The Comprehensive Sourcebook of Bacterial Protein Toxins

The Comprehensive Sourcebook of Bacterial Protein Toxins

Fourth Edition

Joseph Alouf[†]
Institut Pasteur, Paris, France

Daniel Ladant
Institut Pasteur, Unité de Biochimie des Interactions Moléculaires, Paris, France

Michel R. Popoff
Institut Pasteur, Unité des Bactéries anaérobies et Toxines, Paris, France

[†]*Deceased*

ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD
PARIS • SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO

Elsevier
Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK
225 Wyman Street, Waltham, MA 02451, USA

Copyright © 2015 Elsevier Ltd. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices
Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

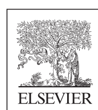
To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

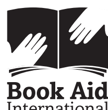
ISBN: 978-0-12-800188-2

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress.

For Information on all Elsevier publications
visit our website at http://store.elsevier.com/





Working together
to grow libraries in
developing countries

www.elsevier.com • www.bookaid.org

List of Contributors

Klaus Aktories Institut für Experimentelle und Klinische Pharmakologie und Toxikologie der Universität Freiburg, Freiburg, Germany

Alberto Alape-Girón Instituto Clodomiro Picado, Facultad de Microbiología; Departamento de Bioquímica, Escuela de Medicina Universidad de Costa Rica, San José, Costa Rica

Julien Barbier Institute of Biology and Technology of Saclay, Gif sur Yvette, France

Joseph T. Barbieri Professor Microbiology and Molecular Genetics Medical College of Wisconsin, Milwaukee, WI, USA

Holger Barth Institute of Pharmacology and Toxicology, University of Ulm Medical Center, Ulm, Germany

Ajit K. Basak Department of Biological Sciences, School of Crystallography, Birkbeck College, Malet Street, London, UK

Roland Benz Department of Life Science and Chemistry, Jacobs University Bremen, Bremen, Germany

Kinga Bercsenyi Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, University College London, London, UK; Molecular NeuroPathobiology Laboratory, Cancer Research UK London Research Institute, London, UK

Jonas Bergan Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Oslo, Norway; Department of Biochemistry, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway

Stefan Bergmann Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany

Sergey M. Bezrukov Program in Physical Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA

Victoria A. Bjørnestad Department of Chemistry, University of Oslo, Oslo, Norway

Patrice Boquet Nice University School of Medicine, Nice, France

Laurent Boyer INSERM, U1065, C3M, Université de Nice Sophia-Antipolis, Equipe Labellisée Ligue Contre le Cancer, Nice, France

Alejandra Bravo Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Amy E. Bryant Infectious Diseases Section, Veterans Affairs Medical Center, Boise, ID, USA; University of Washington School of Medicine, Seattle, WA, USA

Ladislav Bumba Laboratory of Molecular Biology of Bacterial Pathogens, Cell and Molecular Microbiology Division, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Pablo Emiliano Cantón Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Alexandre Chenal Institut Pasteur, CNRS UMR 3528, Unité de Biochimie des Interactions Macromoléculaires, Département de Biologie Structurale et Chimie, Paris, France

Matteo Dal Peraro Institute of Bioengineering, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland

Marcela de Souza Santos Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA

Ulrich Dobrindt Institute of Hygiene, University of Münster, Münster, Germany

J. Oliver Dolly International Centre for Neurotherapeutics, Dublin City University, Dublin, Ireland

J. Daniel Dubreuil Department of Pathology and Microbiology, Université de Montréal, Québec, Canada

Alain Filloux MRC Centre for Molecular Bacteriology and Infection, Department of Life Sciences, Imperial College London, London, UK

Dara W. Frank Professor Microbiology and Molecular Genetics Medical College of Wisconsin, Milwaukee, WI, USA

Marietta Flores-Díaz Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica.

John D. Fraser School of Medical Sciences, University of Auckland and Maurice Wilkins Centre, Auckland, New Zealand

Teresa Frisan Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

Jorge E. Galan Department of Microbial Pathogenesis, Yale University School of Medicine, New Haven, CT, USA

Blanca I. García-Gómez Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Harald Genth Institute of Toxicology, Hannover Medical School, Hannover, Germany

Daniel Gillet Institute of Biology and Technology of Saclay, Gif sur Yvette, France

Isabel Gómez Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Per Einar Granum Norwegian University of Life Sciences, NMBU—School of Veterinary Science, Department of Food Safety and Infection Biology, Oslo, Norway

Jörg Hacker National German Academy of Sciences Leopoldina, Halle/Saale, Germany

Callista B. Harper University of Queensland, Queensland Brain Institute, Clem Jones Centre for Ageing Dementia Research, Brisbane, Australia

Julie E. Heggelund Department of Chemistry, University of Oslo, Oslo, Norway

Mengfei Ho Department of Microbiology, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Eileen M. Hotze Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Jessica Huyet Department of Biological Sciences, School of Crystallography, Birkbeck College, Malet Street, London, UK

Ioan Iacovache Laboratory of Experimental Morphology, Institute of Anatomy, University of Bern, Bern, Switzerland

Thomas Jank Institut für Experimentelle und Klinische Pharmakologie und Toxikologie der Universität Freiburg, Freiburg, Germany

Emmanuel Jover EA-7290 Virulence bactérienne précoce, Fédération de Médecine Translationnelle, Université de Strasbourg, Institut de Bactériologie, Strasbourg, France

Ingo Just Institute of Toxicology, Hannover Medical School, Hannover, Germany

Anne-Marie Krachler Institute of Microbiology and Infection, University of Birmingham, UK

Ute Krengel Department of Chemistry, University of Oslo, Oslo, Norway

Daniel Ladant Institut Pasteur, CNRS UMR 3528, Unité de Biochimie des Interactions Macromoléculaires, Département de Biologie Structurale et Chimie, Paris, France

Ries J. Langley School of Medical Sciences, University of Auckland and Maurice Wilkins Centre, Auckland, New Zealand

Emmanuel Lemichez INSERM, U1065, C3M, Université de Nice Sophia-Antipolis, Equipe Labellisée Ligue Contre le Cancer, Nice, France

Stephen H. Leppla Microbial Pathogenesis Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Peng Li Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA

Toril Lindbäck Norwegian University of Life Sciences, NMBU—School of Veterinary Science, Department of Food Safety and Infection Biology, Oslo, Norway

Anne Berit Dyve Lingelem Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Oslo, Norway; Department of Biochemistry, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway

Shihui Liu Microbial Pathogenesis Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Camille Locht INSERM U1019, Lille, France; CNRS UMR8304, Lille, France; Univ Lille Nord de France, Lille, France; Institut Pasteur de Lille, Lille, France

Diana L. Martínez de Castro Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Jiri Masin Laboratory of Molecular Biology of Bacterial Pathogens, Cell and Molecular Microbiology Division, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Bruce A. McClane Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Adam McCluskey Chemistry, School of Environmental and Life Sciences, University of Newcastle, Callaghan, New South Wales, Australia

Gretel Mendoza Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Frederic A. Meunier University of Queensland, Queensland Brain Institute, Clem Jones Centre for Ageing Dementia Research, Brisbane, QLD, Australia

Mahtab Moayeri Microbial Pathogenesis Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Jordi Molgó CNRS, Institut de Neurobiologie Alfred Fessard – FRC2118, Laboratoire de Neurobiologie – et Développement UPR3294, Gif sur Yvette, France

Laura Monturiol-Gross Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica.

Patrick Munro INSERM, U1065, C3M, Université de Nice Sophia-Antipolis, Equipe Labellisée Ligue Contre le Cancer, Nice, France

Claire E. Naylor Department of Biological Sciences, School of Crystallography, Birkbeck College, Malet Street, London, UK

Ekaterina M. Nestorovich Department of Biology, The Catholic University of America, Washington, DC, USA

Josue Ocelotl Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Janette Onofre Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Joachim H.C. Orth Institut für Experimentelle und Klinische Pharmakologie und Toxikologie, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany

Kim Orth Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA

Radim Osicka Laboratory of Molecular Biology of Bacterial Pathogens, Cell and Molecular Microbiology Division, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Sabino Pacheco Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Andrei P. Pomerantsev Microbial Pathogenesis Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Michel R. Popoff Institut Pasteur, Unité des Bactéries anaérobies et Toxines, Paris, France

Bernard Poulain Institut des Neurosciences Cellulaires et Intégratives, Strasbourg, France

Gilles Prévost EA-7290 Virulence bactérienne précoce, Fédération de Médecine Translationnelle, Université de Strasbourg, Institut de Bactériologie, Strasbourg, France

Thomas Proft School of Medical Sciences, University of Auckland and Maurice Wilkins Centre, Auckland, New Zealand

Vittorio Ricci Department of Molecular Medicine, Unit of Human Physiology, Pavia University School of Medicine, Pavia, Italy

Phillip J. Robinson Children's Medical Research Institute, University of Sydney, Sydney, New South Wales, Australia

Stephanie Rolsma MSTP Trainee, Microbiology and Molecular Genetics Medical College of Wisconsin, Milwaukee, WI, USA

Virginia I. Roxas-Duncan Chief, Select Agent Management Branch, Biosurety Division, U.S. Army Medical Research Institute of Infectious Diseases, MD, USA

Agnes Sagfors MRC Centre for Molecular Bacteriology and Infection, Department of Life Sciences, Imperial College London, London, UK

Dor Salomon Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA

Jorge Sánchez Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Kirsten Sandvig Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Oslo, Norway; Department of Biochemistry, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway; Department of Biosciences, University of Oslo, Oslo, Norway

Christos Savva Department of Biological Sciences, School of Crystallography, Birkbeck College, London, UK

Giampietro Schiavo Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, University College London, London, UK

Gudula Schmidt Institut für Experimentelle und Klinische Pharmakologie und Toxikologie der Albert-Ludwigs-Universität Freiburg, Freiburg, Germany

Nathalie Schmieg Sobell Department of Motor Neuroscience and Movement Disorders, UCL Institute of Neurology, University College London, London, UK; Molecular NeuroPathobiology Laboratory, Cancer Research UK London Research Institute, London, UK

Peter Sebo Laboratory of Molecular Biology of Bacterial Pathogens, Cell and Molecular Microbiology Division, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Sadrick Shah Institute of Hygiene, University of Münster, Münster, Germany

Archana Shrestha Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Tore Skotland Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Oslo, Norway; Department of Biochemistry, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway

Leonard A. Smith Senior Research Scientist (ST) for Medical Countermeasures Technology, Office of Chief Scientist, U.S. Army Medical Research Institute of Infectious Diseases, Maryland, USA

Mario Soberón Instituto de Biotecnologia, Universidad Nacional Autónoma de México, Cuernavaca Morelos, Mexico

Patrizia Sommi Department of Molecular Medicine, Unit of Human Physiology, Pavia University School of Medicine, Pavia, Italy

A.C. Sotomayor-Perez Institut Pasteur, CNRS UMR 3528, Unité de Biochimie des Interactions Macromoléculaires, Département de Biologie Structurale et Chimie, Paris, France

Dennis L. Stevens Infectious Diseases Section and Research & Development Service, Veterans Affairs Medical Center, Boise, ID, USA; University of Washington School of Medicine, Seattle, WA, USA

Bradley G. Stiles Department of Biology, Wilson College, Chambersburg, PA, USA

Mira Y. Tawk EA-7290 Virulence bactérienne précoce, Fédération de Médecine Translationnelle, Université de Strasbourg, Institut de Bactériologie, Strasbourg, France

Richard Titball College of Life and Environmental Sciences, University of Exeter, Exeter, UK

Sarah Tjaden Institute of Hygiene, University of Münster, Münster, Germany

Rodney K. Tweten Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Françoise Gisou van der Goot Global Health Institute, School of Life Sciences, Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Kristin R. Wade Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

Jiafu Wang International Centre for Neurotherapeutics, Dublin City University, Dublin, Ireland

Brenda A. Wilson Department of Microbiology, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Gaëlle Zimmermann-Meisse EA-7290 Virulence bactérienne précoce, Fédération de Médecine Translationnelle, Université de Strasbourg, Institut de Bactériologie, Strasbourg, France

Introduction to the Fourth Edition

In memory of J. E. Alouf (1929–2014)

The first edition of the *Comprehensive Sourcebook of Bacterial Protein Toxins* was published by J. E. Alouf and J. H. Freer in 1991, for the purpose of collecting in a book accessible to scientists, biologists, teachers, and students the basic knowledge of the fascinating world of the bacterial protein toxins. Indeed, these molecules produced by pathogenic and environmental bacteria are extremely diverse in terms of their composition, structure, size, biochemical properties, and mode of interaction with target cells. During the last several decades, numerous bacterial toxins have been described exhibiting characteristics like recognition of novel receptors, novel enzymatic activity, novel mode of entry into cells, or novel intracellular targets. In the first edition, J. E. Alouf and J. H. Freer not only gave detailed descriptions of individual toxins, but also presented pertinent reviews pointing out the common structural and functional aspects of toxin families, as well as the genetic mechanisms regulating toxin expression. Each of the previous editions of this book have included the most recent advances of this rapidly moving world of toxins. This edition, coming out nine years after the last one, is in keeping with this series, with the main objective to update recently acquired knowledge on both previously known and newly discovered toxins, to describe their common features which allow a better understanding of their evolution and their role in the pathogenesis, and to highlight novel applications that have emerged over the past decade. Indeed, the multifaceted aspects of bacterial toxins are the object of multidisciplinary approaches from microbiology, cell biology, molecular biology, genetics, biochemistry, biophysics, and structural biology; and this book discusses multiple applications, including therapeutic tools, development of inhibitors, and countermeasures.

J. E. Alouf was the main force behind the writing of *Comprehensive Sourcebook of Bacterial Protein Toxins*. His encyclopedic formation and education, as well as his interactions with many scientists in the domain of toxins (notably through the organization of the European Workshop on Bacterial Toxins) facilitated the involvement in this project of many of the most eminent specialists in the fields of toxin studies and bacterial pathogenesis. The idea of organizing high-standard scientific workshops in Europe devoted to bacterial toxins was evoked by several scientists and resulted in the creation in 1981 of a steering committee where J. E. Alouf was an active member. He organized the first ETOX meeting in Seillac, France, a small town about 200 km from Paris. The site was very pleasant and quite appropriate for interactive discussions between senior scientists and young students. The first ETOX meeting was a

great success largely due to the selection of the site and organization by J. E. Alouf, as well as the participation of many expert scientists in the toxin field with high standard presentations. The prototype of having a scientific, interactive meeting on toxins with a restricted number of participants allowed frank and open discussions on basic toxin concepts was adopted in the following ETOX meetings, which were organized every two years in different European places. The ETOX meetings remain among the most successful and popular meetings on the topic of bacterial toxins in the world.

The proceedings of the first meetings were published in special issues of *Zentralblatt für Bakteriologie* and then in *International Journal of Medical Microbiology*. However, these publications only discussed the recent advances in toxins, and J. E. Alouf planned to elaborate a book with a wider scope that would collect both basic concepts and recent developments in the bacterial toxin field. He selected the term "Sourcebook" to specify the importance of having basic reference documents that would provide not only an in-depth understanding of each individual toxins, but also comprehensive aspects on their common properties and modes of action. J. E. Alouf was familiar with teaching and wanted to share with other biologists and students the fundamental knowledge in his domain. Indeed, he was the director of a special course on general immunology at the Pasteur Institute, Paris, from 1974 to 1994, in which he included specific modules on bacterial toxins. This course was very appreciated by the students, and J. E. Alouf was very selective when choosing the candidates. He was on the frontier of two scientific domains, toxinology and immunology. He has characterized various bacterial hemolysins, but he was also interested in the antigenic properties of toxins, notably the superantigen activity of streptococcal toxins.

The first edition of the *Comprehensive Sourcebook* was published in 1991, as mentioned, and J. E. Alouf actively participated to the second and third editions. At the beginning of 2013, J. E. Alouf asked us whether we could help him prepare a new edition for Elsevier, even though he had been retired for six years. It was difficult to decline this kind invitation, and until the autumn of 2013, we worked together on the selection of chapters and author invitations. His health declined, but he was always interested in the progress of the book. Unfortunately, he passed away on March 20, 2014, but his contribution to science will continue.

We are very grateful to all the authors who kindly accepted to provide excellent reviews to this fourth edition, allowing the continuity on this exciting adventure in toxins. We also thank the Elsevier staff for their professionalism and patience.

