The role of conditioning film formation and surface chemical changes on *Xylella fastidiosa* adhesion and biofilm evolution

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ABSTRACT

Biofilms are complex microbial communities with important biological functions including enhanced resistance against external factors like antimicrobial agents. The formation of a biofilm is known to be strongly dependent on substrate properties including hydrophobicity/hydrophilicity, structure, and roughness. The adsorption of (macro)molecules on the substrate, also known as conditioning film, changes the physicochemical properties of the surface and affects the bacterial adhesion. In this study, we investigate the physicochemical changes caused by Periwinkle wilt (PW) culture medium conditioning film formation on different surfaces (glass and silicon) and their effect on *X. fastidiosa* biofilm formation. Contact angle measurements have shown that the film formation decreases the surface hydrophilicity degree of both glass and silicon after few hours. Atomic force microscopy (AFM) images show the glass surface roughness is drastically reduced with conditioning film formation. First-layer *X. fastidiosa* biofilm on glass was observed in the AFM liquid cell after a period of time similar to that determined for the hydrophilicity changes. In addition, attenuation total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy supports the AFM observation, since the PW absorption spectra increases with time showing a stronger contribution from the phosphate groups. Although hydrophobic and rough surfaces are commonly considered to increase bacteria cell attachment, our results suggest that these properties are not as important as the surface functional groups resulting from PW conditioning film formation for *X. fastidiosa* adhesion and biofilm development.

1. Introduction

Bacterial biofilms play a crucial role in many fields including biotechnology, biodeterioration, biofouling, immunology, and biomaterial development. Bacteria growing within associated biofilms are more resistant against external factors such as biocides, detergent, antibiotic treatments, and host defense responses than plank-tonic (i.e., free) cells. Biofilm formation is known as a multi-stage process mediated by a number of factors, including surface properties, nutrient solution, pH, and temperature [1]. In current models, biofilm development may be subdivided into the following steps: (1) reversible attachment of the micro-organism to the surface – characterized by non-specific interactions where cells are easily removed by gentle rinse, (2) irreversible attachment – active mechanisms as pili (or fimbriae), adhesion proteins, and exopolymers contribute to a stronger adhesion to the surface through molecular-specific interactions, (3) cell–cell adhesion and proliferation (bacterial colonies), (4) maturation of the biofilm containing an additional polymer matrix, which stabilizes the biofilm against fluctuations, and (5) detachment of cells [2,3].

Adhesion of microbial cells to a surface is an essential step to biofilm formation [1–4]. Nevertheless, the molecular and physical interactions that are involved in the adhesion process have not yet been completely understood. Microbial cells may attach to surfaces via specific and non-specific interactions [2]. Both mechanisms depend on fundamental factors such as surface hydrophobicity/hydrophilicity [5–13], roughness [14–16], charge [16], and also functional groups [17,18]. In literature, the development of

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surface conditioning films as the first step for biofilm formation was also reported [19–22]. Conditioning films are formed due to the adsorption of (macro)molecules on the substrate, thus changing the adhesion conditions for bacteria to this surface. The nature of the conditioning films may be quite different depending on the kind of environment the substrate surface is exposed to. Loeb et al. [22] reported the formation of these conditioning films on surfaces exposed to seawater. The film was first observed after few minutes of exposure with subsequent continuous growth for several hours. Mittelman [23] noticed the influence of conditioning films composed of proteinaceous and polysaccharides from blood, tears, urine, and saliva respiratory secretions on the attachment of bacteria to biomaterials.

Surface hydrophobicity has also been regarded as a determinant factor for microbial cell adhesion [5–9,13]. The concept of hydrophobicity opposes that of surface wettability; hydrophobic surfaces present low wetting. Hydrophobic interactions are essential in life sciences, as they may promote protein folding and aggregation, membrane fusion, and cell adhesion [5]. According to van Oss [6], hydrophobic interactions are usually the strongest of all long-range non-covalent interactions in biological systems. The hydrophobic attraction between two non-polar molecules (including molecules on surfaces), or between one non-polar and one polar molecule in water is considered a consequence of the hydrogen-bonding energy of the water molecules surrounding these molecules [7]. Oliveira et al. [8] determined the relationship between the degree of hydrophobicity of four polymeric materials and the number of attached Staphyloccocus epidermidis cells. They observed that this number increased with the surface hydrophobicity. A similar linear behavior between surface hydrophobicity and the number of adherent cells was observed during the attachment of Alcaligenes denitrificans to polymeric surfaces [9]. Sheng et al. [13] reported that the reduced hydrophobicity of metal surfaces weakens bacterial adhesion.

