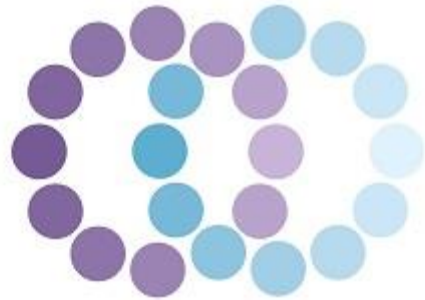


NZMS Annual Conference 2017



*our well-being
& our microbes*

20-23 November, AUT, Auckland



New Zealand
MICROBIOLOGICAL
Society, Inc.

PROGRAMME & ABSTRACTS

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WELCOME

Welcome to the 2017 annual meeting of the **New Zealand Microbiological Society**. This year the theme of the NZ Microbiological Society (NZMS) conference is, **“Our well-being and our microbes”**. We are highlighting the dependence of our economy on maintaining and developing healthy microbial interactions while minimising diseases of concern for humans, livestock and plants. This conference covers the diverse field of microbiology and its disciplines including agricultural, environmental, food, medical and biotechnology sectors that rely on microbial knowledge, management and products.

As our biggest city, Auckland and the surrounding regions has something for everyone, from hot springs to harbours, whale-watching to wine-tasting, mountains to museums and we look forward to hosting your stay here.

CONFERENCE COMMITTEE

Conference Chairs

Gavin Lear (University of Auckland) | g.lear@auckland.ac.nz

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WHILE AT THE CONFERENCE...

- **Registration** desk contact phone number: Rosemary, ph 021 217 8298
- Please wear your **name label** to all conference sessions and social functions. These badges identify you as a NZMS conference participant. The venue is a public space and you may be asked to leave the catering areas if we cannot identify you!
- Please take note of the **location of the exits** in all conference rooms and in your accommodation. Please vacate the building immediately should the alarms sound or when directed to do so by a safety warden.
- Please ask at registration for the **free Wi-Fi** password
- Please do not leave **personal belongings** unattended at any time. If you require temporary storage please ask at the registration desk.
- There are **no refunds** for non-attendance at sessions or social functions.

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PLENARY SPEAKERS



Dr David Bourne | *Molecular Microbial Ecology* | Australian Institute of Marine Science

David is a Senior Lecturer at James Cook University and a Principal Research Scientist at the Australian Institute of Marine Science (AIMS). His teaching and research is focused on the biology of coral reefs and the organisms that reside within these ecosystems. His training is in the area of molecular microbial ecology with his research focused on investigation of microbial diversity, structure and function in complex ecosystems. His research is divided essentially into two areas, the first investigating the normal microbial communities associated with corals and their functional roles in maintaining coral fitness. The second research focus is to elucidate pathogens and mechanism of disease onset in corals and the implications this has on a stressed reef ecosystem in light of climate change being a major driver of coral reef degradation. Current active projects combine use of amplicon sequencing, metagenomic sequencing and transcriptomic sequencing approaches with advanced imaging and chemical analyses to address coral holobiont and coral disease questions. He is also actively engaged in citizen science programs, currently running an EarthWatch project assessing the recovery of reefs around Orpheus Island on the GBR.



Prof. James Paton | *Infectious Disease/Immunology* | University of Adelaide, Australia

James Paton obtained his PhD from the Department of Biochemistry, University of Adelaide, Australia in 1979, and spent 20 years at the Women's and Children's Hospital, Adelaide, where he was Head of the Molecular Microbiology Unit. He took up the Chair of Microbiology at the University of Adelaide in 2000. In 2007, he was awarded one of the inaugural NHMRC Australia Fellowships, and was elected as a Fellow to the Australian Academy of Science in 2013. He is currently a NHMRC Senior Principal Research Fellow and Director of the University of Adelaide's *Research Centre for Infectious Diseases*. For 35 years his research has been focused on the fundamental molecular events involved in the interactions between pathogenic bacteria and their hosts, with particular reference to *Streptococcus pneumoniae*. Early studies focused on identification and characterization of pneumococcal virulence proteins and investigation of their potential as non-serotype-dependent vaccine antigens. More recently, he has been investigating the influence of the host microenvironment on pneumococcal gene expression, and the impact of genetic diversity on virulence profile. Other research interests include Shiga toxin-producing *E. coli* infections and the properties and applications of bacterial AB5 toxins. To date, he has published over 355 scientific papers and book chapters, which collectively have attracted over 23,500 citations.



Colin Murrell

University of East Anglia, UK

Prof. Colin Murrell | *Environmental Microbiology* | Current International Society for Microbial Ecology (ISME) president, University of East Anglia, UK

Colin Murrell is Professor in Environmental Microbiology at the University of East Anglia, Norwich, UK and Director of the Earth and Life Systems Alliance (www.elsa.ac.uk) on the Norwich Research Park. His research encompasses physiology, biochemistry, molecular genetics, biotechnology and molecular ecology of bacteria that grow on methane and other C1 compounds, and the bacterial metabolism of the climate active gas isoprene for which he was recently awarded an ERC Advanced grant. His research over the past 35 years has resulted in ~300 publications and six edited books. Colin is President of the International Society for Microbial Ecology, a Member of the European Molecular Biology Organisation and Member of the European Academy of Microbiology. He serves on the Editorial Boards of *Environmental Microbiology* and *The ISME Journal*, and has Chaired Gordon Research Conferences on C1 Metabolism and Applied and Environmental Microbiology. For more information, see: www.jcmurrell.co.uk



Prof. Emma Allen-Vercoe | *Human Microbiome* | University of Guelph, Canada

Emma obtained her BSc (Hons) in biochemistry in 1993, and her PhD in molecular microbiology in 1999. She has worked with a number of prominent pathogens during her graduate and postgraduate training, including *Salmonella enterica*, enterohemorrhagic *E.coli* and *Mycobacterium tuberculosis*. In 2001 she moved from the UK to Canada to undertake training in cell biology. In 2006, Emma joined the Faculty of Medicine at the University of Calgary, choosing to study the microbes of the human gut, at that time an emerging area of interest. Specifically, she developed a model gut system (dubbed 'Robogut') to emulate the conditions of the human gut and allow communities of microbes to grow together, as they do naturally. Emma moved her lab to the University of Guelph in 2007, and currently runs a lab of 11 people



Prof. Martin Wiedmann | *Food Microbiology/Safety* | Cornell University, USA

Martin received a veterinary degree and a doctorate in Veterinary Medicine from the Ludwig-Maximilians University in Munich in 1992 and 1994, and a Ph.D. in Food Science from Cornell in 1997. He currently is the Gellert Family Professor of Food Safety at Cornell. His research interests focus on farm-to-table microbial food quality and food safety and the application of molecular tools to study the biology and transmission of foodborne pathogens and spoilage organisms. His team has published > 300 peer reviewed publications, which have been cited >10,000 times. He was a member of the Listeria Outbreak Working Group, which was honored by a USDA Secretary's Award for Superior Service in 2000. He also received the Young Scholars award from the American Dairy Science Association in 2002, the Samuel Cate Prescott Award from Institute of Food Technologists' in 2003, the International Life Science Institute North America Future Leaders Award in 2004, and the American Meat Institute Foundation Scientific Achievement Award in 2011. He is a fellow of the Institute of Food Technologists (IFT), a fellow of the American Academy of Microbiology (AAM), and a member of the International Academy of Food Science and Technology. For more information, see: <https://foodscience.cals.cornell.edu/people/martin-wiedmann>



Prof. Joan W. Bennett | *Fungal Genetics* | Rutgers University USA

Joan W. Bennett is Distinguished Professor in the Department of Plant Biology at Rutgers, The State University of New Jersey, USA. She is trained as a fungal geneticist and during much of her career studied the genetics, biosynthesis and molecular biology of aflatoxin production, helping to establish the paradigm that fungal secondary metabolite genes are clustered. In recent years her focus has been on the physiological effects of fungal volatile organic compounds (VOCs) using genetic models. Professor Bennett is a past president of both the Society for Industrial Microbiology and Biotechnology and the American Society for Microbiology, and is a past vice president of the British Mycological Society and the International Union of Microbiological Sciences. Further, she has served as co-editor-in-chief of *Advances in Applied Microbiology* and editor-in-chief of *Mycologia*. She was elected to the National Academy of Sciences (USA) in 2005.

INVITED SPEAKERS



Frank LW Takken
University of Amsterdam, The Netherlands



Xochitl Morgan
University of Otago, New Zealand



Kim Handley
The University of Auckland, New Zealand



Mark Walker
University of Queensland, Australia



Cynthia Whitchurch
University of Technology Sydney, Australia



Paul Williams
University of Nottingham, UK



Gerald Tannock,
University of Otago, New Zealand

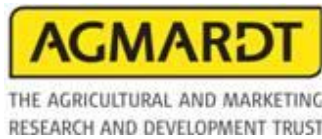
NZMS ORATOR



Stephen On Lincoln University, New Zealand

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NZ Microbiological Society Conference 2017, AUT, AUCKLAND


MONDAY 20th November 2017			
ROOM	WG403	WG308	WG126
12:00-2:10		Student 3 min talks	
2:30-2:45	Conference Opening		
2:45-3:45	Marine Micro Ecology		
	<i>Chair: Gavin Lear</i>		
	Plenary 1 - David Bourne, James Cook University, Australia The role of microbes in coral reef health and disease processes		
	Microbial Ecology	Bioinformatics and Systems Biology	Microbial Physiology
3:45-4:15	<i>Chair: Steven Archer</i>	<i>Chair: Kim Handley</i>	<i>Chair: Carlo Carere</i>
	Invited 1 - Frank LW Takken, University of Amsterdam, Netherlands The good, the bad and the ugly - genetic requirements of beneficial, pathogenic and commensal <i>Fusarium oxysporum</i> strains for colonization of tomato plants.	Invited 2 - Xochitl Morgan, University of Otago Comparative genomics and antibiotic resistance in poultry and clinical vancomycin-resistant enterococci isolates from the post-avoparcin era	
4:15-4:30	#100: Environmental and biogeographical drivers of the <i>Leptospermum scoparium</i> (mānuka) phyllosphere microbiome Anya S. Noble , University of Waikato	#240: Temporal and spatial analysis of <i>Escherichia coli</i> diversity and community structure during colonisation in calves. Adrian L. Cookson , AgResearch Limited, Palmerston North	#226: Investigating the mechanism of pH-mediated antimicrobial potency of gallic acid Ethan Lowry , University of Auckland
4:30-4:45	#221: Phylogeny and genomics of SAUL, an enigmatic bacterial lineage frequently associated with marine sponges Maria del Carmen Astudillo-Garcia , University of Auckland	#176: Metabolic characteristics and genomic epidemiology of <i>Escherichia coli</i> serogroup O145 Rose Collis , Massey University, Palmerston North	#148: Glycocin F: an Antimicrobial Enigma Sean W Bisset , Massey University, Palmerston North
4:45-5:00	#249: Groundwater Health Index: Identifying the risk to drinking water using microbial diversity Louise Weaver , ESR Ltd, Christchurch	#242: De novo hybrid assembly of <i>Escherichia Coli</i> natural isolates using Nanopore sequencing long read technology Georgia Breckell , Massey University, Auckland	#188: Interactions between PvdA and PvdF, enzymes involved in pyoverdine biosynthesis in <i>Pseudomonas aeruginosa</i> PAO1 Priyadarshini Devi Philem , University of Otago
5:00-5:15	#252: The Effects of Ocean Acidification on Microbial Nutrient Cycling and Productivity in Coastal Marine Sediments Shelly Brandt , The University of Waikato	#64: Genome sequence analysis of <i>Streptococcus equinus</i> MDC1, a nisin-producing oral isolate of canine origin Nicholas CK Heng , University of Otago	#140: Microbial metabolism of bioactive compounds and enzymes Michihiko Kobayashi , University of Tsukuba, Japan
5:15-5:30	#251: Microscale coastal sediment responses to ocean acidification Bonnie Laverock , Auckland University of Technology	#225: Novel ncRNAs of the kiwifruit pathogen <i>Psa</i> Bethany R Jose , University of Canterbury	#120: Soft X-ray Tomography: Revealing Cell Structures in the Native State Carolyn A Larabell , University of California, San Francisco, USA


5:30-5:45		#200: The interconnected community: microbial networks in complex and simple natural habitats Shengjing Shi , AgResearch Ltd, Christchurch	#36: The fluorescent protein iLOV outperforms eGFP as a reporter gene in the microaerophilic protozoan <i>Trichomonas vaginalis</i> Shuqi Wang , University of Auckland
5:45-7:15	Exhibition Networking Function & Poster Session #1 - in EXHIBITION & POSTER HALLS		

TUESDAY 21st NOVEMBER		
7:00-8:15	Technicians' Breakfast - Venue: Scarecrow Café	
ROOM	WG403	WG308
	Medical	
	<i>Chair: Thomas Proft</i>	
8:30-9:30	Plenary 2 - James C Paton , University of Adelaide, Australia Pathogenesis of pneumococcal disease	
9:30-10:00	Morning Tea	
	Microbial Ecology ISME Anniversary Session	Medical Microbiology
	 <i>Celebrating 40 years of the International Society for Microbial Ecology Conference</i>	
	<i>Chair: Mike Taylor</i>	<i>Chair: Thomas Proft</i>
10:00-10:30	Invited 3 - Kim Handley , University of Auckland Genome-resolved insights into a reoccurring problem: nutrient-induced blooms of cyanobacteria	Invited 4 - Mark Walker , University of Queensland An experimental group A streptococcal vaccine that reduces pharyngitis and tonsillitis in a non-human primate model
10:30-10:45	#208: pH, temperature and location drive microbial biogeography in 1,000 geothermal springs across New Zealand Jean F Power , GNS Science, Taupo	#83: The Novel Group A <i>Streptococcus</i> antigen SpnA combined with Bead based Immunoassay Technology improves Streptococcal Serology for the Diagnosis of Acute Rheumatic Fever Nicole Moreland , University of Auckland
10:45-11:00	#255: National scale studies of microbial biogeography revisited using highly multiplexed DNA sequence data Gavin Lear , University of Auckland	#23: Mucosal vaccination with pili from Group A <i>Streptococcus</i> expressed on <i>Lactococcus lactis</i> generates protective immune responses Thomas Proft , University of Auckland
11:00-11:15	#129: Ecological insights into unexplored Archaea through environmental ecophysiology, single-cell genomics and cultivation Eva B. Weber , Landcare Research, Lincoln	#22: TeeVax – A multivalent T-antigen-based vaccine against Group A <i>Streptococcus</i> Jacelyn Loh , University of Auckland

11:15-11:30	#202: Influence of dietary treatment on the gut microbiome of a mouse model of autism Giselle Wong , University of Auckland	#246: Using structural biology to inform the design of T-antigen based vaccines for Group A <i>Streptococcus</i> Jeremy Raynes , University of Auckland
11:30-11:45	#109: Whole genome sequencing reveals climate-associated ecotypes in a soil symbiont Anna K. Simonsen , CSIRO, Canberra, Australia	#44: PilVax – a novel peptide antigen delivery strategy for vaccine development Catherine J-Y Tsai , University of Auckland
11:45-12:00		#26: PilVax: a novel peptide carrier for the development of vaccines against tuberculosis Samuel Blanchett , University of Auckland
12:00-1:00	Lunch	
	Microbial Ecology ISME Anniversary Session (cont)	
	<i>Chair: Mike Taylor</i>	
1:00-2:00	Plenary 3 - Colin Murrell - Ecology Microbial degradation of the climate-active trace gas isoprene	
	Microbial Ecology ISME Anniversary Session (cont)	
	<i>Chair: Manpreet Dhani</i>	
	Medical Microbiology (continued)	
	<i>Chair: Nikki Moreland</i>	
2:00-2:15	#217: Network-guided genomic and metagenomic analysis of the faecal microbiota of the critically endangered kakapo Mike Taylor , University of Auckland	#108: Development and preliminary characterisation of a novel poly-Staphylococcal Superantigen like (SSL) fusion vaccine for <i>Staphylococcus aureus</i> Janlin Chan , University of Auckland
2:15-2:30	#65: The beneficial tripartite symbiosis of <i>Piriformospora indica</i> , <i>Rhizobium radiobacter</i> and plants Peter Kaempfer , Justus-Liebig-University Giessen, Germany	#147: A potential role for superantigen producing bacteria in tonsillar hyperplasia Fiona J Radcliff , University of Auckland
2:30-2:45	#230: Paediatric Non-cystic fibrosis Bronchiectasis and the Lung Microbiome David Broderick , University of Auckland	#189: The role of emerging Epsilonproteobacteria in childhood gastroenteritis Angela J Cornelius , ESR, Christchurch
2:45-3:00	#139: Methane-munching microbes - High temperature methanotrophy in geothermal systems Karen M Houghton , University of Waikato	#212: Resolving the heterogeneity in chronic rhinosinusitis: Inflammatory endotypes and microbial associations Kristi Biswas , University of Auckland
3:00-3:30	Afternoon Tea	
	Microbial Ecology ISME Anniversary Session (cont)	
	<i>Chair: Gavin Lear</i>	
	Medical Microbiology (continued)	
	<i>Chair: Simon Swift</i>	
3:30-3:45	#127: Microbial Ecology of Tail Fan Necrosis lesions in New Zealand rock lobster Olga Pantos , ESR, Christchurch	Invited 5 - Cynthia Whitchurch , University of Technology Sydney, Australia

3:45-4:00	#195: One method to rule them all: Optimal DNA extraction approach for multi taxa studies Syrie Hermans , University of Auckland	Morphotype plasticity contributes to antibiotic tolerance by <i>Pseudomonas aeruginosa</i>
4:00-4:15	#142: <i>In vivo</i> transcriptome of the insect pathogen, <i>Yersinia entomophaga</i> MH96T. Amber R Paulson , Massey University, Auckland	#123: A method for the detection and simultaneous typing of multiple <i>Candida albicans</i> strains co-existing at the same human body sites Jan Schmid , Massey University, Palmerston North
4:15-4:30	#155: Mixotrophy drives niche expansion of thermoacidophilic methanotrophs Carlo R. Carere , GNS Science, Taupo	#220: Evaluating the oral microbiota and markers of inflammation in oral and oropharyngeal squamous cell carcinoma Anna Vesty , University of Auckland
4:30-4:45	#172: Functional biogeography of soil bacterial communities across the mountain elevation gradient using 'inferred metagenomes' Jieyun Wu , University of Auckland	#28: Functional Analysis of Streptococcal Virulence Factors Kar Yan Soh , University of Auckland
4:45-5:00	#190: Fungal root endophytes of <i>Agathis australis</i> Mahajabeen Padamsee , Landcare Research, Auckland	#110: Characterization of Mycobacterial Membrane Vesicles Vanessa Chang , University of Auckland
5:00-5:15	#204: Arbuscular mycorrhizal fungal communities across gradients of ecological restoration Julie R Deslippe , Victoria University of Wellington	#146: Rapid selection of an appropriate antibiotic Julia Robertson , University of Auckland
5:15-5:30		#124: Fungi: Key to solving the antibiotic crisis? Tze How Tan , University of Auckland
5:30-5:45		#194: Longitudinal study of the sinonasal microbiota reveals stable, person-specific bacterial communities Brett Wagner Mackenzie , University of Auckland
5:45-7:15	Exhibition Networking Function & Poster Session #2 - in EXHIBITION & POSTER HALLS	
7:15-8:30		Student Networking Function - Room WG308

WEDNESDAY 22nd NOVEMBER			
ROOM	WG403	WG308	WG126
8:30-9:30	<i>Chair: Gerald Tannock</i>		
	Plenary 4 - Emma Allen-Vercoe , University of Guelph, Canada The human gut microbiome: understanding the mechanics of our microbial engine		
9:30-10:30	Morning Tea		
10:00-11:30	Student Oral Competition Finalists		
11:30-12:30	Lunch		
12:00-1:00		NZMS AGM	
1:00-2:00	<i>Chair Brent Seale</i>		
	Plenary 5 - Martin Wiedmann , Cornell University, USA Genomics enabled precision food safety: Moving beyond species to define food safety hazards		
	The Human Microbiome	Food and public health microbiology	Microbial Interactions
			
	<i>Chair: Doug Rosendale</i>	<i>Chair: Brent Seale</i>	<i>Chair: Simon Swift</i>
2:00-2:15	#161: Transcriptional analysis (RNAseq) of a consortium of gut commensals Manuela Centanni , University of Otago	#248: <i>Campylobacter</i> survival in groundwater: Implications for public health Erin McGill , ESR Ltd, Christchurch	Invited 6 - Paul Williams , University of Nottingham, UK Antimicrobial targets and biomarkers - exploiting alkylquinolone-dependent quorum sensing in <i>Pseudomonas aeruginosa</i>
2:15-2:30	#125: Revealing the systemic disorders underlying environmental enteropathy using <i>in vivo</i> proteomics of SILAC-mice, and metabolomics James M McCoy , University of British Columbia, Canada	#151: What induces <i>B. cereus</i> spore germination in food- heat or germinants? Aswathi Soni , University of Otago	
2:30-2:45	#227: The Gut Microbiota During Progression from Health to Type 2 Diabetes in a Transgenic Mouse Model Akarsh Mathrani , University of Auckland	#90: Transcriptome sequencing of a dairy <i>Bacillus licheniformis</i> strain in biofilm and planktonic states reveals insights into biofilm production Faizan Ahmed Sadiq , Massey University, Palmerston North	#97: Molecular anatomy of bacterial membrane vesicles. Simon Swift , University of Auckland
2:45-3:00	#232: Stable isotope probing shows that different redox forms of a sugar are used differently by human gut microbiota in vitro. Doug Rosendale , Plant and Food Research Ltd, Palmerston North	#256: Characterization of the relative abundance of microbiota present in a newly formulated coconut water kefir sourdough using Miseq high throughput sequencing method Mansi Limbad , AUT	#223: Lipid biosensors: investigating the composition of fungal membranes Alyesha Candy , Massey University, Palmerston North
3:00-3:30	Afternoon Tea		

	The Human Microbiome (Continued)	Food and public health microbiology (Continued)	Microbial Ecology and Evolution
			
	<i>Chair: Xochitl Morgan</i>	<i>Chair: Steve Flint</i>	<i>Chair: Heather Hendrickson</i>
3:30-3:45	Invited 7 - Gerald Tannock, University of Otago	#150: <i>Listeria monocytogenes</i> isolates from New Zealand food show differential diversity from international isolates Vathsala Mohan , Plant and Food Research, Auckland	#175: Protozoan Predation Can Influence Bacterial Cell Shape Evolution Danielle Kok , Massey University, Auckland
3:45-4:00	Sustaining the bowel ecosystem of infants	#56: Transcriptome analysis of <i>Listeria monocytogenes</i> exposed to a lethal concentration of nisin Shuyan Wu , Massey University, Palmerston North	#177: Phenotypic Switching in <i>Pseudomonas fluorescens</i> SBW25 Gayle C Ferguson , Massey University, Auckland
4:00-4:15	#235: Elucidating the role of <i>Staphylococcus sp.</i> in chronic rhinosinusitis (CRS) Jesse JB Baker , University of Auckland	#243: Comparison of <i>Listeria monocytogenes</i> inhibitory capability of lactic acid starter cultures during fermentation of shellfish - <i>in vitro</i> and <i>in situ</i> assays Eileen Kitundu , University of Auckland	#216: Evolutionary rescue: quantifying the predictability of the emergence of antibiotic resistance in natural isolates of <i>Escherichia coli</i> . Kelly Hong , Massey University, Auckland
4:15-4:30	#99: A novel system for pectin degradation in <i>Monosphaera pectinilyticus</i> 14T from human faeces Caroline C Kim , Plant and Food Research, Palmerston North	#98: EPA regulates microbes in favour of your wellbeing Manda Safavi , Environmental Protection Authority, Wellington	#152: Probing the Evolutionary History and Species Specificity of Putative Pathogenicity Genes of <i>Pythium insidiosum</i> by Comparative Analyses of Oomycete Genomes Theerapong Krajaejun , Mahidol University, Bangkok, Thailand
			Virology
4:30-4:45	#211: The influence of kiwifruit extract on the human gut microbiota Yao-Chin Liu , University of Auckland	#143: Influence of addition of probiotic bacteria to muesli and storage on consumers' acceptance and bacterial survival Min Min , Lincoln University	#144: From Soil to Sequence: A proof of concept approach using Mycobacteriophage proteins to lyse "from without". Courtney G Davies , Massey University, Auckland
4:45-5:00	#138: Functional Characterization of <i>Faecalibacterium prausnitzii</i> Strains and Its Detection <i>in-vivo</i> Using Culture Independent Technique Sriti Burman , The University of Queensland, Australia	#114: Production of fermented beverages made from whey using <i>Kluyveromyces sp.</i> Naoki Yamahata , Ritsumeikan University, Japan	#158: Geometrical Principles for Understanding the Architecture of Iridoviruses Mihnea Bostina , University of Otago
5:00-5:15	#128: Characterisation of the Gut Microbiome in Depression Bahia Chahwan , University of Technology, Sydney, Australia		#92: Defining The Process Of Sheath Maturation In Anti-feeding prophage (Afp)- A Phage Tailocin Pushpanjali Bhardwaj , University of Auckland
5:15-5:30	#62: Can cooperation within the vaginal microbiome lead to the development of bacterial vaginosis? Nuno Cerca , Universidade do Minho, Braga, Portugal		#193: Targeting Triple Negative Breast Cancer with the Oncolytic Seneca Valley Virus. Laura Burga , University of Otago
6:30-12:00	Conference Dinner - Hilton Hotel		

THURSDAY 23rd NOVEMBER

ROOM	WG403	WG308	WG126
8:30-9:30	<i>Chair: Bevan Weir</i>		
	Plenary 6 - Joan Bennett, Rutgers University, USA Fungal volatile organic compounds: more than just a funky smell		
9:30-10:00	Morning Tea		
	Plant Microbe Interactions	Applied and Industrial Microbiology	Microbial Ecology
	<i>Chair: Bevan Weir</i>		
	<i>Chair: Wei-Qin Zhuang</i>		
	<i>Chair: Mahajabeen Padamsee</i>		
10:00-10:15	#237: More than just plants: A study of biotic stress impacts on the root microbiomes of <i>Trifolium repens</i> Rhys J Jones , AgResearch Ltd, Hamilton	#88: The theory of microbial ubiquity is used by the EPA to make determinations of the “new organism” status of microorganisms Clark Ehlers , EPA, Wellington	#245: Population and single cell analyses reveal sympatric segregation of microbial communities in wastewater treatment plants and microhabitat-dependent predator-prey dynamics Edouard Jurkevitch , The Hebrew University of Jerusalem, Israel
10:15-10:30	#7: Characterizing the Maize Microbiome and Assessing the Risk of GMO-Derived ARG Uptake in Soil Bacteria Courtney M Gardner , Duke University, Durham, USA	#131: “Putting money where the fish’s mouth is” Bikiran Pardesi , University of Auckland	#89: Rapid expansion of <i>Firmicutes</i> phylum under anaerobic alkaline soil pH conditions coincides with dramatic increases in denitrification potential. Craig Anderson , Plant and Food Research, Lincoln
10:30-10:45	#169: Endophyte retention in an artificial <i>Epichloë festucae-Lolium perenne</i> association may come at a price. Flavia Pilar Forte , Aarhus University, Slagelse, Denmark	#133: “When you go fishing, you never know what you will catch: Isolation of previously undiscovered bacteria from the hindgut of the herbivore <i>Kyphosus sydneyanus</i> ” Tony Roberton , University of Auckland	#250: Understanding airborne microorganisms at high temporal resolution Stephen Archer , Auckland University of Technology
10:45-11:00	#253: Genetic determinants of competition and coexistence in the nectar microbiome Manpreet Dhami , Stanford University, CA	#197: There is a spike, but is there a point? Can live cell staining tell us more about bacteria than just bacterial viability? Claire Honney , University of Auckland	#63: Damage to disaster: Cause and effect of tail fan necrosis in spiny lobsters Hua Zha , University of Auckland
11:00-11:15	#222: Oh Boi! How does it work? Ellie L Bradley , Massey University, Palmerston North	#30: Pollutant level influences β -diversity and ring-hydroxylating dioxygenases profile in contaminated soil environment Aliyu Ibrahim Dabai , Queen’s University, Belfast, UK	#254: Investigation of <i>Saccharomyces cerevisiae</i> adaptations during an evolution in emulsion culture Emmelyne Cunningham , Massey University
11:15-11:30	#168: Characterisation of <i>Serratia proteamaculans</i> strain AGR96X encoding an anti-feeding prophage (tailocin) with -activity against grass grub (<i>Costelytra giveni</i>) and manuka beetle (<i>Pyronota spp.</i>) larvae Mark MRH Hurst , AgResearch Ltd, Christchurch	#156: Bacterial Predation of Human Pathogens: Strengths and Limitations Robert J. Mitchell , UNIST, Ulsan, South Korea	
11:30-12:30	NZMS ORATION		
	<i>Chair: Richard Cannon</i>		
	Plenary 7 - Stephen On, University of Lincoln Microbial diagnostics: the germ’s perspective		
12:30-12:40	Conference Closing		
12.40-1.30	Lunch		

Student Oral Presentation Competition Schedule for Preliminary Talks

Room WG308, AUT City Campus

Monday 20 November, 2017

Abstract #	TIME	NAME
225	12:00 - 12:05	Bethany Jose
26	12:05 - 12:10	Samuel Blanchett
36	12:10 - 12:15	Shuqi Wang
227	12:15 - 12:20	Akarsh Mathrani
56	12:20 - 12:25	Shuyan Wu
92	12:25 - 12:30	Pushpanjali Bhardwaj
99	12:30 - 12:35	Caroline Kim
124	12:35 - 12:40	Tze How Tan
235	12:40 - 12:45	Jesse Baker
142	12:45 - 12:50	Amber Paulson
	12:50 - 12:55	BREAK
148	12:55 - 13:00	Sean Bisset
151	13:00 - 13:05	Aswathi Soni
172	13:05 - 13:10	Jieyun Wu
176	13:10 - 13:15	Rose Collis
188	13:15 - 13:20	Priyadarshini Devi Philem
195	13:20 - 13:25	Syrle Hermans
197	13:25 - 13:30	Claire Honney
211	13:30 - 13:35	Yao-Chin Liu
222	13:35 - 13:40	Ellie Bradley
223	13:40 - 13:45	Alyesha Candy
230	13:45 - 13:50	David Broderick
139	13:50 - 13:55	Karen Houghton
216	13:55 - 14:00	Kelly Hong
202	14:00 - 14:05	Giselle Wong

NZMS Conference 2017 Poster Presentations

MONDAY POSTER SESSION - presenters in attendance 5.45pm - 7.15pm

Even number poster; your poster will need to be up from arrival on Monday until Tuesday lunchtime

Poster #	First Name	Last Name	Poster Title
130	Shea	Addison	Bacteriophage-mediated control of <i>Pseudomonas syringae</i> pv. actinidiae on kiwifruit plants
86	Stephen	Ashley	Characterisation of extracellular vesicles from the enteric pathogen <i>Citrobacter rodentium</i>
224	Jian Sheng	Boey	The impact of terrestrial mud inputs on nitrogen cycling microbial communities in the intertidal zone of Waiwera estuary
80	Fabian	Bonk	Comparing ruminal acidosis with acidification of anaerobic digesters to find new therapies and prevention strategies
94	Rosie	Bradshaw	Dothistromin, a fungal secondary metabolite produced during primary growth stage, is regulated by chromatin modification.
214	Marie	Foxwell	Evaluation of strains, carriers and temperatures to develop a spray drying process for <i>Rhizobium leguminosarum</i> microencapsulation
178	Farhad	Golzar	Can Bacterial Virulence Evolve in Response to Protozoan Predation?
174	Tanushree B	Gupta	Occurrence of spore forming bacteria in cattle feed – “ How clean is their food?
38	Chien-Hsun	Huang	Rapid identification of <i>Lactobacillus casei</i> group using MALDI Biotyper combined with ClinproTools
112	Take	Keitaro	Thermostable and acidic chitinase obtained from <i>Streptomyces thermodiastaticus</i>
192	Jisoo	Kwon	Bacterial Predation Reduces Conjugational Transfer of DNA Between Bacteria
238	Donnabella	Lacap-Bugler	Sampling Homogeneities in Aerosol Sampling
258	Huei-Yi	Lai	Experimental evolution uncovers the role of phenotype switching in a newly identified CTG-clade yeast
228	Bonnie	Laverock	Frontal eddies: hotspots for phytoplankton-bacteria interactions and sulphur cycling in the Tasman Sea
170	Van	Le	<i>in vitro</i> Characterization of Furazolidone Resistant <i>Escherichia coli</i> K-12 Isolates to Unveil its Mode of Action
198	Christian	Lux	Efficacy of antibiotic and corticosteroid treatment for chronic rhinosinusitis
82	Daniel	Mulholland	Screening NZ fungi for new antibiotics
184	Hanna-Annette	Peach	The ecology and persistence of thermophilic soil microorganisms through trace-gas oxidation in Tongariro National Park and White Island
234	Janesha	Perera	Quantifying the antifouling and biocidal potential of surfaces.
236	Megan	Petterson	International Collection of Microorganisms from Plants (ICMP)
196	Alannah	Rickerby	Did modern T-DNA evolve from a U-DNA intermediate?
122	Jan	Schmid	<i>Epichloë</i> endophyte-associated antagonism towards an abundant Sordariomycete in the rhizosphere microbiome of perennial ryegrass
122 (a)	Jan	Schmid	A new approach for analysing phosphorous-containing compounds in ryegrass (<i>Lolium perenne</i>) infected with the fungal endophyte <i>Epichloë festucae</i> by ³¹ P NMR
66	Sineenart	Sengyee	Comprehensive analysis of clinical <i>Burkholderia pseudomallei</i> isolates demonstrates conservation of unique lipid A structure and invariable TLR4-dependent innate immune activation
134	Anna	Simonsen	Larger legume hosts have a greater diversity of symbiotic nitrogen-fixing bacteria (rhizobia)
52	Bing	Tian	Biosynthesis of Au and Ag nanoparticles using extracts of <i>Deinococcus</i>
206	Benedict	Uy	Caged infections. Animal work in a PC3
76	Zakieh	Vahdati	The impact of non-tuberculous mycobacterial infection on the immune response of <i>Galleria mellonella</i> .
102	Joni	White	Accurate live and dead bacterial cell enumeration using flow cytometry for <i>Staphylococcus aureus</i>
114	Naoki	Yamahata	Production of fermented beverages from whey
50	Yufeng	Zhang	Identification and understanding the roles of quorum sensing in New Zealand exported lamb

TUESDAY POSTER SESSION - Presenters in attendance 5.45pm - 7.15pm

Odd number poster; your poster will need to be up from Tuesday lunchtime until Thursday

Poster #	First Name	Last Name	Poster Title
37	Haniyeh	Aghababa	Investigations into a novel hypothetical virulence factor from Group A Streptococcus
61	Stephen	Archer	Intercontinental microbial transport to New Zealand from Antarctica
11	Agata	Bancerz-Kisiel	High-Resolution Melting Analysis for SNP Detection In ail Gene of Yersinia enterocolitica Strains Belonging to Different Biotypes
229	Elizabeth	Buckley	Detection and screening of high Ultra Violet resistant microorganisms from the Antarctic and Namib deserts.
47	Nuno	Cerca	Biofilm dormancy enhances antimicrobial tolerance in S. epidermidis
104	Michelle	Challies	Rapid phenotyping of pathogenic and non-pathogenic E. coli O26 using Hyperspectral Imaging
31	Callum	Chalmers	PilVax, a novel peptide antigen delivery system for the development of an effective vaccine against Streptococcus pneumoniae
103	An-Chi	Chen	Use of Natural Products as Preservatives
191	Gayoung	Cho	The Bacterial Predator Bdellovibrio bacteriovorus is Very Sensitive to Detergents
67	Shang	Dai	An Iron Exporter of Deinococcus radiodurans involved in Maintenance of Iron Homeostasis
101	Zachary	Devereux	Fungal Extracellular Vesicles
171	Soeren	Geese	Screening of New Zealand fungi for the discovery of novel antimicrobial compounds.
135	Ting Chun	Hung	Unusual HCV multiple genotype 1b,2,4 mixed infection in the patient on malignant melanoma : Laboratory techniques.
85	Shinnosuke	Ishiyama	Development of a new type of liquor made from whey - selections of Aspergillus species and raw materials for malt, and examination of brewing conditions -
87	Adrina Hema	Khemlani	Streptococcus pyogenes nuclease A (SpnA) mediated virulence does not exclusively depend on nuclease activity
53	Sumeeta	Khurana	Protein Profiling of Acanthamoeba spp. by MALDI-TOF MS for specific identification of Acanthamoeba genotypes
179	Emmanuel	Kyere	Colonisation of 5 different Listeria Monocytogenes strains on hydroponically grown lettuce
141	Kevin	Lee	Does taxonomy matter? Investigating the functional metabolic redundancy within microbial communities across (geo)thermal gradients
121	Tom	Li	Are Plants the Answer to the Global Antibiotic Resistance Crisis?
181	Wonsik	Mun	Cyanide Production by Chromobacterium piscinae Shields It from Bdellovibrio bacteriovorus HD100 Predation
159	Shilpa	Nair	Biocide options to control the transmission of pathogens in our environment
81	Akira	Nakamoto	Construction of D-amino acid derivative production system using Saccharomyces cerevisiae
73	Daiki	Ogiyama	Lactovinegar Production by Ethanol Fermentation of Whey Using Zymomonas mobilis
39	Kim Mizziana	Paras	The Use of PilVax in the Development of Vaccine against Group A Streptococcus
231	Shanthi	Parkar	Variation in chewing behaviour among consumers and its impact on gut microbiome
93	Sarunas	Paskevicius	Plant-Expressed Pyocins As Weapons To Combat Pathogenic Bacteria
239	Jasper	Perry	Investigating the Evolution of mRNA:ncRNA Avoidance in E. coli
49	Masood	Rabbani	Prevalence of soil-borne zoonotic pathogens of public health significance in Punjab province, Pakistan
247	Jeremy	Raynes	Using structural biology to inform the design of T-antigen based vaccines for Group A Streptococcus
71	Chakkaphan	Runchaoren	Whole genome sequencing of ESBL-producing E. coli isolated from patients, farm waste and canals in Thailand
257	Muhammad	Shabbir	Potential relationship between soil characteristics and the occurrence of soil-borne pathogens of public health significance; preliminary findings of a cross-sectional study
137	Hui-Chuan	Shen	Improvement of Abbott Real Time PCR II with a new procedure in Chi-Mei Medical center
203	Nellie	Sibaeva	Exploring the origin of DNA through synthetic biology.
183	Jaspreet Singh	Sidhu	A Comparative Genomics approach to identify secondary metabolite gene clusters that may underlie novel bioactivity in Epichloë endophytes.
105	Christopher	Smith	A biosecurity project: The Ceratocystis fimbriata species complex, Rapid Onset Death and what it means for New Zealand.
107	Mei Lin	Tay	Identifying Autoantibody Targets as New Biomarkers for Acute Rheumatic Fever using Mass Spectrometry and High Content Protein Arrays

43	Georgia	Weaver	The role of helicases in the processing and recovery of DNA replication forks following replication arrest in living <i>Escherichia coli</i> cells
79	Rose	Williams	Winter fungicide sprays impact the dynamics of vineyard <i>Botrytis</i> populations
219	Katie	Worrallo	Impact of estuarine macro-algae on microbial communities and their biogeochemical cycles
185	Dong	Zhang	Next-generation sequencing reveals the seasonal variation of psychrotrophic bacteria in New Zealand raw milk
205	Shuguang	Zhang	Development of vanillin producing Baker'™s yeast

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Joint NZMS / NZSBMB Conference
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Margaret McFall-Ngai
Symbiosis
[USA]



Sylvain Moineau
CRISPR-Cas & Phages
[Canada]



Dan Tawfik
Protein Evolution
[Israel]



Ned Ruby
Bacterial-host interactions
[USA]



Kathryn Holt
Microbial Genomics
[Australia]



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Cryo-EM
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PLENARY & INVITED ABSTRACTS

In order of presentation



PLENARY #1 – David G Bourne

The role of microbes in coral reef health and disease processes

James Cook University and The Australian Institute of Marine Science, Townsville, Australia
e-mail: david.bourne@jcu.edu.au

Coral reefs and the ecosystems they support are in global decline in the face of ever-increasing anthropogenic pressures, including climate change. The design of effective conservation strategies is compromised by our ignorance of the detailed composition of the coral holobiont and how its components interact. Whilst the central role of the symbiotic dinoflagellate *Symbiodinium* in coral metabolism is widely recognised, recent work has also implicated a range of prokaryotes in coral health as well as disease. Transcriptomic evidence suggests that the symbiotic algae may provide not only fixed carbon, but also some amino acids, to their coral hosts. It has frequently been suggested that some of the bacteria closely associated with corals are able to provide essential nutrients, for example, by fixing nitrogen, implying that the microbiome might be critical to holobiont function. However, the true extent of metabolic integration between the various eukaryotic and prokaryotic components of the coral holobiont is unknown. In this talk I will first provide an update of the first coral hologenome, comprising the genomes of the coral host *Porites lutea*, its endogenous *Symbiodinium* strain C15 and detailed insights into the associated bacterial and archaeal communities through reconstruction of 52 population genomes. Analyses of these data are consistent not only with recycling of N, P and S within the system, but also with more complex metabolic interactions between the partners, and suggest that the bacteria may play critical roles in buffering the association against environmental variation. Anthropogenic stresses such as increased sea surface temperatures, nutrient input and sedimentation shift coral-microbiota associations, thereby contributing to reduced coral reef health. Coral diseases are on the rise with disease outbreaks contributing to significant loss of both key reef organisms and coral cover. In this talk I will also explore the microbial dynamics underlying two of the most prevalent coral diseases White Syndrome (WS) and Black Band Disease (BBD). While the causative agent(s) underlying WSs remains elusive, the microbial and cellular processes are beginning to identify and differentiate visually similar but potentially distinct WS etiologies. Black band disease (BBD) is a virulent, globally-distributed infectious disease characterised by a darkly pigmented polymicrobial band at the interface between apparently normal coral tissue and freshly exposed skeleton. Microbial taxonomic profiles for BBD have been extensively characterised, but the underlying microbial metabolic functions that contribute to anoxic and sulphide-rich microenvironments within the lesion and govern its pathogenicity are poorly characterised. Through combined metagenomic and metatranscriptomic profiling of BBD lesions, the development of BBD pathogenicity was linked to relative increases in production of sulfide by sulfate-reducing bacteria, and to photosynthetic CO₂-fixation by cyanobacteria. The latter play a pivotal role in pathogenesis by introducing organic carbon in addition to nutrients derived from dead coral tissue. Furthermore, heterotrophic bacterial degradation of organic carbon produced by cyanobacteria represents a key mechanism for the depletion of dissolved oxygen within BBD lesions to levels harmful to underlying coral tissue, especially in the absence of light. The increased mechanistic understanding of BBD development highlights the complexity of microbial interactions within the polymicrobial communities involved in the pathogenesis of BBD.



