The use of transcriptome and functional analysis to unravel the role played by PhoP and SlyA in the biology of *Pectobacterium brasiliense* 1692

By

Ntombikayise Precious Nkomo

Submitted in partial fulfillment of the requirements for the degree

Doctor of Philosophy (Microbiology)

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

Pretoria

November 2020

SUPERVISOR

Prof. L.N Moleleki Forestry and Agricultural Biotechnology Institute (FABI)

Biochemistry, Genetics& Microbiology (BGM)

University of Pretoria

Lunnon Road, Hatfield

Pretoria

0028

South Africa

CO-SUPERVISORS

Dr. D. Shyntum Forestry and Agricultural Biotechnology Institute (FABI)

Biochemistry, Genetics& Microbiology (BGM)

University of Pretoria

Lunnon Road, Hatfield

Pretoria

0028

South Africa

Dr. D. Bellieny-Rabelo Dept of Ecology & Environmental Sciences

Umea University

6, Linnaeus väg

Umea, Sweden

DECLARATION

I, **Ntombikayise Precious Nkomo,** hereby declare that the thesis is submitted for the PhD. degree in Microbiology at the University of Pretoria, is my own work and has not been previously been submitted by me for a degree at this or any other institution.

SIGNATURE:

DATE:

ACKNOWLEDGEMENTS

First and foremost, I would like to thank God Almighty for being alive and well in these trying times and for making things possible.

Secondly, I want to thank Prof. Moleleki for giving me this opportunity, for helping me grow as a black woman in science. Thank you for the mentorship, guidance, the words of wisdom, most importantly the love. She taught me how to be patient and for that I am truly grateful. I pray God keeps her for many more years to come so that other young girls out there can benefit. Thank you to my co-supervisors Dr. Daniel Bellieny-Rabelo for all the bioinformatics lessons, Dr. Divine Yetafur Shyntum for teaching me all the molecular techniques necessary for me to finish this project. Thank you for the advice and the words of wisdom.

To my family, thank you for the support and the love. Special thanks to Jonathan, Unathi, Nhlamulo and Thabani, you guys are the pillar of my strength, you were there from the beginning. Thank you for your prayers and motivational support.

A special thanks to the Forestry and Agricultural Biotechnology Institute (FABI), Biochemistry, Genetics and Microbiology (BGM) and the University of Pretoria for allowing me to use their facilities.

I am especially grateful to Potato South Africa (PSA) and National Research Foundation (NRF) for the financial support, this work would not have been possible without them.

To my lab mates, Host Pathogen Interactions group (HPI), thank you for the assistance, and support all these years. Special thanks to Dr. Collins Kipngetich Tanui and his wife for prayers. To Jane, Silindile and Brenda thank you for the votes of confidence and prayers.

THESIS SUMMARY

Several members of the soft rot *Pectobacteriaceae* family such as *Pectobacterium brasiliense* 1692 (*Pb*1692) attack a wide range of crops world wide. *P. brasiliense* is a major cause of black leg and soft rot disease in several important plants including beetroot, sugarbeet, cabbage, pepper, cucumber, tomato and potatoes. Currently, no chemical control exists to fight the spread of this disease only methods based on avoiding the pathogen. Despite all the research on *Pectobacterium* species, little is known on how *P. brasiliense* is able to colonise and cause disease in susceptible hosts. Therefore, as part of advancing knowledge on this organism, the objective of this study was to determine the role of two transcriptional regulators SlyA and PhoP in the biology of *Pb*1692.

Chapter One: General literature review on *Pectobacterium* species followed by a review of SlyA and PhoP transcriptional regulators in several *Enterobacteriaceae*.

Chapters Two: In order to better understand the role of the PhoP regulon during the infection process, transcriptional profiles of the wild type *Pb*1692 strain with its isogenic *pho*P deletion mutant were analysed using RNA-sequencing and phenotypic assays. Analysis of the transcriptome data demonstrates that the PhoP regulon is substantially broader than previously suspected affecting the differential expression of more than 400 genes. Additionally, the transcriptome data revealed the repression of T6SS and carbapenem genes, two of the most important traits involved in antibacterial competition, indicating that PhoP negatively regulates T6SS and carbapenem *in planta*. Apart from a role in antibacterial competition, PhoP was shown to play a role in the production of plant cell wall degrading enzymes (PCWDEs), with the repression and activation of some important PCWDEs, PELI/B/Z (pectate lyases) and PEH (polygalacturonase). The broad PhoP regulon included other transcriptional regulators such as EXPR (quorum sensing regulator). In addition to RNA-seq, the study functionally characterized *pho*P mutants based on virulence in susceptible potato tubers and their ability to outcompete *Dickeya dadanti* and attenuated motility. Furthermore, by implementing a time-course RT-qPCR analysis, the study revealed the importance of *pho*P gene in the survival of *Pb*1692 under

acidic conditions encountered in the apoplast. The findings of the RT-qPCR revealed that the relative expression of *pho*P gene was low in the wild type when conditions are still acidic and gradually increased in expression over time when the environment supposedly becomes alkaline. The data demonstrates that PhoP functions as a global regulator in the survival and adaptation of *Pb*1692 *in planta.*

Chapter Three: Bioinformatics analysis was used to understand the evolutionary history and distribution of SlyA in different *Enterobacteriaceae*. In this thesis, multiple sequence alignments of SlyA revealed a 90-100% sequence similarity between the different *Enterobacteriaceae*, indicating that SlyA is conserved among closely related species. Furthermore, the transcriptional profiles of the wild type *Pb*1692 strain with its isogenic *sly*A mutant were analysed using RNA-sequencing and phenotypic assays. Findings from this study revealed a number of genes encoding important virulence factors such as, PCWDEs, biofilm formation as well as other important physiological processes, such as oxidative stress response, assimilation of carbohydrates, iron uptake are all SlyA regulated. The transcriptome data identified genes that are either downregulated or upregulated by *sly*A *in planta*, suggesting that SlyA activated and/or repressed a number of essential genes to adapt and proliferate in a stressful environment. Thus, highlighting that SlyA plays a key role in the adaptive responses of *Pb*1692. This study demonstrated that a functional SlyA is essential for full pathogenicity of *Pb*1692 *in planta.*

Overall, the thesis sheds insights into the regulation of virulence by PhoP and SlyA transcriptional regulators in *Pb*1692 *in planta.*

THESIS OUTPUT

Scientific publications:

Daniel Bellieny-Rabelo**, Ntombikayise Precious Nkomo**, Divine Shyntum, and Lucy Moleleki. Horizontally acquired quorum sensing regulators recruited by the PhoP regulatory network expand host-adaptation repertoire in the phytopathogen *Pectobacterium carotovorum*. **mSystems Vol 5, Issue 1, 24** (DOI: 10.1128/mSystems.00650-19) (Joint first author).

Other Publications:

Divine Yufetar Shyntum, **Ntombikayise Precious Nkomo**, Ntwanano Luann Shingange, Alessanfro Rini Gricia, Daniel Belieny-Rabelo, and Lucy Novungayo Moleleki (2019).The Impact of Type VI Secretion System, Bacteriocins and Antibiotics on Bacterial Competition of *Pectobacterium carotovorum* subsp. *brasiliense* and the Regulation of Carbapenem Biosynthesis by Iron and the Ferric-Uptake Regulator. **Frontiers in Microbiology Vol 10, 2379:1-24.** (DOI: org/10.3389/fmicb.2019.02379).

TABLE OF CONTENTS

SUPERVISOR.. ii

DECLARATION.. iii

ACKNOWLEDGEMENTS.. iv

THESIS SUMMARY..v

LIST OF ABBREVIATIONS.. xii

LIST OF FIGURES..xv

LIST OF TABLES.. xviii

CHAPTER ONE..1

1.1 Introduction..1

1.2 *Enterobacteriaceae*..1

1.3 Stress conditions encountered by bacteria during host infection..5

1.4 Transcription regulation in bacteria..6

1.5 PhoPQ Two Component System (TCS)..8

1.5.1 The role of PhoP in bacterial pathogenesis..9

1.6 MarR family of transcriptional regulators..14

1.6.1 SlyA proteins as regulators of virulence in bacteria..16

1.7 The ‘omics’ era..20

1.8 Importance of the study..21

1.8.1 Study objectives..21

1.9 References..22

CHAPTER TWO..43

PhoP transcriptional regulator of *Pectobacterium brasiliense* 1692 (*Pb*1692) suppresses T6SS and carbapenem production *in planta*..43

2.1 Abstract 43

2.2 Introduction 45

2.3 Results 47

2.3.1 Distribution of PhoP transcriptional regulator in *Dickeya* and *Pectobacterium* species 47

2.3.2 Engineering of *Pb*1692Δ*pho*P mutant strain and whole-transcriptome study.49

2.3.3 *In planta* competition assays on potato tubers 54

2.3.4 PhoP response regulator is required for bacterial motility 55

2.3.5 The PhoP regulators' significance in the early stages of infection 57

2.3.6 Validation of gene expression analysis....................58

2.4 Discussion 60

2.5 Materials and Methods 65

2.5.1 Conditions for growth and bacterial strains 65

2.5.2 *Pb*1692 electro-competent cells....................66

2.5.3 Construction of *Pectobacterium brasiliense* 1692 *pho*P mutant strains....................66

2.5.4 Complementation of the *pho*P mutant 69

2.5.5 *In vitro* growth assays 69

2.5.6 *pho*P gene expression analysis in *Pb*1692 69

2.5.7 RNA extraction from potato tubers and pathogenicity assays....................70

2.5.8 Total RNA quality 70

2.5.9 High-throughput sequencing reads mapping and genome-wide functional annotation 71

2.5.10 Interbacterial *in planta* competition assays in potato tubers 71

2.5.11 Motility assay on agar plates 72

2.5.12 Gene expression analysis....................72

2.5.13 Statistical analysis....................72

2.6 Supplementary data 73

2.7 References 87

CHAPTER THREE 93

The role of SlyA transcriptional regulator in the virulence of *Pectobacterium brasiliense* PBR1692 (*Pb*1692) on potato tubers 93

3.1 Abstract 93

3.2 Introduction 94

3.3 Results 97

3.3.1 MarR/SlyA gene order in *Pectobacterium/ Dickeya* species and the distribution of SlyA homologs in *Enterobacteriaceae* species 97

3.3.2 Generation of *Pb*1692Δ*sly*A mutant strain 100

3.3.3 The mutation of SlyA does not impair bacterial growth 100

3.3.4 Read mapping and transcriptome assembly 101

3.3.5 SlyA is required for pathogenicity of *Pb*1692 and production of some PCWDEs 102

3.3.6 Mutation of *sly*A reduces biofilm formation in *Pb*1692 102

3.3.7 SlyA is essential for an efficient response to H_2O_2 104

3.3.8 Identification and functional annotation of the SlyA regulon in *Pb*1692 during *in planta* infection 105

3.3.9 SlyA regulates the expression of carbohydrate and stress-related genes in *Pb*1692 109

3.3.10 SlyA regulates the expression of metal transport and iron genes in *Pb*1692 109

3.3.11 SlyA regulates the expression of other transcriptional regulators in *Pb*1692 111

3.3.12 SlyA regulates the expression of Hemolysin coregulated protein (Hcp) 111

3.3.13 Expression of virulence-related genes by SlyA 113

3.4 Discussion 115

3.5 Materials and Methods 121

3.5.1 Bacterial strains and growth conditions 121

3.5.2 Construction of a *Pectobacterium brasiliense sly*A mutant 122

3.5.3 Complementation of the *sly*A mutants 124

3.5.4 RNA extraction from potato tubers and pathogenicity assays 124

3.5.5 Cellulase and protease enzyme assays 125

3.5.6 Biofilm formation assay 126

3.5.7 Susceptibility to oxidative stress 126

3.5.8 *In planta* competition assays on potato tubers 126

3.5.9 Library construction, sequencing, reads mapping and genome-wide functional annotation 127

3.5.10 Gene expression analysis (RT-qPCR) 127

3.5.11 Statistical analysis 128

3.6 Supplementary data 129

3.7 References 138

CHAPTER FOUR 146

4.1 Concluding Remarks and Future Perspectives 146

4.2 References 158

LIST OF ABBREVIATIONS

Amp^R	ampicillin resistance marker
AHL	acyl-homoserine-lactone
ASAP	a systematic annotation package for community analysis of genomes
BLAST	basic local alignment search tool
cDNA	complementary DNA
cfu	colony forming unit
CV	crystal violet
DspE/A	effector proteins in *Pectobacterium atrosepticum*
DNA	deoxyribonucleic acid
FABI	forestry and agricultural biotechnology institute
GSEA	Gene set enrichment analysis
hpi	hours post infection
HPI	host-pathogen interaction
H_2O_2	hydrogen peroxide
Kan^R	kanamycin resistance marker
L	ladder
LB	Luria Bertani
LPS	lipopolysaccharides
ml	millilitre
mRNA	messenger RNA
$MgSO_4$	magnesium sulphate

NaCl	sodium chloride
ng	nanogram
NCBI	national centre for biotechnology information
OD	optical density
OCS	One Component System
PCWDEs	Plant cell wall degrading enzymes
PCR	polymerase chain reaction
RT-qPCR	Real time quantitative PCR
QS	Quorum sensing
rpm	revolutions per minute
Rsm	regulator of secondary metabolites
RNA	ribonucleic acid
rRNA	ribosomal RNA
RNA-Seq	RNA sequencing
ROS	Reactive Oxygen Species
subsp	subspecies
SRE	soft rot *Enterobacteriaceae*
SRP	soft rot *Pectobacteriaceae*
spp	species
T1SS	type I secretion system
T2SS	type II secretion system
T3SS	type III secretion system
T6SS	type VI secretion system

TF	transcription factor
TFBS	transcription factor binding site
TCS	Two Component System
V	volts
WT	wild type
pnl	pectin lyase
pel	pectate lyase
peh	polygalacturonase
sec	secretion systems
Prt	protease
°C	degrees Celsius
µg	micro-gram
µl	micro-litre
µm	micro-molar
w/v	weight per volume
WHTH	winged helix-turn-helix
v/v	volume per volume
mM	millimolar
Mg^{2+}	millimolar
v	version
β	beta
α	Alpha
ng/µl	nanogram per microliter

LIST OF FIGURES

Figure 2. 1: Multiple alignment analysis of PhoP response regulator of *Pb*1692, *Dickeya* and *Pectobacterium* species. Sequence alignment done with ClustalOmega and Jalview (Sievers *et al.*, 2011, Waterhouse *et al.*, 2009). Amino acid residues responsible for dimerization and DNA binding were determined by secondary structure analysis in Jalview. The secondary structural elements are shown as red sequences and tubes, while α-helices are shown in red tube and β-strands green tubes. Yellow bars are highly conserved residues. 49

Figure 2. 2 (A): Venn diagram depicting overlap of *Pb*1692 genes between 12 and 24hpi. 238 genes differentially expressed at 12hpi and 137 at 24hpi out of which 116 were common between the two-time points. 50

Figure 2. 3: Pathogenicity assays showing the extent of maceration in potato tubers caused by *Pb*1692Δ*pho*P mutant, *Pb*1692 wild type and *Pb*1692Δ*pho*P-p*pho*P complemented mutant strain after 12 and 24hpi. The standard mean of three biological replicates are represented by the error bars. 53

Figure 2. 4: Interbacterial competition between *Pb*1692 and *D. dadantii* strains on potato tubers. Wild-type *Pb*1692, its isogenic mutant *Pb*1692Δ*pho*P and *Pb*1692Δ*pho*P-p*pho*P complemented strains were co-inoculated with *D. dadantii* for 24 hours. A graph depicting the growth (mean CFU/ml) of *D. dadantii* strain co-inoculated with *Pb*1692 in potato tubers (at a 1:1 ratio). 55

Figure 2.5: Qualitative and quantitative comparison of swimming motility. (**A**)-Attenuated motility of *Pb*1692Δ*pho*P (mutant) relative to *Pb*1692 wild type and *Pb*1692Δ*pho*P-p*pho*P complemented mutant strain. Diameters produced by each bacterial strain on agar plates showing attenuated motility of *Pb*1692Δ*pho*P (mutant) relative to *Pb*1692 wild type and *Pb*1692Δ*pho*P-p*pho*P complemented mutant strain. (**B**)-The diameters were measured in triplicates (three biological replicates) and average values plotted. The statistically significant difference was calculated by the Students't-test. 56

Figure 2. 6: Relative gene expression of the *pho*P gene in *Pb*1692 strain over 24hpi *in planta*. The graph shows the progression of the *pho*P gene from the acidic to alkaline environment. Standard mean deviation is represented by error bars. 58

Figure 2.7: Relative expression levels of genes in the *Pb*1692Δ*pho*P strain relative to *Pb*1692. To calculate the fold change in gene expression levels the cycle threshold (CT) method was used. The fold change in RNA-seq data was determined by (log_2Fold Change). Relative expression calculated using ($2^{-\Delta\Delta CT}$ ± Standard Deviation). 59

Figure S2. 1: PCR amplicons used to generate the *phoP* mutant. Lane 1. DNA ladder 1Kb, 2. Downstream *phoP* PCR fragment, 3. kanamycin cassette PCR product, 4. Upstream *phoP* PCR fragment, 5. Fused product consisting of the downstream, kanamycin and upstream fragment. 6 & 7. Km1 internal kanamycin primers................................... 74

Figure S2.2: Schematic presentation of how *Pb*1692Δ*phoP* mutant strain was generated 75

Figure S2.3: Growth and survival of *Pb*1692 wild type, *Pb*1692Δ*phoP* and *Pb*1692Δ*phoP*-p*phoP* in LB broth for 16 hours at 37°C with agitation at 370 rpm. Growth assay showing that deletion of the *Pb*1692Δ*pho*P gene does not impair the growth of the mutant strain 75

Figure 3. 1(A): Genomic architectures of 100 *Pectobacterium* and *Dickeya* species are represented depicting three genes upstream and three genes downstream of *sly*A gene neighborhoods**.** Arrows represent the direction of transcription. Positive (+) and negative (-) represent direction of transcription of MarR/SlyA. **(B):** Multiple sequence alignment of *sly*A homologs. Leucine (L), Threonine (T), Arginine (R) and Isoleucine (I) residues are similar in all sequences**.** The amino acid sequence alignment was created using ClustalOmega and Jalview. Red rectangles represent SlyA secondary structures (α-helices) and green arrows (β-strands). Yellow bar indicates conserved amino acids. (Sievers *et al.*, 2011, Waterhouse *et al.*, 2009).Generation of *Pb*1692Δ*sly*A mutant strain.................. 100

Figure 3. 2: The growth rate and survival of *Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A in LB broth for 16 hours at 37°C (370 rpm). Three biological replicates were used to plot the growth curve. 101

Figure 3 .3(A): SlyA is essential for the virulence of *Pb*1692 on potato tubers. Virulence assays between *Pb*1692, *Pb*1692Δ*sly*A, and *Pb*1692Δ*sly*A-p*sly*A on potato tubers. Macerated tissue was weighed at 12 and 24hpi. 102

Figure 3. 4 A: Qualitative assays showing biofilm formation after crystal violet staining. **Figure 3.4 B**. Quantitative analysis of biofilm formation by *Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A strains (Pérez-Mendoza, 2011)**.** Three independent experiments were carried out to obtain optical densities (OD_{570}). 103

Figure 3. 5: Reduced survival of *Pb*1692Δ*sly*A mutant in the presence of hydrogen peroxide mimicking ROS. *Pb*1692, *Pb*1692Δ*sly*A, *Pb*1692Δ*sly*A-p*sly*A strains inoculated into LB broth containing 20µM H_2O_2.. 104

Figure 3. 6 (A): Venn diagram depicting overlap of *Pb*1692 genes between 12 and 24hpi. 60 genes were differentially expressed at 12hpi and 102 at 24hpi out of which 13 were common between the two-time points. 106

Figure 3 7: *In planta* competition assays on potato tubers. A graph showing the growth (mean CFUs/ml) of *D. dadantii* 3937 strain when co-inoculated with *Pb*1692 in potato tubers (at a 1:1 ratio). 112

Figure 3.8: Differentially expressed pathogenicity genes in *Pb*1692Δ*sly*A relative to *Pb*1692. cDNA synthesized from 24 hpi RNA samples was used for RT-qPCR validation. The cycle threshold (CT) method was used to analyse the fold change in gene expression levels and data normalization by the ffh gene. The fold change in RNA-seq data determined by (log2FoldChange) was used. Relative expression calculated using ($2^{-\Delta\Delta CT}$ ± Standard Deviation). 114

Figure S3. 1: PCR amplicons used to generate the *slyA* mutant. Lane 1. 1Kb marker, 2. *sly*A downstream PCR fragment, 3. kanamycin cassette PCR product, 4. *sly*A upstream PCR fragment, 5. Fusion product consisting of the downstream, kanamycin and upstream fragment. 6. Km1 primers are internal kanamycin primers. 130

Figure S3.2: Schematic presentation of how *Pb*1692Δ*sly*A mutant strain was generated 131

LIST OF TABLES

Table 2. 1 :List of plasmids and bacterial strains used in the study 65

Table 2. 2: Primers used in this study 67

Table S2 1: Selected DEGs at 12hpi during *in planta* 76

Table S2 2:Summary of selected DEGs during *in planta* infection at 24hpi.................... 80

Table 3. 1:Mapping of RNA-Seq reads from mutant strains on *Pb*1692 reference genome 102

Table 3.2: Total gene count for KEGG annotated genes. (KEGG Orthology) database is designed for molecular functions represented in terms of functional ortholog 110

Table 3.3: List of different bacterial strains and plasmids used in this study 121

Table 3. 4: List of primers used for PCR and qPCR in this study 123

Table S3.1: Differentially expressed genes during *in planta* infection at 12 hpi by SlyA 132

CHAPTER ONE
Literature Review

1.1 Introduction

Potato (*Solanum tuberosum* L.) is one of the staple and well consumed crops in many parts of the world, after maize, rice, and wheat. Potato is a key player in addressing food security worldwide, because it is nutritious and hence the global demand continues to grow (Navarre *et al.*, 2009, Birch *et al.*, 2012) (http://cipotato.org/potato/facts, 2019). In South Africa, potatoes account for approximately 50% of vegetable crops produced (https://www.syngenta.co.za/potatoes). However, almost all potatoes are highly susceptible to some pests and/or pathogens (Oerke *et al.*, 2012). This could be due to severe inbreeding observed over the years, resulting in the accumulation of deleterious and defective alleles (Mullin & Lauer, 1966, Hirsch *et al.*, 2013, Hardigan *et al.*, 2017). Approximately, one hundred and sixty diseases affect potato plants on a daily basis, the majority of these are caused by fungi and viruses while the rest are caused by bacteria (Arora & Khurana, 2004). According to literature, bacteria are regarded as the most serious problem of all pathogens affecting potato seed production (Van der Wolf & De Boer, 2007). Some of the serious problems caused by bacterial pathogens that plaque farmers every year include, blackleg, soft, and stem rot diseases (Charkowski, 2015). Bacterial soft rot symptoms are primarily caused by plant cell wall degrading enzymes (PCWDEs) that degrade the middle lamella together with primary plant cell walls, macerating tissues, and causing a wet, foul smell (Hugouvieux-Cotte-Pattat *et al.*, 2014). Blackleg disease on the other hand, is the spread of the pathogen from a seed tuber to the stem base, causing darkening and decay (Van der Wolf *et al.*, 2017). The following section describes some of the well-studied pathogens that infect potato, causing soft rot and blackleg.

1.2 *Enterobacteriaceae*

The family *Enterobacteriaceae* encompasses a large and diverse group of anaerobic, facultative, and non-spore-forming rod-shaped bacteria (Francino *et al.*, 1992, Oren & Garrity, 2014). The members of *Enterobacteriaceae* are ubiquitous in nature and found in

many different ecological niches, such as soil, water, and in association with living organisms (Brenner & Farmer, 2005, Tyler & Triplett, 2008, Nadarasah & Stavrinides, 2011). Members of the *Enterobacteriaceae* are implicated as pathogens in humans, animals and plants, for example, in pathogens such as *Salmonella enterica, Escherichia coli,* and *Yersinia pestis*, as well as economically important phytopathogens such as members of the genera *Dickeya, Brenneria, Erwinia, Pectobacterium* and *Pantoea* (Bonn & van der Zwet, 2000, Coutinho & Venter, 2009, Croxen & Finlay, 2010, Hauben *et al.*, 1998, Livermore, 2012, Tyler & Triplett, 2008). *Enterobacteriaceae* have been extensively sequenced, accounting for more genome sequences available than any other family (Rosenberg *et al.*, 2013, Charkowski, 2018). However, of importance to this study are the two genera in the *Enterobacteriaceae* family, *Dickeya* and *Pectobacterium*, recently reclassified as Soft Rot *Pectobacteriaceae* (SRP), previously Soft Rot *Enterobacteriaceae* (SRE) (Charkowski *et al.*, 2012, Adeolu *et al.*, 2016, Motyka *et al.*, 2017).

SRP members cause devastating soft rot diseases that affect a variety of plants globally, such as fruits, vegetables, and ornamental plants (Parkinson *et al.*, 2015, Charkowski, 2018, Toth *et al.*, 2003). *Pectobacterium* and *Dickeya* were initially classified in the Erwinia genus in 1920 (*Erwinia carotovora* and *Erwinia chrysanthemi*) (Winslow *et al.*, 1920). Subsequent taxonomic classification within the *Erwinia* taxa prompted the acceptance of the family *Pectobacterium* in 1945 (Waldee, 1945). In 2005, the genus was subsequently divided into *Dickeya* and *Pectobacterium* (Samson *et al.*, 2005). Over the years *Dickeya* and *Pectobacterium* have undergone comprehensive taxonomic revision with each change indicating growth and knowledge gained about phytopathogens (Gardan *et al.*, 2003, Hauben *et al.*, 1998, Zhang *et al.*, 2016). The *Dickeya* genera were split into numerous species, namely *D. chrysanthemi, D. dadantii, D. fangzhongdai, D. dianthicola, D. dieffenbachiae, D. paradisiaca, D. aquatica, D. solani* and *D. zeae* and recently, *D. dieffenbachiae* was renamed *D. dadantii* subsp. *dieffenbachiae* (Samson *et al.*, 2005, Tian *et al.*, 2016, Van Der Wolf *et al.*, 2014, Alič *et al.*, 2017, Parkinson *et al.*, 2014, Brady *et al.*, 2012). The *Pectobacterium* genera were also split into several species, *P. actinidiae, P. aroidearum, P. atrosepticum, P. betavasculorum, P. brasiliense, P. cacticida, P. carotovorum, P. odoriferum, P. parmentieri, P. wasabiae, P. fontis, P. aquaticum, P.*

polaris, P. versatile, P. polonicum, P. peruviense, P. zantedeschiae and a newly proposed species *Candidatus Pectobacterium maceratum* (Zhang *et al.*, 2016, Nabhan *et al.*, 2013, Gardan *et al.*, 2003, Duarte *et al.*, 2004, Alcorn *et al.*, 1991, Hauben *et al.*, 1998, Khayi *et al.*, 2016, Pedron *et al.*, 2019, Oulghazi *et al.*, 2019, Dees *et al.*, 2017, Sarfraz *et al.*, 2018, Shirshikov *et al.*, 2018, Portier *et al.*, 2019, Waleron *et al.*, 2019b, Faye *et al.*, 2018, Waleron *et al.*, 2019a).

As scientists continue to discover, more *Pectobacterium* species have been added to the list and some of the prominent additions include *P. brasiliense*, which was initially isolated from potatoes in Brazil and classified as *E. carotovora* subsp. *brasiliensis* (Duarte *et al.*, 2004). Since its isolation, it has surfaced as a global problem with reports from the United States, Canada, Poland, the Netherlands, Kenya, Switzerland, South Korea, Japan, Italy, Algeria and Mexico (de Werra *et al.*, 2015, Onkendi *et al.*, 2016, Caruso *et al.*, 2016, Naas *et al.*, 2018, Mejía-Sánchez *et al.*, 2019, Onkendi & Moleleki, 2014, De Boer *et al.*, 2012, Leite *et al.*, 2014, Waleron *et al.*, 2015, Choi & Kim, 2013). In 2006/2007 potato growing season, blackleg outbreaks caused by *P. brasiliense* occurred throughout South Africa devastating the potato industry (van der Merwe *et al.*, 2010). In Korea, Choi and colleagues found *P. brasiliense* (*Pb*) growing on paprika (Choi & Kim, 2013). The same pathogen (*Pb*) was later found to cause soft rot in *Daucus carota* subsp. sativus, *Capsicum annum L.,* and *Ornithogalum* spp., (Meng *et al.*, 2017, Nabhan *et al.*, 2012). The name *P. carotovorum* subsp. *brasiliense* has been used in several publications and only recently, *P. brasiliense* was proposed as a species rather than a subspecies of *P. carotovorum* (Glasner *et al.*, 2008, Marquez-Villavicencio *et al.*, 2011, Van der Wolf *et al.*, 2017, Portier *et al.*, 2019). Currently, *P. brasiliense* is a major blackleg and soft rot pathogen in a number of regions and appears to be among the most destructive of the *Pectobacterium* species on potato (Van der Wolf *et al.*, 2017, Onkendi *et al.*, 2016, de Werra *et al.*, 2015, Meng *et al.*, 2017, Onkendi *et al.*, 2014).

Research has indicated that *Pectobacterium* species encompass strains with a wide geographic distribution, diverse metabolic capabilities as well as a wide host range (Ma *et al.*, 2007, Perombelon & Kelman, 1980, Boccara *et al.*, 1991). However, some of the *Pectobacterium* species are an exception and have a narrow host range, for example, *P.*

atrosepticum, P. polaris, and *P. parmentieri* are only limited to potato, while *P. betavasculorum* has only been reported on sugar beet (Khayi *et al.*, 2016, Gardan *et al.*, 2003, Dees *et al.*, 2017). *Dickeya* and *Pectobacterium* can also co-exist as mixed populations in infected tissues, presenting a huge challenge because both these genera tend to infect similar hosts producing similar symptoms, making it nearly impossible to identify the causal agent (Kim *et al.*, 2009, Charkowski, 2018, Ma *et al.*, 2007). In the monocot and dicot families noted as hosts for either *Dickeya* or *Pectobacterium*, just four monocot and six dicot families were accounted for as hosts for the two genera, these include tobacco, broccoli, potato and tomato (Ma *et al.*, 2007, Brady *et al.*, 2012).

Extensive research on *Pectobacterium* species infecting vegetable crops and ornamental plants has resulted in several virulence factors being identified namely, PCWDEs, polysaccharides, motility, iron acquisition systems, bacterial secretion systems, phytotoxins, and the type IV pilus (Davidsson *et al.*, 2013, Nykyri *et al.*, 2012, Toth *et al.*, 2006). SRPs are classified as brute force pathogens based on the ability to produce massive amounts of PCWDEs (Toth *et al.*, 2003). A recent study on 84 *Pectobacterium* genomes revealed that the core pathogenicity factors, PCWDEs were highly conserved, while factors such as siderophores, flagellin, polysaccharides, secretion systems were varied, highlighting the importance of PCWDEs (Li *et al.*, 2018). As reported by Liu and colleagues in 2008, the production of PCWDEs is dependent on the bacterial population through quorum sensing (QS) regulation (Liu *et al.*, 2008). QS, therefore, is able to provide the organism with an upper hand, by holding off on the activation of plant defenses because the presence of PCWDEs activate plant defense responses (Mäe *et al.*, 2001). Apart from the PCWDEs, *Pectobacterium* species are capable of secreting other virulence factors such as antimicrobial compounds, carbapenem, carotovoricin, carocins and toxins (Davidsson *et al.*, 2013, Charkowski *et al.*, 2012, Bell *et al.*, 2004, McGowan *et al.*, 1996, Chan *et al.*, 2009).

Soft rot and blackleg caused by SRP are liable for some of the most devastating losses in potato yield, during harvest, post-harvest as well as during storage (Mansfield *et al.*, 2012, Chung *et al.*, 2013). There are currently no effective control methods available to curb

either soft rot or blackleg (Olsen *et al.*, 2006, Czajkowski *et al.*, 2011). Several methods have been explored, yet the level of progress has been variable, for example, seed certification schemes, avoiding contamination and breeding for resistance have been partially successful (Chung *et al.*, 2013, Czajkowski *et al.*, 2011). Chemical methods, although highly successful, are not desirable due to resistance development, effective methods are therefore required to manage these diseases (Czajkowski *et al.*, 2011). One promising approach currently being explored is phage biocontrol (Gill & Garg, 2014, Cui *et al.*, 2019). Recent research shows that applying specific bacteriophages is gaining momentum as control strategies for phytopathogenic bacteria (Balogh *et al.*, 2010, Czajkowski, 2016, Voronina *et al.*, 2019). Pathogens survive in various environmental conditions, for example on plant surfaces, inside humans, animals as well as in the soil and water. If a pathogen resides on a plant surface, expression of virulence determinants might not be necessary. However, once the pathogen infects and starts reproducing virulence factors are needed for acquiring food, establishing bacterial population, evasion of host defenses, thus, ensuring appropriate disease development (Andresen, 2012). Therefore, survival of a pathogen depends on signals from the environment, coordinated through a complex regulatory network (Charkowski *et al.*, 2012). The following section of this chapter will, therefore, review virulence regulatory mechanisms in some of the *Enterobacteriaceae* species. Before discussing these complex regulatory networks, it is important to note the different environmental conditions encountered by human, animal and plant pathogens from their initial contact with the host.

1.3 Stress conditions encountered by bacteria during host infection

During host cell colonization, invading bacteria encounter different types of stress due to the unfriendly host environmental conditions. For example, in human and animal pathogens acidic pH is found in the gastrointestinal, macrophages and endocytic vesicles, while in plant pathogens acidic and oxidative stress is experienced in the apoplast (Grubor *et al.*, 2006, Foster, 1999, Reverchon & Nasser, 2013). In addition to acidic pH in the apoplast, plant defenses include an influx of calcium ions and a massive efflux of potassium ions, accumulating cytoplasmic solutes inside the host resulting in variable osmotic pressure (Wood, 2010, Garcia-Brugger *et al.*, 2006). On the other hand, human and animal

pathogens are also exposed to osmotic stress during the infection process, affecting the structure and chemistry of bacterial cells (Wood, 2011). Furthermore, human and animal pathogens may also be exposed to reactive oxygen species (ROS), produced as a feature of ordinary aerobic metabolism during respiration, however, high concentrations of ROS are deleterious to several cellular components (Kim *et al.*, 1999, Fones & Preston, 2012). Likewise plant pathogens are also exposed to ROS within the plant, ROS is considered to be the earliest sign of the plant defense mechanism, produced by the oxidative burst, which is a rapid production of ROS in response to invading pathogens (Wojtaszek, 1997). Furthermore, humans, animals and plants produce antimicrobial peptides (AMPs) as part of their defense mechanism (Hancock & Diamond, 2000, Ouellette, 2011, Iqbal *et al.*, 2019). AMPs modify the lipid A domain of the lipopolysaccharides conferring resistance in Gram-negative bacteria (Yeaman & Yount, 2003). Lastly, another important line of host defense is to starve invading pathogens, thereby, limiting the amount of available nutrients creating stressful conditions for the pathogens (Hennigar & McClung, 2016). Therefore, to survive the different environments, bacteria have evolved complex systems that not only detect these stresses but also trigger cellular responses enabling survival under these conditions (Fang *et al.*, 2016). The following section will discuss regulatory mechanisms utilized by bacteria to escape hostile conditions.

1.4 Transcription regulation in bacteria

Transcriptional regulation is fundamental to an organism's development and how it responds to the changing environmental conditions (Gao *et al.*, 2018). Transcriptional gene regulation is controlled by several regulatory components, namely transcription factors (TFs), sigma factors, promoters and transcription binding sites. This review will focus on TFs, which are regulatory proteins that act together with an initiation complex to either activate or repress transcription of target genes via specific binding called transcription factor binding sites (TFBSs) in regulatory regions (Browning & Busby, 2004). TFs are essential cellular components that regulate the first stage of gene expression, the transcription of DNA to RNA, to ensure the correct expression of genes (Balleza *et al.*, 2008). For example, RcsCDB transcription factor is an activator of SPI-2 expression and a repressor of flagellar (*flhDC*) gene expression (Seshasayee *et al.*, 2011, Helmann, 2002,

Gama-Castro *et al.*, 2008, Stormo, 2000, Wang *et al.*, 2007, Weinstein-Fischer & Altuvia, 2007, Erhardt & Dersch, 2015). If functioning as a repressor, the transcription factor binds to DNA targets overlapping important components in the intended promoter regions. This binding occludes access to the RNA polymerase, whereas those functioning as activators bind to DNA targets found upstream of the target promoter, activating transcription initiation (Bintu *et al.*, 2005, Lee *et al.*, 2012, Browning *et al.*, 2019). A distinguishing characteristic of TFs is that they consist of a DNA-binding domain either in the amino or carboxyl terminal and a regulatory domain that is involved in ligand binding. The ligand in question is often a metabolite or physicochemical signal (Babu & Teichmann, 2003, Rivera-Gómez *et al.*, 2011, Martínez-Antonio *et al.*, 2006, Rivera-Gómez *et al.*, 2017). TFs are further classified and grouped based on their structure and DNA-binding domains (Pabo & Sauer, 1992, Kummerfeld & Teichmann, 2006, Perez-Rueda *et al.*, 2018). These structural domains are crucial in recognizing and binding specific DNA sequences as well as providing clues to their functions (Aravind *et al.*, 2005). The most commonly studied family of TFs is the winged helix-turn-helix (WHTH), mainly because they are abundant in both bacteria and archaea (Hernandez-Guerrero *et al.*, 2019, Seshasayee *et al.*, 2006, Berg *et al.*, 2005). To date, studies have shown that transcription factor families are common in bacteria and archaea, indicating that the mechanisms influencing gene expression in each of these groups of prokaryotes could be identical (Lemmens *et al.*, 2019).

Expression of virulence determinants is entirely the ability of a pathogen to sense several environmental signals, for example, pH, osmolarity, metals, or temperature, all of which are encountered upon interaction with or within the host (Thomas & Wigneshweraraj, 2014). Microorganisms have more than one environmental sensing system that enables rapid changes in gene expression and protein function (Miller *et al.*, 2007). These systems range from very simple transcriptional regulators called one component systems (OCS) to more complex ones like the two component system (TCS) (Ulrich *et al.*, 2005). OCS are some of the oldest and simplest signal transduction systems, where the signaling protein has a DNA binding domain (N-terminal input) and a periplasmic sensory output doiman (C-terminal) (Ulrich *et al.*, 2005, Krell, 2018, Cock & Whitworth, 2007). An example of

such a system is CadC transcriptional regulator, found in *E. coli* and *Vibrio cholerae* (Rhee *et al.*, 2005). Another OCS found in *Pectobacteria* species is the quorum-sensing regulator protein ExpR (Whitehead *et al.*, 2001). However, the only challenge with OCS is that they exclusively sense and respond to signals in the cytosol adapting to the conditions internally while neglecting cues coming from the environment (Ulrich *et al.*, 2005). To overcome this challenge, bacteria have evolved more complex environmental sensing systems, such as the TCS.

1.5 PhoPQ Two Component System (TCS)

Complex regulatory networks such as TCS are used by bacteria to regulate multiple virulence determinants at various stages of infection (Charkowski *et al.*, 2012). These TCSs are made up of two key proteins, a membrane sensor histidine kinase (HK) also known as a histidine protein kinase (HPK), and a corresponding response regulator (Robinson *et al.*, 2000, Le Breton *et al.*, 2003). The sensor HPK comprises of an N-terminal periplasmic sensor domain and a cytoplasmic C-terminal kinase core, which harbors the HPK domain (Grebe & Stock, 1999, Zschiedrich *et al.*, 2016). These regulatory systems are found in several organisms and are involved in adaptive responses such as chemotaxis, resistance to antibiotics and control of developmental pathways (Stock *et al.*, 1989, Prost *et al.*, 2007, Bhagirath *et al.*, 2019).

This review, will focus on the PhoPQ TCS, which belongs to the OmpR/PhoB subfamily, present in a diverse group of Gram-negative bacteria, from animals to plant pathogens (Nguyen *et al.*, 2015, Groisman, 2001, Galperin, 2010, Tsai *et al.*, 2019). This is a major subfamily of response regulators, classified based on the sequences found on the C-terminal domain, whose structure has a WHTH motif (Sidote *et al.*, 2008, Nguyen *et al.*, 2015, Kondo *et al.*, 1997, Martínez-Hackert & Stock, 1997). The receiver domain of the OmpR/PhoB response regulators have a highly conserved α4–β5–α5 dimeric interface (Kenney, 2002, Bachhawat & Stock, 2007). A characteristic of the α4–β5–α5 region is that it shares salt bridge interactions and hydrophobic contacts required for homodimerization in various bacterial species. It has also been documented that virulence in bacteria could be sent into disarray by interfering with the highly conserved, α4–β5–α5 region (Gao *et al.*, 2007, Toro-Roman *et al.*, 2005, Menon & Wang, 2011). The sensor PhoQ is made up of a

periplasmic domain that senses environmental changes and transfers the signal to the HPK domain. The HPK domain is the one responsible for the active state of PhoP (DNA binding regulator), phosphorylating PhoP in the process (Choi & Groisman, 2016). Once phosphorylated, PhoP binds specific target genes that control virulence in many Gram-negative pathogens, such as *S. enterica*, *Shigella flexneri*, *Y. pestis, E. coli, Xanthomonas campestris* and *Pectobacterium* species (Fields *et al.*, 1989, Dalebroux & Miller, 2014, Moss *et al.*, 2000b, Oyston *et al.*, 2000, Alteri *et al.*, 2011, Vadyvaloo *et al.*, 2015, Flego *et al.*, 2000).

PhoP response regulator and its kinase sensor PhoQ are widely conserved in bacteria, indicating that this system has evolved to regulate virulence in pathogens and at the same time maintaining its core function in non-pathogenic bacteria (Winfield & Groisman, 2004). PhoP also controls a massive regulatory network in response to different environmental stimuli, namely acidic pH, low magnesium, calcium concentrations and/or presence of AMPs in both human and animal pathogens (Soncini *et al.*, 1996, Choi & Groisman, 2016). Likewise in plant pathogens, PhoP has also been associated with regulation of several virulence factors in response to AMPs, low magnesium and acidic pH present inside the plant as defense mechanisms (Haque & Tsuyumu, 2005, Llama-Palacios *et al.*, 2005b). This indicates that the conditions encountered by plant pathogens are also similar in human and animal pathogens. In addition to direct regulation of several genes, PhoP also regulates other TCS such as (RstA/RstB), (SsrB/SpiR) and (PmrA/PmrB) (Minagawa *et al.*, 2003, Bijlsma & Groisman, 2005, Kato & Groisman, 2004). In the next section, several factors under which PhoP is either activated or repressed during host-pathogen interactions are highlighted.

1.5.1 The role of PhoP in bacterial pathogenesis

The PhoP response regulator is extensively studied in *S. enterica* serovar Typhimurium, where it regulates genes responsible for adaptation to various harsh environmental conditions (Bader *et al.*, 2005, Prost *et al.*, 2007, Miller *et al.*, 1989a, Miller & Mekalanos, 1990, Bearson *et al.*, 1998, Fields *et al.*, 1989, Choi & Groisman, 2016). PhoP is encoded

by the *phoP* locus, initially identified in *Salmonella* as controlling the expression of nonspecific acid phosphatase genes (Kier *et al.*, 1979). PhoP is highly conserved and present in several *Enterobacteriaceae*, controlling both ancestral and some horizontally acquired genes that differ from species to species. (Perez *et al.*, 2009, Perez & Groisman, 2009, Harari *et al.*, 2010). Homologs of PhoP have been identified in *Pseudomonas, E. coli, S. flexneri, Serratia, Yersinia, Erwinia, Dickeya* and in several Gram-negative species, both non-pathogenic and pathogenic (Macfarlane *et al.*, 1999, Groisman *et al.*, 1992, Kasahara *et al.*, 1992, Fields *et al.*, 1989, Groisman *et al.*, 1989, Venkatesh *et al.*, 2006, Haque *et al.*, 2008). In *P. carotovorum,* a homolog of PhoP, PehR was identified (Flego *et al.*, 2000).

The role of PhoP in the pathogenesis of a variety of bacterial pathogens has been the subject of intense study in recent years. As stated by Groisman and colleagues, the primary function of PhoP appears to be the regulation of genes involved in magnesium transport. In *S. enterica* serovar Typhimurium, PhoP controls over 40 genes required for virulence, and one of the genes is *mgt*A, a PhoP-activated gene required for survival in low Mg^{2+} (Groisman, 2001, Prost *et al.*, 2007). Furthermore, a study by Choi and colleagues in 2017 identified a species-specific protein UgtL under the control of PhoP, which was horizontally acquired by *Salmonella*. PhoP activates *ugl*T gene in response to low Mg^{2+} (Choi & Groisman, 2017). Similar to what was observed in *Salmonella*, a transcriptome profile of *Y. pestis* revealed a total of over 700 genes differentially expressed by PhoP under low magnesium conditions, some of these genes included, magnesium transport systems, stress response genes and metabolism-related genes (Zhou *et al.*, 2005).

Many independent studies have found that PhoP is also crucial for the survival of a variety of pathogens in acidic pH. For example, in *E. coli*, PhoP differentially regulates the transcription of hundreds of genes that control acidic pH adaptability (Alteri *et al.*, 2011, Tu *et al.*, 2016). Comparable to PhoP of *E. coli*, in *Salmonella*, PhoP associated genes were activated by the presence of acidic pH (Choi & Groisman, 2016, Lippa & Goulian, 2009). In a study by Liu and colleagues in 2019, PhoP of *Y. pestis* was able to regulate the production of biofilm by controlling transcription of the *waaAE-coaD* operon under acidic

conditions, a major determinant of *Y. pestis* biofilm production (Vadyvaloo *et al.*, 2015, Liu & Zheng, 2019). Also, a *pho*P defective strain of *Y. pestis* is reported to have no abilities to form biofilms i*n vivo* suggesting that PhoP is important for promoting a transmissible infection in fleas under acidic conditions (Rebeil *et al.*, 2013).

Apart from low magnesium and acidic conditions, PhoP was demonstrated to aid the survival of bacteria when exposed to AMPs (Hitchen *et al.*, 2002, Oyston *et al.*, 2000). The expression of over 80 genes in *S. enterica* involved in resistance to AMPs is regulated directly or indirectly through PmrAB TCS by PhoPQ (Gunn, 2008). In *S. enterica* serovar Typhimurium, PhoP controls genes required for resistance to AMPs (Navarre *et al.*, 2005). Additionally, in *Salmonella*. PhoP activates the *ugl*T gene, responsible for resistance to AMPs (Choi & Groisman, 2017). Another PhoP activated gene in *Salmonella*, *mgt*A essential for resistance to polymyxin B, a cationic AMP. The *mgt*A gene was identified as a gene that regulate modifications of phosphate residues in the lipid A portion of lipopolysaccharide (LPS) resulting in resistance to antibiotics (Koprivnjak & Peschel, 2011, Guina *et al.*, 2000, Herrera *et al.*, 2010). In *E. coli,* a transcriptome profiling of PhoB (PhoP homolog) revealed genes associated with palmitoylated lipid A, lipopolysaccharides, outer-membrane proteins, and membrane lipids, all the genes reported to confer resistance to AMPs (Lamarche & Harel, 2010, Yang *et al.*, 2012, Hwan Baek & Yup Lee, 2006).

The PhoP regulator has also been shown to perceive osmotic upshift in *E. coli* and *Salmonella*, another stimulus to which bacteria is exposed to when infecting the host (section 1.3). This osmotic shift was also found to promote the recovery of bacterial cells under hyperosmotic stress in *E. coli* (Zhuge *et al.*, 2018, Yuan *et al.*, 2017). Adaptation to the osmotic shift in *E. coli* was mediated by MgrB membrane protein, which is part of the PhoP regulon (Lippa & Goulian, 2009). In addition to osmotic stress, PhoP regulates genes involved in oxidative stress response (Golubeva & Slauch, 2006). As stated in section 1.3, oxidative stress is another important stress experienced by microorganisms inside the host

(Fones & Preston, 2012). As observed in *Y. pestis*, *pho*P mutants showed increased sensitivity when exposed to oxidative stress, while a transcriptome profile of PhoP regulon in *Salmonella* revealed s*odCI*, as a gene responsible for protection against oxidative stress response (Oyston *et al.*, 2000, Golubeva & Slauch, 2006, Erickson *et al.*, 2011).On the other hand, plant pathogens, encounter a variety of stresses mounted by the plant as a defense mechanism, namely, acidic pH, AMPs, magnesium, oxidative and osmotic stress (Grignon & Sentenac, 1991, Jiang *et al.*, 2016, Wood, 2010, Gloux *et al.*, 2005, Véscovi *et al.*, 1996). Plant pathogens grow and survive in the apoplast where the pH is acidic (Grignon & Sentenac, 1991). In *D. dadantii* (formerly *E. chrysanthemi* strain 3937), PhoP controls the expression of genes associated with the production of PCWDEs in response to acidic pH, such as pectate lyases and polygalacturonases (Haque & Tsuyumu, 2005, Dubey *et al.*, 2016, Wei *et al.*, 2019). *In vitro* studies of extracellular enzymes in *X. campestris* pv. *campestris* showed that PCWDEs, namely polygalacturonase (PG) were diminished significantly in the *pho*P mutants resulting in reduced virulence in citrus trees (Wei *et al.*, 2019). A few studies indicated that polygalacturonases are secreted only when the pH is acidic and therefore required in the early stages of infection (Chun & Huber, 1998, Shevchik *et al.*, 1997). In *Erwinia amylovora,* strains defective of phoP (*pho*P mutants) were resistant to strong acidic pH when compared to the wild type (pH 5 conditions) (Nakka *et al.*, 2010). Conversely in *Xanthomonas* and *D. dadantii* (formerly *E. chrysanthemi*), *pho*P mutants were unable to tolerate strong acidic conditions (Lee *et al.*, 2008). These results suggest that in *E. amylovora*, PhoP acts as a negative regulator while in *Xanthomonas* and *E. chrysanthemi,* PhoP positively regulates genes in acidic conditions.

Regarding AMPs, in plant pathogens, it is widely accepted that AMPs are an essential component of innate immunity in plants (Hancock & Diamond, 2000). Several studies in plant pathogens have demonstrated the role of PhoP in regulating genes involved in resistance to AMPs. For example, in *Xanthomonas*, *pho*P defective strains were susceptible to AMPs whereas the wild type strain was not affected (Lee *et al.*, 2008). Similarly, in *D. dadantii pho*P mutants were also susceptible to AMPs. Likewise, *pho*P mutants of *P. versatile* were also susceptible to an AMP polymyxin B (Llama-Palacios *et al.*, 2005b).

Yet again, in *D. dadantii, pho*P defective strains were more sensitive to the AMP magainin II but this time in the presence of acidic pH (Haque & Tsuyumu, 2005). Similar to *D. dadantii*, *pho*P mutants of *E. amylovora* exposed to cecropin A (an AMP) at acidic pH were more sensitive than the wild type (Nakka *et al.*, 2010). These results suggest that to activate PhoP, both acidic pH and AMPs are required in plant pathogens.

It is well documented that magnesium concentrations in nature is one of the signals that control PhoP, this was observed in some studies where, several genes under the control of PhoP were activated in the presence of low Mg^{2+} (Véscovi *et al.*, 1996, Smith & Maguire, 1998, Minagawa *et al.*, 2003). A study by Llama-Palacios and colleagues suggested that *E. chrysanthemi* (known as *D. dadantii*) encounters low Mg^{2+} in the apoplast inducing PhoP which in turn assists the pathogen to tolerate several stresses. In the same study by Llama-Palacios, *pho*P defective mutants of *D. dadantii* showed impaired growth in low magnesium whereas the wild type was not affected (Llama-Palacios *et al.*, 2005b). Similarly in *P. vesartile, peh*R defective mutants (PhoP homolog), showed impaired growth under Mg^{2+} limiting conditions (Kravchenko *et al.*, 2020).

Past studies have indicated that PhoP is also necessary for the regulation of certain virulence factors, such as the Type III Secretion Systems (T3SS) and motility (Lee *et al.*, 2008, Wei *et al.*, 2019). T3SS is an essential pathogenicity determinant, that translocates effector proteins to suppress host defense mechanisms (Xia *et al.*, 2016). In *E. amylovora*, PhoP negatively regulates T3SS which is in contrast to what was reported for *Xanthomonas*, where PhoP positively regulates T3SS (Nakka *et al.*, 2010, Zhao *et al.*, 2009). Despite numerous studies on the PhoP regulator, there is little information about the regulation of bacterial motility by PhoP. However, in *Salmonella*, where PhoP has been extensively studied, PhoP activated motility by repressing transcription of flagellar genes (Adams *et al.*, 2001, Park *et al.*, 2015). In *E. coli* mutant strains of PhoP were drastically attenuated in motility than the wild type strain (Tu *et al.*, 2016). Similarly, to *pho*P mutants of *E. coli*, *pho*P mutants of *X. citri* subsp. *citri.* were drastically reduced in motility whereas

the wild type was not affected (Wei *et al.*, 2019). Providing proof that PhoP is a regulator of motility in some pathogenic bacteria.

1.6 MarR family of transcriptional regulators

Another equally important family of transcriptional regulators is the multiple antibiotic resistance regulator family (MarR) (Ellison & Miller, 2006). MarR family members control a number of cellular responses, namely resistance to organic solvents, oxidative stress, antibiotics and household disinfectants (Alekshun & Levy, 1999). Members of this family act as sensors of different environmental signals, a trait important for pathogenic bacteria (Wilkinson & Grove, 2006). Homologs of MarR occur throughout bacterial and archeal domains with more than 100 homologs being investigated for their physiological roles (Wilkinson & Grove, 2006). The MarR proteins are members of a WHTH DNA-binding protein family of transcriptional factors. The MarR proteins exist as dimers and bind to specific DNA sequences allowing MarR proteins to recognize palindromic sequences in the target promoters, leading to either transcriptional repression or activation (Wilkinson & Grove, 2006, Zhu *et al.*, 2017). All MarR proteins share a common WHTH motif, however, biochemical analysis has shown that they all have distinct mechanisms depending on the type of ligand involved (Deochand & Grove, 2017, Perera & Grove, 2010). Also, it is more likely that inconsistency in sequences is responsible for differences in DNA sequence recognition and interactions observed within this family (Wu *et al.*, 2003). Almost every MarR protein consist of both α-helices (six) and β-strands (three) with topology α1–α2–β1–α3–α4–β2–β3–α5–α6, except for UrtR (urate-responsive transcriptional regulator) (Perera & Grove, 2011). Previous studies of MarR family crystal structures show that the MarR proteins form a DNA binding lobe and a dimerization region (Bordelon *et al.*, 2006). The regions forming a dimer are said to be hydrophobic, a characteristic highly conserved amongst the MarR family (Kumaraswami *et al.*, 2009).

Homologs of MarR include, (RovA) from *Yersinia* spp., (EmrR, HpaR, HosA, MarR) *E. coli*, (PecS) *E. chrysanthemi* (now known as *D. dadantii*), (Rap) *S. marcescens*, (Hor) *E. carotovora* (known as *P. carotovora),* (SlyA) in *S. enterica, Enterococcus feacalis, D. zeae*

and *D. dadantii* 3739 (Perera & Grove, 2010, Heroven *et al.*, 2004, Reverchon *et al.*, 1994, Cathelyn *et al.*, 2006, Sjöblom *et al.*, 2008, Cabezas *et al.*, 2018, Michaux *et al.*, 2011, Zhou *et al.*, 2016, Zou *et al.*, 2012, Xiong *et al.*, 2000, Galán *et al.*, 2003, Ferrándiz *et al.*, 2005). What makes this family so important is that they react to specific ligands leading to differential gene expression (Perera & Grove, 2010). Some of the ligands known to activate MarR proteins include urate, a ligand for PecS. During host-pathogen interactions, plants counteract the effects of the invading pathogens by producing ROS and generating urate as a byproduct. Urate serves as a signaling compound for plant colonization (Del Río *et al.*, 2002). OhrR, a MarR homolog found in *X. campestris* and *Bacillus subtilis* binds organic hydroperoxide during host-pathogen interactions, utilizing hydrogen peroxide as its ligand to activate OhrR. In *P. carotovorum* SCC3193, (Hor) is activated by an acidic environment in the apoplast, resulting in the activation of genes essential for production of some PCWDEs. (Fuangthong *et al.*, 2001, Sukchawalit *et al.*, 2001). In some of these homologs, specific ligands have not been identified. (Alekshun *et al.*, 2001, Panmanee *et al.*, 2002, Perera & Grove, 2010, Sjöblom *et al.*, 2008).

Gene expression by MarR homologs is most commonly regulated through several mechanisms, for example, it can be regulated either through ligand binding or chemical oxidation of specific cysteine residues, resulting in a conformational change leading to the attenuation of DNA binding (Deochand & Grove, 2017). For example, a MarR homolog in *Yersinia* (RovA) competes with H-NS, a global repressor, for binding to the promoter region of invasin at 26°C (a major adhesion and invasion factor in *Yersinia*). At slightly higher temperatures (37°C), H-NS represses the expression of invasin because RovA is present at low concentrations in the cell and cannot efficiently compete for binding with H-NS. Another, MarR homolog, SlyA utilizes a countersilencing mechanism to positively regulate genes. In this mechanism, SlyA binds to target promoters restricting access to the global repressor H-NS (Will *et al.*, 2014, Perez *et al.*, 2008). This countersilencing role by H-NS has been observed in some Enterobacteria species, such as *E. coli*, *Salmonella, Shigella, Yersinia, Serratia* and *Pectobacterium* (*Erwinia*) (Lithgow *et al.*, 2007, Corbett *et al.*, 2007, Will *et al.*, 2014, Perez *et al.*, 2008, Navarre *et al.*, 2005, Weatherspoon-Griffin & Wing, 2016, Haque *et al.*, 2009, Zou *et al.*, 2012, Thomson *et al.*, 1997, Ellison *et al.*,

2004, Ellison & Miller, 2006, Deochand & Grove, 2017). Furthermore, other MarR homologs, for example, OhrR and SlyA can act as activators through RNA polymerase stabilization or by competing for DNA binding with a repressor (Oh *et al.*, 2007, Di Fiore *et al.*, 2009, Curran *et al.*, 2017).

1.6.1 SlyA proteins as regulators of virulence in bacteria

SlyA, a member of the MarR family of transcriptional regulators belonging to *Enterobacteriaceae* family, is well known for its role in stress response (Spory *et al.*, 2002, Buchmeier *et al.*, 1997, Curran *et al.*, 2017). It was initially discovered in *Salmonella* Typhimurium where it was described as a key component for bacterial survival in macrophages and resistance to oxidative stress response (Buchmeier *et al.*, 1997). Subsequent research has shown that SlyA is an essential regulatory protein controlling the expression of various virulence genes, many of which are yet to be identified (Ludwig *et al.*, 1995, Ballesteros *et al.*, 2018). There are over 130 SlyA homologs identified in bacteria and they appear to have the same crystal structure as the other MarR family members (Michaux *et al.*, 2011). An elucidated structure of SlyA from *E. feacalis* demonstrates that both MarR and SlyA show local divergence in sequence and structure, allowing them to respond to different signal molecules and bind DNA targets using different strategies (Wu *et al.*, 2003). In recent years, the role played by SlyA in the pathogenesis of different bacterial pathogens has been the focus of intense study, with many studies demonstrating that SlyA is crucial for virulence in those pathogens (Reverchon *et al.*, 1994, Thomson *et al.*, 1997, Weatherspoon-Griffin & Wing, 2016).

Several independent studies have explained the significance of SlyA in the pathogenesis of *Salmonella*. In *Salmonella,* during its infective cycle, it faces several stresses, such as oxidative stress response, low iron levels and low pH (Hébrard *et al.*, 2009). To overcome the stresses and adapt to the environment, *Salmonella* utilizes SlyA transcriptional regulator which recognizes inverted repeats in DNA sequences and regulates gene expression (Cabezas *et al.*, 2018). Importantly, transcriptome studies of SlyA in *S.* Typhimurium under oxidative stress conditions revealed the expression of various stress response genes that were either positively or negatively regulated by SlyA (Stapleton *et*

al., 2002, Okada *et al.*, 2007, Cabezas *et al.*, 2018). Furthermore, RNA seq data of *S.* Typhimurium (*sly*A mutant) revealed downregulation of outer membrane proteins when exposed to both sodium hypochlorite (NaOCl) and hydrogen peroxide (H_2O_2) (Cabezas *et al.*, 2018). Further work in the plant pathogen *D. dadantii,* demonstrated that *sly*A mutants were highly sensitive to hydrogen peroxide when compared to the wild type strain, suggesting that SlyA regulate genes involved in resistance to ROS (Haque *et al.*, 2009). Similarly, mutants of the Hor regulator in *P. carotovorum subsp. carotovorum,* were also sensitive to hydrogen peroxide (Thomson *et al.*, 1997). These studies suggest that SlyA is important for oxidative stress tolerance and indicate that several proteins are directly or indirectly regulated by SlyA to protect pathogens from toxic ROS.

