"Identification, diversity and detection of *Xanthomonas* strains associated with pistachio dieback in Australia"

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Dedicated to my son,

'Armin'

Abstract

Bacterial dieback disease was first observed on pistachio (*Pistacia vera*) trees in Australia in 1992 and has not been reported elsewhere. The disease is characterised by shoot death and dieback, limb and trunk lesions and discolouration of woody tissue in shoots more than one year old. *Xanthomonas* strains have been the only micro-organism consistently isolated from diseased trees. Characterisation of a small number of strains of the pathogen has already shown close relatedness to *X. translucens*. At the commencement of this study knowledge about genetic diversity of the pathogen was lacking, characterization of the pathogen and its taxonomic position were rudimentary and there was no efficient, reliable and rapid tool to detect and to recognise the pathogen in planting materials.

In this study, a three-step strategy was undertaken:

First, the genetic diversity of *Xanthomonas* strains isolated from infected pistachio trees from different geographic regions of Australia over several years was examined by repetitive extragenic palindromic-polymerase chain reaction (rep-PCR). Rep-PCR revealed two distinct genotypes, group A and group B, among the strains and showed some variation within group A. There was an association between the two groups and the geographic origin of the strains.

Second, physiological and biochemical tests, including the Biolog microplate™ System, and polyacrylamide gel electrophoresis (PAGE) of whole cell protein analysis were used to characterise and identify the strains. To obtain a sound taxonomic allocation of pistachio strains within the genus *Xanthomonas*, the relatedness of pistachio strains to known *Xanthomonas* species and strains was assessed by comparison of the 16S-23S rDNA internal transcribed spacer (ITS) and 16S rDNA sequences, rep-PCR genomic fingerprints and DNA:DNA homology. Pathogenicity of the strains was assessed on

selected members of the *Poaceae* and *Anacardiaceae*. Results of physiological and biochemical tests including Biolog analysis and protein profiling confirmed the existence of the two groups within the pathogen. Furthermore, the pathogen was identified as *X. translucens*. ITS sequencing confirmed two distinct genotypes among the strains and suggested that the pathogen strains were closely related to *X. t.* pv. *poae*. Based on 16S rDNA sequencing, pistachio strains matched most closely *X. t.* pv. *translucens*. While DNA:DNA homology studies confirmed that pistachio strains from both groups belong to *X. translucens*, rep-PCR showed that xanthomonads from pistachio differ from known pathovars of the species. Pathogenicity and host range studies indicated that the two groups were biologically different and suggested that these strains represent two new pathovars of the species.

Third, specific primers for amplification of DNA of the pathogen were developed based on sequences of the ITS region from strains representing groups A and B. Primers were designed for amplification of DNA sequences specific to each group and a multiplex PCR test was developed that identified and differentiated strains of each group in a single assay. To determine the specificity of the primers, PCR was carried out with DNA from 65 strains of the pathogen, 31 type and reference strains of *Xanthomonas*, and from 191 phytobacteria commonly found in and around pistachio orchards. In the multiplex PCR, a 331 bp fragment was amplified from all strains belonging to group A and a 120 bp fragment from all strains in group B. No PCR products were obtained from the other bacteria tested except for the type strain of *X. translucens* pv. *cerealis*, which has not been found in Australia. The assay was used to detect strains from both groups of the pathogen in pistachio plant material.

This study will contribute to understanding of the epidemiology and management of pistachio dieback.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed: [*Signature in print copy*] Date: 30/12/05

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Abbreviations

°C	degree Celsius
AFLP	amplified fragment length polymorphism
ARMS	amplification refractory mutation system
BSA	bovine serum albumin
BER-PCR	BOX, -ERIC and REP-PCR combined profiles
CFU	colony forming unit
CTAB	hexadecyltrimethylammonium bromide
cv.	cultivar
DNA	deoxyribonucleic acid
DMSO	dimethyl sulphoxide
dNTP	2'-deoxynucleoside 5'-triphosphate
EDTA	ethylenediamine *tetra* acetic acid
ERIC-PCR	enterobacterial repetitive intergenic consensus-based PCR
FAME	fatty acid methyl ester
g, mg, µg, ng	gram, milligram, microgram, nanogram
h	hour(s)
ha	hectare(s)
HR	hypersensitive reaction
ITS	internal transcribed spacer
kb	kilo base
kDa	kilo Dalton
L, mL, µL	litre, millilitre, microlitre
M, mM	molar, millimolar
mA	milliamp
min	minute(s)
mm	millimetre(s)
MQ water	ultrapure milli-Q water
mt	metric ton(s)
MW	molecular weight
NSW	New South Wales
nt	nucleotide
NT	Northern Territory

PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
pv.	pathovar
Qld	Queensland
RAPD	random amplified polymorphic DNA
RNA	ribonucleic acid
rDNA	ribosomal DNA
REP-PCR	repetitive extragenic palindromic-based PCR
rep-PCR	repetitive sequence-based PCR
RFLP	restriction fragment length polymorphism
rpm	revolutions per minute
RT	room temperature
s	second(s)
SA	South Australia
SDS	sodium dodecyl sulphate
TEMED	N-, N'-tetramethylenediamine
TAE	Tris-acetate-EDTA
TE	Tris-EDTA
tRNA	transfer ribonucleic acid
UPGMA	unweighted pair-group method using arithmetic averages
UV	ultra violet
V	volts
v	volume
Vic	Victoria
w	weight