnung†</td>
</tr>
<tr>
<td>BW1506</td>
<td>nfs-1::cat ara Δ(gpt-lac) tna lac</td>
<td>B. Weiss†</td>
</tr>
<tr>
<td>BW1466</td>
<td>wild-type</td>
<td>B. Weiss†</td>
</tr>
</tbody>
</table>

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†Emory University, Atlanta, GA, USA.
§Institut Pasteur, Paris, France.
adhere to glass under anaerobic conditions. Furthermore, this adherence to glass was accompanied by a filamentation response even when the experiments were performed in the absence of oxygen, except for the mutant deficient in SOS induction (DM49) (Fig. 2) and the \( \text{nfi} \) mutant that exhibited no filamentation when it adhered to glass (Table 2).

### SOS response after bacterial adherence to glass in the presence and absence of oxygen

Filamentation is one of the characteristic features of the induction of the SOS response, which is triggered by DNA-damaging agents (Walker, 1985). So, we measured the SOS induction of recovered bacteria that adhered to glass and bacterial cells grown in liquid medium (DMEM), in the presence and absence of oxygen, through an SOS-chromotest assay (Quillardet & Hofnung, 1985; Asad et al., 1997).

In this assay, the level of SOS induced was evaluated by measuring the induction of \( \beta \)-galactosidase in \( E. \ coli \) PQ35 (wild-type strain), in which the \( \text{lacZ} \) gene has been fused to the \( \text{sfiA} \) gene, an SOS gene (Quillardet & Hofnung, 1985). We observed higher \( \beta \)-galactosidase expression in the glass-attached bacteria when compared with DMEM-grown cells under both aerobic and anaerobic conditions (Fig. 3). However, we observed a decrease in \( \beta \)-galactosidase expression in both glass-attached and DMEM-grown bacteria under anaerobic conditions (Fig. 3).

### Quantitative adherence to abiotic surface by \( E. \ coli \) K-12 strains in the presence and absence of oxygen

The ability of \( E. \ coli \) K-12 strains to adhere to a different abiotic surface (polystyrene) was assessed by a quantitative test (Fig. 4). In this assay, it was observed that \( E. \ coli \) K-12 strains (AB1157, BW9091, BW535 and DM49) adhered less to polystyrene than did the EAEC 042 strain, which was used as a positive control. However, the \( \text{nfi} \) mutant showed an increase in biofilm formation when compared with strain EAEC 042 under aerobic and anaerobic conditions (Fig. 5).

Statistical analysis revealed a significant increase in adherence to polystyrene for the wild-type strain (AB1157) and BER mutants (BW9091 and BW535) as compared with the negative control \( E. \ coli \) K-12 DH5z. However, the \( \text{lexA} \) mutant deficient in SOS induction (DM49) did not present any significant difference in adherence to polystyrene in comparison with \( E. \ coli \) K-12 DH5z (Fig. 4).

In aerobiosis, the mutant \( \text{xthA} \) (BW9091) adhered to the polystyrene substratum to a similar degree as the wild-type strain (AB1157) (Fig. 4). However, under the same conditions, the mutant \( \text{xthA} \) showed a decrease in adherence to polystyrene when compared with the wild-type strain (AB1157) (Fig. 4).
conditions, the triple mutant xthA nfo nth (BW535) presented a significant decrease in adherence to polystyrene when compared with the wild-type strain (AB1157) (Fig. 4). Oxygen tension affects the adherence of BER mutants (BW9091 and BW535) to polystyrene as compared with the wild-type strain (AB1157). Our results show that under anaerobic conditions, the mutant xthA (BW9091) and the triple mutant xthA nfo nth (BW535) present a significant decrease in adherence to polystyrene when compared with wild-type strain (AB1157) (Fig. 4). However, the nfi mutant and its wild-type strain did not show any difference in biofilm formed under aerobic or anaerobic conditions (Fig. 5).

