DNA METHODS FOR THE DETECTION OF *PHYTOPHTHORA CINNAMOMI* FROM SOIL

by

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This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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# Table of contents

**CHAPTER 1 LITERATURE REVIEW**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTRODUCTION</td>
<td>11</td>
</tr>
<tr>
<td>1.1 <strong>PHYTOPHTHORA SPECIES</strong></td>
<td>12</td>
</tr>
<tr>
<td>1.1.1 Phytophthora cinnamomi</td>
<td>14</td>
</tr>
<tr>
<td>1.1.2 Biology</td>
<td>15</td>
</tr>
<tr>
<td>1.1.3 Aetiology and epidemiology</td>
<td>17</td>
</tr>
<tr>
<td>1.1.4 Spread of P. cinnamomi</td>
<td>18</td>
</tr>
<tr>
<td>1.1.5 Management of P. cinnamoni</td>
<td>19</td>
</tr>
<tr>
<td>1.2 Detection of <strong>PHYTOPHTHORA SPECIES IN SOIL AND PLANT MATERIAL</strong></td>
<td>22</td>
</tr>
<tr>
<td>1.2.1 Detection by direct plating and baiting</td>
<td>22</td>
</tr>
<tr>
<td>1.2.2 Immunological Detection</td>
<td>24</td>
</tr>
<tr>
<td>1.3 DNA DETECTION METHODS</td>
<td>24</td>
</tr>
<tr>
<td>1.3.1 PCR Detection of <em>P. cinnamoni</em></td>
<td>26</td>
</tr>
<tr>
<td>1.4 Detection of multiple pathogen species by microarray technology</td>
<td>29</td>
</tr>
<tr>
<td>1.4.1 Application of microarrays to analyse microbial populations</td>
<td>30</td>
</tr>
<tr>
<td>1.4.2 The 3D microarray</td>
<td>31</td>
</tr>
<tr>
<td>1.5 SUMMARY</td>
<td>32</td>
</tr>
</tbody>
</table>

*The objectives of this research were:* ...

**CHAPTER 2 PCR DESIGN, OPTIMISATION AND SPECIFICITY**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 INTRODUCTION</td>
<td>35</td>
</tr>
<tr>
<td>2.2 METHODS</td>
<td>36</td>
</tr>
<tr>
<td>2.2.1 Phytophthora isolates used during PCR studies</td>
<td>38</td>
</tr>
<tr>
<td>2.2.2 Maintenance of Phytophthora isolates</td>
<td>38</td>
</tr>
<tr>
<td>2.2.3 Preparation of mycelium for DNA extraction</td>
<td>41</td>
</tr>
<tr>
<td>2.2.4 DNA extraction from mycelial cultures</td>
<td>41</td>
</tr>
<tr>
<td>2.2.5 DNA extraction from soil samples</td>
<td>42</td>
</tr>
<tr>
<td>2.2.6 PCR primer design</td>
<td>43</td>
</tr>
<tr>
<td>2.2.7 PCR analysis</td>
<td>45</td>
</tr>
<tr>
<td>2.2.8 Agarose gel electrophoresis of diagnostic PCR products</td>
<td>45</td>
</tr>
<tr>
<td>2.3 RESULTS</td>
<td>46</td>
</tr>
<tr>
<td>2.3.1 Screening of <em>P. cinnamomi</em> specific primer combinations</td>
<td>46</td>
</tr>
<tr>
<td>2.3.2 Optimisation of $\text{MgCl}_2$ concentrations using three different DNA polymerases</td>
<td>48</td>
</tr>
<tr>
<td>2.3.3 Relative tolerance of three DNA polymerases to co-extracted PCR inhibitors</td>
<td>50</td>
</tr>
<tr>
<td>2.3.4 Optimisation of annealing temperature, dNTP, primer and polymerase concentrations for specific amplification of <em>P. cinnamomi</em> DNA using Tth+ DNA polymerase</td>
<td>52</td>
</tr>
<tr>
<td>2.3.5 Specificity analysis of CIN3A-CINITS4 primers</td>
<td>54</td>
</tr>
<tr>
<td>2.3.6 Selection of nested PCR primers</td>
<td>57</td>
</tr>
<tr>
<td>2.3.7 Re-amplification of primary PCR products with nested primers</td>
<td>59</td>
</tr>
<tr>
<td>2.4 DISCUSSION</td>
<td>60</td>
</tr>
</tbody>
</table>
CHAPTER 3 DEVELOPMENT OF A ROBUST AND SPECIFIC PCR PROTOCOL FOR THE DETECTION OF PHYTOPHTHORA CINNAMOMI FROM SOIL SAMPLES.................................................................................................................................63
3.1 INTRODUCTION ...........................................................................................................64
3.2 MATERIALS AND METHODS ......................................................................................65
3.2.1 Preparation of soil extracts .............................................................................65
3.2.2 PCR amplification ............................................................................................66
3.2.3 PCR Additives; BSA and Formamide .............................................................66
3.2.4 Combination of PCR Additives ........................................................................67
3.3 RESULTS.....................................................................................................................67
3.3.1 Specificity of PCR detection in the presence of BSA and formamide ...........67
3.3.2 Specificity of PCR detection of P. cinnamomi in the presence of soil extract, BSA and Formamide ...............................................................69
3.3.3 Detection sensitivity in the presence of PCR Additives; BSA and Formamide ...........................................................................................................................71
3.3.4 Combination of PCR Additives........................................................................73
3.3.5 PCR inhibition by a range of soil extracts......................................................76
3.4 DISCUSSION ...............................................................................................................79