PLENARY #2 – James C Paton

Pathogenesis of pneumococcal disease

University of Adelaide, Adelaide, SA, Australia

Streptococcus pneumoniae (the pneumococcus) is one of the world's foremost human pathogens responsible for life-threatening infections such as pneumonia, bacteraemia and meningitis, as well as less serious, but highly prevalent infections such as otitis media and sinusitis. It also asymptotically colonises the nasopharynx of a significant proportion of the population, but the mechanism whereby it progresses from colonisation to disease is still poorly understood. For nearly a century after its initial discovery, the virulence of *S. pneumoniae* was almost exclusively attributed to its polysaccharide capsule, of which 97 distinct serotypes have now been recognised. The capsule is strongly anti-phagocytic and enables rapid proliferation of pneumococci in host tissues; loss of the capsule leads to a roughly 10^6 -fold reduction in virulence. In the early 1980s, the vaccine potential of pneumococcal proteins such as Ply and PspA was demonstrated. The advent of recombinant DNA technology then enabled construction of targeted gene knock-out mutants of *S. pneumoniae*, providing definitive evidence for the contribution of these and other putative protein virulence factors to disease pathogenesis in animal models. Analysis of pneumococcal virulence was revolutionised by later advances in genome sequencing and genome-wide screens such as signature-tagged mutagenesis. This established that virulence was impacted not only by toxins or hydrolytic enzymes that directly damage the host (e.g. Ply, NanA), promote adherence to or invasion of host mucosae (e.g. CbpA), or contribute to evasion/subversion of immune responses (e.g. PspA), but also by a plethora of proteins required for nutrient scavenging and uptake, or other metabolic adaptations to the host environment. Recent work shows that quorum sensing regulatory systems play an important role in these adaptations and progression of disease. In recent years a clearer picture of the vast genetic diversity that exists within the species has also emerged. Current research is exploiting genomic, methylomic, transcriptomic, proteomic and metabolomic technologies to further probe the interaction between *S. pneumoniae* and its host, and to understand the molecular basis for distinct virulence properties exhibited even by closely related strains. This will facilitate formulation of vaccines and/or novel therapeutics capable of preventing the full spectrum of pneumococcal infections.



PLENARY #3 – Colin Murrell

Microbial degradation of the climate-active trace gas isoprene

School of Environmental Sciences, University of East Anglia, Norwich, UK

Isoprene (methyl isobutene) is a climate-active volatile organic compound that is released into the atmosphere in similar quantities to that of methane, making it one of the most abundant trace volatiles. Large amounts of isoprene are produced by trees but also substantial amounts are released by microorganisms, including algae in the marine environment. The consequences on climate are complex. Isoprene can indirectly act as a global warming gas but in the marine environment it is also thought to promote aerosol formation, thus promoting cooling through increased cloud formation. We have been studying bacteria that grow on isoprene. These aerobic bacteria appear to be widespread in the terrestrial and marine environment. *Rhodococcus* AD45, our model organism, oxidizes isoprene using a soluble diiron centre monooxygenase which is similar to soluble methane monooxygenase. The physiology, biochemistry and molecular biology of *Rhodococcus* AD45 will be described, together with genome analysis, transcriptome analysis and regulatory mechanisms of isoprene degradation by bacteria. The distribution, diversity and activity of isoprene degraders in both the terrestrial and marine environment has been studied using functional gene probing, DNA-Stable Isotope Probing, metagenomics and metatranscriptomics experiments. Results indicate that isoprene-degrading bacteria are widespread in soils, estuarine sediments and on leaf surfaces and that they are likely to play a major role in the metabolism of isoprene before it escapes to the atmosphere. Web page Murrell Lab: www.jcmurrell.co.uk



PLENARY #4 – Emma Allen Vercoe

The human gut microbiome: understanding the mechanics of our microbial engine

University of Guelph, Guelph, ONTARIO, Canada

It is becoming increasingly clear that our gut microbiota (the collection of microbes resident in our digestive tract), plays a critical role in the maintenance of our health. Disturbances in the gut microbiota are associated with many diseases, including inflammatory bowel diseases and *Clostridioides difficile* infection. Studying the complex interactions of human gut microbial ecosystems is not straightforward, partly because many of the species that comprise these ecosystems are difficult to culture and thus poorly understood. However, contrary to popular belief, many of the bacterial species that live in the gut are culturable, given the right conditions. We have assembled a model platform ('Robogut') in which to culture gut microbial ecosystems to enable *in vitro/ex vivo* study in a simplified and accessible way.

As part of this work we are developing a series of experimental, defined gut microbial ecosystems to explore ecosystem interactions and functions in detail. To understand the gut microbial 'engine', it is necessary to know how each component part fits together. To understand whether the engine is running optimally, it is necessary to look at the 'emissions' - the metabolic output. The Robogut platform for culture of defined ecosystems, coupled with metabolomics approaches to assess ecosystem output, represents a potentially powerful tool for approaching an understanding of the very complex engine of our gut.

In this presentation, I will show some of the work we have done using our platform to model gut microbial ecosystems, and I will demonstrate how this knowledge may be used to develop novel 'microbial ecosystem therapeutics' to treat disease, an approach that is an emerging paradigm in medicine.



PLENARY #5 – Martin Wiedmann

Genomics enabled precision food safety: Moving beyond species to define food safety hazards

Cornell University, USA

Whole genome sequencing (WGS) is starting to be used routinely, by regulatory and public health agencies, to characterize foodborne pathogen isolates obtained from human clinical cases, foods, and food-associated environments. These efforts are significantly improving both outbreak detection as well as detection and identification of outbreak sources. In addition, the large amount of foodborne pathogen WGS data generated will also provide new insights into the diversity and virulence of foodborne pathogens, which may allow for new approaches to food safety ("precision food safety"). This will be illustrated with examples from three pathogens, including *Listeria monocytogenes*, non-typhoidal *Salmonella enterica*, and *Bacillus cereus*. For *L. monocytogenes*, initial phenotypic and single gene sequencing data provided evidence for occurrence of mutations (SNPs) in the virulence gene *inlA* in a large proportion of food isolates with convincing genetic and phenotypic evidence that these mutations significantly reduce human virulence, de facto establishing molecular markers for *L. monocytogenes* strains with reduced virulence. For *Salmonella*, WGS has identified a subset of serotypes and clonal groups that carry a unique cytolethal toxin that appears to cause DNA damage in infected cells, establishing a potentially novel virulence mechanism that may allow a subset of non-typhoidal *Salmonella enterica* to cause unique symptoms and/or long term sequelae. Overall, the examples presented will illustrate that "pathogen species" often represent a number of distinct groups that may need to be addressed with different and more targeted approaches.



PLENARY #6 – Joan W. Bennett

Fungal volatile organic compounds: more than just a funky smell

Distinguished Professor, Department of Plant Science, Rutgers University, New Brunswick, New Jersey, USA

Fungi are known to emit a variety of odors. Enclosed indoor spaces smell musty; bread dough smells sweet and “yeasty;” stinkhorns smell terrible. Our laboratory has pioneered the application of genetic model organisms to study the physiological effects of aroma compounds produced by filamentous fungi. We have focused on several eight-carbon VOCs responsibility for the musty odors found in water-damaged indoor spaces. The toxigenic potential of these fungal VOCs has been explored in *Drosophila melanogaster*, *Arabidopsis thaliana* and *Saccharomyces cerevisiae*. We have found that exposure to mixtures of VOCs emitted by numerous species of molds isolated from flooded homes cause toxicity to third instar larvae of *D. melanogaster*. Further, exposure to chemical standards of 1-octen-3-ol (“mushroom alcohol”) cause neurotoxic symptoms in adult flies that can be dissected at the molecular level using GFP-linked markers, confocal microscopy, and mutant analysis. In other studies using the plant model *Arabidopsis thaliana*, we showed that low concentrations of mushroom alcohol inhibited seed germination and caused bleaching of seedlings. Unexpectedly, we also showed that volatile mixtures emitted from several species of *Trichoderma* enhanced plant growth and contributed to saline resistance. This growth enhancement was dependent on age of the plant, the species of *Trichoderma*, the length of exposure, and the concentration of emitted volatiles. The underlying molecular mechanisms by which 8-carbon VOCs impact organisms have not yet been elucidated. To this end, we screened the yeast knockout library using both lethal and sublethal concentrations of 1-octen-3-ol in order to dissect the molecular mechanisms involved in the physiological processes mediated by this ubiquitous fungal metabolite.

Mycologists and other biologists who study fungi should become more aware that fungal aroma compounds are more than “just peculiar smells.” They function to transmit molecular signals that have numerous physiological effects on fungi and other organisms in the same ecosystem. In particular, future studies on “mycobiomes” of built environments may provide new perspectives about the possible roles of fungal VOCs in the microbial ecosystems associated with indoor environments.



PLENARY #7 – Stephen On

(NZMS ORATOR)

“Microbial diagnostics: the germ’s perspective”

University of Lincoln

Identification of microorganisms to species, and indeed strain level, is essential to inform appropriate control and/or containment procedures, as well as to indicate new or emerging trends in disease, or well-being where beneficial microbes are considered. Significant technical progress has been made in the past decade, with whole genomes now being sequenced within a day and identification of isolates achievable within a heartbeat. Despite these advances, the fact remains that major challenges remain: arguably, because we do not consider “the germ’s perspective” in our processes. This lecture attempts to present such a perspective; and to explain why it is vital to do so, for the sake of all microbial research.



INVITED #1 – Frank LW Takken

The good, the bad and the ugly - genetic requirements of beneficial, pathogenic and commensal *Fusarium oxysporum* strains for colonization of tomato plants.

Martijn Rep¹, Maria E Constatin¹, Francisco J de Lamo¹, Peter van Dam¹, Lotje van der Does¹, Petra M Houterman¹, Mara de Sain¹, Sri Widinugraheni¹, Ji-Ming Li¹, Like Fokkens¹, Frank LW Takken¹

1. University of Amsterdam, Amsterdam, NH, Netherlands

The fungus *Fusarium oxysporum* (*Fo*) is a common resident of the soil. Besides its ability to grow as a saprophyte it is well known for its intimate contacts with living plant roots. The outcomes of these interactions range from beneficial to deleterious. Actually, in a poll among plant pathologists the fungus ranks 5th in top 10 most devastating plant pathogens affecting our crops. It is therefore remarkable that strains of the same fungus are commonly used as biocontrol agent to enhance plant tolerance to (a)biotic stress factors. Co-inoculation of tomato seedlings with a pathogenic and biocontrol *Fo* strain results in colonization of the host vasculature by both strains, but the typical disease symptoms do not emerge - or to a much lesser extent. How this protection is conferred, and what characteristics distinguish a pathogenic strain from a biocontrol strain, are the main focus of our research.

Besides eleven core chromosomes *Fo* typically harbors conditionally dispensable chromosomes. In case of pathogenic strains these extra chromosomes are enriched for effector genes that are correlated with host range. Previously, our lab showed that transfer of one of these pathogenicity chromosomes from a tomato-infecting strain to a biocontrol strain converts it into a tomato pathogen. The same was more recently demonstrated for a strain infecting cucurbits. *Vice versa*, loss of a pathogenicity chromosome can convert a pathogen into a biocontrol strain. To more systematically investigate the genetic requirements distinguishing non-pathogenic from beneficial and pathogenic strains we sequenced the genomes of additional pathogenic strains as well as non-pathogenic strains that vary in either their biocontrol potential or their ability to colonize tomato plants. In our genome comparisons we focus on the effector repertoire of the various strains to identify key effectors required for host colonization and/or pathogenicity. The results of this ongoing analysis will be presented, along with a few examples of *Fo* effector proteins of which we have obtained some insight into the function in the host.



INVITED #2 – Xochitl Morgan

Comparative genomics and antibiotic resistance in poultry and clinical vancomycin-resistant enterococci isolates from the post-avoparcin era.

University of Otago

Vancomycin-resistant enterococci (VRE) are important opportunistic pathogens with limited treatment options. *Enterococcus faecalis* and *Enterococcus faecium* are gut commensals in both humans and chickens, as well as many other agricultural species. Agricultural antibiotic use is well known to induce antimicrobial resistance in animal-associated pathogens and microflora. Historically, the structural similarities between vancomycin and avoparcin cultivated vancomycin resistance within poultry, resulting in the cessation of agricultural avoparcin use in NZ in 2000. In order to better understand the relationships between human and animal-associated VRE strains in the post-avoparcin era, we have sequenced the genomes of a collection of 231 VRE isolates (75 clinical, 156 poultry¹) cultured between 1998 and 2009. Consistently with historical lower-fidelity typing of New Zealand VRE isolates¹, a clonal strain of *E. faecalis* was highly prevalent among both poultry and clinical isolates in the three years following avoparcin discontinuation in 2000. The high genomic conservation and the near-universal presence of bacitracin genes suggest a common poultry origin.

1. Manson, J.M., Keis, S., Smith, J.M. & Cook, G.M. A clonal lineage of VanA-type *Enterococcus faecalis* predominates in vancomycin-resistant *Enterococci* isolated in New Zealand. *Antimicrobial agents and chemotherapy* **47**, 204-10 (2003).

INVITED #3 – Kim Handley



Genome-resolved insights into a reoccurring problem: nutrient-induced blooms of cyanobacteria

Handley KM, Wood S

University of Auckland, New Zealand

Freshwater cyanobacterial biofilms dominated by *Phormidium autumnale* can produce neurotoxins harmful to the health of animals and humans. *Phormidium* establishes mats on the beds of freshwater streams and other water bodies across New Zealand during influxes of high water column nitrogen, resulting in health advisories against swimming, and animal deaths. To gain proteogenomic insights into the structure and functioning of these biofilms, subaqueous biofilms were collected from Wai-iti River throughout a bloom event. Sampling captured early to late developmental stages over a 19-day period. Prokaryotic community diversity decreased during biofilm development, while the eukaryote fraction diversified. Biofilms became increasingly dominated by *Phormidium autumnale* as they matured and thickened. Early stages contained higher relative abundances of bacteria commonly associated with biofilm formation and wastewater bulking, particularly *Sphaerotilus*. While the genomes of these and other bacteria, including several cyanobacteria, were relatively abundant, protein expression profiles were largely attributed to just two *Phormidium autumnale* strains – the primary biofilm architect and another relatively minor, but highly active biofilm constituent. Nitrate transporter and ATP synthase proteins from both these *Phormidium* strains increased during biofilm development, suggesting a stable relationship over time. The strains were evolutionarily close enough to share some overlap in repeat sequences in phage-defense CRISPR arrays, but not captured spacers. Culture experiments predict only one of the *P. autumnale* strains found in *Phormidium*-dominated biofilms is toxic, although both the active Wai-iti strains possess gene analogues encoding anatoxin-a proteins with characteristic neighboring transposases. Understanding the niches occupied by these coexisting *P. autumnale* strains and interactions with other biofilm members may give insights into how *Phormidium* rapidly establishes thick microbial carpets in waterways.

INVITED #4 – Mark J Walker



An experimental group A streptococcal vaccine that reduces pharyngitis and tonsillitis in a non-human primate model

Australian Infectious Diseases Research Centre and School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

Group A *Streptococcus* (GAS) is an important human pathogen responsible for superficial infections of the skin and throat and serious invasive infections including necrotizing fasciitis and streptococcal toxic shock syndrome. Repeated mild infections can also lead to long-term autoimmune complications. Disease and economic burden data support the rationale for the development of a vaccine against GAS. One of the several hurdles in GAS vaccine development is the variability of protection observed in different animal models, particularly in mice. We have used a non-human primate (NHP) model for pharyngeal infection to assess GAS vaccine protective efficacy. The NHP model was validated using full-M1 protein as a vaccine antigen and used to evaluate the protective efficacy of an experimental vaccine (Combo#5) containing streptolysin O, IL-8 protease, C5a peptidase, arginine deiminase and trigger factor. All antigens were adjuvanted with Alum and NHPs were immunised via intramuscular injection on weeks 0, 8 and 17. On week 20, NHPs were challenged with M1T1 GAS strain 5448 via intranasal delivery. High-titre antigen-specific antibody responses were detected in NHP serum samples against all antigens and immunised NHPs showed a reduction in pharyngitis and tonsillitis symptoms compared to control NHPs. This work demonstrates the protective efficacy of a non-M protein combination vaccine in a NHP model of pharyngitis and tonsillitis.



INVITED #5 – Cynthia B Whitchurch

Morphotype plasticity contributes to antibiotic tolerance by *Pseudomonas aeruginosa*

University Of Technology Sydney, Ultimo, NSW, Australia

A major contributor to the ability of pathogenic bacteria to tolerate the actions of antibiotics is their ability to transition between different lifestyles. One of the best studied bacterial lifestyles that confers elevated tolerance to antibiotics is the formation of matrix-encased biofilms. The opportunistic pathogen *Pseudomonas aeruginosa* undergoes two different mechanisms of morphological transitions in which normal rod-shaped (bacillary) cells transition to spherical cells. One of these processes is extremely rapid, occurs in only a sub-set of the population, and leads to explosive cell lysis events that release cellular content including eDNA, moonlighting proteins, membrane vesicles and other public goods into the extracellular milieu. We found that the Lys endolysin, encoded in the R- and F- pyocin (tailocin) gene cluster, is required for explosive cell lysis and biofilm development by *P. aeruginosa*. The second mechanism of spherical cell morphotype transition by *P. aeruginosa*, is slower, occurs *en masse* in response to β -lactam antibiotics, and involves the morphogenesis of cell-wall deficient (CWD) spherical cells which transition to the normal bacillary form upon antibiotic removal. We have recently determined that the β -lactam-induced CWD cells of *P. aeruginosa* are able to proliferate in the presence of high concentrations of β -lactam antibiotics via mechanisms that are similar to those described for bacterial L-forms. Our observations suggest that the ability to reversibly transition to the L-form lifestyle enables *P. aeruginosa* to tolerate high concentrations of β -lactam antibiotics. We are currently exploring whether these mechanisms of morphotype plasticity can be exploited to develop novel therapeutic approaches to combat *P. aeruginosa* infections.



INVITED #6 – Paul Williams

Antimicrobial targets and biomarkers - exploiting alkylquinolone-dependent quorum sensing in *Pseudomonas aeruginosa*

Centre for Biomolecular Sciences, University of Nottingham

In bacterial pathogens such as *Pseudomonas aeruginosa* the expression of multiple virulence and biofilm development genes is often co-ordinately controlled at the transcriptional level by global regulatory systems incorporating quorum sensing (QS). The latter constitutes a cell-to-cell communication network that integrates information at the population level, co-ordinating the metabolic status of the cells with environmental cues. QS depends on the synthesis, secretion and perception of diffusible signalling molecules that enable pathogens to synchronize their behavior. QS signal molecules, although largely considered as effectors of QS-dependent gene expression are also emerging as multi-functional agents that impact on host-pathogen interactions and influence life, development and death in single and mixed microbial populations. QS is a potential antibacterial target in pathogens where strains carrying mutations in key QS genes exhibit highly attenuated pathogenicity in animal infection models. Further indications that QS systems are active during human infections are emerging from clinical studies where QS signal molecules can be detected in patient body fluids. Since QS by definition depends on small molecule ligand/receptor interactions, it offers a direct pharmacological pathway to inhibitor development since the steric requirements for optimal ligand/receptor interactions means that antagonists can be readily obtained through structural modification of native agonists. In this context, advances in our understanding of the molecular and structural biology of alkylquinolone (AQ)-dependent QS in *P. aeruginosa* have led to the identification of multiple 'druggable' targets and small molecule inhibitors. In cystic fibrosis, the presence of AQS in plasma and urine correlates with clinical status highlighting their potential as biomarkers to aid diagnosis and assessment of the response to treatment.



Invited #7 – Gerald Tannock

Sustaining the bowel ecosystem of infants.

Microbiology and Immunology, and Microbiome Otago, University of Otago, Dunedin, OTAGO, New Zealand

A bacterial community (microbiota, microbiome) inhabits the large bowel of infants. This community, during the exclusively milk-fed period of life, is less diverse than that of adults. Microbiota composition is influenced by the nutrition of the child (breast milk or formula-fed) and much research has been devoted to the role of Human Milk Oligosaccharides (HMOs) in the sustenance of the bowel ecosystem. Formula, commonly based on cow's milk, is frequently used in infant nutrition, but HMO-like substances have low concentrations in ruminant milks. The search for new substances that mimic the effects of HMOs and that can be synthesized or extracted from plant or animal sources continues. The role of fatty acids and peptides derived from milk in the nutrition of the bowel microbiota of infants has been neglected and new insights into their importance are needed. New approaches to sustaining the bowel ecosystem of infants may also be required to counteract a perceived change in bifidobacterial ecology in western countries. Interdisciplinary research is needed to understand niche differentiation, consortium formation, and the functioning of the infant microbiota so that model systems can be developed. This will aid the discovery of novel means of sustaining the bowel ecosystem so that it is optimal for infant development and long-term health.

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CONCURRENT SESSION ABSTRACTS

In numerical order

id #7

Characterizing the Maize Microbiome and Assessing the Risk of GMO-Derived ARG Uptake in Soil Bacteria

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Antibiotic resistance rates have increased in both clinical and environmental bacteria over the past several decades. While the causes underlying these trends have been investigated in a clinical setting, little work has been done to estimate the contribution of antibiotic resistance genes (ARGs) derived from non-traditional sources such as transgenic (GM) crops. While ARGs are no longer commonly used as selection markers during plant transformation, historically many commercially available transgenic crops have contained these genes. The main objective of this study was to determine if the decomposition of transgenic maize biomass affected antibiotic resistance propagation in soil bacteria. To this end, endophytic and rhizospheric prokaryotic microbiomes associated with conventional and transgenic maize containing the Cry1Ab gene and *bla* ARG were compared using Illumina MiSeq. In addition, Cry1Ab exudates were measured in the rhizosphere using a quantitative ELISA assay. Internal endophytes were significantly influenced by time and location within the maize, but not by maize type. Nitrogen-cycling bacteria in the rhizosphere of transgenic maize experienced a decrease in diversity at day 56 of maize cultivation, which was moderately linked to the abundance of the Cry1Ab exudates in these soils. *Bla* ARG expression was also found to increase in soils associated with BT maize relative to conventional maize soils. This significant difference in *bla* expression was not explained by bacterial population growth, suggesting that observed increases may be linked to the release of free DNA from decomposing maize. Furthermore, historically competent genera were also consistently detected in the soils associated with both maize types, supporting the possibility that HGT events may have occurred in these soils.

id #22

TeeVax – A multivalent T-antigen-based vaccine against Group A Streptococcus

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The surface of Group A Streptococcus is decorated with hair-like appendages known as pili. These are involved in adhesion and colonisation of the host during infection. The major protein component of the pilus is the T-antigen which multimerises to form the pilus shaft. Recent genomic analysis of *tee* genes from strains circulating in NZ revealed that inclusion of 18 different T-antigens in a vaccine should provide over 90% coverage. The T-antigen therefore represents an attractive target for vaccination. Our aim is to fuse protein domains from up to 6 different T-antigens, and combine up to 3 fusion proteins in the final multivalent vaccine. The first 2 of these fusion proteins have been successfully expressed and purified. Antibody responses were measured by ELISA and an *in vitro* bioluminescent bactericidal assay developed in our lab. Our results show that rabbits immunised with these TeeVax proteins elicit strong antibody responses that are able to mediate opsonophagocytotic killing of Group A Streptococcus. This suggests that TeeVax is a promising vaccine against Group A Streptococcus.

id #23

Mucosal vaccination with pili from Group A Streptococcus expressed on *Lactococcus lactis* generates protective immune responses

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Group A Streptococcus or *Streptococcus pyogenes* is a major human pathogen that causes a range of diseases, from minor skin and throat infections such as impetigo and pharyngitis, to severe invasive infections such as streptococcal toxic shock syndrome and necrotising fasciitis. GAS produces pili, hair-like protrusions from the bacterial cell surface that are involved in adhesion and colonisation of the host. These surface-exposed pili are immunogenic and therefore represent an attractive target for vaccine development. The pilus is encoded in the genomic region known as the fibronectin-collagen-T-

antigen (FCT)-region, of which at least nine different types have been identified. The pilus operon encodes a backbone pilus protein (aka the T antigen), one or two accessory pilus proteins and a pilus assembly sortase enzyme. In this study we investigate expressing two of the most common FCT-types (FCT-3 and FCT-4) in the food-grade bacterium *Lactococcus lactis* for use as a mucosal vaccine. We show that mucosally delivered *L. lactis* expressing GAS pili generate specific antibody responses in rabbits. These antibodies were shown to have both a neutralising effect on bacterial adhesion, as well as facilitate immune-mediated killing of bacteria via opsonophagocytosis. Furthermore, intranasal immunisation of mice improved clearance rates of GAS after nasopharyngeal challenge. These results demonstrate the potential for a novel, pilus-based vaccine to protect against GAS infections

id #26

PilVax: a novel peptide carrier for the development of vaccines against tuberculosis

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PilVax is a peptide delivery strategy for the generation of highly specific mucosal immune responses. The food-grade bacterium *Lactococcus lactis* is used to express selected peptides engineered within the group A streptococcal pilus, thereby allowing for peptide amplification, stabilization, and enhanced immunogenicity. A pilot study showed that mice immunised with PilVax expressing the model peptide Ova₃₂₄₋₃₃₇ presented strong IgG and IgA responses to ovalbumin. The present study aims to demonstrate the suitability of PilVax for the generation of novel peptide vaccines against tuberculosis. Selected peptides (B cell and T cell epitopes), derived from tuberculosis vaccine targets, were genetically engineered into loop regions of the pilus backbone subunit and expressed in *L. lactis*. Western blots confirmed pilus formation on *L. lactis*. Antibody responses from mice immunised intranasally with recombinant *L. lactis* were strong against the pilus backbone, but weak against the target peptide. However, the poor antibody responses to the two peptides tested thus far were expected, due to being primarily T cell epitopes. We are currently also testing PilVax expressing selected B cell epitopes, and analysing the T cell responses of all constructs.

id #28

Functional Analysis of Streptococcal Virulence Factors

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Background: *Streptococcus pyogenes* (Group A Streptococcus, GAS) causes a variety of diseases in humans ranging from pharyngitis and impetigo to more severe invasive diseases including cellulitis, necrotising fasciitis and toxic shock. Investigations of GAS pathomechanisms are hindered by the lack of suitable animal infection models.

Objectives: To characterise the mechanisms of two important GAS virulence factors, *Streptococcus pyogenes* nuclease A (SpnA) and streptococcal 5'-nucleotidase A (S5nA), by investigating the orthologues SpnAi and S5nAi in the fish pathogen *Streptococcus iniae* using a zebrafish infection model

Method: a) Cloning, expression and purification of recombinant proteins (rSpnAi and rS5nAi). Biochemical analysis of rSpnAi and rS5nAi to confirm that they are orthologues b) Deletion of *spnAi* and *s5nAi* genes in *S. iniae* by allelic replacement c) Analysis of *S. iniae* wild-type (WT) and deletion mutants in zebrafish infection models.

Results: Biochemical analysis of rSpnAi and rS5nAi showed that both proteins have very similar reaction conditions compared to SpnA and S5nA. rSpnAi is able to digest linear double-stranded DNA and chromosomal DNA with highest activity at pH 6.5–7.5 and significant activity at 32°C–37°C in the presence of Ca²⁺ and Mg²⁺. While, rS5nAi is able to generate immunomodulatory molecules adenosine and macrophage toxic deoxyadenosine with highest activity at pH 7 and 42°C in the presence of Mg²⁺. A *S. iniae* *spnAi* gene deletion mutant has been generated and is ready for analysis in a zebrafish infection model. Complementation mutants are currently being generated.

Discussion: SpnAi and S5nAi have been confirmed as true orthologues of the GAS proteins SpnA and S5nA, respectively. The study of these proteins in the zebrafish infection model will provide novel insights into the virulence mechanisms that might be correlated to the in-vivo functions of the orthologue GAS proteins in the human host.

id #30

Pollutant level influences β -diversity and ring-hydroxylating dioxygenases profile in contaminated soil environment

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Metagenomic study linked to high-throughput sequencing allow Microbial ecologist to unfold the complex microbial process involved in the degradation of priority pollutant in contaminated soil. In this study, metagenomic analysis was used

to analyse the β -diversity and ring-hydroxylating dioxygenases profile of Niger Delta contaminated soils. A comparison of the β -diversity measures was performed through Principal coordinate (PC) analysis based on unweighted Unifrac matrices. Bray-Curtis analysis and PC analysis was performed to estimate the β -diversity measures of RHDs. The β -diversity measures suggested a clear demarcation between 'new' and 'old' crude oil polluted soil and a tight clustering of the 'new' crude oil polluted samples, despite the fact that geographically the 'N' samples were actually more diverse. The analysis of new and old crude oil polluted soil samples through two-dimensional PCoA generally showed a grouping of the new away from the 'old' samples. When the same analysis was performed with the Illumina data based on PCA plot of the 16S rRNA gene, a clear demarcation was still observed between the 'new' and 'old' crude oil polluted soil samples. The clustering pattern was obtained irrespective of whether the samples were analysed using MEGAN or MG-RAST. When RHDs profiles of the two sets of polluted samples were compared to contaminated urban samples, the polluted urban samples clustered together close to the new polluted Niger Delta samples. The result showed that the RHDs that form clusters had similar sequences contributing to the RHDs OTUS and are distinct from each other. The Bray-Curtis analysis also showed a clear demarcation between the RHDs profiles of the new and old crude oil samples. The result of the PCA plot of the 16S rRNA gene further supported the fact that the crude oil spillage might be of different hence the difference in the microbial community structure. The conclusion from the RHDs pyrosequencing analysis is that there exist a demarcation between the RHDs profile of the two sets of crude oil polluted samples. A link between the chemistry of the site, microbial ecology and RHDs profile seems to be evident from the data analysed in our study.

id #36

The fluorescent protein iLOV outperforms eGFP as a reporter gene in the microaerophilic protozoan *Trichomonas vaginalis*

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Trichomonas vaginalis is a flagellated protozoan causing the most common non-viral sexually transmitted infection worldwide. Due to its anaerobic metabolism, *T. vaginalis* has to be cultured in laboratories under oxygen-deprived conditions.

Traditional green fluorescent protein (GFP) and its derivatives are generally thought unsuitable for anaerobic protozoa, primarily because of the oxygen dependence for maturation of their chromophore. Therefore, an alternative reporter gene that can be stably expressed and readily detectable under an anaerobic cellular milieu is desirable for *T. vaginalis*.

In this study, we chose iLOV protein, a photo-reversible fluorescent reporter derived from *Arabidopsis thaliana* as a fluorescent protein candidate for *T. vaginalis*, as its oxygen independent nature has been proved by earlier studies.

The iLOV coding sequence (CDS) was chemically synthesized and inserted into MasterNeo plasmid (pMN) for expression, driven by the 5' untranslated region (5'UTR) of either α -succinyl CoA synthetase (α -SCS) or ferredoxin (Fd) gene. We compared the maturation rate and fluorescent intensity of iLOV and eGFP in *T. vaginalis*, at transient and stable expression stages respectively, using flow cytometry.

Analysis of transient expression showed that only trichomonas cells transfected with either of the iLOV plasmids (with α -SCS or Fd gene promoter) exhibited positive expression, indicating the production of a green fluorescent signal. In contrast, the two eGFP and control plasmids produced no detectable signal within the first 8 h post-transfection.

Fluorescence intensities at stable expression stage were determined after G418 selection. Once again, stable transfectants carrying the iLOV plasmids displayed higher fluorescence intensities than those carrying the eGFP plasmids. In regards to their promoters, the one with the α -SCS promoter consistently produced higher fluorescent intensity than the Fd counterpart, regardless of the FP type (either iLOV or eGFP).

The better performance of iLOV over eGFP, as observed here, indicates that this protein is better suitable for *T. vaginalis* as a reporter gene. A faster maturation rate during the course of transient expression potentially enables the investigation of ongoing cellular activities of *T. vaginalis*. The high intensity of iLOV signal under oxygen-deprived conditions could improve the understanding of the biology and infection of this protist.

id #44

PilVax – a novel peptide antigen delivery strategy for vaccine development

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Vaccine development has evolved from killed or live attenuated microorganisms to well-defined synthetic vaccines based on individual proteins or peptides. However, peptide antigens are usually poorly immunogenic and sensitive to proteolytic degradation, and thus require conjugation to carrier proteins, and administration with potentially toxic adjuvants. Lactic acid bacteria have become promising vectors to deliver antigens to mucosal tissues. Combining these two research trends, we have developed a novel peptide delivery system by utilising the group A streptococcus (GAS) pilus structure as a carrier for antigenic peptides, and expressing the modified pili on the surface of the non-pathogenic surrogate *Lactococcus lactis*. Advantages of this new technology, termed PilVax, include increased peptide immunogenicity and stability, higher safety and low production cost.

In this proof-of-concept study, we identified several regions within the backbone pilin FctA (Spy0128) of a serotype M1 strain that can be replaced with the model peptide OVA₃₂₄₋₃₃₉ without affecting pilus assembly and display on the surface of *L. lactis*. Intranasal immunisation of mice with the resulting recombinant *L. lactis* strain produced strong Ova-specific antibody responses (IgG and IgA) in serum and bronchoalveolar fluid. Further exploration of the PilVax design has been carried out, including incorporating multiple peptides into the PilVax construct, and using structurally similar but antigenically different pilus type (from M18 serotype) as a platform. This research shows the potential of the PilVax technology for developing safe, effective and inexpensive peptide vaccines for mucosal delivery.

id #56

Transcriptome analysis of *Listeria monocytogenes* exposed to a lethal concentration of nisin

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The persistence of *Listeria monocytogenes* can be defined by the prolonged viability of *L. monocytogenes* following antimicrobial treatment. This poses a risk to food safety. Persister cells of *L. monocytogenes* isolated from a food environment and exposed to lethal concentrations of nisin was used for this experiment. The persister cells had not acquired resistance as they were re-grow to a new population that resulted in a similar proportion of persister cells surviving following nisin treatment as seen in the original culture. The transcriptome of the persister cells from this *L. monocytogenes* food strain was compared with the original population. Hundreds of differentially expressed genes were identified, and subsequent analysis suggested that many biological processes such as cell wall synthesis, ATP-binding cassette transportation, and phosphotransferase system, were involved in the response of *L. monocytogenes* to nisin treatment. This helps us to understand the response of bacteria to long-term environmental stress.

id #62

Can cooperation within the vaginal microbiome lead to the development of bacterial vaginosis?

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Despite being a controversial topic over the past few decades, *Gardnerella vaginalis* has been attributed a central role in bacterial vaginosis (BV) development. An important milestone in BV research was the discovery that the different species involved in BV were associated in a structured polymicrobial biofilm, dominated by *G. vaginalis*. Subsequent studies demonstrated that *G. vaginalis* biofilms display a high resistance to the protective mechanisms of normal vaginal microflora, as well as an increased tolerance to antibiotics. Despite the increased evidence of the pivotal role of *G. vaginalis* in BV biofilm development, the importance of the other BV-associated anaerobes should not be neglected.

There are some observational studies that correlate bacterial co-colonization between *G. vaginalis* and some other specific BV-associated bacteria during BV. Furthermore, synergistic interactions can occur between BV-associated species and *G. vaginalis*, leading to increased biofilm formation in dual-species biofilms. We hypothesized that differential bacterial interactions can occur during BV development. To test our hypothesis, we examined the ecological interactions between *G. vaginalis* and other 15 BV-associated anaerobes that we had previously shown to enhance biofilm formation by *G. vaginalis*, using a dual-species biofilm model.

Bacterial distribution and biofilm structure were evaluated by peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) method and confocal laser scanning microscopy analysis. Furthermore, the bacterial coaggregation ability was determined as well as the gene expression of virulence genes. The total biomass and the bacterial populations of dual-species biofilms were also quantified, using the crystal violet and PNA FISH methods, respectively.

Our results revealed distinct dual-species structures, between the different consortia, with at least 3 unique biofilm morphotypes. Many, but not all consortia, revealed an induction of *G. vaginalis* genes associated to cytotoxicity, biofilm formation, antimicrobial resistance and immune response.

Overall, this important research contributes to our understanding of how multi-species biofilms contribute to the development of BV. Importantly, the detected specific molecular interactions were very specific to each consortium, suggesting that not all BV- secondary anaerobes contribute to enhanced virulence.

id #63

Damage to disaster: Cause and effect of tail fan necrosis in spiny lobsters

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Tail fan necrosis (TFN) is a disorder commonly found in some populations of commercially fished and aquacultured lobsters. The unsightly appearance of affected lobster tails not only dramatically lowers the commercial value of the

lobsters, but may also impacts their health. In this study, six common crustacean immune parameters were assessed in common crayfish (*Jasus edwardsii*) to determine if TFN was impacting their immune status. Reduced total haemocyte count and low phenoloxidase activity in the haemocyte lysate supernatant (HLS) were both found to be associated with TFN, while haemocyte viability, haemolymph bacterial count, the protein content of haemolymph plasma and HLS showed no differences. In addition, a range of bacterial isolates exhibiting proteolytic, lipolytic and especially chitinolytic activity were found to be associated with TFN in lobsters, and might be associated with bacteraemia. Other bacterial characteristics including biofilm forming capability, melanin and siderophore production, were more often associated with bacteria isolated from lobsters with TFN. These results indicate that TFN is likely to be the result of bacteria with characteristics well-suited to exploiting sites of damaged tail fan, potentially causing longer term negative outcomes via bacteraemia and immune suppression.



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Genome sequence analysis of *Streptococcus equinus* MDC1, a nisin-producing oral isolate of canine origin

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Members of the bacterial genus *Streptococcus* inhabit a variety of ecological niches in humans and other animals, with some species being prolific producers of antibacterial proteins (bacteriocins). During a survey of streptococci from the oral cavities of domestic pets, one particular isolate from a healthy dog, *Streptococcus equinus* MDC1, not only possessed unusual growth characteristics but also displayed potent antibacterial activity in a bacteriocin bioassay. The antibacterial agent produced by strain MDC1 inhibits a variety of pathogenic streptococci including *Streptococcus pyogenes*, but not the caries-causing *Streptococcus mutans* or the endodontic pathogen *Enterococcus faecalis*. *Streptococcus uberis* ATCC 27958, a bovine mastitis strain which produces the lantibiotic nisin U was not killed by the MDC1 bacteriocin, indicating that MDC1 may be producing a new member of the nisin class. The primary aim of this study was to characterise the genome of *S. equinus* MDC1, which was achieved using ultra-long-read Pacific Biosciences Single Molecule Real-Time (SMRT) DNA sequencing technology. The sequence data, consisting of 1.46 gigabasepairs (>700-fold coverage) with an average read length of 7,500 basepairs (bp), were assembled using Canu version 1.4 and annotated using the Rapid Annotations by Subsystems Technology (RAST) server. The *S. equinus* MDC1 genome comprises a single 1,936,552-bp chromosome with a G+C content of 37.4%. A total of 1,969 genomic features including 1,879 putative coding sequences, 21 ribosomal RNA genes (organised into 7 operons) and 69 tRNA genes, were detected by RAST. Closer inspection of the genome sequence revealed a 10-gene locus that encodes a nisin-like lantibiotic peptide precursor, lantibiotic modification and export proteins, and putative immunity proteins. The lantibiotic produced by strain MDC1 has since been designated nisin M. Other notable features of the MDC1 genome include an integrated bacteriophage located within a competence (DNA uptake) gene cluster and the peculiar absence of any known class of mobile genetic elements. In conclusion, *Streptococcus equinus* MDC1 represents the first lantibiotic-producing streptococcus of canine origin to be reported and to have its genome sequenced completely. Further sequence analysis will determine if the strain qualifies as a candidate oral probiotic for human or animal use.

The beneficial tripartite symbiosis of *Piriformospora indica*, *Rhizobium radiobacter* and plants

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The mycorrhizal fungi grouped in the order Sebaciales seem to be of high ecological and agricultural significance. Using PCR, denaturing gradient gel electrophoresis and fluorescence in situ hybridization, we detected an intimate association between *Piriformospora indica* and *Rhizobium radiobacter* (syn *Agrobacterium tumefaciens*; “*Agrobacterium fabrum*”), which is frequently detected in low abundance in hypha and spores of fungal laboratory cultures. The endobacterium (strain *RrF4*) can grow in pure culture, but it has not been possible so far to cure the fungal host completely. A partially cured culture of the fungus containing reduced numbers of endobacterial cells showed reduced capacity to form spores in plant roots, showed reduced colonization efficiency and reduced beneficial activity against host plants. The role of the endobacterium in the fungus is still unclear. In contrast to other fungal endobacteria, the genome of strain *RrF4* is not reduced in size. Instead, it shows a high degree of similarity to the genome of the plant pathogenic *A. tumefaciens* strain C58, except vibrant differences in the tumor-inducing (pTi) and accessory (pAt) plasmid, explaining the loss of pathogenicity. Similar to its fungal host, *RrF4* shows plant growth promotion and induced systemic resistance against fungal and bacterial pathogens. Quantitative real-time PCR data confirmed the proliferation of *RrF4* in roots of axenically grown barley, wheat and *Arabidopsis* plants over the time of incubation. The root colonization patterns of *RrF4* and its host *P. indica* were compared by the application of GUS and GFP-tagged *RrF4* cells combined with light, confocal laser scanning, scanning and transmission electron microscopy. It could be shown that *RrF4* colonizes the rhizodermis and cortex tissue of the root hair zone in a similar manner as its fungal host. But, unlike its fungal host, *RrF4* is able to penetrate through the endodermis into the root stele as known for many other plant growth promoting bacteria. Thus, these data, along with the finding that the abundance of endobacterial cells increased during the tripartite symbioses, and that fungi with reduced bacterial cell numbers show reduced beneficial activity suggest that *RrF4* seems to contribute to the beneficial activity that *P. indica* exerts on plants.

