

INFLUENCE OF FATTY ACIDS AND SUGARS RELEASED BY GERMINATING  
SEEDS ON PLANT SPECIES SPECIFIC CONTROL OF *PYTHIUM ULTIMUM* BY  
*ENTEROBACTER CLOACAE*

A Dissertation

Presented to the Faculty of the Graduate School  
of Cornell University

In Partial Fulfillment of the Requirements for the Degree of  
Doctor of Philosophy

by

Pia Sofia T. Windstam

May 2007

© 2007 Pia Sofia T. Windstam

INFLUENCE OF FATTY ACIDS AND SUGARS RELEASED BY GERMINATING SEEDS ON PLANT SPECIES SPECIFIC CONTROL OF *PYTHIUM ULTIMUM* BY *ENTEROBACTER CLOACAE*

Pia Sofia T. Windstam, Ph. D.

Cornell University 2007

*Pythium ultimum* is a devastating pathogen of seeds and seedlings. Germination of pathogen sporangia can be elicited by unsaturated long chain fatty acids that are released by germinating seeds. Sporangial activation and germination are critical for initiating *Pythium* disease development. *Pythium* infection can be prevented by applying the bacterium *Enterobacter cloacae* onto seeds and expression of bacterial fatty acid transport and degradation have been found to be important traits for this control. However, the bacterium is capable of protecting only certain seeds such as cotton and cucumber whereas other seeds, such as corn and pea succumb to *Pythium* infection. It has been postulated that differences in sugar released by seeds may explain this differential protection since sugars are able to repress fatty acid metabolism in *E. cloacae*. Corn and pea seeds are documented as seeds that release high amounts of simple sugars that can repress fatty acid uptake and catabolism. Experiments focused on the temporal release of corn and cucumber seed exudates and their induction of sporangial activation, germination, host colonization and the impact *E. cloacae* had on these pathogen responses while concomitant release of exudate sugars and fatty acids was also analyzed. *E. cloacae* is able to interrupt sporangial activation induced by cucumber seeds, but not in the corn spermosphere. This explains

the differential control by the bacterium, since activation interference directly resulted in suppressed seed colonization. Both corn and cucumber seeds released unsaturated fatty acids as early as 15 min after sowing although quantities from corn exceeded that of cucumber. More importantly, corn seeds released much higher concentrations of simple sugars than cucumber already within 15 min. Quantities detected in corn seed exudate are large enough to completely shut down fatty acid degradation of *E. cloacae*. This provided the first evidence that interference with sporangial activation is the cause for plant protection by *E. cloacae* and seeds not protected by *E. cloacae* are incapable of interfering with sporangial activation. The bacterium does not interfere with sporangial activation because non protected seeds release sugars at such quantities that bacterial fatty acid degradation is repressed.

## BIOGRAPHICAL SKETCH

Pia Sofia Theresa Windstam was born April 23 1976 in Kiruna, Sweden. In this small mining town north of the Arctic circle, she finished high school in 1992 at Parkskolan. She initially attended a 3 year post secondary education program majoring in Nature and Science in Kiruna but later moved to Skellefteå, Sweden where she finished the program in 1995. After a year of working in the local Luossavaara Kirunavaara iron ore mine she started her MS degree at the Swedish University of Agriculture in Alnarp Sweden in 1996. During her MS studies she got funding to do her thesis research in the lab of Eric Nelson at Cornell University in the fall of 2000. After graduating with an MS in Horticulture in 2001 she started the PhD program at Cornell University in the lab of Eric Nelson in August 2001. She completed the requirements for a PhD in the field of Plant Pathology in March 2007.

*To my grandmother Ellen*

*I will always miss you*

## ACKNOWLEDGMENTS

I have always been interested in biology and that was nurtured during my upbringing in a town where most activities revolved around being outside, playing in lakes, woods and the gorgeous mountains found in the north of Sweden. My grandmother Ellen and my father Roger taught me about gardening and the wild berries in the north and whenever we visited my grandparents Ellen would take us outside to fish, pick berries or harvest vegetables. I never imagined as a kid that these interests would some day result in a PhD from Cornell University. My beloved grandmother passed away when I was 12, but I want to believe that she would be proud if she could see me now. I owe her a big thank you for always being patient when teaching an impatient child such as myself.

During my MS studies I got the chance to work with Beatrix Alsanius. She took an interest in me and was the first one to really expose me to research. For that I am forever grateful. I was yet again lucky when coming to Cornell, where I first met with Eric Nelson. Eric has proved to be a fantastic mentor on so many levels. He serves as a great example of what an advisor should be like. Always interested, enthusiastic, encouraging and supportive he also taught me that it is just as important to be a good citizen as it is to be a good scientist.

