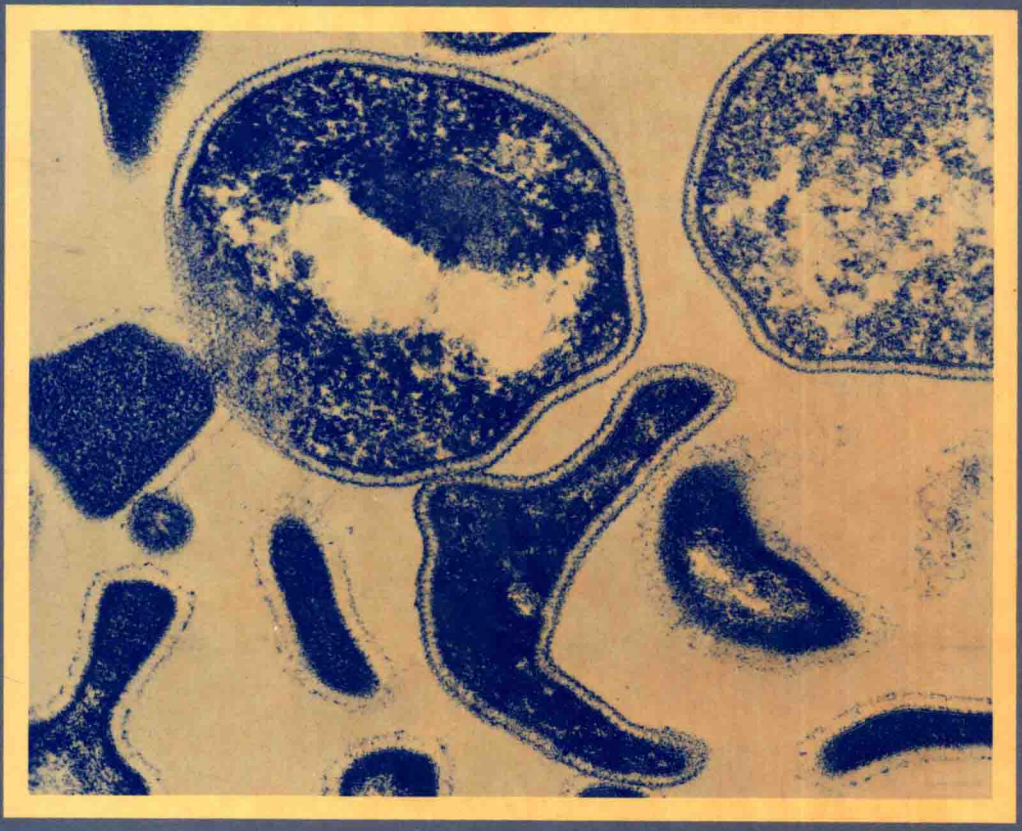
THERMOPHILES



General, Molecular, and Applied Microbiology

Edited by
Thomas D. Brock

Thermophiles

GENERAL, MOLECULAR, AND APPLIED MICROBIOLOGY

edited by

THOMAS D. BROCK
Department of Bacteriology
1550 Linden Drive
University of Wisconsin-Madison
Madison, Wisconsin 53705 USA

A WILEY-INTERSCIENCE PUBLICATION
JOHN WILEY & SONS
New York • Chichester • Brisbane • Toronto • Singapore

Library of Congress Cataloging-in-Publication Data

Thermophiles : general, molecular, and applied microbiology.

(Wiley series in ecological and applied microbiology)
"A Wiley-Interscience publication."
Includes index.
1. Micro-organisms, Thermophilic. 2. Bacteria, Thermophilic. 3. Industrial microbiology. I. Brock, Thomas D. II. Series.
QR84.T46 1986 576 86-7785
ISBN 0-471-82001-6

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

Thermophiles: General, Molecular, and Applied Microbiology

WILEY SERIES IN ECOLOGICAL AND APPLIED MICROBIOLOGY

Edited by *Ralph Mitchell*

MICROBIAL LECTINS AND AGGLUTININS: Properties and Biological Activity
David Mirelman Editor

THERMOPHILES: General, Molecular, and Applied Microbiology
Thomas D. Brock Editor

INNOVATIVE APPROACHES TO PLANT DISEASE CONTROL
Ilan Chet Editor

CONTRIBUTORS

SHUICHI AIBA Department of Fermentation Technology, Faculty of Engineering, Osaka University, Yamada-oka, Suita-shi, Osaka 565, Japan

PIERRE BÉGUIN Unit of Cellular Physiology, Department of Biochemistry and Molecular Genetics, Pasteur Institute, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France

