Hydrogen-Bonding and Long-Range Interactions Involved in the Initial Attachment of Biofilm-Dispersed *Escherichia coli* and *Bacillus subtilis*

F. Pinar Gordesli Duatepe[1], Ayse Ordek[2], Gamze Nur Aspar[2]

[1]Department of Genetics and Bioengineering, Faculty of Engineering, Izmir University of Economics, 35330 Izmir, Turkey
[2]Bioengineering Graduate Program, Graduate School, Izmir University of Economics, 35330 Izmir, Turkey

pinar.gordesli@iue.edu.tr

Biofilms are surface-associated bacterial communities covered by a self-generated extracellular polymeric matrix that protects bacteria against various environmental stresses [1]. Biofilms are significant because of their role in the pathogenesis of many chronic infections and their ability to exhibit resistance to antimicrobial agents [1,2]. Biofilm development can be divided into three distinct stages: initial attachment of planktonic bacterial cells to a surface, growth of the cells into a sessile biofilm form, and dispersal of the cells from the biofilm [2]. The dispersal of the biofilm is the final stage of biofilm development that leads to the spread of the biofilm and hence the transmission of infection [2,3]. Studies have shown that the physiology of cells dispersed from biofilms is quite different from that of planktonic and biofilm cells [3]. Dispersed cells have also been shown to be highly virulent [3], and metabolically more active [4] compared to the planktonic cells. However, the initial attachment of biofilm-dispersed cells to surfaces, the first stage of biofilm development that takes place at the nanoscale, has not been systematically investigated therefore require further examination.

In general, the initial bacterial attachment to a surface is governed by long-range physicochemical interactions, often as described by the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory, and short-range specific molecular interactions such as hydrogen bonds and ligand/receptor bonds [5-8]. The quantitative information on the overall interaction force between the bacterium and the surface can be directly obtained with high resolution using atomic force microscopy (AFM). Moreover, the application of Poisson statistical method to AFM adhesion data allows to decouple the overall interaction force into short-range specific and long-range nonspecific force components [5,7,8]. This method represents one of the only available ways to quantify hydrogen bonding between bacteria and surfaces from AFM data [5,7].

The goal of this study is to provide the necessary information on the fundamental components of the overall interaction forces mediating the initial attachment of biofilm-dispersed Gram-negative *Escherichia coli* and Gram-positive *Bacillus subtilis* cells. For this purpose, first *E. coli* and *B. subtilis* biofilms were grown on glass slides in continuous flow chambers for 24-h. Afterward, a dispersal agent, NO-donor sodium nitroprusside (SNP) was added to the medium bottles at a final concentration of 5 μM. Biofilm dispersion was induced by the continuous flow of the medium supplemented with 5 μM of SNP for 24-h at a rate of 0.4 ml/min (laminar flow) for each investigated. Finally, biofilm-dispersed *E. coli* and *B. subtilis* cells were collected from the waste bottles and directly prepared as samples for AFM measurements. The overall interaction forces (adhesion forces) between silicon nitride AFM tips and the surface molecules of biofilm-dispersed *E. coli* and *B. subtilis* were measured under water by AFM. The adhesion force is related to the number of bonds ruptured during the AFM pull-off event [7,8]. The adhesion forces were then decoupled into hydrogen bonding and long-range force components using the mean equals variance property of the Poisson distribution. Since there are no ligand/receptor bonds to be expected between silicon nitride and the bacterial surfaces in water, the short-range specific forces arise from hydrogen bonding in our system. The hydrogen bonding can arise from the interactions between the surface silanols of silicon nitride and the surface hydroxyl and/or amine groups of the outer membrane molecules of the bacterial cells in water [8].

Consequently, our results indicated that the nature of hydrogen-bonding and long-range forces were attractive for each investigated and, on average, the hydrogen-bonding forces were 2-fold stronger than the long-range forces. The hydrogen-bonding and long-range forces obtained for biofilm-dispersed *E. coli* (0.248 nN and 0.120 nN, respectively) were on average 5-fold higher than those obtained for biofilm-dispersed *B. subtilis* (0.05 nN and 0.02 nN, respectively). These findings together with those of planktonic cells available in the literature [5-8] may guide the design of new strategies aimed at eliminating the initial attachment of biofilm-dispersed Gram-negative and Gram-positive bacterial cells to inert surfaces from a biophysical perspective.

Acknowledgement

This study was financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK) under Grant CAREER (118M404).

References

[1] Donlan RM, Costerton JW. Clinical Microbiology Reviews 2002, 15, 167-193.
[2] Rumbaugh KP, Sauer K. Nature Reviews Microbiology 2020, 18, 571-586.
[3] Chua SL, Liu Y, Yam JK, et al. Nature Communications 2014, 5, 4462.
[4] Guilhen C, Forestier C, Balestrino D. Molecular Microbiology 2017, 105, 188-210.
[5] Abu-Lail NI, Camesano TA. Langmuir 2006, 22, 7296-7301.
[6] Carniello V, Peterson BW, van der Mei HC, Busscher HJ. Advances in Colloid and Interface Science 2018, 261, 1-14.
[7] Chen Y, Busscher HJ, van der Mei HC, Norde W. Applied Environmental Microbiology 2011, 77, 5065−5070.
[8] Gordesli FP, Abu-Lail NI. Environmental Science & Technology 2012, 46, 10089-10098.

Figures

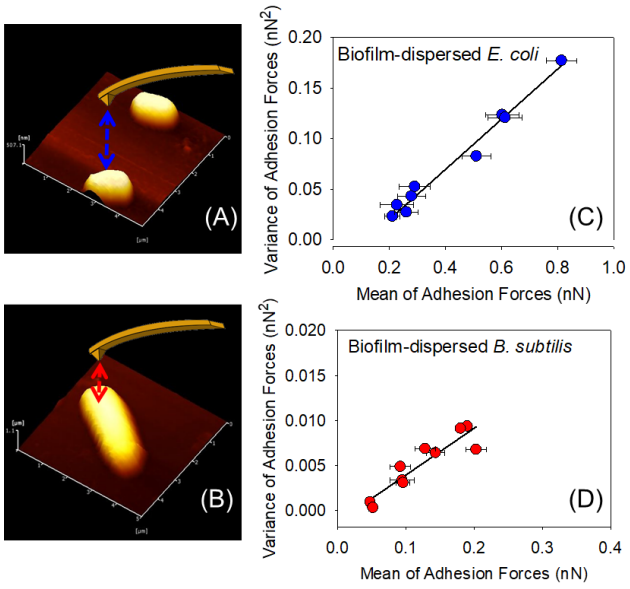

Figure 1. Dynamic fluid mode topographical images of (A) biofilm-dispersed *E. coli* and (B) biofilm-dispersed *B. subtilis* in water, and the representation of the measurement of overall interaction forces between silicon nitride AFM tips and the bacterial cells. (C) and (D) are the linear regressions to the scatter plots of mean versus variance of adhesion forces from which the hydrogen-bonding and long-range forces were determined through the use of Poisson statistical method.