In several plant pathogens, studies show that SlyA has a crucial role in virulence regulation, for example, SlyA controls the production of some PCWDEs (Haque *et al.*, 2009, Zhou *et al.*, 2016). PCWDEs are some of the many virulence determinants catalyzing the disintegration of pectin, the principal component of the plant cell wall. (Mashavha, 2013, Park *et al.*, 2012). In a study by Zhou and colleagues, they found that a mutant of *sly*A increased the expression of protease enzymes. Interestingly, they also detected downregulation of the Type II Secretion System (T2SS) required for secretion of the majority of the PCWDEs (Zhou *et al.*, 2016, Charkowski *et al.*, 2012). Likewise, in *D. dadantii* 3937, a SlyA homolog (PecS) encodes proteins responsible for the production of PCWDEs, such proteases, pectate lyases secreted by the Type I Secretion System (T1SS), pectinases and cellulase secreted by the T2SS (Pédron *et al.*, 2018, Haque *et al.*, 2009, Haque & Tsuyumu, 2005). Also, in *P. carotovora* subsp. *carotovora*, Hor, controls the expression of cellulase and polygalacturonase enzymes (Thomson *et al.,* 1997, Sjöblom *et al.,* 2008).

The role of SlyA in pathogenesis extends beyond the control of PCWDEs. In *D. zeae*, SlyA positively regulated more than 600 genes and negatively controlled the expression of more than 300 genes encoding a variety of cellular functions, for example, efflux systems, biosynthesis of zeamines, c-di-GMP metabolism and secretion systems such as T3SS and T6SS (Zhou *et al.*, 2016). In *D. dadantii*, transcriptome analysis of PecS, a MarR homolog

revealed that the PecS regulon accounts for about 13% of the genome. The PecS regulon encompasses genes involved in metabolism, transport, T3SS and T6SS (Pédron *et al.*, 2018). In a study by Zou and colleagues in 2012, SlyA activated hypersensitive response and pathogenicity genes (*hrp*) together with HrpL (master regulator of T3SS) in *D. dadantii*. The study also revealed that SlyA is key for the formation of pellicles, which was associated with the regulatory effect of SlyA on *hrp* genes (Zou *et al.*, 2012). Similarly, in *X. campestris* pv. c*ampestris,* HpaR (MarR homolog) regulates the transcription of over 400 genes, some of which are involved in pathogenicity, flagellar biosynthesis, metabolism of biomacromolecules and hypersensitive response in plants (Pan *et al.*, 2018). Demonstrating that SlyA controls a diversity of virulence factors.

An equally important virulence determinant in pathogens is motility. It is involved in the survival and virulence of bacteria (Hossain & Tsuyumu, 2006). In *D. dadantii* 3937, SlyA utilized another transcriptional regulator, PhoP, to control motility genes in the presence of low magnesium. In the same study additional RT-qPCR analysis was conducted and further revealed that motility genes (*mot*A and *mot*B) were not under the control of SlyA but PhoP transcriptional regulator in low magnesium concentrations (Haque *et al.*, 2015). In another study profiling PecS of *D. dadantii*, PecS was shown to repress flagellar biosynthesis genes and *pec*S mutants were hypermotile *in vitro* (Hommais *et al.*, 2008). Comparable with *sly*A mutants in *D. zeae*, *sly*A mutants displayed enhanced swimming motility suggesting that SlyA repressed motility genes (Zhou *et al.*, 2016, Hu *et al.*, 2018). Conversely, in *E. coli*, HosA (MarR homolog), positively controlled motility through *fli*C, a flagellin structural gene (Ferrándiz *et al.*, 2005).

Studies on biofilm formation are important towards understanding the effects of biofilms in virulence. Biofilms provide protection against environmental conditions, such as oxidative stress (Gambino & Cappitelli, 2016). Identification of main biofilm determinants in several pathogens has led to possible anti-biofilm strategies (Römling & Balsalobre, 2012, Rendueles & Ghigo, 2012). Previous research has explored the role of transcriptional regulators in biofilm formation (Raetz & Whitfield, 2002, Chalabaev *et al.*, 2014). In the plant pathogen, *D. zeae*, *sly*A mutants abolished the ability of *D. zeae* to form biofilms

(Zhou *et al.*, 2016). While in *Y. pestis* it was shown that RovA, enhances the virulence of this pathogen, by downregulating genes involved in biofilm formation (Liu *et al.*, 2016). In *S.* Typhimurium, SlyA indirectly controls biofilm formation by negatively regulating CsgD, a master regulator involved in biofilm formation under sodium hypochlorite stress (Ogasawara *et al.*, 2011). These studies show that SlyA can regulate different metabolic pathways to enhance bacterial pathogenesis.

SlyA has also been associated with resistance to AMPs (Navarre *et al.*, 2005). Past investigations demonstrate that genes under the control of SlyA encode periplasm proteins, outer membrane and also implicated in AMP resistance, for example in *S. enterica*, transcriptome analysis of SlyA revealed genes with functions associated with bacterial envelope and resistance to AMPs (Navarre *et al.*, 2005). Further studies in *Salmonella* revealed that SlyA controls the transcription of *ugt*L, a gene required for resistance to AMPs (Shi *et al.*, 2004). In *S. marcescens,* Rap (SlyA homolog) regulates carbapenem production, by activating the expression of *carR* gene (Slater *et al.*, 2003). Carbapenems are antibiotics that belong to the β-lactam antibiotic family produced by bacteria and can kill closely related species (Coulthurst *et al.*, 2005). Furthermore, a study done by Shyntum and coworkers, showed that *P. brasiliense* 1692 regulates the production of carbapenem using SlyA in the presence of oxygen and iron (Shyntum *et al.*, 2018). Finally, SlyA appears to regulate similar genes in different species, this could be attributed to the high degree of conservation. SlyA orthologs, Rap (*Serratia*), Hor (*Pectobacterium*) and RovA in *Yersinia*, are conserved in almost every species of *Enterobacteriaceae* (Thomson *et al.*, 1997). The level of conservation suggests that SlyA ancestry plays a key role in the regulatory network organization of *Enterobacteriaceae* (Thomson *et al.*, 1997, Will *et al.*, 2019). The findings from these studies support the view that SlyA is important in the virulence of many pathogens and may have a role to play in regulating other unknown virulence factors. Future studies aimed at unravelling the members of the SlyA regulon will, therefore, help to understand how pathogens can survive and reproduce within the host's hostile environment.

1.7 The 'omics' era

Advances in sequencing technology have revolutionized and rapidly accelerated the rate at which data from genomes is generated (Li *et al*., 2011). To date, complete or draft genome sequences of various *Pectobacterium* spp. are now available (www.genomesonline.org). Information from the genome sequences has provided a significant platform for understanding among other factors, the taxonomy, genetic diversity, horizontal gene transfer (HGT) patterns and the genes responsible for various traits in phytopathogens (Zhang *et al.*, 2015, Chan *et al.*, 2012, Vinatzer *et al.*, 2014, Xu & Wang, 2018). Furthermore, functional and comparative genomics have greatly altered the way in which scientistics currently utilize available genome sequences to understand the mechanisms used by various *Pectobacterium* spp. and other SRP in attacking and colonizing their hosts as well as the defense mechanisms of the hosts (Li *et al.*, 2018, Toth *et al.*, 2006, Pritchard *et al.*, 2016).

Currently, studies focused on profiling the transcriptomes of various phytopathogens are revealing a range of novel information to the scientific community. Transcriptomic studies have the capacity to aid biologists in understanding various activities within cells and tissues, unravelling the set of genes involved in the pathosystem of various pathogens such as *Pectobacterium* spp. and also in understanding the functions of each region of the genome (Wang *et al.*, 2009, Bellieny-Rabelo *et al.*, 2019, Liu *et al.*, 2019, Gorshkov *et al.*, 2018). Over the years studies focusing on transcriptomics have transformed from microarray techniques to RNA-seq technology (Metzker, 2009, Rao *et al.*, 2019). Transcript analysis using microarray and RNA-seq platforms have provided a substantial amount of data from pathogens subjected to various conditions. The RNA-seq technology has the ability to improve on genomic annotation quality, fill in the gaps in genome sequences and also be more sensitive (Croucher & Thomson, 2010, Rai *et al.*, 2018). It also enables one to unravel many novel genetic regions within the bacterial genome as well as to understand both translated and un-translated regions. The ability of RNA-seq to utilize small quantities of RNA, eliminate cloning and cDNA amplification steps (through Helicos technology) has made RNA-seq as the high throughput sequencing method with which the entire transcriptome can be studied (Wang *et al.*, 2009, Han *et al.*, 2015).

1.8 Importance of the study

Recent studies have demonstrated that *P. brasiliense* is a virulent Soft Rot *Pectobacteriaceae* responsible for significant crop losses in potatoes (Waleron *et al.*, 2015, Onkendi *et al.*, 2014). However, up to date, most of these studies have focused only on the etiology, epidemiology and the occurrence of *Pb* (Mejía-Sánchez *et al.*, 2019, Van der Wolf *et al.*, 2017, Czajkowski *et al.*, 2012). A better understanding of regulatory networks controlling virulence determinants in *Pb*1692 is lacking.

1.8.1 Study objectives

Thus, based on the above, the specific objectives of this study were;

1. To construct mutant strains of *Pb*1692 PhoP and *Pb*1692 SlyA transcriptional regulators
2. To identify and comparatively analyze genes under PhoP and SlyA regulation
3. To characterize and investigate the role of PhoP and SlyA regulators in the biology of *Pb*1692

1.9 References

Adams, P., Fowler, R., Kinsella, N., Howell, G., Farris, M., Coote, P., and O'Connor, C.D. (2001) Proteomic detection of PhoPQ-and acid-mediated repression of *Salmonella* motility. *PROTEOMICS: International Edition* **1**: 597-607.

Adeolu, M., Alnajar, S., Naushad, S., and Gupta, R.S. (2016) Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. *Int. J. Syst. Evol. Microbiol.* **66**: 5575-5599.

Adriaenssens, E.M., Ackermann, H.-W., Anany, H., Blasdel, B., Connerton, I.F., Goulding, D., Griffiths, M.W., Hooton, S.P., Kutter, E.M., and Kropinski, A.M. (2012a) A suggested new bacteriophage genus:"Viunalikevirus". *Arch. Virol.* **157**: 2035-2046.

Adriaenssens, E.M., Van Vaerenbergh, J., Vandenheuvel, D., Dunon, V., Ceyssens, P.-J., De Proft, M., Kropinski, A.M., Noben, J.-P., Maes, M., and Lavigne, R. (2012b) T4-related bacteriophage LIMEstone isolates for the control of soft rot on potato caused by '*Dickeya solani*'. *PLoS One* **7**.

Alcorn, S., Orum, T., Steigerwalt, A.G., Foster, J.L., Fogleman, J.C., and Brenner, D.J. (1991) Taxonomy and pathogenicity of *Erwinia cacticida* sp. nov. *Int. J. Syst. Evol. Microbiol.* **41**: 197-212.

Alekshun, M.N., and Levy, S.B. (1999) The mar regulon: multiple resistance to antibiotics and other toxic chemicals. *Trends Microbiol.* **7**: 410-413.

Alekshun, M.N., Levy, S.B., Mealy, T.R., Seaton, B.A., and Head, J.F. (2001) The crystal structure of MarR, a regulator of multiple antibiotic resistance, at 2.3 Å resolution. *Nat. Struct. Mol. Biol.* **8**: 710-714.

Alič, Š., Naglič, T., Tušek-Žnidarič, M., Peterka, M., Ravnikar, M., and Dreo, T. (2017) Putative new species of the genus *Dickeya* as major soft rot pathogens in Phalaenopsis orchid production. *Plant Pathol.* **66**: 1357-1368.

Alteri, C.J., Lindner, J.R., Reiss, D.J., Smith, S.N., and Mobley, H.L. (2011) The broadly conserved regulator PhoP links pathogen virulence and membrane potential in *Escherichia coli. Mol. Microbiol.* **82**: 145-163.

Aravind, L., Anantharaman, V., Balaji, S., Babu, M.M., and Iyer, L.M. (2005) The many faces of the helix-turn-helix domain: transcription regulation and beyond. *FEMS Microbiol. Rev.* **29**: 231-262.

Arora, R., and Khurana, S.P., (2004) Major fungal and bacterial diseases of potato and their management. In: Fruit and vegetable diseases. Springer, pp. 189-231.

Babu, M.M., and Teichmann, S.A. (2003) Functional determinants of transcription factors in *Escherichia coli*: protein families and binding sites. *Trends Genet.* **19**: 75-79.

Bachhawat, P., and Stock, A.M. (2007) Crystal structures of the receiver domain of the response regulator PhoP from *Escherichia coli* in the absence and presence of the phosphoryl analog beryllofluoride. *J. Bacteriol.* **189**: 5987-5995.

Bader, M.W., Sanowar, S., Daley, M.E., Schneider, A.R., Cho, U., Xu, W., Klevit, R.E., Le Moual, H., and Miller, S.I. (2005) Recognition of antimicrobial peptides by a bacterial sensor kinase. *Cell* **122**: 461-472.

Ballesteros, M.F., Lamberti, M.F.T., Farizano, J.V., Pescaretti, M.M., and Delgado, M.A. (2018) Regulatory effect of SlyA on rcsB expression in *Salmonella* Typhimurium. *J. Bacteriol.*

Balleza, E., Lopez-Bojorquez, L.N., Martínez-Antonio, A., Resendis-Antonio, O., Lozada-Chávez, I., Balderas-Martínez, Y.I., Encarnación, S., and Collado-Vides, J. (2008) Regulation by transcription factors in bacteria: beyond description. *FEMS microbiology reviews* **33**: 133-151.

Balogh, B., Jones, J.B., Iriarte, F., and Momol, M. (2010) Phage therapy for plant disease control. *Curr. Pharm. Biotechnol.* **11**: 48-57.

Bearson, B.L., Wilson, L., and Foster, J.W. (1998) A low pH-inducible, PhoPQ-dependent acid tolerance response protects *Salmonella* typhimurium against inorganic acid stress. *J. Bacteriol.* **180**: 2409-2417.

Bell, K., Sebaihia, M., Pritchard, L., Holden, M., Hyman, L., Holeva, M., Thomson, N., Bentley, S., Churcher, L., and Mungall, K. (2004) Genome sequence of the enterobacterial phytopathogen *Erwinia carotovora* subsp. *atroseptica* and characterization of virulence factors. *Proc. Natl. Acad. Sci. U. S. A.* **101**: 11105-11110.

Bellieny-Rabelo, D., Tanui, C.K., Miguel, N., Kwenda, S., Shyntum, D.Y., and Moleleki, L.N. (2019) Transcriptome and comparative genomics analyses reveal new functional insights on key determinants of pathogenesis and interbacterial competition in *Pectobacterium* and *Dickeya* spp. *Appl. Environ. Microbiol.* **85**: e02050-02018.

Berg, G., Krechel, A., Ditz, M., Sikora, R.A., Ulrich, A., and Hallmann, J. (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiol. Ecol.* **51**: 215-229.

Bhagirath, A.Y., Li, Y., Patidar, R., Yerex, K., Ma, X., Kumar, A., and Duan, K. (2019) Two component regulatory systems and antibiotic resistance in Gram-negative pathogens. *Int. J. Mol. Sci.* **20**: 1781.

Bijlsma, J.J., and Groisman, E.A. (2005) The PhoP/PhoQ system controls the intramacrophage type three secretion system of *Salmonella enterica*. *Mol. Microbiol.* **57**: 85-96.

Bintu, L., Buchler, N.E., Garcia, H.G., Gerland, U., Hwa, T., Kondev, J., and Phillips, R. (2005) Transcriptional regulation by the numbers: models. *Curr. Opin. Genet. Dev.* **15**: 116-124.

Birch, P.R., Bryan, G., Fenton, B., Gilroy, E.M., Hein, I., Jones, J.T., Prashar, A., Taylor, M.A., Torrance, L., and Toth, I.K. (2012) Crops that feed the world 8: Potato: are the trends of increased global production sustainable? *Food Security* **4**: 477-508.

Boccara, M., Vedel, R., Lalo, D., Lebrun, M.-H., and Lafay, J.F. (1991) Genetic diversity and host range in strains of *Erwinia chrysanthemi. Mol. Plant-Microbe Interact* **4**: 293-299.

Bonn, W.G., and van der Zwet, T. (2000) Distribution and economic importance of fire blight. *Fire blight: the disease and its causative agent, Erwinia amylovora* **37**: 53.

Bordelon, T., Wilkinson, S.P., Grove, A., and Newcomer, M.E. (2006) The crystal structure of the transcriptional regulator HucR from Deinococcus radiodurans reveals a repressor preconfigured for DNA binding. *J. Mol. Biol.* **360**: 168-177.

Brady, C.L., Cleenwerck, I., Denman, S., Venter, S.N., Rodríguez-Palenzuela, P., Coutinho, T.A., and De Vos, P. (2012) Proposal to reclassify *Brenneria quercina* (Hildebrand and Schroth 1967) Hauben et al. 1999 into a new genus, Lonsdalea gen. nov., as Lonsdalea quercina comb. nov., descriptions of Lonsdalea quercina subsp. quercina comb. nov., Lonsdalea quercina subsp. iberica subsp. nov. and Lonsdalea quercina subsp. britannica subsp. nov., emendation of the description of the genus Brenneria, reclassification of *Dickeya dieffenbachiae* as *Dickeya dadantii* subsp. *dieffenbachiae* comb. nov., and emendation of the description of *Dickeya dadantii. Int. J. Syst. Evol. Microbiol.* **62**: 1592-1602.

Brenner, D., and Farmer, J. (2005) Order XIII."Enterobacteriales". *Bergey's Manual of Systematic Bacteriology* **2**: 587-607.

Browning, D.F., and Busby, S.J. (2004) The regulation of bacterial transcription initiation. *Nature Reviews Microbiology* **2**: 57.

Browning, D.F., Butala, M., and Busby, S.J. (2019) Bacterial Transcription Factors: Regulation by Pick 'N'Mix. *J. Mol. Biol.*

Buchmeier, N., Bossie, S., Chen, C.-Y., Fang, F.C., Guiney, D.G., and Libby, S.J. (1997) SlyA, a transcriptional regulator of *Salmonella* typhimurium, is required for resistance to oxidative stress and is expressed in the intracellular environment of macrophages. *Infect. Immun.* **65**: 3725-3730.

Cabezas, C.E., Briones, A.C., Aguirre, C., Pardo-Esté, C., Castro-Severyn, J., Salinas, C.R., Baquedano, M.S., Hidalgo, A.A., Fuentes, J.A., and Morales, E.H. (2018) The transcription factor SlyA from *Salmonella* Typhimurium regulates genes in response to hydrogen peroxide and sodium hypochlorite. *Res. Microbiol.*

Caruso, A., Licciardello, G., La Rosa, R., Catara, V., and Bella, P. (2016) Mixed infection of *Pectobacterium carotovorum* subsp. *carotovorum* and *P. carotovorum* subsp. *brasiliensis* in tomato stem rot in Italy. *J. Plant Pathol.* **98**: 661-665.

Cathelyn, J.S., Crosby, S.D., Lathem, W.W., Goldman, W.E., and Miller, V.L. (2006) RovA, a global regulator of *Yersinia pestis*, specifically required for bubonic plague. *Proceedings of the National Academy of Sciences* **103**: 13514-13519.

Chalabaev, S., Chauhan, A., Novikov, A., Iyer, P., Szczesny, M., Beloin, C., Caroff, M., and Ghigo, J.-M. (2014) Biofilms formed by gram-negative bacteria undergo increased lipid a palmitoylation, enhancing in vivo survival. *MBio* **5**: e01116-01114.

Chan, J.Z., Halachev, M.R., Loman, N.J., Constantinidou, C., and Pallen, M.J. (2012) Defining bacterial species in the genomic era: insights from the genus A*cinetobacter*. *BMC Microbiol.* **12**: 302.

Chan, Y.-c., Wu, H.-P., and Chuang, D.-y. (2009) Extracellular secretion of Carocin S1 in *Pectobacterium carotovorum* subsp. *carotovorum* occurs via the type III secretion system integral to the bacterial flagellum. *BMC Microbiol.* **9**: 181.

Charkowski, A., Blanco, C., Condemine, G., Expert, D., Franza, T., Hayes, C., Hugouvieux-Cotte-Pattat, N., Solanilla, E.L., Low, D., and Moleleki, L. (2012) The role of secretion systems and small molecules in soft-rot enterobacteriaceae pathogenicity. *Annu. Rev. Phytopathol.* **50**: 425-449.

Charkowski, A.O. (2015) Biology and control of *Pectobacterium* in potato. *American Journal of Potato Research* **92**: 223-229.

Charkowski, A.O. (2018) The changing face of bacterial soft-rot diseases. *Annu. Rev. Phytopathol.* **56**: 269-288.
Choi, J., and Groisman, E.A. (2016) Acidic pH sensing in the bacterial cytoplasm is required for *Salmonella* virulence. *Mol. Microbiol.* **101**: 1024-1038.
Choi, J., and Groisman, E.A. (2017) Activation of master virulence regulator PhoP in acidic pH requires the *Salmonella*-specific protein UgtL. *Sci. Signal.* **10**: eaan6284.
Choi, O., and Kim, J. (2013) *Pectobacterium carotovorum* subsp. *brasiliense* causing soft rot on paprika in Korea. *J. Phytopathol.* **161**: 125-127.
Chun, J.-P., and Huber, D.J. (1998) Polygalacturonase-mediated solubilization and depolymerization of pectic polymers in tomato fruit cell walls: regulation by pH and ionic conditions. *Plant Physiol.* **117**: 1293-1299.
Chung, Y.S., Goeser, N.J., Cai, X., and Jansky, S. (2013) The effect of long term storage on bacterial soft rot resistance in potato. *American journal of potato research* **90**: 351-356.
Cock, P.J., and Whitworth, D.E. (2007) Evolution of prokaryotic two-component system signaling pathways: gene fusions and fissions. *Mol. Biol. Evol.* **24**: 2355-2357.
Corbett, D., Bennett, H.J., Askar, H., Green, J., and Roberts, I.S. (2007) SlyA and H-NS regulate transcription of the *Escherichia coli* K5 capsule gene cluster, and expression of slyA in Escherichia coli is temperature-dependent, positively autoregulated, and independent of H-NS. *J. Biol. Chem.* **282**: 33326-33335.
Coulthurst, S.J., Barnard, A.M., and Salmond, G.P. (2005) Regulation and biosynthesis of carbapenem antibiotics in bacteria. *Nature Reviews Microbiology* **3**: 295.
Coutinho, T.A., and Venter, S.N. (2009) *Pantoea ananatis*: an unconventional plant pathogen. *Mol. Plant Pathol.* **10**: 325-335.
Croucher, N.J., and Thomson, N.R. (2010) Studying bacterial transcriptomes using RNA-seq. *Curr. Opin. Microbiol.* **13**: 619-624.
Croxen, M.A., and Finlay, B.B. (2010) Molecular mechanisms of *Escherichia coli* pathogenicity. *Nature Reviews Microbiology* **8**: 26-38.
Cui, W., He, P., Munir, S., He, P., He, Y., Li, X., Yang, L., Wang, B., Wu, Y., and He, P. (2019) Biocontrol of Soft Rot of Chinese Cabbage Using an Endophytic Bacterial Strain. *Front. Microbiol.* **10**.
Curran, T.D., Abacha, F., Hibberd, S.P., Rolfe, M.D., Lacey, M.M., and Green, J. (2017) Identification of new members of the *Escherichia coli* K-12 MG1655 SlyA regulon. *Microbiology* **163**: 400.
Czajkowski, R. (2016) Bacteriophages of soft rot Enterobacteriaceae—a minireview. *FEMS Microbiol. Lett.* **363**.
Czajkowski, R., De Boer, W., Van Veen, J., and Van der Wolf, J. (2012) Studies on the interaction between the biocontrol agent, S*erratia plymuthica* A30, and blackleg-causing Dickeya sp.(biovar 3) in potato (Solanum tuberosum). *Plant Pathol.* **61**: 677-688.
Czajkowski, R., Ozymko, Z., and Lojkowska, E. (2014a) Isolation and characterization of novel soilborne lytic bacteriophages infecting *Dickeya* spp. biovar 3 ('*D. solani'*). *Plant Pathol.* **63**: 758-772.
Czajkowski, R., Ozymko, Z., Zwirowski, S., and Lojkowska, E. (2014b) Complete genome sequence of a broad-host-range lytic *Dickeya* spp. bacteriophage ϕD5. *Arch. Virol.* **159**: 3153-3155.

Czajkowski, R., Pérombelon, M., Jafra, S., Lojkowska, E., Potrykus, M., Van Der Wolf, J., and Sledz, W. (2015) Detection, identification and differentiation of P*ectobacterium* and *Dickeya* species causing potato blackleg and tuber soft rot: a review. *Ann. Appl. Biol.* **166**: 18-38.

Czajkowski, R., Perombelon, M.C., van Veen, J.A., and van der Wolf, J.M. (2011) Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. *Plant Pathol.* **60**: 999-1013.

Dalebroux, Z.D., and Miller, S.I. (2014) *Salmonellae* PhoPQ regulation of the outer membrane to resist innate immunity. *Curr. Opin. Microbiol.* **17**: 106-113.

Davidsson, P.R., Kariola, T., Niemi, O., and Palva, E.T. (2013) Pathogenicity of and plant immunity to soft rot pectobacteria. *Front Plant Sci* **4**: 191.

De Boer, S., Li, X., and Ward, L. (2012) *Pectobacterium* spp. associated with bacterial stem rot syndrome of potato in Canada. *Phytopathology* **102**: 937-947.

de Werra, P., Bussereau, F., Keiser, A., and Ziegler, D. (2015) First report of potato blackleg caused by *Pectobacterium carotovorum* subsp. *brasiliense* in Switzerland. *Plant Dis.* **99**: 10.1094.

Dees, M.W., Lysøe, E., Rossmann, S., Perminow, J., and Brurberg, M.B. (2017) *Pectobacterium polaris* sp. nov., isolated from potato (Solanum tuberosum). *Int. J. Syst. Evol. Microbiol.* **67**: 5222-5229.

Del Río, L.A., Corpas, F.J., Sandalio, L.M., Palma, J.M., Gómez, M., and Barroso, J.B. (2002) Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. *J. Exp. Bot.* **53**: 1255-1272.

Denner, F., Venter, S., and Niederwieser, J. (2012) Guide to potato production in South Africa. *Agricultural Research Council-Vegetable and Ornamental Plant Institute, Pretoria*.

Deochand, D.K., and Grove, A. (2017) MarR family transcription factors: dynamic variations on a common scaffold. *Crit. Rev. Biochem. Mol. Biol.* **52**: 595-613.

Di Fiore, A., Fiorentino, G., Vitale, R.M., Ronca, R., Amodeo, P., Pedone, C., Bartolucci, S., and De Simone, G. (2009) Structural analysis of BldR from Sulfolobus solfataricus provides insights into the molecular basis of transcriptional activation in Archaea by MarR family proteins. *J. Mol. Biol.* **388**: 559-569.

Dolan, K.T., Duguid, E.M., and He, C. (2011) Crystal structures of SlyA protein, a master virulence regulator of *Salmonella,* in free and DNA-bound states. *J. Biol. Chem.* **286**: 22178-22185.

Duarte, V., De Boer, S., Ward, L., and Oliveira, A. (2004) Characterization of atypical *Erwinia carotovora* strains causing blackleg of potato in Brazil. *J. Appl. Microbiol.* **96**: 535-545.

Dubey, A.K., Yadav, S., Kumar, M., Anand, G., and Yadav, D. (2016) Molecular biology of microbial pectate lyase: a review. *Biotechnology Journal International*: 1-26.

Eayre, C., Bartz, J., and Concelmo, D. (1995) Bacteriophages of *Erwinia carotovora* and *Erwinia* ananas isolated from freshwater lakes. *Plant Dis.* **79**: 801-804.

Ellison, D.W., Lawrenz, M.B., and Miller, V.L. (2004) Invasin and beyond: regulation of *Yersinia* virulence by RovA. *Trends Microbiol.* **12**: 296-300.

Ellison, D.W., and Miller, V.L. (2006) Regulation of virulence by members of the MarR/SlyA family. *Curr. Opin. Microbiol.* **9**: 153-159.

Erhardt, M., and Dersch, P. (2015) Regulatory principles governing Salmonella and *Yersinia* virulence. *Front. Microbiol.* **6**: 949.
Erickson, D.L., Russell, C.W., Johnson, K.L., Hileman, T., and Stewart, R.M. (2011) PhoP and OxyR transcriptional regulators contribute to *Yersinia pestis* virulence and survival within Galleria mellonella. *Microb. Pathog.* **51**: 389-395.
Fang, F.C., Frawley, E.R., Tapscott, T., and Vázquez-Torres, A. (2016) Bacterial stress responses during host infection. *Cell host & microbe* **20**: 133-143.
Faye, P., Bertrand, C., Pédron, J., and Barny, M.-A. (2018) Draft genomes of "*Pectobacterium peruviense*" strains isolated from fresh water in France. *Standards in Genomic Sciences* **13**: 27.
Ferrándiz, M.-J., Bishop, K., Williams, P., and Withers, H. (2005) HosA, a member of the SlyA family, regulates motility in enteropathogenic *Escherichia coli*. *Infect. Immun.* **73**: 1684-1694.
Fields, P., Groisman, E., and Heffron, F. (1989) *Salmonella* locus that controls resistance to antibacterial proteins from phagocytic cells. *Science* **85**: 5189-5193.

Flego, D., Marits, R., Eriksson, A.R., Kõiv, V., Karlsson, M.-B., Heikinheimo, R., and Palva, E.T. (2000) A two-component regulatory system, pehR-pehS, controls endopolygalacturonase production and virulence in the plant pathogen *Erwinia carotovora* subsp. *carotovora*. *Mol. Plant-Microbe Interact.* **13**: 447-455.
Fones, H., and Preston, G.M. (2012) Reactive oxygen and oxidative stress tolerance in plant pathogenic *Pseudomonas*. *FEMS Microbiol. Lett.* **327**: 1-8.
Foster, J.W. (1999) When protons attack: microbial strategies of acid adaptation. *Curr. Opin. Microbiol.* **2**: 170-174.
Francino, M.P., Santos, S.R., and Ochman, H. (1992) Phylogenetic relationships of bacteria with special reference to endosymbionts and enteric species.
Galán, B., Kolb, A., Sanz, J.M., García, J.L., and Prieto, M.A. (2003) Molecular determinants of the hpa regulatory system of Escherichia coli: the HpaR repressor. *Nucleic Acids Res.* **31**: 6598-6609.
Galperin, M.Y. (2010) Diversity of structure and function of response regulator output domains. *Curr. Opin. Microbiol.* **13**: 150-159.
Gama-Castro, S., Jiménez-Jacinto, V., Peralta-Gil, M., Santos-Zavaleta, A., Peñaloza-Spinola, M.I., Contreras-Moreira, B., Segura-Salazar, J., Muniz-Rascado, L., Martinez-Flores, I., and Salgado, H. (2008) RegulonDB (version 6.0): gene regulation model of Escherichia coli K-12 beyond transcription, active (experimental) annotated promoters and Textpresso navigation. *Nucleic Acids Res.* **36**: D120-D124.
Gambino, M., and Cappitelli, F. (2016) Mini-review: Biofilm responses to oxidative stress. *Biofouling* **32**: 167-178.
Gao, R., Mack, T.R., and Stock, A.M. (2007) Bacterial response regulators: versatile regulatory strategies from common domains. *Trends Biochem. Sci.* **32**: 225-234.
Gao, Y., Yurkovich, J.T., Seo, S.W., Kabimoldayev, I., Dräger, A., Chen, K., Sastry, A.V., Fang, X., Mih, N., and Yang, L. (2018) Systematic discovery of uncharacterized transcription factors in Escherichia coli K-12 MG1655. *Nucleic Acids Res.* **46**: 10682-10696.

Garcia-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B., Wendehenne, D., and Pugin, A. (2006) Early signaling events induced by elicitors of plant defenses. *Mol. Plant-Microbe Interact.* **19**: 711-724.

Gardan, L., Gouy, C., Christen, R., and Samson, R. (2003) Elevation of three subspecies of Pectobacterium carotovorum to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavasculorum* sp. nov. and *Pectobacterium wasabiae* sp. nov. *Int. J. Syst. Evol. Microbiol.* **53**: 381-391.

Gill, H.K., and Garg, H., (2014) Pesticides: environmental impacts and management strategies. In: Pesticides-toxic aspects. IntechOpen, pp.

Glasner, J., Marquez-Villavicencio, M., Kim, H.-S., Jahn, C., Ma, B., Biehl, B., Rissman, A., Mole, B., Yi, X., and Yang, C.-H. (2008) Niche-specificity and the variable fraction of the *Pectobacterium* pan-genome. *Mol. Plant-Microbe Interact.* **21**: 1549-1560.

Gloux, K., Touze, T., Pagot, Y., Jouan, B., and Blanco, C. (2005) Mutations of ousA alter the virulence of *Erwinia chrysanthemi. Mol. Plant-Microbe Interact.* **18**: 150-157.

Golubeva, Y.A., and Slauch, J.M. (2006) *Salmonella enterica* serovar Typhimurium periplasmic superoxide dismutase SodCI is a member of the PhoPQ regulon and is induced in macrophages. *J. Bacteriol.* **188**: 7853-7861.

Gorshkov, V., Gubaev, R., Petrova, O., Daminova, A., Gogoleva, N., Ageeva, M., Parfirova, O., Prokchorchik, M., Nikolaichik, Y., and Gogolev, Y. (2018) Transcriptome profiling helps to identify potential and true molecular switches of stealth to brute force behavior in *Pectobacterium atrosepticum* during systemic colonization of tobacco plants. *Eur. J. Plant Pathol.* **152**: 957-976.

Grebe, T.W., and Stock, J.B., (1999) The histidine protein kinase superfamily. In: Adv. Microb. Physiol.: Elsevier, pp. 139-227.

Grignon, C., and Sentenac, H. (1991) pH and ionic conditions in the apoplast. *Annu. Rev. Plant Biol.* **42**: 103-128.

Groisman, E., Heffron, F., and Solomon, F. (1992) Molecular genetic analysis of the E*scherichia coli* phoP locus. *J. Bacteriol.* **174**: 486-491.

Groisman, E.A. (2001) The pleiotropic two-component regulatory system PhoP-PhoQ. *J. Bacteriol.* **183**: 1835-1842.

Groisman, E.A., Chiao, E., Lipps, C.J., and Heffron, F. (1989) *Salmonella* typhimurium phoP virulence gene is a transcriptional regulator. *Proceedings of the National Academy of Sciences* **86**: 7077-7081.

Grubor, B., Meyerholz, D.K., and Ackermann, M.R. (2006) Collectins and cationic antimicrobial peptides of the respiratory epithelia. *Vet. Pathol.* **43**: 595-612.

Guina, T., Eugene, C.Y., Wang, H., Hackett, M., and Miller, S.I. (2000) A PhoP-regulated outer membrane protease of *Salmonella enterica* serovar Typhimurium promotes resistance to alpha-helical antimicrobial peptides. *J. Bacteriol.* **182**: 4077-4086.

Gunn, J.S. (2008) The *Salmonella* PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. *Trends Microbiol.* **16**: 284-290.

Han, Y., Gao, S., Muegge, K., Zhang, W., and Zhou, B. (2015) Advanced applications of RNA sequencing and challenges. *Bioinform. Biol. Insights* **9**: BBI. S28991.

Hancock, R.E., and Diamond, G. (2000) The role of cationic antimicrobial peptides in innate host defences. *Trends Microbiol.* **8**: 402-410.

Haque, M.M., Hirata, H., and Tsuyumu, S. (2015) SlyA regulates motA and motB, virulence and stress-related genes under conditions induced by the PhoP-PhoQ system in *Dickeya dadantii* 3937. *Res. Microbiol.* **166**: 467-475.

Haque, M.M., Kabir, M.S., Aini, L.Q., Hirata, H., and Tsuyumu, S. (2009) SlyA, a MarR family transcriptional regulator, is essential for virulence in *Dickeya dadantii* 3937. *J. Bacteriol.* **191**: 5409-5418.

Haque, M.M., Nahar, K., Rahim, M.A., Gomes, I., and Tsuyumu, S. (2008) PhoP-PhoQ two-component system required for colonization leading to virulence of *Dickeya dadantii* 3937 in planta. *Banglad. J. Microbiol.* **25**: 36-40.

Haque, M.M., and Tsuyumu, S. (2005) Virulence, resistance to magainin II, and expression of pectate lyase are controlled by the PhoP-PhoQ two-component regulatory system responding to pH and magnesium in *Erwinia chrysanthemi* 3937. *Journal of General Plant Pathology* **71**: 47-53.

Harari, O., Park, S.-Y., Huang, H., Groisman, E.A., and Zwir, I. (2010) Defining the plasticity of transcription factor binding sites by deconstructing DNA consensus sequences: the PhoP-binding sites among gamma/enterobacteria. *PLoS Comp. Biol.* **6**.

Hardigan, M.A., Laimbeer, F.P.E., Newton, L., Crisovan, E., Hamilton, J.P., Vaillancourt, B., Wiegert-Rininger, K., Wood, J.C., Douches, D.S., and Farré, E.M. (2017) Genome diversity of tuber-bearing Solanum uncovers complex evolutionary history and targets of domestication in the cultivated potato. *Proceedings of the National Academy of Sciences* **114**: E9999-E10008.

Hauben, L., Moore, E.R., Vauterin, L., Steenackers, M., Mergaert, J., Verdonck, L., and Swings, J. (1998) Phylogenetic position of phytopathogens within the Enterobacteriaceae. *Syst. Appl. Microbiol.* **21**: 384-397.

Hébrard, M., Viala, J.P., Méresse, S., Barras, F., and Aussel, L. (2009) Redundant hydrogen peroxide scavengers contribute to Salmonella virulence and oxidative stress resistance. *J. Bacteriol.* **191**: 4605-4614.

Helmann, J.D. (2002) The extracytoplasmic function (ECF) sigma factors. *Adv. Microb. Physiol.* **46**: 48-111.

Hennigar, S.R., and McClung, J.P. (2016) Nutritional immunity: starving pathogens of trace minerals. *Am. J. Lifestyle Med.* **10**: 170-173.

Hernandez-Guerrero, R., Galán-Vásquez, E., and Pérez-Rueda, E. (2019) The protein architecture in Bacteria and Archaea identifies a set of promiscuous and ancient domains. *PLoS One* **14**.

Heroven, A.K., Nagel, G., Tran, H.J., Parr, S., and Dersch, P. (2004) RovA is autoregulated and antagonizes H-NS-mediated silencing of invasin and rovA expression in *Yersinia pseudotuberculosis*. *Mol. Microbiol.* **53**: 871-888.

Herrera, C.M., Hankins, J.V., and Trent, M.S. (2010) Activation of PmrA inhibits LpxT-dependent phosphorylation of lipid A promoting resistance to antimicrobial peptides. *Mol. Microbiol.* **76**: 1444-1460.

Hirsch, C.N., Hirsch, C.D., Felcher, K., Coombs, J., Zarka, D., Van Deynze, A., De Jong, W., Veilleux, R.E., Jansky, S., and Bethke, P. (2013) Retrospective view of North American potato (Solanum tuberosum L.) breeding in the 20th and 21st centuries. *G3: Genes, Genomes, Genetics* **3**: 1003-1013.

Hitchen, P.G., Prior, J.L., Oyston, P.C., Panico, M., Wren, B.W., Titball, R.W., Morris, H.R., and Dell, A. (2002) Structural characterization of lipo-oligosaccharide (LOS) from *Yersinia pestis:* regulation of LOS structure by the PhoPQ system. *Mol. Microbiol.* **44**: 1637-1650.

Hommais, F., Oger-Desfeux, C., Van Gijsegem, F., Castang, S., Ligori, S., Expert, D., Nasser, W., and Reverchon, S. (2008) PecS is a global regulator of the symptomatic phase in the phytopathogenic bacterium *Erwinia chrysanthemi* 3937. *J. Bacteriol.* **190**: 7508-7522.

Hossain, M.M., and Tsuyumu, S. (2006) Flagella-mediated motility is required for biofilm formation by *Erwinia carotovora* subsp. *carotovora*. *Journal of General Plant Pathology* **72**: 34-39.

Hu, M., Li, J., Chen, R., Li, W., Feng, L., Shi, L., Xue, Y., Feng, X., Zhang, L., and Zhou, J. (2018) *Dickeya zeae* strains isolated from rice, banana and clivia rot plants show great virulence differentials. *BMC Microbiol.* **18**: 136.

Hugouvieux-Cotte-Pattat, N., Condemine, G., and Shevchik, V.E. (2014) Bacterial pectate lyases, structural and functional diversity. *Environ. Microbiol. Rep.* **6**: 427-440.

Hwan Baek, J., and Yup Lee, S. (2006) Novel gene members in the Pho regulon of *Escherichia coli*. *FEMS Microbiol. Lett.* **264**: 104-109.

Iqbal, A., Khan, R.S., Shehryar, K., Imran, A., Ali, F., Attia, S., Shah, S., and Mii, M. (2019) Antimicrobial peptides as effective tools for enhanced disease resistance in plants. *Plant Cell, Tissue and Organ Culture (PCTOC)*: 1-15.

Jiang, X., Zghidi-Abouzid, O., Oger-Desfeux, C., Hommais, F., Greliche, N., Muskhelishvili, G., Nasser, W., and Reverchon, S. (2016) Global transcriptional response of *Dickeya dadantii* to environmental stimuli relevant to the plant infection. *Environ. Microbiol.* **18**: 3651-3672.

Kalischuk, M., Hachey, J., and Kawchuk, L. (2015) Complete genome sequence of phytopathogenic *Pectobacterium atrosepticum* bacteriophage Peat1. *Genome Announc.* **3**: e00760-00715.

Kamau, J.W., Ngaira, J., Kinyua, J., Gachamba, S., Ngundo, G., Janse, J., and Macharia, I. (2019) Occurence of pectinolytic bacteria causing blackleg and soft rot of potato in Kenya. *J. Plant Pathol.*: 1-6.

Kasahara, M., Nakata, A., and Shinagawa, H. (1992) Molecular analysis of the *Escherichia coli* phoP-phoQ operon. *J. Bacteriol.* **174**: 492-498.

Kato, A., and Groisman, E.A. (2004) Connecting two-component regulatory systems by a protein that protects a response regulator from dephosphorylation by its cognate sensor. *Genes Dev.* **18**: 2302-2313.

Kenney, L.J. (2002) Structure/function relationships in OmpR and other winged-helix transcription factors. *Curr. Opin. Microbiol.* **5**: 135-141.

Khayi, S., Cigna, J., Chong, T.M., Quêtu-Laurent, A., Chan, K.-G., Hélias, V., and Faure, D. (2016) Transfer of the potato plant isolates of *Pectobacterium wasabiae* to *Pectobacterium parmentieri* sp. nov. *Int. J. Syst. Evol. Microbiol.* **66**: 5379-5383.

Kier, L., Weppelman, R., and Ames, B. (1979) Regulation of nonspecific acid phosphatase in *Salmonella*: phoN and phoP genes. *J. Bacteriol.* **138**: 155-161.

Kim, H.-S., Ma, B., Perna, N.T., and Charkowski, A.O. (2009) Phylogeny and virulence of naturally occurring type III secretion system-deficient *Pectobacterium* strains. *Appl. Environ. Microbiol.* **75**: 4539-4549.

Kim, Y., Miller, C., and Anderson, A. (1999) Transcriptional regulation by iron and role during plant pathogenesis of genes encoding iron-and manganese-superoxide dismutases of *Pseudomonas syringae* pv. syringae B728a. *Physiol. Mol. Plant Pathol.* **55**: 327-339.

Kondo, H., Nakagawa, A., Nishihira, J., Nishimura, Y., Mizuno, T., and Tanaka, I. (1997) *Escherichia coli* positive regulator OmpR has a large loop structure at the putative RNA polymerase interaction site. *Nat. Struct. Biol.* **4**: 28-31.

Koprivnjak, T., and Peschel, A. (2011) Bacterial resistance mechanisms against host defense peptides. *Cell. Mol. Life Sci.* **68**: 2243-2254.

Korol, N., and Tovkach, F. (2012) Identification of the major proteins of the virions of bacteriophage ZF40 *Pectobacterium carotovorum. Мікробіологічний журнал*: 64-70.

Krauss, A., (2008) II Potato-the hidden treasure. In., pp.

Kravchenko, U., Gogoleva, N., Kolubako, A., Kruk, A., Diubo, J., Gogolev, Y., and Nikolaichik, Y. (2020) The PhoPQ two-component system is the major regulator of cell surface properties, stress responses and plant-derived substrate utilisation during development of *Pectobacterium versatile*-host plant pathosystem. *bioRxiv*.

Krell, T. (2018) Exploring the (almost) unknown: archaeal two-component systems. *J. Bacteriol.* **200**: e00774-00717.

Kubheka, G.C., Coutinho, T.A., Moleleki, N., and Moleleki, L.N. (2013) Colonization patterns of an mCherry-tagged *Pectobacterium carotovorum* subsp. *brasiliense* strain in potato plants. *Phytopathology* **103**: 1268-1279.

Kumaraswami, M., Schuman, J.T., Seo, S.M., Kaatz, G.W., and Brennan, R.G. (2009) Structural and biochemical characterization of MepR, a multidrug binding transcription regulator of the *Staphylococcus aureus* multidrug efflux pump MepA. *Nucleic Acids Res.* **37**: 1211-1224.

Kummerfeld, S.K., and Teichmann, S.A. (2006) DBD: a transcription factor prediction database. *Nucleic Acids Res.* **34**: D74-D81.

Lamarche, M.G., and Harel, J. (2010) Membrane homeostasis requires intact pst in extraintestinal pathogenic *Escherichia coli. Curr. Microbiol.* **60**: 356-359.

Le Breton, Y., Boël, G., Benachour, A., Prévost, H., Auffray, Y., and Rincé, A. (2003) Molecular characterization of *Enterococcus faecalis* two-component signal transduction pathways related to environmental stresses. *Environ. Microbiol.* **5**: 329-337.

Lee, D.H., Lee, J.-H., Shin, H., Ji, S., Roh, E., Jung, K., Ryu, S., Choi, J., and Heu, S., (2012a) Complete genome sequence of *Pectobacterium carotovorum* subsp. *carotovorum* bacteriophage My1. In.: Am Soc Microbiol, pp.

Lee, D.H., Lim, J.-A., Lee, J., Roh, E., Jung, K., Choi, M., Oh, C., Ryu, S., Yun, J., and Heu, S. (2013) Characterization of genes required for the pathogenicity of *Pectobacterium carotovorum* subsp. *carotovorum* Pcc21 in Chinese cabbage. *Microbiology* **159**: 1487-1496.

Lee, D.J., Minchin, S.D., and Busby, S.J. (2012b) Activating transcription in bacteria. *Annu. Rev. Microbiol.* **66**: 125-152.

Lee, J.-H., Shin, H., Ji, S., Malhotra, S., Kumar, M., Ryu, S., and Heu, S., (2012c) Complete genome sequence of phytopathogenic *Pectobacterium carotovorum* subsp. *carotovorum* bacteriophage PP1. In.: Am Soc Microbiol, pp.

Lee, S.-W., Jeong, K.-S., Han, S.-W., Lee, S.-E., Phee, B.-K., Hahn, T.-R., and Ronald, P. (2008) The *Xanthomonas oryzae* pv. oryzae PhoPQ two-component system is required for AvrXA21 activity, hrpG expression, and virulence. *J. Bacteriol.* **190**: 2183-2197.

Leite, L.N., De Haan, E., Krijger, M., Kastelein, P., Van Der Zouwen, P., Van Den Bovenkamp, G., Tebaldi, N., and Van Der Wolf, J. (2014) First report of potato blackleg caused by *Pectobacterium carotovorum* subsp. *brasiliensis* in the Netherlands. *New Dis. Rep* **29**: 2044-0588.2014.

Lemmens, L., Maklad, H.R., Bervoets, I., and Peeters, E. (2019) Transcription regulators in Archaea: homologies and differences with bacterial regulators. *J. Mol. Biol.*

Li, X., Ma, Y., Liang, S., Tian, Y., Yin, S., Xie, S., and Xie, H. (2018) Comparative genomics of 84 *Pectobacterium* genomes reveals the variations related to a pathogenic lifestyle. *BMC Genomics* **19**: 889.

Lim, J.-A., Lee, D.H., and Heu, S. (2015) Isolation and genomic characterization of the T4-Like bacteriophage PM2 infecting *Pectobacterium carotovorum* subsp. *carotovorum. The plant pathology journal* **31**: 83.

Lim, J.-A., Shin, H., Lee, D.H., Han, S.-W., Lee, J.-H., Ryu, S., and Heu, S. (2014) Complete genome sequence of the *Pectobacterium carotovorum* subsp. *carotovorum* virulent bacteriophage PM1. *Arch. Virol.* **159**: 2185-2187.

Lippa, A.M., and Goulian, M. (2009) Feedback inhibition in the PhoQ/PhoP signaling system by a membrane peptide. *PLoS Genet.* **5**.

Lithgow, J.K., Haider, F., Roberts, I.S., and Green, J. (2007) Alternate SlyA and H-NS nucleoprotein complexes control hlyE expression in *Escherichia coli* K-12. *Mol. Microbiol.* **66**: 685-698.

Liu, H., Coulthurst, S.J., Pritchard, L., Hedley, P.E., Ravensdale, M., Humphris, S., Burr, T., Takle, G., Brurberg, M.-B., and Birch, P.R. (2008) Quorum sensing coordinates brute force and stealth modes of infection in the plant pathogen *Pectobacterium atrosepticum*. *PLoS Path.* **4**: e1000093.

Liu, L., Fang, H., Yang, H., Zhang, Y., Han, Y., Zhou, D., and Yang, R. (2016) Reciprocal regulation of *Yersinia pestis* biofilm formation and virulence by RovM and RovA. *Open biology* **6**: 150198.

Liu, L., and Zheng, S. (2019) Transcriptional regulation of *Yersinia pestis* biofilm formation. *Microb. Pathog.*

Liu, M., Wu, F., Wang, S., Lu, Y., Chen, X., Wang, Y., Gu, A., Zhao, J., and Shen, S. (2019) Comparative transcriptome analysis reveals defense responses against soft rot in Chinese cabbage. *Horticulture research* **6**: 1-18.

Livermore, D.M. (2012) Current epidemiology and growing resistance of gram-negative pathogens. *The Korean journal of internal medicine* **27**: 128.

Llama-Palacios, A., López-Solanilla, E., and Rodríguez-Palenzuela, P. (2005) Role of the PhoP-PhoQ system in the virulence of *Erwinia chrysanthemi* strain 3937:

involvement in sensitivity to plant antimicrobial peptides, survival at acid pH, and regulation of pectolytic enzymes. *J. Bacteriol.* **187**: 2157-2162.

Ludwig, A., Tengel, C., Bauer, S., Bubert, A., Benz, R., Mollenkopf, H.-J., and Goebel, W. (1995) SlyA, a regulatory protein from *Salmonella* typhimurium, induces a haemolytic and pore-forming protein in Escherichia coli. *Molecular and General Genetics MGG* **249**: 474-486.

Ma, B., Hibbing, M.E., Kim, H.-S., Reedy, R.M., Yedidia, I., Breuer, J., Breuer, J., Glasner, J.D., Perna, N.T., and Kelman, A. (2007) Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya. Phytopathology* **97**: 1150-1163.

Macfarlane, E.L., Kwasnicka, A., Ochs, M.M., and Hancock, R.E. (1999) PhoP–PhoQ homologues in *Pseudomonas aeruginosa* regulate expression of the outer-membrane protein OprH and polymyxin B resistance. *Mol. Microbiol.* **34**: 305-316.

Mäe, A., Montesano, M., Koiv, V., and Palva, E.T. (2001) Transgenic plants producing the bacterial pheromone N-acyl-homoserine lactone exhibit enhanced resistance to the bacterial phytopathogen *Erwinia carotovora. Mol. Plant-Microbe Interact.* **14**: 1035-1042.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., and Machado, M.A. (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.* **13**: 614-629.

Marquez-Villavicencio, M.d.P., Groves, R.L., and Charkowski, A.O. (2011) Soft rot disease severity is affected by potato physiology and *Pectobacterium* taxa. *Plant Dis.* **95**: 232-241.

Martínez-Antonio, A., Janga, S.C., Salgado, H., and Collado-Vides, J. (2006) Internal-sensing machinery directs the activity of the regulatory network in Escherichia coli. *Trends Microbiol.* **14**: 22-27.

Martínez-Hackert, E., and Stock, A.M. (1997) Structural relationships in the OmpR family of winged-helix transcription factors. *J. Mol. Biol.* **269**: 301-312.

Mashavha, M.L., (2013) Characterisation of *Pectobacterium carotovorum* subsp. *brasiliense* isolates causing blackleg and soft rot diseases of potato in South Africa. In.: University of Pretoria, pp.

McGowan, S., Sebaihia, M., Porter, L., Stewart, G., Williams, P., Bycroft, B., and Salmond, G. (1996) Analysis of bacterial carbapenem antibiotic production genes reveals a novel β-lactam biosynthesis pathway. *Mol. Microbiol.* **22**: 415-426.

Mejía-Sánchez, D., Aranda-Ocampo, S., Nava-Díaz, C., Teliz-Ortiz, D., Livera-Muñoz, M., De La Torre-Almaráz, R., and Ramírez-Alarcón, S. (2019) *Pectobacterium carotovorum* subsp. *brasiliense* Causes Soft Rot and Death of Neobuxbaumia tetetzo in Zapotitlan Salinas Valley, Puebla, Mexico. *Plant Dis.* **103**: 398-403.

Meng, X., Chai, A., Shi, Y., Xie, X., Ma, Z., and Li, B. (2017) Emergence of bacterial soft rot in cucumber caused by *Pectobacterium carotovorum* subsp. *brasiliense* in China. *Plant Dis.* **101**: 279-287.

Menon, S., and Wang, S. (2011) Structure of the response regulator PhoP from *Mycobacterium tuberculosis* reveals a dimer through the receiver domain. *Biochemistry* **50**: 5948-5957.

Metzker, M.L. (2009) Sequencing in real time. *Nat. Biotechnol.* **27**: 150.

Michaux, C., Martini, C., Hanin, A., Auffray, Y., Hartke, A., and Giard, J.-C. (2011) SlyA regulator is involved in bile salts stress response of *Enterococcus faecalis*. *FEMS Microbiol. Lett.* **324**: 142-146.

Miller, S., and Mekalanos, J. (1990) Constitutive expression of the phoP regulon attenuates *Salmonella* virulence and survival within macrophages. *J. Bacteriol.* **172**: 2485-2490.

Miller, S.I., Hoffman, L.R., and Sanowar, S. (2007) Did bacterial sensing of host environments evolve from sensing within microbial communities? *Cell host & microbe* **1**: 85-87.

Miller, S.I., Kukral, A.M., and Mekalanos, J.J. (1989) A two-component regulatory system (phoP phoQ) controls *Salmonella* typhimurium virulence. *Proceedings of the National Academy of Sciences* **86**: 5054-5058.

Minagawa, S., Ogasawara, H., Kato, A., Yamamoto, K., Eguchi, Y., Oshima, T., Mori, H., Ishihama, A., and Utsumi, R. (2003) Identification and molecular characterization of the Mg2+ stimulon of *Escherichia coli*. *J. Bacteriol.* **185**: 3696-3702.

Moss, J.E., Fisher, P.E., Vick, B., Groisman, E.A., and Zychlinsky, A. (2000) The regulatory protein PhoP controls susceptibility to the host inflammatory response in *Shigella flexneri. Cell. Microbiol.* **2**: 443-452.

Motyka, A., Zoledowska, S., Sledz, W., and Lojkowska, E. (2017) Molecular methods as tools to control plant diseases caused by *Dickeya* and *Pectobacterium* spp: A minireview. *New biotechnology* **39**: 181-189.

Mullin, R., and Lauer, F., (1966) Breeding behavior of F1 and inbred potato clones. In: Proceedings of the American Society for Horticultural Science. AMER SOC HORTICULTURAL SCIENCE 701 NORTH SAINT ASAPH STREET, ALEXANDRIA, VA …, pp. 449-&.

Naas, H., Sebaihia, M., Orfei, B., Rezzonico, F., Buonaurio, R., and Moretti, C. (2018) *Pectobacterium carotovorum* subsp. *brasiliense* and *Pectobacterium carotovorum* subsp. *carotovorum* as causal agents of potato soft rot in Algeria. *Eur. J. Plant Pathol.* **151**: 1027-1034.

Nabhan, S., De Boer, S., Maiss, E., and Wydra, K. (2012) Taxonomic relatedness between *Pectobacterium carotovorum* subsp. *carotovorum, Pectobacterium carotovorum* subsp. *odoriferum* and *Pectobacterium carotovorum* subsp. *brasiliense* subsp. nov. *J. Appl. Microbiol.* **113**: 904-913.

Nabhan, S., De Boer, S.H., Maiss, E., and Wydra, K. (2013) *Pectobacteriumaroidearum* sp. nov., a soft rot pathogen with preference for monocotyledonous plants. *Int. J. Syst. Evol. Microbiol.* **63**: 2520-2525.

Nadarasah, G., and Stavrinides, J. (2011) Insects as alternative hosts for phytopathogenic bacteria. *FEMS Microbiol. Rev.* **35**: 555-575.

Nakka, S., Qi, M., and Zhao, Y. (2010) The *Erwinia amylovora* PhoPQ system is involved in resistance to antimicrobial peptide and suppresses gene expression of two novel type III secretion systems. *Microbiol. Res.* **165**: 665-673.

Navarre, D.A., Goyer, A., and Shakya, R., (2009) Nutritional value of potatoes: vitamin, phytonutrient, and mineral content. In: Advances in potato chemistry and technology. Elsevier, pp. 395-424.

Navarre, W.W., Halsey, T.A., Walthers, D., Frye, J., McClelland, M., Potter, J.L., Kenney, L.J., Gunn, J.S., Fang, F.C., and Libby, S.J. (2005) Co-regulation of *Salmonella enterica* genes required for virulence and resistance to antimicrobial peptides by SlyA and PhoP/PhoQ. *Mol. Microbiol.* **56**: 492-508.

Nguyen, M.-P., Yoon, J.-M., Cho, M.-H., and Lee, S.-W. (2015) Prokaryotic 2-component systems and the OmpR/PhoB superfamily. *Can. J. Microbiol.* **61**: 799-810.

Nykyri, J., Niemi, O., Koskinen, P., Nokso-Koivisto, J., Pasanen, M., Broberg, M., Plyusnin, I., Törönen, P., Holm, L., and Pirhonen, M. (2012) Revised phylogeny and novel horizontally acquired virulence determinants of the model soft rot phytopathogen Pectobacterium wasabiae SCC3193. *PLoS Path.* **8**.

Oerke, E.-C., Dehne, H.-W., Schönbeck, F., and Weber, A., (2012) *Crop production and crop protection: estimated losses in major food and cash crops*. Elsevier.

Ogasawara, H., Yamamoto, K., and Ishihama, A. (2011) Role of the biofilm master regulator CsgD in cross-regulation between biofilm formation and flagellar synthesis. *J. Bacteriol.* **193**: 2587-2597.

Oh, S.-Y., Shin, J.-H., and Roe, J.-H. (2007) Dual role of OhrR as a repressor and an activator in response to organic hydroperoxides in *Streptomyces coelicolor*. *J. Bacteriol.* **189**: 6284-6292.

Okada, N., Oi, Y., Takeda-Shitaka, M., Kanou, K., Umeyama, H., Haneda, T., Miki, T., Hosoya, S., and Danbara, H. (2007) Identification of amino acid residues of *Salmonella* SlyA that are critical for transcriptional regulation. *Microbiology* **153**: 548-560.

Olsen, N., Miller, J., and Nolte, P., (2006) *Diagnosis & management of potato storage diseases*. Idaho Agricultural Experiment Station.

Onkendi, E.M., Maluleke, L., and Moleleki, L.N. (2014) First report of *Pectobacterium carotovorum* subsp. *brasiliense* causing soft rot and blackleg of potatoes in Kenya. *Plant Dis.* **98**: 684-684.

Onkendi, E.M., and Moleleki, L.N. (2014) Characterization of *Pectobacterium carotovorum* subsp. *carotovorum* and *brasiliense* from diseased potatoes in Kenya. *Eur. J. Plant Pathol.* **139**: 557-566.

Onkendi, E.M., Ramesh, A.M., Kwenda, S., Naidoo, S., and Moleleki, L. (2016) Draft genome sequence of a virulent *Pectobacterium carotovorum* subsp. *brasiliense* isolate causing soft rot of cucumber. *Genome Announc.* **4**: e01530-01515.

