JÉSSICA CATARINE SILVA DE ASSIS

ELEMENTOS INTEGRATIVOS E CONJUGATIVOS EM BACTÉRIAS FITOPATOGÊNICAS DE IMPACTO ECONÔMICO E CIENTÍFICO

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Magister Scientiae*.

Orientador: Mateus Ferreira Santana

Coorientadoras: Marisa Vieira de Queiroz
Denise Mara Soares Bazzolli

VIÇOSA - MINAS GERAIS
2021

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
A848e
2021

Assis, Jéssica Catarine Silva de, 1994-
Elementos integrativos e conjugativos em bactérias fitopatogênicas de impacto econômico e científico / Jéssica Catarine Silva de Assis. – Viçosa, MG, 2021.
101 f. : il. (algumas color.) ; 29 cm.

Inclui anexos.
Orientador: Mateus Ferreira Santana.
Dissertação (mestrado) - Universidade Federal de Viçosa.
Inclui bibliografia.

1. Bactérias fitopatogênicas. 2. Transferência genética horizontal. 3. Virulência. 4. Adaptação (Biologia).
I. Universidade Federal de Viçosa. Departamento de Microbiologia. Programa de Pós-Graduação em Microbiologia Agrícola. II. Título.

CDD 22. ed. 632.32

Bibliotecário(a) responsável: Bruna Silva CRB6/2552

JÉSSICA CATARINE SILVA DE ASSIS

ELEMENTOS INTEGRATIVOS E CONJUGATIVOS EM BACTÉRIAS FITOPATOGÊNICAS DE IMPACTO ECONÔMICO E CIENTÍFICO

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Microbiologia Agrícola, para obtenção do título de *Magister Scientiae*.

APROVADA: 28 de abril de 2021

Assentimento:

Jéssica Catarine Silva de Assis

Jéssica Catarine Silva de Assis
Autora

Mateus Ferreira Santana
Orientador

AGRADECIMENTOS

A Deus pela coragem, proteção, sabedoria e principalmente saúde dadas para finalizar essa trajetória durante esse período tão conturbado.

À minha base, minha família, meus sinceros agradecimentos. Meus pais Keller e Willian por serem meus maiores exemplos de força e garra, formarem meu caráter, me ensinarem a não desistir frente aos percalços da vida, e incentivar sempre a ir atrás dos meus sonhos. Aos meus maiores companheiros de vida, meus irmãos Lucas, Thamyres, Renan e meu namorado Denner pelo apoio psicológico e por acreditarem em meu potencial, não tenho palavras pra descrever a importância de vocês em minha vida e para que eu fosse capaz de desenvolver esse trabalho. Aos meus avós e tios, em especial a Tia Miriam, por terem sido fundamentais na minha educação e ao meu anjo Vó Theresinha pela proteção e apoio de sempre, eu não chegaria até aqui se não fossem todos vocês.

Agradeço também a minha outra família, meus amigos. Minhas amigas de vida Carla eLarissa, meus amigos Raul, Luiz, Pedro e Henrique, minhas parceiras de graduação Juliana, Bruna, Ana, Letícia e Viviane, obrigada por estarem sempre comigo, mesmo que de longe, me botando para cima e apoiando. À minha “família Viçosense”, que vou chamar pelos apelidos pra não perder nosso jeitinho: Tataly, Ynham, Lelé, e às “Bioagirls”: Cocoly, Pampam, Tatá e Bia. Obrigada por aguentarem firme todos os surtos e pitacos possíveis dessa virginiana durante esses dois anos com muito bom humor e muito companheirismo, meu mestrado não seria o mesmo sem vocês, e obrigada por me deixar ser a “Jéje” de vocês.

Ao professor Mateus Santana, pela orientação, confiança, e conhecimento passados durante o mestrado e aos demais professores do departamento, sobretudo às professoras Marisa Vieira e Denise Bazzoli e ao professor Antônio Galvão do Nascimento. Aos colegas dos laboratórios de Genética Molecular de Fungos e Bactérias, Giarlã, Mísia, Hanna, Patrícia e Osiel entre outros, meus agradecimentos pelo companheirismo, e que com toda a paciência me ensinaram conhecimentos técnicos que vou levar para a vida.

Por fim, deixo meus agradecimentos à Universidade Federal de Viçosa, a coordenação do Programa de Pós Graduação em Microbiologia Agrícola e às agências financiadoras, em especial ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) por permitirem a realização desse trabalho.

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

RESUMO

ASSIS, Jéssica Catarine Silva, M.Sc., Universidade Federal de Viçosa, abril de 2021. **Elementos Integrativos e Conjugativos em Bactérias Fitopatogênicas de Impacto Econômico e Científico.** Orientador: Mateus Ferreira Santana. Coorientadores: Marisa Vieira de Queiroz e Denise Mara Soares Bazzolli.

Bactérias fitopatogênicas causam diversos danos às plantações no mundo todo, gerando grandes prejuízos econômicos. Muitas dessas bactérias possuem uma alta variabilidade genética, genes que codificam diferentes fatores de virulência e resistência à antimicrobianos, que dificultam seu controle. Esses genes podem se espalhar na população por meio da transferência horizontal de genes (THG). Dentre os mecanismos de THG, a conjugação mediada por elementos genéticos móveis auto-transmissíveis, como os elementos integrativos e conjugativos (da sigla em inglês, ICEs = Integrative and Conjugative Elements), tem se mostrado altamente eficiente para a transferência de genes entre as bactérias. Esses elementos permanecem integrados no cromossomo das células bacterianas e possuem estrutura modular, carregando genes responsáveis pela sua integração e excisão do genoma, sua conjugação para novas células, a regulação e genes acessórios capazes de conferir novos fenótipos ao hospedeiro. Neste trabalho, buscou-se conhecer a presença, distribuição e potencial impacto evolutivo desses elementos nas dez espécies de bactérias fitopatogênicas consideradas de maior impacto científico e econômico: *Pseudomonas syringae*, *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas campestris*, *Xanthomonas axonopodis* pv. *manihotis*, *Erwinia amylovora*, *Xylella fastidiosa*, *Dickeya* (*dadantii* e *solani*), *Pectobacterium* (*carotovorum* e *atrosepticum*). Dessa maneira, foi feita uma busca em 300 genomas completos dessas bactérias para a identificação e caracterização dos ICEs. Foram identificados e caracterizados os módulos de integração/excisão, conjugação e manutenção dos ICEs e os genes acessórios com função putativa em virulência, resistência ou adaptação das bactérias. No total foram encontrados 78 ICEs, sendo 45 elementos diferentes e, entre estes, 31 novos ICEs, identificados nos genomas de *A. tumefaciens* (4 ICEs), *D. dadantii* and *solani* (3), P. *carotovorum* and *atrosepticum* (6), *P. syringae* (20), *R. solanacearum species* complex (7), *X. campestris* (2), e *X. fastidiosa* (3), com tamanho variando entre 40 kb (ICEDd.2) a 161 kb (ICEPs.10). No entanto, dentre as bactérias investigadas não foram encontrados ICEs em genomas de *X. oryzae*, *E. carotovora* e *X. campestris*. Os métodos de análise empregados permitiram relatar pela primeira vez a coocorrência de quatro ICEs dentro de um cromossomo em genomas de *P. syringae,* além da identificação do elemento ICEDs.1 em todos os genomas de *Dickeya solani* analisados podendo indicar uma aquisição ancestral desse elemento por essa espécie. Obteve-se uma evidência de ICEs promovendo THG entre espécies diferentes a partir da identificação do ICEPc.2 em isolados de *P. carotovorum* e *S. plymuthica*. Análises comparativas demonstraram que os ICEs compartilham principalmente os módulos de integração e conjugação. Foram caracterizados 396 genes acessórios carregados pelos elementos, sendo que a maioria dos ICEs foram encontrados carregando genes acessórios que podem influenciar na virulência das bactérias hospedeiras, além de adaptação ou resistência a antibióticos e metais pesados. Os fatores de virulência mais encontrados foram genes relacionados ao sistema de secreção tipo III (T3SS). Por fim, análises de dados de RNAseq do isolado SCRI1043 de *P. atrosepticum* mostraram a expressão de genes acessórios relacionados a virulência durante o processo de infecção de plantas de tabaco, carregados pelos elementos ICEPa.1 e ICEPa.2. Esses resultados proporcionam uma visão inicial da interessante relação entre bactérias fitopatogênicas e ICEs, tendo em vista que esses elementos possuem potencial para influenciar na patogenicidade e adaptabilidade de fitobactérias ao seu hospedeiro. Além disso, nossos achados direcionam e abrem caminho para futuros estudos experimentais de ICEs em genomas de fitopatógenos, permitindo um aprofundamento do conhecimento sobre esses elementos intrigantes.

Palavras-chave: Elementos genéticos móveis auto-transmissíveis. Transferência horizontal de genes. Fitopatógenos. Virulência. Adaptação

ABSTRACT

ASSIS, Jéssica Catarine Silva, M.Sc., Universidade Federal de Viçosa, April, 2021. **Integrative and Conjugative Elements in Phytopathogenic Bacteria of Economic and Scientific Impact.** Adviser: Mateus Ferreira Santana. Co-advisers: Marisa Vieira de Queiroz and Denise Mara Soares Bazzolli.

Phytopathogenic bacteria cause several damages to plantations worldwide, generating great economic losses. Many of these bacteria have high genetic variability, genes that encode different factors of virulence, and resistance to antimicrobials, which make them hard to control. Many of these genes can spread to the population through horizontal gene transfer (THG). Among the mechanisms of THG, the conjugation mediated by self-transmitting mobile genetic elements, such as integrative and conjugative elements (ICEs), has been configured highly efficient for a download of genes among bacteria. These elements are integrated into the chromosome of bacterial cells and have a modular structure, carrying genes responsible for their integration and excision of the genome, their conjugation to new host cells, regulation and accessory genes capable of conferring new phenotypes to the host. In this work, we sought to know the presence, distribution and potential evolutionary impact of these elements in the species of phytopathogenic bacteria considered to have the greatest scientific and economic impact: *Pseudomonas syringae*, *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas campestris*, *Xanthomonas axonopodis* pv. *manihotis*, *Erwinia amylovora*, *Xylella fastidiosa*, *Dickeya* (*dadantii* and *solani*), *Pectobacterium* (*carotovorum* and *atrosepticum*). In this way, a search was made in 300 complete genomes of these bacteria for the identification and characterization of the elements. ICEs integration / excision, conjugation and maintenance modules and accessory genes with putative function in virulence, resistance or adaptation of bacteria were identified and characterized. In total, 78 ICEs were found, of which 45 were distinct elements and, among these, 31 new ICEs, identified in the genomes of *A. tumefaciens* (4 ICEs), *D. dadantii* and *solani* (3), *P. carotovorum* and *atrosepticum* (6), *P. syringae* (20), *R. solanacearum* species complex (7), *X. campestris* (2), and *X. fastidiosa* (3), ranging in size from 40 kb (ICEDd.2) to 161 kb (ICEPs.10). However, among the investigated bacteria, ICEs were not found in the genomes of *X. oryzae*, *E. carotovora* and *X. campestris*. The analysis methods employed allowed the first reporting of the co-occurrence of four ICEs within a chromosome in *P. syringae* genomes, in addition to the identification of the ICEDs.1 element in all predicted *D. solani* genomes, which may indicate an ancestral acquisition of this element. by that species. Evidence of ICEs promoting THG among different species was obtained from the identification of ICEPc.2 in evidence of *P. carotovorum* and *S. plymuthica*. Comparative analyzes have shown that ICEs mainly share their integration and conjugation modules. 396 accessory genes carried by the elements were characterized, with the majority of ICEs found carrying genes that can lead to the virulence of host bacteria, in addition to adaptation or resistance to antibiotics and heavy metals. The most commonly found virulence factors were genes related to the type III secretion system (T3SS). Finally, analysis of RNAseq data from isolate SCRI1043 of *P. atrosepticum* illustrated the expression of accessory genes related to virulence during the process of infection of tobacco plants, carried by the elements ICEPa.1 and ICEPa.2. These results provide an initial view of the interesting relationship between phytopathogenic bacteria and ICEs, considering that these elements have the potential to influence the pathogenicity and adaptability of phytobacteria to their host. In addition, our findings direct and pave the way for future experimental studies of ICEs in phytopathogen genomes, allowing a deeper understanding of these intriguing elements.

Keywords: Self-transmissible mobile elements. Horizontal gene transfer. Phytopathogens. Virulence. Adaptation

SUMÁRIO

CAPÍTULO 1 - INTRODUÇÃO GERAL **9**

Introdução Geral 10

Bibliografia **18**

CAPÍTULO 2 - Integrative and Conjugative Elements (ICEs) Potentially Influence the Lifestyle of MostRelevant Phytopathogenic Bacteria **25**

Abstract 26

Introduction **26**

Methods **28**

Identification and characterization of Integrative and Conjugative Elements 28

Differential expression analysis 29

Comparative Analysis 29

Results **30**

Identification of ICEs in bacterial chromosomes. 30

Agrobacterium tumefaciens 36

Dickeya (*dadantii* and *solani*) 36

Pectobacterium carotovorum (and *atrosepticum*) 37

Pseudomonas syringae pathovars 39

Ralstonia solanacearum species complex 42

Xanthomonas campestris pathovars 42

Xylella fastidiosa 43

Discussion **44**

Bibliography **50**

Supplementary Material **62**

CAPÍTULO 1

INTRODUÇÃO GERAL

Introdução Geral

Em 2012, a convite da revista Molecular Plant Pathology, fitobacteriologistas elencaram os dez fitopatógenos bacterianos mais importantes do ponto de vista científico e econômico (Mansfield et al., 2012). As principais características dessas bactérias em sua ordem na lista estão citadas abaixo:

Pseudomonas syringae são bactérias Gram negativas, aeróbias estritas, de grande importância para os estudos de patogênese em plantas por se tratar de um grupo de patógenos de extrema relevância econômica, capazes de causar necroses em partes aéreas das plantas como folhas, frutas e caules, além de serem disseminados e sobreviverem em diversos habitats e nichos (Morris et al., 2007; Green et al., 2010; Mansfield et al., 2012). Além disso, algumas cepas de *P. syringae* possuem uma fase epifítica em plantas, e também podem estar envolvidas no ciclo da água participando do processo de nucleação do gelo (Lindemann, 1984; Morris et al., 2007; Monteil et al., 2014). Essa espécie apresenta alta variabilidade genética, compreendendo pelo menos 50 patovares que se diferenciam pela gama de hospedeiros, podendo causar doenças em monocotiledôneas e dicotiledôneas herbáceas ou arbóreas, possuindo também diversos fatores de virulência (Kennelly et al., 2007; Green et al., 2010).

O complexo de espécies *Ralstonia solanacearum* (RSSC) compreende linhagens geneticamente distintas de bactérias aeróbias e gram-negativas, sendo considerado o complexo fitobacteriano mais impactante no mundo (Genin, 2010; Mansfield et al., 2012). Espécies do RSSC infectam principalmente as raízes de cerca de 250 espécies de plantas dentro de 54 famílias diferentes, invadindo e se multiplicando os vasos do xilema, vindo a causar murcha generalizada e morte das plantas infectadas (Salanoubat et al., 2002; Denny, 2006; Prior et al., 2016). Além de linhagens de *R. solanacearum* provenientes do solo, também faziam parte do complexo RSSC a espécie *Ralstonia syzygii*, capaz de causar o Mal de Sumatra em craveiro-da-Índia e "*Blood Disease Bacterium*" (BDB), um organismo ainda não classificado e agente causador da *banana blood disease* (Prior et al., 2016). Em 2014 foi proposta uma revisão taxonômica do complexo de espécies *Ralstonia solanacearum*, sendo reconhecidas três espécies: *Ralstonia solanacearum* compreendendo os isolados do filótipo II dessa espécie, *Ralstonia syzygii,* os isolados previamente caracterizadas como BDB e do filótipo IV de *R. solanacearum* e por último foram classificadas como *Ralstonia pseudosolanacearum* os isolados dos filótipos I e III (Safni et al., 2014).

A bactéria *Agrobacterium tumefaciens* conhecida como "engenheira genética natural", causa a doença galha de coroa em uma ampla gama de espécies de plantas, além de ter grande importância biotecnológica por ser usada como veículo da transferência de genes para fungos e células vegetais e a criação de plantas transgênicas (Gelvin, 2003). *A. tumefaciens* induz a formação de galhas, principalmente na região do colo e no sistema radicular (Salanoubat et al., 2002; Guo et al., 2019;) Além dos dois cromossomos, *A. tumefaciens* possui um plasmídeo com 200 kb chamado plasmídeo Ti (indutor de tumor), que carrega a sequência de DNA que será transferida para a célula hospedeira (T-DNA) e os genes responsáveis pela transferência de DNA presentes na região de virulência (*vir*) (Zambryski, 1992). A transferência do T-DNA para o genoma da planta hospedeira ocasiona alterações no metabolismo celular causando o crescimento desordenado das células transformadas (Escobar & Dandekar, 2003).

Xanthomonas oryzae pv. oryzae (Xoo) causa o crestamento bacteriano do arroz (*Oryza sativa*), uma das três doenças mais sérias dessa cultura (Niño-Liu et al., 2006; Midha et al., 2017). Essa doença ocorre na maioria das regiões de crescimento de arroz como a América, África, Austrália e na Ásia, onde causa grandes impactos econômicos (Swings et al., 1990; Doucouré et al., 2018). A partir da entrada nos tecidos por feridas e hidatódio essa bactéria Gram negativa infecta o xilema das plantas, utilizando nesse processo de patogênese proteínas de virulência da família de efetores do tipo de ativador de transcrição, conhecidos como efetores TAL (Doucouré et al., 2018).

Xanthomonas campestris pv. campestris, *Xanthomonas euvesicatoria e X. axonopodis* pv. malvacearum anteriormente caracterizadas como patovares de *Xanthomonas campestris* também foram selecionadas por causarem doenças agronômicas relevantes e terem sido usadas em estudos de grande impacto científico (Mansfield et al., 2012). *Xanthomonas campestris* pv. campestris (Xcc) causa a podridão negra dos crucíferos, principalmente em plantações de repolho (*Brassica oleracea*). Apesar dessa característica de patógeno, Xcc também é importante como produtor de xantana, um polissacarídeo utilizado nas indústrias alimentícia, farmacêutica e petrolífera (Vicente et al., 2001; Mansfield et al., 2012) *Xanthomonas campestris* pv. vesicatoria (Xcv), atualmente classificada como *Xanthomonas euvesicatoria*, é responsável por causar a mancha bacteriana do pimentão e pimentas (*Capsicum* spp.) e principalmente em tomate (*Lycopersicon* spp.) (Swings & Civetta, 2012). Já, *X. campestris* pv. malvacearum (agora classificado como *X. axonopodis* pv. malvacearum) causa a mancha-angular do algodoeiro (*Gossypium* spp) uma doença com ocorrência generalizada em todas regiões produtoras de algodão, de grande potencial destrutivo, e que também foi importante em diversos estudos de interação de interações bactéria-planta (Gabriel et al., 1986; Vicente et al., 2001; Mansfield et al., 2012).