My committee has some awesome members and many thanks go to them for their time and effort whenever I have asked for it. Janice Thies and Stephen Winans have been great as my official representatives for the minor concentrations of study and Anthony Hay, although not an official member is truly a jewel for stepping in during the A-exam and whenever I have asked him since. There are so many other faculty members that have provided such support throughout the years and I have to thank all of them.

In the Nelson lab, there are some truly great people that I will miss when

leaving Ithaca. Mary Ann, our lab manager, promised me that she would stay throughout my PhD and she has stayed true to her word. She makes sure that the lab is kept organized which makes it very easy to get work done. I have enjoyed many good conversations with the other grads that have passed through the lab and the ones that remain. I feel especially grateful to Mary, Fernando, Mei-Hsing and Allison that were not just labmates but they are also great friends. Thanks also to Megan, Tom, Koji, Daisuke, Holly, Eric, and others that have been in the lab at point or another.

All the friends that I have met during my time living in Ithaca have made my life so much more enjoyable. Thanks to Andrea, Janet (Stickane and Sherman), Doris, Norma, Ana Maria, Evan, Meghan, Dave & Jessie, Mary & Gordon, and all my fellow graduate students in the department. Please forgive me if there is someone that I have forgotten! To Petter and Shannon who have already left Ithaca; you are the best! You guys are so great and supportive. Just getting to know you have made the stay in Ithaca completely worthwhile.

There are folks in the department that are very helpful whenever I have approached them, and without their insight or generosity at one point or another my Cornell experience would not have been the same. Thanks to Monica, Kevin, Jean, and Kent. Before thanking the most important people in my life, my family, I also have to express my gratitude and gratefulness over the opportunity to be a grad student in Ithaca, NY. I will miss Ithaca, the University, the ISSO, and the library dearly. Getting a PhD at Cornell is an exclusive experience and I feel blessed and humbled to be one of the participants.

Much love and thanks to my parents, Roger and Pirjo, and my siblings Maria, Staffan and Heidi for their unconditional love and support through all the years of schooling. I feel extremely grateful to my father for giving up so much so that I and my siblings would have opportunities and chances that were lost to him. I am also

extremely lucky to have become a member of the Ward clan. Barbara, Eileen, Kevin and Penny (and your wild band of boys), Chris – much love goes to you for being so caring and generous. Last, but always first in my heart, I owe everything that I ever have to my love, min käresta, älskling Kim.

## TABLE OF CONTENTS

BIOGRAPHICAL SKETCH	iii
DEDICATION	iv
ACKNOWLEDGEMENTS	v
CHAPTER ONE: INTRODUCTION	
INTRODUCTION TO THE SEED- <i>PYTHIUM ULTIMUM</i> - <i>ENTEROBACTER CLOACAE</i> MODEL SYSTEM	1
<i>PYTHIUM</i> BIOLOGY AND PATHOGENESIS IN THE SPERMOSPHERE	2
<i>Pythium ultimum</i> biology in the spermosphere	2
Temporal dynamics of <i>P. ultimum</i> pathogenesis in the spermosphere	7
Plant derived fatty acid elicitors of <i>P. ultimum</i> sporangia germination	9
<i>ENTEROBACTER CLOACAE</i> CONTROL OF <i>PYTHIUM</i> INFECTION	11
<i>E. cloacae</i> distribution and proliferation in the spermosphere	11
Fatty acid degradation as a control mechanism of <i>E. cloacae</i>	13
Seed exudate components with potential to regulate plant species specificity of <i>E. cloacae</i> protection	15
STATEMENT OF PROBLEM AND HYPOTHESES	21
RESEARCH OBJECTIVES	22
LITERATURE CITED	23

CHAPTER TWO: DIFFERENTIAL INTERFERENCE WITH *PYTHIUM ULTIMUM*  
SPORANGIUM ACTIVATION AND GERMINATION BY *ENTEROBACTER*  
*CLOACAE* IN THE CORN AND CUCUMBER SPERMOSPHERE

ABSTRACT	39
INTRODUCTION	40
MATERIALS AND METHODS	42
Plant material	42
Production and germination of <i>Pythium ultimum</i> sporangia	43
Production of <i>Enterobacter cloacae</i> cells	43
<i>P. ultimum</i> sporangia activation and germination assays	44
Seed colonization incidence	45
Quantification of <i>P. ultimum</i> biomass in the spermosphere by qPCR	45
Statistical analysis	48
RESULTS	49
Seed characterizations	49
Activation and germ tube emergence of <i>P. ultimum</i> sporangia in the spermosphere	49
Effects of cell density and <i>fad</i> mutants of <i>E. cloacae</i> on <i>P. ultimum</i> sporangial activation in the spermosphere	50
<i>P. ultimum</i> colonization of seeds treated with <i>E. cloacae</i>	53
<i>P. ultimum</i> biomass development on corn and cucumber seeds treated with <i>E. cloacae</i>	58
Relationship between sporangium activation and <i>P. ultimum</i> biomass on cucumber seeds	59
DISCUSSION	62
LITERATURE CITED	71

