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Bacteriophages: Practical Applications for Nature's Biocontrol

First and foremost, I give thanks to the Almighty God for giving me the knowledge, the strength and the direction to complete this summary of my life's work on bacteriophages. I dedicate this book to my wife Ghazal, and my three daughters: Maryam, Sarah and Amna in thanks for their unwavering support and understanding. In recognition for his dedication and assistance in articulating this work, I have named my friend Richard Limoges as my co-author. Last but not least, I share this information as guidance to researchers and students who seek a greater understanding of natural biological processes and wish to serve mankind with their knowledge and talents.

Sabah A.A. Jassim

Preface

We find ourselves in the twenty-first century with a world of disenchantment, a self-imposed return to the dark ages of medicine! Most antibiotics are unable to treat multidrug-resistant bacteria, which are causing serious diseases. Prior to the discovery of penicillin, there were fewer bacteria that caused diseases, fewer bacterial mutations, less food poisoning, less water contamination. Our own interventions have caused bacterial mutations resulting in more lethal bacteria with fewer remedies. Throughout much of the twentieth century, antibiotics have been our primary defense against bacterial diseases. The excessive and inappropriate use of antibiotics particularly in animal husbandry is at the root of this problem and threatening their efficacy. The pharmaceutical industry appears unlikely to offer the necessary countermeasures because of the objective difficulties with synthesis of new antibiotics. The inexorable rise in the incidence of antibiotic resistance in bacterial pathogens, coupled with the low rate of emergence of new, clinically useful antibiotics, have encouraged researchers to revisit the bacteriophage and the potential utility of bacteriophages in biocontrol and for preventing or treating human and animal bacterial diseases.

The proper use of lytic 'virulent' bacteriophages through dietary and environmental application shows promise in livestock and poultry in particular. Bacteriophages may also be used to enhance or rekindle the effectiveness of antibiotics in numerous applications. Bacteriophages are known to have some advantages associated with human therapy over the use of antibiotics. However, we urge caution since the mechanism that caused the spread of antibiotic resistance genes between bacteria occurs most often through lysogenic bacteriophage-mediated transduction. Inappropriate use of bacteriophages could similarly lead to bacterial development of bacteriophage resistance. Furthermore, bacteriophage proteins including those that are genetically modified for commercial purposes, may also integrate into human and animal society with unknown effect. Therefore, it would be wise to approach such methodologies with caution in order to avoid repeating mistakes that were made with the improper use of antibiotics.

We suggest the use of properly developed and highly virulent lytic bacteriophages for environmental biocontrol to selectively reduce or eliminate problematic

bacteria from sensitive environments. Bacteriophages can be effective in decontamination and sanitation of both natural and manmade environments, including farms, factories, in workplaces, crowded places, and healthcare settings or in the laboratory. When strategically applied, they can be used without harmful effect on and around people and animals to eliminate harmful bacteria while supporting beneficial microflora. The ability of bacteriophages to recognize precisely their target hosts, renders them as favorable antibacterial agents compared to broad-spectrum antibiotics which kill target bacteria along with other beneficial bacteria. In this book we discuss the safe use of bacteriophages as antidotes or as a biocontrol from farm to fork and as a biodefence or to prevent biothreats while recognizing the obstacles associated with their use.

Windsor, ON, Canada
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Sabah A.A. Jassim
Richard G. Limoges

About the Authors

Professor Sabah A.A. Jassim Adjunct Professor, Civil and Environmental Engineering, University of Windsor, is CEO of Applied Bio Research Inc., Canada. His research and academic contributions span 29 years. Sabah was awarded his M. Phil and Ph.D. degrees from Nottingham University and Loughborough University, respectively, both in the UK. He has worked as a faculty research fellow and an adjunct professor at Nottingham University, UK and University of Guelph in Canada, respectively, focussing in phage biotechnologies. He was also a visiting professor at Universiti Putra Malaysia supervising a research postdoctoral team working on phage design technology. Sabah has also worked extensively in the private sector focussing on practical applications for his scientific research, especially relating to biota as well as bacteriophages and related topics.

Sabah was also once listed 13th of Power 500 the World's Most Influential Arabs/Middle East by Arabian Business Journal. Winner of several best research awards, he made trend-setting achievements to the state of the art in bacteriophage breeding and design technology to produce large-scale highly lytic phages for biocontrol systems. These include using phages for rapid bacterial detection, rapid drug susceptibility testing, biocontrol, alternative therapy, molecular detection and characterization of bacterial pathogens, control of pathogens in environmental industries, microbial bioluminescence, deletion of bacterial biofilm, bacterial stress response, controlling harmful algal blooms and novel methods in wastewater treatment.

His more recent innovative phage programming technology represents a model for smart phages to gain a high-speed infection against their counterpart bacterial pathogens which can play a significant role in decreasing bacterial pathogenic risk, preventing loss of life and reducing the use of antibiotics in animal agriculture industries. Dr. Jassim holds 18 international patents in several biological sciences including phage biotechnologies. Three of these technologies have been transferred to industrial practice. He has published extensively in prestigious journals and conferences including peer-reviewed research and review articles as well as book chapters and is a consistent leader in R&D to enhance bacteriophages infectious activity to their target bacteria. Sabah has devoted much of his research to using

phages as a novel, environmentally friendly biocontrol, particularly in agricultural applications from farm to fork.

