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Pseudomonas grimontii biofilm protects food contact surfaces from *Escherichia coli* colonization

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ABSTRACT

Escherichia coli typically colonizes food contact surfaces in the presence of other bacterial strains. The aim of this work was to evaluate the influence of a resident strain isolated from a fresh-cut salad industry (*Pseudomonas grimontii* 13A10) on the development of a model pathogen (*E. coli*) on bare stainless steel (SST) and stainless steel coated with diamond-like carbon (DLC) films, a-C:H:Si:O designated by SICON® and a-C:H:Si designated by SICAN. The bacterial composition and spatial organization of single- and dual-species biofilms were analyzed by confocal laser scanning microscopy (CLSM). Biofilms were developed for 1 and 3 days at 10 °C and it was observed that the biovolume of *E. coli* biofilms in the presence of *P. grimontii* was lower than in axenic conditions, suggesting that the isolate can protect food contact surfaces from pathogen colonization. After 3 days, the dual-species biofilms contained essentially *P. grimontii* cells and no preferential vertical distribution of bacterial strains was observed. The use of a-C:H:Si:O coated surfaces reduced the short-term colonization of the model pathogen in single- and dual-species biofilms, whereas decreased colonization by the non-pathogenic strain was only observed after 3 days.

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1. Introduction

Escherichia coli is a common foodborne bacterial pathogen (Giaouris et al., 2015) that can be found in vegetable processing industries and ready-to-eat products (Srey, Jahid, & Ha, 2013). When a pathogenic bacterium such as *E. coli* encounters a surface, it can interact with other microorganisms of the surface-associated community. It is well documented that *E. coli* is able to form multi-species biofilms with most common bacterial genera occurring on food processing surfaces, including both Gram negative (e.g. *Pseudomonas*, *Acinetobacter* and *Salmonella*) and Gram positive species (e.g. Staphylococci and *Bacillus*) (Castonguay et al., 2006; Chen, Zhao, & Doyle, 2015; Habimana, Heir, Langsrud, Asli, & Moretro, 2010; Kuznetsova, Maslennikova, Karpunina, Nesterova, & Demakov, 2013; Liu, Nou, Lefcourt, Shelton, & Lo, 2014; Marouani-Gadri, Augier, & Carpentier, 2009). Species interactions in biofilms are generally categorized as synergistic, antagonistic or neutral by comparing dual-species biofilms with axenic conditions (Dai, 2010). Bacteria isolated from food processing environments have been shown to stimulate biofilm formation of *E. coli* (Castonguay et al., 2006; Habimana et al., 2010; Klayman, Volden, Stewart, & Camper, 2009; Liu et al., 2014; Marouani-Gadri et al., 2009; Uhlich, Rogers, & Mosier, 2010). All but one of 20 collected bacterial isolates obtained after cleaning and disinfection of a beef processing plant increased the counts of attached *E. coli* O157:H7 in dual-culture biofilms (Marouani-Gadri et al., 2009). Under flow conditions, adhesion of *Acinetobacter calcoaceticus* (Habimana et al., 2010), *Pseudomonas aeruginosa* (Klayman et al., 2009) and *Pseudomonas putida* (Castonguay et al., 2006) also stimulated *E. coli* adhesion. Liu et al. (2014) found that strong biofilm-forming plant-associated bacteria promoted the incorporation of *E. coli* O157:H7 into dual-species biofilms. In contrast, some other isolates were shown to reduce the *E. coli* population (Liu et al., 2014). Other authors have also shown an antagonistic effect in mixed-species biofilms with *E. coli* (Castonguay et al., 2006; Chen et al., 2015; Dai, 2010; Kuznetsova et al., 2013; Liao, 2007). Liao (2007) reported that the native microflora recovered from fresh-peeled baby carrots had an inhibitory effect on the viability and growth of *E. coli* O157:H7. *E. coli* biofilm formation on solid surfaces was also significantly reduced in dual-species cultures with *Staphylococcus*

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epidermidis (Castonguay et al., 2006), *Stenotrophomonas maltophilia* (Dai, 2010) and *P. aeruginosa* (Kuznetsova et al., 2013). Chen et al. (2015) reported that the count of *E. coli* O157:H7 cells in dual-species biofilms formed with a *Salmonella* strain was approximately 10-fold lower than in the single-species biofilm.

Besides the particular bacterial species or strain, biofilm onset and resistance in food processing environments may also be affected by the properties of the contact surface (Lemos, Gomes, Mergulhão, Melo, & Simões, 2015; Moreira, Gomes, Simões, Melo, & Mergulhão, 2015; Oosterik, Tuntufye, Butaye, & Goddeeris, 2014). In food plants, it is common to find critical zones, such as crevices, corners, joints and valves, with low fluid velocities, making these zones suitable places for biofilm development (Lemos et al., 2015). One of the approaches to reduce bacterial adhesion and improve cleanability in these regions is the modification of the energetic and topographic surface properties (Boxler, Augustin, & Scholl, 2013a). In particular, diamond-like carbon (DLC) coatings, approved as food contact surfaces, have been investigated as alternative to stainless steel due to their thermal conductivity, low friction, smoothness, wear resistance and anti-fouling properties (Boxler, Augustin, & Scholl, 2013b).

