

ASM Summer Student Research Awards: 2019



Priscilla Jobanesen

ASM Student and Early Career
Microbiologist Engagement
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This year the society awarded nine ASM Summer Student Research Awards. These awards are run as a collaboration between national office and state branches and are a fantastic opportunity for students to complete a research project in a laboratory over the summer vacation period. This activity allows students to gain valuable experience in a microbiology research laboratory. This year the successful awardees were: Joshua Dubowsky from South Australia; Amy Griffith and Mikaela G Bell from Queensland; Cassandra Stanton, Joshua J Vido and Yinglei Hua from Victoria; and Wenna Lee, Andrew Vaitekenas, and Alicia Tan Yi Jia from Western Australia. As you will see from the student's abstracts, awardees performed research on wide-ranging areas of microbiology from microbial communities in a tropical rainforest ecosystem through to human and veterinary microbial pathogens. The society congratulates all of the winners of the 2019 awards.

South Australia

Investigating transcriptional disparity in components of the complement alternative pathway between different cell types infected with dengue virus



Joshua Dubowsky

Supervised by: Associate Professor Jill Carr

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Dengue virus (DENV) is the most significant arbovirus affecting human health worldwide yet there is currently no approved treatment to combat the disease it causes. The severe symptoms that can result from DENV infection have clinical features similar to those caused by complement alternative

pathway (AP) overactivity and our laboratory has previously shown that DENV infection induces complement factor H (FH) mRNA. This mRNA could encode for full length FH, or a shorter FH-like protein (FHL-1), which has different properties in regulating the AP. The aim of this project was to quantify the levels of these two alternative transcripts, in DENV infected cells. DENV infection in both HeLa and MDM significantly increased mRNA transcripts from the 5' end of FH however, significant increases in further 3' and full-length FH transcripts were only observed in DENV-MDM. Furthermore, DENV-MDM but not HeLa demonstrated a significant increase in FHL-1 mRNA. MDM represent the natural *in vivo* target for DENV infection and demonstrates that the full transcriptional response of FH to DENV-infection is not mimicked by routine laboratory cell lines such as HeLa. However, HeLa remain suitable for investigating mechanisms of the FH mRNA induction at the level of the promoter. Establishing this methodology will benefit further characterisation of DENV induction of FH and FHL-1 mRNA in other target cells for DENV replication, such as endothelial cells.

Queensland

Characterising outer membrane vesicles (OMVs) of *Moraxella catarrhalis*



Amy Griffith

Supervised by: Dr Aimee Tan and Associate Professor Kate Seib

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Moraxella catarrhalis is an important human restricted pathogen associated with otitis media in children and chronic obstructive pulmonary disease (COPD) exacerbations in adults. Outer membrane vesicles (OMVs) are naturally produced from the outer membrane of Gram-negative organisms including *M. catarrhalis* and have roles in virulence. This study characterised genes involved in OMV biogenesis, protein composition of OMVs and the effects of OMV formation on virulence mechanisms including biofilm formation and serum survival. We identified one gene involved in OMV formation, with ~2–4-fold increase in OMV production in the *M. catarrhalis* 195ME and 25239 mutant strains lacking this gene. This study also characterised OMV composition

of the wild-type *M. catarrhalis* 195ME and 25239 strains, identifying strain dependent differences in composition. In addition, data from phenotypic assays suggested that serum survival is altered by OMV concentration; *M. catarrhalis* 25239 has decreased serum survival when cells are washed prior to the assay representing a hypovesiculating phenotype and have increased serum survival when exogenous OMVs are added. This information helps to understand OMV biogenesis and pathogenesis of *M. catarrhalis*, while also aiding in the characterisation of *M. catarrhalis* OMV protein composition.

Development of a novel *Stenotrophomonas maltophilia* real-time PCR assay



Mikaela G Bell^{A,B}

Supervisors and co-authors: Dr Tamieka A Fraser^{A,B}, Dr Patrick Harris^{C,D}, Professor Scott C Bell^E, Mr Haakon Bergh^C, Professor Graeme Nimmo^C, Dr Derek S Sarovich^{A,B} and Dr Erin P Price^{A,B}

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Stenotrophomonas maltophilia is a Gram-negative, multi-drug-resistant environmental bacterium that is emerging as an important respiratory pathogen in those afflicted with the multiorgan disease, cystic fibrosis (CF). *S. maltophilia* is also recognised as a threatening nosocomial pathogen, especially among the paediatric population, and its presence in those afflicted with CF is associated with more advanced diseases. With a history of being overlooked as a pathogen in its own right, it is currently unclear how widespread *S. maltophilia* is amongst CF cases, so its role in disease pathogenesis remains enigmatic. Currently, there are no rapid, highly-accurate methods for the detection of *S. maltophilia*, meaning that its true prevalence is likely being underestimated. The goal for this study was to use large-scale comparative genomics of *S. maltophilia* and near-neighbour species to identify a highly-specific genetic target for this emerging pathogen, and to subsequently develop and test a newly designed PCR assay for its specific detection. The assay designed was successfully able to detect all 89 clinical *S. maltophilia* samples with 100% accuracy.