Roughness has also been reported as an important property affecting cell attachment. Several reports show that the number of attached cells increases with roughness [14,15]. Characklis et al. [14] observed that the extent of microbial colonization appears higher as the surface roughness increases. Oh et al. [15] noticed a lower number of Pseudomonas aeruginosa bacterial cells attached to the surface when the roughness of the substrate decreases. Although several studies [7–9,13–15] have considered hydrophobicity and roughness as fundamentals properties for cell attachment, only a few of them [17,18] have targeted the question how functional groups at the surface may influence the adhesion process. Gubner and Beech [17] studied the effect of the conditioning film formed by capsular, planktonic, and biofilm exopolymers produced by marine Pseudomonas species in continuous cultures. They found that the chemistry and the concentration of exopolymers (EPS) on the surface play a more important role in cell adhesion than the surface hydrophobicity or roughness.

In addition, the irreversible cell attachment to a surface is followed by biofilm development, which may be pathogenic in several cases. This is the case of the Gram-negative bacteria Xylella fastidiosa. This bacterium is responsible for several diseases in economically important plants, such as citrus, grapevine, plum, almond, peach and coffee. In Brazil, it is responsible for the citrus variegated chlorosis, a disease that causes annual losses of more than $100 million to the citrus agroindustry [24,25]. X. fastidiosa is the first phytopathogenic bacterium for which a complete genome sequence was determined [26]. The mechanism of pathogenicity is largely attributed to the occlusion of xylem vessels by aggregation of X. fastidiosa and biofilm formation [27]. Consequently, understanding the factors affecting the adhesion process is a key issue in any effort aiming at the identification of mechanisms to prevent biofilm formation.

In the present study, we investigated the conditioning film formation by Periwinkle wilt (PW) culture medium [28] and its effect on different surface properties. X. fastidiosa biofilms were observed from the early formation along a development cycle of 20 days. Surface hydrophobicity degree, roughness, and chemical changes are discussed with respect to their role in attachment and development of X. fastidiosa biofilms.

2. Materials and methods

2.1. Bacteria strain and growth conditions

The 9a5c bacterial strain of X. fastidiosa subspecies pauca [29] was used in this study. Bacterial cells were inoculated into the Citrus sinensis plant to maintain their pathogenicity state and avoid attenuation due to successive passages in the axenic medium. Petioles and stems were aseptically ground in phosphate-buffered saline (PBS), and the suspension was spread onto Periwinkle wilt medium (PW) broth [28].

In order to obtain X. fastidiosa biofilms, we used an experimental protocol developed by Souza et al. [27]. X. fastidiosa cells were incubated at 28 °C on autoclaved glass and silicon surfaces immersed in PW broth without replenishing the medium.

2.2. Periwinkle wilt culture medium (PW)

Bovine serum albumin (BSA) is among the relevant PW broth compounds [28]. Davis et al. [28] reported that BSA protein is necessary in PW for X. fastidiosa growth; Galvani et al. [30], however, observed a much slower X. fastidiosa growth in BSA absence and thus concluded that BSA is not essential for the growth of bacteria. In addition, BSA is a protein known for spontaneous adsorption onto different surfaces [31,32]. To investigate the BSA's influence on the conditioning film properties, and consequently, on the attachment and development of X. fastidiosa biofilms, we used PW with and without BSA, as described in each case. Aqueous solutions from individual compounds of the PW broth for ATR-FTIR spectra acquisition were prepared at the same concentration used within the actual PW broth.

2.3. Preparation of glass and silicon surfaces

Round glass coverslips (15 mm diameter, 0.13–0.17 mm thickness – Waldemar Knittel Glasbearbeitungs – GmbH Germany) were used on AFM experiments where the temperature-controlled liquid cell was required (see Section 2.5); uncoated sterile glass-bottom 35-mm culture dishes (10 mm glass diameter, 0.13–0.16 mm thickness) with high-quality borosilicate coverslips (MatTek Corporation Ashland MA, USA) were employed in all other cases. Round glass coverslips and silicon surfaces ($\langle 1\,0\,0 \rangle$, cut into square shapes of approximately 2×2 cm^2 from originally 10 cm diameter wafers), were rinsed with acetone, 2-propanol, and deionized water to remove the organic contamination; these surfaces were subsequently sterilized by autoclaving procedure. For contact angle measurements, both glass surfaces were used; similar results were observed for both cases.

