POLYMERASE CHAIN REACTIONTECHNIQUES FOR THE STUDY OF RUMEN BACTERIAL ECOLOGY AND BIOTECHNOLOGY.

By

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I certify that then substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have bee acknowledged in this thesis.

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ABSTRACT.

The Polymerase Chain Reaction (PCR) was investigated as a suitable technique for tracing rumen bacteria *in vitro*. Target sequences for specific bacterial strains were characterised and tested, proving to be specific to the organism targeted. The technique proved highly sensitive, only limited by the amount of template material, which could be included in the PCR reaction. It proved possible to detect specific bacterial strains down to a level of 10^3/ml in a rumen sample containing about 10^{10} bacteria/ml.

PCR techniques were used to monitor bacterial strains introduced into the rumen both in the field, and under animal house conditions. The results of these tests showed that it was possible to introduce foreign strains of bacteria into the rumen, as well as to return strains from the laboratory to the rumen. It was found that bacterial populations fluctuate widely within and between animals, and that not all the variation observed could be related to the animal's diet, although this had a significant effect on the levels of some bacterial strains.

Quantitation techniques were developed for PCR amplifications of rumen bacteria, based on competitive PCR. Several variations of the basic competitive PCR method were developed and compared. Quantitiation methods were used to estimate bacterial numbers in rumen samples, and to estimate the genome size of two bacterial strains.

Finally, PCR methods for various other biotechnological applications were developed, or modified from published methods. These included production of chimaeric molecules, investigation of 16S rRNA genes, testing bacterial transformants, and production of highly specific radiolabeled probes.