The Novel Group A *Streptococcus* antigen SpnA combined with Bead-based Immunoassay Technology improves Streptococcal Serology for the Diagnosis of Acute Rheumatic Fever

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Streptococcal serological tests provide evidence of prior infection by Group A Streptococcus (GAS). These tests are crucial to the diagnosis of the post-infectious immune sequelae acute rheumatic fever (ARF) and glomerulonephritis since these syndromes develop several weeks after a GAS infection. Current tests measure anti-streptolysin-O (ASO) and anti-DNaseB (ADB) antibodies. Though widely used, these tests have limitations including a slow rate of decay in antibody response that can increase the risk of false positives in settings where GAS is endemic, and incompatible methodology, requiring the two assays to be run in parallel. In this study, the utility of a novel GAS antigen, SpnA, was assessed in a multiplex bead-based assay that also incorporated streptolysin-O and DNaseB. Recombinant streptolysin-O, DNaseB and SpnA were conjugated to polystyrene beads for measurement of serum antibody binding in a Cytometric Bead Array. Multiplex assays were run on sera samples collected from participants in three groups: ARF; ethnically matched healthy children; and healthy adults. The ability of the antigens to detect a previous GAS exposure for ARF diagnosis was assessed using the 80th centile of the healthy children group as a cut-off (upper limit of normal). Using these experimentally determined cut-offs SpnA had the highest sensitivity at 88% compared to 75% for ASO and 56% for DNaseB. In conclusion, SpnA has favourable immunokinetics for streptococcal serology, and the combination of SpnA with ASO and ADB in a multiplex assay should improve the efficiency and accuracy of streptococcal serology.

id #88

The theory of microbial ubiquity is used by the EPA to make determinations of the “new organism” status of microorganisms

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The Environmental Protection Authority (EPA) is the government agency responsible for giving effect to specific environmental management legislation in New Zealand. The EPA's New Organisms team is responsible for assessing and managing the risks associated with the importation, development, field testing and release of new microorganisms in New Zealand in accordance with the provisions of the Hazardous Substances and New Organisms (HSNO) Act 1996. Researchers must obtain approval from the EPA to study new microorganisms in containment or to release those organisms in the environment. This paper presents an overview of the EPA's approach to making determinations of the “new organism” status of microorganisms under the HSNO Act. In 2014, the EPA introduced the theory of microbial ubiquity to inform scientific assessments and decision making regarding the status of microbes under the Act. As a result, EPA Decision-making Committees have used evidence that microbial species are commonly found in multiple environments internationally, for which there are no or limited New Zealand evidence available, to make decisions that they are not new for the purpose of the Act. This paper presents an update of all statutory determinations the EPA assessed since 2014 using microbial ubiquity. We discuss the nature of the supporting evidence and summarise the results for 47 microbes that are frequently found in soils, foods, food production and industrial environments assessed in seven statutory determination applications. Consequently, no further EPA approvals would be required to import, research or release these microbes.

id #89

Rapid expansion of *Firmicutes* phylum under anaerobic alkaline soil pH conditions coincides with dramatic increases in denitrification potential.

Craig Anderson¹, Michelle Peterson¹, Rebekah Frampton¹, Simon Bulman¹, Denis Curtin¹

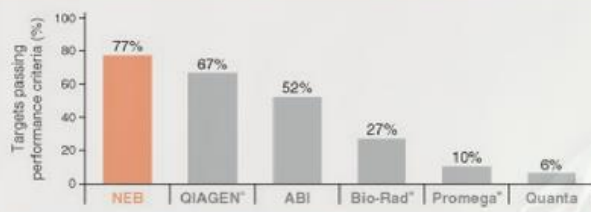
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Urine (urea-N) is an important source of N pollution in agricultural systems, contributing to both nitrate (NO_3^-) leaching and greenhouse gas (N_2O) emissions. Denitrification is an integral part of the soil N-cycle, having an important role in removing excess NO_3^- while also reducing N_2O to N_2 , depending on soil physicochemical conditions. We sought to establish the short-term dynamics of microbial community change under rapid soil pH change and to establish the denitrification potential immediately post urine deposition. We used potassium hydroxide (KOH) to emulate the rapid soil pH change associated with the production of NH_4OH during urea ammonification in soil without adding confounding sources of N. Soil pH was elevated from pH 4.7 to 6.7, 8.3 and 8.8 and then incubated anaerobically for up to 48 hours before measurement of denitrification enzyme activity (DEA) and microbial community change. KOH addition was accompanied by release of up to 240-fold more native dissolved organic matter (DOM) to counterbalance changes in dissolved cation equivalents and charge balance equilibrium. The maximum respiration rate recorded was $\sim 1600 \text{ ng CO}_2\text{-C g}^{-1} \text{ h}^{-1}$. Broad changes in microbial community structure were observed within 16 to 24 hours, dominated by two OTUs from the *Firmicutes* phylum, while few changes were observed in the fungal communities. DEA increased up to 25-fold, with the highest rates occurring at pH 8.3 after 24-hours incubation. Nitrous oxide reductase ($\text{N}_2\text{O-R}$) activity was low at pH <6.7 while $>3000 \text{ ng N}_2\text{O-N g}^{-1} \text{ h}^{-1}$ was reduced at pH 8.3. Our results indicate that microbial communities adapt within 24 hours to the large changes in soil pH and chemistry expected after urine deposition on soil. Suitable conditions for full denitrification to N_2 can develop soon after urine is deposited, with this early denitrification driven by microbes generally not associated with ‘classical’ soil denitrification processes.



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id #90

Transcriptome sequencing of a dairy *Bacillus licheniformis* strain in biofilm and planktonic states reveals insights into biofilm production

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Bacillus licheniformis is the most prevalent spore-forming microbial contaminant of the dairy industry throughout the world. A variety of studies suggest that the ability of this species to form biofilms is the key reason for its widespread prevalence. However, despite its apparent prevalence and spoilage potential, there is no information regarding the molecular determinants involved in biofilm formation by *B. licheniformis*. We used transcriptome sequencing (RNA-seq) technology to reveal over- and underrepresented transcripts in the transition from the planktonic (logarithmic culture) to the biofilm state (24 h old) in a high biofilm forming *B. licheniformis* dairy strain. In total, 1212 differentially expressed genes (DEG) were up regulated at the biofilm stage as compared to the planktonic stage. Similarly, 500 DEG were up regulated at the planktonic stage as compared to the biofilm stage. The gene expression patterns of several groups of genes differed significantly ($P < 0.05$) under the two growth conditions. KEGG pathway impact analysis shows that the genes related to metabolic pathways were significantly upregulated in the biofilm state as compared to the planktonic culture which shows that metabolic pathways could be important for biofilm formation. The genes related to peptidoglycan, amino sugar and nucleotide sugar metabolism, ABC transport system and production of secondary metabolites were particularly more expressed in biofilms as compared to the planktonic state. The genes related to chemotaxis proteins (CheA, CheB, CheC, CheD, CheV, CheW, CheY, MotA, MotB) and flagellar assembly were exclusively upregulated in the planktonic phenotype. Interestingly, the genes related to sporulation and extracellular polymeric substances were concomitantly expressed in the biofilm state which suggests that sporulation is coupled with the biofilm formation in this species similar to *Bacillus subtilis*. The high expression of sortase A (SrtA) in biofilms as compared to the planktonic stage indicates its role in adhesion on the surface. Similarly, filamentous hemagglutinin and poly- β -1,6-GlcNAc (PGA) may serve as important adhesins for the attachment of *B. licheniformis*. Collectively, our results provide a comprehensive insight into biofilm formation in *B. licheniformis* that will be helpful for future research into mechanisms and targets.

Defining The Process Of Sheath Maturation In Anti-feeding prophage (Afp)- A Phage Tailocin

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The Anti-feeding prophage (Afp) is a contractile phage tailocin that causes cessation of feeding resulting in a chronic infection called amber disease of grass grub *Costelytra giveni*, a major pasture pest in New Zealand. The Afp comprises of 18 different proteins. The Afp can be defined as a cell-free variant of the type VI secretion system based on its predicted-function data, bioinformatics work and structural studies. Recently, the term tailocin has been used to describe pyocins and Afp-like structures. Tailocin is defined as a defective phage that unlike a bacteriophage lacks head and contains no DNA. The proteins Afp1-15 combine to form a structure called Tube Baseplate Complex (TBC), whereas Afp1-16 form a sheathed Afp particle of 110nm in length. Contrary to bacteria active- pyocins, or the T4 bacteriophage, Afp has three sheath proteins - Afp2, Afp3 and Afp4. Afp length is determined by the Afp14 ruler protein, while Afp16 is a tail termination protein (TrP) that is also involved in the process of sheath maturation by interacting with sheath proteins.

Using targeted mutagenesis combined with *trans*-complementation of the resultant Afp variants and transmission electron microscopy (TEM), several key mutations were identified that exhibited alterations in the sheath maturation process. One key sheath mutation resulted in the formation of TBC like particles of mature length. The central tube in at least 70% of these, observed TBCs was seen surrounded by a polymerising material. Interestingly, the mutation also yielded a high frequency of dissociated baseplates and tail fibres. The mutation was able to complement successfully thereby restoring both the structure and the biological activity of the Afp. These observations along with mutations in other component Afp proteins, combined with protein-protein interactions of various Afp sheath components resulted in a refinement of the model of Afp sheath maturation that will be discussed.

In summary, the Afp tailocin holds a great promise for the tailored delivery of insect active proteins.

Molecular anatomy of bacterial membrane vesicles.

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Bacteria release membrane vesicles (MV) that can carry a cargo of effectors. Using uropathogenic *Escherichia coli* (UPEC) as a model we have purified MVs and have characterised their molecular composition, finding that they carry a range of proteins, RNA species and lipopolysaccharide. We further show that iron starvation or supply *in vitro*, mimicking a key signal in the progression of infection, impacts the molecular composition of MVs. An RNAseq approach has identified carriage of rRNA, tRNAs, other small RNAs as well as full-length protein coding mRNAs. A proteomic approach has characterised MV protein composition in different media and in comparison to whole cells and other *E. coli*.

What roles might the MVs perform? We show that UPEC MVs are taken up by tissue cultured bladder epithelial cells and that they deliver their RNA content. A microarray approach has characterised the transcriptional responses of the cultured bladder epithelial cells to whole MVs and MV RNA delivered by lipofectamine. The results of these studies combined allow us to hypothesise a role for the molecular MV contents in the subversion of antimicrobial responses from the host cell, and a role for the vesicle as a vehicle for cross-kingdom communication.

EPA regulates microbes in favour of your wellbeing

Manda Safavi¹

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Sample6 is a food safety kit that contains 6 genetically modified (GM) bacteriophages to detect *Listeria* in food and environmental samples. To import and hold Sample6 GM bacteriophages (which are new organisms) in containment, a risk assessment was carried out by the Environmental Protection Authority (EPA), the administrator of the Hazardous Substances and New Organisms (HSNO) Act. The purpose of the HSNO Act is to protect the environment, and the health and safety of people and communities, by preventing or managing the adverse effects of hazardous substances and new organisms. The risk assessment for Sample6 took into account New Zealand's native biota, ecosystems, public health, the relationship of Māori and their culture and tradition, the economy, and New Zealand's international obligations. After assessing the risks, costs and benefits of this application, an approval was granted. Similarly, before new organisms may be developed in New Zealand (including genetic modification, regeneration, fermentation), the EPA is required to undertake a risk assessment. For example, an application to develop microbes to use phage display techniques for protein interaction to specifically recognise targets of relevance to human disease, met the HSNO Act criteria for low-risk genetic modification, and was assessed under a rapid pathway which takes 10 working days. Applications that do not meet the low-risk criteria, are subject to a full assessment. Both rapid and full assessments of development approvals take into account the associated risks, costs and benefits.

A novel system for pectin degradation in *Monosphaera pectinilyticus* 14^T from human faeces

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Pectin is abundantly consumed in modern day diets, as it constitutes the middle lamellas and approximately one third of the dry carbohydrate weight of the plant cell wall (PCW) in vegetables and fruit. Our current understanding of pectin degradation in the human colon has largely derived from studying the generalist model provided by the luminal *Bacteroides* spp. However, the ecological niche for particulate-bound pectinolytic bacteria has remained vacant due to a lack of cultured species to study, creating a 'missing link' in our overall understanding of the hierarchical deconstruction of PCW by our gut microbiota. Here we show that *Monosphaera pectinilyticus* is the first human gut bacterium with a focused glyco biome capability to exclusively degrade pectin, presumably modulated by cell-surface S-layer homology (SLH) domain-containing proteins which occur outside the usual context of lignocellulolytic systems of clostridial isolates from environmental samples. The *M. pectinilyticus* genome encodes 42 extracellular SLH proteins, amongst the most in any anaerobic bacterial genomes sequenced to date. Among these SLH proteins, 8 contain domains of pectin-degrading polysaccharide lyases (PLs), carbohydrate esterases (CEs), and a glycoside hydrolase (GH), and these proteins were often differentially up-regulated in response to the availability of pectin. These cell surface proteins of *M. pectinilyticus* are distinct from the constituents of the well-known microbial carbohydrate degradation systems reported from the human colon (e.g. cellulosome), adding a new layer of complexity to understanding microbe-carbohydrate interactions. Furthermore, the prevalence of *M. pectinilyticus* and related uncultured bacteria from the gastrointestinal systems of humans and animals suggests that organisms from this lineage are ubiquitous terrestrial gut commensals, prompting a wider search for the taxon and its pectinolytic potentials.



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Environmental and biogeographical drivers of the *Leptospermum scoparium* (mānuka) phyllosphere microbiome

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New Zealand's mānuka honey is renowned for its unique healing properties and highly sought after by consumers worldwide. With the export revenue of mānuka honey predicted to rise to \$1.2 billion by 2028, mānuka has become the new buzz-word in New Zealand economy. A substantial body of research exists on the physiology and genetics of the mānuka plant; however, attempts to elucidate the driving factors of honey quality have so far proven unsuccessful.

The surface of leaves, defined as the phyllosphere, provides an enormous habitat for microbial life on Earth and is dominated by diverse communities of epiphytic bacteria. Recent research has demonstrated these bacterial communities exhibit significant variation in composition and diversity with respect to spatial and temporal biotic and abiotic factors as well as host plant genotype. Furthermore, the phyllosphere microbiome is reported to mediate the health and fitness of individual host plants as well as important ecological interactions that facilitate positive terrestrial ecosystem function and productivity. Despite the emerging ecological significance of such epiphytic communities, the phyllosphere of many New Zealand natives, including *Leptospermum scoparium* (mānuka), have not yet been investigated from a microbiological perspective.

This research provides the first exploration of bacterial communities that comprise the phyllosphere of natural mānuka across the North Island. Five discrete mānuka populations were chosen for study, from which leaf samples were collected during flowering season. Culture-independent methods, including DNA extraction and 16s rRNA gene PCR amplicon sequencing analysis, were used to characterise bacterial communities in a hierarchical manner, i.e., within single trees, between different trees, and across different mānuka populations. The data generated will also be used in conjunction with information provided by Steens Mānuka Honey Ltd to examine whether a relationship exists between phyllosphere communities and the quality of mānuka honey derived from each sampled population. The results from this research will provide new insight pertaining to the bacterial communities of the mānuka phyllosphere and will shed light on a potential driving factor of mānuka honey quality. Moreover, this research has the potential to open new avenues in both manuka and microbial ecology fields of investigation within New Zealand.

Development and preliminary characterisation of a novel poly-Staphylococcal Superantigen like (SSL) fusion vaccine for *Staphylococcus aureus*

Janlin Chan, Ries Langley, Fiona Radcliff, Fiona Clow, John Fraser

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major human pathogen first discovered in 1960 as a notorious causative agent of opportunistic infection in nosocomial settings. Fast-forward four decades and *S. aureus* infections have disseminated beyond healthcare facilities to reflect in a global rise of community-associated MRSA (CA-MRSA) cases. Emergence of multi-drug resistant *S. aureus* in recent years further exacerbates the demand for new treatment. Currently, New Zealand has the highest incidence of *S. aureus* infection amongst the developed countries with indigenous populations bearing much of the disease burden. With the dearth of new antibiotics, prophylaxis in the form of vaccination is essential to curtail the rise of resistant strains. In 2000, a new group of *S. aureus* virulence factors known as Staphylococcal superantigen-like proteins (SSLs) was discovered. The fourteen member *ssl* cluster was identified in every *S. aureus* strain with various SSLs playing key roles in host immune dampening and evasion. SSL3, SSL7 and SSL11 are among the best characterised of the SSL proteins secreted to confound host toll-like receptors, the complement cascade and myeloid cells during disease establishment. The overall goal of my research is to synthesize, characterize and ultimately produce a poly-SSL vaccine to alleviate disease symptoms in immunocompromised individuals and carriers prone to recurrent *S. aureus* infections.

Wild-type and mutant (attenuated) forms of *ssl3*, *ssl7* and *ssl11* were cloned into an expression vector to produce two poly-SSL proteins, SSL7-11 and SSL3-7-11 for *in vitro* studies. Binding and functional assays were performed to confirm that the fusion vaccine components had comparable activity to the individual control SSL proteins and that the attenuated SSL7-11 had reduced functionality relative to wild-type SSL7-11. Fusion of SSL7 to SSL11 had no apparent effect on the functionality of either protein and the site-directed mutagenesis of key conserved active sites in *ssl7* and *ssl11* resulted in an attenuated SSL7-11 protein. My next goal is to conduct functionality assays for assessment of wildtype SSL3-7-11 and attenuated SSL3-7-11 activity, before then determining whether vaccination with these constructs can raise antibodies that are able to neutralize SSL activity and impair survival of *S. aureus* in *in vitro* assays.

Whole genome sequencing reveals climate-associated ecotypes in a soil symbiont

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Adaptation, via natural selection, is often assumed as a critical mechanism that enables natural soil microbe populations to rapidly respond to climate change, yet few studies have explicitly tested whether climate influences microbial adaptation in wild populations primarily because complex heterogeneous environmental factors across the landscapes make detection of adaptive signatures difficult. If climate is important in structuring adaptive variation, we predicted that micro-evolutionary forces acting on closely related bacterial populations will generate distinct ecotypes along a climate gradient. Here, we sampled 380 whole genomes of symbiotic nitrogen fixing bacteria, *Bradyrhizobium japonicum* (i.e. rhizobia) originating from *Acacia* hosts along a climate gradient in Southwest Australia. Using a novel analytical approach, we clustered rhizobia genomes according to their shared response to the environment, allowing the discovery of climate-associated ecotype clusters among closely related rhizobial strains. We found that although localized soil conditions and legume host characteristics strongly influence the distribution patterns of *B. japonicum* ecotypes, climate is an important factor in structuring the distribution patterns of genomic variants across the landscape. We found numerous putative ecotypes that occur exclusively in arid and wet conditions, and one arid ecotype was distributed throughout the sampled region providing evidence that this genomic clusters likely harbours a genetic background that has enabled specialization in arid conditions. Our study provides evidence that landscape level climate patterns have differentiated natural nitrogen-fixing symbiont populations into distinct ecotypes, despite the influence of local heterogeneous environmental variation on population structure.

id #110

Characterization of Mycobacterial Membrane Vesicles

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Tuberculous and non-tuberculous Mycobacteria release membrane vesicles (MMVs) that contain lipoproteins and polar lipid. It is hypothesized that membrane vesicles facilitate both the delivery of virulence factors and function as “immune decoys”. To better understand the biology of MMVs we undertook the analysis of mycobacterial species: *Mycobacterium smegmatis* (non-pathogenic, fast-grower), *Mycobacterium abscessus* (human pathogen, fast-grower), *Mycobacterium marinum* (fish and opportunistic human pathogen, slow-grower) and *Mycobacterium tuberculosis* (pathogenic, slow grower).

Mycobacteria were grown in Sauton’s medium with growth measured as optical density and viable counting, and culture viability measured by live/dead staining. MMVs were purified using ultrafiltration and density gradient centrifugation (DGC). Nanoparticle tracking analysis and electron microscopy techniques were used to determine MMV concentration, size and cell viability. DNA, RNA and protein content was quantified using commercial kits. Protein content was further analysed using SDS-PAGE. THP-1 monocytes were grown in RPMI1640 with serum and a Presto Blue viability assay was performed after MMV challenge.

MMVs were observed to be produced throughout growth, with most produced at the transition between exponential and stationary phase. Live/dead staining showed that MMVs were produced by cultures comprising predominantly live cells, and suggested that vesicle production could be an active, selective, biological process. MMVs were isolated with mean diameters between 80-200nm. Different amounts of DNA and RNA were isolated from each species also suggesting the existence of a selective packaging mechanism. Different DNA, RNA and protein patterns observed across the density layers obtained from DGC suggest heterogeneity among species, a notion supported by the interspecies differences observed in SDS-PAGE protein profiles. *In vitro* experiments challenging THP-1 cells with *M. marinum* vesicles showed that MMVs had a dose dependent effect on THP-1 cell viability. The effect of MMVs on these cultured immune cells support a role in modulating host responses and future work elucidating their effects on these cells might contribute to a better understanding of disease pathogenesis.

id #114

Production of fermented beverages made from whey using *Kluyveromyces* sp.

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The aim of this study is to develop new whey beverages brewed by lactose-fermenting yeast, *Kluyveromyces lactis* NBRC 433, *Kluyveromyces marxianus* NBRC 1735, and *Candida kefyr* NBRC 8. Whey is a main by-product of the cheese production and it contains lactose, protein, ash, fat, and other bioactive components. The major industrial usage of whey is a dietary supplement of whey protein. However, about 75% of dry weight of whey is lactose, on the other hand, that of protein is 13% and the utilization of lactose from whey is limited. In addition, the common brewing yeast, *Saccharomyces cerevisiae*, cannot consume lactose so that it is difficult to use *S. cerevisiae* for ethanol fermentation from whey. *Kluyveromyces* sp. and *C. kefyr*, an anamorph of *K. marxianus*, are the typical lactose-fermenting yeasts. Since these strains isolated from food were used in this study, their usage for brewing whey beverages is acceptable.

Strain *K. marxianus* NBRC 1735 produced ethanol faster than other two strains from standard medium which contains 15 w/v% lactose. The maximum amount of ethanol produced by the strain NBRC 1735 was 10 v/v% for 7 days, whereas *K. lactis* NBRC 433 produced only 6.1 v/v% ethanol for 14 days from the same medium. To increase the fermentation ability of the strain NBRC 433, we made fused yeast between the strain NBRC 433 and *S. cerevisiae* K7 using protoplast fusion method with polyethylene glycol (PEG). In 272 colonies obtained in selection medium, 120 isolates showed blue colony in

the standard medium containing X-gal. Among 4 fused strains produced ethanol more than 1.5 times as much as parental strain, NBRC 433, at further screening, fused strain named NY108 produced 9.3 v/v% ethanol from standard medium for 10 days. This result indicated that the fermentation ability of strain NY108 is slightly lower than NBRC 1735, and higher than NBRC 433. The fused yeast strain NY108 which consumes lactose and produces ethanol faster than parental strain was obtained. It is expected to be used for production of fermented beverage made from whey.



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id #120

Soft X-ray Tomography: Revealing Cell Structures in the Native State

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Soft X-ray tomography (SXT) is similar in concept to the well-established medical diagnostic technique, computed axial tomography (CAT), except SXT is capable of imaging with a spatial resolution of 50 nm or better. In SXT, cells are illuminated with x-ray photons from within a region of the spectrum known as the 'water window' (284 – 543eV). 'Water window' x-ray photons are absorbed an order of magnitude more strongly by carbon- and nitrogen-containing organic material than by water. Consequently, variation in biomolecule composition and concentration gives rise to quantitative, high-contrast images of intact, fully hydrated cells without the need to use contrast-enhancing agents. Cells imaged by SXT are, therefore, highly representative of the cell in its native, functional state. Attenuation of soft x-rays, as they pass through the specimen, adheres to the Beer-Lambert Law. Attenuation is, therefore, a function of chemical composition and concentration of organic material, yielding unique quantitative Linear Absorption Coefficient (LAC) measurements for cellular components. To image molecules with respect to cell structures, we developed high numerical aperture cryogenic fluorescence tomography (CFT) for correlated imaging studies. This multi-modal approach - imaging the same cell using both CFT and SXT - allows localization of genetically encoded fluorescent molecules directly in the context of a high-resolution 3-D tomographic reconstruction of the cell. Imaging with SXT has broad applications, ranging from small microbes and microbial communities in their normal environmental conditions, to algae, plants, and viral- or bacterial-infected cells. LAC values are enormously powerful in terms of quantifying alterations in cell structures during events such as cell differentiation, progression or etiology of disease states, genetic manipulation, and application of exogenous agents. We will present examples of a number of different cell types imaged using SXT and CFT-SXT to demonstrate the power of imaging with these technologies.

A method for the detection and simultaneous typing of multiple *Candida albicans* strains co-existing at the same human body sites

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The yeast *Candida albicans* is an important opportunistic human pathogen, and *C. albicans* strains from human samples are often typed and assessed for antifungal drug susceptibility. For this, a single colony is normally used. This seemed justified because previous studies suggested that colonization of a site by multiple strains may be infrequent and usually involves highly similar derivatives of one strain. However, because of the small number of colonies per sample tested in these studies, their results may have underestimated the incidence of clinical samples containing multiple strains. I.e. multiple strains could be present in patient samples, and clinical decisions based on incomplete information, more often than currently assumed. We therefore developed 100+1 NGS-MLST, a next-generation sequencing (NGS) modification of the existing *C. albicans* MLST (Multi Locus Strain Typing) method that reliably detects multiple strains in a sample. In 100+1 NGS-MLST, DNA is extracted from a pool of colonies from a sample, and also from one of the colonies. MLST loci are amplified from both DNA preparations and subjected to high-throughput sequencing. Using base call frequencies, our bespoke DALMATIONS software determines the MLST type of the single colony, then uses this information, plus base call frequencies for the colony pool amplicons, to detect the presence of an additional strain in the sample and infers its MLST type. In mixes of previously typed strains, 100+1 NGS-MLST reliably detected a second strain when present at a frequency of $\geq 10\%$; indirectly inferred MLST types of second strains were always more similar to real MLST types of added strains than to those of any of 55 other strains (24/32 were perfect matches). Applying 100+1 NGS-MLST to 58 samples from 49 individuals, we found that 9 (16%) contained two distinct strains. Our results indicate that it is advisable to test clinical samples for the presence of multiple strains and that 100+1 NGS-MLST is an effective tool for this purpose.

Fungi: Key to solving the antibiotic crisis?

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Antibiotics have been one of the most successful medical treatments in the history of medicine, however our overuse and misuse of antibiotics has placed selective pressure on bacteria leading to the rise of antibiotic resistance. *Pseudomonas aeruginosa*, one of the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogens, has been recently put on the World Health Organization priority list of pathogens for the development of new antibiotics. As New Zealand has been geographically isolated from the rest of the world, much of the native flora and fauna are unique. We hypothesise that New Zealand fungi will produce novel antibiotics. My main objective is to screen New Zealand fungi for metabolites with antibacterial properties against *P. aeruginosa*, and then extract and purify the antibacterial compounds. To achieve my objective, reservoirs are made in the fungi obtained from Landcare Research using punch biopsies. Bioluminescent *P. aeruginosa* is placed in the holes, and light production is measured at time 0, 6, and 24 hours. Fungal biopsies are also used to measure zones of inhibition from the fungi. Initial experiments showed approximately 10% of the 150 fungi screened reduce or eliminate the bioluminescence from *P. aeruginosa*, suggesting the presence of a potentially antibacterial compound. Approximately 5% of 150 fungi screened result in a zone of *P. aeruginosa* growth inhibition. Fungi showing activity against *P. aeruginosa* are now undergoing extraction of potentially antibacterial compounds. Once the extraction is completed, I will perform the Minimum Inhibitory Concentration and Minimum Bactericidal Concentration to test out the concentration needed to inhibit or kill the bacteria.

Revealing the systemic disorders underlying environmental enteropathy using in vivo proteomics of SILAC-mice, and metabolomics

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Environmental Enteropathy (EE) is an inflammatory disease of the small intestine. While typically being subclinical, it is a major cause of persistent malnutrition in children, especially in developing nations. However, the aetiology of EE still remains largely mysterious. Our group recently showed EE is caused by a dysfunction in the gut microbiome, and developed a mouse model of EE. In this model, malnourished mice are orally exposed to a defined mixture of Bacteroidales and *Escherichia coli* bacteria; species which are part of the normal gut microbiome. This combination of malnutrition and non-pathogenic bacteria induce growth stunting, intestinal permeability, and inflammation. Unexpectedly, liver from EE mice also showed significant fat deposits, which is indicative of metabolic dysfunction. This suggests that oral exposure to certain bacterial species can precipitate metabolic disorders, which may exacerbate symptoms of malnutrition.

Stable Isotope Labelling with Amino acids in Cell culture (SILAC) has become a standard tool in quantitative proteomics. However, it has several limitations for studying disease, because its use is restricted to simple cell culture models. As a powerful alternative, mice can be fed diets containing $^{13}\text{C}_6$ -substituted lysine to completely label their proteome. Mouse models of disease can then be used to ask new, more medically relevant questions.

We conducted a multi-omics study of livers of EE mice. By combining quantitative proteomics of EE SILAC-mice with metabolomics data, we have begun to unravel the signalling pathways that underlie the systemic disorders that underlie EE. Proteomics data show for the first time that several key enzymes in liver metabolism are dysregulated during EE. Furthermore, metabolomics data link this dysregulation to changes in metabolism of fatty-acid precursors. This multi-omics dataset shows the wide-ranging implications of "pathogenic" microbiomes, and will enable improved treatment of at-risk children in malnourished environments.

id #127

Microbial Ecology of Tail Fan Necrosis lesions in New Zealand rock lobster

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Lobsters are the basis of highly valuable fisheries in many countries, including New Zealand, where the rock lobster *Jasus edwardsii* (colloquially known as 'crayfish') has both economic and cultural significance. Since the mid to late 1990s lobsters off the coast of the North Island of New Zealand have been found with blackened and disfigured tail fans. This syndrome, known as Tail Fan Necrosis (TFN), is also seen in southern Australia but is predominantly associated with aquaculture facilities, with only very low levels occurring in wild populations. Although it does not pose a risk to human health, the symptoms are aesthetically displeasing, and therefore renders the lobsters unmarketable. While the aetiology of this and other crustacean shell diseases remain unknown there is general consensus that the prevalence and severity of necrosis is likely due to a combination of internal and external factors.

As part of a larger study looking at the factors involved in the elevated occurrence of TFN at particular sites, the microbial communities associated with healthy tail fans and TFN lesions were determined by 16S metabarcoding. The microbial communities associated with healthy tail fans were significantly different between sites with or without TFN, and between lobsters with and without lesions. At sites where TFN was prevalent, although the communities of healthy and lesioned tail fans were significantly different, both were dominated by chitinolytic species and had lower levels of richness and evenness.

These results suggest that even in the absence of visual symptoms of TFN, changes in the associated microbial communities may indicate that environmental or physiological factors, such as water quality or physiological state of the animal, are involved in TFN occurrence.

id #128

Characterisation of the Gut Microbiome in Depression

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Publish consent withheld

id #129

Ecological insights into unexplored Archaea through environmental ecophysiology, single-cell genomics and cultivation

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Unexplored soil microorganisms present a potential reservoir of novel metabolisms that may contribute to important ecosystem processes. One example of unexplored soil microorganisms is Group 1.1c Thaumarchaeota that inhabit many

acidic soils and have unknown metabolic potential. To gather insights into the environmental role of Group 1.1c Thaumarchaeota, several strategies were employed in this study.

The ecophysiology of Group 1.1c Thaumarchaeota was investigated in Scottish pine forest soil microcosms. Although growth was observed, it was not associated with ammonia oxidation and the functional gene *amoA* could not be detected. In addition, environmental studies and genomic predictions suggested existence of both aerobic and anaerobic metabolisms in these microorganisms. However, a microcosm experiment performed in this study demonstrated only aerobic growth of Group 1.1c Thaumarchaeota originating from both aerobic and anaerobic soils.

The metabolic pathways of Group 1.1c Thaumarchaeota were then investigated by developing and applying a targeted single-cell genomic approach. The genomic data obtained indicated four metabolic pathways that may be important for cellular growth: fatty acid β -oxidation, nitrate assimilation, ammonification and taurine oxidation. Based on these metabolic predictions, a specific archaeal cultivation medium was designed and its inoculation with pine forest soil led to the enrichment of the first Group 1.1c thaumarchaeotal representative. This extends the known metabolisms of soil Thaumarchaeota and suggests the involvement of Thaumarchaeota in both autotrophic and heterotrophic soil processes.

id #131

“Putting money where the fish’s mouth is”

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Alternate sources of fish feed for finfish aquaculture are required to meet the growing demand for fish production to feed humanity. Bacteria living in the gut of the seaweed eating fish - Silver drummer (*Kyphosus sydneyanus*) degrade/ferment seaweed, thus facilitating growth and nutrition of the fish. We plan to exploit this and mimic processes inside the fish gut to ferment seaweed in-vitro to obtain nutrients and bacterial biomass that can be used to feed cultured fish. Our aim is thus to develop batch fermentation processes that generate single-cell microbial protein based on these hindgut communities. We have followed two approaches to achieve this. In the bottom-up approach, we have designed and tested over fifty bespoke media (liquid and solid), and have successfully cultured bacteria from the phyla (*Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Proteobacteria*) which are predominant in the inoculum used, i.e. whole gut contents, as identified by 16S ribosomal DNA (rDNA) gene sequencing. Most of these bacteria are previously uncultured and belong to undescribed species and in some cases also genera. We now plan to use a combination of these isolated bacteria to test seaweed degradation in-vitro. In the top-down approach, freshly homogenized seaweed was anaerobically incubated with whole fish-gut contents. In the incubations which looked most degraded, bacteria were identified using 16S rDNA gene sequencing. Preliminary results look promising but these experiments require further optimisation, as we have discovered, in some of our incubations, an increase in groups of bacteria that are less abundant in the fish gut. Future work will focus on improving anaerobic conditions for these seaweed incubations. 16S rDNA gene sequencing of gut contents has shown great variation in the microbial communities in the gut of individual fishes, suggesting that different combinations of bacteria degrade seaweed in wild-type gut communities. Future work will involve getting whole genome sequences of some of our isolates to identify genes that are involved in seaweed degradation.

id #133

“When you go fishing, you never know what you will catch: Isolation of previously undiscovered bacteria from the hindgut of the herbivore *Kyphosus sydneyanus*”

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The herbivorous fish *Kyphosus sydneyanus* feeds mainly on brown algal seaweed. This food source is largely degraded by the bacterial microflora in the fermentative hindgut, yielding short chain fatty acids and bacterial biomass. Microflora composition changes during the passage of material from anterior to posterior hindgut. There is also a large inter-fish variation in community composition, suggesting that different combinations of bacteria can achieve the same overall degradation process to provide the fish with nutrition. As in the rumen and in the human large intestine, the main bacterial phyla present are *Firmicutes* and *Bacteroidetes*. Following studies on various compositions of media that were able to support the growth of mixed cultures of bacteria in broth culture, we have used loop streaking on agar plates with the same compositions of media to grow isolated pure colonies. Reducing and anaerobic conditions were maintained, using an anaerobic chamber and plates were incubated in anaerobic jars. We have obtained over 100 isolates, and carried out 16S rDNA sequencing to confirm identity of isolates. In the 16S rDNA analyses of gut fluid microbiome from section IV L fish hindgut fluid, the *Firmicutes* comprised ~ 71 % of the OTUs, and *Bacteroidetes* ~7.5 % of the total. When the classifications of the microbiome in *K. sydneyanus* were analysed at the family level, the major bacterial types are *Rickenellaceae* (phylum *Bacteroidetes*), *Lachnospiraceae*, *Ruminococcaceae* and *Erysipelotrichaceae* (phylum *Firmicutes*), often some members of the *Tenericutes* (bacteria lacking rigid cell walls) and (genus) *Akkermansia* in the phylum *Verrucomicrobia*, particularly at the mucosal surface of the terminal hindgut. Other bacterial types often seen are the *Vibrionaceae* (phylum *Proteobacteria*) and bacteria from the phylum *Tennericutes*. We have isolates from families *Rickenellaceae*, *Lachnospiraceae*, *Ruminococcaceae* and *Erysipelotrichaceae* and *Vibrionaceae* in pure culture, hopefully representing the

major bacterial types involved in seaweed degradation. If one looks at the 16s sequences of most of these isolates and compares them to database sequences, many of them appear likely to represent new species or new genera, though this can only be confirmed with further studies needed to publish their existence as new bacterial types.



id #138

Functional Characterization of *Faecalibacterium prausnitzii* Strains and Its Detection *in-vivo* Using Culture Independent Technique

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Background and Aims: *Faecalibacterium prausnitzii* is commonly present in healthy adults, and is now widely thought of as a biomarker for gut homeostasis and health. Its attributes include the production of butyrate and other anti-inflammatory factors, including peptides derived from the product of the *mam* gene. However, very little is known about the genetic and functional diversity inherent to this bacterial genus, its anti-inflammatory capabilities, and its persistence in an inflammatory environment. Here, we describe our approaches to overcome these knowledge gaps using a combination of *in-vitro* and *in-vivo* assays.

Methods: The immunomodulatory potential of two characterised *F. prausnitzii* strains (A2-165 and KLE-1255) as well as two new strains isolated from Australian subjects (HMI19 and AHMP21) were determined by quantifying the suppression of NFkB-directed release of IL-8 from human intestinal epithelial (Caco-2) cells. Based on these results, strain AHMP-21 was then used to inoculate BALB/c ZAP70^{W163C}-mutant (SKG) mice by routine oral gavage of viable cells. Colonization and persistence was determined in the fecal and/or cecal contents baseline and end point (day 48), by qPCR using strain-specific (AHMP21) and species-specific primers.

Results: The *mam* gene is constitutively expressed in all 4 strains, but the AHMP21 *mam* gene product is distinctly different to the cognate genes from the other three strains. Additionally, *F. prausnitzii* strain AHMP21 cell extracts were most suppressive of NFkB-directed IL-8 release from Caco-2 cells (~50%), compared to extracts from A2-165, KLE-1255 and HMI19 (~25-40% suppression). The qPCR efficiency of the AHMP21-specific primers was optimised and a detectable amplicon was produced from cecal and fecal samples from SKG mice that received AHMP21 gavage but not control mice, suggesting their successful and specific colonization and persistence in these mice.

Conclusion: The observed differences between *F. prausnitzii* strains in their suppression of NFkB-directed activation of IL-8 are more likely to reflect differences in Mam protein/peptide profile rather than differences in *mam* gene expression. The *mam* gene is both highly conserved and restricted to *Faecalibacterium* spp. and our results suggest the *mam* gene can be used for both qPCR and strain profiling approaches.

id #139

Methane-munching microbes - High temperature methanotrophy in geothermal systems

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Methane plays an important role in global warming, being 25 times more effective at absorbing infra-red radiation than carbon dioxide. Both aerobic and anaerobic microorganisms (methanotrophs) have evolved mechanisms to exploit this energy source prior to its release into the atmosphere, thereby acting as biofilters to reduce emissions of this potent greenhouse gas.

Research on methane oxidisers has been concentrated on low temperature environments, and there are currently only a few isolated methanotrophs that are known to be thermophilic with $T_{\text{opts}} > 45^{\circ}\text{C}$. However, geothermal systems are significant sources for atmospheric gas emissions, with New Zealand fields degassing up to 27 % v/v methane of the total gas released. The methane flux in these ecosystems suggests that thermophilic methanotrophs are likely taking advantage of this abundant, high potential energy source. To assess the possibility of substantial thermophilic methane oxidising populations in geothermal ecosystems, we undertook a study of geothermal systems in the Taupō Volcanic Zone (TVZ) using both culture-independent and culture-dependent methods.

16S rRNA gene amplicon sequencing confirmed the presence of known aerobic methanotrophs in all 59 geothermal samples. Methane oxidation was observed in 53 % of soil and sediment microcosms, and in 63 % of enrichment cultures derived from these microcosms, at temperatures up to 75 °C. Novel bacterial strains, including *Methylacidiphilum*, *Methylocaldum*, *Methylococcus* and *Methylocystis* species, were isolated from microcosms and characterised, including preferred carbon and nitrogen sources, specific growth rates on methane, and the identity of key genes related to methane oxidation, including *pmoA*, *mxoF* and *mmoX*.

Data from this study will be used to advance understanding of the microbial ecology of these climate-impacting species and to determine the physicochemical conditions that support methanotrophy in geothermal environments.

id #140

Microbial metabolism of bioactive compounds and enzymes

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We have been involved in studies on microbial metabolism of various compounds¹⁻⁵⁾ including man-made compounds. Recently, we have been interested in microbial metabolism of natural ingredients in foods. Here, I show you two examples: microbial metabolism of sesamin and piperine, both of which contain a methylenedioxyphenyl group, the structure of which includes a unique ring. We have looked for microorganisms that degrade each of them and have investigated enzymes involved in the degradation of the target compounds.

Sesamin is a type of lignin and is included in a sesame seed. It is a biologically active compound with antioxidative, cholesterol-lowering, lipid-lowering, antihypertensive and anti-inflammatory properties, and so on. While some microbial metabolites of sesamin have been identified, sesamin-metabolic pathways remain unclear at both the enzyme and gene levels. By using the enrichment culture technique, we isolated a bacterium, *Sinomonas* sp. growing on a medium containing sesamin as a sole carbon source. The purified enzyme from the strain cleaved the methylenedioxy bridge of sesamin, and catalyzed the conversion of sesamin to sesamin mono-catechol, and conversion of the resultant sesamin mono-catechol to sesamin di-catechol. Interestingly, tetrahydrofolate was found to be required for higher enzyme activity. The enzyme showed the activity toward (+)-episesamin, (-)-asarinin, sesaminol, and (+)-sesaminol. *Escherichia coli* transformant carrying the enzyme gene the enzyme are expected to be good tools for the production of useful compounds such as sesamin mono-catechol and sesamin di-catechol. Based on site-directed mutagenesis and biochemical analysis, we propose a new and unique catalytic mechanism of the enzyme.