Oren, A., and Garrity, G.M. (2014) Then and now: a systematic review of the systematics of prokaryotes in the last 80 years. *Antonie Van Leeuwenhoek* **106**: 43-56.

Ouellette, A.J. (2011) Paneth cell α-defensins in enteric innate immunity. *Cell. Mol. Life Sci.* **68**: 2215-2229.

Oulghazi, S., Cigna, J., Lau, Y.Y., Moumni, M., Chan, K.G., and Faure, D. (2019) Transfer of the waterfall source isolate *Pectobacterium carotovorum* M022 to *Pectobacterium fontis* sp. nov., a deep-branching species within the genus *Pectobacterium*. *Int. J. Syst. Evol. Microbiol.* **69**: 470-475.

Oyston, P.C., Dorrell, N., Williams, K., Li, S.-R., Green, M., Titball, R.W., and Wren, B.W. (2000) The response regulator PhoP is important for survival under conditions of macrophage-induced stress and virulence in *Yersinia pestis*. *Infect. Immun.* **68**: 3419-3425.

Pabo, C.O., and Sauer, R.T. (1992) Transcription factors: structural families and principles of DNA recognition. *Annu. Rev. Biochem.* **61**: 1053-1095.

Pan, Y., Liang, F., Li, R.-J., and Qian, W. (2018) MarR-Family Transcription Factor HpaR Controls Expression of the vgrR-vgrS Operon of *Xanthomonas campestris* pv. *campestris. Mol. Plant-Microbe Interact.* **31**: 299-310.

Panmanee, W., Vattanaviboon, P., Eiamphungporn, W., Whangsuk, W., Sallabhan, R., and Mongkolsuk, S. (2002) OhrR, a transcription repressor that senses and responds to changes in organic peroxide levels in *Xanthomonas campestris* pv. *phaseoli*. *Mol. Microbiol.* **45**: 1647-1654.

Park, S.-Y., Pontes, M.H., and Groisman, E.A. (2015) Flagella-independent surface motility in *Salmonella enterica* serovar Typhimurium. *Proceedings of the National Academy of Sciences* **112**: 1850-1855.

Park, T.-H., Choi, B.-S., Choi, A.-Y., Choi, I.-Y., Heu, S., and Park, B.-S. (2012) Genome sequence of *Pectobacterium carotovorum* subsp. *carotovorum* strain PCC21, a pathogen causing soft rot in Chinese cabbage. *J. Bacteriol.* **194**: 6345.

Parkinson, N., DeVos, P., Pirhonen, M., and Elphinstone, J. (2014) *Dickeya aquatica* sp. nov., isolated from waterways. *Int. J. Syst. Evol. Microbiol.* **64**: 2264-2266.

Parkinson, N., Pritchard, L., Bryant, R., Toth, I., and Elphinstone, J. (2015) Epidemiology of *Dickeya dianthicola* and *Dickeya solani* in ornamental hosts and potato studied using variable number tandem repeat analysis. *Eur. J. Plant Pathol.* **141**: 63-70.

Pedron, J., Bertrand, C., Taghouti, G., Portier, P., and Barny, M.-A. (2019) *Pectobacterium aquaticum* sp. nov., isolated from waterways.

Pédron, J., Chapelle, E., Alunni, B., and Van Gijsegem, F. (2018) Transcriptome analysis of the *Dickeya dadantii* PecS regulon during the early stages of interaction with Arabidopsis thaliana. *Mol. Plant Pathol.* **19**: 647-663.

Perera, I.C., and Grove, A. (2010) Molecular mechanisms of ligand-mediated attenuation of DNA binding by MarR family transcriptional regulators. *J. Mol. Cell. Biol.* **2**: 243-254.

Perera, I.C., and Grove, A. (2011) MarR homologs with urate-binding signature. *Protein Sci.* **20**: 621-629.

Perez-Rueda, E., Hernandez-Guerrero, R., Martinez-Nuñez, M.A., Armenta-Medina, D., Sanchez, I., and Ibarra, J.A. (2018) Abundance, diversity and domain architecture variability in prokaryotic DNA-binding transcription factors. *PLoS One* **13**.

Perez, J.C., and Groisman, E.A. (2009) Transcription factor function and promoter architecture govern the evolution of bacterial regulons. *Proceedings of the National Academy of Sciences* **106**: 4319-4324.

Perez, J.C., Latifi, T., and Groisman, E.A. (2008) Overcoming H-NS-mediated transcriptional silencing of horizontally acquired genes by the PhoP and SlyA proteins in *Salmonella enterica*. *J. Biol. Chem.* **283**: 10773-10783.

Perez, J.C., Shin, D., Zwir, I., Latifi, T., Hadley, T.J., and Groisman, E.A. (2009) Evolution of a bacterial regulon controlling virulence and Mg2+ homeostasis. *PLoS Genet.* **5**.

Perombelon, M.C., and Kelman, A. (1980) Ecology of the soft rot erwinias. *Annu. Rev. Phytopathol.* **18**: 361-387.

Portier, P., Pédron, J., Taghouti, G., Fischer-Le Saux, M., Caullireau, E., Bertrand, C., Laurent, A., Chawki, K., Oulgazi, S., and Moumni, M. (2019) Elevation of *Pectobacterium carotovorum* subsp. *odoriferum* to species level as *Pectobacterium*

odoriferum sp. nov., proposal of *Pectobacterium brasiliense* sp. nov. and *Pectobacterium actinidiae* sp. nov., emended description of *Pectobacterium carotovorum* and description of *Pectobacterium versatile* sp. nov., isolated from streams and symptoms on diverse plants. *Int. J. Syst. Evol. Microbiol.* **69**: 3207-3216.

Pritchard, L., Glover, R.H., Humphris, S., Elphinstone, J.G., and Toth, I.K. (2016) Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. *Analytical Methods* **8**: 12-24.

Prost, L.R., Daley, M.E., Le Sage, V., Bader, M.W., Le Moual, H., Klevit, R.E., and Miller, S.I. (2007) Activation of the bacterial sensor kinase PhoQ by acidic pH. *Mol. Cell* **26**: 165-174.

Raetz, C.R., and Whitfield, C. (2002) Lipopolysaccharide endotoxins. *Annu. Rev. Biochem.* **71**: 635-700.

Rai, M.F., Tycksen, E.D., Sandell, L.J., and Brophy, R.H. (2018) Advantages of RNA-seq compared to RNA microarrays for transcriptome profiling of anterior cruciate ligament tears. *Journal of Orthopaedic Research®* **36**: 484-497.

Rao, M.S., Van Vleet, T.R., Ciurlionis, R., Buck, W.R., Mittelstadt, S.W., Blomme, E.A., and Liguori, M.J. (2019) Comparison of RNA-seq and microarray gene expression platforms for the toxicogenomic evaluation of liver from short-term rat toxicity studies. *Frontiers in genetics* **9**: 636.

Ravensdale, M., Blom, T., Gracia-Garza, J., Svircev, A., and Smith, R. (2007) Bacteriophages and the control of *Erwinia carotovora* subsp. *carotovora. Canadian Journal of Plant Pathology* **29**: 121-130.

Rebeil, R., Jarrett, C.O., Driver, J.D., Ernst, R.K., Oyston, P.C., and Hinnebusch, B.J. (2013) Induction of the *Yersinia pestis* PhoP-PhoQ regulatory system in the flea and its role in producing a transmissible infection. *J. Bacteriol.* **195**: 1920-1930.

Rendueles, O., and Ghigo, J.-M. (2012) Multi-species biofilms: how to avoid unfriendly neighbors. *FEMS Microbiol. Rev.* **36**: 972-989.

Resibois, A., Colet, M., Faelen, M., Schoonejans, E., and Toussaint, A. (1984) φEC2, a new generalized transducing phage of *Erwinia chrysanthemi*. *Virology* **137**: 102-112.

Reverchon, S., and Nasser, W. (2013) *Dickeya* ecology, environment sensing and regulation of virulence programme. *Environ. Microbiol. Rep.* **5**: 622-636.

Reverchon, S., Nasser, W., and Robert-Baudouy, J. (1994) pecS: a locus controlling pectinase, cellulase and blue pigment production in *Erwinia chrysanthemi*. *Mol. Microbiol.* **11**: 1127-1139.

Rhee, J.E., Kim, K.-S., and Choi, S.H. (2005) CadC activates pH-dependent expression of the Vibrio vulnificus cadBA operon at a distance through direct binding to an upstream region. *J. Bacteriol.* **187**: 7870-7875.

Rivera-Gómez, N., Martínez-Núñez, M.A., Pastor, N., Rodriguez-Vazquez, K., and Perez-Rueda, E. (2017) Dissecting the protein architecture of DNA-binding transcription factors in bacteria and archaea. *Microbiology* **163**: 1167-1178.

Rivera-Gómez, N., Segovia, L., and Pérez-Rueda, E. (2011) Diversity and distribution of transcription factors: their partner domains play an important role in regulatory plasticity in bacteria. *Microbiology* **157**: 2308-2318.

Robinson, V.L., Buckler, D.R., and Stock, A.M. (2000) A tale of two components: a novel kinase and a regulatory switch. *Nature Structural and Molecular Biology* **7**: 626.

Römling, U., and Balsalobre, C. (2012) Biofilm infections, their resilience to therapy and innovative treatment strategies. *J. Intern. Med.* **272**: 541-561.

Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., and Thompson, F., (2013) *The prokaryotes: applied bacteriology and biotechnology*. Springer.

Samson, R., Legendre, J., and Christen, R. (2005) Fischer Le Saux M., Achouak W., Gardan L. Transfer of *Pectobacterium chrysanthemi* (Burkholder et al. 1953) Brenner et al. 1973 and Brenneria paradisiacal to the genus *Dickeya* gen. nov. as *Dickeya chrisanthemi* comb. nov. and *Dickeya* paradisiacal comb. nov. and delineation of four novel species, Dickeya dadantii sp. nov., and Dickeya dianthicola sp. nov., *Dickeya dieffenbachiae* sp. nov., and *Dickeya zeae* sp. nov. *International Journal of Systematic Evolution and Microbiology*.

Sarfraz, S., Riaz, K., Oulghazi, S., Cigna, J., Sahi, S.T., Khan, S.H., and Faure, D. (2018) *Pectobacterium punjabense* sp. nov., isolated from blackleg symptoms of potato plants in Pakistan. *Int. J. Syst. Evol. Microbiol.* **68**: 3551-3556.

Seshasayee, A.S., Bertone, P., Fraser, G.M., and Luscombe, N.M. (2006) Transcriptional regulatory networks in bacteria: from input signals to output responses. *Curr. Opin. Microbiol.* **9**: 511-519.

Seshasayee, A.S.N., Sivaraman, K., and Luscombe, N.M., (2011) An overview of prokaryotic transcription factors. In: A Handbook of Transcription Factors. Springer, pp. 7-23.

Shevchik, V.E., Robert-Baudouy, J., and Hugouvieux-Cotte-Pattat, N. (1997) Pectate lyase PelI of *Erwinia chrysanthemi* 3937 belongs to a new family. *J. Bacteriol.* **179**: 7321-7330.

Shi, Y., Latifi, T., Cromie, M.J., and Groisman, E.A. (2004) Transcriptional control of the antimicrobial peptide resistance ugtL gene by the *Salmonella* PhoP and SlyA regulatory proteins. *J. Biol. Chem.* **279**: 38618-38625.

Shirshikov, F.V., Korzhenkov, A.A., Miroshnikov, K.K., Kabanova, A.P., Barannik, A.P., Ignatov, A.N., and Miroshnikov, K.A. (2018) Draft genome sequences of new genomospecies "*Candidatus Pectobacterium maceratum"* strains, which cause soft rot in plants. *Genome Announc.* **6**: e00260-00218.

Shyntum, D.Y., Nkomo, N., Gricia, A.R., Shigange, N.L., Bellieny-Rabelo, D., and Moleleki, L.N. (2018) The impact of type VI secretion system, bacteriocins and antibiotics on competition amongst Soft-Rot Enterobacteriaceae: Regulation of carbapenem biosynthesis by iron and the transcriptional regulator Fur. *bioRxiv*: 497016.

Sidote, D.J., Barbieri, C.M., Wu, T., and Stock, A.M. (2008) Structure of the *Staphylococcus aureus* AgrA LytTR domain bound to DNA reveals a beta fold with an unusual mode of binding. *Structure* **16**: 727-735.

Sjöblom, S., Harjunpää, H., Brader, G., and Palva, E.T. (2008) A novel plant ferredoxin-like protein and the regulator Hor are quorum-sensing targets in the plant pathogen *Erwinia carotovora. Mol. Plant-Microbe Interact.* **21**: 967-978.

Slater, H., Crow, M., Everson, L., and Salmond, G.P. (2003) Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and-independent pathways. *Mol. Microbiol.* **47**: 303-320.

Smith, R.L., and Maguire, M.E. (1998) Microbial magnesium transport: unusual transporters searching for identity. *Mol. Microbiol.* **28**: 217-226.

Soleimani-Delfan, A., Etemadifar, Z., Emtiazi, G., and Bouzari, M. (2015) Isolation of *Dickeya dadantii* strains from potato disease and biocontrol by their bacteriophages. *Braz. J. Microbiol.* **46**: 791-797.

Soncini, F.C., Vescovi, E.G., Solomon, F., and Groisman, E.A. (1996) Molecular basis of the magnesium deprivation response in *Salmonella* typhimurium: identification of PhoP-regulated genes. *J. Bacteriol.* **178**: 5092-5099.

Spory, A., Bosserhoff, A., von Rhein, C., Goebel, W., and Ludwig, A. (2002) Differential regulation of multiple proteins of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium by the transcriptional regulator SlyA. *J. Bacteriol.* **184**: 3549-3559.

Stapleton, M.R., Norte, V.A., Read, R.C., and Green, J. (2002) Interaction of the *Salmonella* typhimurium transcription and virulence factor SlyA with target DNA, and identification of members of the SlyA regulon. *J. Biol. Chem.*

Stock, J., Ninfa, A., and Stock, A. (1989) Protein phosphorylation and regulation of adaptive responses in bacteria. *Microbiol. Rev.* **53**: 450-490.

Stormo, G.D. (2000) DNA binding sites: representation and discovery. *Bioinformatics* **16**: 16-23.

Thomas, M.S., and Wigneshweraraj, S. (2014) Regulation of virulence gene expression. *Virulence* **5**: 832-834.

Thomson, N., Cox, A., Bycroft, B., Stewart, G., Williams, P., and Salmond, G. (1997) The rap and hor proteins of *Erwinia, Serratia* and *Yersinia:* a novel subgroup in a growing superfamily of proteins regulating diverse physiological processes in bacterial pathogens. *Mol. Microbiol.* **26**: 531-544.

Tian, Y., Zhao, Y., Yuan, X., Yi, J., Fan, J., Xu, Z., Hu, B., De Boer, S.H., and Li, X. (2016) *Dickeyafangzhongdai* sp. nov., a plant-pathogenic bacterium isolated from pear trees (Pyrus pyrifolia). *Int. J. Syst. Evol. Microbiol.* **66**: 2831-2835.

Toro-Roman, A., Mack, T.R., and Stock, A.M. (2005) Structural analysis and solution studies of the activated regulatory domain of the response regulator ArcA: a symmetric dimer mediated by the α4-β5-α5 face. *J. Mol. Biol.* **349**: 11-26.

Toth, I.K., Bell, K.S., Holeva, M.C., and Birch, P.R. (2003) Soft rot erwiniae: from genes to genomes. *Mol. Plant Pathol.* **4**: 17-30.

Toth, I.K., Pritchard, L., and Birch, P.R. (2006) Comparative genomics reveals what makes an enterobacterial plant pathogen. *Annu. Rev. Phytopathol.* **44**: 305-336.

Tsai, K.-C., Hung, P.-P., Cheng, C.-F., Chen, C., and Tseng, T.-S. (2019) Exploring the mode of action of inhibitors targeting the PhoP response regulator of *Salmonella enterica* through comprehensive pharmacophore approaches. *RSC advances* **9**: 9308-9312.

Tu, J., Huang, B., Zhang, Y., Zhang, Y., Xue, T., Li, S., and Qi, K. (2016) Modulation of virulence genes by the two-component system PhoP-PhoQ in avian pathogenic *Escherichia coli*. *Pol. J. Vet. Sci.* **19**: 31-40.

Tyler, H.L., and Triplett, E.W. (2008) Plants as a habitat for beneficial and/or human pathogenic bacteria. *Annu. Rev. Phytopathol.* **46**: 53-73.

Ulrich, L.E., Koonin, E.V., and Zhulin, I.B. (2005) One-component systems dominate signal transduction in prokaryotes. *Trends Microbiol.* **13**: 52-56.

Vadyvaloo, V., Viall, A.K., Jarrett, C.O., Hinz, A.K., Sturdevant, D.E., and Hinnebusch, B.J. (2015) Role of the PhoP–PhoQ gene regulatory system in adaptation of *Yersinia pestis* to environmental stress in the flea digestive tract. *Microbiology* **161**: 1198-1210.

van der Merwe, J.J., Coutinho, T.A., Korsten, L., and van der Waals, J.E. (2010) *Pectobacterium carotovorum* subsp. *brasiliensis* causing blackleg on potatoes in South Africa. *Eur. J. Plant Pathol.* **126**: 175-185.

Van der Wolf, J., De Haan, E., Kastelein, P., Krijger, M., De Haas, B., Velvis, H., Mendes, O., Kooman-Gersmann, M., and Van Der Zouwen, P. (2017) Virulence of *Pectobacterium carotovorum* subsp. *brasiliense* on potato compared with that of other *Pectobacterium* and *Dickeya* species under climatic conditions prevailing in the Netherlands. *Plant Pathol.* **66**: 571-583.

Van der Wolf, J.M., and De Boer, S.H. (2007) Bacterial pathogens of potato. *Potato Biology and Biotechnology, Advances and Perspectives. Oxford, UK: Elsevier*: 595-619.

Van Der Wolf, J.M., Nijhuis, E.H., Kowalewska, M.J., Saddler, G.S., Parkinson, N., Elphinstone, J.G., Pritchard, L., Toth, I.K., Lojkowska, E., and Potrykus, M. (2014) *Dickeya solani* sp. nov., a pectinolytic plant-pathogenic bacterium isolated from potato (Solanum tuberosum). *Int. J. Syst. Evol. Microbiol.* **64**: 768-774.

Venkatesh, B., Babujee, L., Liu, H., Hedley, P., Fujikawa, T., Birch, P., Toth, I., and Tsuyumu, S. (2006) The *Erwinia chrysanthemi* 3937 PhoQ sensor kinase regulates several virulence determinants. *J. Bacteriol.* **188**: 3088-3098.

Véscovi, E.G., Soncini, F.C., and Groisman, E.A. (1996) Mg2+ as an extracellular signal: environmental regulation of *Salmonella* virulence. *Cell* **84**: 165-174.

Vinatzer, B.A., Monteil, C.L., and Clarke, C.R. (2014) Harnessing population genomics to understand how bacterial pathogens emerge, adapt to crop hosts, and disseminate. *Annu. Rev. Phytopathol.* **52**: 19-43.

Voronina, M.V., Bugaeva, E.N., Vasiliev, D.M., Kabanova, A.P., Barannik, A.P., Shneider, M.M., Kulikov, E.E., Korzhenkov, A.A., Toschakov, S.V., Ignatov, A.N., and Miroshnikov, K.A. (2019) Characterization of *Pectobacterium carotovorum* subsp. *carotovorum* Bacteriophage PP16 Prospective for Biocontrol of Potato Soft Rot. *Microbiology* **88**: 451-460.

Waldee, E.L. (1945) Comparative studies of some peritrichous phytopathogenic bacteria.

Waleron, M., Misztak, A., Waleron, M., Franczuk, M., Jońca, J., Wielgomas, B., Mikiciński, A., Popović, T., and Waleron, K. (2019a) *Pectobacterium zantedeschiae* sp. nov. a new species of a soft rot pathogen isolated from Calla lily (Zantedeschia spp.). *Syst. Appl. Microbiol.* **42**: 275-283.

Waleron, M., Misztak, A., Waleron, M., Jonca, J., Furmaniak, M., and Waleron, K. (2019b) Pectobacterium polonicum sp. nov. isolated from vegetable fields. *Int. J. Syst. Evol. Microbiol.* **69**: 1751-1759.

Waleron, M., Waleron, K., and Lojkowska, E. (2015) First Report of *Pectobacterium carotovorum* subsp. *brasiliense* Causing Soft Rot on Potato and Other Vegetables in Poland. *Plant Dis.*

Wang, Q., Zhao, Y., McClelland, M., and Harshey, R.M. (2007) The RcsCDB signaling system and swarming motility in *Salmonella enterica* serovar Typhimurium: dual regulation of flagellar and SPI-2 virulence genes. *J. Bacteriol.* **189**: 8447-8457.

Wang, Z., Gerstein, M., and Snyder, M. (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nature reviews genetics* **10**: 57.

Weatherspoon-Griffin, N., and Wing, H.J. (2016) Characterization of SlyA in *Shigella flexneri* identifies a novel role in virulence. *Infect. Immun.* **84**: 1073-1082.

Wei, C., Ding, T., Chang, C., Yu, C., Li, X., and Liu, Q. (2019) Global Regulator PhoP is Necessary for Motility, Biofilm Formation, Exoenzyme Production and Virulence of *Xanthomonas citri* Subsp. *citri* on Citrus Plants. *Genes* **10**: 340.

Weinstein-Fischer, D., and Altuvia, S. (2007) Differential regulation of *Escherichia coli* topoisomerase I by Fis. *Mol. Microbiol.* **63**: 1131-1144.

Whitehead, N.A., Barnard, A.M., Slater, H., Simpson, N.J., and Salmond, G.P. (2001) Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol. Rev.* **25**: 365-404.

Wilkinson, S.P., and Grove, A. (2006) Ligand-responsive transcriptional regulation by members of the MarR family of winged helix proteins. *Curr. Issues Mol. Biol.* **8**: 51.

Will, W.R., Bale, D.H., Reid, P.J., Libby, S.J., and Fang, F.C. (2014) Evolutionary expansion of a regulatory network by counter-silencing. *Nature communications* **5**.

Will, W.R., Brzovic, P., Le Trong, I., Stenkamp, R.E., Lawrenz, M.B., Karlinsey, J.E., Navarre, W.W., Main-Hester, K., Miller, V.L., and Libby, S.J. (2019) The evolution of SlyA/RovA transcription factors from repressors to countersilencers in Enterobacteriaceae. *MBio* **10**: e00009-00019.

Winfield, M.D., and Groisman, E.A. (2004) Phenotypic differences between *Salmonella* and *Escherichia coli* resulting from the disparate regulation of homologous genes. *Proceedings of the National Academy of Sciences* **101**: 17162-17167.

Winslow, C.-E., Broadhurst, J., Buchanan, R., Krumwiede Jr, C., Rogers, L., and Smith, G. (1920) The families and genera of the bacteria: final report of the committee of the Society of American Bacteriologists on characterization and classification of bacterial types. *J. Bacteriol.* **5**: 191.

Wojtaszek, P. (1997) Oxidative burst: an early plant response to pathogen infection. *Biochem. J.* **322**: 681-692.

Wood, J.M. (2010) Osmotic stress. *Bacterial Stress Responses*: 133-156.

Wood, J.M. (2011) Bacterial osmoregulation: a paradigm for the study of cellular homeostasis. *Annu. Rev. Microbiol.* **65**: 215-238.

Wu, R.-y., Zhang, R.-g., Zagnitko, O., Dementieva, I., Maltzev, N., Watson, J.D., Laskowski, R., Gornicki, P., and Joachimiak, A. (2003) Crystal structure of *Enterococcus faecalis* SlyA-like transcriptional factor. *J. Biol. Chem.* **278**: 20240-20244.

Wu, W., Liu, Y., Huang, X., and Zhang, L. (2017) First report of summer canker caused by *Pectobacterium carotovorum* subsp. *actinidiae* on kiwifruit in China's Sichuan Province. *Plant Dis.* **101**: 1540.

Xia, T., Li, Y., Sun, D., Zhuo, T., Fan, X., and Zou, H. (2016) Identification of an extracellular endoglucanase that is required for full virulence in *Xanthomonas citri* subsp. *citri*. *PLoS One* **11**.

Xiong, A., Gottman, A., Park, C., Baetens, M., Pandza, S., and Matin, A. (2000) The EmrR protein represses the *Escherichia coli* emrRAB multidrug resistance operon by directly binding to its promoter region. *Antimicrob. Agents Chemother.* **44**: 2905-2907.

Xu, J., and Wang, N. (2018) Where are we going with genomics in plant pathogenic bacteria? *Genomics*.

Yang, C., Huang, T.-W., Wen, S.-Y., Chang, C.-Y., Tsai, S.-F., Wu, W.-F., and Chang, C.-H. (2012) Genome-wide PhoB binding and gene expression profiles reveal the hierarchical gene regulatory network of phosphate starvation in *Escherichia coli*. *PLoS One* **7**.

Yeaman, M.R., and Yount, N.Y. (2003) Mechanisms of antimicrobial peptide action and resistance. *Pharmacol. Rev.* **55**: 27-55.

Yuan, J., Jin, F., Glatter, T., and Sourjik, V. (2017) Osmosensing by the bacterial PhoQ/PhoP two-component system. *Proceedings of the National Academy of Sciences* **114**: E10792-E10798.

Zhang, Y., Fan, Q., and Loria, R. (2016) A re-evaluation of the taxonomy of phytopathogenic genera *Dickeya* and *Pectobacterium* using whole-genome sequencing data. *Systematic and applied microbiology* **39**: 252-259.

Zhang, Y., Jalan, N., Zhou, X., Goss, E., Jones, J.B., Setubal, J.C., Deng, X., and Wang, N. (2015) Positive selection is the main driving force for evolution of citrus canker-causing *Xanthomonas*. *The ISME journal* **9**: 2128-2138.

Zhao, Y., Sundin, G.W., and Wang, D. (2009) Construction and analysis of pathogenicity island deletion mutants of *Erwinia amylovora*. *Can. J. Microbiol.* **55**: 457-464.

Zhou, D., Han, Y., Qin, L., Chen, Z., Qiu, J., Song, Y., Li, B., Wang, J., Guo, Z., and Du, Z. (2005) Transcriptome analysis of the Mg2+-responsive PhoP regulator in *Yersinia pestis*. *FEMS Microbiol. Lett.* **250**: 85-95.

Zhou, J.N., Zhang, H.B., Lv, M.F., Chen, Y.F., Liao, L.S., Cheng, Y.Y., Liu, S.Y., Chen, S.H., He, F., and Cui, Z.N. (2016) SlyA regulates phytotoxin production and virulence in *Dickeya zeae* EC1. *Mol. Plant Pathol.*

Zhu, R., Hao, Z., Lou, H., Song, Y., Zhao, J., Chen, Y., Zhu, J., and Chen, P.R. (2017) Structural characterization of the DNA-binding mechanism underlying the copper (II)-sensing MarR transcriptional regulator. *JBIC Journal of Biological Inorganic Chemistry* **22**: 685-693.

Zhuge, X., Sun, Y., Xue, F., Tang, F., Ren, J., Li, D., Wang, J., Jiang, M., and Dai, J. (2018) A novel PhoP/PhoQ regulation pathway modulates the survival of extraintestinal pathogenic *Escherichia coli* in macrophages. *Front. Immunol.* **9**: 788.

Zou, L., Zeng, Q., Lin, H., Gyaneshwar, P., Chen, G., and Yang, C.-H. (2012) SlyA regulates type III secretion system (T3SS) genes in parallel with the T3SS master regulator HrpL in *Dickeya dadantii* 3937. *Appl. Environ. Microbiol.* **78**: 2888-2895.

Zschiedrich, C.P., Keidel, V., and Szurmant, H. (2016) Molecular mechanisms of two-component signal transduction. *J. Mol. Biol.* **428**: 3752-3775.

CHAPTER TWO

PhoP transcriptional regulator of *Pectobacterium brasiliense* 1692 (*Pb*1692) suppresses T6SS and carbapenem production *in planta*

2.1 Abstract

Host-pathogen interaction is a complicated process involving entry into the host tissues, apoplastic colonization, host defense suppression and ultimately development of disease symptoms. The mechanism of disease development is affected by a variety of virulence factors, such as several PCWDEs and secretion systems to ensure successful infection of plant hosts. The objective of this particular study, was to functionally characterise the role of *Pb*1692 PhoP transcriptional regulator *in planta* using RNA-seq and phenotypic assays. RNA-sequencing analysis generated approximately 1.64 million reads and 87% of the reads were exclusively mapped to the *Pb*1692 genome. Furthermore, the results showed a total of 23 Type VI secretion system (T6SS) related genes and carbapenem genes overexpressed in the absence of PhoP. These are some of the genes implicated in antibacterial activity. Based on the transcriptome data, competition assays between *D. dadantii* 3937 and *Pb*1692Δ*pho*P mutants were conducted. Competition assays showed a 5-fold (CFU/ml) reduction in the survival of *D. dadantii* 3937 when co-inoculated with *pho*P mutants, and a 3-fold (CFU/ml) reduction of *D. dadantii* when co-inoculated with *Pb*1692 wild type. In the *Pb*1692Δ*pho*P mutant, some of the genes associated with the production of PCWDEs such as pectate lyases were upregulated, while polygalacturonases were down-regulated. In addition to PCWDEs, a quorum-sensing regulator, ExpR was differentially expressed in the *Pb*1692*pho*P mutant. These findings suggest that PhoP communicates with a vast signaling network to regulate numerous virulence determinants responsible for bacterial adaption to the host environment during infection. Analysis of the transcriptome data demonstrates that the PhoP regulon is substantially broader than previously suspected affecting the differential expression of more than 400 genes. Furthermore, a time-course RT-qPCR analysis, revealed the importance of *pho*P gene in the survival of *Pb*1692 under acidic conditions encountered in the apoplast. The findings of the RT-qPCR revealed that the relative expression of *pho*P gene was low in the wild type, which is in agreement with the regulator's decreased cellular demand after

alkalization of the environment due to progressive cell lysis. The data confirms that PhoP functions as a global regulator in *Pb*1692 and it is essential in the early stages of infection. In conclusion, these results suggest that following plant infection, *Pb*1692 activates virulence factors that are key in defense response against various plant defenses. Overall this study provides the first insight of defense mechanisms in *Pb*1692 during *in planta* infection using PhoP transcriptional regulator.

2.2 Introduction

Pectobacterium species are Gram-negative necrotrophic phytopathogens causing soft rot diseases on several economically important plants by secreting a battery of extracellular enzymes such as cellulases, pectinases, and proteases breaking down the plant cell wall (Charkowski *et al.*, 2012). Apart from secreting PCWDEs as their primary virulence factor, *Pectobacterium* species also possess several other virulence factors, namely flagella, lipopolysaccharides, siderophores, Nep1-like proteins and *hrp* genes (Toth *et al.*, 2003, Hancock *et al.*, 1995, López-Solanilla *et al.*, 1998, Czajkowski *et al.*, 2015, Li *et al.*, 2018). Contribution of these virulence determinants to pathogenicity and how they are regulated is a complicated process involving several signaling transduction mechanisms. One of the widely distributed signal transduction system utilized by bacteria is represented by the TCS (Dalebroux & Miller, 2014). TCSs are used by bacteria to sense and generate a response to specific environmental signals, through changes in gene expression (Beier & Gross, 2006). Some of the well-studied TCS belong to the OmpR/PhoB and NarL/FixJ subfamily of response regulators. This family is further characterised based on the sequence similarity of the C-terminal DNA binding domain, whose structure contains a WHTH domain (Stock & Da Re, 2000, Cheung & Hendrickson, 2010, Groisman, 2016, Groisman, 2001, Noriega *et al.*, 2010, Nguyen *et al.*, 2015). A typical TCS involves a response regulator which is specifically activated through the interaction with its cognate HPK (Gao & Stock, 2009, Tiwari *et al.*, 2017). Upon specific signal-recognition, a phosphoryl group from the HPK is transferred to the response regulator which elicits a cellular response ranging from transcriptional regulation through DNA binding to enzymatic catalysis (Batchelor *et al.*, 2008, Romling *et al.*, 2005, Cheung & Hendrickson, 2010). Suggesting that gene regulation occurs through alterations in the response regulator protein, thereby regulating its affinity for DNA binding in the promoter region of target genes (Beier & Gross, 2006). Furthermore over 4,000 TCS have been identified in more than 145 bacterial genomes (Haque & Tsuyumu, 2005). In Soft Rot *Pectobacteriaceae*, several TCS have been characterised, for example, ExpA-ExpS, PehR-PehS, PmrB-PmrA and PhoPQ. In many Gram-negative bacteria, namely *S. enterica* serovar Typhimurium, *Y. pestis, S. flexineri* and *D. dadantii,* PhoPQ is one of the well-studied TCS (Miller *et al.*, 1989b, Vadyvaloo *et al.*, 2015, Banda *et al.*, 2018, Haque *et al.*, 2009). PhoP was originally reported in *S.*

enterica serovar Typhimurium, where its major role appears to be the regulation of genes involved in adaptation to magnesium availability and low acidic pH (Lin *et al.*, 2018, Groisman, 2001). Since then, PhoP has been reported to regulate a variety of virulence factors in several other Gram-negative bacteria (Banda *et al.*, 2018, Lin *et al.*, 2017). For example, PhoP of *Y. pestis* regulates physiological adaptation in response to acidic surroundings when colonizing the flea gut (Vadyvaloo *et al.*, 2015). Similarly, tolerance to acidic pH in *S. flexneri* was also reported to be modulated by PhoP, this observation was also demonstrated by *pho*P defective strains which were attenuated in virulence (Lin *et al.*, 2018, Moss *et al.*, 2000a).

In addition to human and animal pathogens, PhoP was also characterized in plant pathogens such as *D. dadantii, D. chrysanthemi, X. citri* subsp. *citri* and *P. versatile* where it regulates multiple virulence factors (Babujee *et al.*, 2007, Wei *et al.*, 2019, Kravchenko *et al.*, 2020, Llama-Palacios *et al.*, 2005a). In a study conducted by Llama and colleagues on *D. chrysanthemi* 3937 exposed to acidic pH conditions, *pho*P mutants of *D. chrysanthemi* 3937 were not sensitive to acidic pH which is in contrast with what was reported for *pho*P mutants of *D. chrysanthemi* strain EC16 and other *Enterobacteriaceae*, whose *pho*P mutants were sensitive to acidic pH (Llama-Palacios *et al.*, 2005a, Llama-Palacios *et al.*, 2003, Bearson *et al.*, 1998, Oyston *et al.*, 2000). According to Llama-Palacios, the differences observed between the two *D. chrysanthemi* strains (3937 and EC16) could be attributed to strain-specific strategies used by *D. chrysanthemi* to cope with acidic pH in the apoplast. Llama and colleagues further concluded that *D. chrysanthemi* 3937 does not rely on the PhoP regulatory network while *D. chrysanthemi* EC16 seems to depend on it (Llama-Palacios *et al.*, 2005a). These two studies indicate that PhoP regulatory mechanisms differ among pathogenic bacteria, including those of the same genus. PhoP has recently been found to control the expression of multiple virulence determinants in *X. citri* subsp. *citri* and *P. versatile* adaptations, such as the production of PCWDEs, bacterial motility and the formation of biofilms (Wei *et al.*, 2019, Kravchenko *et al.*, 2020). Even though the role of PhoP has been deciphered in other plant pathogens such as *D. dadantii*, however, the complete regulon of PhoP in the SRPs is yet to be determined. The objective of this study was therefore to determine the regulon of PhoP in *Pb*1692 and to functionally characterise its role in the biology of this plant pathogen.

2.3 Results

2.3.1 Distribution of PhoP transcriptional regulator in *Dickeya* and *Pectobacterium* species

To evaluate the evolutionary relationship between protein sequence of PhoP transcriptional regulator in *Pectobacterium* and *Dickeya* species, multiple sequence alignment of the PhoP protein was done using ClustalOmega and Jalview. PhoP protein from *Pb*1692 was aligned with over 100 species of *Dickeya* and *Pectobacterium* (Figure 2.1). This comparison revealed high sequence identity and common secondary structural features between the species of *Pectobacterium* and *Dickeya.* The PhoP proteins from *Pb*1692, *Dickeya* and other *Pectobacterium* show high sequence similarity of >90% between the species. The yellow bars indicate highly conserved regions. The alignment also revealed a total of 8 α-helices and 8 β-strands. The β-strands consist of mainly isoleucine, leucine and valine residues. These residues are hydrophobic and form part of the hydrophobic core. The α-helices appear to be amphipathic as it is evident by the number of conserved amphipathic residues namely histidine, tryptophan, tyrosine and methionine residues (His-14, 15; Trp-63,81; Tyr-138; Met-105, 155&166). Also essential aspartate residue 55 (D55) is conserved in all the aligned species.

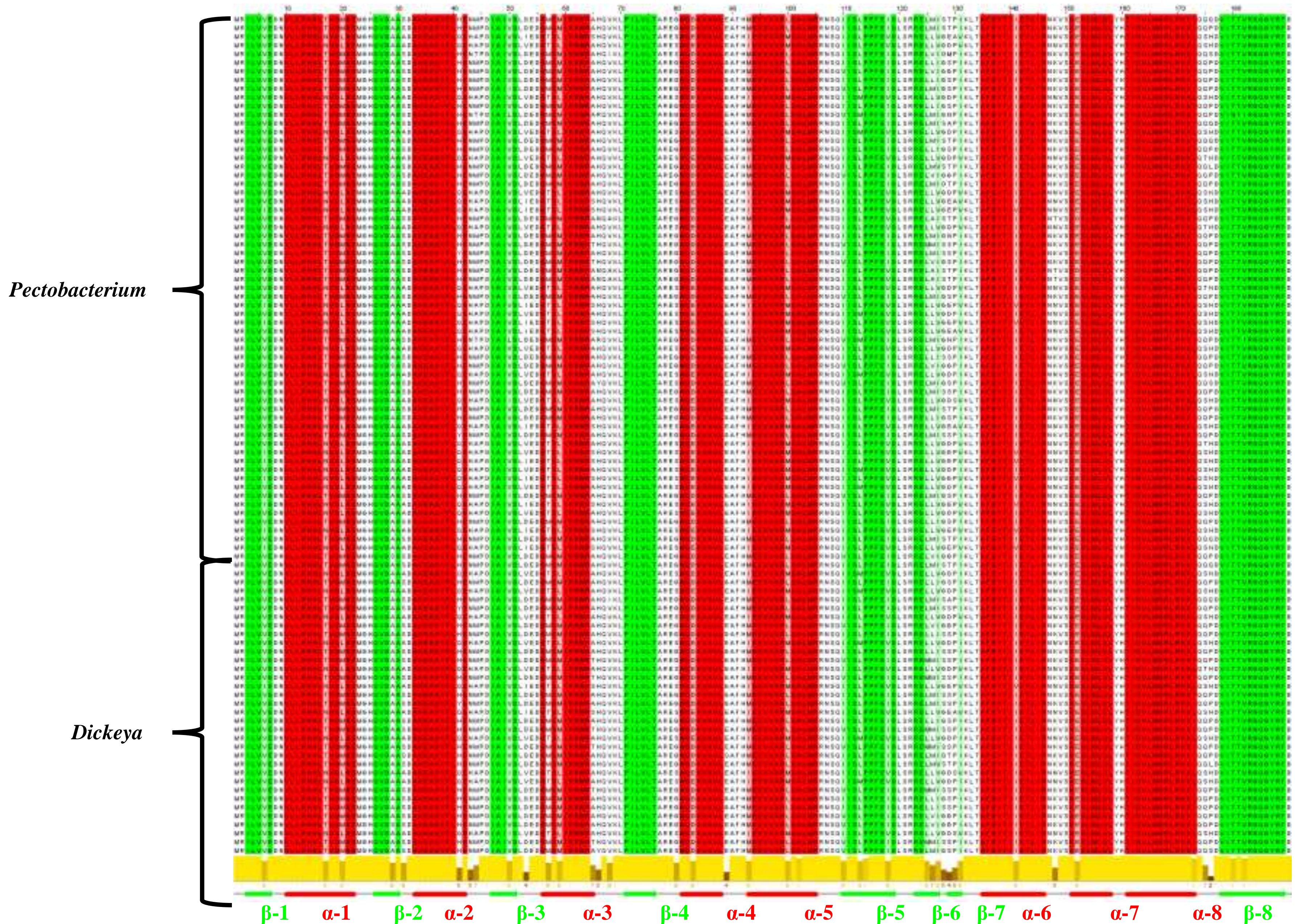

Pectobacterium
Dickeya
β-1
α-1
β-2
α-2
β-3
α-3
β-4
α-4
α-5
β-5
β-6
β-7
α-6
α-7
α-8
β-8

Figure 2. 1: Multiple alignment analysis of PhoP response regulator of *Pb*1692, *Dickeya* and *Pectobacterium* species. Sequence alignment done with ClustalOmega and Jalview (Sievers *et al.*, 2011, Waterhouse *et al.*, 2009). Amino acid residues responsible for dimerization and DNA binding were determined by secondary structure analysis in Jalview. The secondary structural elements are shown as red sequences and tubes, while α-helices are shown in red tube and β-strands green tubes. Yellow bars are highly conserved residues.

2.3.2 Engineering of *Pb*1692Δ*pho*P mutant strain and whole-transcriptome study

To investigate the functional characteristics of PhoP in *Pb*1692 regulatory programs, the lambda recombination technique by Datsenko and Werner was used to generate a *Pb*1692Δ*pho*P mutant (see 'Experimental Procedure' for details 2.5.3) (Datsenko & Wanner, 2000). The integrity of the mutants was confirmed by DNA sequencing together with PCR analyses (Figure S2.1 and S2.2). Furthermore, growth assay analysis between the wild type and *Pb*1692Δ*pho*P showed that deletion of *pho*P gene does not affect the mutant's ability to grow (Figure S2.3). Next, the effect caused by *pho*P deletion in the *Pb*1692 genome was dissected in an entire transcriptome database consisting of ~164 million RNA-seq reads. Furthermore, the analysis deduced transcriptional regulation effected by the PhoP network by comparing significant gene expression between *Pb*1692 and *Pb*1692Δ*pho*P. For this analysis, eight mRNA libraries were sequenced depicting both Δ*pho*P strain and wild-type *Pb*1692 collected from two different time points after infection of potato tubers: 12 and 24hpi.

The dataset yielded good quality reads, as 92% (~151 million) of the reads were matched on the *Pb*1692 genome. Out of these, 87.1% (~132 million reads) was exclusively mapped to the *Pb*1692 genome. Further analysis between *Pb*1692 and *Pb*1692Δ*pho*P identified a total of 491 genes affected (up-/down-regulation) by the PhoP network at 12 and 24hpi (Figure 2.2A, Table S2.1 & S2.2). The stringent P-value of > 0.005 was used for the classification of differential expressed genes between *Pb*1692 wild type and *Pb*1692Δ*pho*P mutant. The KEGG database was also used to show genes in various signaling pathways that were enriched ($p < 0.05$) in different pathways.

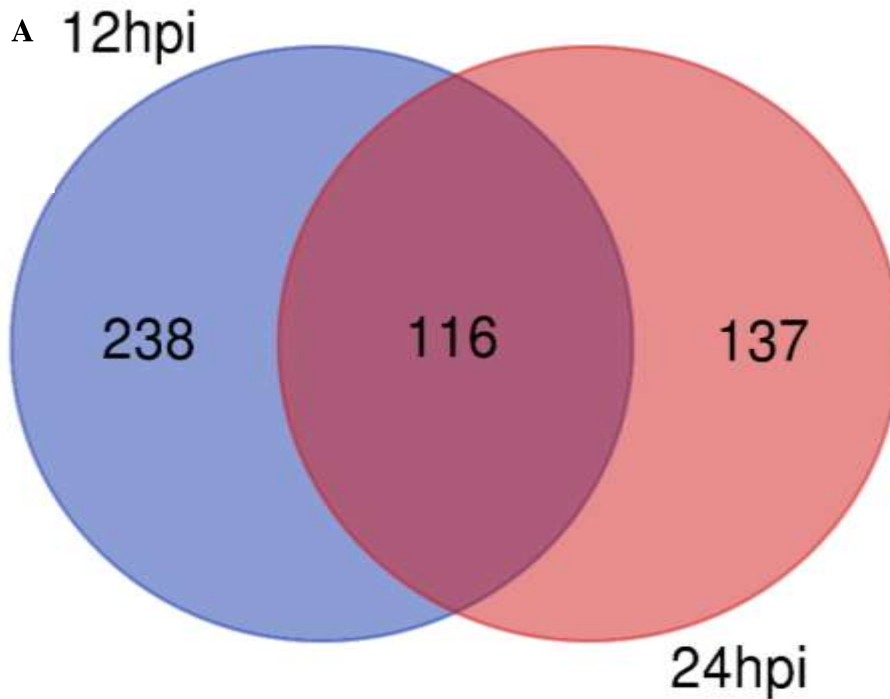
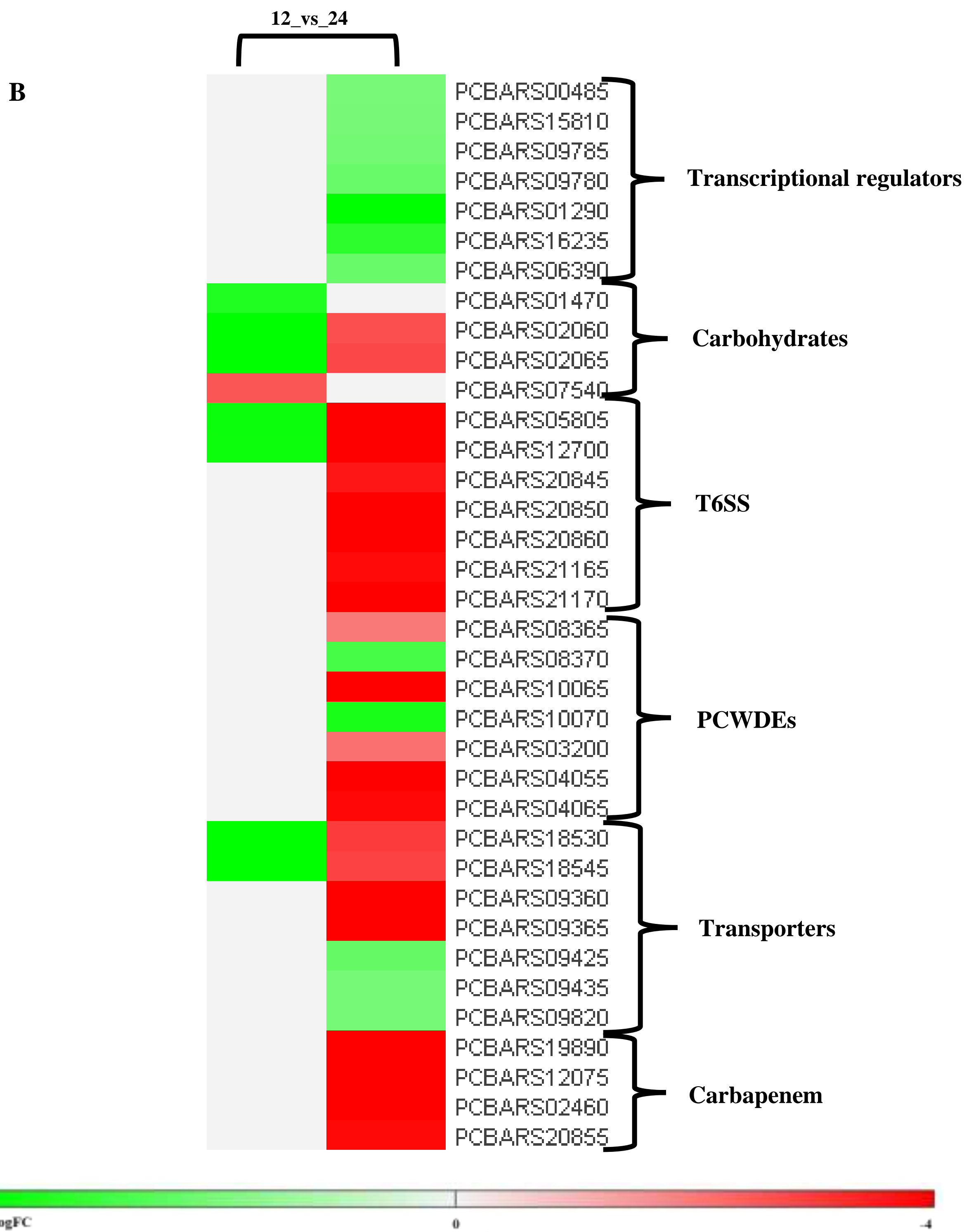


Figure 2. 2 (A): Venn diagram depicting overlap of *Pb*1692 genes between 12 and 24hpi. 238 genes differentially expressed at 12hpi and 137 at 24hpi out of which 116 were common between the two-time points.

Furthermore, a heat map showing the distribution of 12 and 24hpi DEGs was constructed using Gitools software (Figure 2.2 B). The differentially expressed genes were functionally grouped into the following functional groups; T6SS, T3SS, transport, carbohydrates, PCWDEs, fimbrial and transcriptional regulators. Through functional annotation of *Pb*1692 sequences, (Material and Methods 2.5.3) the presence of key virulence themes from *Pb*1692 within the PhoP regulon were identified. The results revealed DEGs involved in the regulation of virulence either up/or down-regulated. This observation includes regulation of 37 and 38 genes, respectively, in 12 and 24hpi assigned to the 'secretion

system' (KEGG: ko02044) class (Table S2.1 & S2.2). Gene set enrichment analysis (GSEA) was done to identify genes that are over-represented and might be associated with disease development (Subramanian *et al.*, 2005). In addition, regulation of secretion systems-associated genes is enriched in the *pho*P knockout-strain during infection and encompasses genes associated with several secretion systems including T6SS (Table S2.1 & 2.2). The study also detected enrichment in the regulation of genes associated with carbapenem biosynthesis (KEGG: ko00332) in the *Pb*1692Δ*pho*P mutant strain at 12 and 24hpi showing 5 out of 8 annotated genes in this pathway.

Utilizing the subsequent time-course RNA-seq information, the differential expression patterns of selected genes were evaluated. The analysis identified genes either down/-up regulated by PhoP when infection potato tubers. At 24hpi a total of seven transcriptional regulators were downregulated by PhoP. At 12hpi, majority of carbohydrate genes were downregulated and at 24hpi the same genes were upregulated, suggesting that carbohydrates are utilized by *Pb*1692 early in the infection process to establish bacterial populations. The entire T6SS cluster and carbapenem genes were upregulated at 24hpi by PhoP, repressing the activation of these genes. PCWDEs, namely cellulase and polygalacturonase were downregulated at 24hpi while pectate lyases were upregulated. Demonstrating how PhoP times the virulent stages relative to the appropriate environmental cues and host physiology. These results suggest that PhoP differentially expressed an assortment of genes to defend against plant defence responses.



12_vs_24
B
PCBARS00485
PCBARS15810
PCBARS09785
PCBARS09780
PCBARS01290
PCBARS16235
PCBARS06390
PCBARS01470
PCBARS02060
PCBARS02065
PCBARS07540
PCBARS05805
PCBARS12700
PCBARS20845
PCBARS20850
PCBARS20860
PCBARS21165
PCBARS21170
PCBARS08365
PCBARS08370
PCBARS10065
PCBARS10070
PCBARS03200
PCBARS04055
PCBARS04065
PCBARS18530
PCBARS18545
PCBARS09360
PCBARS09365
PCBARS09425
PCBARS09435
PCBARS09820
PCBARS19890
PCBARS12075
PCBARS02460
PCBARS20855
Transcriptional regulators
Carbohydrates
T6SS
PCWDEs
Transporters
Carbapenem
4 LogFC
0
-4

Fingure 2.2(B): Heat-map showing transcriptional profiles of selected differentially expressed genes *in planta* between 12 and 24hpi, following inoculation with *Pb*1692 compared to *Pb*1692Δ*pho*P samples. Red shows up-regulated genes and green down-regulated genes.

Furthermore, major genes encoding PCWDEs are represented in the PhoP regulon, such as pectate lyases (PL), cellulases (CELA/B) and the pectin lyase enzyme (PNL) (Table S2.1 & 2.2). On the basis of these findings, the impact of *Pb*1692Δ*pho*P mutant was investigated in potato tubers by measuring the maceration extent after 12 and 24hpi. The *Pb*1692Δ*pho*P mutant was drastically reduced (threefold) in virulence when compared to *Pb*1692, and the *Pb*1692Δ*pho*P-p*pho*P complementation restored the production of the PCWDEs to the wild type level. (Figure 2.3). In the subsequent sections, the phenotypic outcomes caused directly or indirectly by the perturbation of the PhoP regulatory network mainly focusing on central mechanisms of pathogenicity will be discussed.

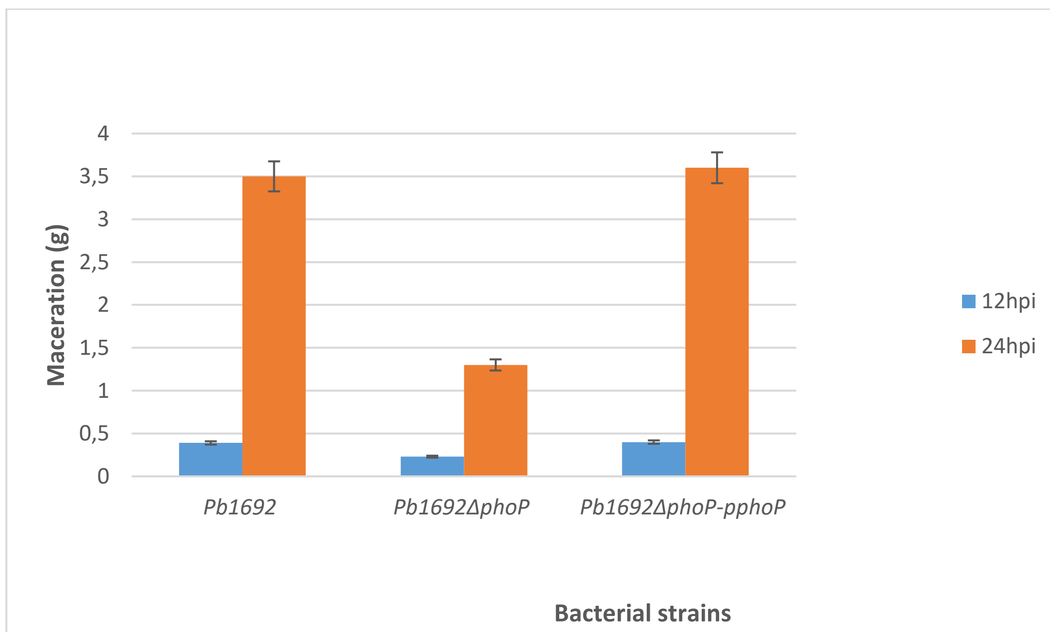


Figure 2. 3: Pathogenicity assays showing the extent of maceration in potato tubers caused by *Pb*1692Δ*pho*P mutant, *Pb*1692 wild type and *Pb*1692Δ*pho*P-p*pho*P complemented mutant strain after 12 and 24hpi. The standard mean of three biological replicates are represented by the error bars.

2.3.3 *In planta* competition assays on potato tubers

Evidence from the RNA seq revealed that T6SS and carbapenem genes were upregulated in the *Pb*1692Δ*pho*P mutant at 24hpi. These results suggest that *Pb*1692 PhoP transcriptional regulator represses T6SS and the production of carbapenem *in planta*. Based on these results, a hypothesis was formulated, that the growth of *D. dadantii* in potato tubers would be inhibited by a Δ*pho*P mutant strain. To verify this hypothesis, bacterial competition assays were conducted *in planta*. In a previous study in our laboratory, *Pb*1692 was found to outcompete *D. dadantii* when co-inocuated *in planta* (Shyntum *et al.*, 2018). However, in the present study a 5-fold reduction (CFU/ml) in the survival of *D. dadantii* was observed when co-inoculated with *Pb*1692Δ*pho*P (mutant) and only a 3-fold reduction when co-inoculated with *Pb*1692 and the ability to inhibit *D. dadantii* was restored in the complemented strain (Figure 2.4). As hypothesized, *Pb*1692Δ*pho*P (mutant) outcompeted *D. dadantii* significantly better than the *Pb*1692 wild type strain. In this regard, the findings confirm that PhoP negatively regulates T6SS *in planta* as seen in the transcriptome data.

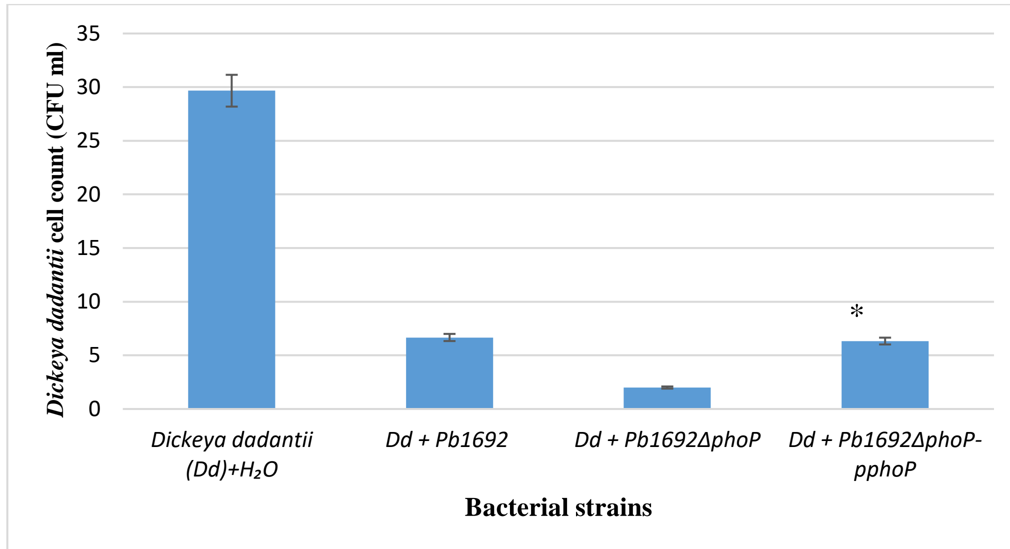


Figure 2. 4: Interbacterial competition between *Pb*1692 and *D. dadantii* strains on potato tubers. Wild-type *Pb*1692, its isogenic mutant *Pb*1692Δ*pho*P and *Pb*1692Δ*pho*P-p*pho*P complemented strains were co-inoculated with *D. dadantii* for 24 hours. A graph depicting the growth (mean CFU/ml) of *D. dadantii* strain co-inoculated with *Pb*1692 in potato tubers (at a 1:1 ratio).

2.3.4 PhoP response regulator is required for bacterial motility

RNA seq data identified genes associated with motility (ko02035) in the *Pb*1692Δ*pho*P mutant. These results suggest that *Pb*1692 PhoP transcriptional regulator may control motility *in planta*. The effect of deleting the *pho*P gene in *Pb*1692 was further examined on motility agar plates. The halos formed by individual strains were measured. Motility was drastically impaired in the *Pb*1692Δ*pho*P compared to *Pb*1692 (*Pb*1692Δ*pho*P mutant had diameter width of 3 mm compared to 22 mm displayed by the *Pb*1692 and 20mm in the *Pb*1692Δ*pho*P-p*pho*P complemented mutant strain). In the complemented strain, motility was restored to the wild type levels (Figure 2.5A and 2.5B). The majority of the genes activated in the transcriptome data at 12hpi were methyl accepting genes (PCBA_RS05895, -1.3), (PCBA_RS16315,-1.4), (PCBA_RS20715,-1.2),

PCBA_RS02830, -1.4). These results emphasize the importance of motility and chemotaxis during the invasion process. The combined ability of PhoP to down-regulate both motility and chemotaxis genes might explain why the mutant strain is impaired in virulence.

