

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**Identification and functional analysis of *Pseudomonas syringae* pv.
actinidiae effector-triggered immunity in *Nicotiana spp.* and
Arabidopsis thaliana.**

A thesis presented in partial fulfilment of the requirements for
the degree of Doctor of Philosophy in Plant Science
at Massey University, Manawatu, New Zealand.

Sera Choi

2017

© This copy of the thesis has been supplied on the condition that anyone who consults it is understood to recognize that its copyright rests with the author and that no quotation from the thesis, nor any information derived therefrom, may be published without the author's prior written consent.

Abstract

Pseudomonas syringae pv. *actinidiae* (*Psa*) is the causal agent of bacterial canker in commercially important cultivars of kiwifruit (*Actinidia deliciosa* and *A. chinensis*) worldwide, including New Zealand. Like many gram-negative pathogens, *Psa* is expected to utilise type III effectors to promote virulence in host plants. In order to better understand *Psa* effector-triggered immunity and susceptibility, we aimed to investigate multiple molecular characteristics of *Psa* type III effectors and their recognition mechanisms in model plants, *Nicotiana* spp. and *Arabidopsis thaliana*.

Nicotiana tabacum and *N. benthamiana* are widely-used model plants for *Agrobacterium*-mediated transient expression (agroinfiltration) of effectors for functional characterization. Firstly, we screened multiple characteristics of effectors from two *Psa* strains, *Psa* NZ V13 and *Psa* NZ LV5. The former is a strongly virulent and the latter is a weakly virulent strain in kiwifruit. By using agroinfiltration in *Nicotiana* spp. to express individual effector proteins, we observed diverse subcellular localisation for *Psa* effectors. Additionally, we identified multiple *Psa* effectors that can trigger HR-like cell death (HCD) in both *N. tabacum* and *N. benthamiana*. Using virus-induced gene silencing (VIGS), we identified that some *Psa* effector-triggered HCD requires the immunity regulator *SGT1*, suggesting that the *Psa* effector-triggered HCD could be a result of immunity activation.

We focused on one *Psa* NZ V13 effector, HopZ5, which belongs to the YopJ-like acetyltransferase family. HopZ5 triggers hypersensitive response (HR) in *Arabidopsis* accession, Ct-1. Another *Arabidopsis* accession, Col-0, does not develop an HR but shows immunity in response to HopZ5. The gene that confers HopZ5-triggered HR in Ct-1 was identified as *SOBER1* (*SUPPRESSOR OF AVRBS-T-ELICITED RESISTANCE 1*) by using recombinant inbred lines derived from two parental accessions, Ct-1 and Col-0. *SOBER1* is a known suppressor of *Xanthomonas* effector AvrBsT-triggered immunity. Interestingly, AvrBsT also belongs to YopJ family. Uniquely, *SOBER1* specifically suppressed HCD triggered by several YopJ-like acetyltransferase effectors in *N. benthamiana*, including HopZ5 and HopZ3 from *Psa*. This suggests a common mechanism shared between a subset of YopJ-like acetyltransferase effectors is suppressed by *SOBER1*.

Finally, we identified one *Arabidopsis* accession, Ga-0, which carries a truncated *SOBER1* variant but does not develop an HR upon HopZ5 delivery. Using bulked-segregant analysis of an F₂ population derived from a cross between Ct-1 and Ga-0, we mapped the locus conferring HopZ5-recognition in Ct-1 to the upper arm of Chromosome 3.

Acknowledgements

First of all, I would like to thank my primary supervisor, Prof. Kee Hoon Sohn for great advices and scientific encouraging on me during my PhD. Delightful and enlightening discussions with him really helped me a lot to carry on my project. I feel grateful for his comments and supports throughout the degree. Also, I want to show my gratitude to our former senior postdoc Prof. Cecile Segonzac for being an excellent mentor in the lab. I appreciate her for sharing her knowledge and guiding us from her experiences not only in the lab, but also outside of the lab. I also want to thank my co-supervisors, Dr. Rosie Bradshaw, Dr. Janet Reid, Dr. Matt Templeton and Dr. Erik Rikkerink for their supports and help.

I also would like to thank all KSL members, in New Zealand and in Korea for being excellent companions for the long journey. I was so lucky to have such nice PhD students, Toby, Maxim and Jay as colleagues also as friends. I especially thank my project mate, Jay, for being a nicest mentor I've ever had and for his support. I really appreciate his enthusiasm on science which drove me also into the project.

I sincerely thank my partner, Maxim for encouraging me to continue whenever I face difficulties, for inspiring me by his enthusiasm into science and holding my hands always.

Lastly, I appreciate endless supports and help from my family. Even though we were far away from each other, my mother and sister always made me feel like I was with them. Without their help, I wouldn't have been able to achieve this journey. I also want to thank my little cat, Tori for being my late-night mate in New Zealand.

Table of contents

Abstract	2
Acknowledgements.....	3
Table of contents.....	4
Chapter 1. General introduction	8
1.1 Introduction.....	8
1.2 <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> in kiwifruit	9
1.3 Plant innate immune system	10
1.3.1 PAMP-triggered immunity (PTI).....	10
1.3.2 Effector triggered immunity (ETI)	14
1.4. The roles of effectors in pathogenicity.....	23
1.4.1 Effector delivery systems.....	23
1.4.2 Roles of effectors in pathogenicity	24
1.5. YopJ family effectors in plant.....	28
1.5.1. Targets of YopJ family effectors in plants.....	30
1.5.2. Examples of recognition of YopJ family effectors in plants	32
1.6. Suppressors of effector-triggered immunity in plants	32
1.7. Aims of the study.....	33
Chapter 2. Materials and Methods	35
2.1 Bacterial materials.....	35
2.1.1 List of bacteria strains.....	35
2.1.2 Media.....	36
2.1.3 Antibiotics.....	36
2.1.4 Triparental conjugation	36
2.1.5 Electroporation.....	37
2.2 Plant materials	37
2.2.1 List of plant materials	37
2.2.2 Growth condition of plants.....	39
2.3 Plasmid constructs.....	40
2.3.1 List of vectors used in this study.....	40