In this study, a model *E. coli* pathogen (*E. coli* SS2 GFP) and an isolate from a fresh-cut salad process (*Pseudomonas grimontii* 13A10) were used to form biofilms on bare stainless steel (SST) and on two DLC-coated SST surfaces, a-C:H:Si:O designated by SICON® and a-C:H:Si designated by SICAN, under either single- and dual-species conditions, for 1 (early biofilm) and 3 days (mature biofilm). Confocal laser scanning microscopy (CLSM) was applied to evaluate the biofilm-forming capacity of both strains in co-culture and in single-species biofilms. The effectiveness of the modified DLC surfaces in reducing biofilm formation was also assessed in both conditions. Additionally, we wondered how these surfaces may influence the bacterial composition and spatial distribution of biofilms. The information gathered from this study provides clues to understand the pathogen persistency on food contact surfaces and to develop more efficient microbiological control strategies in fresh-produce processing environments.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The bacterial strains used in this study were a model pathogen - *E. coli* SS2 (kindly provided by Manuel Simões, University of Porto) expressing the green fluorescent protein (GFP) -, and an industrial isolate from a fresh-cut salad process - *P. grimontii* 13A10 (kindly provided by Stéphanie Lacarbona, ACTALIA). For the construction of *E. coli* SS2 GFP, the previously described pCM11 plasmid (Pmid 19610092) carrying the gene encoding superfolder GFP (sGFP) and conferring ampicillin resistance in *E. coli* was introduced in the SS2 strain by the heat shock method (Sambrook, Fritsch, & Maniatis, 1989). Stock cultures were maintained at −80 °C in Tryptone Soy Broth (TSB, BioMérieux, Marcy-l'Étoile, France) containing 200 mL/L glycerol. Prior to each experiment, frozen cells were subcultured twice in TSB at 30 °C. Ampicillin at 0.1 g/L final concentration was used to maintain pCM11 in *E. coli* SS2.

2.2. Surface preparation

Round coupons (1 cm diameter) made from electropolished stainless steel (SST; AISI 316L/X2CrNiMo17-12-2/1.4404), SICON® and SICAN were tested. The coatings were prepared by the Fraunhofer Institute for Surface Engineering and Thin Films (IST) in Braunschweig, Germany, and a detailed description of the SICON® and SICAN preparation methods can be found elsewhere (Corbella, Bialuch, Kleinschmidt, & Bewilogua, 2009; Grischke, Hieke, Morgenweck, & Dimigen, 1998).

Surfaces were soaked for 20 min in a solution of an industrial disinfectant (at 20 mL/L) that is commonly used in the salad-washing industry (TEGO 2000®, Goldschmidt AG, Essen, Germany) under strong agitation and then aseptically rinsed twice in distilled water for 20 min. The sterility of the coupons was confirmed by the absence of bacterial growth in the coupon surface after a 1-day incubation in TSB at 30 °C.

2.3. Biofilm formation

For single-species biofilms of *E. coli* SS2 GFP and *P. grimontii* 13A10, 2 mL of an overnight culture in TSB adjusted to an optical density (OD) of 0.01 at 600 nm (1:100 dilution from an initial cell suspension at $OD_{600\ nm} = 1$) were added to each well of 24-well polystyrene, flat-bottomed TPP® tissue culture plates (Sigma-Aldrich, Saint-Quentin-Fallavier, France) containing the coupons of different materials. For dual-species biofilms formed by *E. coli* SS2 GFP in the presence of the competing isolate *P. grimontii* 13A10, overnight cultures of strains were adjusted to an OD_{600nm} of 0.01, mixed at a ratio of 1:1 and transferred to 24-well plates. The microplates were then kept at 10 °C for 1 h to enable bacteria to attach to the surface materials. After this adhesion step, the wells were emptied and refilled with 2 mL of sterile TSB, and the microplates were incubated for 1 (early biofilm) and 3 days (mature biofilm) at 10 °C without shaking to allow biofilm development.

2.4. Confocal Laser Scanning Microscopy (CLSM) image acquisition and analysis

Both single- and dual-species biofilms which developed after 1 and 3 days on all tested surfaces were observed using a Leica SP2 AOBS CLSM (Leica Microsystems, Nanterre, France) at the INRA MIMA2 microscopy platform (www6.jouy.inra.fr/mima2). *E. coli* cells were pinpointed from the GFP expression, whereas *P. grimontii* 13A10 was counterstained in red with 5×10^6 mol/L Syto61 (Invitrogen, Paris, France), a cell-permeant fluorescent nucleic acid marker. Stained coupons were rinsed once with TSB to eliminate any free floating bacteria and dye in excess. Each coupon was inverted, mounted on a coverslip and scanned using a 40× water immersion objective lens at excitation wavelengths of 488 nm (argon laser) and 633 nm (helium-neon laser). The emitted fluorescence was recorded within the range of 500–580 nm to collect the GFP emission fluorescence and 640–730 nm to collect the Syto61 emitted fluorescence. To generate images of the biofilms, a minimum of nine *z* image series with a 4 μm step were acquired for each single- and dual-species biofilms on three different coupons.

Three- and two-dimensional projections (Figs. 1 and 3, respectively) of biofilm structures were reconstructed using the "Easy 3D" and "Section" functions of the IMARIS 7.0 software (Bitplane, Zürich, Switzerland), respectively, directly from the *xyz* images series. Biofilm biovolumes were calculated using PHLIP (Xavier, White, & Almeida, 2003), a freely available Matlab-based image analysis toolbox (http://sourceforge.net/projects/phlip/). The biovolumes represent the overall volume of cells (μm^3) and can be used to estimate the total biomass of the biofilm. The biovolume was defined as the number of foreground pixels in an image stack multiplied by the voxel volume, which is a product of the squared pixel size and the scanning step size (Kuehn et al., 1998). Biovolume values were normalized by the surface unit ($\mu m^3/\mu m^2$). A control experiment was performed in which biofilms of *E. coli* SS2 GFP were stained with Syto61 and the biovolumes were determined using the GFP or the Syto61 channel. Similar results were obtained, which indicates that cells were effectively expressing GFP.

