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Social behaviour and making attachments: a report from the fifth 'Young Microbiologists Symposium on Microbe Signalling, Organisation and Pathogenesis'

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Abstract

The fifth Young Microbiologists Symposium was held in Queen's University Belfast, Northern Ireland, in late August 2018. The symposium, focused on 'Microbe signalling, organization and pathogenesis', attracted 121 microbiologists from 15 countries. The meeting allowed junior scientists to present their work to a broad audience, and was supported by the European Molecular Biology Organization, the Federation of European Microbiological Societies, the Society of Applied Microbiology, the Biochemical Society and the Microbiology Society. Sessions covered recent advances in areas of microbiology including gene regulation and signalling, secretion and transport across membranes, infection and immunity, and antibiotics and resistance mechanisms. In this Meeting Report, we highlight some of the most significant advances and exciting developments communicated during talks and poster presentations.

INTRODUCTION

The fifth Young Microbiologists Symposium (YMS) on 'Microbe Signalling, Organisation and Pathogenesis' was held in Belfast on 27 and 28 August 2018. The meeting was organised by Shi-qi An (Queen's University of Belfast, UK), Laura Hobley (University of Nottingham, UK), Joana Sa Pessoa (Queen's University of Belfast, UK) and Liang Yang (Nanyang Technological University, Singapore). One hundred and twenty-one participants attended, most of them junior principal investigators, postdoctoral researchers, and students from institutions in Asia, Australia, Europe and North and South America. The programme of 34 talks and 57 poster presentations provided junior scientists a platform to showcase their work, engage with their peers,and foster collaborations.

In 2009 the first YMS was held at University College Cork [1], signalling to the microbiology community the need to provide a forum for early career microbiologists not just to attend meetings, but also to be involved in the organization, delivery and presentation of the meeting. Like the four meetings before, the Belfast meeting had topical theme-based sessions discussing microbial intracellular signalling, antibiotic resistance, bacterial secretion and host–microbe interactions [1–4]. Each session was topped-and-tailed by talks from renowned experts (a mix of invited junior and senior principal investigators), while most of the talks were given by young researchers from leading laboratories worldwide. Three keynote lectures featured eminent academics that have made substantial contributions to microbiology.

The meeting highlighted the extraordinary range of functions associated with bacterial life, and emphasized recent discoveries regarding signalling and regulatory processes in bacterial development and virulence. In this report, we summarize the work presented in oral and poster presentations.

GENE REGULATION AND INTRACELLULAR SIGNALLING

The first session on Gene Regulation and Intracellular Signalling featured bacterial second-messenger molecules including cyclic-di-GMP and cyclic-di-AMP. The studied organisms ranged from soil-dwelling Gram-positive bacteria to human-restricted Gram-negative pathogens. Zhao-xun Liang (Nanyang Technological University) explained the characterization of MapZ, a single-domain PilZ protein in *Pseudomonas aeruginosa*, as a c-di-GMP-binding adaptor protein that modulates flagellar motor switching through interaction with a chemotaxis methyltransferase CheR1[5]. MapZ–CheR1 interaction is important for efficient surface attachment, biofilm formation and dissemination of

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P. aeruginosa. He presented new structural data on the CheR1/MapZ/c-di-GMP ternary complex, revealing dramatic rearrangements in MapZ upon c-di-GMP binding, which allow the adaptor to recognize and inhibit its target protein CheR1[6].

Andreas Latoscha (talk) and Sara Neumann (poster), Humboldt-Universität zu Berlin, presented their findings on the role of c-di-AMP in regulating the complex life cycle of *Streptomyces venezuelae*. They identified a novel c-di-AMP hydrolysing phosphodiesterase lacking the known domains of c-di-AMP degrading enzymes that is highly conserved in Actinobacteria. Deleting the gene for this enzyme resulted in pleiotropic phenotypes including slow growth and reduced pigmentation.

Returning to c-di-GMP, Przemyslaw Olejnik (University of Tübingen, Germany) presented his work on the role of the diguanylate cyclase SadC from *P. aeruginosa*, which was needed for the induction of alginate production under anaerobic conditions. He proposed that the presence of a PilZ domain within the Alg44 alginate exporter may link c-di-GMP synthesis by SadC with the increased production of alginate.

