Chapter 5:

Concluding remarks:

The search for biological compounds worthy of production as antibiotics has been a continuous and ever challenging journey in science. The search for useful molecules has been narrowed to bacteria that are cable of producing various compounds that are useful in terms of medicine. *Pseudomonads* are known to produce a wide range of molecules with bactericidal, bioremedial and biosurfactant properties. An environmental *Pseudomonas* isolate (derived from 16s rDNA sequencing) was identified to produce an inhibitory activity towards *M. smegmatis*. The active molecule was isolated in the form of a crude ethyl acetate extract and its inhibitory activity characterised in terms of bacterial range and structural properties. It was found that this inhibitory molecule was only produced during the stationary phase of the *Pseudomonas* growth cycle on 7H10 media (low nutrient medium). The possibility that the production of this molecule is related to quorum sensing was also raised but further experiments are needed to determine if there is any significance between the two. The crude extract yielded showed a yellow colour which could be significant since many pyo compound molecules do exhibit a colour. The extractions yielded crude extracts that ranged from a yellow colour to an orange- brown colour. We found that there was a difference in the activity of the bacteria that the crude extract was tested against. However this could also be attributed to how the test organism reacts to organic solvents. Sine the crude extracts were not purified, the results attained from the bacterial range could be misleading. Purification of the crude extract was attempted so that the active component or molecule could be identified. TLC analysis and staining techniques revealed that the compound was hydrophobic and did not consist of known amino acids or a carbohydrate component. UV analysis of the fractions revealed that there was a component that was absorbing light at 414nm. This was consistent with results from the bioassay and the MS results which showed the presence of unique markers to the respective fractions. The MS results would need to be repeated with a proper negative control that is an extract from a mutant lacking the antimycobacterial activity. Transposon mutagenesis was attempted

to achieve this, however the technique has proven to be quite cumbersome. The reversion rate with this particular isolate was too high (> 50%), therefore the technique was deemed unreliable and inefficient. However, the technique did produce some kanamycin mutants which were screened for the lack of inhibitory activity. It was found that the high reversion rate could have been due to the instability of the Tn5 transposon. Further experiments involving the amplification of the IS50 L and IS50R regions of the plasmid pRL1063a needs to be done in order to determine the presence of the entire transposon. One mutant M1 was cloned and sequenced to determine if the transposition event had occurred. The sequencing revealed that no genomic DNA was present in the clone confirming that transposition of Tn5 had not taken place. The plasmid is most likely to be replicating at a very low copy number or maintaining itself within the bacteria. Characterisation of the antimycobacterial compound was only partially achieved. Crude extracts with a greater concentration of the compound of interest and a HPLC purified extract as well as more ESI-MS analysis is needed to elucidate the structure of the molecule which would ultimately lead to identification of the molecule.