Future work will entail screening of non-*S. maltophilia* strains, and determine the limits of detection and quantitation for this new assay.

Victoria

The genetic manipulation of bacteriophage, CSP3, for the detection of *Burkholderia cepacia*



Cassandra Stanton

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A phage infective for two clinical strains of *B. cepacia*, CSP3, was previously isolated and characterised. In this study, CSP3 was used for the development of a biosensor through the addition of a fluorophore (GFP) onto the N-terminus of Orf25, the predicted major capsid protein. Two homology arms were PCR amplified from the CSP3 genome and cloned in either side of *gfp* with no stop codon to create the vector pBBR1-NW. pBBR1-NW was then conjugated into *B. cepacia* and grown with wildtype CSP3. It was hypothesised that as CSP3 infected *B. cepacia* containing pBBR1-NW, the regions of homology between the phage and vector would undergo a double cross-over homologous recombination event, with *gfp* being inserted into CSP3. GFP would form a fusion protein at the N-terminus of Orf25, resulting in translation and expression of GFP during phage replication. Plaques were screened by PCR assays to detect recombinant phages. This study presents a method for producing phage biosensors able to detect *B. cepacia* or to be further applied to other phages and pathogenic bacteria for their sensitive detection.

Exploring the influence of soil abiotic components on microbial community structure within a tropical rainforest ecosystem



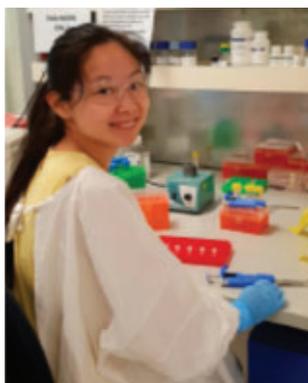
Joshua J Vido

Supervisors: Dr Jennifer Wood and Associate Professor Ashley Franks

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Tropical rainforests harbour the greatest amount of plant diversity of all naturally occurring ecosystems on earth. However, the rapidly changing climate is placing a significant threat on the biodiversity within these ecosystems. Observations into the microbial community dynamics within soil may provide an understanding of how tropical rainforests may be altered from climate change. This is due to microbial communities responding rapidly to environmental change, while the response may be delayed in above-ground organisms such as mature plants. This study aimed to analyse abiotic factors (moisture, pH and electrical conductivity) of soil samples collected from a tropical rainforest in far North Queensland, Australia, in order to investigate the relationship between these factors as well as their influence on bacterial, fungal and oomycete communities. The elevation within the study site where each soil sample was taken was observed to influence microbial community structure, resulting in high beta diversity along the elevation gradient, it was proposed that pH was a major contributor as a strong negative correlation between pH and elevation was observed. This provides an understanding of the soil dynamics underpinning microbial community structure which can be furthered explored through more comprehensive analysis techniques such as next-generation sequencing.

Investigation of pneumococcal gene expression associated with pneumonia pathogenesis



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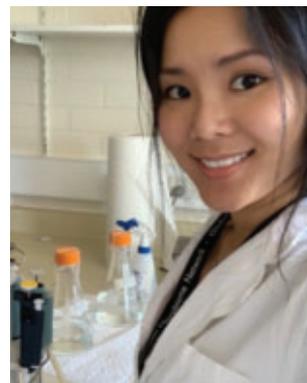
Institute for Infection and Immunity, The University of Melbourne, Parkville, Vic., Australia; ^CDepartment of Paediatrics, The University of Melbourne, Parkville, Vic., Australia

Streptococcus pneumoniae (the pneumococcus) is the most common cause of community acquired pneumonia. However, it is also commonly found as an asymptomatic coloniser of the upper respiratory tract. It is not clear how this pathogen may change from the colonisation state to causing disease. This project investigated pneumococcal gene expression in the nasopharynx and the lung using a unique collection of paired nasopharyngeal swab and lung aspirate samples. It aimed to elucidate the molecular

processes by which the pneumococcus can transition from the carriage to infection state, and identify potential genes involved in pathogenesis of pneumococcal pneumonia. RNA was extracted directly from clinical samples and transcriptomic changes were examined by reverse transcription quantitative PCR (RT-qPCR). Previously, expression of pneumococcal virulence genes has been examined using RNA from five pairs of culture-positive nasopharyngeal swabs and lung aspirates. For the remaining culture-negative clinical samples, sufficient pneumococcal RNA was obtained from nasopharyngeal swabs, but not from culture-negative lung aspirates, for RT-qPCR analysis. In the future, gene expression in the nasopharyngeal swabs from pneumonia patients will be compared with the swabs collected from healthy controls, to investigate whether the pneumococcus changes its behaviour dramatically in the nasopharynx of pneumonia patients compared with healthy controls.

