Bacterial Toxins: Methods and Protocols

METHODS IN MOLECULAR BIOLOGY™

Bacterial Toxins

Methods and Protocols

Edited by

Otto Holst

Research Center Borstel, Germany

Humana Press Totowa, New Jersey

999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

This publication is printed on acid-free paper. ∞
ANSI Z39.48-1984 (American Standards Institute)
Permanence of Paper for Printed Library Materials.

Cover design by Patricia Cleary.
Cover illustration from: Chapter 6, Structure–Function Analysis of Cysteine-Engineered Entomopathogenic Toxins, by J.-L. Schwartz and L. Masson.

For additional copies, pricing for bulk purchases, and/or information about other Humana titles, contact Humana at the above address or at any of the following numbers: Tel.: 973-256-1699; Fax: 973-256-8341; E-mail: humana@humanapr.com; or visit our Website: http://humanapress.com

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

Library of Congress Cataloging in Publication Data

Bacterial toxins : methods and protocols / edited by Otto Holst.
p. cm. -- (Methods in molecular biology ; 145)
Includes bibliographical references and index.
ISBN 0-89603-604-9 (alk. paper)
1. Bacterial toxins--Research--Methodology. I. Holst, Otto.
II. Series: Methods in molecular biology (Clifton, N.J.) ; v. 145.
QP632.B3B334 2000
579.3'165--dc21

