CAPILLARY ELECTROPHORESIS OF RIBOSOMAL RNA FOR CHARACTERISATION OF MICROBIAL COMMUNITIES

by

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Declaration

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of the my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Yi Heng Nai

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Statement of Co-Authorship

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5. New CE approaches for Characterization of Microbial Communities.
6. **CE-RNA-SSCP: A New Approach for Characterization of Microbial Communities.**


7. **In search of Sieving Polymers for Separation of Ribonucleic acids by Conformation**


8. **In search of Sieving Polymers for Non denaturing Capillary Electrophoresis Separation of Ribonucleic acid (RNA)**

ABSTRACT

This thesis documents research on new capillary electrophoresis (CE) based rRNA fingerprint approaches for characterisation of low diversity microbial communities.

In the first body of work, an alternative approach for sieving polymer synthesis through reversible addition fragmentation chain transfer (RAFT) polymerisation is presented. Sieving polymer matrices are typically synthesised by conventional free radical polymerisation. This thesis describes the first synthesis of a high molecular weight poly(n,n-dimethylacrylamide) (PDMA) in which both the molar mass and polydispersity distribution were controlled by RAFT polymerisation. A multi-step chain extension is detailed and the physical properties and separation performance of DNA/RNA using this RAFT polymer are described.

The second body of work deals with the development of new approach for characterisation of microbial communities using CE. The new approach involves conformational separation of microbial 16S ribosomal RNA (rRNA) molecules containing the highly variable regions present in 16S rRNA. Single stranded conformation polymorphism (SSCP) is a separation technique based on the principle that for nucleic acid fragments of equal lengths, variation in sequences can affect nucleic acid folding and hence can be separated due to the difference in electrophoretic mobility. While CE DNA-SSCP has been commonly applied in clinical mutation diagnostic tests and studies of microbial diversity, CE rRNA-SSCP has yet to be demonstrated. In this work, an enzymatic based RNA-oligonucleotide cleavage method was employed to cleave the 16S rRNA (~1542 bases) to smaller fragments of similar length (~340 bases). This strategy uses a eubacterial ‘scissor’ probe to target and
hybridise highly conserved sites within the rRNA flanking highly variable regions (e.g. V1, V2 or V3). As rRNA is synthesised only by actively-growing cells, together with its role as the marker molecule for assigning sequences to genera and species, it can thus be used to correlate to the functioning members of microbial communities. Taking advantage of these unique properties, CE-rRNA-SSCP circumvents the need for polymerase chain reaction (PCR) amplification and retains the quantitative information regarding to the evenness of the microbial community that is important for ecological studies that were otherwise lost during PCR step. Compared to gel electrophoresis based approach, CE- rRNA SSCP significantly decreased the analysis time from 24 hours to 60 min and the use of a fluorescently labelled hybridisation probe for detection decreased the sample requirement by ten-fold. The combination of fast analysis time, low sample requirement and sensitive fluorescence detection makes CE-rRNA-SSCP an appealing new approach for characterising low diversity microbial communities.

The third body of work deals with the conception and development of a novel characterisation approach termed multiplex cleavage microbial community analysis (MCMCA), which is a potential method to simultaneously link the phylogeny of multiple groups of metabolically active microorganisms to their respective metabolic activity and relative abundance within a community. MCMCA utilizes the similar sequence-specific cleavage of rRNA molecules with oligonucleotides and RNase H employed in previous approach but differs by the use of multiple taxon specific probes selected to specifically cut the 16S rRNA into discrete fragments varying in length. The cleaved rRNA mixture is subsequently mixed with a fluorescently labelled locked nucleic acid (LNA) universal hybridisation probe and resolved using denaturing CE size separation. The feasibility of this rational is tested using model microbial strains,
followed by optimisation of the cleavage procedure to achieve multiplex cleavage in a
model microbial community. This approach was then applied to characterise a
hydrocarbon degrading enrichment community derived from soil.
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