Second, piperine is included in a black pepper and shows a wide range of biological properties such as antioxidant, antiinflammatory, antitumor, antimycobacterial and insecticidal activities. We obtained piperine-degrading No.14 strain (which was identified as *Rhodococcus* sp.) that grew on media containing piperine as a sole carbon source. Piperine was found to be metabolized into piperic acid in the No.14 strain. The purified piperine-degrading enzyme was found to cleave the carbon-nitrogen bond in piperine. This is a new enzyme; there have been no reports concerning tertiary amide-degrading enzymes.

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id #142

***In vivo* transcriptome of the insect pathogen, *Yersinia entomophaga* MH96T.**

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Pathogenic bacteria have evolved specialized virulence factors (VFs) that enable entry and persistence within host tissues. Here we investigated the *in vivo* transcriptome of a novel insect pathogenic bacterium, *Yersinia entomophaga* MH96T, to identify key VFs involved during different stages of infection (early, middle and late) and at different temperatures (25 and 37 °C) within the hemocoel of larval insect host, *Galleria mellonella*. Originally, *Y. entomophaga* was isolated from the cadaver of *Costelytra giveni* (Coleoptera: Scarabaeidae) larva, which is an endemic and notorious pasture pest of New Zealand. Development of *Y. entomophaga* as a biopesticide has proven consistent pathogenesis by *per os* challenge against *C. giveni*, as well as a wide range of coleopteran, lepidopteran, and orthopteran species. Additionally, a median lethal dose of at least three bacterial cells is sufficient to kill larvae of the greater wax moth *G. mellonella* within 4 days of injection with similar levels of mortality observed at both 25°C and 37°C. *In vivo* transcriptome analysis using time-series differential expression and fuzzy clustering identified upregulation of an usher-chaperone fimbrial cluster during early infection, which likely plays an important role in host cell attachment during initial hemolymph colonization. Furthermore, a number of hemoceolic factors, including hemolysins, chitin-binding proteins and iron acquisition mechanisms, were also identified as highly expressed *in vivo*. Striking temperature-dependent regulation was observed for several key VFs, including insecticidal toxin complex (Yen-TC), type VI secretion system and flagellum, all of which were completely down-regulated at 37 °C compared to 25 °C. Similar to mammalian pathogens, the *in vivo* transcriptome of *Y. entomophaga* uncovered a complex network of non-coding RNAs, which likely contributes to the regulation of VFs during pathogenesis. Further work identifying possible RNA-RNA interactions using secondary structure and transcriptional start-site predictions indicated extensive overlapping between non-coding RNAs and potential target mRNAs, characteristic of a highly redundant and intricate regulatory network. This work provides critical insight into the pathobiology and in-host gene expression of a potentially important biopesticide, with focus on novel VF discovery.

id #143

Influence of addition of probiotic bacteria to muesli and storage on consumers' acceptance and bacterial survival

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This study investigated the effect of *B. longum* ATCC 15707 addition and 14 days storage on the sensory characteristics and survival of *B. longum* in muesli. Ninety-two consumers participated in this sensory evaluation to test four samples (probiotic muesli on day 1 and day 14, and plain muesli on day 1 and day 14) and scored the degree of liking for appearance, flavour, texture and overall using a 9-point hedonic scale. They also ranked the four samples in order of overall preference. Microbiological quality was tested by enumerating viable cell counts (CFU g⁻¹) of *B. longum* after storage at 20 °C and 50% relative humidity for 14 days. Overall, a decrease of 2 logs CFU g⁻¹ and 3 logs CFU g⁻¹ at the end of the storage was recorded for probiotic muesli samples made by hands and by using a mixer ($p < 0.05$), respectively. No significant difference in the survival of *B. longum* was observed in probiotic muesli samples between sealed in plastic and vacuum packed in foil. All mean consumers' scores of probiotic muesli samples were not significantly different compared with plain muesli samples on day 1. However, significant differences were noted for appearance, flavour and overall liking of mueslis after 14 days storage ($p < 0.05$). Overall liking of the four samples was highly correlated to flavour (0.87), texture (0.82) and to a less extent to appearance (0.65). There was no significant difference on consumers' overall preference for the four samples by using a Friedman ranking test. In general, 51% of consumers gave a score greater than 5 (mean score 6.99) for overall liking of probiotic mueslis, while 62% of consumers scored plain mueslis over 5 (mean score 6.89). The overall results indicate a moderate degree of consumers' acceptance of probiotic mueslis.

id #144

From Soil to Sequence: A proof of concept approach using Mycobacteriophage proteins to lyse "from without".

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The Mycobacterium genus includes over 190 species with pathogens such as *M. bovis* posing a current and increasing threat to the New Zealand dairy industry. Whilst bacteriophages, the natural parasites of bacteria are a promising line of defense against such pathogens but live bacteriophages evolve, initiating a resistance-evolution race with their bacterial

hosts. We are investigating the potential of harnessing lytic enzymes from Mycobacterium specific bacteriophages and attaching them to biodegradable microbeads in order to use these as a barrier against infection in this genus. We are using the massive publicly available mycobacteriophage database (PhagesDB.org) to bioinformatically explore potential lysis agents to infect Mycobacterial hosts specifically, as a proof of concept approach. Preliminary results and previous literature propose a combination of endolysin and holin domains as practical yet simple tools to carry out the infection process, from outside of the bacterial cell. Each of the 1,500 mycobacteriophage in this database contains at least two genes that are potential candidates. We are currently screening candidates for further molecular analysis including mycobacterial host range efficiency. This research will serve as a proof of concept for a potentially time-effective, cost-efficient and simple-to-implement solution to an array of Mycobacterial species both within New Zealand and beyond.

id #146

Rapid selection of an appropriate antibiotic

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Antibiotic resistance is a serious threat to public health. The empiric use of the wrong antibiotic can select for resistant pathogens, so how can science help clinicians make the right choice? We have developed a fibre-optic device (optrode) to measure fluorescence from stained cells that can be used to enumerate the bacterial load. We have extended this concept to enumerate dual labelled mixtures of differentially stained live and dead cells. For calibration, we have developed a flow cytometry method to accurately enumerate down to 2.5% live cells in a population of dead cells. We propose a device based on the optrode that will take bacteria in a clinical sample, treat with a panel of antibiotics and measure live/dead ratios to determine the best bactericidal choice. We present progress to date indicating the inoculum size and the incubation time required. We further consider the effect of blood cells in the inoculum sample, the effect of mixed cultures, and the parameters required for lytic and non-lytic bactericidal antibiotics.

id #147

A potential role for superantigen producing bacteria in tonsillar hyperplasia

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The tonsils are lymphoid organs that act as sentinels for the nasopharyngeal region and can be prone to recurrent infection, particularly throughout early childhood. We hypothesise that some cases of tonsillar hyperplasia may be driven by local production of bacterial superantigens (SAg), potent toxins that activate T cells and are best known as the causal agents of toxic shock syndrome and food poisoning. *Staphylococcus aureus* and Group A Streptococcus (GAS), which are both common colonisers of the nasopharyngeal region, produce multiple SAGs. Tonsil tissue collected from 81 patients undergoing surgery for recurrent tonsillitis or obstructive sleep apnoea was cultured for aerobic bacteria using standard techniques. Tissue from a subset of 40 patients was also examined using 16S rRNA gene-targeted amplicon sequencing. Analysis of these data to genus level indicated that the bacterial microbiome varied substantially between individuals, with no discernible differences attributable to recent antibiotic consumption or reason for surgery. *S. aureus* was cultured from 41/81 (50%) and GAS from 8/81 (10%) of patients. The *S. aureus* isolates were profiled for SAg genes by multiplex PCR and 22 contained genes for potent Staphylococcal Enterotoxins (SE). Notably, SEC was detected in 11/22 of these isolates. Culture supernatants from isolates containing genes encoding for one or more potent staphylococcal SAg genes were verified as highly mitogenic in a T cell proliferation assay. *S. aureus* and GAS were confirmed to occupy an invasive location by immuno-histology, either in micro-colonies within the tonsillar tissue, or scattered throughout the tonsil tissue. Collectively these preliminary results suggest potential involvement of bacterial SAGs, particularly staphylococcal SAGs, in tonsillar hyperplasia. A better understanding of the significance of SAGs – and the possibility that *S. aureus* is a major source of these toxins in tonsil tissues – may lead to improved medical treatments for both recurrent tonsillitis and tonsillar hyperplasia.

id #148

Glycocin F: an Antimicrobial Enigma

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Glycocin F (GccF) is a 43 amino acid, diglycosylated bacteriocin produced by *Lactobacillus plantarum*. GccF is modified with two N-acetylglucosamine (GlcNAc) moieties which are required for its activity. GccF exhibits a potent yet reversible bacteriostatic effect on multiple Gram positive species, including *L. plantarum*, *Enterococcus faecalis*, and *E. faecium*. This

bacteriostatic effect, as well as the wide range of species its targets, makes GccF unique among bacteriocins, which are generally noted for their narrow and specific antimicrobial spectrum. The GlcNAc-specific phosphoenolpyruvate phosphotransferase system (PTS) has previously been identified as a possible target for GccF, although the mechanism of action of this bacteriocin has yet to be elucidated. Understanding the mechanism by which GccF initiates bacteriostasis in cells will ideally provide a new avenue through which to inhibit pathogenic bacteria, and contribute to the fight against growing antibiotic resistance.

Recently, the protein which confers immunity to this bacteriocin in producing strains has been identified. Expression of this immunity factor, GccH, has been demonstrated to provide complete resistance when expressed in susceptible non-producer *L. plantarum* strains. To this end, GccH has been modified with an N-terminal FLAG tag, and protein pull-down assays carried out in cells exposed and not exposed to GccF in an effort to determine the mechanism of immunity, and by extension, the mechanism of action of GccF. The results of this work will be presented, including a proposed mechanism of action.

id #149

Sustaining the bowel ecosystem of infants.

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A bacterial community (microbiota, microbiome) inhabits the large bowel of infants. This community, during the exclusively milk-fed period of life, is less diverse than that of adults. Microbiota composition is influenced by the nutrition of the child (breast milk or formula-fed) and much research has been devoted to the role of Human Milk Oligosaccharides (HMOs) in the sustenance of the bowel ecosystem. Formula, commonly based on cow's milk, is frequently used in infant nutrition, but HMO-like substances have low concentrations in ruminant milks. The search for new substances that mimic the effects of HMOs and that can be synthesized or extracted from plant or animal sources continues. The role of fatty acids and peptides derived from milk in the nutrition of the bowel microbiota of infants has been neglected and new insights into their importance are needed. New approaches to sustaining the bowel ecosystem of infants may also be required to counteract a perceived change in bifidobacterial ecology in western countries. Interdisciplinary research is needed to understand niche differentiation, consortium formation, and the functioning of the infant microbiota so that model systems can be developed. This will aid the discovery of novel means of sustaining the bowel ecosystem so that it is optimal for infant development and long-term health.

id #150

Listeria monocytogenes isolates from New Zealand food show differential diversity from international isolates

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Introduction and objectives: *Listeria monocytogenes* is a human pathogen that causes serious illnesses including encephalitis, meningitis, septicaemia, abortion, and stillbirth but the disease is also manifested as gastrointestinal illness. Infections are largely food borne, *L. monocytogenes* being widespread in the environment and often isolated from ready-to-eat food products. Molecular typing tools, population genetics and evolutionary studies have played major roles in understanding the transmission, virulence and evolution of *L. monocytogenes* in different niches. Here, 123 *L. monocytogenes* isolates from New Zealand seafood, horticultural and clinical sources were compared with the international strains available in the National Centre for Biotechnology Information database, to better understand the characteristics of New Zealand isolates and their relationships with those present internationally using a variety of molecular typing tools.

Experimental methods: Pulse field gel electrophoresis (PFGE), multi-locus sequence typing (MLST), multi-virulence-locus sequence typing (MvLST) and genome wide analysis of single nucleotide polymorphisms (SNPs) were used to compare *L. monocytogenes* isolates.

Results & conclusion: PFGE analysis showed that the seafood isolates from New Zealand grouped separately from isolates identified globally. The minimum spanning tree conducted using seven MLST alleles also revealed that most New Zealand isolates formed unique MLST clonal complexes (MLST CC), although some grouped closely with the clonal complexes containing international human pathogenic lineage I strains. MLST CC 14 was the major clone belonging to lineage II. Consistent with PFGE and MLST, genome wide SNP analysis revealed that most of the seafood isolates clustered in lineage II and some with lineage I, whereas horticultural isolates were identified as belonging to lineage III or IV. MvLST also showed unique sequence types (ST) from New Zealand that were not reported from other countries. In conclusion, the results of molecular typing indicated that New Zealand has unique *L. monocytogenes* strains, consistent with other studies showing geographical separation. The distinct isolates present in New Zealand raises speculation that they may have evolved differently, adapting to New Zealand conditions.

What induces *B. cereus* spore germination in food- heat or germinants?**Aswathi Soni¹, Phil Bremer¹, Patrick Silcock¹, Indrawati Oey¹***1. University of Otago, Dunedin, OTAGO, New Zealand*

Bacillus cereus spores remain dormant in food for long periods of time until they encounter a trigger to germinate. On germination, and subsequent outgrowth into vegetative cells, *B. cereus* strains produce 2 types of toxins, an emetic toxin (heat-stable cereulide) in food and diarrheal enterotoxins (Nhe, CytK, Hbl) in the human intestine. Understanding the factors that trigger germination of *B. cereus* spores in food could help to prevent germination in preserved food or to induce germination prior to inactivation using technologies designed to kill vegetative cells. The minimum temperature and time of exposure required for inducing germination in *B. cereus* spores in the presence or absence of germinants is unclear.

This study investigated the efficiency of a germinant (L-alanine) and heat separately or together in different combinations to induce germination of *B. cereus* on incubation at the optimum temperature of growth (30 °C). The germination of *Bacillus* spores is a two-step process: first, there is release of dipicolinic acid (DPA) and loss of heat resistance, followed by resumption of metabolic activity and elongation. The first stage or onset of germination in *B. cereus* spores was monitored by following DPA release and the completion was monitored by obtaining the cell and spore numbers before and after heat killing the vegetative cells. The structural changes were assessed using scanning electron microscopy (SEM). Heat activation triggered significantly highest release of DPA that is 125.8µg/ml within 30 min of treatment. No vegetative cells were detected in control (no treatment) or in the presence of L-alanine (0.9mg/ml) alone but were found to be 2 Log CFU/mL and 3.4 Log CFU/ml with heat activation alone and in combination with L-alanine. Slow germination (1 Log CFU/ml) was observed when the heat activated spores were incubated at 30 °C for another 1 hour. Morphological changes on the spore surface were most prominent with spores exposed to heat. In summary, heat activation (70 °C) for 30 min with or without germinant (L-alanine) induces 99% germination in *B. cereus* spores and has the potential to be used both as a hurdle step before inactivation or a preventive step against germination.

Probing the Evolutionary History and Species Specificity of Putative Pathogenicity Genes of *Pythium insidiosum* by Comparative Analyses of Oomycete Genomes**Thidarat Rujirawat¹, Preecha Patumcharoenpol², Tassanee Lohnoo¹, Wanta Yingyong¹, Yothin Kumsang¹, Penpan Payattikul¹, Sithichoke Tangphatsornruang³, Prapat Suriyaphol⁴, Onrapak Reamtong⁵, Gagan Garg⁶, Weerayuth Kittichotirat², Theerapong Krajaeju⁷***1. Research Center, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand**2. Systems Biology and Bioinformatics Research Group, Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkhuntien, Bangkok, Thailand**3. Genomic Research Laboratory, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani, Thailand**4. Bioinformatics and Data Management for Research, Office for Research and Development, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand**5. Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand**6. CSIRO Agriculture and Food, Centre for Environment and Life Sciences, Floreat, WA, Australia**7. Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand***Publish consent withheld****Mixotrophy drives niche expansion of thermoacidophilic methanotrophs****Carlo R. Carere¹, Kiel Hards², Karen M. Houghton¹, Jean F. Power¹, Ben McDonald³, Christophe Collet³, Daniel J. Gapes³, Richard Sparling⁴, Eric S. Boyd⁵, Gregory M. Cook^{2,6}, Chris Greening^{2,6}, Matthew B. Stott^{1,7}***1. Geomicrobiology Research Group, GNS Science, Taupo, New Zealand**2. Department of Microbiology and Immunology, University of Otago, Dunedin, New Zealand**3. Scion, Rotorua, New Zealand**4. Department of Microbiology, University of Manitoba, Winnipeg, Canada**5. Department of Microbiology and Immunology, Montana State University, Bozeman, Montana, USA**6. Centre for Geometric Biology and School of Biological Sciences, Monash University, Melbourne, Australia**7. School of Biological Sciences, University of Canterbury, Christchurch, New Zealand*

Methanotrophic bacteria have evolved a specialist lifestyle dependent on consumption of methane and other short-chain carbon compounds. However, their apparent substrate specialism runs contrary to the high relative abundance of these microorganisms in dynamic environments, where the availability of methane and oxygen fluctuates. In this work, we

provide *in situ* and *ex situ* evidence that thermoacidophilic methanotrophs are mixotrophs. Verrucomicrobia-dominated soil communities from an acidic geothermal field in Rotokawa, New Zealand rapidly oxidised methane and hydrogen gases simultaneously. We isolated and characterised a verrucomicrobial strain from these soils, *Methylacidiphilum* sp. RTK17.1, and showed that it constitutively oxidises molecular hydrogen. Genomic analysis confirmed this strain encoded two [NiFe]-hydrogenases (group 1d and 3b), and biochemical assays revealed it used hydrogen as an electron donor for aerobic respiration and carbon fixation. While the strain could grow heterotrophically on methane or autotrophically on hydrogen, it grew optimally by combining these metabolic strategies. Hydrogen oxidation was particularly important for adaptation to methane and oxygen limitation. Our findings illustrate that verrucomicrobial methanotrophs have evolved to simultaneously utilise hydrogen and methane from geothermal sources to meet energy and carbon demands where nutrient flux is dynamic. This mixotrophic lifestyle is likely to have facilitated expansion of the niche space occupied by these microorganisms, allowing them to become dominant in geothermally-influenced surface soils. Genes encoding oxygen-tolerant uptake [NiFe]-hydrogenases were identified in all publicly-available methanotroph genomes, suggesting hydrogen oxidation is a general metabolic strategy in this guild.

id #156

Bacterial Predation of Human Pathogens: Strengths and Limitations

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Bacterial predators, of which *Bdellovibrio bacteriovorus* is the best characterized, actively attack, kill and consume other bacterial cells. To date, the activity of *B. bacteriovorus* has been demonstrated with over 100 human pathogens, including *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Escherichia coli* (1). Research has shown that predation can reduce the prey population by several log when performed *in vitro* (1, 2). Not only do predatory bacteria kill pathogens, they remove the cellular DNA, as demonstrated by my group using a recombinant β -lactamase producing strain of *E. coli* (2). Moreover, several recent studies have touted their gentle nature towards human cell cultures (3, 4). One of these studies from my group demonstrated that an exposure to 1,100 predatory bacteria cells per human cell did not elicit strong cytokine responses (IL-6, IL-8, IL-10, TNF α) nor cause cell death (3). Moreover, in another study we found they protected human cells from a pathogenic bacterium (5). Although bacterial predators are very active in *in vitro* systems, they are more constrained when applied *in vivo*. Consequently my group has sought to understand how different environmental factors, particularly those found *in vivo*, affect predation and its efficacy. One example of this is the severely mitigated predatory activities seen within human blood serum. We discovered that serum albumin and the osmolality both negatively impact bacterial predation (6), the former by binding to and coating the predatory strain and the latter by inhibiting *B. bacteriovorus* motility and protease production levels. Similarly, the concentration of indole within the human gut can reach 1.2 mM, a level that we have found to be inhibitory for both *B. bacteriovorus*' activity as well as its development within the prey (7). As such, predatory bacteria offer many benefits, including a broad spectrum of activity against priority human pathogens, but several hurdles need to be overcome for them to be effective as an antimicrobial within the human body.

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id #158

Geometrical Principles for Understanding the Architecture of Iridoviruses

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Megavirales are a group of extremely complex double-stranded DNA viruses with intriguing three dimensional architectures. Numerous structural proteins and the large dimension of the capsid makes them a difficult task for structural biologists. Their basic capsid structure shows a combination of pentasymetrons (PS) and trisymetrons (TS) which assemble into an icosahedral capsid protecting a membrane enclosed genome. We used cryo-electron microscopy and phase plate cryo-electron tomography to study the structure of *Wiseana Iridovirus* (WIV), one of the smallest members of the large DNA virus family. WIV has a 212 kbp genome and infects insects whose larvae become iridescent upon viral capsid

accumulation. Our analysis shows that WIV has a T=147 capsid assembled from PS (formed by 31 capsomers) and TS (formed by 55 capsomers). A long flexible fibre emanates from inside each trimeric capsomer. The major capsid protein has the expected double jelly-roll structure with prominent loops at the exterior. Tomograms show that the membrane interacts with several TS via specific proteins occupying only part of the interior of the capsid. We also obtained a structure of an empty capsid at subnanometer resolution. Comparison between the two structures suggests a mechanism for capsid assembly. Our hypothesis is that the TS are capable of independent assembly in the absence of membranes, while the PS are added to preformed capsids together with a chain of zipper proteins which stabilize the capsid.

id #161

Transcriptional analysis (RNAseq) of a consortium of gut commensals

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The human colon harbours a complex microbial community (microbiome). A range of syntrophic interactions takes place among different commensal species, leading to niche differentiation based on resource sharing in the habitat. Some commensals can degrade an available substrate that is metabolically inaccessible to other species. Partially degraded substrate and hydrolytic products then become available to other species.

Complex plant polysaccharides (dietary fibre) present in the human diet represent a rich banquet for bowel commensals because they reach the colon undegraded, and may form the nucleus for the assembly of bacterial consortia. A simple consortium of bowel bacteria has been built *in vitro* so as to unravel the syntrophic interactions among gut commensals. Previous screening experiments allowed the selection of the plant polysaccharide and bacterial species for this consortium. *Bacteroides ovatus* hydrolyses barley beta-glucan, releasing oligosaccharides that then become growth substrates for *Hungatella hathewayi* and *Subdoligranulum variabile*. Transcriptomics of pure cultures of *B. ovatus* growing on beta-glucan, and *H. hathewayi* and *S. variabile* growing on beta-glucan-derived-oligosaccharides, showed changes in genetic expression during the exponential growth phase, with genes encoding specific glycosidases and carbohydrate transporters expressed at different time points. These observations were consistent with data from chromatographic analysis. RNAseq analysis of the three microorganisms in co-culture showed transcriptional responses of these commensals living together and sharing resources.

This bacterial consortium can be viewed as an example of how bowel bacteria assemble around a dietary fibre and respond to food sources available in their environment. Different plant fibres could drive the formation of consortia containing different bacterial species, with subsequent changes to the bowel ecosystem.

id #166

The human gut microbiome: understanding the mechanics of our microbial engine

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It is becoming increasingly clear that our gut microbiota (the collection of microbes resident in our digestive tract), plays a critical role in the maintenance of our health. Disturbances in the gut microbiota are associated with many diseases, including inflammatory bowel diseases and *Clostridioides difficile* infection. Studying the complex interactions of human gut microbial ecosystems is not straightforward, partly because many of the species that comprise these ecosystems are difficult to culture and thus poorly understood. However, contrary to popular belief, many of the bacterial species that live in the gut are culturable, given the right conditions. We have assembled a model platform ('Robogut') in which to culture gut microbial ecosystems to enable *in vitro/ex vivo* study in a simplified and accessible way.

As part of this work we are developing a series of experimental, defined gut microbial ecosystems to explore ecosystem interactions and functions in detail. To understand the gut microbial 'engine', it is necessary to know how each component part fits together. To understand whether the engine is running optimally, it is necessary to look at the 'emissions' - the metabolic output. The Robogut platform for culture of defined ecosystems, coupled with metabonomics approaches to assess ecosystem output, represents a potentially powerful tool for approaching an understanding of the very complex engine of our gut.

In this presentation, I will show some of the work we have done using our platform to model gut microbial ecosystems, and I will demonstrate how this knowledge may be used to develop novel 'microbial ecosystem therapeutics' to treat disease, an approach that is an emerging paradigm in medicine.

id #168

Characterisation of *Serratia proteamaculans* strain AGR96X encoding an anti-feeding prophage (tailocin) with -activity against grass grub (*Costelytra giveni*) and manuka beetle (*Pyronota* spp.) larvae

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A highly virulent *Serratia proteamaculans* strain, AGR96X, exhibiting specific pathogenicity against larvae of the New Zealand grass grub (*Costelytra giveni*; Coleoptera: Scarabaeidae; formerly *C. zealandica*) and the New Zealand manuka beetle (*Pyronota festiva* and *P. setosa*; Coleoptera: Scarabaeidae), was isolated from a diseased grass grub larva. Following ingestion of a high dose of AGR96X, death occurred within 5–12 days. Pot trials assessing AGR96X found the bacterium to be effective at controlling larvae of both grass grub and manuka beetle, giving comparable, if not greater protection than chemical insecticides. The rapid kill of AGR96X suggests a mode of action more similar to an insecticide, than to the slow disease progression of the commercialised grass grub specific bacterium *Serratia entomophila*, which can take 3–4 months to kill the larvae. In contrast to *S. entomophila*, AGR96X rapidly multiplied within the insect host and gained entry to the haemocoelic cavity, leading to a bacterial load of approximately 2×10^8 cells per larvae within 3 days of ingestion. Genome sequencing of AGR96X revealed a plasmid encoding a variant of the *S. entomophila* anti-feeding prophage (Afp), a tailocin designated AfpX. Unlike the Afp, AfpX contains two Afp16 tail-length termination protein orthologues and two putative toxin components. A 37-kb DNA fragment encoding the AfpX-associated region was cloned, transformed into *Escherichia coli*, and fed to *C. giveni* and *Pyronota* larvae, causing mortality. In addition, deletion of the *afpX15* putative chaperone component abolished the virulence of AGR96X. Unlike the *S. entomophila* Afp, the AfpX tailocin could be induced by mitomycin C. Transmission electron microscopy analysis revealed the presence of Afp-like particles of varying lengths. Grass grub and Manuka larvae fed with purified AfpX tailocin showed similar symptoms to those fed AGR96X. The rapid lethality and broader host range of AGR96X make this bacterium a viable alternative to *S. entomophila* for pest control.

id #169

Endophyte retention in an artificial *Epichloë festucae-Lolium perenne* association may come at a price.

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Epichloë festucae var. *lolii* is a fungal endophyte which forms a mutualistic symbiosis with perennial ryegrass (*Lolium perenne*). In pastures, endophyte promotes plant fitness through conferring protection against biotic and abiotic stresses. The usefulness of new artificial plant-endophyte associations depends on reliable endophyte seed transmission from one plant generation to the next. To date, the mechanisms leading to compatible and maintainable artificial associations have not been investigated. We studied a high-grade seed maintenance programme of a novel ryegrass-endophyte association, that, by frequent selection for infection, achieved a high proportion of infected seed (89–99%). We compared two generations (G2 and G6) for plant and fungal characteristics by phenotyping and physiological analysis of five plant genotypes per generation grown under controlled conditions. Plants from G6 had more hyphae but smaller diameter hyphae than G2 plants ($P < 0.032$). Endophyte colonisation of vascular bundles, usually associated with incompatible or pathogenic interactions, occurred in 40% of G6 plants analysed, compared with 11% in G2. There was a moderate decrease in plant growth performance in G6 compared with G2 (tillering and photosynthetic rate/intercellular CO_2). The expression of six plant genes involved in biotrophic defence response indicated variability within each generation due to genotype effects. However, on average G6 plants exhibited a lower expression than G2 plants of almost all the genes, as could be expected in plants with reduced defences because of adaptation to its endophyte symbiont. Furthermore, opportunist exposure to fungal disease and an insect pest (of a total of 323 plant genotypes in a greenhouse) indicated increased susceptibility of G6, especially to insect infestation ($P < 0.046$); in both generations endophyte-positive plants were affected nearly 8 x more often than endophyte-free plants. Our data so far suggest that a seed multiplication process in which high endophyte transmission is achieved may come with trade-offs in terms of fungal colonisation and plant fitness. However, the protection afforded by reliable endophyte transmission outweighs the potential reduction in plant fitness. Further work is required to explore effects of intergenerational endophyte adaptation to the host relative to other effects such as harvest season.

id #172

Functional biogeography of soil bacterial communities across the mountain elevation gradient using ‘inferred metagenomes’

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DNA sequencing can produce a large amount of genomic data, providing useful insight into the diverse functional traits of microbial communities. However, metagenomics is rarely used to study the biogeography of microbial communities across large scales, due to the high cost of DNA sequencing. Here, we explored the use of ‘inferred metagenomic methods’ to investigate the relationships between environmental conditions and the presumed abundance of genomes and functional gene categories (e.g., N cycling genes). This method allows the abundance of all possible genomes in each soil sample to be

predicted and compared across environmental gradients from the analysis of 16S rRNA gene data. We collected 405 soil samples across a continuous ridge on Mt. Cardrona, New Zealand (every 100 m across an elevation gradient of almost 1,500 m). Climatic factors (i.e., temperature and soil moisture), soil physicochemistry (i.e., pH, total C, total N, available nitrate, available ammonium and organic phosphorous) and plant community data (i.e., above-ground plant biomass) were also measured at each sample site. We retrieved approximately 400 bacterial genomes from 17,000 distinct bacterial operational taxonomic units (97% OTUs). We found a highly similar elevational pattern in bacterial community composition from the subset of our community for which genome data were available, as compared to the pattern obtained using our original full 16S rRNA dataset. This suggests that data from our 'inferred metagenomic method' represents the actual composition of bacterial 16S rRNA data to a degree. From the genome data we collated, we found the distribution of certain functional categories of gene were associated with variations in critical soil conditions (e.g., total carbon, total nitrogen), and particularly in response to gradients in disturbance by livestock grazing. Our inferred metagenomics approach therefore sheds light on the functional biogeography of soil bacterial communities and particularly how soil microbial functioning varies with environmental factors. Our method can be seen as a test case, providing scientists a general picture of spatial variation in soil microbial genes/functional potential without the need to conduct true shotgun metagenomic sequencing.

id #175

Protozoan Predation Can Influence Bacterial Cell Shape Evolution

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Cell shape is a fundamental characteristic of single-celled organisms, affecting replication, nutrient acquisition and growth. Phylogenetic mapping of bacteria based on 16S rRNA revealed all bacteria evolved from a filamentous or rod-shaped ancestor. This ancestral rod-like, bacteria adopted spherical cell shape over 14 times in the phylogenetic record, losing a single gene, *mreB* in each case. Ironically, spherical cell shape is a disadvantage in terms of motility, nutrient exchange and DNA segregation. Most model bacteria are inviable as *mreBΔ* mutants. However, *Pseudomonas fluorescens* SBW25 is a rod-like bacterium that is capable of surviving the initial step in the evolution to coccal cell shape. Startlingly, we observe that these cells are not only spherical, but have a dramatic 4-fold increase in cell volume. Suggesting an alternative narrative for the adaptation conferred by deletion of *mreB*: large cell size.

We hypothesise that this large size intermediate may confer an advantage under protozoan predation, leading to the observation of evolved coccal cells in nature. We are therefore experimentally assessing the potential of protozoan predation as a driver of cell shape evolution. We have tested *Naegleria gruberi*, *Dictyostelium discoideum* and *Caenorhabditis elegans* for predation preferences between three different cell morphologies of otherwise isogenic mutants of *P. fluorescens* SBW25. Our experimental results suggest predation by physically smaller protozoa, has a 10-fold preference for WT cells over their larger *mreBΔ* derivatives. However, the larger predator prefers larger cells, giving smaller evolved *mreBΔ* cells an advantage over their WT predecessors under predation.

Experimental conditions in the laboratory can only hint at the effect of protozoan predation to drive evolution of cell shape in nature. An agent-based model was developed, allowing us to take a wide range of possible conditions including predator preference, growth disadvantage and predator abundance into account and determined a minimum predator population of 10 and a 4-fold preference of WT over *mreBΔ* cells will favour the increase of *mreBΔ* cells in mixed populations. This work allows us to speculate about ecological parameters in which *mreBΔ* cells might have a transient adaptation, leading to long-term changes in cell shape in bacteria.

id #176

Metabolic characteristics and genomic epidemiology of *Escherichia coli* serogroup O145

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Shiga toxin-producing *Escherichia coli* (STEC) are a global public health concern, and cause human disease of varying severity from diarrhoea to life-threatening kidney failure. Ruminants are asymptomatic reservoirs of STEC, shedding this pathogen via their faeces. There is 'zero tolerance' for the Top7 STEC serogroups (O26, O45, O103, O111, O121, O145 and O157) in ground beef products exported to the USA. Even with good hygienic practices during processing, STEC can contaminate carcasses, therefore STEC are a major regulatory concern for New Zealand's meat industry. A previous study investigating the prevalence of STEC in young calves throughout New Zealand identified STEC O145 as the most prevalent serogroup (43%) at the dairy farm level compared to the other Top7 serogroups. This high prevalence underlines STEC O145 as a public health concern and an issue for the meat industry.

Current culture-based methods for the detection of STEC are not fully discriminatory due to the lack of consistent differential characteristics between STEC and non-pathogenic *E. coli*. This study aims to (i) investigate metabolic characteristics of serogroup O145 to facilitate the differential culture of this serogroup and (ii) understand the genomic epidemiology of *E. coli* serogroup O145 using whole genome sequencing (WGS). Carbon utilisation profiles were determined with the Biolog phenotypic microarray system, which measures microbial cell respiration. For WGS, in-house

library preparations were used and sequencing performed with Illumina HiSeq technology. Comparative genome analysis was conducted using a range of analysis tools. Our results indicate that *E. coli* serogroup O145 are genetically and metabolically diverse, based on carbon utilisation. These metabolic and genomic analyses indicated no correlation between isolation source and toxigenic or non-toxigenic strains. However, the analysis indicates clustering of O145 strains based on multi locus sequence-type and at the level of *eae* subtype, a gene encoding the protein intimin which is involved in bacterial attachment to intestinal epithelial cells. These findings highlight characteristics which can be used to differentiate within serogroup O145 and demonstrates that O145 strains are metabolically and genetically diverse. Future work will involve linking specific genes associated with defined metabolic pathways to observed substrate utilisation activities; i.e. phenotype-genotype correlations.

id #177

Phenotypic Switching in *Pseudomonas fluorescens* SBW25

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When challenged with repeated selection cycles through two contrasting environments, the bacterium *Pseudomonas fluorescens* SBW25 evolved, *de novo*, the ability to switch repeatedly between two bistable states - one where cells secrete an extracellular colanic acid-like polymer (cap+), and an ancestral, uncapsulated state (cap-) ¹. Subsequent characterisation showed the switch to be epigenetic, though underpinned by mutation at the start of the pyrimidine biosynthesis pathway ².

Taking a genetic approach, we sought to identify the ultimate cause of bistability. Exploration of the genotype-phenotype map through a combination of transposon mutagenesis screens, reversion and genome re-sequencing, transcriptome analysis, and gene deletion revealed a complex network of 'players' whose activities modulate Cap+/Cap- switching. Among these are the Gac/Rsm signalling pathway and the stringent response, specifically, RpoD and the nucleotide alarmone ppGpp, recently shown to underpin another bistable phenotype - that of antibiotic 'persisters'. Moreover, several lines of evidence indicate that expression of ribosome components is up-regulated in the switchers (Cap+) compared to wild-type cells.

We present evidence in support of aberrant regulation of ribosome production as the underlying cause of Cap+/Cap-bistability in SBW25 'switcher' mutants

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id #188

Interactions between PvdA and PvdF, enzymes involved in pyoverdine biosynthesis in *Pseudomonas aeruginosa* PAO1

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Background: During infections, *Pseudomonas aeruginosa* secretes an iron scavenging compound, pyoverdine. Pyoverdine consists of a peptide group containing the unusual amino acid formylhydroxyornithine attached to a chromophore and an acyl side chain. Formylhydroxyornithine is formed from the conversion of ornithine to hydroxyornithine by PvdA and subsequently to formyl hydroxyornithine by PvdF. The instability of hydroxyornithine indicates substrate channeling between PvdA and PvdF. This research investigates the interaction of PvdA and PvdF.

Methods: A co-purification pull-down method was used to determine whether PvdA and PvdF interact. Hexahistidine-tagged PvdA was co-expressed with untagged PvdF, and vice versa. Interaction was tested by looking for the presence of the binding partner following purification of the His-tagged protein by affinity chromatography. Protein identities were confirmed by protein mass spectrometry. Bacterial two hybrid system was used to further confirm the interaction of PvdA and PvdF; PvdA and PvdF were cloned into pKNT25 and pUT18C plasmids, containing the complementary fragments of adenylate cyclase. The plasmids were then co-transformed into *E. coli* DHM1. The interaction was detected by plating the transformants on LB-X-gal plates.

Results: When the proteins were co-expressed in *E. coli*, PvdF co-purified with Histidine-tagged-PvdA demonstrating protein-protein interactions. PvdA showed little or no co-purification with PvdF that has an N-terminal hexahistidine tag. When *E. coli* DHM1 (pUT18C::PvdF) (pKNT25::PvdA) were plated on LB-Xgal plates, blue colonies were observed indicating that PvdA and PvdF interact to allow T18 and T25 to form a functional adenylate cyclase and consequent upregulation of the β -galactosidase encoding lacZ gene.

Conclusions: These results demonstrate interaction between PvdA and PvdF. The histidine tag at the N-terminal of PvdF may interfere with interaction with PvdA. The quantification of the interaction can be carried out by measuring the β -

galactosidase activity in liquid cultures. Interaction between PvdA and PvdF is likely to increase the efficiency of formyl hydroxyornithine enabling efficient pyoverdine synthesis.

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id #189

The role of emerging Epsilonproteobacteria in childhood gastroenteritis

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Each year 1.5 billion cases of acute gastroenteritis occur globally in children under 5 years, with an estimated 1.5-2.5 million deaths. Current laboratory methods fail to find an aetiological agent for up to 80% of faecal samples examined. The bacterial class Epsilonproteobacteria contains several species known to cause gastroenteritis but the role many more species play in this illness is unclear. To better understand this relationship, a Multiplex Ligation-dependent Probe Amplification (MLPA) assay for the detection of 28 taxa within the Epsilonproteobacteria was developed and applied to a collection of DNA extracts from a Belgian childhood gastroenteritis case control study. Using culture-based methods, Epsilonproteobacteria were isolated from 17.4% (32/184) of cases and 5.1% (9/175) of controls. *Campylobacter jejuni* was isolated from 26 cases and 3 controls, while *C. concisus* was isolated from 6 cases and 4 controls. In contrast, MLPA detected Epsilonproteobacteria in 21.3% (37/174) of cases and 10.4% (17/163) of controls. *C. concisus* was the most common species detected by MLPA in both cases (23, 13.2%) and controls (14, 8.6%). *C. jejuni* was detected in 17 (9.8%) cases and 1 (0.61%) control. *C. concisus* genomospecies 2 (GS2), a group of genetically-related *C. concisus* strains that is distinct from the type strain, was detected in 15 (8.6%) of cases and 6 (3.7%) of controls. The odds of being a case compared to a control was significantly higher for samples positive for *C. jejuni* by culture ($p = <0.001$, Odds Ratio [OR] = 11.6) and MLPA ($p = 0.011$, OR = 13.9). The odds of being a case compared to a control for samples positive for *C. concisus* was not significantly different by either culture or MLPA. The odds of being a case compared to a control was significantly higher for samples positive for *C. concisus* GS2 ($p = 0.023$, OR = 4.3). These results support *C. jejuni* as the most common Epsilonproteobacterial cause of childhood gastroenteritis in Belgium and provide some evidence that *C. concisus* GS2 may also be an important enteric pathogen for children.

id #190

Fungal root endophytes of *Agathis australis*

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Kauri (*Agathis australis*, Araucariaceae) is restricted in distribution to the Northern tip of the North Island of New Zealand. Living for 1,500 years or more and having trunks up to 3 m diam., *A. australis* exerts enormous influence on surrounding forest composition and structure and provides varying habitat niches for complex fungal communities. However, information on the diversity of fungi associated with *A. australis* is sparse. Additionally, since the 1970s these trees have been under threat from the exotic invasive pathogen, *Phytophthora agathidicida* that causes kauri dieback. Our study aimed to characterise the fungal root endophytes of *A. australis* and investigate the subsequent impact of kauri dieback on these fungi. We obtained root samples from three locations and 32 kauri trees in the Waitakere ranges. We isolated over 300 cultures from kauri roots and obtained DNA sequences from all of the fungal cultures. We clustered the sequences to OTUs and assigned identities using BLAST. The 354 sequences clustered into 34 OTUs with 63 singletons using a 97% cut-off. We compared the OTUs on the basis of location and disease status. The results give us our first glimpse at the enormous influence of *A. australis* on the fungal community and suggest what the impacts on fungal diversity might be after invasion by *P. agathidicida*.

id #193

Targeting Triple Negative Breast Cancer with the Oncolytic Seneca Valley Virus.

