How Bugs Kill Bugs: Progress and Challenges in Bacteriocin Research

A Biochemical Society Focused Meeting held at University of Nottingham, U.K., 16–18 July 2012. Organized and Edited by Colin Kleanthous (Oxford, U.K.), Chris Penfold (Nottingham, U.K.) and Dan Walker (Glasgow, U.K.).

How Bugs Kill Bugs: Progress and Challenges in Bacteriocin Research

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Abstract

A Biochemical Society Focused Meeting on bacteriocins was held at the University of Nottingham on 16–18 July 2012 to mark the retirement of Professor Richard James and honour a scientific career of more than 30 years devoted to an understanding of the biology of colicins, bacteriocins produced by *Escherichia coli*. This meeting was the third leg of a triumvirate of symposia that included meetings at the Île de Bendor, France, in 1991 and the University of East Anglia, Norwich, U.K., in 1998, focused on bringing together leading experts in basic and applied bacteriocin research. The symposium which attracted 70 attendees consisted of 18 invited speakers and 22 selected oral communications spread over four themes: (i) Role of bacteriocins in bacterial ecology, (ii) Mode of action of bacteriocins, (ii) Mechanisms of bacteriocin import across the cell envelope, and (iv) Biotechnological and biomedical applications of bacteriocins. Speakers and poster presenters travelled from around the world, including the U.S.A., Japan, Asia and Europe, to showcase the latest developments in their scientific research.

Introduction

How time flies! It's been 14 and 21 years respectively since meetings at the University of East Anglia, U.K., and Île de Bendor, off the south coast of France (Figure 1) brought together scientists from around the globe to discuss developments in our understanding of the organization, structure and function of genes and gene products that control the synthesis and secretion of bacteriocins. Questions being asked then included 'How are bacteriocins synthesized?', 'What is the mechanism of immunity to a bacteriocin?', 'What are the functional domains of bacteriocins?' and 'How does the structure of a bacteriocin relate to its function?' Since then, answers to many of these questions have been forthcoming while also throwing up new questions that, during the ensuing years, have led to great advances in bacteriocin knowledge and understanding through novel experimental design and techniques. Flicking through the meeting transactions of the Île de Bendor symposium in 1991 [1], diagrams of restriction maps and SDS/PAGE gels have nowadays been replaced by figures illustrating bacteriocin structures [2–7] or structures of complexes of bacteriocins with binding ligands that represent efforts to elucidate the mechanisms of bacteriocin import and mode of action [8–11].

Richard James's preface to the Île de Bendor meeting transactions makes interesting reading, not least for his recommendation that a follow-up meeting should have been organized 2 years later! As 14 years have passed since the last meeting at the University of East Anglia, Norwich, U.K., another meeting was overdue to bring together scientists interested in bacteriocin research. A meeting was organized by Colin Kleanthous, Chris Penfold and Daniel Walker in collaboration with the Biochemical Society in Nottingham on 16–18 July 2012 (Figure 2) to discuss advances in bacteriocin research and mark the impending retirement of Professor Richard James in December 2012. The meeting was designed as four half-day sessions over 3 days to address the following topics: (i) Role of bacteriocins in bacterial ecology, (ii) Mode

Key words: bacterial ecology, bacteriocin, colicin, enterocin, lectin, microbial competition.

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Figure 1 | **Bacteriocins, Microcins and Lantibiotics: delegates of the NATO ASI conference on the Île de Bendor, France, in 1991.**
Photo courtesy of Dr Roland Lloubès, CNRS Marseille, France.

of action of bacteriocins, (iii) Mechanisms of bacteriocin import across the cell envelope, and (iv) Biotechnological and biomedical applications of bacteriocins, encompassing a combination of invited speakers and selected talks.