X. axonopodis pv. *manihotis* (Xam), foi outra bactéria do gênero Xanthomonas selecionada por ser uma espécie Gram-negativa, causadora da bacteriose ou murcha bacteriana da mandioca (CBB, do inglês: *Cassava bacterial blight*), doença endêmica em áreas tropicais e subtropicais incluindo países da América Latina e África e que pode causar em variedades suscetíveis perdas que podem variar de 50 a 100%, quando as condições climáticas são favoráveis (Lozano & Sequeira, 1974; Verdier et al., 2004). Os sintomas da doença incluem lesões nas folhas, murcha, presença de exsudatos e cancro no caule, sendo que a penetração do patógeno ocorre por estômatos ou feridas nas folhas (Vicente et al., 2001; Mansfield et al., 2012). A patogenicidade de Xam está relacionada principalmente com a translocação de efetores do tipo III para as células da planta via sistemas de secreção do tipo III, que provocam alterações no metabolismo das células ou à supressão das respostas de defesa (López & Bernal, 2012).

Erwinia amylovora é o agente causal do fogo bacteriano das pomáceas, doença que afeta de forma destrutiva plantas cultiváveis e silvestres da família *Rosaceae,* como amora, framboesa e principalmente pera e maçã (Vanneste, 2000; Piqué et al., 2015). Esse patógeno gram-negativo, membro da família *Enterobacteriaceae,* está distribuído principalmente nas regiões temperadas, onde seu controle é realizado via tratamento com antibióticos durante o período de surgimento das flores, porém isolados resistentes já foram identificadas nas quais foram relatadas mutações ou plasmídeos e transposons que conferem a resistência a antibióticos_(McManus et al., 2002). *Erwinia amylovora* penetra nas plantas por feridas ou aberturas naturais, sobretudo pelos nectários nas flores, causando infecções sistêmicas, e matando a planta a partir da evolução dos sintomas como murcha e necrose dos tecidos suculentos (Vanneste, 2000).Por fim, esse fitopatógeno tem grande importância histórica por ter sido a primeira bactéria associada a doença em plantas, sendo descrita como a “primeira bactéria fitopatogênica” (Mansfield et al., 2012).

Xylella fastidiosa possui uma ampla gama de hospedeiros, sendo responsável por doenças de grande importância econômica: a doença de Pierce em videira, a clorose variegada dos citros (CVC), e a escaldadura das folhas da amendoeira, sendo as duas primeiras de maior importância econômica (Chatterjee et al., 2008; Mansfield et al., 2012). A colonização dessa bactéria é restrita ao xilema, ou seja, coloniza e se espalha nesse tecido vascular e bloqueia os vasos ao formar biofilmes bloqueando a translocação de água e nutrientes, sendo obrigatoriamente transmitida por insetos vetores que se alimentam da seiva (Purcell & Hopkins, 1996; Chatterjee et al., 2008; Mansfield et al., 2012). *Xylella fastidiosa* não possui genes relacionados com o sistema de secreção do tipo III e aparentemente também não possui efetores do tipo III, fatores importantes para a supressão das defesas da planta pelo patógeno. Porém possui os sistemas de secreção do tipo I e II ativos, que podem ser responsáveis pelas bombas de efluxo e pela secreção de enzimas hidrolíticas importantes para a sua virulência (Chatterjee et al., 2008; Mansfield et al., 2012).

As bactérias pertencentes ao gênero *Dickeya,* anteriormente caracterizadas pela espécie *Erwinia chrysanthemi*, causam doenças em uma ampla gama de plantas gerando grandes perdas economicamente importantes pelo mundo, com sintomas como apodrecimento e infecções vasculares que levam à murchas (Parkinson et al., 2009). Dentro desse gênero, Mansfield et al. em 2012 selecionaram duas espécies de fitopatógenos para ocupar a nona posição de sua lista, *Dickeya dadantii* e *Dickeya solani,* que de acordo com os autores, essas bactérias foram selecionadas por motivos distintos.

Dickeya dadantii é um patógeno oportunista que causa a doença da podridão mole em diversas plantas, incluindo cultivos de importância econômica, com a contribuição efetores translocados pelo sistema de secreção do tipo III. Essa bactéria foi incluída na lista de top 10 pelo fato do isolado 3937, membro de *Dickey*a, ter sido utilizado em diversos estudos de mecanismos moleculares da fitopatogenicidade bacteriana (Yang et al., 2002; Grenier et al., 2006; Mansfield et al., 2012). '*Dickeya solani'* é um nome proposto para isolados de *Dickeya*responsáveis pelo aumento repentino de doenças com sintomas de murcha lenta em plantações de batata (*Solanum tuberosum*) na Europa e Israel, com contribuição de enzimas de degradação de parede celular de plantas, como pectinases e celulases, sendo por isso selecionada para a lista de top 10 bactérias fitopatogênicas, porém, pouco se sabe sobre esse patógeno e estudos estão sendo feitos para suprir essa necessidade e permitir seu controle (Toth et al., 2011; Mansfield et al., 2012; van der Wolf et al., 2014; Pritchard et al., 2016).

Pectobacterium carotovorum (Pcc) e *Pectobacterium atrosepticum* (Pca), anteriormente classificadas como as subespécies de *Erwinia carotovora*, *carotovora* e *atroseptica* respectivamente, foram as últimas espécies de fitopatógenos adicionadas à lista, sendo consideradas patógenos "modelos" durante os primeiros estudos genéticos de fitopatogenicidade e responsáveis por causar sintomas de podridão mole em diversas culturas (Gardan et al., 2003; Mansfield et al., 2012). Essas bactérias produzem enzimas pectinases extracelulares em grandes quantidades, que são capazes de macerar o tecido parenquimatoso das plantas causando os sintoma de podridão mole (Perombelon & Kelman, 1980; Pérombelon, 2002). *Pectobacterium carotovorum* infecta uma ampla gama de hospedeiros espalhados pelo mundo, enquanto Pca é restrita às plantações de batata em regiões de climas temperados, levando à doença da canela preta, responsável por grandes perdas econômicas (Perombelon & Kelman, 1980; Pérombelon, 2002).

Além da sua importância econômica e científica, as bactérias supracitadas possuem ainda em comum o fato de apresentarem alta variabilidade genética (Mansfield et al., 2012). Variabilidade genética em bactérias é determinada em parte pela presença de elementos genéticos móveis. Alguns desses elementos, além do impacto óbvio da própria movimentação no genoma, estão envolvidos em processos de transferência horizontal de genes (Mira et al., 2002). A transferência horizontal de genes (THG) tem papel de extrema importância no direcionamento da evolução dos procariotos contribuindo para a adaptação genética desses microrganismos a alterações ambientais e a ocupação de novos nichos (Burrus & Waldor, 2004). A THG em bactérias ocorre a partir de três mecanismos, sendo eles transdução, transformação e conjugação (Wozniak & Waldor, 2010). Uma THG completa ocorre em três etapas: (i) a transferência do DNA entre as células, podendo envolver uma atuação viral (transdução), contato entre as células (conjugação) ou incorporação de material genético livre no ambiente (transformação); (ii) a disseminação da sequência de DNA adquirida por replicação desse material genético, seja por replicação autônoma ou concomitante a replicação do cromossomo e (iii) adaptação ao ambiente dependente de funções vantajosas codificadas pelo DNA adquirido, permitindo um sucesso evolutivo para a população (Bellanger et al., 2014). Nesse contexto, os elementos genéticos móveis autotransmissíveis têm papel fundamental por se tratar de sequências de DNA que codificam proteínas capazes de promover a HGT (Frost et al., 2005). Três tipos principais de elementos genéticos móveis autotransmissíveis são capazes de exercer THG: os bacteriófagos, os plasmídeos conjugativos e os elementos integrativos e conjugativos (da abreviação em inglês, *Integrative and Conjugative Eements* = ICEs) (Burrus, 2017).

Os ICEs são elementos móveis autotransmissíveis que variam em tamanho de 18 a mais de 600 kb, de modo semelhante aos plasmídeos conjugativos, codificam a maquinaria utilizada para a sua transferência por conjugação. Entretanto, esses elementos são normalmente encontrados integrados no cromossomo de seu hospedeiro e dessa forma codificam proteínas envolvidas no controle e regulação da sua integração, assim como na sua excisão do cromossomo (Johnson & Wozniak & Waldor, 2010; Grossman, 2015; Burrus, 2017). Sua propagação pode ocorrer passivamente, de forma simultânea com a replicação do cromossomo da célula hospedeira, ou em alguns casos, os ICEs podem ser capazes dereplicação autônoma semelhante à de plasmídeos (Johnson & Grossman, 2015).

Esses elementos são formados por sequências de estrutura modular típica de outros elementos móveis autotransmissíveis como plasmídeos e fagos, nesse caso, os genes que codificam proteínas com funções similares permanecem agrupados e podem ser recombinados entre ICEs ou entre o ICE e o cromossomo levando à novos elementos potenciais (Burrus & Waldor, 2004; Johnson & Grossman, 2015; Burrus, 2017). Os principais módulos encontrados nos ICEs são os responsáveis por mediar sua integração e excisão do cromossomo hospedeiro, conjugação e regulação, que podem ter conteúdo variável nos diferentes ICEs levando a diferentes mecanismos a partir de uma diversa gama de genes (Toussaint & Merlin, 2002; Wozniak & Waldor, 2010). Os ICEs possuem regiões necessárias para o processamento do DNA que será transferido, porém os genes responsáveis por essa etapa não ficam ativos no período em que o ICE está integrado no cromossomo, devido a uma repressão de sua transcrição, mas são induzidos em algumas condições específicas como a resposta SOS, *quorum sensing*, durante a fase estacionária ou induzida por alguma substância (Johnson & Grossman, 2015). Além dessas características, já foram relatados na literatura, os ICEs chamados tripartidos, possuem três regiões integradas separadamente no cromossomo hospedeiro que, após o processo de excisão se associam por processos de recombinação entre as regiões flanqueadoras formando uma única região que é transferida por conjugação como um único elemento circular (Haskett et al., 2017).

Quando integrado no cromossomo a sequência do ICE é flanqueada por duas sequências específicas de repetições diretas que possuem entre 10 e 60 pares de bases, denominadas *attR* (à direita) e *attL* (à esquerda) importantes para o processo de excisão do cromossomo (Wozniak & Waldor, 2010; Delavat et al., 2017). Dessa forma, a indução da expressão dos genes permite a excisão do ICE a partir de um evento de recombinação entre as sequências *attR* e *attL* mediada pela proteína integrase, levando à formação de uma molécula circular de DNA dupla fita, e gerando as sequências *attP* (presente na ICE) e *attB* (no cromossomo).

Proteínas de formação de pares de acasalamento (do inglês *Mating pair formation*) pertencentes ao sistema de secreção do tipo IV (T4SS), codificadas pelo ICE, formam o poro de acoplamento responsável pela transferência do ICE (Wozniak & Waldor, 2010; Johnson &

Grossman, 2015) A partir desse ponto, outras proteínas codificadas pelo ICE, como a relaxase, reconhecem a sequência de origem de transferência (*oriT*) presente no ICE e processam esse DNA extracromossomal, um processo semelhante ao processamento de plasmídeos, levando a formação de um complexo linear de DNA fita simples ligado às proteínas, chamado DNA de transferência, a partir do processo de replicação em círculo rolante (Wozniak & Waldor, 2010; Johnson & Grossman, 2015). Esse complexo é levado para a célula receptora pelas proteínas do T4SS, onde servirá de molde para que a DNA polimerase possa formar novamente a molécula circular de dupla fita que se integrará no novo cromossomo hospedeiro a partir de recombinação sítio-específica entre as regiões *attB* e *attP*, um processo também catalisado pela integrase ou transposase DDE em alguns casos (Johnson & Grossman, 2015; Delavat et al., 2017). Similar aos plasmídeos conjugativos, os ICEs também podem codificar proteínas de exclusão de entrada que inibem a transferência de DNA repetitivo. São proteínas pequenas que se localizam na membrana da célula hospedeira e impedem que uma célula que já possui o elemento o receba novamente, podendo levar à morte celular (Auchtung et al., 2007; Marrero & Waldor, 2007; Avello et al., 2019) .

Nesse processo de transferência, onde ocorre a replicação em círculo rolante, os ICEs não aumentam em número de cópias em seu hospedeiro, logo sendo não replicativo (Wozniak & Waldor, 2010). No entanto, alguns desses elementos podem ter formas replicativas, sendo capazes de se duplicar de forma autônoma quando estão excisados do cromossomo hospedeiro, o que pode levar a uma maior estabilidade e maior frequência de transferência, já que cada elemento individualmente irá iniciar um novo processo de conjugação individualmente (Wozniak & Waldor, 2010 Carraro & Burrus, 2015).

Os ICEs carregam frequentemente genes acessórios que não possuem papel na sua transmissão ou em outras funções principais do elemento, mas que podem conferir um fenótipo distinto a seu hospedeiro, gerando vantagens adaptativas em algumas circunstâncias (Carraro & Burrus, 2015; Burrus, 2017). O estudo dos genes acessórios carregados pelos ICEs permitiu o descobrimento desses elementos, já que os fenótipos atribuídos como resistência à antibióticos, facilitaram sua identificação inicial. Recentemente, com o avanço e barateamento das tecnologias de sequenciamento de DNA o estudo desses elementos pode ser feito de forma ampla por meio de análises de genomas, porém as análises *in silico* isoladamente não fornecem prova do benefício dos genes acessórios para o hospedeiro, demandando a necessidade adicionais de análises funcionais (Johnson & Grossman, 2015).

Entre os fenótipos conferidos pelos genes acessórios presentes em ICEs já foi caracterizada a resistência à antibióticos, como na ICETn43716061 de *Pseudomonas aeruginosa* que carrega o gene *bla*SPM-1, que tem como produto uma metalo- β-lactamase, que confere resistência aos antibióticos carbapenêmicos (Fonseca et al., 2015) e o ICE*Pmu*1 de *Pasteurella multocida* que carrega 11 genes que codificam proteínas ativas contra vários antibióticos (Michael et al., 2012). Estudos também apresentam a resistência à metais pesados sendo conferida por ICEs, como em cepas de *Pseudomonas syringae* pv. *actinidiae* isoladas da Nova Zelândia, que carregavam sequências de diferentes ICEs conferindo resistência à arsênio, e ao cobre (Colombi et al., 2017). Alguns desses elementos também já foram descritos conferindo fenótipos como o aumento da adaptabilidade do hospedeiro, e a capacidade de fixação de nitrogênio (Sullivan & Ronson, 1998).

Já foi documentada a influência de genes acessórios presentes em ICEs na virulência de bactérias hospedeiras de humanos, como o ICE*Pm1* encontrado de forma conservada em *Proteus mirabilis*, *Providencia stuartii* e *Morganella morgani*, bactérias agentes causais de infecção urinária associada à cateter. O *ICEPm1* carrega genes que codificam proteínas como adesina protease e um sistema de aquisição de ferro que contribuem para a virulência nessas bactérias (Flannery et al., 2009, 2011; Alamuri et al., 2010; Himpsl et al., 2010). Em relação às bactérias fitopatogênicas, Bell et al. (2004) sequenciaram o genoma da cepa SCRI1043 de *Pectobacterium atrosepticum* e identificaram nessa bactéria um *cluster* de genes de biossíntese de ácido coronafácico, um fator de virulência de grande importância, sendo carregado pelo ICE HAI2. Posteriormente foi demonstrado que a presença do ICE carregando esse cluster gênico aumenta a virulência da bactéria em cultivos de batata, causando murcha ou podridão do caule dessas plantas (Panda et al., 2016). Nosso grupo de pesquisa também tem caracterizado *in silico* os ICEs nos genomas pertencentes ao complexo *Ralstonia solanacearum*. Um desses ICEs, o ICETn4371, foi identificado na linhagem modelo *Ralstonia solanacearum* GM1000, recentemente reclassificada como *R. pseudosolanacearum* (Safni et al., 2014; Prior et al., 2016). O ICETn4371 codifica enzimas hidrolíticas e proteínas de degradação de parede celular do hospedeiro, o que revela um possível impacto na virulência desse isolado (Salanoubat et al., 2002; Gonçalves et al., 2020).

Dessa forma, o estudo de ICEs em bactérias fitopatogênicas é importante para entender a função desses elementos nas doenças causadas pelos patógenos e na sua adaptação ao ambiente, e com isso indicar a importância de ICEs para a transferência horizontal de genes relevantes para essas bactérias.

Bibliografia

Alamuri, P., Löwer, M., Hiss, J. A., Himpsl, S. D., Schneider, G., & Mobley, H. L. T. (2010). Adhesion, invasion, and agglutination mediated by two trimeric autotransporters in the human uropathogen *Proteus mirabilis*. *Infection and Immunity*, *78*(11), 4882–4894. https://doi.org/10.1128/IAI.00718-10

Auchtung, J. M., Lee, C. A., Garrison, K. L., & Grossman, A. D. (2007). Identification and characterization of the immunity repressor (ImmR) that controls the mobile genetic element ICEBs1 of *Bacillus subtilis*. *Molecular Microbiology*, *64*(6), 1515–1528. https://doi.org/10.1111/j.1365-2958.2007.05748.x

Avello, M., Davis, K. P., & Grossman, A. D. (2019). Identification, characterization and benefits of an exclusion system in an integrative and conjugative element of *Bacillus subtilis*. *Molecular Microbiology*, *112*(4), 1066–1082.https://doi.org/10.1111/mmi.14359

Bellanger, X., Payot, S., Leblond-Bourget, N., & Guédon, G. (2014). Conjugative and mobilizable genomic islands in bacteria: Evolution and diversity. *FEMS Microbiology Reviews*, *38*(4), 720–760. https://doi.org/10.1111/1574-6976.12058

Burrus, V. (2017). Mechanisms of stabilization of integrative and conjugative elements. *Current Opinion in Microbiology*, *38*, 44–50. https://doi.org/10.1016/j.mib.2017.03.014

Burrus, V., & Waldor, M. K. (2004). Shaping bacterial genomes with integrative and conjugative elements. *Research in Microbiology*, *155*(5), 376–386. https://doi.org/10.1016/j.resmic.2004.01.012

Carraro, N., & Burrus, V. (2015). The dualistic nature of integrative and conjugative elements. *Mobile Genetic Elements*, *5*(6), 98–102.https://doi.org/10.1080/2159256x.2015.1102796

Chatterjee, S., Almeida, R. P. P., & Lindow, S. (2008). Living in two worlds: The plant and insect lifestyles of *Xylella fastidiosa*. *Annual Review of Phytopathology*, *46*, 243–271. https://doi.org/10.1146/annurev.phyto.45.062806.094342

Colombi, E., Straub, C., Künzel, S., Templeton, M. D., McCann, H. C., & Rainey, P. B. (2017). Evolution of copper resistance in the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* through acquisition of integrative conjugative elements and plasmids. *Environmental Microbiology*, *19*(2), 819–832. https://doi.org/10.1111/1462-2920.13662

Delannoy, E., Lyon, B. R., Marmey, P., Jalloul, A., Daniel, J. F., Montillet, J. L., Essenberg, M., & Nicole, M. (2005). Resistance of cotton towards *Xanthomonas campestris* pv. *malvacearum*. *Annual Review of Phytopathology*, *43*(5), 63–82. https://doi.org/10.1146/annurev.phyto.43.040204.140251

Delavat, F., Miyazaki, R., Carraro, N., Pradervand, N., & van der Meer, J. R. (2017). The hidden life of integrative and conjugative elements. *FEMS Microbiology Reviews*, *41*(4), 512–537. https://doi.org/10.1093/femsre/fux008

Denny, T. P. (2006). Plant pathogenic Ralstonia species. In *Plant-Associated Bacteria* (pp. 573–644).