2.4. ATR-FTIR spectroscopy

ATR-FTIR spectra were recorded with a Vector 70 spectrometer (Bruker Optics, Ettlingen, Germany) equipped with a BioATRCell II (Bruker Optics, Ettlingen, Germany), which provides a ZnSe ATR crystal with a thin silicon layer on top. For spectra acquisition, the BioATRCell II was filled with 20 μL of PW broth or aqueous solution of its individual compounds at 28 °C (same conditions as

for the biofilm growth). At least 100 scans with a spectral resolution of 4 cm^{-1} were averaged in the range between 4000 and 800 cm^{-1}. The spectrum of deionized water was used to remove the spectral background during all spectroscopic studies.

Spectra from the PW broth with and without BSA were acquired every 5 min for 8 h. For the spectra evaluation, the relevant absorption peak heights were plotted as a function of time for both PW broths. The absorption rate (α) of each band at the surface was calculated from the angular coefficient of the intensity vs. time curve for each peak of interest within the obtained spectra.

2.5. Atomic force microscopy

AFM images were acquired with an Agilent AFM system Model 5500 (Agilent Technologies, Chandler, AZ, USA) in non-contact mode using conical Si tips with a radius less than 10 nm and a length of ∼20 μm (NSC14/AIBS MikroMash, Tallin, Estonia). The spring constant of these cantilevers was typically inside the range 1.8–12.5 Nm^{-1}, and their resonance frequency was in the range 110–220 kHz. To observe possible surface changes by the PW medium and biofilm formation, the images were acquired in solution in real time at temperature-controlled (28 °C) samples using the AFM liquid cell.

To evaluate roughness changes on the surface in contact with PW broth, the root-mean-squared roughness (RMS) [33] was determined over areas of 5 μm^2 for each sample. Indentation experiments were also performed. In order to produce the indentation, a smaller scan area (5×5 μm^2) image was acquired with a set point at 0 V (tip surface in contact) followed by a new image with a larger area (20×20 μm^2 scan size).

2.6. Electron microscopy

Secondary electron images were acquired using a dual-beam FIB/SEM system (Quanta 3D FEG, FEI Company, Eindhoven, NL). The sample preparation included a gentle water rinse to remove only the PW medium and not the adherent cells; the samples were then dried at room temperature overnight.

2.7. Surface contact angle measurements

Contact angle measurements were performed using the sessile drop method and a goniometer (Ramé Hart 100-00) at room temperature. Drop images were collected by a digital photograph camera and analyzed by a curve fitting method using the tangent approximation. For complete wetting, we have considered a contact angle lower than 10°. For these measurements, the samples were washed with deionized water and dried at room temperature overnight.

3. Results and discussion

3.1. Biofilm development on different surfaces and conditions

Biofilms were grown on silicon and glass surfaces using PW broth with BSA and observed by optical microscopy (data not shown) and electron microscopy (Fig. 1). In a previous work [34], X. fastidiosa biofilm formation on glass surfaces using PW broth without BSA was investigated by characterizing changes in the morphology, size, and nearest-neighbor distance with growth time. Most biofilms revealed a circular perimeter (compact pattern) in the initial and final growth stages, while irregular shapes (branch patterns) were characteristic of the maturation stage. The observed changes were associated with two main factors: nutrient concentration and EPS formation. In agreement with this

previous study [34], we have also observed compact circular patterns – figure a, d (initial stage) and c, e (final stage) – and branched (figure b – maturation stage) shapes for biofilms along the 20-day cycle. These results indicate that the presence of BSA in the PW broth is not a necessary condition for X. fastidiosa biofilm development. Moreover, the biofilm growth shows similar behaviors on different surfaces, i.e., glass and silicon.

First-layer biofilms on silicon (Fig. 1d–f) were also observed for all samples (after 5, 10, 15 and 20 days), which is indicative of a continuous biofilm formation process on different regions of the surface. Moreover, this suggests new adhesion events even at older stages since the biofilm growth is mainly driven by cell division and not by free cell attachment [34,35].

