Molecular Microbiology Programme

Programme leader: Geoffrey Tompkins

Molecular Microbiology research within the SJWRI encompasses microbiological investigations applied to a variety of disciplines including endodontics, periodontics and implantology, cariology and treatment with antimicrobials, antifungal drug development, microbial genomics and forensics.

Major funding supporting research within the Theme during 2014-2016 came from the Marsden Fund (Royal Society of New Zealand), University of Otago Research Committee, New Zealand Dental Research Foundation, Ministry of Health Oral Health Research Fund, New Zealand Health Research Council, Maurice and Phyllis Paykel Trust, the Fuller Scholarship, Otago Innovation Ltd., Dentsply, Syngenta and other industrial concerns.

Personnel

Faculty and Staff

Associate Professor Vincent Bennani
Professor Richard Cannon
Dr Peter Cathro
Professor Mauro Farella
Dr Nick Heng
Dr Ann Holmes
Dr Mikhail Keniya
Dr Erwin Lamping
Dr Hee Ji Lee
Associate Professor Jonathan Leichter
Professor Karl Lyons
Dr Li Mei
Associate Professor Brian Monk
Dr Don Schwass
Dr Andrew Tawse-Smith
Associate Professor Geoffrey Tompkins
Dr Rajni Wilson
Dr Mathew Woods

Postgraduate Students

Peter Cathro (PhD; graduated 2016)
Gemma Cotton (PhD)
Sarah Davies (PhD; graduated 2016)
Golnoush Madani (PhD)
Bikiran Pardesi (PhD; graduated 2015)
Alia Sagatova (PhD; graduated 2016)
Syarida Safii (PhD)
Amira Salem (PhD)
Don Schwass (PhD)
Josh Dunn (MSc)
Shreya Aggarwala (DClinDent; graduated 2016)
Gareth Benic (DClinDent; graduated 2016)
James Dawson (DClinDent; graduated 2016)
Arpana Devi (DClinDent; graduated 2016)
Siddhanta Dhrupad (DClinDent; graduated 2016)
Nivea Kamalendran (DClinDent; graduated 2015)
Lydia Meredith (DClinDent; graduated 2015)

DClinDent student Gareth Benic was the winner of the Best Oral Presentation Award in the Molecular Microbiology session of the 2016 SJWRI Research Symposium.

Summer students and Honours Students

Nancy Chen (BDS summer student, 2015)
Nicholas Choo (BDS summer student, 2014)
Danyon Graham (BDS summer student 2014, 2015)
Harith Hassan (BDS summer student, 2015)
Chuen Lin Hong (BDS summer student, 2015)
Allen Hu (BDS Honours, 2016)
Kenny Kim (BDS honours, 2015)
Joanne Lee (BDS summer student, 2014)
Fay Yan (BDS summer student, 2015)

Visiting Scientists and students

Dr Mohammed Alqumber, Albaha University, Saudi Arabia

Dr Francesca Fabiano, University of Messina, Messina, Italy

Camille Herhusky, University of California at Berkeley, San Francisco, USA

Extramural Collaborators

Dr Stewart Bisset, AgResearch, Palmerston North

Dr Ariya Chindamporn, Chulalongkorn University, Bangkok, Thailand

Dr Edmund Fleischer, MicroCombiChem, Weisbaden, Germany

Dr Anette Klinger, MicroCombiChem, Weisbaden, Germany

Dr Michael Gottesman, National Cancer Institute, NIH, Bethesda, USA

Professor Susumu Kajiwara, Tokyo Institute of Technology, Tokyo, Japan

Dr Kurt Lackovic, Walter and Eliza Hall Institute, Melbourne, Australia

Associate Professor Alok Mitra, Auckland University, Auckland

Professor Rajendra Prasad, Jawaharlal Nehru University, New Delhi, India

Dr Jan Schmid, Massey University, Palmerston North

Professor Larry Sklar, University of New Mexico, Albuquerque, USA

Professor Robert Stroud, UCSF, San Francisco, USA

Dr Thomas Tomasiak, UCSF, San Francisco, USA

Dr Silas Villas-Bôas University of Auckland, Auckland

Associate Professor Maggie-Lee Huckabee, University of Canterbury, Christchurch

Associate Professor Marina Bakri, Malaysia, University of Malaya, Kuala Lumpur

Professor Pete Magee, University of Minnesota, Minneapolis, USA

Dr Masakazu Niimi, Chulalongkorn University, Bangkok, Thailand

Professor Amarila Malik, Universitas Indonesia, Depok, Indonesia

Associate Professor Koshy Philip, Universiti Malaya, Kuala Lumpur, Malaysia

Current research

Structure-directed antimicrobial discovery

Principal Investigator: Brian Monk

Structure-directed discovery of next-generation antifungals

There is a paucity of structural information on existing antifungal targets and there is an emerging problem of antifungal resistance that affects both medicine and agriculture. These problems are being addressed by overexpressing in yeast the azole drug target lanosterol 14α-demethylase, the terbinafine drug target squalene monooxygenase, the echinocandin drug target glucan synthase and drug efflux pumps from the ATP binding cassette and major facilitator superfamily. These constructs provide proteins for purification and structural resolution by X-ray crystallography plus key tools that enable targeted screens for antifungals and valuable tests of antifungal efficacy.