CORALE L. BRIERLEY Advanced Mineral Technologies, Inc., 5920 McIntyre, Golden, Colorado 80403 USA

JAMES A. BRIERLEY Advanced Mineral Technologies, Inc., 5920 McIntyre, Golden, Colorado 80403 USA

THOMAS D. BROCK Department of Bacteriology, 1550 Linden Drive, University of Wisconsin-Madison, Madison, Wisconsin 53706 USA

TADAYUKI IMANAKA Department of Fermentation Technology, Faculty of Engineering, Osaka University, Yamada-oka, Suita-shi, Osaka 565, Japan

WILLIAM R. KENEALY Central Research and Development Department, E.I. du Pont de Nemours and Company, Experimental Station, Wilmington, Delaware 19898 USA

THOMAS A. LANGWORTHY Department of Microbiology, School of Medicine, University of South Dakota, Vermillion, South Dakota 57069 USA

JACQUELINE MILLET Unit of Cellular Physiology, Department of Biochemistry and Molecular Genetics, Pasteur Institute, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France

THOMAS K. NG Central Research and Development Department, E.I. du Pont de Nemours and Company, Experimental Station, Wilmington, Delaware 19898 USA

TAIRO OSHIMA Department of Life Science, Tokyo Institute of Technology, Ookayama, Tokyo 152, Japan

JEAN L. POND Department of Microbiology, School of Medicine, University of South Dakota, Vermillion, South Dakota 57069 USA

KARL O. STETTER Institute for Microbiology, University of Regensburg, 8400 Regensburg, Universitätsstrasse 31, Federal Republic of Germany

T.K. SUNDARAM Department of Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science and Technology, Manchester M60 1QD, United Kingdom

PAUL J. WEIMER Central Research and Development Department, Building 402, E.I. du Pont de Nemours and Company, Experimental Station, Wilmington, Delaware 19898 USA

JUERGEN WIEGEL Department of Microbiology and Center for Biological Resource Recovery, University of Georgia, Athens, Georgia 30602 USA

STEPHEN H. ZINDER Department of Microbiology, Stocking Hall, Cornell University, Ithaca, New York 14853 USA

SERIES PREFACE

The Ecological and Applied Microbiology series of monographs and edited volumes is being produced to facilitate the exchange of information relating to the microbiology of specific habitats, biochemical processes of importance in microbial ecology, and evolutionary microbiology. The series will also publish texts in applied microbiology, including biotechnology, medicine, and engineering, and will include such diverse subjects as the biology of anaerobes and thermophiles, paleomicrobiology, and the importance of biofilms in process engineering.

During the past decade we have seen dramatic advances in the study of microbial ecology. It is gratifying that today's microbial ecologists not only cooperate with colleagues in other disciplines but also study the comparative biology of different habitats. Modern microbial ecologists, investigating ecosystems, gain insights into previously unknown biochemical processes, comparative ecology, and evolutionary theory. They also isolate new microorganisms with application to medicine, industry, and agriculture.

Applied microbiology has also undergone a revolution in the past decade. The field of industrial microbiology has been transformed by new techniques in molecular genetics. Because of these advances, we now have the potential to utilize microorganisms for industrial processes in ways microbiologists could not have imagined 20 years ago. At the same time, we face the challenge of determining the consequences of releasing genetically engineered microorganisms into the natural environment.

New concepts and methods to study this extraordinary range of exciting problems in microbiology are now available. Young microbiologists are increasingly being trained in ecological theory, mathematics, biochemistry, and genetics. Barriers between the disciplines essential to the study of modern microbiology are disappearing. It is my hope that this series in Ecological and Applied Microbiology will facilitate the reintegration of microbiology and stimulate research in the tradition of Louis Pasteur.

In recent years interest in the biology of thermophiles has increased dramatically. Scientists working in widely divergent disciplines are studying

this fascinating group of organisms. Questions are being raised about their mode of survival, ecological significance, and evolutionary strategies.

Thomas Brock's pioneering research on thermophiles has led to a new understanding of the adaptive behavior of microorganisms living in extreme environments. In this volume he has brought together chapters by leading authorities on the molecular, physiological, and genetic properties of thermophiles, as well as discussions of their importance in ecology and biotechnology.

RALPH MITCHELL

Cambridge, Massachusetts
April 1986

PREFACE

Thermophiles are a fascinating group of microorganisms which have received considerable interest in recent years because of their potential biotechnological applications. However, thermophiles are more than biotechnological wonders. Thermophiles are of considerable general interest because of the fascinating problems in basic biology which they present. Biologists are conditioned to expect high temperatures to be harmful to living organisms, yet here is a group of organisms that not only tolerates high temperatures, but actually thrives under these extreme conditions. The existence of thermophiles immediately raises evolutionary and molecular questions of considerable importance.