3.2. Biofilm formation: initial adhesion

Several studies have reported [36–38] AFM imaging of bacterial cells using artificial immobilization procedures of the cells to a surface or dried samples where the effect of dehydration can be observed on the bacteria. In this study, the natural adhesion of X. fastidiosa was utilized to observe the early biofilm stages without additional immobilization procedures. Thus, X. fastidiosa cells were inoculated in the PW broth without BSA on glass surfaces into the AFM liquid cell. Fig. 2 shows a first-layer biofilm found on the surface after 6 h of inoculation, as well as a few isolated cells around the biofilm. AFM images were acquired continuously since surface was inoculated; however, no cells or biofilm layers were found before 6 h of observation. Moreover, we were able to acquire these images without removing or damaging the biofilm/cells, suggesting an irreversible attachment of this first-layer biofilm. Considering that growth and division process of X. fastidiosa takes at least 10 h, the first layer of biofilm – observed only 6 h after inoculation – is probably due to the adhesion of aggregated cells (as opposed to a single cell) onto the surface. Indeed, in our SEM experiments (Figure S1, Supplementary material) with dry samples, adhesion of both few cells and aggregates was observed after four (Fig. S1a) and six (Fig. S1b) hours of inoculation, consistent with our AFM data. After two hours, however, no biofilms were found. In addition, AFM phase images (Fig. 2b and d) revealed different contrast variations which we associate to deposits of extracellular material around and on top of both the biofilm layer and isolated