99-41237
CIP

Bacterial Toxins

METHODS IN MOLECULAR BIOLOGY™

John M. Walker, SERIES EDITOR

145. **Bacterial Toxins:** *Methods and Protocols*, edited by *Otto Holst, 2000*
144. **Calpain Methods and Protocols**, edited by *John S. Elce, 2000*
143. **Protein Structure Prediction:** *Methods and Protocols*, edited by *David Webster, 2000*
142. **Transforming Growth Factor-Beta Protocols**, edited by *Philip H. Howe, 2000*
141. **Plant Hormone Protocols**, edited by *Gregory A. Tucker and Jeremy A. Roberts, 2000*
140. **Chaperonin Protocols**, edited by *Christine Schneider, 2000*
139. **Extracellular Matrix Protocols**, edited by *Charles Streuli and Michael Grant, 2000*
138. **Chemokine Protocols**, edited by *Amanda E. I. Proudfoot, Timothy N. C. Wells, and Christine Power, 2000*
137. **Developmental Biology Protocols, Volume III**, edited by *Rocky S. Tuan and Cecilia W. Lo, 2000*
136. **Developmental Biology Protocols, Volume II,** edited by *Rocky S. Tuan and Cecilia W. Lo, 2000*
135. **Developmental Biology Protocols, Volume I,** edited by *Rocky S. Tuan and Cecilia W. Lo, 2000*
134. **T Cell Protocols:** *Development and Activation*, edited by *Kelly P. Kearse, 2000*
133. **Gene Targeting Protocols,** edited by *Eric B. Kmiec, 2000*
132. **Bioinformatics Methods and Protocols,** edited by *Stephen Misener and Stephen A. Krawetz, 2000*
131. **Flavoprotein Protocols,** edited by *S. K. Chapman and G. A. Reid, 1999*
130. **Transcription Factor Protocols,** edited by *Martin J. Tymms, 2000*
129. **Integrin Protocols,** edited by *Anthony Howlett, 1999*
128. **NMDA Protocols,** edited by *Min Li, 1999*
127. **Molecular Methods in Developmental Biology:** Xenopus *and Zebrafish*, edited by *Matthew Guille, 1999*
126. **Adrenergic Receptor Protocols,** edited by *Curtis A. Machida, 2000*
125. **Glycoprotein Methods and Protocols:** *The Mucins*, edited by *Anthony P. Corfield, 2000*
124. **Protein Kinase Protocols,** edited by *Alastair D. Reith, 2000*
123. ***In Situ* Hybridization Protocols (2nd ed.),** edited by *Ian A. Darby, 2000*
122. **Confocal Microscopy Methods and Protocols,** edited by *Stephen W. Paddock, 1999*
121. **Natural Killer Cell Protocols:** *Cellular and Molecular Methods*, edited by *Kerry S. Campbell and Marco Colonna, 2000*
120. **Eicosanoid Protocols,** edited by *Elias A. Lianos, 1999*
119. **Chromatin Protocols,** edited by *Peter B. Becker, 1999*
118. **RNA–Protein Interaction Protocols,** edited by *Susan R. Haynes, 1999*
117. **Electron Microscopy Methods and Protocols,** edited by *M. A. Nasser Hajibagheri, 1999*
116. **Protein Lipidation Protocols,** edited by *Michael H. Gelb, 1999*
115. **Immunocytochemical Methods and Protocols (2nd ed.),** edited by *Lorette C. Javois, 1999*
114. **Calcium Signaling Protocols,** edited by *David G. Lambert, 1999*
113. **DNA Repair Protocols:** *Eukaryotic Systems*, edited by *Daryl S. Henderson, 1999*
112. **2-D Proteome Analysis Protocols,** edited by *Andrew J. Link, 1999*
111. **Plant Cell Culture Protocols,** edited by *Robert D. Hall, 1999*
110. **Lipoprotein Protocols,** edited by *Jose M. Ordovas, 1998*
109. **Lipase and Phospholipase Protocols,** edited by *Mark H. Doolittle and Karen Reue, 1999*
108. **Free Radical and Antioxidant Protocols,** edited by *Donald Armstrong, 1998*
107. **Cytochrome P450 Protocols,** edited by *Ian R. Phillips and Elizabeth A. Shephard, 1998*
106. **Receptor Binding Techniques,** edited by *Mary Keen, 1999*
105. **Phospholipid Signaling Protocols,** edited by *Ian M. Bird, 1998*
104. **Mycoplasma Protocols,** edited by *Roger J. Miles and Robin A. J. Nicholas, 1998*
103. ***Pichia* Protocols,** edited by *David R. Higgins and James M. Cregg, 1998*
102. **Bioluminescence Methods and Protocols,** edited by *Robert A. LaRossa, 1998*
101. **Mycobacteria Protocols,** edited by *Tanya Parish and Neil G. Stoker, 1998*
100. **Nitric Oxide Protocols,** edited by *Michael A. Titheradge, 1998*
99. **Stress Response:** *Methods and Protocols*, edited by *Stephen M. Keyse, 2000*
98. **Forensic DNA Profiling Protocols,** edited by *Patrick J. Lincoln and James M. Thomson, 1998*
97. **Molecular Embryology:** *Methods and Protocols*, edited by *Paul T. Sharpe and Ivor Mason, 1999*
96. **Adhesion Protein Protocols,** edited by *Elisabetta Dejana and Monica Corada, 1999*
95. **DNA Topoisomerases Protocols:** *II. Enzymology and Drugs*, edited by *Mary-Ann Bjornsti and Neil Osheroff, 2000*
94. **DNA Topoisomerases Protocols:** *I. DNA Topology and Enzymes*, edited by *Mary-Ann Bjornsti and Neil Osheroff, 1999*
93. **Protein Phosphatase Protocols,** edited by *John W. Ludlow, 1998*
92. **PCR in Bioanalysis,** edited by *Stephen J. Meltzer, 1998*
91. **Flow Cytometry Protocols,** edited by *Mark J. Jaroszeski, Richard Heller, and Richard Gilbert, 1998*
90. **Drug–DNA Interaction Protocols,** edited by *Keith R. Fox, 1998*
89. **Retinoid Protocols,** edited by *Christopher Redfern, 1998*
88. **Protein Targeting Protocols,** edited by *Roger A. Clegg, 1998*
87. **Combinatorial Peptide Library Protocols,** edited by *Shmuel Cabilly, 1998*
86. **RNA Isolation and Characterization Protocols,** edited by *Ralph Rapley and David L. Manning, 1998*
85. **Differential Display Methods and Protocols,** edited by *Peng Liang and Arthur B. Pardee, 1997*
84. **Transmembrane Signaling Protocols,** edited by *Dafna Bar-Sagi, 1998*
83. **Receptor Signal Transduction Protocols,** edited by *R. A. John Challiss, 1997*
82. **Arabidopsis Protocols,** edited by *José M Martinez-Zapater and Julio Salinas, 1998*
81. **Plant Virology Protocols:** *From Virus Isolation to Transgenic Resistance*, edited by *Gary D. Foster and Sally Taylor, 1998*
80. **Immunochemical Protocols (2nd. ed.),** edited by *John Pound, 1998*

