The Australian Society for Microbiology
bringing Microbiologists together
NSW-ACT Branch

Syntrophy

Volume 22 Issue 3 2021

From the editor

By Jim Manos

Dear members,

Welcome to the May 2021 edition of Syntrophy. This issue is largely dedicated to the ASM summer research scholarships.

The National ASM initiative for summer scholarships was launched in October 2020 on a nationwide basis, with National funding one scholarship in each state and branches asked if they could sponsor at least one additional scholarship (each worth $1500). Our branch decided to fund four scholarships for this round as we had few other sponsored events due to the COVID restrictions.

This issue also contains a report from Maurizio on the Zoom Seminar by Dr Yann Boucher from Singapore.

We hope this issue makes for pleasant reading!

Best Regards

Jim

Contact Details

ASM National Office
9/397 Smith Street
Fitzroy Victoria 3065
Australia
Tel 1300 656 423
Fax 1300 655 841
www.theasm.org.au
admin@theasm.com.au

ASM NSW-ACT Branch Chair
Jim Manos
Tel +61 2 93518942
jim.manos@sydney.edu.au

ASM NSW-ACT Branch Secretary
Tim Newsome
Tel +61 2 93512907
tim.newsome@sydney.edu.au

ASM NSW-ACT Branch Treasurer
Christopher Harmer
Tel +61 2 93516028
christopher.harmer@sydney.edu.au

Syntrophy is distributed via email to ASM members located in NSW and the ACT using details included on the ASM National Office Database.

Not yet a member? Join today!
www.theasm.org.au/membership

Submissions and enquiries can be directed to the Syntrophy Coordinator via the ASM NSW-ACT Branch Secretary.

Organisations with research opportunities, or companies seeking to fill positions are welcome to place an advertisement in an upcoming issue of Syntrophy. Please contact the Syntrophy Coordinator with your details for inclusion.

Report on ASM Summer Research Scholarships

By Jim Manos

The ASM Summer Scholarship was open to final year undergraduate microbiology students, and an 'expression of interest' was circulated to members asking prospective candidates to submit their outline for a proposed research project with the support of their sponsoring supervisor, including a project background and description, aims, methodology, and references. They also needed to supply a sponsor's letter of support, CV, and a statement of interest and motivation.

We received 11 submissions by the closing date (December 14), and these were ranked for quality by a subcommittee set up by the branch. Five finalists were chosen based on the following assessment; CV (25%), statement of interest and motivation (25%), sponsor's letter of support (25%), and research project description (25%).

Finalists were then expected to undertake their research project over the summer period and submit a publication-quality research report for examination by March 31. The guidelines for the final report were set by ASM National Office for all participants, as follows:

- Title
- Abstract (200 words maximum), including author name(s) (student awardee and supervisors) and affiliation(s). The abstract should be of sufficient standard to be publishable in Syntrophy and in Microbiology Australia.
- Background or Introduction
- Methods and Materials
- Results
- Discussion
- References. These must be used throughout, listed at the end of the report, and should be formatted in the style of the *Journal of Bacteriology*
- The document must be formatted using 2 cm margins, 12-point font, double spaced throughout, and not exceed five A4 pages.

Four of the finalists submitted their report within the March deadline. The reports were examined by a subcommittee of the branch and reviewer's comments provided. After finalisation of the reports, the finalists were awarded their prize of $1500 (three by the Branch and one by National) plus a certificate signed by the Branch chair.

We are pleased to present you on the following pages the abstracts of our four finalists:

Lucy O'Shannessy – The University of Sydney

Tsung-Yu Pai – Westmead Institute of Medical Research

Daniel Neville – The University of New South Wales

Callum Kay – The Australian National University

ASM Summer Research Scholarships Abstracts

Investigation of mechanism of phagocytosis of *Coxiella burnetii* by bovine mammary epithelial cells.