M. R. Popoff
D. Ladant

Evolutionary aspects of toxin-producing bacteria

1

Brenda A. Wilson, Mengfei Ho
Department of Microbiology, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Introduction

It has long been believed that microbes capable of causing human disease evolve over long periods of time through complex interactions between the microbes and their hosts. The prevailing view was that maintenance and amplification of mutations that might lead to increased virulence required a strong or persistent selective pressure, which could be applied only specifically from exposure to the host environment. However, new evidence suggests that acquisition of large DNA segments by horizontal gene transfer (HGT) may account for a much more rapid evolution of pathogens than was previously thought [1–4], particularly in terms of the origin of virulence factors such as toxins. Indeed, the abundance of toxin genes associated with "foreign" DNA segments, called *pathogenicity islands (PAIs)*, in the genomes of most of the sequenced toxin-producing pathogens compared to their nontoxigenic and nonpathogenic counterparts suggests that HGT must occur at a relatively high frequency in the real world [1–4]. Consequently, scientists are beginning to ask two questions: How many of the emerging or reemerging bacterial diseases are surfacing because of the acquisition of new toxin-containing PAIs, new combinations, or recombinations of PAI-encoded toxin genes? And if HGT, with or without selective pressure, is a major driving force in the evolution of toxins and bacterial pathogens, where is it taking place?

Molecular ecology of toxin-producing bacteria

Pathogenicity islands, horizontal gene transfer, and the prevalence of toxins

Strong evidence now points to an important role for HGT (in the form of mobile PAIs) in contributing to genome variability and the evolution of pathogens [1–4]. Given a strong selective pressure such as that encountered in the host environment, a bacterium that has acquired new genes can rapidly undergo pathoadaptation to become more virulent [5]. The structural genes encoding most bacterial protein toxins are located on PAIs in the form of extrachromosomal plasmids, or within genomes as part of temperate bacteriophages, putative transposons, integrated conjugative plasmids, or remnants of these mobile elements (see Table 1.1). Possible mechanisms through which HGT

The Comprehensive Sourcebook of Bacterial Protein Toxins. DOI: http://dx.doi.org/10.1016/B978-0-12-800188-2.00001-X
© 2015 Elsevier Ltd. All rights reserved.

Table 1.1 **Selected PAI-encoded protein toxins**

Protein toxins	Gene	Location	Bacterial host	References
Clostridial neurotoxins				
BoNT/A	*botA1*	Chromosome, plasmid	*C. botulinum*	[9]
BoNT/B	*botB*	Chromosome, plasmid	*C. botulinum*	
BoNT/C1	*botC1*	Prophage	*C. botulinum*	
BoNT/D	*botD*	Prophage	*C. botulinum*	
BoNT/Dsa	*botC/D*	Phage	*C. botulinum*	
BoNT/E	*botE*	Plasmid, phage	*C. botulinum, C. butyricum*	
BoNT/F	*botF*	Chromosome, plasmid	*C. botulinum, C. baratii*	[9,10]
BoNT/G	*botG*	Plasmid	*C. botulinum*	[9]
BoNT/H	*botH*		*C. botulinum*	[11]
TeNT	*tet*	Plasmid	*C. tetani, C. argentinense*	[9]
ADP-ribosylating toxins				
Diphtheria toxin (DT)	*tox*	corynephages α, β, P, π, δ, L, h, ω, (*tox*$^+$), γ (*tox*$^-$)	*C. diphtheriae, C. ulcerans, C. pseudotuberculosis*	[12–14]
Cholera toxin (CT)	*ctxAB*	CTXϕ, VPI	*V. cholerae, V. mimicus*	[7,15–18]
Heat-labile enterotoxin (HLT)	*elt, etx*	Plasmid, chromosome	*E. coli*	[13]
Pertussis toxin (PT)	*ptxA-E*	Chromosome PAI	*Bordetella pertussis*	[19]
Cholix toxin (ChxA)	*chxA*	Chromosome PAI	*V. cholerae* (non-O1/ non-O139)	[20,21]

28S rRNA-depurinating toxins				
Shiga toxin (ST)	*stx*	Phages	*S. dysenteriae, E. coli*	[22–24]
Shiga-like toxins (SLT)	*stx1*	ϕ 19B	*E. coli*	[25]
	stx2	ϕ933W	*E. coli*	[26]
	stx2c	ϕ2851	*E. coli*	[27]
	stx	ϕ7888	*S. sonnei*	[28]
	stx1c	ϕ6220	*E. coli*	[29]
RTX (MARTX toxins)	*VcRTX*	Chromosome	*V. cholerae*	[30,31]
	VvRTX	chromosome	*V. vulnificus*	[32]
Cytolethal distending toxins (CDT)				
CdtA, CdtC (B subunits)	*cdtABC*	Prophage	*E. coli*	[33]
CdtB (DNase A subunit)		Plasmid pVir	*E. coli*	[34]
Typhoid A_2B_5 toxin	*pltBA, cdtB*	Phage	*S. enterica* serovar Typhi	[35]
Deamidating toxins				
Cytotoxic necrotizing factors				
CNF1	*cnf1*	Chromosome (PAI)	*E. coli*	[36,37]
CNF2	*cnf2*	Plasmid pVir		[34]
CNF3	*cnf3*	Transposon		[38,39]
CNFy	*cnfY*	PAI	*Y. enterocolitica, Y. pseudotuberculosis*	[40]
CNFp	*cnfP*	PAI	*P. damselae*	EMBL: EEZ39234.1
CNFm	*cnfM*	PAI	*M. viscosa*	[41]
Burkholderia lethal factor (Blf1)	*blf1*	Chromosome (PAI)	*B. pseudomallei*	[42,43]
Cell cycle–inhibiting factor (Cif)	*cif*	λ Prophage	*E. coli* (EPEC, EHEC)	[44,45]
Cif homologue *Yersinia* (CHYP)	*cif*	Chromosome (PAI)	*Y. pseudotuberculosis*	[46]

(*Continued*)

Table 1.1 **(Continued)**

Protein toxins	Gene	Location	Bacterial host	References
Cif homologue *Photorhabdus* (CHP)	*cif*	Chromosome (PAI)	*P. asymbiotica, P. luminescens*	[46]
Cif homologue *B. pseudomallei* (CHBP)	*cif*	Chromosome (PAI)	*B. pseudomallei*	[44]
Cif homologue *Shigella* (OspI)	*ospI*	Plasmid	*S. flexneri*	[47]
Pasteurella dermonecrotic toxin (PMT)	*toxA*	λ-like linear dsDNA phage	*P. multocida*	[48]
Bordetella dermonecrotic toxin (DNT)	*dnt*	Chromosome PAI	*B. pertussis, B. bronchiseptica, B. parapertussis*	[49]
Photorhabdus asymbiotica toxin (PaTox)	*tox*	PAI	*P. asymbiotica*	[50]
Anthrax and Cereus toxins				
Protective antigen (PA)	*pag*	Plasmid (pX01)	*B. anthracis*	[51]
Lethal factor (LF)	*lef*			
Edema factor (EF)	*cya*			
Certhrax toxin (CerADPRT)	*cer*	Plasmid (pBC218)	*B. cereus*	[52,53]
Staphylococcal toxins				
enterotoxins	*sea, sep*	ϕN315, ϕMu50A	*S. aureus*	[54,55]
	seg, sen, sei, sem, seo	PAI		
	sel, sek, sec3	TSST-1 PAIs		
exotoxins	*set1-15*	PAI		
leukotoxins	*lukD, lukE*	PAI		
exfoliative toxins	*eta*	ϕETA		[56]
	etb	Plasmid		[57]
	etd	PAI		[58]

leukocidins	*pvl, lukD, lukE, lukF, lukM, lukS*	ϕPVL, ϕPV83, ϕSLT		[54,55,59,60]
hemolysins	*hla, hld, hlg*	PAI		[61]
toxic shock syndrome toxin-1 (TSST-1)	*tst*	TSST-1 PAIs		[62]
Streptococcal toxins				
superantigen A	*ssa*	Prophages	*S. pyogenes*	[63,64]
exotoxins	*speA, speB, speC, speG, speH, speI, speK, speL, speM*			
streptolysin O (SLO)	*slo*	Chromosome		[65]
streptolysin S (SLS)	*sls*	Chromosome		
Heat-stabile enterotoxins (SLT)	*estA, estB*	Transposon	*E. coli*	[66]
	ast (EAST-1)	Plasmid		
Pore-forming toxins				
α-hemolysin	*hlyI,II*	Chromosome (PAI), Plasmid	*E. coli*	[67,68]
enterohemolysins (Ehly 1, 2)	*ehl 1,2*	Phage	*E. coli*	[36,37]
Pseudomonas cytotoxin	*ctx*	ϕCTX, PS21	*P. aeruginosa*	[69,70]

contributes to bacterial pathogenesis include exchange and recombination of toxin and other virulence genes among different bacterial populations; one-step acquisition of toxins and other virulence genes to increase colonization and survival against the host's immune system or to provide means for dissemination within the host or between hosts; and provision of mechanisms to enhance survival in the external environment when outside the host body. Phages or lysogenic bacterial strains carrying toxin prophages might serve as the natural reservoir for toxin genes, with lysogenization and conversion processes taking place not only in the human or animal host (such as in the lungs or intestines), but also in the external environment, such as in food, water, soil, or other vectors or hosts (e.g., insects, amoeba, or plants) [1,6–8].

HGT under any of these conditions could produce new pathogenic strains and may account for the prevalence of related toxins among diverse pathogens. Examples can readily be found in some of the emerging or reemerging pathogens, including heterogeneity of the genes for diphtheria toxin, *tox*, and its iron-dependent regulator, *dtxR*, in clinical isolates of *Corynebacterium diphtheriae* from epidemic outbreaks [71]; production of superantigen variants among group A streptococci [63], exotoxin-diversity among community-acquired versus hospital-acquired methicillin-resistant *Staphylococcus aureus* strain lineages [72]; cholera toxin production by *Vibrio cholerae* isolates [7,73]; heat-labile enterotoxin (a homologue of cholera toxin) production by enterotoxigenic *Escherichia coli* strains (ETEC) [74]; and the widespread production of Shiga toxin variants among the *Shigella* and *E. coli* strains [75–77]. Indeed, studies on Shiga toxin (*stx*) gene–containing phages indicate that they are transmitted not only by temperate bacteriophages between different bacteria *in vivo* (i.e., in the intestines of humans and animals [78–80]), but also extraintestinally in aquatic environments, such as oceans [81], sewage, and other fecally contaminated water sources [76,82,83], and irrigation water, soil, and crops [84,85].

In addition, new studies indicate that phage biology may contribute to bacterial pathogenesis by allowing for export or release of toxins mediated by phage lysis or for toxin gene expression upon phage induction, particularly when optimal promoters for the toxin gene are lacking in the new host. Many phage-encoded toxin genes are located near the phage attachment site, supporting acquisition by a transduction mechanism. For instance, corynebactriophages of *C. diphtheriae* carry the *tox* gene near the phage attachment site [86]. The Panton-Valentine-leukocidin (PVL) toxin genes, *lukS-lukF*, are also located close to the phage attachment site *attP* and integrase (*int*) gene encoded by a mitomycin C–inducible temperate prophage, ΦPVL, in *S. aureus* strain V8 [87]. However, the Shiga or Shiga-like toxin (*stx*) genes are an exception. The *stx1* and *stx2* genes are downstream of the λ-like transcriptional activator Q homologue and upstream of the phage lysis and morphogenesis genes in the H-19B and 933W phages, respectively [25,88,89]. Several studies have demonstrated that expression and release of the Stx proteins from the bacteria is mediated by phage induction and lysis [26,90,91]. This came as a surprise since several groups had previously identified a functional Fur-like promoter that was directly upstream of the *stx1* gene and had reported that toxin expression was regulated by iron through this promoter [88]. However, subsequent studies indicated that phage induction by agents such as mitomycin C dramatically increased the production and release of Stx

by Shiga toxin–producing *E. coli* (STEC) strains far above that observed under iron regulation [90,91]. This was further confirmed by mutational analysis of the promoter regions [26]. The CT genes, *ctxAB*, are located on a filamentous prophage, CTXΦ, in *V. cholerae*. CT production is likewise regulated by two promoters: the P_{ctxAB} promoter, which is located immediately upstream of the *ctxAB* operon; and the P_{rstA} promoter, which is located 5 kilobases (kbp) upstream of the *ctxAB* operon and regulates the phage virion structural genes [7].

Phage induction may also contribute to the release of toxins when specific protein secretion systems do not exist for the toxin, or when the toxin is too large for efficient export. Bacterial stress responses, including SOS-inducing antibiotics and oxidative stress, are known to trigger phage induction [92,93] and subsequent release of Shiga-like toxins in *E. coli* [94,95], as well as the large clostridial toxins, ToxA and ToxB, from *C. difficile* responsible for nosocomial antibiotic-induced enterocolitis [92,96]. There is additional evidence that the introduction and induction of other lysogenic phages can regulate toxin gene expression in *C. difficile* during infection [97,98]. The toxin-encoding regions of different *C. difficile* strains are part of a transmissible pathogenicity locus (PaLoc) that appears to be evolving [99–102]. Promoters that control the phage lytic-lysogenic switch of the ΦSa3ms phage found in hypervirulent community-acquired *S. aureus* strain 476 also control the production of four phage-encoded virulence genes for staphylococcal enterotoxins, SEA, SEG, and SEK, and finbrinolytic staphylokinase (Sak) [103]. Another example where this may be relevant, albeit not yet demonstrated, is for the *Pasteurella multocida* toxin (PMT), which is responsible for the symptoms of atrophic rhinitis in animals, a disease that is most prevalent and more severe when animals are under stressful conditions or during coinfections [104]. The PMT gene (*toxA*) was reported to reside on a temperate bacteriophage [48].

Toxins encoded by plasmids, bacteriophages, and other pathogenicity islands

The clinical profile of diarrheal diseases caused by pathogenic *E. coli* strains is a composite of the various toxins and other virulence factors produced by those strains [105,106]. ETEC strains are characterized by the presence of two different plasmid-encoded enterotoxins, the Type I heat-labile toxin (HLT-I), which is an AB_5-type ADP-ribosylating toxin closely related to CT, and the heat-stable toxin (HST), which is one of several related small peptide toxins produced by different strains of *E. coli* that bind and activate intestinal guanylate cyclase receptors [107]. Some ETEC strains produce a second type of HLT (HLT-II), which is not neutralized by antisera against CT or HLT-I. HLT-II toxins are chromosomally encoded within predicted lambdoid-like prophage PAIs and are much more diverse with evidence of having arisen via multiple recombination events [74]. ETEC and extraintestinal strains of *E. coli* from animals often carry another Vir plasmid, which encodes the gene for cytotoxic necrotizing factor 2 (*cnf2*) [34,108], as well as the gene for cytolethal distending

toxin (*cdt*) [109]. Uropathogenic *E. coli* (UPEC) strains, on the other hand, carry the *cnf1* gene, which is homologous to the *cnf2* gene but appears along with the gene for α-hemolysin (*hly*) on a chromosomal PAI [67,110,111]. *E. coli* strains associated with intestinal or extraintestinal infections in humans and animals that produce CNF1 or CNF2 are also sometimes referred to as *necrotoxigenic E. coli (NTEC)* [112]. Many extraintestinal *E. coli* strains contain variants of the α-hemolysin gene on different plasmids or chromosomal PAIs [67,113]. The plasmid-encoded enterohemolysin (*ehxABD*) of enterohemorrhagic *E. coli* (EHEC) strains also has strong homology to α-hemolysin, but the N- and C-termini are different in the two proteins [114].

STEC strains have emerged as a major group of food-borne pathogens [115,116]. Nearly all of the Shiga toxin genes are carried by temperate bacteriophages [22,23]. Indeed, it appears that no two sequenced Stx-encoding phages are the same, and indeed, other than the *stx* genes remaining linked to the λ-like transcriptional activator Q gene, nearly all of the phages are mosaics showing appreciable evidence of genomic recombination and gene-swapping events [77]. The two classes of Shiga toxins found in STEC, Stx1 and Stx2, have similar structures and mechanisms of action. The *stx1* gene is highly conserved and nearly identical to the *stx* gene from *Shigella dysenteriae*, whereas the *stx2* gene shares about 58% DNA sequence homology with *stx1* and has many distinct variants among different STEC isolates, with some containing multiple variants [117,118]. Some *stx* genes contain mosaic structures, suggesting that recombination between *stx*-phages can also occur in nature. In 2011, a large outbreak of diarrhea and hemolytic-uremic syndrome occurred in Germany due to an unusual, highly virulent strain of enteroaggregative hemorrhagic *E. coli* (EAHEC) serotype O104:H4 that had acquired through HGT a prophage encoding Stx-2, as well as additional virulence factors [119,120]. There is some evidence that the Stx-encoding phage may have emerged from a bovine reservoir of STEC [24].