Fundamental and applied research on thermophiles is underway in a large number of laboratories throughout the world. It is the purpose of the present book to present the current status and future direction of thermophile research. The authors of the chapters in this book have made important research contributions on thermophiles, and they are also knowledgeable about the current research in other laboratories. We are thus fortunate in having such an expert collection of authors for this important and timely book.

The initial discussion on the content and authorship of this book was done in association with J. Gregory Zeikus, who was originally to have been a coeditor and coauthor. Subsequently, Dr. Zeikus' commitments became so extensive that he could not be an active participant in this project, but his advice and counsel are greatly appreciated.

Editorial/production supervision of this book was competently handled by Dr. Katherine M. Brock of Science Tech, Inc. and I greatly appreciate the extensive contributions which she made to make this book possible. My work as editor of this book was supported in part by the College of Agricultural and Life Sciences of the University of Wisconsin-Madison, the National Science Foundation, and the Wisconsin Alumni Research Foundation through a grant to the Graduate School of the University of Wisconsin-Madison. I hope that *Thermophiles: General, Molecular, and Applied*

Microbiology will be a useful book for all those with an interest in basic and applied microbiology.

THOMAS D. BROCK

Madison, Wisconsin, USA
April 1986

CONTENTS

1

INTRODUCTION: AN OVERVIEW OF THE THERMOPHILES

THOMAS D. BROCK

Department of Bacteriology, University of Wisconsin, 1550 Linden Drive, Madison, WI 53706

Thermophiles are defined as organisms living at high temperatures. They have fascinated scientists for many years, but there has been a burst of interest in these organisms over the past generation, spurred on by the discoveries of bacteria living at or even above the boiling point of water.

Thermophiles are of interest both from fundamental and applied points of view. Basic research on thermophiles deals with molecular biology, genetics, biochemistry, evolution, taxonomy, ecology, and origins. From an applied or biotechnological point of view, thermophiles are of interest as sources of unique enzymes with unusual properties, as the active agents in high-temperature fermentations, in waste-treatment processes, and in mineral leaching. Although research in all of these areas is already extremely active, it seems clear from the articles in this book that the future holds vast promise for both basic and applied research on thermophilic microorganisms.

Because of the current excitement and extensive research on thermophilic microorganisms, the present time is apt for a book which provides a synthesis of current knowledge. In the present volume, some of the most active research workers have provided careful, considered overviews of thermophile research. This book can be viewed in two ways: 1) as a description of the current status of the field; 2) as a collection of guideposts to future work.

In the present book we deal almost exclusively with thermophilic bacteria (i.e., procaryotes), the microbial group capable of growth at the highest temperatures of any group of living organisms. Thermophilic bacteria have been of most biotechnological interest and have also been most extensively studied biochemically. Work on thermophilic algae is not dealt with to any extent in this book, but is well covered in Brock (1) and Castenholz (2). Thermophilic fungi, another major group of thermophiles, have been reviewed by Tansey and Brock (3).

1. THE DEFINITION OF A THERMOPHILE

The term *thermophile* has been used in a variety of ways, and there is no universally accepted definition. Simply put, a thermophile is an organism capable of growth at high temperature. How high a temperature? This will depend on the group of organisms under consideration, as illustrated in Table 1. In this table, the upper temperature limits for the growth of various groups of organisms are given. Note in this table that for any given group of organisms only a *few* species are capable of living close to the upper limit for the group; most organisms in the group live only at lower temperatures. Using Table 1 as a guide, we can construct the following definition: *a thermophile is an organism capable of living at temperatures at or near the maximum for the taxonomic group of which it is a part.* Thus, a thermophilic vertebrate would be one capable of living near 37°C, a thermophilic fungus one capable of living near 60°C, a thermophilic cyanobacterium one capable of living near 70°C, and so on. This definition has the advantage that it emphasizes the taxonomic distinctions of thermophily in different groups of organisms, but it has the disadvantage that it is imprecise and says nothing about the temperature range or optimum of the organism.