Lucy O'Shannessy[1], Katrina Bosward[1], Paul Sheehy[1]

[1]*School of Veterinary Science, The University of Sydney*

Q fever is a globally important zoonotic disease caused by the bacterium *Coxiella burnetii*. Livestock are reservoirs for human infection, they shed bacteria transmitted primarily *via* inhalation of contaminated aerosols, while ingestion is a less common and poorly understood transmission route. *Coxiella burnetii* shows high prevalence in unpasteurised dairy products worldwide, thereby presenting a possible public health risk. Bovine mammary epithelial cells (bMECs) have been hypothesised to be a replicative niche in the mammary gland, hence this study aimed to characterise the mechanism mediating their uptake of *C. burnetii*. Expression of Leukocyte Response Integrin (αVβ3) and Complement Receptor 3 (CR3) (receptors mediating uptake of *C. burnetii* in phagocytic cells) was confirmed on the bMEC line, MAC-T, in 2D and 3D organoid culture by immunohistochemical analysis, using monoclonal antibodies targeting CD61 and CD11b (integrins forming αVβ3 and CR3, respectively). A phagocytosis assay was then conducted by pre-incubating MECs with antibody to block αVβ3 and CR3, followed by inoculation with fixed and fluorescently labelled Nine Mile phase II (Clone 4) *C. burnetii*. Despite coincubation with antibody, *C. burnetii* was still taken up, highlighting the complexity of the uptake mechanism. Future work is required to optimise the competitive inhibition model and allow characterisation of uptake mechanisms.

The alcohol dehydrogenase *adhE* encodes a regulatory small RNA within the 3'UTR that is induced by heat shock in enterohaemorrhagic *E. coli*

Daniel Neville[1], Brandon Sy[1], Jai Tree[1]

[1]*School of Biotechnology and Biomolecular Sciences, UNSW*

Enterohaemorrhagic *E. coli* (EHEC) is the most prevalent etiological agent of infectious haemolytic uremic syndrome. EHEC elaborates a Shiga toxin that antagonises the renal endothelium, preventing protein synthesis that leads to cell apoptosis and potentially renal failure. Post-transcriptional regulation plays an important role in EHEC pathogenesis and is controlled by regulatory small RNAs (sRNA). A novel sRNA (here termed AdhU) was identified within the 3'UTR of the highly conserved bifunctional aldehyde/alcohol dehydrogenase AdhE. Deletion of *adhE* attenuates EHEC pathogenesis. The mechanism of this attenuation is unknown and we hypothesise that AdhU may play an important role in virulence gene regulation. Here we have demonstrated that RNase E is not required for the AdhU biogenesis but in doing so, observed heat shock-dependent induction of AdhU expression in EHEC. This indicates that AdhU is controlled by a heat shock responsive promoter. We have used RLM-RACE to verify the presence of multiple RNA 5'-ends upstream of the *adhE* 3'UTR and RNA-seq experiments to identify the regulatory targets of AdhU are ongoing. These are expected to further elucidate the role of AdhU in EHEC pathogenesis.

ASM Summer Research Scholarships Abstracts

NLRP3 inflammasome activation by the bacterial toxin phospholipase C

Callum Kay[1,2] and Si Ming Man[1]

[1] *Department of Immunology and Infectious Disease, The John Curtin School of Medical Research, The Australian National University, Canberra, Australia.*
[2] *Medical School, The Australian National University, Canberra, Australia.*

Inflammasome signalling is a central pillar of innate immunity leading to inflammation and cell death. Identifying and characterising new activators of the inflammasome is critical in elucidating the molecular basis of innate immune recognition of pathogens and to inform the development of novel therapeutics. Previously, I screened a panel of toxins from phylogenetically diverse organisms and identified phospholipase C (PLC) from the bacterial pathogen *Clostridium perfringens* as an activator of the NLRP3 inflammasome. My existing data suggest that phagocytosis and endo-lysosomal trafficking of the toxin are required for PLC-mediated inflammasome activation. PLC then likely induces lysosomal membrane disruption and potassium efflux as a signal to trigger NLRP3 inflammasome assembly. In this study, I obtained new data to strengthen this model. In particular, I used correlative light and electron microscopy to demonstrate that PLC localised with vesicular structures resembling lysosomes in macrophages. I also verified PLC as a bona fide NLRP3 activator using recombinant PLC produced by an alternative commercial source. Additionally, I examined the role of the plasma membrane rupture mediator protein NINJ1 in PLC-mediated cell death. Together, these data further elucidate the mechanism by which a pathogen-derived phospholipase can be detected by the mammalian innate immune system.