Doucouré, H., Pérez-Quintero, A. L., Reshetnyak, G., Tekete, C., Auguy, F., Thomas, E., Koebnik, R., Szurek, B., Koita, O., Verdier, V., & Cunnac, S. (2018). Functional and genome sequence-driven characterization of tal effector gene repertoires reveals novel variants with altered specificities in closely related malian *Xanthomonas oryzae* pv. *oryzae* strains. *Frontiers in Microbiology*, *9*(AUG), 1–17. https://doi.org/10.3389/fmicb.2018.01657

Escobar, M. A., & Dandekar, A. M. (2003). *Agrobacterium tumefaciens* as an agent of disease. *Trends in Plant Science*, *8*(8), 380–386. https://doi.org/10.1016/S1360- 1385(03)00162-6

Flannery, E. L., Antczak, S. M., & Mobley, H. L. T. (2011). Self-transmissibility of the integrative and conjugative element ICEPm1 between clinical isolates requires a functional integrase, relaxase, and type IV secretion system. *Journal of Bacteriology*, *193*(16), 4104–4112. https://doi.org/10.1128/JB.05119-11

Flannery, E. L., Mody, L., & Mobley, H. L. T. (2009). Identification of a modular pathogenicity island that is widespread among urease-producing uropathogens and shares features with a diverse group of mobile elements. *Infection and Immunity*, *77*(11), 4887– 4894. https://doi.org/10.1128/IAI.00705-09

Fonseca, E. L., Marin, M. A., Encinas, F., & Vicente, A. C. P. (2015). Full characterization of the integrative and conjugative element carrying the metallo-β-lactamase blaSPM-1 and bicyclomycin bcr1 resistance genes found in the pandemic *Pseudomonas aeruginosa* clone SP/ST277. *Journal of Antimicrobial Chemotherapy*, *70*(9), 2547–2550. https://doi.org/10.1093/jac/dkv152

Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: The agents of open source evolution. *Nature Reviews Microbiology*, *3*(9), 722–732. https://doi.org/10.1038/nrmicro1235

Gabriel, D. W., Burges, A., & Lazo, G. R. (1986). Gene-for-gene interactions of five cloned avirulence genes from *Xanthomonas campestris* pv. *malvacearum* with specific resistance genes in cotton. *Proceedings of the National Academy of Sciences*, *83*(17), 6415–6419. https://doi.org/10.1073/pnas.83.17.6415

Gardan, L., Gouy, C., Christen, R., & 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. *International Journal of Systematic and Evolutionary Microbiology*, *53*(2), 381–391. https://doi.org/10.1099/ijs.0.02423-0

Gelvin, S. B. (2003). Agrobacterium-Mediated Plant Transformation: the Biology behind the "Gene-Jockeying" Tool. *Microbiology and Molecular Biology Reviews*, *67*(1), 16–37. https://doi.org/10.1128/mmbr.67.1.16-37.2003

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytologist*, *187*(4), 920–928. https://doi.org/10.1111/j.1469-8137.2010.03397.x

Gonçalves, O. S., de Queiroz, M. V., & Santana, M. F. (2020). Potential evolutionary impact of integrative and conjugative elements (ICEs) and genomic islands in the *Ralstonia solanacearum* species complex. *Scientific Reports*, *10*(1), 1–13. https://doi.org/10.1038/s41598-020-69490-1

Green, S., Studholme, D. J., Laue, B. E., Dorati, F., Lovell, H., Arnold, D., Cottrell, J. E., Bridgett, S., Blaxter, M., Huitema, E., Thwaites, R., Sharp, P. M., Jackson, R. W., & Kamoun, S. (2010). Comparative genome analysis provides insights into the evolution and adaptation of *Pseudomonas syringae* pv. *aesculi* on *Aesculus hippocastanum*. *PLoS ONE*, *5*(4), 1–14. https://doi.org/10.1371/journal.pone.0010224

Grenier, A. M., Duport, G., Pagès, S., Condemine, G., & Rahbé, Y. (2006). The phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) is a pathogen of the pea aphid. *Applied and Environmental Microbiology*, *72*(3), 1956–1965. https://doi.org/10.1128/AEM.72.3.1956-1965.2006

Guo, M., Ye, J., Gao, D., Xu, N., & Yang, J. (2019). Agrobacterium-mediated horizontal gene transfer: Mechanism, biotechnological application, potential risk and forestalling strategy. *Biotechnology Advances*, *37*(1), 259–270.https://doi.org/10.1016/j.biotechadv.2018.12.008

Haskett, T. L., Ramsay, J. P., Bekuma, A. A., Sullivan, J. T., O'Hara, G. W., & Terpolilli, J. J. (2017). Evolutionary persistence of tripartite integrative and conjugative elements. *Plasmid*, *92*(June), 30–36. https://doi.org/10.1016/j.plasmid.2017.06.001

Himpsl, S. D., Pearson, M. M., Arewng, C. J., Nusca, T. D., Sherman, D. H., & Mobley, H. L. T. (2010). Proteobactin and a yersiniabactin-related siderophore mediate iron acquisition in *Proteus mirabilis*. *Molecular Microbiology*, *78*(1), 138–157. https://doi.org/10.1111/j.1365-2958.2010.07317.x

Johnson, C. M., & Grossman, A. D. (2015). Integrative and Conjugative Elements (ICEs): What They Do and How They Work. *Annual Review of Genetics*, *49*, 577–601.

https://doi.org/10.1146/annurev-genet-112414-055018

Kennelly, M. M., Cazorla, F. M., Vicente, A. de, Ramos, C., & Sundin, G. W. (2007). *Pseudomonas syringae* Diseases of Fruit Trees - Progress toward Understanding and Control. *Plant Disease*, *91*(1), 4–17.

Lindemann, J. (1984). Epiphytic Populations of *Pseudomonas syringae* pv. *syringae* on Snap Bean and Nonhost Plants and the Incidence of Bacterial Brown Spot Disease in Relation to Cropping Patterns In *Phytopathology* (Vol. 74, Issue 11, p. 1329). https://doi.org/10.1094/phyto-74-1329

López, C. E., & Bernal, A. J. (2012). Cassava Bacterial Blight: Using Genomics for the Elucidation and Management of an Old Problem. *Tropical Plant Biology*, *5*(1), 117–126. https://doi.org/10.1007/s12042-011-9092-3

Lozano, J. C., and Luis Sequeira. "Bacterial blight of cassava in Colombia: epidemiology and control." *Phytopathology* 64.1 (1974): 83-88.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., & Foster, G. D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, *13*(6), 614–629. https://doi.org/10.1111/j.1364-3703.2012.00804.x

Marrero, J., & Waldor, M. K. (2007). Determinants of entry exclusion within Eex and TraG are cytoplasmic. *Journal of Bacteriology*, *189*(17), 6469–6473.https://doi.org/10.1128/JB.00522-07

McManus, P. S., Stockwell, V. O., Sundin, G. W., & Jones, A. L. (2002). Antibiotic use in plant agriculture. *Annual Review of Phytopathology*, *40*(18), 443–465. https://doi.org/10.1146/annurev.phyto.40.120301.093927

Michael, G. B., Kadlec, K., Sweeney, M. T., Brzuszkiewicz, E., Liesegang, H., Daniel, R., Murray, R. W., Watts, J. L., & Schwarz, S. (2012). ICEPmu1, an integrative conjugative element (ICE) of *Pasteurella multocida*: Analysis of the regions that comprise 12 antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy*, *67*(1), 84–90. https://doi.org/10.1093/jac/dkr406

Midha, S., Bansal, K., Kumar, S., Girija, A. M., Mishra, D., Brahma, K., Laha, G. S., Sundaram, R. M., Sonti, R. V., & Patil, P. B. (2017). Population genomic insights into variation and evolution of *Xanthomonas oryzae* pv. *oryzae*. *Scientific Reports*, *7*(January), 1–13. https://doi.org/10.1038/srep40694

Mira, A., Klasson, L., & Andersson, S. G. E. (2002). Microbial genome evolution: Sources of variability. *Current Opinion in Microbiology*, *5*(5), 506–512. https://doi.org/10.1016/S1369-

5274(02)00358-2

Monteil, C. L., Bardin, M., & Morris, C. E. (2014). Features of air masses associated with the deposition of *Pseudomonas syringae* and *Botrytis cinerea* by rain and snowfall. *ISME Journal*, *8*(11), 2290–2304. https://doi.org/10.1038/ismej.2014.55

Morris, C. E., Kinkel, L. L., Xiao, K., Prior, P., & Sands, D. C. (2007). Surprising niche for the plant pathogen *Pseudomonas syringae*. *Infection, Genetics and Evolution*, *7*(1), 84– 92. https://doi.org/10.1016/j.meegid.2006.05.002

Niño-Liu, D. O., Ronald, P. C., & Bogdanove, A. J. (2006). *Xanthomonas oryzae* pathovars: Model pathogens of a model crop. *Molecular Plant Pathology*, *7*(5), 303–324. https://doi.org/10.1111/j.1364-3703.2006.00344.x

Panda, P., Vanga, B. R., Lu, A., Fiers, M., Fineran, P. C., Butler, R., Armstrong, K., Ronson, C. W., & Pitman, A. R. (2016). *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* harbor distinct, independently acquired integrative and conjugative elements encoding coronafacic acid that enhance virulence on potato stems. *Frontiers in Microbiology*, *7*(MAR), 1–13. https://doi.org/10.3389/fmicb.2016.00397

Parkinson, N., Stead, D., Bew, J., Heeney, J., Tsror, L., & Elphinstone, J. (2009). Dickeya species relatedness and clade structure determined by comparison of recA sequences. *International Journal of Systematic and Evolutionary Microbiology*, *59*(10), 2388–2393. https://doi.org/10.1099/ijs.0.009258-0

Pérombelon, M. C. M. (2002). Potato diseases caused by soft rot erwinias: An overview of pathogenesis. *Plant Pathology*, *51*(1), 1–12. https://doi.org/10.1046/j.0032

Perombelon, M. C. M., & Kelman, A. (1980). Ecology of the Soft Rot Erwinias. *Annual Review of Phytopathology*, *18*(1), 361–387.https://doi.org/10.1146/annurev.py.18.090180.002045

Piqué, N., Miñana-Galbis, D., Merino, S., & Tomás, J. M. (2015). Virulence factors of *Erwinia amylovora*: A review. *International Journal of Molecular Sciences*, *16*(6), 12836–12854. https://doi.org/10.3390/ijms160612836

Prior, P., Ailloud, F., Dalsing, B. L., Remenant, B., Sanchez, B., & Allen, C. (2016). Genomicand proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. *BMC Genomics*, *17*(1), 1–11.https://doi.org/10.1186/s12864-016-2413-z

Pritchard, L., Glover, R. H., Humphris, S., Elphinstone, J. G., & Toth, I. K. (2016). Genomicsand taxonomy in diagnostics for food security: Soft-rotting enterobacterial plant pathogens. *Analytical Methods*, *8*(1), 12–24. https://doi.org/10.1039/c5ay02550h

Purcell, A. H., & Hopkins, D. L. (1996). Fastidious xylem-limited bacterial plant pathogens. *Annual Review of Phytopathology*, *34*, 131–151.

https://doi.org/10.1146/annurev.phyto.34.1.131

Safni, I., Cleenwerck, I., De Vos, P., Fegan, M., Sly, L., & Kappler, U. (2014). Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: Proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains *as Ralstonia syzygii* subsp. *syzygii* subsp. nov., R. s. *International Journal of Systematic and Evolutionary Microbiology*, *64*, 3087–3103. https://doi.org/10.1099/ijs.0.066712-0

Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottiert, P., Camus, J. C., Cattolico, L., Chandler, M., Choisne, N., Claudel-Renard, C., Cunnac, S., Demange, N., Gaspin, C., Lavie, M., Moisan, A., Robert, C., Boucher, C. A. (2002). Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature*, *415*(6871), 497–502. https://doi.org/10.1038/415497a

Sullivan, J. T., & Ronson, C. W. (1998). Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(9), 5145–5149. https://doi.org/10.1073/pnas.95.9.5145

Swings, Jean, and Lucia Civetta. *Xanthomonas*. *Springer Science & Business Media*, 2012.

Swings, J., Van Den Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T. W., & Kersters, K. (1990). Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. rev. *International Journal of Systematic Bacteriology*, *40*(3), 309–311. https://doi.org/10.1099/00207713-40-3-309

Toth, I. K., van der Wolf, J. M., Saddler, G., Lojkowska, E., Hélias, V., Pirhonen, M., Tsror (Lahkim), L., & Elphinstone, J. G. (2011). Dickeya species: An emerging problem for potato production in Europe. *Plant Pathology*, *60*(3), 385–399. https://doi.org/10.1111/j.1365-3059.2011.02427.

Toussaint, A., & Merlin, C. (2002). Mobile elements as a combination of functional modules. *Plasmid*, *47*(1), 26–35. https://doi.org/10.1006/plas.2001.1552

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., Potrykus, M., Waleron, M., de Vos, P., Cleenwerck, I., Pirhonen, M., Garlant, L., Hélias, V., Pothier, J. F., Pflüger, V., Duffy, B., Manulis, S. (2014). *Dickeya solani* sp. nov., a pectinolytic plant- pathogenic bacterium isolated from potato (*Solanum tuberosum*). *International Journal of Systematic and*

Evolutionary Microbiology, *64*(PART 3), 768–774. https://doi.org/10.1099/ijs.0.052944-0

Vanneste, J.L. Fire blight: The disease and its causative agent. In *Erwinia Amylovora*;

CABI Publishing: Oxfordshire, UK, 2000

Verdier, V., Restrepo, S., Mosquera, G., Jorge, V., & Lopez, C. (2004). Recent progress in the characterization of molecular determinants in the *Xanthomonas axonopodis* pv. *manihotis*-cassava interaction. *Plant Molecular Biology*, *56*(4), 573–584. https://doi.org/10.1007/s11103-004-5044-8

Vicente, J. G., Conway, J., Roberts, S. J., & Taylor, J. D. (2001). Identification and origin of *Xanthomonas campestris* pv. *campestris* Races and related pathovars. *Phytopathology*, *91*(5), 492–499. https://doi.org/10.1094/PHYTO.2001.91.5.492

Wozniak, R. A. F., & Waldor, M. K. (2010). Integrative and conjugative elements: Mosaic mobile genetic elements enabling dynamic lateral gene flow. *Nature Reviews Microbiology*, *8*(8), 552–563. https://doi.org/10.1038/nrmicro2382

Yang, C. H., Gavilanes-Ruiz, M., Okinaka, Y., Vedel, R., Berthuy, I., Boccara, M., Wei-Ta Chen, J., Perna, N. T., & Keen, N. T. (2002). hrp genes of *Erwinia chrysanthemi* 3937 are important virulence factors. *Molecular Plant-Microbe Interactions*, *15*(5), 472–480. https://doi.org/10.1094/MPMI.2002.15.5.472

Zambryski, P. C. (1992). Chronicles from the Agrobacterium-plant cell DNA transfer story. *Annual Review of Plant Physiology and Plant Molecular Biology*, *43*(1), 465–490. https://doi.org/10.1146/annurev.pp.43.060192.002341

CAPÍTULO 2

Integrative and Conjugative Elements (ICEs) Potentially Influence the Lifestyle of Most Relevant Phytopathogenic Bacteria

Integrative and Conjugative Elements (ICEs) Potentially Influence the Lifestyle of Most Relevant Phytopathogenic Bacteria

Abstract

ICEs are mobile elements that remain integrated into bacterial chromosomes and play an important role in horizontal gene transmission. These elements also carry accessory genes that have the potential to provide interesting phenotypes for the host bacteria such as antibiotic resistance and virulence. In our work, we seek to demonstrate the presence of ICEs in 300 complete genomes of phytopathogenic bacteria with the greatest scientific and economic impact. A total of 78 ICEs (45 different elements) were identified and characterized in chromosomes of *A. tumefaciens* (4 ICEs), *D. dadantii* and *D. solani* (3), *P. carotovorum* and

P. atrosepticum (6), *P. syringae* (20), *R. solanacearum* species complex (7), and *X. campestris* (2). Intriguingly, we were able to track down the co-occurrence of four ICEs in genomes of some *P. syringae* isolates, but also no elements were found in the genomes of *X. oryzae*, *E. amylovora* and *X. axonopodis* analyzed. Moreover, our work has identified 31 novel elements, among them ICEPc2, uncover in genomes of *P. carotovorum* and *S. plymuthica,* as an evidence of horizontal gene transfer between different species, and the ICE ICEDs.1 was spotted in all *Dickeya solani* genomes available, indicating a possible ancestral acquisition of the element. Among the 396 cargo genes characterized, most identified elements carry genes with potential influence on virulence and fitness, such as genes coding for functions related to T3SS, T6SS, cell wall degradation, toxin, superficial adhesion, detoxification, and resistance to heavy metals. We also present the expression of cargo genes related to virulence of the known element ICEPa.1 and the novel ICEPa.2 in *P. atrosepticum* SCI1043, evidencing the role of these genes in the infection process of tobacco plants. Furthermore, this paper has highlighted the potential of ICEs in influencing the pathogenicity and lifestyle of phytopathogens, establishing knowledge about these elements and bacteria.