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Triple negative breast cancer (TNBC), characterised by the absence of markers for targeted therapy (i.e. estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2), is considered the most aggressive form of breast cancer. Currently, there is scientific consensus that targeting cancer stem cells in TNBC would reduce multi-drug resistance, malignancy, and the relapse of disease. Oncolytic virotherapy has become a promising cancer treatment with high affinity for tumour cells and low patient toxicity. Seneca Valley Virus (SVV), a member of the Picornaviridae family, has emerged as a very promising oncolytic virus targeting a range of tumours. We recently identified Tumour Endothelial Marker 8 (TEM8) as the cellular receptor for SVV. TEM8 is also expressed in TNBC, and was found to be associated with breast cancer stem cells. This makes it a promising target for SVV virotherapy. In this study, we screened a panel of TNBC cell lines for permissivity to SVV infection. Using an MTT cytotoxicity assay we identified several permissive TNBC cell lines. Furthermore, we characterized and established the efficacy of SVV to specifically infect and lyse these cancer cells. Using high pressure freezing – freeze substitution followed by electron microscopy, we were able to visualize changes in the cellular architecture of infected cells. In conclusion, this study confirms the capacity of SVV to infect certain TNBC cells. In order to become a clinical tool in TNBC treatment, further research is necessary to understand the mechanism of action and the factors involved in SVV infection.

id #194

Longitudinal study of the sinonasal microbiota reveals stable, person-specific bacterial communities

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The stability of the healthy sinonasal microbiota over a prolonged period of time is largely unknown. Establishing a baseline of normal variation and quantifying long-term bacterial community stability is vital for the appropriate interpretation of shifts away from a healthy sinonasal microbiota that occurs in conditions such as sinusitis. In this study, we sampled the left and right middle meatuses of four individuals every month for one year, then once every three months for an additional year. Illumina MiSeq amplicon sequencing of bacterial 16S rRNA genes revealed stable, person-specific bacterial communities that were mainly dominated by an abundance of *Corynebacterium*. Each of the four individuals were consistently differentiated by the abundance ($p < 0.01$) of a secondary bacterial group: *Dolosigranulum*, *Propionibacterium*, *Enterobacteriaceae* or *Staphylococcus*. Differences between subjects contributed the most to overall observed variation (11%, $p = 0.005$), while seasonal differences only contributed to variation in the context of an individual subject (9%, $p = 0.02$). Antibiotics and corticosteroids were administered to one subject during the course of the study for an episode of acute bacterial sinusitis that was characterised by a significant decrease in bacterial diversity and near-complete dominance by members of the genus *Haemophilus*. Interestingly, the bacterial community recovered within four months to the initial community composition observed before this episode. These results suggest that while some seasonal differences can be observed within individuals, the sinonasal bacterial community remains relatively stable across two years and may quite quickly return to baseline after perturbation by acute infection and antibiotic treatment.

id #195

One method to rule them all: Optimal DNA extraction approach for multi taxa studies

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Continuing advances in DNA sequencing technologies allow for microbial communities to be studied at larger scales than ever before. This provides exciting opportunities to explore the relationships between both bacterial and fungal organisms present in different environmental samples. Since different sample types present unique challenges for the extraction of DNA, different methods are often used. However, there is ample evidence of different DNA extraction techniques not only resulting in different quantities and qualities of DNA, but also impacting measures of community richness, diversity and composition. This evidence has been obtained by comparing the ability of multiple extraction methods to obtain DNA from a single sample substrate, leaving us unable to determine if there is one extraction technique that can be used on a wide range of sample substrates to detect bacterial and fungal organisms. We therefore tested the ability of six commonly used DNA extraction kits to obtain high-quality DNA from a variety of different sample media, including stream water, sediment, leaf litter, soil, and invertebrates. The construction of a 'mock community' which is composed of a range of known microbial species, also allowed us to evaluate each kit's ability to extract DNA from a range of cell types, and determine if there are biases that alter the perceived abundance of certain microorganisms. Additionally, we assessed each kit for its ability to detect macro-organisms, thereby allowing the direct comparison of microbial and macroinvertebrate eDNA, extracted using the same procedure. Through this study, we aim to recommend a single extraction technique which performs optimally for use on a wide range of sample media, and obtains DNA from both macro and microorganisms. This would be greatly beneficial to the overall field of eDNA research, and allow better integration of molecular methods to study the total biodiversity present in our environment.

There is a spike, but is there a point? Can live cell staining tell us more about bacteria than just bacterial viability?

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Rapid identification of microorganisms present in a sample can provide important information for downstream decision making. These rapid methods should be faster or more accurate than what is currently used. But often rapid methods of bacterial classification have an increased cost, which can be a barrier to widespread usage.

SYTO9 is a commercially available fluorescent DNA stain commonly used to detect both "live" and "dead" bacteria in a sample. We have developed a fibre-optic device ("the optrode") to measure fluorescence from SYTO9 stained cells that can be used to enumerate the bacterial load in near real time. By using the emission spectrum from SYTO9 stained cells we attempt to identify specific spectral signatures that can be used to identify the microorganism present.

Using five different organisms; *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica*, and *Staphylococcus aureus* at different growth phases; lag, log, and stationary, we have measured the emission spectrum from SYTO9 stained cells excited using a 473 nm laser. A total of 27 spectra were collected from the test bacteria and the data was put through a machine learning algorithm to see if the machine could accurately predict the species, gram stain, and the growth phase, of an unknown sample. The spectra were then processed using principal component analysis to reduce the dimensions used to for machine learning to again train and test the algorithm.

The results demonstrate an ability to differentiate some bacterial species based on their SYTO9 emission spectrum using machine learning. Thus opening up the possibility of a rapid, low cost method for accurately identifying bacteria using the optrode.

The interconnected community: microbial networks in complex and simple natural habitats

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Microbes do not live alone in nature, instead they live in the interconnected community. The interactions among microbes are an important characteristic of the community, however, very little is known about microbial networks owing to the lack of appropriate experimental data and analytical tools. Recent advances in metagenomics technologies (such as high throughput sequencing) has provided revolutionary tools for analyzing microbial community networks. We used random matrix theory (RMT)-based network analysis to identify potential bacterial interactions (co-occurrence patterns) in two different natural systems: diverse rhizosphere communities associated with wild oat (*Avena fatua*) and relatively simple legume (*Medicago sativa* L.) nodule endophytic communities. Rhizosphere networks were substantially more complex than those in surrounding bulk soils. Network complexity increased as plants grew, even as diversity indices decreased, highlighting that interactions are a crucial dimension of community organization overlooked by univariate diversity. Consistent with the hypothesis that extensive mutualistic interactions occur among rhizosphere bacteria, covariations were predominantly positive (>80%); we identified quorum-based signaling as one potential rhizosphere interaction strategy. Putative keystone species often had low relative abundances, suggesting low abundance taxa may significantly contribute to rhizosphere structure and function. Currently, we are analysing the microbial network in a legume nodule endophytic communities. Although nodule communities contain much fewer microbial taxa compared to a rhizosphere community, defined microbial networks are evident. These studies show that network complexity appears to be a defining characteristic of the interconnected microbiome, and is a previously uncharacterised property of these habitats.

Influence of dietary treatment on the gut microbiome of a mouse model of autism

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Autism spectrum disorder (ASD) is a neurodevelopmental condition typically characterised by impairments in social interactions and stereotypical repetitive behaviours. Sequence variations in a wide range of genes have been implicated in individuals with ASD. Some of these genes encode for proteins which play a pivotal role at the synapse, and mouse models which lack functional versions of these proteins can provide novel insights into the mechanisms which underlie ASD. Given recent evidence of links between the gut microbiota and the brain in ASD and other neurological conditions, we have been studying the gut microbiome of a mouse model based on a deficient synaptic protein. These mice exhibit ASD-like behaviours and deficiencies in brain synapse structure, both of which can be ameliorated *via* changes in diet. We hypothesise that gut microbes may play an important role in mediating this effect. Gut tissue (ileum, caecum, colon) and faecal samples were collected, and the PCR-amplified bacterial 16S rRNA gene was sequenced using the Illumina MiSeq platform. A subset of the faecal samples were also sequenced using shotgun metagenomics to explore the functional potential of these gut microbes. The 16S rRNA gene amplicon sequencing revealed that the dominant bacterial phyla are *Firmicutes*, *Bacteroidetes* and *Verrucomicrobia*, with notable genera including the mucin degrader *Akkermansia* and 'Homeothermaceae' (family S24-7), a widely found homeothermic gut microbe. At the overall community level, the mouse gut microbiota did not exhibit major changes in composition among treatment groups, which is attributable at least in part to high microbiota variability among different individual mice. Although bacterial community *structure* did not vary greatly among different treatment types, our ongoing research with this mouse model is exploring whether *functional* changes are occurring.

Arbuscular mycorrhizal fungal communities across gradients of ecological restoration

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Arbuscular mycorrhizal fungi (AMF) are obligate and ubiquitous symbionts of plants. Trophic mutualists that exchange plant growth-limiting nutrients from soils for photosynthetic carbon, their functions profoundly influence the assembly and persistence of plant communities. Despite these crucial roles, the taxonomy and ecology of AMF remain poorly understood. This deficit limits our capacity to use AMF effectively to achieve production and environmental gains. For example, more than half of all trees planted in ecological restoration projects in the Wellington region die in the first year after planting. While laudable community-led efforts, these planting campaigns are typically poorly informed by knowledge of plant and soil biology. Here I discuss our work examining the AMF communities of native plant species in the context of wetland restoration in the Wairarapa Valley, lower North Island. Focusing on the canopy emergent podocarp species kahikatea (*Dacrycarpus dacrydioides*) and the early successional, but persistent monocot harakeke (New Zealand flax, *Phorium tenax*) I describe the spatial and temporal patterns of AMF community composition as revealed by Illumina Mi-Seq sequencing of coding and non-coding regions of fungal rRNA genes. Finally, I discuss the implications of these data for the success of wetland restoration projects.

pH, temperature and location drive microbial biogeography in 1,000 geothermal springs across New Zealand

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There is a lack of consensus on the role of both deterministic and neutral processes in shaping microbial community structures across a host of global environments. Geothermal springs are an ideal model to clarify these processes as they i) represent discrete, easily definable habitats; ii) are abundant across vast geographical scales; iii) are physicochemically-unique, homogenous environments across broad geochemical gradients; and iv) have comparatively simple community structures. We analysed bacterial and archaeal community composition (via 16S rRNA gene amplicon sequencing) and 46 physicochemical variables in nearly 1,000 geothermal springs across New Zealand's principal geothermal region, the Taupō Volcanic Zone, to determine parameters driving microbial biogeography. All data is available to view via <http://1000springs.org.nz/>. The most abundant and prevalent taxa were Aquificae (genera *Venenivibrio*, *Hydrogenobaculum* and *Aquifex*), followed closely by the proteobacterium *Acidithiobacillus*. While pH was the main driver

selecting microbial diversity and community structure, temperature only significantly affected alpha diversity above 70 °C. Exemplar geochemical signatures, within some geothermal fields, were also predictive of community composition, with specific taxa-geochemical associations evident for some genera. Overall, we show that the local physicochemical environment selected community composition within neighbouring springs, while a regional distance-decay pattern indicated geography limited microbial dispersal. Finally, genus-level endemism of *Venenivibrio stagnispumantis* within the New Zealand archipelago suggests allopatric speciation is occurring within the Aquificales order, most likely due to the geographic isolation of the islands in the South Pacific Ocean. This study, which is the largest survey to date of geothermal environments, combines large-scale sample density across many geographic locations, greatly expanding our knowledge of life in extreme habitats. It also definitively identifies pH and geography as major players behind microbial diversity patterns, and provides a sound baseline for future microbial biogeography research across all ecosystems.

id #209

The good, the bad and the ugly - genetic requirements of beneficial, pathogenic and commensal *Fusarium oxysporum* strains for colonization of tomato plants.

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The fungus *Fusarium oxysporum* (*Fo*) is a common resident of the soil. Besides its ability to grow as a saprophyte it is well known for its intimate contacts with living plant roots. The outcomes of these interactions range from beneficial to deleterious. Actually, in a poll among plant pathologists the fungus ranks 5th in top 10 most devastating plant pathogens affecting our crops. It is therefore remarkable that strains of the same fungus are commonly used as biocontrol agent to enhance plant tolerance to (a)biotic stress factors. Co-inoculation of tomato seedlings with a pathogenic and biocontrol *Fo* strain results in colonization of the host vasculature by both strains, but the typical disease symptoms do not emerge - or to a much lesser extent. How this protection is conferred, and what characteristics distinguish a pathogenic strain from a biocontrol strain, are the main focus of our research.

Besides eleven core chromosomes *Fo* typically harbors conditionally dispensable chromosomes. In case of pathogenic strains these extra chromosomes are enriched for effector genes that are correlated with host range. Previously, our lab showed that transfer of one of these pathogenicity chromosomes from a tomato-infecting strain to a biocontrol strain converts it into a tomato pathogen. The same was more recently demonstrated for a strain infecting cucurbits. *Vice versa*, loss of a pathogenicity chromosome can convert a pathogen into a biocontrol strain. To more systematically investigate the genetic requirements distinguishing non-pathogenic from beneficial and pathogenic strains we sequenced the genomes of additional pathogenic strains as well as non-pathogenic strains that vary in either their biocontrol potential or their ability to colonize tomato plants. In our genome comparisons we focus on the effector repertoire of the various strains to identify key effectors required for host colonization and/or pathogenicity. The results of this ongoing analysis will be presented, along with a few examples of *Fo* effector proteins of which we have obtained some insight into the function in the host.

id #211

The influence of kiwifruit extract on the human gut microbiota

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The first validated health claim generated by the New Zealand food and beverage industry was that eating two whole kiwifruit each day improves gut comfort (regularity). The fibre, nutrients and bioactives contained within render the berry beneficial for alleviating bowel discomfort, along with providing many other health benefits. The gut microbiota likely plays a role in mediating these effects, and indeed it has been demonstrated that kiwifruit consumption can influence the composition of gut bacterial communities.

Consumption of kiwifruit in an extract form is a convenient alternative to the whole fruit, but whether it confers the same health benefits remains unclear. Similarly, the influence of kiwifruit extracts on the gut microbiota has also not been determined up until now.

In this study, we utilized faecal samples collected during a clinical trial to examine the efficacy of kiwifruit extract on constipation. A double-blinded, randomised cross-over trial was conducted on 40 constipated individuals, but no significant improvement was seen in constipation symptoms. Our aim here was to determine whether, despite the lack of a clinically relevant effect, the kiwifruit extracts had an influence on the composition of the gut microbiota. We used PCR to amplify bacterial 16S rRNA genes from DNA extracted from faecal samples, then sequenced the obtained amplicons using Illumina MiSeq. Consistent with previous studies, bacterial communities were dominated by members of the phyla *Bacteroidetes* and *Firmicutes*, and selected key members of the microbiota were targeted for quantification using real-time PCR. This study contributes to existing knowledge about the influence of kiwifruit on the gut microbiota. This in turn may be used to derive underlying mechanisms.

Resolving the heterogeneity in chronic rhinosinusitis: Inflammatory endotypes and microbial associations

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A complex mix of inflammatory and microbial associations underscores chronic rhinosinusitis (CRS), and the etiology remains poorly understood. Recent work has begun to address the underlying heterogeneity by delineating between variants (endotypes) of CRS on the basis of inflammatory biomarkers. This study aimed to assess inflammatory patterns in CRS phenotypes and to identify putative endotypes of CRS. Ten cytokines and 6 inflammatory cell types were assessed in mucosal biopsies from 93 CRS subjects and 17 controls via cytometric bead array and immunohistochemical techniques. Putative endotypes were identified via cluster analysis of subjects on the basis of inflammatory markers and comorbidities including polyposis, asthma, and aspirin sensitivity. Finally, previously published bacterial data for this cohort were reanalyzed to evaluate associations with inflammatory markers and CRS variants. Inflammatory patterns were highly variable within standard CRS phenotypes, including mixed inflammatory types within phenotypes and individual subjects. Eight distinct subject clusters were identified, with strong delineation on the basis of polyposis and asthma, but also subtle distinctions in several inflammatory markers. An association was also identified between depletion of several 'health-associated' bacterial taxa, reduced bacterial diversity and increased overall bacterial load, with markers of inflammation and clinical severity. This study contributes to ongoing efforts to define distinct endotypes of CRS on the basis of underlying inflammatory processes, and also offers compelling evidence of a link between bacterial community dysbiosis and inflammation in CRS. Further resolving the heterogeneity of CRS is vital to inform clinical management and personalized treatment approaches.

Evolutionary rescue: quantifying the predictability of the emergence of antibiotic resistance in natural isolates of *Escherichia coli*.

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The successful resolution of bacterial infections can be compromised by the evolution of resistance in bacteria. If the evolution of antibiotic resistance were predictable, it might be possible to tailor treatment to decrease the probability that resistance evolves. Here we use laboratory evolution of natural isolates of *Escherichia coli* to test the predictability with which antibiotic resistance evolves. We evolved replicate populations of 12 natural isolates of *E. coli*, as well as the model lab strain *E. coli* K12, to increasing levels of three different antibiotics: a total of 1536 populations. 40 daily transfers were performed, with a 35% increase in antibiotic concentration per day. After this evolutionary regime, many strains were able to grow well in media with drug concentrations at more than 10,000X MIC. We found strong evidence that resistance emerged in a predictable fashion, and the evolution of resistance to one drug often conferred cross resistance to a second. These results have considerable implications for effective prevention and control of resistance development in pathogens.

Network-guided genomic and metagenomic analysis of the faecal microbiota of the critically endangered kakapo

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The kakapo is a critically endangered, herbivorous parrot endemic to New Zealand. The kakapo hindgut hosts a dense microbial community of low taxonomic diversity, typically dominated by *Escherichia fergusonii*, which has proven to be remarkably stable, displaying little variation in core membership over years of study. To elucidate mechanisms underlying this robustness, we performed 16S rRNA gene-based co-occurrence network analysis to identify interactions between *E. fergusonii* and the wider bacterial community. Genomic and metagenomic sequencing was employed to facilitate interpretation of interactions observed in the network. *E. fergusonii* maintained very few connections with other members of the microbiota, and isolates possessed genes for the generation of energy from a wide range of carbohydrate sources, including plant fibres such as cellulose. We surmise that this dominant microorganism is abundant not due to ecological interaction with other members of the microbiota, but its ability to metabolise a wide range of nutrients in the gut. This research represents the first concerted effort to understand the functional roles of the kakapo microbiota, and leverages metagenomic data to contextualise co-occurrence patterns.

Evaluating the oral microbiota and markers of inflammation in oral and oropharyngeal squamous cell carcinoma

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Comparative analyses of oral microbial communities in oral and oropharyngeal squamous cell carcinoma (OSCC/OPSCC) patients have focused on characterising differences relative to a healthy control group. Oral cancer patients often present with poor oral health, which in turn is linked to oral inflammation. Accordingly, we designed a study to compare the oral microbiome and salivary inflammatory cytokine levels of OSCC/OPSCC patients with a group of dentally compromised but oral cancer-free patients, in addition to a group of healthy controls. The oral microbiome and mycobion of the three groups were analysed based on Illumina amplicon sequencing of the V3 – V4 region of the bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS) 1 region from dental plaque and saliva samples. Bacterial diversity was significantly higher in plaque compared with saliva samples, and sample type was a significant driver of beta diversity. Within a given sample type, no significant differences in alpha or beta diversity were identified between the OSCC/OPSCC patients and the dentally compromised, oral cancer-free group. Fungal communities in plaque and saliva were largely dominated by *Candida*, irrespective of cohort. Linear discriminant analysis (LDA) effect size (LEfSE) suggested that several bacterial OTUs differentiated the groups when plaque and saliva were independently analysed. A cytometric bead array was used to quantify inflammatory markers in saliva, and indicated the concentrations of inflammatory cytokines interleukin (IL)-1 β and IL-8 were higher in the OSCC/OPSCC and dentally-compromised patients, when independently compared to the healthy control group. Our findings suggest that oral microbial communities in OSCC/OPSCC patients are comparable to those seen in dentally compromised patients, which may be causally related to these groups having similar inflammatory cytokine profiles.

Phylogeny and genomics of SAUL, an enigmatic bacterial lineage frequently associated with marine sponges

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Many marine sponges contain dense and diverse communities of associated microorganisms. The so-called “sponge-associated unclassified lineage” (SAUL) clade of bacteria are frequently observed in sponges, yet virtually nothing is known about their physiology. Here we investigated the distribution and phylogenetic status of SAUL. A meta-analysis of the available literature revealed the widespread distribution of this clade and its association with taxonomically varied sponge hosts. Phylogenetic analyses, conducted using both 16S rRNA gene-based phylogeny and concatenated marker protein sequences, revealed that SAUL is a monophyletic sister clade of the candidate phylum “*Latescibacteria*”. Furthermore, we conducted a comprehensive analysis of two draft genomes assembled from sponge metagenomes, revealing novel insights into the physiology of this symbiont. Metabolic reconstruction suggested that SAUL members are aerobic bacteria with facultative anaerobic metabolism, with the capacity to degrade multiple sponge- and algae-derived carbohydrates. Finally, we described for the first time in a sponge symbiont the putative capacity to transport phosphate into the cell and to produce and store polyphosphate granules, presumably constituting a phosphate reservoir for the sponge host in deprivation periods. Also revealed were genes encoding symbiosis factors such as eukaryotic-like repeats and CRISPR-Cas defence systems, as well as the genomic capability of secondary metabolite production.

Oh Boi! How does it work?

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Reactive oxygen species (ROS) produced by the fungal NADPH oxidase (Nox) complex play an important role in regulating both mutualistic and antagonistic fungal-plant interactions. In the mutualistic symbiotic interaction between the fungal

endophyte *Epichloë festucae* and its grass host *Lolium perenne*, loss of ROS signalling through disruption of *noxA* leads to a dramatic change in the host-interaction phenotype. Hyphae are defective in cell-cell fusion and undergo proliferative, instead of restrictive, growth in the leaves of the host. Plants infected with $\Delta noxA$ are severely stunted and prematurely senesce. The fungal NoxA complex consists of membrane bound NoxA and NoxD components and cytosolic subunits NoxR, RacA, BemA and Cdc24, which are recruited to the membrane in response to some as yet unidentified signal. Activation of the mammalian Nox2 (gp91phox) complex involves changes in the cell membrane phosphoinositides, which leads to recruitment of the PX-domain cytosolic components. Although fungal BemA contains a putative phosphoinositide-binding PX domain, this has been shown to be dispensable for membrane localisation in *Neurospora crassa*. Instead, we hypothesize that recruitment of BemA occurs via interaction with the phosphoinositide-binding Boi1-like protein, which is known to interact with Bem1 in yeast. *E. festucae* contains two Boi1-like proteins, designated BoiA and BoiB. Phylogenetic analysis suggests *E. festucae* genes encoding these proteins arose from a common ancestor shared with *Saccharomyces cerevisiae* *BOI1* and *BOI2*. Transcriptome studies have shown *boiA* is highly expressed *in planta* compared to in culture, and up-regulated in mutants that disrupt the mutualistic interaction, suggesting an important role for *boiA* in symbiotic maintenance. Microscopy analysis showed that in comparison to wild-type, $\Delta boiA$ deletion mutants revealed a loss of hyphal cell-cell fusion in culture, while *in planta* the $\Delta boiA$ mutants exhibited multiple hyphae per intercellular space, intrahyphal hyphae, and convoluted hyphal structures within the leaves. The resulting $\Delta boiA$ infected plants had a significant reduction in tiller length compared to wild-type infected plants. These results show BoiA is required for establishment of a mutualistic symbiotic interaction between *E. festucae* and *L. perenne*.

1. Tanaka et al. 2006
2. Schurg et al. 2012
3. Eaton et al. 2015

id #223

Lipid biosensors: investigating the composition of fungal membranes

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In plant-associated fungi, the production of reactive oxygen species (ROS) by the NADPH oxidase (Nox) complex plays an integral role in controlling the growth of both beneficial and pathogenic fungi within their plant host. Therefore, specific regulation of the Nox complex is essential to maintaining a successful symbiotic relationship. Whilst much is known about the importance of ROS in plant-fungal interactions, comparatively little is known about how its production is regulated. In plant and mammalian systems, production of ROS by the Nox complex is regulated via the recruitment of its cytosolic components. This recruitment is controlled by lipid signalling, with cytosolic components containing sequences which target them to specific phospholipids enriched at certain locations within the plasma membrane. It is hypothesised that similar regulation also occurs in fungi.

Very little is known about the lipid composition of fungal membranes, so the aim of this project is to comprehensively analyse membrane lipid composition in the beneficial fungal symbiont of ryegrass, *Epichloë festucae*, using a suite of biosensors. These biosensors consist of highly specific mammalian lipid binding domains fused to a fluorophore, enabling live cell imaging of phospholipid localisation via fluorescence microscopy. This approach also allows for analysis of membrane lipid composition of hyphae growing *in planta*. Using a phospholipase C-eGFP biosensor, phosphatidylinositol 4,5-bisphosphate (PI[4,5]P₂) was detected in the plasma membrane and at septa of hyphae grown in culture and *in planta*. Similar to yeast and mammalian systems, phosphatidylinositol 4-phosphate (PI[4]P), detected with mCherry-Pleckha3 and Pleckha3-eGFP, was found to be localised to golgi vesicles in culture grown hyphae. Biosensors to other key modified phospholipids, namely phosphatidylinositol 3-phosphate (PI[3]P), phosphatidylinositol 3,4-bisphosphate (PI[3,4]P₂), and phosphatidylinositol 3,4,5-triphosphate (PI[3,4,5]P₃), localised to the cytoplasm of hyphae in culture and *in planta*, suggesting that these phospholipids are absent under these growth conditions in *E. festucae*.

id #225

Novel ncRNAs of the kiwifruit pathogen *Psa*

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Successful pathogenic bacteria must alter gene expression in response to changing and hostile environments. Non-coding RNAs (ncRNAs) contribute to adaptability and pathogenicity by forming complex regulatory networks, and include riboswitches, cis-regulatory elements and sRNAs. Despite their important biological function, the annotation and discovery of ncRNAs is hindered by a lack of sequence conservation and other distinguishing signals.

Pseudomonas syringae pv. *actinidiae* (*Psa*), the causal agent of kiwifruit canker disease, has emerged as a major threat to kiwifruit agriculture during pandemic outbreaks in the past decade. As of yet no major studies have been undertaken to

extensively characterise ncRNA genes in *Psa*. We have generated transcriptomes of *Psa* in multiple growth conditions to identify and characterise novel ncRNAs, with the aim of identifying those involved in infection and virulence.

id #226

Investigating the mechanism of pH-mediated antimicrobial potency of gallic acid

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Microbial evolution keeps pace with the discovery and application of novel antimicrobial compounds, pushing the limits of the worldwide research capacity and driving the need for innovation. We hypothesise that we can reduce the total biocide content of a product—thus reducing ecotoxicity and other side-effects— while retaining inhibitory or biocidal efficacy and sidestepping microbial resistance by using secondary ‘enhancing’ molecules to support the activity of a primary effector.

During Minimum Inhibitory/Biocidal Concentration experiments, an enhanced antimicrobial activity was observed in gallic acid, a natural phenolic compound, against *Escherichia coli* and *Staphylococcus aureus* when the pH of the solution was adjusted to neutral or alkaline (rather than an acidic pH of ~3) prior to bacteria inoculation. Gallic acid in aqueous solution will promote the conversion of dissolved oxygen to hydrogen peroxide, a process that is significantly accelerated as pH rises. We investigated whether hydrogen peroxide formation is a significant component of the antimicrobial mechanism of gallic acid. In support, catalase and antioxidant treatments protected bacteria against gallic acid killing in pH-enhanced solutions, and the antimicrobial effect of gallic acid was lost in anaerobic conditions. In addition, both gallic acid and hydrogen peroxide affected MIC/MBC similarly when applied in combination with a panel of synthetic and inorganic biocides. However, quantification of hydrogen peroxide in bacteriostatic/bactericidal solutions of gallic acid revealed a level approximately 100 times below the MIC.

Taken together these results indicate a crucial role for hydrogen peroxide and oxidative stress in the lethal action of gallic acid, but is this the whole story? Future studies will examine whether or not this effect is transient or instantaneous (due to a prolonged flux of hydrogen peroxide or a single initial dose) and we will conduct metabolomic and transcriptomic analyses to confirm or further develop our hypothesis.

id #227

The Gut Microbiota During Progression from Health to Type 2 Diabetes in a Transgenic Mouse Model

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Changes in human diet and lifestyle have led to an increasing incidence of metabolic disorders such as obesity and type 2 diabetes (T2D). Responsible globally for almost 5 million deaths each year, these two diseases represent a significant public health crisis around the world.

There exists considerable evidence for a link between T2D, obesity and the human gut microbiota. However, it has proven challenging to elucidate underlying mechanisms, due at least in part to the inherent microbiota variation among human individuals, so our group has been utilising a transgenic mouse model of T2D (as well as non-transgenic control mice) in an attempt to decipher the complex interplay between the host, diet and the gut microbiota. In this study we used Illumina MiSeq sequencing of 16S rRNA genes amplified by PCR from faecal DNA extracts to follow changes in the microbiota during progression from health, through pre-diabetes, to full T2D.

Consistent with previous studies, our results indicate the mouse gut microbiota to be composed mainly of two phyla, *Bacteroidetes* and *Firmicutes*. Particularly dominant were members of the S24-7 family (*Bacteroidetes*), which are common gut commensals within mice and humans. S24-7 bacteria dominated irrespective of disease state, constituting on average 62.6% of gut-derived sequences. Additionally, except for a single outlier, members of the *Akkermansia* genus of mucin-degrading bacteria were only detected at levels over 0.3% in non-diseased mice. Having previously been linked with health in European populations, one member of this genus, *Akkermansia muciniphila* is of particular interest. The alpha-diversity of the gut microbiomes between non-transgenic and transgenic mice did not vary significantly.

These results represent the first microbiome data from this globally unique murine model of type 2 diabetes, with ongoing research set to explore the influence of prebiotics on disease progression and the microbiome.

id #228

Frontal eddies: hotspots for phytoplankton-bacteria interactions and sulphur cycling in the Tasman Sea

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Dimethylsulfoniopropionate (DMSP) is a globally important sulphur compound produced by a diverse range of phytoplankton and subsequently degraded by marine bacteria via one of two pathways: demethylation, by which sulphur is incorporated into cellular protein; and cleavage to dimethyl sulphide (DMS), a volatile gas that acts as a precursor for cloud condensation nuclei. Interactions between DMSP-producing phytoplankton and marine bacteria are critical drivers of marine microbial carbon and sulphur cycling, but we currently have little insight into how regional scale oceanography may affect these processes. Mechanisms driving the bacterial cycling of DMSP and DMS were investigated within 2 distinct water masses in the Tasman Sea, off the east coast of Australia: (i) a large cold-core eddy (CCE), and (ii) a small frontal CCE. For each water mass, samples were collected at 3 depths (surface, chlorophyll maximum and sub-mixed layer) along an east-west transect. We found that the small, frontal CCE contained higher concentrations of DMSP, but lower concentrations of DMS, in comparison to the large CCE. Bacterial genes for DMSP demethylation (*dmdA*) and cleavage (*dddP*), quantified using qPCR, had higher relative abundance within the frontal CCE. High-throughput sequencing was performed on 16S rRNA amplicons to identify bacterial and eukaryotic chloroplast OTUs, and specific variations in the SAR11 Alphaproteobacteria were explored using 16S rRNA oligotyping and amplicon sequencing of the *dmdA* gene. Network analysis revealed DMSP to be a key driver of ecological interactions in the Tasman Sea, correlating with chlorophyll-a concentrations, bacterial carbon production rates, *Roseobacter*-like *dddP*, two SAR11 *dmdA* clades, and several highly abundant bacterial OTUs. We hypothesise that frontal CCEs can act as hotspots for marine carbon and sulphur cycling, driven by “bloom-like” dynamics within the phytoplankton community that influence the abundance and distribution of key bacterial OTUs. These findings indicate that mesoscale oceanographic processes play an important role in driving environmental and ecological interactions between marine microorganisms, potentially influencing the production of climatically important DMS.

id #230

Paediatric Non-cystic fibrosis Bronchiectasis and the Lung Microbiome

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Background: Non-cystic fibrosis bronchiectasis is a growing worldwide health problem which is particularly prevalent in New Zealand. Bronchiectasis results from permanent dilation of the lung airways, leading to mucus build-up which results in coughs, wheezing and recurrent infections. The pathophysiology of bronchiectasis is poorly understood, but infections and inflammation are believed to play a role. Here we described differences between pulmonary bacterial communities in newly diagnosed children compared to healthy controls.

Methods: Bronchoalveolar lavage samples were collected from 25 affected and 10 healthy children with a median age of 2.9 years. For affected children at least two lung sites were sampled, whereas only one sample was taken for members of the healthy cohort. DNA was extracted from pelleted lavage material and 16S rRNA gene sequencing (targeting the V3-V4 region) was conducted using Illumina MiSeq. Sequence data were analysed using a combination of USEARCH and QIIME.

Results: A total of 57 lavage samples from the 35 children yielded sequence numbers above the threshold of 3000. The most common phylum among both affected and unaffected children was *Proteobacteria*, with 60.7% average sequence abundance among healthy individuals compared to 60.2% among those with bronchiectasis. The most dominant genera were *Haemophilus* and *Neisseria*. Bacterial richness varied slightly but non-significantly between healthy and affected individuals. The mean microbiota dissimilarity was 0.72 among healthy controls and 0.71 among newly diagnosed individuals, whereas mean dissimilarity within one individual from the newly diagnosed cohort (where more than one sample was provided) was only 0.26. Together with the largely overlapping microbiotas between the healthy affected cohorts, this suggests that the strongest determinant of lung microbiota composition at this early stage of the disease is the individual rather than disease status.

Conclusion: The lungs of healthy children and those who are newly diagnosed with bronchiectasis were similar in both composition and diversity. In both cohorts the same phyla and genera were dominant, with differences between groups overshadowed by inter-individual variation.

id #232

Stable isotope probing shows that different redox forms of a sugar are used differently by human gut microbiota *in vitro*.

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Introduction: Foods contain different amounts of sugars, sugar acids and sugar alcohols, which differ in their redox consequences for intestinal bacteria that may ferment them. The demands for the reduction of the glycolysis co-factor nicotinamide dinucleotide (NAD⁺) back to NADH force fermentative anaerobic bacteria to generate oxidized byproducts such as lactate or ethanol, representing a loss of potential biomass carbon. The NAD cost for sugars, sugar alcohols and sugar acids is 2, 3 and 0 NAD, respectively, suggesting different substrates will be differentially incorporated or produced as byproducts, depending on this redox demand.

We specifically hypothesised that complex gut microbial communities will respond to substrates glucose, glucitol (sorbitol) and glucuronic acid according to this cost, and this will be reflected in the relative amounts of substrates incorporated into biomass (in order: glucuronic acid, glucose, glucitol) or secreted as byproducts (relative to glucuronic acid: glucose, increased lactate; glucitol, increased ethanol).

Methods: We anaerobically cultured mixed human faecal inocula with universally ¹³C-labelled glucuronic, glucose or glucitol substrates and, using stable isotope probing, we identified the bacteria predominantly utilizing these substrates by fractionating total RNA in isopycnic buoyant density gradients followed by 16S rRNA sequencing. Major ¹³C-labelled fermentation byproducts were identified by gas chromatography with mass spectrometry.

Results: Fermentation of ¹³C-glucose predictably resulted in ¹³C-lactate production and a concomitant increase in *Erysipelotricaceae*, known for glucose utilization; ¹³C-glucitol did not result in a ¹³C ethanol increase, suggesting that redox balance was not the major driving force in microbial response to this substrate, and that perhaps substrate uptake was limited. ¹³C-glucuronic acid resulted in a relative increase in *Ruminococcaceae*, known for complex plant polysaccharide utilization (a dietary source of uronic acids), and an increase in ¹³C-threonate/erythronate production, suggesting alternative usage or detoxification of this more oxidized substrate.

Conclusion: This indicates that sugar utilization by the gut microbiota is a combination of factors such as acquisition and detoxification in addition to fermentative redox balancing. This knowledge may be important when attempting to deliberately engineer specific gut microbial outcomes through consumption of sugar alcohols from non-digestible food additives, or uronic acid-rich plant or seaweed cell wall polysaccharides.

id #235

Elucidating the role of *Staphylococcus* spp. in chronic rhinosinusitis (CRS)

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Chronic rhinosinusitis (CRS) involves prolonged inflammation (>12 weeks) of the sinonasal mucosa and affects roughly 5% of the western population. Common symptoms include nasal obstruction and discharge, facial pain and olfactory dysfunction. Although the etiology of CRS remains unclear, a role for the bacterium *Staphylococcus aureus* and more recently the sinonasal microbiota as a whole has been proposed. However, little is known about the interplay between *S. aureus* and its congener *S. epidermidis*, the relationship between these bacteria and the wider sinus microbiota and ultimately how these interactions could affect CRS pathogenesis.

In this study we therefore aimed to: (1) describe differences in sinonasal microbiota composition between healthy and CRS-affected individuals, and (2) quantify and compare abundance of *S. aureus* and *S. epidermidis* between healthy and CRS-affected individuals. Genomic DNA was extracted from swabs collected from the left and right middle meatus of 14 healthy and 43 CRS-affected individuals during endoscopic sinus surgery. Illumina MiSeq was used to sequence PCR-amplified 16S rRNA genes. Relative abundances of *S. aureus* and *S. epidermidis* were inferred using real-time qPCR. The most common phyla were *Actinobacteria*, *Firmicutes* and *Proteobacteria* whilst the most prevalent genera included *Corynebacterium*, *Staphylococcus*, *Haemophilus*, *Moraxella* and *Dolosigranulum*. CRS patients displayed an increase in *Haemophilus* and decrease in *Staphylococcus* (genus) abundance compared to healthy controls. CRS and healthy patients exhibited no significant differences in alpha-diversity. Our results contribute to a better understanding of the influence of *S. aureus* and *S. epidermidis* load on sinus microbiota taxonomic composition and yield insights into the role of these bacterial species in CRS pathogenesis.

id #237

More than just plants: A study of biotic stress impacts on the root microbiomes of *Trifolium repens*

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New Zealand pasture plants are subject to a multitude of biotic and abiotic stresses. While the pasture plants themselves are well-studied, the microbes associated with the plants are not. In two trials, *Trifolium repens* (white clover), the key legume component of New Zealand pastoral agriculture, was grown in different soils from around the country exposed to several biotic treatments. We then used next generation sequencing to sequence variable regions of the ITS and 16S ribosomal DNA from the root microbiome of the white clover plants. The microbiome was partitioned in two compartments: the endosphere and rhizosphere. Multivariate analyses such as canonical correlation analysis were then used to compare the ribosomal DNA proportions to the biotic and abiotic factors challenging the plants. Data from the two trials was compared to assess robustness of the methodologies. We will present new insights into the interactions of white clover and its microbiome to biotic factors.

id #240

Temporal and spatial analysis of *Escherichia coli* diversity and community structure during colonisation in calves.

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Current culture methods to investigate changes in *Escherichia coli* community structure are often slow and laborious. Genes such as *gnd* (6-phosphogluconate dehydrogenase) have a highly variable nucleotide sequence and provide a target for *E. coli* microbiome analysis using culture-independent methods. The objective of this study was to investigate the impact of dairy farm and age on the community structure and diversity of *E. coli* in newly born calves using *gnd* as a target. Barcoded PCR primers were used to generate separate amplicon libraries from calves born on two separate farms in the Manawatu region during the 2016 autumn calving season for high throughput sequencing. Recto-anal mucosal swab (RAMS) samples were obtained from ten calves on each farm, with each animal sampled a total of nine times between one day and seven weeks of age. Each RAMS was enriched in modified Tryptone Soya Broth and a crude boiled lysate of washed bacterial growth was used as a template for PCR. Two mock control libraries were prepared containing equimolar concentrations of *gnd* amplicons from DNA extractions of serotyped *E. coli* strains to determine sequencing error rates. Illumina MiSeq 250bp paired end sequencing was performed on two pooled library preparations representative of amplicons obtained from calves sampled on each farm. Overlapped sequence pairs were mapped to a *gnd* database containing 320 separate sequences from various serotypes.

All calves were rapidly colonised by *E. coli* (<24 hours) with individual animals often harbouring >50 separate *E. coli* strains. However, bacterial communities were dominated (>1%) by 5 to 25 strains alongside a large pool of subdominant strains present at low abundances. Only rarely were dominant *E. coli* strains maintained in consecutive weekly samples from the same calf, suggesting a rapid turnover of *E. coli* types in the developing calf gut. Amplicon sequencing data were used for targeted culture of Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157) for whole genome sequencing and single nucleotide polymorphism analysis which separated isolated strains at the farm level. This work indicates that the *E. coli* microbiota associated with distinct dairy farms is driven by environmental and calf management factors.

id #242

De novo hybrid assembly of *Escherichia coli* natural isolates using Nanopore sequencing long read technology

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Until recently *Escherichia coli* was believed to exist predominantly in the human gut, and was routinely used as an indicator of water faecal contamination. However, new data suggest that some strains exist for long periods of time outside the host environment. Such naturalised strains may present new diversity to the *E. coli* species complex. Recently, a collection of these strains was isolated from soil samples in northern Minnesota, USA. As a first step toward further understanding the genomic changes underlying the divergence from host dependence, we used Illumina short read data and Oxford Nanopore long read data to generate hybrid genome assemblies. We focus on using different assembly methods including both short read only and long read only assembly approaches to generate multiple draft assemblies. Further to this the long-read data is subsampled based on both quality and length in order to optimize the data for bacterial assembly. Using this approach multiple draft genomes are produced and comparative analysis of assembly length, coverage, and structure has allowed us to determine an appropriate pipeline for *de novo* hybrid bacterial genome assembly using Nanopore long reads.

id #243

Comparison of *Listeria monocytogenes* inhibitory capability of lactic acid starter cultures during fermentation of shellfish - *in vitro* and *in situ* assays

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Fermentation is the preservation of food involving microorganisms that create an environment unsuitable for pathogen growth and without negative impact on the sensory quality of the product. Many commercial, concentrated starter cultures have been produced by different companies, but currently none have been used for the fermentation of shellfish. In this study, we examined the suitable starter culture for inhibition of *Listeria monocytogenes* in shellfish. Five different

starter cultures were obtained from Christian Hansen Ltd: BFL-F02, SM-194, F-LC, BFL-F04 and T-SC-150. These starter cultures contain different combinations of *Pediococcus* spp., *Staphylococcus* spp., *Lactobacillus* spp. and *Debaryomyces*, spp. All starter cultures were compared for their inhibition capability against *Listeria monocytogenes*. Starter cultures were grown for 18 hours at 30 °C and centrifuged at 4000 rpm 15 minutes. The supernatant was then filter-sterilized, pasteurized and used to examine for inhibitory activity on microplate inhibition assay, minimum inhibitory concentration method and a spot-on-lawn assay. Live starter culture also were used on spot-on-lawn assay. For an *in situ* assay, New Zealand green shell mussels were boiled for 7 minutes to open the shells, the meat minced, inoculated with *L. monocytogenes* and fermented. Filtrate extract from F-LC, BFL-F04 and T-SC-150 shown extended lag phase for *L. monocytogenes* and T-SC-150 showed no growth until 48 hours of incubation. On the spot-on-lawn assay F-LC, BFL-F04 and T-SC-150 shown inhibitory ability when both filtrate and live cultures tested. All cultures filtrates showed minimum inhibitory concentration at 10⁶ dilution (original dilution being 10⁸) after 18 hours of incubation. *In situ*, the T-SC-150 shown high inhibition capability, after four days of fermentation CFU was 60/ml and after one week storage in both room and fridge temperature no *Listeria* was observed. All other cultures shown growth of *Listeria*. The modern concentrated starter cultures, the F-LC, BFL-F04 and T-SC-150 have shown promising anti-*Listeria* activity and T-SC-150 being the best for application in food systems as effective biopreservatives.

id #244

Morphotype plasticity contributes to antibiotic tolerance by *Pseudomonas aeruginosa*

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A major contributor to the ability of pathogenic bacteria to tolerate the actions of antibiotics is their ability to transition between different lifestyles. One of the best studied bacterial lifestyles that confers elevated tolerance to antibiotics is the formation of matrix-encased biofilms. The opportunistic pathogen *Pseudomonas aeruginosa* undergoes two different mechanisms of morphological transitions in which normal rod-shaped (bacillary) cells transition to spherical cells. One of these processes is extremely rapid, occurs in only a sub-set of the population, and leads to explosive cell lysis events that release cellular content including eDNA, moonlighting proteins, membrane vesicles and other public goods into the extracellular milieu. We found that the Lys endolysin, encoded in the R- and F- pyocin (tailocin) gene cluster, is required for explosive cell lysis and biofilm development by *P. aeruginosa*. The second mechanism of spherical cell morphotype transition by *P. aeruginosa*, is slower, occurs *en masse* in response to β -lactam antibiotics, and involves the morphogenesis of cell-wall deficient (CWD) spherical cells which transition to the normal bacillary form upon antibiotic removal. We have recently determined that the β -lactam-induced CWD cells of *P. aeruginosa* are able to proliferate in the presence of high concentrations of β -lactam antibiotics via mechanisms that are similar to those described for bacterial L-forms. Our observations suggest that the ability to reversibly transition to the L-form lifestyle enables *P. aeruginosa* to tolerate high concentrations of β -lactam antibiotics. We are currently exploring whether these mechanisms of morphotype plasticity can be exploited to develop novel therapeutic approaches to combat *P. aeruginosa* infections.

id #245

Population and single cell analyses reveal sympatric segregation of microbial communities in wastewater treatment plants and microhabitat-dependent predator-prey dynamics

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Publish consent withheld

id #246

Using structural biology to inform the design of T-antigen based vaccines for Group A Streptococcus

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Group A Streptococcus (GAS) is a globally important pathogen causing a broad range of human disease and significant morbidity and mortality. There are no vaccines for GAS currently available but vaccine candidates based on the T-antigen, which forms the pilus backbone, are in pre-clinical development. T-antigens have previously been shown to have protective properties in mice and relatively low antigenic variation (GAS cluster into 18 major *tee*-types). This project aims to investigate antibody-T-antigen interactions and provide 3-dimensional structural data of T-antigens to inform structured, vaccine design efforts.

