INVESTIGATION OF DNA PROFILING METHODS FOR FORENSIC EXAMINATION OF SOIL EVIDENCE.

By

James M. Waters, B.Btec. (Hons.)

Being a thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy.

The Flinders University of South Australia
School of Biological Sciences
Bedford Park, South Australia

September 2004

Table of Contents

Summary

In this thesis, an investigation of the potential of two DNA based profiling techniques for the analysis of forensic soil evidence is presented. These profiling techniques were:

- The previously described technique, Terminal Restriction Fragment Length Polymorphism Analysis (TRFLP) of 16S DNA (Chpt 3.1) and
- A semi-novel profiling technique, Arbitrarily Amplified DNA (AAD) profiling (Chpts 3.2 and 3.3), analysed by both:
    - *conventional* length polymorphism of DNA fingerprints (AADLP) (Chpt 3.2) and
    - *a completely novel method*, DNA sequence similarity (AADSS) (Chpts 3.2 and 3.3), which was investigated using
        - Southern Hybridisation (Chpt 3.2) and
        - Microarray Technology (Chpt 3.3).

These methods were successful at distinguishing samples of soil to varying degrees. TRFLP analysis was capable of generating low but significant differences in similarity statistics between replicate and distinct soil profiles, while both AAD analyses (AADLP and AADSS) generated large and significant differences in similarity statistics between replicate and distinct soil profiles. The potential for significant differences between similarity statistics to be generated enables classification of soil samples as either having common origins (matching, if there is no significant difference) or not (excluded from matching, if there is a significant difference).
The affect of several technical, environmental and practical variables on the biological profiles generated using these techniques was investigated further. These variables included sampling and processing of soils (assessed with TRFLP, AADLP and AADSS), time *in situ* (TRFLP and AADSS), time *ex situ* under a number of storage conditions, as well as spatial variations of microbial communities over small distances (AADSS only).

The most capable method for distinguishing soils of different origin was AADSS, closely followed by AADLP, with TRFLP obtaining only marginal success relative to the other two methods. The preferred format of the AADSS technique is the microarray technology as it is capable of generating a good deal more data from soil DNA profiles than Southern hybridisation. However, as both are capable of distinguishing soils, the low cost Southern hybridisation technique may provide a suitable entry point technology for many forensic laboratories.
The molecular mechanism of the arbitrary amplification profiling system (AAD) was investigated (Chpt 3.4), allowing insight into the way these profiles are generated and potential ways to control the process in order to optimise profiles for various purposes. Many potential improvements and developments are suggested which may further enhance the utility of the techniques presented in this thesis.
The findings presented in this thesis demonstrate the potential for biological profiling of soil communities as a relatively simple, high resolution, objective tool that permits stringent statistical analysis, is not reliant on expert interpretation and is complementary to existing strategies for the forensic examination of soil evidence.

Declaration

I certify that this thesis does not incorporate, without acknowledgement, any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

James M Waters
September 2004

I believe that this thesis is properly presented, conforms to the specifications for a thesis and is sufficient to be, *prima facie*, worthy of examination.

Leigh A Burgoyne
September 2004

Acknowledgements

This thesis would not have been completed without the help and support of many people, too numerous to mention individually. There are however, a few people who should be thanked and acknowledged:

Professor Leigh A. Burgoyne, my supervisor, for his passionate support and involvement throughout the entire duration of this research project. I have been considerably expanded by our many discussions, scientific and otherwise;

Professor David E. A. Catcheside, my co-supervisor, for his help editing manuscripts and this thesis, for supplying a laboratory bench and a great working environment;

Dr. Paul Kirkbride, Assistant Director of Forensic Science Service, South Australia and Manager of operational programs and research of National Institure of Forensic Science (NIFS), my co-supervisor, for his help and enthusiasm with the project.

members of the Catcheside laboratory; Fred Bowring, P. Jane Yeadon, Lin Koh, J. Paul Rassmussen, Steve Henderson, Graham Eariss and Russell Stainer for their seemingly endless source of wisdom, support, entertainment and friendship;

my family, for their unwavering support, love and faith

and my partner Megan, who knows why.

Abbreviations

A_{600}	absorbancy at 600nm
AFLP	alternate fragment length polymorphism
AP-PCR	arbitrarily primed polymerase chain reaction
Bp	base-pair(s)
BSA	bovine serum albumin
cDNA	copied *or* cloned deoxyribonucleic acid
CTAB	hexadecyltrimethylammonium bromide
CV	coefficient of variance
DGGE	denaturation gradient gel electrophoresis
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
A	adenine
C	cytosine
G	guanine
T	thymine
dNTP	deoxynucleoside triphosphate
dsDNA	double stranded deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
FAM	6-carboxyfluorescein
Kbp	kilobase pairs
OTU	operational taxonomic unit
PCR	polymerase chain reaction
PLFA	phospholipid fatty acid
RAPD	random amplified polymorphic DNA
rDNA	ribosomal deoxyribonucleic acid
RFLP	restriction fragment length polymorphism
RFU	relative fluorescence unit
RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
SD	standard deviation
SDS	sodium dodecyl sulphate *or* sodium lauryl sulphate
SMIPS	structurally mediated inter-primer selectivity
SI	similarity index
TAE	Tris acetate and EDTA
Taq	*Thermus aquaticus*
TGGE	temperature gradient gel electrophoresis
TRF	terminal restriction fragment
TRFLP	terminal restriction fragment length polymorphism
UV	ultraviolet