Influence of oxygen tension on the viability of E. coli K-12 strains during adherence to glass

To verify whether the reduction in the number of BER mutants adherent to polystyrene under anaerobic conditions was related to the death of the bacterial strains, viability tests were performed using the FilmTracer live/dead biofilm viability kit. Thus, bacteria with intact cell membranes (i.e. live) would stain fluorescent green, whereas bacteria with damaged membranes (i.e. dead) would stain fluorescent red. Our results showed that the presence or absence of oxygen did not directly affect the viability of strains, since the number of viable bacteria observed was greater than the number of dead bacteria under aerobic as well as anaerobic conditions, for all strains tested (Fig. 6).

Scanning electron microscopy of E. coli K-12 strains that adhered to glass

Our results showed that the wild-type strain (AB1157) and xthA mutant (BW9091) presented microcolony formation on a glass surface surrounded by a structure suggestive of an extracellular matrix under aerobic conditions (Fig. 7a, c). However, under anaerobic conditions, the matrix-enclosed microcolonies were absent in both strains (Fig. 7b, d). Moreover, the triple mutant xthA nfo nth (BW535) formed microcolonies on a glass surface, but did not present a structure suggestive of an extracellular matrix under aerobic or anaerobic conditions (Fig. 7e, f). For this strain, intense filamentation was also observed under both conditions (Fig. 7e, f).

DISCUSSION

We showed previously that induction of the SOS response occurs when E. coli K-12 strains interact with epithelial cells
Recently, a clear link was shown between the activation of the SOS response and the adherence to abiotic surfaces by *L. monocytogenes* and *P. aeruginosa* (van der Veen & Abee, 2010; Gotoh et al., 2010). Filamentation is a consequence of the SOS response. Clearly, the involvement in SOS response in adherence to abiotic surfaces raises an interesting question about colonization surfaces (Gotoh et al., 2010).

It is known that other systems inducible by ROS are not only involved in the defence against environmental stresses, but also involved in the adherence of *E. coli* to surfaces. For example, mutation of the *E. coli* oxidative stress response regulator gene *oxyR* results in the overexpression of an adhesin related to adherence to abiotic surfaces (Ag43). As a result, *E. coli oxyR* mutants display an increased adherence to abiotic surfaces and autoaggregation in addition to a decrease in motility (Ulett et al., 2006). Moreover, *rpoS*, another regulator gene involved in several stress responses, may affect cell adherence (Dong et al., 2009).

Studies investigating the role of oxygen in bacterial interaction are of great importance, because the primary niche of *E. coli in vivo* is the gastrointestinal tract where conditions are anaerobic or of low oxygen tension (Bjergbaek et al., 2007).

Our results show that under anaerobic conditions the bacterial strains tested had the ability to adhere to glass and form filamentous growth. However, a reduction in the filamentation was observed when wild-type strain (AB1157) and BER mutants (BW9091 and BW535) were submitted to anaerobiosis. This reduction in filamentation in the absence of oxygen was consistent with the decrease in SOS induction in this condition, when compared with that observed under aerobic conditions. These results suggest that even under anaerobic conditions, other agents such as nitrous agents can be produced, which can be responsible for the DNA damage of these strains, inducing the SOS response and observable filamentation. The wild-type strain proficient in endonuclease V showed a decrease in filamentation under anaerobic conditions.

The mutant *nfi* presented higher biofilm formation in aerobic and anaerobic conditions. Endonuclease V is an enzyme that is involved in DNA repair of nitrosative lesions (Weiss, 2001). Probably, these lesions caused by nitrosating agents are generated independently of oxygen tension. Weiss (2001) observed that under aerobic conditions the frequency of mutation in the *nfi* mutant is similar to what is seen under anaerobic conditions. Because in the *nfi* mutant the base damage is not removed from DNA and gaps are not formed, the SOS response is not induced and the associated filamentation does not occur. However, the wild-type strain presented an SOS response and filamentation.