Metal-based antifungal drug testing in insect model (*Galleria mellonella*)

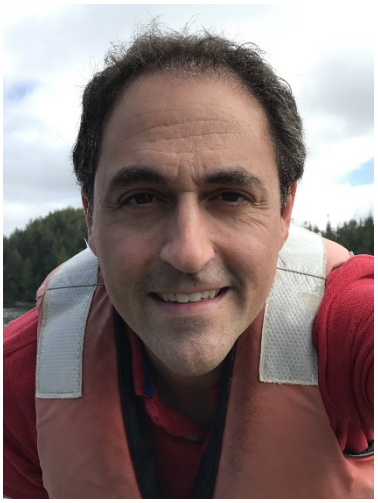
Tsung-Yu Pai[1], Alex Kan[1], Wieland Meyer[1]

[1] Molecular Mycology Research Laboratory, Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research, Westmead NSW.

There is an urgent need for new therapeutic options to address the pressing issue of antibiotic resistance in fungal pathogens. In previous work, cobalt-based complexes were found to present antifungal activity *in vitro* in previous work. In the current study, various cobalt complexes were tested for their potential therapeutic effect against four clinically important fungal species (*Candida albicans, C. auris, Cryptococcus gattii* and *C. neoformans*) in the moth larval model, *Galleria mellonella*. Due to the short timeframe and the limited moth larvae supply, only two test compounds could be tested once. The results showed no significant difference in the survival rates of moth larvae between fluconazole treated and non-treated groups, expect for one test with *C. albicans*, indicating the moth larval mortality was likely to occur before fluconazole could act. Hence, while the inoculating concentration of one strain was optimised, those of other fungal strains need to be further refined in subsequent experiments, and more replicates and additional compounds will be included.

Report on Yann Boucher seminar

By Maurizio Labbate



On the 14th of May, The ASM NSW-ACT Branch hosted A/Prof. Yann Boucher from the National University of Singapore for his presentation entitled "*Vibrio paracholerae: hiding in the shadow of cholera for the last century*". Yann and his team sampled in Dhaka, Bangladesh and identified a new species of *Vibrio* closely related to and associated with the diarrhoeal cholera-causing pathogen, *Vibrio cholerae*. He presented evidence that *V. paracholerae* has caused sporadic diarrhoeal disease in humans and regularly shares DNA with *V. cholerae*. For more details, take a look at the abstract and biography below and, don't forget that we will be hosting more fantastic microbiologists so please keep an eye on our website and Twitter feed (@ASM_NSWACT) for upcoming presentations!

Abstract

Dhaka, the capital city of Bangladesh, is known for recurrent outbreaks of the diarrheal disease cholera. Although this disease and its causative agent, *Vibrio cholerae,* have been studied in details, there is yet to be a study linking the presence of this species in drinking water and the occurrence of an outbreak. To look for this link, we undertook a year-long molecular monitoring of *V. cholerae* populations in the water reservoirs of Dhaka. The lineage of this species linked with the current cholera pandemic (serogroups O1/O139) was present at stable but dangerous levels throughout the year, with no indication of spikes before outbreaks. Most surprisingly however, a novel lineage was even more abundant in the most densely populated and polluted part of Dhaka city. Phylogenomic analysis revealed that isolates from this novel lineage formed a cluster at the base of the *V. cholerae* species, sufficiently differentiated genetically and phenotypically to form a novel species. Numerous cases of infections in the USA, previously identified as ambiguous, also correspond to this novel species. Strains from this species have been anecdotally isolated from around the world, and what has been believed to be one of the oldest *V. cholerae* strains in culture, originating from a soldier suffering choleraic diarrhea in 1916 Egypt, actually belongs to this species. To honor this historical origin, we have named it *Vibrio paracholerae*, a name that was unofficially given to its oldest isolate in 1935.

Biography

Yann is an Associate Professor at the National University of Singapore Saw Swee Hock School of Public Health and a Principal Research Fellow at the Singapore Center for Environmental Life Sciences Engineering. He was previously an Associate Professor in the Department of Biological Sciences, University of Alberta and a Senior Fellow of the Integrated Microbial Biodiversity Program of the Canadian Institute for Advanced Research. He holds a B.Sc. in Biochemistry from Université Laval and obtained his Ph.D in Biochemistry and Molecular Biology from Dalhousie University, both in Canada. He moved to Australia to study mobile genetic elements as a Macquarie University Research Fellow before going to the Massachusetts Institute of Technology as a Merck-MIT Computational and Systems Biology Fellow.

Upcoming / ongoing events