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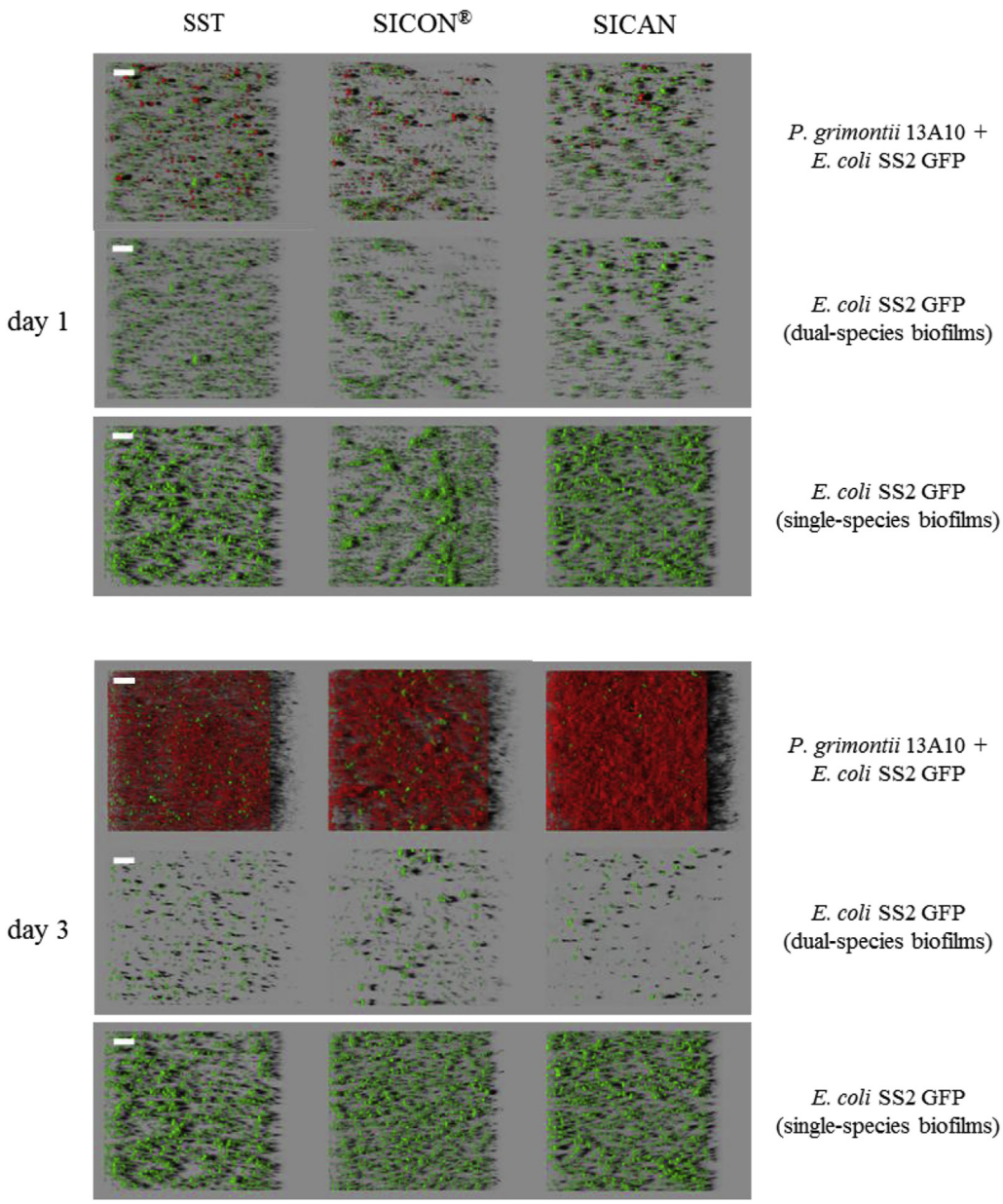

Fig. 1. Dual-species biofilms of *P. grimontii* 13A10 and *E. coli* SS2 GFP, and single-species biofilms of *E. coli* SS2 GFP. Biofilms were formed on stainless steel (SST), SICON® and SICAN for 1 and 3 days. These representative images were obtained from confocal *z* stacks using IMARIS software and present an aerial view of biofilm structures, with the shadow projection on the right. The first row of each day presents the combination of red and green filters (*P. grimontii* 13A10 + *E. coli* SS2 GFP), while the second row of each day corresponds only to the green filter (*E. coli* SS2 GFP). The third row of each day corresponds to single-species biofilms of *E. coli* SS2 GFP. Scale bars are 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5. Statistical analysis

One-way ANOVA was performed using Statgraphics v6.0 software (Manugistics, Rockville, USA) and differences in biofilm biovolumes were reported as significant for *P* values lower than 0.05.

3. Results

Fig. 1 shows the three-dimensional structure of dual- (*P. grimontii* 13A10 + *E. coli* SS2 GFP) and single-species biofilms (*E. coli* SS2 GFP) formed on the different materials observed by CLSM. The first row for each day presents the simultaneous localization of *P. grimontii* (in red) and *E. coli* cells (in green) within the dual-species biofilms, while the second row corresponds to the spatial distribution of only *E. coli* cells in the same biofilms. Within 1 day of incubation, it was possible to visualize sparse biofilms of less than 30 μm in thickness composed of small, isolated cell clusters, where *P. grimontii* and *E. coli* populations appear to have similar densities, regardless of the surface material. The biomass of *P. grimontii* increased from day 1 to day 3 and thicker biofilms with a smoother appearance were formed, where the dominant strain was clearly *P. grimontii*. A very small number of *E. coli* cells were occasionally observed (to a lesser extent than in 1-day-old biofilms developed on all contact surfaces) suggesting a protective effect of *P. grimontii* against pathogenic *E. coli* colonization. When comparing the confocal images of *E. coli* in dual- and single-species biofilms (second and third rows of each day, respectively), it is noticeable that the *E. coli* colonization was reduced in mixed

biofilms.

