Biofilm formation and local electrostatic force characteristics of Escherichia coli O157:H7 observed by electrostatic force microscopy

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Biofilm formation and local electrostatic force characteristics of *Escherichia coli* O157:H7 observed by electrostatic force microscopy

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The authors report growth media dependence of electrostatic force characteristics in *Escherichia coli* O157:H7 biofilm through local measurement by electrostatic force microscopy (EFM). The difference values of electrostatic interaction between the bacterial surface and the abiotic surface show an exponential decay behavior during biofilm development. In the EFM data, the biofilm in the low nutrient media shows a faster decay than the biofilm in the rich media. The surface potential in the bacterial cells was changed from 957 to 149 mV. Local characterization of extracellular materials extracted from the bacteria reveals the progress of the biofilm formation and functional complexities. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2719030]

Understanding the mechanisms involved in bacterial adhesion to fresh produce surface may lead to improved technology for removing or inactivation pathogenic bacteria from fresh produce.[1] Bacterial adhesion on biomaterial surfaces is the initial step in establishing infections and leads to the formation of biofilms. Adhesion depends partly on the surface properties of the bacteria such as van der Waals forces, polar or Lewis acid base, and electrostatic interactions.[2] The interactions originate both from the entire cell body and from more specific, localized adhesion sites, such as proteins on the cell surface. The superstructural components of the surface are comprised of macromolecules containing carboxylate, phosphate, and amino functions which are ionized as a function of the environmental *p*H, thereby conferring electrostatic charge to the cell periphery.[3] Bacterial surface charge can also play a role in bacterial interaction with solid surfaces. Since nearly all surfaces occurring in nature carry a net negative charge under physiological conditions, electrostatic interactions in bacterial adhesion are mostly repulsive and have to be overcome by attractive van der Waals, hydrophobic, and specific interaction forces.[4] Bacterial surface charge is an important factor for bacterial adhesion. Generally, cell surface charge, as a result of charged functional groups on lipopolysaccharides (LPSs) of *E. coli*, is not measured directly. Using electrostatic force microscopy (EFM), however, local electrical property of the cell surface can be addressed directly. The EFM data are obtained by a local interaction between a tip and an outer sample surface while the conventional zeta-potential measurement is a sum of large area signals. Here, the spatial resolution is an order of 10 nm. This scanning probe approach has recently been used to assess functional group heterogeneity on bacteria surfaces. Moreover, with the development of EFM, it is now possible to directly inspect and quantify charge distribution on surfaces as well as topographic imaging.

This study was undertaken to characterize the surface properties of *E. coli* O157:H7, a virulent food-borne pathogen,[5] and the influence of different media on their ability to adhere and grow on abiotic surface. Also the difference of cell surface charge in different media was investigated. *Escherichia coli* O157:H7 strain (ATCC 43894) was transformed with pKEN2GFPmut2 for easy visualization of cells.[6] Cells were grown either in minimal (M9) glucose medium or Luria-Bertani (LB) medium for 16–18 h (approximately 10^9 CFU) with aeration at 28–30 °C. Details of the experimental method were published in other report.[7]

The biofilm was rinsed in a phosphate-buffer (*p*H 7.4) and lightly blowing the liquid off with a pure nitrogen gas. Biofilms were cultured at 25 °C and harvested at various time intervals in order to study the effects of the biofilm maturity and morphology. The biofilm was imaged either in noncontact mode or contact mode in an ambient environment, using a commercial atomic force microscopy (AFM) (Nanofocus Inc.). EFM was used to monitor topography and EFM image of biofilm simultaneously. In our experiments, the surface charge of the cell surface was acquired in ambient air by small ac voltage with an amplitude of 2–4 V (peak to peak) and a resonant frequency of cantilever is between 45 and 115 kHz while the tip was scanning the biofilm surface. We used a Pt coated Si cantilever or heavily doped Si cantilever with a spring constant of 0.2 N/m. Fresh cantilevers were used for each experiment to prevent sample contamination. The probing tip, with an apex radius of about 10 nm, was in mechanical contact with biofilm surface during the measurements. Cantilever vibration depending on the surface charge was detected using a conventional lock-in technique. Details on the experimental setup and ideas of dynamic contact mode EFM were published earlier.[8] The attachment of *E. coli* O157:H7 cells to abiotic surface and cell-to-cell attachment in the biofilms were examined by AFM. Studies were conducted initially by minimal media (M9) and complex media (LB) containing nutrients. Growth and biofilm development *E. coli* O157:H7 on abiotic surface in the M9 and the LB media are shown in Fig. 1.