FVB/n Mice and NZ white rabbits were vaccinated with recombinant T18 antigen (found in M18, M71 and M217 strains). Sera from these animals were screened by ELISA using an array of T-antigens that covers all major circulating *tee*-types to elucidate cross-reactivity patterns. The dominant antibody cross-reactivity observed was to T3.2 and T13. To visualise

antibody cross-reactivity at a molecular level the structures of the three T-antigens (T3.2, T13 and T18.1) were solved using X-ray crystallography. Each T-antigen features two immunoglobulin-like domains and, despite low overall sequence identity, show significant structural homology with the previously published T1 antigen. Structural overlays reveal that T-antigens share a highly conserved core decorated with variable loop regions. Purification of T-antigen specific IgG from the animal sera, together with the isolation of high affinity (<50 nanomolar) monoclonal antibodies from the same animals, has enabled the patterns of antibody specificity to be mapped onto T-antigen structures. These structural maps of antibody-T-antigen interactions will inform the design of T-antigen based vaccines.

id #248

***Campylobacter* survival in groundwater: Implications for public health**

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Aim

Following the recent *Campylobacter* outbreak in Havelock North it was recognised that there was a lack of data on the survival of *Campylobacter* in groundwater. To close this knowledge gap we designed an experiment to measure the survival of an outbreak strain of *Campylobacter* and the type strain in an oxic and an anoxic groundwater.

Methodology

A laboratory scale system was set up to investigate the survival of an outbreak strain (HN16) and type strain of *Campylobacter* in both an oxic and anoxic groundwater. The two groundwater types were held at 12°C (average temperature of local groundwater and the dissolved oxygen was maintained in both systems throughout the experiment. Samples were taken at specific time intervals over the period and analysed for *Campylobacter* using selective media.

Results

The results show that *Campylobacter* can survive in groundwater for over two weeks. Differences were observed in the rate of die off between the two strains of *Campylobacter* studied. The outbreak strain of *Campylobacter* showed higher survival rate compared with the type strain of *Campylobacter* in anoxic groundwater. Initially (first 7 days), the two strains of *Campylobacter* died off at different rates, and were not affected by the different types of groundwater. The type strain showed die off after 7 days, with a log drop in concentration present, irrespective of groundwater type. In comparison, the outbreak strain showed very little die off. After 7 days, the type of groundwater appeared to have an effect on the survival of both strains of *Campylobacter*. The outbreak strain continued to survive well in anoxic groundwater and showed less than a log drop in concentration present after 17 days. The type strain also showed less die off in anoxic groundwater over days 7 to 17. It is interesting to note that there appeared to be a plateauing of the die off occurring in both strains of *Campylobacter* occurring in oxic groundwater.

Conclusions

Campylobacter is a microaerophilic microorganism which grows optimally in low oxygen levels. The low oxygen concentration (maintained at less than 2 mg/L) could play a role in the survival of *Campylobacter* in groundwater.

id #249

Groundwater Health Index: Identifying the risk to drinking water using microbial diversity

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Groundwater is a vital source of drinking water both in New Zealand and globally. Although groundwater represents a small proportion of the global water, it is a predominant source of drinking water. The perception, often, is that groundwater is a pristine, sterile environment. There are, however, a vast array of organisms thriving under our feet. These complex ecosystems protect our groundwater by removing contaminants that enter the groundwater through anthropogenic activities on the surface. There is however, as with all things a balance to maintain and there is a point which tips the balance. In these environments this can mean a drinking water is no longer protected from contaminants.

Current methods for assessment of the quality of groundwater are reactive; tests taken at point source that indicate a past problem. Our research is aimed at gaining a better understanding of these vital ecosystems and how they respond to contaminants in order to develop a proactive method of assessing groundwater health. The novel toolbox we are developing will assess the presence or absence of keystone species (micro and macro) to give a better picture of the health of the groundwater ecosystem and how it is able to protect the water we drink. We are using cutting edge technologies to develop the toolbox due to the inherent difficulties of sampling below the ground.

We will present our findings so far, identifying key microbial groups present in groundwaters of differing chemistries and changes occurring both spatially and temporally. This is the first step towards a groundwater health index similar to the MCI for surface waters.

Understanding airborne microorganisms at high temporal resolution

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The air contains a diverse and constantly changing microbiological community with known impacts to atmospheric events, biogeography and human health. However, due to the technological challenges of biomass retrieval, studies to date have relied on coarse temporal samples of several hours to typically days. Our primary objective was to create tools capable of gaining high spatial and temporal resolution samples of airborne environmental DNA in the hopes of understanding the ephemeral nature of airborne microorganisms.

We have developed methodology that can reliably characterise the total airborne microbial community using high-volume vortex air samplers and sensitive DNA extraction protocols. Successful DNA yields suitable for next generation amplicon and shotgun metagenomic sequencing have been consistently gained from one hour long samplings making the investigation of the airborne microbial community changes throughout the day possible.

Preliminary bacterial 16S and eukaryal ITS amplicon sequencing results from a continuous 24-hour period in Auckland, New Zealand have identified a relatively consistent airborne microbial community throughout the day whose community structure changes to varying extents hourly. Typical dominant Bacterial phyla include Proteobacteria, Firmicutes, Cyanobacteria, Bacteroidetes, Actinobacteria and Tenericutes. We found the samples to be surprisingly diverse with as many as 1776 unique bacterial OTUs in a single sample and a total of 3091 unique OTUs. Ten eukarya phyla were identified representing 6891 OTUs, however, Blastocladiomycota and Ascomycota were strongly dominant.

This methodology has proven to be ideal to gain high-temporal resolution bioaerosol samples and for environmental based field work where long term installation of equipment isn't practical. As we build a more robust data set, we hope to link community changes to environmental variables and air mass source, evaluate ideal minimum sampling durations to capture the environmental diversity and determine variation in aerosol communities over various time scales.

Microscale coastal sediment responses to ocean acidification

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Microbial processes occurring within the top few millimetres of sediment drive the functioning of coastal marine ecosystems. For example, cycling of the nutrients nitrogen and phosphorus is coupled between the sediment and the overlying seawater, underpinning primary production and trophic interactions. This coupling, however, is perturbed by the ocean's increasing absorption of anthropogenic CO₂, a process that alters seawater carbonate chemistry (ocean acidification, OA). Despite the far-reaching consequences of such perturbations, we still know very little about the biogeochemical mechanisms underlying the sediment's response to OA. Understanding these mechanisms is complicated by the small spatial scale at which various microbial processes in the sediment interact, and the complex interactions between microorganisms, sediment-inhabiting fauna, and microalgae. Tackling such challenges therefore requires an approach that can resolve complex biological interactions at the microscale, and link cellular response with ecosystem function. Here, we will introduce a new project (Acidification Responses of Marine Sediments, ARMS) that uses such an approach in a series of increasingly inclusive and complex experiments. Combining microscale measurements of the porewater chemical environment with molecular analyses of microbial community structure and activity, we are able to interpret alterations to microbial metabolic processes at each successive level of environmental complexity. We can then scale these observations to ecosystem level by linking changes in the sediment-seawater flux of, e.g., inorganic nitrogen with microbial processes, such as nitrification and denitrification. Our approach provides a powerful tool for understanding the functional response of coastal marine ecosystems to global environmental change.

The Effects of Ocean Acidification on Microbial Nutrient Cycling and Productivity in Coastal Marine Sediments

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2. *Auckland University of Technology, Auckland, New Zealand*

Ocean acidification (OA) refers to the reduction in seawater pH over time, primarily caused by the uptake of CO₂ from the atmosphere. The chemical process of OA is thoroughly defined and increasingly well documented in current field data. Since the start of the industrial era, the concentration of atmospheric CO₂ has increased by an estimated 40% from 280 ppmv (parts per million volume) to 384 ppmv. The Intergovernmental Panel on Climate Change (IPCC) has classified 800 ppmv as the projected end-of-the-century concentration operating under the business-as-usual CO₂ emissions scenario.

Previous studies surrounding marine biogeochemistry have demonstrated that pH is *a*, if not *the* critical environmental parameter governing microbial community composition, structure, and function. Coastal sediments are highly stratified habitats and are typically characterized as having a thin oxic surface layer, an anoxic layer, and a sulphidic zone dominated

by sulfate reduction. Microorganisms are inherently linked to the functionality of the ecosystem based on the duties they perform in mediating the biological fluxes of key elements: carbon (C), nitrogen (N), phosphorus (P), and sulphur (S). The biogeochemical sequence of marine sediments has been thoroughly investigated across a wide range of applications, however information regarding the stratification of microbial communities and the effect of reduced pH on their functional role within the system is highly limited. This highlights a significant gap in knowledge for understanding microbial resilience and function on a regional and global scale.

This study aims to provide a first look at how microbial communities will respond to inevitable environmental perturbations. The experiment was conducted *in situ* using a specially designed mesocosm system. Parameters for pH were based on current IPCC projections. Microbial communities were characterised using 16S rRNA gene PCR amplicon sequencing to assess changes in microbial community composition. Bioinformatics tools were used to trim and analyse the data. Preliminary pipeline results indicate no significant changes occurring at the phylum, family, or genus level. Further analysis demonstrated compositional changes at the OTU level within the top 10% most abundant OTUs. Subtle changes may be an indication of microbial resilience. Future directions involve metagenomic and metatranscriptomic analyses.

id #253

Genetic determinants of competition and coexistence in the nectar microbiome

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Floral nectar is a harsh environment that hosts a community of microbes, including yeasts and bacteria. In this nitrogen-limited and osmotically-stressed environment, these microbes compete fiercely for resources. We surveyed a 200 km range of floral nectar from host plant sticky monkeyflower in California and assessed the distribution patterns of the nectar microbiome, in relation to guild membership and nectar chemistry. We further analyzed the prevalence of the dominant yeast *Metschnikowia reukaufii*, and identified genetic and physiological trade-offs that may play a role in facilitating coexistence in the nectar microbiome. Direct microcosm competition experiments against other species evaluated the competitive strength of *M. reukaufii* strains to explain the observed distribution patterns.

The nectar microbiome is a low diversity system, predominantly occupied by *Metschnikowia* yeasts and few bacterial species. Across the landscape, bacteria and yeasts were rarely found co-habiting the same flowers, such that flowers with high density of yeasts harbored fewer bacteria and vice versa. Population-level analysis of *M. reukaufii* revealed multiple local genotypes but two distinct phenotypic assignments. Variation in metabolic and physiological traits suggest that different *M. reukaufii* strains may occupy different niches in the nectar environment. Direct competition assays against other species further confirmed the disparity in competitive strength between the two *M. reukaufii* phenotypes and provided one explanation for its observed dominance in the nectar microbiome

id #254

Investigation of *Saccharomyces cerevisiae* adaptations during an evolution in emulsion culture

Emelyne Cunnington and Thomas Pfeiffer

Massey University, New Zealand

A trade-off, in biology, is defined as a constraint that link two traits. Improving one trait has a negative impact on the other trait. While there is an increased interest in trade-offs, and their roles in shaping the traits of organisms, it remains difficult to demonstrate their presence and relevance in lab experiments. My study focuses on a trade-off between yield (biomass production per resource) and rate (biomass production over time), which have been proposed based on theoretical reason (Pfeiffer *at all*, 2011). This theory is based on a trade-off between rate and yield of ATP production in energy metabolism. One system where this trade-off arises is sugar degradation by Crabtree-positive yeasts, such as *Saccharomyces cerevisiae*. There are 2 pathways to produce ATP from sugar: fermentation and respiration. Fermentation has a low ATP production yield while respiration has a high yield in ATP production. In presence of oxygen, Crabtree-positive yeasts use the fermentation pathway in addition to respiration. Compared to the exclusive use of respiration in ATP production, this is expected to decrease ATP yield but increase ATP rate. To test this idea we conducted an experimental evolution study with *Saccharomyces cerevisiae* in an emulsion culture that has previously been shown to select for yield (Bachmann *et al*, 2013). We observed that 4 of our 6 lines increased in growth yield during evolution. While this increase was not correlated with a decrease in growth rate, our study allowed us to uncover adaptations of yeasts to this specific environment. Pfeiffer, T., Schuster, S. & Bonhoeffer, S. Cooperation and competition in the evolution of ATP-producing pathways. *Science* **292**, 504–7 (2001).

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National scale studies of microbial biogeography revisited using highly multiplexed DNA sequence data

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Bacterial communities are a fundamental component of virtually every ecosystem on Earth. However, little remains known of the patterns and processes that determine their distribution and the existence of even the most basic patterns such as latitudinal gradients in their taxonomic richness and range remain unclear. We sought to determine the relative influences of spatial and environmental factors on bacterial community structure and individual community members based at various taxonomic levels. Using a highly multiplexed DNA sequencing approach we explored the diversity and biogeography of soil and freshwater bacterial communities at over 500 locations within New Zealand. The geographical range of bacterial taxa was strongly and positively correlated with latitude, also coinciding with a greater than 5-fold increase in bacterial taxon richness across the 1,000 km latitudinal gradient. These gradients were observed despite enormous variability in soil physical, chemical and land use attributes among sites, and also when restricting our analysis to samples collected only from native forest catchments. The biogeographic range of microbial taxa also appeared to increase in correlation with average genome size at higher latitudes, reflecting the greater functional plasticity needed to tolerate a broader range of environmental conditions. Despite these strong spatial gradients in bacterial community composition, variables such as soil pH and concentration of available phosphorous explain a major component of the observed variation in bacterial community attributes. Altogether, our results highlight the strong relationship between the environment and both bacterial community composition as well as the abundance of specific community members. This could have significant implications for how we monitor our environment in the future, as it suggests that there is scope to use the abundance of individual community members as biologically relevant indicators of the condition of the soil and freshwater resources.

Characterization of the relative abundance of microbiota present in a newly formulated coconut water kefir sourdough using Miseq high throughput sequencing method

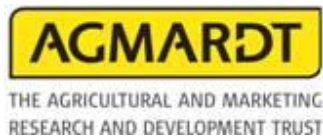
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To characterise the microorganisms present in the sourdough, formulated using probiotic kefir culture, Miseq sequencing was performed in association with culture-dependent methods. The different diversity of the lactic acid bacteria (LAB) was investigated, in addition to enumeration, pH, total titratable acidity (TTA), D-, L- Lactic acid and rise in volume. The LAB counts obtained varied from 3.01 log₁₀ CFU/ml at the start of coconut water kefir sourdough incubation to 5.42 log₁₀ CFU/ml at the end of 96 hours of incubation. The total yeast counts were between 2.04 log₁₀ CFU/ml to 3.87 log₁₀ CFU/ml from 0 hour to 96 hours, respectively, for coconut water kefir sourdough. There was drop in pH and rise in TTA and D-/L- Lactic acid concentrations during dough rise. To extract the dough DNA, a high speed stomacher was used. The DNA was quantified then amplified by PCR for 16S metagenomics library preparation and sequencing. The 16srRNA sequences were analysed and assembled for operational taxonomic units (OTU's) identification. The research highlights are the detection of the OTU's such as *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Lactobacillus casei*, *Lactobacillus murinus*, *Lactococcus lactis*, *Lactobacillus brantae* over the period of dough incubation, indicating the change in the microbial ecological succession over time for lactic acid bacteria.

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POSTER ABSTRACTS

In numerical order

id #11

High-Resolution Melting Analysis for SNP Detection In *ail* Gene of *Yersinia enterocolitica* Strains Belonging to Different Biotypes

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The *Yersinia enterocolitica* species is classified into six biotypes, divided into three groups according to their pathogenicity: non-pathogenic biotype 1A, weakly pathogenic biotypes 2, 5, and highly pathogenic biotype 1B. The *ail* gene is an important chromosomal virulence marker of *Y. enterocolitica* which encodes Ail, a 17-kDa outer membrane protein that promotes attachment and invasion. The aim of the study was to analyze the presence of the *ail* gene single nucleotide polymorphism (SNP) in *Y. enterocolitica* strains belonging to different biotypes.

The presence of the correlations between the sequence of the *ail* gene and the biotype of the analyzed *Y. enterocolitica* (1A, 1B, 2 and 4) was determined using high resolution melting (HRM) analysis and DNA sequencing. The HRM-PCR profile was as follows: initial denaturation at 95°C for 5 min, followed by 40 cycles at 95°C for 10 s and 52°C for 30 s. The melt analysis was conducted by measuring fluorescence at the temperature ramp of 65°C to 90°C at 0.1°C intervals.

Sequence alignment of *Y. enterocolitica* strains belonging to biotype 1B (genotype 1B) revealed 99.8% similarity between the examined *ail* gene nucleotide sequences and the reference *Y. enterocolitica* AM286415 (GenBank) sequence. Only one mutation . transition G2007848T . was detected, whereas an analysis of *Y. enterocolitica* strains belonging to biotype 1A (genotype 1A) revealed the presence of two SNPs: transitions G2007848T and G2008088T. An analysis of *Y. enterocolitica* strains belonging to biotypes 2 and 4 (genotype 2/4) revealed that their *ail* gene nucleotide sequences were identical in 100%. However, in comparison with *Y. enterocolitica* AM286415, the presence of 14 SNPs was characteristic for each of the strain belonging to biotypes 2 or 4.

Amplicon genotyping based on HRM analysis supports rapid identification of *ail* SNPs correlated with biotype and biotype-dependent pathogenicity group of *Y. enterocolitica*. The assay developed in this study is a sensitive and reliable method of fast screening *Y. enterocolitica* strains, including *ail*-positive biotype 1A isolates.

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id #31

PilVax, a novel peptide antigen delivery system for the development of an effective vaccine against *Streptococcus pneumoniae*

Callum Chalmers, Thomas Proft, Jacelyn Loh, Catherine Tsai, Samuel Blanchett, Adrina Hema Jethanand-Khemlani, Kim Paras

Background

Streptococcus pneumoniae is a human nasopharyngeal pathogen which is the leading cause of the severe infections pneumonia, meningitis and sepsis. Mortality due to these infections remains high despite available vaccines. Current polysaccharide-based vaccines suffer poor immunogenicity, limited serotype coverage and serotype replacement meaning vaccines against more conserved protein targets, such as Pneumococcal Surface Protein A (PspA), are needed. A novel protein vaccine technology suitable for such a vaccine is the PilVax expression platform. This platform incorporates peptide epitopes into the polymeric backbone of the Group A *Streptococcus* M1 pilus, amplifying them and improving their immunogenicity. This recombinant pilus is then expressed on the surface of the food-grade bacterium *Lactococcus lactis* to generate a safe, effective vaccine.

Objectives

To vaccinate mice with conserved PspA epitopes presented on the novel peptide carrier platform PilVax and analyse the epitope-specific immune response.

Methods

Selected PspA B cell epitopes were cloned into a plasmid-borne copy of the backbone monomer gene of the M1 pilus. The complete M1 pilus operon was then heterologously expressed in *L. lactis*. Western blotting and flow cytometry with specific antibodies against the pilus monomer were used to analyse pilus expression. Groups of mice will be vaccinated intranasally with the PilVax constructs or controls. The controls will include *L. lactis* expressing the M1 pilus without the PspA epitopes, synthetic peptides (with and without adjuvants) and peptide-flagellin fusion proteins. After intranasal vaccination of mice with the recombinant *L. lactis*, the IgG and IgA responses against full length PspA will be analysed in serum, saliva and bronchoalveolar lavage fluid using ELISA.

Results

Both Western blotting and flow cytometry showed that incorporation of the peptides did not interfere with pilus expression and assembly on the surface of *L. lactis*. Once vaccination is complete, the titres of IgG and IgA will be used to judge the immunogenicity of the vaccine.

Discussion

Initial results suggest that the PspA epitopes do not disrupt pilus formation and are therefore suitable for immunisation using the PilVax platform. Further experiments are needed to evaluate whether this vaccine is immunogenic and protective.

Investigations into a novel hypothetical virulence factor from Group A Streptococcus

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Group A Streptococcus (GAS) or *Streptococcus pyogenes* is a major human pathogen that causes a range of diseases, from minor skin and throat infections such as impetigo and pharyngitis, to severe invasive infections such as streptococcal toxic shock syndrome and necrotising fasciitis. This is facilitated by a large arsenal of virulence factors that contribute to colonisation of host tissue, invasive spreading and immune evasion.

We have identified an open reading frame encoding for a hypothetical 221 amino acid protein that is predicted to be secreted by the bacteria. The protein is highly conserved in GAS, but not found in related species suggesting a more specialised function.

The complete gene without the predicted N-terminal signal peptide sequence was amplified from the GAS SF370 (serotype M1) strain by PCR and cloned into a pProEx-Hta expression vector. The recombinant protein was expressed as a maltose binding protein (MBP) fusion in *E. coli* BL21 and purified by immobilised metal chelate affinity chromatography (IMAC) using a NTA-Ni²⁺ resin. The MBP was removed by using recombinant tobacco etch virus (rTEV) protease and the GAS protein was further purified by size exclusion chromatography.

The purified GAS protein is currently being investigated for specific binding to host factors using pull-down experiments with human serum and cell extracts.

Rapid identification of *Lactobacillus casei* group using MALDI Biotyper combined with ClinProTools

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In this study, we applied matrix-assisted laser desorption ionization. time of flight mass spectrometry (MALDI-TOF MS) to rapid discriminate of LCG strains by creating an analytical in-house database (IHDB), and to develop a classification model for subspecies-level differentiation based on the biomarkers mass spectra using ClinProTools bioinformatics software. The genotypic methods (housekeeping gene sequencing and species-specific PCR) were also established for validation of MALDI-TOF MS platform. A total of 47 LCG reference strains were correctly identified to the species level and had high score values (mean score: 2.46±0.1) using MALDI-TOF MS with an IHDB, which was in accordance with *mutL* gene sequencing and specific PCR-based methods. However, one strain identified as *L. casei* with relatively low score value (2.02±0.02) and lower sequence similarities (*mutL*: 90.4%), and failed to amplify species-specific amplicon, which may represent an undescribed novel species. In addition, after implementation of the classification model (contained two biomarker peaks: *m/z* 4,930 and 5,303), the *L. paracasei* strains could be clearly and easily differentiated to subspecies level. Our data demonstrate the high resolution performance of MALDI-TOF MS for fast and accurate demarcation of LCG strains by using IHDB and coupled with ClinProTools, which can as an alternative methodology for quality control of the probiotic products.

The Use of PilVax in the Development of Vaccine against Group A Streptococcus

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Background:

PilVax is a novel peptide delivery system that utilises a food-grade bacterium, *Lactococcus lactis* expressing group A streptococcus (GAS) pili on its surface for use as a mucosal vaccine. Peptides are incorporated within the pilus backbone protein (BP), otherwise known as the T-antigen, of which 50-100 are covalently linked to form hair-like appendages. The homopolymerisation of the BP and stability of the pilus structure make it an excellent platform for antigen delivery. Preliminary studies in mice have shown that intranasal immunisation of PilVax can elicit specific mucosal and systemic immune responses to model peptides engineered within the BP.

Objective:

To generate PilVax constructs which have immunogenic GAS epitopes for intranasal immunisations in mice and to analyse mucosal and systemic immune responses to GAS in mice.

Method:

Four linear epitopes of 2 GAS virulence factors, Spy0469 and Spy1228 were incorporated into the E/F loop region of BP and expressed in *L. lactis*. Pilus expression was determined using Western blot and quantified using flow cytometry. Intranasal vaccination with the recombinant *L. lactis* will be carried out and serum from immunised mice will be tested for GAS-specific IgA and IgG antibodies.

Results:

Western blot and flow cytometry showed pili expression on *L. lactis* after incorporation of different epitopes within the BP.

Discussion:

Two of these PilVax constructs will be selected for immunisation of mice and generated mucosal and systemic immune responses will be investigated.

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2. Mortensen, R., Nissen, T. N., Fredslund, S., Rosenkrands, I., Christensen, J. P., Andersen, P., & Dietrich, J. (2016). Identifying protective Streptococcus pyogenes vaccine antigens recognized by both B and T cells in human adults and children. Scientific Reports, 6(1).

The role of helicases in the processing and recovery of DNA replication forks following replication arrest in living *Escherichia coli* cells

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DNA replication is essential for all forms of life and must be completed accurately for the duplication of a cell. Despite being a precise and rapid process, the replisome is often impeded by barriers, arresting the progression of replication and potentially threatening genome integrity. Such arrest may lead to the dissociation of replisome components and the collapse of the replication fork. A major source of replication fork stalling is the encounter of the replisome with proteins bound to DNA. In *E. coli*, a multitude of recombination proteins have been implicated in the processing of arrested replication forks, including several helicases that may reverse the stalled fork into a Holliday junction. In this study, the early processes that a cell undergoes to restore a replication fork structure have been assessed. The main focus has been to investigate the significant helicases RecG, RuvABC and RecQ for their roles in DNA repair and fork restoration.

In order to achieve this, an *in vivo* repressor/operator system has been established to create a site-specific nucleoprotein replication block in the *E. coli* chromosome. The addition of temperature sensitive replisome components can be used to induce replication fork collapse. The formation of the replication block and its subsequent release can be visualised using fluorescence microscopy, cell viability assays and 2-dimensional agarose gel electrophoresis to analyse the DNA structures present at the nucleoprotein blockage and subsequently evaluate the effect of deleting key helicases on replication fork processing and recovery. A novel role of RecQ has been elucidated and the relative contributions of RecG and RuvABC to fork and HJ processing have been determined. These findings alter the current perception of the helicase roles in fork recovery following collapse.

Biofilm dormancy enhances antimicrobial tolerance in *S. epidermidis*

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Indwelling medical devices have been increasingly used in modern medicine and have saved millions of lives worldwide. However, they can also be an important source of infections, most commonly caused by coagulase negative-staphylococci, particularly by biofilm forming *Staphylococcus epidermidis*. A key feature of biofilms is its enhanced tolerance to antibiotics. Several mechanisms have been proposed to contribute to this phenomenon. We recently developed an *in vitro* model able to stimulate the induction or prevention of biofilm dormancy. Herein, we used that model to determine if biofilms with induced dormancy presented a distinct antimicrobial tolerance profile than biofilms with prevented dormancy. Both clinical or commensal isolates were included and a total of 43 unique isolates, from different parts of the world were tested. Biofilms were exposed to tetracycline, vancomycin and rifampicin and were analysed by flow cytometry, CFU counts and CLSM. Three unique observations were obtained. First, biofilm dormancy was found as a widespread condition in both clinical and commensal isolates, suggesting this is a fundamental process not only related to the infectious process. Second, while vancomycin did not present any significant effect on the tested biofilms, tetracycline and rifampicin significantly reduced the number of CFUs in biofilms with prevented dormancy tested (up to 4 log killing under 8 h), but were significantly less effective in biofilms with induced dormancy. The third and more curious observation was that the very high reduction in cultivable bacteria was not correlated with the reduction of total and viable cells. Overall, our data suggests in one hand that biofilms with induced dormancy are more tolerant to tetracycline and rifampicin and that those antibiotics further induce dormancy in biofilms, instead of effectively eliminating the biofilm bacteria.

Prevalence of soil-borne zoonotic pathogens of public health significance in Punjab province, Pakistan

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2. The Pennsylvania State University, State College, Pennsylvania, USA

Introduction: Utilizing molecular detection assays, surveillance for Extremely Dangerous Pathogens (EDPs) from a complex ecological niche (soil) is imperative to devise appropriate interventions for public and animal health. Such an approach is important for a particular setting such as Pakistan where appropriate bio-containment facilities for culturing and archiving EDPs are not available.

Methods: We conducted a cross-sectional study to estimate genomic prevalence of *Burkholderia mallei*, *Coxiella burnetii*, *Francisella tularensis*, and *Bacillus anthracis* in soil representing select districts (n=09) of Punjab province, Pakistan. The study included 485 villages of districts Lahore (n=29), Sheikhpura (n=295), Gujranwala (n=360), Faisalabad (n=370), Sahiwal (n=255), Attock (n=225), Sargodha (n=370), Chakwal (n=190) and DG Khan (n=215). A total of 2,425 soil samples representing different geographical locations were processed; 05 were collected from each village comprising of 04 from different livestock barns and 01 from a nearby agriculture land with no apparent animal and human interaction. Genomic DNA was extracted and processed using well-optimized and validated molecular assay (real time PCR) for chromosomal gene of *B. mallei*, transposase gene (ISIII) for *C. burnetii*, lipoprotein gene (Tul4) for *F. Tularensis*, and both capsular (CapB) and protective antigen (PA) for *B. anthracis*.

Results: We found varying prevalence of select pathogens among study districts. The occurrence of genome of *F. tularensis* was more frequent (n=93, 3.84%, 95% CI: 3.15 . 4.68) followed by *C. burnetii* (n=47, 1.94%, 95% CI: 1.46 . 2.57) *B. mallei* (n=13, 0.54%, 95% CI: 0.32 . 0.92), *B. anthracis* (CapB) (n=13, 0.54%, 95% CI: 0.32 . 0.92) and *B. anthracis* (PA) (n=04, 0.16%, 95% CI: 0.06 . 0.42).

Conclusion: The study provides first-ever evidence of select pathogens in soil from Pakistan. The gained outcome ascertain further rigorous analytical and epidemiological studies to determine genetic nature and molecular diversity of prevailing pathogens together with seroconversion in human and animals.

id #50

Identification and understanding the roles of quorum sensing in New Zealand exported lamb

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Meat is contaminated with microorganisms from the animal source and the abattoir facilities. Microbial growth leading to spoilage or sensory issues can occur during the handling transportation, distribution and storage of meat. Microorganisms communicate with each other during growth through quorum sensing (QS) which may regulate the physiological activities of spoilage bacteria. New Zealand is the largest exporter of sheep meat in the world and it brings substantial amount of revenue to the country. Vacuum-packing and chilling lamb meat reduce microbial growth, but spoilage still occurs via the interactions of *Enterobacteriaceae* and lactic acid bacteria (LAB). Lamb is very different from beef in pH and nutrients, present work aims to understand QS process in chilled lamb stored under vacuum packaging, used for distant markets and several factors that influence QS production.

A group of meat spoilage bacteria including *Brochothrix* spp., *Carnobacterium* spp., *Enterobacteriaceae* and *Shewanella putrefaciens* were used for QS analysis. These cold-tolerant organisms grow when oxygen is absent. A sample of spoiled chilled lamb stored under vacuum packaging for over 120 days was also used for comparison. QS signalling molecules acyl homoserine lactones (AHLs) were sensed by biosensor strains *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NTL4/pZLR4. Agar plating assay was used to detect these signal molecules and chromatography further differentiated and characterised the AHLs produced. Another QS signalling molecule, autoinducer-II (AI-2), was detected by a bioluminescence assay. Different growth conditions, such as growth in lamb loin and shoulder juices with different nutrients and pH values under anaerobic/aerobic environment and at different temperatures, were investigated.

In this project, only Gram-negative organisms were found to produce a variety of AHL molecules and 3-oxo-C6-HSL was demonstrated as the major AHL molecule produced. This was also found in spoiled vacuum-packed chilled lamb. AI-2 molecule was found to be universal in all the spoilage bacteria and spoiled meat. It was also clearly exhibited that oxygen, high pH, high temperature, low fat content and high glucose level enhanced QS production. Also in this study, *Enterobacteriaceae* were found to produce AHLs at 10⁵ CFU/mL in lamb meat juices i.e., much lower concentrations than generally associated with spoilage.

id #52

Biosynthesis of Au and Ag nanoparticles using extracts of *Deinococcus*

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Gold and silver nanoparticles have attracted significant interests due to their conspicuously unique properties, such as size-related electronic, optical, thermal and catalytic properties. Herein we investigated biosynthesis of Au and Ag nanoparticles using the extracts of *Deinococcus* bacteria, which are known for their strong resistance to radiation, oxidants, chemical mutagens as well as strong ability of reducing heavy metal ions. The cultures of *D. geothermalis* and *D. radiopugnans* were investigated for their biosynthesis ability of Au nanoparticles (AuNps) and Ag nanoparticles (AgNps) using gold and silver ion in solution, respectively. Under the same conditions, *D. geothermalis* had the relatively short time to reach reaction equilibrium of nanoparticle formation (approximately 10 h for AuNps and 25 h for AgNps). The AuNPs and AgNPs were characterized by various techniques including scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), X-ray diffraction (XRD), dynamic light scattering (DLS) and Fourier transform infrared spectroscopy (FTIR). The nanoparticles were mainly spherical or ellipsoid, while a minority of them was irregular-shaped. Some functional groups, such as C-O groups, amide and carboxyl groups from polypeptides or proteins were involved in metal ion reduction and formation of AuNPs/AgNPs, indicating that proteins might be used as the capping agents on the surface of nanoparticles. The results provided a basis for further research of nanomaterials synthesis and biosynthesis mechanism by the extreme microorganisms.

id #53

Protein Profiling of *Acanthamoeba* spp. by MALDI-TOF MS for specific identification of *Acanthamoeba* genotypes

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Objective : Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI TOF MS) is a recent rapid, accurate and cost effective method for reliable identification and classification of organisms. The aim of our study was to create *Acanthamoeba* spp. specific profiles and compare it with 18S rDNA identification.

Materials and methods: Twenty-five *Acanthamoeba* isolates from clinical and environmental settings were included in this study, and grown monoxenically on non-nutrient agar. All the *Acanthamoeba* isolates were previously genetically characterized targeting 18S rDNA gene. Twelve out of 25 isolates belonged to T4 genotype, 6 belonged to T11 genotype, 4 belonged to T5 genotype, 2 belonged to T3 and 1 belonged to T10 genotype. *E.coli* were separated from *Acanthamoeba* by three methods: heat killing, ultra-violet ray (UV) killing and membrane filtration. Both the trophozoite and cyst stages were used to create the

spectral profile. Reference databases were created in Biotyper 3.0 software as Main Spectrum Profile (MSP) and blind testing of coded samples was done.

Results : Homogenous spectra were obtained with cyst form of *Acanthamoeba* and membrane filtration method was the best for separation of *E. coli* from *Acanthamoeba*. Nine MSPs were generated in Biotyper v 3.0 software, four for T4, two for T5, one each for T3, T10 and T11 genotype. When MALDI TOF MS identification was compared with the 18S rDNA sequencing, the results were concordant except in 2 isolates which were incorrectly identified by MALDI TOF MS. Thus, the sensitivity of MALDI TOF MS was 92%.

Conclusions: MALDI TOF MS is a rapid technique compared to the 18S rDNA sequencing for identification of species and genotype of *Acanthamoeba*: less time is required for sample processing and *Acanthamoeba* species can be identified in hours. This helps in early initiation of the appropriate treatment as drug susceptibility was found to be variable in different species with some species more susceptible while some are resistant.

id #61

Inter- Continental Microbial Transport to New Zealand from Antarctica.

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Antarctica represents one of the last unexplored biomes on earth. We have only recently developed the molecular tools that enable us to gain in-depth insights into the microbial biodiversity that is present there. Antarctica provides a unique opportunity to study transport of microorganisms due to its unparalleled level of isolation and dominance of microbiota in its simple ecosystems. Antarctica is warming rapidly due to climate change, and so is vulnerable to colonisation by invasive species. Aerial transport has the potential to distribute microbes worldwide in short time frames. However, sampling from the air remains challenging due to low biomass, especially in regions such as Antarctica. Current methods for microbial air-sample collection have been shown to be biased and require extended sampling times. A solution to this is to use high volume samplers with liquid collection mediums. However, these devices are not designed for sub-zero conditions which precludes the usage of these superior methods in cold locations. We have developed and tested a method using a liquid cyclone high volume air- sampler which can operate in sub-zero conditions. Our method produces reliable DNA yields for further analysis, greatly expanding our tool kit for understanding life in very cold environments. We have field tested and optimised our method in Antarctica and NZ, achieving improved DNA yields in greatly reduced sampling times. We plan to utilise our method for data collection in Summer 2017 in both Antarctica and NZ. We will use shotgun metagenomics and 16S rRNA gene amplicon data to examine the microbial communities in both locations and elucidate patterns of microbial diversity and transport between Antarctica and NZ.

id #66

Comprehensive analysis of clinical *Burkholderia pseudomallei* isolates demonstrates conservation of unique lipid A structure and invariable TLR4-dependent innate immune activation

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Publish consent withheld

id #67

An Iron Exporter of *Deinococcus radiodurans* involved in Maintenance of Iron Homeostasis

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Iron acquisition by bacteria is well described; however, iron export from bacteria is far from understood even though it is likely to play an important role in iron homeostatic and is essential for intracellular redox cycling. Here, we identified an iron exporter gene (*dr1440*) from *Deinococcus radiodurans*, which is known for its extreme resistance to oxidants and radiation. A *dr1440* mutant showed higher sensitivity to ferrous iron (Fe²⁺), hydrogen peroxide, hypochlorous acid, gamma irradiation and UV irradiation than the wild-type strain. ICP-MS analysis showed that the mutant contains approximately 2 fold than the wild type. The high intracellular concentration of iron caused more serious protein carbonylation as detected using western blot assays. By using quantitative real time PCR, we found that the expression level of *dr1440* was up-regulated under iron and hydrogen peroxide stress, respectively. Deletion of *dr1440* also led to up-regulation of iron acquisition gene such as ABC-type hemin transporter gene (*drb0016*), ABC-type Fe(III)-siderophore transporter gene (*drb0017*), two Fe(II) transporters gene (*dr1219*, *dr1220*), and DNA protection protein (Dps) genes (*dr2263*, *drb0092*), which might contribute to the increase of cellular iron content. The findings suggest that the iron exporter plays an important role in iron homeostasis and stress resistance of *D. radiodurans*.

id #71

Whole genome sequencing of ESBL-producing *E. coli* isolated from patients, farm waste and canals in Thailand

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Publish consent withheld

id #73

Lactovinegar Production by Ethanol Fermentation of Whey Using *Zymomonas mobilis*

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Whey is generated from milk during cheese production. Although it is nutrient rich, the use of whey has been mostly abandoned because of its low preservation potential and unpleasant taste. The development of products with high additional value from whey has been a subject of considerable interest.

We describe the production of milk vinegar (also called; lactovinegar) with high nutrient content using whey to effectively utilize this resource. The use of *Zymomonas mobilis* for baking has been reported to result in change of flavor. Thus, seasoning using this species is expected to change the flavor of vinegar.

Lactovinegar fermentation comprised the following 3 steps: First, lactose and protein in the whey solution were hydrolyzed by *Aspergillus oryzae* enzymes. Next, ethanol was produced by *Z. mobilis*. Finally, acetic acid was produced from ethanol by *Acetobacter pasteurianus*. After pasteurization, lactovinegar was obtained. Ethanol and acetic acid were measured by gas chromatography. The acidity and concentration of calcium and magnesium ions were measured by titration, and 17 amino acids were quantitatively analyzed by high performance liquid chromatography. Antioxidant ability was evaluated by DPPH methods.

Total acidity of the lactovinegar produced was over 40 g/L. The concentrations of calcium and magnesium ions were higher than those of conventional rice vinegar. The amounts of 17 amino acids in lactovinegar were several times higher than those in conventional rice vinegar. In addition, D-Ala which has an anti-aging effect was included to some extent in lactovinegar. These results indicate that lactovinegar is rich in nutrients such as minerals and amino acids derived from whey. Antioxidant ability was approximately 1600 m in terms of Trolox. It was about 7 times higher than those in conventional rice vinegar, and it was nearly equal antioxidant ability of kiwi fruit and strawberry. This is the first report on lactovinegar production from whey using *Z. mobilis*.

id #76

The impact of non-tuberculous mycobacterial infection on the immune response of *Galleria mellonella*.

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Over the past decade, larvae of the Greater Wax Moth *Galleria mellonella* have become widely used as a surrogate host for studying pathogenic microorganisms. The larvae can be housed at 37°C (human body temperature) and have an innate immune system that has much in common with its mammalian equivalent. We have recently established *G. mellonella* as a host for the opportunistic human bacterium *Mycobacterium marinum*, which is frequently used to model *M. tuberculosis*, the causative agent of the lung disease tuberculosis (TB).