bacteria on the surface. This additional material was also observed around the biofilms on silicon surfaces, as evidenced in the electron microscopy images (Fig. 1f, arrows and Fig. S1). Several authors [37–39] attribute these additional deposits to EPS, which contributes to irreversible adhesion and biofilm protection. Although aggregated cells are not usually considered to pioneer biofilm formation [40], their role should not be neglected once irreversible adhesion takes place. Our results altogether suggest no irreversible attachment occurs before 4 h of inoculation and that aggregates may also attach to the surface and pioneer biofilm development.

3.3. Conditioning film: PW broth effects

Fig. 3 shows AFM topography images of glass before and after contact with pure and inoculated PW broth without BSA for 3 h at 28 °C. Morphology changes are evident for both PW media conditions; furthermore, the RMS surface roughness decreases significantly during this process. Moreover, the deposition of material was observed on silicon surfaces after 2 h of contact with the PW broth containing BSA (data not shown), although the roughness is not significantly altered in this case. The same experiment was carried out on glass surfaces for samples after 5, 15, and 20 days. From AFM indentation studies, it is estimated that the film thickness increases from few (∼5–10) nanometers after 2 h to
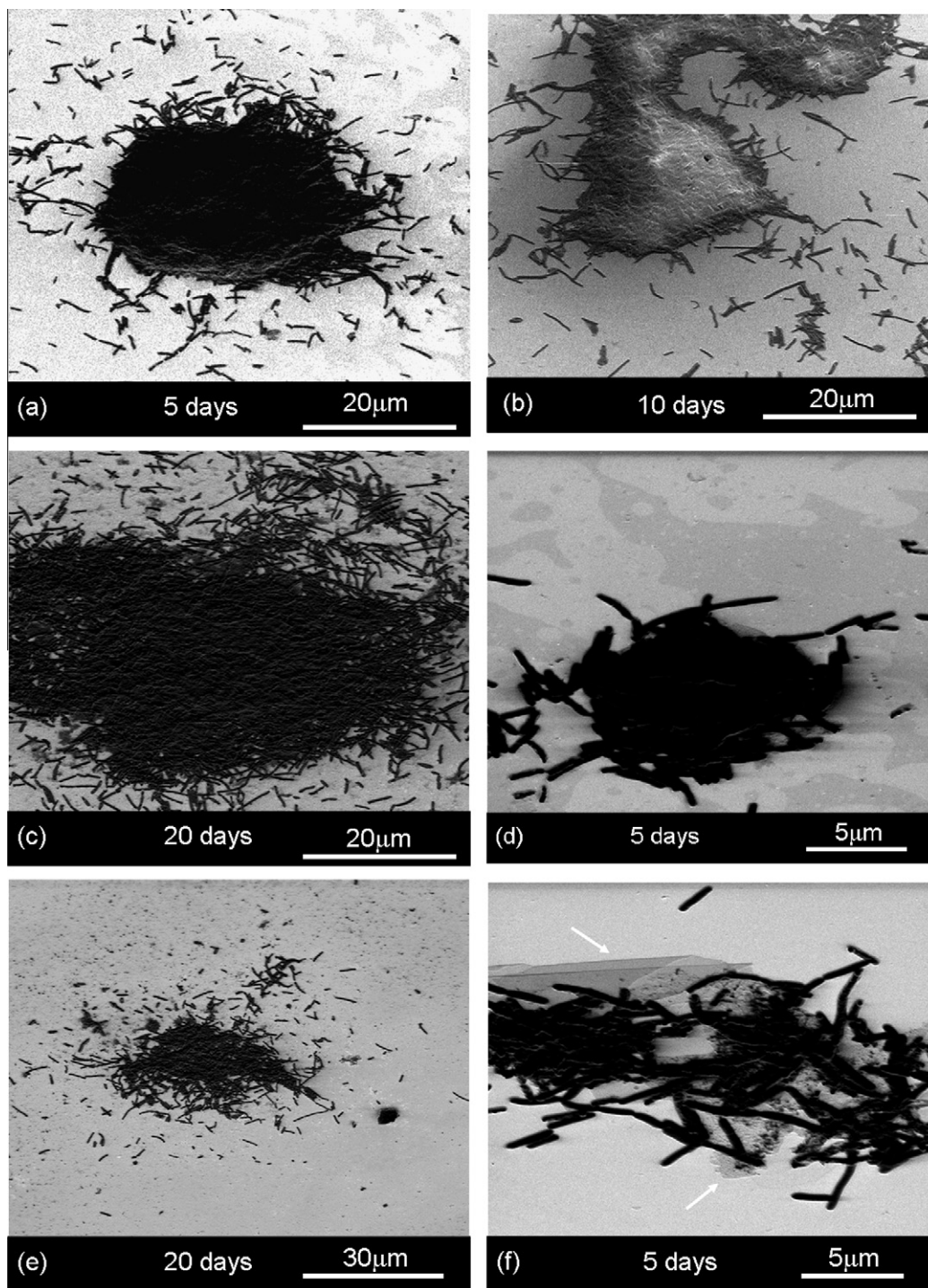
Fig. 1. Electron microscopy images of *X. fastidiosa* biofilms grown on silicon surfaces using PW broth with BSA. Biofilms were grown for 5 days (a, d, f), 10 days (b) and 20 days (c and e). Notice the different magnification scales used for the images.

