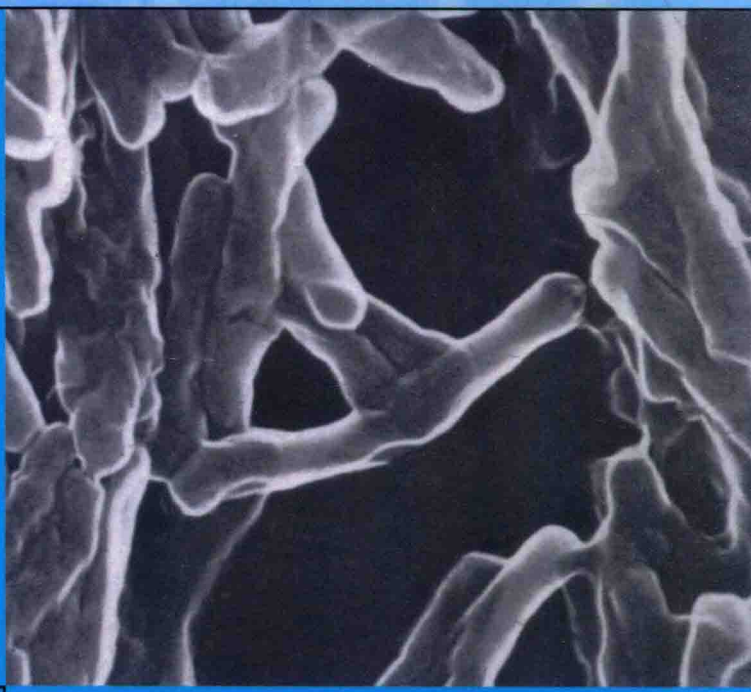
ADVANCES IN MOLECULAR AND CELLULAR MICROBIOLOGY

AMCM

Dormancy and Low-Growth States in Microbial Disease



Edited by
Anthony R. M. Coates

CAMBRIDGE

Advances in Molecular and Cellular Microbiology 3

Dormancy and Low-Growth States in Microbial Disease

EDITED BY
Anthony R. M. Coates
St. George's Hospital Medical School, London.

CAMBRIDGE
UNIVERSITY PRESS

CAMBRIDGE UNIVERSITY PRESS
Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore,
São Paulo, Delhi, Dubai, Tokyo, Mexico City

Cambridge University Press
The Edinburgh Building, Cambridge CB2 8RU, UK

Published in the United States of America by Cambridge University Press, New York

www.cambridge.org
Information on this title: www.cambridge.org/9780521187848

First published 2003
First paperback edition 2011

A catalogue record for this publication is available from the British Library

Library of Congress Cataloguing in Publication data
Dormancy and low-growth states in microbial disease / edited by
Anthony R. M. Coates.
p. cm. – (Advances in molecular and cellular microbiology ; 3)
Includes bibliographical references and index.
ISBN 0-521-80940-1 (hardback)
1. Medical microbiology. 2. Dormancy (Biology) I. Coates, Anthony R. M.
II. Series.
QR46 .D624 2003
616'.014 – dc21 2002035070

ISBN 978-0-521-80940-5 Hardback
ISBN 978-0-521-18784-8 Paperback

Additional resources for this publication at www.cambridge.org/9780521187848

Dormancy and Low-Growth States in Microbial Disease

Organisms multiply only when the conditions are beneficial, and when not multiplying, they concentrate on survival of environmental stress. Many bacteria that harm humans survive for most of the period of infection in a low-growth state. This book addresses the basic scientific aspects of microbial dormancy and low-growth states, and places them in the context of human medicine. The book introduces basic scientific aspects of bacterial growth, non-growth, culturability, and viability. Later chapters cover the crucial relationship between low-growth states and survival of stress, the survival of the immune response, and interbacterial signalling. This is followed by chapters on aspects that are of direct importance to medicine, namely antibiotic resistance arising in stationary phase, biofilms, tuberculosis, and the bacteria, which cause gastric ulcers.

ANTHONY R. M. COATES is currently Professor of Medical Microbiology and Chairman of the Department of Medical Microbiology at St. George's Hospital Medical School, London. His research background is in the molecular pathogenesis of infection with a particular interest in tuberculosis.