To characterise the cellular and humoral immune response of *G. mellonella* larvae to *M. marinum* infection, larvae were injected with 10⁶-10⁷ colony forming units (CFU) of *M. marinum*. At various time points during infection, groups of wax worms were euthanized to determine their bacterial burden, as well as measure the innate immune parameters, including haemocyte determination and subpopulation, haemocyte quantification, phagocytosis, nodulation and melanisation. Our results demonstrate that *M. marinum* is pathogenic to *G. mellonella* wax worms, with bacterial numbers steadily rising until the death of the larvae. Histological examination revealed that immune cells are recruited to the site of infection and form nodules, a process known as nodulation. These results indicate that *G. mellonella* is a viable model host to study the pathogenesis of non-tuberculous mycobacteria.

id #79

Winter fungicide sprays impact the dynamics of vineyard *Botrytis* populations

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Botrytis populations in vineyards often show seasonal differences. Early season populations tend to be less pathogenic than those at harvest. This change probably reflects differences in competitive ability in winter versus summer conditions. Such a seasonal pattern was observed in a Waipara vineyard from 2008 to 2012. The population at flowering was dominated by a *Botrytis cinerea* low pathogenicity haplotype; the population at harvest was dominated by a high pathogenicity haplotype. Since the 2013/2014 season there has been a sudden change in this dynamic, with the high pathogenicity haplotype now dominant at both flowering and harvest. This change in the seasonal dynamic was confirmed using microsatellite analysis. A possible explanation for the change in behaviour of the *Botrytis* populations was a change in management practice, with the addition over the past 2-3 seasons of a winter GelSeal spray for control of vascular pathogens. GelSeal is a triazole fungicide effective against *Botrytis*. The change to the vineyard spray programme may have disrupted the competitive advantage the low-pathogenicity population previously enjoyed over winter.

Comparing ruminal acidosis with acidification of anaerobic digesters to find new therapies and prevention strategies

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Rumens and anaerobic digesters for biogas production share many features. In particular, they have a common problem of high economic importance called ruminal acidosis in veterinary medicine or acidification in the field of environmental biotechnology. In both cases, it means the sudden accumulation of carboxylic acids resulting in a decrease of pH and an inhibition of the microbiota. In the worst case, the consequences for animal and anaerobic digester are death and process failure, respectively. In this review, we compare the reasons and mechanisms of acidosis in both systems, as well as prevention and therapy strategies. In both systems for example, the addition of buffer and neutralizing agents is applied as a therapy. An example for a common prevention method in both systems is the gradual adaptation to a new feed composition. An example for a prevention method that has only been tested in anaerobic digesters is the decrease of feeding intervals while keeping organic loading rate constant. The thereby induced stresses on the microbiota made the digester more stable against acidification by organic overload. In addition to experimental studies, we compare mathematical models of both systems in their ability to represent acidosis scenarios and to predict the influence of prevention and therapy strategies. In particular, we focus on mechanistic models of microbial growth and on novel methods such as dynamic flux balance analyses based on meta-omics data. We see important gaps in the mechanistic modelling of flux distributions between anabolisms, catabolism and maintenance under different stresses, such as pH decrease and carboxylic acid accumulation that occur during acidosis. A better mechanistic description in this regard can benefit both fields of veterinary medicine and environmental biotechnology.

Construction of D-amino acid derivative production system using *Saccharomyces cerevisiae*

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Publish consent withheld

Screening NZ fungi for new antibiotics

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Antibiotic-resistant bacteria are a leading cause of difficult-to-treat infections, especially in patients with weakened natural defences. Several species have emerged that are resistant to multiple antibiotics, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. We are currently screening a large collection of NZ-isolated fungi for new kinds of antibiotics against these organisms. To do this, we grow constitutively bioluminescent strains of target bacteria alongside test fungi. Suppression of luminescence is taken as a possible indication of fungal antibiotic production. Crude extracts are then prepared from active fungi and tested for antibacterial activity. Active components of crude extracts are isolated via thin layer chromatography and bioluminescence-based bioautography. Compounds present in active fractions are identified via MS-NMR. So far, we have screened 238 fungi, identifying 18 that selectively inhibited *S. aureus* luminescence. A further 23 isolates inhibited the luminescence of all target bacteria. Out of 10 crude extracts prepared from these fungi, the most active have been from a common plant pathogenic fungus called *Cercospora Fresen*. These extracts showed a minimum inhibitory concentration of 16 µg/ml against *S. aureus*, but greater than 1 mg/ml against *E. coli* and *P. aeruginosa*. Fractionation and MS-NMR revealed that the active compound was a cercosporin. While the antibacterial properties of cercosporins have already been reported, our data demonstrates that our bioluminescence-based screening method works, and has the potential to discover new kinds of antibiotics in the future. We are currently expanding the screening to include two additional pathogens of clinical importance, *Klebsiella pneumoniae* and *Acinetobacter baumannii*.

Development of a new type of liquor made from whey - selections of *Aspergillus* species and raw materials for malt, and examination of brewing conditions -

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First, we determined combination of koji mold and raw material, which gave high activity of α -galactosidase to hydrolyze lactose in whey. As a result, two strains of *Aspergillus oryzae* with high liquefaction ability and high saccharification ability were selected as koji molds, respectively. Wheat bran was selected as a raw material. High liquefaction koji and high saccharification koji prepared with 150% water content showed the highest α -galactosidase activity. Then, these koji preparations were used for brewing. We brewed using two koji preparations and *Saccharomyces cerevisiae*. These koji preparations were separately added to sterilized and deproteinized whey. After the hydrolysis, koji residues were taken out from whey with filtration and yeast was added for ethanol fermentation. Sampling was carried out at every 24 hours after the addition of yeast, and reducing sugar concentration, ethanol concentration, α -galactosidase, and protease activities were measured. As a result, the ethanol concentration reached 8.5% in the liquefaction koji preparation and 7.6% in the strongly saccharified koji preparation. All the reducing sugars were consumed in both preparations within 10 days. After brewing, sensory test of the products were performed. Based on these analyses, we evaluated the quality of the brewed products as a new type of liquor made from whey.

Characterisation of extracellular vesicles from the enteric pathogen *Citrobacter rodentium*

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Bacteria are masters of adaptation, capable of adapting to environments as diverse and challenging as boiling hot geysers, oil slicks, as well as mammalian hosts. Pathogenic strains of *Escherichia coli* remain a global health issue, with enteropathogenic *E. coli* (EPEC) causing high mortality in the developing world and enterohaemorrhagic *E. coli* (EHEC) causing sporadic outbreaks in the developed world. *Citrobacter rodentium* is a murine enteropathogen which infects mice using the same mode of operation as EPEC and EHEC, allowing many aspects of pathogenesis to be explored. An emerging field in microbiology is the study of Extracellular Vesicles (EVs), which are suggested to play a role in survival and pathogenesis of bacteria. Prior studies have shown EV production by *E. coli*, presenting a new avenue of research into *C. rodentium*. The question remains: what role do EVs play in *C. rodentium* infection, and how do EVs change following evolution?

I am seeking to answer this question by comparing the production of EVs by the ancestral *C. rodentium* strain and an evolved isolate which has undergone subsequent infections of mice for 5 months. Total EV production is measured throughout growth in different environmental conditions, as well as characterising the EV composition. EVs are isolated and purified from samples taken during growth of the bacteria, with EV numbers and size determined using Nanosight, protein concentration measured via BCA assay, and nucleic acids examined using Qubit.

Preliminary data indicates that *C. rodentium* produces a high concentration of vesicles per cell, and that the evolved *C. rodentium* produces EVs with a higher DNA content than the ancestor strain. These results will give us a greater insight into the evolution of bacteria, showing how changes in EVs may contribute to bacterial pathogenesis.

Streptococcus pyogenes nuclease A (SpnA) mediated virulence does not exclusively depend on nuclease activity

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Streptococcus pyogenes, or Group A Streptococcus (GAS), is a human pathogen that causes a wide range of diseases, including pharyngitis, necrotizing fasciitis and toxic shock syndrome. This bacterium produces a large arsenal of virulence factors, including the cell wall-anchored *Streptococcus pyogenes* nuclease A (SpnA), which facilitates immune evasion by degrading the DNA backbone of neutrophil extracellular traps. SpnA consists of a C-terminal endo/exonuclease domain and a N-terminal domain of unknown function. Site-directed mutagenesis of SpnA has been carried out to further define the mechanism of the nuclease. The ability to degrade DNA by these recombinant SpnA mutants were either abolished or reduced when predicted metal-binding and catalytic site residues were mutated. To investigate the role of SpnA in virulence *in vivo*, *Galleria mellonella* (wax moth) larvae were used as an infection model. A GAS *spnA* deletion mutant showed reduced virulence in this model, with the *spnA wt* complementation completely restoring virulence. Interestingly, complementation with the *spnA* catalytic site mutant SpnA H716A only partially restored virulence. Our results outline the critical role of several predicted residues in enzymatic activity and demonstrate that nuclease activity is not exclusively responsible for SpnA-mediated GAS virulence in a *Galleria mellonella* infection model.

Plant-Expressed Pyocins As Weapons To Combat Pathogenic Bacteria

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Pseudomonas aeruginosa is an ubiquitous gram-negative bacterium belonging to Gamma Proteobacteria class, which persists in the environment as well as inside human body. This opportunistic pathogen can cause life-threatening infections in patients with compromised immune system. *P. aeruginosa* is one of the six pathogens causing hospital ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) infections, which readily develop resistance to antibiotics. Recently, the WHO published a list of bacteria for which new antibiotics are urgently needed, and carbapenem-resistant *Pseudomonas aeruginosa* was declared a problem of critical importance. In such context, a new generation of antimicrobial substances is urgently needed.

Bacteriocins are instruments of microbial warfare in competition for ecological niches. *P. aeruginosa* strains rival with each other by secreting various activity spectrum antibacterial proteins called pyocins: deoxyribonucleases, ribonucleases, pore-forming proteins, peptidoglycan synthesis-blocking proteins, lectin-like proteins, and bacteriophage tail-like protein complexes. We attempted to express pyocins of all known types in highly efficient protein synthesis platform – a plant transient gene expression system. Pyocins involved in cell wall synthesis, pore-forming pyocins and lectin-like pyocins were expressed most efficiently reaching 10-50% of total soluble leaf protein. Pyocins S5, PaeM, three lectin-like pyocins (L1, L2 and L3) and one new pyocin, PaeM4, were purified to homogeneity and their antibacterial activity was tested in several assays. Since pyocins tend to be strain specific, we evaluated the spectrum of their activity with a collection of one hundred clinical strains. By using only three pyocins (S5, PaeM and PaeM4), we were able to target as much as 68% of all tested strains, including multidrug resistant isolates. Among the three tested pyocins, PaeM4 targeted the largest number (53%) of clinical isolates.

Plant-produced pyocins reduced *P. aeruginosa* CFU counts in liquid culture assays by several orders of magnitude and efficiently reduced biofilm growth. We further demonstrated the ability of plant-produced pyocins to protect *Galleria mellonella* larvae against lethal *P. aeruginosa* infection. Also, mutation rates by fluctuation analysis were determined for pyocins. We propose that plant-produced pyocins should be considered as a viable alternative to antibiotics for the control of pathogenic *P. aeruginosa*.

Dothistromin, a fungal secondary metabolite produced during primary growth stage, is regulated by chromatin modification.

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Dothistromin is a polyketide virulence factor in Dothistroma needle blight of pines and is structurally similar to a precursor of aflatoxin. It is produced by the fungal pathogen *Dothistroma septosporum* during early exponential growth in culture and at a corresponding stage *in planta* when disease lesions begin to form. Production during primary growth stage is unusual for a secondary metabolite, and a further unusual feature is that genes for dothistromin biosynthesis are dispersed across a chromosome instead of being clustered. Dothistromin biosynthesis was previously shown to be regulated by pathway and global regulator proteins such as AfIR and LaeA. The objective of this investigation was to determine if production of dothistromin is also regulated by chromatin modification in a similar way to other fungal secondary metabolite gene clusters, despite its unusual features. The methylation and acetylation status of histones at dothistromin gene promoters was investigated over a time course using chromatin immunoprecipitation (ChIP)-PCR and the results compared with gene expression analysis in chromatin modification mutants of *D. septosporum*. Our results showed that histone 3 acetylation at lysine 9 (H3K9ac) was associated with higher dothistromin production during early exponential growth whilst histone 3 methylation (H3K9me3 and H3K27me3) was associated with a subsequent decline in the rate of dothistromin production during mid-exponential stage. Dothistromin genes at five loci dispersed across chromosome 12 were co-regulated by alterations in H3K27me3 status. However alterations in H3K9ac/me3 occurred mainly at the centrally-located AfIR pathway regulator gene, providing indirect control of other dispersed genes. These results show that fragmented gene clusters can be coordinately controlled at the chromatin level. We also show that modifications in the timing of secondary metabolism can occur through chromatin-level control. We suggest that the early production of dothistromin is an adaptation to its role as a virulence factor in enabling lesion expansion in pine needles.

Fungal Extracellular Vesicles

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The extracellular vesicles (EVs) produced by bacteria are becoming well characterised for their many biological roles, but the study of fungal EVs has lagged behind that of their bacterial counterparts. We hypothesise that yeasts (*Candida* species) and moulds (*Aspergillus* species) produce EVs, and will be good models to study the biology of EVs produced by fungi. We demonstrate the production of EVs in different culture media and purify them using density gradient centrifugation to characterise their size, morphology, quantity and composition. Nanoparticle tracking analysis is applied to quantify and size EVs; transmission electron microscopy is applied to visualise EV morphology and to estimate sizes; RNA and DNA isolation and fluorometric analyses are applied to measure RNA and DNA concentrations; bicinchoninic acid assays are applied to measure total protein concentration; and SDS-PAGE is applied to compare EV protein banding patterns. We conclude that fungi produce EVs and consider what biological roles they might fulfil.

Accurate live and dead bacterial cell enumeration using flow cytometry for *Staphylococcus aureus*

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Flow cytometry (FCM) is based on the detection of scattered light and fluorescence to quickly and reliably identify particular cell characteristics. However, most FCM cannot precisely control the flow through its interrogation point and hence the volume and concentration of the sample cannot be immediately obtained. The easiest, most reliable and inexpensive way of obtaining absolute counts with FCM is by using reference beads. Recently, a method for absolute count using FCM of the gram negative rod *Escherichia coli* was published with promising applications. Results showed FCM measurements correlated well with plate counts. We have now investigated whether adaptations of this method are required for analysing different bacterial species, by evaluating the accuracy of this method for the analysis of gram positive coccus *Staphylococcus aureus*. We used FCM with reference beads to measure live and dead *S. aureus* bacterial mixtures over the concentration range of 10⁶ to 10⁸ cells/mL and live:dead ratios of 0.1, 0.25, 0.5, 0.75 and 1.0.

S. aureus solutions with differing ratios of live:dead cells were stained with fluorescent dyes SYTO 9 and propidium iodide (PI), which label live and dead cells, respectively. Samples were measured using a LSR II Flow Cytometer (BD Biosciences); using 488 nm excitation with 20 mW power. Both SYTO 9 and PI fluorescence were collected and threshold was set to side scatter. Traditional culture-based plate count was done in parallel to the FCM analysis. The concentration of live bacteria from FCM was compared to that obtained by plate counts. We expect that this method may be extended to a wider concentration range and for studying kill kinetics and other cell characteristics.

Use of Natural Products as Preservatives

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Synthetic preservatives are used in many products, but they are known to associate with allergies and more serious conditions. An alternative approach to prevent spoilage is to use natural products, like plant extracts, as natural preservatives. The aim of this project is to combine plant extracts with antibacterial and antifungal properties together, with and without synthetic preservatives, to determine synergy effects and to reduce or eliminate the use of synthetic preservatives.

We have tested twelve plant extracts against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus brasiliensis* and *Penicillium citrinum* using standardized antibacterial and antifungal susceptibility tests to find minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC). Plant extracts are tested in combination to determine synergy using a checkerboard approach. Our findings suggest candidate plant extracts with good potential to replace or reduce the use of synthetic preservatives in various consumer products.

id #104

Rapid phenotyping of pathogenic and non-pathogenic *E. coli* O26 using Hyperspectral Imaging

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Rapid phenotyping of spoilage and pathogenic microbes is an international research priority that will have a direct impact on the NZ Red Meat industry. For this study *Escherichia coli* (*E. coli*) O26 was selected as an exemplar of high interest to the NZ Red Meat Industry due to its presence in the Top 7 Shiga-toxin producing *E. coli* (STEC) group of serotypes prohibited on beef products imported into the US, such that if it is detected it would significantly disrupt access to their market. *E. coli* O26 can be grouped by the presence or absence of two genes (*stx* and *eae*) without which the bacteria is relatively benign and non-pathogenic, but whose presence creates a potentially lethal pathogenic form. Distinctive spectral shapes and absorbance in the Near Infrared (NIR) region of the electromagnetic spectrum is found to differentiate between pathogenic and non-pathogenic *E. coli* O26 based on their metabolite chemistry. Isolates of *E. coli* O26 from faecal and soil samples and with the virulence profiles of interest (*eae+stx+*, *eae+stx-*, *eae-stx-*) were grown as pure and mixed cultures on modified Rainbow® Agar O157 (mRBA). Hyperspectral images (600-1700nm) were captured at 24 hours of growth, where NIR spectra for each pixel in a high resolution image were processed, analysed and calibrations for classification of virulence groups were built and tested. Preliminary results show classification performance at a low colony density (<100 per plate) with few well separated colonies was best with a rate of 92% correct classifications, while at a higher colony density (<1000 per plate) where colonies were more densely distributed, the classification rate reduced to 70%. Spectral differences in the NIR region were detected between the soil and faecal isolates used in this trial indicating potential application for lineage tracing. The spatial aspect of the hyperspectral image shows promise for further investigation of metabolic changes across the body of a single colony.

id #105

A biosecurity project: The *Ceratocystis fimbriata* species complex, Rapid Ohia Death and what it means for New Zealand.

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First identified in 2015, Rapid Ohia Death (ROD) is the colloquial name for the disease caused by the ascomycete fungus *Ceratocystis fimbriata* that is currently spreading throughout Hawaii. ROD is infecting and killing the Ohia tree (*Metrosideros polymorpha*), the dominant forest tree in Hawaii. This plant pathogen is an important biosecurity concern for New Zealand as 12 of our native tree species are in the *Metrosideros* genus, including the iconic Pohutukawa (*Metrosideros excelsa*) and all of the Rata.

Throughout the world there are numerous strains of *C. fimbriata*, with the most common isolate infecting sweet potato (*Ipomoea batatas*) being found with a global distribution. It is believed this was spread clonally through distribution of sweet potato and it is this strain that is found in New Zealand. However, other host specific strains have been identified that infect a range of species, such as mango trees (*Mangifera indica*) and a variety of others. How these strains are related is poorly understood. Recent molecular research indicates that these different strains are closely related cryptic species, with at least two being described as separate species; *C. platani* and *C. cacaofunesta*. We have sequenced and assembled 11 isolates from Hawaii and other countries to better understand the origin of ROD and how it is related to other strains of *C. fimbriata*. Phylogenetic analysis was undertaken through Bayesian inference of a set of ~3,300 core genes identified using BUSCO. Of the three isolates of ROD, two group together with *C. platani* and an isolate collected from a *Syngonium* sp at a Hawaiian nursery, whereas the third ROD isolate groups separately into a clade with *C. pirilliformis*. This indicates that what is being called ROD is actually two phylogenetically distinct groups. Further investigation may yield insight into the origin of ROD. Given the cryptic nature of the *C. fimbriata* species complex an accurate molecular identification test is necessary. As part of this goal we have developed a PCR-based assay that can positively identify ROD and can distinguish it from the strain of *C. fimbriata* that is already present in New Zealand.

id #107

Identifying Autoantibody Targets as New Biomarkers for Acute Rheumatic Fever using Mass Spectrometry and High Content Protein Arrays

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Acute rheumatic fever (ARF) and associated rheumatic heart disease (RHD) are serious sequelae of Group A Streptococcus (GAS) infection. Rates of ARF/RHD remain unacceptably high in the developing world and in indigenous populations in certain developed countries such as Australia and New Zealand. Specific diagnostic tests for ARF are lacking, with diagnosis instead relying on a set of clinical criteria. This presents a major hurdle in disease control efforts, with an accurate diagnosis requiring a series of assessments over a period of days. Our aim is to identify novel human targets of auto-antibodies in patients with ARF that can serve as biomarkers for diagnosis. Firstly, an optimised western blot protocol has been developed to test for heart reactive antibodies from the sera of ARF patients with carditis. Reactivity of ARF sera was screened in whole heart lysates, as well as aorta and mitral valve lysates. Specific bands detected in ARF sera and not healthy control sera have been subject to

mass spectrometry for identification. Secondly, high content array technology has been applied to identify potential autoantibody targets in an unbiased fashion. Arrays containing 9000 (Protoarray) and 15000 (HuProt Array) human proteins have been screened with ARF patient sera (with and without carditis) and matched healthy controls. Analysis has revealed a panel of proteins that have potential as biomarkers in ARF. Validation of these potential hits in immunoassays with larger patient numbers will be presented as a route to assessing the utility of the proteins in clinical diagnosis of ARF.

id #112

Thermostable and acidic chitinase obtained from *Streptomyces thermodiastaticus*

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Chitin, a β -(1,4)-linked polymer of *N*-acetyl-D-glucosamine, is the second most abundant carbohydrate polymer and can be a source of biomass. Chitinases, which catalyze the hydrolysis of chitin, have been used in the bioconversion of chitin. Many industrial processes operate under extreme pH and at elevated temperatures; thus, the enzyme utilized must function under these process conditions.

We identified the strain *Streptomyces thermodiastaticus* HF 3-3 that produces various thermostable glycolytic enzymes. One of chitinases from *S. thermodiastaticus* was purified and characterized and some of its properties were investigated to evaluate the potential application of the enzyme in the chitin industry. The effect of media components was investigated to optimize chitinase production of *S. thermodiastaticus*. Chitinase with molecular mass of 40 kDa was purified by ion-exchange column chromatography from culture broth. Purified chitinase, designated as chitinase1 (Chi1), was characterized using acid-swollen chitin as the substrate. Chi1 obtained from *S. thermodiastaticus* was active under pH 1.5–6.0, exhibiting maximum activity at pH 5.5; the enzyme was stable in a broad range of pH from 1.5 to 9.0. The enzyme showed a temperature optimum at 65°C. When incubated for 3 h at 65°C, Chi1 retained approximately 100% of its residual activity. Chi1 retained 87% of the original activity in the presence of 15% NaCl. Chi1 activity was inhibited by Ag⁺, Mn²⁺, but other chemicals had no significant effect on the activity. TLC analysis of the enzyme reaction products showed that diacetylchitobiose was mainly produced. Chi1 was found to be more stable in acidic conditions than other *Streptomyces* sp. Chi1 is a unique enzyme because it has high stability under extremely acidic conditions and under a broad range of pH values with a high optimum temperature and thermal stability. Furthermore, Chi1 showed resistance to various chemicals on the enzyme activity. This enzyme will be useful in the industrial bioconversion of chitin.

id #114

Production of fermented beverages made from whey using *Kluyveromyces* sp.

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The aim of this study is to develop new whey beverages brewed by lactose-fermenting yeast, *Kluyveromyces lactis* NBRC 433, *Kluyveromyces marxianus* NBRC 1735, and *Candida kefir* NBRC 8. Whey is a main by-product of the cheese production and it contains lactose, protein, ash, fat, and other bioactive components. The major industrial usage of whey is a dietary supplement of whey protein. However, about 75% of dry weight of whey is lactose, on the other hand, that of protein is 13% and the utilization of lactose from whey is limited. In addition, the common brewing yeast, *Saccharomyces cerevisiae*, cannot consume lactose so that it is difficult to use *S. cerevisiae* for ethanol fermentation from whey. *Kluyveromyces* sp. and *C. kefir*, an anamorph of *K. marxianus*, are the typical lactose-fermenting yeasts. Since these strains isolated from food were used in this study, their usage for brewing whey beverages is acceptable.

Strain *K. marxianus* NBRC 1735 produced ethanol faster than other two strains from standard medium which contains 15 w/v% lactose. The maximum amount of ethanol produced by the strain NBRC 1735 was 10 v/v% for 7 days, whereas *K. lactis* NBRC 433 produced only 6.1 v/v% ethanol for 14 days from the same medium. To increase the fermentation ability of the strain NBRC 433, we made fused yeast between the strain NBRC 433 and *S. cerevisiae* K7 using protoplast fusion method with polyethylene glycol (PEG). In 272 colonies obtained in selection medium, 120 isolates showed blue colony in the standard medium containing X-gal. Among 4 fused strains produced ethanol more than 1.5 times as much as parental strain, NBRC 433, at further screening, fused strain named NY108 produced 9.3 v/v% ethanol from standard medium for 10 days. This result indicated that the fermentation ability of strain NY108 is slightly lower than NBRC 1735, and higher than NBRC 433. The fused yeast strain NY108 which consumes lactose and produces ethanol faster than parental strain was obtained. It is expected to be used for production of fermented beverage made from whey.

id #121

Are Plants the Answer to the Global Antibiotic Resistance Crisis?

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New Zealand has one of the highest incidence rates of *Staphylococcus aureus* infections in the developed world, and as rates of infection and resistance to existing antibiotics continue to increase there is an urgent need for novel *S. aureus* treatments. New Zealand's isolated geography and unique climate have allowed for the evolution of many endemic plant species, which we hypothesise produce novel antimicrobial compounds that can be incorporated into a topical cream treatment for *S. aureus* skin infections.

A panel of twelve unknown plant extracts and placebos were screened for the possibility of antimicrobial activity against a spectrum of pathogenic micro-organisms: *S. aureus*, *Escherichia coli*, *Candida albicans* and *Candida auris*. Broth microdilution assays involving the inoculation of plant extracts with microbes were performed in Müller-Hinton and RPMI-1640 broth for bacteria and yeasts respectively, and the minimum inhibitory concentration (MIC) determined. The contents were also plated onto nutrient agar plates and incubated overnight to find the minimum bactericidal/fungicidal concentrations (MBC/MFC). The tests were repeated for extracts exhibiting activity towards *S. aureus* and *E. coli* against MRSA and ESBL-producing *E. coli* to

investigate whether their antibacterial effects are extended to drug-resistant strains. Extracts were identified with potent activity against drug-sensitive staphylococci and drug resistant staphylococci (MRSA), *E. coli* and drug resistant *E. coli* (ESBLs), *C. albicans* and drug resistant *C. auris*. Antibacterial potency was further assessed by time to kill assays, with some extracts able to kill 99.9% of an inoculum within 10 minutes. These results demonstrate that plant species possess a reservoir of compounds which could provide a valuable source for new antimicrobials.

id #122

***Epichloë* endophyte-associated antagonism towards an abundant Sordariomycete in the rhizosphere microbiome of perennial ryegrass**

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Although they do not colonize roots, foliar *Epichloë* endophytes can alter community composition of the root-associated rhizosphere microbiome of their grass hosts. It has been shown that *Epichloë festucae* var. *lolii* infection is associated with alterations in the rhizosphere microbiome of its host *Lolium perenne*, but the exact nature of such changes remains to be elucidated. To do so, we regenerated perennial ryegrass plantlets from sterilely micropropagated meristems of an *L. perenne* clone (NuiD), and infected some of them with *Epichloë festucae* var. *lolii* strain Lp19. In two experiments, sets of infected and uninfected plantlets were placed in soil freshly collected from a ryegrass pasture. After two months of growth under controlled climatic conditions (the two experiments differed in terms of collection of soil, soil compaction and watering) bacterial and fungal rhizosphere microbiomes of endophyte were analyzed by high-throughput metabarcoding. Both the number and the size of most endophyte infection-associated differences were small compared to differences between the two experiments, and the vast majority of endophyte infection-associated differences occurred only in one experiment or the other. A striking exception was an endophyte infection-associated reduction of the most prominent fungal OTU, highly enriched in the root (27x relative to the soil). Reads mapping to this OTU were of significantly lower abundance in endophyte-infected plants in both experiments (average 35.9±13.6% in uninfected versus 19.2±11.1% in infected plants across all 2 x 6 microbiomes analysed; one-sided *P* of 0.0264 in regression analysis with differences between experiments considered first). A BLASTn search places the OTU amongst the Sordariomycetes, with 74-79% homology, across 95% of its internal transcribed spacer sequence, to reference sequences belonging to the families Magnaporthaceae, Nectriaceae and Ceratocystideaceae. All of these families contain well-known plant pathogens. Under our relatively stress-free conditions there were no striking difference in performance of endophyte-infected and uninfected plants. However it is conceivable that, under field conditions, reducing the abundance of this and similar potentially pathogenic fungal OTUs in the root microbiome could contribute to the enhanced fitness of *E. festucae*-infected *L. perenne*.

id #122a

A new approach for analysing phosphorous-containing compounds in ryegrass (*Lolium perenne*) infected with the fungal endophyte *Epichloë festucae* by ³¹P NMR

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It is well established that infection with the fungal endophyte *Epichloë festucae* var. *lolii* increases the fitness of the perennial ryegrass *Lolium perenne* in New Zealand pastures, but how it does so is only partially understood. Transcriptomics analyses on the well-characterized symbiosis between *E. festucae* var. *lolii* strain Lp19 and ryegrass clone Nui D suggest that in infected plants the symbionts may jointly synthesize phosphonates or phosphinates. These molecules possess carbon-phosphorus (C-P) bonds and comprise a diverse class of natural products with herbicidal, antimicrobial and insecticidal properties. C-P compounds could thus potentially play a role in enhancing the fitness of endophyte-infected plants. However, these compounds, as well as extraction methods to obtain them from plant material remain poorly characterized. ³¹P Nuclear Magnetic Resonance (³¹P NMR) is a sensitive and reliable analytical tool to investigate phosphorous-containing compounds from biological samples. Therefore this technique was used to establish if endophyte-infected perennial ryegrass does contain C-P compounds and if their presence is associated with endophyte infection. Foliar samples were obtained from Lp19-infected and uninfected NuiD plants, grown and harvested as the material used in the abovementioned transcriptomics analyses (tillers from exponentially tillering plants maintained at 15 °C, 12h light/12h dark, 630 μmol x m⁻² x s⁻¹, 70% RH). Ten tillers from two infected plants and two uninfected plants (20 total) were harvested, and freeze-dried samples were extracted with two different methods: solvent mix extraction (Methanol:Chloroform:Water - MCW) and NaOH-EDTA extraction. Both methods recovered various phosphorous compounds from the sample, but the MCW method yielded better results (i.e. a higher number of peaks after ³¹P NMR analysis). Recorded spectra showed that samples contained a variable number and amount of C-P compounds but did not suggest major differences between infected and uninfected plants. This study is the first attempt to characterize C-P compounds in ryegrass plants and we recommend fine-tuning this technique to further investigate the effect of microorganisms on the production of C-P compounds in plant systems.

id #130

Bacteriophage-mediated control of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit plants

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Bacterial plant pathogens, such as *Pseudomonas syringae* pv. actinidiae (Psa), can be responsible for devastating crop losses. Bacteriophages (phages) hold promise as environmentally-friendly biocontrol agents for plant pathogens. However, effective phage application in an orchard or other outdoor setting still remains a challenge. In 2011/2012 a collection of over 200 phages with activity against Psa were isolated from New Zealand soil and sewage samples. Two phages were selected from this collection based on their host range, and suitability for biocontrol. We are now testing the ability of these phages to control Psa on kiwifruit plants. We developed a plantlet based assay to assess the effectiveness of phages under controlled conditions. Kiwifruit plantlets were grown on agar growth medium from tissue culture. The plantlets were flooded with Psa culture at approximately three months old. This method of inoculation ensured even coverage of the plantlets. Symptoms, such as leaf lesions, were visible after eight days. Phages were applied to the plantlets by spraying. This technique was chosen as it resembles what would be used in an orchard setting. Different timings and concentrations of phage were tested. Leaf discs were taken from the plantlets and sampled for Psa and phage numbers. Establishing this plantlet based assay allowed us to test different factors in order to better understand the action of phages on plant surfaces. The impact of phage application on Psa numbers and symptom development was determined. Both phages were still detected on leaf surfaces two weeks after application but at reduced levels compared to application. We are continuing to optimise the assay conditions in order to improve the phage stability and effectiveness.

id #134

Larger legume hosts have a greater diversity of symbiotic nitrogen-fixing bacteria (rhizobia)

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A major goal in microbial ecology is to understand the environmental factors that structure bacterial communities across space and time. Previous studies have shown that the identity of the plant species is important in structuring soil microbe communities in the rhizosphere, but traits of individual plant hosts may also have an effect. For microbes with symbiotic relationships with plants, an important host characteristic which may influence their communities is size or age. Using tree diameter size as a proxy for age, we quantified the diversity of rhizobia that associate with an endemic legume, *Acacia acuminata*, of variable size across a climate gradient in Southwest Australia. We examined the 16S diversity (V1 hypervariable region) of rhizobia at the taxonomic level and at higher sequence level diversity within taxonomic groups. We identified 3 major taxonomic clades that associated with *Acacia*, *Bradyrhizobiaceae*, *Rhizobiaceae*, and *Burkholderiaceae*. Within these groups, we found extensive genetic variability, especially within *Bradyrhizobiaceae*. Using binomial multivariate statistical models that controlled for other factors that could potentially influence tree size and rhizobia communities (climate and local soil characteristics), we determined that soil sampled at the base of larger *Acacia* trees had a higher probability of containing a greater number of taxonomic clades. Furthermore, our models also showed that larger *Acacia* trees had a greater probability of containing more genetic variants within each taxonomic clade. These results could be driven by several important non-mutually exclusive ecological processes. Larger legume hosts may house a greater rhizobia diversity because they create more habitat spaces that accepts a greater immigration of all rhizobia diversity (island biogeography hypothesis) or larger hosts have a greater diversity because they have more micro-habitats within the same soil space (niche diversity hypothesis). Larger and thus generally older hosts may also have had more time to influence the soil to promote in-situ diversity (diversity accumulation hypothesis).

id #135

Unusual HCV multiple genotype 1b,2,4 mixed infection in the patient on malignant melanoma : Laboratory techniques.

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[Background]:

According to WHO Global Hepatitis Report describes in 2017, Hepatitis C virus (HCV) is a major pathogens of liver disease. Direct-acting antivirals (DAAs) are effective in treatment of different hepatitis C virus genotypes.

[Objectives]:

Molecular techniques are the most common diagnostic tools for HCV genotypes

The epidemiological trends of HCV genotypes obtained in this case can serve as important references for physicians in clinical diagnosis.

[Methods]:

Sample was collected from a rare case of HCV-infected patient who combined with malignant melanoma. HCV genotyping was analyzed by two laboratory methods. Using 5'UTR & Core region sequencing and Abbott Real-Time HCV genotype II.

[Results]:

We survey the genotype of HCV from patients (n=1392) in our laboratory, the results showed that one patient (0.07%) was infected with mixed-genotype 1b,2 and 4.Ct value obtained by Real-Time HCV genotype II and showed a rare mixed-genotype in this case : genotype 1b(Ct value:19.48), genotype 2(Ct value:18.18) and genotype 4(Ct value:18.22). In order to reconfirm the accuracy of performance, genotype 1b,2 and 4

were also identified by sequencing method.

[Conclusions]:

In this case, the co-infections by three HCV genotypes was detected and never seen before. Although HCV mixed-genotype was detected in this case by both real time PCR and sequencing method, the minor sequence peak was noted in genotype 4.

Comparison of sequencing and Real-Time HCV genotype II assay, we summarize three reasons in this case. First, The Abbott Real Time HCV Genotype II assay uses four sets of PCR primers, these primers are designed to amplify the 5' untranslated region (UTR) and non structural 5b (NS5b) region of the HCV genome. However the sequencing method amplify the sequence on 5' untranslated region (UTR) and core region. Therefore, the different result in different HCV sequence. Second, the cross-

reaction rate between genotype 1 and 4 was considered to occur in about 1.9%. Third, the higher sensitivity of genotype 4 using sequencing assay than real time PCR kit.

Although the PCR/Sequencing technique presently used in laboratories is highly accurate, its complicated procedure and multiple steps make the analysis time-consuming. In addition, the multiplex real-time PCR technique was determined to decrease the human error rate and improve the test quality as well as resultant satisfaction.

id #137

Improvement of Abbott Real Time PCR II with a new procedure in Chi-Mei Medical center

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Background: The genotyping of the hepatitis C virus (HCV) plays an important role in the treatment of HCV because genotype determination has recently been incorporated into the treatment guidelines for HCV infections. Although direct sequencing is a gold standard for HCV genotyping, this approach is time consuming and not suitable for routine use in our clinical laboratories. We conduct a Abbott RealTime HCV Genotype II assay (GT II). The assay amplifies the HCV genome to determine the genotype in a single step by targeting the 5 untranslated region (5'UTR) for genotypes 2a, 2b, 3, 4, 5 and 6 and the NS5B region for genotypes 1a and 1b. However, the assay has reportedly misclassified genotype 1 as genotype 6 on occasion. Thus, we develop a procedure to have a better service for the clinical need.

Methods: We develop a new procedure to classify HCV genotype in our laboratory. As the Fig.1. Using three assays: Abbott RealTime HCV Genotype II (GT II) test, Abbott HCV Genotype Plus RUO (Plus) assay and DNA sequencing in 5'UTR/core region.

Results: We collected an annual data to see the distribution for HCV genotype between DNA sequencing (2015/Jan~2015/Dec) and the new procedure (2016/Jan~2016/Dec). Sequencing results for 3.7%, 29.9%, 46.8%, 0.5% and 19.1% of gt1a, gt1b, gt2, gt3 and gt6, New Procedure results for 3.3%, 38.3%, 39.6%, 0.2%, 18.2% and 0.2% of gt1a, gt1b, gt2, gt3, gt6 and mix type.

Conclusion: HCV genotyping remains challenging for genotyping and subtype assignment by commercial assays but also for sequencing, and no perfect method currently exists. We use the new procedure to provide better service for the clinical need.

id #141

Does taxonomy matter? Investigating the functional metabolic redundancy within microbial communities across (geo)thermal gradients

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Microorganisms often share similar functional metabolic characteristics in spite of evolutionary distance. This functional redundancy has recently been described in some ecosystems (e.g. the human gut microbiome), where despite the large variations in microbial community compositions, overall community metabolic profile remained similar between individuals. However, the underlying mechanisms of redundancy (i.e. what metabolic functions are redundant in a community and how are they maintained?) and the limits to this (i.e. how dissimilar can communities be while still exhibiting similar metabolic processes?) remains poorly understood. To address these questions, we use a metagenomics approach to examine the relationship between taxonomic and functional metabolic diversity across a (geo)thermal gradient.

Samples were collected from two geothermal springs in the Taupō Volcanic Zone, New Zealand, that vary thermally ($\pm 50^\circ\text{C}$) through time (via discrete temperature cycles) or space (via an outflow stream). Shotgun metagenomic sequencing was performed on 10 aqueous and sediment samples using Illumina HiSeq. Taxonomic classification has identified temperature-dependent community structures, corroborating previous amplicon-based community surveys from the same springs. Metagenomic assembly was performed using MEGAHIT and metaSPAdes with the NeSI Pan cluster, and analysed with MG-RAST and IMG/ERM systems. The *de novo* co-assembly successfully produced long contigs resembling genome segments from known species (e.g. *Chloroflexus auranticus* and *Caldilinea aerophila*), as well as contigs from novel uncultivated species which are only distantly related to described taxa.

Microbiology has often relied on identifying ecosystem function through a determination of the microbial species present in a community using taxonomic associations. If functional redundancy is more prevalent than previously believed, the conventional orthodoxy of how we define a microbial community may require reevaluation. This research will explore the limits of functional redundancy and, in doing so, prepare a framework for describing community assemblages through metabolic capability. The results from this study are anticipated to affect how we model and value microbial communities in the future, and their contribution to ecosystem services.

id #159

Biocide options to control the transmission of pathogens in our environment

Shilpa Saseendran Nair, Simon Swift, Siouxsie Wiles

Antibiotic-resistant pathogens are a growing concern to public health worldwide. Research data suggests that contaminated fomites are a risk factor for nosocomial infections. The inanimate hospital environment (e.g. surfaces and medical equipment) can become contaminated with nosocomial pathogens such as *Clostridium difficile*, Enterococci, *Escherichia coli*, and *Staphylococcus aureus*, which may also be resistant to antibiotics. In this project, we aim to reduce transmission of infectious agents by applying environmentally-friendly, non-toxic biocides to surfaces. We have established methodologies to quantify the antibacterial activity of surfaces in wet and dry conditions using *S. aureus* and *E. coli*, based on the industry standard JIS Z 2801 method. We have tested these methodologies using plastic and glass protected with a commercially available siloxane anchored quaternary ammonium-based biocide. Our results show that in wet fomite conditions antimicrobial surfaces can

reduce the burden of *S. aureus* below our limits of detection within 10 minutes and of *E. coli* in 30 minutes. In dry fomite conditions, both *E. coli* and *S. aureus* were reduced below our limits of detection within 10 minutes. Now that we have established these assays, future studies will investigate the activity of novel biocides against antibiotic-resistant strains of *S. aureus* and *E. coli* and against endospores of *Bacillus cereus*. The testing has been extended to establish whether a commercially available biocide is fit for purpose by testing the microbial burden of nine frequently touched areas of our laboratory before and after application of the biocide. The activity of the antimicrobial surface in laboratory based test conditions supports the extension of testing to real world hospital applications.

id #170

***in vitro* Characterization of Furazolidone Resistant *Escherichia coli* K-12 Isolates to Unveil its Mode of Action**

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Furazolidone (FZ) is a 5-nitrofurantoin-derived antimicrobial agent which was developed in the late 1940s. This drug is used to treat bacterial diarrhoea, giardiasis and is sometimes included as a component in *Helicobacter pylori* treatment. FZ and other nitrofurantoin antibacterial agents are effective in killing antibiotic-resistant Gram-negative bacteria against which there is a small number of available antibiotics, such as carbapenem-resistant and ESBL-producing enterobacteria. In the model organism *Escherichia coli*, it is widely known that FZ is a prodrug, and requires reductive activation by nitroreductases NfsA and/or NfsB which have redundant reduction activities on this drug. An intermediate in the reduction pathway of FZ, a reactive hydroxylamino intermediate, is thought to be the active form of FZ, causing DNA damage and interfering in protein synthesis, resulting in bacterial cell death. We set out to investigate the FZ activation and mechanism of action in the light of surprising scarcity of molecular studies devoted to this antibacterial agent. Our preliminary results showed that the minimum inhibitory concentration for FZ in the double nitroreductase knock-out ($\Delta nfsA \Delta nfsB$) *E. coli* K-12 was quite low, 16 $\mu\text{g/ml}$, suggesting the presence of other activating enzymes in *E. coli* and/or antibacterial activity of the unreduced form of FZ. To identify targets or additional activating enzymes in the absence of nitroreductases NfsA and NfsB, we selected for mutants of $\Delta nfsA \Delta nfsB$ *E. coli* K-12 strain that have become resistant to FZ concentration that kills the parent. Fifteen independently selected mutants were isolated; all having an MIC for FZ of 20 $\mu\text{g/ml}$. We analysed ten of these mutations by combining whole genome sequencing and a toolset of bioinformatics. The ten analysed mutants harbour each a different loss-of-function mutation in the same gene, *ahpF*, which encodes for the peroxiredoxin reductase subunit AhpF in the antioxidant alkyl hydroperoxide reductase AhpCF system (in addition to existing $\Delta nfsA \Delta nfsB$ mutations). We propose that AhpF may play a role in the activation of FZ besides the two well studied enzymes NfsA and NfsB. Further genetic and biochemical assays are required to shed more light on the role of AhpF in the mode of action of furazolidone.

id #171

Screening of New Zealand fungi for the discovery of novel antimicrobial compounds.