Shreya Dasgupta (National Institute of Cholera and Enteric Diseases) discussed her findings on the role of (p)ppGpp in virulence regulation of the human-restricted *Salmonella* Typhi, which is synthesized by two enzymes encoded by the *relA* and *spoT* genes. Using a *relA*/*spoT* deletion strain, she identified flagellar motility and Vi capsule production as new virulence-associated traits regulated by ppGpp in *S.* Typhi. Also, deletion of *relA*/*spoT* strongly reduced capsule production, which rendered the pathogen sensitive to human serum killing.

The talk by Susanne Gebhard (University of Bath, UK) revealed a novel form of intracellular signalling involving a transporter and sensor kinase that form a sensory complex, the BceAB transporter, a known resistance determinant against the antibiotic bacitracin [7]. By combining genetics and molecular modelling, she showed that activity of the BceAB transporter tightly controls the conformation of the sensor kinase BceS and the activity of the kinase domain.

Finally, an exciting talk by Regine Hengge (Humboldt-University, Berlin), who gave the EMBO Lecture, highlighted the importance of c-di-GMP in establishing distinct spatial zones of *E. coli* biofilm architecture through c-di-GMP-driven regulation of global transcription patterns (Fig. 1). In the first part of her talk, she explained how to visualize global gene expression patterns in different spatial biofilm zones using GFP reporter fusions, and to correlate these with naturally existing nutrient gradients and extracellular matrix architecture [8]. In the second part she described PdeR, the prototype of a class of enzymes called trigger phosphodiesterases (trigger PDEs) [9], and its role in determining spatial biofilm organization. As a trigger PDE, PdeR is a bifunctional enzyme; it binds and degrades c-di-GMP but also inhibits the transcription factor MlrA through direct interaction, which results in the transcriptional repression of the biofilm regulator CsgD. Intriguingly, this latter activity of PdeR is inhibited by c-di-GMP. Hence, with increasing levels of c-di-GMP during entry into stationary phase, PdeR activity is inhibited and transcriptional repression of MlrA is relieved. This switches on CsgD activity and drives production of amyloid curli fibres and cellulose, important extracellular components of the outer, nutrient-starved layer of the biofilm.

SECRETION AND TRANSPORT ACROSS MEMBRANES

The cell envelope represents the outermost layer of the bacterial cell, functioning as a scaffold for holding macromolecules that contribute to the assembly of the various secretion systems (Fig. 2). This session focused on the roles of secretion in the interactions of bacterial cells with their environment. Andrew Roe (University of Glasgow, UK) presented new work linking 1,2-propanediol metabolism to the regulation of the LEE Type 3 secretion system (T3SS) locus [10]. Using the substitute *Citrobacter rodentium* infection model (mimicking that of EHEC/EPEC), he compared the metabolic signatures *in vivo* to those *in vitro*, revealing significant induction of 1,2-propanediol metabolism as a metabolic signature specific to the host environment. Addition of either 1,2-propanediol or propionate (a product of the same pathway) induced LEE expression. Furthermore, Andrew showed that the *pdu* cluster (involved in 1,2-propanediol metabolism) helps fine-tuning virulence gene regulation resulting in maximal colonization *in vivo*.

The link between secreted proteins and metabolism continued with a talk on the amyloid fibre-forming protein, TasA, present in the biofilm matrix of *Bacillus subtilis*. Jesús Camara-Almirón (Universidad de Málaga, Spain) showed that a Δ*tasA* mutant had a defect in biofilm morphology, altered metabolism and accelerated growth phase progression. When inoculated onto plant leaves, the Δ*tasA* was impaired in survival and fitness but retained anti-fungal activity with increased level of fengycin production in the mutant.

Andrew Fenton (University of Sheffield, UK) described his work identifying regulators of the cell wall synthases PBP1a and PBP2a in *Streptococcus pneumoniae*. By applying TnSeq, he had previously identified CozE as a component of the MreCD complex in *S. pneumoniae* required for correct activity of PBP1 [11]. Andrew then described his recently published work identifying MacP as an activator of PBP2a in *S. pneumoniae* [12] and how phosphorylation of MacP is required for PBP2a function.

