University of Pretoria etd – Brady, C L (2005)

Taxonomy of *Pantoea* associated with bacterial blight of *Eucalyptus*

by

Carrie Louise Brady

Submitted in partial fulfilment of the
requirements for the degree
Magister Scientiae

in

The Faculty of Natural and Agricultural Sciences
University of Pretoria
Pretoria

Supervisor: Dr S.N. Venter
Co-supervisor: Prof T.A. Coutinho

University of Pretoria etd – Brady, C L (2005)

Taxonomy of *Pantoea* associated with bacterial blight of *Eucalyptus*

by

Carrie Louise Brady

Supervisor: Dr S.N. Venter
Co-supervisor: Prof T.A. Coutinho
Department: Microbiology and Plant Pathology
Degree: MSc (Microbiology)

SUMMARY

The genus *Pantoea* has seven species and two sub-species, isolated from diverse geographical and ecological sources. The majority of *Pantoea* species are plant-associated and cause a wide variety of diseases on a range of hosts. *P. ananatis* causes disease on many agricultural crops including onion, maize, sudangrass, honeydew melon and pineapple. *P. ananatis* has been identified as the causal agent of a serious bacterial blight and dieback of *Eucalyptus* in South Africa. Bacterial isolates have also been recovered from Eucalypts in South America and Uganda exhibiting *Pantoea ananatis*-like symptoms. These isolates have not been identified. Identification of *P. ananatis*, based on phenotypic analysis, is difficult due to similarities in phenotypic characteristics between *Pantoea* species and related *Enterobacteriaceae*.

Regular isolations of *P. ananatis* have highlighted the need for a rapid, molecular-based identification technique for the pathogen. A PCR assay, based on amplification of a partial region of the 16S-23S ITS gene with species-specific primers, was evaluated for the rapid identification

of *P. ananatis*. Authentic strains of *P. ananatis* were included in the study, along with the unidentified isolates from South America and Uganda, and authentic strains of all species of the genus *Pantoea*. All authentic strains of *P. ananatis* produced a single PCR product of 398 bp following amplification. Only one unidentified isolate from South America produced the 398 bp PCR product. The only other species to be detected by the primers was *P. stewartii* subsp. *indologenes*. For the 16S-23S ITS-PCR assay to successfully detect only *P. ananatis*, the species-specific primers will have to be modified to increase their specificity.

Little is known concerning the genetic relatedness between species and strains of *Pantoea*, and no standardised molecular typing system exists for the genus. The entire 16S-23S ITS gene was evaluated for a genetic relatedness study for the genus *Pantoea*. Universal primers were used to amplify the entire spacer region. Multiple amplification products were visible for all *Pantoea* strains and the unidentified isolates. This indicated that the *Pantoea* genome contains multiple copies of the rRNA operon and a high degree of similarity exists among the rRNA operons of species of the genus *Pantoea*. Therefore it is not possible to determine the genetic relatedness of *Pantoea* species based on a typing technique targeting the 16S-23S ITS region.

The entire genome was then screened by AFLP analysis to examine the genetic relatedness of the genus *Pantoea*. The AFLP technique was found to be successful and distinct clusters were visible for each *Pantoea* species in the dendrogram. The majority of the South American and Ugandan isolates formed three separate clusters from *P. ananatis*. Representative strains were chosen from among the unidentified isolates for 16S rRNA sequencing. Based on the resulting phylogram, it is clear that at least two new *Pantoea* species or subspecies exist among the South American and Ugandan isolates, and more than one *Pantoea* species may be associated with bacterial blight and dieback of *Eucalyptus*. DNA-DNA hybridisations will be performed on these isolates to determine their correct taxonomic position within the genus *Pantoea*.

ACKNOWLEDGMENTS

- I would like to thank my parents, Garry and Yvonne, for giving me the opportunity to further my studies and for never holding me back. They have supported me unwaveringly in all endeavours and have always had confidence in me. For this I am extremely grateful, and blessed to have parents who care so much.

- To Alan, for his patience, support and love. Also for his assistance with all the computer graphics.

- All the students from the pathology lab, many of whom became friends. Lisa, Michelle and Sonya, thanks for all the laughs and good times.

- Dr S.N. Venter and Prof T.A. Coutinho, for your guidance, patience and advice. Thank you for the incredible opportunity which this project turned out to be.

- Prof M.J. Wingfield for collection of the South American isolates.

- Marc Vancanneyt and Ilse Cleenwerck for their kindness, advice and hospitality in Gent.

- DoL/NRF for the financial support over the past three years.

CONTENTS

LIST OF ABBREVIATIONS

AFLP	-	amplified fragment length polymorphism
ATP	-	adenosine triphosphate
bp	-	basepair
ºC	-	degrees Celsius
CHEF	-	contour-clamped homogenous electric field
CYVD	-	cucurbit yellow vine disease
dATP	-	deoxyadenine triphosphate
dCTP	-	deoxycytosine triphosphate
dGTP	-	deoxyguanine triphosphate
dTTP	-	deoxythymine triphosphate
DGGE	-	denaturing gradient gel electrophoresis
DNA	-	deoxyribonucleic acid
DTT	-	dithiothreitol
EDTA	-	ethylenediaminetetraacetic acid
EMBL	-	European Molecular Biology Laboratory

ERIC	-	enterobacterial repetitive intergenic consensus
fig.	-	figure
G + C mol %	-	moles percent guanosine plus cytosine
ITS	-	internally transcribed spacer
KAc	-	potassium acetate
kb	-	kilobase
KZN	-	KwaZulu/Natal
M	-	molar
Mb	-	megabase
MgAc	-	magnesium acetate
$MgCl_2$	-	magnesium chloride
mg/mL	-	milligrams/millilitre
MLEE	-	multilocus enzyme electrophoresis
µL	-	microlitre
µM	-	micromolar
MLST	-	multilocus sequence typing

mM	-	millimolar
ng	-	nanogram
PAGE	-	polyacrylamide gel electrophoresis
PFGE	-	pulsed-field gel electrophoresis
pmol	-	picomole
PCR	-	polymerase chain reaction
REP	-	repetitive extragenic palindromic
RFLP	-	restriction fragment length polymorphism
RNA	-	ribonucleic acid
rRNA	-	ribosomal ribonucleic acid
RS-HP	-	ribosomal spacer-heteroduplex polymorphism
R.S.A.	-	Republic of South Africa
SE-AFLP	-	single enzyme-AFLP
sp.	-	species
ssp.	-	subspecies
subsp.	-	subspecies

TAE	-	Tris.HCl-Sodium acetate-EDTA
TBE	-	Tris-Boric acid-EDTA
TEMED	-	N,N,N',N'-tetramethylethylene diamine
Tris HAc	-	Tris (hydroxymethyl)aminomethane acetate
U	-	unit
UPGMA	-	unweighted pair-group method with arithmetic mean
U.S.A.	-	United States of America
UV	-	ultraviolet
V	-	volt
W	-	watt