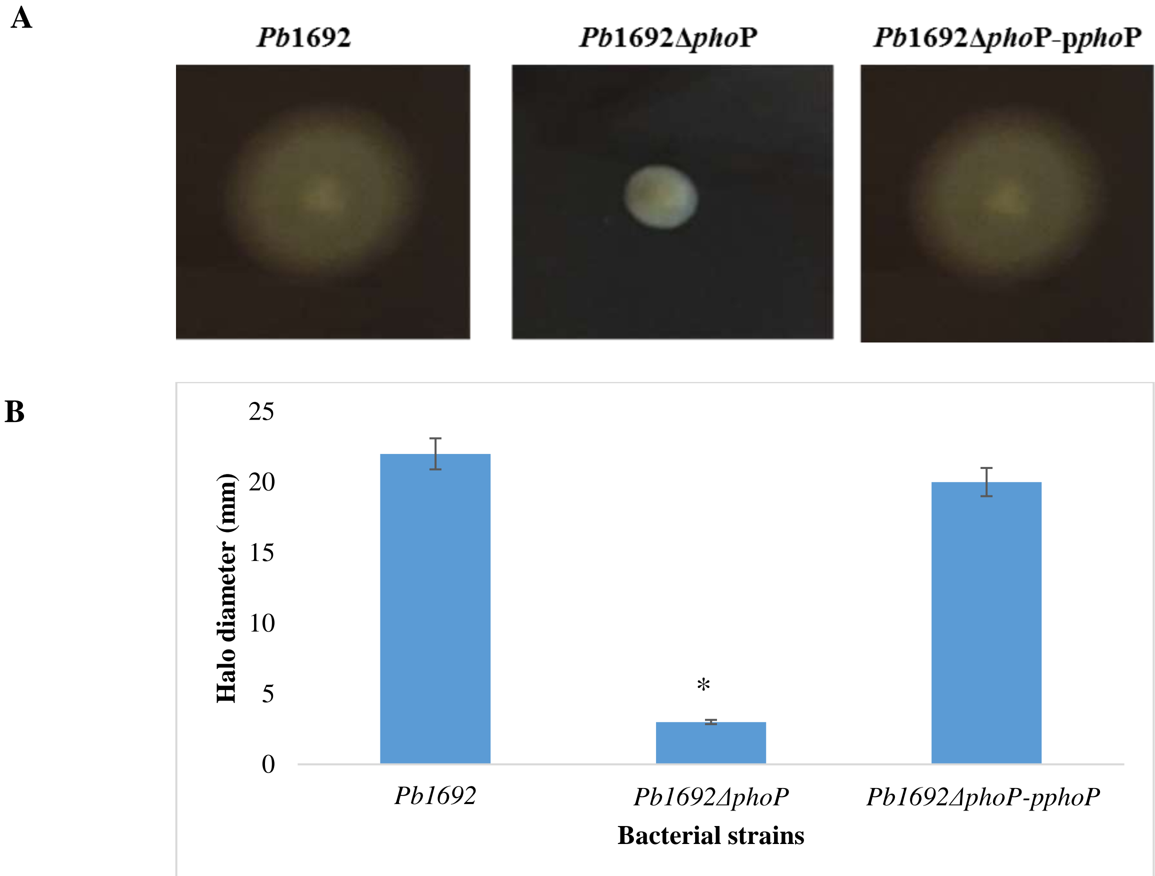


Figure 2.5: Qualitative and quantitative comparison of swimming motility. (**A**)-Attenuated motility of *Pb*1692Δ*pho*P (mutant) relative to *Pb*1692 wild type and *Pb*1692Δ*pho*P-p*pho*P complemented mutant strain. Diameters produced by each bacterial strain on agar plates showing attenuated motility of *Pb*1692Δ*pho*P (mutant) relative to *Pb*1692 wild type and *Pb*1692Δ*pho*P-p*pho*P complemented mutant strain. (**B**)-The diameters were measured in triplicates (three biological replicates) and average values plotted. The statistically significant difference was calculated by the Students't-test.

2.3.5 The PhoP regulators' significance in the early stages of infection

In the RNA-seq data, there was a reduction in the number of genes differentially expressed at 12hpi (238 transcripts) while at 24hpi a total of 137 genes were differentially expressed. ccording to literature, in the early stages of infection the environment is acidic, therefore in this study, a hypothesis that PhoP transcriptional regulator is essential in the early stages of infection when the environment is still acidic (apoplast) was tested. To test this hypothesis, the number of differentially expressed genes between 12 and 24hpi was compared. A maximum of 491 genes were differentially expressed, 238 of these genes were expressed at 12hpi and only 137 at 24hpi; PhoP deletion also affected about 116 of these genes at both time points (Figure 2.2 A). To further understand the shift in transcripts between 12 and 24hpi, *Pb*1692 was inoculated into potato tubers and harvested bacterial samples at 8, 12, 16, 20 and 24hpi. To analyse *pho*P gene expression in *Pb*1692 wild type, RT-qPCR was used. Although the transcriptome data was at 12 and 24hpi, a hypothesis was tested, that changes may have occurred earlier, thus the 8hpi sampling point. The findings of the RT-qPCR revealed that the relative expression of *pho*P gene was low in the wild type at 8hpi (0.830879 - fold) while at 12, 16, 20 the relative expression of *pho*P gene increased 1.889347, 1.163316 and 1.140652 -fold respectively. At 24hpi *pho*P relative gene expression levels were significantly reduced (0.445606 fold), confirming that PhoP is essential in the early stages of infection (Figure 2.6).

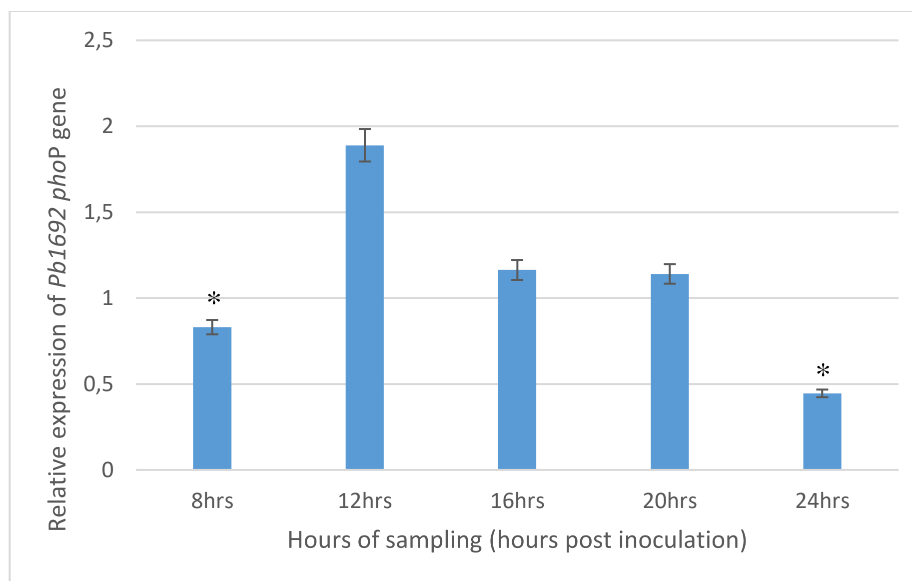


Figure 2. 6: Relative gene expression of the *pho*P gene in *Pb*1692 strain over 24hpi *in planta*. The graph shows the progression of the *pho*P gene from the acidic to alkaline environment. Standard mean deviation is represented by error bars.

2.3.6 Validation of gene expression analysis

The transcriptome results were also validated using RT-qPCR (Figure 2.7). Five genes were used for validation namely *fla*A (flagellin), *car*C (carbapenem synthase), *tss*E and *tss*C (T6SS) and *mot*B (motility). cDNA for RT-qPCR was synthesized from the 12 hour-time point RNA. The RT-qPCR findings showed that the relative gene expression of *fla*A genes was drastically reduced in the *Pb*1692Δ*pho*P in comparison to *Pb*1692. The relative expression of *car*C, *tss*E, and *tss*C were significantly increased. Similar results were observed in the transcriptome data as an upregulation of genes involved in carbapenem production and T6SS while motility genes were downregulated.

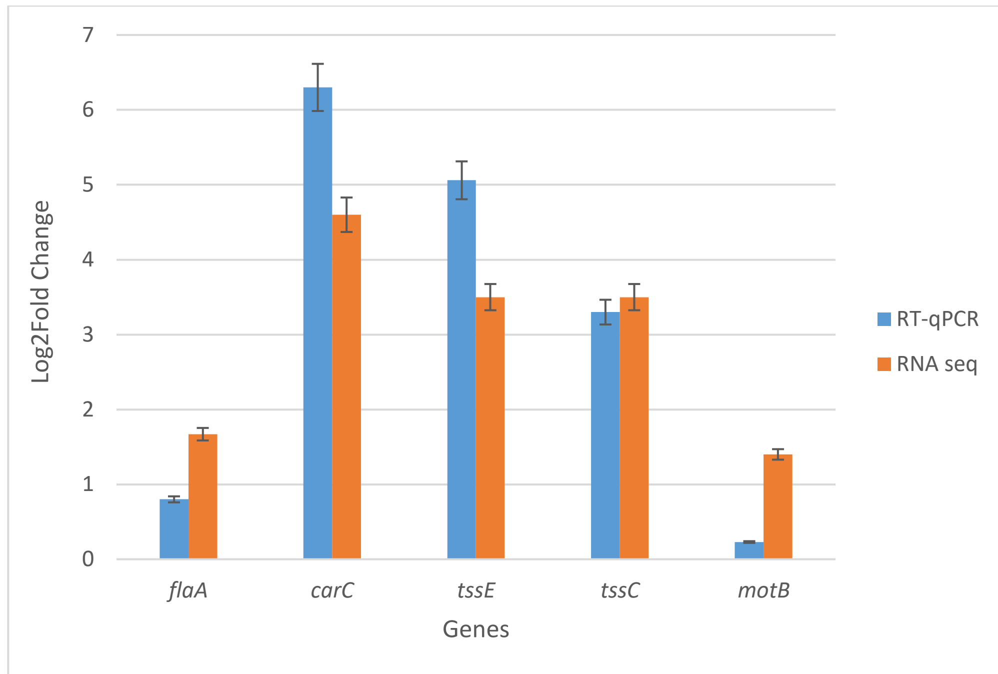


Figure 2.7: Relative expression levels of genes in the *Pb*1692Δ*pho*P strain relative to *Pb*1692. To calculate the fold change in gene expression levels the cycle threshold (CT) method was used. The fold change in RNA-seq data was determined by (log_2Fold Change). Relative expression calculated using ($2^{-\Delta\Delta CT}$ ± Standard Deviation).

2.4 Discussion

A new emerging, highly virulent pathogenic SRP *Pectobacterium brasiliense* is becoming a major concern for plant growers worldwide (Waleron *et al.*, 2015, Onkendi *et al.*, 2014, Moraes *et al.*, 2017). Currently, there are no effective control strategies for the control of soft rot pathogens, only preventative measures such as the use of certified seeds. (Czajkowski *et al.*, 2011). Due to the distruction caused by *Pb*, this work is aimed at understanding the regulatory mechanisms utilized by *Pb*1692 when infecting potato tubers, specifically the role of PhoP response regulator. PhoP response regulator has been characterised in several Gram-negative bacteria and its regulatory mechanism appears to be a key feature of pathogenicity in bacteria (Vescovi *et al.*, 1994, Shprung *et al.*, 2012, Llama-Palacios *et al.*, 2005a, Wei *et al.*, 2019). PhoP was shown to enhance bacterial survival under stressful conditions, such as acidic pH, AMPs and magnesium availability (Bearson *et al.*, 1998, Choi & Groisman, 2017, Nakka *et al.*, 2010).

The contribution of PhoP to the adaptation and virulence of *Pb*1692 was investigated using both RNA sequencing and phenotypic assays. RNA seq identified PhoP as another regulator responsible for the regulation of PCWDEs in *Pb*1692. In 2017 a study by Moleleki and colleagues on *Pb*1692, ExpI was found to be a regulator responsible for the production of PCWDEs (Moleleki *et al.*, 2017). The RNA seq analysis, revealed that at 12 and 24hpi, a number of PCWDEs were differentially expressed. Specifically, PELI/B/Z (pectate lyases) and CELA/B (cellulases) were upregulated, suggesting that PhoP repressed the production of these enzymes, while activating PEH (polygalactronase enzymes). Similarly, in a study conducted by Shevchik in *E. chrysanthemi* 3937 (now known as *D. dadantii* 3937), revealed that polygalacturonases necessitated the survival of this pathogen when the pH was acidic and pectate lyases were only active at an alkaline pH (Shevchik *et al.*, 1997). These results are therefore in accordance with what was observed by Shevchik's in *D. dadantii* 3937. In addition Bellieny-Rabelo and colleagues in 2019, found that pectate lyase and cellulase enzymes were solely expressed at 72 hpi when the environment has changed from acidic to alkaline (Bellieny-Rabelo *et al.*, 2019). Another possible

explaination for the activation and repression of enzymes could be due to bacterial cell population densities. A study by Barnard and colleagues demonstrated that PCWDEs are only synthesized when the bacterial population density surpass a particular threshold, for example, if the population is very small, bacteria will suspend the production of some enzymes until they are absolutely required (Barnard *et al.*, 2007). Moreover, in this study a quorum sensing regulator, ExpR (PCBA_RS15665) was upregulated. ExpR, senses the increased levels of AHL molecules and activate genes encoding PCWDEs (Liu *et al.*, 2008, Sjöblom *et al.*, 2006, Põllumaa *et al.*, 2012). Based on the observations noted in the transcriptome data generated in this study, further virulence assays *in planta* were conducted between *Pb*1692 (wild type) and *Pb*1692Δ*pho*P (mutant). A notable reduction in tissue maceration was observed in *Pb*1692Δ*pho*P relative to *Pb*1692 ($p < 0.05$). The results further highlight the significance of PhoP in activation and repression of important PCWDEs.

The importance of the PhoP response regulator is further highlighted in previous studies that reported on the conservation of the PhoP proteins in both pathogenic and non-pathogenic *Enterobacteriaceae* (Alteri *et al.*, 2011). In this study, to better understand the conservation of the PhoP protein in *Pb*1692, *Dickeya* and *Pectobacterium*, multiple sequence alignment of the PhoP protein using ClustalOmega and Jalview was conducted (Sievers *et al.*, 2011, Waterhouse *et al.*, 2009). This alignment did not only reveal more than 90% sequence similarities between these species, it also revealed a total of 8-α-helices and 8-β-strands. In comparison to other *Enterobacteriaceae* such as, *S*. Typhimurium and *E. coli* where PhoP is highly conserved. PhoP protein of *Pb*1692, *Dickeya* and other *Pectobacterium* is equally conserved, suggesting that PhoP might have a similar role in all these species (Winfield & Groisman, 2004). A study by Winfield and coworkers, showed that conserved genes are able to mediate phenotypic traits, which could explain all the phenotypic changes displayed by PhoP in *Pb*1692 (Winfield & Groisman, 2004). Furthemore, according to literature, the presence of the α-helices and β-strands in the PhoP

molecule is crucial for their interaction with other molecules thereby stabilizing and maintaining the PhoP homodimer (Tsai *et al.*, 2019). In addition to the overall conservation of the PhoP protein in *Pb*1692, *Dickeya* and other *Pectobacterium*, the essential aspartate residue 55 (D55) is also conserved in all the species. This aspartate residue on the PhoP protein is responsible for either activating or repressing target genes after the transfer of the phosphate group to PhoP (Galperin, 2010).

In addition to PhoP regulating PCWDEs, transcriptome analysis further revealed PhoP represses T6SS and carbapenem genes *in planta,* arguably two of the most important traits involved in antibacterial competition. To support the findings observed for T6SS and carbapenem, RT-qPCR analysis was performerd and the results revealed an upregulation of T6SS and carbapenem genes in the *pho*P mutant, confirming the RNA seq data. In addition to RT-qPCR, *in planta* interbacterial competition assays were conducted on potato tubers in order to verify the effects of PhoP during interbacterial competition. In the competition assays, a 5-fold reduction (CFU/ml) in *D. dadantii* was observed when co-inoculated with *Pb*1692Δ*pho*P mutant, and a 3-fold reduction (CFU/ml) in *D. dadantii* when inoculated with *Pb*1692 wild type. These results showed that the deletion of *pho*P resulted in the mutant strain outcompeting *D. dadantii* significantly better than *Pb*1692 wild type, which further confirms the upregulation of T6SS seen in the RNA seq data and RT-qPCR. All these results demonstrate that PhoP negatively regulates both T6SS and carbapenem genes *in planta.* On a separate study by Shyntum and colleagues, looking specifically at T6SS, a *Pb*1692 mutant strain of T6SS was co-inoculated with *D. dadantii* and the results indicate that the mutant (T6SS) was unable to inhibit *D. dadantii*, suggesting that *Pb*1692 uses T6SS *in planta* to kill competing bacteria (Shyntum *et al.*, 2018).

Another significant role for PhoP in *Pb*1692's virulence is the regulation of motility. Interestingly, the transcriptome data did not only reveal bacterial motility proteins, a number of methyl accepting proteins involved in chemotaxis were also activated at 24hpi.

In support of observations made from the transcriptome data, motility assays further revealed that *pho*P mutants were diminished in motility when compared to the *Pb*1692 and *Pb*1692ΔphoP-p*pho*P strains. Furthermore, RT-qPCR showed a reduction in gene expression levels of *flaA* and *motB* genes, highlighting that PhoP positively regulates flagellar and motility genes *in planta*. Likewise, in *Xanthomonas oryzae* chemotaxis and motility genes were activated for entry and host colonization (Kumar Verma *et al.*, 2018). These findings further suggest that chemotaxis and motility are critical in virulence as bacteria searches for nutrients or evades toxic and harsh conditions by utilizing motility and chemotaxis genes.(Stock & Baker, 1996, Reverchon & Nasser, 2013).

Another major role of PhoP in *Enterobacteriaceae* is the regulation of genes under acidic conditions. PhoP controls virulence factors in an acidic environment, the same conditions likely to be encountered by *Pb*1692 when it infects potato tubers (Nachin & Barras, 2000, Llama-Palacios *et al.*, 2005b). The plant apoplast presents a challenge to the pathogen because of organic acids such as citric acid or malic acid as well as protons from nearby cells making the environment acidic (Grignon & Sentenac, 1991). In the transcriptome data, there was a reduction in the number of genes differentially expressed at 12hpi (238 transcripts) and relative to those differentially expressed at 24hpi (137 transcripts). To account for this shift in transcripts, RT-qPCR was done with the intention of quantifying *pho*P gene expression in the wild type strain over time during the early stages of infection. In the RT-qPCR data, the expression of *pho*P gene at 8hpi was relatively low (0.830879-fold) when compared with 12hpi (1.889347-fold), 16hpi (1.163316 -fold) and 20hpi (1.140652-fold). At 24hpi, there was a drastic reduction in the expression of *pho*P (0.445606 - fold). These results show a shift in the PhoP regulon between 12 and 24 hours, which correlates with what was observed in the transcriptome data. According to literature, PhoP is often linked with transcriptional responses to acidic environments (Peng *et al.*, 2017, Groisman, 2001). Therefore, in the early stages of infection, the increased expression of the *pho*P gene is probably a response to the acidic conditions present in the apoplast. By comparison, *pho*P gene expression decreased between 20 and 24hpi, which is in agreement

with the regulator's decreased cellular demand after alkalization of the environment due to progressive cell lysis.

Several studies in other *Enterobacteriaceae* such as *Y. pestis, S. enterica* serovar Typhimurium*, S. flexineri*, *D. dadantii* and *P. versatile* have reported the importance of PhoP response regulator in controlling multiple virulence determinants (Miller *et al.*, 1989b, Vadyvaloo *et al.*, 2015, Banda *et al.*, 2018, Haque *et al.*, 2009, Kravchenko *et al.*, 2020). The i*n planta* transcriptome data generated for PhoP in *Pb*1692 in this study serves as an initial attempt to expand molecular knowledge of this pathogen as well as other SRPs. The present study has also added critical virulence factors such as T6SS and carbapenem to the PhoP regulon. It has also revealed the role of PhoP as the regulator of genes involved in motility and production of some PCWDEs. Transcriptome analyses revealed that PhoP in *Pb*1692 has both positive and negative effects on the expression of several important genes. Our observations reveal that PhoP likely has more extensive functions in the virulence of *Pb*1692. Nevertheless, more studies are needed to characterize individual genes under the PhoP regulon, and these genes could provide insights into the mechanisms utilized by *Pb*1692 to survive and proliferate inside potato tubers.

2.5 Materials and Methods

2.5.1 Conditions for growth and bacterial strains

All bacterial and plasmids strains were cultured in Luria-Bertani (LB) broth and agar plates at 37°C. Different antibiotics namely, ampicillin (100µg/ml) or kanamycin (50µg/ml) were supplemented in the media (as required). Reagents were used in accordance with the manufacturer's instructions Table 2.1.

Table 2. 1 :List of plasmids and bacterial strains used in the study

Strains	Description	Sources
Pectobacterium brasiliense 1692 (*Pb*1692)	Initially isolated from potato in Brazil, sequenced strain	Professor A. Charkowski, Wisconsin University, (Duarte *et al.*, 2004)
*Pb*1692Δ*pho*P-p*pho*P	*Pb*1692Δ*pho*P expressing the phoP gene from the p-JET-T plasmid; Amp^r	This study
*Pb*1692Δ*pho*P	*Pectobacterium. brasiliense* 1692 *pho*P, Kan^r	This study
Dickeya dadantii 3937 LMG 25991^T	*Pelargonium capitatum*, Comoros, sequenced,	(Samson *et al.*, 2005)
Plasmids		
pKD4	Plasmid containing a Kan^r cassette	(Datsenko & Wanner, 2000)
pJET1.2/blunt	Bacterial cloning vector containing the *pho*P gene insert, Amp^r	Thermofischer
pJET-*pho*P	Bacterial expression vector expressing *pho*P gene, Amp^r	This study
pKD20	Temperature sensitive replication ori (repA101ts); encodes lambda Red genes (exo, bet, gam)	(Datsenko & Wanner, 2000)

Kan^r, $Gent^r$, Amp^r = Resistance to kanamycin, gentamicini and ampicillin

2.5.2 *Pb*1692 electro-competent cells

*Pb*1692 harboring pKD20 plasmid obtained from *E. coli* DH5α were cultured on LB agar plates at 37°C for 24 hours. Thereafter, 100 ml of sterile LB was inoculated with one colony and grown overnight at 37°C (200 rpm). This was done to obtain sufficient bacterial cells to provide an OD_{600} of 0.1. Overnight culture of *Pb*1692 (100µl) harboring a curable pKD20 plasmid were cultured into 100 ml of LB broth and incubated at 31°C until OD_{600} of 0.4 is reached. Approximately 10 µM of arabinose was added to the bacterial culture and incubated at 31°C for 30 min. Bacterial cells were subdivided into falcon tubes (50 ml) and chilled for 20 min on ice before centrifuging them for 8 min at 6000 rpm, 4°C. The *Pb*1692 cell pellets were washed once in 20 ml and 40 ml of ice-cold, sterile 10% (*v/v*) glycerol using the previous centrifugation settings. The cell pellets were thereafter re-suspended in 4 ml of ice-cold, sterile 10% (*v/v*) glycerol, pooled together to form 8 ml cell suspensions in sterile 10% (*v/v*) glycerol before being centrifuged for 8 min at 6000 rpm, 4°C. Next, the cell pellets were re-suspended in 1 ml of 10% (*v/v*) glycerol and 100 µl aliquots of the cells flush frozen in liquid nitrogen before being stored at -80°C.

2.5.3 Construction of *Pectobacterium brasiliense* 1692 *pho*P mutant strains

The *Pb*1692Δ*pho*P mutant was constructed using a strategy by Datsenko & Wanner in 2000. (Datsenko & Wanner, 2000). The regions flanking *pho*P (up and downstream regions, approx.1000bp) were amplified by PCR (polymerase chain reaction) from the *Pb*1692 genomic DNA using primer combination (upstream- PhoPF and PhoPKanR) and (downstream-PhoPR and PhoPKanF), respectively (Table 2.2). PCR conditions were set up as follows: initial denaturation 95°C for 3 minutes, 25 denaturation cycles at 98°C for 20 seconds, annealing temperature 65°C for 15 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 2 minutes. PKD4 plasmid was used to amplify the kanamycin resistance gene cassette with KanF and KanR primers with the following PCR

conditions: initial denaturation at 95°C for 3 minutes, followed by 30 denaturation cycles at 98°C for 30seconds, annealing at 60°C for 15 seconds, extension at 72°C for 2minutes and final extension at 72°C for 4 minutes. To purify the three PCR fragments, Genomic DNA Purification Kit-Thermo fisher was used. Fragments were combined (15 ng of each fragment) and then amplified using the PhoPF and PhoPR primers generating a hybrid PCR amplicon consisting of upstream *pho*P, kanamycin and downstream *pho*P, with the same PCR conditions only adjusting the annealing temperature to 62°C. After successfully fusing the mutagenesis cassette, electrocompetent cell aliquots (50 µl) of *Pb*1692 were mixed with 1.0 µl (20 ng) of the mutagenesis cassette in a 2 mm gap electroporation cuvette. The cassette was electroporated using micropulser electroporator (Biorad).

Table 2. 2: Primers used in this study

Primer name	**Primer sequence 5' to 3'**	**Length (bp)**	**References**
***PhoP* primers**			
PhoPTest-F	GCAAGATTAATGGTGCCGTCGG	22	This study
PhoPTest-R	CAGAGCGCACGCTGGCCCGATG	22	This study
PhoPF	CTGGGTATCAACTGGAACCCGT	22	This study
PhoPR	GCCGGTTCGCAATGCACGTAACG	23	This study
KanF	GCGATGCGGATTCTGGTCGTTGTGTAGGCTGGAGCTGC	38	This study
PhoPKanR	GCAGCTCCAGCCTACACAACGACCAGAATCCGCATCGC	38	This study
PhoPKanF	CTAAGGAGGATATTCATATGTTGATCCCCCTTCAGGCTC	39	This study
KanR	GAGCCTGAAGGGGGATCAACATATGAATATCCTCCTTAG	39	This study
CompPhoPF	CCGTTCTCAAACCCGAACGTGAC	23	This study
CompPhoPR	GCGAGAAGGGCAGCTTCTTATCG	23	This study

qPCR Primers			
FfhF	TGGCAAGCCAATTAAATTCC	20	(Tanui *et al.*, 2017)
FfhR	TCCAGGAAGTCGGTCAAATC	20	(Tanui *et al.*, 2017)
TssEf	GGCGATCCGACAGTGTATCT	20	This study
TssEr	TTGAAAGAGGCAACCTGCTC	20	This study
TssCf	AAGAACAGGTTCAGGCAGGA	20	This study
TssCr	CTGCTGCATTACCGCTATCA	20	This study
CarF	ACTCGGCTTACGGTACGATCG	21	This study
CarR	AGCCAGAGCATGGTCGCATACC	22	This study
Mot B –R	ACAGCGTTTTACGACCGAAT	20	This study
Mot B –F	TTGCCTACGGTTTTGTCTCC	20	This study
laaF	CGTTAAACCGGAACCAACTG	20	This study
FlaaR	AACCACCGTACTGCCTTTTG	20	This study

Thereafter, cells were immediately diluted in 1000 µl of LB broth and then transferred into 1.5 ml eppendorf tubes. The resultant mixture was incubated for 3 h at 31°C in a 120 rpm shaker and plated on plates containing kanamycin (50 µg/ml) before finally incubating the plates overnight at 37°C. The *pho*P gene disruption was confirmed with PCR using flanking primers (PhoPTest-F and PhoPTest-R), accompanied by amplicon sequencing. PCR conditions: initial denaturation at 96°C for 5 minutes, 25 denaturing cycles at 98°C for 20 seconds, annealing at 59°C for 15 seconds, extension at 72°C for 2 minutes and a final extension at 72° for 2 minutes. All primers synthesized by Inqaba Biotech T (South Africa).

2.5.4 Complementation of the *pho*P mutant

The cloning vector (pJET1.2/blunt) was used for complementation of the mutant strains. The *pho*P gene with its putative promoter sequence was PCR amplified from *Pb*1692 using primers (CompPhoPF and CompPhoPR) (Table 2.2). Gel Extraction Kit from Thermo Scientific was used to excise and purify the resulting fragments from the agarose gel. Each fragment was cloned into p-JET-T to construct pJET-*pho*P (Table 2.1). The pJET-*pho*P plasmid was electroporated into electrocompetent *Pb*1692Δ*pho*P strains and then transformants (*Pb*1692Δ*pho*P-p*pho*P) grown on LB plates augmented with 100 µg/ml ampicillin and confirmed using PCR and sequencing.

2.5.5 *In vitro* growth assays

*G*rowth assays of *Pb*1692, *Pb*1692Δ*pho*P and *Pb*1692Δ*pho*P-p*pho*P strains were evaluated by growing for 16 hours at 37°C shaking at 370 rpm in LB broth augmented with kanamycin and ampicillin for mutant and complemented strains, respectively. The cultures were adjusted to OD_{600} equal to 0.1 then diluted one to two hundred in fresh LB broth and volume adjusted to 200 ml. The optical density at 600 nm (OD_{600}) was measured every hour for 16 hours using a spectrophotometer to determine the growth of bacteria.

2.5.6 *pho*P gene expression analysis in *Pb*1692

*Pb*1692 was inoculated into disinfected potato tubers and harvested every 4 hours for a period of 24 hours (section 2.5.7). SuperScript IV First-Strand Synthesis System (invitrogen) was used to reverse transcribe 5 µg of total RNA to cDNA. Real Time-quantitative PCR (RT-qPCR) was conducted using the QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems™). Primers for RT-qPCR are listed in (Table 2.2), with the following cycling parameters used: 95°C for 3 minutes followed by 40 cycles of 95°C for 60 seconds, 55°C for 45 seconds and 72°C for 60seconds, followed by 72°C for 7

minutes. The $2^{\Delta\Delta ct}$ method was used for statistical analysis of relative gene expression (Livak & Schmittgen, 2001).

2.5.7 RNA extraction from potato tubers and pathogenicity assays

A 10% (v/v) sodium hypochlorite was utilized to disinfect potato tubers (cv. Mondial, a susceptible cultivar), from that point potatoes were washed with distilled water and air dried. Potatoes were wounded with a sterile pipette (1cm holes). 10-µl aliquot of the bacterial suspension with OD_{600} comparable to 1 (*Pb*1692, *Pb*1692Δ*pho*P and *Pb*1692Δ*pho*P-p*pho*P) were pipetted into the wounds. Magnesium Sulphate (10 mM $MgSO_4$) was used as the control for the experiment. Petroleum jelly was used to seal the holes and then placed in damp plastic containers (incubated at 25°C for 72 hours to create moisture). Followed by scooping and weighing of macerated tissue at 12 and 24hpi.

RNA was extracted from macerated potato tubers inoculated with (*Pb*1692 and *Pb*1692Δ*pho*P) at 12 and 24hpi (incubated at 25 °C). A pestle and mortar was used to grind macerated tissue to harvest bacterial cells using approximately 20 ml of double distilled water. Potato debris were removed at 10,000 rpm for 1 minute by centrifuging the grinded tissue. The bacterial cells were present in the supernatant. The supernatant was then taken out into 50 ml Falcon tubes with RNA stabilization buffer (Qiagen, Hilden, Germany). All the samples were subjected to DNAse treatment for the removal of DNA contaminants.

2.5.8 Total RNA quality

The concentration and purity of each RNA sample extracted was measured at a ratio of 230/260nm using spectrophotometric analysis (NanoDrop® technologies, Wilmington, DE). To evaluate RNA quality, 1% (w/v) agarose gel electrophoresis was used together with 1% TAE buffer at 100 volts for 30 minutes. The final image was developed and viewed using Gel Doc EZ system (Bio-Rad Laboratories). The Bioanalyzer from Inqaba Biotech, was used to analyze the final RNA concentration.

2.5.9 High-throughput sequencing reads mapping and genome-wide functional annotation

The reads quality was done using a *fastqc* software. Low quality segment reads were trimmed by Trimmomatic v 0.36 and *hisat2* v 2.1.0 was used to align the reads to the reference genome of *Pb*1692 (GCF_000173135.1) (Bolger *et al.*, 2014). Aligned reads were then computed by *featureCounts* package. DGE thereafter analyzed by the EdgeR package and the FDR < 0.05 was assigned as differentially expressed (Liao *et al.*, 2014, Robinson *et al.*, 2010). Up and down regulation of genes was assigned based on log2fold-change transcriptional variation. Additionally, eggNOG was used to functionally annotate sequences. KEGG-library hierarchy (KEGG B and A) was used to retrieve sequences with higher annotation (Kanehisa & Goto, 2000, Huerta-Cepas *et al.*, 2017). Comparison between *Pb*1692Δ*pho*P relative to *Pb*1692 were done at12 and 24hpi. A more elevated level of annotation was achieved by inspecting conserved protein sequence domains using the Pfam database and HMMER3 (Finn *et al.*, 2015, Finn *et al.*, 2010).

2.5.10 Interbacterial *in planta* competition assays in potato tubers

Interbacterial competition was carried out in potato tubers as demonstrated by Axelrood in 1988 with minor adjustments (Axelrood *et al.*, 1988, Shyntum *et al.*, 2018). Potato tubers were disinfected with 10% sodium hypochlorite, rinsed with distilled water and then wounded with a sterile pipette (1cm depth). *Pb*1692, *Pb*1692Δ*pho*P, *Pb*1692Δ*pho*P-p*pho*P and *D. dadantii* with OD_{300} equivalent to 0.3 were cultured overnight and mixed in a 1:1 ratio with the target strain (*D. dadantii* carrying a plasmid that confers gentamycin resistance) and inoculated into potato tubers. The wounds were filled with petroleum jelly and placed in plastic containers at 25°C for 24 hours. The subsequent macerated tissue was weighed to standardize the cultures. The target strain (*D. dadantii* CFU/ml) was enumerated in LB plate's augumented with gentamycin (15µg/ml) through serial dilutions.

2.5.11 Motility assay on agar plates

Bacterial strains (*Pb*1692, *Pb*1692Δ*pho*P and *Pb*1692Δ*pho*P-p*pho*P) were cultured overnight at 37°C agitating at 370 rpm on LB. The optical densities of each strain was adjusted to 0.4 (OD_{600}=0.4). Motility assay was conducted on a semi-solid LB medium (0.3% agar) using the normalized strains. The plates were inoculated with bacterial strains by stabbing with a sterile toothpick and incubating at 37°C for 24 h (Tanui *et al.*, 2017).

2.5.12 Gene expression analysis

The RT-qPCR was conducted using five genes (selected randomly) in order to substantiate the results observed from the RNA-seq analysis. Gene expression analysis was performed with the same samples used in RNA sequencing. The SuperScript IV First-Strand Synthesis System (invitrogen) was used to reverse transcribe 5 µg of total RNA into first-strand cDNA. QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems™) was used for RT-qPCR (Primers for RT-qPCR, Table 2.2). The accompanying cycling parameters were as follows: 95°C for 3 min followed by 40 cycles of 95°C for 60s, 55°C for 45s and 72°C for 60s, followed by 72°C for 7 min; *ffh* (house-keeping gene control) were used to standardize gene expression. The following genes were analyzed: *fla*A (flagellin), *car*C (carbapenem synthase), *tss*E, *tss*C (T6SS) and *mot*B (motility). For statistical analysis of relative expression, the CT method was used (Livak & Schmittgen, 2001).

2.5.13 Statistical analysis

Student's t-tests were used to assess the importance of statistics. Statistically significant was a *p*-value of <0.05.

2.6 Supplementary data

Figure S2.1 : PCR amplicons used to generate the *phoP* mutant.

Lane 1. DNA ladder 1Kb, 2. Downstream *phoP* PCR fragment, 3. kanamycin cassette PCR product, 4. Upstream *phoP* PCR fragment, 5. Fused product consisting of the downstream, kanamycin and upstream fragment. 6. Km1 internal kanamycin primers.

Figure S2.2: Schematic presentation of how *Pb*1692Δ*phoP* mutant strain was generated

Figure S2.3: Growth and survival of *Pb*1692 wild type*, Pb*1692Δ*phoP* and *Pb*1692Δ*phoP*-p*phoP* in LB broth for 16 hours at 37°C with agitation at 370 rpm. Growth assay showing that deletion of the *Pb*1692Δ*pho*P gene does not impair the growth of the mutant strain.
Table S2.1: Selected DEGs at 12hpi during *in planta*

Table S2.2: Summary of selected DEGs during *in planta* infection at 24hpi

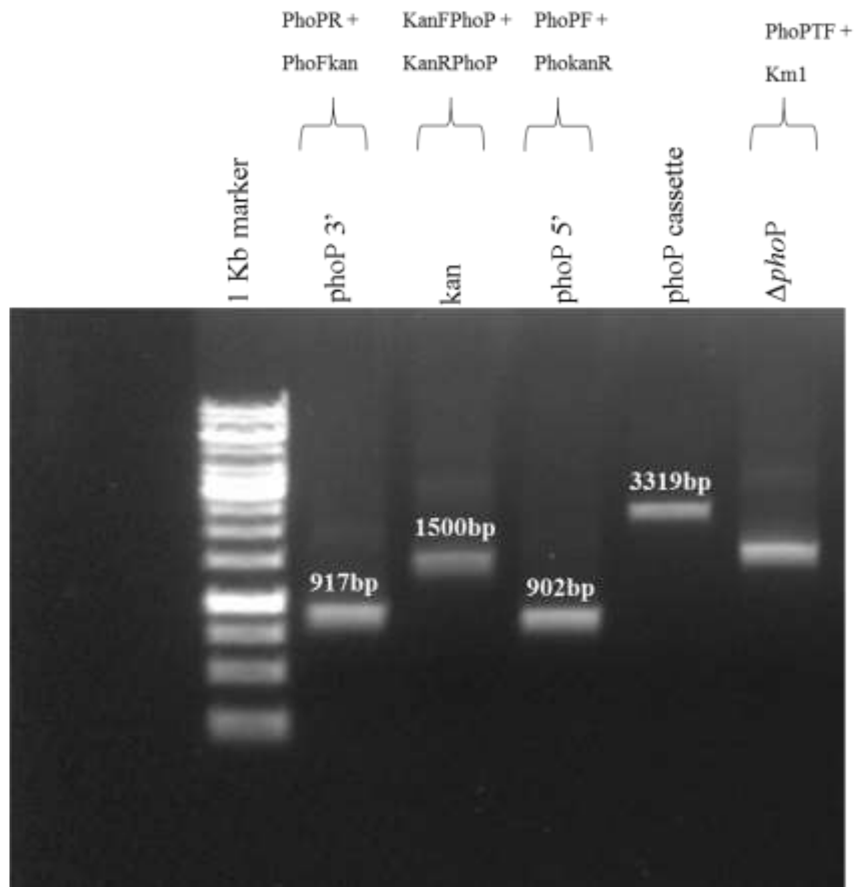


Figure S2. 1: PCR amplicons used to generate the *phoP* mutant. Lane 1. DNA ladder 1Kb, 2. Downstream *phoP* PCR fragment, 3. kanamycin cassette PCR product, 4. Upstream *phoP* PCR fragment, 5. Fused product consisting of the downstream, kanamycin and upstream fragment. 6 & 7. Km1 internal kanamycin primers.

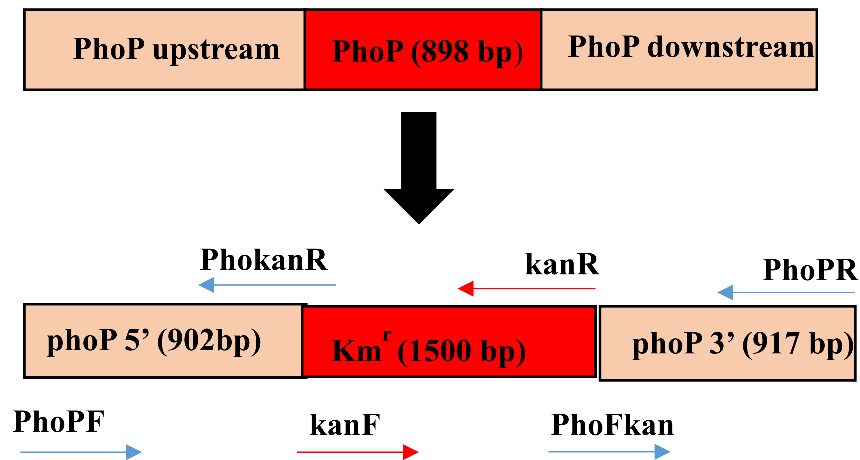


Figure S2.2: Schematic presentation of how *Pb*1692Δ*phoP* mutant strain was generated

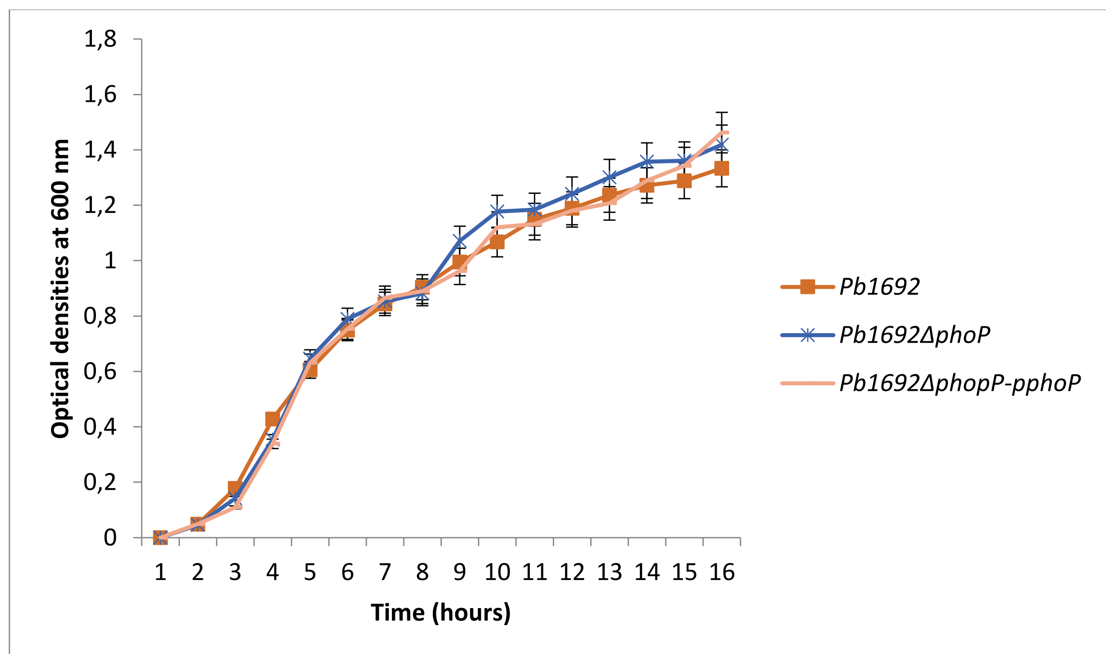


Figure S2.3: Growth and survival of *Pb*1692 wild type, *Pb*1692Δ*phoP* and *Pb*1692Δ*phoP*-p*phoP* in LB broth for 16 hours at 37°C with agitation at 370 rpm. Growth assay showing that deletion of the *Pb*1692Δ*pho*P gene does not impair the growth of the mutant strain

Table S2 1: Selected DEGs at 12hpi during *in planta*

Locus_tag	logFC	eggNOG_model_annotation
	Down regulated genes	
	Chemotaxis	
PCBA_RS05895	-1.291515312	methyl-accepting chemotaxis
PCBA_RS16315	-1.38366062	methyl-accepting chemotaxis sensory transducer
PCBA_RS20715	-1.189387287	methyl-accepting chemotaxis sensory transducer
PCBA_RS02830	-1.423686634	methyl-accepting chemotaxis sensory transducer with pas pac sensor
	Metal uptake	
PCBA_RS08425	-1.721666513	molybdenum cofactor biosynthesis protein b
PCBA_RS08440	-1.609673253	molybdopterin
PCBA_RS08320	-1.146811822	molybdopterin biosynthesis
PCBA_RS08315	-1.202987983	molybdopterin biosynthesis protein (moeb)
PCBA_RS08435	-1.541525157	molybdopterin converting factor subunit 1
	PCWDE	
PCBA_RS10070	-5.855483552	polygalacturonase
	Carotovoracins	
PCBA_RS12365	-1.537792177	entericidin ecna/b family
PCBA_RS01805	-1.106918861	antibiotic biosynthesis monooxygenase
	PTS system	
PCBA_RS02065	-1.942927768	pts system
PCBA_RS01000	-1.928288684	pts system
PCBA_RS07485	-1.285788949	pts system
PCBA_RS09330	-2.072584151	pts system beta-glucoside-specific transporter subunit iiabc
PCBA_RS01005	-1.793534236	pts system mannose fructose sorbose family iic subunit
PCBA_RS01010	-1.730017508	pts system mannose-specific transporter subunit iid
PCBA_RS01470	-1.312318058	pts system, lactose cellobiose family iic
PCBA_RS08795	-1.16125321	pts system, lactose cellobiose family iic
	Iron uptake	
PCBA_RS17525	-1.374403554	receptor
PCBA_RS09920	-1.32998935	receptor
PCBA_RS04935	-2.089405782	heme iron utilization protein
PCBA_RS17520	-4.295435104	heme-binding protein a (hasa)
PCBA_RS00835	-1.864034772	hemerythrin hhe cation binding
PCBA_RS17395	-2.043985592	periplasmic protein
PCBA_RS13855	-1.83187563	permease protein
	Secretion systems	

	T1SS	
PCBA_RS17510	-2.036333129	type i secretion membrane fusion protein
PCBA_RS17505	-2.105115536	type i secretion outer membrane protein
PCBA_RS17515	-2.067228193	type i secretion system atpase
	T2SS	
PCBA_RS17600	-1.213439041	type ii secretion system protein e
	T3SS	
PCBA_RS14140	-1.866710035	type iii secretion
PCBA_RS14110	-1.555762966	type iii secretion
PCBA_RS14210	-1.317016339	type iii secretion
PCBA_RS14125	-1.26679651	type iii secretion
PCBA_RS14135	-1.218772677	type iii secretion
PCBA_RS14185	-1.16393794	type iii secretion
PCBA_RS14090	-1.349907914	type iii secretion
PCBA_RS14200	-1.332174568	hrpf protein
PCBA_RS14235	-1.135756738	hrpw-specific chaperone
PCBA_RS14225	-1.342627524	hrpz
PCBA_RS14175	-1.757629347	(ttss, hrp secretion system)
	T5SS	
PCBA_RS12655	-1.553846095	hemolysin activator protein
	carbohyrate metabolism	
PCBA_RS11925	-2.25653782	uronic isomerase
PCBA_RS02570	-2.413515409	xylose isomerase
PCBA_RS09210	-1.113758401	beta-glucosidase ec 3.2.1.21
PCBA_RS08915	-1.053291873	6-phospho-beta-glucosidase
PCBA_RS09325	-1.587071934	6-phospho-beta-glucosidase
PCBA_RS17700	-1.454738756	rhamnulose kinase
PCBA_RS10070	-5.855483552	polygalacturonase
PCBA_RS09325	-1.587071934	6-phospho-beta-glucosidase
PCBA_RS08790	-1.279044812	beta-glucosidase ec 3.2.1.21
PCBA_RS10825	-1.202611326	catalyzes the conversion of acetate into acetyl-coa (accoa)
PCBA_RS00510	-2.295164358	catalyzes the conversion of l-arabinose to l-ribulose
PCBA_RS20070	-1.074282664	catalyzes the conversion of uracil and 5-phospho-alpha- d-ribose 1-diphosphate
PCBA_RS10060	-1.639281409	catalyzes the dehydration of d-mannonate
PCBA_RS04340	-1.15982697	catalyzes the isomerization of 5-dehydro-4-deoxy-d-glucuronate
PCBA_RS18515	-1.263321823	d-galactose-1-phosphate
PCBA_RS17330	-1.315955997	glucarate dehydratase
PCBA_RS11065	-1.91930465	glycerol dehydrogenase
PCBA_RS09490	-1.593763222	glycerol uptake facilitator protein
PCBA_RS02060	-2.151205151	glycoside hydrolase family protein
PCBA_RS13720	-1.401442115	glycosyl hydrolase family 88

PCBA_RS00385	-1.582007005	glycosyltransferase involved in cell wall biogenesis
PCBA_RS02655	-1.615427166	hydrolase, family 31
PCBA_RS18530	-1.867683444	involved in the transport of maltose and maltodextrins
PCBA_RS00500	-2.39969376	l-arabinose-binding periplasmic protein
PCBA_RS17695	-1.947307095	l-rhamnose isomerase
PCBA_RS02605	-1.044501359	l-ribulose-5-phosphate 4-epimerase
PCBA_RS17685	-1.883892492	involved in the anomeric conversion of l-rhamnose
PCBA_RS04695	-1.748971162	mandelate racemase muconate lactonizing
PCBA_RS00380	-2.123336066	mannosyl-3-phosphoglycerate phosphatase
PCBA_RS00635	-1.470799974	oligogalacturonate-specific porin
PCBA_RS19150	-1.92764535	oligosaccharide h symporter, major facilitator superfamily (mfs)
PCBA_RS06515	-1.869471282	oligosaccharide h symporter, major facilitator superfamily (mfs)
PCBA_RS04475	-1.183980915	phosphorylase is an important allosteric enzyme in carbohydrate metabolism.
PCBA_RS01475	-1.870350706	phosphotransferase system pts, lactose cellobiose-specific iia subunit
PCBA_RS04535	-2.021772917	pirin domain protein
PCBA_RS06205	-2.036711763	alcohol dehydrogenase
PCBA_RS00715	-1.744202751	alcohol dehydrogenase
PCBA_RS11095	-2.020806415	arabinogalactan endo-1,4-beta-galactosidase
PCBA_RS17675	-2.112803666	atpase, p-type (transporting), had superfamily, subfamily ic
PCBA_RS16310	-1.876892337	alpha/beta hydrolase fold
PCBA_RS06520	-2.496378719	alpha-galactosidase
PCBA_RS13100	-1.74998448	alpha-n-arabinofuranosidase
PCBA_RS19155	-1.94738284	beta-galactosidase
PCBA_RS17690	-1.860416189	catalyzes the reversible cleavage of l-rhamnulose-1-phosphate
	Amino acid metabolism	
PCBA_RS04280	-2.200213245	cysteine synthase
PCBA_RS13295	-1.209290716	cystine transporter subunit
	Stress response	
PCBA_RS01580	-1.434677762	glutaredoxin 2
PCBA_RS02495	-2.134733731	glutathione s-transferase
PCBA_RS11875	-1.795423942	glutathione s-transferase
PCBA_RS02610	-2.00798023	carbon starvation protein
PCBA_RS03520	-1.304721587	cold shock protein
	Transcriptional regulators	
PCBA_RS06265	-2.416121912	transcriptional regulator
PCBA_RS00485	-1.827697233	transcriptional regulator (arac
	Citrate	

PCBA_RS04300	-2.33701485	citrate lyase beta
	upregulated	
	PCWDE	
PCBA_RS04070	1.7144558	pectate lyase
PCBA_RS04065	2.1285943	pectate lyase
PCBA_RS04055	3.4302853	pectate lyase
PCBA_RS19200	4.6291887	pectate lyase
PCBA_RS10065	4.6563672	pectate lyase
PCBA_RS02710	2.9709954	cellulase (ec 3.2.1.4)
	Carotovoracins	
PCBA_RS20625	1.731778	Phage baseplate protein
PCBA_RS20635	1.3400193	phage tail protein
PCBA_RS20640	1.473562	phage tail protein
PCBA_RS20630	1.3940916	Baseplate
	Iron uptake	
PCBA_RS04365	2.8505423	2fe-2s iron-sulfur cluster binding domain
PCBA_RS02815	1.4655568	ferredoxin
	Secretion system	
	T2SS	
PCBA_RS13945	1.3693091	general secretion pathway protein
	T6SS	
PCBA_RS11135	3.5776638	type IV secretion system protein
PCBA_RS11145	4.3430375	type VI secretion
PCBA_RS20860	3.7107833	type VI secretion ATPase, ClpV1 family
PCBA_RS11160	4.5537493	type VI secretion protein
PCBA_RS21165	3.2077879	type VI secretion protein IcmF
PCBA_RS21170	3.6853179	type VI secretion protein IcmF
PCBA_RS11170	3.9203425	type VI secretion protein, EvpB VC_A0108 family
PCBA_RS11175	3.9870344	type VI secretion protein, VC_A0107 family
PCBA_RS11155	4.5559458	type VI secretion protein, VC_A0111 family
PCBA_RS11140	3.8975283	type VI secretion protein, VC_A0114 family
PCBA_RS05805	3.8397012	type VI secretion system effector, Hcp1 family
PCBA_RS18055	4.0517312	type VI secretion system effector, Hcp1 family
PCBA_RS12700	4.705179	type VI secretion system effector, Hcp1 family
PCBA_RS11165	4.3809962	type VI secretion system, lysozyme-related protein
PCBA_RS20850	3.788277	type VI secretion-associated protein, VC_A0118 family
PCBA_RS20845	3.5422885	type VI secretion-associated protein, VC_A0119 family
PCBA_RS18070	3.1640443	impA domain protein
PCBA_RS18075	3.4536981	impA domain protein
PCBA_RS05770	1.5571228	paar motif

PCBA_RS18045	2.5375678	paar repeat-containing protein
PCBA_RS18050	3.0039504	rhs element vgr protein
PCBA_RS05800	3.9928881	rhs element vgr protein
PCBA_RS21085	4.662942	rhs element vgr protein
	Carbohyrate metabolism	
PCBA_RS02075	6.9022093	trehalose-6-phosphate hydrolase
PCBA_RS12600	2.1706386	responsible for the transport of dicarboxylates such as succinate, fumarate
PCBA_RS11790	2.276361	membrane protein terc
PCBA_RS11130	3.4870503	phosphoenolpyruvate--protein phosphotransferase
PCBA_RS13735	4.0068071	aldehyde dehydrogenase
	amino acid metabolism	
PCBA_RS14015	2.4530825	pyoverdine biosynthesis protein
PCBA_RS18005	3.6164722	pyoverdine biosynthesis protein
	Transcriptional regulators	
PCBA_RS02460	6.5173099	transcription regulator
PCBA_RS04390	2.1002601	transcriptional
PCBA_RS11550	1.7530367	transcriptional regulator
PCBA_RS15665	2.0374003	transcriptional regulator
PCBA_RS20855	3.9538212	transcriptional regulator

Table S2 2:Summary of selected DEGs during *in planta* infection at 24hpi

Locus_tag	**logFC**	**eggNOG_model_annotation**
	Down regulated	
	Transport	
PCBA_RS13855	-1.034505255	permease protein
PCBA_RS08485	-1.537958804	transport system permease
PCBA_RS08480	-1.386135824	transport system permease protein
	Carbohydrates Metabolism	
PCBA_RS02940	-1.174217172	acyltransferase
PCBA_RS16310	-1.5851022	alpha/beta hydrolase fold
PCBA_RS08490	-1.548739339	atp-binding protein
PCBA_RS00385	-1.643542207	glycosyltransferase involved in cell wall biogenesis
PCBA_RS02560	-1.390371111	transketolase
PCBA_RS04695	-1.505720807	mandelate racemase muconate lactonizing
PCBA_RS00380	-1.891608214	mannosyl-3-phosphoglycerate phosphatase
PCBA_RS10070	-1.79678204	polygalacturonase

PCBA_RS12660	-1.201460679	possible hemagglutinin (duf637)
PCBA_RS02570	-1.451577935	xylose isomerase
	Anti-feci	
PCBA_RS18380	-1.002255506	anti-feci sigma factor, fecr
PCBA_RS06385	-1.181023257	anti-feci sigma factor, fecr
	Biotin metabolism	
PCBA_RS08390	-1.183946323	atp- dependent insertion of co2 between the n7 and n8 nitrogen atoms of 7,8-diaminopelargonic acid (dapa)
PCBA_RS08375	-1.473884552	catalyzes the conversion of dethiobiotin (dtb) to biotin
PCBA_RS08385	-1.282257291	converts the free carboxyl group of a malonyl-thioester to its methyl ester
	PCWDE	
PCBA_RS08370	-1.424124429	cellulose
PCBA_RS10070	-1.79678204	polygalacturonase
	Stress response	
PCBA_RS16320	-1.982565788	hemolysin activator protein
PCBA_RS02755	-1.01299446	hemolysin activator protein
PCBA_RS18375	-2.04763851	resistance protein
	Adhesins/Attachment	
PCBA_RS11610	-3.449309908	fimbrial protein
PCBA_RS11590	-2.129345772	fimbrial protein
PCBA_RS17580	-1.060282191	flp pilus assembly protein cpab
PCBA_RS17570	-1.063242164	flp/fap pilin component
PCBA_RS17575	-1.078719668	peptidase a24a, prepilin type iv
PCBA_RS11595	-2.566945589	may be involved in a fimbrial system chaperoned
	Chemotaxis	
PCBA_RS16315	-1.660041786	methyl-accepting chemotaxis sensory transducer
	Toxins	
PCBA_RS14265	-1.274832583	hemolysin activator protein
PCBA_RS12655	-1.935994878	hemolysin activator protein
	Metal uptake	
PCBA_RS19820	-1.272097304	metal-binding protein

	Transcriptional regulators	
PCBA_RS15810	-1.018572625	transcriptional regulator lysr family
PCBA_RS09785	-1.04494453	signal transduction histidine kinase, nitrogen specific
PCBA_RS09780	-1.142153824	two component, sigma54 specific, transcriptional regulator, fis family
PCBA_RS01290	-5.028296551	two component transcriptional regulator, winged helix family
PCBA_RS16235	-1.629077351	transcriptional regulator
PCBA_RS00485	-1.010077393	transcriptional regulator (arac)
PCBA_RS06390	-1.146264592	ecf subfamily rna polymerase
	Secretion systems	
	T3SS	
PCBA_RS14180	-2.07467694	type III secretion apparatus protein, YscI HrpB family
PCBA_RS14190	-1.30817957	type III secretion protein
PCBA_RS14120	-1.670663705	type III secretion protein
PCBA_RS14195	-1.464375717	type III secretion protein
PCBA_RS14140	-1.394311613	type III secretion protein
PCBA_RS14130	-2.317354773	type III secretion protein
PCBA_RS14185	-1.760081509	type III secretion protein
PCBA_RS14135	-1.759647454	type III secretion protein
PCBA_RS14105	-1.810643797	type III secretion protein
PCBA_RS14125	-1.671253398	type III secretion protein
PCBA_RS14200	-1.904420652	hrpf protein
PCBA_RS14235	-1.239492034	hrpw-specific chaperone
PCBA_RS14175	-2.200606989	major structure protein of the hrp pilus, which is a component of the type III secretion system (TTSS, Hrp secretion system)
	Transporters	
PCBA_RS01470	-1.237194798	pts system, lactose cellobiose family iic
PCBA_RS09820	-1.032188374	permease
PCBA_RS13860	-1.364558275	periplasmic binding protein
PCBA_RS08475	-1.179344744	periplasmic binding protein
PCBA_RS08470	-1.030536786	receptor
PCBA_RS03755	-1.293730593	receptor
PCBA_RS17525	-1.815776559	receptor
PCBA_RS06380	-1.235549818	receptor
PCBA_RS04130	-1.506018598	polar amino acid abc transporter, inner membrane subunit
PCBA_RS04125	-1.423806991	polar amino acid abc transporter, inner membrane subunit

PCBA_RS13850	-1.264515141	abc transporter
PCBA_RS09425	-1.172479573	abc transporter
PCBA_RS20270	-1.721162524	abc transporter
PCBA_RS09435	-1.030648514	abc transporter (permease)
PCBA_RS04135	-1.611976892	abc transporter atp-binding protein
	Up-regulated	
	Citrate	
PCBA_RS03300	1.214528763	(citrate (pro-3s)-lyase ligase)
PCBA_RS03290	1.041420441	citrate lyase beta
PCBA_RS03285	1.14560839	citrate lyase, alpha
	Iron uptake	
PCBA_RS04365	2.007880425	2fe-2s iron-sulfur cluster binding domain
PCBA_RS02295	1.173091136	quinol dehydrogenase periplasmic component
	Carbohydrates Metabolism	
PCBA_RS06455	1.450744721	acetolactate synthase
PCBA_RS06770	1.339801838	alcohol dehydrogenase
PCBA_RS07385	1.529810992	alcohol dehydrogenase
PCBA_RS06775	1.431036984	aldo keto reductase
PCBA_RS20955	1.688551905	alkyl hydroperoxide reductase
PCBA_RS06450	1.52335319	alpha-acetolactate decarboxylase
PCBA_RS18560	1.273126333	arabinogalactan endo-1,4-beta-galactosidase
PCBA_RS19155	1.270836904	beta-galactosidase
PCBA_RS02060	1.360871433	glycoside hydrolase family protein
PCBA_RS04375	4.661788394	taurine catabolism dioxygenase taud, tfda family
PCBA_RS07540	1.101555529	uDP-glucose 6-dehydrogenase
PCBA_RS17665	1.577731384	carbonic anhydrase
PCBA_RS04360	1.947588611	sulfatase-modifying factor enzyme 1
PCBA_RS02075	1.723099912	trehalose-6-phosphate hydrolase
	PCWDE	
PCBA_RS08365	1.005213199	cellulase
PCBA_RS02710	2.532699555	cellulase (EC 3.2.1.4)
PCBA_RS12620	1.080569529	cellulose biosynthesis protein BcsE
PCBA_RS12630	1.059315855	cellulose synthase operon protein YhjQ
PCBA_RS03200	1.086994844	pectate lyase
PCBA_RS04055	2.836680087	pectate lyase

PCBA_RS04065	1.939878215	pectate lyase
PCBA_RS10065	6.217310112	Pectate lyase
PCBA_RS19200	2.857636177	pectate lyase
	Amino Acids	
PCBA_RS07520	1.24898801	catalyzes the deformylation of 4-deoxy-4-formamido-L-arabinose-phosphoundecaprenol
PCBA_RS03305	1.245319018	fumarylacetoacetate (FAA) hydrolase
PCBA_RS14015	2.736587037	pyoverdine biosynthesis protein
PCBA_RS04370	3.907503934	proline dehydrogenase
PCBA_RS05205	1.485895749	decarboxylase
PCBA_RS20660	1.468144234	d-isomer specific 2-hydroxyacid dehydrogenase
	Stress response	
PCBA_RS07535	1.07345107	polymyxin and cationic amps
PCBA_RS07530	1.186006324	polymyxin and cationic amps
PCBA_RS05775	1.060283563	cell division cycle 123 homolog (s. cerevisiae)
PCBA_RS19690	2.715343182	glyoxalase bleomycin resistance protein dioxygenase
PCBA_RS00230	1.399174079	osmotically inducible protein
PCBA_RS11460	1.252529466	thioredoxin 2
	Methyl Accepting proteins	
PCBA_RS20690	3.351752165	methyl-accepting chemotaxis sensory transducer
PCBA_RS17350	1.299745696	methyl-accepting chemotaxis sensory transducer with pas pac sensor
	Toxins	
PCBA_RS13815	1.69803924	necrosis inducing
	Immunity protein	
PCBA_RS18000	1.218576499	immunity protein
	Carotovorocin	
PCBA_RS20585	2.978702858	tail collar domain protein
	Transcriptional regulators	
PCBA_RS19890	2.846277748	transcriptional regulator
PCBA_RS12075	2.930164215	transcriptional regulator
PCBA_RS02460	5.820460165	transcription regulator

PCBA_RS20855	1.932009184	transcriptional regulator
PCBA_RS04390	2.052260197	transcriptional
PCBA_RS15665	1.421424839	transcriptional regulator
	Secretion systems	
	T6SS	
PCBA_RS17775	4.828987516	protein of unknown function (duf796)
PCBA_RS18055	4.574923454	type VI secretion system effector, Hcp1 family
PCBA_RS12700	5.51718833	type VI secretion system effector, Hcp1 family
PCBA_RS05805	4.949751541	type VI secretion system effector, Hcp1 family
PCBA_RS11160	3.186674669	type VI secretion protein
PCBA_RS11155	3.790252043	type VI secretion protein, VC_A0111 family
PCBA_RS11140	3.720518184	type VI secretion protein, VC_A0114 family
PCBA_RS11175	3.765595366	type VI secretion protein, VC_A0107 family
PCBA_RS11145	3.428916821	type VI secretion
PCBA_RS20850	2.078313371	type VI secretion-associated protein, VC_A0118 family
PCBA_RS11170	3.720409885	type VI secretion protein, EvpB VC_A0108 family
PCBA_RS20860	2.284050217	type VI secretion ATPase, ClpV1 family
PCBA_RS18070	1.643346396	impa domain protein
PCBA_RS18075	1.815989801	impa domain protein
PCBA_RS21085	4.237178267	rhs element vgr protein
PCBA_RS18050	3.721707404	rhs element vgr protein
PCBA_RS05800	4.565270533	rhs element vgr protein
PCBA_RS21170	2.647905626	type VI secretion protein IcmF
PCBA_RS21165	1.922556566	type VI secretion protein IcmF
PCBA_RS20845	1.823706544	type VI secretion-associated protein, VC_A0119 family
PCBA_RS11165	3.433007227	type VI secretion system, lysozyme-related protein
PCBA_RS11135	3.048316926	type IV VI secretion system protein, DotU family
	Transporters	
PCBA_RS17660	1.515845118	sulfate transporter
PCBA_RS18545	1.454779992	periplasmic protein
PCBA_RS18530	1.506132381	involved in the transport of maltose and maltodextrins (by similarity)
PCBA_RS19140	2.363864469	major facilitator
PCBA_RS11130	1.955028612	phosphoenolpyruvate--protein phosphotransferase
PCBA_RS09365	3.195468851	transporter
PCBA_RS07415	1.560129654	tripartite ATP-independent periplasmic transporter, DctQ component
PCBA_RS09360	2.993469052	transporter

PCBA_RS19150	1.722999357	oligosaccharide H symporter, major facilitator superfamily (MFS)
	Others	
PCBA_RS04385	5.801238031	domain of unknown function (duf1933)
PCBA_RS20310	3.43796739	peptide synthetase
PCBA_RS04380	5.507912305	enoyl-coa hydratase/isomerase family
PCBA_RS14010	2.120183685	fad monooxygenase phea tfdb family
PCBA_RS11150	3.783856556	fha domain-containing protein
PCBA_RS05785	2.732922975	inherit from cog: yd repeat protein
PCBA_RS20720	1.813650162	inherit from firmnog: transcriptional regulator
PCBA_RS05605	3.026392058	inherit from pronog: sir2 family nad-dependent protein deacetylase
PCBA_RS17745	1.053501044	inner membrane protein yagu
PCBA_RS05770	1.071074298	paar motif
PCBA_RS18045	2.065582503	paar repeat-containing protein
PCBA_RS21090	4.338693319	domain of unknown function (duf1795)
PCBA_RS21095	3.986245463	domain of unknown function (duf1795)
PCBA_RS12570	2.214195039	membrane
PCBA_RS05610	2.996215484	mth538 tir-like domain (duf1863)
PCBA_RS22220	2.103349269	protein of unknown function (duf2645)
PCBA_RS12625	1.453610681	protein of unknown function (duf2629)

2.7 References

Alteri, C.J., Lindner, J.R., Reiss, D.J., Smith, S.N., and Mobley, H.L. (2011) The broadly conserved regulator PhoP links pathogen virulence and membrane potential in *Escherichia coli*. *Mol. Microbiol.* **82**: 145-163.