The spore-forming, neurotoxin-producing *Clostridia* are strict anaerobic Gram-positive bacteria that are found ubiquitously in the environment. Neurotoxin-producing strains of *Clostridium botulinum* are defined by which of the closely related but antigenically distinct botulinum neurotoxins (BoNTs) that they produce (A1-A5, B1-B5, C1, CD, D, DC, E1-E8, F1-F7, G, or H), which differ at the amino acid level by up to 30% [9,121]. These toxins are also related to the tetanus neurotoxin (TeNT) produced by *Clostridium tetani*. Comparative phylogenic analysis has revealed the strong likelihood that HGT of BoNT genes has occurred via phage, mobile plasmids, and transposons not only within the *C. botulinum* species, but also with other clostridial species, including *Clostridium butyricum, Clostridium baratti*, and *Clostridium argentinense* [9]. The diverse locations of the genes for the BoNT toxins are illustrative of the appreciable degree of HGT that has occurred during their evolution [9,121].

The gene clusters for BoNT/A1, BoNT/A2, BoNT/B, and BoNT/F are often located on the chromosome [9,122,123]; the gene for BoNT/G is plasmid-encoded [124]; the genes for BoNT/C1 and BoNT/D are encoded by prophages [125,126]; and the gene for BoNT/E has been found on both phages and plasmids [127,128]. Gene clusters for BoNT/A3, BoNT/A4, or BoNT/B have also been found on large plasmids [129,130]. Certain strains of *C. botulinum* have been reported to contain

mixtures of toxin types. For example, there are reports of strains that harbor both *botB* and *botF* gene clusters, as well as strains that harbor both *botA* and *botF* clusters or both *botA* and *botB* clusters [9]. Many type A strains have both the *botA1* gene cluster and a *botB* cluster, although the *botB* cluster in these cases often has a cryptic *botB* gene [131]. A *C. botulinum* strain has been reported harboring two chromosomally located gene clusters, encoding BoNT/A2 and BoNT/F4, and a large plasmid encoding the gene cluster for BoNT/F5 [10]. Strains containing a mosiac of toxins that are composites between *botC* and *botD* genes (e.g., combinations of BoNT/Dsa, BoNT/CD, or BoNT/DC) have also been reported [132–134]. A clinical strain has recently been isolated that possesses two chromosomal gene clusters: one for a BoNT/B2-like toxin and another for a new BoNT serotype H [11,135]. All of the *bot* genes, but not the *tet* genes, have an upstream, cognate *ntnh* gene encoding a 130-kDa nontoxic nonhemagglutinin (NTNH) protein [9,123], which has been shown to function as a molecular chaperone or partner in a complex with the secreted toxin that protects the toxin from harsh environments such as the stomach [136]. Recent structural studies have revealed that the serotype A NTNH forms an interlocked complex with BoNT/A and that the NTNH structure has a conformation remarkably similar to that of BoNT/A [137,138].

PAI-encoded toxins delivered by specialized secretion systems

Another class of PAI-encoded virulence factors that modulate signaling, metabolic processes, or both in host cells, includes the type III, IV, and VI secretion systems (T3SS, T4SS, and T6SS, respectively) and their translocated effector proteins (see Table 1.2). Although they are not toxins per se, these effector proteins can be considered as such since they mediate cytopathic or cytotoxic effects in host cells upon being directly delivered to the host cell cytosol by the secretion apparatus. Virulence plasmids of many pathogens, such as *Salmonella, Shigella*, *E. coli*, and *Yersinia*, harbor conserved clusters of genes for one or more secretion system and their associated effector proteins that modulate host responses [139]. Computational methods based on protein structural motif and sequence analyses have been developed to predict the effectors from T3SS [140–143], T4SS [143–145], and T6SS [146,147].

The three pathogenic strains of *Yersinia* (*Yersinia pestis, Yersinia enterocolitica*, and *Yersinia pseudotuberculosis*) all contain a large virulence plasmid (pYV) that encodes the T3SS and associated *Yersinia* outer protein (Yop) effector proteins [155], which modulate trafficking and innate immune functions once delivered to the macrophage by the T3SS [156,157]. An analogous set of T3SS (Mxi/Spa) and effector (Ipa) proteins [154], which is involved in manipulation of intestinal innate and adaptive immune responses [158], are encoded on the large invasion plasmid (pInv, pWR100 in *Shigella flexneri*) harbored by *Shigella* and enteroinvasive *E. coli* (EIEC) strains [159,160]. EHEC and enteropathogenic *E. coli* (EPEC) deliver toxic effector proteins into host cells via a T3SS encoded by the locus of enterocyte effacement

Table 1.2 **Selected PAI-encoded effector proteins**

Effectors	Gene	Location	Bacterial host	References
***Pseudomonas* T3SS effectors**				
ExoS	*exoS*	Chromosome (putative PAI)	*P. aeruginosa*	[148]
ExoT	*exoT*	Chromosome (putative PAI)		[148]
ExoY	*exoY*	Chromosome (putative PAI)		[149]
ExoU	*exoU*	Chromosome (putative PAI)		[150]
***Salmonella* T3SS Effectors**				
Sop proteins	*sopE*	SopE ϕ	*S. enterica* serovars Typhimurium, Typhi	[151–153]
	sopE2	Chromosome (phage?)		
	sspH2	Chromosome (phage?)		
	gogB	GIFSY-1		
	ssel (*gtgB, sfrH*)	GIFSY-2		
	sspH1	GIFSY-3 (phage remnant)		
	sipA-D, sptP, avrA	Chromosome (SPI-1)		
	sseA-G, ssaB	Chromosome (SPI-2)		
	sopB	Chromosome (SPI-5)		
	sopA, slrP, sopD, sopD2, sseJ, sifA, sifB	Chromosome		
	sopE	Chromosome (λ-like phage remnant)	*S. enterica* serovars Hadar, Gallinarum, Dublin, Enteritidis	
***Shigella* T3SS effectors**				
Ipa proteins	*ipaA-D*	Plasmid	*S. flexneri*	[154]
Enterotoxin	*senA*	Plasmid		
***Yersinia* T3SS effectors**				
Yop proteins	*yopE, yopB, yopD, yopH, yopO, yopT, yopM, yopP/J*	Plasmid	*Y. pestis, Y. enterocolitica, Y. pseudotuberculosis*	[155]

(LEE) PAI [161]. One of these T3SS effectors, Cif, although tightly associated with the LEE, is encoded outside the LEE by a lambdoid prophage, which is present in a wide range of EHEC and EPEC isolates and is also highly diverse in terms of other encoded effector genes [162].

Strains of the *Salmonella enterica* serovar Typhimurium harbor a number of PAIs and prophages, two of which contain genes that encode distinct T3SS apparati, which directly inject cytopathic effector proteins into the host cell at different stages of the infection cycle [163] to cause a variety of intracellular effects on the host cell [151]. The effectors secreted by the T3SS encoded by the *Salmonella* pathogenicity island-1 (SPI-1) are involved in mediating the invasion of macrophages [164], while the effectors secreted by the T3SS encoded by SPI-2 are required for survival within the macrophage and during systemic infection [165]. The effector proteins injected by the SPI-1 T3SS, all of which affect various intracellular signaling processes in the macrophage, include SopB (phosphatidylinositolphosphatase), SopE2 (guanine nucleotide exchange factor, encoded by SPI-2), AvrA (deubiquitinase), SipB-SipC (translocon inserted into host cell membrane), SptP (tyrosine phosphatase), SipA (actin polymerization), SspH1 (E3 ubiquitin ligase, encoded by the GIFSY-3 prophage), SspH2 (encoded by a phage remnant found in most strains), SopD, SlrP, SopA, and sometimes SopE (encoded by another phage in some strains) [152,163,166–168].

Over 30 effector proteins are translocated by the SPI-2 T3SS [153], although not all *S. enterica* serovars contain the full repertoire of SPI-2 effectors, which are mostly encoded by different prophages in distinct regions on the chromosome [152,153,163,166–168]. These 30 SPI-2 effector proteins have a variety of reported intracellular enzymatic activities, including E3 ubiquitin ligase (SspH1, SspH2, SlrP), deubiquitinase (SseL), cholesterol acyltransferase (SseJ), *Salmonella*-induced filament (Sif) formation and extension (SifA, SopD2, PipB2, SseF), protease (GtgE), actin adenosine diphosphate (ADP)–ribosyltransferase (SpvB), kinase (SteC), and phosphothreonine lyase (SpvC) [153]. Sequence comparison of *Salmonella* isolates has revealed that different strains of *Salmonella* can harbor different sets of effectors, since frequently these effector proteins are themselves encoded by genes on different bacteriophages [169]. For example, analysis of the *sopE* gene among various *S. enterica* strains indicates that HGT can occur between different phage families [169,170], suggesting that shuffling of the T3SS effector protein repertoires in *Salmonella* species has created new epidemic strains [168]. Further, the *Salmonella* strain SL1344 has *sopE* located on a prophage ϕSopE that is not found in strain 14028, while strain 14028 has *sspH1* located on prophage ϕGifsy-3, but does not have *sopE* [171]. Comparative genome analyses among various *Salmonella* isolates are now enabling the identification of additional T3SS effectors present on other lysogenic phages, such as the SseK/NleB T3SS effectors found on ϕST64B lysogens in the genome sequence of *Salmonella* strain SL1344, but not in the genome sequence of the commonly used laboratory strain LT2 [172].

Some Gram-negative bacteria utilize T4SSs to achieve the exchange of genetic material or translocation of virulence plasmids or effector proteins into the extracellular medium or into their eukaryotic hosts [173,174]. Both the AB_5 and the B_5 forms

of the pertussis toxin (PT) complex are assembled in the periplasm of *Bordetella pertussis*, where they are then secreted into the extracellular medium via the Ptl-T4SS [175,176]. The nine structural genes for Ptl T4SS (*ptlA-ptlH*) are all located within the same operon downstream of the five *ptx* structural genes for the S1-S5 subunits of PT [177]. In *Helicobacter pylori*, the Cag-PAI encodes the oncogenic and immunomodulating effector CagA, which is delivered into host cells directly using the Cag-PAI-encoded T4SS [178,179]. The plant pathogen *Agrobacterium tumefaciens* has a VirB/VirD4-T4SS system to deliver a range of virulence-associated effector proteins [180]. Some effectors, VirD2 and VirE2, are involved in processing and protection of the Ti plasmid for T4SS-dependent transfer and integration [173], while others, such as the transcription factor VIP1, suppress plant defense responses [181].

Many intracellular pathogens, such as *Bartonella*, *Brucella*, *Coxiella*, and *Legionella*, utilize T4SSs to deliver effectors from the bacteria-containing vacuole into the host cell cytosol [182]. For example, *Bartonella henselae* has seven VirB/VirD4-T4SS-translocated effectors, BepA-BepG, required for cytopathic reactions and establishment of chronic infection [183]. The effector genes appear to have evolved via multiplication and subsequent functional divergence from a single ancestral *bep* gene [184]. A total of 11 VirB-T4SS effectors, BspA-BspK, have been identified in *Brucella abortus*, some of which are known to target secretory pathways in host cells [185]. *Legionella pneumophila* utilizes Dot/Icm T4SS to deliver over 300 effectors, targeting multiple host-signaling pathways and manipulating transcription and translation machinery during intracellular growth in its own vacuolar compartment [186,187]. *Coxiella burnetii* encodes a T4SS that is homologous to the *Legionella* T4SS and that likewise delivers effectors required for intracellular replication and formation of its own vacuolar compartment [188]. T4SSs have now also been identified in *Streptococcus* and other Gram-positive bacteria, suggesting additional T4SS effectors may be associated with these Gram-positive bacterial T4SSs [189].

Many Gram-negative bacteria use T6SSs for delivery of effectors into neighboring bacteria through a contact-dependent mechanism [190,191]. T6SS is prevalent in interbacterial competition within the infected host environment [191], where the T6SS effectors are frequently coproduced with their specific immunity proteins in the donor bacteria and are toxic only to the recipient bacteria. To date, there are only a limited number of reported T6SS effectors known to target eukaryotic host cells directly. The C-terminal actin-cross-linking domain (ACD) of VgrG1 in *V. cholerae* has cytotoxicity against the amoebae *Dictyostelium* and mammalian J774 macrophages [192]. The VgrG1 in *Aeromonas hydrophila* has a VIP-2 like, C-terminal domain that ADP-ribosylates actin, causing cells to be rounded up [193]. The C-terminal domain (CTD) of VgrG5 from *Burkholderia* species has been found to cause complex effects on eukaryotic cells, which may help the pathogen in immune evasion and cell-to-cell spread, including fusogenic activity [194], multinucleation [195], and Rho-activation [196]. Like the T3SS and T4SS effectors, there may be an arsenal of yet-to-be-identified T6SS cytotoxic effectors harbored by bacteria to manipulate their hosts.

Molecular evolution of toxins through genetic exchange

Genetic exchange and toxin evolution

If HGT is a major player in the evolution of toxin-producing pathogens, where do most of these transfers occur? And what about recombination events? Could it be that the host body is not the only setting where these types of evolution occur? This is of particular interest because many pathogens, including those that produce toxins, spend a substantial amount of their life cycle outside the host body.

Toxin evolution and transmission in the host

Microbes are defined by their environment, and for most pathogens, that environment is believed to be predominantly the human or animal host. Pathogenic bacteria establish infections in widely diverse host environments, ranging from the skin to various mucosal surfaces, such as the oral cavity, lungs, gut, and vagina. Consequently, specific host *in vivo* signals might be expected to modulate bacterial gene transfer events [197]. There are ample studies demonstrating that transfer of antibiotic resistance genes among different Gram-negative and Gram-positive bacteria can occur in the intestinal tracts of humans and animals [198–201]. A number of genetic analyses of the plasmid profiles from clinical fecal isolates indicate that HGT occurs rampantly within the human gut [198,199,202,203].

Conjugal gene transfer between different *Salmonella* strains has also been shown to occur within cultured human epithelial cells [204], suggesting that the phagocytic vacuoles or cytosol of animal cells could be a niche for HGT if bacteria conjugate within the intracellular environment. A comparative genome analysis of *L. pneumophila* has revealed an extensive degree of recombination and HGT events among bacteria and from eukaryotes to bacteria (e.g., in shaping the genomes of *L. pneumophila* [205]). An interesting example of HGT can be found in the genetic evidence for homologous recombination within the vacuolating cytotoxin gene, *vacA*, which occurred among different *H. pylori* strains coinhabiting the stomach and resulted in chimeric *vacA* genes [206,207].

Genetic evidence supports that evolution of toxin genes in the Gram-positive staphylococci is still occurring via phage-mediated HGT [54,208], particularly in relation to the various toxin gene clusters for superantigens (*set*), exfoliative toxins (*eta*, *etb*), and toxic shock syndrome toxins (*tst*) found in pathogenic strains. All human isolates (>8000 tested so far) of pathogenic *S. aureus* and Group A *Streptococcus* (GAS) produce superantigens [209]. Many of the widespread *S. aureus* pathogenicity islands (SaPIs) encode two or more superantigen toxins, with frequent recombination events within genes also occurring [55]. The most common superantigen gene found in SaPIs of clinical isolates is the *tst* gene, which encodes the toxic shock syndrome toxin-1 (TSST-1), which is responsible for menstral-assoicated toxic shock syndrome [55]. Besides TSST-1, there are over 19 different superantigens, including the staphylococcal enterotoxins (SEA, SEBn, SECn, SED, SEE, and SEG, where n denotes multiple variants) and the SE-like superantigens (SE-l H, SE-l I, SE-l J through SE-l X)

reported from S. aureus strains [209–211]. Comparative genome sequencing analysis uncovered several virulence factor–encoding PAIs in a human clinical isolate of *S. aureus*, strain MRSA252, which are shared with *S. aureus* strains associated with bovine mastitis [212]. This strongly suggests that zoonotic contact can lead to HGT events and emergence of new virulence properties.