Fig. 2 presents the biovolumes of *E. coli* SS2 GFP and *P. grimontii* 13A10 on SST, SICON® and SICAN coupons, at different incubation days (1 and 3 days), under single- or dual-species conditions. The biofilm biovolumes were estimated from the confocal image analysis.

Regardless of the contact surface and incubation time, *P. grimontii* formed higher amounts of biofilm in single-species experiments than *E. coli* (biovolumes ranged from 9 to 51 $\mu m^3/\mu m^2$ for *P. grimontii* and from 1.3 to 1.9 $\mu m^3/\mu m^2$ for *E. coli*). Concerning the model *E. coli* pathogen, it is possible to observe that single-species biofilms formed after 3 days had approximately the same biovolume ($P > 0.05$) as biofilms formed after 1 day, regardless of the material tested. Looking at the 1-day results (Fig. 2A), the

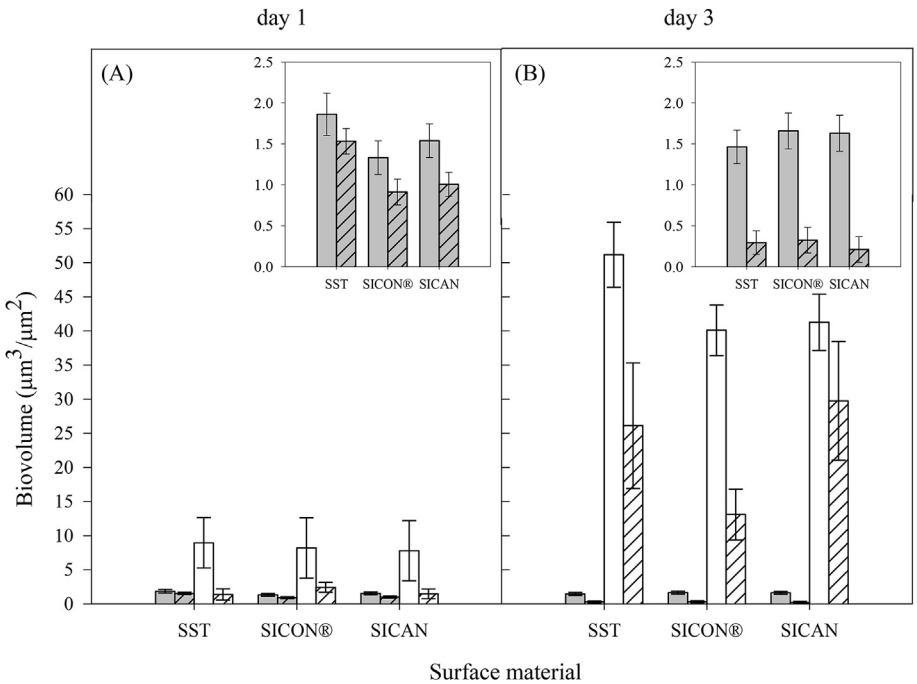

Fig. 2. Biovolumes of *E. coli* SS2 GFP (▭) and *P. grimontii* 13A10 (▭) populations in single-species biofilms, and of *E. coli* SS2 GFP (▨) and *P. grimontii* 13A10 (▨) in dual-species biofilms formed on stainless steel (SST), SICON® and SICAN. (A) 1 and (B) 3 days of biofilm formation. The biovolumes were obtained from confocal image series using the PHLIP tool. The averages ± standard errors (95% confidence) for three independent experiments are illustrated.