approximately 50–100 nm after a few days (observations were carried out up to 20 days). The relatively small difference in thicknesses measured over this period indicates that most of the deposition occurs within a few days of growth and then saturates; the composition of the surface film should thus be richer in the less-soluble compounds used for the media. Moreover, the results are similar for both investigated substrates and could explain the similar development of *X. fastidiosa* biofilms on different surfaces.

In addition, although silicon and glass surfaces present hydrophilic (angle < 90°) surfaces, contact angle measurements (Table 1) show that their surface properties change from moderate to complete wetting character after 3 h of contact with PW broth containing BSA. This period increases almost 10-fold (approx. 30 h) if no BSA is added to the solution according to the data presented in Table 1. Thus, the obtained results reveal that the PW conditioning film on silicon and glass surfaces is responsible for reducing their hydrophilic character as well as the glass roughness; at the same time, the adhesion of *X. fastidiosa* occurs. The first layer of the biofilm is observed after periods (~6 h, as shown in Fig. 2) corresponding to those expected for initial changes in hydrophilicity and roughness associated with a thin film covering the entire substrate surface.
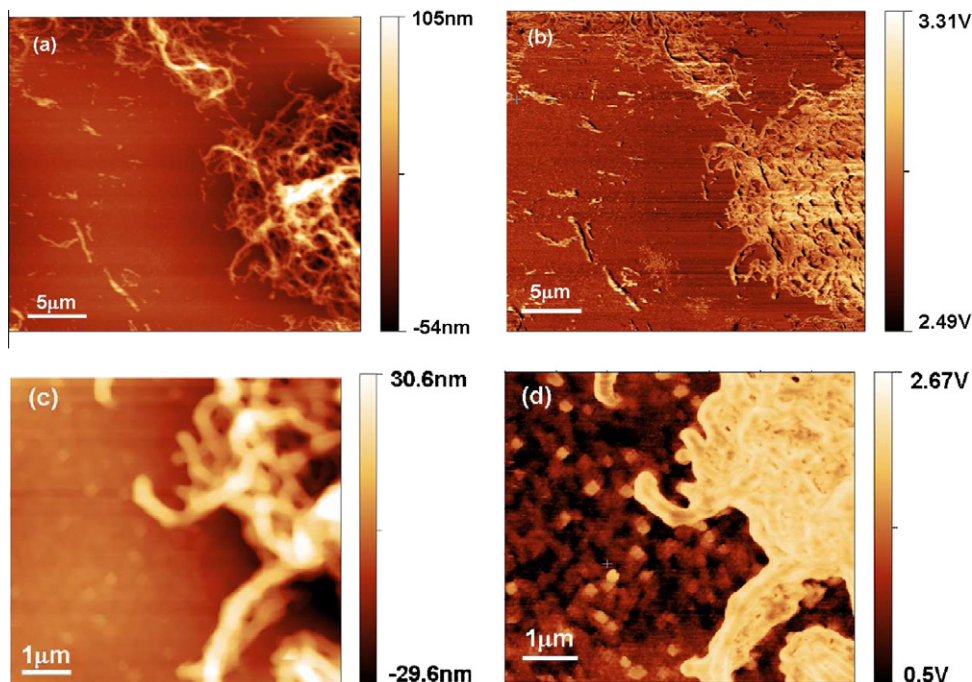
Fig. 2. AFM topography (a and c) and phase (b and sd) images of typical *X. fastidiosa* first-layer biofilm on a glass surface after 6-h growth into AFM liquid cell. PW without BSA was used as culture medium.
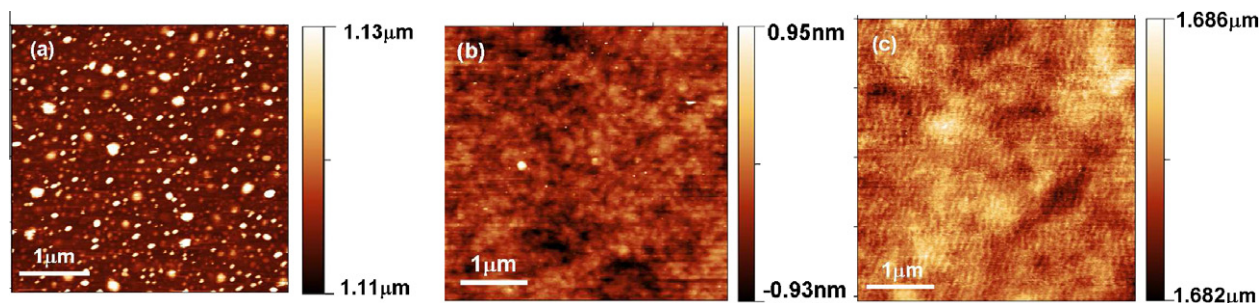

Fig. 3. AFM topography images of glass: (a) in air (RMS ∼ 3.8 nm), (b) after contact with pure PW medium for 3 h (RMS ∼ 0.2 nm) and (c) after contact with inoculated PW medium for 3 h (RMS ∼ 0.2 nm). Images (b) and (c) were acquired in PW medium at 28 °C. No BSA was used in this experiment.

Table 1
Contact angle measurements for different surfaces.

Time in contact with PW medium	Contact angle (°)		
	Glass (PW without BSA)	Glass (PW with BSA)	Silicon (PW with BSA)
Without contact	82.2 ± 0.1	82.2 ± 0.1	59.4 ± 0.1
3 h	74.3 ± 0.1	<10	<10
6 h	71.2 ± 0.1	<10	<10
12 h	63.5 ± 0.1	<10	<10
24 h	59.9 ± 0.7	<10	<10
3 days	<10	<10	<10

In contrast to the results presented herein, many reports in literature usually attribute enhanced bacteria adhesion and biofilm formation to surfaces with hydrophobic termination and high roughness properties [5–15]. However, the presence of the conditioning film and the lack of substrate specificity for *X. fastidiosa* adhesion suggest an important role of the chemical surface composition in this process. Indeed, Kefford and Marshall [41] observed that the adhesion of *Leptospira biflexa serovar patoc 1* (*L. patoc*)

was significantly larger on inert hydrophobic surfaces than on hydrophilic surfaces but continued to increase despite the reduction in surface hydrophobicity when BSA protein coated surfaces were used.