A similar evolutionary story of high variability and extensive HGT is told for the streptococci. GAS strains can produce one or more of the streptococcal pyrogenic exotoxins (SpeA, SpeC, SpeG through SpeM), which are related to the staphylococcal superantigen family, streptococcal superantigen (Ssa), streptococcal mitogenic exotoxin Zn (SmeZn, where *n* denotes multiple variants), or SpeB, which is a cysteine protease with controversial superantigenic activity [209,210]. The prophage-encoded genes for SpeA (the scarlet fever toxin) and SpeC are variably present in clinical GAS isolates, and there is strong genetic evidence that they undergo HGT and recombination [63,213]. Comparative genome sequence analysis of 12 *Streptococcus pyogenes* strains revealed the presence of multiple different prophages within the otherwise relatively conserved (90%) genomes [214]. The human pharyngeal cell has been shown to release a soluble factor that stimulates the lytic activation of *S. pyogenes* prophage [215]. Indeed, using a mouse model of *S. pyogenes* infection, the mammalian host was shown to promote both efficient phage induction at the mucosal surface, as well as subsequent lysogenic conversion of the nontoxigenic streptococci occupying the same niche [216].

The *ctxAB* genes that encode the A and B subunits of CT were found to be located on a filamentous prophage CTXϕ in naturally occurring clinical and environmental *V. cholerae* and *Vibrio mimicus strains* [7,15–17,217]. What was even more remarkable was the finding that the phage CTXϕ uses as its receptor the toxin coregulated pilus (TCP), which itself is a colonization factor in the gut [218]. The genes for TCP are encoded by another putative prophage, VPIϕ, present in CTXϕ-positive *Vibrio* isolates. While classical pandemic strains of *V. cholerae* express TCP under a variety of conditions, more recent El Tor pandemic strains express TCP only in the infected mammalian host [7,219,220]. The vast majority of epidemic *V. cholerae* strains, which are responsible for the massive diarrheal disease cholera, do not produce CT or TCP pili when they are outside the human or animal host. Under certain laboratory conditions, they can undergo lysogenic conversion, but the most efficient conversion occurred within host intestines [18]. In addition, generalized transduction of CTXϕ by yet another phage (CP-T1) was found to be an alternative mechanism by which CTXϕ-carrying strains could transfer the CTXϕ prophage into nontoxigenic strains [221]. There is evidence for recent HGT of CTXϕ and VPIϕ between *V. cholerae* and *V. mimicus*, suggesting that in the environment, *V. mimicus* might serve as a reservoir for these phages and the potential emergence of new pathogenic isolates [7,15].

Pathogenic *Bordetella* species (*B. pertussis, Bordetella parapertussis*, and *Bordetella bronchiseptica*) are closely related Gram-negative bacteria that colonize the respiratory tracts of mammals [222]. *Bordetella petrii* is the only known environmental species of the *Bordetella* genus, and interestingly, lacks all of the known toxins found in the pathogenic species [223]. *B. pertussis* is a strict human pathogen responsible for whooping cough; *B. parapertussis* is a recently emerged variant that infects both humans and

sheep; and *B. bronchiseptica* is a broad host-range pathogen that infects primarily animals and occasionally humans. Comparative genome sequence analysis suggests that *B. pertussis* and *B. parapertussis* evolved from *B. bronchiseptica* in the recent past [224,225]. Interestingly, many of the genes encoding virulence factors, including the genes for PT (*ptxA-E*), adenylate cyclase-hemolysin (*cya*), dermonecrotic toxin (*dnt*), and tracheal cytotoxin (*tct*), can be found on distinct regions of the chromosomes in all three species. It was previously thought that PT expression differences in the strains were due to inactivating mutations in the promoter regions in *B. parapertussis* and *B. bronchiseptica* [226]. However, genetic analysis suggests that PT may be more tightly regulated in *B. parapertussis* and *B. bronchiseptica* and therefore is expressed only *in vivo* by these strains, while more recent mutations in the promoter region increased the transcription of the gene in *B. pertussis* [227].

Toxin evolution and transmission in the soil environment

Many bacterial pathogens, even those for which humans or animals can serve as the natural or primary reservoir, spend a substantial amount of time outside the host body in the external environment. This suggests that the evolution of pathogens through HGT may not occur only in the host environment. For example, it is now well recognized that the selective pressure of xenobiotic pollutants in soil and water can lead to the acquisition by soil bacteria of plasmids encoding xenobiotic-degrading enzymes [228,229]. There is even some evidence that natural electrotransformation, as might occur during a thunderstorm, might be a feasible mechanism for increasing the frequency of HGT in soil microcosms [230]. The impact of HGT via phages on the diversity and evolution of bacteria is also well established now [231–233].

Bacillus species are common spore-forming soil bacteria barely distinguishable at the genome sequence level [234–236], yet this group of bacteria differs considerably in their virulence properties. *Bacillus subtilis* is commonly used as a nonpathogenic laboratory model system for studying bacterial sporulation; *Bacillus thuringiensis* produces a number of insecticidal toxins widely used as pesticides in agriculture and in genetically modified plants to confer insect resistance; *Bacillus cereus* is a food-borne pathogen capable of causing human and animal gastrointestinal disease; and *Bacillus anthracis* is a human and animal pathogen that causes anthrax and has received much attention because of its potential use as a bioterror agent. The genes encoding the major virulence factors of *B. anthracis* responsible for anthrax, the anthrax lethal toxin and edema toxin genes (*pag, lef, cya*) and the poly-D-glutamate capsule biosynthetic genes (*capBCA*), reside on two large plasmids, pXO1 and pXO2, respectively [51,236,237]. Loss of the pXO2 plasmid resulted in the greatly attenuated Sterne vaccine strain [235]. Although it does not appear that these plasmids are self-transmissible, there are reports suggesting that conjugative plasmids from other *Bacillus* species might be able to supply the conjugal transfer functions in trans for these two virulence plasmids [238,239]. If this is true, then it is conceivable that other *Bacillus* species may serve as environmental reservoirs for the anthrax toxin genes [235]. And, indeed, HGT is reported to be quite common among *Bacillus* species [239–243].

Toxin evolution and transmission in aquatic environments

Numerous studies have shown that the concentrations of bacteriophages in natural aquatic environments can be high enough to be conducive for phage transduction, but they can be especially high in contaminated water systems [6,76,82–85]. The emergence, evolution, and spread of antibiotic resistance has been firmly linked to HGT in aquatic environments [244,245]; similar findings have emerged regarding toxin genes [1,6,82–85]. As mentioned previously, there is now strong genetic evidence that epidemic strains of *V. cholerae*, the etiological agent of cholera, have acquired CT, TCP, and other virulence factor genes through bacteriophage transduction and conjugal transfer in aquatic environments, possibly through *V. mimicus* reservoirs [7,15–17,217]. A broad range of *E. coli* strains harboring *stx2* (and to a lesser extent *stx1*) genes are commonly found in municipal sewage and various animal wastewaters [82,85]. Moreover, of the *E. coli* strains testing positive for toxin genes (only 1 out of 59 representative strains was typed as O157:H7), about 50% from animal wastewater and 10% from human sewage were also able to produce the toxin proteins [82]. These findings not only indicate the presence of a significant exchange of these genes between bacterial populations in these environments, but also suggest a potential health risk, in that the bacteria also make the toxins.

Since HGT occurs at maximal frequencies when bacterial densities of potential recipients are high [246–251], one might ask how genetic exchange can occur in aquatic environments, where some bacteria, such as *Vibrio*, spend most of their time and where their concentrations are expected to be too low to be conducive to HGT. Studies have shown, however, that bacteria found in marine environments are often adherent to marine animals or particles, such as zooplankton or marine snow [252–254]. Indeed, *Vibrio* strains are often found in close association with zooplankton as dense bacterial biofilms [254–257]. This finding correlates with the observation that zooplankton blooms frequently precede cholera outbreaks and that simple water filtration to remove zooplankton can significantly reduce the incidence of cholera cases in endemic areas [258]. Similar findings have been observed for other environmental bacteria [259]. The presence of particulate matter in aqueous environments was found to increase potential pathogenic bacteria-phage interactions and thereby stimulate transduction [6,259,260], the outcome of which has been proposed to supply virulence factors, such as toxins, that benefit both the bacteria and phage and contribute to their evolution as pathogens [261].

Toxin evolution and transmission in the phyllosphere

Other environmental settings could also serve as ideal locations for biofilm formation and consequent HGT [260,262]. One such important setting is the phyllosphere (i.e., plant surfaces). Numerous examples of relatively high conjugal transfer rates on the phylloplane of plants have been observed within and between species of *Pseudomonas* and *Rhizobium*, especially when humidity is high [263–265]. The soil bacterium *Ralstonia solanacearum*, a plant pathogen, even develops a state of natural competence in plant tissues and readily exchanges genetic material with plants and

other bacteria [266–268]. Aggregation or clustering of the bacteria into microhabitats in the interstices and stomata of the leaf surface has been found to stimulate bacterial survival and conjugal transfer [269,270]. HGT between plants and bacteria, such as *Acintobacter baylyi*, in the phyllosphere have also been visualized in situ using fluorescence microscopy [271].

Evidence for phage-mediated transduction among bacterial strains on the phylloplane has also been documented for a number of human pathogens [272,273], the plant and opportunistic human pathogens *Pseudomonas aeruginosa*, *Serratia marcescens* and *Serratia liquefaciens*, plant pathogens [274–276], and indigenous plant microbes, *Pseudomonas fluorescens, Pseudomonas syringae, Pseudomonas viridiflava*, and *S. liquefaciens* [231,277]. The transmission of *E. coli* O157:H7 and *Salmonella* species from manure-contaminated soil and irrigation water to various vegetables (lettuce, spinach, potatoes, carrots), sprouts (alfalfa, radish), and other plants, as well as the subsequent disease outbreaks, has been thoroughly documented [272,273]. Indeed, the toxin-producing bacteria were found not only on the plant surfaces, but also throughout the root system and within the edible portions of the plant tissues [278]. The implications of this finding, in terms of the spread of human disease through fresh produce, are alarming and may account for the emergence of new pathogens and disease outbreaks [273].

Toxin evolution in the guts of insects and other vectors

Bacteriophages and other mobile genetic elements also play important evolutionary roles in bacterial endosymbiont systems of insect hosts [279–282]. The extensive genome diversity found among parasitic arthropod bacterial symbionts (*Wolbachia, Rickettsia*) was shown to reside primarily in regions originating from HGT between *Wolbachia* strains that coinfect a diverse set of insect host cells [280,282,283]. Amoeba have also been found to serve as the environmental reservoir for a number of pathogenic bacteria that can facilitate HGT between the bacterial coinhabitants, as well as the bacteria and their amoeba host [279]. Recently, *mcf*-like genes were identified in the fungal genomes of the *Epichloë* species, which are intercellular symbionts of grasses and help protect the host grasses from insects [284]. These *mcf*-like genes are homologues of *mcf1* and *mcf2* found in the nematode symbiont *Photorhabdus luminescence* and *fitD* from plant-associated *Pseudomonas* species. The pore-forming domains of these insecticidal toxins share homology with the large modular toxins from *Clostridium difficile*, TcdA and TcdB. These results suggest that interdomain genetic exchange involving bacteria, fungi, insects, and plants can occur in an external environment.

B. thuringiensis produces a considerable arsenal of toxins directed against insects and nematodes, with multiple toxin-encoding genes on plasmids and various mobilizable genetic elements on the chromosome [285]. Selective pressure and HGT, combined with recombination and shuffling between toxin genes (resulting in domain swapping) and sequence divergence, has yielded a wide range of host specificities for these insecticidal toxins [235,285–287]. The genes encoding the crystal protein toxins, for example, are frequently clustered on different transmissible plasmids or

transposable elements [235,285–288], and conjugation between different strains has been observed in the soil environment and within insect guts [289–292]. Individual toxins have insecticidal activity only against a limited range of insect species (i.e., usually only within certain insect orders). The composite of the toxins produced by a particular strain thus defines the total insecticidal specificity and activity spectrum of that bacterial strain [285,286,288].

Accumulating evidence has lead some researchers to put forth the proposal that HGT within the guts of insects commonly associated with domestic animals may represent a key link to the transmission of antibiotic resistance and other pathogenic traits in bacteria found in contaminated environments [293]. For example, houseflies and blowflies have been reported to carry toxinogenic *E. coli* O157:H7 [294–299], as well as other pathogens such as *Salmonella* and *Listeria* [300,301]. Plasmid transfer from *Erwinia herbicola* to *Enterobacter cloacae* was observed in the guts of silkworms [302]. Both conjugative and mobilizable plasmids from *E. coli* can be transferred to a wide variety of *Proteobacteria* within the gut of soil microarthropods [303,304], which could play host to HGT of toxin genes in *E. coli*-contaminated water or soil. *Y. pestis*, the causative agent of plague, has also been demonstrated to be capable of interacting with and acquiring plasmids from *E. coli* in the midgut of the flea at transfer frequencies of about 10^{-3} per recipient [305]. One might ask whether toxin gene–encoding plasmids, phages, or other mobile elements might also be transferred within the guts of insects, and, indeed, such transfers can occur and have occurred. For example, efficient gene transfer of plasmids encoding δ-endotoxin has been observed between different strains of *B. thuringiensis* in various arthropod hosts [306].

Toxin evolution in biofilms and regulation by quorum sensing

Evidence is accumulating that HGT and biofilm formation are interconnected [307–314]. It appears that the high rates of HGT observed in bacterial biofilms may be attributable to quorum-sensing control of the conjugation and production of exoenzymes, toxins, and other virulence factors, in addition to biofilm formation [247,251,263,307,315–317]. *Roseobacter* species and other environmental bacteria besides *Vibrio* associate with marine snow and also produce acyl homoserine lactones. Hence, these bacteria use quorum sensing to regulate phenotypic traits (e.g., biofilm formation, metabolite/toxin production, conjugation) when their populations in the marine snow environment reach adequate densities [318,319]. Indeed, quorum-sensing autoinducers produced by other bacteria within a polymicrobial biofilm have been shown to promote HGT among *Vibrio* species [315].

High levels of HGT and genetic recombination also occur *in vivo* within the host environment, such as during nasopharyngeal carriage and cocolonization of *S. pneumoniae* strains in biofilms, suggesting that the environment of the nasopharynx is highly conducive for promoting transformation [320]. The strong conservation of virulence determinants (e.g., toxins) among clinical and environmental isolates of *P. aeruginosa* supports the notion that conserved selective pressures for the maintenance of the virulence traits exist in the environmental reservoir [321]. Evidence suggests that quorum-sensing regulation of the formation of biofilms and the production of toxins may have

played a critical evolutionary advantage in the environment by providing a protective mechanism through which the bacteria are able to resist flagellate and other protozoan predation and thereby persist outside the human host [322–325]. Similarly, the effector protein toxins that are delivered by the Icm/Dot type IV secretion system of *L. pneumophila* appear to be equally effective in mammalian cells and in amoeba [186].

Another well-characterized example of biofilm and toxin regulation through quorum sensing can be found for the opportunistic pathogens *P. aeruginosa* and *Burkholderia cepacia* complex in the lungs of cystic fibrosis (CF) patients [316,326–329], where both bacteria form aerobic and anaerobic biofilms that differ significantly in virulence and phenotypic properties [323,324,330]. Indeed, there is strong evidence for distinct pathoadaptive mutations occurring over time in these systems [316,326,328,331,332], including evidence of the pathogens interacting and exchanging virulence-associated genes with each other. Other bacteria are emerging as pathogens in the CF lung as well [333,334]. For example, in the CF lung, quorum-sensing systems in *P. aeruginosa* regulate the formation of biofilms, as well as toxin/exoenzyme production. Interestingly, whereas the expression of exotoxin A (ExoA) and various protease and phospholipase exoenzymes are up-regulated by quorum sensing in biofilms, the T3SS and its translocated effectors (ExoS, ExoT, ExoY, and ExoU), which are important only when the bacteria are in direct contact with the host cells, are down-regulated by quorum sensing [335,336]. One exception to the strong conservation of virulence genes in *P. aeruginosa* is the presence of the genes for exotoxin S (ExoS) and exotoxin U (ExoU), with isolates from different clinical and environmental settings possessing one or the other of the *exoS* or *exoU* genes, but not both [321,337]. It has been suggested that one possible explanation may be that expression and delivery of one or the other toxin might provide a selective advantage in a particular, as-yet-unidentified, target host or tissue site [321,338].

