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Tuberculosis (TB) has been described by the World Health Organisation as a global epidemic. The bacterium that caused TB *Mycobacterium tuberculosis* is thought to be carried by one third of the world's population. Whilst the incidence and notification rates of TB in New Zealand are relatively low (~10 per 100,000) these rates have been steadily increasing over the last decade. The problem of TB has recently been compounded by the dramatic increase in multi-drug resistant (MDR) strains of *M. tuberculosis* and the increased susceptibility of HIV-infected individuals. The alarming global statistics and the synergy between TB and HIV make the search for new TB antibiotics an urgent priority.

Much research has been published on the biochemistry, structure and function of PIN-domain proteins. Remarkably, *M. tuberculosis* has 48 PIN-domain proteins (in striking contrast to other species of bacteria) and the hypothesis of our research group is that the biochemical function of these proteins is linked to the persistence of *M. tuberculosis*. PIN domain proteins are ribonucleases and we hypothesise that this activity causes auto-toxicity and/or growth arrest in *M. tuberculosis*. Thus the PIN-domains could be described as TB auto-toxins. To control the auto-toxicity, each PIN domain has an inhibitor or antitoxin associated with it, and the genes for toxin and anti-toxin are adjacent on the genome forming a Toxin-Antitoxin (TA) operon.

In the past six months, my main focus has been on expressing the toxin-antitoxin complex from the bacterium *Mycobacterium smegmatis*, then subsequent purification of the protein to get into an ultra pure form for crystallisation trials and other functional analysis. I have successfully purified the TA complex, but the toxin alone proved more problematic. The toxin appears to be toxic to the cell, so there is high selection pressure to mutate the gene in the overexpression plasmid, so its toxic effect is not observed. This has been investigated in more detail with the use of DNA sequencing to look for changes in the toxin gene sequence. Alternative protein expression systems are also being pursued for toxin expression.

DNA binding of the TA complex from *M. smegmatis* is being investigated. The complex is thought to bind to DNA upstream of the TA operon, thereby affecting gene regulation. I have conducted DNA binding experiments to see if this is the case.

I have also been continuing work on the RNase activity of the *Pyrobaculum aerophilum* PIN-domain toxin proteins PAE0151 & PAE2754. I have shown that these proteins require magnesium or manganese as cofactors, and have tested their RNase activity against a variety of RNA substrates.

A major achievement has been the recent publication of a paper of which I am a co-author in the journal *Proteins*. Bunker, Richard, McKenzie, Joanna L., Baker, Edward N., Arcus, Vickery L. 'Crystal structure of PAE0151 from *Pyrobaculum aerophilum*, a PIN-domain (VapC) protein from a toxin-antitoxin operon', *Proteins* (2008).

This is a very exciting, busy time in my research. I am very close to crystal trials of the TA complex (this is very exciting as gaining a crystal structure for the TA complex is one of the main objectives of my research) and feel I have greater understanding of the function of these proteins, although there is still much to learn!