The normal smooth surfaces of the *E. coli* O157:H7 biofilm grown in the M9 media for 0 and 2 days are shown in Figs. 1(a) and 1(b). Clustering of cells was observed together with a few scattered individual cells. Cells were embedded in a layer of extracellular materials (ECMs), as shown in Fig. 1(c). Despite a light rinsing of the cells to remove excess growth media and planktonic cells, some of this ECM remained, especially after 2 days, around and under the cells.

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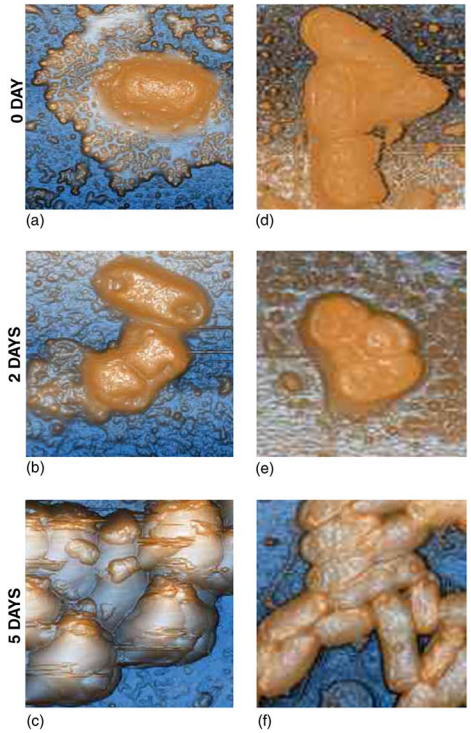

FIG. 1. (Color online) AFM images of *E. coli* O157:H7 biofilm grown on the abiotic surface. (a) Biofilm grown in the M9 minimal media for 0 day, (b) for 2 days, and (c) for 5 days. (d) Biofilm grown in the LB complex media for 0 day, (e) for 2 days, and (f) for 5 days. All images are 5×5 μm^2 area.

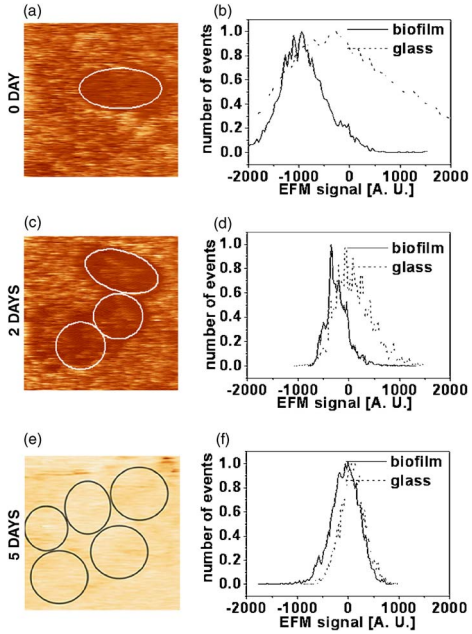

FIG. 2. (Color online) EFM images of *E. coli* O157:H7 biofilm grown on an abiotic surface. (a) Biofilms were 0 day old, (c) 2 days old, and (e) 5 days old in the M9 minimal media. All the EFM images simultaneously obtained with the topographic images in Figs. 1(a)–1(c). A plot of surface charge distribution obtained from each EFM image. All images are 5×5 μm^2 area.