Day 1: Role of bacteriocins in bacterial ecology

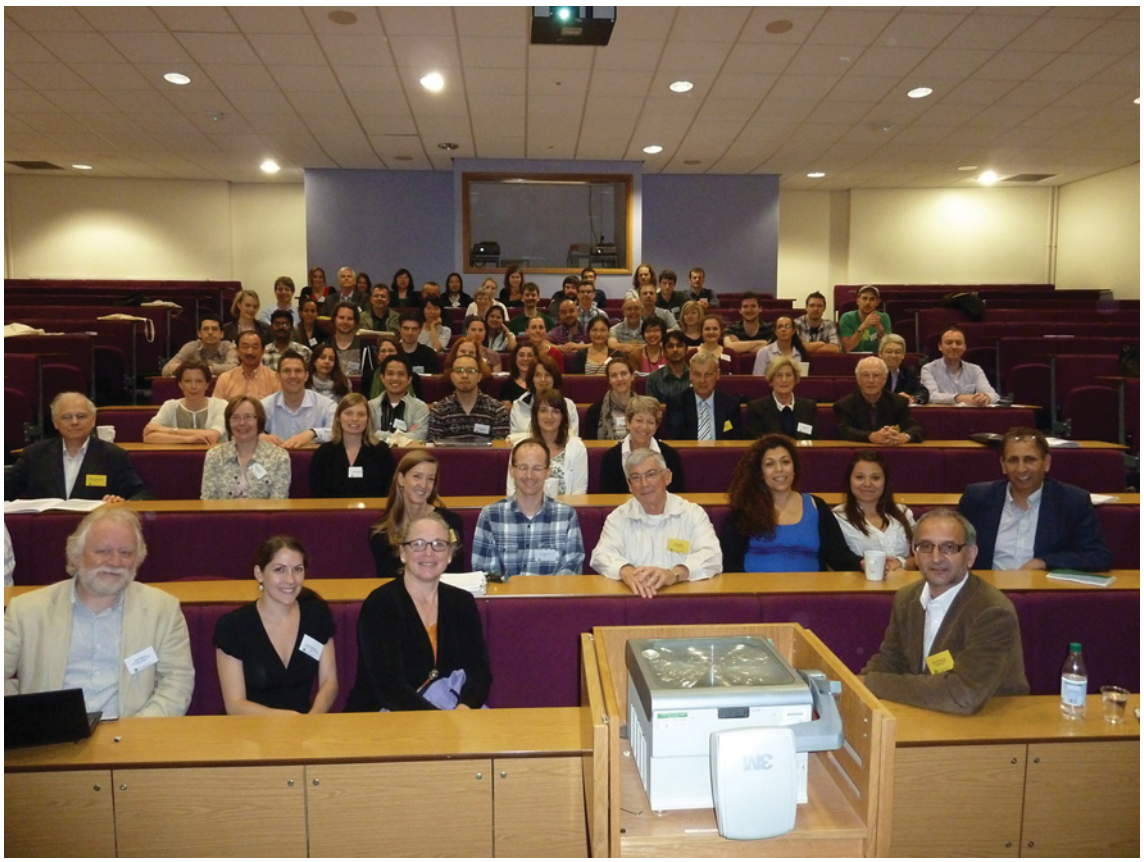
Professor James was invited to present the opening keynote speech and chose to present a history of his involvement in colicin research that has spanned more than 30 fruitful years and all started through serendipity from an undergraduate science practical and a few chicken guts. His engaging and amusing presentation detailed 'the early work with colicins – BC' (before cloning!) in the 1980s that showed *Escherichia coli* strains often contain tandem immunity genes to different colicins as a means of cross protection, before progressing through many structure–function studies involving the determination of X-ray structures of colicins and colicin complexes, and finishing with his present interest in the role of autoinduction of colicins in social interactions of bacteria and bacterial warfare.

The session on the 'Role of bacteriocins in bacterial ecology' followed the keynote speech and maintained the uplifting start to the meeting providing new insights into bacteriocin-mediated interactions of bacterial populations from a range of organisms including *E. coli* [12], *Streptococcus pneumoniae* [13], *Pseudomonas aeruginosa* and *Salmonella* spp. The session was eloquently opened by Margaret (Peg) Riley who, in her relaxed but gripping style, treated the audience to a compelling argument for the use of bacteriocins in antimicrobial chemotherapy despite the reluctance of 'Big Pharma' to adopt this approach because of the narrow spectrum activities of individual bacteriocins [14].

Day 2: Mode of action and mechanisms of bacteriocin import

Day 2 was devoted to sessions on the mechanistic aspects of bacteriocin biology. How bacteriocins penetrate sensitive cells and then proceed to kill the cell through a precise catalytic process. Invited talks by Karen Jakes [15], Volkmar Braun [16], Bill Cramer [17] and Roland Lloubès [18], all attendees at the original meeting in Île de Bendor, were supported by several academics and junior members of established laboratories that presented recent research accomplishments on the diverse range of mechanisms that bacteriocins use to traverse the cell membranes and navigate their way through host defences to their cytotoxic site of action. It was pleasing to listen to presentations on a variety of bacteriocin types from bacteria occupying taxonomically distant phyla. The majority of the presentations were on colicins where mechanisms for crossing the outer membrane of *E. coli* cells [19,20] were complemented by models of colicin translocation through the cell periplasm [21], methods for inserting and traversing the inner membrane [22,23], and novel modes of action [24]. Other talks by Sylvie Rebuffat [25] and Konstantinos Beis [26] presented exciting new data on the structure–function studies of a variety of microcins, and Kenji Sonomoto who provided a through overview of

Figure 2 | Delegates at the How Bugs Kill Bugs conference, Nottingham, U.K., 2012



the work that his group has been doing to elucidate the mechanisms of action of type-A(II) lantibiotics [27].