Vaccines and toxin evolution

Despite high vaccination coverage and good, long-lasting immunogenicity in many populations [339], the once nearly eradicated diseases diphtheria and pertussis have unfortunately reemerged as a global health threat, even in developed countries [340–347]. One possible explanation for this reemergence is that the constant selective pressure imposed by immunization might have resulted in increased antigenic divergence in the remaining bacterial population. Consequently, the effects of vaccination on toxin evolution are beginning to be examined [340,341,343,348–353].

Comparative genetic analysis of the genes for *B. pertussis* PT and pertactin (an outer-membrane protein) between epidemic isolates and the vaccine strains revealed that expansion of strains antigenically distinct from vaccine strains has occurred [340,341,348–350,352–354]. These findings strongly implicate vaccination as a strong driving force in the continuous evolution of the *B. pertussis* population and may forebode the emergence of novel variants resistant to vaccination. Moreover, since pertactin and PT are the primary bacterial components in acellular pertussis vaccines (ACVs) that were introduced in the 1970s and have replaced the whole-cell vaccines (WCVs) in some countries, these findings throw into question the long-term

efficacy of ACVs. Indeed, the recent pertussis outbreaks in the United States have been linked primarily to switching WCVs for ACVs, which occurred in the 1990s, and waning immunity associated with ACVs [340,341,343]. The practice of nonmedical exemption of vaccination also appears to enhance the risk of pertussis [355]. While the efficacy of ACV is been reevaluated [345] and the pathogenesis of *Bordetella* is being thoroughly scrutinized [356], these recurring pertussis outbreaks could further exacerbate the selective pressure for the evolution of antigens.

As a result of longstanding immunization programs using the diphtheria toxoid, most current isolates of *C. diphtheriae* or *Corynebacterium ulcerans* are nontoxigenic [354], yet toxigenic strains are still present [344,347,354,357,358]). While nontoxigenic strains are devoid of the *tox* gene, there is the fear that these strains could potentially be converted into toxigenic strains by exposure to a corynebacteriophage β carrying the *tox* gene. There is also the fear that the continued presence of nontoxigenic strains in immunized populations and the potential import of toxigenic strains from endemic areas such as Russia might allow phage conversion to occur [344,354,357,359]. In addition, it has been shown that recombination events are possible between strains harboring phages that contain *tox* genes and nontoxigenic strains that harbor phage with defective *tox* genes or by reversion of the defective *tox* gene through spontaneous mutation [360], such that any of these scenarios might result in reversion to full toxigenicity.

The high potential of phage-mediated or other HGT-mediated conversion of nontoxigenic bacterial strains, particularly within the host environment, has also raised serious safety concerns regarding the use of live bacterial for potential biomedical applications such as live-cell vaccine development, probiotics, or other therapeutics [361,362]. While it was well known that the DT gene could be easily transmitted via temperate phage between toxigenic and nontoxigenic strains of *C. diphtheriae*, this was not thought to be a problem for bacteria that did not appear to be able to transfer their genes between pathogenic and benign strains. However, this perception was contradicted by the finding that the CT gene, which was previously thought to be on a putative prophage PAI that was no longer transmissible, could actually be transferred *in vivo* in the host environment [18]. Recently, it was demonstrated that the chromosomally located PaLoc PAI of *C. difficile*, which encodes the large glycosyltransferase toxins, TcdA and TcdB, could convert via conjugation a nontoxigenic strain into a toxin-producing strain [361]. This finding has broader implications regarding the safety of using nontoxigenic, avirulent strains as therapeutic delivery vehicles.

Modular recombination of bacterial toxins

Bacterial toxins with structurally and functionally homologous activity domains but different binding domains, such as DT and *Pseudomonas* ExoA [363], or with similar binding domains but structurally and functionally unrelated activity domains, such as STx, PT, CT, and HLT [364], have been known since the early days of bacterial toxin research. The occurence of these related toxins suggests a possible process of modular recombination in toxin evolution. With the rapid growth in the volume of available

microbial genomes, a vast number of additional examples of structurally or functionally related bacterial toxins have emerged. This modular recombination process could involve both HGT and additional insertion-deletion processes.

One interesting example of modular recombination is the prevalence of the CNF-like domain in toxins or putative toxins found in a variety of bacteria. CNF1 in uropathogenic *E. coli* (UPEC) strains, CNF2 in EPEC strains, and the dermonecrotic toxin DNT in *B. bronchiseptica* were first identified as Rho-modifying factors in both human and animal clinical isolates [365,366]. The C-terminal (about 300 residues of these toxins) harbors a Rho-modifying γ-glutamyl-deamidase activity [367–369]. Additional CNF homologues have now been found in NTEC strains of *E. coli* (CNF3) [38], *Y. pseudotuberculosis* (CNFy) [40], *Photobacterium damselae* (CNFp, EMBL: EEZ39234.1) and *Moritella viscosa* (CNFm) [41]. All CNF homologues share a similar N-terminal fragment of about 700 amino acid residues that also exhibits homology with the N-terminus of PMT, while the N-terminus of DNT has homology to the N-terminus of a putative toxin from *Photorhabdus* carrying an unrelated protease effector domain. In addition to CNFs and DNTs, the CNF-like Rho-activating domain has been found in other classes of toxin effectors.

This diverse recombination allows microbes to explore various forms of entry pathways into host cells, including receptor-mediated endocytosis by AB toxin systems, insecticidal toxin complex systems, T3SSs, T6SSs, and other as-yet-undefined, large effector systems (Figure 1.1A). Sequence comparison of all CNF domains found among various effectors showed a wide spectrum of heterogeneity (Figure 1.1B). Among these, the CNF and DNT clades are most dissimilar. The large number of CNF-related T3SS effectors clustered into two clades (*Vibrio parahaemolyticus* VopC and *P. luminescens* Pnf). With such a diversity of CNF activity domain sequences, it is likely that CNF-like effectors could have evolved differential substrate selectivities. For example, the T3SS effector VopC only activates Rac and Cdc42, but not RhoA [372], while all CNFs and DNTs tested activate RhoA, Rac, and Cdc42 [373].

Multifunctional autoprocessing RTX toxins (MARTXs) of *V. cholerae* and *Vibrio vulnificus* strains are another case of modular recombination. Related MARTX sequences have been identified in many *Vibrio* species and a diverse number of other bacteria [374]. MARTXs consist of conserved N-terminal A repeats, B repeats, and C-terminal C repeats. The conserved cysteine protease domain (CPD) responsible for autoprocessing is located immediately before the C repeats. Between the B repeats and CPD are up to five multifunctional effector domain inserts, including an actin-crossing domain (ACD), a Rho GTPase-inactivation domain (RID), and other domains of unknown function.

There are a number of bacterial mono-ADP-ribosylating toxins (mADPRTs) and effectors that play important roles in pathogenesis. Based on sequence similarity, these mADPRTs have been classified into four families: DT/ExoS-like toxins, CT/PT-like toxins, C2-like toxins, and C3-like toxins [52]. Combining a structure-based data-mining approach with a yeast growth-defect phenotyping test for activity verification, new types of domain assembly for mADPRTs have been identified [53,375,376]. Similar bioinformatic approaches should be able to uncover even more examples of new toxins derived from modular domain recombination.

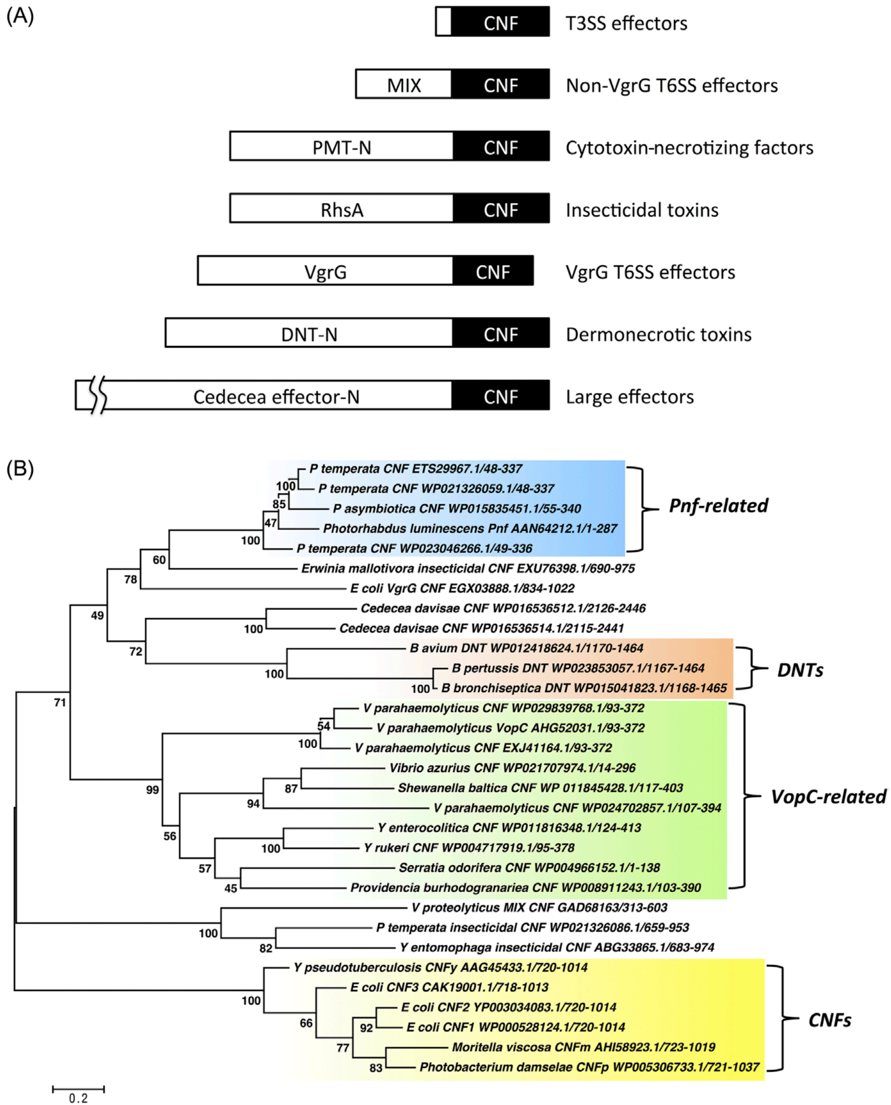


Figure 1.1 Modular organization of CNF-related bacterial effector toxins. (A) Shown is a schematic diagram depicting different classes of proteins with CNF-related effector modules, such as the T3SS effectors from *V. parahaemolyticus* VopC and *P. luminescens* Pnf; cytotoxic necrotizing factors such as *E. coli* CNF1, CNF2, and CNF3, and *Y. pseudotuberculosis* CNFy; insecticidal toxins such as *Y. entomophaga* YenC1, *E. mallotivora* TccC3; T6SS factors from *E. coli* such as VgrG-CNF; dermonecrotic toxins such as *B. pertussis* DNT; and large CNF-like domain-containing protein effectors such as *C. davisae* effector. (B) A MEGA5 maximum likelihood phylogenetic tree [370] was constructed from MUSCLE alignment [371] of protein sequences homologous to the CNF1 activity domain, as defined in PDB entry *1hq0*. Numbers on each node was calculated by a bootstrap method after 500 replicates. The GenBank accession number and the range of residues used for comparison are listed for each sequence.

Conclusion

The evolution of bacterial toxins involves HGT and recombination through exposure to a combination of both the host and external environments. Most toxin genes are located on PAIs on plasmids or in the bacterial chromosome as prophages or other transmissible elements. Genome sequencing and genetic analysis of toxin genes in epidemiological isolates may provide more information about their extent and limitations, as well as regulatory mechanisms that influence HGT and recombination and their impact on toxin evolution.

References

[1] Casas V, Maloy S. Role of bacteriophage-encoded exotoxins in the evolution of bacterial pathogens. Future Microbiol 2011;6:1461–73.
[2] Groisman EA, Ochman H. Pathogenicity islands: bacterial evolution in quantum leaps. Cell 1996;87:791–4.
[3] Heesemann J. Darwin's principle of divergence revisited: small steps and quantum leaps set the path of microbial evolution. Int J Med Microbiol 2004;294:65–6.
[4] Schmidt H, Hensel M. Pathogenicity islands in bacterial pathogenesis. Clin Microbiol Rev 2004;17:14–56.
[5] Miskinyte M, Sousa A, Ramiro RS, de Sousa JA, Kotlinowski J, Caramalho I, et al. The genetic basis of Escherichia coli pathoadaptation to macrophages. PLoS Pathog 2013;9:e1003802.
[6] Casas V, Magbanua J, Sobrepena G, Kelley ST, Maloy SR. Reservoir of bacterial exotoxin genes in the environment. Int J Microbiol 2010;2010:754368.
[7] Faruque SM, Mekalanos JJ. Phage-bacterial interactions in the evolution of toxigenic Vibrio cholerae. Virulence 2012;3:556–65.
[8] Fortier LC, Sekulovic O. Importance of prophages to evolution and virulence of bacterial pathogens. Virulence 2013;4:354–65.
[9] Popoff MR, Bouvet P. Genetic characteristics of toxigenic Clostridia and toxin gene evolution. Toxicon 2013;75:63–89.
[10] Dover N, Barash JR, Hill KK, Davenport KW, Teshima H, Xie G, et al. Clostridium botulinum strain Af84 contains three neurotoxin gene clusters: bont/A2, bont/F4 and bont/F5. PLoS One 2013;8:e61205.
[11] Dover N, Barash JR, Hill KK, Xie G, Arnon SS. Molecular characterization of a novel botulinum neurotoxin type H gene. J Infect Dis 2014;209:192–202.
[12] Buck GA, Cross RE, Wong TP, Loera J, Groman N. DNA relationships among some tox-bearing corynebacteriophages. Infect Immun 1985;49:679–84.
[13] Leong D, Murphy JR. Characterization of the diphtheria tox transcript in Corynebacterium diphtheriae and Escherichia coli. J Bacteriol 1985;163:1114–9.
[14] Ratti G, Rappuoli R, Giannini G. The complete nucleotide sequence of the gene coding for diphtheria toxin in the corynephage omega (tox+) genome. Nucleic Acids Res 1983;11:6589–95.
[15] Boyd EF, Moyer KE, Shi L, Waldor MK. Infectious CTXPhi and the vibrio pathogenicity island prophage in Vibrio mimicus: evidence for recent horizontal transfer between V. mimicus and V. cholerae. Infect Immun 2000;68:1507–13.
[16] Faruque SM, Asadulghani Alim AR, Albert MJ, Islam KM, Mekalanos JJ. Induction of the lysogenic phage encoding cholera toxin in naturally occurring strains of toxigenic Vibrio cholerae O1 and O139. Infect Immun 1998;66:3752–7.
[17] Faruque SM, Rahman MM, Asadulghani Nasirul Islam KM, Mekalanos JJ. Lysogenic conversion of environmental Vibrio mimicus strains by CTXPhi. Infect Immun 1999;67:5723–9.
[18] Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. Science 1996;272:1910–4.
[19] Locht C, Keith JM. Pertussis toxin gene: nucleotide sequence and genetic organization. Science 1986;232:1258–64.
[20] Jorgensen R, Purdy AE, Fieldhouse RJ, Kimber MS, Bartlett DH, Merrill AR. Cholix toxin, a novel ADP-ribosylating factor from Vibrio cholerae. J Biol Chem 2008;283:10671–8.
[21] Purdy AE, Balch D, Lizarraga-Partida ML, Islam MS, Martinez-Urtaza J, Huq A, et al. Diversity and distribution of cholix toxin, a novel ADP-ribosylating factor from Vibrio cholerae. Environ Microbiol Rep 2010;2:198–207.