CHAPTER THREE: TEMPORAL EXUDATION OF FATTY ACIDS AND  
SUGARS FROM IMBIBING CORN AND CUCUMBER SEEDS: IMPLICATIONS  
FOR SUGAR REGULATION OF FATTY ACID DEGRADATION BY  
*ENTEROBACTER CLOACE*

ABSTRACT	74
INTRODUCTION	75
MATERIALS AND METHODS	78
Production and germination evaluation of <i>Pythium ultimum</i> sporangia	78
Collection of seed exudate	78
Seed exudate fatty acid extraction, derivatization and analysis	79
Seed exudate sugar derivatization and analysis	80
Statistical analysis	80
RESULTS	81
Corn and cucumber seed exudate yields	81
<i>P. ultimum</i> sporangial germination in response to corn and cucumber seed exudates	81
Fatty acids in seed exudates	83
Sugars in seed exudates	86
DISCUSSION	89
LITERATURE CITED	95
GENERAL CONCLUSIONS	99
APPENDIX	105
Sporangial activation in the corn and cucumber spermosphere at 10% water content	105

Sporangial germination in the corn and cucumber spermosphere at 10% water content	106
<i>inaZ</i> expression in response to fructose and sucrose in culture	107
<i>inaZ</i> expression in response to negative control sugars in culture	108
<i>inaZ</i> expression of a fructose and sucrose responsive promoter in the corn and cotton spermosphere	109
LITERATURE CITED	110

## LIST OF FIGURES

Figure 1.1	Model of seed- <i>Pythium-Enterobacter</i> interactions in the spermosphere	20
Figure 2.1	Temporal imbibition dynamics of corn and cucumber seeds	51
Figure 2.2	<i>P. ultimum</i> sporangial activation in the corn and cucumber spermosphere	52
Figure 2.3	<i>P. ultimum</i> sporangial germination in the corn and cucumber spermosphere	55
Figure 2.4	<i>P. ultimum</i> sporangial activation in the presence of different densities of <i>E. cloacae</i> and <i>E. cloacae</i> strains L1 ( <i>fadL</i> Δ), 31 ( <i>fadAB</i> Δ) and 501 (wt)	56
Figure 2.5	Frequency of corn and cucumber seeds colonized by <i>P. ultimum</i> after being treated with <i>E. cloacae</i> strains L1 ( <i>fadL</i> Δ), 31 ( <i>fadAB</i> Δ) and 501 (wt)	57
Figure 2.6	<i>P. ultimum</i> biomass development on corn and cucumber seeds treated with <i>E. cloacae</i> strains 31 ( <i>fadAB</i> Δ) and 501 (wt)	60
Figure 2.7	<i>P. ultimum</i> biomass development on cucumber seeds treated with <i>E. cloacae</i> strains 31 ( <i>fadAB</i> Δ) and 501 (wt) added 2 hrs post sowing, and seeds treated with 0-600 sporangia per seed	63
Figure 3.1	<i>P. ultimum</i> sporangial germination in response to corn and cucumber seed exudate	85
Figure 3.2	Oleic and linoleic acid released by corn and cucumber seeds	87
Figure 3.3	Saturated fatty acids released by corn and cucumber seeds	88

Figure 3.4 Fructose, glucose, sucrose, and other sugars in corn and  
cucumber seed exudate

93

## LIST OF TABLES

Table 2.1	Regression statistics of the sporangial activation and germination response in the corn and cucumber spermosphere	54
Table 2.2	Regression statistics of <i>P. ultimum</i> sporangial activation, colonization and biomass development on corn and cucumber seeds treated with <i>E. cloacae</i> strains L1 ( <i>fadL</i> Δ), 31 ( <i>fadAB</i> Δ), and 501 (wt)	61
Table 2.3	Regression statistics of <i>P. ultimum</i> biomass on cucumber seeds inoculated with <i>E. cloacae</i> strains 31 ( <i>fadAB</i> Δ) and 501 (wt) 2 hrs post sowing	64
Table 2.4	Regression statistics of <i>P. ultimum</i> biomass on cucumber seeds inoculated with 0-600 sporangia per seed	67
Table 3.1	Corn and cucumber seed exudate yields	82
Table 3.2	Sporangia EC <sub>50</sub> values in response to corn and cucumber seed exudate	84
Table 3.3	Total amount of fatty acids and sugars released by corn and cucumber seed	94