2.3.2. Module constructs in pUC19B for Golden Gate assembly.....	41
2.3.3. Constructs in binary vector.....	47
2.3.3 Constructs in broad host range vector	47
2.4 Plant pathology	47
2.4.1 Bacterial infiltration in <i>Arabidopsis thaliana</i> and Hypersensitive Response assay	47
2.4.2 <i>Agrobacterium</i> -mediated transformation	48
2.4.3 Ion leakage assay	49
2.4.4 Bacterial growth assay in <i>Arabidopsis thaliana</i>	49
2.4.5 Virus-induced gene silencing (VIGS) in <i>Nicotiana benthamiana</i>	50
2.5 Molecular Biology methods	51
2.5.1 DNA.....	51
2.5.2. RNA	61
2.5.2.1. Trizol RNA purification.....	61
2.5.3. Protein	62
2.5.4. Genetic mapping.....	63
Chapter 3. Multiple type III effectors from <i>Pseudomonas syringae</i> pv. <i>actinidiae</i> induce programmed cell death in <i>Nicotiana</i> species.	68
3.1. Objectives and contributions	68
3.1.1 Objectives	68
3.1.2 Contributions	69
3.2 Results.....	69
3.2.1 Establishment of <i>Psa</i> effector library	69
3.2.2 Subcellular localization of <i>Psa</i> effectors in <i>N. benthamiana</i> leaf epidermal cells	73
3.2.3 Multiple <i>Psa</i> effectors induce HR-like cell death in <i>Nicotiana</i> spp.	77
3.2.4 HR-like cell death induced by multiple <i>Psa</i> effectors partially requires <i>SGT1</i> in <i>Nicotiana benthamiana</i>	81
3.3 Discussion	84
3.3.1. Diverse localization of effectors implicates their various targets in the host cell	85
3.3.2. From HCD-triggering effectors to developing resistance in kiwifruit	88
3.3.3. Comparative analysis of type III effectors from <i>Psa</i> and other <i>Pseudomonas</i> <i>strains</i>	90

Chapter 4 Identification of SOBER1 (Suppressor of AvrBsT-elicited Resistance 1) as the Suppressor of HopZ5-triggered Immunity in <i>Arabidopsis thaliana</i>	92
4.1 Objectives	92
4.2 Results.....	93
4.2.1 The <i>Psa</i> effector HopZ5, a YopJ family acetyltransferase effector, triggers hypersensitive response in <i>Arabidopsis</i> Ct-1	93
4.2.2 Genetic analysis of HopZ5-triggered HR	97
4.2.3 The locus containing the suppressor of HopZ5-triggered HR harbours <i>SOBER1</i> . ..	99
4.2.4. <i>SOBER1</i> suppresses HopZ5-triggered HR but not immunity in <i>Arabidopsis</i> accession Col-0.	107
4.2.5 <i>SOBER1</i> , a putative phospholipase (or α/β carboxylase), requires conserved Ser106 and His192 to suppress HopZ5-triggered HR-like cell death in <i>Nicotiana</i>	111
4.2.6 Natural variation of <i>SOBER1</i> in <i>Arabidopsis</i> accessions strongly correlates with HopZ5-triggered HR.....	115
4.2.7 <i>SOBER1</i> suppresses HCD induced by multiple YopJ family acetyltransferases in <i>Nicotiana</i>	120
4.2.8 Autoacetylation of HopZ5 is stabilized by <i>SOBER1</i> in <i>N. benthamiana</i>	124
4.2.9 <i>Pto</i> DC3000 delivery of HopZ5 does not trigger HR in <i>sober1-3</i> Col.....	126
4.3 Discussion	128
4.3.1. Natural variation in <i>SOBER1</i> -mediated suppression of HR and immunity.	128
4.3.2. Mechanisms by which <i>SOBER1</i> suppresses plant immunity.....	130
4.3.3. Interplay between effectors from pathogens.....	135
Chapter 5 Identification of the genomic locus conferring HopZ5-triggered immunity in <i>Arabidopsis thaliana</i>.....	136
5.1. Objectives and contributions	136
5.1.1. Objectives	136
5.1.2. Contributions.....	136
5.2 Results.....	137
5.2.1. Different genetic components are required for HopZ5-triggered HR and growth restriction in <i>Arabidopsis</i>	137
5.2.2 <i>Arabidopsis</i> accession Ga-0 carries a truncated <i>SOBER1</i> variant and does not trigger HR in response to HopZ5	140
5.2.3 Two different genes may be involved in HopZ5-triggered immunity independent of <i>SOBER1</i>	142

5.2.4 The dominant locus that confers HopZ5 recognition is located on the upper arm of chromosome 3.....	145
5.3 Discussion	147
5.3.1. HopZ5-triggered immunity but not HR may involve EDS1/PAD4-dependent SA regulatory signalling.	147
5.3.2. Two <i>R</i> genes in Ct-1, one <i>R</i> gene in Col-0: do Ct-1 and Col-0 share the same recessive gene?	148
Chapter 6. General discussion and future directions	151
6.1 Understanding the diversity of <i>Psa</i> effectors; dynamic cellular environmental changes by effector proteins	151
6.2 Different signalling pathways are required for HR and immune responses with shared regulators	152
6.3. Possible roles of SOBER1 and SOBER1-like proteins in plant immunity.....	156
References	158