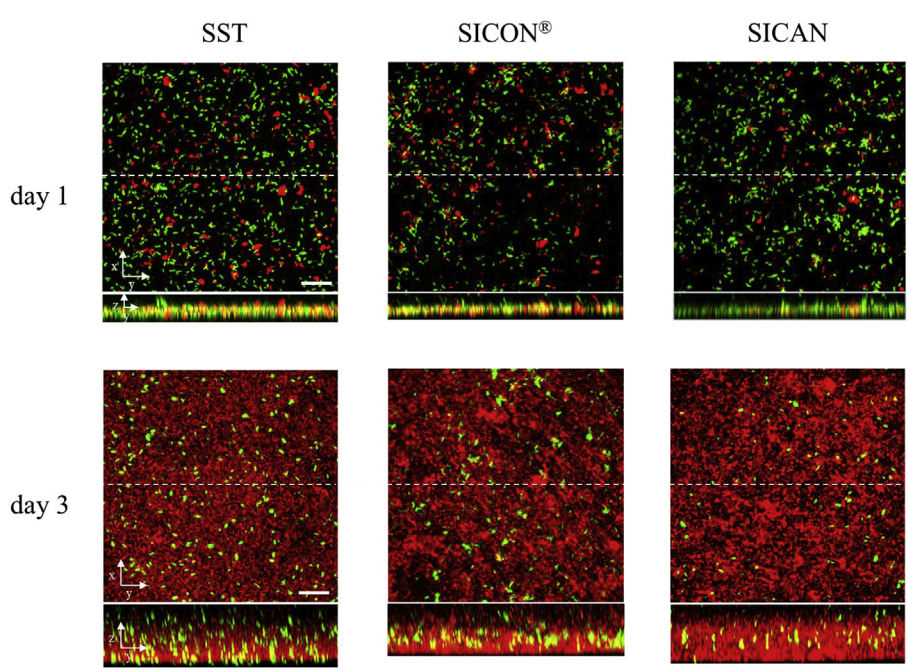

Fig. 3. Localization of *P. grimontii* 13A10 (in red) and *E. coli* SS2 GFP (in green) within 1- and 3-day-old biofilms formed on stainless steel (SST), SICON® and SICAN. These images derived from the same confocal *z*-stacks used to generate Fig. 1. Dotted lines indicate vertical sections. Scale bars are 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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E. coli biovolume on the SICON® surface was 28% lower than on SST ($P < 0.05$), however there was no statistically significant difference between the biovolumes on SICAN and SST. By day 3 (Fig. 2B), *E. coli* biofilms with similar biovolume had developed on all three surface materials ($P < 0.05$). Measurements of biovolume of single-species biofilms formed by *P. grimontii* revealed that following 1 day of incubation (Fig. 2A) the biovolume was quite similar ($P > 0.05$) on all surfaces examined, with an average biovolume of 8 $\mu m^3/\mu m^2$. Following 3 days of incubation (Fig. 2B), the biovolume of *P. grimontii* biofilms increased approximately 90%. Nevertheless, the amount of biofilm formed on SICON® and SICAN was 21% lower than that formed on SST ($P < 0.05$), but it was similar between both modified surfaces ($P > 0.05$).

It was observed that the simultaneous presence of both bacterial species in the biofilm community led to a reduction of their sessile populations compared to single-species biofilms ($P < 0.05$ for 10 out of 12 cases, Fig. 2). In general, under single-species conditions, *E. coli* reached an average biovolume of 1.6 $\mu m^3/\mu m^2$, and this was reduced by approximately 30% in day 1 and 80% in day 3 in dual-species conditions (Fig. 2A and B).

As for single-species biofilms, *E. coli* growth in multi-species biofilms was not affected by the type of surface material on day 3, although on day 1 it was reduced on SICON® and SICAN when compared to SST (on average 37%, $P < 0.05$) (Fig. 2A and B). Regarding *P. grimontii*, its sessile population decreased under dual-species conditions by 79% at day 1 and 48% at day 3 compared to monoculture biofilms. The SICON® surface reduced colonization by this strain (50%) on day 3 when compared to SST ($P < 0.05$) (Fig. 2B).

On the first day of incubation, *P. grimontii* dominated in the dual-species biofilms formed on SICON® (corresponding to 90% of the total biovolume, data not shown). The *P. grimontii* biovolume was similar to the *E. coli* biovolume in biofilms formed on the remaining materials ($P > 0.05$, Fig. 2A). However, after 3 days of incubation, all dual-species biofilms were composed by on average by 99% of *P. grimontii* cells (data not shown), suggesting a strong antagonistic behavior by this bacterial strain towards the model pathogen. The CLSM study for 1 h of initial adhesion indicated that the initial ratio of the strains in dual-species biofilms was different between surface materials (data not shown). However, the confocal images revealed that the strains are co-located, which was quantitatively validated by the fluorescence profiles along the biofilm (see Fig. 1S of Supplementary material). This observation confirms that the prevalence of *P. grimontii* in mature biofilms was not achieved by *E. coli* cell coverage ("blanketing" phenomenon) in the initial stages of biofilm formation.

Overall, SICON® was more effective than SICAN since it reduced the biofilm biovolume in 25% of the cases when compared to SST, while the SICAN surface was only more effective in 13% of the cases. Significant biovolume reductions were found for SICON® at day 1 for *E. coli* in single- and dual-species biofilms. For mature biofilms, reductions were only found for *P. grimontii* (in single culture with both modified surfaces) and in mixed biofilms for SICON®.

The vertical distribution of the two bacterial strains within biofilms was also determined by CLSM (Fig. 3) in order to check whether the protective role of *P. grimontii* is related to a preferential localization of this bacterium in a specific region of the mixed biofilm. Results show that *P. grimontii* and *E. coli* cells were mixed together throughout the biofilm volume (co-aggregation), independently of the materials tested.

4. Discussion

For the single-species biofilms, although modified surfaces did not affect the development of mature biofilms of *E. coli* SS2 GFP (the model pathogen), biofilm formation by *P. grimontii* (the industrial isolate) was significantly reduced on SICON® and SICAN when compared to SST. Thus, in this case, it seems that cell adhesion is being dominated by biological factors likely those associated with bacterial cell wall decorations, as mentioned by Moreira, Fulgêncio, Alves et al. (2016). Our research group recently demonstrated that bacterial adhesion (up to 6 h) and biofilm formation (from 1 to 5 days) were similar on SICAN and stainless steel (Moreira, Fulgêncio, Alves et al, 2016), but significantly reduced on SICON® (Moreira, Fulgêncio, Oliveira et al., 2016). In general, the number of culturable cells and the biofilm thickness was lower (on average 1 log10 and 18%, respectively) on SICON® than on stainless steel (Moreira, Fulgêncio, Oliveira et al., 2016). In contrast to the present work, these results were obtained at 30 °C under flow conditions using axenic cultures of *E. coli* JM109(DE3) (Moreira, Fulgêncio, Alves et al., 2016; Moreira, Fulgêncio, Oliveira et al., 2016). Although the environmental conditions and bacterial strains used here were different from those previously used in order to better simulate the process conditions on a fresh-cut vegetable processing facility (lower temperature and higher nutrient load), the results confirm that SICON® surfaces may be beneficial to reduce the formation of *E. coli* single-species biofilms. To the best of our knowledge, there are only two works published on these modified stainless steel surfaces concerning bacterial fouling (Moreira, Fulgêncio, Alves et al., 2016; Moreira, Fulgêncio, Oliveira et al., 2016). Although other authors have worked with SICON® and SICAN, they have investigated the performance of these coatings against abiotic fouling (Augustin, Geddert, & Scholl, 2007; Boxler et al., 2013b, 2013a; Geddert, Augustin, & Scholl, 2011), concluding that they are a valid approach to reduce fouling and increase the removal of deposits formed in food industry.