Preface

The interest of investigators across a broad spectrum of scientific disciplines has been steadily stimulated by the field of bacterial toxin research, an area that makes use of a large variety of biological, chemical, physicochemical, and medically oriented approaches. Researchers studying bacterial toxins need to be acquainted with all these disciplines in order to work effectively in the field. To date, there has been no published collection offering detailed descriptions of the techniques and methods needed by researchers operating across the field's diverse areas. The present volume *Bacterial Toxins: Methods and Protocols*, is intended to fill this gap.

Bacterial Toxins: Methods and Protocols consists of two sections: one on protein toxins (15 chapters) and one on endotoxins (5 chapters). Each section is introduced by an overview article (Chapters 1 and 16). The protocols collected represent state-of-the-art techniques that each have high impact on future bacterial toxin research. All methods are described by authors who have regularly been using the protocol in their own laboratories. Included in each chapter is a brief introduction to the method being described.

Since the goal of the book this to outline the practical steps necessary for successful application of the methods, the major part of each chapter provides a step-by-step description of the method treated. Each chapter also possesses a Notes section, which deals with difficulties that may arise when using the method, and with the modifications and limitations of the technique. In sum, our volume, *Bacterial Toxins: Methods and Protocols* should prove useful to a broad spectrum of researchers, including those without any previous experience with a particular technique.

Otto Holst

Contributors

DAVID W. K. ACHESON • *Division of Geographic Medicine and Infectious Diseases, New England Medical Center Hospital, Boston, MA*

JOSEPH E. ALOUF • *Institut Pasteur de Lille, Lille, France*

CHRISTIAN ALTENBACH • *Jules Stein Eye Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, CA*

PETER AMERSDORFER • *Phylos Inc., Lexington, MA*

MARIE-FRANCE BADER • *INSERM U338, Strasbourg, France*

R. JOHN COLLIER • *Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA*

GREGORY DE CRESCENZO • *Biotechnology Research Institute, National Research Council Canada, Montréal, Canada*

MAURO DALLA SERRA • *CNR-ITC, Centro di Fisica Degli Stati Aggregati, Povo, Italy*

MIKAEL DOHLSTEN • *AstraZeneca, Lund, Sweden*

ALI EL BAYÀ • *Institut für Infektiologie, Westfälische Wilhelms-Universität Münster, Münster, Germany*

VOLKER T. EL-SAMALOUTI • *Department of Immunology and Cell Biology, Research Center Borstel, Borstel, Germany*

HANS-DIETER FLAD • *Department of Immunology and Cell Biology, Research Center Borstel, Borstel, Germany*

MICHAEL C. GOODNOUGH • *Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, WI*

LUTZ HAMANN • *Department of Immunology and Cell Biology, Research Center Borstel, Borstel, Germany*

KEN-ICHI HARADA • *Laboratory of Instrumental Analytical Chemistry, Faculty of Pharmacy, Meijo University, Nagoya, Japan*

OTTO HOLST • *Division of Medical and Biochemical Microbiology, Research Center Borstel, Borstel, Germany*

WAYNE L. HUBBELL • *Jules Stein Eye Institute and Department of Chemistry and Biochemistry, University of California, Los Angeles, CA*

ERIC A. JOHNSON • *Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, WI*

ANNE V. KANE • *Center for Gastroenterology Research on Absorptive and Secretory Processes, New England Medical Center Hospital, Boston, MA*

GERALD T. KEUSCH • *Division of Geographic Medicine and Infectious Diseases, New England Medical Center Hospital, Boston, MA*

KARIN KRISTENSSON • *Active Biotech, Lund, Sweden*

LINDA LAWTON • *School of Applied Sciences, Robert Gordon University, Aberdeen, UK*

BUKO LINDNER • *Division of Biophysics, Research Center Borstel, Borstel, Germany*

CARL J. MALIZIO • *Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, WI*

JAMES D. MARKS • *SFGH-Department of Anesthesia, University of California, San Francisco, CA*