These emitted materials can be made up of heterogeneous polysaccharides, DNA, proteins, and other macromolecules secreted by bacteria.[9–11] Figures 1(d) and 1(e) show a simple biofilm, the bacterial growth in small cluster or groups. In Fig. 1(f), bacteria formed a near-continuous layer on the abiotic surface. As compared with biofilm grown in the minimal media, AFM images of biofilm grown in the complex media showed cells to be packed at different densities in different regions of the biofilm. The difference in ability to form biofilms between LB media and M9 media is due to the existence of ECM.[12–14]

Using EFM, we acquired surface charges of bacterial cell and biofilm surface directly. Figures 2(a), 2(c), and 2(e) show EFM images of the biofilm which grown for 0, 2, and 5 days. Each circle indicates a region of cell existence, as shown in Fig. 1. The topographic and surface charge image of the biofilm surface was taken simultaneously. A dark area shows negative surface charges in contrast bright area shows positive surface charges. Figures 2(b), 2(d), and 2(f) display a plot of surface charge distribution of the cell surface and abiotic surface as a function of probability. The value of surface charge was obtained by averaging the EFM signals throughout the cell surface region and abiotic surface region, respectively. Figure 2(a) presents EFM images of a cell surface with an overall homogeneous negative charge distribution on the abiotic surface. Initial interactions between bacterial cells and the substrate are governed in part by electrostatic interaction. The substrate is abiotic and covered with ECM; the initial interactions that occur between the cell and the surface are greatly influenced by the overall surface charge of the bacterial cell which is governed by the composition of lipopolysaccharides. One of the contributing factors to the overall negative charge of the bacterial cell is its LPS composition. In Figs. 2(d) and 2(f), the value is decreased so that the bacterial cell surface charge on the abiotic surface with biofilm formed by ECM displayed an inhomogeneous surface charge distribution.

As microcolonies increase in size, bacteria are thought to begin to secrete exopolysaccharides (EPSs) and other substances that encase the cells in a stabilizing, protective matrix. EPS in nature are homopolysaccharides, such as cellulose, which consists of repeating glucose unit. Homopolysaccharide are either anionic or neutral in overall charge. Neutrally charged EPS is characterized by EFM images. Figure 3 presents EFM images of a cell surface with an overall homogeneous negative charge on the abiotic surface and a plot of a surface charge distribution. Figures 3(a) and 3(b) show a low negative EFM signal on the bacterial surface. Figures 3(c) and 3(e) show that negative cell surface charge decreases slowly compared with biofilm surface charge grown in the M9 media. ECM is a key factor of variation of cell surface charges.

Figures 4(a)–4(c) show each line profile obtained from EFM images of the biofilm shown in Fig. 2. EFM signals on the bacterial surface present homogeneous in Fig. 4(a). But in Fig. 4(b), bacteria do not induce the production of extracellular polymeric substances on their surface regularly. They irregularly secrete EPS on the specific region of the bacteria surface and these materials exhibit different behaviors of the electrostatic interaction. Figure 4(d) shows variation of the charge difference between biofilm surface region and abiotic surface region as a function of time. ΔQ is described by

$$\Delta Q = \frac{\sum_{c=0}^{N} q_c}{N} - \frac{\sum_{g=0}^{M} q_g}{M}, \tag{1}$$

where q_c are surface charges on the biofilm, q_g are surface charges on the abiotic surface, and N and M are numbers of

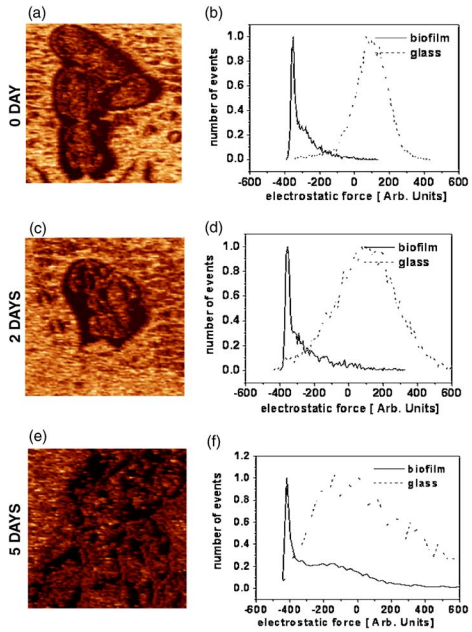

FIG. 3. (Color online) EFM images of *E. coli* O157:H7 biofilm grown on an abiotic surface. (a) Biofilms were 0 day old, (c) 2 days old, and (e) 5 days old in the LB complex media. All the EFM images simultaneously obtained with AFM images in Figs. 1(d)–1(f). A plot of surface charge distribution obtained from each EFM image. All images are 5×5 μm^2 area.