[22] Herold S, Karch H, Schmidt H. Shiga toxin-encoding bacteriophages—genomes in motion. Int J Med Microbiol 2004;294:115–21.
[23] Unkmeir A, Schmidt H. Structural analysis of phage-borne stx genes and their flanking sequences in shiga toxin–producing Escherichia coli and Shigella dysenteriae type 1 strains. Infect Immun 2000;68:4856–64.
[24] Beutin L, Hammerl JA, Reetz J, Strauch E. Shiga toxin-producing Escherichia coli strains from cattle as a source of the Stx2a bacteriophages present in enteroaggregative Escherichia coli O104:H4 strains. Int J Med Microbiol 2013;303:595–602.
[25] Huang A, Friesen J, Brunton JL. Characterization of a bacteriophage that carries the genes for production of Shiga-like toxin 1 in Escherichia coli. J Bacteriol 1987;169:4308–12.
[26] Tyler JS, Mills MJ, Friedman DI. The operator and early promoter region of the Shiga toxin type 2-encoding bacteriophage 933W and control of toxin expression. J Bacteriol 2004;186:7670–9.
[27] Strauch E, Schaudinn C, Beutin L. First-time isolation and characterization of a bacteriophage encoding the Shiga toxin 2c variant, which is globally spread in strains of Escherichia coli O157. Infect Immun 2004;72:7030–9.
[28] Strauch E, Lurz R, Beutin L. Characterization of a Shiga toxin–encoding temperate bacteriophage of Shigella sonnei. Infect Immun 2001;69:7588–95.
[29] Koch C, Hertwig S, Appel B. Nucleotide sequence of the integration site of the temperate bacteriophage 6220, which carries the Shiga toxin gene stx(1ox3). J Bacteriol 2003;185:6463–6.
[30] Lin W, Fullner KJ, Clayton R, Sexton JA, Rogers MB, Calia KE, et al. Identification of a vibrio cholerae RTX toxin gene cluster that is tightly linked to the cholera toxin prophage. Proc Natl Acad Sci USA 1999;96:1071–6.
[31] Sheahan KL, Cordero CL, Satchell KJ. Identification of a domain within the multifunctional Vibrio cholerae RTX toxin that covalently cross-links actin. Proc Natl Acad Sci USA 2004;101:9798–803.
[32] Chen CY, Wu KM, Chang YC, Chang CH, Tsai HC, Liao TL, et al. Comparative genome analysis of Vibrio vulnificus, a marine pathogen. Genome Res 2003;13:2577–87.
[33] Janka A, Bielaszewska M, Dobrindt U, Greune L, Schmidt MA, Karch H. Cytolethal distending toxin gene cluster in enterohemorrhagic Escherichia coli O157:H- and O157:H7: characterization and evolutionary considerations. Infect Immun 2003;71:3634–8.
[34] Oswald E, Sugai M, Labigne A, Wu HC, Fiorentini C, Boquet P, et al. Cytotoxic necrotizing factor type 2 produced by virulent *Escherichia coli* modifies the small GTP-binding proteins Rho involved in assembly of actin stress fibers. Proc Natl Acad Sci USA 1994;91:3814–8.
[35] Song J, Gao X, Galan JE. Structure and function of the Salmonella Typhi chimaeric A(2)B(5) typhoid toxin. Nature 2013;499:350–4.
[36] Beutin L, Stroeher UH, Manning PA. Isolation of enterohemolysin (Ehly2)–associated sequences encoded on temperate phages of Escherichia coli. Gene 1993;132:95–9.
[37] Stroeher UH, Bode L, Beutin L, Manning PA. Characterization and sequence of a 33-kDa enterohemolysin (Ehly 1)–associated protein in Escherichia coli. Gene 1993;132:89–94.
[38] Stoll T, Markwirth G, Reipschlager S, Schmidt G. A new member of a growing toxin family—Escherichia coli cytotoxic necrotizing factor 3 (CNF3). Toxicon: Off J Int Soc Toxinol 2009;54:745–53.
[39] Orden JA, Dominguez-Bernal G, Martinez-Pulgarin S, Blanco M, Blanco JE, Mora A, et al. Necrotoxigenic Escherichia coli from sheep and goats produce a new type of cytotoxic necrotizing factor (CNF3) associated with the eae and ehxA genes. Int Microbiol: Off J Span Soc Microbiol 2007;10:47–55.
[40] Lockman HA, Gillespie RA, Baker BD, Shakhnovich E. Yersinia pseudotuberculosis produces a cytotoxic necrotizing factor. Infect Immun 2002;70:2708–14.
[41] Tunsjo HS, Wiik-Nielsen CR, Grove S, Skjerve E, Sorum H, L'Abee-Lund TM. Putative virulence genes in Moritella viscosa: activity during in vitro inoculation and in vivo infection. Microb Pathog 2011;50:286–92.
[42] Cruz-Migoni A, Hautbergue GM, Artymiuk PJ, Baker PJ, Bokori-Brown M, Chang CT, et al. A Burkholderia pseudomallei toxin inhibits helicase activity of translation factor eIF4A. Science 2011;334:821–4.
[43] Hautbergue GM, Wilson SA. BLF1, the first Burkholderia pseudomallei toxin, connects inhibition of host protein synthesis with melioidosis. Biochem Soc Trans 2012;40:842–5.
[44] Cui J, Yao Q, Li S, Ding X, Lu Q, Mao H, et al. Glutamine deamidation and dysfunction of ubiquitin/NEDD8 induced by a bacterial effector family. Science 2010;329:1215–8.
[45] Marches O, Ledger TN, Boury M, Ohara M, Tu X, Goffaux F, et al. Enteropathogenic and enterohaemorrhagic Escherichia coli deliver a novel effector called Cif, which blocks cell cycle G2/M transition. Mol Microbiol 2003;50:1553–67.

[46] Jubelin G, Chavez CV, Taieb F, Banfield MJ, Samba-Louaka A, Nobe R, et al. Cycle-inhibiting factors (CIFs) are a growing family of functional cyclomodulins present in invertebrate and mammal bacterial pathogens. PLoS One 2009;4:e4855.
[47] Sanada T, Kim M, Mimuro H, Suzuki M, Ogawa M, Oyama A, et al. The Shigella flexneri effector OspI deamidates UBC13 to dampen the inflammatory response. Nature 2012;483:623–6.
[48] Pullinger GD, Bevir T, Lax AJ. The *Pasteurella multocida* toxin is encoded within a lysogenic bacteriophage. Mol Microbiol 2004;51:255–69.
[49] Walker KE, Weiss AA. Characterization of the dermonecrotic toxin in members of the genus *Bordetella*. Infect Immun 1994;62:3817–28.
[50] Jank T, Bogdanovic X, Wirth C, Haaf E, Spoerner M, Bohmer KE, et al. A bacterial toxin catalyzing tyrosine glycosylation of Rho and deamidation of Gq and Gi proteins. Nat Struct Mol Biol 2013;20:1273–80.
[51] Okinaka RT, Cloud K, Hampton O, Hoffmaster AR, Hill KK, Keim P, et al. Sequence and organization of pXO1, the large Bacillus anthracis plasmid harboring the anthrax toxin genes. J Bacteriol 1999;181:6509–15.
[52] Simon NC, Aktories K, Barbieri JT. Novel bacterial ADP-ribosylating toxins: structure and function. Nat Rev Microbiol 2014;12:599–611.
[53] Fieldhouse RJ, Turgeon Z, White D, Merrill AR. Cholera- and anthrax-like toxins are among several new ADP-ribosyltransferases. PLoS Comp Biol 2010;6:e1001029.
[54] Novick RP. Mobile genetic elements and bacterial toxinoses: the superantigen-encoding pathogenicity islands of Staphylococcus aureus. Plasmid 2003;49:93–105.
[55] Novick RP, Subedi A. The SaPIs: mobile pathogenicity islands of Staphylococcus. Chem Immunol Allergy 2007;93:42–57.
[56] Yamaguchi T, Hayashi T, Takami H, Nakasone K, Ohnishi M, Nakayama K, et al. Phage conversion of exfoliative toxin A production in Staphylococcus aureus. Mol Microbiol 2000;38:694–705.
[57] Yamaguchi T, Hayashi T, Takami H, Ohnishi M, Murata T, Nakayama K, et al. Complete nucleotide sequence of a Staphylococcus aureus exfoliative toxin B plasmid and identification of a novel ADP-ribosyltransferase, EDIN-C. Infect Immun 2001;69:7760–71.
[58] Yamaguchi T, Nishifuji K, Sasaki M, Fudaba Y, Aepfelbacher M, Takata T, et al. Identification of the Staphylococcus aureus etd pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B. Infect Immun 2002;70:5835–45.
[59] Kaneko J, Kamio Y. Bacterial two-component and hetero-heptameric pore-forming cytolytic toxins: structures, pore-forming mechanism, and organization of the genes. Biosci Biotechnol Biochem 2004;68: 981–1003.
[60] Narita S, Kaneko J, Chiba J, Piemont Y, Jarraud S, Etienne J, et al. Phage conversion of Panton-Valentine leukocidin in Staphylococcus aureus: molecular analysis of a PVL-converting phage, phiSLT. Gene 2001;268:195–206.
[61] Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP, Enright MC, et al. Complete genomes of two clinical Staphylococcus aureus strains: evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci USA 2004;101:9786–91.
[62] Lindsay JA, Ruzin A, Ross HF, Kurepina N, Novick RP. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in Staphylococcus aureus. Mol Microbiol 1998;29:527–43.
[63] Banks DJ, Beres SB, Musser JM. The fundamental contribution of phages to GAS evolution, genome diversification, and strain emergence. Trends Microbiol 2002;10:515–21.
[64] Beres SB, Sylva GL, Barbian KD, Lei B, Hoff JS, Mammarella ND, et al. Genome sequence of a serotype M3 strain of group A Streptococcus: phage-encoded toxins, the high-virulence phenotype, and clone emergence. Proc Natl Acad Sci USA 2002;99:10078–83.
[65] Sierig G, Cywes C, Wessels MR, Ashbaugh CD. Cytotoxic effects of streptolysin o and streptolysin s enhance the virulence of poorly encapsulated group a streptococci. Infect Immun 2003;71:446–55.
[66] Lee CH, Hu ST, Swiatek PJ, Moseley SL, Allen SD, So M. Isolation of a novel transposon which carries the Escherichia coli enterotoxin STII gene. J Bacteriol 1985;162:615–20.
[67] Blum G, Falbo V, Caprioli A, Hacker J. Gene clusters encoding the cytotoxic necrotizing factor type 1, Prs-fimbriae, and alpha-hemolysin form the pathogenicity island II of the uropathogenic Escherichia coli strain J96. FEMS Microbiol Lett 1995;126:189–95.
[68] Falbo V, Pace T, Picci L, Pizzi E, Caprioli A. Isolation and nucleotide sequence of the gene encoding cytotoxic necrotizing factor 1 of *Escherichia coli*. Infect Immun 1993;61:4909–14.
[69] Elsabbagh H, Xiong G, Lutz F. Nucleotide sequence of attP and cos sites of phage CTX and expression of cytotoxin in Pseudomonas aeruginosa PA158. Mol Gen Genet 1993;237:421–8.

[70] Hayashi T, Kamio Y, Hishinuma F, Usami Y, Titani K, Terawaki Y. Pseudomonas aeruginosa cytotoxin: the nucleotide sequence of the gene and the mechanism of activation of the protoxin. Mol Microbiol 1989;3:861–8.

[71] Nakao H, Pruckler JM, Mazurova IK, Narvskaia OV, Glushkevich T, Marijevski VF, et al. Heterogeneity of diphtheria toxin gene, tox, and its regulatory element, dtxR, in Corynebacterium diphtheriae strains causing epidemic diphtheria in Russia and Ukraine. J Clin Microbiol 1996;34:1711–6.

[72] Fossum Moen AE, Saltyte Benth J, Alm-Kristiansen K, Bukholm G. Exotoxin-encoding gene content in community-acquired and hospital-acquired methicillin-resistant Staphylococcus aureus. Clin Microbiol Infect 2009;15:1139–45.

[73] Davis BM, Waldor MK. Filamentous phages linked to virulence of Vibrio cholerae. Curr Opin Microbiol 2003;6:35–42.

[74] Jobling MG, Holmes RK. Type II heat-labile enterotoxins from 50 diverse Escherichia coli isolates belong almost exclusively to the LT-IIc family and may be prophage encoded. PLoS One 2012;7:e29898.

[75] Allison HE. Stx-phages: drivers and mediators of the evolution of STEC and STEC-like pathogens. Future Microbiol 2007;2:165–74.

[76] Muniesa M, Imamovic L, Jofre J. Bacteriophages and genetic mobilization in sewage and faecally polluted environments. Microb Biotechnol 2011;4:725–34.

[77] Smith DL, Rooks DJ, Fogg PC, Darby AC, Thomson NR, McCarthy AJ, et al. Comparative genomics of Shiga toxin encoding bacteriophages. BMC Genomics 2012;13:311.

[78] De Paepe M, Leclerc M, Tinsley CR, Petit MA. Bacteriophages: an underestimated role in human and animal health? Front Cell Infect Microbiol 2014;4:39.

[79] Martinez-Castillo A, Quiros P, Navarro F, Miro E, Muniesa M. Shiga toxin 2–encoding bacteriophages in human fecal samples from healthy individuals. Appl Environ Microbiol 2013;79:4862–8.

[80] Mills S, Shanahan F, Stanton C, Hill C, Coffey A, Ross RP. Movers and shakers: influence of bacteriophages in shaping the mammalian gut microbiota. Gut Microbes 2013;4:4–16.

[81] Paul JH. Prophages in marine bacteria: dangerous molecular time bombs or the key to survival in the seas? ISME J 2008;2:579–89.

[82] Garcia-Aljaro C, Muniesa M, Jofre J, Blanch AR. Prevalence of the stx2 gene in coliform populations from aquatic environments. Appl Environ Microbiol 2004;70:3535–40.

[83] Muniesa M, Jofre J. Abundance in sewage of bacteriophages infecting Escherichia coli O157:H7. Methods Mol Biol 2004;268:79–88.

[84] Cooley MB, Jay-Russell M, Atwill ER, Carychao D, Nguyen K, Quinones B, et al. Development of a robust method for isolation of Shiga toxin–positive Escherichia coli (STEC) from fecal, plant, soil, and water samples from a leafy greens production region in California. PLoS One 2013;8:e65716.

[85] Cooley MB, Quinones B, Oryang D, Mandrell RE, Gorski L. Prevalence of Shiga toxin producing Escherichia coli, Salmonella enterica, and Listeria monocytogenes at public access watershed sites in a California central coast agricultural region. Front Cell Infect Microbiol 2014;4:30.

[86] Holmes RK. Biology and molecular epidemiology of diphtheria toxin and the tox gene. J Infect Dis 2000;181(Suppl. 1):S156–67.

[87] Kaneko J, Kimura T, Narita S, Tomita T, Kamio Y. Complete nucleotide sequence and molecular characterization of the temperate staphylococcal bacteriophage phiPVL carrying Panton-Valentine leukocidin genes. Gene 1998;215:57–67.

[88] Calderwood SB, Mekalanos JJ. Iron regulation of Shiga-like toxin expression in Escherichia coli is mediated by the fur locus. J Bacteriol 1987;169:4759–64.

[89] Jackson MP, Newland JW, Holmes RK, O'Brien AD. Nucleotide sequence analysis of the structural genes for Shiga-like toxin I encoded by bacteriophage 933J from Escherichia coli. Microb Pathog 1987;2:147–53.

[90] Wagner PL, Livny J, Neely MN, Acheson DW, Friedman DI, Waldor MK. Bacteriophage control of Shiga toxin 1 production and release by Escherichia coli. Mol Microbiol 2002;44:957–70.

[91] Wagner PL, Neely MN, Zhang X, Acheson DW, Waldor MK, Friedman DI. Role for a phage promoter in Shiga toxin 2 expression from a pathogenic Escherichia coli strain. J Bacteriol 2001;183:2081–5.

[92] Gerber M, Walch C, Loffler B, Tischendorf K, Reischl U, Ackermann G. Effect of sub-MIC concentrations of metronidazole, vancomycin, clindamycin and linezolid on toxin gene transcription and production in Clostridium difficile. J Med Microbiol 2008;57:776–83.

[93] Kohler B, Karch H, Schmidt H. Antibacterials that are used as growth promoters in animal husbandry can affect the release of Shiga toxin-2–converting bacteriophages and Shiga toxin 2 from Escherichia coli strains. Microbiology 2000;146(Pt 5):1085–90.