Identification of novel effector proteins and their targets of the type VI secretion system (T6SS) was another major theme through the meeting. Fabiana Bisaro (Queen's University Belfast, UK) described her work on the T6SS effector protein TecA from *Burkholderia cenocepacia*. TecA mediates cytoskeletal rearrangement in infected macrophages

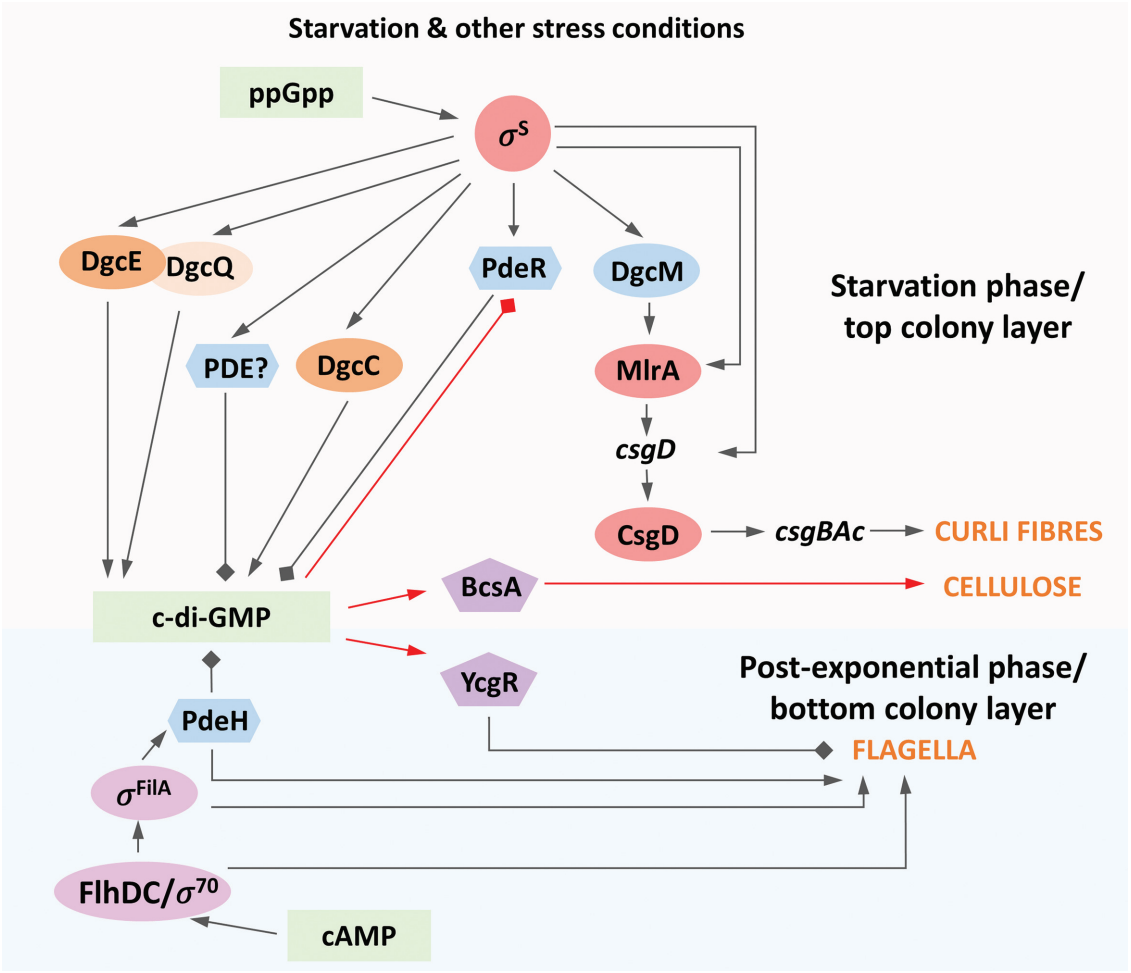

Fig. 1. The role of cyclic di-GMP signalling in *E. coli* biofilm formation. For further details please see Klauck *et al.*, [8].

and induces the deamidation of RhoA and RacI GTPases [13, 14]. She also showed that the formation of the ASC complex by *B. cenocepacia* depends on T6SS. Fabiana also described how TecA interacts with the T6SS Hcp needle, but that none of the known T6SS chaperones are required for TecA secretion.