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Antibiotic resistance is an important issue, and with very few new antibiotics being developed, the discovery of new antibacterial drugs is of critical importance. Of equal importance, is the discovery of compounds which are able to enhance the activity of existing antibiotics. One way in which this can be achieved is by resensitising antibiotic resistance bacteria by circumventing resistance mechanisms. Resensitising agents can then be used in combination with existing antibiotics to restore their activity against problematic pathogens.

Due to the country's geographical isolation, many of New Zealand's fungi are unique with around half of the described indigenous species found nowhere else (1). NZ fungi, in particular, have not been extensively characterized making them a valuable and largely untapped resource, particularly for novel antibiotics and resensitising agents. Fungi are important sources of biodiversity because of their ability to produce numerous secondary metabolites making them ideal for screening for antibiotic compounds.

Fungal isolates are acquired by punch biopsies and placed on an agar plate which is then overlaid by bacteria. Zones of no bacterial growth surrounding individual fungal isolates indicate the production of antibiotic compounds. This method allows for up to six fungal isolates to be screened at once and for the use of a mixture of sensitive and resistant bacteria.

Resensitising agents will be identified by co-culturing methicillin resistant *Staphylococcus aureus* and fungal isolates both in the presence and absence of methicillin. Clearance of bacteria only in the presence of methicillin will indicate the production of resensitising compounds by the fungal isolates, while bacterial death on both plates indicates the production of an antibacterial compound. Once either an antibiotic or resensitising compound has been discovered it will then be extracted and identified.

id #174

Occurrence of spore forming bacteria in cattle feed – How clean is their food?

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It's a big challenge to control bacterial contamination of the food chain and involves huge investments. However, there is a bigger challenge to overcome the food safety with respect to spore forming bacteria. Spore forming bacteria are of major concern as these are common causes of food spoilage and the spores are resistant to heat and various disinfectants. Spores of *Clostridium* and *Bacillus* species are ubiquitous and both the vegetative as well as spore forms can contaminate raw milk via sources along the whole dairy food chain e.g., water, soil, faeces, milking equipment etc. Feed type and quality have been implicated as the major primary source of spore forming bacteria in bulk tanker milk. Raw milk becomes contaminated through herd consumption of poor quality feed e.g. silage, straw, hay etc., followed by the survival of spores in the gastrointestinal tract

resulting in contaminated faeces. Subsequent faecal contamination of teats and udder surfaces may result in contamination of raw milk, particularly if good hygienic practice is not followed. Therefore, it's imperative to investigate the main sources of contamination into our food. Our present study is aimed at surveying and characterising spore forming bacteria from different feeds from 4 different farms over two seasons. We investigated the types of spore formers present in these samples using Sheep Blood agar, Shahidi- Ferguson Perfringens (SFP) with egg yolk agar media and 16s rDNA PCR. Our data show a variety of aerobic and anaerobic species present in different feed tested, some being food spoilage and food borne pathogens. However, broader analysis and collection of more on-farm and dairy processing samples needs to be carried out to better understand the food spoilage risk and potential sources of contamination.

id #178

Can Bacterial Virulence Evolve in Response to Protozoan Predation?

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The evolution of bacterial virulence is often considered in the context of multicellular hosts. However, many of the qualities specific to bacterial pathogens such as biofilm formation, toxin secretion systems and intracellular growth strategies can evolve outside of the context of plants or animals (1). Protozoan predators are a widespread phenomenon in diverse environments (2). These single-celled eukaryotes are capable of decreasing bacterial populations nearly 60% per day in soil and have a key role in bacterial adaptation (3). It has been hypothesised that the development of bacterial pathogenicity can emerge in response to protozoan predation. This hypothesis suggests that the evolution of anti-predatory traits as defence against protozoan predators may lead to the emergence of infection in multicellular eukaryotic hosts (4).

Our study investigates the degree to which a common soil based protozoan predator can affect the evolution of pathogenic characteristics in non-pathogenic bacteria. In order to do this, we are using a long-term experimental evolution approach. Parallel populations of *Pseudomonas fluorescens* SBW25 were evolved for many generations on a rich solid media in the presence of a protozoan predator, *Naegleria gruberi*. Experimental evolution allows us to do this in many parallel lines and importantly, to preserve a permanent collection of the co-evolved bacterial and protozoan populations over time for further studies (5). This methodology will allow us to sequence the bacterial populations to determine what genetic changes have allowed the bacteria to adapt to predation. We anticipate that these mutations are anti-predatory behaviours that bacteria establish to escape or damage protozoan predators.

To date, our preliminary experiments have revealed bacteria with colony morphology changes indicative of genetic changes in our evolved prey populations after as little as 20 days of co-evolution. Intriguingly, some of these colonies are similar to the wrinkly spreader+ phenotypes observed in mutants that are capable of colonising the air-liquid interface in standing liquid cultures (6,7). Future work, will further characterise the mutations, the degree to which these mutations promote escape or attack strategies in response to the predators and determine whether these evolved traits contribute to virulence against multicellular hosts.

id #179

COLONIZATION OF 5 DIFFERENT *LISTERIA MONOCYTOGENES* STRAINS ON HYDROPONICALLY GROWN LETTUCE

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Listeria monocytogenes is commonly associated with fresh-produce related food borne outbreaks. Most previous studies about the survival of *L. monocytogenes* on lettuce leaves investigated leaves detached from their parent plant. In this study, the colonization of 5 different strains of *L. monocytogenes* on a hydroponically grown lettuce was investigated after 24 and 48 hours. *L. monocytogenes* strains; O8A05, O8A06 (isolated from coleslaw), O8A07 (isolated from cabbage), O8A08 and O8A10 (isolated from a coleslaw producing facility) were used. An initial inoculum of 6 log CFU/leaf of each *L. monocytogenes* strain was inoculated on the upper surface of the lettuce leaf, at room temperature and dried for 3 hours. Drying resulted in a decrease in all strains to 2-3 log CFU/leaf. Strains O8A05, O8A06, O8A07 and O8A10 decreased to less than 1 log after 24 hours, however *L. monocytogenes* O8A08 decreased from 6 log CFU/leaf to 2 log CFU/leaf after 24 hours and had not decreased further after 48 hours. This indicates that the number of *L. monocytogenes* surviving on a hydroponic lettuce leaf surface under the conditions used in this trial reduce with time and the amount of reduction is strain dependent.

id #181

Cyanide Production by *Chromobacterium piscinae* Shields It from *Bdellovibrio bacteriovorus* HD100 Predation

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Predation of *Chromobacterium piscinae* by *Bdellovibrio bacteriovorus* HD100 was inhibited in dilute nutrient broth (DNB) but not in HEPES. Experiments showed the effector responsible was present in the media, as cell-free supernatants retained the ability to inhibit predation, and that it was not toxic to *B. bacteriovorus*. Violacein, a bisindole, secondary metabolite produced by *C. piscinae*, however, was not responsible. Further characterization of the *C. piscinae* found this strain produces sufficient concentrations of cyanide (202 µM) when grown in DNB to inhibit the predatory activity of *B. bacteriovorus*, but in HEPES the cyanide concentrations were negligible (19 µM). The antagonistic role of cyanide was further confirmed as the addition of hydroxocobalamin, which chelates cyanide, allowed predation to proceed. The activity of cyanide against *B. bacteriovorus* was found to be two-fold, depending on the life-cycle stage of this predator. For the attack-phase predatory cells, it caused them to lose motility and tumble, while, for intraperiplasmic predators, development and lysis of the prey cell was halted. These findings suggest cyanogenesis in nature may be employed by the bacterial strains that produce this compound to prevent and reduce their predation by *B. bacteriovorus*.

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id #183

A Comparative Genomics approach to identify secondary metabolite gene clusters that may underlie novel bioactivity in *Epichloë* endophytes.

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Epichloë fungi form symbiotic associations with their host plants, including perennial ryegrass which is the major pasture grass in New Zealand. These endophytes provide resistance to both biotic and abiotic stresses. They offer bioactivity against insects and different *Epichloë* strains show variation in their bioactivity. This bioactivity is attributed to secondary metabolites produced by the endophytes, normally encoded in gene clusters. There are four main classes of secondary metabolites produced by *Epichloë*; Ergot alkaloids, lolines, indole diterpenes and peramine. All of these classes of alkaloids have been demonstrated to provide protection from insect pests, but some, including the ergot alkaloid ergovaline and the indole diterpenes Lolitrem B, are also mammalian toxins. Research to identify strains that provide protection to forage grasses against insect pests, whilst being non-toxic to grazing animals, has identified that some strains have bioactivity that cannot be attributed to the 4 main alkaloid classes. Genome sequencing of *Epichloë* has identified many other potential secondary metabolite pathways that may underlie this novel bioactivity. We have therefore used a comparative genomics approach to look for unique sequences which may be linked to novel bioactivity in different *Epichloë* strains. We have targeted, in the first instance, non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) genes that are commonly found in fungal secondary metabolite pathways. We compared the genomes of 6 different *Epichloë* strains and have identified a number of candidate secondary metabolite genes that are unique to certain strains and might contribute to observed novel bioactivity.

id #184

The ecology and persistence of thermophilic soil microorganisms through trace-gas oxidation in Tongariro National Park and White Island

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Microbial populations are subjected to extreme environmental perturbations (e.g. seasonal weather variations, nutrient limitation, eruption events) in alpine and volcanic environments. We propose that microbial populations survive these challenges in various states of dormancy, achieved through persistence mechanisms, such as trace-gas oxidation of molecular hydrogen (H₂) and carbon monoxide (CO) as energy sources. The oxidation of trace-gases can provide microorganisms the maintenance energy needed for dormancy in sub-optimal conditions, and may explain the persistence of thermophilic species in extreme soil environments.

To test this hypothesis, we collected soil and gas samples from 7 sites in Tongariro National Park, White Island, and Kaimanawa Forest Park, at surface level and 10 cm depth. Soils were incubated at 4 °C with 100 ppm H₂, 100 ppm CH₄ and 10 ppm CO. Gas chromatography was used to measure temporal trace-gas oxidation in the resident microbial communities. Soil gas concentrations and select physicochemistry were also measured *in situ*, and community diversity was determined by 16S rRNA gene amplicon sequencing. Results from these analyses will be presented, along with community profiles, trace-gas consumption rates, and biogeographical patterns across the range of landscapes sampled.

Previous studies of extreme environments, such as Antarctic dry valleys, hot deserts, and geothermal springs reported that these areas harbour a surprising amount of biodiversity. Recent findings also indicate that thermophiles can persist by scavenging trace-gas from the atmosphere, thereby demonstrating metabolic flexibility and contribution to global biogeochemical cycles. Our study will expand this novel finding to include other extreme environments on the planet, and in doing so, disseminate the survival strategies undertaken by these diverse and resilient microorganisms.

id #185

Next-generation sequencing reveals the seasonal variation of psychrotrophic bacteria in New Zealand raw milk

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The psychrotrophic bacterial contamination of raw milk is one of the key factors determining the quality of processed dairy products. We used next generation sequencing technology to investigate the effect of seasonal variation on the diversity of psychrotrophic bacteria in raw milk collected from six different major milking regions in New Zealand during four different seasons. After storing raw milk at 7°C for 5 days, genomic DNA was extracted and subjected to 16S rDNA high-throughput sequencing. The results showed that *Pseudomonas* was the most dominant genus in 23 milk samples out of a total of 24 samples collected during four seasons. The sub-dominant genera varied in milk samples with different seasons:

Enterobacteriaceae in autumn, Enterobacteriaceae and Lactococcus in winter, Lactococcus in spring, and Acinetobacteria in summer. The results showed a seasonal variation in the microbial composition of raw milk. Factors contributing to these differences could be the use of different feed and temperature variations. This is the first report of the use of a high-throughput DNA technique to analyze the seasonal microbiota of New Zealand raw milk. It is known that seasonal variations in milk occur (e.g. fat content and flavour). This work shows that microbial variation also occurs with different seasons which can influence the spoilage potential in final dairy products.

id #191

The Bacterial Predator *Bdellovibrio bacteriovorus* is Very Sensitive to Detergents

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Publish consent withheld

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id #192

Bacterial Predation Reduces Conjugal Transfer of DNA Between Bacteria

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Publish consent withheld

id #196

Did modern T-DNA evolve from a U-DNA intermediate?

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The extreme conservation of DNA makes it difficult to trace the deep evolution of this fundamental molecule. The precursors of DNA are produced from the constituent RNA precursors through ribonucleotide reduction, leading to the logical conclusion that DNA evolved after RNA. Thymine (T) is produced by further processing of deoxyuracil (dU), which suggests that the evolution of modern DNA might have been via a uracil-containing intermediate form of DNA. We therefore propose that DNA evolution proceeded in the following order: RNA → U-DNA → T-DNA. We have previously argued that the transition from U-DNA to T-DNA was driven by cytosine deamination, which would lead to mutagenic generation of U in the genome via C→U deamination events. However, a transition to T is an indirect fix to this problem; it enables identification of mutagenic U:G pairs arising from cytosine deamination, but it would not enable repair. Thus T does not deal with the issue of cytosine deamination itself. We have proposed that the U → T transition may have been driven by inefficient repair of cytosine deamination¹. We are undertaking experiments to establish whether a uracil-repair enzyme that targeted all uracils in the genome, irrespective of whether or not these are correctly incorporated, drove the evolution of thymine. We will describe our model in detail and explain how we are using microbial experimental evolution techniques to examine the drivers for the U to T transition. This project therefore aims to test the plausibility of a hypothetical U-DNA intermediate, and to assess the plausibility for a key step in the evolutionary transition from RNA to DNA.

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id #198

Efficacy of antibiotic and corticosteroid treatment for chronic rhinosinusitis

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Chronic rhinosinusitis (CRS) is a morbid condition of the paranasal sinuses which severely impairs patients' quality of life. Symptoms of this inflammatory disease include nasal obstruction or discharge, facial pain or pressure, loss of smell and fatigue. In standard practice, CRS is initially treated with a combination of systemic or topical corticosteroids and antibiotics, which may go on for years. While various microorganisms have been implicated in CRS, evidence for the efficacy of antibiotics in its treatment is scarce and several clinical guidelines do not routinely recommend their use. Inappropriate or excessive

antibiotic treatment may alter the structure of the natural commensal community, making the sino-nasal mucosa more susceptible to colonisation by pathogenic microorganisms. Additionally, it contributes to development of antibiotic resistance.

The main objective of this study is to investigate the effectiveness of the current medical management for CRS by determining the effects of antibiotics and corticosteroids on the sinus microbiome, host inflammation and clinical outcome in CRS. In order to achieve this goal we aim to establish a unilateral inflammatory sinus model in rabbits that accurately reflects the chronic nature of CRS. This animal model will be applied to determine the effects of commonly prescribed antibiotics and corticosteroids on the microbiome, host inflammatory response and disease progression. Additionally, we plan to investigate the effects of antibiotics and corticosteroids on the sinus microbiome of human CRS patients by analysing microbiome and clinical data sets for over 200 patients and relating these to an accurate database of the medications prescribed to each of these patients.

Ultimately, the findings of this work will help in developing a better understanding of the medical management of CRS. Implementation of better treatment strategies will improve the quality of life of CRS patients.

id #203

Exploring the origin of DNA through synthetic biology.

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The scope of synthetic biology has come to include minimal genome and protocell engineering, emphasising the relevance of this field to evolution and origin of life research. Our group is using a synthetic biology approach to investigate the evolution of DNA genomes. We are creating a novel essential metabolic pathway for the synthesis deoxyribonucleotides *in vivo*, consequently generating a cell with a metabolism which does not have a known counterpart in nature. Deoxyribonucleotide metabolism of modern biology relies on ribonucleotide reduction as the sole *de novo* route for the synthesis of deoxyribonucleotides *in vivo*. Chemical complexity of this reaction suggests that transition to DNA genomes may have occurred relatively late, possibly after the primary lineages of modern life began to diverge. However, interdomain horizontal gene transfer obscures the evolutionary history of ribonucleotide reductases (RNRs). An alternative, chemically simpler, pathway for the synthesis of deoxyribonucleotides has been suggested where production of deoxyribose is catalysed by deoxyriboaldolase (DERA). This pathway naturally runs in the catabolic direction. Our goal is to establish the operation of the DERA pathway in the synthetic direction *in vivo*, aiming for complete functional replacement of ribonucleotide reduction. If achieved, this will be the only free living modern organism to produce its deoxyribonucleotides *de novo* without RNRs, reinforcing the plausibility of an earlier transition to DNA genomes by simpler chemistry. Our group has generated knockouts of all RNRs in *Escherichia coli*, abolishing ribonucleotide reduction activity and creating strains completely dependent on deoxyribonucleosides in the growth medium. We are screening for conditions where the DERA pathway reverts the cells to being able to synthesise deoxyribonucleotides in the absence of RNRs. This research highlights the value of synthetic biology for experimentally testing hypotheses on the origin of life.

id #205

Development of vanillin producing Baker's yeast

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Vanillin (3-methoxy-4-hydroxybenzaldehyde) is one of the world's most important and frequently used flavour components in the foods, beverages and cosmetic industries with a global market of 180 million dollars. Natural vanillin is extracted from the seedpods of the plant *Vanilla planifolia* but this takes less than 1% in total and most of the vanillin on the market is chemically synthesised. Current studies on the genomics and synthetic biology have made it possible to bio-synthesize vanillin with recombinant approaches. Hansen *et al* (2009) constructed recombinant fission yeast (*Schizosaccharomyces pombe*) and Baker's yeast (*Saccharomyces cerevisiae*) constructs by integration of an engineered gene cluster which encodes a 3-dehydroshikimate dehydratase of *Podospora pauciseta*, an aromatic carboxylic acid reductase (ACAR) of *Nocardia*, an O-methyltransferase of *Homo sapiens* and a phosphopantetheinyl transferase of *Corynebacterium glutamicum* (the last gene is required only for *S. cerevisiae*) and successfully produced vanillin from glucose. Gallage, *et al* (2014) found that a single enzyme, vanillin synthase (VpVAN), from *V. planifolia* catalyses the conversion of ferulic acid and its glucoside into vanillin and its glucoside, respectively *in vitro* and *in vivo* (in *S. cerevisiae*).

For our project, we are targeting at developing recombinant vanillin-producing yeast for the production of vanillin by industrial-scale fermentation using vanillic acid or ferulic acid as the substrates. Two gene clusters, of which cluster A (on plasmid pIRL-72) contains Gen^R, opACAR and OpPPTcg-1 genes and cluster B (on plasmid pIRL-75), contains Gen^R and VpScDspVAN genes, respectively were designed with the aid of the synthetic biology tool GenoCAD and synthesized commercially. After transformation into haploid and diploid *S. cerevisiae* strains, our preliminary screening with a TLC method showed that at a 10 ml cultivation scale, among five haploid transformants transformed with plasmid pIRL-75, three transformants h75-1, h75-3, h75-5 converted ferulic acid to vanillin and among 6 diploid transformants transformed with plasmid pIRL-75, three transformants d75-1, d75-2, d75-5 converted ferulic acid to vanillin. The TLC method also showed the only haploid transformant transformed with plasmid pIRL-72, h72-a, converted vanillic acid to vanillin. These positive candidates will be further identified/confirmed by HPLC before any scale up work with our bioreactors.

id #206

Caged infections. Animal work in a PC3

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Microorganisms are classified into four different risk groups depending on the pathogenicity, host range, mode of transmission, and treatment options available. Four levels of physical containment (PC) are specified for laboratories working with each of these risk groups; for example risk group 2 microbes must be handled in a PC2 or higher laboratory. The bacterium *Mycobacterium tuberculosis* is designated as a risk group 3 microorganism, as it can cause lethal disease in humans, is airborne and presents a significant risk to laboratory workers. New Zealand has many PC1 and PC2 laboratories, and a handful of PC3 laboratories. The PC3 facility in the University of Auckland has been operating since 2012 and is New Zealand's first PC3 laboratory capable of infecting, housing, and monitoring of animals infected with risk group 3 microorganisms. The facility has developed protocols for the monitoring and housing of animals infected with *M. tuberculosis*.

id #214

Evaluation of strains, carriers and temperatures to develop a spray drying process for *Rhizobium leguminosarum* microencapsulation

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leguminosarum bv. trifolii has been applied as a clover seed inoculant over wide areas in New Zealand, primarily in coated seed formulations. Rhizobia survive well in the moist peat media they are grown in but die rapidly after inoculation onto seed due to factors such as desiccation, temperature stress or toxins produced by seeds. Alternative formulations that enhance survival on seed are strongly desirable to improve success of the symbiosis and hence increase clover nitrogen fixation and yield. Microencapsulation of cells may improve their survival by providing a protective structure for immobilization and functionalization of cells. The aim of this study was to examine the behavior of two *R. leguminosarum* strains cultured on two different carrier materials under conditions required to produce microcapsules by spray drying. Commercial strains TA1 and CC275e were cultured on peat and diatomaceous earth (DE) carriers for 14 days, then air dried at 25°C for 6 hours. The viability, and moisture content were evaluated at two hourly intervals. The viability of TA1 and CC275e decreased by one logarithmic unit over 6 hours in both peat and DE. The moisture content reduced more quickly in DE decreasing from 58 % to 3 % compared to 45 % to 11 % with peat. Heat tolerance of both rhizobia strains inoculated on both carriers was tested at 40, 60 and 80°C. Viability was measured after 5 and 10 minutes. No significant differences in survival were found between strains, however the carrier had a significant effect on survival for both strains at 40 and 60°C. Rhizobia did not survive at 80 °C. Finally, both strains inoculated on peat were formulated into microcapsules using a lab scale spray drier. Survival of both strains immediately after spray drying was around 10⁸ CFU/g, two logarithmic units lower than the theoretical value, showing that both strains were similarly affected by the process. Currently, we are testing the viability of microencapsulated rhizobia stored at 20°C and 4°C. In conclusion, both strains inoculated on peat are able to be used in a microencapsulation process by spray drying if temperatures higher than 60°C are avoided.

id #219

Impact of estuarine macro-algae on microbial communities and their biogeochemical cycles

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The Hutt river estuary has extensive covers of intertidal nutrient-promoted macro-algae and has been described as eutrophic. With increases in agriculture and urbanization, eutrophication is becoming a significant issue; it is therefore important to understand the impact eutrophication may have on microbial communities as they play vital roles in biogeochemical cycling within the estuarine environment. To assess differences in microbial communities caused by the presence of algae, surface sediment samples (0-2 cm depth) were collected from three sites in the estuary. Two of these were from areas of well-established cover and one from an area not affected by algal cover. Analysis of the covered sites showed elevated ammonium and sulphide concentrations compared to over lying water column and the uncovered site, whereas nitrate was higher in uncovered sites. The microbial communities could be distinguished based on their association with macro-algae. Areas with macro-algae had higher relative abundances of bacteria involved in sulphate reduction and sulfur oxidation, suggesting the establishment of a sulfur cycling community. Conversely, areas that had experienced no long-term algal cover had higher populations of bacteria and archaea associated with aerobic and anaerobic ammonium oxidation. These results suggest that the organic carbon provided by the macro-algae shifted the surface sediment communities from nitrogen cycling processes towards sulfur cycling processes.

We also used experimental plots to test the impact of uncovering long-term macroalgal areas and covering areas that had not experienced algal cover. Experiments were conducted for a 10 day period and indicated that the sediment communities were compositionally and structurally resilient to short term loss or gain of macro-algae; although a small increase in ammonium concentrations was seen in short term covered sites suggesting a shift towards macroalgal impacted conditions. Analysis of genomic and transcriptomic data will be used to confirm taxonomy-based predictions of community functioning.

id #224

The impact of terrestrial mud inputs on nitrogen cycling microbial communities in the intertidal zone of Waiwera estuary

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Estuaries are highly productive and heterogeneous ecosystems that vary dynamically. As interface between fresh and seawater they are constantly perturbed by natural and human derived activity. Anthropogenic disturbances, such as increased sedimentation caused by erosion and land use changes are concerning due to their pronounced and lasting effects, e.g. habitat displacement. To investigate the effects of terrestrial mud inputs on estuarine microbial community structure and impacts on nitrogen cycling members of the community, we sampled an intertidal sand-mud gradient in the bar-built Waiwera

estuary. Samples of each sediment type were collected at two different spatial scales at <1 metre apart and at tens of metres apart. We amplified and sequenced 16S rRNA genes, archaeal (AOA) and bacterial (AOB) *amoA* genes for ammonia oxidizers, and *nosZ* genes for denitrifiers. Results indicate that whole communities measured by 16S exhibited strong conservation of composition and structure, but only at highly localised spatial scales, and exhibited no correlation to mud content. However, heterotrophic denitrifier and ammonia oxidizer communities grouped strongly based on mud content, suggesting estuarine mud inputs influence the microbial nutrient cycle. Interestingly, ammonia oxidizing bacteria were more diverse in sand than in mud, while the opposite trend was observed for denitrifiers and ammonia oxidizing archaea. Preliminary quantitative PCR results indicate that AOB, while more diverse in sand, are significantly more abundant in muddy sites. On-going qPCR work will be used to look at the other N-cycling gene abundances and also gene expression levels between the mud impacted and unimpacted sites. To test how resilient sand or mud associated communities are to additional estuarine contaminants, we set up incubation experiments with Waiwera estuary sediment of varying mud and sand contents. Sediments were incubated under diurnal light conditions for one or two weeks with organic carbon (intertidal macroalgae), nitrate, a combination of both, or nothing. Early results tentatively indicate that sand associated communities are less resilient to introduced stressors and that the separation between nitrogen cycling bacteria and archaea in muddy and sandy sediments became more distinct after being introduced to these stressors.

id #228

Frontal eddies: hotspots for phytoplankton-bacteria interactions and sulphur cycling in the Tasman Sea

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Dimethylsulfoniopropionate (DMSP) is a globally important sulphur compound produced by a diverse range of phytoplankton and subsequently degraded by marine bacteria via one of two pathways: demethylation, by which sulphur is incorporated into cellular protein; and cleavage to dimethyl sulphide (DMS), a volatile gas that acts as a precursor for cloud condensation nuclei. Interactions between DMSP-producing phytoplankton and marine bacteria are critical drivers of marine microbial carbon and sulphur cycling, but we currently have little insight into how regional scale oceanography may affect these processes. Mechanisms driving the bacterial cycling of DMSP and DMS were investigated within 2 distinct water masses in the Tasman Sea, off the east coast of Australia: (i) a large cold-core eddy (CCE), and (ii) a small frontal CCE. For each water mass, samples were collected at 3 depths (surface, chlorophyll maximum and sub-mixed layer) along an east-west transect. We found that the small, frontal CCE contained higher concentrations of DMSP, but lower concentrations of DMS, in comparison to the large CCE. Bacterial genes for DMSP demethylation (*dmdA*) and cleavage (*dddP*), quantified using qPCR, had higher relative abundance within the frontal CCE. High-throughput sequencing was performed on 16S rRNA amplicons to identify bacterial and eukaryotic chloroplast OTUs, and specific variations in the SAR11 Alphaproteobacteria were explored using 16S rRNA oligotyping and amplicon sequencing of the *dmdA* gene. Network analysis revealed DMSP to be a key driver of ecological interactions in the Tasman Sea, correlating with chlorophyll-a concentrations, bacterial carbon production rates, *Roseobacter*-like *dddP*, two SAR11 *dmdA* clades, and several highly abundant bacterial OTUs. We hypothesise that frontal CCEs can act as hotspots for marine carbon and sulphur cycling, driven by bloom-like dynamics within the phytoplankton community that influence the abundance and distribution of key bacterial OTUs. These findings indicate that mesoscale oceanographic processes play an important role in driving environmental and ecological interactions between marine microorganisms, potentially influencing the production of climatically important DMS.

id #229

Detection and screening of high Ultra Violet resistant microorganisms from the Antarctic and Namib deserts.

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Research into the environmental effects of Ultra Violet Radiation (UVR) on terrestrial ecosystems has been widely investigated since the discovery of the ozone layer depletion over Antarctica. Continuous exposure to high levels of radiation causes microbial deoxyribonucleic acid (DNA) to suffer considerable and often irreparable damage. To survive, microorganisms must adapt to this stress by reallocating resources for survival and away from growth pathways. Developing a stronger understanding of how microorganisms have adapted to survive high UVR environments such as Antarctica and the Namib Desert will lead to greater insight of microbial physiological responses to this stress. Soil samples were obtained from surface soils from the Antarctic Dry Valleys, and Namib Desert. Isolates were cultured and isolated by spread plate method on nutrient agar, Luria Bertani agar, Reasoner's 2A agar and tryptic soy agar at 4, 15, 25, 35, 45 °C. Isolates were then screened for their resistance to different wavelengths and intensities of UVR using a modified drop plate method. UVR wavelengths used were 254 (UVC), 302 (UVB) and 365nm (UVA). To date, 145 organisms have been isolated from six locations in the Antarctica Dry Valleys and 166 organisms have been isolated from five locations along the Namib Desert. Of these, 21 isolates have been identified as resistant to high levels of UVB (15W/m²) for 10 minutes. All isolates are resistant to UVA at 30W/m² for 10 minutes. A total of 14 isolates from Antarctica and 5 isolates from the Namib Desert have been identified as resistant to high levels of UVC for 10 minutes at 5W/m²; which is 5 times the amount of UVC radiation that reaches the surface of the Antarctic Dry Valleys (1W/m²). The initial screening step in this research has found a number of UV resistant microorganisms in order to carry out further work into determining their molecular survival responses to UVR.

Variation in chewing behaviour among consumers and its impact on gut microbiome

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There is a large interest in the relationship between food structure, digestion and human nutrition, and many studies explore how food structures are broken down as they traverse the entire gastro-intestinal tract. However, the first stage of digestion, mastication, is often assumed to be trivial. In our recent research, we have assessed the chewing behaviour of 120 people and investigated the influence of chewing behaviour on digestion using *in-vitro* foregut digestion and gut fermentation models. Participants masticated samples of brown rice and expectorated just before the urge to swallow. Chewing behaviour measured by video observations and chewing outcome (i.e. bolus particle size, saliva addition to bolus) varied significantly among individuals, resulting in differences in the digestion of carbohydrates. Thorough chewers produced a higher glucose release in the gastro-intestinal digestion due to the increase in the food surface area and more saliva addition to bolus, whereas shorter chewers (who chew their food for lesser time) produced a higher amount of total short chain fatty acids (particularly lactate) in colonic fermentation due to the larger amount of undigested carbohydrate available for fermentation. Next generation sequencing analysis of a subset of the individuals revealed that the shorter chewers microbiome showing an increase in the relative abundance of *Bifidobacterium* and *Lactobacillus*. Our results indicate the potential role of particle structure and the availability of substrates thereof in influencing gut microbiota. This implies alternative strategies to shape the gut microbiome by changing the way people chew, without altering diets. This is an important contribution to the growing recognition that the microbiota of the large intestine play an important role in metabolic, nutritional, physiological and immunological processes in the human body. In this presentation, we will present this recent finding addressing the importance of understanding variance in consumer chewing behaviour for designing food products that deliver desired functionalities for target market segments.

Quantifying the antifouling and biocidal potential of surfaces.

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Micro-organisms on surfaces can be problem as their growth can damage the surface, or its effectiveness, and their presence can be a reservoir for disease and transmission to other surfaces. Antifouling surfaces are less easily colonised, and biocidal surfaces kill any micro-organisms that are able to colonise the surface. In this study we develop a methodology to challenge and assess the effectiveness of antifouling and biocidal surfaces.

Antimicrobial surfaces on 13mm diameter coverslips were challenged with *Escherichia coli* 25922 growing in Tryptic Soy broth from 1×10^6 CFU/ml in a 24 well plate. The plates were incubated at 37 C for 24 hrs, re-inoculated and incubated for a further 24 hrs. After 48 hrs of incubation, surfaces were rinsed three times with 0.9% w/v saline and stained with SYTO9, (staining live cells green), and propidium iodide, (staining dead cells red). Surfaces were placed within a CoverWell™ and were imaged by fluorescence microscopy. Triplicate coverslips were tested on three occasions for each surface, and six images were taken of each surface. Images were processed with ImageJ freeware to quantify live, dead and the total number of bacteria on the surface. Control samples of an untreated glass surface were run in parallel.

Effective antifouling surfaces were identified by a reproducible reduction in the surface coverage. Biocidal surfaces were further characterised by an increase in the proportion of red stained dead cells.

International Collection of Microorganisms from Plants (ICMP)

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The International Collection of Microorganisms from Plants (ICMP) is New Zealand's national culture collection of living Bacteria, Fungi, and Chromists. The collection and associated databases considered Nationally Significant by the government, and in part publicly funded.

The ICMP holds 20,000 cultures predominantly from plant, soil, and water in the natural environment, as well as important reference and type cultures of the world's plant pathogenic fungi and bacteria.

All cultures are databased and available online at <https://SCD.LandcareResearch.co.nz> cultures are available for a fee to cover retrieval costs. New accessions into the collection are welcome, and recommended when publishing papers on microbes to provide a stable permanent resource for future researchers.

The cultures are preserved under liquid nitrogen or in freeze dried ampoules. The ICMP containment and transitional facility conforms to enhanced PC2 Containment criteria, with generic permits to import quarantine and unwanted organisms into New Zealand.

1. Johnston, P. R.; Weir, B. S.; Cooper, J. A. (2017) Open data on fungi and bacterial plant pathogens in New Zealand. Mycology 1-8 doi:10.1080/21501203.2016.1278409

Sampling Homogeneities in Aerosol Sampling

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To date, metagenomic techniques have been widely used to study of microbial communities from various niche such as soil, ocean, sediment and fresh water. Nonetheless, the metagenomics of airborne microbial communities remains technically challenging, partly owing to the limited biomass of collectable atmospheric particulate matter (PM). While various methods for extracting genomic DNA (gDNA) from collected PM have been examined in previous studies, the effect of sampling durations on extraction efficiency is poorly understood. Here we present a comparative study on gDNA extraction method for PM collected over a 6 month period (August 2016 to January 2017) in the Central Business District of Auckland, New Zealand and Kigali, Rwanda. PM samples less than 2.5 μ m in diameter (PM_{2.5}) and less than 10 μ m in diameter (PM₁₀) were collected using a high volume air sampler fitted with a glass fibre filter over five sampling periods: seven days, five days, twenty-four hours, twelve hours, and six hours. PM_{2.5} and PM₁₀ filters were cut into different sizes (half/quarter/eighth of the original filter), and gDNA was extracted following the phenol extraction and CTAB extraction protocols. The amount of gDNA (ng/ μ l) recovered correlates linearly with the sampling duration of twenty-four hours, twelve hours, and six hours in both locations and extraction protocols. However, the total amount of gDNA was relatively similar in both five (4.1 ng/ μ l) and seven days (4.2 ng/ μ l) period. The CTAB extraction protocol was found to have a higher gDNA yield. The analysis also revealed that the amount of gDNA recovered was relatively higher in PM₁₀ than in PM_{2.5} in all sampling durations. The shorter sampling duration, twenty-four hours, twelve hours, and six hours, showed heterogeneous distribution of particulate matters collected on the filter. Therefore, a larger sampling area (at least half of the whole filter) would be necessary for DNA extraction. This methodology provides novel insight on gDNA extraction from atmospheric particulate matter, which is the bottleneck in bioaerosol metagenomics study.

id #239

Investigating the Evolution of mRNA:ncRNA Avoidance in *E. coli*

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The central dogma of molecular biology states the DNA is transcribed into RNA, which in turn is translated into protein. Thus, there is an expectation that RNA and protein levels should correlate relatively well. However, this is not always the case. RNA and protein levels may be imperfectly correlated. Two explanations for this discrepancy are mRNA secondary structure and codon usage bias, however at best these two explanations only account for 40% of the total variation in mRNA and protein abundances.

Recently a new explanation has been put forward that may better explain this phenomenon. Non-coding RNAs (ncRNAs) in principle ought to stochastically bind mRNAs thereby impacting translation and lowering protein expression. It follows then that for highly expressed mRNAs, or those that are essential for cellular function, the affinity for interaction with ncRNAs should be lower, thus these mRNAs avoid interaction. This signal for %avoidance+ was detected across the tree of life by a team at the University of Canterbury consisting of Sinan Umu, Ant Poole, Ren Dobson and Paul Gardner.

While evidence for this signal is clear the model for the evolution of mRNA:ncRNA avoidance remains untested. Understanding how this mechanism of avoidance evolved is highly important as a key application of this work is the ability to modulate the expression of proteins with extreme accuracy. While it has already been demonstrated that factoring avoidance into gene design can generate strains with a desired level of expression, we want to know whether selection will promote and maintain such changes under standard culturing conditions.

We aim to address this using a combination of *in silico* design and experimental evolution. We will therefore design a gene with high affinity for interaction with native ncRNAs in *E.coli*. Provided the survival of our bacterial populations is contingent on the expression of this gene we can create a selective pressure to drive the evolution of avoidance. We will then characterise any changes that may occur at the DNA/RNA level. Thus, this research will test the ability for natural selection to drive the evolution of avoidance of ncRNAs with essential genes.

id #247

Using structural biology to inform the design of T-antigen based vaccines for Group A Streptococcus

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id #257

Potential relationship between soil characteristics and the occurrence of soil-borne pathogens of public health significance; preliminary findings of a cross-sectional study

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Background: A little is known of soil characteristics that may facilitate occurrence of *Francisella tularensis*, *Coxiella burnetii* and *Burkholderia mallei* in natural environment.

Methods: Subsequent to screening soil of select districts (n=09) of Punjab province of Pakistan, we determined potential association between their occurrence in soil and its predictors (n=21) i.e. macro- and micro-nutrients using Independent t-test, Man-Whitney U-test, Pearson's Chi square test and binary logistic regression modal. The soil (n=302) represented different locations where genome of study pathogen was and was not detected, and were comprised of *F. tularensis* (n=186, 93 vs 93), *C. burnetii* (n=94, 47 vs 47) and *B mallei* (n=22, 11 vs 11).

Results: Univariate analysis revealed a non-significant difference for either the presence or absence of genome of *B. mallei* to any of soil characteristics. Soil pH, soluble salt, organic matter, magnesium, cobalt, sodium, calcium, potassium, nitrogen and silt, clay, soluble salt, organic matter, nitrogen, zinc, chromium, and manganese revealed significant differences for *C. burnetii* and *F. tularensis*, respectively. Odds of detecting *C. burnetii* was increased with a unit increase in organic matter [2.511 (95% CI: 1.453. 4.340), $p = 0.001$] and sodium [1.013 (95% CI: 1.005. 1.022), $p = 0.001$], whereas, calcium [0.984 (95% CI: 0.975. 0.994), $p = 0.002$] and potassium [0.994 (95% CI: 0.990. 0.999), $p = 0.011$] had protective effect. For *F. tularensis*, odds of occurrence was increased with a unit increase in clay [1.085 (95% CI: 1.049 . 1.122), $p = 0.000$], soluble salt [1.296 (95% CI: 1.047 . 1.604), $p = 0.017$], and zinc [4.728 (95% CI: 1.111 . 20.12), $p = 0.002$].

Conclusion: The data provides a preliminary relationship of soil-characteristic to occurrence of study pathogens in natural environment. Further studies are necessary to ascertain the study outcomes and determine the potential role of each analyte in a more systematic manner.

id #258

Experimental evolution uncovers the role of phenotype switching in a newly identified CTG-clade yeast

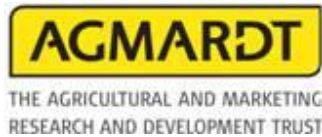
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Phenotype switching between cell types in microbes provides opportunities to occupy different ecological niches. Switching between white and opaque colonies in *Candida albicans* is a well-known example of how an opportunistic pathogen can escape host immune systems and cause different infections. Studying the evolutionary origins and the ecological significance of microbial phenotype switching can greatly improve our understanding in microbial diversity. Here, we report an undocumented switching of colony morphology in an ascomycetous yeast, *Kodamaea ohmeri*, which causes rare infections in human. In rich and undefined medium, *K. ohmeri* appears as smooth colonies with sporadic wrinkled colonies, which remain stable for generations with occasional reversion to smooth. We experimentally evolved *K. ohmeri* in defined minimal medium for 1500 generations and found that the proportions of smooth and wrinkled colonies in all the six evolved lines have changed and, moreover, that the colony morphology in the evolved lines differs from that in the ancestor and depends more on culture conditions. The wrinkled colony of evolved lines becomes less wrinkled, and cells derived from the wrinkled colony form smooth colonies on rich and undefined agar plates. Furthermore, whole genome sequencing revealed that, like *C. albicans*, *K. ohmeri* carries a Ser-tRNACAG, which translates the CUG codon into serine instead of leucine. Investigating the connection of phenotype switching in *K. ohmeri* to that in other CTG clade yeasts provides a opportunity to uncover the evolutionary origins of phenotype switching in the fungal CTG clade.

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