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Note

Extracellular polymeric bacterial coverages as minimal area surfaces

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Abstract

Surfaces formed by extracellular polymeric substances enclosing individual and some small communities of *Acidithiobacillus ferrooxidans* on plates of hydrophobic silicon and hydrophilic mica are analyzed by means of atomic force microscopy imaging. Accurate nanoscale descriptions of such coverage surfaces are obtained. The good agreement with the predictions of a rather simple but realistic theoretical model allows us to conclude that they correspond, indeed, to minimal area (constant mean curvature) surfaces enclosing a given volume associated with the encased bacteria. This is, to the best of our knowledge, the first shape characterization of the coverage formed by these biomolecules, with potential applications to the study of biofilms.

Keywords: Extracellular polymeric substances; Bacterial coverage; Surface tension; Minimal area surfaces

Extracellular polymeric substances (EPS) are produced by microorganisms during the process of adhesion to an environmental surface, acting mainly to protect yet them and to facilitate their interactions [1]. The exact functions of EPS have not been yet completely elucidated because of their extremely heterogeneous nature. It is known, however, that EPS play significant role in the formation and function of microbial aggregates, including matrix structure formation and microbial physiological processes [2]. In this Note, we report an analysis, based experimentally on atomic force microscopy (AFM) imaging, of the EPS bacterial coverage produced in communities of *Acidithiobacillus ferrooxidans* adhered to flat plates of silicon (hydrophobic) and mica (hydrophilic). AFM has the ability to image the coverage surface morphology in aqueous conditions, without any chemical fixation. In particular, AFM has recently proved to be useful in imaging the morphology of bacteria [3], liposomes [4], and DNA molecules [5] on solid surfaces. Beech et al. [6], furthermore, showed that AFM allows the estimation of the width and height of bacterial exopolymeric capsule and bacterial flagella. We notice also that AMF has been recently used to characterize wetting morphologies on microstructured surfaces [7].

As we will show, AFM can be also used to determine the shape of different EPS coverage patterns of individual bacterium and some small communities of *A. ferrooxidans*. The appearance of the minimal area phenomena on extracellular polymeric coverage is associated with the need of the bacteria to prevent losing water under drying conditions. The EPS secreted in solution or after fixation will have to cover the bacteria if they are going to survive. Since EPS production costs resources and energy to the bacteria, it would be natural to expect that EPS coverage surfaces should obey some variational principle, implying, therefore, that the observed surfaces should be *minimal* with respect to some criteria. At this scale (~ 1 μm), on the other hand, one does not expect any other force to be relevant besides the surface tension [8–10]. Consequently, the observed surfaces should correspond to minimal area (constant mean curvature) surfaces enclosing a given and fixed volume, associated, of course, with the encased bacteria. In this way, the observed surfaces would minimize both the potential elastic energy and the total amount of EPS necessary to form them. Our analyses confirm this hypothesis, EPS coverages of *A. ferrooxidans* adhered to mica and silicon plates can be indeed understood as minimal area surfaces enclosing some fixed volumes. For a

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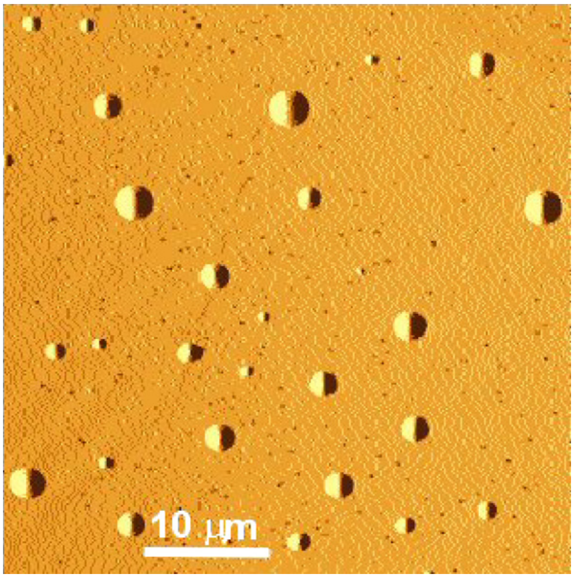

Fig. 1. *A. ferrooxidans* adhered to a hydrophilic mica plate observed in air. For all bacteria, the covering material form a cap-like structure.

review of the biological significance of *free* (i.e., without any volume constraint, zero mean curvature) minimal surfaces, see [9,10]. Our conclusions are in agreement with the recently reported studies [7] on the minimal area surfaces associated to different wetting morphologies on microstructured surfaces.