Western Australia

Molecular characterisation of canine haemotropic *Mycoplasma* in Australia



Wenna Lee

Supervisors: Dr Charlotte Oskam, Dr Amanda Barbosa and Professor Peter Irwin

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Emerging infectious diseases (EIDs) are a serious threat to public health and a quarter of EIDs are

zoonotic, directly caused by arthropod vectors. However, efforts to control outbreaks of EIDs are sometimes limited by a lack of information, such as identification of aetiological agents, genetic diversity and transmission dynamics. Ticks are important arthropod vectors that transmit causative agents associated with tick-borne diseases (TBDs), such as *Babesia*, *Anaplasma* and *Mycoplasma* spp. This study aimed to molecularly characterise haemotropic *Mycoplasma* spp. in a group of dogs (blood samples $n = 10$) and haematophagous ticks (*Rhipicephalus sanguineus*; $n = 20$). While all ticks tested were negative for *Mycoplasma*, six out of 10 (60%) dogs screened were positive. Sanger sequencing revealed two dogs were infected with *Mycoplasma haemocanis* and four dogs were infected with *Mycoplasma haematoparvum*; both species were genetically identical (100%) to GenBank references. While *R. sanguineus* is a known vector for mycoplasmas overseas, the *R. sanguineus* analysed in this study were all negative. However, this is the first study to report 60% prevalence of mycoplasmas in dogs from an anonymous kennel.

Further research should focus on determining if the prevalence observed in this study is representative of other kennel dwelling dogs across Australia.

Bacteriophage to treat *Pseudomonas aeruginosa* infection in cystic fibrosis' patient lungs



Andrew Vaitekenas

Supervisors: Associate Professor Anthony Kicic and Dr Stephanie Trend

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Cystic fibrosis is characterised by recurrent bacterial infections principally with *Pseudomonas aeruginosa*. Treatment of infections is currently possible, but antibiotic-resistant bacteria, of which *P. aeruginosa* is a high priority, threaten to make this impossible. Bacteriophages are candidates for the treatment of antibiotic-resistant infections. Propagation is one of the first steps in preparing bacteriophages for therapeutic purposes and screening. Commercial and academic sources of *P. aeruginosa* bacteriophages and propagation conditions were identified for later use. A stock of bacteriophage E79 had a titre of 7.2×10^6 , ~2 logs less than when it was prepared in 2016, indicating that it was relatively stable at 4°C over time. High multiplicity of infections (the ratio of bacteriophage to bacteria; MOI) was tested in a pilot experiment, which suggested that smaller MOIs (e.g. 0.001) were optimum and that initially high bacteriophage density impeded production of progeny. Changes to the protocol that included MOIs from 0.1–0.001 and infection of log-phase bacteria, did not have a marked effect on E79 titre but could be implemented to decrease the time propagation takes.

Using bioinformatics to identify novel virulence factors responsible for the persistence and threat of penicillin-resistant serogroup W *Neisseria meningitidis* in Western Australia



Alicia Tan Yi Jia

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Neisseria meningitidis causes invasive meningococcal disease

(IMD), which presents as septicaemia and/or meningitis. During asymptomatic carriage of *N. meningitidis* in the nasopharynx, acquisition of DNA via homologous recombination from closely related bacteria is frequent, resulting in a highly dynamic population structure. In Western Australia, IMD is predominantly caused by MenW:cc11 meningococci that belongs to two phylogenetically distinct clusters, A and B, which contain penicillin-susceptible and penicillin-resistant isolates, respectively. Interestingly, Cluster B has expanded more rapidly than Cluster A. In this comparative genomics project, we identified horizontal gene transfer events responsible for the emergence and evolution of the penicillin-resistant meningococci. Potential novel virulence factors were described and the prevalence of novel virulence factors in other genetic lineages of *N. meningitidis* was also studied. Various virulence factors such as genes involved in metabolism, iron uptake and transport, amino acid biosynthesis, lipooligosaccharide assembly and modification were identified. Homology to proteins in commensals and pathogens colonising the nasopharynx was observed, with possible acquisition of virulence factors *via* homologous recombination. Possible factors responsible for the global increase in reduced penicillin susceptibility have also been defined. These factors should be studied in mutagenic assays and targeted to address the fitness and persistence of penicillin-resistant meningococci.

Who said this?

But when it has been shown by the researches of Pasteur that the septic property of the atmosphere depended not on the oxygen, or any gaseous constituent, but on minute organisms suspended in it, which owed their energy to their vitality, it occurred to me that decomposition in the injured part might be avoided without excluding the air, by applying as a dressing some material capable of destroying the life of the floating particles. Upon this principle I have based a practice.

Who else but Sir Joseph Lister (1827–1912), a British surgeon and a pioneer of antiseptic surgery. While working at Glasgow Infirmary, Lister introduced the practice of sterilising surgical instruments and cleaning wounds with carbolic acid, now known as phenol. Lister had observed that the stench of fields irrigated with sewage was reduced by the use of carbolic acid. Cows subsequently grazing on the grass did not appear to suffer any side-effects so he concluded it would be a safe effective disinfectant. No TGA in those days!

His patients suffered fewer post-operative infections and he became Britain's leading surgical authority, so much so that even in his late years, after suffering a stroke, he was consulted by surgeons called on to carry out an emergency appendectomy on King Edward VII, two days before his scheduled coronation. Lister advised them in the latest antiseptic surgical methods, which they followed, and the King survived.

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