Axelrood, P.E., Rella, M., and Schroth, M.N. (1988) Role of antibiosis in competition of *Erwinia* strains in potato infection courts. *Appl. Environ. Microbiol.* **54**: 1222-1229.

Babujee, L., Venkatesh, B., Yamazaki, A., and Tsuyumu, S. (2007) Proteomic analysis of the carbonate insoluble outer membrane fraction of the soft-rot pathogen *Dickeya dadantii* (syn. *Erwinia chrysanthemi*) strain 3937. *J. Proteome Res.* **6**: 62-69.

Banda, M.M., Lopez, C., Manzo, R., Rico-Perez, G., Garcia, P., Rosales-Reyes, R., De la Cruz, M.A., Soncini, F.C., Garcia-Del Portillo, F., and Bustamante, V.H. (2018) HilD and PhoP independently regulate the expression of grhD1, a novel gene required for *Salmonella* Typhimurium invasion of host cells. *Sci Rep* **8**: 4841.

Barnard, A.M., Bowden, S.D., Burr, T., Coulthurst, S.J., Monson, R.E., and Salmond, G.P. (2007) Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **362**: 1165-1183.

Batchelor, J.D., Doucleff, M., Lee, C.J., Matsubara, K., De Carlo, S., Heideker, J., Lamers, M.H., Pelton, J.G., and Wemmer, D.E. (2008) Structure and regulatory mechanism of Aquifex aeolicus NtrC4: variability and evolution in bacterial transcriptional regulation. *J Mol Biol* **384**: 1058-1075.

Bearson, B.L., Wilson, L., and Foster, J.W. (1998) A low pH-inducible, PhoPQ-dependent acid tolerance response protects *Salmonella* typhimurium against inorganic acid stress. *J. Bacteriol.* **180**: 2409-2417.

Beier, D., and Gross, R. (2006) Regulation of bacterial virulence by two-component systems. *Curr. Opin. Microbiol.* **9**: 143-152.

Bellieny-Rabelo, D., Tanui, C.K., Miguel, N., Kwenda, S., Shyntum, D.Y., and Moleleki, L.N. (2019) Transcriptome and comparative genomics analyses reveal new functional insights on key determinants of pathogenesis and interbacterial competition in *Pectobacterium* and *Dickeya* spp. *Appl. Environ. Microbiol.* **85**: e02050-02018.

Bolger, A.M., Lohse, M., and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114-2120.

Charkowski, A., Blanco, C., Condemine, G., Expert, D., Franza, T., Hayes, C., Hugouvieux-Cotte-Pattat, N., Solanilla, E.L., Low, D., and Moleleki, L. (2012) The role of secretion systems and small molecules in soft-rot enterobacteriaceae pathogenicity. *Annu. Rev. Phytopathol.* **50**: 425-449.

Cheung, J., and Hendrickson, W.A. (2010) Sensor domains of two-component regulatory systems. *Curr. Opin. Microbiol.* **13**: 116-123.

Choi, E., Groisman, E.A., and Shin, D. (2009) Activated by different signals, the PhoP/PhoQ two-component system differentially regulates metal uptake. *J. Bacteriol.* **191**: 7174-7181.

Choi, J., and Groisman, E.A. (2017) Activation of master virulence regulator PhoP in acidic pH requires the *Salmonella*-specific protein UgtL. *Sci. Signal.* **10**: eaan6284.

Czajkowski, R., Pérombelon, M., Jafra, S., Lojkowska, E., Potrykus, M., Van Der Wolf, J., and Sledz, W. (2015) Detection, identification and differentiation of *Pectobacterium* and *Dickeya* species causing potato blackleg and tuber soft rot: a review. *Ann. Appl. Biol.* **166**: 18-38.

Czajkowski, R., Perombelon, M.C., van Veen, J.A., and van der Wolf, J.M. (2011) Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species: a review. *Plant Pathol.* **60**: 999-1013.

Datsenko, K.A., and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences* **97**: 6640-6645.

Finn, R.D., Clements, J., Arndt, W., Miller, B.L., Wheeler, T.J., Schreiber, F., Bateman, A., and Eddy, S.R. (2015) HMMER web server: 2015 update. *Nucleic Acids Res* **43**: W30-38.

Finn, R.D., Mistry, J., Tate, J., Coggill, P., Heger, A., Pollington, J.E., Gavin, O.L., Gunasekaran, P., Ceric, G., Forslund, K., Holm, L., Sonnhammer, E.L., Eddy, S.R., and Bateman, A. (2010) The Pfam protein families database. *Nucleic Acids Res* **38**: D211-222.

Galperin, M.Y. (2010) Diversity of structure and function of response regulator output domains. *Curr. Opin. Microbiol.* **13**: 150-159.

Gao, R., and Stock, A.M. (2009) Biological insights from structures of two-component proteins. *Annu. Rev. Microbiol.* **63**: 133-154.

Grignon, C., and Sentenac, H. (1991) pH and ionic conditions in the apoplast. *Annu. Rev. Plant Biol.* **42**: 103-128.

Groisman, E.A. (2001) The pleiotropic two-component regulatory system PhoP-PhoQ. *J. Bacteriol.* **183**: 1835-1842.

Groisman, E.A. (2016) Feedback control of two-component regulatory systems. *Annu. Rev. Microbiol.* **70**: 103-124.

Hancock, R.E., Falla, T., and Brown, M., (1995) Cationic bactericidal peptides. In: Adv. Microb. Physiol.: Elsevier, pp. 135-175.

Haque, M.M., Kabir, M.S., Aini, L.Q., Hirata, H., and Tsuyumu, S. (2009) SlyA, a MarR family transcriptional regulator, is essential for virulence in *Dickeya dadantii* 3937. *J. Bacteriol.* **191**: 5409-5418.

Haque, M.M., Nahar, K., Rahim, M.A., Gomes, I., and Tsuyumu, S. (2008) PhoP-PhoQ two-component system required for colonization leading to virulence of *Dickeya dadantii* 3937 in planta. *Banglad. J. Microbiol.* **25**: 36-40.

Haque, M.M., and Tsuyumu, S. (2005) Virulence, resistance to magainin II, and expression of pectate lyase are controlled by the PhoP-PhoQ two-component regulatory system responding to pH and magnesium in *Erwinia chrysanthemi* 3937. *Journal of General Plant Pathology* **71**: 47-53.

Harshey, R.M. (2003) Bacterial motility on a surface: many ways to a common goal. *Annual Reviews in Microbiology* **57**: 249-273.

Huerta-Cepas, J., Forslund, K., Coelho, L.P., Szklarczyk, D., Jensen, L.J., von Mering, C., and Bork, P. (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Molecular biology and evolution* **34**: 2115-2122.

Kanehisa, M., and Goto, S. (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research* **28**: 27-30.

Kim, D., Langmead, B., and Salzberg, S.L. (2015) HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* **12**: 357-360.

Kravchenko, U., Gogoleva, N., Kolubako, A., Kruk, A., Diubo, J., Gogolev, Y., and Nikolaichik, Y. (2020) The PhoPQ two-component system is the major regulator of cell surface properties, stress responses and plant-derived substrate utilisation during development of Pectobacterium versatile-host plant pathosystem. *bioRxiv*.

Kumar Verma, R., Samal, B., and Chatterjee, S. (2018) *Xanthomonas oryzae* pv. *oryzae* chemotaxis components and chemoreceptor Mcp2 are involved in the sensing of constituents of xylem sap and contribute to the regulation of virulence-associated functions and entry into rice. *Mol. Plant Pathol.* **19**: 2397-2415.

Li, X., Ma, Y., Liang, S., Tian, Y., Yin, S., Xie, S., and Xie, H. (2018) Comparative genomics of 84 *Pectobacterium* genomes reveals the variations related to a pathogenic lifestyle. *BMC Genomics* **19**: 889.

Liao, Y., Smyth, G.K., and Shi, W. (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**: 923-930.

Lin, Z., Cai, X., Chen, M., Ye, L., Wu, Y., Wang, X., Lv, Z., Shang, Y., and Qu, D. (2017) Virulence and Stress Responses of *Shigella flexneri* Regulated by PhoP/PhoQ. *Front Microbiol* **8**: 2689.

Lin, Z., Cai, X., Chen, M., Ye, L., Wu, Y., Wang, X., Lv, Z., Shang, Y., and Qu, D. (2018) Virulence and stress responses of *Shigella flexneri* regulated by PhoP/PhoQ. *Front. Microbiol.* **8**: 2689.

Liu, H., Coulthurst, S.J., Pritchard, L., Hedley, P.E., Ravensdale, M., Humphris, S., Burr, T., Takle, G., Brurberg, M.-B., and Birch, P.R. (2008) Quorum sensing coordinates brute force and stealth modes of infection in the plant pathogen *Pectobacterium atrosepticum*. *PLoS Path.* **4**: e1000093.

Livak, K.J., and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods* **25**: 402-408.

Llama-Palacios, A., Lopez-Solanilla, E., and Rodriguez-Palenzuela, P. (2005a) Role of the PhoP-PhoQ system in the virulence of *Erwinia chrysanthemi* strain 3937: involvement in sensitivity to plant antimicrobial peptides, survival at acid Hh, and regulation of pectolytic enzymes. *J Bacteriol* **187**: 2157-2162.

Llama-Palacios, A., López-Solanilla, E., and Rodríguez-Palenzuela, P. (2005b) Role of the PhoP-PhoQ system in the virulence of *Erwinia chrysanthemi* strain 3937: involvement in sensitivity to plant antimicrobial peptides, survival at acid pH, and regulation of pectolytic enzymes. *J. Bacteriol.* **187**: 2157-2162.

Llama-Palacios, A., López-Solanilla, E., Poza-Carrión, C., García-Olmedo, F., and Rodríguez-Palenzuela, P. (2003) The *Erwinia chrysanthemi* phoP-phoQ operon

plays an important role in growth at low pH, virulence and bacterial survival in plant tissue. *Mol. Microbiol.* **49**: 347-357.

López-Solanilla, E., García-Olmedo, F., and Rodríguez-Palenzuela, P. (1998) Inactivation of the sapA to sapF locus of *Erwinia chrysanthemi* reveals common features in plant and animal bacterial pathogenesis. *The Plant Cell* **10**: 917-924.

Miller, S.I., Kukral, A.M., and Mekalanos, J.J. (1989) A two-component regulatory system (phoP phoQ) controls *Salmonella* typhimurium virulence. *Proc Natl Acad Sci U S A* **86**: 5054-5058.

Moraes, A., Souza, E., Mariano, R., Silva, A., Lima, N., Peixoto, A., and Gama, M. (2017) First report of *Pectobacterium aroidearum* and *Pectobacterium carotovorum* subsp. *brasiliensis* causing soft rot of Cucurbita pepo in Brazil. *Plant Dis.* **101**: 379.

Moss, J.E., Fisher, P.E., Vick, B., Groisman, E.A., and Zychlinsky, A. (2000) The regulatory protein PhoP controls susceptibility to the host inflammatory response in *Shigella flexneri. Cell Microbiol* **2**: 443-452.

Nachin, L., and Barras, F. (2000) External pH: an environmental signal that helps to rationalize pel gene duplication in *Erwinia chrysanthemi. Mol. Plant-Microbe Interact.* **13**: 882-886.

Nakka, S., Qi, M., and Zhao, Y. (2010) The *Erwinia amylovora* PhoPQ system is involved in resistance to antimicrobial peptide and suppresses gene expression of two novel type III secretion systems. *Microbiol. Res.* **165**: 665-673.

Nguyen, M.-P., Yoon, J.-M., Cho, M.-H., and Lee, S.-W. (2015) Prokaryotic 2-component systems and the OmpR/PhoB superfamily. *Can. J. Microbiol.* **61**: 799-810.

Noriega, C.E., Lin, H.Y., Chen, L.L., Williams, S.B., and Stewart, V. (2010) Asymmetric cross-regulation between the nitrate-responsive NarX–NarL and NarQ–NarP two-component regulatory systems from Escherichia coli K-12. *Mol. Microbiol.* **75**: 394-412.

Onkendi, E.M., Maluleke, L., and Moleleki, L.N. (2014) First report of *Pectobacterium carotovorum* subsp. *brasiliense* causing soft rot and blackleg of potatoes in Kenya. *Plant Dis.* **98**: 684-684.

Oyston, P.C., Dorrell, N., Williams, K., Li, S.-R., Green, M., Titball, R.W., and Wren, B.W. (2000) The response regulator PhoP is important for survival under conditions of macrophage-induced stress and virulence in *Yersinia pestis*. *Infect. Immun.* **68**: 3419-3425.

Peng, B.-Y., Pan, Y., Li, R.-J., Wei, J.-W., Liang, F., Wang, L., Wang, F.-F., and Qian, W. (2017) An essential regulatory system originating from polygenic transcriptional rewiring of PhoP-PhoQ of *Xanthomonas campestris. Genetics* **206**: 2207-2223.

Põllumaa, L., Alamäe, T., and Mäe, A. (2012) Quorum sensing and expression of virulence in pectobacteria. *Sensors* **12**: 3327-3349.

Reverchon, S., and Nasser, W. (2013) *Dickeya* ecology, environment sensing and regulation of virulence programme. *Environ. Microbiol. Rep.* **5**: 622-636.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**: 139-140.

Romling, U., Gomelsky, M., and Galperin, M.Y. (2005) C-di-GMP: the dawning of a novel bacterial signalling system. *Mol Microbiol* **57**: 629-639.

Shevchik, V.E., Robert-Baudouy, J., and Hugouvieux-Cotte-Pattat, N. (1997) Pectate lyase PelI of *Erwinia chrysanthemi* 3937 belongs to a new family. *J. Bacteriol.* **179**: 7321-7330.

Shprung, T., Peleg, A., Rosenfeld, Y., Trieu-Cuot, P., and Shai, Y. (2012) Effect of PhoP-PhoQ activation by broad repertoire of antimicrobial peptides on bacterial resistance. *J. Biol. Chem.* **287**: 4544-4551.

Shyntum, D.Y., Nkomo, N., Gricia, A.R., Shigange, N.L., Bellieny-Rabelo, D., and Moleleki, L.N. (2018) The impact of type VI secretion system, bacteriocins and antibiotics on competition amongst Soft-Rot Enterobacteriaceae: Regulation of carbapenem biosynthesis by iron and the transcriptional regulator Fur. *bioRxiv*: 497016.

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., and Söding, J. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **7**.

Sjöblom, S., Brader, G., Koch, G., and Palva, E.T. (2006) Cooperation of two distinct ExpR regulators controls quorum sensing specificity and virulence in the plant pathogen Erwinia carotovora. *Mol. Microbiol.* **60**: 1474-1489.

Stock, J., and Da Re, S. (2000) Signal transduction: response regulators on and off. *Curr. Biol.* **10**: R420-R424.

Stock, J.B., and Baker, M. (1996) Chemotaxis.

Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., and Lander, E.S. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences* **102**: 15545-15550.

Tanui, C.K., Shyntum, D.Y., Priem, S.L., Theron, J., and Moleleki, L.N. (2017) Influence of the ferric uptake regulator (Fur) protein on pathogenicity in *Pectobacterium carotovorum* subsp. *brasiliense*. *PLoS One* **12**: e0177647.

Tiwari, S., Jamal, S.B., Hassan, S.S., Carvalho, P.V., Almeida, S., Barh, D., Ghosh, P., Silva, A., Castro, T.L., and Azevedo, V. (2017) Two-component signal transduction systems of pathogenic bacteria as targets for antimicrobial therapy: an overview. *Front. Microbiol.* **8**: 1878.

Toth, I.K., Bell, K.S., Holeva, M.C., and Birch, P.R. (2003) Soft rot erwiniae: from genes to genomes. *Mol. Plant Pathol.* **4**: 17-30.

Tsai, K.-C., Hung, P.-P., Cheng, C.-F., Chen, C., and Tseng, T.-S. (2019) Exploring the mode of action of inhibitors targeting the PhoP response regulator of *Salmonella enterica* through comprehensive pharmacophore approaches. *RSC advances* **9**: 9308-9312.

Ud-Din, A.I.M.S., and Roujeinikova, A. (2017) Methyl-accepting chemotaxis proteins: a core sensing element in prokaryotes and archaea. *Cell. Mol. Life Sci.* **74**: 3293-3303.

Vadyvaloo, V., Viall, A.K., Jarrett, C.O., Hinz, A.K., Sturdevant, D.E., and Hinnebusch, B.J. (2015) Role of the PhoP–PhoQ gene regulatory system in adaptation of Yersinia pestis to environmental stress in the flea digestive tract. *Microbiology* **161**: 1198-1210.

Vescovi, E.G., Soncini, F., and Groisman, E. (1994) The role of the PhoP/PhoQ regulon in Salmonella virulence. *Res. Microbiol.* **145**: 473-480.

Waleron, M., Waleron, K., and Lojkowska, E. (2015) First Report of *Pectobacterium carotovorum* subsp. *brasiliense* Causing Soft Rot on Potato and Other Vegetables in Poland. *Plant Dis.*

Waterhouse, A.M., Procter, J.B., Martin, D.M., Clamp, M., and Barton, G.J. (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**: 1189-1191.

Wei, C., Ding, T., Chang, C., Yu, C., Li, X., and Liu, Q. (2019) Global Regulator PhoP is Necessary for Motility, Biofilm Formation, Exoenzyme Production and Virulence of *Xanthomonas citri* Subsp. *citri* on Citrus Plants. *Genes* **10**: 340.

Winfield, M.D., and Groisman, E.A. (2004) Phenotypic differences between *Salmonella* and *Escherichia coli* resulting from the disparate regulation of homologous genes. *Proceedings of the National Academy of Sciences* **101**: 17162-17167.

CHAPTER THREE

The role of SlyA transcriptional regulator in the virulence of *Pectobacterium brasiliense* PBR1692 (*Pb*1692) on potato tubers

3.1 Abstract

Pectobacterium brasiliense is an important plant pathogen affecting potato crops worldwide. It is currently not clear how this pathogen is able to colonize and cause disease on susceptible hosts. In a few bacterial species SlyA has been associated with virulence and adaptation. To date, SlyA's regulatory role in *Pb*1692 has not been well characterised, therefore, the present study aims to characterise the function of SlyA transcriptional regulator in *Pb*1692 in the early stages of infection using a *sly*A deletion mutant (Δ*sly*A). The study found that the deletion of *sly*A led to a total of 175 differentially expressed genes (DEGs) *in planta*. Intriguingly, more genes were downregulated in both time points (activated 61 at 12hpi and 72 at 24hpi) and fewer genes upregulated in both time points (repressed 12 at 12hpi and 43 at 24hpi). The majority of these genes were associated with the production of extracellular enzymes, biofilm formation, and oxidative stress response. In addition, the study showed that SlyA regulates the expression of the phosphotransferase system (PTS). Furthermore, GSEA suggested that the DEGs were enriched in carbohydrate metabolism, transporters, stress response, bacterial secretion systems, transcriptional regulators and biofilm formation. To verify the transcriptome data, target genes were randomly selected for RT-qPCR. The target genes included, *sod*C (encoding a superoxide dismutase), *tss*C (T6SS), *tss*E (T6SS needle hub protein), *PTS* IIA (encoding the phosphotransferase system IIA), *PTS* IIC (encoding the phosphotransferase system IIC) together with two unannotated proteins *hrp*V (negative regulator for hrp) and *big*A (Big Association gene). Together the findings from this study expanded the knowledge on how *Pb*1692 colonise and adapt to the environment using SlyA transcriptional regulator. Conclusions can be drawn from the results that at least *Pb*1692 is able to persist inside the host partly due to the presence of SlyA.

3.2 Introduction

MarR family of regulators, are detectors of environmental signals, a critical trait for pathogenic bacteria (Wilkinson & Grove, 2006). Members of this family are made up of a diversified group of TFs that govern genes encoding a broad spectrum of biological processes, such as stress response, export of phenolic compounds, antibiotics, virulence factors and metabolic pathways (Aravind *et al.*, 2005, Davis *et al.*, 2013). In the MarR family, *mar*R was the first gene described in the multidrug-resistant strain of *E.coli* K-12 as a gene responsible for the multi-antibiotic resistance phenotype (George & Levy, 1983). The MarR family homologs exist all over bacterial and archaeal domains and studies have demonstrated that this family could have emerged prior to the divergence of these two domains (Pérez-Rueda & Collado-Vides, 2001, Pérez-Rueda *et al.*, 2004, Wilkinson & Grove, 2006). Some of the well-studied homologs in this family include, (RovA) from *Yersinia* spp., (MarR) *E. coli*, (PecS) *E. chrysanthemi* (now known as *D. dadantii),* (Rap) *S. marcescens*, (Hor) *E. carotovora* (known as *P. carotovora),* (SlyA) *S. enterica, E. feacalis, D. zeae and D. dadantii* 3739 (Perera & Grove, 2010, Heroven *et al.*, 2004, Reverchon *et al.*, 1994, Cathelyn *et al.*, 2006, Sjöblom *et al.*, 2008, Cabezas *et al.*, 2018, Michaux *et al.*, 2011, Zhou *et al.*, 2016, Zou *et al.*, 2012).

SlyA was initially identified in *S. enterica* serovar Typhimurium as a gene responsible for the induction of hemolytic and cytotoxic phenotypes in *E. coli* (Ludwig *et al.*, 1995). Successive work demonstrated that SlyA is vital for the regulation of several genes in a number of *Enterobacteriaceae* (Reverchon *et al.*, 1994, Thomson *et al.*, 1997, Weatherspoon-Griffin & Wing, 2016, Navarre *et al.*, 2005). A dominant feature of the SlyA regulator is that they are members of a WHTH family of TFs, with the WHTH motif attached to a β-wing used for DNA binding and α-helical dimerization domain (Alekshun *et al.*, 2001, Lim *et al.*, 2002, Wu *et al.*, 2003). SlyA proteins occur as dimers and bind to target DNA sequences by recognizing specific palindromic sequences. This recognition leads to the attenuation of gene expression by obstructing the binding of RNA polymerase to the target promoters, resulting in either transcriptional activation or repression

(Wilkinson & Grove, 2006, Zhu *et al.*, 2017, Kim *et al.*, 2016). The mode of action in different SlyA homologs differs in response to host-derived signals. This mode of action is also attributed to differences at the amino acid level resulting in each homolog reacting differently to various signaling molecules (Ellison & Miller, 2006, Dolan *et al.*, 2011). For example, PecS in *D. dadantii* is activated by urate which is produced as a byproduct of reactive oxygen species (ROS) during host-pathogen interactions resulting in the activation of secretion systems and some PCWDEs, while in *X. campestris*, hydroperoxide activates OhrR during host-pathogen interactions resulting in the activation of oxidative stress response genes (Pédron *et al.*, 2018, Panmanee *et al.*, 2002). As much as the mode of action differs from homolog to homolog, a conserved mechanism exist amongst regulators, which is competition for DNA binding sites with other binding proteins, for example, the global repressor protein H-NS (histone-like nucleoid) (Perez *et al.*, 2008).

In animal and human pathogenic bacteria, SlyA was shown to be directly implicated in the survival and virulence of *E. faecalis* inside peritoneal macrophages and persistence in mouse kidneys and liver. Similarly, in *S. enterica* serovar Typhimurium, SlyA was required for adaptation inside mice and in macrophages (Michaux *et al.*, 2011, Buchmeier *et al.*, 1997). Furthermore, in *S. enterica* serovar Typhimurium and *E. coli*, SlyA activated the expression of hemolysin, flagella production and was also associated with the regulation of capsular polysaccharide, protecting cells from engulfment by macrophages (Libby *et al.*, 1994, Wyborn *et al.*, 2004, Norte *et al.*, 2003, Corbett *et al.*, 2007). In addition to animal and human pathogens, SlyA was also implicated in the regulation of virulence determinants such as the formation of biofilms, bacterial motility, production of proteases, polygalacturonases and pectate lyases in plant-pathogenic bacteria *D. zeae* (Zhou *et al.*, 2016). Likewise, in *D. dadantii* 3937, SlyA also controlled motility genes by regulating flagellar motor genes (Haque *et al.*, 2015). In *P. carotovorum* SCC3193, Hor, a SlyA homolog, controls the expression of cellulase and polygalacturonase production, while in *P. carotovorum* subsp. *carotovorum*, SlyA was characterised as an essential gene for carbapenem antibiotic production (Thomson *et al.*, 1997, Sjöblom *et al.*, 2008). These

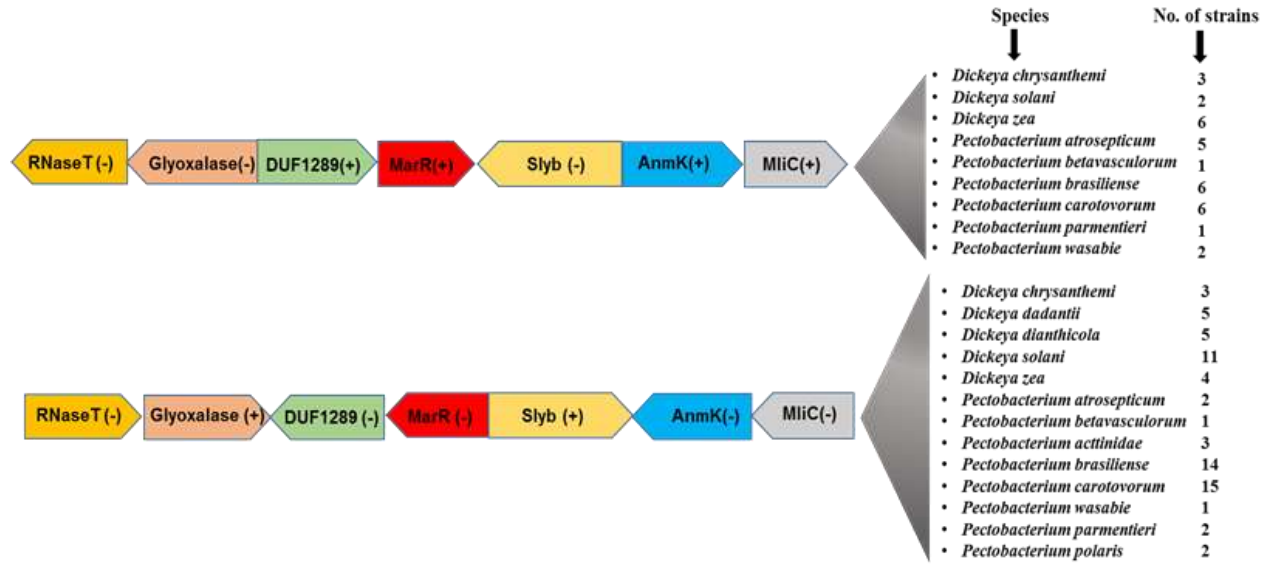
studies indicate that the genes activated by SlyA differ from species to species and this could be due to the differences in the amino acid levels observed in SlyA homologs (Dolan *et al.*, 2011). In this work, using phenotypic and transcriptome profiling through RNA-sequencing, the role of SlyA in *Pb* strain PBR 1692 (*Pb*1692) was characterised *in planta*. This was done by comparing changes in transcripts between *Pb*1692 wild-type and *Pb*1692Δ*sly*A mutant strain.

3.3 Results

3.3.1 MarR/SlyA gene order in *Pectobacterium/ Dickeya* species and the distribution of SlyA homologs in *Enterobacteriaceae* species

Using publicly available genome sequences of 100 Soft Rot *Pectobacteriaceae*, gene organization and conservation analysis were conducted. The analysis focused on *Pectobacterium* and *Dickeya* species to determine the gene order and conservation in these species. Proximal to the *sly*A/*mar*R gene, each species shows a high degree of conservation in gene order organization, sequence identity as well as the direction of transcription. The genome sequences of all 100 SRP's contain the same gene order and only differ in the direction of transcription (Figure 3.1A). This architecture depicts three genes upstream and three genes downstream of the *sly*A gene. The gene order organization also indicates that if *sly*A is transcribed positively (*mar*R+) then the next gene downstream of *mar*R is transcribed negatively (*sly*b), while the gene upstream of *mar*R is transcribed positively (DUF1289+). Conversely if *mar*R is transcribed negatively (*mar*R-) then *sly*b is transcribed positively. This orientation appears to be conserved throughout *Dickeya* and *Pectobacterium*, highlighting the degree of conservation. Furthermore, multiple sequence alignment of SlyA homologs using ClustalOmega and Jalview shows 90-100% identity particularly in Leucine (L-12, 37, 52, 63, 67, 70), Arginine (R-65), Threonine (T-36) and Isoleucine (I-40). The alignment also revealed a total of six α-helices and two β-strands. The alignment of *Pb*1692 SlyA is similar to other SlyA proteins in the *Enterobacteriaceae* family (Figure 3.1B). In this analysis, regions of similarity (α-helices and β-strands) were identified that could be SlyA's functional, structural or even evolutionally relationships (Figure 3.1 B)

. **(A)**



(B)

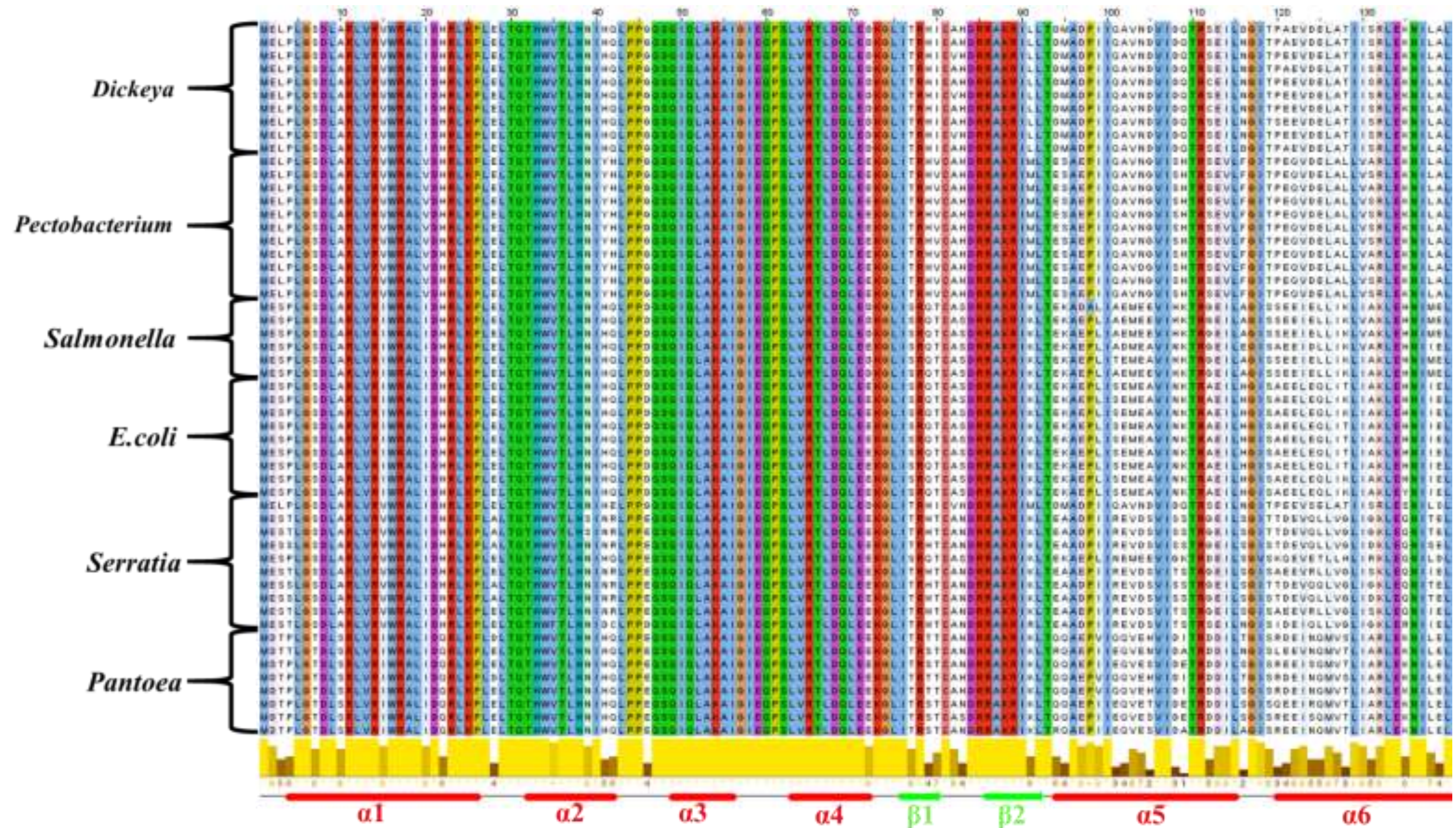


Figure 3. 1(A): Genomic architectures of 100 *Pectobacterium* and *Dickeya* species are represented depicting three genes upstream and three genes downstream of *sly*A gene neighborhoods**.** Arrows represent the direction of transcription. Positive (+) and negative (-) represent direction of transcription of MarR/SlyA. **(B):** Multiple sequence alignment of *sly*A homologs. Leucine (L), Threonine (T), Arginine (R) and Isoleucine (I) residues are similar in all sequences**.** The amino acid sequence alignment was created using ClustalOmega and Jalview. Red rectangles represent SlyA secondary structures (α-helices) and green arrows (β-strands). Yellow bar indicates conserved amino acids. (Sievers *et al.*, 2011, Waterhouse *et al.*, 2009).Generation of *Pb*1692Δ*sly*A mutant strain

To investigate the functional characteristics of SlyA in *Pb*1692 regulatory programs, a *Pb*1692Δ*sly*A mutant was generated. Firstly, *sly*A was identified in the genome of *Pb*1692. Next, *Pb*1692Δ*sly*A mutant was generated using the lambda recombination one-step PCR inactivation technique (see 'Experimental Procedure' for details 3.5.2). The end product was a *Pb*1692Δ*sly*A mutant strain where the *sly*A gene was disrupted using a kanamycin resistance marker gene. The mutagenesis cassette, (kanamycin cassette) was confirmed by PCR. Primers flanking *sly*A gene were used (Figure S3.1 and S3.2). The resulting mutant was also sequenced to confirm insertion. A complementation strain was also constructed by amplifying the *sly*A gene with its putative promoter region. The complemented *sly*A was cloned into pJET plasmid and confirmed by nucleotide sequencing (results not shown). The recombinant plasmid was kept in the *Pb*1692Δ*sly*A mutant.

3.3.2 The mutation of SlyA does not impair bacterial growth

To elucidate the role of SlyA in *Pb*1692, a *sly*A knockout mutant *Pb*1692Δ*sly*A was successfully constructed, together with its complementation strain *Pb*1692Δ*sly*A-p*sly*A (Figure 3.2). After successfully generating a *sly*A mutant, a growth curve was constructed to ensure that the mutation of *sly*A had no effect on the growth and proliferation of *Pb*1692. Comparing the wild-type *Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A, no notable difference was observed in the growth rate of these strains, therefore any phenotype

observed will most likely be as a result of the *sly*A gene knockout and not different growth rates (Figure 3.2).

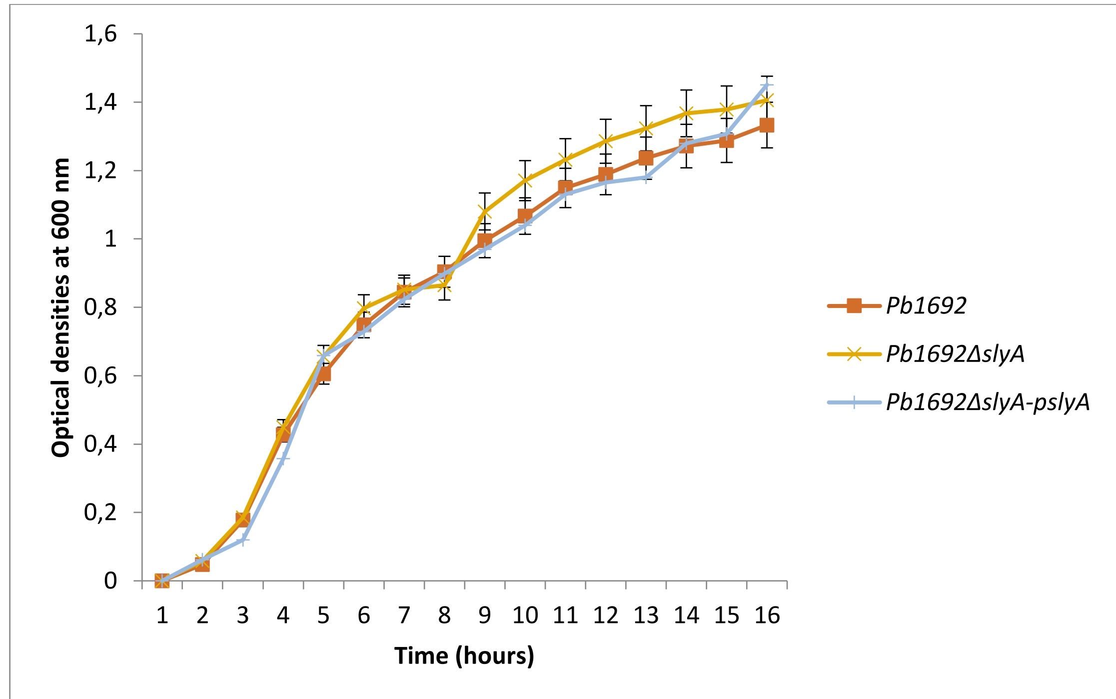


Figure 3. 2: The growth rate and survival of *Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A in LB broth for 16 hours at 37°C (370 rpm). Three biological replicates were used to plot the growth curve.

3.3.3 Read mapping and transcriptome assembly

RNA sequencing was used to analyse differences between *Pb*1692 (reference sample) and the *Pb*1692Δ*sly*A (test sample). The global effect caused by the deletion of *sly*A in the *Pb*1692 genome *in planta* was analysed using an entire transcriptome data sets consisting of ~164 million reads (Table 3.1). Approximately 90% of these reads were matched successfully to the *Pb*1692 genome. Out of these, 87.1% (~132 million reads) were mapped on the *Pb*1692 genome. The analysis was done by comparing Δ*sly*A with the wild-type *Pb*1692 collected from infected potatoes at 12 and 24hpi.

Table 3. 1:Mapping of RNA-Seq reads from mutant strains on *Pb*1692 reference genome

Sample	Unmapped Reads	Multiple Matches	Uniquely Mapped Reads	% Uniquely Mapped	Total Mapped reads	% Total Mapped	Raw Reads Inputs
Wt-12hpi	2,103,480	1,579,707	14,658,939	90.27	16,238,646	88.5	18,342,126
Wt-24hpi	1,791,915	2,172,838	14,593,401	87.04	16,766,239	90.3	18,558,153
Δ*sly*A-12hpi	1,879,481	1,480,426	15,456,620	91.3	16,937,046	90.0	18,816,527
Δ*sly*A-24hpi	1,992,419	1,680,424	14,492,035	89.6	16,172,459	89.0	18,164,877
** hpi*							

3.3.4 SlyA is required for pathogenicity of *Pb*1692 and production of some PCWDEs

3.3.5 Mutation of *sly*A reduces biofilm formation in *Pb*1692

Formation of biofilms is one of the crucial virulence determinants for the pathogenesis of plant and animal bacterial pathogens because they allow bacteria to attach to surfaces and provide protection against adverse conditions. Gene ontology functional enrichment of pathways (Gene set enrichment analysis) revealed a total of 45 genes regulated by SlyA at 12 and 24hpi that were associated with biofilm formation. Based on the information from Gene set enrichment analysis, a hypothesis was formulated, that a SlyA defective mutant will be unable to form biofilms. To test this hypothesis, biofilm formation was analysed using the crystal violet staining assay. *Pb*1692 wild type and *Pb*1692*ΔslyA*-p*sly*A complementation strains demonstrated visual evidence of biofilm formation, while *Pb*1692*ΔslyA* mutant strain showed no biofilm formation, indicating that SlyA positively regulates genes involved in biofilm formation (Figure 3.4 A and B).

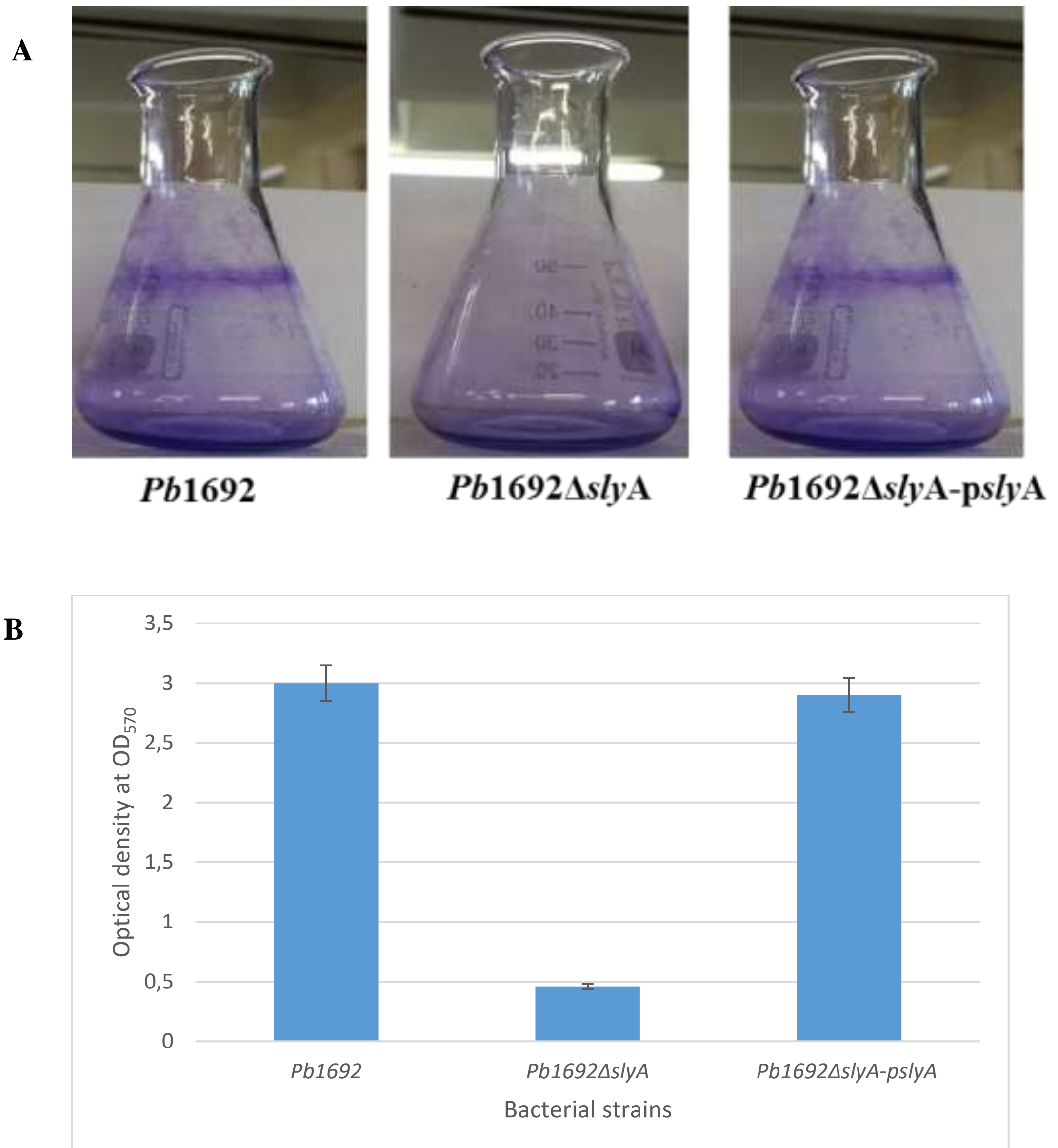


Figure 3. 4 A: Qualitative assays showing biofilm formation after crystal violet staining. **B**. Quantitative analysis of biofilm formation by *Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A strains (Pérez-Mendoza, 2011). Three independent experiments were carried out to obtain optical densities (OD_{570}).

3.3.6 SlyA is essential for an efficient response to H_2O_2

At 12hpi, RNA seq data revealed genes associated with protection against oxidative stress under the regulation of SlyA, such as: PCBA_RS02495 (-1.6), PCBA_RS11660 (-1.8), PCBA_RS18515 (-2.6), PCBA_RS18520 (-2.5), PCBA_RS18530 (-2.5) (Table S3.1). The results lead us to hypothesize that SlyA is essential for survival of *Pb*1692 in oxidative stress, prompting us to subject the three bacterial strains (*Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A to (20µM) to hydrogen peroxide (H_2O_2). After 12 hours of incubation, the strains were serially diluted to 10^8 CFU/ml, viable cells were determined by serial dilutions. *Pb*1692Δ*sly*A was highly sensitive to H_2O_2 when compared to the *Pb*1692 and *Pb*1692Δ*sly*A-p*sly*A. The survival of *Pb*1692Δ*sly*A mutant strain when cultured with H_2O_2 compared to *Pb*1692 displayed a 60% reduction (Figure 3.5). The transcriptome data further revealed a downregulation of NADPH dehydrogenase, this enzyme belongs to the family of oxidoreductases and provides protection against ROS.These results imply that SlyA positively regulates genes involved in oxidative stress response.

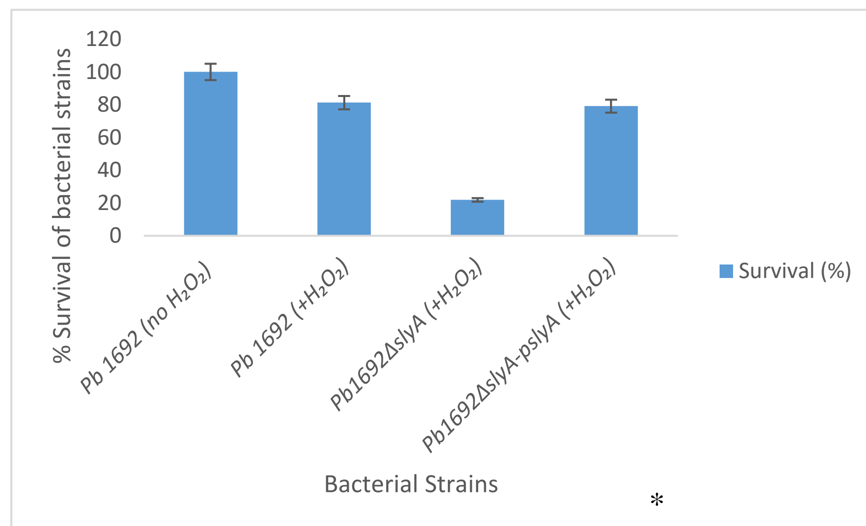


Figure 3. 5: Reduced survival of *Pb*1692Δ*sly*A mutant in the presence of hydrogen peroxide mimicking ROS. *Pb*1692, *Pb*1692Δ*sly*A, *Pb*1692Δ*sly*A-p*sly*A strains inoculated into LB broth containing 20µM H_2O_2

3.3.7 Identification and functional annotation of the SlyA regulon in *Pb*1692 during *in planta* infection

A total of 175 genes were affected (up-/down-regulation) by SlyA in two-time points. Seventy-three were differentially expressed at 12hpi and 115 at 24hpi (Figure 3.6 A). In the 12-hour time point, 12 transcripts showed increased abundance (repressed) while 61 had decreased abundance (activated). A total of 43 RNA transcripts displayed increased abundance while 72 transcripts had decreased abundance at 24hpi. In both time points (12 and 24hpi) appears there is a high amount of DEGs being activated by SlyA when compared to the DEGs that are repressed. In this study, differential expressed genes between *Pb*1692 and *Pb*1692Δ*sly*A were classified, identified and enriched based on the GO database using a stringent P-value of > 0.005. KEGG database analysis showed genes that were significantly enriched in six signaling pathways, carbohydrate metabolism, transporters, stress response, bacterial secretion systems, transcriptional regulators and biofilm formation (Table S3.1 and S3.2). Furtheremore, a heat map showing the distribution of 12 and 24 DEGs was constructed using Gitools software. Figure 3.6 (B). The next section of the results will interrogate these categories in more detail.

(A)

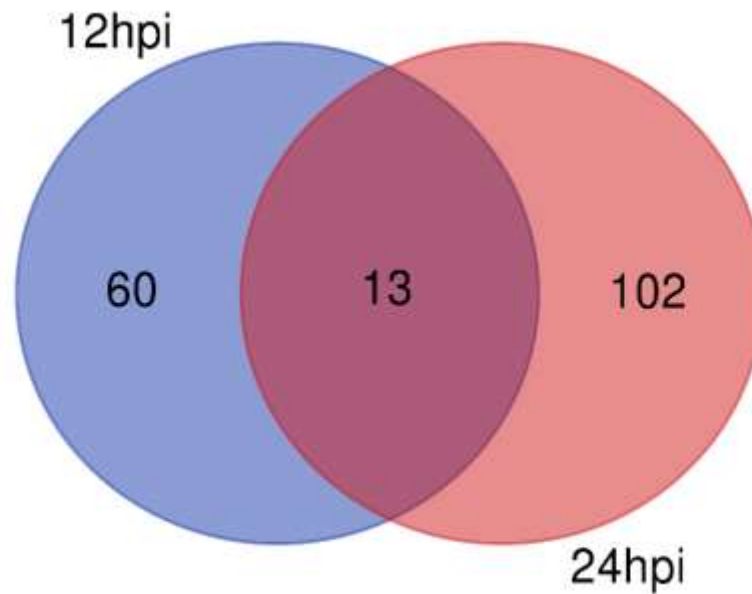
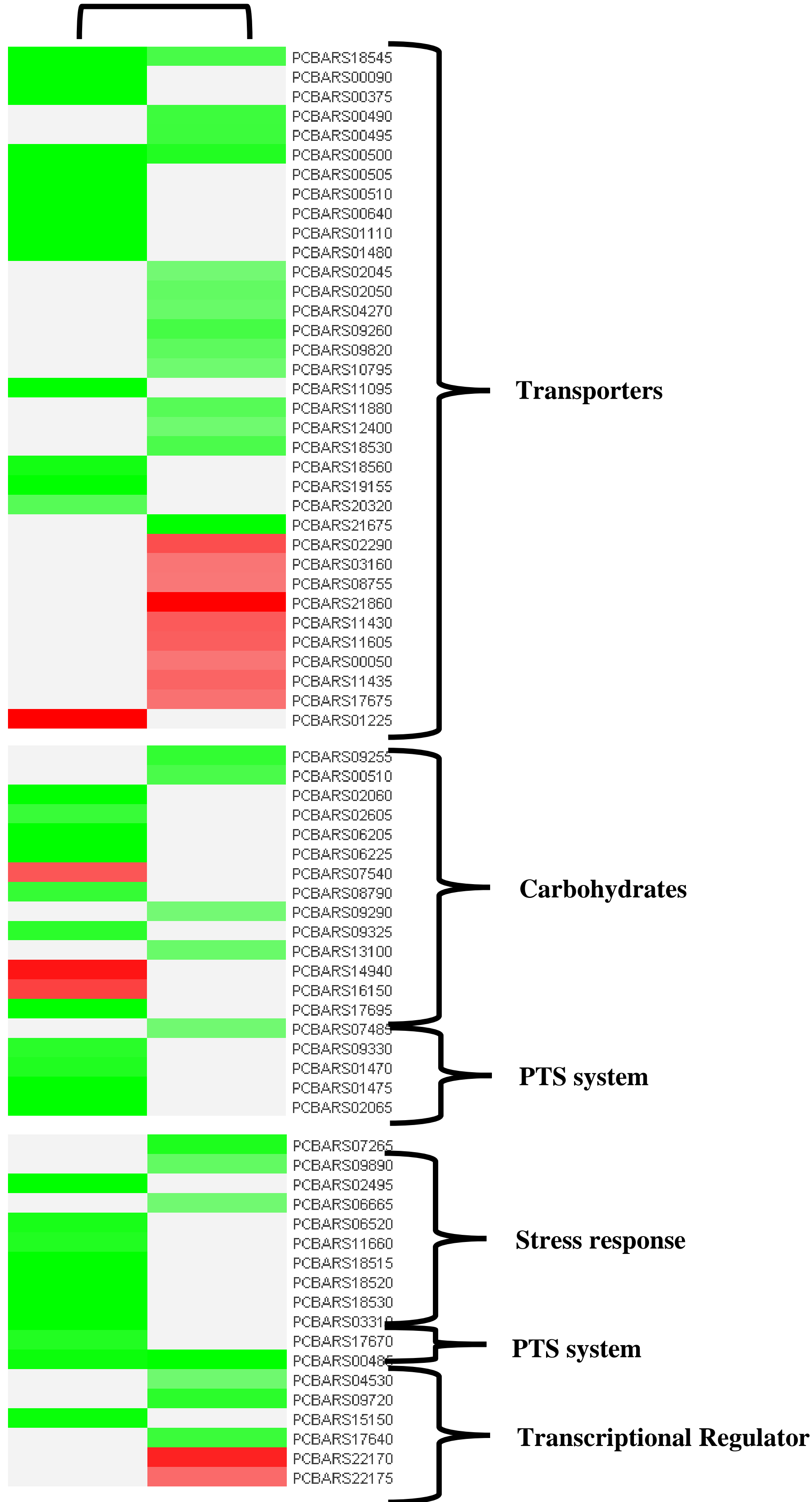


Figure 3. 6 (A): Venn diagram depicting overlap of *Pb*1692 genes between 12 and 24hpi. 60 genes were differentially expressed at 12hpi and 102 at 24hpi out of which 13 were common between the two-time points.


