

The Bacteriocins

Current Knowledge and Future Prospects

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Preface

From the fine-scale biophysics of protein folding to the mathematical modelling of antagonism and altruism in microbial communities, the bacteriocins have proven an unexpectedly rich entryway into multiple subdisciplines within the life sciences. This structurally, functionally, and phylogenetically diverse class of proteins, first described nearly a century ago, has steadily attracted the attention of talented investigators around the world.

As this volume makes clear, these proteins continue to generate unexpected insights and rich hypotheses at multiple scales of biological organization. At the molecular level, every aspect of bacteriocin function – from their synthesis, to their translocation across the membrane of target bacteria, to their rapid killing activity – justly elicits our amazement. While we have learned a great deal about structure–function relationships from the bacteriocins, their stability, folding kinetics and possible interactions with other proteins are not yet fully elucidated. At the cellular level, the regulation of bacteriocin synthesis and activity has attracted increasing attention. We have known for some time that bacteriocin production is often a component of the stress response in a number of bacterial species, but we are only now beginning to fully understand the regulatory networks in which bacteriocins are embedded. Conversely, while the bactericidal effects of these proteins are well known, the potential functional consequences of sublethal bacteriocin concentrations remain unexplored. Finally, these proteins raise rich ecological and evolutionary riddles: What role do bacteriocins play in the emerging portrait of the microbiome? Why do virtually all Bacteria and Archaea examined to date produce some kind of bacteriocin-like protein? Why do some, but not all, bacteriocins exhibit a narrow killing spectrum?

These and other questions are tantalizing in their own right, but understanding the biology of bacteriocins has taken on a new urgency. Although their potential as therapeutic molecules was foreshadowed decades ago, it is only recently that they have emerged as serious lead compounds for drug design. The renaissance of interest in the applied biology of bacteriocins has been driven, at least in part, by the growing realization that we are reaching the end of the conventional antibiotic era. As the clinical and economic costs of antibiotic resistance continue to mount, as the pipeline for new antibiotic classes slows to a trickle, and as the importance of healthy microbiome snaps into focus, interest in the therapeutic potential of the bacteriocins has grown. As this volume makes clear, evolution has been confronting and solving both the challenges of evolving novel antibiotics and of outpacing emerging resistance. As our species, *Homo sapiens*, confronts those very same challenges, it behoves us to consider that potential answers may be hidden in plain sight – if only we are astute enough to see them. This volume, we hope, represents a step in that direction, and

honours a colleague whose career has been dedicated to understanding bacteriocins in all their complex and messy beauty. We dedicate this book to our colleague Richard James and to the generation of researchers and students that he has inspired.

Robert Dorit, Margaret Riley and Sandra Roy

Foreword

It was a very pleasant surprise when Peg Riley and Rob Dorit informed me that this Caister Academic Press volume on bacteriocins was to be dedicated in my honour. This accolade follows a Biochemical Society Focussed Meeting¹ in Nottingham in 2012 organized by Colin Kleanthous, Chris Penfold and Dan Walker in honour of my retirement. I am very grateful for the recognition of the work of my research group with bacteriocins conferred by my peers by both events. In this preface I will provide some background on my 34 years of working with bacteriocins and my perspective on the bacteriocin research field.

When I was a final-year undergraduate student in London in 1970 I gave a short talk on antibiotics to my fellow students at the graduation dinner of my BSc (Hons) Applied Biology course in which I had specialized in microbiology. Antibiotics was a subject that had fascinated me during my undergraduate degree; little did I know that I would spend much of the rest of my academic career working in this field. My PhD was carried out at the Middlesex Hospital Medical School in London in a small lab working on the radiation biology of *E. coli* bacteria, led by Dr Neil Gillies. This work introduced me to mutations such as *lon* which make *E. coli* cells exquisitely sensitive to low doses of radiation by inhibiting cell division and inducing the formation of long filamentous cells.

In 1973, my interest in bacterial cell division then took me to work as a Postdoctoral Fellow with Professor Arthur Pardee at Princeton University, New Jersey, USA, for 2 years. One of the major lines of research in the lab was the use of the new beta-lactam antibiotic FL1060 (mecillinam) which caused the rounding up of cells. Another English postdoc, Brian Spratt, and I speculated that mecillinam may inhibit murein synthesis required for cell elongation as a rod, in contrast to other beta-lactams such as cephalixin which caused filament formation by inhibiting murein synthesis required for the formation of cross walls during septation. Brian and I separately published a number of papers which explained the mechanism of inhibition of cell division by mecillinam.