In order to evaluate potential molecular details of this conditioning film, ATR-FTIR spectra were acquired for the PW broth. Fig. 4 shows a typical spectrum of the PW broth with and without BSA, as well as of some of the individual PW broth compounds. For both cases, the relevant IR absorption bands were found between 1700 and 950 cm^{-1}. It is evident that the dominating contribution in the spectrum of the PW broth results from the proteins – BSA and glutamine – and the polyphosphate groups (potassium phosphate, K_2HPO_4). The peaks observed at 1656.8 cm^{-1} and 1666.4 cm^{-1} were assigned to the folded and helical protein structures [42], and those at 1578.6 cm^{-1} and 1547.8 cm^{-1} were attributed to the N–H bending, C–N stretching, and asymmetric stretching for deprotonated COO$^-$, while the peak at 1408.9 cm^{-1} corresponds to the symmetric stretching for deprotonated COO$^-$ [43]. The P=O stretching vibration of the phosphodiester and polyphosphate products is located around 1077.2 cm^{-1} [42]. The peaks observed at 1454.9 cm^{-1} and 1300.9 cm^{-1} correspond to the CH$_2$/CH$_3$ bending and C–N vibrations, respectively. The symmetric
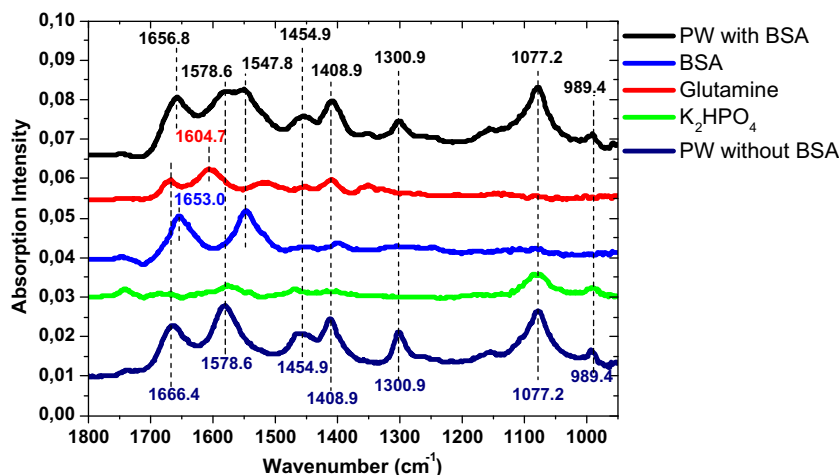
Fig. 4. ATR-FTIR spectra of PW medium solution with and without BSA and some of its compounds (BSA, glutamine, and K_2PO_4).
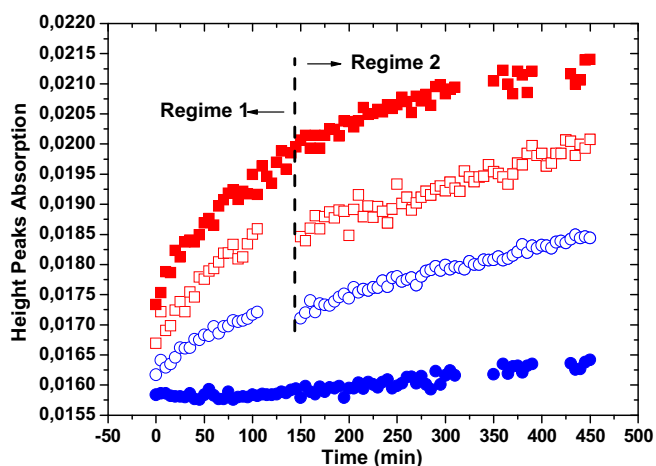

Fig. 5. Absorption peak heights for PW medium with (close symbols) and without (open symbols) BSA as a function of time. Square and circle (dots) symbols represent the peaks at 1077.2 and 1578.6 cm^{-1}, respectively.

Table 2
Absorption rate (α) for PW wavenumbers with and without BSA.

Wavenumber (cm^{-1})	Absorption rate (a) [10^{-6}]			
	PW with BSA		PW without BSA	
	Regime 1	Regime 2	Regime 1	Regime 2
1656.8/1666.4	6.8 ± 0.4	1.9 ± 0.1	8.3 ± 0.2	3.9 ± 0.1
1578.6	1.3 ± 0.1	–	9.1 ± 0.4	3.9 ± 0.1
1454.9/1456.2	1.06 ± 0.01	–	3.6 ± 0.4	0.9 ± 0.1
1408.9	2.2 ± 0.1	–	8.1 ± 0.4	3.8 ± 0.1
1300.9	4.9 ± 0.2	1.8 ± 0.2	9.1 ± 0.4	3.7 ± 0.1
1077.2	15.5 ± 0.1	4.1 ± 0.1	16.2 ± 0.1	4.9 ± 0.1
989.4	8.7 ± 0.6	2.4 ± 0.3	15 ± 1	4.6 ± 0.5

Following this transient period, the absorption rate stabilizes (regime 2), indicating that a dynamic equilibrium is reached for the concentration of constituents within the initial surface film. Most importantly, it is evident from Table 2 that the absorption rate is always larger for bands attributed to polyphosphate products and independent of the presence of BSA in the media.

