Biofilm Development with an Emphasis on *Bacillus subtilis*

K. P. Lemon, A. M. Earl, H. C. Vlamakis, C. Aguilar, and R. Kolter

Abstract Our understanding of the molecular mechanisms involved in biofilm formation has increased tremendously in recent years. From research on diverse bacteria, a general model of bacterial biofilm development has emerged. This model can be adjusted to fit either of two common modes of unicellular existence: nonmotile and motile. Here we provide a detailed review of what is currently known about biofilm formation by the motile bacterium *Bacillus subtilis*. While the ability of bacteria to form a biofilm appears to be almost universal and overarching themes apply, the combination of molecular events necessary varies widely, and this is reflected in the other chapters of this book.

In most natural settings, bacteria are found predominantly in biofilms (Henrici 1933; Costerton et al. 1999; Hall-Stoodley et al. 2004). The widespread recognition that biofilms impact myriad environments, from water pipes to indwelling devices in hospital patients, led to an increased interest in investigating the molecular mechanisms underlying the formation and maintenance of these communities. As a consequence, we have recently witnessed much growth in our knowledge of biofilms. The ability to form biofilms, once considered the domain of a few species, is now seen as a nearly universal attribute of microorganisms. It has also become evident that the pathways utilized by bacteria to build biofilms are extremely...
diverse, varying enormously among different species and under different environmental conditions. There are, however, several common features among all biofilms examined to date:

1. Constituent cells are held together by an extracellular matrix composed of exopolysaccharides (EPS), proteins, and sometimes nucleic acids (Whitchurch et al. 2002; Branda et al. 2005; Lasa 2006).

2. Biofilm development occurs in response to extracellular signals, both environmental and self-produced (Kolter and Greenberg 2006; Spoering and Gilmore 2006).

3. Biofilms afford bacteria with protection from a wide array of environmental insults, as diverse as antibiotics (Mah and O’Toole 2001), predators (Kadouri et al. 2007), and the human immune system (Singh et al. 2000; Fedtke et al. 2004; Leid et al. 2005).

Initial studies on bacterial biofilms were predominantly descriptive. By applying novel microscopic approaches, most notably laser scanning confocal microscopy, a whole new universe of biofilm architecture became apparent. These approaches, coupled with time-lapse video microscopy and microsensors, have given us a more complete view of the complex structure of biofilms. These descriptive advances have been followed in the last decade by an outburst in the number of molecular genetic analyses carried out in biofilms. Today, investigators are applying a wide range of molecular biological approaches to the study of the regulatory processes that underlie biofilms. As is made clear in the chapters of this book, there are many examples where molecular genetics has had great impact on biofilm research. For almost every organism that has been investigated, we can now draw working genetic models for the steps in the pathways of biofilm development. There are genes expressed during each step of development that can serve as reporters of that stage and there are genes whose functions are essential for each particular step to be completed successfully. In spite of these advances, one can argue that biofilm genetics is still in its infancy. Investigators continue to identify novel genes that are either essential for, or expressed during, biofilm formation in many different organisms. Even among genes that have been previously identified, we have only recently begun to elucidate exactly how some of these genes’ products contribute to biofilm development, maintenance, and dissolution.

1 How Do We Study Biofilms in the Laboratory?

Pipelines, catheters, teeth, plant roots, and the lungs of cystic fibrosis patients are but a few of the most widely recognized surfaces where the effects of biofilms are readily apparent. The biofilms that form on such surfaces almost invariably house a complex mixture of species, rendering them not particularly amenable to molecular genetic studies. To be able to address questions regarding the molecular basis of biofilm formation, investigators have developed artificial biofilm model systems that are easy to control and reproducible from laboratory to laboratory.