

# METHODS IN MOLECULAR BIOLOGY

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# **Microbial Biofilms**

## **Methods and Protocols**

Edited by

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## Preface

Starting from the late 1970s, the biofilm's pioneers Bill Costerton and Niels Hoiby have provided significant information on the ability of microorganisms to stick on biotic and abiotic surfaces and to build communities of cells closely interacting with each other within a self-produced exopolysaccharide matrix. However, only since the early 1990s it has been possible to observe, by a confocal laser scanning microscope, living biofilms of *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Vibrio parahaemolyticus*, stained with viable fluorescent probes. Biofilms were found to be highly hydrated open structures constituted of 73 to 98 % of extracellular substances and large void spaces allowing the circulation of nutrients and signaling molecules and the removal of microbial catabolites. Thus, the so-called mushroom model was proposed to schematically represent the tridimensional structure of these microbial communities, the dynamics of their sessile growth, and the main interactions among the cells and the surrounding environment.

This novel view of the microbial world has led us in the last decades to the consciousness of the predominance of biofilms not only in natural or engineered ecosystems but also in the human body. As biofilms in the different niches are concerned, a new awareness has been acquired on the pivotal role that these sessile-growing communities of microorganisms play in a number of environmental processes: from the biofouling to the biocorrosion of the pipelines of concrete wastewater pipes, to the clogging of the pipelines in the dairy industry, to the deterioration of stones, frescoes, paintings, books, and other ancient remains. And again, the understanding that most of the chronic infections in humans, including the oral, lung, vaginal, and foreign body-associated infections, are biofilm-based, has prompted the need to design new and properly focused preventive and therapeutic strategies for these diseases. In this framework, the consensus conference organized in 2013 by Niels Hoiby under the umbrella of the Study Group for Biofilms of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) deserves to be mentioned. The objective of this initiative, made possible by the active contribution of a selected number of scientists working on biofilms of medical interest, has been to draft the "ESCMID guidelines for the diagnosis and treatment of biofilm infections" to be published early in 2014. Of course, the detailed description of most of the better established and validated experimental procedures to investigate microbial biofilms contained in the present book will be of paramount importance for all of those involved in the practical application of the abovementioned guidelines.

In fact, most of the currently available methods and protocols to investigate bacterial and fungal biofilms have been exhaustively illustrated and critically annotated in the 25 chapters by authors well known for their relevant experience in the respective fields. The book has joined together microbiologists and specialists in infectious diseases, hygiene, and public health involved in exploring different aspects of microbial biofilms as well as in designing new methods and/or developing innovative laboratory protocols. Chapters have been subgrouped by dividing the experimental approaches suitable for studying biofilms in health and disease from those more appropriate to assay antibiofilm compounds or evaluate antimicrobial strategies and from those regarding the application of methods to detect

biofilms growing in the environment or affecting manufacturing plants. In the whole, readers will have at their disposal a precious working tool to perform experiments focused on both the structural and functional properties of single- and multi-species biofilms as well as their response to matrix-dissolving agents, biocides, sanitizers, and antimicrobial molecules. In this regard, advanced techniques such as the multiplex fluorescence in situ hybridization and the chip calorimetry, and innovative antibiofilm strategies as the photodynamic therapy or the bacteriophage attack, are described. Microbiological methods for in vitro screening of bacterial biofilm inhibitors and antifungal compounds are also detailed. Researchers interested in methods based on in vitro or in vivo biofilm observations, in static or dynamic conditions, by fluorescence, confocal, and scanning electron microscopy, will find in this book all the relative information provided by expert guides, each chapter being rich of useful practical suggestions and warnings. Specific chapters also deal with the most advanced animal models, including the nonmammalian ones, to investigate bacterial and fungal biofilms. Other contributions of particular interest are those related to assay protocols for staphylococcal and enterococcal quorum sensing systems, to study the pharmacokinetics and pharmacodynamics of antibiotics in biofilm-related infections, and to evaluate the efficacy of antibiotic-loaded polymers and polymeric nanoparticles.

I am sure that all the “biofilm’s lovers” will enjoy this book.

*Rome, Italy*

*Gianfranco Donelli*

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