pixel of selected area in EFM images. The value of charge difference was obtained by averaging surface charge throughout each specified region. In 0 day old biofilm grown in the M9 media, ΔQ is larger than other value of surface charge. In contrast, biofilms grown in the LB media show a similar ΔQ value between 0 day old biofilm and 5 day old biofilm. The following is a fitting function:

$$\Delta Q = \alpha + \beta \exp\left[\frac{-t}{\gamma}\right], \tag{2}$$

where t is a time, α and β are constants, ΔQ is a difference charge between biofilm and abiotic surface, and γ is the constant of an exponential decay. Biofilm grown in the M9 media shows $\alpha=125.7545$, $\beta=831.7675$, and $\gamma=1.4016$ and biofilm grown in the LB media shows $\alpha=322.9671$, $\beta=64.3479$, and $\gamma=2.1796$, respectively. In the minimal media, surface charge decays exponentially while secreting ECM and formed mature biofilm. The differences seen above indicate that initial cell-to-surface interactions are influenced by bacterial LPS but do not depend on bacterial surface structure. Rozhok and Holz reported that a negative potential of −1000 mV to the electrochemically attached *E. coli* cells can be applied on gold surface, indicating that the electrochemical attachment process is only partially reversible.[15] In our experimental results, the estimated value of the surface potential between the bacteria cells and the abiotic surface is about 900 mV. Obtaining zeta potential is accomplished by measuring distance between colloidal particles inside of the liquid surrounding bacterial cells, which is different from the direct measurement of the surface potential in the dehydrate surface in our case. In general, zeta potential is known to have an order of 1–10 mV around *p*H 7.0.[16]

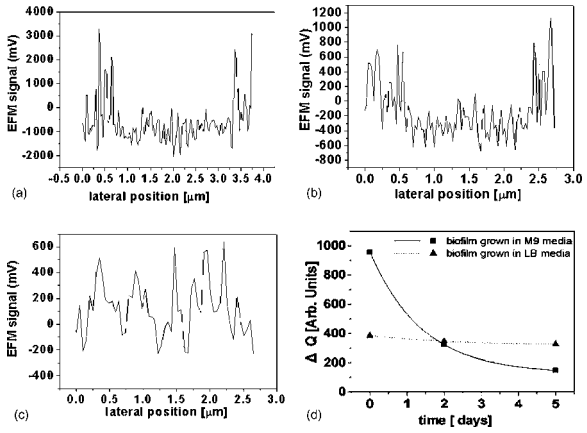

FIG. 4. (a) Cross-sectional line profile of the bacterial surface charge obtained from Fig. 2(a), (b) obtained from Fig. 2(c), and (c) obtained from Fig. 2(e). Decay behavior of the average charge difference between the biofilm surface and the abiotic surface grown in the M9 media and the LB media.

In summary, we report the effect of nutrient on the *E. coli* o157:H7 biofilm and surface charges of the biofilm. It is shown that biofilm developed faster with ECM in the low nutrient media. In addition, the surface charge of biofilm represents the interaction of cell through ECM and potential differences. As the biofilm is getting matured, the value of charge difference seems to be smaller, indicating that electrostatic force is a sign of ECM existence. The most important function in the biofilm is protection. And bacteria in ECM are generally more resistant than the other single bacteria to toxic substances in the environment, including antibiotics because they make their surface neutral condition. For this neutral condition, if they formed a biofilm, bacteria protect from external interference such as medicinal delivery.

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