[94] Kimmitt PT, Harwood CR, Barer MR. Toxin gene expression by shiga toxin-producing Escherichia coli: the role of antibiotics and the bacterial SOS response. Emerg Infect Dis 2000;6:458–65.

[95] Nassar FJ, Rahal EA, Sabra A, Matar GM. Effects of subinhibitory concentrations of antimicrobial agents on Escherichia coli O157:H7 Shiga toxin release and role of the SOS response. Foodborne Pathog Dis 2013;10:805–12.
[96] Ketley JM, Haslam SC, Mitchell TJ, Stephen J, Candy DC, Burdon DW. Production and release of toxins A and B by Clostridium difficile. J Med Microbiol 1984;18:385–91.
[97] Meessen-Pinard M, Sekulovic O, Fortier LC. Evidence of in vivo prophage induction during Clostridium difficile infection. Appl Environ Microbiol 2012;78:7662–70.
[98] Sekulovic O, Meessen-Pinard M, Fortier LC. Prophage-stimulated toxin production in Clostridium difficile NAP1/027 lysogens. J Bacteriol 2011;193:2726–34.
[99] Rupnik M. Clostridium difficile toxinotyping. Methods Mol Biol 2010;646:67–76.
[100] Rupnik M, Avesani V, Janc M, von Eichel-Streiber C, Delmee M. A novel toxinotyping scheme and correlation of toxinotypes with serogroups of Clostridium difficile isolates. J Clin Microbiol 1998;36:2240–7.
[101] Stabler RA, Gerding DN, Songer JG, Drudy D, Brazier JS, Trinh HT, et al. Comparative phylogenomics of Clostridium difficile reveals clade specificity and microevolution of hypervirulent strains. J Bacteriol 2006;188:7297–305.
[102] Stabler RA, He M, Dawson L, Martin M, Valiente E, Corton C, et al. Comparative genome and phenotypic analysis of Clostridium difficile 027 strains provides insight into the evolution of a hypervirulent bacterium. Genome Biol 2009;10:R102.
[103] Sumby P, Waldor MK. Transcription of the toxin genes present within the Staphylococcal phage phiSa3ms is intimately linked with the phage's life cycle. J Bacteriol 2003;185:6841–51.
[104] Wilson BA, Ho M. Pasteurella multocida: from zoonosis to cellular microbiology. Clin Microbiol Rev 2013;26:631–55.
[105] Clarke SC. Diarrhoeagenic Escherichia coli—an emerging problem? Diagn Microbiol Infect Dis 2001;41:93–8.
[106] Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev 1998;11:142–201.
[107] Fleckenstein JM, Hardwidge PR, Munson GP, Rasko DA, Sommerfelt H, Steinsland H. Molecular mechanisms of enterotoxigenic Escherichia coli infection. Microbes Infect 2010;12:89–98.
[108] Oswald E, de Rycke J, Lintermans P, van Muylem K, Mainil J, Daube G, et al. Virulence factors associated with cytotoxic necrotizing factor type 2 in bovine diarrheic and septicemic strains of Escherichia coli. J Clin Microbiol 1991;29:2522–7.
[109] Mainil JG, Jacquemin E, Oswald E. Prevalence and identity of cdt-related sequences in necrotoxigenic Escherichia coli. Vet Microbiol 2003;94:159–65.
[110] Bogyiova E, Siegfried L, Kmetova M, Sandorcinova Z, Liptakova A, Biros E. Occurrence and genetic association of selected virulence factors in clinical Escherichia coli isolates. Folia Microbiol (Praha) 2002;47:73–7.
[111] Landraud L, Gauthier M, Fosse T, Boquet P. Frequency of Escherichia coli strains producing the cytotoxic necrotizing factor (CNF1) in nosocomial urinary tract infections. Lett Appl Microbiol 2000;30:213–6.
[112] Mainil JG, Jacquemin E, Herault F, Oswald E. Presence of pap-, sfa-, and afa-related sequences in necrotoxigenic Escherichia coli isolates from cattle: evidence for new variants of the AFA family. Can J Vet Res 1997;61:193–9.
[113] Muller D, Hughes C, Goebel W. Relationship between plasmid and chromosomal hemolysin determinants of Escherichia coli. J Bacteriol 1983;153:846–51.
[114] Schmidt H, Kernbach C, Karch H. Analysis of the EHEC hly operon and its location in the physical map of the large plasmid of enterohaemorrhagic Escherichia coli O157:h7. Microbiology 1996;142(Pt 4):907–14.
[115] Hussein HS. Prevalence and pathogenicity of Shiga toxin–producing Escherichia coli in beef cattle and their products. J Anim Sci 2007;85:E63–72.
[116] Smith JL, Fratamico PM, Gunther IV NW. Shiga toxin–producing Escherichia coli. Adv Appl Microbiol 2014;86:145–97.
[117] Asakura H, Makino S, Kobori H, Watarai M, Shirahata T, Ikeda T, et al. Phylogenetic diversity and similarity of active sites of Shiga toxin (stx) in Shiga toxin–producing Escherichia coli (STEC) isolates from humans and animals. Epidemiol Infect 2001;127:27–36.
[118] Melton-Celsa A, Mohawk K, Teel L, O'Brien A. Pathogenesis of Shiga toxin–producing Escherichia coli. Curr Top Microbiol Immunol 2012;357:67–103.
[119] Bielaszewska M, Mellmann A, Zhang W, Kock R, Fruth A, Bauwens A, et al. Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. Lancet Infect Dis 2011;11:671–6.

[120] Rasko DA, Webster DR, Sahl JW, Bashir A, Boisen N, Scheutz F, et al. Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany. N Engl J Med 2011;365:709–17.
[121] Popoff MR. Botulinum neurotoxins: more and more diverse and fascinating toxic proteins. J Infect Dis 2014;209:168–9.
[122] Dineen SS, Bradshaw M, Johnson EA. Neurotoxin gene clusters in Clostridium botulinum type A strains: sequence comparison and evolutionary implications. Curr Microbiol 2003;46:345–52.
[123] Jacobson MJ, Lin G, Raphael B, Andreadis J, Johnson EA. Analysis of neurotoxin cluster genes in Clostridium botulinum strains producing botulinum neurotoxin serotype A subtypes. Appl Environ Microbiol 2008;74:2778–86.
[124] Zhou Y, Sugiyama H, Nakano H, Johnson EA. The genes for the Clostridium botulinum type G toxin complex are on a plasmid. Infect Immun 1995;63:2087–91.
[125] Eklund MW, Poysky FT. Interconversion of type C and D strains of Clostridium botulinum by specific bacteriophages. Appl Microbiol 1974;27:251–8.
[126] Hauser D, Gibert M, Marvaud JC, Eklund MW, Popoff MR. Botulinal neurotoxin C1 complex genes, clostridial neurotoxin homology and genetic transfer in Clostridium botulinum. Toxicon 1995;33:515–26.
[127] Hauser D, Gibert M, Boquet P, Popoff MR. Plasmid localization of a type E botulinal neurotoxin gene homologue in toxigenic Clostridium butyricum strains, and absence of this gene in non-toxigenic C. butyricum strains. FEMS Microbiol Lett 1992;78:251–5.
[128] Zhou Y, Sugiyama H, Johnson EA. Transfer of neurotoxigenicity from Clostridium butyricum to a nontoxigenic Clostridium botulinum type E–like strain. Appl Environ Microbiol 1993;59:3825–31.
[129] Marshall KM, Bradshaw M, Pellett S, Johnson EA. Plasmid encoded neurotoxin genes in Clostridium botulinum serotype A subtypes. Biochem Biophys Res Commun 2007;361:49–54.
[130] Smith TJ, Hill KK, Foley BT, Detter JC, Munk AC, Bruce DC, et al. Analysis of the neurotoxin complex genes in Clostridium botulinum A1-A4 and B1 strains: BoNT/A3, /Ba4 and /B1 clusters are located within plasmids. PLoS One 2007;2:e1271.
[131] Hutson RA, Zhou Y, Collins MD, Johnson EA, Hatheway CL, Sugiyama H. Genetic characterization of Clostridium botulinum type A containing silent type B neurotoxin gene sequences. J Biol Chem 1996;271:10786–92.
[132] Moriishi K, Koura M, Abe N, Fujii N, Fujinaga Y, Inoue K, et al. Mosaic structures of neurotoxins produced from Clostridium botulinum types C and D organisms. Biochim Biophys Acta 1996;1307:123–6.
[133] Nakamura K, Kohda T, Umeda K, Yamamoto H, Mukamoto M, Kozaki S. Characterization of the D/C mosaic neurotoxin produced by Clostridium botulinum associated with bovine botulism in Japan. Vet Microbiol 2010;140:147–54.
[134] Takeda M, Tsukamoto K, Kohda T, Matsui M, Mukamoto M, Kozaki S. Characterization of the neurotoxin produced by isolates associated with avian botulism. Avian Dis 2005;49:376–81.
[135] Barash JR, Arnon SS. A novel strain of Clostridium botulinum that produces type B and type H botulinum toxins. J Infect Dis 2014;209:183–91.
[136] Simpson L. The life history of a botulinum toxin molecule. Toxicon 2013;68:40–59.
[137] Gu S, Jin R. Assembly and function of the botulinum neurotoxin progenitor complex. Curr Top Microbiol Immunol 2013;364:21–44.
[138] Gu S, Rumpel S, Zhou J, Strotmeier J, Bigalke H, Perry K, et al. Botulinum neurotoxin is shielded by NTNHA in an interlocked complex. Science 2012;335:977–81.
[139] Raymond B, Young JC, Pallett M, Endres RG, Clements A, Frankel G. Subversion of trafficking, apoptosis, and innate immunity by type III secretion system effectors. Trends Microbiol 2013;21:430–41.
[140] Arnold R, Brandmaier S, Kleine F, Tischler P, Heinz E, Behrens S, et al. Sequence-based prediction of type III secreted proteins. PLoS Pathog 2009;5:e1000376.
[141] Dong X, Zhang YJ, Zhang Z. Using weakly conserved motifs hidden in secretion signals to identify type-III effectors from bacterial pathogen genomes. PLoS One 2013;8:e56632.
[142] Lower M, Schneider G. Prediction of type III secretion signals in genomes of gram-negative bacteria. PLoS One 2009;4:e5917.
[143] McDermott JE, Corrigan A, Peterson E, Oehmen C, Niemann G, Cambronne ED, et al. Computational prediction of type III and IV secreted effectors in gram-negative bacteria. Infect Immun 2011;79:23–32.
[144] Wang Y, Wei X, Bao H, Liu SL. Prediction of bacterial type IV secreted effectors by C-terminal features. BMC Genomics 2014;15:50.
[145] Zou L, Nan C, Hu F. Accurate prediction of bacterial type IV secreted effectors using amino acid composition and PSSM profiles. Bioinformatics 2013;29:3135–42.
[146] Salomon D, Kinch LN, Trudgian DC, Guo X, Klimko JA, Grishin NV, et al. Marker for type VI secretion system effectors. Proc Natl Acad Sci USA 2014;111:9271–6.

[147] Whitney JC, Beck CM, Goo YA, Russell AB, Harding BN, De Leon JA, et al. Genetically distinct pathways guide effector export through the type VI secretion system. Mol Microbiol 2014;92:529–42.
[148] Frank DW. The exoenzyme S regulon of Pseudomonas aeruginosa. Mol Microbiol 1997;26:621–9.
[149] Yahr TL, Vallis AJ, Hancock MK, Barbieri JT, Frank DW. ExoY, an adenylate cyclase secreted by the Pseudomonas aeruginosa type III system. Proc Natl Acad Sci USA 1998;95:13899–904.
[150] Sato H, Frank DW. ExoU is a potent intracellular phospholipase. Mol Microbiol 2004;53:1279–90.
[151] Dean P. Functional domains and motifs of bacterial type III effector proteins and their roles in infection. FEMS Microbiol Rev 2011;35:1100–25.
[152] Agbor TA, McCormick BA. Salmonella effectors: important players modulating host cell function during infection. Cell Microbiol 2011;13:1858–69.
[153] Figueira R, Holden DW. Functions of the Salmonella pathogenicity island 2 (SPI-2) type III secretion system effectors. Microbiology 2012;158:1147–61.
[154] Buchrieser C, Glaser P, Rusniok C, Nedjari H, D'Hauteville H, Kunst F, et al. The virulence plasmid pWR100 and the repertoire of proteins secreted by the type III secretion apparatus of Shigella flexneri. Mol Microbiol 2000;38:760–71.
[155] Cornelis GR, Boland A, Boyd AP, Geuijen C, Iriarte M, Neyt C, et al. The virulence plasmid of Yersinia, an antihost genome. Microbiol Mol Biol Rev 1998;62:1315–52.
[156] Bliska JB, Wang X, Viboud GI, Brodsky IE. Modulation of innate immune responses by Yersinia type III secretion system translocators and effectors. Cell Microbiol 2013;15:1622–31.
[157] Cornelis GR. The type III secretion injectisome, a complex nanomachine for intracellular "toxin" delivery. Biol Chem 2010;391:745–51.
[158] Phalipon A, Sansonetti PJ. Shigella's ways of manipulating the host intestinal innate and adaptive immune system: a tool box for survival? Immun Cell Biol 2007;85:119–29.
[159] Le Gall T, Mavris M, Martino MC, Bernardini ML, Denamur E, Parsot C. Analysis of virulence plasmid gene expression defines three classes of effectors in the type III secretion system of Shigella flexneri. Microbiology 2005;151:951–62.
[160] Parsot C. Shigella spp. and enteroinvasive Escherichia coli pathogenicity factors. FEMS Microbiol Lett 2005;252:11–18.
[161] Schmidt MA. LEEways: tales of EPEC, ATEC, and EHEC. Cell Microbiol 2010;12:1544–52.
[162] Loukiadis E, Nobe R, Herold S, Tramuta C, Ogura Y, Ooka T, et al. Distribution, functional expression, and genetic organization of Cif, a phage-encoded type III-secreted effector from enteropathogenic and enterohemorrhagic Escherichia coli. J Bacteriol 2008;190:275–85.
[163] Galan JE. Salmonella interactions with host cells: type III secretion at work. Annu Rev Cell Dev Biol 2001;17:53–86.
[164] Lostroh CP, Lee CA. The Salmonella pathogenicity island-1 type III secretion system. Microbes Infect 2001;3:1281–91.
[165] Kuhle V, Hensel M. Cellular microbiology of intracellular Salmonella enterica: functions of the type III secretion system encoded by Salmonella pathogenicity island 2. Cell Mol Life Sci 2004;61:2812–26.
[166] Miao EA, Miller SI. A conserved amino acid sequence directing intracellular type III secretion by Salmonella typhimurium. Proc Natl Acad Sci USA 2000;97:7539–44.
[167] Miao EA, Scherer CA, Tsolis RM, Kingsley RA, Adams LG, Baumler AJ, et al. Salmonella typhimurium leucine-rich repeat proteins are targeted to the SPI1 and SPI2 type III secretion systems. Mol Microbiol 1999;34:850–64.
[168] Zhang S, Santos RL, Tsolis RM, Mirold S, Hardt WD, Adams LG, et al. Phage mediated horizontal transfer of the sopE1 gene increases enteropathogenicity of Salmonella enterica serotype Typhimurium for calves. FEMS Microbiol Lett 2002;217:243–7.
[169] Mirold S, Ehrbar K, Weissmuller A, Prager R, Tschape H, Russmann H, et al. Salmonella host cell invasion emerged by acquisition of a mosaic of separate genetic elements, including Salmonella pathogenicity island 1 (SPI1), SPI5, and sopE2. J Bacteriol 2001;183:2348–58.
[170] Pelludat C, Mirold S, Hardt WD. The SopEPhi phage integrates into the ssrA gene of Salmonella enterica serovar Typhimurium A36 and is closely related to the Fels-2 prophage. J Bacteriol 2003;185:5182–91.
[171] Figueroa-Bossi N, Bossi L. Resuscitation of a defective prophage in Salmonella cocultures. J Bacteriol 2004;186:4038–41.
[172] Brown NF, Coombes BK, Bishop JL, Wickham ME, Lowden MJ, Gal-Mor O, et al. Salmonella phage ST64B encodes a member of the SseK/NleB effector family. PLoS One 2011;6:e17824.
[173] Christie PJ, Whitaker N, Gonzalez-Rivera C. Mechanism and structure of the bacterial type IV secretion systems. Biochim Biophys Acta 2014;1843:1578–91.
[174] Wallden K, Rivera-Calzada A, Waksman G. Type IV secretion systems: versatility and diversity in function. Cell Microbiol 2010;12:1203–12.