12_vs_24
PCBARS18545
PCBARS00090
PCBARS00375
PCBARS00490
PCBARS00495
PCBARS00500
PCBARS00505
PCBARS00510
PCBARS00640
PCBARS01110
PCBARS01480
PCBARS02045
PCBARS02050
PCBARS04270
PCBARS09260
PCBARS09820
PCBARS10795
PCBARS11095
PCBARS11880
PCBARS12400
PCBARS18530
PCBARS18560
PCBARS19155
PCBARS20320
PCBARS21675
PCBARS02290
PCBARS03160
PCBARS08755
PCBARS21860
PCBARS11430
PCBARS11605
PCBARS00050
PCBARS11435
PCBARS17675
PCBARS01225
Transporters
PCBARS09255
PCBARS00510
PCBARS02060
PCBARS02605
PCBARS06205
PCBARS06225
PCBARS07540
PCBARS08790
PCBARS09290
PCBARS09325
PCBARS13100
PCBARS14940
PCBARS16150
PCBARS17695
Carbohydrates
PCBARS07485
PCBARS09330
PCBARS01470
PCBARS01475
PCBARS02065
PTS system
PCBARS07265
PCBARS09890
PCBARS02495
PCBARS06665
PCBARS06520
PCBARS11660
PCBARS18515
PCBARS18520
PCBARS18530
Stress response
PCBARS03310
PCBARS17670
PTS system
PCBARS00485
PCBARS04530
PCBARS09720
PCBARS15150
PCBARS17640
PCBARS22170
PCBARS22175
Transcriptional Regulator

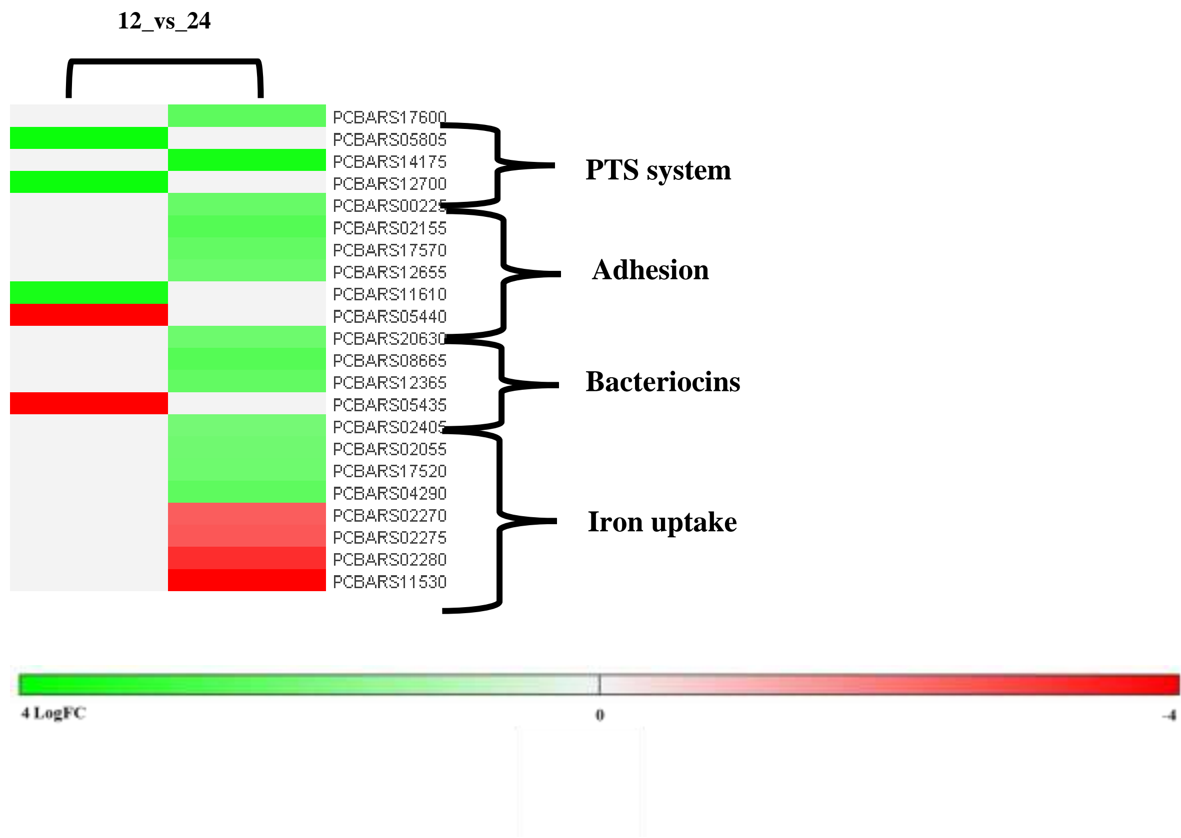


Figure 3.6 (B): Heat-map showing transcriptional profiles of selected differentially expressed genes *in planta* between 12 and 24hpi, following inoculation with *Pb*1692 compared to *Pb*1692Δ*sly*A samples. Red shows up-regulated genes and green down-regulated genes.

3.3.8 SlyA regulates the expression of carbohydrate and stress-related genes in *Pb*1692

Transcriptome analysis in both 12 and 24hpi revealed that SlyA positively regulated carbohydrate associated genes such as the PTS (PCBA_RS01470, -1.7), (PCBA_RS01475, -3.2), (PCBA_RS02065, -3.5), (PCBA_RS09330, -1.7), (PCBA_RS07485, -1.1), (PCBA_RS09330, -1.0) (Table S3.1 and S3.2). The PTS system which is involved in the uptake of carbohydrate such as glucose, mannose, fructose, cellobiose, and control of carbon metabolism was down-regulated at 12 and 24hpi. PTS is critical in bacterial signaling allowing bacteria to efficiently utilize essential carbohydrate sources. The results also showed a set of stress response genes that are under SlyA regulation. SlyA also positively regulated stress response genes, these genes are responsible for the protection of bacteria against general and oxidative stress responses (PCBA_RS02495, -1.6), (PCBA_RS11660, -1.7), (PCBA_RS18515, -2.5), (PCBA_RS18520, -2.4), (PCBA_RS18530, -2.4), (PCBA_RS02495-1.6), (PCBA_RS06665, -1.1), (PCBA_RS06520, -1.8), (PCBA_RS11660, -1.7) (Table S3.1).

3.3.9 SlyA regulates the expression of metal transport and iron genes in *Pb*1692

Gene ontology functional enrichment analyses coupled with KEGG orthology database (KEGG B and C), further suggested that DEGs were enriched in membrane transport and ABC transporters (Table 3.2). KEGG database is used to understand the variation and conservation of genes at a cellular level (Kanehisa & Goto, 2000). Additionally, metal ABC transporters were activated by SlyA *in planta* at 24hpi (PCBA_RS00505, -2.2), (PCBA_RS00090, -2.3), (PCBA_RS00375, -2.4), (PCBA_RS00510, -3.5). Furthermore, the activation of metal transporters also suggests that *Pb*1692 utilizes transporters to acquire and expel excess trace metals like iron, zinc, copper, and manganese to avoid toxicity and maintain homeostasis. In addition to ABC and metal transporters, iron transporters Fe-S-binding protein, Hemin iron-containing porphyrin, Heme-binding protein A (HasA) and tonB-dependent siderophore receptor were down-regulated (Table

S3.1). These genes are responsible for the transport of iron in *Pb*1692, indicating the importance of iron and transporters during *in planta* infection.

Table 3.2: Total gene count for KEGG annotated genes. (KEGG Orthology) database is designed for molecular functions represented in terms of functional ortholog

KEGG_B	**Annotated in Target**	**Annotated in Background**	**P-value**
09131-Membrane transport	17	833	0.2546
KEGG_C	**Annotated in Target**	**Annotated in Background**	**P-value**
05111-Biofilm formation - [PATH:ko05111]	1	45	0.5148
02010-ABC transporters [PATH:ko02010]	3	308	0.476
02000-Transporters [BR:ko02000]	14	697	0.303

3.3.10 SlyA regulates the expression of other transcriptional regulators in *Pb*1692

Six transcriptional regulators were activated by SlyA *in planta* at 12 and 24hrs. These include (PCBA_RS00485 transcriptional regulator, AraC), PCBA_RS15150 LacI transcriptional regulator, PCBA_RS04260 ParB-like nuclease domain, PCBA_RS04530 transcriptional regulator, PCBA_RS09720 transcriptional regulator, PCBA_RS17640 Ig family of transcriptional regulators). Some of these transcriptional regulators control stress response genes, transport many sugar-based solutes and they are also primary receptors of chemotaxis. On the other hand, only four transcriptional regulators were repressed by SlyA, PCBA_RS22170 transcriptional regulator, RpiR family, PCBA_RS02575 transcriptional regulator, PCBA_RS16145 transcriptional regulator, PCBA_RS22175 transcriptional regulator (Table S3.1 and S3.2).

3.3.11 SlyA regulates the expression of Hemolysin coregulated protein (Hcp)

An important component of the T6SS, hemolysin coregulated protein (Hcp) was activated by SlyA *in planta* at 12 and 24hpi (PCBA_RS05805, -1.90, PCBA_RS12700, -1.9, PCBA_RS05805, -1.1) (Table S3.1 and S3.2). Only three *hcp* genes from the T6SS gene cluster were activated by SlyA and none of the other T6SS components were activated, therefore, a hypothesis was formulated that *sly*A defective mutants will have the same killing effects as *Pb*1692 wild type strain because not all major components of the T6SS were activated. To test this hypothesis, *in planta* competition assays were conducted as previously described (Shyntum *et al.*, 2018). Target bacteria *D. dadantii* 3937 was co-inoculated with *Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A to test if a *sly*A defective mutant strain can inhibit the growth of *D. dadantii* 3937. The result showed a 100 % survival of *D. dadantii* 3937 when co-inoculated with water (control). A 5-6 fold reduction was observed in the survival of *D. dadantii* 3937 when co-inoculated with *Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A. These results show that the mutant strain displayed the same killing ability as the wild type and complemented strain, indicating that there are

no significant differences between the *Pb*1692 and *Pb*1692Δ*sly*A strain (Figure 3.7). Thus, the conclusion is that knocking out *sly*A does not affect the killing ability of T6SS..

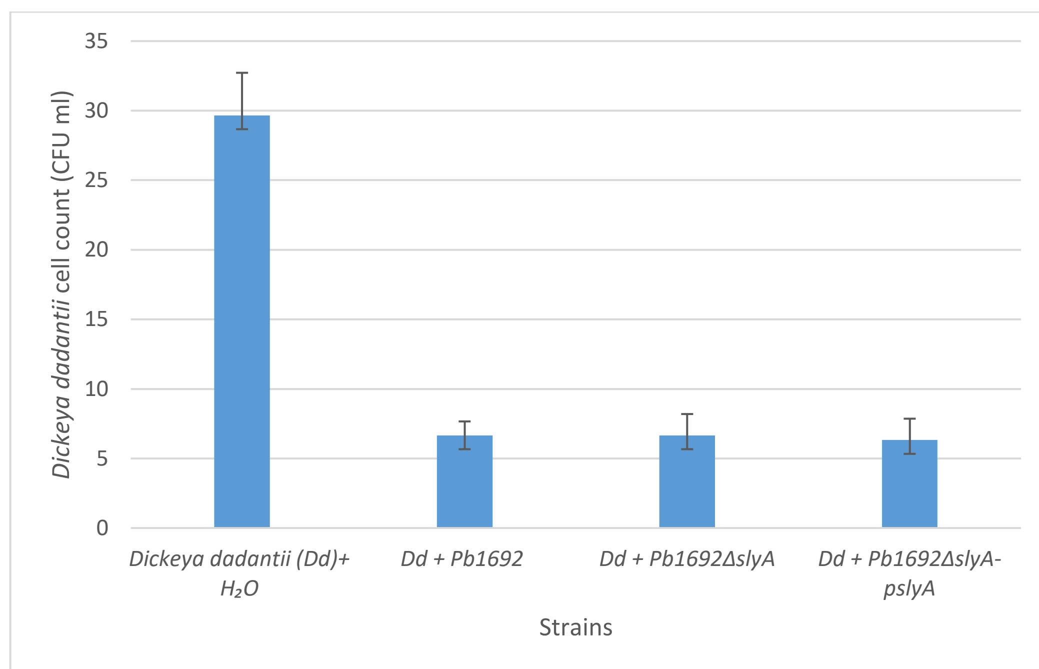


Figure 3 7: *In planta* competition assays on potato tubers. A graph showing the growth (mean CFUs/ml) of *D. dadantii* 3937 strain when co-inoculated with *Pb*1692 in potato tubers (at a 1:1 ratio).

3.3.12 Expression of virulence-related genes by SlyA

To validate the RNA seq data, genes were randomly selected and subjected to RT-qPCR analysis. cDNA for RT-qPCR was synthesized from the 12 hour time point RNA samples. The target genes included, *sod*C (encoding a superoxide dismutase), *tss*C (T6SS), *tss*E (T6SS needle hub protein), *PTS* IIA (encoding the phosphotransferase system IIA), *PTS* IIC (encoding the phosphotransferase system IIC) together with two unannotated proteins *hrp*V (negative regulator for hrp) and *big*A (Big Association gene) (Figure 3.8). The *ffh* gene was used to standardise gene expression, this gene encodes a signal recognition particle protein (Takle *et al.*, 2007). The results indicated that *sod*C (0.614208), *tss*C (0.901565), *tss*E (0.816342), *PTS* IIA (0.834021), *PTS*IIC (0.936278), *hrp*V (negative regulator for hrp) (0.382102) and *big*A (Big Association gene) (0.570176) were significantly reduced in *Pb*1692Δ*sly*A compared to *Pb*1692 strain, which correlated with the transcriptome data. The reduction of *sod*C confirms the role of SlyA protein in stress response, *sod*C decrease oxidative stress during aerobic respiration, while *PTS II* A and C confirms the role of SlyA in carbohydrate metabolism.

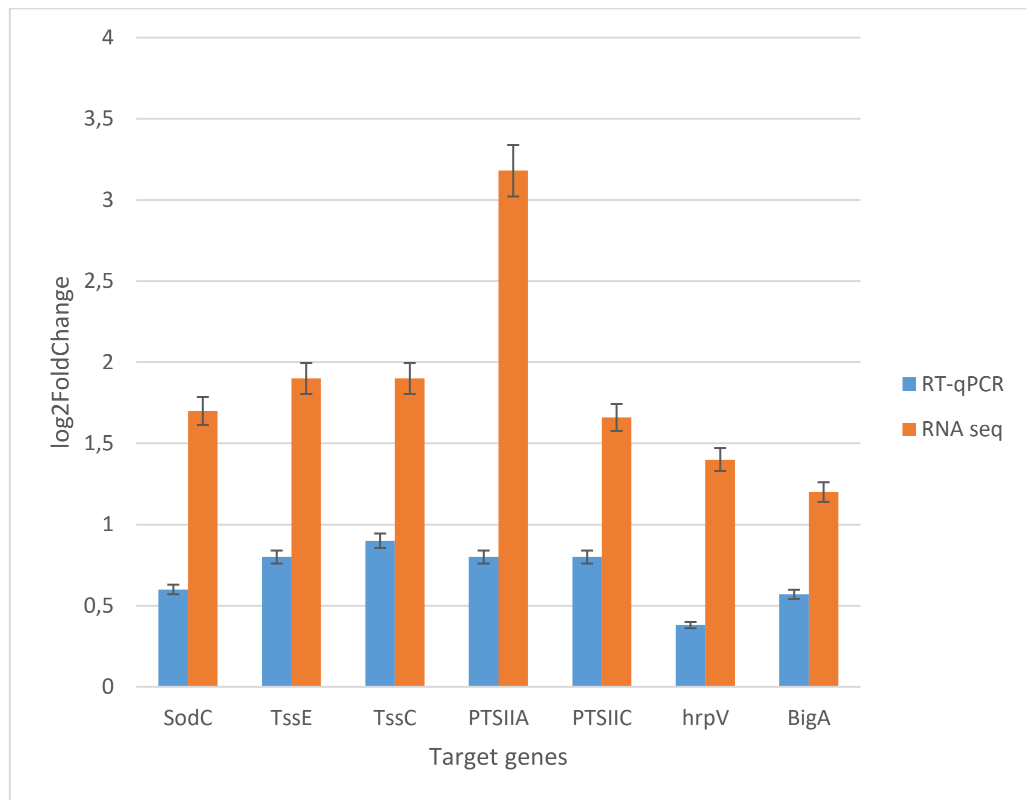


Figure 3.8: Differentially expressed pathogenicity genes in *Pb*1692Δ*sly*A relative to *Pb*1692. cDNA synthesized from 24 hpi RNA samples was used for RT-qPCR validation. The cycle threshold (CT) method was used to analyse the fold change in gene expression levels and data normalization by the ffh gene. The fold change in RNA-seq data determined by (log2FoldChange) was used. Relative expression calculated using ($2^{-\Delta\Delta CT}$ ± Standard Deviation).

3.4 Discussion

The presence of several stress conditions, namely oxidative stress, nutrient limitation, alterations in pH, toxins and AMPs presents a challenge for the survival of bacteria (Foster & Spector, 1995, Wojtaszek, 1997, Iqbal *et al.*, 2019, Hennigar & McClung, 2016, Fones & Preston, 2012). This study presents evidence that SlyA is one of the essential regulators required by *Pb*1692 to survive *in planta*. Using phenotypic, bioinformatics and transcriptome analysis the aim was to identify SlyA-regulated genes *in planta*. A strategy by Datsenko and Wanner was used to construct a *sly*A mutant, and pairwise comparison was done between the wild type and the mutant (Datsenko & Wanner, 2000).

Next, using *in silico* analysis, gene neighborhoods of *sly*A gene across *Pectobacterium* and *Dickeya* genomes were inspected. This analysis indicated a significant similarity between the two species as they share a synteny of genes encoding an outer membrane lipoprotein gene *slyB*. Furthermore, *sly*A gene appears to be transcribed either positively or negatively in different strains of *Pectobacterium* and *Dickeya*. These results suggest that *sly*A is conserved among these closely related species indicating a functional relationship between the genes. In addition to gene neighborhood organisation, more *in silico* analyses were conducted using ClustalOmega and Jalview to understand the conservation level of SlyA in different SRP species. Multiple sequence alignment of different SlyA homologs revealed a total of six α-helices and two β-strands with 90-100% identity in Leucine (L-12, 37, 52, 63, 67, and 70), Arginine (R-65), Threonine (T-36) and Isoleucine (I-40). In a site-directed mutagenesis experiment conducted by Okada and colleagues in 2007, major amino acid residues of SlyA classified as important for its transcriptional regulatory activity were identified. These amino residues included, (R86, V64, L-6, K88L67, L70 and R65) which are associated with DNA binding and residues (L12 and L126) are required for efficient dimer formation. Another study revealed the importance of Leu-37, T-36, Leu-70, Ile-40, Leu-52, Leu-63 and Leu-67. Some of these residues have roles in DNA binding, dimerization and structural integrity (Okada *et al.*, 2007, Haider *et al.*, 2008). Interestingly, some of the major residues highlighted in other studies, namely Leucine 12 and 63,

Arginine 65 and Threonine 36 are also conserved in SlyA of *Pb*1692. Suggesting that SlyA transcriptional regulator is implicated in DNA binding and dimer formation, allowing efficient interaction with promoter regions of its regulated genes in *Pb*1692. Moreover, as evident from the alignment between SlyA in *Pb*1692 and other SlyA proteins with known structures, SlyA in *Pb*1692 might have similar functions as that of other resolved SlyA molecules. These results further demonstrate that SlyA is highly conserved among *Enterobacteriaceae* species. This level of conservation demonstrates that SlyA ancestry has a crucial role in the regulatory mechanisms of *Enterobacteriaceae* species (Dale & Maudlin, 1999).

Another critical virulence factor in plant pathogenic bacteria is the production PCWDEs. PCWDEs in *Pectobacterium* and *Dickeya* species have been well studied for their role in the disease development (Matsumoto *et al.*, 2003). In this study, the transcriptome data, revealed a downregulation of T2SS. As part of the virulence mechanism of phytopathogenic bacteria, they secrete a massive amount of PCWDEs such as cellulases, proteases and pectinases using T2SS (Barras *et al.*, 1994, Jha *et al.*, 2005). In light of the transcriptome data, protease and cellulase plate assays were conducted and these results demonstrate that the activity of the extracellular enzymes cellulase and protease was drastically decreased in *Pb*1692Δ*sly*A compared to *Pb*1692. In addition to the enzyme assays, pathogenicity assays also showed a drastic reduction in macerated tissue of the Δ*sly*A mutant in comparison to the wild-type strain when inoculated onto potato tubers at 12 and 24hpi. These results undoubtedly show that SlyA regulates the production of some PCWDEs. Likewise in, *D. dadantii* and *P. carotovorum* subsp. *carotovorum* SCC3193, it was shown that SlyA is essential for the production of extracellular enzyme (Haque *et al.*, 2009, Sjöblom *et al.*, 2008).

Biofilms are complex aggregates of bacterial species that cushion individual cells from physical, chemical, predators and/or biological harm (Kostakioti *et al.*, 2013, Davey & O'toole, 2000). Besides protection from various forms of assault, biofilm structures in bacterial pathogens play a critical role in pathogenesis during the infection process (Branda

et al., 2005, Malamud *et al.*, 2013). Closely related *Pectobacterium* spp. have been extensively studied on pathogenicity determinants such as N-acyl homoserine lactones (AHLs) and formation of biofilms (Liu *et al.*, 2008, Lee *et al.*, 2013). Furthermore, a connection was made between the formation of biofilms and quorum sensing, where a quorum sensing defective mutant strain lost the ability to form bacterial aggregates on stems of susceptible potatoes or produce acyl-homoserine lactone resulting in a *Pb*1692 strain attenuated in virulence (Moleleki *et al.*, 2017). In susceptible potato plants, *Pb*1692 colonizes the host by forming biofilm-like aggregates leading to the obstruction of the xylem, indicating that bacterial aggregates formed by *Pb*1692 enable the pathogen to survive harsh environmental conditions during host-pathogen interactions (Kubheka *et al.*, 2013). In a study conducted by Zhou in 2016, on a plant pathogen *D. zeae,* a defective Δ*sly*A mutant was unable to form biofilms in liquid media, suggesting that SlyA is essential for the formation of biofilm (Zhou *et al.*, 2016). In this particular study, disruption of *sly*A abolished biofilm formation. Comparable to the changed phenotype observed (lack of biofilm formation), gene ontology functional enrichment of pathways revealed enriched genes associated with biofilm formation under the control of SlyA.

Generally, the role of SlyA has been comprehensively studied in the regulation of genes involved in protection against toxic compounds (Cabezas *et al.*, 2018, Ellison & Miller, 2006, Curran *et al.*, 2017). In pathogenic bacteria, ROS is one of the toxic compounds produced by the host as a defense mechanism, and ROS include hydroxyl radicals, hydrogen peroxide (H_2O_2) and superoxide (O_2) (Lehmann *et al.*, 2015). These species damage cell components such as lipids, proteins and nucleic acids (Halliwell, 2006). Several enzymes have been studied for their effects on the production of ROS in the apoplast following pathogen attack, for example, NADPH dehydrogenase. This enzyme is vital for anti-oxidative stress response in microorganisms and are activated following recognition of fungal and bacterial pathogens (Minard & McAlister-Henn, 2005, Singh *et al.*, 2008, Chittoor *et al.*, 1997). In the current study NADPH dehydrogenase enzyme and glutathione were activated at 12 and 24hpi, leading us to speculate that when *Pb*1692 was exposed to ROS in the apoplast, it activated stress response genes in order to cope with the

stress. The study further speculated that hydrogen peroxide as one of the ROS, will inhibit the growth of *sly*A mutant. As hypothesized, *Pb*1692*sly*A were more sensitive to H_2O_2 than *Pb*1692 strains, an indication that SlyA transcriptional regulator might be regulating genes involved in oxidative stress response. Similar to a study in *D. dadantii* Δ*sly*A mutants were highly sensitive to H_2O_2 in comparison to the wild type (Haque *et al.*, 2009). The current study, in addition to the transcriptome data and *in vitro* assays, RT-qPCR was conducted to validate RNA-seq data. In the RT-qPCR analysis, *sod*C gene was selected to check if SlyA positively regulate genes involved in protection against hydrogen peroxide. Superoxide dismutase (*sod*C) is one of the genes required by the organism to overcome ROS stress (Brioukhanov & Netrusov, 2004).

SlyA in *Pb*1692 also regulated carbohydrate associated genes. Interestingly, more carbohydrate associated genes were positively regulated in the 12-hour time point when compared to 24 hours, thus indicating that when *Pb*1692 is not well adapted inside the host, it activates carbohydrate associated genes to assimilate nutrients from the plant. Carbohydrate genes activated included the phosphotransferase system (PTS). This system is important for the transport and phosphorylation of essential carbohydrates. In *Salmonella,* PTS is responsible for transporting a number of carbohydrates utilized by this pathogen (Miller *et al.*, 2013). Whereas in *E. coli*, the phosphocarrier protein HPr, an important component of the PTS system, is responsible for controlling energy metabolism by directly interacting with several carbohydrate-metabolizing enzymes (Rodionova *et al.*, 2017). *Xanthomonas fuscans* subsp. *aurantifolii,* a plant pathogen, also contains a specific fructose PTS, responsible for the uptake of important sugars (Moreira *et al.*, 2010). Similar to the studies above, in the current study, activation of PTS implies that SlyA activates PTS to enable *Pb*1692 to assimilate carbohydrates inside the host ensuring its survival and establishment of infection.

The transcriptome data further revealed that transporters were differentially expressed at 12 and 24hpi. Notably are the ion transporters namely, Fe-S-binding protein, Hemin iron-containing porphyrin, Heme-binding protein A (HasA), tonB-dependent siderophore

receptor which were differentially expressed at 24hpi while metal ABC transporters were expressed at 12hpi. Transporters allow the cells to accumulate nutrients while excreting unwanted by-products and maintaining cell turgor for growth and development (Du *et al.*, 2015). Furthermore, to defend against foreign objects, bacteria utilize several metal ABC transport systems, restricting the availability of essential metals in the host environment (Payne *et al.*, 2016). In this study, it appears that for *Pb*1692 to adapt inside the host, iron sequestration genes are activated and once enough iron has been acquired, iron is transported to the cytoplasm by transport proteins such as ABC transporters. Similar to *Xanthomonas. campestris* pv. *Campestris*, HpaR, a MarR regulator was found to directly control virulence, by binding to promoter regions of genes encoding virulence factors, such ABC transporters and TonB dependent (Pan *et al.*, 2018). To fulfill the need for trace metal accumulation, *Pb*1692 tightly regulates metal uptake, ensuring enough trace metals are absorbed while allowing for elimination of excess metals to avoid accumulation of toxins.

SlyA also affected the expression of other transcriptional regulators, which also control other sets of genes. Several transcriptional regulators with a wide variety of regulatory functions were affected by SlyA. SlyA appears to be a master regulator that regulates the expression of specific genes and at the same time, regulating other pathways by activating other transcriptional regulators such as AraC and LacI family of transcriptional regulators. AraC is important for virulence while, LacI is a transcriptional regulator that transports essential sugars (Gallegos *et al.*, 1997, Ravcheev *et al.*, 2014). AraC transcriptional regulators have been reported in *E. amylovora* as regulators of virulence genes whereas LacI regulates pathogenicity of *D. dadantii* (Pletzer *et al.*, 2014, Hommais *et al.*, 2008). This could mean SlyA indirectly controls other virulence genes by tapping into other regulatory pathways in *Pb*1692.

Finally, this study revealed that SlyA activated three Hcp components of the T6SS, a hemolysin-coregulated protein considered to be one of the main components of the T6SS. T6SS is involved in the pathogenicity of a number of bacteria such *Vibrio cholerae*, *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Pectobacterium brasiliense*

(Pukatzki *et al.*, 2009, Suarez-Rodriguez *et al.*, 2007, Mougous *et al.*, 2006, Shyntum *et al.*, 2018). T6SS is known to contribute to bacterial virulence, either by directly depositing protein substrates to host cells or secreting toxins into neighbouring bacteria to eliminate them from competing for specific nutrients (Green & Mecsas, 2016). In this particular study, based on the transcriptome data, competition assays were performed using potato tubers in order to verify the effects of SlyA during interbacterial competition. Initially, the study hypothesized that *sly*A mutants will behave the same way as the wild type because only three *hcp* genes were activated by SlyA and not the entire T6SS cluster. *D. dadantii* 3937 (target strain) was co-inoculated with *Pb*1692 and *sly*A mutant, and as speculated no notable differences were observed between *Pb*1692 and *sly*A, as the killing effect between the two strains was the same. These results suggest that SlyA does not control T6SS directly. In a study previously conducted in our lab, *Pb*1692 inhibited the growth of *D. dadantii* by up to 6-fold reduction in CFU/ml ($p < 0.01$). Additionally, this study also revealed that a mutant strain of *Pb*1692ΔT6 was unable to inhibit the growth of *D. dadantii*, *Pectobacterium carotovorum carotovorum*, or *D. chrysanthemi in vitro* (Shyntum *et al.*, 2018). Further highlighting that it is T6SS that inhibits competing bacteria and not SlyA. In conclusion, SlyA transcriptional regulator has been shown to be important for the survival of *Pb*1692 *in planta* and it appears to control a complex network of genes and other regulators. Further experiments aimed at characterising the role of the SlyA regulon will provide insights into the mechanisms by which *Pb*1692 persists and survives *in planta*.

3.5 Materials and Methods

3.5.1 Bacterial strains and growth conditions

Overnight bacterial strains growing on agar plates growing at 37°C were transferred to fresh LB broth (Luria-Bertani), supplemented with different antibiotics: ampicillin (100µg/ml) and/or kanamycin (50µg/ml) (Sigma Aldrich) (Table 3.3) *In vitro* growth of *Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692*Δsly*A-p*sly*A strains was assessed by incubation in liquid LB broth (supplemented with kanamycin or ampicillin for mutant and complemented strains, respectively) at 37°C for 16 hours (370 rpm). The bacterial strains were adjusted to OD_{600} equal to 0.1 then diluted one to two hundred in fresh LB broth and volume adjusted to 200 ml. Spectrophotometer was used to measure optical density at 600nm (OD_{600}) at one-hour intervals for sixteen hours to determine bacteria growth.

Table 3.3: List of different bacterial strains and plasmids used in this study

Bacterial strains	**Description**	**Sources**
Pectobacterium brasiliense 1692 (*Pb*1692)	Initially isolated from potato in Brazil, sequenced strain	Professor A. Charkowski, Wisconsin University (Duarte *et al.*, 2004)
*Pb*1692Δ*sly*A	*P. brasiliense* 1692*sly*A, Kan^r	This study
*Pb*1692Δ*sly*A-p*sly*A	*Pb*1692Δ*sly*A expressing the *sly*A gene from the p-JET-T plasmid; Amp^r	This study
Dickeya dadantii 3937 LMG 25991^T	*Pelargonium capitatum*, Comoros, sequenced.	(Samson *et al.*, 2005)
Plasmids		
pKD4	Plasmid containing a Kan^r cassette	(Datsenko & Wanner, 2000)
pJET-*sly*A	Bacterial expression vector containing the *sly*A gene insert, Amp^r	This study
pJET1.2/blunt	Commercial cloning vector, f1 ori, Ampr	Thermofischer
pKD20	Temperature-sensitive replication ori; encodes lambda Red genes (exo, bet, gam).	(Datsenko & Wanner, 2000)

Kan^r, $Gent^r$, Amp^r = Resistance to kanamycin, gentamicini and ampicillin

3.5.2 Construction of a *Pectobacterium brasiliense sly*A mutant

The ASAP database was used to identify the *sly*A gene in the *Pb*1692 genome. A strategy developed by Datsenko and Wanner was used to construct a *Pb*1692*ΔslyA* mutant (Datsenko & Wanner, 2000). Uostream and downstream regions flanking the *sly*A gene (approx.1000bp) were amplified from *Pb*1692 genomic DNA using PCR with primer combinations (upstream-SlyAF and SlyAKanR) or (downstream-SlyAR and SlyAKanF), respectively (Table 3.4). The PCR conditions are similar to the conditions set out in section 2.5.3 with the exception of the annealing temperature which was set at 60°C for 15 sec. Kanamycin resistance gene cassette was amplified from plasmid DNA pKD4 using primers KanF and KanR with PCR conditions similar to the conditions set out in section 2.5.3. The three PCR fragments were purified (Genomic DNA Purification Kit-Thermo fisher) combined (15 ng of each fragment) and then amplified using SlyAF and SlyAR primers to construct a hybrid PCR amplicon comprising of SlyA upstream, kanamycin and SlyA downstream, with the same PCR conditions only adjusting the annealing temperature to 62°C. After successfully fusing the mutagenesis cassette, electrocompetent cell aliquots (Section 2.5.2) (50 µl) of *Pb*1692 were mixed with 1.0 µl (20 ng) of the mutagenesis cassette in a 2 mm gap electroporation cuvette. The cassette was electroporated using micropulser electroporator (Biorad). Thereafter, cells were immediately diluted in 1000 µl of LB broth and then transferred into 1.5 ml eppendorf tubes. The resultant mixture was incubated for 3 h at 31°C in a 120 rpm shaker and plated on plates containing kanamycin (50 µg/ml) before finally incubating the plates overnight at 37°C. Disruption of the *sly*A gene with kanamycin was confirmed by PCR amplification using combinations of *sly*A gene flanking primers (SlyATest-F and SlyATest-R), followed by sequencing of the amplicon. PCR conditions: initial denaturation at 96°C for 5 min, followed by 25 cycles of denaturing at 98°C for 20s, annealing at 62°C for 15 sec, extension at 72°C for 2 min and a final extension at 72° for 2 min. All primers were synthesized by Inqaba Biotech T (South, Africa).

Table 3. 4: List of primers used for PCR and qPCR in this study

Primer name	Primer sequence 5' to 3'	Length (bp)	References
SlyA primers			
SlyATest-F	GTCAACGCCCAGCGCGATATG	21	This study
SlyATest-R	CTGGTGCCGGACATTACGCC	20	This study
SlyAF	GCGCATGCCCAGGATTTGGGTATAG	20	This study
SlyAR	GTGGCATGCTACACTGAGCC	20	This study
KanF	GCTAACAATAAGGAGGGGTTGTGTAGGCTGGAGCTGCTTCG	41	This study
SlyAKanR	CGAAGCAGCTCCAGCCTACACAACCCCTCCTTATTGTTAGC	41	This study
SlyAKanF	GGAACTTCGGAATAGGAACTAAGGAGGATATTCATATGCG	40	This study
KanR	CGCATATGAATATCCTCCTTAGTTCCTATTCCGAAGTTCC	40	This study
CompFSlyA	CAGCAGAGTCCCGTCAGCCAT	21	This study
CompRSlyA	GCGGCGATCGTCACAACGAATAAT	24	This study
qPCR Primers			
ffhF	TGGCAAGCCAATTAAATTCC	20	(Tanui *et al.*, 2017)
ffhR	TCCAGGAAGTCGGTCAAATC	20	(Tanui *et al.*, 2017)
tssEf	GGCGATCCGACAGTGTATCT	20	This study
tssEr	TTGAAAGAGGCAACCTGCTC	20	This study
SodCF	TAAATCAGTTCCCGCTCTGG	20	This study
SodCR	GCCAGAATTGGGTAGGTTGA	20	This study

tsscF	AAGAACAGGTTCAGGCAGGA	20	This study
tsscR	CTGCTGCATTACCGCTATCA	20	This study
PTSIICF	CTGGCAGTCGATAGCAACCA	20	This study
PTSIICR	GGATGGCTGGAACCACTCTC	20	This study
PTSIIAF	AATGCGCTCCATCACTCCAA	20	This study
PTSIIAR	CTGAGTTTCCCGTTGGACGA	20	This study
bigAF	TGACCGAGAAAATCACCCCT	20	This study
bigAR	GGGTACATCTCTTCCAGCGT	20	This study
hrpVF	GCTCGACATTACCCCGGTAT	20	This study
hrpVR	AGTTCGTCGATTGCCACAAC	20	This study

3.5.3 Complementation of the *sly*A mutants

CompFSlyA and CompRSlyA primers were used to amplify the *sly*A gene with its native promoter from *Pb*1692. The pJET1.2/blunt cloning vector was used for complementation of mutant strains (Table 3.3). The Thermo Scientific Gel Extraction Kit was used to cut and purify the fragments from an agarose gel. The fragments were each cloned into p-JET to construct pJET-*sly*A (Table 3.3). The pJET-*sly*A plasmid was electroporated into electrocompetent *Pb*1692Δ*sly*A mutant strains (see materials and methods 3.5.2), transformants (*Pb*1692Δ*sly*A-p*sly*A) selected on LB agar plates containing 100 µg/ml ampicillin.

3.5.4 RNA extraction from potato tubers and pathogenicity assays

A 10% (v/v) sodium hypochlorite was utilized to disinfect potato tubers (cv. Mondial, a susceptible cultivar), from that point potatoes were washed twice with distilled water and air dried. Potatoes were wounded with a 1cm sterile pipette. Bacterial suspension (10-µl aliquot) with OD_{600} comparable to 1 (*Pb*1692 wild type, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A) were pipetted into the wounds. Magnesium sulphate (10 mM $MgSO_4$) was used as a control. Petroleum jelly was used to seal the holes and then kept in plastic containers at 25°C for 72 hours. Followed by scooping and weighing of macerated tissue at 12 and 24hpi.

RNA was extracted from macerated potato tubers inoculated with (*Pb*1692 and *Pb*1692Δ*sly*A) at 12 and 24hpi (incubated at 25 ºC). A pestle and mortar was used to grind macerated tissue to harvest bacterial cells using approximately 20 ml of double distilled water. Potato debris were removed at 10,000 rpm for 1 minute by centrifuging the grinded tissue. The bacterial cells were present in the supernatant. The supernatant was then taken out into a new Falcon tubes (50ml) with RNA stabilization buffer. All the samples were subjected to DNAse treatment for the removal of DNA contaminants.

The purity and concentration of each RNA sample extracted was measured at a ratio of 230/260nm using spectrophotometric analysis (NanoDrop® technologies). To evaluate RNA quality, 1% (w/v) agarose gel electrophoresis was used together with 1% TAE buffer at 100 volts for 30 minutes. The image was viewed and developed using Gel Doc EZ system (Bio-Rad Laboratories). The Bioanalyzer from Inqaba Biotech, was used to analyze the final RNA concentration.

3.5.5 Cellulase and protease enzyme assays

To evaluate the role of SlyA in the production of extracellular enzymes, an enzyme activity assay as previously done by Matsumoto was conducted (Matsumoto *et al.*, 2003, Chatterjee *et al.*, 2009). Holes were made on both cellulase and protease assay plates using stop corker, 80 μl of each strain inoculated into individual holes (*Pb*1692, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A). The cellulase assay plates were incubated for 72 hours and then stained with 0.1% (w/v) Congo red solution (Sigma-Aldrich). Additionally, the plates washed three times with 1M NaCl until a clear zone became visible around the holes. The protease assay plates were incubated for three days and inspected for clear zones around the holes with no further treatment. Experiment were performed three independent times, in triplicates.

3.5.6 Biofilm formation assay

To assess the ability of Δ*sly*A mutants in formation of biofilms, 100µl of the overnight cultures (*Pb*1692 wild type, *Pb*1692Δ*sly*A and *Pb*1692Δ*sly*A-p*sly*A) were inoculated into 100ml LB. The cultures were allowed to grow for 48 hours at 37°C. The cultures were removed by aspiration and biofilm formation was visualized by staining with 0.5% crystal violet for 30 minutes after washing with water. The test was conducted in triplicates, two independent times.

3.5.7 Susceptibility to oxidative stress

Susceptibility to oxidative stress was assayed by exposing individual strains to 20 µM H_2O_2, which corresponds to a lethal dose (Tanui *et al.*, 2017). Bacterial strains (*Pb*1692 wild type, *Pb*1692Δ*sly*A mutant, *Pb*1692Δ*sly*A-p*sly*A complemented mutant strain) were grown in LB for 16 h at 37°C with shaking (OD_{600}=1). Thereafter, each culture was inoculated into 100 ml of LB broth augumented with 20 µM H_2O_2 and incubated for 2 h with agitation (370 rpm) at 37°C and viable bacteria enumerated by serial dilution and plating onto nutrient agar plates.

3.5.8 *In planta* competition assays on potato tubers

Interbacterial competition was carried out in potato tubers as demonstrated by Axelrood in 1988 with minor adjustments (Axelrood *et al.*, 1988, Shyntum *et al.*, 2018). Potato tubers were disinfected with 10% sodium hypochlorite, rinsed twiced with distilled water, air dried and then wounded with a sterile pipette (1cm depth). *Pb*1692 wild type, *Pb*1692Δ*sly*A, *Pb*1692Δ*sly*A-p*sly*A and *D. dadantii* with OD_{300} equivalent to 0.3 were cultured overnight and mixed in a 1:1 ratio with the target strain (*D. dadantii*) and inoculated into potato tubers. The wounds were filled with petroleum jelly and incubated in moist plastic containers at 25°C for 24 hours. The subsequent macerated tissue was

weighed to standardize the cultures. The target strain (*D. dadantii* CFU/ml) was enumerated in LB augumented with gentamycin (15μg/ml) through serial dilutions.

3.5.9 Library construction, sequencing, reads mapping and genome-wide functional annotation

The reads quality was done using a *fastqc* software. Reads exhibiting low quality segments were trimmed by Trimmomatic v 0.36 and *hisat2* v 2.1.0 was used to align the reads to the reference genome of *Pb*1692 (GCF_000173135.1) (Bolger *et al.*, 2014). Aligned reads were then computed by *featureCounts* package. DGE thereafter analyzed by the EdgeR package and the FDR < 0.05 was assigned as differentially expressed (Liao *et al.*, 2014, Robinson *et al.*, 2010). Up and down regulation of genes was assigned based on log2fold-change transcriptional variation. Additionally, eggNOG was used to functionally annotate sequences. KEGG-library hierarchy (KEGG B and A) was used to retrieve sequences with higher annotation (Kanehisa & Goto, 2000, Huerta-Cepas *et al.*, 2017). Comparison between *Pb*1692Δ*sly*A relative to *Pb*1692 were done at12 and 24hpi. A more elevated level of annotation was achieved by inspecting conserved protein sequence domains using the Pfam database and HMMER3 (Finn *et al.*, 2015, Finn *et al.*, 2010).

3.5.10 Gene expression analysis (RT-qPCR)

To substantiate the RNA-seq data, RT-qPCR analysis was conducted with seven randomly selected genes. The SuperScript IV First-Strand Synthesis System (invitrogen) was used to reverse transcribe 5 μg of total RNA into first-strand cDNA. QuantStudio™ 12K Flex Real-Time PCR System (Applied Biosystems™) was used for gene expression analysis. The PCR parameters used are similar to the ones used in section 2.5.12, with the exception of the annealing stage (60°C for 45s). House-keeping gene (*ffh*) were used to standardize gene expression. The gene expression levels of the following genes were analyzed: included *sod*C (encoding a superoxide dismutase), *tss*C (T6SS), *tss*E (T6SS needle hub protein), *PTS* IIA (encoding the phosphotransferase system IIA), *PTS* IIC (encoding the phosphotransferase system IIC) together with two unannotated proteins *hrp*V (negative

regulator for hrp) and *big*A (Big Association gene). Statistical analysis of relative gene expression, the $2^{\Delta\Delta CT}$ method was used (Livak & Schmittgen, 2001).

3.5.11 Statistical analysis

Student's t-tests was used to determine statistical significance. A *p*-value <0.05 was considered to be statistically significant.

3.6 Supplementary data

Figure S3.1: PCR amplicons used to generate the *slyA* mutant.

Lane 1. 1Kb marker, 2. *sly*A downstream PCR fragment, 3. kanamycin cassette PCR product, 4. *sly*A upstream PCR fragment, 5. Fusion product consisting of the downstream, kanamycin and upstream fragment. 6. Km1 primers are internal kanamycin primers.

Figure S3.2: Schematic presentation of how *Pb*1692Δ*sly*A mutant strain was generated

Table S3.1 : Differentially expressed genes during *in planta* infection at 12hpi by SlyA

Table S3.2 : Some of the selected differentially expressed genes (DEGs) during *in planta* infection at 24hpi by SlyA

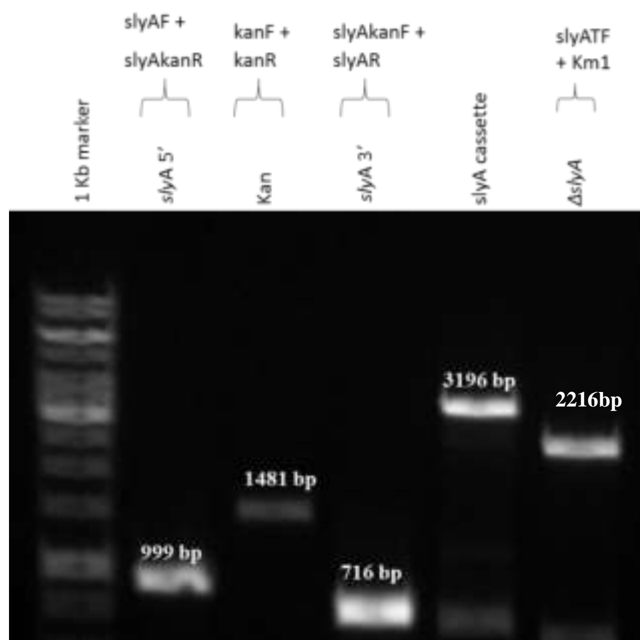


Figure S3. 1: PCR amplicons used to generate the *slyA* mutant. Lane 1. 1Kb marker, 2. *sly*A downstream PCR fragment, 3. kanamycin cassette PCR product, 4. *sly*A upstream PCR fragment, 5. Fusion product consisting of the downstream, kanamycin and upstream fragment. 6. Km1 primers are internal kanamycin primers.

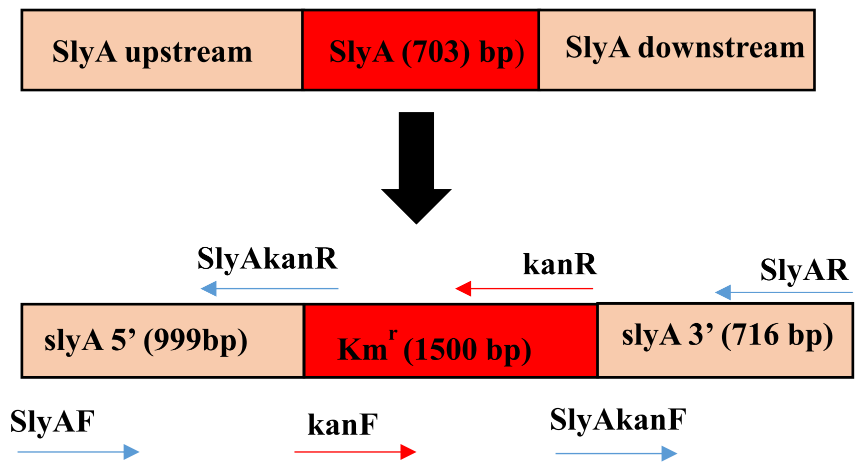


Figure S3.2: Schematic presentation of how *Pb*1692Δ*sly*A mutant strain was generated

\

Table S3.1: Differentially expressed genes during *in planta* infection at 12 hpi by SlyA

Locus_tag	logFC	eggNOG_model_annotation
	Down-regulated genes	
	Transporters	
PCBA_RS18545	-2.436390992	extracellular solute-binding protein
PCBA_RS01480	-2.41651425	abc transporter atp-binding protein
PCBA_RS00500	-2.848369946	arabinose abc transporter substrate-binding protein
PCBA_RS11095	-2.279454934	efflux rnd transporter periplasmic
PCBA_RS18560	-1.842287393	magnesium transporter
PCBA_RS19155	-2.311195179	major family facilitator
PCBA_RS00505	-2.193264116	metal abc transporter permease
PCBA_RS00090	-2.248068367	metal abc transporter substrate-binding protein
PCBA_RS00375	-2.382571994	metal abc transporter substrate-binding protein
PCBA_RS00510	-3.514135859	metal abc transporter substrate-binding protein
PCBA_RS01110	-2.541156385	oligogalacturonate-specific porin
PCBA_RS00640	-2.814651428	outer membrane protein w
PCBA_RS20320	-1.29556131	transmembrane protein
	Carbohydrates	
PCBA_RS09325	-1.646424618	6-phospho-beta-glucosidase
PCBA_RS06205	-2.438500243	alcohol dehydrogenase
PCBA_RS08790	-1.563202976	beta-glucosidase ec 3.2.1.21
PCBA_RS02060	-4.135251606	glycoside hydrolase family protein
PCBA_RS17695	-2.526289436	l-rhamnose isomerase
PCBA_RS02605	-1.54081825	l-ribulose-5-phosphate 4-epimerase
PCBA_RS06225	-3.1185113	phosphoglucosamine mutase
	Phosphotransferase system	
PCBA_RS01470	-1.736005788	pts system, lactose cellobiose family iic
PCBA_RS01475	-3.180321413	phosphotransferase system pts
PCBA_RS02065	-3.513434399	phosphotransferase system pts
PCBA_RS09330	-1.665820899	pts system subunit iiabc
	Stress response	
PCBA_RS06520	-1.798653332	nadp-dependent oxidoreductase
PCBA_RS11660	-1.731602418	nadph dehydrogenase
PCBA_RS18515	-2.529066492	organic hydroperoxide resistance protein
PCBA_RS18520	-2.43223572	organic hydroperoxide resistance protein

PCBA_RS18530	-2.429182991	organic hydroperoxide resistance protein
PCBA_RS02495	-2.053171362	glutathione s-transferase

	Chemotaxis	
PCBA_RS03310	-2.151630799	methyl-accepting chemotaxis protein
PCBA_RS17670	-1.723348503	methyl-accepting chemotaxis
	Transcriptional regulator	
PCBA_RS00485	-1.912880308	transcriptional regulator (arac
PCBA_RS15150	-1.922899189	laci transcriptional regulator
	T6SS/Hcp	
PCBA_RS05805	-1.911116617	type vi secretion system effector, hcp1 family
PCBA_RS12700	-1.900165473	type vi secretion system effector, hcp1 family
	Exported protein	
PCBA_RS06395	-1.740782133	exported protein
	Adhesion	
PCBA_RS11610	-1.801372002	fimbrial protein
Up-Regulated genes		
	Transcriptional regulators	
PCBA_RS02575	1.531706	transcriptional regulator
PCBA_RS16145	1.657965	transcriptional regulator
	Transporters	
PCBA_RS01225	2.795431	major facilitator superfamily
	Bacteriocins	
PCBA_RS05435	3.538905	beta-lactamase domain-containing protein
	Adhesion	
PCBA_RS05440	2.547646	protein-disulfide isomerase
	Carbohydrates	
PCBA_RS07540	1.288761	nucleotide sugar dehydrogenase
PCBA_RS14940	1.838881	glucose-1-phosphatase inositol phosphatase
PCBA_RS16150	1.463208	short-chain dehydrogenase reductase

Table S3. 2: Some of the selected differentially expressed genes (DEGs) during *in planta* infection at 24hpi by SlyA		
Locus_tag	**logFC**	**eggNOG_model_annotation**
Down-Regulated genes		
	Transporters	
PCBA_RS18545	-1.411317576	extracellular solute-binding protein
PCBA_RS00500	-1.723180675	arabinose abc transporter substrate-binding protein
PCBA_RS10795	-1.086115648	efflux transporter periplasmic
PCBA_RS11880	-1.291615605	inner membrane protein yqjf
PCBA_RS18530	-1.382506427	involved in the transport of maltose and maltodextrins
PCBA_RS00495	-1.503389661	l-arabinose abc transporter atp-binding protein arag
PCBA_RS00490	-1.494730557	l-arabinose transporter permease
PCBA_RS21675	-2.477659308	lipopolysaccharide abc transporter
PCBA_RS04270	-1.14347937	major facilitator superfamily
PCBA_RS02050	-1.194581541	periplasmic
PCBA_RS09820	-1.238109473	permease
PCBA_RS02045	-1.051087562	permease protein
PCBA_RS02045	-1.051087562	permease protein membrane transporter
PCBA_RS09260	-1.434228261	sugar abc transporter substrate-binding protein
PCBA_RS12400	-1.090308731	transporter
PCBA_RS07485	-1.070102459	pts system
PCBA_RS09330	-1.009256299	pts system transporter subunit iiabc
	Carbohydrates	
PCBA_RS09255	-1.585283743	2-dehydrogenase
PCBA_RS13100	-1.13627807	alpha-n-arabinofuranosidase
PCBA_RS00510	-1.391803174	catalyzes the conversion of l-arabinose to l-ribulose
PCBA_RS09290	-1.045735955	myoinositol catabolism
	Stress response	
PCBA_RS07265	-1.759172148	extradiol ring-cleavage dioxygenase, class iii
PCBA_RS09890	-1.194524399	universal stress protein a

PCBA_RS02495	-1.596433327	glutathione s-transferase
PCBA_RS06665	-1.069632553	glutathione transferase (ec 2.5.1.18)

	Transcriptional regulators	
PCBA_RS00485	-2.026793691	transcriptional regulator (arac)
PCBA_RS04530	-1.082978784	transcriptional regulator
PCBA_RS09720	-1.645475786	transcriptional regulator
PCBA_RS15150	-1.281090802	laci transcriptional regulator
PCBA_RS17640	-1.51854965	ig family of transcriptional regulators
	T2SS	
PCBA_RS17600	-1.231075506	type ii secretion system protein e
	T6SS/Hcp	
PCBA_RS05805	-1.055008339	type vi secretion system effector, hcp1 family
	T3SS	
PCBA_RS14175	-1.824627137	type iii secretion system (ttss, hrp secretion system)
	Adhesion	
PCBA_RS00225	-1.14889506	rare lipoprotein a
PCBA_RS02155	-1.306140395	major outer membrane lipoprotein
PCBA_RS17570	-1.186431346	flp/fap pilin component
PCBA_RS12655	-1.114170284	hemolysin activator protein
	Amino acid metabolism	
PCBA_RS04280	-1.277314362	cysteine synthase
PCBA_RS16550	-1.259562851	amino acid adenylation domain-containing protein
	Carotovorocin	
PCBA_RS20630	-1.10332579	baseplate
PCBA_RS08665	-1.301361466	tail tube protein
PCBA_RS12365	-1.195953708	entericidin ecna/b family
	Iron uptake	
PCBA_RS02405	-1.038888418	(fe-s)-binding protein

PCBA_RS02055	-1.076098551	hemin iron containing porphyrin
PCBA_RS17520	-1.09767326	heme-binding protein a (hasa)
PCBA_RS04290	-1.22734149	tonb-dependent siderophore receptor
Up-Regulated genes-24hpi		
	Iron transport	
PCBA_RS02275	1.285904	cytochrome c-type biogenesis protein
PCBA_RS02280	1.630569447	cytochrome C-type protein
PCBA_RS11530	3.615803778	biopolymer transport protein ExbD TolR
	Nitrate metabolism	
PCBA_RS02285	1.398029889	periplasmic nitrate reductase complex NapAB
	Transcriptional regulators	
PCBA_RS22170	1.719931444	transcriptional regulator, RpiR family
PCBA_RS22175	1.12446209	transcriptional regulator
	Transporters	
PCBA_RS02290	1.359062492	quinol dehydrogenase membrane component
PCBA_RS03160	1.033362118	exported protein
PCBA_RS08755	1.020182766	transporter
PCBA_RS21860	3.247900739	abc-type nitrate sulfonate bicarbonate transport
PCBA_RS11430	1.262526243	branched-chain amino acid transport protein (azld)
PCBA_RS11605	1.228098606	outer membrane usher protein
PCBA_RS00050	1.036378743	major facilitator
PCBA_RS11435	1.174413032	multidrug efflux mfs transporter
PCBA_RS17675	1.071129556	atpase, p-type (transporting), had superfamily, subfamily ic
	Nitrate metabolism	
PCBA_RS02960	2.508996751	nitrate reductase, alpha subunit
PCBA_RS02965	2.44027241	nitrate reductase
PCBA_RS02970	2.266066805	nitrate reductase
PCBA_RS02975	1.486241832	respiratory nitrate reductase
	Stress response	
PCBA_RS05475	1.151711952	cytochrome p450
PCBA_RS05890	1.434765205	dsrB protein
	PCWDEs	
PCBA_RS10070	1.04764971	Polygalacturonase

Fold change represent the mean change of mRNA levels for each gene under the paired growth conditions (*sly*A mutant versus WT strain 12 and 24hpi). The positive number stands for fold up-regulated (repressed), while minus down-regulated (activated).

3.7 References

Alekshun, M.N., Levy, S.B., Mealy, T.R., Seaton, B.A., and Head, J.F. (2001) The crystal structure of MarR, a regulator of multiple antibiotic resistance, at 2.3 Å resolution. *Nat. Struct. Mol. Biol.* **8**: 710-714.

Aravind, L., Anantharaman, V., Balaji, S., Babu, M.M., and Iyer, L.M. (2005) The many faces of the helix-turn-helix domain: transcription regulation and beyond. *FEMS Microbiol. Rev.* **29**: 231-262.

Axelrood, P.E., Rella, M., and Schroth, M.N. (1988) Role of antibiosis in competition of *Erwinia* strains in potato infection courts. *Appl. Environ. Microbiol.* **54**: 1222-1229.

Barras, F., van Gijsegem, F., and Chatterjee, A.K. (1994) Extracellular enzymes and pathogenesis of soft-rot Erwinia. *Annu. Rev. Phytopathol.* **32**: 201-234.

Bolger, A.M., Lohse, M., and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114-2120.

Branda, S.S., Vik, Å., Friedman, L., and Kolter, R. (2005) Biofilms: the matrix revisited. *Trends Microbiol.* **13**: 20-26.

Brioukhanov, A., and Netrusov, A. (2004) Catalase and superoxide dismutase: distribution, properties, and physiological role in cells of strict anaerobes. *Biochemistry (Moscow)* **69**: 949-962.

Buchmeier, N., Bossie, S., Chen, C.-Y., Fang, F.C., Guiney, D.G., and Libby, S.J. (1997) SlyA, a transcriptional regulator of *Salmonella* typhimurium, is required for resistance to oxidative stress and is expressed in the intracellular environment of macrophages. *Infect. Immun.* **65**: 3725-3730.

Cabezas, C.E., Briones, A.C., Aguirre, C., Pardo-Esté, C., Castro-Severyn, J., Salinas, C.R., Baquedano, M.S., Hidalgo, A.A., Fuentes, J.A., and Morales, E.H. (2018) The transcription factor SlyA from *Salmonella* Typhimurium regulates genes in response to hydrogen peroxide and sodium hypochlorite. *Res. Microbiol.*

Cathelyn, J.S., Crosby, S.D., Lathem, W.W., Goldman, W.E., and Miller, V.L. (2006) RovA, a global regulator of *Yersinia pestis*, specifically required for bubonic plague. *Proceedings of the National Academy of Sciences* **103**: 13514-13519.

Charkowski, A., Blanco, C., Condemine, G., Expert, D., Franza, T., Hayes, C., Hugouvieux-Cotte-Pattat, N., Solanilla, E.L., Low, D., and Moleleki, L. (2012) The role of secretion systems and small molecules in soft-rot enterobacteriaceae pathogenicity. *Annu. Rev. Phytopathol.* **50**: 425-449.

Chatterjee, A., Cui, Y., and Chatterjee, A.K. (2009) RsmC of *Erwinia carotovora* subsp. *carotovora* negatively controls motility, extracellular protein production, and virulence by binding FlhD and modulating transcriptional activity of the master regulator, FlhDC. *J. Bacteriol.* **191**: 4582-4593.

Chittoor, J.M., Leach, J.E., and White, F.F. (1997) Differential induction of a peroxidase gene family during infection of rice by *Xanthomonas oryzae* pv. oryzae. *Mol. Plant-Microbe Interact.* **10**: 861-871.

Corbett, D., Bennett, H.J., Askar, H., Green, J., and Roberts, I.S. (2007) SlyA and H-NS regulate transcription of the *Escherichia coli* K5 capsule gene cluster, and

expression of slyA in *Escherichia coli* is temperature-dependent, positively autoregulated, and independent of H-NS. *J. Biol. Chem.* **282**: 33326-33335.
Curran, T.D., Abacha, F., Hibberd, S.P., Rolfe, M.D., Lacey, M.M., and Green, J. (2017) Identification of new members of the *Escherichia coli* K-12 MG1655 SlyA regulon. *Microbiology* **163**: 400.
Dale, C., and Maudlin, I. (1999) Sodalis gen. nov. and Sodalis glossinidius sp. nov., a microaerophilic secondary endosymbiont of the tsetse fly Glossina morsitans morsitans. *Int. J. Syst. Evol. Microbiol.* **49**: 267-275.
Datsenko, K.A., and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences* **97**: 6640-6645.
Davey, M.E., and O'toole, G.A. (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.* **64**: 847-867.
Davis, J.R., Brown, B.L., Page, R., and Sello, J.K. (2013) Study of PcaV from *Streptomyces coelicolor* yields new insights into ligand-responsive MarR family transcription factors. *Nucleic Acids Res.* **41**: 3888-3900.
Dolan, K.T., Duguid, E.M., and He, C. (2011) Crystal structures of SlyA protein, a master virulence regulator of *Salmonella,* in free and DNA-bound states. *J. Biol. Chem.* **286**: 22178-22185.
Du, D., van Veen, H.W., Murakami, S., Pos, K.M., and Luisi, B.F. (2015) Structure, mechanism and cooperation of bacterial multidrug transporters. *Curr. Opin. Struct. Biol.* **33**: 76-91.
Duprey, A., Reverchon, S., and Nasser, W. (2014) Bacterial virulence and Fis: adapting regulatory networks to the host environment. *Trends Microbiol.* **22**: 92-99.
Ellison, D.W., and Miller, V.L. (2006) Regulation of virulence by members of the MarR/SlyA family. *Curr. Opin. Microbiol.* **9**: 153-159.
Finn, R.D., Clements, J., Arndt, W., Miller, B.L., Wheeler, T.J., Schreiber, F., Bateman, A., and Eddy, S.R. (2015) HMMER web server: 2015 update. *Nucleic Acids Res* **43**: W30-38.
Finn, R.D., Mistry, J., Tate, J., Coggill, P., Heger, A., Pollington, J.E., Gavin, O.L., Gunasekaran, P., Ceric, G., Forslund, K., Holm, L., Sonnhammer, E.L., Eddy, S.R., and Bateman, A. (2010) The Pfam protein families database. *Nucleic Acids Res* **38**: D211-222.
Fones, H., and Preston, G.M. (2012) Reactive oxygen and oxidative stress tolerance in plant pathogenic Pseudomonas. *FEMS Microbiol. Lett.* **327**: 1-8.
Foster, J.W., and Spector, M.P. (1995) How *Salmonella* survive against the odds. *Annu. Rev. Microbiol.* **49**: 145-174.
Gallegos, M.-T., Schleif, R., Bairoch, A., Hofmann, K., and Ramos, J.L. (1997) Arac/XylS family of transcriptional regulators. *Microbiol. Mol. Biol. Rev.* **61**: 393-410.
George, A., and Levy, S. (1983) Gene in the major cotransduction gap of the *Escherichia coli* K-12 linkage map required for the expression of chromosomal resistance to tetracycline and other antibiotics. *J. Bacteriol.* **155**: 541-548.
Green, E.R., and Mecsas, J. (2016) Bacterial secretion systems–an overview. *Microbiology spectrum* **4**.