The T6SS of *Klebsiella pneumoniae* was the focus of Daniel Storey (Queen's University Belfast, UK). He described the intricate regulation of T6SS activity in *K. pneumoniae*, and its contribution to both intra- and inter-bacterial species killing. He also described the antibacterial effects of a new type of VgrG protein, and the discovery of the cognate immunity protein, as well as the identification of the domain essential for the antibacterial phenotype.

The Arthur Mitchell Brown Memorial Lecture, sponsored by the School of Medicine, Dentistry and Biomedical Sciences, Queen's University, was delivered by Alain Filloux (Imperial College London). He highlighted his recent work on *Pseudomonas aeruginosa* T6SS antibacterial toxins and their cognate immunity proteins,showing that the T6SS toxin arsenal includes a diverse array of activities affecting distinct subcellular compartments of the prey bacterium. One T6SS toxin is a DNase of the GGH2 family, which is fitted on the T6SS tip, the VgrG protein through its N-terminal PAAR domain. Another toxin, which is an 'evolved VgrG' with a catalytic C-terminal extension, acts in the periplasm inducing severe cell division and cell morphology defects. Finally, he described an unbiased screening strategy using TraDIS to systematically identify all T6SS toxins from *P. aeruginosa*, and provided an example of one novel toxin which might interfere with tRNA biogenesis and protein synthesis.

INFECTION AND IMMUNITY

The Infection and Immunity session covered a range of strategies by pathogens to avoid the immune system by manipulating host pathways that promote survival. Suzana Salcedo (University of Lyon, France) presented a novel strategy of the intracellular pathogen *Brucella* to inhibit host

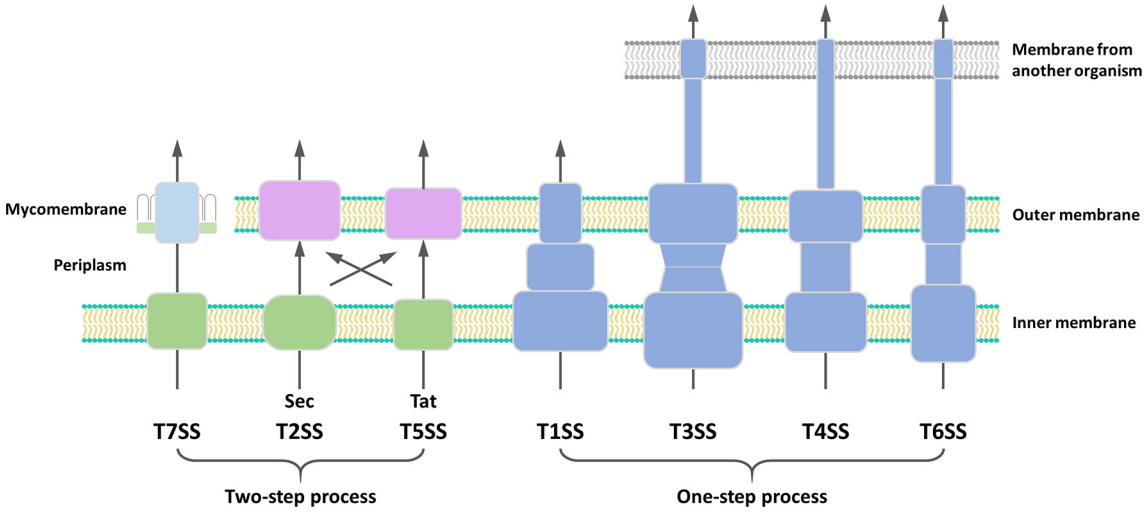

Fig. 2. Overview of bacterial secretion systems I–VII. For further details please see Maffei, Francetic and Subtil [23].

immunity. Two nuclear effectors, BnpA and BnpB, which associate with promyelocytic leukaemia bodies in the nucleus, hijack specific components of the SUMOylation machinery, affecting both post-translational modifications and the subcellular localization of different nuclear proteins during infection. Control of spatial dynamics of these proteins by BnpA and BnpB is necessary for efficient intracellular replication of *Brucella*.