LUKE MASSON • *Biotechnology Research Institute, National Research Council Canada, Montréal, Canada*

ALBERTO MAZZA• *Biotechnology Research Institute, National Research Council Canada, Montréal, Canada*

GIANFRANCO MENESTRINA • *CNR-ITC, Centro di Fisica Degli Stati Aggregati, Povo, Italy*

JUSSI MERILUOTO • *Department of Biochemistry and Pharmacy, Åbo Akademi University, Turku, Finland*

JORDI MOLGÓ • *Laboratoire de Neurobiologie Cellulaire et Moléculaire, Gif-sur-Yvette, France*

JOHN R. MURPHY • *Department of Medicine, Boston University School of Medicine, Boston, MA*

KYOUNG JOON OH • *Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA*

WILLIAM D. PICKING • *Department of Molecular Biosciences, University of Kansas, Lawrence, KS*

BERNARD POULAIN • *Laboratoire de Neurobiologie Cellulaire, Strasbourg, France*

KRISTIAN RIESBECK • *Active Biotech, Lund, Sweden, and Department of Medical Microbiology, University Hospital MAS, Malmoe, Sweden*

ALEXANDER ROSENDAHL • *AstraZeneca, Lund, Sweden*

JAMES C. RICHARDS • *Institute for Biological Sciences, National Research Council of Canada, Ottawa, Canada*

M. ALEXANDER SCHMIDT • *Institut für Infektiologie, Westfälische Wilhelms-Universität Münster, Münster, Germany*

JEAN-LOUIS SCHWARTZ • *Biotechnology Research Institute, National Research Council,and Groupe de Recherche en Transport Membranaire, Université de Montréal, Montréal, Canada*

ULRICH SEYDEL • *Division of Biophysics, Research Center Borstel, Borstel, Germany*

PIERRE THIBAULT • *Institute for Biological Sciences, National Research Council of Canada, Ottawa, Canada*

ARTUR J. ULMER • *Department of Immunology and Cell Biology, Research Center Borstel, Borstel, Germany*

ANDRE WIESE • *Division of Biophysics, Research Center Borstel, Borstel, Germany*

JOHANNA C. VANDERSPEK • *Department of Medicine, Boston University School of Medicine, Boston, MA*

LARS VON OLLESCHIK-ELBHEIM • *Institut für Infektiologie, Westfälische Wilhelms-Universität Münster, Münster, Germany*

Contents

1

Bacterial Protein Toxins

An Overview

Joseph E. Alouf

To the physiologist the poison becomes an instrument which dissociates and analyzes the most delicate phenomenon of living structures and by attending carefully to their mechanism in causing death, he can learn indirectly much about the physiological processes of life.

Claude Bernard, *La Science Experimentale*
Paris, 1878

1. Introduction

This short overview attempts to highlight the current state of the art relevant to bacterial protein toxins. In particular we outline the major achievements in this field during the past decade and briefly describe some significant hallmarks of toxinological research since the advent of modern methodologies elucidating the biochemistry, genetics, and cell biology of these fascinating bacterial effectors.

Valuable information on the progress of our knowledge during the past 15 yr can be found in recently published books *(1–13)* and in the series (eight to date) *Bacterial Protein Toxins* (European Workshops Books) published by Academic Press (London, 1983) and thereafter every other year by Gustav Fischer Verlag (Stuttgart/Jena).

Valuable general reviews have also been published *(14–37)* that may help the reader to find specific information and appropriate bibliography.

2. What are Bacterial Toxins?

In microbiology, the term bacterial toxin, coined 110 yr ago by Roux and Yersin *(38)*, designates exclusively the special class of bacterial macromolecu-

From: *Methods in Molecular Biology, vol. 145: Bacterial Toxins: Methods and Protocols*
Edited by: O. Holst © Humana Press Inc., Totowa, NJ

Table 1
Repertoire of Bacterial Protein/Peptide Toxins (as of June, 1999: 323)

148 (46%) from Gram-positive bacteria
175 (54%) from Gram-negative bacteria
Extracellular toxins: 75%
Intracellular toxins : 25%
Membrane damaging/pore-forming cytolysins: 110 (approx 35% of protein toxins)

lar substances that when produced during natural or experimental infection of the host or introduced parenterally, orally (bacterial food poisoning), or by any other route in the organism results in the impairment of physiological functions or in overt damage to tissues. These unfavorable effects may lead to disease and even to the death of the individual.

