CONCLUSIONS

Characterisation of microorganisms belonging to the *Enterobacteriaceae* is difficult as a high degree of phenotypic similarity exists in this family. In the past, identification of enterobacteria was based on colony morphology, physiological tests, fatty acid analysis and quinone composition. However, misidentification of isolates often occurred due to the phenotypic resemblance between the enterobacteria. More recently, characterisation of bacteria has been through genotypic methods as well as phenotypic characteristics. Sequencing of the 16S rRNA gene is considered a standard part of the description of bacterial taxa, although it is widely accepted that this gene is highly conserved particularly among the *Enterobacteriaceae*. For this reason, the initial molecular identification technique for *Pantoea ananatis* was based on amplification of the 16S-23S ITS region as it was thought to be more variable.

The 16S-23S ITS region can be used for identification of *P. ananatis* if the species-specific primers are redesigned. When the entire 16S-23S ITS gene was amplified with universal primers, it became clear that the *Pantoea* genome contains multiple copies of the rRNA operon. A high degree of similarity was noted among the number and size of rRNA operons of *Pantoea* species, meaning the genetic relatedness could not be examined using this technique. Possible methods for a taxonomy study of the genus *Pantoea* included both rep-PCR and PFGE, but access to a LI-COR automated sequencer made AFLP an obvious choice. AFLP analysis proved to be a reliable and reproducible method for typing of *Pantoea* species.

The majority of the seven species clustered in distinct groups with their type strains. From the manner in which the South American and Ugandan isolates clustered, it is clear that they belong to either a new *Pantoea* species or subspecies. DNA hybridisations will be performed on these strains to finalise their identity. The conclusions from this research project can be briefly summarised as follows:

- A rapid molecular identification technique for *P. ananatis*, based on the 16S-23S ITS region, is possible providing the species-specific primers are redesigned to exclude amplification of *P. stewartii* subsp. *indologenes* (Chapter 3).

- Examination of a partial region of the 16S-23S ITS gene is not sufficient for the determination of the geographical spread of *P. ananatis* (Chapter 3).

- The genomes of *Pantoea* species contain multiple copies of the rRNA operon, which is in keeping with organisms belonging to the family *Enterobacteriaceae* (Chapter 4).

- It is not possible to determine the genetic relatedness of *Pantoea* species, based on DNA fingerprints produced from amplification of the entire 16S-23S ITS region (Chapter 4).

- A high degree of similarity appears to exist among the rRNA operons of species of the genus *Pantoea* (Chapter 4).

- AFLP analysis provides an efficient method for differentiation of *Pantoea* species, even at the strain level (Chapter 5).

- There is little correlation between results produced by AFLP analysis and 16S rRNA gene sequencing of *Pantoea* species (Chapter 5).

- The possibility of more than one species of *Pantoea* causing disease on *Eucalyptus* should be investigated (Chapter 5).

- DNA-DNA hybridisation must be performed on the South American and Ugandan isolates to determine their position within the genus *Pantoea* (Chapter 5).

- The South American and Ugandan isolates should undergo pathogenicity tests to determine whether they are disease-causing organisms or epiphytes (Chapter 5).

- The species *P. citrea* and *P. terrea* require further examination due to the unusual grouping of their type strains in the AFLP dendrogram (Chapter 5).

Although AFLP analysis provided an accurate portrayal of the genetic relatedness of *Pantoea* species, the epidemiology of *P. ananatis* strains is still unclear. MLST is currently the most discriminative typing method for epidemiological studies and has been used successfully to examine the biodiversity of Vibrios (Thompson *et al*., 2004). If the global epidemiology of *Pantoea ananatis* is to be determined, MLST would provide a viable method.

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