

Taxonomic evaluation of the genus *Pantoea* based on a multigene approach

by

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or in part, been submitted to any other university for a degree.

Signature:

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Summary

The genus *Pantoea* contains seven validly-published species, which are primarily known as plant-associated or phytopathogenic bacteria, but some have also been linked with human infections. Over the past six years, there have been increasing isolations of *Pantoea* strains from a range of hosts and locations which can not be conclusively identified. In South America and Uganda, *Pantoea* strains were isolated from *Eucalyptus* infected with bacterial blight. In South Africa, *Pantoea* strains were isolated from maize suffering from brown stalk rot and from onion seed. This has highlighted the need for a rapid, molecular-based technique to conclusively characterize these *Pantoea* strains.

In the literature, two key taxonomic problems were identified within the genus *Pantoea*. The first involves three species isolated from fruit and soil samples in Japan, namely *P. citrea*, *P. punctata* and *P. terrea*, known as the “Japanese” *Pantoea* species. A recent review of the genus noted that *Pantoea* can be separated into two

groups: the *Pantoea* “core” containing *P. agglomerans*, *P. ananatis*, *P. dispersa* and *P. stewartii* and the “Japanese” species. It was also stated that more taxonomic work is required to justify the assignment of the “Japanese” species to the genus *Pantoea*. The second taxonomic issue raised concerns over four DNA hybridization groups from a study of a large number of clinical strains belonging to the *Erwinia herbicola*-*Enterobacter agglomerans* complex. It had been previously suggested that these four DNA hybridization groups should belong to the genus *Pantoea*. The phylogenetic relationship between the *Pantoea* “core” species, the “Japanese” species and the four DNA hybridization groups is not clear.

Multilocus sequence analysis (MLSA) was selected for a taxonomic study of the genus *Pantoea*. The MLSA scheme was based on the sequences of four housekeeping genes: *rpoB*, *atpD*, *gyrB* and *infB*. It was found that a phylogenetic tree of the concatenated sequences could differentiate all seven validly-published species as well as ten groups of strains from *Eucalyptus*, maize, onion, flowering shrubs and clinical isolates. The phylogenetic trees also confirmed the separation of *Pantoea* into two groups of species and indicated a close affiliation of the “Japanese” species with the genus *Tatumella*. The clusters observed in the MLSA phylogenetic trees were confirmed by DNA-DNA hybridization.

Using the MLSA data for support, four novel species were proposed from plant hosts: *Pantoea vagens*, *Pantoea eucalypti*, *Pantoea deleyii* and *Pantoea anthophila*. Four additional species were proposed for the clinical strains from the *Erwinia herbicola*-*Enterobacter agglomerans* complex, contained in three DNA hybridization groups: *Pantoea septica*, *Pantoea eucrina*, *Pantoea brenneri* and *Pantoea conspicua*. Phenotypic information and DNA-DNA hybridization values confirmed that the “Japanese” species were more similar to *Tatumella* than to *Pantoea*. This was demonstrated phylogenetically in the MLSA trees and gave support to transfer the “Japanese” species to the genus *Tatumella*. Additionally, a novel species was proposed for strains thought to belong to *T. citrea*, causing pink disease of pineapple: *Tatumella morbirosei*. Therefore, MLSA was not only proven to rapidly and successfully identify *Pantoea* strains, but also resolved the two major taxonomic issues within the genus.

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List of Abbreviations

AAI	-	average amino acid identity
adj.	-	adjective
AFLP	-	Amplified fragment length polymorphism
ANI	-	average nucleotide identity
ATCC	-	American Type Culture Collection
<i>atpA</i>	-	gene encoding ATP synthase α subunit
<i>atpD</i>	-	gene encoding ATP synthase β subunit
BCC	-	Bacterial Culture Collection, Forestry and Agricultural Biotechnology Institute (FABI)
BCCM/LMG	-	Belgian Coordinated Collection of Microorganisms/Laboratory of Microbiology, Ghent University
BD	-	Bacterial Disease, Plant Pathogenic and Plant Protecting Bacteria (PPPPB) Culture Collection
Bp	-	base pair
$^{\circ}\text{C}$	-	degrees Celsius
<i>carA</i>	-	gene encoding carbamoyl phosphate synthase
CCUG	-	Culture Collection, University of Göteborg
CDC	-	Centres for Disease Control
cm	-	centimetre
comb. nov.	-	combination nova
ΔT_m	-	thermal denaturation midpoint
dim.	-	diminutive
DKGA	-	2,5-diketo-D-gluconic acid
DNA	-	deoxyribonucleic acid
dNTP's	-	deoxynucleotide triphosphate
EMBL	-	European Molecular Biology Laboratory
ERIC	-	enterobacterial repetitive intergenic consensus
FABI	-	Forestry and Agricultural Biotechnology Institute
fem.	-	feminine



Fig.	-	figure
gen.	-	genitive
Gr.	-	Greek
<i>groEL</i>	-	gene encoding heat shock protein
GTR	-	general time reversible
<i>gyrB</i>	-	gene encoding DNA gyrase
HPLC	-	high performance liquid chromatography
HR	-	hypersensitivity reaction
<i>hsp60</i>	-	gene encoding heat shock protein 60
H ₂ S	-	Hydrogen sulphide
IJSEM	-	International Journal of Systematic and Evolutionary Microbiology
<i>infB</i>	-	gene encoding initiation translation factor 2
K3P	-	Kimura
KCN	-	Potassium cyanide
L.	-	Latin
LMG	-	Laboratory of Microbiology, Ghent University
μl	-	microlitre
μm	-	micrometre
μM	-	micromolar
M.L.	-	medieval Latin
MLSA	-	multilocus sequence analysis
MLST	-	multilocus sequence typing
mol %	-	moles percent guanosine plus cytosine
n.	-	noun
NCPPB	-	National Collection of Plant Pathogenic Bacteria
Neut.	-	neuter
N.L.	-	new Latin
NRF	-	National Research Foundation
ONPG	-	<i>o</i> -Nitrophenyl-β-D-galactopyranoside
PCR	-	polymerase chain reaction
RBR	-	relative binding ratio
<i>recA</i>	-	gene encoding recombinase A
<i>recN</i>	-	gene encoding recombinase N



rep-PCR	-	repetitive extragenic palindromic-PCR
rRNA	-	ribosomal ribonucleic acid
<i>rpoB</i>	-	gene encoding RNA polymerase β subunit
sp. nov.	-	species nova
ssp.	-	subspecies
ST	-	sequence type
subsp.	-	subspecies
THRIP	-	Technology and Human Resources for Industry Programme
TN93	-	Tamura-Nei
TPCP	-	Tree Protection Co-operative Programme
tRNA	-	transfer ribonucleic acid
TSI	-	triple sugar iron
<i>tuf</i>	-	gene encoding elongation factor
UPGMA	-	unweighted pair groups method using arithmetic average
U.S.A.	-	United States of America
v.	-	verb
V	-	volt

Prepared Manuscripts

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