For simplicity, most of the research on biofilms has focused on single-species biofilms. However, biofilms in food environments comprise multiple species and it is known that the interactions between them can shape the development, structure and function of such communities (Giaouris et al., 2015). In recent years, the study of multi-species biofilms composed of pathogenic bacteria has increased the understanding of the dynamics of surface-attached bacteria and biofilms under conditions relevant to food processing (Chorianopoulos, Giaouris, Skandamis, Haroutounian, & Nychas, 2008; Habimana et al., 2010; Kostaki, Chorianopoulos, Braxou, Nychas, & Giaouris, 2012; Marouani-Gadri et al., 2009; Saá Ibusquiza, Herrera, Vázquez-Sánchez, & Cabo, 2012; Sanchez-Vizuete, Orgaz, Aymerich, Le Coq, & Briandet, 2015; van der Veen & Abee, 2011). Bacterial interactions may lead either to the accumulation of pathogenic species or to the delay of their growth. In this work, it was demonstrated that *P. grimontii*, a bacterial strain isolated from a fresh-cut processing facility, reduced the *E. coli* presence in dual-species biofilms. Similarly, other authors have shown a decrease in *E. coli* biofilm formation on solid surfaces in the presence of *Staphylococcus epidermidis* (Castonguay et al., 2006), *Stenotrophomonas maltophilia* (Dai, 2010), *P. aeruginosa* (Kuznetsova et al., 2013) and a Salmonella strain (Chen et al., 2015). *P. grimontii* was first isolated from natural mineral waters and characterized by Baïda, Yazourh, Singer, and Izard (2002). The available information about this bacterial strain is scarce and, to the best of our knowledge, there are no published studies on its ability to form biofilms. However, the observed dominance of *P. grimontii* over the model *E. coli* pathogen may be related with the well-known ability of Pseudomonads to produce a variety of extracellular polymeric substances (EPS), such as cellulose, alginate, and PeI and PsI exopolysaccharides, which help them to form strong biofilm communities on abiotic surfaces (Chang et al., 2007; Ude, Arnold, Moon, Timms-Wilson, & Spiers, 2006; Yang et al., 2011). This result also suggest that there may be competition for essential nutrients between the two species or that *P. grimontii*, like other

Pseudomonas strains, produces molecules that inhibit *E. coli* growth (Culotti & Packman, 2014).

Since the isolate outcompeted and protected the food surfaces from pathogenic colonization, it was expected that the dominance of *P. grimontii* was achieved by covering surface-attached *E. coli* cells, a phenomenon termed "blanketing" (An, Danhorn, Fuqua, & Parsek, 2006). Although it has been shown that *P. grimontii* sp. is able to switch to anaerobic growth using nitrate (Baïda et al., 2002), the growth medium used in this work does not contain the electron donors necessary for this switch. Since *E. coli* is a facultative anaerobe, the different oxygen requirements could have contributed to a layered-biofilm structure. Curiously, the images captured by CLSM revealed that *P. grimontii* and *E. coli* cells were mixed together throughout the biofilm, which indicates that limitations on mass transfer (nutrients and oxygen) were not affecting the spatial organization of both microorganisms within dual-species biofilms, as well as the pathogen growth on food surfaces.

Concerning the influence of DLC surfaces on dual-species biofilm formation, SICON® was efficient to reduce the *P. grimontii* biomass. However, both SICON® and SICAN did not affect the *E. coli* colonization in mature biofilms when compared to bare SST. Hence, in this case, the fate of *E. coli* pathogens in multi-species biofilms was more affected by the composition of the bacterial community than by the surface properties. So far, few studies have been performed addressing the impact of different materials on the development of mixed biofilms (Almeida, Azevedo, Santos, Keevil, & Vieira, 2011; Arnold & Silvers, 2000; Moreira, Fulgêncio, Alves et al., 2016; Paterson et al., 2016; Saá Ibusquiza et al., 2012) and most of them do not contain detailed information on the involved microorganisms nor their proportions in biofilms formed in the tested materials. Almeida et al. (2011) presented a full characterization of *Salmonella enterica*/*L. monocytogenes*/*E. coli* single, dual and tri-species biofilms on different support materials. They found that all strains maintained the same profile for six out of seven materials in single- and multi-species biofilms, concluding that the type of adhesion material was not a major determinant on the amount of biofilm produced (Almeida et al., 2011). Regarding SICON® and SICAN surfaces and mixed biofilms, Moreira, Fulgêncio, Alves et al. (2016) studied cell adhesion for 6 h using industrial water collected from a salad washing line and the same industrial water spiked with *E. coli*. It was observed that the unknown microorganisms belonging to the natural flora present in the industrial water were capable of adhering equally to the stainless steel and modified surfaces. Furthermore, addition of significant amounts of *E. coli* did not potentiate further cell adhesion in any material (Moreira, Fulgêncio, Alves et al., 2016).