Published titles

1. *Bacterial Adhesion to Host Tissues.* Edited by Michael Wilson 0521801079
2. *Bacterial Evasion of Host Immune Responses.* Edited by Brian Henderson and Petra Oyston 0521801737

Forthcoming titles in the series

Susceptibility to Infectious Diseases. Edited by Richard Bellamy 0521815258
Mammalian Antimicrobial Peptides. Edited by Deirdre Devine and Robert Hancock 0521822203
The Dynamic Bacterial Genome. Edited by Peter Mullany 0521821576
Bacterial Protein Toxins. Edited by Alistair Lax 052182091X
Bacterial Invasion of Host Cells. Edited by Richard Lamont 0521809541

Over the past decade, the rapid development of an array of techniques in the fields of cellular and molecular biology has transformed whole areas of research across the biological sciences. Microbiology has perhaps been influenced most of all. Our understanding of microbial diversity and evolutionary biology, and of how pathogenic bacteria and viruses interact with their animal and plant hosts at the molecular level, for example, has been revolutionized. Perhaps the most exciting recent advance in microbiology has been the development of the interface discipline of cellular microbiology, a fusion of classic microbiology, microbial molecular biology, and eukaryotic cellular and molecular biology. Cellular microbiology is revealing how pathogenic bacteria interact with host cells in what is turning out to be a complex evolutionary battle of competing gene products. Molecular and cellular biology are no longer discrete subject areas but vital tools and integrated parts of current microbiological research. As part of this revolution in molecular biology, the genomes of a growing number of pathogenic and model bacteria have been fully sequenced, with immense implications for our future understanding of micro-organisms at the molecular level.

Advances in Molecular and Cellular Microbiology is a series edited by researchers active in these exciting and rapidly expanding fields. Each volume will focus on a particular aspect of cellular or molecular microbiology, and will provide an overview of the area, as well as examining current research. This series will enable graduate students and researchers to keep up with the rapidly diversifying literature in current microbiological research.

ADVANCES IN MOLECULAR AND CELLULAR MICROBIOLOGY

AMCM

Contributors

M. R. Barer
Department of Microbiology and Immunology
Medical Sciences Building
PO Box 138
University Road
Leicester LE1 9HN
UK

Michael R. W. Brown
Department of Pharmacy and Pharmacology
University of Bath
Claverton Down
Bath BA2 7AY
UK

Anthony R. M. Coates
Department of Medical Microbiology
St George's Hospital Medical School
London SW17 0RE
UK

Petra Dersch
Institute für Biologie, Mikrobiologie
Freie Universität Berlin
Königin-Luise-Str. 12-16
14195 Berlin
Germany

C. Stewart Goodwin
Department of Oncology, Gastroenterology
Endocrinology and Metabolism
St. George's Hospital Medical School
London SW17 0RE
UK

Regine Hengge-Aronis
Institute für Biologie, Mikrobiologie
Freie Universität Berlin
Königin-Luise-Str. 12-16
14195 Berlin
Germany

Yanmin Hu
Department of Medical Microbiology
St. George's Hospital Medical School
London SW17 0RE
UK

Gerald C. Johnston
Department of Microbiology and Immunology
Dalhousie University
Halifax Nova Scotia B3H 4H7
Canada

David R. Katz
Department of Immunology and Molecular Pathology
Windeyer Institute, University College London
46 Cleveland Street
London W1T 4JF
UK

Valerie Mizrahi
MRC/NHLS/WITS Molecular Mycobacteriology Research Unit
National Health Laboratory Service
PO Box 1038
Johannesburg 2000
South Africa

Gabriele Pollara
Department of Immunology and Molecular Pathology
Windeyer Institute, University College London
46 Cleveland Street
London W1T 4JF
UK

Hugh W. Pritchard
Seed Conservation Department
Royal Botanic Gardens, Kew
Wellcome Trust Millennium Building
Wakehurst Place
Ardingly
West Sussex RH17 6TN
UK

Richard A. Singer
Department of Biochemistry and Molecular Biology
Dalhousie University
Halifax Nova Scotia B3H 4H7
Canada