Since 2014 the group has deposited in the Protein Data Bank over 25 crystal structures of wild type and mutant lanosterol 14α-demethylase from *Saccharomyces cerevisiae* in complex with a range of azole drugs and agrochemicals plus the first crystal structure of a full-length lanosterol 14α-demethylase from a fungal pathogen (*Candida glabrata*). This information, together with our recent determination of the crystal structure of *Candida albicans* lanosterol 14α-demethylase, is being used to design chimeric antifungals that combined the best attributes of existing antifungals and has enabled computer-based screens of large compound libraries in efforts to discover novel antifungals.

The group published 6 papers in the 2015-2016, with an additional research paper and a book chapter review in press. The group completed a Marsden Fund grant (2010-2015), a 2 year research collaboration with the agrochemical company Bayer AG (2014-2016) and a Health Research Council of New Zealand grant (2013-2016) that led to the award of a further Health Research Council grant (2016-2019) entitled "Structure-directed discovery of next-generation antifungals". In 2016 Associate Professor Monk was also awarded a grant from Lotteries Health Research to purchase an advanced HPLC machine used for protein purification. Research collaborations involve Associate Professor Joel Tyndall in the New Zealand's National School of Pharmacy, the laboratory of Professor Robert Stroud at UCSF (San Francisco), the combinatorial chemistry company MicroCombichem (Wiesbaden, Germany) and Bayer AG Crop Protection Division (Monheim, Germany and Lyon, France). PhD student Alia Sagatova and DClinDent students Shreya Aggarwala and

Arpana Devi completed their studies in 2016, with Alia awarded a Thomas Kay Sidey postdoctoral fellowship. Summer student Danyon Graham won the 2015 junior poster competition at the IADR conference in Dunedin and the Otago Medical Research Foundation summer student speaker competition in 2016.

Candida adherence and drug-resistance

Principal investigator: Richard Cannon

The increased incidence of infections caused by drug resistant microorganisms is a major global health concern. While the multidrug resistance of bacteria is most prominent, drug resistance of fungi is also of great importance. The main cause of high-level azole drug resistance in the most common oral fungal pathogen, *Candida albicans*, is over-expression of ATP-binding cassette (ABC) membrane proteins that efflux the drugs from cells. We have used our patented *Saccharomyces cerevisiae* system for heterologously expressing membrane proteins to study *C. albicans* efflux pump Cdr1 function. Site-directed mutagenesis has been used to investigate the role of amino acids, particularly cysteines, in pump function. We have also used the expression system to study ABC efflux pumps from other important fungal species such as *Candida utilis* and *Penicillium marneffei*.

C. albicans is a diploid fungus that can mate but does so infrequently. By studying the growth rates of fusants formed *in vitro* and the survival of fusants *in vivo* we found that mating generates genotypes superior to existing strains often enough to be under slight positive selection.

In 2014-2015, this research was funded from the following sources: the Marsden Fund of the Royal Society of New Zealand, the Maurice and Phyllis Paykel Trust, the New Zealand Dental Association Research Foundation, and the University of Otago Research Committee.