Introduction

Integrative and conjugative elements (ICEs) are self-transmissible mobile elements that play a central role in bacterial adaptation processes; hence they can directly affect the evolution of their host (Carraro & Burrus, 2015; Burrus, 2017). These widely distributed elements are currently found integrated into the bacterial chromosome, as they are capable of performing their excision, by recombination of direct repetition sequences (DRs) that flank the element (attachment sites), and transference by conjugation, carrying their machinery in a modular structure (Guglielmini et al., 2011; Wozniak & Waldor, 2010; Johnson & Grossman, 2015). The main genetic modules found in ICEs include genes that encode functions related to mediation of their integration and excision from host chromosome, conjugation and regulation, the modules have variable content, leading to a performance of these functions by different mechanisms from a diverse range of genes (Toussaint & Merlin, 2002; Wozniak & Waldor, 2010). ICEs often carry cargo genes conferring significant phenotypes like virulence, resistance to antibiotics, and heavy metals that are important to bacteria fitness, for instance, ICE$_{\text{Tn}4371}$6061 discovered on *Pseudomonas aeruginosa,* providing resistance against carbapenem antibiotics, and ICE*Pm1* of *Proteus mirabilis*, *Providencia stuartii*, and *Morganella morgani*, that carries genes that encode a adhesion protease and an iron acquisition system that contribute to virulence in these bacteria (Flannery et al., 2009, 2011; Fonseca et al., 2015; Johnson & Grossman, 2015)

ICEs are broadly distributed in bacterial chromosomes, and some studies demonstrate their presence in phytopathogenic bacteria, microorganisms that are involved in major crop losses by host tissue invasion using virulence factors as biofilm formation and toxins (Mole et al., 2007; Guglielmini et al., 2011). For instance, different integrative and conjugative elements were found in strains of *Pseudomonas syringae* pv. *actinidiae* conferring resistance to heavy metals, and ICE HAI2 of *Pectobacterium atrosepticum* that transmit genes that codify a biosynthetic cluster of an important virulence factor (Panda et al., 2016; Colombi et al., 2017). Among these phytopathogenic bacteria, the most relevant were classified by their importance in science and economic impact by Mansfield et al., in 2012, in a ranking composed by *Pseudomonas syringae, Ralstonia solanacearum, Agrobacterium tumefaciens, Xanthomonas oryzae pv. oryzae, Xanthomonas campestris, Xanthomonas azonopodis pv. manihotis, Erwinia amylovora, Xylella fastidiosa, Dickeya* (*dadantii* and *solani*) and *Pectobacterium* (*carotovorum* and *atrosepticum*) (Mansfield et al., 2012).

A large part of pioneer studies involving ICEs was based only on phenotypes conferred by cargo genes, not providing a broader knowledge about these elements (Johnson & Grossman, 2015). However, the development of Whole-Genome Sequencing (WGS) efforts leading to the large availability of complete genome sequences has enabled the investigation to better understand the role of ICEs in bacterial evolution (Panda et al., 2016; Botelho & Schulenburg, 2020). Thus, here we search for ICEs integrated into 300 complete genomes of major phytopathogenic bacteria, analyzing putative cargo genes and their potential role in virulence or adaptation, starting to establish a relationship between theseelements and phytopathogenic bacteria.

Methods

Data

Three hundred complete genomes of phytopathogenic bacteria of economic and scientific impact (Mansfield et al., 2012) were downloaded from the National Center for Biotechnology Information (NCBI - http://ftp.ncbi.nih.gov/assembly) in July 2020, and the chromosome sequences provided by GenBank were used to search for ICEs (Supplementary Table 1-10).

Identification and characterization of Integrative and Conjugative Elements

To uncover sequences of ICEs in bacterial chromosomes, we resort to a methodsimilar to what was applied by Gonçalves and Santana (2021). Thus, we perform a search ofthe element sequences using BLASTn (Altschul et al., 1990) against known ICEs deposited inthe ICEberg database (M. Liu et al., 2019), and only sequences that obtained an E-value lessor equal to 10^{-5} and coverage more or equal to 50% were selected. We submit the nucleotidesequencesof ICEs to the software ICEfinder (https://dbmml.sjtu.edu.cn/ICEfinder/ICEfinder.html), an online tool provided by ICEberg 2.0 thatidentifies signature features of integrative and conjugative elements as integrase gene, type IVsecretion system (T4SS), and directed repeats sequences (DRs) in bacterial genomes (M. Liu et al., 2019), OriTfinder (Li et al., 2018) (https://toolmml.sjtu.edu.cn/oriTfinder/oriTfinder.html) that identifies transfer origin sequences inbacterial chromosomes, among other features, which shows an indication of the presence ofthe ICE, MOBscan (https://castillo.dicom.unican.es/mobscan/) that identifies relaxase MOB families, and CONJScan (https://galaxy.pasteur.fr/root?tool_id=toolshed.pasteur.fr/repos/odoppelt/conjscan/ConjScan) that identifies conjugative systems in bacterial genomes searching for Type IV secretion systems (Cury et al., 2020). We also execute a manual search on the sequences of complete and annotated chromosomes looking for ICEs signature genes: genes that are part of the T4SS involved in conjugative transfer such as *tra*, *vir* or *trb,* and Integrase (*int*) (Wozniak & Waldor, 2010). The delimitation of elements and identification of *att* regions that were not detected by ICEfinder were made using BLASTn. Initially we delimited the element by looking for genes of integrases close to tRNAs, after that region between these genes was selected to make the BLAST looking for a repeated sequence in another position of the genome that was close to the coordinates the possible final portion of the element given by ICEfinder.

Protein sequence annotation was realized with Uniprot (https://www.uniprot.org/) (Bateman, 2019), and Pfam (http://pfam.xfam.org/) (Bateman et al., 2004) protein databases. Putative functions of accessory genes were investigated performing a BLASTp against Pathogen-host Interactions database (http://www.phi-base.org/) (Urban et al., 2017), Virulence Factor Database (http://www.mgc.ac.cn/VFs/) (B. Liu et al., 2019), and Type III secretion system effectors database (http://effectors.bic.nus.edu.sg/blast.php) (Tay et al., 2010). The parameters used to identify sequences coding proteins were: e-value less or equal to 10^{-5}, and amino acid identity greater than 30% (Gonçalves et al., 2020).

Subsequently, the nucleotide sequences of identified elements were downloaded in GenBank format and analyzed using the Geneious® software (Biomatters Ltd.) for the characterization of the excision and integration, conjugation, regulation, and maintenance modules. ICEs were named following patterns already described (Burrus et al., 2002).

Differential expression analysis

Differential expression analysis was performed using RNA seq data from *P. atrosepticum* isolate SCRI1043 corresponding to two stages of infection in tobacco plant (asymptomatic and symptomatic) and an *in vitro* culture. The data are available in NCBI BioProject (accession number PRJNA403794) (Gorshkov et al., 2018). The analysis was done using the Geneious software following the Expression Analysis tutorial with default parameters. To study the gene expression of the ICEs present in the genome, the element sequence was used as reference to map the reads and differential expression was measured. Reads per kilobase per million (RPKM) values were plotted in GraphPad Prism version 8.4.3 to generate heatmaps.

Comparative Analysis

Nucleotide sequences of ICEs identified were submitted to ClustalW (Larkin et al., 2007) to generate Pairwise Identity Matrices for Heatmaps creation, performed using

GraphPad Prism version 8.4.3 for Windows. The sequences were also submitted to Mauve (Darling et al., 2004),and clinker clustermap.js (Gilchrist & Chooi, 2020) to generate gene cluster comparison and synteny analyses.

Results

Identification of ICEs in bacterial chromosomes.

Knowing the significance of these bacteria for large plant cultivations around the world, we seek to investigate the presence of ICEs in their genomes. After an extensive search on chromosomal sequences, a total of 78 putative elements were found in nine species of phytopathogenic bacteria, being them: *D. dadantii, D. solani, P. atrosepticum, P. carotovorum, A. tumefaciens, P. syringae, X. campestris, X. fastidiosa* and *R. solanacearum species* complex (Figure 1 and Table S11). Among these elements, 45 are distinct, and *P. syringae* was the species where a greater number of ICEs were found: 33 in total and 20 distinct elements (Figure 2-A). No ICEs were found into complete chromosomal sequences of *X. oryzae, X. axonopodis,* and *E. amylovora.*

The elements exhibited a great variation in sequence size, the largest was the ICEPs.10 element of *P. syringae* with 161.009 base pairs (bp) and the smallest, ICEDd.2 of *Dickeya dadantii* with 40.117 bp. The average size of the elements was 80.187 bp. The GC content varied from 40,07 (ICEPa.2 – *P. atrosepticum*) to 66,4 (ICEXf.2 – *X. fastidiosa*), and there was an average of 55,07 in all characterized elements (Table S12). As we expected, no relationship was found between the increase in the size of the host genome and the presence of the elements (Figure 2-B).

Most elements (68 elements -88,3%) were found inserted in tRNA sequences, whilst nine were found in other genes. Attachment sites (*att*) were identified in 49 ICEs, however our search methods did not allow to find the *att* sites in any of the elements found in *R. solanacearum* species complex (Table S13). Among the types of Integrase and Relaxase, the Tyr and MOBh types predominated, present in a total of 58 and 34 ICEs respectively (Figure 2-C, D- Table S14).

Interestingly, not all elements found are unique to their species. We identified ICEPc.2 of *P. carotovorum* in the genome of C-1 isolate of *Serratia plymuthica*, and comparative analyses of the elements presented 99.97% of nucleotide identity between the sequences. In addition to that, some of our ICEs showed high similarity with elements of other species such as ICEPc.1that presented 82.34% of nucleotide identity with a novel ICE of *Pectobacterium aroidearum* identified in L6 isolate, and ICEXc.1, with a novel ICE of *Xanthomonas arboricola* pv. juglandis isolate Xaj 417 (88.79% of nucleotide identity). Among the bacterial species studied in this work, two of our ICEs, ICEDd.1of *Dickeya dadantii* and Tn4371 of *Ralstonia pseudosolanacearum* were previously classified in the same family, named Tn4371(Gonçalves et al., 2020; Toussaint et al., 2003). However, sequence comparativeanalyses indicated a low similarity between these elements (45.36% of nucleotide identity).

The genes carried by the elements were also investigated, and classified by their putative role. As expected, a greater number of hypothetic and genes with unknown function were identified, followed by conjugation and cargo genes in general (Figure 3A). Between the cargo genes specifically, those encoding functions of oxidation-reduction processes and resistance appeared in a greater number of ORFs in the elements, followed by virulence factors (Figure 3B). Putative virulence and adaption roles were verified in most ICEs: genes codifying virulence functions were tracked down in 28 elements and with putative adaption role were spotted in 12 ICEs, among other important roles such as metal and antibiotic resistance (Table S15). Regarding virulence, genes were also organized by their putative function, and the most significant class of virulence genes were genes that encodes proteins translocated by Type III Secretion System (T3SS) and components of this system, followed by genes that encodes hydrolytic enzymes involved in host cell wall degradation (Figure 3C).

These results demonstrate a significant presence of ICEs in genomes of high impact phytopathogenic bacteria. Specific results will be presented by group of bacteria.

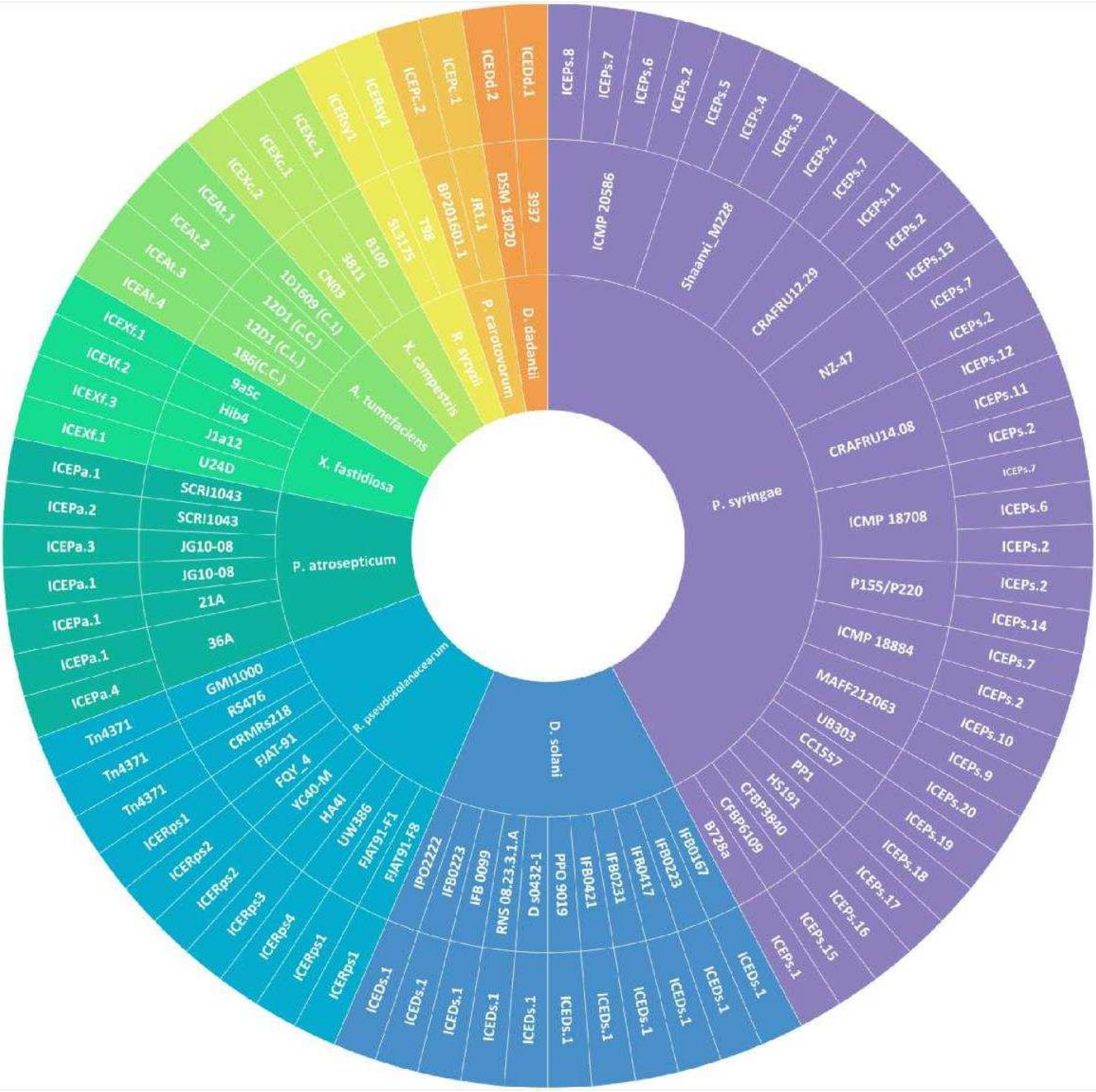


Figure 1: Distribution of ICEs among bacterial isolates. Solar explosion chart indicating the elements present in all isolates. Bacterial species were arranged from the species with the largest number of elements to the species with the least number of elements and separated by color: Lilac: *P. syringae;* Dark blue: *D. solani*; Light blue: *R. pseudosolanacearum*; Green (from the darkest to the lightest respectively): *P. atrosepticum, X. fastidiosa, A. tumefaciens, X. campestris*; Yellow: *R. syzigii*; Light orange: *P. carotovorum*; Dark orange: *D. dadantii*. From the inside out of the chart: The bacterial species, name of the isolated and the identified elements present in each isolated.

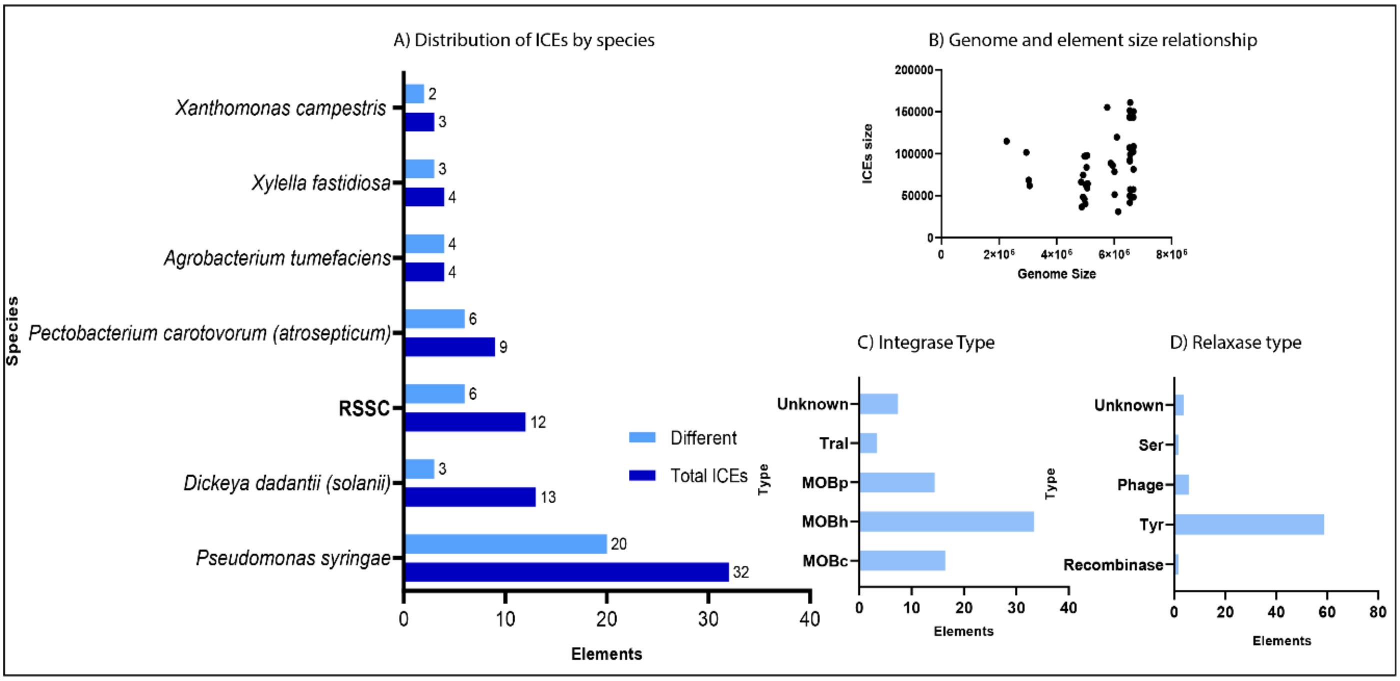


Figure 2: General ICES identification results. A) Bar chart of ICEs number distribution by groups of bacteria (dark blue: total elements, light blue: different elements); B) Distribution chart of genome size compared to the size of ICEs; C and D) Bar chart of Integrase and Relaxase types in all elements respectively.