Consequently, the obtained results suggest that functional surface groups resulting from the conditioning film formation play a more important role in X. fastidiosa adhesion processes than hydrophobicity and roughness. This role is, however, strongly dependent on the charge distribution at the bacteria cell surface. Several models have been suggested to explain X. fastidiosa cell adhesion at charged surfaces. Leite et al. [44] proposed a model to explain the adhesion of X. fastidiosa to xylem vessels. In this model, divalent cations could bridge negatively charged substrates on the xylem wall and on the X. fastidiosa surface (which is presumed to be negatively charged). In the present study, the availability of magnesium and potassium in the PW broth could assist the formation of bonds between X. fastidiosa cells and the phosphate groups enriched at the substrate surface. In turn, Osiro et al. [45] proposed a kinetic model where the adhesion process is dependent on the electrostatic attraction between positively charged surface proteins and negatively charged host surfaces. In this case, they considered that the number of positively charged amino acids (lysines, arginines, and histidines) exceeds the negative charges (glutamic and aspartic acids) in X. fastidiosa surface protein sequences. In this model as well, the presence of polyphosphate groups at the substrate could also contribute to the bacteria cell adhesion. In addition, Wolfe et al. [46] have demonstrated that the presence of acetyl phosphate may act as a signal that permits an orderly switch between the (flagella-dependent) reversible

stretching vibration of the phosphoryl groups usually gives rise to the signal at 989.4 cm^{-1}. Fig. 5 shows a typical temporal evolution of absorption peak heights with and without BSA for two selected cases, i.e., the peaks at 1077 cm^{-1} and 1578.6 cm^{-1}. Despite the lower absorption rate for some peaks (1578.6 cm^{-1}, 1454.9/1456.2 cm^{-1}, and 1408.9 cm^{-1}, see Table 2), an increase in absorption intensity with time for all bands is observed independent of the presence of BSA, thus providing further evidence for the conditioning film formation. Most of the peaks[4] present two growth regimes, as indicated in Fig. 5. The first non-linear regime is most likely associated with the initial coverage of the surface, since it is observed during the first 3 h, which – according to AFM analysis – corresponds to a film with a thickness of few nanometers. Furthermore, the average absorption rate for all peaks in the spectra was calculated for both regimes whenever data permitted (Table 2). The first regime, which corresponds to the first 2 h of spectra acquisition, clearly reveals higher absorption rates than the second regime, indicating a fast growth of species on the surface, which in turn concurs with the estimates of thickness in the present study.

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[4] The first regime is not observed for the 1578.6 cm^{-1} peak with BSA, since the absorption was too low and convoluted with the band at 1547 cm^{-1}.

and (type I fimbriae-dependent) irreversible attachment phases of biofilm formation. In a recent review, Monds and O'Toole [40] discussed the role of inorganic phosphate in the regulation of secretion and/or localization of adhesin LapA, a protein necessary for adhesion and biofilm formation of *Pseudomonas fluorescens.* Thus, phosphate groups within the PW conditioning film could influence surface adhesion process and biofilm development not just facilitating the surface–cell interaction but also as a regulator for the cell signaling.

4. Conclusions

A large number of factors contribute for the adhesion processes leading to biofilm formation. Hydrophobic surfaces with larger roughness usually show an increase in cell attachment and biofilm evolution. In contrast to these observations, *X. fastidiosa* cell adhesion only occurs after roughness and hydrophobicity are minimized due to the formation of a conditioning film on glass and silicon substrates. The lack of surface specificity for these substrates and the observed similarity in biofilm evolution suggests an important role of the chemical nature of the surface, which was further analyzed via infrared spectroscopy. Our results indicate that the presence of phosphate groups at the substrate surface resulting from the composition of the conditioning film appears more relevant for facilitating adhesion than surface roughness or hydrophobic surface properties. This interpretation is in agreement with recent works in literature [40,46], which considered the role of phosphate groups as a regulator for the secretion of surface proteins, essential for biofilm formation. However, to date, the role of the nutrient solution and the formation of a conditioning film affecting the chemical composition of the surface is frequently neglected. In the present case, it is confirmed that chemical surface changes are extensively involved in facilitating biofilm growth, which correlates well with current models for *X. fastidiosa* cell adhesion.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jcis.2011.03.066.

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