On my return to England I was offered a Lectureship in the School of Biological Sciences at the University of East Anglia (UEA) teaching mostly medical microbiology. I was happy to offer a postdoctoral position to work in my laboratory to Dr Pearl Cooper, a microbial geneticist, and this was serendipitously to lead to my interest in colicins, bacteriocins produced by *E. coli*. Pearl, who had taught microbiology at UEA previously, was helping to run a practical class which she had designed in which students isolated bacteria from the intestines

¹ How bugs kill bugs: progress and challenges in bacteriocin research. *Biochem. Soc. Trans.* 40, 1433–1565 (2012).

of chickens and then characterized them in terms of aerobic/anaerobic growth, antibiotic sensitivity and bacteriocin production. Bacteriocin-producing strains were further characterized by the students in an attempt to identify the group of bacteriocin being produced. One pair of students identified a J strain which exhibited an unusual pattern of production of a colicin. In subsequent work back in the lab Pearl determined that J contained seven different plasmids and produced both an E colicin and colicin M. Using transformation and conjugation methods, Pearl demonstrated that two of the small plasmids encoded different E colicins, and a large plasmid encoded colicin M production and colicin B immunity. This single strain turned out to be a treasure trove of science which kept researchers in my laboratory busy for the next 30 years. I always used the story of this strain as an example to academics of the potential value to them of teaching students.

E. coli transformed with one of the two E colicin encoding plasmids was then tested for immunity to the seven known E colicin-encoding strains (ColE1 to ColE7). The basis of immunity testing is that each of the seven strains are immune to killing by strains producing the same E colicin but are sensitive to the other six E colicins. This immunity testing confirmed that J encoded two new E colicins (ColE8 and ColE9). The immunity testing also highlighted an unusual non-reciprocal immunity in which a ColE3-producing strain was immune to both ColE3 and ColE8, whilst a ColE9-producing strain was immune to both ColE9 and ColE5. I speculated that the observed non-reciprocal immunity could be due to a high homology between ColE3 and ColE8 (or between ColE5 and ColE9), such that the ColE3 immunity gene protected against both ColE3 and ColE8. An alternative explanation was that the ColE3 plasmid encoded two different immunity genes, one of which gave protection against ColE3 and the other against ColE8. In order to answer this question we had to learn the new techniques of gene cloning and transposon mutagenesis in the early 1980s so that we could clone and analyse fragments of E colicin-encoding plasmids. Kin Chak in my laboratory showed unequivocally that the ColE3-CA38 plasmid carried a separate, tandem immunity gene that confers immunity to ColE8. A follow-on paper by Kin Chak in 1986 confirmed that the ColE9 plasmid isolated from J carried two tandem immunity genes, one providing protection to ColE9 and the other to ColE5. Subsequent DNA sequence analysis suggested a close evolutionary relationship between the ColE5 and ColE9 plasmids in which a segment of DNA which encodes the last 73 amino acids of the colicin E9 structural gene, the E9 immunity gene and the E9 lysis gene has been transferred into the colicin E5 structural gene at a site 48 amino acids from the C-terminus.

Pearl Cooper and I then turned our attention to the bacteriocins produced by *Klebsiella pneumoniae* strains of the Edmondson and Cooke klebicin typing panel. Many younger researchers may not know that in the 1970s bacteriocin typing was a widely used technique for distinguishing strains of important pathogens. As a result several bacteriocin typing panels of strains were available. We demonstrated that the pP5a plasmid that encodes klebicin A1 and immunity to klebicin A1, also encodes immunity to colicin E6, with both immunities being encoded by the same gene. In contrast, the pP5b plasmid that encodes klebicin A2 and immunity to klebicin A2 has a tandem immunity gene to colicin E3, and the pP3 plasmid that encodes klebicin A3 and immunity to klebicin A3 also has a tandem immunity gene to colicin E3. *K. pneumoniae* strains are not killed by E colicins, as they lack the BtuB outer-membrane receptor, and strains are not killed by klebicins as they lack the aerobactin receptor. It is therefore difficult to envisage how either a single or tandem

immunity genes encoding immunity to both a klebicin and an E colicin could evolve in the absence of selection pressure.

Having had the good fortune of isolating two new members of the E colicin family (ColE8 and ColE9), we now had four homologous DNase type E colicins (ColE2, ColE7, ColE8 and ColE9) along with their immunity genes (Im2, Im7, Im8 and Im9). I realized that this would be an excellent model system for a research grant call to study the emerging field of the molecular recognition of protein–protein interactions. Understanding the basis of molecular recognition between an E colicin and its immunity protein required knowledge of (1) the amino-acids which act as specificity determinants both in the DNase domain of the colicin and in the Im protein; (2) the kinetics of binding between each DNase domain and the four different Im proteins; and (3) the structures of the four DNase/Im protein complexes. I setup a long term collaboration at UEA with two talented colleagues, Geoff Moore and Colin Kleanthous, which was able to contribute important data in all three areas, and has also provided significant insights into the basis of molecular recognition of proteins in other biological systems.