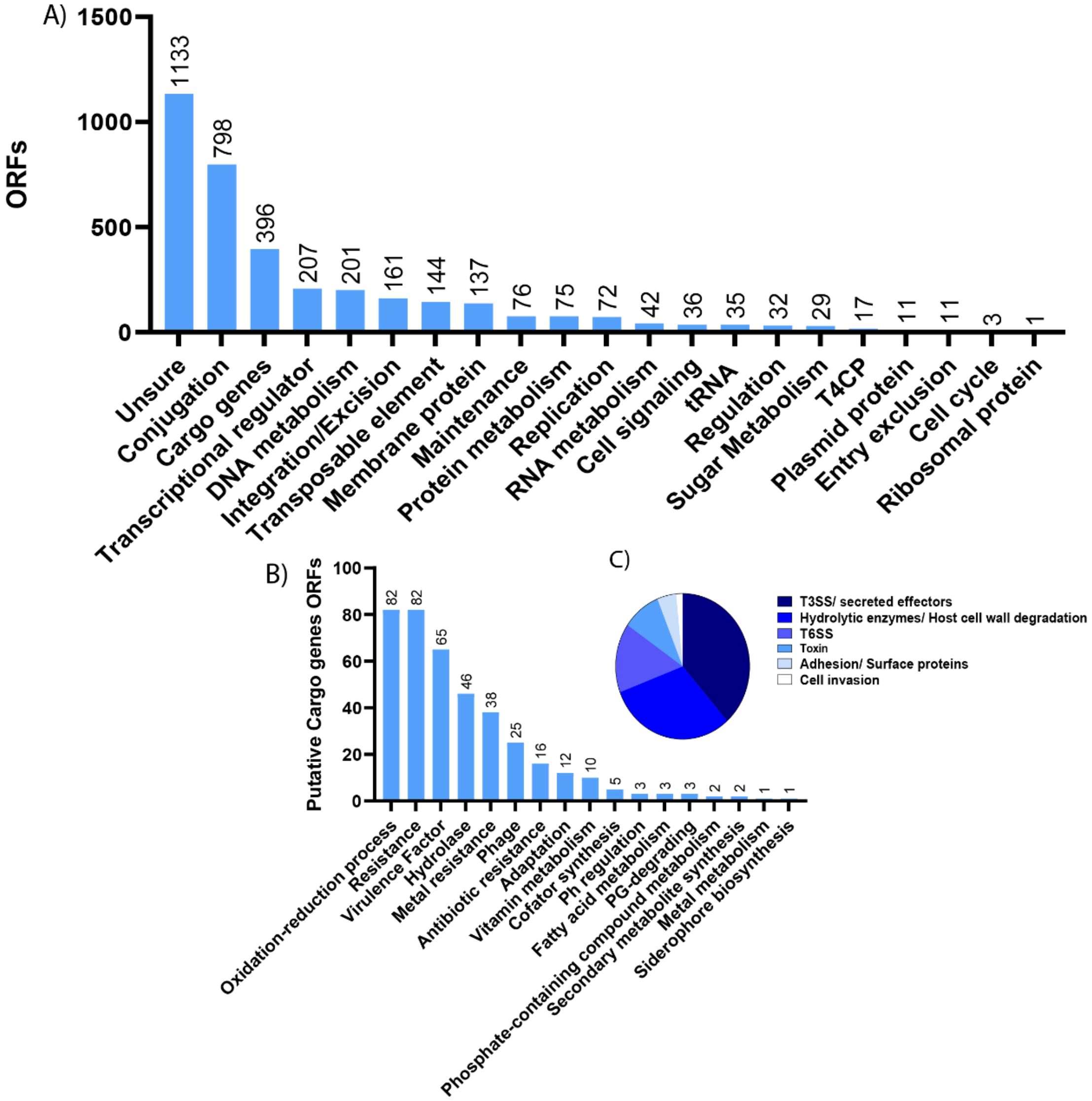


Figure 3: Putative functions of ICEs genes. A) Bar chart of putative roles codified by ICEs genes separated by categories (Unsure category comprises Hypothetical protein, Domain of unknown function (DUF genes), and genes with undetermined function). B) Bar chart of Cargo genes divided by putative roles. C) Pie chart representing putative roles of Virulence factors carried by ICEs.

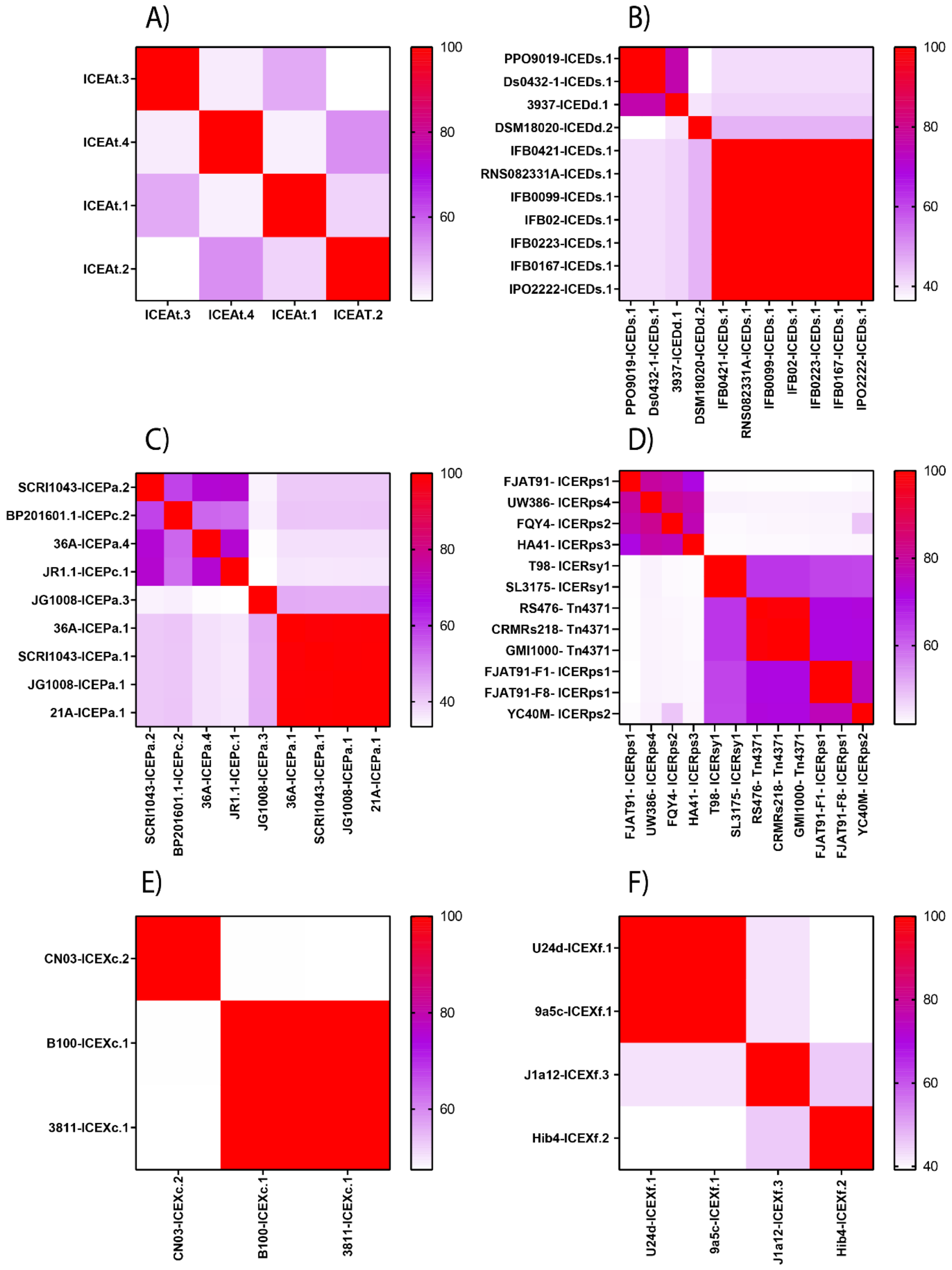


Figure 4: ICEs identity matrix heatmaps. Heatmaps are divided by groups of bacteria; red color represents 100% of nucleotide identity A) *A. tumefaciens*; B) *Dickeya* (*dadantii* and *solani*); C) *P. carotovorum* (and *atrosepticum*); D) *R. solanacearum* species complex; E) *X. campestris*; F) *X.fastidio*

Agrobacterium tumefaciens

Initially, we investigate chromosome sequences of seventeen isolates available in NCBI database, each one of them has two chromosomes, so we search for ICEs in 32 sequences of *A. tumefaciens* chromosomes (Table S3). Our methods allowed the identification of four novel elements in sequences of three isolates: ICEAt.1 identified in chromosome named I of 1D1609, ICEAt.2, and ICEAt.3 in the circular and linear chromosomes of 12D1 respectively, and at last, ICEAt.4 in the circular chromosome of isolate 186 (Figure 1). These elements had a mean size of 86.773 bp; ICEAt.3 was the element with a larger sequence, presenting 114.964 bp, followed from ICEAt.4 (101.577 bp), ICEAt.2 (68.662 bp), and the smallest element was ICEAt.1 with 61.887 bp (Table S-12). The mean GC content of the elements was 60,4 and varied from 58.1 (ICEAt.3) to 63.7 (ICEAt.1), only the elements ICEAt.2 and ICEAt.3 of 12D1 showed lower GC content than the genome: 58,7 and 58,1 respectively (Table S12). The attachment sites of ICEAt.3 and ICEAt.4 were identified, and regarding the integration site, both of these elements and ICEAt.2 are inserted in tRNA sequences, and ICEAt.1 is inserted in *guaA* gene (Table S13-14).

The ICEs sequence alignment showed a bigger similarity between ICEAt.2 and ICEAt.4 with 53,78% of nucleotide identity, and syntenic analysis presents that all elements share gene clusters that represent their conjugation and integration modules mainly (Figure 4A – S1).

We were able to identify cargo genes in *A. tumefaciens* ICEs that encodes proteins that may have important functions such as Cysteine hydrolase and Glycosidase on ICEAt.1, and Endo-1,4-beta-xylanase on ICEAt.4 with a putative virulence role; likewise, Alkene reductase and Glutathione S-transferase on ICEAt.1, and Universal stress protein on ICEAt.3 that may have a putative role in the adaption of these bacteria. Also, we identify genes coding for a L.D

– transpeptidase of ICEAt.3 and MBL fold metalo-hydrolase on ICEAt.4 that allows putative resistance to antibiotics (Table S15).

Dickeya (dadantii and solani)

We search for ICEs in chromosomal sequences of thirteen *Dickeya* genomes, thus, two of them were *D. dadantii*, and eleven genomes belonging to the *D. solani* species (Table S9). In those sequences we were able to find three distinct elements, and thirteen ICEs on total: ICEDd.1 was found in the chromosomal sequence of isolate 3937, ICEDd.2 on the chromosome of DSM 18020, and interestingly, ICEDs.1 was present in all of the eleven isolates of *Dickeya solani*, hence, all of the investigated strains harbor ICEs (Figure 1). Of these elements, only ICEDd.1 has been cited in the literature, as an element of Tn4371 family (Table S16) (Toussaint et al., 2003).

Regarding the size of those elements, ICEDd.1 was the biggest element, with 74.610 bp, followed by ICEDs.1 (48.378 bp) and ICEDd.2 was the smallest with 40.117 bp (Table S12). The GC content of all elements was lower than the content of the genomes, with a mean of 51,46: 52,1 of ICEDs.1, 52,9 of ICEDd2 and 49,9 of ICEDs.1 (Table S12). Both ICEDd.1 and ICEDs.1 had their attachment sites identified and are inserted in tRNA sequences, ICEDd.2 was located inserted in *bamE* gene (Table S13-14). The different elements showed low similarity between each other (< 50% of nucleotide identity) (Figure 4B). However, clusters of syntenic genes were observed between all three elements, connecting its recombinase gene, some transcriptional regulators, and cargo genes related to Type VI secretion system (T6SS) described above. Furthermore, ICEDd.1 and ICEDs.1 may also share their conjugation and regulation modules since syntenic gene clusters comprising genes of T4SS and a toxin-antitoxin system were verified (Figure S2).

Between the main cargo genes of these ICEs we identify a putative Tellurium resistance (*terB*) and an entry exclusion (*eexN*) in ICEDd.1. Moreover, component genes of T6SS, *hcp* effectors, and *tssI* - *vgrG* with putative virulence role was verified in all three different elements (Table S15).

Pectobacterium carotovorum (and atrosepticum)

The chromosomic sequences of eight genomes were investigated, four from *P. carotovorum* (Pcc) and four from *P. atrosepticum* (Pca), enabling the identification of six distinct ICEs in a total of nine elements (Table S10 – Figure1). As a result, in Pcc we found ICEPc.1 and ICEPc.2 in genomes of JR1.1 and BP201601.1 isolates respectively, and in Pca we observed a co-occurrence of ICEs, in other words, a single chromosome harboring more than one element: in the chromosome of SCRI1043 we localize ICEPa.1 and ICEPa.2, we observed JG10-08 carrying the elements ICEPa.1 and ICEPa.3, and the isolate 36A, the elements ICEPa.1 and ICEPa.4. The element ICEPa.1 by himself was identified in the chromosome sequence of 21A isolate, and was the only element that has been studied in the literature (Table S16 - Figure1) (Bell et al., 2004; Vanga et al., 2015; Panda et al., 2016).

The alignment of ICEs sequences showed similarity between ICEPa.2 and ICEPc.1 with 69,54% of nucleotide identity, and ICEPa.2 and ICEPa.4 with a nucleotide identity percentage of 68,94 (Figure 4C). Above all, syntenic analyses showed that with the except for ICEPa.1, all elements seem to share their conjugation modules, which is demonstrated by a highly syntenic gene cluster comprising conjugation and T4SS genes present in the elements, also, the results indicate a similarity between integration genes of ICEPc.1, ICEPa2, ICEPc.2, and ICEPa.4 (Figure S3).

Regarding the main cargo genes of these elements, the coronafacic acid biosynthesis cluster of ICEPa.1 is known for its importance for the virulence in SCRI1043 isolate (Panda et al., 2016). Genes encoding proteins with putative virulence role were also found in ICEPa.2 (Phospholipase D), ICEPa.4 (Arginase family protein), and ICEPc.2 (Glutathione peroxidase). Moreover, we identify putative entry exclusion genes in ICEPa.2, ICEPa.4 and ICEPc.1, and genes encoding proteins that may confer antibiotic resistance: MBL fold metallo-hydrolase in ICEPa.3 and ICEPc.2, and *mipA/ompV* family protein of ICEPa.4 (Table S15).

As presented by Gorshkov et al., 2018, our analysis of differential gene expression confirmed a higher expression of the gene cluster of coronafacic acid biosynthesis carried by ICEPa.1 during the asymptomatic stage of *P. atrosepticum* SCRI1043 infection in tobacco, represented by a higher RPKM value in comparison with symptomatic stage and *in vitro* culture (Fig 5-A). As already described, the isolate SCRI1043 also has ICEPa.2, and the expression of its putative virulence gene, Phospholipase D were verified, presenting the same pattern displayed by the genes of ICEPa.1, with a higher RPKM value in the asymptomatic stage of infection (Fig 5-B). Finally, we analyzed the expression of relaxase genes carried by ICEPa.1 (loci tag ECA_RS03055) and ICEPa.2 (loci tag ECA_RS08300) to indicate the stage of infection where the mobilization of these elements occurs. A different pattern of results was verified, the relaxase of ICEPa.1 presented a greater expression in the symptomatic stage of infection, followed by the *in vitro* culture, and lower expression in the asymptomatic stage (Fig 5-D). The relaxase of ICEPa.2 exhibited an upregulation of the expression in the *in vitro* culture, followed by the expression on the symptomatic phase (Fig 5-C).

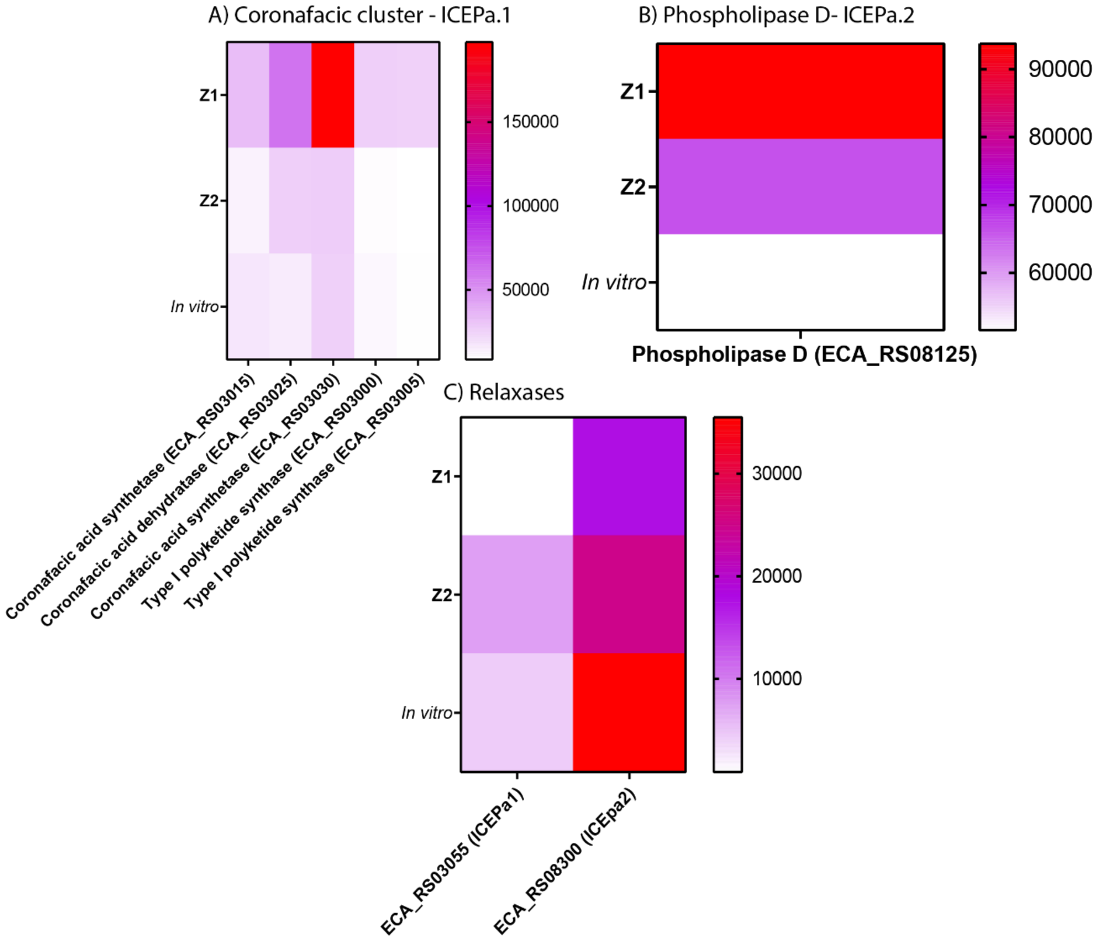


Figure 5: Different Expression Analyses - RPKM Heatmaps: Significant genes of *P. atrosapticum* elements ICEPa.1 and ICEPa.2 (SCRI1043 isolate) in tobacco plants - Z1: Asymptomatic zone, Z2: Symptomatic zone. Red color represents high expression A) Coronafacic acid biosynthesis gene cluster carried by ICEPa.1 B) Cargo gene of ICEPa.2 with putative Virulence role – Phospholipase D; C) Relaxases of ICEPa.1 and ICEPa.2.

Pseudomonas syringae pathovars

This group of bacteria showed interesting results since it was where most elements were found; chromosomes of 28 isolates were used to look for ICEs, and 20 distinct elements were found, from a total of 33 elements (Table S1 – Figure1). Regarding the distribution of these elements in bacterial genomes, it was verified that ICEPs.2 was the most common element, present in nine isolates, followed by ICEPs.7 detected in seven isolates (Figure 1). We were able to track down seven *P. syringae* ICEs that have already been cited in the literature, therefore, this work brings thirteen novel putative elements (Table S16).