Haider, F., Lithgow, J.K., Stapleton, M.R., Norte, V.A., Roberts, R.E., and Green, J. (2008) DNA recognition by the *Salmonella enterica* serovar Typhimurium transcription factor SlyA. *Int. Microbiol.* **11**: 245.

Halliwell, B. (2006) Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol.* **141**: 312-322.

Haque, M.M., Hirata, H., and Tsuyumu, S. (2015) SlyA regulates motA and motB, virulence and stress-related genes under conditions induced by the PhoP-PhoQ system in *Dickeya dadantii* 3937. *Res. Microbiol.* **166**: 467-475.

Haque, M.M., Kabir, M.S., Aini, L.Q., Hirata, H., and Tsuyumu, S. (2009) SlyA, a MarR family transcriptional regulator, is essential for virulence in *Dickeya dadantii* 3937. *J. Bacteriol.* **191**: 5409-5418.

Hennigar, S.R., and McClung, J.P. (2016) Nutritional immunity: starving pathogens of trace minerals. *Am. J. Lifestyle Med.* **10**: 170-173.

Heroven, A.K., Nagel, G., Tran, H.J., Parr, S., and Dersch, P. (2004) RovA is autoregulated and antagonizes H-NS-mediated silencing of invasin and rovA expression in *Yersinia pseudotuberculosis. Mol. Microbiol.* **53**: 871-888.

Hommais, F., Oger-Desfeux, C., Van Gijsegem, F., Castang, S., Ligori, S., Expert, D., Nasser, W., and Reverchon, S. (2008) PecS is a global regulator of the symptomatic phase in the phytopathogenic bacterium *Erwinia chrysanthemi* 3937. *J. Bacteriol.* **190**: 7508-7522.

Huerta-Cepas, J., Forslund, K., Coelho, L.P., Szklarczyk, D., Jensen, L.J., von Mering, C., and Bork, P. (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Molecular biology and evolution* **34**: 2115-2122.

Iqbal, A., Khan, R.S., Shehryar, K., Imran, A., Ali, F., Attia, S., Shah, S., and Mii, M. (2019) as effective tools for enhanced disease resistance in plants. *Plant Cell, Tissue and Organ Culture (PCTOC)*: 1-15.

Jha, G., Rajeshwari, R., and Sonti, R.V. (2005) Bacterial type two secretion system secreted proteins: double-edged swords for plant pathogens. *Mol. Plant-Microbe Interact.* **18**: 891-898.

Kanehisa, M., and Goto, S. (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research* **28**: 27-30.

Kim, D., Langmead, B., and Salzberg, S.L. (2015) HISAT: a fast spliced aligner with low memory requirements. *Nat Methods* **12**: 357-360.

Kim, Y., Joachimiak, G., Bigelow, L., Babnigg, G., and Joachimiak, A. (2016) How aromatic compounds block DNA binding of HcaR catabolite regulator. *J. Biol. Chem.* **291**: 13243-13256.

Kostakioti, M., Hadjifrangiskou, M., and Hultgren, S.J. (2013) Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb. Perspect. Med.* **3**: a010306.

Kubheka, G.C., Coutinho, T.A., Moleleki, N., and Moleleki, L.N. (2013) Colonization patterns of an mCherry-tagged *Pectobacterium carotovorum* subsp. *brasiliense* strain in potato plants. *Phytopathology* **103**: 1268-1279.

Lee, D.H., Lim, J.-A., Lee, J., Roh, E., Jung, K., Choi, M., Oh, C., Ryu, S., Yun, J., and Heu, S. (2013) Characterization of genes required for the pathogenicity of *Pectobacterium carotovorum* subsp. *carotovorum* Pcc21 in Chinese cabbage. *Microbiology* **159**: 1487-1496.

Lehmann, S., Serrano, M., L'Haridon, F., Tjamos, S.E., and Metraux, J.-P. (2015) Reactive oxygen species and plant resistance to fungal pathogens. *Phytochemistry* **112**: 54-62.

Liao, Y., Smyth, G.K., and Shi, W. (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**: 923-930.

Libby, S.J., Goebel, W., Ludwig, A., Buchmeier, N., Bowe, F., Fang, F.C., Guiney, D.G., Songer, J.G., and Heffron, F. (1994) A cytolysin encoded by *Salmonella* is required for survival within macrophages. *Proceedings of the National Academy of Sciences* **91**: 489-493.

Lim, D., Poole, K., and Strynadka, N.C. (2002) Crystal structure of the MexR repressor of themexRAB-oprM multidrug efflux operon of *Pseudomonas aeruginosa*. *J. Biol. Chem.* **277**: 29253-29259.

Liu, H., Coulthurst, S.J., Pritchard, L., Hedley, P.E., Ravensdale, M., Humphris, S., Burr, T., Takle, G., Brurberg, M.-B., and Birch, P.R. (2008) Quorum sensing coordinates brute force and stealth modes of infection in the plant pathogen *Pectobacterium atrosepticum*. *PLoS Path.* **4**: e1000093.

Livak, K.J., and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**: 402-408.

Ludwig, A., Tengel, C., Bauer, S., Bubert, A., Benz, R., Mollenkopf, H.-J., and Goebel, W. (1995) SlyA, a regulatory protein from *Salmonella* typhimurium, induces a haemolytic and pore-forming protein in Escherichia coli. *Molecular and General Genetics MGG* **249**: 474-486.

Malamud, F., Homem, R.A., Conforte, V.P., Yaryura, P.M., Castagnaro, A.P., Marano, M.R., Do Amaral, A.M., and Vojnov, A.A. (2013) Identification and characterization of biofilm formation-defective mutants of *Xanthomonas citri* subsp. citri. *Microbiology* **159**: 1911-1919.

Matsumoto, H., Muroi, H., Umehara, M., Yoshitake, Y., and Tsuyumu, S. (2003) Peh production, flagellum synthesis, and virulence reduced in *Erwinia carotovora* subsp. *carotovora* by mutation in a homologue of cytR. *Mol. Plant-Microbe Interact.* **16**: 389-397.

Michaux, C., Martini, C., Hanin, A., Auffray, Y., Hartke, A., and Giard, J.-C. (2011) SlyA regulator is involved in bile salts stress response of *Enterococcus faecalis*. *FEMS Microbiol. Lett.* **324**: 142-146.

Miller, K.A., Phillips, R.S., Mrázek, J., and Hoover, T.R. (2013) *Salmonella* utilizes D-glucosaminate via a mannose family phosphotransferase system permease and associated enzymes. *J. Bacteriol.* **195**: 4057-4066.

Minard, K.I., and McAlister-Henn, L. (2005) Sources of NADPH in yeast vary with carbon source. *J. Biol. Chem.* **280**: 39890-39896.

Moleleki, L.N., Pretorius, R.G., Tanui, C.K., Mosina, G., and Theron, J. (2017) A quorum sensing-defective mutant of *Pectobacterium carotovorum* ssp. *brasiliense* 1692 is attenuated in virulence and unable to occlude xylem tissue of susceptible potato plant stems. *Mol. Plant Pathol.* **18**: 32-44.

Moreira, L.M., Almeida, N.F., Potnis, N., Digiampietri, L.A., Adi, S.S., Bortolossi, J.C., da Silva, A.C., da Silva, A.M., de Moraes, F.E., and de Oliveira, J.C. (2010) Novel insights into the genomic basis of citrus canker based on the genome sequences of two strains of *Xanthomonas fuscans* subsp. *aurantifolii. BMC Genomics* **11**: 238.

Mougous, J.D., Cuff, M.E., Raunser, S., Shen, A., Zhou, M., Gifford, C.A., Goodman, A.L., Joachimiak, G., Ordoñez, C.L., and Lory, S. (2006) A virulence locus of *Pseudomonas aeruginosa* encodes a protein secretion apparatus. *Science* **312**: 1526-1530.

Navarre, W.W., Halsey, T.A., Walthers, D., Frye, J., McClelland, M., Potter, J.L., Kenney, L.J., Gunn, J.S., Fang, F.C., and Libby, S.J. (2005) Co-regulation of *Salmonella enterica* genes required for virulence and resistance to antimicrobial peptides by SlyA and PhoP/PhoQ. *Mol. Microbiol.* **56**: 492-508.

Norte, V.A., Stapleton, M.R., and Green, J. (2003) PhoP-responsive expression of the *Salmonella enterica* serovar typhimurium slyA gene. *J. Bacteriol.* **185**: 3508-3514.

Okada, N., Oi, Y., Takeda-Shitaka, M., Kanou, K., Umeyama, H., Haneda, T., Miki, T., Hosoya, S., and Danbara, H. (2007) Identification of amino acid residues of *Salmonella* SlyA that are critical for transcriptional regulation. *Microbiology* **153**: 548-560.

Pan, Y., Liang, F., Li, R.-J., and Qian, W. (2018) MarR-Family Transcription Factor HpaR Controls Expression of the vgrR-vgrS Operon of *Xanthomonas campestris* pv. *campestris. Mol. Plant-Microbe Interact.* **31**: 299-310.

Panmanee, W., Vattanaviboon, P., Eiamphungporn, W., Whangsuk, W., Sallabhan, R., and Mongkolsuk, S. (2002) OhrR, a transcription repressor that senses and responds to changes in organic peroxide levels in *Xanthomonas campestris* pv. *phaseoli. Mol. Microbiol.* **45**: 1647-1654.

Payne, S.M., Mey, A.R., and Wyckoff, E.E. (2016) Vibrio iron transport: evolutionary adaptation to life in multiple environments. *Microbiol. Mol. Biol. Rev.* **80**: 69-90.

Pédron, J., Chapelle, E., Alunni, B., and Van Gijsegem, F. (2018) Transcriptome analysis of the *Dickeya dadantii* PecS regulon during the early stages of interaction with Arabidopsis thaliana. *Mol. Plant Pathol.* **19**: 647-663.

Perera, I.C., and Grove, A. (2010) Molecular mechanisms of ligand-mediated attenuation of DNA binding by MarR family transcriptional regulators. *J. Mol. Cell. Biol.* **2**: 243-254.

Pérez-Rueda, E., and Collado-Vides, J. (2001) Common history at the origin of the position–function correlation in transcriptional regulators in archaea and bacteria. *J. Mol. Evol.* **53**: 172-179.

Pérez-Rueda, E., Collado-Vides, J., and Segovia, L. (2004) Phylogenetic distribution of DNA-binding transcription factors in bacteria and archaea. *Comput. Biol. Chem.* **28**: 341-350.

Perez, J.C., Latifi, T., and Groisman, E.A. (2008) Overcoming H-NS-mediated transcriptional silencing of horizontally acquired genes by the PhoP and SlyA proteins in *Salmonella enterica. J. Biol. Chem.* **283**: 10773-10783.

Pletzer, D., Schweizer, G., and Weingart, H. (2014) AraC/XylS family stress response regulators rob, SoxS, PliA, and OpiA in the fire blight pathogen *Erwinia amylovora*. *J. Bacteriol.* **196**: 3098-3110.

Pukatzki, S., McAuley, S.B., and Miyata, S.T. (2009) The type VI secretion system: translocation of effectors and effector-domains. *Curr. Opin. Microbiol.* **12**: 11-17.

Ravcheev, D.A., Khoroshkin, M.S., Laikova, O.N., Tsoy, O.V., Sernova, N.V., Petrova, S.A., Rakhmaninova, A.B., Novichkov, P.S., Gelfand, M.S., and Rodionov, D.A. (2014) Comparative genomics and evolution of regulons of the LacI-family transcription factors. *Front. Microbiol.* **5**: 294.

Reverchon, S., Nasser, W., and Robert-Baudouy, J. (1994) pecS: a locus controlling pectinase, cellulase and blue pigment production in *Erwinia chrysanthemi*. *Mol. Microbiol.* **11**: 1127-1139.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**: 139-140.

Rodionova, I.A., Zhang, Z., Mehla, J., Goodacre, N., Babu, M., Emili, A., Uetz, P., and Saier, M.H. (2017) The phosphocarrier protein HPr of the bacterial phosphotransferase system globally regulates energy metabolism by directly interacting with multiple enzymes in *Escherichia coli. J. Biol. Chem.* **292**: 14250-14257.

Shyntum, D.Y., Nkomo, N., Gricia, A.R., Shigange, N.L., Bellieny-Rabelo, D., and Moleleki, L.N. (2018) The impact of type VI secretion system, bacteriocins and antibiotics on competition amongst Soft-Rot Enterobacteriaceae: Regulation of carbapenem biosynthesis by iron and the transcriptional regulator Fur. *bioRxiv*: 497016.

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., and Söding, J. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **7**.

Singh, R., Lemire, J., Mailloux, R.J., and Appanna, V.D. (2008) A novel strategy involved anti-oxidative defense: the conversion of NADH into NADPH by a metabolic network. *PLoS One* **3**: e2682.

Sjöblom, S., Harjunpää, H., Brader, G., and Palva, E.T. (2008) A novel plant ferredoxin-like protein and the regulator Hor are quorum-sensing targets in the plant pathogen *Erwinia carotovora*. *Mol. Plant-Microbe Interact.* **21**: 967-978.

Suarez-Rodriguez, M.C., Adams-Phillips, L., Liu, Y., Wang, H., Su, S.-H., Jester, P.J., Zhang, S., Bent, A.F., and Krysan, P.J. (2007) MEKK1 is required for flg22-induced MPK4 activation in Arabidopsis plants. *Plant Physiol.* **143**: 661-669.

Takle, G.W., Toth, I.K., and Brurberg, M.B. (2007) Evaluation of reference genes for real-time RT-PCR expression studies in the plant pathogen *Pectobacterium atrosepticum. BMC Plant Biol.* **7**: 50.

Tanui, C.K., Shyntum, D.Y., Priem, S.L., Theron, J., and Moleleki, L.N. (2017) Influence of the ferric uptake regulator (Fur) protein on pathogenicity in *Pectobacterium carotovorum* subsp. *brasiliense*. *PLoS One* **12**: e0177647.

Thomson, N., Cox, A., Bycroft, B., Stewart, G., Williams, P., and Salmond, G. (1997) The rap and hor proteins of *Erwinia, Serratia and Yersinia*: a novel subgroup in a growing superfamily of proteins regulating diverse physiological processes in bacterial pathogens. *Mol. Microbiol.* **26**: 531-544.

Waterhouse, A.M., Procter, J.B., Martin, D.M., Clamp, M., and Barton, G.J. (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**: 1189-1191.

Weatherspoon-Griffin, N., and Wing, H.J. (2016) Characterization of SlyA in *Shigella flexneri* identifies a novel role in virulence. *Infect. Immun.* **84**: 1073-1082.

Wilkinson, S.P., and Grove, A. (2006) Ligand-responsive transcriptional regulation by members of the MarR family of winged helix proteins. *Curr. Issues Mol. Biol.* **8**: 51.

Wojtaszek, P. (1997) Oxidative burst: an early plant response to pathogen infection. *Biochem. J.* **322**: 681-692.

Wu, R.-y., Zhang, R.-g., Zagnitko, O., Dementieva, I., Maltzev, N., Watson, J.D., Laskowski, R., Gornicki, P., and Joachimiak, A. (2003) Crystal structure of *Enterococcus faecalis* SlyA-like transcriptional factor. *J. Biol. Chem.* **278**: 20240-20244.

Wyborn, N.R., Stapleton, M.R., Norte, V.A., Roberts, R.E., Grafton, J., and Green, J. (2004) Regulation of *Escherichia coli* hemolysin E expression by H-NS and *Salmonella* SlyA. *J. Bacteriol.* **186**: 1620-1628.

Zhou, J.N., Zhang, H.B., Lv, M.F., Chen, Y.F., Liao, L.S., Cheng, Y.Y., Liu, S.Y., Chen, S.H., He, F., and Cui, Z.N. (2016) SlyA regulates phytotoxin production and virulence in *Dickeya zeae* EC1. *Mol. Plant Pathol.*

Zhu, R., Hao, Z., Lou, H., Song, Y., Zhao, J., Chen, Y., Zhu, J., and Chen, P.R. (2017) Structural characterization of the DNA-binding mechanism underlying the copper (II)-sensing MarR transcriptional regulator. *JBIC Journal of Biological Inorganic Chemistry* **22**: 685-693.

Zou, L., Zeng, Q., Lin, H., Gyaneshwar, P., Chen, G., and Yang, C.-H. (2012) SlyA regulates type III secretion system (T3SS) genes in parallel with the T3SS master regulator HrpL in *Dickeya dadantii* 3937. *Appl. Environ. Microbiol.* **78**: 2888-2895.

CHAPTER FOUR

4.1 Concluding Remarks and Future Perspectives

Pectobacterium brasiliense 1692 (*Pb*1692) is a Gram-negative opportunistic pathogen of plants; a leading cause of soft rot and blackleg infections in agriculturally important plants. The main aim of this project was to characterise two transcriptional regulators, PhoP and SlyA, through transcriptome and phenotypic analyses. The analyses in this study have expanded our knowledge on the role of *Pb*1692 PhoP and SlyA as regulators of important virulence factors contributing to the development of soft rot disease in potatoes. This is the first transcriptome study characterising the role of PhoP and SlyA specifically in *Pb*1692 *in planta*. The majority of studies on PhoP and SlyA have centered on *in vitro* analysis under specific conditions (Lamarche & Harel, 2010, Yang *et al.*, 2012, Hwan Baek & Yup Lee, 2006, Groisman, 2001, Prost *et al.*, 2007, Rebeil *et al.*, 2013, Cabezas *et al.*, 2018, Navarre *et al.*, 2005, Shyntum *et al.*, 2018). The current study has demonstrated that in the absence of PhoP and SlyA, *Pb*1692 was unable to cause full maceration on potato tubers, suggesting that both these regulators control some important PCWDEs (section 2.3.2 and 3.3.5). In addition to PhoP regulating PCWDEs, it also controls motility, carbapenem production, carbohydrate genes and T6SS, while SlyA regulates genes involved in biofilm formation, iron metabolism, carbohydrates and survival in oxidative stress response. In addition to the phenotypic assays, transcriptome analysis also revealed that both PhoP and SlyA regulate the transcription of the phosphotransferase system (PTS) genes, responsible for transporting a number of carbohydrates from the host to the pathogen (PCBA_RS01470) (Table S2.1, S2.2, S3.1 and 3.2 supplementary data).

In Chapter 2 and 3 (section 2.3.2 and 3.3.5) the pathogenicity assays revealed that PhoP and SlyA potentially regulate pectate lyase enzymes. In some pathogens, such as *D. dadantii*, pectate lyases are considered main virulence factors leading to the development of soft rot disease (Hugouvieux-Cotte-Pattat *et al.*, 2014). The current study further suggests that a number of determinants contribute to the development of soft rot symptoms

in potato tubers. Analyzing some of these contributing factors, PhoP and SlyA regulons at 12 and 24hpi were thoroughly examined. Carbohydrate genes were differentially expressed by both PhoP and SlyA at 12 and 24hpi supporting the growth and development of infection by *Pb*1692. Furthermore, PhoP activated expression of motility genes, chemotaxis genes while repressing carbapenem genes, T6SS, some transporters, ExpR and a SlyA transcriptional regulator. On the other hand SlyA controlled the expression of stress response genes, biofilm formation, metal and iron transporters (Table S2.1, S2.2, S3.1 and 3.2 supplementary data). This means that for *Pb*1692 to cause soft rot disease, multiple virulence factors are activated and repressed by PhoP and SlyA. To put this into perspective, *Pb*1692 infects potato tubers through natural openings and once inside it is confronted with several adverse conditions, such as acidic pH, oxidative stress, AMPs and iron limitation (Jiang *et al.*, 2016). Once *Pb*1692 is inside the host PhoP and SlyA are activated by different cues and *Pb*1692 begin to assimilate easily metabolized plant soluble sugars by activating carbohydrate genes. At the same time PhoP activates motility and chemotaxis genes to escape the low acidic environment in the apoplast while SlyA activates stress response genes to neutralize the effects of ROS. *Pb*1692 then moves into the nearby tissues and continue to grow until a quorum density is reached, initiating the synthesis of PCWDEs. Resulting in the break-down of the plant cell wall causing the appearance of soft rot symptoms.

Apart from PhoP and SlyA regulating PCWDEs, *Pb*1692 strains lacking *pho*P abolished motility and strains lacking *sly*A were highly sensitive to oxidative stress and formed no biofilms (section 2.3.4, 3.3.6 and 3.3.7). Suggesting that each regulator regulates a different set of genes required by *Pb*1692 to survive and adapt inside the host. Independent regulation of genes by PhoP and SlyA is further made evident by the number and different types of genes expressed by PhoP and SlyA at 12 and 24hpi, demonstrating the precision with which *Pb*1692 initiate disease development. For example, at 12hpi, when the environment is supposedly acidic, PhoP regulated over 400 genes while SlyA only affected 70 genes. These findings suggest that PhoP has a much bigger regulon required in the early

stages of infection to directly respond to the poor nutritional conditions found in the apoplast. Comparing PhoP to the SlyA regulon, it appears that in the early stages of infection, SlyA does not play a major role in the acidic environment. This is further illustrated by the RNA-seq data, at 24hpi the PhoP regulon shrinks to half of what was observed in the acidic environment (approximately 253 transcripts), while the SlyA regulon almost doubles it size (116 transcripts) (Table S2.1, S2.2, S3.1 and 3.2 supplementary data). This may be due to the fact that the major role of SlyA is the regulation of genes required for survival in oxidative stress response and not in the acidic environment. In plant pathogens, such as *D. dadantii*, low acid pH has been shown to promote the export of protons while co-inducing oxidative stress, likely through the increased production of oxygen radicals (Jiang *et al.*, 2016).

The *in planta* transcriptome analysis of PhoP and SlyA identified a number and identity of virulence genes varying between the two regulators. The two regulators, PhoP and SlyA, induce the expression of essential virulence-associated genes. PhoP regulates genes such as T6SS, carbapenem, motility genes, PELI/B/Z (pectate lyases), CELA/B (cellulases) and the EXPR regulator, while SlyA regulates biofilm formation, Hcp (a structural component of T6SS), oxidative stress response genes and T2SS in *Pb*1692. From the virulence-associated genes mentioned above, nothing appears to be common between PhoP and SlyA. However there is an overlap of genes that are regulated by both PhoP and SlyA, namely phosphotransferase system (PTS), glycolysis (PCBA_RS02065, PCBA_RS01470, PCBA_RS02060, vitamins (PCBA_RS02605), and the transcriptional regulator AraC (PCBA_RS00485) essential for the transport and phosphorylation of essential carbohydrates and vitamins (Table S2.1, S2.2, S3.1 and 3.2 supplementary data). This might be due to what is required by *Pb*1692 at a given time under a particular stress. Demonstrating precise precision with which PhoP and SlyA regulate gene expression. The output of the RNA seq data has provided additional knowledge on the PhoP and SlyA regulators in *Pectobacterium* spp.

PhoP also negatively regulated the entire T6SS cluster while SlyA positively regulates only 3 Hcps (a component of the T6SS). To substantiate the RNA-seq data, interbacterial competition assays were conducted. The SlyA competition results revealed that there was no significant difference in CFU/ml between the *sly*A mutant and *Pb*1692 wild type when coinoculated with *D. dadanti*, while *pho*P mutants outcompeted *D. dadantii* significantly better than the wild type (section 2.3.3 and 3.5.9). These observations suggest that PhoP and SlyA work together to achieve full virulence, where SlyA activates a component of T6SS (3 *hcp* gene and PhoP represses the entire T6SS cluster based on the current conditions experienced by *Pb*1692 *in planta*). Which could mean that for SlyA to completely outcompete *D. dadantii* the entire T6SS cluster should have been activated.

The virulence and adaptation of *Pb*1692 *in planta* appears to be complex, in this study several traits under the control of PhoP and SlyA such as motility, interbacterial competition, pectate lyases, polygalacturonase enzymes, stress response genes and biofilm formation were utilized to overcome stressful conditions inside potato tubers. Based on the information presented, the study could explain at least in part, how *Pb*1692 utilises PhoP and SlyA in the early stages of infection to colonize potato tubers and adapt during disease development. In *Enterobacteriaceae* species, such as *Salmonella, E. coli, S. flexneri, Serratia, Yersinia* and *Erwinia* PhoP has been studied under different environmental conditions where it regulates a variety of virulence genes, from motility to stress response (Choi *et al.*, 2009, Choi & Groisman, 2017, Zhou *et al.*, 2005). The majority of these studies were carried out under low acidic pH, low magnesium, osmolarity and presence of AMPs, conditions reported to activate PhoP (Groisman, 2001, Liu & Zheng, 2019, Macfarlane *et al.*, 1999, Tu *et al.*, 2016). As for SlyA, it has been investigated in *Salmonella, E. coli, Serratia, Yersinia* and *Erwinia*, where it was implicated in resistance to AMPs, survival within macrophages, and oxidative stress (Reverchon *et al.*, 1994, Thomson *et al.*, 1997, Weatherspoon-Griffin & Wing, 2016, Navarre *et al.*, 2005, Slater *et al.*, 2003, Will *et al.*, 2019). Therefore, for this particular study, the role of PhoP and SlyA was investigated in potato tubers because potato is *Pb*1692's natural environment. *In vitro* studies are only

mimicry, the plethora of factors that constantly change between the pathogen and host during infection cannot really be duplicated *in vitro*. It is always important to analyze plant-pathogen interactions in their native state. The results of this thesis serve to additionally institute the roles and hierarchies of PhoP and SlyA in virulence regulation, where PhoP influences the transcription of numerous virulence-associated genes in contrast to SlyA. The regulons of proteins interacting with PhoP and SlyA organization, for example, ExpR, remain to be fully characterized and hierarchically positioned in the overall systemic regulation of soft rot bacteria.

Further *in vitro* transcriptomic analysis looking at individual stresses encountered by *Pb*1692 could help develop this regulatory map. For example, exposing *pho*P and *sly*A mutants to acidic pH, osmotic stress, magnesium limitations, oxidative stress and compare the outcomes to the *in planta* analysis. The information from the individual stresses will allow us to compare genes expressed *in planta* and *in vitro,* mostly because this study have not been able to specifically pinpoint which genes were activated by which stress because the plant cell environment harbors a number of different stresses. Future work therefore needs to look at which of these genes are recruited for specific stress conditions. To answer this question, mutants of the two transcriptional regulators (Δ*sly*A and Δ*pho*P) and the wild type will have to be exposed to individual stresses, mimicking stresses that are encountered by *Pectobacterium brasiliense* when it invades potato tubers. The next step is to use bioinformatics to analyse the transcriptome data generated from individual stress responses and compare these to the *in planta* results from the current study. From this information, it's safe to hypothesize that both the *in planta* and *in vitro* data could share some critical stress response genes under SlyA and PhoP regulators. These important genes can then be paired with specific stress conditions, for example, genes responsive to low magnesium, acidic pH, oxidative stress and AMPs. Furthermore, transcriptional regulators work on the basis of DNA binding and recognition of specific ligands to either activate or repress gene expression. What would be interesting is identifying natural ligands for PhoP and SlyA. To identify specific ligands, cultures of *Pb*1692 wild type, *pho*P and *sly*A mutants must be

exposed to exogenous stresses, such as hydrogen peroxide or acidic pH to evaluate their effect on the transcription of PhoP and SlyA. Exposed *Pb*1692 wild type, *pho*P and *sly*A mutants will be further subjected to quantitative RT-qPCR to check the transcription levels of s*ly*A and *pho*P when exposed to different stresses. An increase in *sly*A gene when exposed to hydrogen peroxide for example suggest that hydrogen peroxide functions as a ligand for SlyA. Ligands serves as a signal required for the colonization of a host, like the case of urate binding MarR homologs. Most natural ligands for SlyA are unknown and identification of natural ligands presents a huge challenge for researchers because testing of individual ligands is time consuming.

4.2 References

Adams, P., Fowler, R., Kinsella, N., Howell, G., Farris, M., Coote, P., and O'Connor, C.D. (2001) Proteomic detection of PhoPQ-and acid-mediated repression of Salmonella motility. *PROTEOMICS: International Edition* **1**: 597-607.

Adeolu, M., Alnajar, S., Naushad, S., and Gupta, R.S. (2016) Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. *Int. J. Syst. Evol. Microbiol.* **66**: 5575-5599.

Alcorn, S., Orum, T., STEIGERWALT, A.G., FOSTER, J.L., FOGLEMAN, J.C., and BRENNER, D.J. (1991) Taxonomy and pathogenicity of *Erwinia cacticida* sp. nov. *Int. J. Syst. Evol. Microbiol.* **41**: 197-212.

Alekshun, M.N., and Levy, S.B. (1999) The mar regulon: multiple resistance to antibiotics and other toxic chemicals. *Trends Microbiol.* **7**: 410-413.

Alekshun, M.N., Levy, S.B., Mealy, T.R., Seaton, B.A., and Head, J.F. (2001) The crystal structure of MarR, a regulator of multiple antibiotic resistance, at 2.3 Å resolution. *Nat. Struct. Mol. Biol.* **8**: 710-714.

Alič, Š., Naglič, T., Tušek-Žnidarič, M., Peterka, M., Ravnikar, M., and Dreo, T. (2017) Putative new species of the genus Dickeya as major soft rot pathogens in Phalaenopsis orchid production. *Plant Pathol.* **66**: 1357-1368.

Alteri, C.J., Lindner, J.R., Reiss, D.J., Smith, S.N., and Mobley, H.L. (2011) The broadly conserved regulator PhoP links pathogen virulence and membrane potential in Escherichia coli. *Mol. Microbiol.* **82**: 145-163.

Andresen, L., (2012) Regulation of virulence in plant-pathogenic pectobacteria. In., pp.

Aravind, L., Anantharaman, V., Balaji, S., Babu, M.M., and Iyer, L.M. (2005) The many faces of the helix-turn-helix domain: transcription regulation and beyond. *FEMS Microbiol. Rev.* **29**: 231-262.

Arora, R., and Khurana, S.P., (2004) Major fungal and bacterial diseases of potato and their management. In: Fruit and vegetable diseases. Springer, pp. 189-231.

Axelrood, P.E., Rella, M., and Schroth, M.N. (1988) Role of antibiosis in competition of Erwinia strains in potato infection courts. *Appl. Environ. Microbiol.* **54**: 1222-1229.

Babu, M.M., and Teichmann, S.A. (2003) Functional determinants of transcription factors in Escherichia coli: protein families and binding sites. *Trends Genet.* **19**: 75-79.

Babujee, L., Venkatesh, B., Yamazaki, A., and Tsuyumu, S. (2007) Proteomic analysis of the carbonate insoluble outer membrane fraction of the soft-rot pathogen Dickeya dadantii (syn. Erwinia chrysanthemi) strain 3937. *J. Proteome Res.* **6**: 62-69.

Bachhawat, P., and Stock, A.M. (2007) Crystal structures of the receiver domain of the response regulator PhoP from Escherichia coli in the absence and presence of the phosphoryl analog beryllofluoride. *J. Bacteriol.* **189**: 5987-5995.

Bader, M.W., Sanowar, S., Daley, M.E., Schneider, A.R., Cho, U., Xu, W., Klevit, R.E., Le Moual, H., and Miller, S.I. (2005) Recognition of antimicrobial peptides by a bacterial sensor kinase. *Cell* **122**: 461-472.

Ballesteros, M.F., Lamberti, M.F.T., Farizano, J.V., Pescaretti, M.M., and Delgado, M.A. (2018) Regulatory effect of SlyA on rcsB expression in Salmonella Typhimurium. *J. Bacteriol.*

Balleza, E., Lopez-Bojorquez, L.N., Martínez-Antonio, A., Resendis-Antonio, O., Lozada-Chávez, I., Balderas-Martínez, Y.I., Encarnación, S., and Collado-Vides, J. (2008) Regulation by transcription factors in bacteria: beyond description. *FEMS microbiology reviews* **33**: 133-151.

Balogh, B., Jones, J.B., Iriarte, F., and Momol, M. (2010) Phage therapy for plant disease control. *Curr. Pharm. Biotechnol.* **11**: 48-57.

Banda, M.M., Lopez, C., Manzo, R., Rico-Perez, G., Garcia, P., Rosales-Reyes, R., De la Cruz, M.A., Soncini, F.C., Garcia-Del Portillo, F., and Bustamante, V.H. (2018) HilD and PhoP independently regulate the expression of grhD1, a novel gene required for Salmonella Typhimurium invasion of host cells. *Sci Rep* **8**: 4841.

Barnard, A.M., Bowden, S.D., Burr, T., Coulthurst, S.J., Monson, R.E., and Salmond, G.P. (2007) Quorum sensing, virulence and secondary metabolite production in plant soft-rotting bacteria. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **362**: 1165-1183.

Barras, F., van Gijsegem, F., and Chatterjee, A.K. (1994) Extracellular enzymes and pathogenesis of soft-rot Erwinia. *Annu. Rev. Phytopathol.* **32**: 201-234.

Batchelor, J.D., Doucleff, M., Lee, C.J., Matsubara, K., De Carlo, S., Heideker, J., Lamers, M.H., Pelton, J.G., and Wemmer, D.E. (2008) Structure and regulatory mechanism of Aquifex aeolicus NtrC4: variability and evolution in bacterial transcriptional regulation. *J Mol Biol* **384**: 1058-1075.

Bearson, B.L., Wilson, L., and Foster, J.W. (1998) A low pH-inducible, PhoPQ-dependent acid tolerance response protects Salmonella typhimurium against inorganic acid stress. *J. Bacteriol.* **180**: 2409-2417.

Beier, D., and Gross, R. (2006) Regulation of bacterial virulence by two-component systems. *Curr. Opin. Microbiol.* **9**: 143-152.

Bell, K., Sebaihia, M., Pritchard, L., Holden, M., Hyman, L., Holeva, M., Thomson, N., Bentley, S., Churcher, L., and Mungall, K. (2004) Genome sequence of the enterobacterial phytopathogen Erwinia carotovora subsp. atroseptica and characterization of virulence factors. *Proc. Natl. Acad. Sci. U. S. A.* **101**: 11105-11110.

Bellieny-Rabelo, D., Tanui, C.K., Miguel, N., Kwenda, S., Shyntum, D.Y., and Moleleki, L.N. (2019) Transcriptome and comparative genomics analyses reveal new functional insights on key determinants of pathogenesis and interbacterial competition in Pectobacterium and Dickeya spp. *Appl. Environ. Microbiol.* **85**: e02050-02018.

Berg, G., Krechel, A., Ditz, M., Sikora, R.A., Ulrich, A., and Hallmann, J. (2005) Endophytic and ectophytic potato-associated bacterial communities differ in

structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiol. Ecol.* **51**: 215-229.

Bhagirath, A.Y., Li, Y., Patidar, R., Yerex, K., Ma, X., Kumar, A., and Duan, K. (2019) Two component regulatory systems and antibiotic resistance in Gram-negative pathogens. *Int. J. Mol. Sci.* **20**: 1781.

Bijlsma, J.J., and Groisman, E.A. (2005) The PhoP/PhoQ system controls the intramacrophage type three secretion system of Salmonella enterica. *Mol. Microbiol.* **57**: 85-96.

Bintu, L., Buchler, N.E., Garcia, H.G., Gerland, U., Hwa, T., Kondev, J., and Phillips, R. (2005) Transcriptional regulation by the numbers: models. *Curr. Opin. Genet. Dev.* **15**: 116-124.

Birch, P.R., Bryan, G., Fenton, B., Gilroy, E.M., Hein, I., Jones, J.T., Prashar, A., Taylor, M.A., Torrance, L., and Toth, I.K. (2012) Crops that feed the world 8: Potato: are the trends of increased global production sustainable? *Food Security* **4**: 477-508.

Boccara, M., Vedel, R., Lalo, D., Lebrun, M.-H., and Lafay, J.F. (1991) Genetic diversity and host range in strains of Erwinia chrysanthemi. *Mol. Plant-Microbe Interact* **4**: 293-299.

Bolger, A.M., Lohse, M., and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**: 2114-2120.

Bonn, W.G., and van der Zwet, T. (2000) Distribution and economic importance of fire blight. *Fire blight: the disease and its causative agent, Erwinia amylovora* **37**: 53.

Bordelon, T., Wilkinson, S.P., Grove, A., and Newcomer, M.E. (2006) The crystal structure of the transcriptional regulator HucR from Deinococcus radiodurans reveals a repressor preconfigured for DNA binding. *J. Mol. Biol.* **360**: 168-177.

Brady, C.L., Cleenwerck, I., Denman, S., Venter, S.N., Rodríguez-Palenzuela, P., Coutinho, T.A., and De Vos, P. (2012) Proposal to reclassify Brenneria quercina (Hildebrand and Schroth 1967) Hauben et al. 1999 into a new genus, Lonsdalea gen. nov., as Lonsdalea quercina comb. nov., descriptions of Lonsdalea quercina subsp. quercina comb. nov., Lonsdalea quercina subsp. iberica subsp. nov. and Lonsdalea quercina subsp. britannica subsp. nov., emendation of the description of the genus Brenneria, reclassification of Dickeya dieffenbachiae as Dickeya dadantii subsp. dieffenbachiae comb. nov., and emendation of the description of Dickeya dadantii. *Int. J. Syst. Evol. Microbiol.* **62**: 1592-1602.

Branda, S.S., Vik, Å., Friedman, L., and Kolter, R. (2005) Biofilms: the matrix revisited. *Trends Microbiol.* **13**: 20-26.

Brenner, D., and Farmer, J. (2005) Order XIII.“Enterobacteriales”. *Bergey’s Manual of Systematic Bacteriology* **2**: 587-607.

Brioukhanov, A., and Netrusov, A. (2004) Catalase and superoxide dismutase: distribution, properties, and physiological role in cells of strict anaerobes. *Biochemistry (Moscow)* **69**: 949-962.

Browning, D.F., and Busby, S.J. (2004) The regulation of bacterial transcription initiation. *Nature Reviews Microbiology* **2**: 57.

Browning, D.F., Butala, M., and Busby, S.J. (2019) Bacterial Transcription Factors: Regulation by Pick ‘N’Mix. *J. Mol. Biol.*

Buchmeier, N., Bossie, S., Chen, C.-Y., Fang, F.C., Guiney, D.G., and Libby, S.J. (1997) SlyA, a transcriptional regulator of Salmonella typhimurium, is required for resistance to oxidative stress and is expressed in the intracellular environment of macrophages. *Infect. Immun.* **65**: 3725-3730.

Cabezas, C.E., Briones, A.C., Aguirre, C., Pardo-Esté, C., Castro-Severyn, J., Salinas, C.R., Baquedano, M.S., Hidalgo, A.A., Fuentes, J.A., and Morales, E.H. (2018) The transcription factor SlyA from Salmonella Typhimurium regulates genes in response to hydrogen peroxide and sodium hypochlorite. *Res. Microbiol.*

Caruso, A., Licciardello, G., La Rosa, R., Catara, V., and Bella, P. (2016) Mixed infection of Pectobacterium carotovorum subsp. carotovorum and P. carotovorum subsp. brasiliensis in tomato stem rot in Italy. *J. Plant Pathol.* **98**: 661-665.

Cathelyn, J.S., Crosby, S.D., Lathem, W.W., Goldman, W.E., and Miller, V.L. (2006) RovA, a global regulator of Yersinia pestis, specifically required for bubonic plague. *Proceedings of the National Academy of Sciences* **103**: 13514-13519.

Chalabaev, S., Chauhan, A., Novikov, A., Iyer, P., Szczesny, M., Beloin, C., Caroff, M., and Ghigo, J.-M. (2014) Biofilms formed by gram-negative bacteria undergo increased lipid a palmitoylation, enhancing in vivo survival. *MBio* **5**: e01116-01114.

Chan, J.Z., Halachev, M.R., Loman, N.J., Constantinidou, C., and Pallen, M.J. (2012) Defining bacterial species in the genomic era: insights from the genus Acinetobacter. *BMC Microbiol.* **12**: 302.

Chan, Y.-c., Wu, H.-P., and Chuang, D.-y. (2009) Extracellular secretion of Carocin S1 in Pectobacterium carotovorum subsp. carotovorum occurs via the type III secretion system integral to the bacterial flagellum. *BMC Microbiol.* **9**: 181.

Charkowski, A., Blanco, C., Condemine, G., Expert, D., Franza, T., Hayes, C., Hugouvieux-Cotte-Pattat, N., Solanilla, E.L., Low, D., and Moleleki, L. (2012) The role of secretion systems and small molecules in soft-rot enterobacteriaceae pathogenicity. *Annu. Rev. Phytopathol.* **50**: 425-449.

Charkowski, A.O. (2015) Biology and control of Pectobacterium in potato. *American Journal of Potato Research* **92**: 223-229.

Charkowski, A.O. (2018) The changing face of bacterial soft-rot diseases. *Annu. Rev. Phytopathol.* **56**: 269-288.

Chatterjee, A., Cui, Y., and Chatterjee, A.K. (2009) RsmC of Erwinia carotovora subsp. carotovora negatively controls motility, extracellular protein production, and virulence by binding FlhD and modulating transcriptional activity of the master regulator, FlhDC. *J. Bacteriol.* **191**: 4582-4593.

Cheung, J., and Hendrickson, W.A. (2010) Sensor domains of two-component regulatory systems. *Curr. Opin. Microbiol.* **13**: 116-123.

Chittoor, J.M., Leach, J.E., and White, F.F. (1997) Differential induction of a peroxidase gene family during infection of rice by Xanthomonas oryzae pv. oryzae. *Mol. Plant-Microbe Interact.* **10**: 861-871.

Choi, E., Groisman, E.A., and Shin, D. (2009) Activated by different signals, the PhoP/PhoQ two-component system differentially regulates metal uptake. *J. Bacteriol.* **191**: 7174-7181.

Choi, J., and Groisman, E.A. (2016) Acidic pH sensing in the bacterial cytoplasm is required for Salmonella virulence. *Mol. Microbiol.* **101**: 1024-1038.

Choi, J., and Groisman, E.A. (2017) Activation of master virulence regulator PhoP in acidic pH requires the Salmonella-specific protein UgtL. *Sci. Signal.* **10**: eaan6284.

Choi, O., and Kim, J. (2013) Pectobacterium carotovorum subsp. brasiliense causing soft rot on paprika in Korea. *J. Phytopathol.* **161**: 125-127.

Chun, J.-P., and Huber, D.J. (1998) Polygalacturonase-mediated solubilization and depolymerization of pectic polymers in tomato fruit cell walls: regulation by pH and ionic conditions. *Plant Physiol.* **117**: 1293-1299.

Chung, Y.S., Goeser, N.J., Cai, X., and Jansky, S. (2013) The effect of long term storage on bacterial soft rot resistance in potato. *American journal of potato research* **90**: 351-356.

Cock, P.J., and Whitworth, D.E. (2007) Evolution of prokaryotic two-component system signaling pathways: gene fusions and fissions. *Mol. Biol. Evol.* **24**: 2355-2357.

Corbett, D., Bennett, H.J., Askar, H., Green, J., and Roberts, I.S. (2007) SlyA and H-NS regulate transcription of the Escherichia coli K5 capsule gene cluster, and expression of slyA in Escherichia coli is temperature-dependent, positively autoregulated, and independent of H-NS. *J. Biol. Chem.* **282**: 33326-33335.

Coulthurst, S.J., Barnard, A.M., and Salmond, G.P. (2005) Regulation and biosynthesis of carbapenem antibiotics in bacteria. *Nature Reviews Microbiology* **3**: 295.

Coutinho, T.A., and Venter, S.N. (2009) Pantoea ananatis: an unconventional plant pathogen. *Mol. Plant Pathol.* **10**: 325-335.

Croucher, N.J., and Thomson, N.R. (2010) Studying bacterial transcriptomes using RNA-seq. *Curr. Opin. Microbiol.* **13**: 619-624.

Croxen, M.A., and Finlay, B.B. (2010) Molecular mechanisms of Escherichia coli pathogenicity. *Nature Reviews Microbiology* **8**: 26-38.

Cui, W., He, P., Munir, S., He, P., He, Y., Li, X., Yang, L., Wang, B., Wu, Y., and He, P. (2019) Biocontrol of Soft Rot of Chinese Cabbage Using an Endophytic Bacterial Strain. *Front. Microbiol.* **10**.

Curran, T.D., Abacha, F., Hibberd, S.P., Rolfe, M.D., Lacey, M.M., and Green, J. (2017) Identification of new members of the Escherichia coli K-12 MG1655 SlyA regulon. *Microbiology* **163**: 400.

Czajkowski, R. (2016) Bacteriophages of soft rot Enterobacteriaceae—a minireview. *FEMS Microbiol. Lett.* **363**.

Czajkowski, R., De Boer, W., Van Veen, J., and Van der Wolf, J. (2012) Studies on the interaction between the biocontrol agent, Serratia plymuthica A30, and blackleg-causing Dickeya sp.(biovar 3) in potato (Solanum tuberosum). *Plant Pathol.* **61**: 677-688.

Czajkowski, R., Pérombelon, M., Jafra, S., Lojkowska, E., Potrykus, M., Van Der Wolf, J., and Sledz, W. (2015) Detection, identification and differentiation of Pectobacterium and Dickeya species causing potato blackleg and tuber soft rot: a review. *Ann. Appl. Biol.* **166**: 18-38.

Czajkowski, R., Perombelon, M.C., van Veen, J.A., and van der Wolf, J.M. (2011) Control of blackleg and tuber soft rot of potato caused by Pectobacterium and Dickeya species: a review. *Plant Pathol.* **60**: 999-1013.

Dale, C., and Maudlin, I. (1999) Sodalis gen. nov. and Sodalis glossinidius sp. nov., a microaerophilic secondary endosymbiont of the tsetse fly Glossina morsitans morsitans. *Int. J. Syst. Evol. Microbiol.* **49**: 267-275.

Dalebroux, Z.D., and Miller, S.I. (2014) Salmonellae PhoPQ regulation of the outer membrane to resist innate immunity. *Curr. Opin. Microbiol.* **17**: 106-113.

Datsenko, K.A., and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proceedings of the National Academy of Sciences* **97**: 6640-6645.

Davey, M.E., and O'toole, G.A. (2000) Microbial biofilms: from ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.* **64**: 847-867.

Davidsson, P.R., Kariola, T., Niemi, O., and Palva, E.T. (2013) Pathogenicity of and plant immunity to soft rot pectobacteria. *Front Plant Sci* **4**: 191.

Davis, J.R., Brown, B.L., Page, R., and Sello, J.K. (2013) Study of PcaV from Streptomyces coelicolor yields new insights into ligand-responsive MarR family transcription factors. *Nucleic Acids Res.* **41**: 3888-3900.

De Boer, S., Li, X., and Ward, L. (2012) Pectobacterium spp. associated with bacterial stem rot syndrome of potato in Canada. *Phytopathology* **102**: 937-947.

de Werra, P., Bussereau, F., Keiser, A., and Ziegler, D. (2015) First report of potato blackleg caused by Pectobacterium carotovorum subsp. brasiliense in Switzerland. *Plant Dis.* **99**: 10.1094.

Dees, M.W., Lysøe, E., Rossmann, S., Perminow, J., and Brurberg, M.B. (2017) Pectobacterium polaris sp. nov., isolated from potato (Solanum tuberosum). *Int. J. Syst. Evol. Microbiol.* **67**: 5222-5229.

Del Río, L.A., Corpas, F.J., Sandalio, L.M., Palma, J.M., Gómez, M., and Barroso, J.B. (2002) Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. *J. Exp. Bot.* **53**: 1255-1272.

Deochand, D.K., and Grove, A. (2017) MarR family transcription factors: dynamic variations on a common scaffold. *Crit. Rev. Biochem. Mol. Biol.* **52**: 595-613.

Di Fiore, A., Fiorentino, G., Vitale, R.M., Ronca, R., Amodeo, P., Pedone, C., Bartolucci, S., and De Simone, G. (2009) Structural analysis of BldR from Sulfolobus solfataricus provides insights into the molecular basis of transcriptional activation in Archaea by MarR family proteins. *J. Mol. Biol.* **388**: 559-569.

Dolan, K.T., Duguid, E.M., and He, C. (2011) Crystal structures of SlyA protein, a master virulence regulator of Salmonella, in free and DNA-bound states. *J. Biol. Chem.* **286**: 22178-22185.

Du, D., van Veen, H.W., Murakami, S., Pos, K.M., and Luisi, B.F. (2015) Structure, mechanism and cooperation of bacterial multidrug transporters. *Curr. Opin. Struct. Biol.* **33**: 76-91.

Duarte, V., De Boer, S., Ward, L., and Oliveira, A. (2004) Characterization of atypical Erwinia carotovora strains causing blackleg of potato in Brazil. *J. Appl. Microbiol.* **96**: 535-545.

Dubey, A.K., Yadav, S., Kumar, M., Anand, G., and Yadav, D. (2016) Molecular biology of microbial pectate lyase: a review. *Biotechnology Journal International*: 1-26.
Ellison, D.W., Lawrenz, M.B., and Miller, V.L. (2004) Invasin and beyond: regulation of Yersinia virulence by RovA. *Trends Microbiol.* **12**: 296-300.
Ellison, D.W., and Miller, V.L. (2006) Regulation of virulence by members of the MarR/SlyA family. *Curr. Opin. Microbiol.* **9**: 153-159.
Erhardt, M., and Dersch, P. (2015) Regulatory principles governing Salmonella and Yersinia virulence. *Front. Microbiol.* **6**: 949.
Erickson, D.L., Russell, C.W., Johnson, K.L., Hileman, T., and Stewart, R.M. (2011) PhoP and OxyR transcriptional regulators contribute to Yersinia pestis virulence and survival within Galleria mellonella. *Microb. Pathog.* **51**: 389-395.
Fang, F.C., Frawley, E.R., Tapscott, T., and Vázquez-Torres, A. (2016) Bacterial stress responses during host infection. *Cell host & microbe* **20**: 133-143.
Faye, P., Bertrand, C., Pédron, J., and Barny, M.-A. (2018) Draft genomes of "Pectobacterium peruviense" strains isolated from fresh water in France. *Standards in Genomic Sciences* **13**: 27.
Ferrándiz, M.-J., Bishop, K., Williams, P., and Withers, H. (2005) HosA, a member of the SlyA family, regulates motility in enteropathogenic Escherichia coli. *Infect. Immun.* **73**: 1684-1694.
Fields, P., Groisman, E., and Heffron, F. (1989) Salmonella locus that controls resistance to antibacterial proteins from phagocytic cells. *Science* **85**: 5189-5193.
Finn, R.D., Clements, J., Arndt, W., Miller, B.L., Wheeler, T.J., Schreiber, F., Bateman, A., and Eddy, S.R. (2015) HMMER web server: 2015 update. *Nucleic Acids Res* **43**: W30-38.
Finn, R.D., Mistry, J., Tate, J., Coggill, P., Heger, A., Pollington, J.E., Gavin, O.L., Gunasekaran, P., Ceric, G., Forslund, K., Holm, L., Sonnhammer, E.L., Eddy, S.R., and Bateman, A. (2010) The Pfam protein families database. *Nucleic Acids Res* **38**: D211-222.
Flego, D., Marits, R., Eriksson, A.R., Kõiv, V., Karlsson, M.-B., Heikinheimo, R., and Palva, E.T. (2000) A two-component regulatory system, pehR-pehS, controls endopolygalacturonase production and virulence in the plant pathogen Erwinia carotovora subsp. carotovora. *Mol. Plant-Microbe Interact.* **13**: 447-455.
Fones, H., and Preston, G.M. (2012) Reactive oxygen and oxidative stress tolerance in plant pathogenic Pseudomonas. *FEMS Microbiol. Lett.* **327**: 1-8.
Foster, J.W. (1999) When protons attack: microbial strategies of acid adaptation. *Curr. Opin. Microbiol.* **2**: 170-174.
Foster, J.W., and Spector, M.P. (1995) How Salmonella survive against the odds. *Annu. Rev. Microbiol.* **49**: 145-174.
Francino, M.P., Santos, S.R., and Ochman, H. (1992) Phylogenetic relationships of bacteria with special reference to endosymbionts and enteric species.
Galán, B., Kolb, A., Sanz, J.M., García, J.L., and Prieto, M.A. (2003) Molecular determinants of the hpa regulatory system of Escherichia coli: the HpaR repressor. *Nucleic Acids Res.* **31**: 6598-6609.

Gallegos, M.-T., Schleif, R., Bairoch, A., Hofmann, K., and Ramos, J.L. (1997) Arac/XylS family of transcriptional regulators. *Microbiol. Mol. Biol. Rev.* **61**: 393-410.

Galperin, M.Y. (2010) Diversity of structure and function of response regulator output domains. *Curr. Opin. Microbiol.* **13**: 150-159.

Gama-Castro, S., Jiménez-Jacinto, V., Peralta-Gil, M., Santos-Zavaleta, A., Peñaloza-Spinola, M.I., Contreras-Moreira, B., Segura-Salazar, J., Muniz-Rascado, L., Martinez-Flores, I., and Salgado, H. (2008) RegulonDB (version 6.0): gene regulation model of Escherichia coli K-12 beyond transcription, active (experimental) annotated promoters and Textpresso navigation. *Nucleic Acids Res.* **36**: D120-D124.

Gambino, M., and Cappitelli, F. (2016) Mini-review: Biofilm responses to oxidative stress. *Biofouling* **32**: 167-178.

Gao, R., Mack, T.R., and Stock, A.M. (2007) Bacterial response regulators: versatile regulatory strategies from common domains. *Trends Biochem. Sci.* **32**: 225-234.

Gao, R., and Stock, A.M. (2009) Biological insights from structures of two-component proteins. *Annu. Rev. Microbiol.* **63**: 133-154.

Gao, Y., Yurkovich, J.T., Seo, S.W., Kabimoldayev, I., Dräger, A., Chen, K., Sastry, A.V., Fang, X., Mih, N., and Yang, L. (2018) Systematic discovery of uncharacterized transcription factors in Escherichia coli K-12 MG1655. *Nucleic Acids Res.* **46**: 10682-10696.

Garcia-Brugger, A., Lamotte, O., Vandelle, E., Bourque, S., Lecourieux, D., Poinssot, B., Wendehenne, D., and Pugin, A. (2006) Early signaling events induced by elicitors of plant defenses. *Mol. Plant-Microbe Interact.* **19**: 711-724.

Gardan, L., Gouy, C., Christen, R., and Samson, R. (2003) Elevation of three subspecies of Pectobacterium carotovorum to species level: Pectobacterium atrosepticum sp. nov., Pectobacterium betavasculorum sp. nov. and Pectobacterium wasabiae sp. nov. *Int. J. Syst. Evol. Microbiol.* **53**: 381-391.

George, A., and Levy, S. (1983) Gene in the major cotransduction gap of the Escherichia coli K-12 linkage map required for the expression of chromosomal resistance to tetracycline and other antibiotics. *J. Bacteriol.* **155**: 541-548.

Gill, H.K., and Garg, H., (2014) Pesticides: environmental impacts and management strategies. In: Pesticides-toxic aspects. IntechOpen, pp.

Glasner, J., Marquez-Villavicencio, M., Kim, H.-S., Jahn, C., Ma, B., Biehl, B., Rissman, A., Mole, B., Yi, X., and Yang, C.-H. (2008) Niche-specificity and the variable fraction of the Pectobacterium pan-genome. *Mol. Plant-Microbe Interact.* **21**: 1549-1560.

Gloux, K., Touze, T., Pagot, Y., Jouan, B., and Blanco, C. (2005) Mutations of ousA alter the virulence of Erwinia chrysanthemi. *Mol. Plant-Microbe Interact.* **18**: 150-157.

Golubeva, Y.A., and Slauch, J.M. (2006) Salmonella enterica serovar Typhimurium periplasmic superoxide dismutase SodCI is a member of the PhoPQ regulon and is induced in macrophages. *J. Bacteriol.* **188**: 7853-7861.

Gorshkov, V., Gubaev, R., Petrova, O., Daminova, A., Gogoleva, N., Ageeva, M., Parfirova, O., Prokchorchik, M., Nikolaichik, Y., and Gogolev, Y. (2018) Transcriptome profiling helps to identify potential and true molecular switches of

stealth to brute force behavior in Pectobacterium atrosepticum during systemic colonization of tobacco plants. *Eur. J. Plant Pathol.* **152**: 957-976.

Grebe, T.W., and Stock, J.B., (1999) The histidine protein kinase superfamily. In: Adv. Microb. Physiol.: Elsevier, pp. 139-227.

Green, E.R., and Mecsas, J. (2016) Bacterial secretion systems–an overview. *Microbiology spectrum* **4**.

Grignon, C., and Sentenac, H. (1991) pH and ionic conditions in the apoplast. *Annu. Rev. Plant Biol.* **42**: 103-128.

Groisman, E., Heffron, F., and Solomon, F. (1992) Molecular genetic analysis of the Escherichia coli phoP locus. *J. Bacteriol.* **174**: 486-491.

Groisman, E.A. (2001) The pleiotropic two-component regulatory system PhoP-PhoQ. *J. Bacteriol.* **183**: 1835-1842.

Groisman, E.A. (2016) Feedback control of two-component regulatory systems. *Annu. Rev. Microbiol.* **70**: 103-124.

Groisman, E.A., Chiao, E., Lipps, C.J., and Heffron, F. (1989) Salmonella typhimurium phoP virulence gene is a transcriptional regulator. *Proceedings of the National Academy of Sciences* **86**: 7077-7081.

Grubor, B., Meyerholz, D.K., and Ackermann, M.R. (2006) Collectins and cationic antimicrobial peptides of the respiratory epithelia. *Vet. Pathol.* **43**: 595-612.

Guina, T., Eugene, C.Y., Wang, H., Hackett, M., and Miller, S.I. (2000) A PhoP-regulated outer membrane protease of Salmonella enterica serovar Typhimurium promotes resistance to alpha-helical antimicrobial peptides. *J. Bacteriol.* **182**: 4077-4086.

Gunn, J.S. (2008) The Salmonella PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. *Trends Microbiol.* **16**: 284-290.

Haider, F., Lithgow, J.K., Stapleton, M.R., Norte, V.A., Roberts, R.E., and Green, J. (2008) DNA recognition by the Salmonella enterica serovar Typhimurium transcription factor SlyA. *Int. Microbiol.* **11**: 245.

Halliwell, B. (2006) Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol.* **141**: 312-322.

Han, Y., Gao, S., Muegge, K., Zhang, W., and Zhou, B. (2015) Advanced applications of RNA sequencing and challenges. *Bioinform. Biol. Insights* **9**: BBI. S28991.

Hancock, R.E., and Diamond, G. (2000) The role of cationic antimicrobial peptides in innate host defences. *Trends Microbiol.* **8**: 402-410.

Hancock, R.E., Falla, T., and Brown, M., (1995) Cationic bactericidal peptides. In: Adv. Microb. Physiol.: Elsevier, pp. 135-175.

Haque, M.M., Hirata, H., and Tsuyumu, S. (2015) SlyA regulates motA and motB, virulence and stress-related genes under conditions induced by the PhoP-PhoQ system in Dickeya dadantii 3937. *Res. Microbiol.* **166**: 467-475.

Haque, M.M., Kabir, M.S., Aini, L.Q., Hirata, H., and Tsuyumu, S. (2009) SlyA, a MarR family transcriptional regulator, is essential for virulence in Dickeya dadantii 3937. *J. Bacteriol.* **191**: 5409-5418.

Haque, M.M., Nahar, K., Rahim, M.A., Gomes, I., and Tsuyumu, S. (2008) PhoP-PhoQ two-component system required for colonization leading to virulence of Dickeya dadantii 3937 in planta. *Banglad. J. Microbiol.* **25**: 36-40.

Haque, M.M., and Tsuyumu, S. (2005) Virulence, resistance to magainin II, and expression of pectate lyase are controlled by the PhoP-PhoQ two-component regulatory system responding to pH and magnesium in Erwinia chrysanthemi 3937. *Journal of General Plant Pathology* **71**: 47-53.

Harari, O., Park, S.-Y., Huang, H., Groisman, E.A., and Zwir, I. (2010) Defining the plasticity of transcription factor binding sites by deconstructing DNA consensus sequences: the PhoP-binding sites among gamma/enterobacteria. *PLoS Comp. Biol.* **6**.

