Genotypic and phenotypic analysis of plant-associated *Pseudomonas*

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Abstract

The ecological success of *Pseudomonas* in plant environments is largely determined by the phenotypes that it expresses: efficient utilization of plant-derived nutritional substrates is fundamentally important for bacterial competitive growth. Not surprisingly then, *Pseudomonas* up-regulates the expression of many genes involved in nutrient scavenging when colonizing the plant surfaces. A typical example is the *hut* genes dedicated to the utilization of histidine and urocanate (the first intermediate of the histidine degradation pathway) in the model organism of *P. fluorescens* SBW25. Previous work has defined the genes involved in the histidine/urocanate uptake, degradation and regulation. This study aims to extend our understanding of histidine/urocanate utilization to the population level.

A total of 230 *Pseudomonas* strains were isolated from the phyllosphere of sugar beets grown in Oxford (UK) and Auckland (New Zealand) and their ability to grow on histidine and urocanate was tested. The results revealed considerable variation of phenotypes, for example, strains were capable of growing on histidine but not on urocanate (His^+, Uro^-, 11%) and vice versa (His^-, Uro^+, 13%). Interestingly, His^+, Uro^- strains were commonly found in the Auckland population, whereas His^-, Uro^+ strains were more prevalent in the Oxford population. Introduction of cloned copies of the histidine- and urocanate-specific transporter genes (*hutTh* and *hutTu)* from *P. fluorescens* SBW25 restored the ability of many naturally His^- and Uro^- strains to utilize histidine and urocanate, respectively. Together, the data indicate that *Pseudomonas* populations are polymorphic with respect to the transporters.

The genetic relatedness of the two *Pseudomonas* populations from Oxford and Auckland was estimated using multi-locus sequence analysis (MLSA) of three genes (*gapA*, *gltA* and *acnB*). For each of the three genes, oligonucleotide primers were designed to amply the DNA fragment (~600 nt) which was subjected to subsequent DNA sequencing. The DNA sequences of three genes (615 nt for *gltA*, 303 nt for *gapA*, 273 nt for *acnB*) were concatenated and used for phylogenetic analysis. Results showed that the *Pseudomonas* population

from Auckland is phylogenetically distinct from that of Oxford; there is a clear correlation between the MLSA genotypes and the phenotypes (i.e., utilization of histidine vs. urocanate).

Taken together, my data show that the two *Pseudomonas* populations colonizing the phyllosphere of sugar beets in Oxford and Auckland are genetically diverse and display distinct phenotypes in terms of their ability to grow on histidine and urocanate as the sole source of carbon and nitrogen. Furthermore, the observed phenotypic diversity is attributable to variation in histidine- and urocanate-specific transports, not genes for histidine catabolism.

[Of note, the results reported in this thesis on the polymorphism of histidine and urocanate utilization in plant-associated *Pseudomonas* has been published in the journal of *Environmental Microbiology*, wherein I am the second author (see Appendix B).]

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Table of Abbreviations

Abbreviation	Meaning
µl	microlitre
BLAST	basic local alignment search tool
bp	base pairs
dNTP	dinucleotide triphosphate
h	hour
IPTG	isopropyl-β-D-thiogalactoside
kb	kilobase pairs
Km	kanamycin
LB	luria-bertaini
g	gram/gravity
His	histidine
Uro	urocanate
M	molar
mg	milligram
nt	nucleotide
min	minute
OD	optical density
nm	nanometre
PCR	polymerase chain reaction
rpm	rotation per minute
NF	nitrofurantonin
TBE	tris-borate-EDTA
Tc	tetracycline
Gen	gentamicin
UV	ultraviolet
X-gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside
MLSA	multi-locus sequence analysis
LCMSMS	liquid chromatography-mass spectrometry
HPLC	high pressure liquid chromatography
WT	wild type
ICP-MS	Inductively Coupled Plasma Mass Spectrometry