Interesting events of ICE co-occurrence have been verified in *P. syringae* chromosomal sequences, mainly in sequences Shaanxi_M228, and ICMP 20586, where we were able to uncover four monopartite elements in each one. Furthermore, we spot the co-occurrence of three monopartite ICEs in genomes of CRAFRU14.08, NZ-47, and CRAFRU12.29; and two elements in MAFF212063, ICMP 18708, ICMP 18884, and P155

(Figure 1). Some of those elements could be seen grouped *in tandem* on the chromosome sequence with the same attachment sites, their arrangement was outlined in Figure 6A. This may be the first time in literature that more than three elements are track down in onegenome.

The size of *P. syringae* elements varied from 30.968 bp of ICEPs.20 to 161.009 bp of ICEPs.10, with a mean of 99.073 pb (Table S12), and their GC content had a mean of 55,13 ranging from 57,2 (ICEPs.2) to 50,7 (ICEPs.20) (Table S12). Attachment sites of fifteen elements were identified and all the 32 elements were localized integrated next to a tRNA sequence (Table S13-14). Between the elements, a bigger similarity was seen among ICEPs.4 and ICEPs.11 with 85% of nucleotide identity followed by ICEPs.1 and ICEPs.14 with 82% of nucleotide identity (Figure 6B). Comparative analysis of gene clusters illustrated highly syntenic ICEs in *P. syringae*, and enabled us to divide these elements into three groups, based on clusters of syntenic genes. Group 1 comprises the ICEPs.3, ICEPs.15, ICEPs.16, ICEPs.18 and ICEPs.19, as a result these elements mainly share their conjugation and integration modules with some IS sequences (Figure S4). Group 2 is the bigger one, comprising the ICEPs.1, ICEPs.4, ICEPs.6, ICEPs.9, ICEPs.11, ICEPs.13, ICEPs.14, ICEPs.17 and

ICEPs.20; this group, in addition to sharing the conjugation and integration modules as seen in group one, also share cargo gene encoding relevant functions, such as copper and arsenic resistance gene clusters among other cargo genes, except forICEPs.20, the most different element among the *P. syringae* ICEs, that only shares an integrase and a maintenance gene (*ardR*) (Figure S5). Finally, group three comprises the ICEPs.2, ICEPs.5, ICEPs.7, ICEPs.10, and ICEPs.12; all of the elements also share the main modules (integration and conjugation), moreover, ICEPs.2 and ICEPs.10 also had a syntenic gene cluster that contains T3SS effectors, a cellulase, a transporter gene cluster, chemotaxis gene clusters that may be involved in element regulation as other cargo genes. With this comparison analysis, we were also able to verify sequence differences between ICEPs.2 of ICMP20586 and Shaanxi_M228 due to IS insertions in the elements (Figure S6).A large number of cargo genes encoding putative significative roles were identified in

P. syringae ICEs: Between the most important, copper and arsenic resistance gene clusters of ICEPs.8 and ICEPs.13 has been described in the literature (Colombi et al., 2017) but here we report those gene clusters in ICEPs.1 and ICEPs.14 (Cooper and arsenic resistance), ICEPs.4 and ICEPs.11 (only Arsenic resistance). A great number of genes encoding putative virulence roles were found in some of *P. syringae* elements, such as T3SS effector genes, that were found in ICEPs.2, ICEPs.5, ICEPs.7, ICEPs.9, ICEPs.10, ICEPs.12, and ICEPs.18. Cargo genes conferring putative antibiotic resistance were also identified, such as *crpP* familyprotein that is carried by twelve elements, and *mupB* of ICEPs.18. Moreover, a gene cluster encoding putative Tellurium resistance was identified in ICEPs.2 (Table S15).

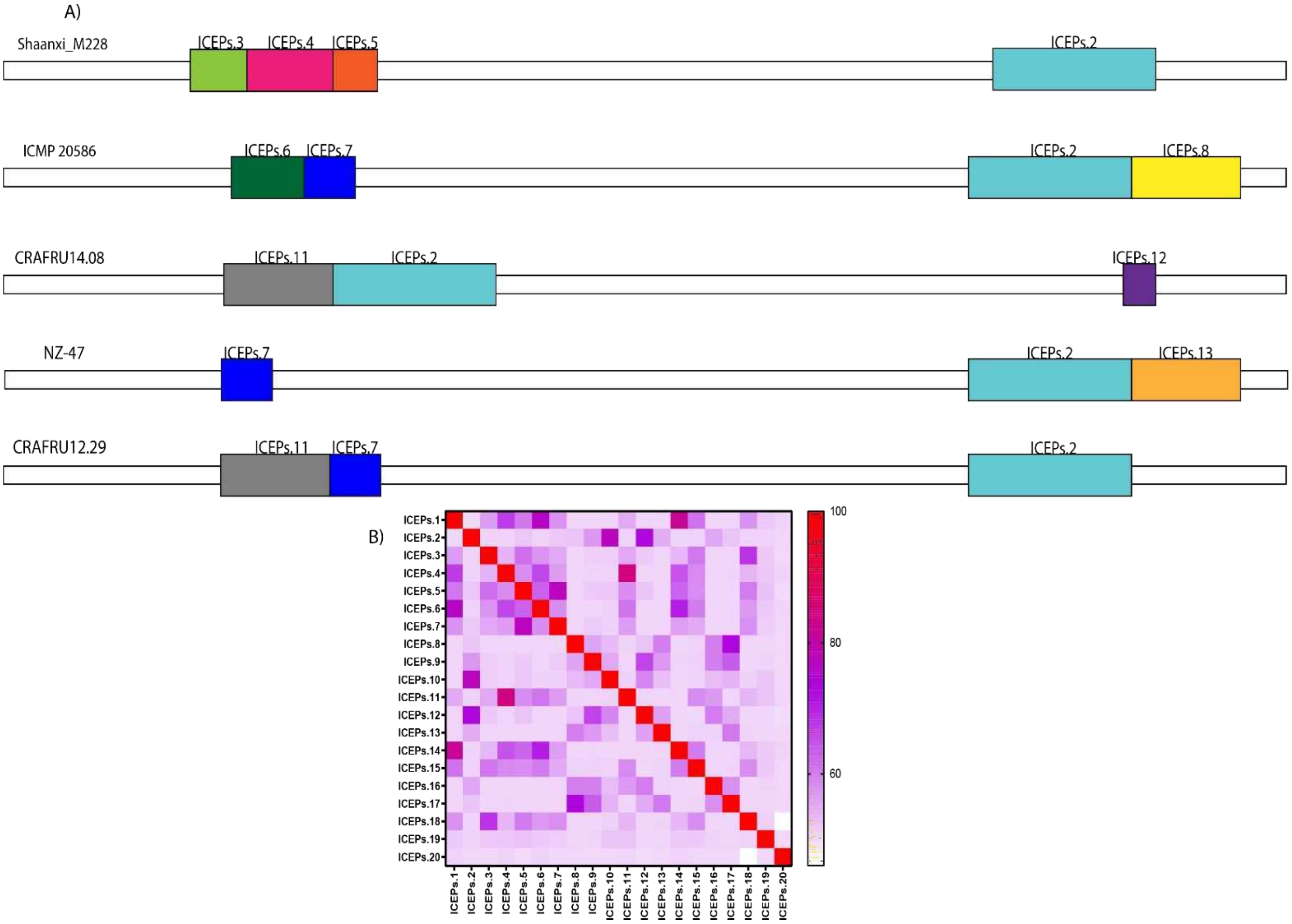


Figure 6: *P. syringae* ICEs. A) *P. syringae* ICEs co-occurrence and *in-tandem* configuration**.** (White rectangles: bacterial chromosomes – small colored rectangles: ICEs, the colors represents different elements); B) *P.syringae* ICEs identity matrix heatmap: red - high identity, purple -intermediate white-low identity

Ralstonia solanacearum species complex

Chromosome sequences of one hundred *R. solanacearum* isolates were investigated, leading to a result of seven distinct ICEs detected, being six from *R. pseudosolanacearum* and one ICE spotted in *R syzygii*, from a total of twelve elements (Table S2- Figure 1). Most of those elements had already been described in another work of our research group (Gonçalves et al., 2020) (Table S16), and here we report the presence of a known element (ICERps.1) in two more isolates, FJAT91-F1 and FJAT91-F8, and a novel element, ICERps.4, detected in UW386. The novel element, ICERps.4, have a percentual GC of 62,4, and size of 56.386 bp and it is inserted in a tRNA sequence (Tables S12-14). The alignment of the sequences showed similarity between ICERps.4 and ICERps.1, ICERps.2, and ICERps.3 with nucleotide identity percentages of 79,7, 81,52 and 78,53 respectively (Figure 3D) that was illustrated with syntenic gene clusters between these elements comprising the conjugation module and other cargo genes (Figure S7).

These elements carry cargo genes that encode putative role in bacterial adaption such as Glutathione S-transferase from Tn4371 and ICERps.3 and Gamma-glutamylcyclotransferase carried by ICERps.1. Likewise, we were able to identify genes with putative influence in virulence, like Amidohydrolase of ICERsy.1, and SDR family oxidoreductase present in Tn4371 and ICERps.3, at last ICERps.1 also carries a Superoxide dismutase gene with putative antioxidant resistance role (Table S15).

Xanthomonas campestris pathovars

We started our research with chromosomal sequences of eighteen *X. campestris* isolates, in which we track down two different ICEs, from a total of three novel elements: ICEXc.1 was found in the chromosomes of B100 and 3811 isolates and ICEXc.2 in the chromosome of CN03 (Table S5- Figure 1). The size of the element ICEXc.1 varied on the different isolates: 64.106 bp in B100 and 64.534 bp in 3811, moreover, the size of the ICEXc.2 element was 83.757 bp; the GC content was 61,7 and 60,1 respectively (Table S12). The attachment sites of the elements were described, and both were localized inserted in tRNA sequences (Table S13-14). Curiously the different elements shared low similarity, with a nucleotide identity percentage of 47.41 (Figure 3E) but are highly syntenic as presented by gene clusters comparison analyses (Figure S8).

Both elements identified in *X. campestris* carry cargo genes encoding putative roles in virulence. In ICEXc.1, we find genes encoding an aminotransferase, and a lytic mureintransglycosylase in ICEXc.2. This element also carries two putative avirulence effector genes and cargo genes such as Inositol hexakisphosphate that may be related to the adaption of thesebacteria (Table S15).

Xylella fastidiosa

Chromosomes of twenty-one *X. fastidiosa* isolates were analyzed, enabling the discovery of three distinct ICEs from a total of four elements (Table S8 – Figure1). The element's mean size was 108.150 bp ranging from 88.202 bp of ICEXf.1 to 158.355 of ICEXf.3 and the mean GC content was 56,7, varying from 54,6 (ICEXf.3) to 66,4 (ICEXf.2) (Table S12). The insertion of the element ICEXf.1 diverged from the others, since this element was found integrated next to ABC transporter gene; ICEXf.2 and ICEXf.3 were found integrated next to tRNA sequences, moreover, only the attachment sites of ICEXf.2 was identified (Table S13-14). Up till now, only ICEXf.2 of Hib4 has been identified in the literature (Denancé et al., 2019), hence our work presents two novel elements in *X. fastidiosa*. The alignment analysis indicates low similarity between the elements with nucleotide identity percentage less or equal to 42,63 (Figure 3F). Gene clusters comparison analyses were able to demonstrate significative syntenic groups shared by ICEXf.3 and ICEXf.1, comprising their conjugation and maintenance modules, represented by T4SS genes and a toxin-antitoxin gene cluster, in addition to other cargo genes; ICEXf.2 exhibit low similarity with the other elements in this analysis as well (Figure S9).

Cargo genes with putative roles in virulence were found in all three *X. fastidiosa* elements such as Virulence factors encountered in ICEXf.1 and ICEXf.3; also, genes conferring a putative antibiotic resistance were found in both elements (MBL fold metallo-hydrolase- ICEXf.1) and (UDP-3-O-(3-hydroxymyristoyl- glucosamine N-acyltransferase - ICEXf.3). We identify genes conferring a putative antioxidant resistance (Superoxide dismutase) and with putative role in adaption such as coproporphyrinogen III oxidase in ICEXf.2. Furthermore, a putative Entry exclusion gene was identified in ICEXf.3 (Table S15).

Discussion

The restrict knowledge about ICEs engender by the study bias of phenotypes conferred by these elements has been overcame by the greater the availability of complete genome sequences in recent years (Wozniak & Waldor, 2010; Johnson & Grossman, 2015; Botelho & Schulenburg, 2020). Some researchers have addressed the presence of ICEs in phytopathogenic bacteria but most of these works did not deal in depth, thus much still needs to be done to fully understand the relationship between these elements and bacteria and economically important phytobacteria (Mole et al., 2007; Guglielmini et al., 2011).

Through an *in-silico* investigation of ICEs using 300 genomes available at NCBI, we have identified and characterized 78 putative ICEs in genomes of ten phytopathogenic bacteria, and as expected, most of the ICEs were found integrated into tRNA sequences (Johnson & Grossman, 2015). The modules of ICEs were determined by putative gene functions: integrase and relaxase genes were placed in Integration/excision modules, T4SS and mating-pair genes in conjugation module, Toxin-antitoxin gene clusters and anti-restriction (*ardR*) genes in maintenance module, as already seen in literature, even though it is known that the content of these modules varies between elements (Wozniak & Waldor, 2010; Johnson & Grossman, 2015). The regulation module was restricted to some putative SOS response and chemotaxis genes, as also already seen in the literature, because it is a module that needs better experimental proof of the gene functions (Burrus & Waldor, 2004; Wozniak & Waldor, 2010; Johnson & Grossman, 2015). Some identified elements also carry Entry exclusion genes (*eexn* and *traG*), that can avoid redundant transfer of ICEs leading to host energy savings (Marrero & Waldor, 2007; Dunny, 2019).

Our search methods did not allow the identification of ICEs in *X. oryzae* pv. oryzae*,* *X. axonopodis,* and *E. amylovora.* This result may have occurred due to a bias in the software used, since we are dealing with highly diverse elements and therefore difficult to identify. Regarding to *E. amylovora* and *X. axonopodis* another bias involves the low number of complete genomes available (nine and three genomes respectively) promoting a reduced view of the variability of these genomes. However, we search for ICEs in 83 complete genomes of *X. oryzae* pv. oryzae and despite its large repertoire of insertion sequences (Salzberg et al., 2008; Cesbron et al., 2015), no ICE was found. Similarly to that, we investigate one hundred sequences of RSSC complete genomes (40 new more genomes than the analyzed by Gonçalves et al., 2020), and only found one novel ICE (ICERps.4), besides reporting one element already known in two new genomes (ICERps.1 in FJAT91-F1 and FJAT91-F8). We hypothesize that the present variation of ICEs in genomes of different bacterial species maybe related to a type of mechanism to control the entry of these elements in cells, which can be more or less rigorous, allowing or not the acquisition of ICEs by host cells.

Most of the identified elements carry genes with putative functions for adaptation and virulence. Putative antibiotic resistance genes were also identified, but although they have not been classified in this way, they can be related to the ICEs maintenance module. Regardingthe virulence factors found, a greater number of T3SS ORFs was observed in ICEs. This secretion system is an important virulence tool of phytobacteria, allowing the translocation of effector proteins into plant cells changing their metabolism or suppressing defenses. Among the genera of bacteria that have this system are *Pseudomonas*, *Ralstonia,* and *Xanthomonas* (Cornelis & van Gijsegem, 2000; Alfano & Collmer, 2004). Furthermore, we also foundgenes that encodes hydrolytic enzymes involved in host cell wall degradation as the second most prevalent group of virulence factors in our ICEs. Thus, our work highlights the importance of ICEs for a possible function on parasitism evolution, directing the spread of significant putative virulence genes in phytopathogenic bacteria. The specific results in bacterial groups will be discussed above:

A. tumefaciens is a phytopathogenic bacteria with economical and scientificsignificance, since it can cause the crown gall disease in a wide variety of plants and is also animportant biotechnological tool of gene transfer into in plants cells (Gelvin, 2003). Similarly to other facultative phytopathogens, *A tumefaciens* needs to deal with the variation between taking shelter in the environment or host, leading to a great heterogeneity of habitats and consequently to great selective pressure and genetic variability in those bacteria (Barton et al., 2018). Among the results presented, we found four putative ICEs in *A. tumefaciens* genomes, three of them carrying genes such as glycosidase and xylanase, with a putative virulence role, acting on plant cell wall degradation, similarly to the activity indicated by similar genesshown by Mathews et al., in 2019. Cargo genes with putative adaption role also were found, such as Glutathione S- transferase (GST) of ICEAt.1, that were identified not only in *A. tumefaciens* ICEs but in *P. atrosepticum* (ICEPa.3), and *R. pseudosolanacearum* (Tn4371 and ICERps.3). Bacterial GSTs are known by its role in detoxification processes (Allocati et al., 2009; Gullner et al., 2018).

D. dadantii and *D. solani* are two bacterial species selected by Mansfield et al., in2012 to occupy the same position in their ranking, however, they were selected for different reasons. The phytopathogen *D. dadantii* is responsible for soft rot disease in crops around the world, therefore, its isolate 3937 was used to study plant pathogenicity for many years

(Grenier et al., 2006; Mansfield et al., 2012). Otherwise, the pectinolytic bacterium *D. solani* can cause wilt in potato crops (Toth et al., 2011; van der Wolf et al., 2014). We were able to track down three putative different ICEs in *D. dadantii* and *D. solani*, being two novel elements and one (ICEDd.1) already been described (Toussaint et al., 2003). Interestingly, the

D. solani element, ICEDs.1 were identified in all of the complete genomes available in NCBI database, of isolates from different European countries, which can indicate an ancestral acquisition of this ICE, or a recent spread event of these bacteria in potato crops around Europe. Pulse field gel electrophoresis and multi locus sequence analyses with some *D. solani* strains from different regions suggested a high degree of clonality between them (van der Wolf et al., 2014), a feature that may explain the presence of the same ICE in all isolates. All three elements (ICEDd.1, ICEDd.2, and ICEDs.1) share the *Hcp* effector gene as a cargo gene related to virulence, *Hcp* belongs to the T6SS, and it is a part of a tubular structure facilitating the transport of other effectors into the host cells, jointly, *tssI* is another component of this system present in *Dickeya* ICEs (Silverman et al., 2012; Russell et al., 2014; Cianfanelli et al., 2016).