Oral Bacteriology

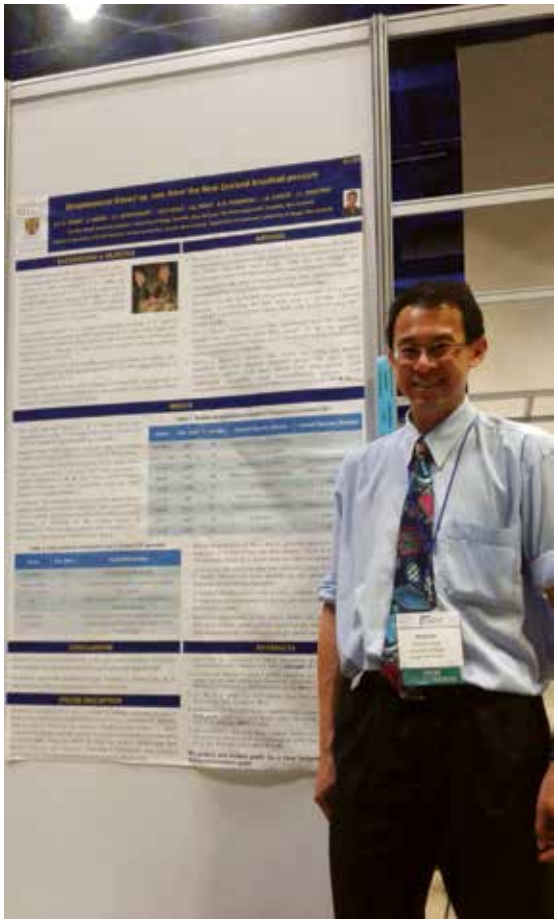
Principal investigator: Geoffrey Tompkins

Bacteria are involved in various diseases affecting the teeth and gingival tissues. Current projects in this group include: (i) development of new antimicrobials directed at the extremely alkaline-tolerant bacteria that cause root canal treatments to fail; (ii) evaluation of lasers to remove biofilms from dental implants; (iii) investigation into how periodontal bacteria acquire heme; (iv) the involvement of dental plaque bacteria in the development of aspiration pneumonia in stroke patients; (v) the effect of various antimicrobials, including chlorhexidine, manuka honey and silver-based antimicrobials affect oral microbial ecology.

Microbial profiling and bacterial genome sequencing using next-generation DNA sequencing technology

Principal investigator: Nick Heng

The oral cavity of each human and animal harbours its own distinctive community of microbes, termed the "oral microbiota". The human oral microbiota alone is estimated to comprise over 700 species of microbes. Many species have long been associated with disease such as *Streptococcus mutans* (dental caries) and *Porphyromonas gingivalis* (periodontal disease). Bacterial profiling of oral samples from healthy or diseased participants using next-generation DNA sequencing technology have helped identify some species that may either contribute to disease progression or are associated with good oral health. This research group is also interested in revealing the genomic secrets of cultured species such as the antimicrobial-producing *Streptococcus salivarius* (from humans) and new oral streptococcal species isolated from other animals.



Dr Nick Heng presenting his research at the 2016 IADR General Session in Seoul.

Microbial biofilms

Principal investigators: Vincent Bennani and Li Mei

Most microorganisms live within biofilms and in the mouth these biofilms can cause diseases such as dental caries, periodontitis and peri-implantitis. We are interested in how biofilms form on oral surfaces including denture acrylic, implant titanium, and orthodontic appliances - and measuring how effective methods are for removing these biofilms. We have also investigated the use of *Streptococcal salivarius* strains as probiotics to inhibit the growth of oral pathogens and improve oral health in orthodontic patients.

Other research programmes undertaken within the Molecular Microbiology Theme overlap with and are described in respective staff members' profiles elsewhere in this volume.

Highlights

Research Prizes

Alia Sagatova : Thomas Kaye Sidey postdoctoral fellowship (2016)

Danyon Graham: Junior poster competition at the IADR conference (Dunedin, 2015); Otago Medical Research Foundation summer student speaker competition (2016).

Gareth Benic, Sir John Walsh Research Institute Research Symposium Oral Presentation prize

Chuen Lin Hong, IADR NZ Section poster prize and IADR ANZ Division Colgate poster prize

Joanne Lee, IADR NZ Section poster prize

Notable Publications

Aung, H. L., Samaranayaka, C. U. K., Enright, R., Beggs, K. T., & Monk, B. C. (2015). Characterisation of the DNA gyrase from the thermophilic eubacterium *Thermus thermophilus. Protein Expression & Purification,* 107, 62-67. doi: 10.1016/j.pep.2014.11.009

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Seymour, G.J., Cullinan, M.P. and Heng, N.C.K. (2016) Oral Biology: Molecular Techniques and Applications, Second Edition. Methods in Molecular Biology, volume 1537.

Tawse-Smith, A., Atieh, M. A., Tompkins, G., Duncan, W. J., Reid, M. R., & Stirling, C. H. (2015). The effect of piezoelectric ultrasonic instrumentation on titanium discs: A microscopy and trace elemental analysis in vitro study. *International Journal of Dental Hygiene*. Advance online publication. doi: 10.1111/idh.12142

Walker, G.V., Heng, N.C.K., Carne, A., Tagg, J.R. and Wescombe, P.A. (2016) Salivaricin E and abundant dextranase activity may contribute to the anti-cariogenic potential of the probiotic candidate *Streptococcus salivarius* JH. *Microbiology* 162:476-486

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