5. Conclusions

Although the DLC coatings have been presented as promising alternatives to the traditional stainless steel used in the food industry, little is known about their antifouling ability. In this study, SICON® coated surfaces were only more effective than the SST surfaces in reducing the pathogen colonization in early biofilms and the isolate growth in mature biofilms. Hence, it has been concluded that the fate of *E. coli* and *P. grimontii* in multi-species biofilms was more affected by the bacterial composition than by the surface properties. Additionally, it was shown that the presence of a resident biofilm of *P. grimontii* inhibits the sessile growth of *E. coli* under the typical process conditions found in fresh produce industries. Such observations expand our knowledge on the physiology of multi-species pathogenic biofilms and suggest that a guided microbial ecology of industrial surfaces could be a sustainable and efficient control strategy to be used in the food industry.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2017.03.005.

References

Almeida, C., Azevedo, N. F., Santos, S., Keevil, C. W., & Vieira, M. J. (2011). Discriminating multi-species populations in biofilms with peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH). *PLoS ONE, 6*(3), e14786.

An, D., Danhorn, T., Fuqua, C., & Parsek, M. R. (2006). Quorum sensing and motility mediate interactions between *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens* in biofilm cocultures. *Proceedings of the National Academy of Sciences of the United States of America, 103*(10), 3828–3833.

Arnold, J. W., & Silvers, S. (2000). Comparison of poultry processing equipment surfaces for susceptibility to bacterial attachment and biofilm formation. *Poultry Science, 79*(8), 1215–1221.

Augustin, W., Geddert, T., & Scholl, S. (2007). Surface treatment for the mitigation of whey protein fouling. Paper presented at the Heat Exchanger Fouling and Cleaning VII, Portugal. ECI Symposium Series. In H. Müller-Steinhagen, M. R. Malayeri, & A. P. Watkinson (Eds.). *Proceedings of 7th International Conference on Heat Exchanger Fouling and Cleaning - Challenges and Opportunities* (Volume RP5), ISBN 978-1-61567-000-0.

Baïda, N., Yazourh, A., Singer, E., & Izard, D. (2002). *Pseudomonas grimontii* sp. nov. *International Journal of Systematic and Evolutionary Microbiology, 52*(5), 1497–1503.

Boxler, C., Augustin, W., & Scholl, S. (2013a). Cleaning of whey protein and milk salts soiled on DLC coated surfaces at high-temperature. *Journal of Food Engineering, 114*(1), 29–38.

Boxler, C., Augustin, W., & Scholl, S. (2013b). Fouling of milk components on DLC coated surfaces at pasteurization and UHT temperatures. *Food and Bioproducts Processing, 91*(4), 336–347.

Castonguay, M.-H., van der Schaaf, S., Koester, W., Krooneman, J., van der Meer, W., Harmsen, H., et al. (2006). Biofilm formation by *Escherichia coli* is stimulated by synergistic interactions and co-adhesion mechanisms with adherence-proficient bacteria. *Research in Microbiology, 157*(5), 471–478.

Chang, W.-S., van de Mortel, M., Nielsen, L., Nino de Guzman, G., Li, X., & Halverson, L. J. (2007). Alginate production by *Pseudomonas putida* creates a hydrated microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions. *Journal of Bacteriology, 189*(22), 8290–8299.

Chen, D., Zhao, T., & Doyle, M. P. (2015). Single- and mixed-species biofilm formation by *Escherichia coli* O157:H7 and *Salmonella*, and their sensitivity to levulinic acid plus sodium dodecyl sulfate. *Food Control, 57*, 48–53.

Chorianopoulos, N. G., Giaouris, E. D., Skandamis, P. N., Haroutounian, S. A., & Nychas, G. J. (2008). Disinfectant test against monoculture and mixed-culture biofilms composed of technological, spoilage and pathogenic bacteria: bactericidal effect of essential oil and hydrosol of *Satureja thymbra* and comparison with standard acid-base sanitizers. *Journal of Applied Microbiology, 104*(6), 1586–1596.

Corbella, C., Bialuch, I., Kleinschmidt, M., & Bewilogua, K. (2009). Up-scaling the production of modified a-C: H coatings in the framework of plasma polymerization processes. *Solid State Sciences, 11*(10), 1768–1772.

Culotti, A., & Packman, A. I. (2014). *Pseudomonas aeruginosa* promotes *Escherichia coli* biofilm formation in nutrient-limited medium. *PLoS ONE, 9*(9), e107186.

Dai, D. (2010). *The effects and genetic mechanisms of bacterial species interactions on biofilm formation*. PhD thesis. Ann Arbor: University of Michigan.

Geddert, T., Augustin, W., & Scholl, S. (2011). Induction time in crystallization fouling on heat transfer surfaces. *Chemical Engineering & Technology, 34*(8), 1303–1310.

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Giaouris, E. D., Heir, E., Desvaux, M., Hébraud, M., Møretrø, T., Langsrud, S., … Simões, M. (2015). Intra- and inter-species interactions within biofilms of important foodborne bacterial pathogens. *Frontiers in Microbiology, 6*, 841.

Grischke, M., Hieke, A., Morgenweck, F., & Dimigen, H. (1998). Variation of the wettability of DLC-coatings by network modification using silicon and oxygen. *Diamond and Related Materials, 7*(2–5), 454–458.

Habimana, O., Heir, E., Langsrud, S., Asli, A. W., & Moretro, T. (2010). Enhanced surface colonization by *Escherichia coli* O157:H7 in biofilms formed by an *Acinetobacter calcoaceticus* isolate from meat-processing environments. *Applied and Environmental Microbiology, 76*(13), 4557–4559.

Klayman, B. J., Volden, P. A., Stewart, P. S., & Camper, A. K. (2009). *Escherichia coli* O157:H7 requires colonizing partner to adhere and persist in a capillary flow cell. *Environmental Science & Technology, 43*(6), 2105–2111.