The "model" pathogens for genetic phytopathogenic studies, *Pectobacterium carotovorum* (Pcc) and *Pectobacterium atrosepticum* (Pca) were posteriorly classified by *Erwinia carotovora* subspecies *carotovora* and *atrosepticum,* respectively, and together with *Dickeya* genera can cause soft rot diseases in several plant species (Perombelon & Kelman, 1980; Gardan et al., 2003; Ma et al., 2007). In our work, we identify five putative new ICEs in Pcc and Pca, and one known element, ICEPa.1(Bell et al., 2004). Regarding the cargo genes of these elements, we found an Arginase family protein in ICEPa.4 that may influence bacterial virulence, it has been shown that an Arginase gene (*rocF*) is associated to virulence in *X. oryzae pv. oryzae*, influencing EPS production, biofilm formation and hydrogen peroxide resistance (Zhang et al., 2019). Furthermore, Pcc and Pca are known for their fabrication of cell- wall-degrading enzymes that macerate plant tissues causing soft rot symptoms (Barras et al., 1985), connected to that, we found ICEPa.2 carrying a putative Phospholipase D that can influence virulence from the same process. Moreover, ICEPa.1, described in the literature as HAI2, is known for its biosynthetic coronafacic acid gene cluster that has verified a significant relationship with his bacterial host virulence (Panda et al., 2016). A previous study with the isolate SCRI1043 that infects tobacco plants showed that the cluster is upregulated in asymptomatic plant zones similarly to our results, the authors suggest a relationship between coronafacic acid and a pathogen behavior change, causing the disease (Gorshkov et al., 2018). Our differential expression analysis of phospholipase D carried by

ICEPa.2 revealed the same pattern of expression seen for coronafacic acid biosynthesis genes, indicating a possible similar role of this gene in the process of developing symptoms development during tobacco infection. Our results of expression of the relaxase genes of these elements possibly indicate that the mobilization of ICEPa.1 and ICEPa.2 in SCRI1043 isolate does not occur during the asymptomatic phase of infection due to its downregulation in this stage. These results correlate with the knowledge that the genes related to the mobilization of the ICE normally remain inactive and are induced by specific signals and conditions such as SOS response, and during the growth phase of the bacterial host (Johnson & Grossman, 2015), what can explain the upregulation of these genes in the symptomatic stage of infection and *in vitro* culture seen in our work. Due to the low quality of the RNAseq sequences obtained, only the data of one of the two repetitions were used to develop our graphics.

The *P. syringae* species comprises a group of bacterial pathovars that are capable of causing necrosis on aerial plant parts in a large part of the economically important plants around the world, occupying the first place of Mansfield's ranking (Hirano & Upper, 1990; Mansfield et al., 2012; Xin et al., 2018), moreover, these bacteria are able to survive and multiply in diverse habitats, not only acting as phytopathogen (Morris et al., 2007). Such interesting features of this bacterium reflects in our findings: about 42% (33) of the ICEs detected were found in their genomes, plus the largest element of this work was also detected in *P. syringae* (ICEPs.10 – 161.009 bp.), also, some of these elements appear together on a single host chromosome in events of co-occurrence and some configurations show ICEs *in tandem* (Figure 5). Gene clusters comparison of the elements illustrates another interesting feature, they are highly syntenic, and share gene clusters "blocks" that vary between ICEs. *P. syringae* ICEs already had been studied, but its nomenclature and the dissemination of information seems confusing, for that matter we were able to locate only seven elements identified in this work in literature. Among the elements already known, ICEPs.8 and ICEPs.13 (Psa$_{NZ45}$ICE_Cu and Psa$_{NZ47}$ICE_Cu respectively) were experimentally studied presenting copper and arsenic resistance gene clusters, that were used later as a way to control these bacteria, therefore the work illustrates the importance of ICEs in the spread of resistance genes in bacterial populations (Colombi et al., 2017). In our work, we contribute to this information by detecting putative copper and arsenic resistance gene clusters in ICEPs.1 and ICEPs.14, and arsenic resistance gene cluster in ICEPs.4 and ICEPs.11. Like other phytopathogens, Type III Secretion System plays a fundamental role in pathogenesis in *P. syringae* (Cunnac et al., 2009), reflecting the importance of the seven ICEs carrying putative T3SS genes, such as effectors and chaperones.

Together with other important putative cargo genes related to resistance and adaptation of these bacteria, we detected a putative Tellurium resistance gene cluster in ICEPs.2. This chemical element is lethal for some microorganisms, and the resistance against its oxyanion has been described in *P. syringae* (Summers & Jacoby, 1977; Cournoyer et al., 1998; Cunha et al., 2009). A putative *terb* gene related to Tellurium resistance was also founded in ICEDd.1. Curiously, the presence of these genes may play a different role as the Tellurium resistance expected, due to the concentration of the element in nature, hence they can act conferring other phenotypes for these bacteria (Cournoyer et al., 1998; Zannoni et al., 2008).

The *X. campestris* pathovars cause economic impact being the source of diseases in many crops around the world and were represented in the ranking of top 10 plant pathogens by *X. campestris* pv. campestris (Xcc) that causes disease in brassicas, *X. campestris* pv. vesicatoria (Xcv - reclassified by *X. euvesicatoria*) causing disease in pepper and tomato, and *X. campestris* pv. malvacearum (Xcm – reclassified by *X. axonopodis* pv. malvacearum) that causes disease in cotton plantations (Jones et al., 2000; Akello & Hillocks, 2002; Mansfield et al., 2012; Kyeon et al., 2016). Here we tracked down two new ICEs in *X. campestris* genomes, which interestingly are highly syntenic but have low nucleotide identity. In particular ICEXc.2 carries some avirulence genes, among them we identified the gene that codifies AvrXccC effector, that besides its role activating plant immune system, it also acts in virulence interfering with abscisic acid plant immune response (Ho et al., 2013).

We also found ICEs in *X. fastidiosa*, a vector-transmitted phytopathogen accountable for economic losses due to several different diseases that it can cause in crops, such as citrus variegated chlorosis and the disease of grape (Chatterjee et al., 2008). We identified three different ICEs in *X. fastidiosa* genomes, and we spotted a putative Superoxide dismutase gene carried by ICEXf.2, that can influence virulence providing defense against host's immune responses (Lambais et al., 2000). Moreover, the lack of material about virulence factors in *X. fastidiosa* ICEs may be explained by the information that although it is a pathogen limited to plants xylem, it uses different virulence factors from other vascular pathogens, which may explain the absence of virulence factors carried by their elements that were common in other ICEs described in this work, such as T3SS effectors and hydrolases (Wells et al., 1987; Chatterjee et al., 2008).

Finally, we discovered one complete putative ICE being shared by different species, ICEPc.2 present in *P. carotovorum* and in the rhizobacteria *S. plymuthica.* This evidence of ICEs transfer between different bacterial genera brings attention to the potential for gene spreading function of these elements, which may be of concern in the case of virulence and antibiotic resistance genes. In addition to that, ICEPc.1 and ICEXc.1 shared high nucleotide similarity with two novel ICEs of *P. aroidearum and X. arboricola* pv. juglandis respectivelly. In conclusion, our results suggest a putative association between ICEs and phytobacteria fitness. Our *in-silico* work opens the doors to further experimental investigations that need to be done to help better understand the role of ICEs and their cargo genes in phytopathogenic bacteria.

Bibliography

Akello, B., & Hillocks, R. J. (2002). Distribution and races of *Xanthomonas axonopodis* pv. malvacearum on cotton (*Gossypium hirsutum*) in Uganda. *Journal of Phytopathology*, *150*(2), 65–69. https://doi.org/10.1046/j.1439-0434.2002.00718.x

Alamuri, P., Löwer, M., Hiss, J. A., Himpsl, S. D., Schneider, G., & Mobley, H. L. T. (2010). Adhesion, invasion, and agglutination mediated by two trimeric autotransporters in the human uropathogen *Proteus mirabilis*. *Infection and Immunity*, *78*(11), 4882–4894. https://doi.org/10.1128/IAI.00718-10

Alfano, J. R., & Collmer, A. (2004). Type III secretion system effector proteins: Double agents in bacterial disease and plant defense. *Annual Review of Phytopathology*, *42*, 385–414. https://doi.org/10.1146/annurev.phyto.42.040103.110731

Allocati, N., Federici, L., Masulli, M., & Di Ilio, C. (2009). Glutathione transferases in bacteria. *FEBS Journal*, *276*(1), 58–75. https://doi.org/10.1111/j.1742-4658.2008.06743.x

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Auchtung, J. M., Lee, C. A., Garrison, K. L., & Grossman, A. D. (2007). Identification and characterization of the immunity repressor (ImmR) that controls the mobile genetic element ICEBs1 of *Bacillus subtilis*. *Molecular Microbiology*, *64*(6), 1515–1528. https://doi.org/10.1111/j.1365-2958.2007.05748.x

Avello, M., Davis, K. P., & Grossman, A. D. (2019). Identification, characterization and benefits of an exclusion system in an integrative and conjugative element of *Bacillus subtilis*. *Molecular Microbiology*, *112*(4), 1066–1082.https://doi.org/10.1111/mmi.14359

Barras, F., van Gijsegem, F., & Chatterjee, A. K. (1985). Extracellular Enzymes and Pathogenesis of Soft-Rot Erwinia. *Compr Biotech, The Princip. Appl and Regal ofBiotechnol in Ind, Agric and Med*, *1*, 587–604.https://doi.org/10.1128/9781555816513.ch39

Barton, I. S., Fuqua, C., & Platt, T. G. (2018). Ecological and evolutionary dynamics of a model facultative pathogen: Agrobacterium and crown gall disease of plants. E*nvironmental Microbiology*, *20*(1), 16–29. https://doi.org/10.1111/1462-2920.13976

Bateman, A. (2019). UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Research*, *47*(D1), D506–D515. https://doi.org/10.1093/nar/gky1049

Bateman, A., Coin, L., Durbin, R., Finn, R. D., Hollich, V., Griffiths-Jones, S., Khanna, A., Marshall, M., Moxon, S., Sonnhammer, E. L. L., Studholme, D. J., Yeats, C., & Eddy, S.

R. (2004). The Pfam protein families database. *Nucleic Acids Research*, *32*(DATABASE ISS.), 138–141. https://doi.org/10.1093/nar/gkh121

Bell, K. S., Sebaihia, M., Pritchard, L., Holden, M. T. G., Hyman, L. J., Holeva, M. C., Thomson, N. R., Bentley, S. D., Churcher, L. J. C., Mungall, K., Atkin, R., Bason, N., Brooks, K., Chillingworth, T., Clark, K., Doggett, J., Fraser, A., Hance, Z., Hauser, H., Toth, I. K. (2004). Genome sequence of the enterobacterial phytopathogen *Erwinia carotovora subsp. atroseptica* and characterization of virulence factors. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(30), 11105– 11110. https://doi.org/10.1073/pnas.0402424101

Bellanger, X., Payot, S., Leblond-Bourget, N., & Guédon, G. (2014). Conjugative and mobilizable genomic islands in bacteria: Evolution and diversity. *FEMS Microbiology Reviews*, *38*(4), 720–760. https://doi.org/10.1111/1574-6976.12058

Botelho, J., & Schulenburg, H. (2020). The Role of Integrative and Conjugative Elements in Antibiotic Resistance Evolution. *Trends in Microbiology*, 1–11. https://doi.org/10.1016/j.tim.2020.05.011

Burrus, V. (2017). Mechanisms of stabilization of integrative and conjugative elements. *Current Opinion in Microbiology*, *38*, 44–50. https://doi.org/10.1016/j.mib.2017.03.014

Burrus, V., Pavlovic, G., Decaris, B., & Guédon, G. (2002). Conjugative transposons: The tip of the iceberg. *Molecular Microbiology*, *46*(3), 601–610. https://doi.org/10.1046/j.1365-2958.2002.03191.x

Burrus, V., & Waldor, M. K. (2004). Shaping bacterial genomes with integrative and conjugative elements. *Research in Microbiology*, *155*(5), 376–386. https://doi.org/10.1016/j.resmic.2004.01.012

Carraro, N., & Burrus, V. (2015). The dualistic nature of integrative and conjugative elements. *Mobile Genetic Elements*, *5*(6), 98–102.https://doi.org/10.1080/2159256x.2015.1102796

Cesbron, S., Briand, M., Essakhi, S., Gironde, S., Boureau, T., Manceau, C., Fischer-Le Saux,M., & Jacques, M. A. (2015). Comparative genomics of pathogenic and nonpathogenic strains of *Xanthomonas arboricola* unveil molecular and evolutionary events linked to pathoadaptation. *Fro*

Chatterjee, S., Almeida, R. P. P., & Lindow, S. (2008). Living in two worlds: The plant and insect lifestyles of *Xylella fastidiosa*. *Annual Review of Phytopathology*, *46*, 243–271. https://doi.org/10.1146/annurev.phyto.45.062806.094342

Cianfanelli, F. R., Monlezun, L., & Coulthurst, S. J. (2016). Aim, Load, Fire: The Type VI Secretion System, a Bacterial Nanoweapon. *Trends in Microbiology*, *24*(1), 51–62. https://doi.org/10.1016/j.tim.2015.10.005

Colombi, E., Straub, C., Künzel, S., Templeton, M. D., McCann, H. C., & Rainey, P. B. (2017). Evolution of copper resistance in the kiwifruit pathogen *Pseudomonas syringae* pv. actinidiae through acquisition of integrative conjugative elements and plasmids. *Environmental Microbiology*, *19*(2), 819–832. https://doi.org/10.1111/1462-2920.13662

Cornelis, G. R., & van Gijsegem, F. (2000). Assembly and function of type III secretory systems. *Annual Review of Microbiology*, *54*, 735–774.

Cournoyer, B., Watanabe, S., & Vivian, A. (1998). A tellurite-resistance genetic determinant from phytopathogenic pseudomonads encodes a thiopurine methyltransferase: Evidence of a widely-conserved family of methyltransferases. *Biochimica et Biophysica Acta - Gene Structure and Expression*, *1397*(2), 161–168. https://doi.org/10.1016/s0167- 4781(98)00020-7

Cunha, R. L. O. R., Gouvea, I. E., & Juliano, L. (2009). A glimpse on biological activities of tellurium compounds. *Anais Da Academia Brasileira de Ciencias*, *81*(3), 393–407. https://doi.org/10.1590/S0001-37652009000300006

Cunnac, S., Lindeberg, M., & Collmer, A. (2009). *Pseudomonas syringae* type III secretion system effectors : repertoires in search of functions. *Current Opinion in Microbiology*, *Table 1*. https://doi.org/10.1016/j.mib.2008.12.003

Darling, A. C. E., Mau, B., Blattner, F. R., & Perna, N. T. (2004). Mauve: Multiple alignment of conserved genomic sequence with rearrangements. *Genome Research*, *14*(7), 1394– 1403. https://doi.org/10.1101/gr.2289704

Delannoy, E., Lyon, B. R., Marmey, P., Jalloul, A., Daniel, J. F., Montillet, J. L., Essenberg, M., & Nicole, M. (2005). Resistance of cotton towards *Xanthomonas campestris* pv. malvacearum. *Annual Review of Phytopathology*, *43*(5), 63–82. https://doi.org/10.1146/annurev.phyto.43.040204.140251

Delavat, F., Miyazaki, R., Carraro, N., Pradervand, N., & van der Meer, J. R. (2017). The hidden life of integrative and conjugative elements. *FEMS Microbiology Reviews*, *41*(4), 512–537. https://doi.org/10.1093/femsre/fux008

Denancé, N., Briand, M., Gaborieau, R., Gaillard, S., & Jacques, M. A. (2019). Identification of genetic relationships and subspecies signatures in *Xylella fastidiosa*. *BMC Genomics*, *20*(1), 1–21. https://doi.org/10.1186/s12864-019-5565-9

Denny, T. P. (2006). Plant pathogenic Ralstonia species. In *Plant-Associated Bacteria* (pp. 573–644).

Doucouré, H., Pérez-Quintero, A. L., Reshetnyak, G., Tekete, C., Auguy, F., Thomas, E., Koebnik, R., Szurek, B., Koita, O., Verdier, V., & Cunnac, S. (2018). Functional and genome sequence-driven characterization of tal effector gene repertoires reveals novel variants with altered specificities in closely related malian *Xanthomonas oryzae* pv. oryzae strains. *Frontiers in Microbiology*, *9*(AUG), 1–17. https://doi.org/10.3389/fmicb.2018.01657

Dunny, G. M. (2019). A new flavor of entry exclusion in ICE elements provides a selective advantage for the element and its host. *Micro Commentary 112*(July), 1061–1065. https://doi.org/10.1111/mmi.14342

Escobar, M. A., & Dandekar, A. M. (2003). *Agrobacterium tumefaciens* as an agent of disease. *Trends in Plant Science*, *8*(8), 380–386. https://doi.org/10.1016/S1360- 1385(03)00162-6

Flannery, E. L., Antczak, S. M., & Mobley, H. L. T. (2011). Self-transmissibility of the integrative and conjugative element ICEPm1 between clinical isolates requires a functional integrase, relaxase, and type IV secretion system. *Journal of Bacteriology*, *193*(16), 4104–4112. https://doi.org/10.1128/JB.05119-11

Flannery, E. L., Mody, L., & Mobley, H. L. T. (2009). Identification of a modular pathogenicity island that is widespread among urease-producing uropathogens and shares features with a diverse group of mobile elements. *Infection and Immunity*, *77*(11), 4887– 4894. https://doi.org/10.1128/IAI.00705-09

Fonseca, E. L., Marin, M. A., Encinas, F., & Vicente, A. C. P. (2015). Full characterization of the integrative and conjugative element carrying the metallo-β-lactamase blaSPM-1 and bicyclomycin bcr1 resistance genes found in the pandemic *Pseudomonas aeruginosa* clone SP/ST277. *Journal of Antimicrobial Chemotherapy*, *70*(9), 2547–2550. https://doi.org/10.1093/jac/dkv152

Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: The agents of open source evolution. *Nature Reviews Microbiology*, *3*(9), 722–732. https://doi.org/10.1038/nrmicro1235

Gabriel, D. W., Burges, A., & Lazo, G. R. (1986). Gene-for-gene interactions of five cloned avirulence genes from *Xanthomonas campestris* pv. malvacearum with specific resistance genes in cotton. *Proceedings of the National Academy of Sciences*, *83*(17), 6415–6419. https://doi.org/10.1073/pnas.83.17.6415

Gardan, L., Gouy, C., Christen, R., & 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. *International Journal of Systematic and Evolutionary Microbiology*, *53*(2), 381–391. https://doi.org/10.1099/ijs.0.02423-0

Gelvin, S. B. (2003). Agrobacterium-Mediated Plant Transformation: the Biology behind the "Gene-Jockeying" Tool. *Microbiology and Molecular Biology Reviews*, *67*(1), 16–37.

https://doi.org/10.1128/mmbr.67.1.16-37.2003

Genin, S. (2010). Molecular traits controlling host range and adaptation to plants in *Ralstonia solanacearum*. *New Phytologist*, *187*(4), 920–928. https://doi.org/10.1111/j.1469-8137.2010.03397.x

Gilchrist, C. L. M., & Chooi, Y. H. (2020). clinker & clustermap.js: Automatic generation of gene cluster comparison figures. *BioRxiv*. https://doi.org/10.1101/2020.11.08.370650

Gonçalves, O. S., de Queiroz, M. V., & Santana, M. F. (2020). Potential evolutionary impact of integrative and conjugative elements (ICEs) and genomic islands in the *Ralstonia solanacearum* species complex. *Scientific Reports*, *10*(1), 1–13. https://doi.org/10.1038/s41598-020-69490-1

Gorshkov, V., Gubaev, R., Petrova, O., Daminova, A., Gogoleva, N., Ageeva, M., Parfirova, O., Prokchorchik, M., Nikolaichik, Y., & 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. *European Journal of Plant Pathology*, *152*(4), 957–976. https://doi.org/10.1007/s10658- 018-1496-6

Green, S., Studholme, D. J., Laue, B. E., Dorati, F., Lovell, H., Arnold, D., Cottrell, J. E., Bridgett, S., Blaxter, M., Huitema, E., Thwaites, R., Sharp, P. M., Jackson, R. W., & Kamoun, S. (2010). Comparative genome analysis provides insights into the evolution and adaptation of *Pseudomonas syringae* pv. aesculi on *Aesculus hippocastanum*. *PLoS ONE*, *5*(4), 1–14. https://doi.org/10.1371/journal.pone.0010224

Grenier, A. M., Duport, G., Pagès, S., Condemine, G., & Rahbé, Y. (2006). The phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) is a pathogen of the pea aphid. *Applied and Environmental Microbiology*, *72*(3), 1956–1965. https://doi.org/10.1128/AEM.72.3.1956-1965.2006

Guglielmini, J., Quintais, L., Garcillán-Barcia, M. P., de la Cruz, F., & Rocha, E. P. C. (2011). The repertoire of ICE in prokaryotes underscores the unity, diversity, and ubiquity of conjugation. *PLoS Genetics*, *7*(8).https://doi.org/10.1371/journal.pgen.1002222

Gullner, G., Komives, T., Király, L., & Schröder, P. (2018). Glutathione S-transferase enzymes in plant-pathogen interactions. *Frontiers in Plant Science*, *871*(December), 1– 19. https://doi.org/10.3389/fpls.2018.01836

Guo, M., Ye, J., Gao, D., Xu, N., & Yang, J. (2019). Agrobacterium-mediated horizontal gene transfer: Mechanism, biotechnological application, potential risk and forestalling strategy. *Biotechnology Advances*, *37*(1), 259–270.https://doi.org/10.1016/j.biotechadv.2018.12.008

Haskett, T. L., Ramsay, J. P., Bekuma, A. A., Sullivan, J. T., O'Hara, G. W., & Terpolilli, J.