Klebsiella pneumoniae, an extracellular pathogen, also manipulates host post-translational modifications. Ciara Ross (Queen's University Belfast, Uk) reported her studies on how *K. pneumoniae* affects ISGylation. ISGylation plays an essential role in antiviral responses, but there is limited information on whether it plays any role in bacteria–host interactions. She showed that *K. pneumoniae* impairs ISGylation and the activation of IRF3, the transcriptional factor governing the expression of *isg15*. By targeting these post-translational modifications, *K. pneumoniae* results in decreased inflammation during infection.

Bacteria can also target host transcription. Sarah McCormack (University College Dublin), studies the *Burkholderia cepacia* complex, one of the pathogens that chronically infect cystic fibrosis patients. She reported the identification of an immunogenic protein upregulated during chronic infection and indirectly involved in attachment of *Burkholderia* to host cells. Proteomics of the mutant strain and structural analysis of the protein revealed its potential role as a DNA mimic. This protein, dubbed *erp* (electronegative regulator protein), may be a global bacterial regulator maintaining redox homeostasis and DNA supercoiling.

Ana Monserrat-Martinez (University of New South Wales, Australia) used single-molecule spectroscopy (SMS) to investigate the interaction of enteropathogenic *Escherichia coli* (EPEC) with its host. Interaction of immune proteins with EPEC effector proteins, as measured by SMS, revealed how these effectors lead to the formation of higher-order assembly structures associated with host-cell death pathways. This information can be used in the development of new drugs against EPEC bacterial infection.

Clare Bryant (University of Cambridge, UK) demonstrated the assembly of the supramolecular organizing centre MyDDosome that coordinates inflammatory signalling [15]. Using *S.* Typhimurium infections and *in vivo* models of innate and adaptive immunity, her group investigates the role of Pattern Recognition Receptors (PRRs) and inflammasome components in pathogen clearance. PRR and inflammasome-deficient mice restrict growth of the bacterium in the host but cannot completely clear the infection, as the activation of inflammasome components is crucial in maintaining a memory $CD4^+$ T-cell response. Clare ended by concluding that for clearance of *Salmonella* infection *in vivo*, both apoptosis and necroptosis cell death pathways seem to be necessary.

ANTIBIOTIC RESISTANCE AND STRESS RESPONSE

The fourth session covered mechanisms of resistance and response to antimicrobials, providing insights into the development of treatment strategies against infection. Kimberly Kline (Nanyang Technological University, Singapore), presented her work on *Enterococcus faecalis*, an important pathogen associated with bacteraemia, endocarditis and wounds and urinary tract infections. She showed that HtrA, a highly conserved serine protease, contributes to membrane stress response in *E. faecalis*, and is also implicated in colonization and host tissue infection. Her transcriptomic studies also revealed that the two-component system, CroR–CroS, ias required to sense endogenous stress and regulate cell morphology. These findings suggested that the