Kostaki, M., Chorianopoulos, N., Braxou, E., Nychas, G.-J., & Giaouris, E. (2012). Differential biofilm formation and chemical disinfection resistance of sessile cells of *Listeria monocytogenes* strains under monospecies and dual-species (with *Salmonella enterica*) conditions. *Applied and Environmental Microbiology, 78*(8), 2586–2595.

Kuehn, M., Hausner, M., Bungartz, H.-J., Wagner, M., Wilderer, P. A., & Wuertz, S. (1998). Automated confocal laser scanning microscopy and semiautomated image processing for analysis of biofilms. *Applied and Environmental Microbiology, 64*(11), 4115–4127.

Kuznetsova, M. V., Maslennikova, I. L., Karpunina, T. I., Nesterova, L. Y., & Demakov, V. A. (2013). Interactions of *Pseudomonas aeruginosa* in predominant biofilm or planktonic forms of existence in mixed culture with *Escherichia coli* in vitro. *Canadian Journal of Microbiology, 59*(9), 604–610.

Lemos, M., Gomes, I., Mergulhão, F., Melo, L., & Simões, M. (2015). The effects of surface type on the removal of *Bacillus cereus* and *Pseudomonas fluorescens* single and dual species biofilms. *Food and Bioproducts Processing, 93*(0), 234–241.

Liao, C. H. (2007). Inhibition of foodborne pathogens by native microflora recovered from fresh peeled baby carrot and propagated in cultures. *Journal of Food Science, 72*(4), M134–M139.

Liu, N. T., Nou, X., Lefcourt, A. M., Shelton, D. R., & Lo, Y. M. (2014). Dual-species biofilm formation by *Escherichia coli* O157:H7 and environmental bacteria isolated from fresh-cut processing facilities. *International Journal of Food Microbiology, 171*, 15–20.

Marouani-Gadri, N., Augier, G., & Carpentier, B. (2009). Characterization of bacterial strains isolated from a beef-processing plant following cleaning and disinfection - influence of isolated strains on biofilm formation by Sakai and EDL 933 *E. coli* O157:H7. *International Journal of Food Microbiology, 133*(1–2), 62–67.

Moreira, J. M. R., Fulgêncio, R., Alves, P., Machado, I., Bialuch, I., Melo, L. F., … Mergulhão, F. J. (2016). Evaluation of SICAN performance for biofouling mitigation in the food industry. *Food Control, 62*, 201–207.

Moreira, J. M. R., Fulgêncio, R., Oliveira, F., Machado, I., Bialuch, I., Melo, L. F., … Mergulhão, F. J. (2016). Evaluation of SICON® surfaces for biofouling mitigation in critical process areas. *Food and Bioproducts Processing, 98*, 173–180.

Moreira, J. M. R., Gomes, L. C., Simões, M., Melo, L. F., & Mergulhão, F. J. (2015). The impact of material properties, nutrient load and shear stress on biofouling in food industries. *Food and Bioproducts Processing, 95*, 228–236.

Oosterik, L. H., Tuntufye, H. N., Butaye, P., & Goddeeris, B. M. (2014). Effect of serogroup, surface material and disinfectant on biofilm formation by avian pathogenic *Escherichia coli*. *The Veterinary Journal, 202*(3), 561–565.

Paterson, J. S., Ogden, S., Smith, R. J., Delpin, M. W., Mitchell, J. G., & Quinton, J. S. (2016). Surface modification of an organic hessian substrate leads to shifts in bacterial biofilm community composition and abundance. *Journal of Biotechnology, 219*, 90–97.

Saá Ibusquiza, P., Herrera, J. J. R., Vázquez-Sánchez, D., & Cabo, M. L. (2012). Adherence kinetics, resistance to benzalkonium chloride and microscopic analysis of mixed biofilms formed by *Listeria monocytogenes* and *Pseudomonas putida*. *Food Control, 25*(1), 202–210.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Protocol 25: Preparation and transformation of competent *E. coli* using calcium chloride. In *Molecular cloning: A laboratory manual* (pp. 116–118). New York: Cold Spring Harbor.

Sanchez-Vizuete, P., Orgaz, B., Aymerich, S., Le Coq, D., & Briandet, R. (2015). Pathogens protection against the action of disinfectants in multispecies biofilms. *Frontiers in Microbiology, 6*, 705.

Srey, S., Jahid, I. K., & Ha, S.-D. (2013). Biofilm formation in food industries: a food safety concern. *Food Control, 31*(2), 572–585.

Ude, S., Arnold, D. L., Moon, C. D., Timms-Wilson, T., & Spiers, A. J. (2006). Biofilm formation and cellulose expression among diverse environmental Pseudomonas isolates. *Environmental Microbiology, 8*(11), 1997–2011.

Uhlich, G. A., Rogers, D. P., & Mosier, D. A. (2010). *Escherichia coli* serotype O157:H7 retention on solid surfaces and peroxide resistance is enhanced by dual-strain biofilm formation. *Foodborne Pathogens and Disease, 7*(8), 935–943.

van der Veen, S., & Abee, T. (2011). Mixed species biofilms of *Listeria monocytogenes* and *Lactobacillus plantarum* show enhanced resistance to benzalkonium chloride and peracetic acid. *International Journal of Food Microbiology, 144*(3), 421–431.

Xavier, J. B., White, D. C., & Almeida, J. S. (2003). Automated biofilm morphology quantification from confocal laser scanning microscopy imaging. *Water Science & Technology, 47*(5), 31–37.

Yang, L., Hu, Y., Liu, Y., Zhang, J., Ulstrup, J., & Molin, S. (2011). Distinct roles of extracellular polymeric substances in *Pseudomonas aeruginosa* biofilm development. *Environmental Microbiology, 13*(7), 1705–1717.

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