CHAPTER 4 COMPARISON OF PCR BASED DETECTION OF PHYTOPHTHORA CINNAMOMI WITH DETECTION BY SOIL BAITING FROM NATURALLY INFESTED SOIL SAMPLES .................................................................................................83
4.1 INTRODUCTION ..........................................................................................................84
4.2 METHODS...................................................................................................................86
4.2.1 Sampling ...........................................................................................................86
4.2.2 Sample preparation and storage .....................................................................87
4.2.3 PCR Analysis ....................................................................................................87
4.2.4 Baiting detection of Phytophthora species .....................................................87
4.2.5 NARPH antibiotic selective medium ...............................................................88
4.2.6 Population Density Index Analysis ................................................................88
4.2.7 Direct PCR analysis of P. cinnamomi isolations .............................................89
4.2.8 Sample handling and cross contamination assessment .................................90
4.2.9 DNA Sequencing of products amplified from field soil samples...............91
4.3 RESULTS.....................................................................................................................92
4.3.1 Comparison of detection sensitivity between baiting and nested PCR from naturally infested soils .................................................................92
4.3.2 Variation in pathogen detection in response to periods of adverse conditions ........................................................................................................................................94
4.3.3 Baiting vs DNA detection from field sites .....................................................96
4.3.4 Consistency of detection ................................................................................99
4.3.5 Cross contamination analysis ........................................................................99
4.3.6 Sequence Confirmation ..................................................................................100
4.4 DISCUSSION .............................................................................................................102
CHAPTER 5  USE OF HYBRIDIZATION MELTING KINETICS FOR DETECTING PHYTOPHTHORA SPECIES USING THREE-DIMENSIONAL MICROARRAYS: DEMONSTRATION OF A NOVEL CONCEPT FOR THE DIFFERENTIATION OF DETECTION TARGETS ................................................................. 107

5.1 INTRODUCTION ........................................................................................................... 108
5.2 MATERIALS AND METHODS .................................................................................... 109
  5.2.1 Sourcing and maintenance of Phytophthora isolates ........................................ 109
  5.2.2 Design of Microarray Probes ......................................................................... 110
  5.2.3 Preparation of Target DNA .......................................................................... 113
  5.2.4 Microarray Hybridisation ............................................................................. 114
  5.2.5 Image Analysis .............................................................................................. 115
5.3 RESULTS ................................................................................................................... 117
5.4 DISCUSSION ............................................................................................................. 117

CHAPTER 6  CHARACTERISTICS OF PROBE-TARGET DUPLEX FORMATION ON THREE DIMENSIONAL MICROARRAYS ................................................. 129