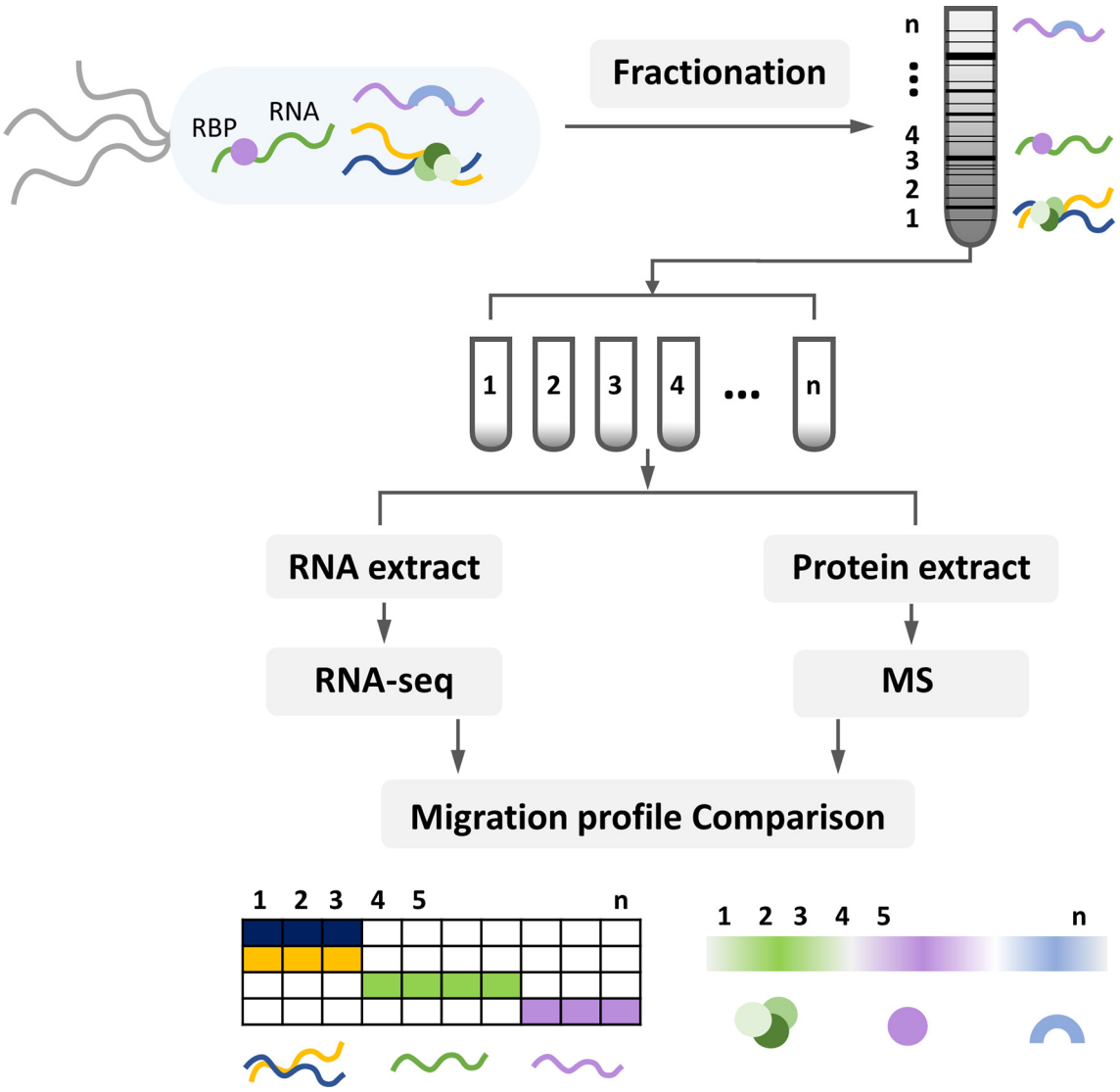

Fig. 3. Schematic of Grad-seq experimental strategy. For further details please see Smirnov *et al.* [20].

cell envelope response links both antibiotic resistance and virulence factor assembly in *E. faecalis*.

Continuing with the theme of host adaptation, Sergii Krysenko (University of Tübingen, Germany) described his work characterizing a novel gamma-glutamylpolyamine synthetase GlnA3, which is responsible for polyamine utilization in *Streptomyces* [16]. Sergii showed that deletion of the gene encoding GlnA3 results in defective growth, with polyamines as the sole nitrogen source and reduced resistance to high concentrations of polyamines. He also found that Rv1878, a *Mycobacterium tuberculosis* GlnA3 homologue, plays a similar role in polyamine metabolism by allowing *M. tuberculosis* to better utilize host-derived polyamines for growth and propagation during infection. This suggests that GlnA3 may be a therapeutic target used to control *M. tuberculosis* infections.