Paradoxically, the *nfi* mutant has an increased rate of survival when compared with the wild-type strain. The increase in double-stranded DNA breaks in the wild-type strain is more lethal than the unrepaird DNA mutagenic lesions in the *nfi* mutant (1506) (Guo and Weiss, 1998). This fact may explain the decrease in biofilm formation of the wild-type strain (1466) compared with the *nfi* mutant.

We also measured the SOS induction of bacteria grown in liquid medium (DMEM), and a decrease in SOS response was observed in the presence or absence of oxygen when
compared with the SOS induction of bacteria that adhered to glass under both conditions. These results suggest that bacterial cells that adhered to abiotic surfaces, such as glass, present an increased SOS expression when compared with those in planktonic form.

In order to quantify the adhesion to the abiotic surface, tests were performed on another surface (polystyrene). Although the number of bacteria that attached to polystyrene for each strain (AB1157, BW9091 and BW535) was lower than that measured for the EAEC 042 strain, which was used as a positive control in the test, it was observed that the strains tested in this study adhered more than *E. coli* K-12 DH5α (not forming biofilm), which was used as a negative control in this type of experiment (Mohamed et al., 2007). This result indicates that these *E. coli* K-12 strains were able to form biofilms under aerobic or anaerobic conditions. Our results also show that the *nfi* mutant had a higher biofilm formation than the positive control EAEC 042 under aerobic and anaerobic conditions.

Gotoh et al. (2010) showed that a *lexA* mutant deficient in SOS induction did not present any biofilm formation in *P. aeruginosa*. Here, it was also observed that the *lexA* mutant deficient in SOS induction (DM49) did not present any significant difference in adherence to polystyrene when compared with *E. coli* K-12 DH5α, indicating that induction of the SOS system can affect the adherence by *E. coli* K-12 to abiotic surfaces. Interestingly, despite the fact that the *nfi* mutant did not show increased filamentation, it showed an increase in biofilm formation.

It is known that oxygen influences biofilm formation in *E. coli* K-12, since oxygen tension has been identified as one of the factors that contribute to the dispersion of mature biofilms.
biofilms (Karatan & Watnick, 2009). Nevertheless, some authors have shown that *E. coli* K-12 strains are able to form biofilms under anaerobic conditions, depending on the conditions of the experiment (Cabellos-Avelar et al., 2006; Bjergbaek et al., 2007).

In this study, we report on *E. coli* strains (AB1157, BW9091 and BW535) with the ability to adhere to polystyrene in the absence of oxygen, but at different levels. In aerobiosis, the mutant *xthA* (BW9091) adhered to the polystyrene substratum to a similar degree to that of the wild-type strain (AB1157), but in anaerobiosis, a significant decrease was observed in its adherence to polystyrene. The triple mutant *xthA nfo nth* (BW535) presented a significant decrease in adherence to polystyrene when compared with the wild-type strain (AB1157) under both conditions. However, we can attest that the reduction in the number of BER mutants adherent to polystyrene under anaerobic conditions was not related to the death of the bacterial strains. In this case, lesions generated in DNA of BER strains may lead to filamentation and SOS induction in biofilms. Probably, BER participates subsequently, removing the terminal left by the action of endonuclease V. In this way, the gaps formed by endonuclease V are not repaired in the mutants deficient in BER, leading to increased frequency of mutations, which could reduce biofilm formation.

Several authors have found that biofilm growth can produce extensive genetic diversity in bacterial populations even though no external stress or mutagen is applied. Diverse genetic variants are generated by biofilms of *P. aeruginosa, Pseudomonas fluorescens, V. cholerae, Streptococcus pneumoniae* and *Staphylococcus aureus*, and the formation of bacterial subpopulations interferes with the characteristics of biofilms (Déziel et al., 2001; Waite et al., 2001; Boles et al., 2004; Kirisits et al., 2005; Allegrucci & Sauer, 2007; Palmer & Stoodley, 2007; Yarwood et al., 2007). Boles & Singh (2008) showed that the presence of double-stranded DNA breaks generated under stress conditions increased the adaptability to environmental changes in biofilms of the pathogen *P. aeruginosa* and that this was due to an increase in mutation frequencies leading to the formation of genetic variants when these breaks were repaired by a mutagenic mechanism involving recombinatorial DNA repair genes.