6.1 GENERAL INTRODUCTION ....................................................................................... 130
6.2 REPRODUCIBILITY OF MICROARRAY DATA .......................................................... 131
  6.2.1 Introduction ....................................................................................................... 131
  6.2.2 Method .............................................................................................................. 131
  6.2.3 Results .............................................................................................................. 132
  6.2.4 Discussion ....................................................................................................... 135
6.3 RATE OF DUPLEX FORMATION .............................................................................. 136
  6.3.1 Introduction ....................................................................................................... 136
  6.3.2 Methods ............................................................................................................ 136
  6.3.3 Results ............................................................................................................. 136
  6.3.4 Discussion ....................................................................................................... 140
6.4 TARGET AMPLIFICATION STRATEGY FOR MICROARRAY ANALYSIS .................. 142
  6.4.1 Introduction ....................................................................................................... 142
  6.4.2 Methods ............................................................................................................ 142
  6.4.3 Results ............................................................................................................. 143
  6.4.4 Discussion ....................................................................................................... 145
6.5 PURIFICATION OF TARGET DNA .......................................................................... 146
  6.5.1 Introduction ....................................................................................................... 146
  6.5.2 Methods ............................................................................................................ 146
  6.5.3 Results ............................................................................................................. 146
  6.5.4 Discussion ....................................................................................................... 148
6.6 USE OF SPACER MOLECULES ............................................................................. 149
  6.6.1 Introduction ....................................................................................................... 149
  6.6.2 Methods ............................................................................................................ 150
  6.6.3 Results ............................................................................................................. 152
  6.6.4 Discussion ....................................................................................................... 154
6.7 PROBE DESIGN STRATEGIES .............................................................................. 156
  6.7.1 Introduction ....................................................................................................... 156
  6.7.2 Methods ............................................................................................................ 156
  6.7.3 Results ............................................................................................................. 157
  6.7.4 Discussion ....................................................................................................... 159
6.8 ANALYSIS OF MIXED TARGET DNA .................................................................. 160
6.8.1 Introduction .................................................................................................... 160
6.8.2 Observation..................................................................................................... 160
6.8.3 Discussion ....................................................................................................... 161
6.9 Influence of Secondary Structure on the Hybridisation Capacity of Immobilised Probes .............................................................................................. 163
6.9.1 Introduction .................................................................................................... 163
6.9.2 Methods ........................................................................................................... 164
6.9.3 Results ............................................................................................................. 164
6.9.4 Discussion ....................................................................................................... 173
6.10 Probe-Target Duplex General Discussion ........................................................... 176

CHAPTER 7 General Discussion ............................................................................... 177

7.1 Overview of Major Outcomes ............................................................................... 178
7.2 Diagnostic PCR of P. CINNAMOMI in Soil ............................................................. 179
7.3.1 Overcoming PCR inhibition .......................................................................... 179
7.3.2 Comparison of detection of P. cinnamomi by nested PCR and baiting........ 181
7.4 Diagnostic Microarrays .......................................................................................... 183
7.4.1 Limitations of diagnostic microarray research ............................................... 185
7.4.2 Future research directions.............................................................................. 186
7.5 Conclusions .......................................................................................................... 187

REFERENCES ............................................................................................................... 188

APPENDIX 1: Melting curves of specific and non-specific duplexes formed with each probe during microarray analysis Error! Bookmark not defined.
Declaration

The work described in this thesis was undertaken while I was an enrolled student for the degree of Doctor of Philosophy at Murdoch University, Perth, Western Australia. I declare that this thesis is my own account of my research and contains, as its main contents work which has not previously been submitted for a degree at any tertiary education institution. To the best of my knowledge, contains no material or work performed by others, published or unpublished without due reference being made within the text.

SIGNED: _________________________________   DATE: _______________
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Abstract

This project assesses two aspects of DNA detection of Phytophthora species from soil samples. Firstly, a nested PCR protocol was established with both primary and nested PCR specific for P. cinnamomi detection. PCR amplification of P. cinnamomi DNA isolated from soil was optimised with the addition of bovine serum albumin and formamide. This was found to improve both the specificity and sensitivity of PCR amplification of DNA in the presence of inhibitors co-extracted along with the target DNA from soil samples. The application of diagnostic nested PCR with the addition of BSA and formamide was verified by comparison with routine culture based detection methods. In all cases, nested PCR detection incorporating BSA and formamide was found to be considerably more sensitive than the culture based detection methods.

The second component of this thesis investigates the simultaneous detection of multiple species of Phytophthora using microarray analysis. Microarray based detection has been previously limited by variable and inconsistent hybridisation intensities across the diversity of probes used in each array. In this study a novel concept for the differentiation of detection targets using duplex melting kinetics is introduced. A microarray assay was developed on a PamChip® microarray enabling the differentiation of target Phytophthora species using the melting kinetics of probe-target duplexes. In the majority of cases the hybridization kinetics of target and non-target duplexes differed significantly. Analysis of the melting kinetics of duplexes formed by probes with target and non-target DNA was found to be an effective method for determining specific hybridization and was independent
of fluctuations in hybridization signal intensity. This form of analysis was more robust than the traditional approach based on hybridisation intensity, and allowed the detection of individual *Phytophthora* species and mixtures there of.