Biofilm formation was discussed as a result of altered c-di-GMP, in the gene regulation and signalling session. Carly Webb (Queen's University Belfast, UK) shared her investigations into the molecular mechanisms underpinning biofilm formation in the bacterial pathogen *K. pneumoniae*, showing that the organism produces multiple polysaccharides that alter the biofilm formation capabilities of this organism.

Acquired antibiotic resistance was also discussed in this session by Yi-chen Ding (Nanyang Technological University, Singapore), who discovered the transfer of Msr(E), a macrolide antibiotic resistance gene, into the genomes of a group of *P. aeruginosa* clinical isolates. He further showed that acquisition of Msr(E) by *P. aeruginosa* causes this pathogen to resist macrolide-mediated quorum-sensing inhibition, which may provide a novel treatment strategy [17]. Yi-chen

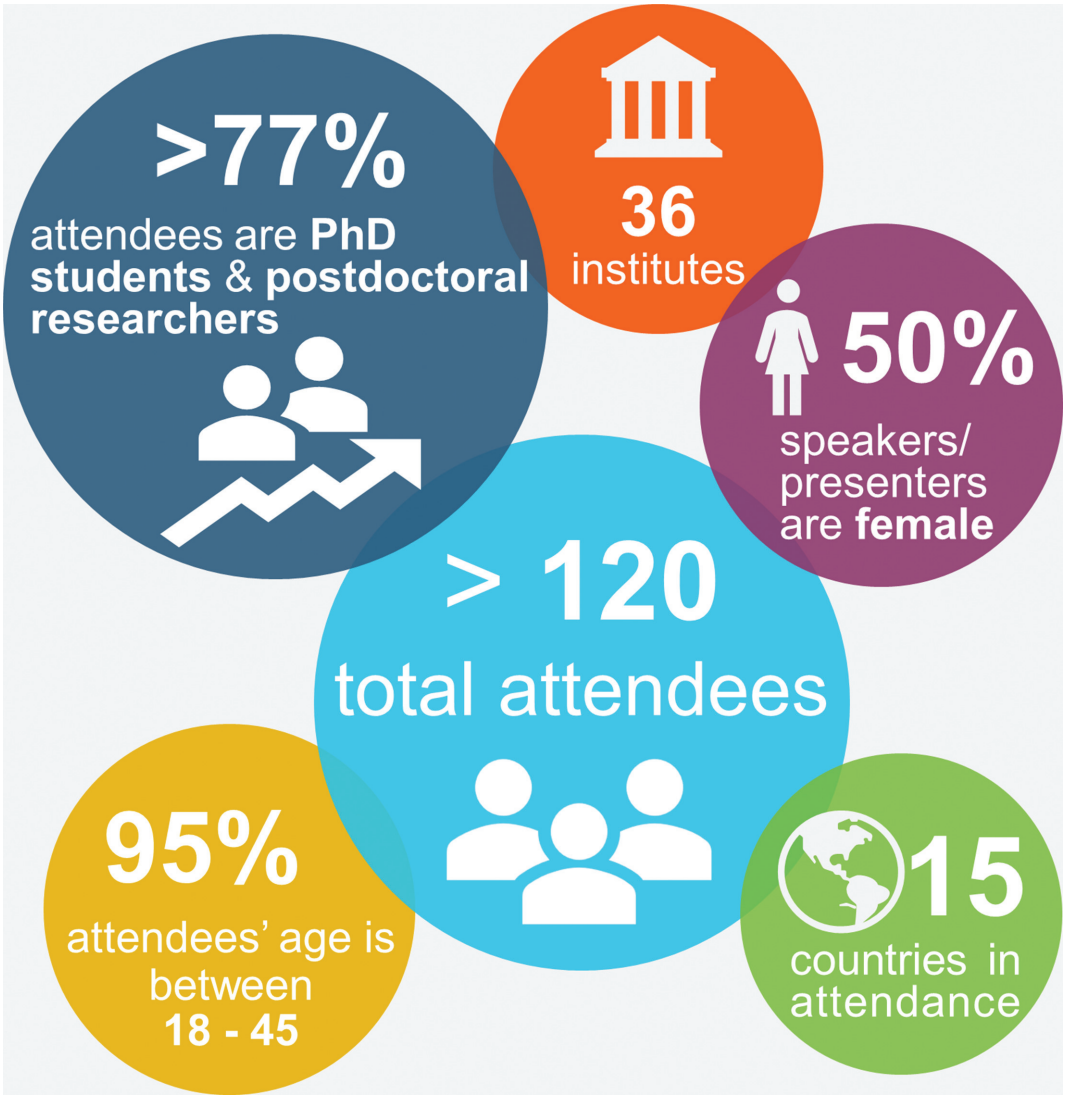

Fig. 4. Summary of meeting statistics associated with attendees and participants.

showed the cryo-EM structure of Msr(E)-bound ribosomes, which provided most direct evidence that Msr(E), and probably other ABC-F-type antibiotic resistance proteins, mediates antibiotic resistance by ribosomal protection [18].

In a poster talk, July Fong (Nanyang Technological University, Singapore) raised a concern about the current quorum-sensing (QS) inhibiting strategies. Using a mathematic simulation and chemical biology experiments, July demonstrated that a single small molecule targeting the LuxR type QS receptors for AHLs could not fully suppress the multiple AHL QS systems that often coexist in Gram-negative bacteria. A combination of LuxR-specific inhibitors and quorum-quenching enzymes was effective in fully inhibiting the double AHL-based QS system in *P. aeruginosa* [19].

Fiona Walsh (Maynooth University, Ireland) described her work on a novel gene for resistance to nalidixic acid identified in an unknown soil bacterium. This gene has no known homologues, except for a newly sequenced gene encoding a protein of unknown function. Fiona described her ongoing investigations to elucidate the mode of action of this novel resistance protein.

The meeting ended with a keynote lecture sponsored by the Microbiology Society, which was delivered by Jörg Vogel (University of Würzburg, Germany). He described his group's recent work on bacterial RNA-binding proteins (RBPs), especially the development of the gradient sequencing (Grad-seq) approach. An example of the power of this strategy was the discovery of ProQ, a globally acting RNA