Mr. Richard G. Limoges currently a businessman, is operating two successful small businesses in Windsor, Ontario where he met Sabah several years ago. Mr. Limoges now acts as Chief Administrative Officer of Applied Bio Research, Inc., a company dedicated to the commercialization of Dr. Jassim's various innovative technologies. Rick has a long history of community service having served for 14 years (5 terms) as a Member of Windsor City Council and Chair of numerous Local Boards and Committees. He was next elected Member of Parliament, Windsor-St. Clair in Canada's 36th Parliament. Prior to his election as an M.P., Mr. Limoges worked as a Senior Manager in one of Canada's largest banks. Rick is a graduate of the University of Windsor with Honours in Business Administration, and has applied his communication skills to assist Dr. Jassim in disseminating his research and life's work into several peer-reviewed publications in scientific journals and now this book. In recognition of Rick's efforts and dedication to assisting Dr. Jassim with this work, he is honored to be named as co-author in several of Sabah's publications.

Acronyms and Abbreviations

AAP	American Academy of Pediatrics
ABHRs	Alcohol-Based Hand Rubs
AGPs	Antibiotic Growth-Promoters
AMR	Antimicrobial Resistance
ATP	Adenosine Triphosphate
BGA	Blue-Green Algae
BoNT	*Clostridium botulinum* Neurotoxin
BSL-2	Biosafety Level-2
BTA	Biothreat Alarm System
BZ	Burst Size
CA	Community-Associated
CCN	Conical Cyanophage Net
CDC	Centers for Diseases Control and Prevention
CFR	Code of Federal Regulations
CFU	Colony-Forming Units
$CHAP_K$	Cysteine- and Histidine-Dependent Amidohydrolase/Peptidase
CoNS	Coagulase-Negative Staphylococci
CoPS	Coagulase-Positive Staphylococci
CRE	Carbapenem-Resistant Enterobacteriaceae
CyanoHABs	Cyanobacterial Harmful Algal Blooms
DFPS	Dry Fog Phage System
DNA	Deoxyribose Nucleic Acid
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EHEC	Enterohemorrhagic *E. coli*
ELISA	Enzyme-Linked Immunosorbent Assay
EMRSA	Epidemic MRSA
EPA	Environmental Protection Agency
ESBL	Multidrug-Resistant Extended-Spectrum β-Lactamase
ESR	Institute for Environmental Science and Research

EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FIGE	Field Inversion Gel Electrophoresis
FnBPA	Fibronectin-Binding Protein A
FSEP	Food Safety Enhancement Program
FSIS	Food Safety Inspection Services
GFP	Green Fluorescent Protein
GHG	Greenhouse Gas
GMP	Good Manufacturing Practices
GRAS	Generally Recognized as Safe
HA	Hospital-Associated or Acquired
HABs	Harmful Algal Blooms
HACCP	Hazard Analysis Critical Control Point
HCW	Healthcare Worker
HGT	Horizontal Gene Transfer
HICPAC	Healthcare Infection Control Practices Advisory Committee
ICMSF	International Commission for Microbiological Safety of Foods
IDSA	Infectious Diseases Society of America
IR	Infective Ratio
LA	Luria Agar
LA-MRSA	Livestock-Associated MRSA
LB	Luria Broth
LEAD	Livestock, Environment and Development
LODs	Limits of detections
	LuxAB-PASA
	LuxAB-Phage Anthracis Spore Alarm
MDRB	Multidrug-Resistant Bacteria
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MRSP	Methicillin-Resistant *Staphylococcus pseudintermedius*
MSSA	Methicillin-Susceptible *Staphylococcus aureus*
NDM	New Delhi Metallo-β-Lactamase
OIE	World Organisation for Animal Health
PAD	Phage Alarm and Detector
PBHR	Phage-Based Hand Rubs
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PDA	Phage Alarm and Detector
PFGE	Pulsed-Field Gel Electrophoresis
PFU	Plaque Forming Units
PIA	Polysaccharide Intercellular Adhesion
PLPs	Phage-Like Particles
PRE	Pomegranate Rind Extract
PVL	Panton-Valentine Leukocidin
Q&Q	Qualitative and Quantitative

QRA	Quantitative Risk Assessment
RNA	Ribonucleic Acid
SE	*Salmonella enterica* serovar Enteritidis
SEs	Staphylococcal Enterotoxins
SHEA	Society for Healthcare Epidemiology of America
STEC	Shiga-Toxin producing *E. coli*
SUR	Solar Ultraviolet Radiations
UK	United Kingdom
US FDA	United States Food and Drug Administration
USA	United States of America
USDA	United States Department of Agriculture
USDA-FSIS	United States Department of Agriculture- Food Safety and Inspection Service
US-FSIS	US-Food Safety and Inspection Service
UTIs	Urinary Tract Infections
UV	Ultraviolet
VFA	Volatile Fatty Acids
WHO	World Health Organization

Contents

Chapter 1
Enhanced Bacteriophages

Abstract The bacterial cell wall is the most important part of the bacterial structure for bacteriophage attachment, which is required to initiate infection. The rapid and precise attachment of the phage onto a susceptible host cell is the first step of infection. In this chapter, methods are described to control phage-host interactions and to produce highly lytic phages with no or far less phage-resistant mutants, along with broad host targeting capabilities. These methods do not employ genetic modification to breed 're-tailored' wild phages with auxiliary mechanisms for phage adherence, adsorption, binding and uptake which are critical for plaque formation. The purpose of these tactics is to gain new sub-strains of phages that are able to infect previously resistant bacteria and to play an important role in future applications.

Keywords Bacteriophage · Phage design · Phage breeding · Phage reprogramming technology

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