Anthony W. Smith
Department of Pharmacy and Pharmacology
University of Bath
Claverton Down
Bath BA2 7AY
UK

Simon Swift
Division of Molecular Medicine and Pathology
Faculty of Medical and Health Sciences
University of Auckland
Private Bag 92019 Auckland
New Zealand

Peter E. Toorop
Seed Conservation Department
Royal Botanic Gardens, Kew
Wellcome Trust Millennium Building
Wakehurst Place
Ardingly
West Sussex RH17 6TN
UK

Digby F. Warner
MRC/NHLS/WITS Molecular Mycobacteriology Research Unit
National Health Laboratory Service
PO Box 1038
Johannesburg 2000
South Africa

Preface

All cellular life forms can exist in multiplying, non-multiplying, and slowly multiplying states. In the case of bacteria, slowing of growth is associated with tolerance to a wide range of stresses, such as heat, cold, and antibiotics. This makes sense because organisms multiply only when the conditions are beneficial, and when not multiplying, the organism concentrates on survival in the face of environmental threats.

Many different species of microorganisms live in or on humans. Most are harmless or even beneficial. A few are pathogens. Persistence in a slowly multiplying or non-multiplying form is very common. In some situations, slow multiplication is the dominant phase for bacteria. This book addresses the basic scientific aspects of microbial dormancy and low-growth states and places them in the context of human medicine. An introduction to aspects of growth, non-growth, culturability, and viability is provided in Chapter 1. In the next chapter, the crucial relationship of low-growth states with survival of stress is discussed, with detail on the molecular mechanisms which are involved in the stress response. Chapter 3 sets the scenario for survival of the immune response, and many examples are provided of low-growth states that are associated with different survival mechanisms in animals and in humans. The next two chapters deal with interbacterial signalling in dormancy and mutations in stationary phase, respectively. These are two relatively new areas of study, which are becoming increasingly important. Chapter 6 describes biofilms and their relationship to dormancy and antibiotic resistance. Biofilms are most important in medicine, because many bacterial infections persist as biofilms on, for example, indwelling catheters. Unfortunately, such infections are difficult to eradicate because organisms within the biofilm are tolerant to antibiotics. In the Chapter 7, tuberculosis is discussed. Of all the bacterial infections, this is the classic persistence type, surviving the immune

system for decades and requiring six months of chemotherapy due to sub-populations of antibiotic-tolerant bacteria. A similar situation is discussed in Chapter 8, namely gastric ulcers, which are caused by a bacterium which persists in the stomach for years. The final two chapters are on dormancy in eukaryotes. The reason for including these is to show that all cellular life forms have low-growth states which are stress resistant when compared to the multiplying phase. Low growth in yeast is dealt with in Chapter 9, and dormancy in plants in Chapter 10.

Anthony R. M. Coates

Contents

The plate facing page 82 is available for download in color from www.cambridge.org/9780521187848

CHAPTER 1

Physiological and molecular aspects of growth, non-growth, culturability and viability in bacteria

M. R. Barer

INTRODUCTION

Infection requires growth of pathogens in host tissues or on host epithelia. Cessation of growth is generally correlated with control of infection. Clinically latent infections may reflect microbial growth balanced by host control mechanisms such that the interaction remains below the threshold of detection. Alternatively, the pathogen may have genuinely ceased growth and survive in some form of stasis. In most cases we cannot distinguish between these possibilities. However, there have been important recent advances in our understanding of bacterial populations in which net growth cannot be detected and in recognising the limitations of *in vitro* culture as a means of determining the presence and viability of bacteria. These advances present new opportunities to study the role of non-growing and dormant bacteria in infection and to consider the degree to which culture-based methods may give a false impression of the absence of pathogens during infection, clinical latency and treatment.

The progress of molecular methods in microbiology challenges us to determine the molecular basis of growth and its regulation and to develop such methods to detect growth and viability. In the present context, the long-term aim must be to recognise growth states of microbial populations in the human host.

BACTERIAL GROWTH

Growth involves the accumulation of biomass and may include genomic replication, cell division and an increase in the number of propagules of the organism concerned. For most bacteria it is generally held that, after division,

a newly formed cell placed in an environment favourable to growth will double its mass then divide to form two equal-sized progeny via binary fission. This process has been subjected to detailed analysis and is discussed from a highly selective viewpoint here. For more comprehensive and introductory discussions, the reader is referred to recent reviews (35, 55, 57, 66, 82, 104).