Bacterial toxins are differentiated into two major classes on the basis of their chemical nature, regardless of their cellular location and the staining features of the bacteria that produce them: bacterial protein toxins and the toxic lipopolysaccharide complexes present at the surface of the outer membrane of the cell walls of Gram-negative bacteria.

The protein toxins that are the subject of this chapter constitute a wide collection of more than 300 distinct entities (**Table 1**) which are mostly released from bacterial cells during growth and therefore are considered as exotoxins. However, ca. 25% of protein toxins remain either intracytoplasmic or more or less firmly associated to the cell surface. Their eventual release outside the bacterial cell takes place during the decline of bacterial growth or after cell death, generally through autolytic processes.

2.1. Historical Background

The concept that pathogenic bacteria might elaborate harmful substances to the infected host emerged shortly after the discovery of these microorganisms as etiological agents of human diseases. In 1884, Robert Koch suggested that cholera was elicited by a bacterial component released by *Vibrio cholerae*, but parenteral injection of bacterial culture filtrates in animals did not produce any toxic effect and the idea of an extracellular cholera poison was abandoned. Then 4 yr later, Roux and Yersin (Institute Pasteur, Paris) discovered the first bacterial toxin (diphtheria toxin) in the culture filtrate of *Corynebacterium diphtheriae (38)* after the failure of Loeffer 1 yr earlier to prove the release of this toxin. The Institute Pasteur investigators thus brought up the prototype of a new class of extraordinarily toxic, pharmacologically and physiologically

active factors of immense potential for medicine, microbiology, immunology, molecular and cellular biology, and the neurosciences.

Two other major toxins were soon to follow: tetanus toxin discovered in 1890 independently by Faber, Briedel and Frankel, Tizzoni and Cattani, and botulinum toxin discovered in 1896 by Van Ermengem *(38)*. From that time through the end of the 1940s, about 60 toxins were identified, among them clostridial toxins as a result of the experience gained during World War I gas gangrenes. The onset of World War II stimulated further research into toxinogenic anaerobes. A milestone was the discovery by Macfarlane and Knight that *C. perfringens* α-toxin was a phospholipase C. This toxin became the first for which a biochemical mode of action was recognized at the molecular level. It is now the prototype of the group of at least 14 cytolytic toxins that disrupt eucaryotic cell membranes by hydrolysis of their constitutive phospholipids *(23)*. The beginning of the 1950s witnessed the discovery of *Bacillus anthracis* toxin (anthrax toxin) by H. Smith and his co-workers *(40)*. A great advance in our understanding was the observation in India in 1953 by De and co-workers that the injection of living *V. cholerae* or cell-free filtrates into the lumen of a ligated loop of rabbit ileum caused accumulation of a large amount of fluid having gross similarity to cholera. This led to the discovery of cholera toxin which revived and experimentally confirmed the old Koch prediction of the reality of an enteropathogenic cholera exotoxin. However, it was some 17 yr after De's initial work that the putative enterotoxin was isolated and purified in 1969 by Finkelstein and his co-workers *(29,38)*. Cholera toxin, a 84-kDa oligomeric protein is the prototype of a wide family of biochemically, immunologically, and pharmacologically related toxins found in human and porcine *E. coli* strains, *V. cholerae*, *V. mimicus*, non-01 *Aeromonas hydrophila*, *Campylobacter jejuni*, *Salmonella enterica* sv. *typhi* and sv. *typhimurium*, and *Plesiomonas shigelloides (29,34)*. From 1970 to 1983 the bacterial toxin repertoire encompassed about 220 proteins and peptides. At present (1999) it comprises 323 different members (**Table 1**).

One notes that the discovery of this considerable class of toxins over more than one century was the combined fruit of rational design based on technological advances and also chance or serendipity to a certain degree. This was particularly the case for diphtheria and cholera toxins.

