The ecological genetics of *Pseudomonas syringae* in the kiwifruit phyllosphere

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Abstract

The impact of disease-causing bacteria on their hosts is shaped by interactions with co-occurring microbes, but such interactions are rarely studied. *Pseudomonas syringae* is a ubiquitous and significant plant pathogen infecting a wide range of plants, often of agricultural importance. The community context of *P. syringae* in infected plant hosts has been little explored. I determined the population structure and genetic diversity of *Pseudomonas syringae* strains collected from infected and uninfected orchards over the course of a growing season during the current outbreak of bacterial canker of kiwifruit (*P. syringae* pv. *actinidiae, Psa*) in New Zealand. A total of 148 strains comprising Phylogroups 1, 2, 3 and 5 were characterised by Multi Locus Sequence Typing (MLST). The overall population structure was clonal, but with a low level of recombination for single housekeeping genes within phylogroups. More than half of the isolates belonged to a new Phylogroup 3 clade (PG3a) that was also commonly found on kiwifruit leaves in China and previously reported from kiwifruit leaves in Japan. To understand the ecological basis of the co-occurrence of PG3a and PG1 (*Psa*) I looked for evidence of niche specialisation by performing reciprocal invasion from rare assays of a selected representative from each lineage both *in vitro* and *in planta*. *P. syringae* G33C (PG3a) demonstrates antagonistic behaviour towards *Psa* NZ54, whereas *Psa* NZ54 exhibits a beneficial effect on growth of *P. syringae* G33C; an effect that could not be attributed to virulence activity encoded by the Type 3 Secretion System. Given this antagonistic

behaviour, I explored the virulence repertoire in these commensal strains to determine their potential in the emergence of future more virulent types of *Psa*. In addition, I used comparative genomics to unravel the phylogenetic resolution of the novel *P. syringae* clade in context with known representatives of *P. syringae* PG3. Together my data draw attention to the community context of disease and demonstrate the value of incorporating an ecological dimension into the study of the genetic structure of pathogen populations.

Table of Abbreviations

Abbreviation	**Meaning**
AHL	*N*-acyl derivatives of homoserine lactone
Avr	Avirulence
bp	Basepair
CDS	Coding sequence
CEL	Conserved effector locus
cfu	Colony forming unit
CDI	Contact-dependent inhibition
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Dinucleotide triphosphate
dpi	Days post inoculation
EEL	Exchangeable effector locus
EPS	Extracellular polysaccharides
ETI	Effector triggered immunity
HGT	Horizontal Gene Transfer
Hop	Hrp outer protein
HR	Hypersensitive response
IAA	Indole 3-acetic acid
ICE	Integrative conjugative element
Ice+/-	Ice-nucleation active/negative bacteria
INA	Ice-nucleation activity
Kan	Kanamycin

Abbreviation	**Meaning**
KB	King's B medium
LB	Lysogeny broth
M9	M9 medium
MAMP	Microbial associated molecular patterns
Mbp	Megabasepairs
MCMC	Markov chain Monte Carlo
ML	Maximum Likelihood
MLST	Multi Locus Sequence Typing
MST	Minimum Spanning Tree
NRPS	Non-ribosomal peptide synthetases
OD	Optical density
P.	*Pseudomonas*
PAI	Pathogenicity island
PAMP	Pathogen associated molecular patterns
PCR	Polymerase Chain Reaction
PG	Phylogroup
PGPR	Plant growth promoting bacteria
PRR	Pattern-recognition receptors
Psa	*Pseudomonas syringae* pv. *actinidiae*
PTI	Pattern triggered immunity
pv.	Pathovar
R genes	Resistance genes
RK	Receptor kinase
RLP	Receptor- like protein

Abbreviation	**Meaning**
ROS	Reactive Oxygen Species
SE	Standard error
ST	Sequence type
T3SE	Type 3 Secretion Effectors
T3SS	Type 3 Secretion System
WGS	Whole genome sequencing
WT	Wildtype

Contents

List of Figures

List of Tables