Our current understanding of bacterial growth derives overwhelmingly from studying selected organisms in broth cultures. Liquid cultures are convenient; most variables can be precisely controlled, and the scale can be adjusted to provide sufficient biomass for almost any form of analysis. In achieving reproducible results between laboratories, the development of chemically defined media, consistent inocula and the recognition of growth states that can be detected by sequential optical density or turbidity measurements have provided a platform for further development. The widely accepted terminology of lag, exponential (or log) and stationary phases of growth in batch culture provides essential physiological points of reference and these are often applied, with scant justification, to bacterial cells and populations outside the highly defined laboratory environments indicated.

A detailed analysis of the energetics and stoichiometry of bacterial growth has been made possible by analysing bacterial populations growing at constant rates in chemostat or turbidostat cultures (35, 57, 93). These systems provide a relatively reproducible gold standard in which a state referred to as "balanced exponential growth" can be achieved for extended times. The resultant population of cells is generally believed to be uniform and growing at similar rates. Thus it is considered legitimate that analyses of cells in balanced exponential growth can be divided equally amongst all the cells present in the sample to yield estimates of content or activity per cell present.

An important alternative approach has been to start by considering the bacterial cell cycle, which starts with the birth of a cell by binary fission of a parental cell and ends with the division of the new cell. This kind of work draws substantially on our understanding of the eukaryotic cell cycle, where the biochemical and physiological events have been separated into distinct phases (G_1, S, G_2, and M with or without G_0), and has been pursued using techniques that provide large populations of cells that are all at the same stage of the cycle. While some controversy continues, it is generally thought that events that are considered critical for progression through the cell cycle in eukaryotes (e.g., initiation and termination of DNA synthesis) are not similarly regulated in bacteria. Rather, the short-term fate of a cell is determined by the rate at which it accumulates biomass and by the particular size:growth

rate ratios at which division is initiated (18). Recently, however, Walker and colleagues (120) have suggested that the *umuDC* component of the bacterial SOS response functions in a manner analogous to the eukaryotic S phase checkpoint. The analogy is complicated by the fact that rapidly growing bacteria initiate new rounds of chromosome synthesis before the last has finished. The authors also point out that the associated checkpoint and DNA repair systems are well suited to dealing with DNA damage accumulated during stationary phase at the time of re-entry into the growth cycle (92).

Most biochemical knowledge obtained with these methods refers to large cell populations ($>10^7$) of readily culturable bacteria in exponential growth phase. Here, we are primarily concerned with the behaviour of pathogens during infections. Not only will these organisms rarely be in a simple suspension phase but also it seems most unlikely that the environment will be conducive to unimpeded exponential growth. Evidently, the degree to which most of our knowledge of bacterial growth is applicable to the environments that primarily concern us must be limited.

Laboratory studies on bacterial growth have also provided limited information regarding growth in colonies on or in solidified laboratory media (74, 125) and in biofilms (37, 75). While information on the growth of bacteria in colonies and in broth may be valuable in designing isolation and culture media for medically important bacteria (35), growth in biofilms is probably a principal mode of bacterial propagation in natural communities. In infections involving fluid-filled spaces (e.g., cystitis) it is plausible that the growth phases recognised in broth culture may be applicable and the relevance of biofilm growth to colonization of intravascular devices also seems certain. However, beyond these examples, assignment of *in vitro*-defined growth phases to pathogens at various stages in infection is largely speculative.

Molecular Information Related to Bacterial Growth

Studies on carefully defined broth cultures remain the principal reliable source of information on the molecular basis of bacterial growth. As key genes involved in growth and its regulation have been identified through recent pre- and post-genomic studies, the possibility of determining the importance of these genes to infection through deletion, over-expression and reporter studies has been extensively exploited. In the context of infection, it is conspicuous that technologies applied to detection of genes essential for growth *in vivo*, such as signature tagged mutagenesis, have often detected genes that appear integral to growth and metabolism (as opposed to classical