2.2. Structural and Genetic Aspects

2.2.1. Molecular Topology

A striking feature of bacterial protein toxins is the broad variety of molecular size and topological features in contrast to the more homogeneous structures of protein effectors of eucaryotic origin (e.g., hormones, neuropeptides, cytokines, growth factors).

2.2.1.1. Single-Chain Molecules

Most protein toxins occur as single-chain holoproteins varying from approx 2–3 kDa such as the *E. coli* 17/18-amino-acid residue thermostable enterotoxin *(29)* and the *S. aureus* 26-amino-acid residue δ-toxin *(39)*, to the 150-kDa tetanus and botulinum neurotoxins *(26)* up to the approx 300-kDa *Clostridium difficile* toxins A (308 kDa) and B (270 kDa), which are the largest single-chain bacterial proteins hitherto identified *(35)*.

2.2.1.2. Oligomeric Molecules

Several toxins occur as multimolecular complexes comprising two or more noncovalently bonded different subunits. Cholera toxin, *E. coli* thermolabile enterotoxins I and II (LT-I, LT-II), and other related enterotoxins form an heterohexamer AB5 composed of one 28-kDa A-subunit (the ADP-ribosylating moiety of the toxin) and five identical 11.8 kDa B-subunits *(29,34)*. Shiga toxin and *E. coli* shiga-like toxins (verotoxins) are also composed of a single 32-kDa A-subunit in association with a pentamer of 7.7-kDa B-subunits. The A-subunit is the holotoxin component that acts as an *N*-glycosidase to cleave a single adenine residue from the 28S rRNA component of the eucaryotic ribosomal complex *(18)*. Pertussis toxin has the most complex structure known so far among protein toxins *(41)*. The toxin is an A–B type hexamer composed of five dissimilar subunits, S1–S5. S1 is the enzymatic ADP-ribosylating A-subunit (M_r = 26220) and the B oligomeric moiety contains the S2 (21.9 kDa), S3 (21.8 kDa), S4 (12 kDa), and S5 (11.7 kDa) protomers complexed in a 1:1:2:1 molar ratio.

2.2.1.3. Macromolecular Complexes of Toxins Associated to Nontoxic Moieties

This situation is known for the 150-kDa botulinum neurotoxins (BoNT) found in bacterial cultures and contaminated foodstuffs. These complexes, referred to as progenitor toxins, comprise three different forms: M toxin (300 kDa), L toxin (500 kDa) and LL toxin (900 kDa). The smaller M toxin is composed of a BoNT molecule (150 kDa) in association with a similarly sized nontoxic protein (150 kDa). The larger L and LL progenitor toxins additionally contain an undefined number of proteins with hemagglutinin (HA) activity. The form of progenitor toxin found varies between the different toxinogenic types, and more than one form may be produced by a single strain. All three forms have been found in type A *Clostridium botulinum* strains. *C. botulinum* type G strains produce the L toxin. The botulinum toxin of type E and F strains is composed exclusively of M progenitor toxin *(8)*.

2.2.1.4. Multifactorial Toxins

A number of toxins designated *binary toxins* are composed of two independent single chains not joined by either covalent or noncovalent bonds. In this respect, they differ from oligomeric toxins, the protomers of which are assembled in a defined structure (holotoxin). The moieties of binary toxins should act in concert to be efficient. Each individual protein separately expresses little or no toxicity.

Binary toxins are produced by a variety of Gram-positive bacteria, for example, *S. aureus* leucocidin and γ-toxin ***(42,43)***, *Enterococcus faecalis* hemolysin/bacteriocin ***(44)***, *Clostridium botulinum* C2 toxin, *Cl. perfringens* iota-toxin, and *Cl. spiroforme* iota-like toxin ***(35,45)***. *Bacillus anthracis* three-component toxin is more complex. Two different sets of active toxin result from the combination of either the lethal factor (LF) and protective antigen (PA) leading to the metalloprotease lethal toxin, or the combination of PA and edema factor (EF) which constitutes the calmodulin-dependent adenyl cyclase ***(46–48)***.