chaperone in *E. coli* and the bacterial pathogen *Salmonella enterica* [20] (Fig. 3).

SUMMARY

Concerning science, the symposium brought much new insight in maintaining the track record of previous YMS meetings [1–4], and also provided an excellent forum for those with an interest in microbiology to interact and gain exposure to a wide range of topics. The lively and stimulating discussions between senior academics and early career scientists during oral and poster sessions clearly emphasized the quality of work on display. To get a flavour of the atmosphere of the meeting, see our short video overview here: Video

After the final keynote lecture, various awards were presented. Carlos Molina-Santiago (Universidad de Málaga, Spain) was awarded the *Portland Press* prize for his presentation entitled, 'Overtake, resilience and escape: three ecological strategies that define the *Pseudomonas-Bacillus* interactions', and Chao-ying Deng (Chinese Academy of Sciences) was given the *Nature Reviews Microbiology* prize for her talk entitled, 'Proteolytic cleavage sequesters histidine kinase signalling and promotes bacterial stress tolerance'. The poster prizes, which were sponsored by *Molecular Microbiology* and *Trends in Microbiology*, respectively, were awarded to Danny Ward (John Innes Centre, UK) and Martina Pasqua (Sapienza Università di Roma, Italy). Besides the various scientific highlights of this meeting, there were entertaining social events, including a conference dinner and a traditional Irish céilí.

Overall, the feedback from attendees was very positive; participants appreciated the quality of the scientific programme and the intimate atmosphere of the small conference. Recent data collection has demonstrated strong and sustained male dominance in invited speakers to most microbiology meetings, and this is also true for meetings promoting junior scientists [21, 22]. Post-meeting statistics showed that over 50 % of invited speakers, organizers, session chairs and total attendance of the meeting was female (Fig. 4). Our survey illustrated that 92.7 % of the survey participants found the scientific programme 'good' or 'very good' and 96.4 % were interested in attending a future Young Microbiologists Symposium conference. There were also some excellent suggestions for improvement and delivery of the next meeting. This bodes well for another iteration of the meeting planned for 2020.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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