**Fig. 7.** Scanning electron micrographs of *E. coli* K-12 strains that adhered to glass in aerobiosis (a, c, e) and anaerobiosis (b, d, f), after 18 h of incubation at 37 °C in DMEM. (a, b) Wild-type strain (AB1157), (c, d) *xthA* mutant (BW9091) and (e, f) triple mutant *xthA nfo nth* (BW535) showing intense filamentation (arrows) under both conditions. Bars, 5 μm.
The presence of an extracellular matrix involving bacteria is an important factor for the development of a mature biofilm, and the composition of this matrix is highly complex and influenced by many environmental factors as well as the characteristics of the strains tested (Ghannoum & O’Toole, 2001; Beloin et al., 2008).

The scanning electron micrographs showed that the strains tested (AB1157, BW9091 and BW535) formed microcolonies on glass surfaces under aerobic and anaerobic conditions. However, a structure suggestive of an extracellular matrix was observed only in the wild-type strain (AB1157) and the xthA mutant (BW9091) in aerobicosis. Bjergbaek et al. (2007) showed that oxygen tension affects the morphology of biofilms. Müskin et al. (2008) observed that enterohaemorrhagic E. coli (EHEC) expressed a type of fimbriae only under anaerobic conditions. Moreover, as mentioned, the oxygen tension also regulates the expression of an adhesion (Ag43) (Schembri & Klemm, 2001).

In this study, no extracellular matrix structure was observed in the triple mutant xthA nfo nth (BW535), and this mutant formed less biofilm, both in aerobicosis and in anaerobiosis. Janion et al. (2003) showed that a triple mutant for the xth, nfo and nth genes, deficient in BER, which chronically triggered the SOS system, exhibited a strong filament formation. The fact that this mutant chronically triggered the SOS system may reflect both the quantity and morphology of the biofilm formation for this strain.

Recently, it has been shown that induction of the SOS response influences biofilm formation. Gotoh et al. (2010) showed that a mutant of P. aeruginosa, which chronically triggered the SOS system, exhibited a decrease in biofilm formation. Gotoh et al. (2008) showed that different environmental stresses lead to the induction of the SOS system; the induction of this system is responsible for increasing the ability of bacterial strains to form biofilms. In our work, we showed that the induction of the SOS system occurs in biofilms, even without the influence of an external inducing agent.

Studies have shown that filamentation should not be considered merely the response of the overstressed, sick and dying members of the population, but also a form of survival of the bacterial community. Uropathogenic E. coli (UPEC) employ filamentation as a means to evade immune response. A study by Justice et al. (2006) proposed that components of the innate immune response such as oxidative radicals may induce the SOS response and bacterial filamentation.

All data presented in the current work show the influence of bacterial interaction with the abiotic surface, leading to the induction of the SOS system with the associated filamentation response. Furthermore, we showed that BER mutants present more filaments as compared with the isogenic wild-type strain in the biofilm formed. The relevance of these data highlights that responses such as the SOS are involved in the defence of the genome of planktonic cells against environmental agents and that they can be triggered when micro-organisms adhere to abiotic surfaces, which is essential for colonization of commensal bacteria and/or pathogens. By contrast, the nfi mutant did not induce the SOS response and associated filamentation under either aerobic or anaerobic conditions, but this mutant was able to form biofilms more intensely than the EAEC 042 strain used as positive control. Therefore, other pathways besides induction of the SOS system could participate in biofilm formation.

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