2.2.1.5. Protoxin Forms

Several protein toxins are secreted in their mature form into the culture medium as inactive protoxins similarly to several proenzymes (zymogens). These protoxins are converted to active toxins by proteolytic enzymes present in the medium or by treatment with proteases that split off small fragments from the precursor, for example, *C. perfringens* ε- and iota-toxins, the C2-toxin of *C. botulinum* (component C2-II), and the membrane damaging toxin aerolysin of *Aeromonas hydrophila*.

2.2.1.6. Three-Dimensional Crystal Structure

Since the elucidation in 1986 of the three-dimensional structure of *P. aeruginosa* exotoxin A, that of 28 other toxins (among them 10 of the family of Gram-positive cocci superantigens) has been established so far (**Table 2**).

2.2.2. Molecular Genetics

The past 15 yr (1983–1998) may be considered as the golden age of the molecular genetics of bacterial protein. More than 150 structural genes have been cloned and sequenced (vs only 10 by the end of 1982). About 85% of the genes are chromosomal. The other genes are located on mobile genetic elements: bacteriophages, plasmids, and transposons. Bacteriophagic genes were found to encode, among other toxins: diphtheria toxin ***(38,49)***, cholera toxin ***(50)***, *S. pyogenes* erythrogenic toxins A and C and *S. aureus* enterotoxins A and E (***16*** and **Table 3**), *C. botulinum* toxins C1 and D ***(8,26)***, and *E. coli*

Table 2
Three-Dimensional Structure of Crystallized Toxins[a]

1. *P. aeruginosa* exotoxin A (**ref. *76***)
2. *E. coli* LT-1 toxin (**refs. *77,78***)
3. *Bacillus thuringiensis* δ-toxin (**ref. *79***)
4. Oligomer B of *E. coli* shiga-like toxin (verotoxin) (**ref. *80***)
5. Diphtheria toxin (**refs. *81,82***)
6. *Aeromonas hydrophila* proaerolysin (**ref. *83***)
7. *Pertussis* toxin (**refs. *84,85***)
8. *Shigella dysenteriae* toxin (**ref. *86***)
9. Oligomer B of the choleragenoid form of cholera toxin (**ref. *87***)
10. Cholera toxin (holotoxin) (**ref. *88***)
11. *S. aureus* α-toxin (**ref. *89***)
12. *S. aureus* exfoliative toxin (**refs. *90,91***)
13. Anthrax toxin P component (protective antigen) (**ref. *92***)
14. Hc fragment of tetanus neurotoxin (**ref. *93***)
15. Perfringolysin O (**ref. *94***)
16. *Clostridium perfringens* α-toxin (**ref. *95***)
17. *Clostridium botulinum* neurotoxin A (**ref. *96***)
18. *S. aureus* leucocidin (LukE-PV) (**ref. *96a***)
19. *S. aureus* leukocidin (LukE) (**ref. *96b***)

Superantigens

20. *S. aureus* enterotoxin B (**ref. *97***) Enterotoxin B—CMH of class II complex (**ref. *98***)
21. *S. aureus* enterotoxin C1 (**ref. *99***)
22. *S. aureus* enterotoxin C2 (**ref. *100***)
23. *S. aureus* enterotoxin A (**refs. *101,102***)
24. *S. aureus* enterotoxin D (**ref. *103***)
25. *S. aureus* toxic-shock syndrome toxin-1 (TSST-1) (**refs. *104,105***)
26. TSST-1—CMH class II complex (**ref. *106***)
27. *S. pyogenes* erythrogenic (pyrogenic) exotoxin C (**ref. *107***)
28. *S. pyogenes* erythrogenic (pyrogenic) exotoxin A (**ref. *107a***)
29. Streptococcal super antigen (SSA) (**ref. *107b***)

[a]For general references see *(10,11)*.

shiga-like toxins I and II *(18)*. Plasmid-borne genes encode tetanus toxin *(8,26)*; anthrax toxin complex PA, EF and LF *(46–48)*; *S. aureus* enterotoxin D *(16)*; and *E. coli* heat-labile and heat-stable enterotoxins *(29,34)*. Heat-stable enterotoxin was also shown to be encoded by the transposon gene. The determination of the nucleotide sequences of toxin genes made it possible to deduce the primary structure of the relevant encoded proteins, thereby paving the way for