EPS form a highly entangled hydrated structure, composed basically by sugars and water linked by hydrogen bonds, in agreement with our optical observations suggesting that the EPS coverage behaves as an isotropic gel-like structure. Also, the typical EPS coverage has a volume 20 times larger than the encased bacterial volume. The details about the bacteria growing conditions, EPS chemical composition analysis, and the experimental setup are given in the Section material and methods of the supplementary material.

Fig. 1 shows a typical distribution of bacteria adhering to a hydrophilic mica plate at the center of the deposited droplet (∼5 mL, 3.0 pH), corresponding to a view on an area of ∼2500 μm^2. We notice that, typically, each bacterium is isolated from the others and the area is almost uniformly covered. The shapes of the covering structures are shown in detail in Fig. 2, where top views of individual covered bacteria are displayed; all bacteria show a cap-like structure formed by the covering material. Also, images recorded after scanning large areas provide direct evidence for the presence of a continuous layer covering part of the substrate. Although layers as thin as ∼20 nm are observed, most of the covered substrate has thicker layers (∼600 nm) of deposited material. We remind that bacteria shape is determined by their membranes structure, which typically have highly fluid shapes, varying from cylindrical when in a solution to some flat prolate structures when deposited on a substrate, as shown, for instance, in the dried sample depicted in Fig. 2c.

The problem of finding a minimal area surface enclosing a given volume is a classical isoperimetric (isovolume) variational problem, and several mathematical and computational tools are available to solve it in the most generic contexts. Further details of our mathematical analysis can be found in the Section minimal area surfaces of the supplementary material. For the case of axisymmetric surfaces, analytical solutions are available, whereas for the non-symmetric case we had to use some approximations or iterative numerical methods. For the first case, one has yet two qualitative distinct cases according to the nature of the support region $\mathcal{D}$. For simply connected $\mathcal{D}$, it is

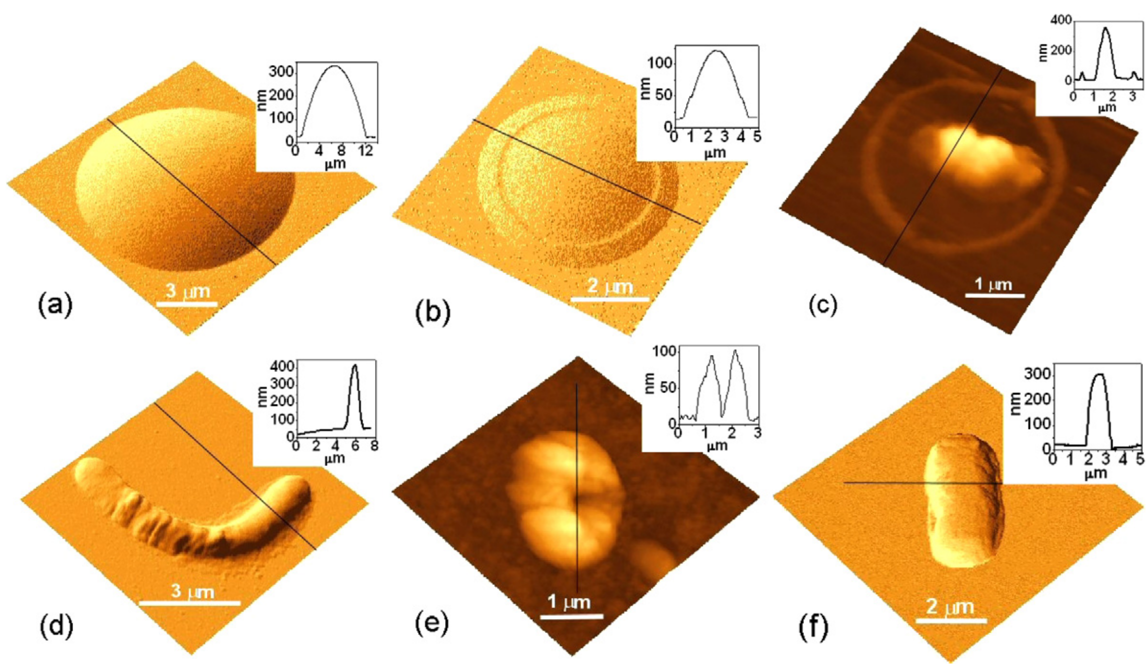

Fig. 2. Some AFM images of the EPS coverage of single *A. ferrooxidans* adhered to hydrophilic mica (a–c) and hydrophobic silicon (d–f) plates. The curves correspond to the slices indicated in the images. All images except (c) were obtained in aqueous condition. (c) corresponds to a rinsed sample similar to (b), where one can see clearly the circular ring corresponding to the thicker basis of the coverage. See the text for further details.