[175] Farizo KM, Huang T, Burns DL. Importance of holotoxin assembly in Ptl-mediated secretion of pertussis toxin from Bordetella pertussis. Infect Immun 2000;68:4049–54.
[176] Locht C, Coutte L, Mielcarek N. The ins and outs of pertussis toxin. Febs J 2011;278:4668–82.
[177] Weiss AA, Johnson FD, Burns DL. Molecular characterization of an operon required for pertussis toxin secretion. Proc Natl Acad Sci USA 1993;90:2970–4.
[178] Backert S, Ziska E, Brinkmann V, Zimny-Arndt U, Fauconnier A, Jungblut PR, et al. Translocation of the Helicobacter pylori CagA protein in gastric epithelial cells by a type IV secretion apparatus. Cell Microbiol 2000;2:155–64.
[179] Odenbreit S, Puls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. Science 2000;287:1497–500.
[180] Pitzschke A, Hirt H. New insights into an old story: Agrobacterium-induced tumour formation in plants by plant transformation. EMBO J 2010;29:1021–32.
[181] Pitzschke A, Djamei A, Teige M, Hirt H. VIP1 response elements mediate mitogen-activated protein kinase 3-induced stress gene expression. Proc Natl Acad Sci USA 2009;106:18414–9.
[182] Voth DE, Broederdorf LJ, Graham JG. Bacterial type IV secretion systems: versatile virulence machines. Future Microbiol 2012;7:241–57.
[183] Dehio C. Infection-associated type IV secretion systems of Bartonella and their diverse roles in host cell interaction. Cell Microbiol 2008;10:1591–8.
[184] Engel P, Salzburger W, Liesch M, Chang CC, Maruyama S, Lanz C, et al. Parallel evolution of a type IV secretion system in radiating lineages of the host-restricted bacterial pathogen Bartonella. PLoS Genet 2011;7:e1001296.
[185] Myeni S, Child R, Ng TW, Kupko III JJ, Wehrly TD, Porcella SF, et al. Brucella modulates secretory trafficking via multiple type IV secretion effector proteins. PLoS Pathog 2013;9:e1003556.
[186] Isaac DT, Isberg R. Master manipulators: an update on Legionella pneumophila Icm/Dot translocated substrates and their host targets. Future Microbiol 2014;9:343–59.
[187] Rolando M, Buchrieser C. Legionella pneumophila type IV effectors hijack the transcription and translation machinery of the host cell. Trends Cell Biol 2014.
[188] Weber MM, Chen C, Rowin K, Mertens K, Galvan G, Zhi H, et al. Identification of Coxiella burnetii type IV secretion substrates required for intracellular replication and Coxiella-containing vacuole formation. J Bacteriol 2013;195:3914–24.
[189] Bhatty M, Laverde Gomez JA, Christie PJ. The expanding bacterial type IV secretion lexicon. Res Microbiol 2013;164:620–39.
[190] Coulthurst SJ. The type VI secretion system—a widespread and versatile cell targeting system. Res Microbiol 2013;164:640–54.
[191] Ho BT, Dong TG, Mekalanos JJ. A view to a kill: the bacterial type VI secretion system. Cell Host Microbe 2014;15:9–21.
[192] Pukatzki S, Ma AT, Sturtevant D, Krastins B, Sarracino D, Nelson WC, et al. Identification of a conserved bacterial protein secretion system in Vibrio cholerae using the Dictyostelium host model system. Proc Natl Acad Sci USA 2006;103:1528–33.
[193] Suarez G, Sierra JC, Erova TE, Sha J, Horneman AJ, Chopra AK. A type VI secretion system effector protein, VgrG1, from Aeromonas hydrophila that induces host cell toxicity by ADP ribosylation of actin. J Bacteriol 2010;192:155–68.
[194] Toesca IJ, French CT, Miller JF. The type VI secretion system spike protein VgrG5 mediates membrane fusion during intercellular spread by pseudomallei group Burkholderia species. Infect Immun 2014;82:1436–44.
[195] Schwarz S, Singh P, Robertson JD, LeRoux M, Skerrett SJ, Goodlett DR, et al. VgrG-5 is a Burkholderia type VI secretion system-exported protein required for multinucleated giant cell formation and virulence. Infect Immun 2014;82:1445–52.
[196] Xu H, Yang J, Gao W, Li L, Li P, Zhang L, et al. Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. Nature 2014.
[197] Mel SF, Mekalanos JJ. Modulation of horizontal gene transfer in pathogenic bacteria by in vivo signals. Cell 1996;87:795–8.
[198] Shoemaker NB, Vlamakis H, Hayes K, Salyers AA. Evidence for extensive resistance gene transfer among Bacteroides spp. and among Bacteroides and other genera in the human colon. Appl Environ Microbiol 2001;67:561–8.
[199] Sommer MO, Church GM, Dantas G. The human microbiome harbors a diverse reservoir of antibiotic resistance genes. Virulence 2010;1:299–303.
[200] Szmolka A, Nagy B. Multidrug resistant commensal Escherichia coli in animals and its impact for public health. Front Microbiol 2013;4:258.

[201] Wichmann F, Udikovic-Kolic N, Andrew S, Handelsman J. Diverse antibiotic resistance genes in dairy cow manure. MBio 2014;5:e01017.
[202] Coyne MJ, Zitomersky NL, McGuire AM, Earl AM, Comstock LE. Evidence of extensive DNA transfer between bacteroidales species within the human gut. MBio 2014;5:e01305–e01314.
[203] Schjorring S, Krogfelt KA. Assessment of bacterial antibiotic resistance transfer in the gut. Int J Microbiol 2011;2011:312956.
[204] Ferguson GC, Heinemann JA, Kennedy MA. Gene transfer between Salmonella enterica serovar Typhimurium inside epithelial cells. J Bacteriol 2002;184:2235–42.
[205] Gomez-Valero L, Rusniok C, Jarraud S, Vacherie B, Rouy Z, Barbe V, et al. Extensive recombination events and horizontal gene transfer shaped the Legionella pneumophila genomes. BMC Genomics 2011;12:536.
[206] Aviles-Jimenez F, Letley DP, Gonzalez-Valencia G, Salama N, Torres J, Atherton JC. Evolution of the Helicobacter pylori vacuolating cytotoxin in a human stomach. J Bacteriol 2004;186:5182–5.
[207] Gangwer KA, Shaffer CL, Suerbaum S, Lacy DB, Cover TL, Bordenstein SR. Molecular evolution of the Helicobacter pylori vacuolating toxin gene vacA. J Bacteriol 2010;192:6126–35.
[208] Chen J, Novick RP. Phage-mediated intergeneric transfer of toxin genes. Science 2009;323:139–41.
[209] Spaulding AR, Salgado-Pabon W, Kohler PL, Horswill AR, Leung DY, Schlievert PM. Staphylococcal and streptococcal superantigen exotoxins. Clin Microbiol Rev 2013;26:422–47.
[210] Bohach GA, Fast DJ, Nelson RD, Schlievert PM. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit Rev Microbiol 1990;17:251–72.
[211] Thomas D, Chou S, Dauwalder O, Lina G. Diversity in Staphylococcus aureus enterotoxins. Chem Immunol Allergy 2007;93:24–41.
[212] Brody T, Yavatkar AS, Lin Y, Ross J, Kuzin A, Kundu M, et al. Horizontal gene transfers link a human MRSA pathogen to contagious bovine mastitis bacteria. PLoS One 2008;3:e3074.
[213] Malachowa N, DeLeo FR. Mobile genetic elements of Staphylococcus aureus. Cell Mol Life Sci 2010;67:3057–71.
[214] Fischetti VA. In vivo acquisition of prophage in Streptococcus pyogenes. Trends Microbiol 2007;15:297–300.
[215] Broudy TB, Pancholi V, Fischetti VA. Induction of lysogenic bacteriophage and phage-associated toxin from group a streptococci during coculture with human pharyngeal cells. Infect Immun 2001;69:1440–3.
[216] Broudy TB, Fischetti VA. In vivo lysogenic conversion of Tox(-) Streptococcus pyogenes to Tox(+) with lysogenic Streptococci or free phage. Infect Immun 2003;71:3782–6.
[217] Dalsgaard A, Serichantalergs O, Forslund A, Lin W, Mekalanos J, Mintz E, et al. Clinical and environmental isolates of Vibrio cholerae serogroup O141 carry the CTX phage and the genes encoding the toxin-coregulated pili. J Clin Microbiol 2001;39:4086–92.
[218] Karaolis DK, Somara S, Maneval Jr. DR, Johnson JA, Kaper JB. A bacteriophage encoding a pathogenicity island, a type-IV pilus, and a phage receptor in cholera bacteria. Nature 1999;399:375–9.
[219] Mekalanos JJ, Rubin EJ, Waldor MK. Cholera: molecular basis for emergence and pathogenesis. FEMS Immunol Med Microbiol 1997;18:241–8.
[220] O'Shea YA, Reen FJ, Quirke AM, Boyd EF. Evolutionary genetic analysis of the emergence of epidemic Vibrio cholerae isolates on the basis of comparative nucleotide sequence analysis and multilocus virulence gene profiles. J Clin Microbiol 2004;42:4657–71.
[221] Boyd EF, Waldor MK. Alternative mechanism of cholera toxin acquisition by Vibrio cholerae: generalized transduction of CTXPhi by bacteriophage CP-T1. Infect Immun 1999;67:5898–905.
[222] Preston A, Parkhill J, Maskell DJ. The bordetellae: lessons from genomics. Nat Rev Microbiol 2004;2:379–90.
[223] Gross R, Guzman CA, Sebaihia M, dos Santos VA, Pieper DH, Koebnik R, et al. The missing link: Bordetella petrii is endowed with both the metabolic versatility of environmental bacteria and virulence traits of pathogenic Bordetellae. BMC Genomics 2008;9:449.
[224] Park J, Zhang Y, Buboltz AM, Zhang X, Schuster SC, Ahuja U, et al. Comparative genomics of the classical Bordetella subspecies: the evolution and exchange of virulence-associated diversity amongst closely related pathogens. BMC Genomics 2012;13:545.
[225] Parkhill J, Sebaihia M, Preston A, Murphy LD, Thomson N, Harris DE, et al. Comparative analysis of the genome sequences of Bordetella pertussis, Bordetella parapertussis and Bordetella bronchiseptica. Nat Genet 2003;35:32–40.
[226] Arico B, Rappuoli R. Bordetella parapertussis and Bordetella bronchiseptica contain transcriptionally silent pertussis toxin genes. J Bacteriol 1987;169:2847–53.
[227] Hausman SZ, Cherry JD, Heininger U, Wirsing von Konig CH, Burns DL. Analysis of proteins encoded by the ptx and ptl genes of Bordetella bronchiseptica and Bordetella parapertussis. Infect Immun 1996;64:4020–6.

[228] Demaneche S, Kay E, Gourbiere F, Simonet P. Natural transformation of Pseudomonas fluorescens and Agrobacterium tumefaciens in soil. Appl Environ Microbiol 2001;67:2617–21.
[229] Lu N, Mylon SE, Kong R, Bhargava R, Zilles JL, Nguyen TH. Interactions between dissolved natural organic matter and adsorbed DNA and their effect on natural transformation of Azotobacter vinelandii. Sci Total Environ 2012;426:430–5.
[230] Ceremonie H, Buret F, Simonet P, Vogel TM. Isolation of lightning-competent soil bacteria. Appl Environ Microbiol 2004;70:6342–6.
[231] Ashelford KE, Day MJ, Fry JC. Elevated abundance of bacteriophage-infecting bacteria in soil. Appl Environ Microbiol 2003;69:285–9.
[232] Nasir A, Forterre P, Kim KM, Caetano-Anolles G. The distribution and impact of viral lineages in domains of life. Front Microbiol 2014;5:194.
[233] Srinivasiah S, Bhavsar J, Thapar K, Liles M, Schoenfeld T, Wommack KE. Phages across the biosphere: contrasts of viruses in soil and aquatic environments. Res Microbiol 2008;159:349–57.
[234] Han CS, Xie G, Challacombe JF, Altherr MR, Bhotika SS, Brown N, et al. Pathogenomic sequence analysis of Bacillus cereus and Bacillus thuringiensis isolates closely related to Bacillus anthracis. J Bacteriol 2006;188:3382–90.
[235] Kolsto AB, Tourasse NJ, Okstad OA. What sets Bacillus anthracis apart from other Bacillus species? Annu Rev Microbiol 2009;63:451–76.
[236] Read TD, Peterson SN, Tourasse N, Baillie LW, Paulsen IT, Nelson KE, et al. The genome sequence of Bacillus anthracis Ames and comparison to closely related bacteria. Nature 2003;423:81–6.
[237] Okinaka R, Cloud K, Hampton O, Hoffmaster A, Hill K, Keim P, et al. Sequence, assembly and analysis of pX01 and pX02. J Appl Microbiol 1999;87:261–2.
[238] Andrup L, Jorgensen O, Wilcks A, Smidt L, Jensen GB. Mobilization of “nonmobilizable” plasmids by the aggregation-mediated conjugation system of Bacillus thuringiensis. Plasmid 1996;36:75–85.
[239] Pannucci J, Okinaka RT, Sabin R, Kuske CR. Bacillus anthracis pXO1 plasmid sequence conservation among closely related bacterial species. J Bacteriol 2002;184:134–41.
[240] Hu X, Van der Auwera G, Timmery S, Zhu L, Mahillon J. Distribution, diversity, and potential mobility of extrachromosomal elements related to the Bacillus anthracis pXO1 and pXO2 virulence plasmids. Appl Environ Microbiol 2009;75:3016–28.
[241] Kiel JL, Parker JE, Holwitt EA, McCreary RP, Andrews CJ, De Los Santos A, et al. Geographical distribution of genotypic and phenotypic markers among Bacillus anthracis isolates and related species by historical movement and horizontal transfer. Folia Microbiol (Praha) 2008;53:472–8.
[242] Papazisi L, Rasko DA, Ratnayake S, Bock GR, Remortel BG, Appalla L, et al. Investigating the genome diversity of B. cereus and evolutionary aspects of B. anthracis emergence. Genomics 2011;98: 26–39.
[243] Zwick ME, Joseph SJ, Didelot X, Chen PE, Bishop-Lilly KA, Stewart AC, et al. Genomic characterization of the Bacillus cereus sensu lato species: backdrop to the evolution of Bacillus anthracis. Genome Res 2012;22:1512–24.
[244] Lupo A, Coyne S, Berendonk TU. Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Front Microbiol 2012;3:18.
[245] Rizzo L, Manaia C, Merlin C, Schwartz T, Dagot C, Ploy MC, et al. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review. Sci Total Environ 2013;447:345–60.
[246] Dunny GM. The peptide pheromone-inducible conjugation system of Enterococcus faecalis plasmid pCF10: cell-cell signalling, gene transfer, complexity and evolution. Philos Trans R Soc Lond B Biol Sci 2007;362:1185–93.
[247] Hausner M, Wuertz S. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. Appl Environ Microbiol 1999;65:3710–3.
[248] Hohnstock AM, Stuart-Keil KG, Kull EE, Madsen EL. Naphthalene and donor cell density influence field conjugation of naphthalene catabolism plasmids. Appl Environ Microbiol 2000;66:3088–92.
[249] Lilley AK, Bailey MJ, Barr M, Kilshaw K, Timms-Wilson TM, Day MJ, et al. Population dynamics and gene transfer in genetically modified bacteria in a model microcosm. Mol Ecol 2003;12:3097–107.
[250] Singh PK, Meijer WJ. Diverse regulatory circuits for transfer of conjugative elements. FEMS Microbiol Lett 2014;358:119–28.
[251] Wetzel ME, Kim KS, Miller M, Olsen GJ, Farrand SK. Quorum-dependent mannopine-inducible conjugative transfer of an Agrobacterium opine-catabolic plasmid. J Bacteriol 2014;196:1031–44.
[252] Azam F, Long RA. Sea snow microcosms. Nature 2001;414(495):7–8.
[253] Takemura AF, Chien DM, Polz MF. Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. Front Microbiol 2014;5:38.