Hardigan, M.A., Laimbeer, F.P.E., Newton, L., Crisovan, E., Hamilton, J.P., Vaillancourt, B., Wiegert-Rininger, K., Wood, J.C., Douches, D.S., and Farré, E.M. (2017) Genome diversity of tuber-bearing Solanum uncovers complex evolutionary history and targets of domestication in the cultivated potato. *Proceedings of the National Academy of Sciences* **114**: E9999-E10008.

Hauben, L., Moore, E.R., Vauterin, L., Steenackers, M., Mergaert, J., Verdonck, L., and Swings, J. (1998) Phylogenetic position of phytopathogens within the Enterobacteriaceae. *Syst. Appl. Microbiol.* **21**: 384-397.

Hébrard, M., Viala, J.P., Méresse, S., Barras, F., and Aussel, L. (2009) Redundant hydrogen peroxide scavengers contribute to Salmonella virulence and oxidative stress resistance. *J. Bacteriol.* **191**: 4605-4614.

Helmann, J.D. (2002) The extracytoplasmic function (ECF) sigma factors. *Adv. Microb. Physiol.* **46**: 48-111.

Hennigar, S.R., and McClung, J.P. (2016) Nutritional immunity: starving pathogens of trace minerals. *Am. J. Lifestyle Med.* **10**: 170-173.

Hernandez-Guerrero, R., Galán-Vásquez, E., and Pérez-Rueda, E. (2019) The protein architecture in Bacteria and Archaea identifies a set of promiscuous and ancient domains. *PLoS One* **14**.

Heroven, A.K., Nagel, G., Tran, H.J., Parr, S., and Dersch, P. (2004) RovA is autoregulated and antagonizes H-NS-mediated silencing of invasin and rovA expression in Yersinia pseudotuberculosis. *Mol. Microbiol.* **53**: 871-888.

Herrera, C.M., Hankins, J.V., and Trent, M.S. (2010) Activation of PmrA inhibits LpxT-dependent phosphorylation of lipid A promoting resistance to antimicrobial peptides. *Mol. Microbiol.* **76**: 1444-1460.

Hirsch, C.N., Hirsch, C.D., Felcher, K., Coombs, J., Zarka, D., Van Deynze, A., De Jong, W., Veilleux, R.E., Jansky, S., and Bethke, P. (2013) Retrospective view of North American potato (Solanum tuberosum L.) breeding in the 20th and 21st centuries. *G3: Genes, Genomes, Genetics* **3**: 1003-1013.

Hitchen, P.G., Prior, J.L., Oyston, P.C., Panico, M., Wren, B.W., Titball, R.W., Morris, H.R., and Dell, A. (2002) Structural characterization of lipo-oligosaccharide (LOS) from Yersinia pestis: regulation of LOS structure by the PhoPQ system. *Mol. Microbiol.* **44**: 1637-1650.

Hommais, F., Oger-Desfeux, C., Van Gijsegem, F., Castang, S., Ligori, S., Expert, D., Nasser, W., and Reverchon, S. (2008) PecS is a global regulator of the symptomatic phase in the phytopathogenic bacterium Erwinia chrysanthemi 3937. *J. Bacteriol.* **190**: 7508-7522.

Hossain, M.M., and Tsuyumu, S. (2006) Flagella-mediated motility is required for biofilm formation by Erwinia carotovora subsp. carotovora. *Journal of General Plant Pathology* **72**: 34-39.

Hu, M., Li, J., Chen, R., Li, W., Feng, L., Shi, L., Xue, Y., Feng, X., Zhang, L., and Zhou, J. (2018) Dickeya zeae strains isolated from rice, banana and clivia rot plants show great virulence differentials. *BMC Microbiol.* **18**: 136.

Huerta-Cepas, J., Forslund, K., Coelho, L.P., Szklarczyk, D., Jensen, L.J., von Mering, C., and Bork, P. (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Molecular biology and evolution* **34**: 2115-2122.

Hugouvieux-Cotte-Pattat, N., Condemine, G., and Shevchik, V.E. (2014) Bacterial pectate lyases, structural and functional diversity. *Environ. Microbiol. Rep.* **6**: 427-440.

Hwan Baek, J., and Yup Lee, S. (2006) Novel gene members in the Pho regulon of Escherichia coli. *FEMS Microbiol. Lett.* **264**: 104-109.

Iqbal, A., Khan, R.S., Shehryar, K., Imran, A., Ali, F., Attia, S., Shah, S., and Mii, M. (2019) Antimicrobial peptides as effective tools for enhanced disease resistance in plants. *Plant Cell, Tissue and Organ Culture (PCTOC)*: 1-15.

Jha, G., Rajeshwari, R., and Sonti, R.V. (2005) Bacterial type two secretion system secreted proteins: double-edged swords for plant pathogens. *Mol. Plant-Microbe Interact.* **18**: 891-898.

Jiang, X., Zghidi-Abouzid, O., Oger-Desfeux, C., Hommais, F., Greliche, N., Muskhelishvili, G., Nasser, W., and Reverchon, S. (2016) Global transcriptional response of Dickeya dadantii to environmental stimuli relevant to the plant infection. *Environ. Microbiol.* **18**: 3651-3672.

Kanehisa, M., and Goto, S. (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research* **28**: 27-30.

Kasahara, M., Nakata, A., and Shinagawa, H. (1992) Molecular analysis of the Escherichia coli phoP-phoQ operon. *J. Bacteriol.* **174**: 492-498.

Kato, A., and Groisman, E.A. (2004) Connecting two-component regulatory systems by a protein that protects a response regulator from dephosphorylation by its cognate sensor. *Genes Dev.* **18**: 2302-2313.

Kenney, L.J. (2002) Structure/function relationships in OmpR and other winged-helix transcription factors. *Curr. Opin. Microbiol.* **5**: 135-141.

Khayi, S., Cigna, J., Chong, T.M., Quêtu-Laurent, A., Chan, K.-G., Hélias, V., and Faure, D. (2016) Transfer of the potato plant isolates of Pectobacterium wasabiae to Pectobacterium parmentieri sp. nov. *Int. J. Syst. Evol. Microbiol.* **66**: 5379-5383.

Kier, L., Weppelman, R., and Ames, B. (1979) Regulation of nonspecific acid phosphatase in Salmonella: phoN and phoP genes. *J. Bacteriol.* **138**: 155-161.

Kim, H.-S., Ma, B., Perna, N.T., and Charkowski, A.O. (2009) Phylogeny and virulence of naturally occurring type III secretion system-deficient Pectobacterium strains. *Appl. Environ. Microbiol.* **75**: 4539-4549.

Kim, Y., Joachimiak, G., Bigelow, L., Babnigg, G., and Joachimiak, A. (2016) How aromatic compounds block DNA binding of HcaR catabolite regulator. *J. Biol. Chem.* **291**: 13243-13256.

Kim, Y., Miller, C., and Anderson, A. (1999) Transcriptional regulation by iron and role during plant pathogenesis of genes encoding iron-and manganese-superoxide dismutases of Pseudomonas syringae pv. syringae B728a. *Physiol. Mol. Plant Pathol.* **55**: 327-339.

Kondo, H., Nakagawa, A., Nishihira, J., Nishimura, Y., Mizuno, T., and Tanaka, I. (1997) Escherichia coli positive regulator OmpR has a large loop structure at the putative RNA polymerase interaction site. *Nat. Struct. Biol.* **4**: 28-31.

Koprivnjak, T., and Peschel, A. (2011) Bacterial resistance mechanisms against host defense peptides. *Cell. Mol. Life Sci.* **68**: 2243-2254.

Kostakioti, M., Hadjifrangiskou, M., and Hultgren, S.J. (2013) Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb. Perspect. Med.* **3**: a010306.

Kravchenko, U., Gogoleva, N., Kolubako, A., Kruk, A., Diubo, J., Gogolev, Y., and Nikolaichik, Y. (2020) The PhoPQ two-component system is the major regulator of cell surface properties, stress responses and plant-derived substrate utilisation during development of Pectobacterium versatile-host plant pathosystem. *bioRxiv*.

Krell, T. (2018) Exploring the (almost) unknown: archaeal two-component systems. *J. Bacteriol.* **200**: e00774-00717.

Kubheka, G.C., Coutinho, T.A., Moleleki, N., and Moleleki, L.N. (2013) Colonization patterns of an mCherry-tagged Pectobacterium carotovorum subsp. brasiliense strain in potato plants. *Phytopathology* **103**: 1268-1279.

Kumar Verma, R., Samal, B., and Chatterjee, S. (2018) Xanthomonas oryzae pv. oryzae chemotaxis components and chemoreceptor Mcp2 are involved in the sensing of constituents of xylem sap and contribute to the regulation of virulence-associated functions and entry into rice. *Mol. Plant Pathol.* **19**: 2397-2415.

Kumaraswami, M., Schuman, J.T., Seo, S.M., Kaatz, G.W., and Brennan, R.G. (2009) Structural and biochemical characterization of MepR, a multidrug binding transcription regulator of the Staphylococcus aureus multidrug efflux pump MepA. *Nucleic Acids Res.* **37**: 1211-1224.

Kummerfeld, S.K., and Teichmann, S.A. (2006) DBD: a transcription factor prediction database. *Nucleic Acids Res.* **34**: D74-D81.

Lamarche, M.G., and Harel, J. (2010) Membrane homeostasis requires intact pst in extraintestinal pathogenic Escherichia coli. *Curr. Microbiol.* **60**: 356-359.

Le Breton, Y., Boël, G., Benachour, A., Prévost, H., Auffray, Y., and Rincé, A. (2003) Molecular characterization of Enterococcus faecalis two-component signal transduction pathways related to environmental stresses. *Environ. Microbiol.* **5**: 329-337.

Lee, D.H., Lim, J.-A., Lee, J., Roh, E., Jung, K., Choi, M., Oh, C., Ryu, S., Yun, J., and Heu, S. (2013) Characterization of genes required for the pathogenicity of Pectobacterium carotovorum subsp. carotovorum Pcc21 in Chinese cabbage. *Microbiology* **159**: 1487-1496.

Lee, D.J., Minchin, S.D., and Busby, S.J. (2012) Activating transcription in bacteria. *Annu. Rev. Microbiol.* **66**: 125-152.

Lee, S.-W., Jeong, K.-S., Han, S.-W., Lee, S.-E., Phee, B.-K., Hahn, T.-R., and Ronald, P. (2008) The Xanthomonas oryzae pv. oryzae PhoPQ two-component system is required for AvrXA21 activity, hrpG expression, and virulence. *J. Bacteriol.* **190**: 2183-2197.

Lehmann, S., Serrano, M., L'Haridon, F., Tjamos, S.E., and Metraux, J.-P. (2015) Reactive oxygen species and plant resistance to fungal pathogens. *Phytochemistry* **112**: 54-62.

Leite, L.N., De Haan, E., Krijger, M., Kastelein, P., Van Der Zouwen, P., Van Den Bovenkamp, G., Tebaldi, N., and Van Der Wolf, J. (2014) First report of potato blackleg caused by Pectobacterium carotovorum subsp. brasiliensis in the Netherlands. *New Dis. Rep* **29**: 2044-0588.2014.

Lemmens, L., Maklad, H.R., Bervoets, I., and Peeters, E. (2019) Transcription regulators in Archaea: homologies and differences with bacterial regulators. *J. Mol. Biol.*

Li, X., Ma, Y., Liang, S., Tian, Y., Yin, S., Xie, S., and Xie, H. (2018) Comparative genomics of 84 Pectobacterium genomes reveals the variations related to a pathogenic lifestyle. *BMC Genomics* **19**: 889.

Liao, Y., Smyth, G.K., and Shi, W. (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**: 923-930.

Libby, S.J., Goebel, W., Ludwig, A., Buchmeier, N., Bowe, F., Fang, F.C., Guiney, D.G., Songer, J.G., and Heffron, F. (1994) A cytolysin encoded by Salmonella is required for survival within macrophages. *Proceedings of the National Academy of Sciences* **91**: 489-493.

Lim, D., Poole, K., and Strynadka, N.C. (2002) Crystal structure of the MexR repressor of themexRAB-oprM multidrug efflux operon of Pseudomonas aeruginosa. *J. Biol. Chem.* **277**: 29253-29259.

Lin, Z., Cai, X., Chen, M., Ye, L., Wu, Y., Wang, X., Lv, Z., Shang, Y., and Qu, D. (2017) Virulence and Stress Responses of Shigella flexneri Regulated by PhoP/PhoQ. *Front Microbiol* **8**: 2689.

Lin, Z., Cai, X., Chen, M., Ye, L., Wu, Y., Wang, X., Lv, Z., Shang, Y., and Qu, D. (2018) Virulence and stress responses of Shigella flexneri regulated by PhoP/PhoQ. *Front. Microbiol.* **8**: 2689.

Lippa, A.M., and Goulian, M. (2009) Feedback inhibition in the PhoQ/PhoP signaling system by a membrane peptide. *PLoS Genet.* **5**.

Lithgow, J.K., Haider, F., Roberts, I.S., and Green, J. (2007) Alternate SlyA and H-NS nucleoprotein complexes control hlyE expression in Escherichia coli K-12. *Mol. Microbiol.* **66**: 685-698.

Liu, H., Coulthurst, S.J., Pritchard, L., Hedley, P.E., Ravensdale, M., Humphris, S., Burr, T., Takle, G., Brurberg, M.-B., and Birch, P.R. (2008) Quorum sensing coordinates brute force and stealth modes of infection in the plant pathogen Pectobacterium atrosepticum. *PLoS Path.* **4**: e1000093.

Liu, L., Fang, H., Yang, H., Zhang, Y., Han, Y., Zhou, D., and Yang, R. (2016) Reciprocal regulation of Yersinia pestis biofilm formation and virulence by RovM and RovA. *Open biology* **6**: 150198.

Liu, L., and Zheng, S. (2019) Transcriptional regulation of Yersinia pestis biofilm formation. *Microb. Pathog.*

Liu, M., Wu, F., Wang, S., Lu, Y., Chen, X., Wang, Y., Gu, A., Zhao, J., and Shen, S. (2019) Comparative transcriptome analysis reveals defense responses against soft rot in Chinese cabbage. *Horticulture research* **6**: 1-18.

Livak, K.J., and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods* **25**: 402-408.

Livermore, D.M. (2012) Current epidemiology and growing resistance of gram-negative pathogens. *The Korean journal of internal medicine* **27**: 128.

Llama-Palacios, A., Lopez-Solanilla, E., and Rodriguez-Palenzuela, P. (2005a) Role of the PhoP-PhoQ system in the virulence of Erwinia chrysanthemi strain 3937: involvement in sensitivity to plant antimicrobial peptides, survival at acid Hh, and regulation of pectolytic enzymes. *J Bacteriol* **187**: 2157-2162.

Llama-Palacios, A., López-Solanilla, E., and Rodríguez-Palenzuela, P. (2005b) Role of the PhoP-PhoQ system in the virulence of Erwinia chrysanthemi strain 3937: involvement in sensitivity to plant antimicrobial peptides, survival at acid pH, and regulation of pectolytic enzymes. *J. Bacteriol.* **187**: 2157-2162.

Llama-Palacios, A., López-Solanilla, E., Poza-Carrión, C., García-Olmedo, F., and Rodríguez-Palenzuela, P. (2003) The Erwinia chrysanthemi phoP-phoQ operon plays an important role in growth at low pH, virulence and bacterial survival in plant tissue. *Mol. Microbiol.* **49**: 347-357.

López-Solanilla, E., García-Olmedo, F., and Rodríguez-Palenzuela, P. (1998) Inactivation of the sapA to sapF locus of Erwinia chrysanthemi reveals common features in plant and animal bacterial pathogenesis. *The Plant Cell* **10**: 917-924.

Ludwig, A., Tengel, C., Bauer, S., Bubert, A., Benz, R., Mollenkopf, H.-J., and Goebel, W. (1995) SlyA, a regulatory protein from Salmonella typhimurium, induces a haemolytic and pore-forming protein in Escherichia coli. *Molecular and General Genetics MGG* **249**: 474-486.

Ma, B., Hibbing, M.E., Kim, H.-S., Reedy, R.M., Yedidia, I., Breuer, J., Breuer, J., Glasner, J.D., Perna, N.T., and Kelman, A. (2007) Host range and molecular phylogenies of the soft rot enterobacterial genera Pectobacterium and Dickeya. *Phytopathology* **97**: 1150-1163.

Macfarlane, E.L., Kwasnicka, A., Ochs, M.M., and Hancock, R.E. (1999) PhoP–PhoQ homologues in Pseudomonas aeruginosa regulate expression of the outer-membrane protein OprH and polymyxin B resistance. *Mol. Microbiol.* **34**: 305-316.

Mäe, A., Montesano, M., Koiv, V., and Palva, E.T. (2001) Transgenic plants producing the bacterial pheromone N-acyl-homoserine lactone exhibit enhanced resistance to the bacterial phytopathogen Erwinia carotovora. *Mol. Plant-Microbe Interact.* **14**: 1035-1042.

Malamud, F., Homem, R.A., Conforte, V.P., Yaryura, P.M., Castagnaro, A.P., Marano, M.R., Do Amaral, A.M., and Vojnov, A.A. (2013) Identification and characterization of biofilm formation-defective mutants of Xanthomonas citri subsp. citri. *Microbiology* **159**: 1911-1919.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., and Machado, M.A. (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.* **13**: 614-629.

Marquez-Villavicencio, M.d.P., Groves, R.L., and Charkowski, A.O. (2011) Soft rot disease severity is affected by potato physiology and Pectobacterium taxa. *Plant Dis.* **95**: 232-241.

Martínez-Antonio, A., Janga, S.C., Salgado, H., and Collado-Vides, J. (2006) Internal-sensing machinery directs the activity of the regulatory network in Escherichia coli. *Trends Microbiol.* **14**: 22-27.

Martínez-Hackert, E., and Stock, A.M. (1997) Structural relationships in the OmpR family of winged-helix transcription factors. *J. Mol. Biol.* **269**: 301-312.

Mashavha, M.L., (2013) Characterisation of Pectobacterium carotovorum subsp. brasiliense isolates causing blackleg and soft rot diseases of potato in South Africa. In.: University of Pretoria, pp.

Matsumoto, H., Muroi, H., Umehara, M., Yoshitake, Y., and Tsuyumu, S. (2003) Peh production, flagellum synthesis, and virulence reduced in Erwinia carotovora subsp. carotovora by mutation in a homologue of cytR. *Mol. Plant-Microbe Interact.* **16**: 389-397.

McGowan, S., Sebaihia, M., Porter, L., Stewart, G., Williams, P., Bycroft, B., and Salmond, G. (1996) Analysis of bacterial carbapenem antibiotic production genes reveals a novel β-lactam biosynthesis pathway. *Mol. Microbiol.* **22**: 415-426.

Mejía-Sánchez, D., Aranda-Ocampo, S., Nava-Díaz, C., Teliz-Ortiz, D., Livera-Muñoz, M., De La Torre-Almaráz, R., and Ramírez-Alarcón, S. (2019) Pectobacterium carotovorum subsp. brasiliense Causes Soft Rot and Death of Neobuxbaumia tetetzo in Zapotitlan Salinas Valley, Puebla, Mexico. *Plant Dis.* **103**: 398-403.

Meng, X., Chai, A., Shi, Y., Xie, X., Ma, Z., and Li, B. (2017) Emergence of bacterial soft rot in cucumber caused by Pectobacterium carotovorum subsp. brasiliense in China. *Plant Dis.* **101**: 279-287.

Menon, S., and Wang, S. (2011) Structure of the response regulator PhoP from Mycobacterium tuberculosis reveals a dimer through the receiver domain. *Biochemistry* **50**: 5948-5957.

Metzker, M.L. (2009) Sequencing in real time. *Nat. Biotechnol.* **27**: 150.

Michaux, C., Martini, C., Hanin, A., Auffray, Y., Hartke, A., and Giard, J.-C. (2011) SlyA regulator is involved in bile salts stress response of Enterococcus faecalis. *FEMS Microbiol. Lett.* **324**: 142-146.

Miller, K.A., Phillips, R.S., Mrázek, J., and Hoover, T.R. (2013) Salmonella utilizes D-glucosaminate via a mannose family phosphotransferase system permease and associated enzymes. *J. Bacteriol.* **195**: 4057-4066.

Miller, S., and Mekalanos, J. (1990) Constitutive expression of the phoP regulon attenuates Salmonella virulence and survival within macrophages. *J. Bacteriol.* **172**: 2485-2490.

Miller, S.I., Hoffman, L.R., and Sanowar, S. (2007) Did bacterial sensing of host environments evolve from sensing within microbial communities? *Cell host & microbe* **1**: 85-87.

Miller, S.I., Kukral, A.M., and Mekalanos, J.J. (1989a) A two-component regulatory system (phoP phoQ) controls Salmonella typhimurium virulence. *Proceedings of the National Academy of Sciences* **86**: 5054-5058.

Miller, S.I., Kukral, A.M., and Mekalanos, J.J. (1989b) A two-component regulatory system (phoP phoQ) controls Salmonella typhimurium virulence. *Proc Natl Acad Sci U S A* **86**: 5054-5058.

Minagawa, S., Ogasawara, H., Kato, A., Yamamoto, K., Eguchi, Y., Oshima, T., Mori, H., Ishihama, A., and Utsumi, R. (2003) Identification and molecular characterization of the Mg2+ stimulon of Escherichia coli. *J. Bacteriol.* **185**: 3696-3702.

Minard, K.I., and McAlister-Henn, L. (2005) Sources of NADPH in yeast vary with carbon source. *J. Biol. Chem.* **280**: 39890-39896.

Moleleki, L.N., Pretorius, R.G., Tanui, C.K., Mosina, G., and Theron, J. (2017) A quorum sensing-defective mutant of Pectobacterium carotovorum ssp. brasiliense 1692 is attenuated in virulence and unable to occlude xylem tissue of susceptible potato plant stems. *Mol. Plant Pathol.* **18**: 32-44.

Moraes, A., Souza, E., Mariano, R., Silva, A., Lima, N., Peixoto, A., and Gama, M. (2017) First report of Pectobacterium aroidearum and Pectobacterium carotovorum subsp. brasiliensis causing soft rot of Cucurbita pepo in Brazil. *Plant Dis.* **101**: 379.

Moreira, L.M., Almeida, N.F., Potnis, N., Digiampietri, L.A., Adi, S.S., Bortolossi, J.C., da Silva, A.C., da Silva, A.M., de Moraes, F.E., and de Oliveira, J.C. (2010) Novel insights into the genomic basis of citrus canker based on the genome sequences of two strains of Xanthomonas fuscans subsp. aurantifolii. *BMC Genomics* **11**: 238.

Moss, J.E., Fisher, P.E., Vick, B., Groisman, E.A., and Zychlinsky, A. (2000a) The regulatory protein PhoP controls susceptibility to the host inflammatory response in Shigella flexneri. *Cell Microbiol* **2**: 443-452.

Moss, J.E., Fisher, P.E., Vick, B., Groisman, E.A., and Zychlinsky, A. (2000b) The regulatory protein PhoP controls susceptibility to the host inflammatory response in Shigella flexneri. *Cell. Microbiol.* **2**: 443-452.

Motyka, A., Zoledowska, S., Sledz, W., and Lojkowska, E. (2017) Molecular methods as tools to control plant diseases caused by Dickeya and Pectobacterium spp: A minireview. *New biotechnology* **39**: 181-189.

Mougous, J.D., Cuff, M.E., Raunser, S., Shen, A., Zhou, M., Gifford, C.A., Goodman, A.L., Joachimiak, G., Ordoñez, C.L., and Lory, S. (2006) A virulence locus of Pseudomonas aeruginosa encodes a protein secretion apparatus. *Science* **312**: 1526-1530.

Mullin, R., and Lauer, F., (1966) Breeding behavior of F1 and inbred potato clones. In: Proceedings of the American Society for Horticultural Science. AMER SOC HORTICULTURAL SCIENCE 701 NORTH SAINT ASAPH STREET, ALEXANDRIA, VA …, pp. 449-&.

Naas, H., Sebaihia, M., Orfei, B., Rezzonico, F., Buonaurio, R., and Moretti, C. (2018) Pectobacterium carotovorum subsp. brasiliense and Pectobacterium carotovorum subsp. carotovorum as causal agents of potato soft rot in Algeria. *Eur. J. Plant Pathol.* **151**: 1027-1034.

Nabhan, S., De Boer, S., Maiss, E., and Wydra, K. (2012) Taxonomic relatedness between Pectobacterium carotovorum subsp. carotovorum, Pectobacterium carotovorum subsp. odoriferum and Pectobacterium carotovorum subsp. brasiliense subsp. nov. *J. Appl. Microbiol.* **113**: 904-913.

Nabhan, S., De Boer, S.H., Maiss, E., and Wydra, K. (2013) Pectobacteriumaroidearum sp. nov., a soft rot pathogen with preference for monocotyledonous plants. *Int. J. Syst. Evol. Microbiol.* **63**: 2520-2525.

Nachin, L., and Barras, F. (2000) External pH: an environmental signal that helps to rationalize pel gene duplication in Erwinia chrysanthemi. *Mol. Plant-Microbe Interact.* **13**: 882-886.

Nadarasah, G., and Stavrinides, J. (2011) Insects as alternative hosts for phytopathogenic bacteria. *FEMS Microbiol. Rev.* **35**: 555-575.

Nakka, S., Qi, M., and Zhao, Y. (2010) The Erwinia amylovora PhoPQ system is involved in resistance to antimicrobial peptide and suppresses gene expression of two novel type III secretion systems. *Microbiol. Res.* **165**: 665-673.

Navarre, D.A., Goyer, A., and Shakya, R., (2009) Nutritional value of potatoes: vitamin, phytonutrient, and mineral content. In: Advances in potato chemistry and technology. Elsevier, pp. 395-424.

Navarre, W.W., Halsey, T.A., Walthers, D., Frye, J., McClelland, M., Potter, J.L., Kenney, L.J., Gunn, J.S., Fang, F.C., and Libby, S.J. (2005) Co-regulation of Salmonella enterica genes required for virulence and resistance to antimicrobial peptides by SlyA and PhoP/PhoQ. *Mol. Microbiol.* **56**: 492-508.

Nguyen, M.-P., Yoon, J.-M., Cho, M.-H., and Lee, S.-W. (2015) Prokaryotic 2-component systems and the OmpR/PhoB superfamily. *Can. J. Microbiol.* **61**: 799-810.

Noriega, C.E., Lin, H.Y., Chen, L.L., Williams, S.B., and Stewart, V. (2010) Asymmetric cross-regulation between the nitrate-responsive NarX–NarL and NarQ–NarP two-component regulatory systems from Escherichia coli K-12. *Mol. Microbiol.* **75**: 394-412.

Norte, V.A., Stapleton, M.R., and Green, J. (2003) PhoP-responsive expression of the Salmonella enterica serovar typhimurium slyA gene. *J. Bacteriol.* **185**: 3508-3514.

Nykyri, J., Niemi, O., Koskinen, P., Nokso-Koivisto, J., Pasanen, M., Broberg, M., Plyusnin, I., Törönen, P., Holm, L., and Pirhonen, M. (2012) Revised phylogeny and novel horizontally acquired virulence determinants of the model soft rot phytopathogen Pectobacterium wasabiae SCC3193. *PLoS Path.* **8**.

Oerke, E.-C., Dehne, H.-W., Schönbeck, F., and Weber, A., (2012) *Crop production and crop protection: estimated losses in major food and cash crops*. Elsevier.

Ogasawara, H., Yamamoto, K., and Ishihama, A. (2011) Role of the biofilm master regulator CsgD in cross-regulation between biofilm formation and flagellar synthesis. *J. Bacteriol.* **193**: 2587-2597.

Oh, S.-Y., Shin, J.-H., and Roe, J.-H. (2007) Dual role of OhrR as a repressor and an activator in response to organic hydroperoxides in Streptomyces coelicolor. *J. Bacteriol.* **189**: 6284-6292.

Okada, N., Oi, Y., Takeda-Shitaka, M., Kanou, K., Umeyama, H., Haneda, T., Miki, T., Hosoya, S., and Danbara, H. (2007) Identification of amino acid residues of

Salmonella SlyA that are critical for transcriptional regulation. *Microbiology* **153**: 548-560.

Olsen, N., Miller, J., and Nolte, P., (2006) *Diagnosis & management of potato storage diseases*. Idaho Agricultural Experiment Station.

Onkendi, E.M., Maluleke, L., and Moleleki, L.N. (2014) First report of Pectobacterium carotovorum subsp. brasiliense causing soft rot and blackleg of potatoes in Kenya. *Plant Dis.* **98**: 684-684.

Onkendi, E.M., and Moleleki, L.N. (2014) Characterization of Pectobacterium carotovorum subsp. carotovorum and brasiliense from diseased potatoes in Kenya. *Eur. J. Plant Pathol.* **139**: 557-566.

Onkendi, E.M., Ramesh, A.M., Kwenda, S., Naidoo, S., and Moleleki, L. (2016) Draft genome sequence of a virulent Pectobacterium carotovorum subsp. brasiliense isolate causing soft rot of cucumber. *Genome Announc.* **4**: e01530-01515.

Oren, A., and Garrity, G.M. (2014) Then and now: a systematic review of the systematics of prokaryotes in the last 80 years. *Antonie Van Leeuwenhoek* **106**: 43-56.

Ouellette, A.J. (2011) Paneth cell α-defensins in enteric innate immunity. *Cell. Mol. Life Sci.* **68**: 2215-2229.

Oulghazi, S., Cigna, J., Lau, Y.Y., Moumni, M., Chan, K.G., and Faure, D. (2019) Transfer of the waterfall source isolate Pectobacterium carotovorum M022 to Pectobacterium fontis sp. nov., a deep-branching species within the genus Pectobacterium. *Int. J. Syst. Evol. Microbiol.* **69**: 470-475.

Oyston, P.C., Dorrell, N., Williams, K., Li, S.-R., Green, M., Titball, R.W., and Wren, B.W. (2000) The response regulator PhoP is important for survival under conditions of macrophage-induced stress and virulence in Yersinia pestis. *Infect. Immun.* **68**: 3419-3425.

Pabo, C.O., and Sauer, R.T. (1992) Transcription factors: structural families and principles of DNA recognition. *Annu. Rev. Biochem.* **61**: 1053-1095.

Pan, Y., Liang, F., Li, R.-J., and Qian, W. (2018) MarR-Family Transcription Factor HpaR Controls Expression of the vgrR-vgrS Operon of Xanthomonas campestris pv. campestris. *Mol. Plant-Microbe Interact.* **31**: 299-310.

Panmanee, W., Vattanaviboon, P., Eiamphungporn, W., Whangsuk, W., Sallabhan, R., and Mongkolsuk, S. (2002) OhrR, a transcription repressor that senses and responds to changes in organic peroxide levels in Xanthomonas campestris pv. phaseoli. *Mol. Microbiol.* **45**: 1647-1654.

Park, S.-Y., Pontes, M.H., and Groisman, E.A. (2015) Flagella-independent surface motility in Salmonella enterica serovar Typhimurium. *Proceedings of the National Academy of Sciences* **112**: 1850-1855.

Park, T.-H., Choi, B.-S., Choi, A.-Y., Choi, I.-Y., Heu, S., and Park, B.-S. (2012) Genome sequence of Pectobacterium carotovorum subsp. carotovorum strain PCC21, a pathogen causing soft rot in Chinese cabbage. *J. Bacteriol.* **194**: 6345.

Parkinson, N., DeVos, P., Pirhonen, M., and Elphinstone, J. (2014) Dickeya aquatica sp. nov., isolated from waterways. *Int. J. Syst. Evol. Microbiol.* **64**: 2264-2266.

Parkinson, N., Pritchard, L., Bryant, R., Toth, I., and Elphinstone, J. (2015) Epidemiology of Dickeya dianthicola and Dickeya solani in ornamental hosts and potato studied using variable number tandem repeat analysis. *Eur. J. Plant Pathol.* **141**: 63-70.

Payne, S.M., Mey, A.R., and Wyckoff, E.E. (2016) Vibrio iron transport: evolutionary adaptation to life in multiple environments. *Microbiol. Mol. Biol. Rev.* **80**: 69-90.

Pedron, J., Bertrand, C., Taghouti, G., Portier, P., and Barny, M.-A. (2019) Pectobacterium aquaticum sp. nov., isolated from waterways.

Pédron, J., Chapelle, E., Alunni, B., and Van Gijsegem, F. (2018) Transcriptome analysis of the Dickeya dadantii PecS regulon during the early stages of interaction with Arabidopsis thaliana. *Mol. Plant Pathol.* **19**: 647-663.

Peng, B.-Y., Pan, Y., Li, R.-J., Wei, J.-W., Liang, F., Wang, L., Wang, F.-F., and Qian, W. (2017) An essential regulatory system originating from polygenic transcriptional rewiring of PhoP-PhoQ of Xanthomonas campestris. *Genetics* **206**: 2207-2223.

Perera, I.C., and Grove, A. (2010) Molecular mechanisms of ligand-mediated attenuation of DNA binding by MarR family transcriptional regulators. *J. Mol. Cell. Biol.* **2**: 243-254.

Perera, I.C., and Grove, A. (2011) MarR homologs with urate-binding signature. *Protein Sci.* **20**: 621-629.

Pérez-Rueda, E., and Collado-Vides, J. (2001) Common history at the origin of the position–function correlation in transcriptional regulators in archaea and bacteria. *J. Mol. Evol.* **53**: 172-179.

Pérez-Rueda, E., Collado-Vides, J., and Segovia, L. (2004) Phylogenetic distribution of DNA-binding transcription factors in bacteria and archaea. *Comput. Biol. Chem.* **28**: 341-350.

Perez-Rueda, E., Hernandez-Guerrero, R., Martinez-Nuñez, M.A., Armenta-Medina, D., Sanchez, I., and Ibarra, J.A. (2018) Abundance, diversity and domain architecture variability in prokaryotic DNA-binding transcription factors. *PLoS One* **13**.

Perez, J.C., and Groisman, E.A. (2009) Transcription factor function and promoter architecture govern the evolution of bacterial regulons. *Proceedings of the National Academy of Sciences* **106**: 4319-4324.

Perez, J.C., Latifi, T., and Groisman, E.A. (2008) Overcoming H-NS-mediated transcriptional silencing of horizontally acquired genes by the PhoP and SlyA proteins in Salmonella enterica. *J. Biol. Chem.* **283**: 10773-10783.

Perez, J.C., Shin, D., Zwir, I., Latifi, T., Hadley, T.J., and Groisman, E.A. (2009) Evolution of a bacterial regulon controlling virulence and Mg2+ homeostasis. *PLoS Genet.* **5**.

Perombelon, M.C., and Kelman, A. (1980) Ecology of the soft rot erwinias. *Annu. Rev. Phytopathol.* **18**: 361-387.

Pletzer, D., Schweizer, G., and Weingart, H. (2014) AraC/XylS family stress response regulators rob, SoxS, PliA, and OpiA in the fire blight pathogen Erwinia amylovora. *J. Bacteriol.* **196**: 3098-3110.

Põllumaa, L., Alamäe, T., and Mäe, A. (2012) Quorum sensing and expression of virulence in pectobacteria. *Sensors* **12**: 3327-3349.

Portier, P., Pédron, J., Taghouti, G., Fischer-Le Saux, M., Caullireau, E., Bertrand, C., Laurent, A., Chawki, K., Oulgazi, S., and Moumni, M. (2019) Elevation of

Pectobacterium carotovorum subsp. odoriferum to species level as Pectobacterium odoriferum sp. nov., proposal of Pectobacterium brasiliense sp. nov. and Pectobacterium actinidiae sp. nov., emended description of Pectobacterium carotovorum and description of Pectobacterium versatile sp. nov., isolated from streams and symptoms on diverse plants. *Int. J. Syst. Evol. Microbiol.* **69**: 3207-3216.

Pritchard, L., Glover, R.H., Humphris, S., Elphinstone, J.G., and Toth, I.K. (2016) Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. *Analytical Methods* **8**: 12-24.

Prost, L.R., Daley, M.E., Le Sage, V., Bader, M.W., Le Moual, H., Klevit, R.E., and Miller, S.I. (2007) Activation of the bacterial sensor kinase PhoQ by acidic pH. *Mol. Cell* **26**: 165-174.

Pukatzki, S., McAuley, S.B., and Miyata, S.T. (2009) The type VI secretion system: translocation of effectors and effector-domains. *Curr. Opin. Microbiol.* **12**: 11-17.

Raetz, C.R., and Whitfield, C. (2002) Lipopolysaccharide endotoxins. *Annu. Rev. Biochem.* **71**: 635-700.

Rai, M.F., Tycksen, E.D., Sandell, L.J., and Brophy, R.H. (2018) Advantages of RNA-seq compared to RNA microarrays for transcriptome profiling of anterior cruciate ligament tears. *Journal of Orthopaedic Research®* **36**: 484-497.

Rao, M.S., Van Vleet, T.R., Ciurlionis, R., Buck, W.R., Mittelstadt, S.W., Blomme, E.A., and Liguori, M.J. (2019) Comparison of RNA-seq and microarray gene expression platforms for the toxicogenomic evaluation of liver from short-term rat toxicity studies. *Frontiers in genetics* **9**: 636.

Ravcheev, D.A., Khoroshkin, M.S., Laikova, O.N., Tsoy, O.V., Sernova, N.V., Petrova, S.A., Rakhmaninova, A.B., Novichkov, P.S., Gelfand, M.S., and Rodionov, D.A. (2014) Comparative genomics and evolution of regulons of the LacI-family transcription factors. *Front. Microbiol.* **5**: 294.

Rebeil, R., Jarrett, C.O., Driver, J.D., Ernst, R.K., Oyston, P.C., and Hinnebusch, B.J. (2013) Induction of the Yersinia pestis PhoP-PhoQ regulatory system in the flea and its role in producing a transmissible infection. *J. Bacteriol.* **195**: 1920-1930.

Rendueles, O., and Ghigo, J.-M. (2012) Multi-species biofilms: how to avoid unfriendly neighbors. *FEMS Microbiol. Rev.* **36**: 972-989.

Reverchon, S., and Nasser, W. (2013) Dickeya ecology, environment sensing and regulation of virulence programme. *Environ. Microbiol. Rep.* **5**: 622-636.

Reverchon, S., Nasser, W., and Robert-Baudouy, J. (1994) pecS: a locus controlling pectinase, cellulase and blue pigment production in Erwinia chrysanthemi. *Mol. Microbiol.* **11**: 1127-1139.

Rhee, J.E., Kim, K.-S., and Choi, S.H. (2005) CadC activates pH-dependent expression of the Vibrio vulnificus cadBA operon at a distance through direct binding to an upstream region. *J. Bacteriol.* **187**: 7870-7875.

Rivera-Gómez, N., Martínez-Núñez, M.A., Pastor, N., Rodriguez-Vazquez, K., and Perez-Rueda, E. (2017) Dissecting the protein architecture of DNA-binding transcription factors in bacteria and archaea. *Microbiology* **163**: 1167-1178.

Rivera-Gómez, N., Segovia, L., and Pérez-Rueda, E. (2011) Diversity and distribution of transcription factors: their partner domains play an important role in regulatory plasticity in bacteria. *Microbiology* **157**: 2308-2318.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**: 139-140.

Robinson, V.L., Buckler, D.R., and Stock, A.M. (2000) A tale of two components: a novel kinase and a regulatory switch. *Nature Structural and Molecular Biology* **7**: 626.

Rodionova, I.A., Zhang, Z., Mehla, J., Goodacre, N., Babu, M., Emili, A., Uetz, P., and Saier, M.H. (2017) The phosphocarrier protein HPr of the bacterial phosphotransferase system globally regulates energy metabolism by directly interacting with multiple enzymes in Escherichia coli. *J. Biol. Chem.* **292**: 14250-14257.

Römling, U., and Balsalobre, C. (2012) Biofilm infections, their resilience to therapy and innovative treatment strategies. *J. Intern. Med.* **272**: 541-561.

Romling, U., Gomelsky, M., and Galperin, M.Y. (2005) C-di-GMP: the dawning of a novel bacterial signalling system. *Mol Microbiol* **57**: 629-639.

Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., and Thompson, F., (2013) *The prokaryotes: applied bacteriology and biotechnology*. Springer.

Samson, R., Legendre, J., and Christen, R. (2005) Fischer Le Saux M., Achouak W., Gardan L. Transfer of Pectobacterium chrysanthemi (Burkholder et al. 1953) Brenner et al. 1973 and Brenneria paradisiacal to the genus Dickeya gen. nov. as Dickeya chrisanthemi comb. nov. and Dickeya paradisiacal comb. nov. and delineation of four novel species, Dickeya dadantii sp. nov., and Dickeya dianthicola sp. nov., Dickeya dieffenbachiae sp. nov., and Dickeya zeae sp. nov. *International Journal of Systematic Evolution and Microbiology*.

Sarfraz, S., Riaz, K., Oulghazi, S., Cigna, J., Sahi, S.T., Khan, S.H., and Faure, D. (2018) Pectobacterium punjabense sp. nov., isolated from blackleg symptoms of potato plants in Pakistan. *Int. J. Syst. Evol. Microbiol.* **68**: 3551-3556.

Seshasayee, A.S., Bertone, P., Fraser, G.M., and Luscombe, N.M. (2006) Transcriptional regulatory networks in bacteria: from input signals to output responses. *Curr. Opin. Microbiol.* **9**: 511-519.

Seshasayee, A.S.N., Sivaraman, K., and Luscombe, N.M., (2011) An overview of prokaryotic transcription factors. In: A Handbook of Transcription Factors. Springer, pp. 7-23.

Shevchik, V.E., Robert-Baudouy, J., and Hugouvieux-Cotte-Pattat, N. (1997) Pectate lyase PelI of Erwinia chrysanthemi 3937 belongs to a new family. *J. Bacteriol.* **179**: 7321-7330.

Shi, Y., Latifi, T., Cromie, M.J., and Groisman, E.A. (2004) Transcriptional control of the antimicrobial peptide resistance ugtL gene by the Salmonella PhoP and SlyA regulatory proteins. *J. Biol. Chem.* **279**: 38618-38625.

Shirshikov, F.V., Korzhenkov, A.A., Miroshnikov, K.K., Kabanova, A.P., Barannik, A.P., Ignatov, A.N., and Miroshnikov, K.A. (2018) Draft genome sequences of new

genomospecies “Candidatus Pectobacterium maceratum” strains, which cause soft rot in plants. *Genome Announc.* **6**: e00260-00218.

Shprung, T., Peleg, A., Rosenfeld, Y., Trieu-Cuot, P., and Shai, Y. (2012) Effect of PhoP-PhoQ activation by broad repertoire of antimicrobial peptides on bacterial resistance. *J. Biol. Chem.* **287**: 4544-4551.

Shyntum, D.Y., Nkomo, N., Gricia, A.R., Shigange, N.L., Bellieny-Rabelo, D., and Moleleki, L.N. (2018) The impact of type VI secretion system, bacteriocins and antibiotics on competition amongst Soft-Rot Enterobacteriaceae: Regulation of carbapenem biosynthesis by iron and the transcriptional regulator Fur. *bioRxiv*: 497016.

Sidote, D.J., Barbieri, C.M., Wu, T., and Stock, A.M. (2008) Structure of the Staphylococcus aureus AgrA LytTR domain bound to DNA reveals a beta fold with an unusual mode of binding. *Structure* **16**: 727-735.

Sievers, F., Wilm, A., Dineen, D., Gibson, T.J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., and Söding, J. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* **7**.

Singh, R., Lemire, J., Mailloux, R.J., and Appanna, V.D. (2008) A novel strategy involved anti-oxidative defense: the conversion of NADH into NADPH by a metabolic network. *PLoS One* **3**: e2682.

Sjöblom, S., Brader, G., Koch, G., and Palva, E.T. (2006) Cooperation of two distinct ExpR regulators controls quorum sensing specificity and virulence in the plant pathogen Erwinia carotovora. *Mol. Microbiol.* **60**: 1474-1489.

Sjöblom, S., Harjunpää, H., Brader, G., and Palva, E.T. (2008) A novel plant ferredoxin-like protein and the regulator Hor are quorum-sensing targets in the plant pathogen Erwinia carotovora. *Mol. Plant-Microbe Interact.* **21**: 967-978.

Slater, H., Crow, M., Everson, L., and Salmond, G.P. (2003) Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in Serratia via both quorum-sensing-dependent and-independent pathways. *Mol. Microbiol.* **47**: 303-320.

Smith, R.L., and Maguire, M.E. (1998) Microbial magnesium transport: unusual transporters searching for identity. *Mol. Microbiol.* **28**: 217-226.

Soncini, F.C., Vescovi, E.G., Solomon, F., and Groisman, E.A. (1996) Molecular basis of the magnesium deprivation response in Salmonella typhimurium: identification of PhoP-regulated genes. *J. Bacteriol.* **178**: 5092-5099.

Spory, A., Bosserhoff, A., von Rhein, C., Goebel, W., and Ludwig, A. (2002) Differential regulation of multiple proteins of Escherichia coli and Salmonella enterica serovar Typhimurium by the transcriptional regulator SlyA. *J. Bacteriol.* **184**: 3549-3559.

Stapleton, M.R., Norte, V.A., Read, R.C., and Green, J. (2002) Interaction of the Salmonella typhimurium transcription and virulence factor SlyA with target DNA, and identification of members of the SlyA regulon. *J. Biol. Chem.*

Stock, J., and Da Re, S. (2000) Signal transduction: response regulators on and off. *Curr. Biol.* **10**: R420-R424.

Stock, J., Ninfa, A., and Stock, A. (1989) Protein phosphorylation and regulation of adaptive responses in bacteria. *Microbiol. Rev.* **53**: 450-490.

Stock, J.B., and Baker, M. (1996) Chemotaxis.
Stormo, G.D. (2000) DNA binding sites: representation and discovery. *Bioinformatics* **16**: 16-23.
Suarez-Rodriguez, M.C., Adams-Phillips, L., Liu, Y., Wang, H., Su, S.-H., Jester, P.J., Zhang, S., Bent, A.F., and Krysan, P.J. (2007) MEKK1 is required for flg22-induced MPK4 activation in Arabidopsis plants. *Plant Physiol.* **143**: 661-669.
Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., and Lander, E.S. (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences* **102**: 15545-15550.
Takle, G.W., Toth, I.K., and Brurberg, M.B. (2007) Evaluation of reference genes for real-time RT-PCR expression studies in the plant pathogen Pectobacterium atrosepticum. *BMC Plant Biol.* **7**: 50.
Tanui, C.K., Shyntum, D.Y., Priem, S.L., Theron, J., and Moleleki, L.N. (2017) Influence of the ferric uptake regulator (Fur) protein on pathogenicity in Pectobacterium carotovorum subsp. brasiliense. *PLoS One* **12**: e0177647.
Thomas, M.S., and Wigneshweraraj, S. (2014) Regulation of virulence gene expression. *Virulence* **5**: 832-834.
Thomson, N., Cox, A., Bycroft, B., Stewart, G., Williams, P., and Salmond, G. (1997) The rap and hor proteins of Erwinia, Serratia and Yersinia: a novel subgroup in a growing superfamily of proteins regulating diverse physiological processes in bacterial pathogens. *Mol. Microbiol.* **26**: 531-544.
Tian, Y., Zhao, Y., Yuan, X., Yi, J., Fan, J., Xu, Z., Hu, B., De Boer, S.H., and Li, X. (2016) Dickeyafangzhongdai sp. nov., a plant-pathogenic bacterium isolated from pear trees (Pyrus pyrifolia). *Int. J. Syst. Evol. Microbiol.* **66**: 2831-2835.
Tiwari, S., Jamal, S.B., Hassan, S.S., Carvalho, P.V., Almeida, S., Barh, D., Ghosh, P., Silva, A., Castro, T.L., and Azevedo, V. (2017) Two-component signal transduction systems of pathogenic bacteria as targets for antimicrobial therapy: an overview. *Front. Microbiol.* **8**: 1878.
Toro-Roman, A., Mack, T.R., and Stock, A.M. (2005) Structural analysis and solution studies of the activated regulatory domain of the response regulator ArcA: a symmetric dimer mediated by the α4-β5-α5 face. *J. Mol. Biol.* **349**: 11-26.
Toth, I.K., Bell, K.S., Holeva, M.C., and Birch, P.R. (2003) Soft rot erwiniae: from genes to genomes. *Mol. Plant Pathol.* **4**: 17-30.
Toth, I.K., Pritchard, L., and Birch, P.R. (2006) Comparative genomics reveals what makes an enterobacterial plant pathogen. *Annu. Rev. Phytopathol.* **44**: 305-336.
Tsai, K.-C., Hung, P.-P., Cheng, C.-F., Chen, C., and Tseng, T.-S. (2019) Exploring the mode of action of inhibitors targeting the PhoP response regulator of Salmonella enterica through comprehensive pharmacophore approaches. *RSC advances* **9**: 9308-9312.
Tu, J., Huang, B., Zhang, Y., Zhang, Y., Xue, T., Li, S., and Qi, K. (2016) Modulation of virulence genes by the two-component system PhoP-PhoQ in avian pathogenic Escherichia coli. *Pol. J. Vet. Sci.* **19**: 31-40.

Tyler, H.L., and Triplett, E.W. (2008) Plants as a habitat for beneficial and/or human pathogenic bacteria. *Annu. Rev. Phytopathol.* **46**: 53-73.

Ulrich, L.E., Koonin, E.V., and Zhulin, I.B. (2005) One-component systems dominate signal transduction in prokaryotes. *Trends Microbiol.* **13**: 52-56.

Vadyvaloo, V., Viall, A.K., Jarrett, C.O., Hinz, A.K., Sturdevant, D.E., and Hinnebusch, B.J. (2015) Role of the PhoP–PhoQ gene regulatory system in adaptation of Yersinia pestis to environmental stress in the flea digestive tract. *Microbiology* **161**: 1198-1210.

van der Merwe, J.J., Coutinho, T.A., Korsten, L., and van der Waals, J.E. (2010) Pectobacterium carotovorum subsp. brasiliensis causing blackleg on potatoes in South Africa. *Eur. J. Plant Pathol.* **126**: 175-185.

Van der Wolf, J., De Haan, E., Kastelein, P., Krijger, M., De Haas, B., Velvis, H., Mendes, O., Kooman-Gersmann, M., and Van Der Zouwen, P. (2017) Virulence of Pectobacterium carotovorum subsp. brasiliense on potato compared with that of other Pectobacterium and Dickeya species under climatic conditions prevailing in the Netherlands. *Plant Pathol.* **66**: 571-583.

Van der Wolf, J.M., and De Boer, S.H. (2007) Bacterial pathogens of potato. *Potato Biology and Biotechnology, Advances and Perspectives. Oxford, UK: Elsevier*: 595-619.

Van Der Wolf, J.M., Nijhuis, E.H., Kowalewska, M.J., Saddler, G.S., Parkinson, N., Elphinstone, J.G., Pritchard, L., Toth, I.K., Lojkowska, E., and Potrykus, M. (2014) Dickeya solani sp. nov., a pectinolytic plant-pathogenic bacterium isolated from potato (Solanum tuberosum). *Int. J. Syst. Evol. Microbiol.* **64**: 768-774.

Venkatesh, B., Babujee, L., Liu, H., Hedley, P., Fujikawa, T., Birch, P., Toth, I., and Tsuyumu, S. (2006) The Erwinia chrysanthemi 3937 PhoQ sensor kinase regulates several virulence determinants. *J. Bacteriol.* **188**: 3088-3098.

Vescovi, E.G., Soncini, F., and Groisman, E. (1994) The role of the PhoP/PhoQ regulon in Salmonella virulence. *Res. Microbiol.* **145**: 473-480.

Véscovi, E.G., Soncini, F.C., and Groisman, E.A. (1996) Mg2+ as an extracellular signal: environmental regulation of Salmonella virulence. *Cell* **84**: 165-174.

Vinatzer, B.A., Monteil, C.L., and Clarke, C.R. (2014) Harnessing population genomics to understand how bacterial pathogens emerge, adapt to crop hosts, and disseminate. *Annu. Rev. Phytopathol.* **52**: 19-43.

Voronina, M.V., Bugaeva, E.N., Vasiliev, D.M., Kabanova, A.P., Barannik, A.P., Shneider, M.M., Kulikov, E.E., Korzhenkov, A.A., Toschakov, S.V., Ignatov, A.N., and Miroshnikov, K.A. (2019) Characterization of Pectobacterium carotovorum subsp. carotovorum Bacteriophage PP16 Prospective for Biocontrol of Potato Soft Rot. *Microbiology* **88**: 451-460.

Waleron, M., Misztak, A., Waleron, M., Franczuk, M., Jońca, J., Wielgomas, B., Mikiciński, A., Popović, T., and Waleron, K. (2019a) Pectobacterium zantedeschiae sp. nov. a new species of a soft rot pathogen isolated from Calla lily (Zantedeschia spp.). *Syst. Appl. Microbiol.* **42**: 275-283.

Waleron, M., Misztak, A., Waleron, M., Jonca, J., Furmaniak, M., and Waleron, K. (2019b) Pectobacterium polonicum sp. nov. isolated from vegetable fields. *Int. J. Syst. Evol. Microbiol.* **69**: 1751-1759.

Waleron, M., Waleron, K., and Lojkowska, E. (2015) First Report of Pectobacterium carotovorum subsp. brasiliense Causing Soft Rot on Potato and Other Vegetables in Poland. *Plant Dis.*

Wang, Q., Zhao, Y., McClelland, M., and Harshey, R.M. (2007) The RcsCDB signaling system and swarming motility in Salmonella enterica serovar Typhimurium: dual regulation of flagellar and SPI-2 virulence genes. *J. Bacteriol.* **189**: 8447-8457.

Wang, Z., Gerstein, M., and Snyder, M. (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nature reviews genetics* **10**: 57.

Waterhouse, A.M., Procter, J.B., Martin, D.M., Clamp, M., and Barton, G.J. (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**: 1189-1191.

Weatherspoon-Griffin, N., and Wing, H.J. (2016) Characterization of SlyA in Shigella flexneri identifies a novel role in virulence. *Infect. Immun.* **84**: 1073-1082.

Wei, C., Ding, T., Chang, C., Yu, C., Li, X., and Liu, Q. (2019) Global Regulator PhoP is Necessary for Motility, Biofilm Formation, Exoenzyme Production and Virulence of Xanthomonas citri Subsp. citri on Citrus Plants. *Genes* **10**: 340.

Weinstein-Fischer, D., and Altuvia, S. (2007) Differential regulation of Escherichia coli topoisomerase I by Fis. *Mol. Microbiol.* **63**: 1131-1144.

Whitehead, N.A., Barnard, A.M., Slater, H., Simpson, N.J., and Salmond, G.P. (2001) Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol. Rev.* **25**: 365-404.

Wilkinson, S.P., and Grove, A. (2006) Ligand-responsive transcriptional regulation by members of the MarR family of winged helix proteins. *Curr. Issues Mol. Biol.* **8**: 51.

Will, W.R., Bale, D.H., Reid, P.J., Libby, S.J., and Fang, F.C. (2014) Evolutionary expansion of a regulatory network by counter-silencing. *Nature communications* **5**.

Will, W.R., Brzovic, P., Le Trong, I., Stenkamp, R.E., Lawrenz, M.B., Karlinsey, J.E., Navarre, W.W., Main-Hester, K., Miller, V.L., and Libby, S.J. (2019) The evolution of SlyA/RovA transcription factors from repressors to countersilencers in Enterobacteriaceae. *MBio* **10**: e00009-00019.

Winfield, M.D., and Groisman, E.A. (2004) Phenotypic differences between Salmonella and Escherichia coli resulting from the disparate regulation of homologous genes. *Proceedings of the National Academy of Sciences* **101**: 17162-17167.

Winslow, C.-E., Broadhurst, J., Buchanan, R., Krumwiede Jr, C., Rogers, L., and Smith, G. (1920) The families and genera of the bacteria: final report of the committee of the Society of American Bacteriologists on characterization and classification of bacterial types. *J. Bacteriol.* **5**: 191.

Wojtaszek, P. (1997) Oxidative burst: an early plant response to pathogen infection. *Biochem. J.* **322**: 681-692.

Wood, J.M. (2010) Osmotic stress. *Bacterial Stress Responses*: 133-156.

Wood, J.M. (2011) Bacterial osmoregulation: a paradigm for the study of cellular homeostasis. *Annu. Rev. Microbiol.* **65**: 215-238.

Wu, R.-y., Zhang, R.-g., Zagnitko, O., Dementieva, I., Maltzev, N., Watson, J.D., Laskowski, R., Gornicki, P., and Joachimiak, A. (2003) Crystal structure of Enterococcus faecalis SlyA-like transcriptional factor. *J. Biol. Chem.* **278**: 20240-20244.

Wyborn, N.R., Stapleton, M.R., Norte, V.A., Roberts, R.E., Grafton, J., and Green, J. (2004) Regulation of Escherichia coli hemolysin E expression by H-NS and Salmonella SlyA. *J. Bacteriol.* **186**: 1620-1628.

Xia, T., Li, Y., Sun, D., Zhuo, T., Fan, X., and Zou, H. (2016) Identification of an extracellular endoglucanase that is required for full virulence in Xanthomonas citri subsp. citri. *PLoS One* **11**.

Xiong, A., Gottman, A., Park, C., Baetens, M., Pandza, S., and Matin, A. (2000) The EmrR protein represses the Escherichia coli emrRAB multidrug resistance operon by directly binding to its promoter region. *Antimicrob. Agents Chemother.* **44**: 2905-2907.

Xu, J., and Wang, N. (2018) Where are we going with genomics in plant pathogenic bacteria? *Genomics*.

Yang, C., Huang, T.-W., Wen, S.-Y., Chang, C.-Y., Tsai, S.-F., Wu, W.-F., and Chang, C.-H. (2012) Genome-wide PhoB binding and gene expression profiles reveal the hierarchical gene regulatory network of phosphate starvation in Escherichia coli. *PLoS One* **7**.

Yeaman, M.R., and Yount, N.Y. (2003) Mechanisms of antimicrobial peptide action and resistance. *Pharmacol. Rev.* **55**: 27-55.

Yuan, J., Jin, F., Glatter, T., and Sourjik, V. (2017) Osmosensing by the bacterial PhoQ/PhoP two-component system. *Proceedings of the National Academy of Sciences* **114**: E10792-E10798.

Zhang, Y., Fan, Q., and Loria, R. (2016) A re-evaluation of the taxonomy of phytopathogenic genera Dickeya and Pectobacterium using whole-genome sequencing data. *Syst. Appl. Microbiol.* **39**: 252-259.

Zhang, Y., Jalan, N., Zhou, X., Goss, E., Jones, J.B., Setubal, J.C., Deng, X., and Wang, N. (2015) Positive selection is the main driving force for evolution of citrus canker-causing Xanthomonas. *The ISME journal* **9**: 2128-2138.

Zhao, Y., Sundin, G.W., and Wang, D. (2009) Construction and analysis of pathogenicity island deletion mutants of Erwinia amylovora. *Can. J. Microbiol.* **55**: 457-464.

Zhou, D., Han, Y., Qin, L., Chen, Z., Qiu, J., Song, Y., Li, B., Wang, J., Guo, Z., and Du, Z. (2005) Transcriptome analysis of the Mg2+-responsive PhoP regulator in Yersinia pestis. *FEMS Microbiol. Lett.* **250**: 85-95.

Zhou, J.N., Zhang, H.B., Lv, M.F., Chen, Y.F., Liao, L.S., Cheng, Y.Y., Liu, S.Y., Chen, S.H., He, F., and Cui, Z.N. (2016) SlyA regulates phytotoxin production and virulence in Dickeya zeae EC1. *Mol. Plant Pathol.*

Zhu, R., Hao, Z., Lou, H., Song, Y., Zhao, J., Chen, Y., Zhu, J., and Chen, P.R. (2017) Structural characterization of the DNA-binding mechanism underlying the copper (II)-sensing MarR transcriptional regulator. *JBIC Journal of Biological Inorganic Chemistry* **22**: 685-693.

Zhuge, X., Sun, Y., Xue, F., Tang, F., Ren, J., Li, D., Wang, J., Jiang, M., and Dai, J. (2018) A novel PhoP/PhoQ regulation pathway modulates the survival of extraintestinal pathogenic Escherichia coli in macrophages. *Front. Immunol.* **9**: 788.

Zou, L., Zeng, Q., Lin, H., Gyaneshwar, P., Chen, G., and Yang, C.-H. (2012) SlyA regulates type III secretion system (T3SS) genes in parallel with the T3SS master regulator HrpL in Dickeya dadantii 3937. *Appl. Environ. Microbiol.* **78**: 2888-2895.

Zschiedrich, C.P., Keidel, V., and Szurmant, H. (2016) Molecular mechanisms of two-component signal transduction. *J. Mol. Biol.* **428**: 3752-3775.