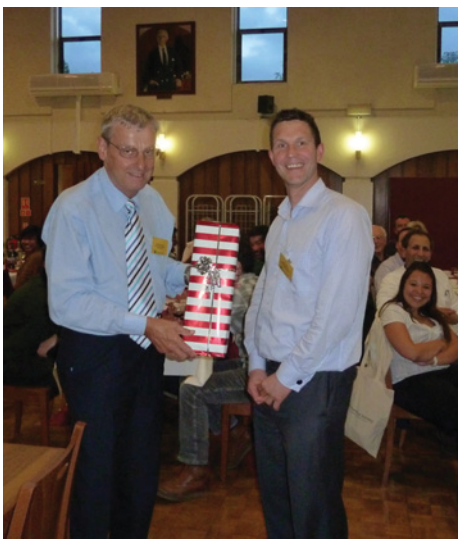
Day 3: Biotechnological and biomedical applications

The final session of the meeting was designed to consolidate all of the research that had been presented in the previous half-day sessions into an applied focus for potential uses of bacteriocin production. The session was opened by Colin Hill who presented work conducted by his group on the bioengineering and derivatization of lantibiotics for improved food security against organisms such as *Listeria monocytogenes*, and antimicrobial efficacy against *Shigella* spp., *Pseudomonas* spp. and *Salmonella* spp. [28]. Other invited speakers included Susan Buchanan and Daniel Walker. Dan Walker introduced the novel concept of using narrow range bacteriocins to control agriculturally important phytopathogenic bacteria [29], and Susan Buchanan presented work that combined structural biology with bioengineering to create a modified phage lysin of pesticin and T4 lysozyme that has enhanced activity against Gram-negative bacteria [30]. Some of the structural work was also completed independently by Kornelius Zeth who presented his studies on the structural and mechanistic studies of pesticin [31]. Maarten Ghequire presented some structure–function studies and possible applications of mannose-binding lectins from monocot plants with potential antimicrobial activities against medically important fungi and bacteria [32], and Ross Williams presented data on expression levels of anti-listerial enterocins under various batch culture conditions for the use in controlling *Listeria monocytogenes* in infected food stuffs [33]. Carla Brown showed that colicins and pyocins are highly effective against antibiotic-tolerant biofilms formed by *E. coli* and *Ps. aeruginosa* respectively [34].

Concluding remarks

Presentations at the meeting were supported and enhanced by a large number of very high quality posters which enabled lively debate and many delegate interactions discussing complementary interests and forging meaningful collaborations. The interest generated in all delegates at the poster sessions and the conference dinner was evident by the large levels of noise emanating from each of these rooms. It is clear that technological advances over the last 20–30 years have led to many answers on the structural organization of bacteriocins, the participating proteins involved in bacteriocin import and interactions with bacteriocin domains, and how certain bacteriocins kill cells once they have invaded

Figure 3 | **Professor Richard James (left) receiving a bottle of champagne from Dr Chris Penfold (right) at the How Bugs Kill Bugs conference dinner**



them. However, these advances have thrown up even more questions as scientists seek opportunities to solve many of the more ambitious or tantalizing questions related to bacteriocin biology through novel experimental approach. Challenges ahead are numerous and include (i) an understanding of the role of bacteriocins in sculpting the human microbiome and how this affects microbial competition, (ii) an understanding of the interactions of bacteriocins with host proteins, (iii) the sequence of events that occur *in vivo* after binding of the bacteriocin to the host cell, (iv) the role of energy in the entry process, (v) the mechanism of transport across lipid membranes and the role of transmembrane proteins in membrane transport, (vi) the use of bacteriocins in biomedical applications, and (vii) the use of genetic engineering for improving bacteriocin diversity and antimicrobial activities.

It was fitting that Professor Richard James was presented with a personalized magnum of champagne detailing the meeting and honouring his contribution to bacteriocin research over the last 30 years (Figure 3). His enthusiasm for colicins has certainly not been tempered over the ensuing years, nor has his love for Provence and French wine since his initial introduction to the region at the Île de Bendor meeting in 1991.

Acknowledgements

We acknowledge the immense contributions of Frances van Klaveren and Rowena Mitchell from the Biochemical Society for all their hard work in making the conference possible and our sponsors for the event, *Biochemical Society Transactions*, The Society of General Microbiology, Unilever, *Nature Reviews Microbiology*, Portland Press Ltd and Scientific Laboratory Supplies (SLS). We also acknowledge Dr Mireille Vankemmelbeke for helpful comments.

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Received 24 September 2012
doi:10.1042/BST20120253