Studying colicin biology is like a gift that never stops giving as it keeps throwing up other important properties that are of wider relevance in biology. Examples include (1) Im9 is one of the fastest folding proteins in nature; (2) the complex of ColE9 DNase with Im9 is one of the highest affinity interactions observed in nature; (3) Im9 does not bind at the active site of the DNase; (4) the DNase domain of E colicins form channels in lipid bilayers; (5) the similarity of the HNH domain of the DNase to Caspase-activated DNase (CAD) in eukaryotes; and (6) the mechanism of translocation of E colicins.

In order to kill, the folded, functional DNase domain of E colicins has to be delivered from the outside to the inside of an cell, a process that involves interactions with a surface receptor and Tol proteins. This translocation process is a unique event in prokaryotic biology. An understanding of this process requires knowledge of (1) the receptor binding process; (2) how colicin domains cross membranes; (3) the specificity determinants of colicins and Tol proteins; (4) the kinetics of binding of Tol binding domains and Tol proteins; and (5) the structures of colicin/Tol protein complexes. Though Colin Kleanthous moved to York (and now Oxford), I moved to Nottingham, and Geoff Moore remained at UEA, we managed to maintain a very productive collaboration, also involving Chris Penfold and Mireille Vankemmelbeke in Nottingham, that has provided valuable information on the translocation process.

One other aspect of our research with colicins to mention is that the study of any biological system requires the use of novel tools. I was very keen on developing novel tools or adapting existing tools to facilitate the study of bacteriocins in the lab. Examples of this include: promoter mapping vectors and transposon mutagenesis (with Kin Chak); chemical mutagenesis, PCR mutagenesis and alanine scanning mutagenesis (with Carole Garinot-Schneider); chimeric proteins with GFP and introducing disulphide locks into colicin domains to study their flexibility (with Chris Penfold); SOS promoter–*lux* fusions to monitor DNA damage (with Mireille Vankemmelbeke); Surface Plasmon resonance to study the affinity of protein–protein interactions (with Sarah Hands); introducing enterokinase cleavage sites to study surface accessibility of colicin molecules (with Ying Zhang); and Alexa Fluor labelled Im proteins to study their release from colicin-Im protein complexes (with Mireille Vankemmelbeke).

It was 1991 that Claude Lazdunski, Franc Pattus and I organized a meeting on the Île de Bendor, an island close to Marseille in France, to bring scientists from around the globe to discuss bacteriocins. Many of the participants at this meeting, including Volkmar Braun, Bill Cramer, Colin Hill, Karen Jakes, Roland Lloubes and Bauke Oudega, attended a follow up bacteriocin meeting that I organized in Nottingham 7 years later, and my retirement meeting in 2012. I would like to thank all members of the bacteriocin community for making possible the exciting progress that has happened in the last 24 years in so many aspects of the biology of these fascinating protein antibiotics. I have been privileged to make so many good friends who share my love for bacteriocins.

During my academic career at both UEA and Nottingham I have taught medical microbiology to undergraduates as well as masters and medical students. In all cases I provided lecture courses on both the mechanism of action of antibiotics and the variety of mechanisms by which bacteria acquire resistance to antibiotics. With each year that passed I was spending more time talking about the latter topic, concentrating a lot on what could be done to counter the threat to healthcare systems of antibiotic resistance. In January 2007, I founded the Centre for Healthcare-associated Infections (CHAI) which involved researchers from seven Schools of the University of Nottingham together with colleagues in Nottingham University Hospital. The aim of CHAI was to carry out research on the treatment and prevention of healthcare-associated infections, many of which were due to antibiotic-resistant, bacterial pathogens. On the day of the CHAI launch Symposium I was accused of being a 'sensationalist and scaremonger' about the problems of antibiotic resistance by the Chief Nursing Officer at the Department of Health, UK. As I expected, antibiotic resistance was a very important topic and I was asked to give over 300 media interviews over the next three years, and was awarded the Communications Award of the Society for Applied Microbiology in 2008 for helping to inform the public of the scale of the problem. When the Chief Medical Officer (England) stated in 2013 that 'antimicrobial resistance poses a catastrophic threat that is as serious as global warming' I was satisfied that at last the scale of the threat had been recognized.

With the increasing awareness of the paucity of new antibiotics combined with increasing incidence of antibiotic resistance to current antibiotics, it would be hoped that the large number of diverse bacteriocins that have been characterized could help provide new options for treating serious bacterial infections. Nisin, a lantibiotic produced by lactic acid bacteria, is widely used as a preservative in the food industry in processed cheese, dairy and canned products. Research in Colin Hill's laboratory has shown exciting prospects for the bioengineering of nisin to enhance its antibacterial activity, especially against Gram-negative pathogens. The potential immunological problems of using protein antibiotics like bacteriocins to treat systemic infections is seen as a significant impediment to their development as new antibiotics, but there are some examples of the treatment of topical infections such as UTIs, which have been shown by Peg Riley to have some promise. It would be so exciting to those who have worked with bacteriocins over many years if they became useful treatment options for a wide range of antibiotic-resistant infections.

Richard James