TABLE 1. Upper temperature limits for growth

Group	Approximate upper temperature (°C)
Animals	
Fish and other aquatic vertebrates	38
Insects	45–50
Ostracods (crustaceans)	49–50
Plants	
Vascular plants	45
Mosses	50
Eucaryotic microorganisms	
Protozoa	56
Algae	55–60
Fungi	60–62
Procaryotic microorganisms	
Cyanobacteria (blue-green algae)	70–73
Photosynthetic bacteria	70–73
Chemolithotrophic bacteria	>100
Heterotrophic bacteria	>100

From Brock (1)

2. TERMINOLOGY OF THERMOPHILIC BACTERIA

Unfortunately, terminology has proliferated for the thermophilic bacteria, and many of the terms have been ill defined. The problem can be discussed with reference to Figure 1. Some bacteria grow over a fairly wide range; such organisms are called *eurythermal.* Other bacteria, called *stenothermal,* grow only over a narrow temperature range. The concepts of *stenothermal* and *eurythermal* do not apply specifically to thermophiles but are broad terms describing the temperature/growth relationships of any organism. In general, organisms living in environments of constant temperature tend to be stenothermal, whereas organisms living in habitats of fluctuating temperatures tend to be eurythermal.

The *thermophile boundary* of 55 to 60°C defined in Figure 1 is arbitrary, but has some ecological and evolutionary basis. Temperatures lower than 50°C are widespread on earth, associated with sun-heated habitats, whereas temperatures greater than 55 to 60°C are much rarer in nature, being associated almost exclusively with geothermal habitats. Another reason for defining a thermophile boundary for bacteria at a temperature around 60°C is that 60°C is the upper temperature limit for eucaryotic life (Table 1), so that at temperatures above 60°C only procaryotes are found.

In Figure 1b, several terms frequently used with reference to thermophiles are illustrated. The terms described in Figure 1b may or may not be useful,

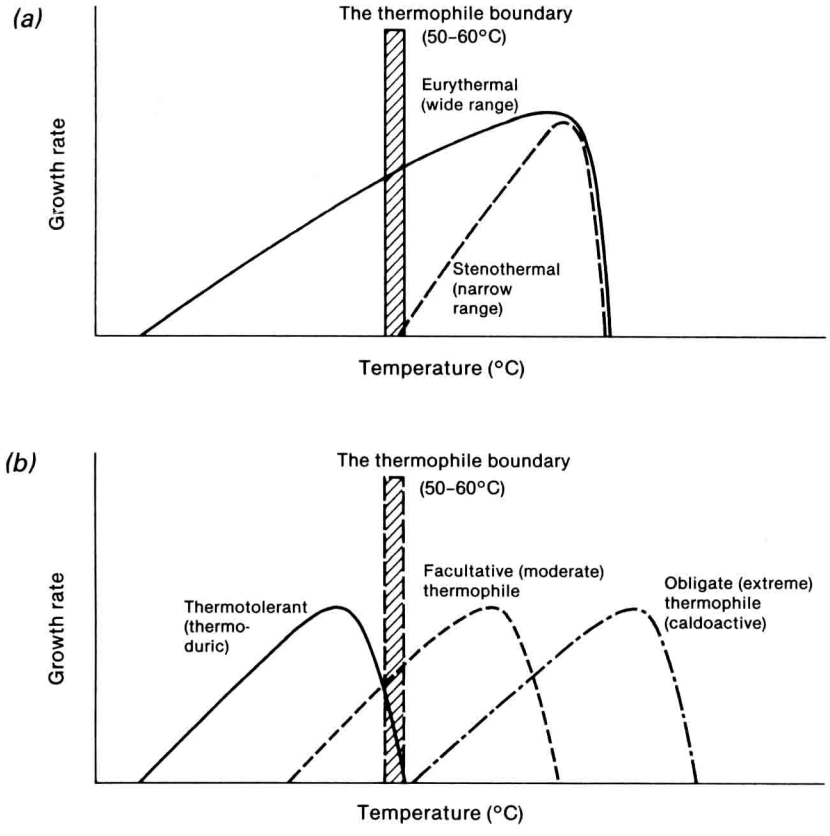


Figure 1. The relationship between growth rate and temperature for various bacteria, illustrating the thermophile boundary and the terminology which has been used to describe organisms exhibiting different temperature responses. For definitions of some of these terms, see Williams (4).

depending on circumstances. It is of some importance to emphasize that there are no real boundaries, but only a continuum. Thermophilic bacteria have been described, for instance, with temperature optima of 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, and 105°C, and at many temperatures in between those listed. Furthermore, the temperature optimum of an organism may change a few degrees depending on the culture medium and conditions in which the organism is grown (see Chapter 4, this volume). For any careful description of a thermophilic bacterium, the precise temperature range over which it grows (and the culture conditions used) must be described (see Chapter 2).

Another important point is that the temperature responses listed in Figure 1 are for growth, *not* survival. Many bacteria, especially those capable of forming endospores, can *tolerate* temperatures much higher than the tem-