J. (2017). Evolutionary persistence of tripartite integrative and conjugative elements. *Plasmid*, *92*(June), 30–36. https://doi.org/10.1016/j.plasmid.2017.06.001

Himpsl, S. D., Pearson, M. M., Arewng, C. J., Nusca, T. D., Sherman, D. H., & Mobley, H. L. T. (2010). Proteobactin and a yersiniabactin-related siderophore mediate iron acquisition in *Proteus mirabilis*. *Molecular Microbiology*, *78*(1), 138–157. https://doi.org/10.1111/j.1365-2958.2010.07317.x

Hirano, S. S., & Upper, C. D. (1990). Population biology and epidemiology of *Pseudomonas syringae*. *Annual Review of Phytopathology. Vol. 28*, 155–177. https://doi.org/10.1146/annurev.py.28.090190.001103

Ho, Y. P., Tan, C. M., Li, M. Y., Lin, H., Deng, W. L., & Yang, J. Y. (2013). The AvrB-AvrC domain of AvrXccC of *Xanthomonas campestris* pv. campestris is required to elicit plant defense responses and manipulate ABA homeostasis. *Molecular Plant-Microbe Interactions*, *26*(4), 419–430. https://doi.org/10.1094/MPMI-06-12-0164-R

Johnson, C. M., & Grossman, A. D. (2015). Integrative and Conjugative Elements (ICEs): What They Do and How They Work. *Annual Review of Genetics*, *49*, 577–601. https://doi.org/10.1146/annurev-genet-112414-055018

Jones, J. B., Bouzar, H., Stall, R. E., Almira, E. C., Roberts, P. D., Bowen, B. W., Sudberry,J., Strickler, P. M., & Chun, J. (2000). Systematic analysis of xanthomonads (Xanthomonas spp.) associated with pepper and tomato lesions. *International Journal of Systematic and Evolutionary Microbiology*, *50*(3), 1211–1219. https://doi.org/10.1099/00207713-50-3-1211

Kennelly, M. M., Cazorla, F. M., Vicente, A. de, Ramos, C., & Sundin, G. W. (2007). *Pseudomonas syringae* Diseases of Fruit Trees - Progress toward Understanding and Control. *Plant Disease*, *91*(1), 4–17.

Kyeon, M. S., Son, S. H., Noh, Y. H., Kim, Y. E., Lee, H. I., & Cha, J. S. (2016). *Xanthomonas euvesicatoria* causes bacterial spot disease on pepper plant in Korea. *Plant Pathology Journal*, *32*(5), 431–440. https://doi.org/10.5423/PPJ.OA.01.2016.0016

Lambais, M. R., Goldman, M. H. S., Camargo, L. E. A., & Goldman, G. H. (2000). A genomic approach to the understanding of *Xylella fastidiosa*. *Current Opinion in Microbiology* 3.5 (2000): 459–462.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., Mcgettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., & Higgins, D. G. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics*, *23*(21), 2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Li, X., Xie, Y., Liu, M., Tai, C., Sun, J., Deng, Z., & Ou, H. Y. (2018). OriTfinder: A web- based tool for the identification of origin of transfers in DNA sequences of bacterial mobile genetic elements. *Nucleic Acids Research*, *46*(W1), W229–W234. https://doi.org/10.1093/nar/gky352

Lindemann, J. (1984). Epiphytic Populations of *Pseudomonas syringae* pv. syringae on Snap Bean and Nonhost Plants and the Incidence of Bacterial Brown Spot Disease in Relation to Cropping Patterns In *Phytopathology* (Vol. 74, Issue 11, p. 1329). https://doi.org/10.1094/phyto-74-1329

Liu, B., Zheng, D., Jin, Q., Chen, L., & Yang, J. (2019). VFDB 2019: A comparativepathogenomic platform with an interactive web interface. *Nucleic Acids Research*, *47*(D1), D687–D692. https://doi.org/10.1093/nar/gky1080

Liu, M., Li, X., Xie, Y., Bi, D., Sun, J., Li, J., Tai, C., Deng, Z., & Ou, H. Y. (2019). ICEberg 2.0: An updated database of bacterial integrative and conjugative elements. *Nucleic Acids Research*, *47*(D1), D660–D665. https://doi.org/10.1093/nar/gky1123

López, C. E., & Bernal, A. J. (2012). Cassava Bacterial Blight: Using Genomics for the Elucidation and Management of an Old Problem. *Tropical Plant Biology*, *5*(1), 117–126. https://doi.org/10.1007/s12042-011-9092-3

Ma, B., Hibbing, M. E., Kim, H. S., Reedy, R. M., Yedidia, I., Breuer, J., Breuer, J., Glasner, J. D., Perna, N. T., Kelman, A., & Charkowski, A. O. (2007). Host range and molecular phylogenies of the soft rot enterobacterial genera Pectobacterium and Dickeya. *Phytopathology*, *97*(9), 1150–1163. https://doi.org/10.1094/PHYTO-97-9-1150

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., & Foster, G. D. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, *13*(6), 614–629. https://doi.org/10.1111/j.1364-3703.2012.00804.x

Marrero, J., & Waldor, M. K. (2007). Determinants of entry exclusion within Eex and TraG are cytoplasmic. *Journal of Bacteriology*, *189*(17), 6469–6473. https://doi.org/10.1128/JB.00522-07

Mathews, S. L., Hannah, H., Samagaio, H., Martin, C., Rodriguez-rassi, E., & Matthysse, A. G. (2019). *crossm Glycoside Hydrolase Genes Are Required for Virulence of*. *85*(15), 1– 15.

McManus, P. S., Stockwell, V. O., Sundin, G. W., & Jones, A. L. (2002). Antibiotic use in plant agriculture. *Annual Review of Phytopathology*, *40*(18), 443–465. https://doi.org/10.1146/annurev.phyto.40.120301.093927

Michael, G. B., Kadlec, K., Sweeney, M. T., Brzuszkiewicz, E., Liesegang, H., Daniel, R., Murray, R. W., Watts, J. L., & Schwarz, S. (2012). ICEPmu1, an integrative conjugative element (ICE) of *Pasteurella multocida*: Analysis of the regions that comprise 12 antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy*, *67*(1), 84–90. https://doi.org/10.1093/jac/dkr406

Midha, S., Bansal, K., Kumar, S., Girija, A. M., Mishra, D., Brahma, K., Laha, G. S., Sundaram, R. M., Sonti, R. V., & Patil, P. B. (2017). Population genomic insights into variation and evolution of *Xanthomonas oryzae* pv. oryzae. *Scientific Reports*, *7*(January), 1–13. https://doi.org/10.1038/srep40694

Mira, A., Klasson, L., & Andersson, S. G. E. (2002). Microbial genome evolution: Sources of variability. *Current Opinion in Microbiology*, *5*(5), 506–512. https://doi.org/10.1016/S1369-5274(02)00358-2

Mole, B. M., Baltrus, D. A., Dangl, J. L., & Grant, S. R. (2007). Global virulence regulation networks in phytopathogenic bacteria. *Trends in Microbiology*, *15*(8), 363–371. https://doi.org/10.1016/j.tim.2007.06.005

Monteil, C. L., Bardin, M., & Morris, C. E. (2014). Features of air masses associated with the deposition of *Pseudomonas syringae* and *Botrytis cinerea* by rain and snowfall. *ISME Journal*, *8*(11), 2290–2304. https://doi.org/10.1038/ismej.2014.55

Morris, C. E., Kinkel, L. L., Xiao, K., Prior, P., & Sands, D. C. (2007). Surprising niche for the plant pathogen *Pseudomonas syringae*. *Infection, Genetics and Evolution*, *7*(1), 84– 92. https://doi.org/10.1016/j.meegid.2006.05.002

Niño-Liu, D. O., Ronald, P. C., & Bogdanove, A. J. (2006). *Xanthomonas oryzae* pathovars: Model pathogens of a model crop. *Molecular Plant Pathology*, *7*(5), 303–324. https://doi.org/10.1111/j.1364-3703.2006.00344.x

Panda, P., Vanga, B. R., Lu, A., Fiers, M., Fineran, P. C., Butler, R., Armstrong, K., Ronson, C. W., & Pitman, A. R. (2016). *Pectobacterium atrosepticum* and *Pectobacterium carotovorum* harbor distinct, independently acquired integrative and conjugative elements encoding coronafacic acid that enhance virulence on potato stems. *Frontiers in Microbiology*, *7*(MAR), 1–13. https://doi.org/10.3389/fmicb.2016.00397

Parkinson, N., Stead, D., Bew, J., Heeney, J., Tsror, L., & Elphinstone, J. (2009). Dickeya species relatedness and clade structure determined by comparison of recA sequences. *International Journal of Systematic and Evolutionary Microbiology*, *59*(10), 2388–2393. https://doi.org/10.1099/ijs.0.009258-0

Pérombelon, M. C. M. (2002). Potato diseases caused by soft rot erwinias: An overview of pathogenesis. *Plant Pathology*, *51*(1), 1–12. https://doi.org/10.1046/j.0032- 0862.

Perombelon, M. C. M., & Kelman, A. (1980). Ecology of the Soft Rot Erwinias. *Annual Review of Phytopathology*, *18*(1), 361–387.https://doi.org/10.1146/annurev.py.18.090180.002045

Piqué, N., Miñana-Galbis, D., Merino, S., & Tomás, J. M. (2015). Virulence factors of *Erwinia amylovora*: A review. *International Journal of Molecular Sciences*, *16*(6), 12836–12854. https://doi.org/10.3390/ijms160612836

Prior, P., Ailloud, F., Dalsing, B. L., Remenant, B., Sanchez, B., & Allen, C. (2016). Genomicand proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. *BMC Genomics*, *17*(1), 1–11.https://doi.org/10.1186/s12864-016-2413-z

Pritchard, L., Glover, R. H., Humphris, S., Elphinstone, J. G., & Toth, I. K. (2016). Genomicsand taxonomy in diagnostics for food security: Soft-rotting enterobacterial plant pathogens. *Analytical Methods*, *8*(1), 12–24. https://doi.org/10.1039/c5ay02550h

Purcell, A. H., & Hopkins, D. L. (1996). Fastidious xylem-limited bacterial plant pathogens. *Annual Review of Phytopathology*, *34*, 131–151. https://doi.org/10.1146/annurev.phyto.34.1.131

Russell, A. B., Peterson, S. B., & Mougous, J. D. (2014). Type VI secretion system effectors: Poisons with a purpose. *Nature Reviews Microbiology*, *12*(2), 137–148. https://doi.org/10.1038/nrmicro3185

Safni, I., Cleenwerck, I., De Vos, P., Fegan, M., Sly, L., & Kappler, U. (2014). Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: Proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. syzygii subsp. nov., R. s. *International Journal of Systematic and Evolutionary Microbiology*, *64*, 3087–3103. https://doi.org/10.1099/ijs.0.066712-0

Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottiert, P., Camus, J. C., Cattolico, L., Chandler, M., Choisne, N., Claudel-Renard, C., Cunnac, S., Demange, N., Gaspin, C., Lavie, M., Moisan, A., Robert, C., … Boucher, C. A. (2002). Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature*, *415*(6871), 497–502. https://doi.org/10.1038/415497a

Salzberg, S. L., Sommer, D. D., Schatz, M. C., Phillippy, A. M., Rabinowicz, P. D., Tsuge,S., Furutani, A., Ochiai, H., Delcher, A. L., Kelley, D., Madupu, R., Puiu, D., Radune, D., Shumway, M., Trapnell, C., Aparna, G., Jha, G., Pandey, A., Patil, P. B., … Bogdanove, A. J. (2008). Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. oryzae PXO99A. *BMC Genomics*, *9*(May), 1–16. https://doi.org/10.1186/1471-2164-9-204

Silverman, J. M., Brunet, Y. R., Cascales, E., & Mougous, J. D. (2012). Structure andregulation of the type VI secretion system. *Annual Review of Microbiology*, *66*, 453–472. https://doi.org/10.1146/annurev-micro-121809-151619

Sullivan, J. T., & Ronson, C. W. (1998). Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(9), 5145–5149. https://doi.org/10.1073/pnas.95.9.5145

Summers, A. O., & Jacoby, G. A. (1977). Plasmid determined resistance to tellurium compounds. *Journal of Bacteriology*, *129*(1), 276–281.https://doi.org/10.1128/jb.129.1.276-281.1977

Swings, J., Van Den Mooter, M., Vauterin, L., Hoste, B., Gillis, M., Mew, T. W., & Kersters, K. (1990). Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* pv. oryzae) and bacterial leaf streak (*Xanthomonas campestris* pv. oryzicola) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. nov., nom. rev. *International Journal of Systematic Bacteriology*, *40*(3), 309–311. https://doi.org/10.1099/00207713-40-3-309

Tay, D. M. M., Govindarajan, K. R., Khan, A. M., Ong, T. Y. R., Samad, H. M., Soh, W. W., Tong, M., Zhang, F., & Tan, T. W. (2010). T3SEdb: Data warehousing of virulence effectors secreted by the bacterial Type III Secretion System. *BMC Bioinformatics*, *11*(SUPPL. 7). https://doi.org/10.1186/1471-2105-11-S7-S4

Toth, I. K., van der Wolf, J. M., Saddler, G., Lojkowska, E., Hélias, V., Pirhonen, M., Tsror (Lahkim), L., & Elphinstone, J. G. (2011). Dickeya species: An emerging problem for potato production in Europe. *Plant Pathology*, *60*(3), 385–399. https://doi.org/10.1111/j.1365-3059.2011.02427.x

Toussaint, A., & Merlin, C. (2002). Mobile elements as a combination of functional modules. *Plasmid*, *47*(1), 26–35. https://doi.org/10.1006/plas.2001.1552

Toussaint, A., Merlin, C., Monchy, S., Benotmane, M. A., Leplae, R., Mergeay, M., & Springael, D. (2003). The biphenyl- and 4-chlorobiphenyl-catabolic transposon Tn4371,a member of a new family of genomic islands related to IncP and Ti plasmids. *Applied and Environmental Microbiology*, *69*(8), 4837–4845.https://doi.org/10.1128/AEM.69.8.4837-4845.2003

Urban, M., Cuzick, A., Rutherford, K., Irvine, A., Pedro, H., Pant, R., Sadanadan, V., Khamari, L., Billal, S., Mohanty, S., & Hammond-Kosack, K. E. (2017). PHI-base: A new interface and further additions for the multi-species pathogen-host interactions database. *Nucleic AcidsResearch*, *45*(D1), D604–D610.https://doi.org/10.1093/nar/gkw1089

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., Potrykus, M., Waleron, M., de Vos, P., Cleenwerck, I., Pirhonen, M., Garlant, L., Hélias, V., Pothier, J. F., Pflüger, V., Duffy, B., … Manulis, S. (2014). *Dickeya solani* sp. nov., a pectinolytic plant- pathogenic bacterium isolated from potato (*Solanum tuberosum*). *International Journal of Systematic and Evolutionary Microbiology*, *64*(PART 3), 768–774. https://doi.org/10.1099/ijs.0.052944-0

Vanga, B. R., Ramakrishnan, P., Butler, R. C., Toth, I. K., Ronson, C. W., Jacobs, J. M. E., & Pitman, A. R. (2015). Mobilization of horizontally acquired island 2 is induced in planta in the phytopathogen *Pectobacterium atrosepticum* SCRI1043 and involves the putative relaxase ECA0613 and quorum sensing. *Environmental Microbiology*, *17*(11), 4730– 4744. https://doi.org/10.1111/1462-2920.13024

Verdier, V., Restrepo, S., Mosquera, G., Jorge, V., & Lopez, C. (2004). Recent progress in the characterization of molecular determinants in the *Xanthomonas axonopodis* pv. manihotis-cassava interaction. *Plant Molecular Biology*, *56*(4), 573–584. https://doi.org/10.1007/s11103-004-5044-8

Vicente, J. G., Conway, J., Roberts, S. J., & Taylor, J. D. (2001). Identification and origin of *Xanthomonas campestris* pv. campestris Races and related pathovars. *Phytopathology*, *91*(5), 492–499. https://doi.org/10.1094/PHYTO.2001.91.5.492

Wells, J. M., Raju, B. C., Hung, H., Weisburg, W. G., Mandelco-paul, L., & Brenner, D. O. N. J. (1987). Limited, Fastidious Plant Bacteria Related to Xanthomonas spp. *International Journal of Systematic Bacteriology*, *37*(2), 136–143.

Wozniak, R. A. F., & Waldor, M. K. (2010). Integrative and conjugative elements: Mosaic mobile genetic elements enabling dynamic lateral gene flow. *Nature Reviews Microbiology*, *8*(8), 552–563. https://doi.org/10.1038/nrmicro2382

Xin, X. F., Kvitko, B., & He, S. Y. (2018). *Pseudomonas syringae*: What it takes to be a pathogen. *Nature Reviews Microbiology*, *16*(5), 316–328.https://doi.org/10.1038/nrmicro.2018.17

Yang, C. H., Gavilanes-Ruiz, M., Okinaka, Y., Vedel, R., Berthuy, I., Boccara, M., Wei-Ta Chen, J., Perna, N. T., & Keen, N. T. (2002). hrp genes of *Erwinia chrysanthemi* 3937 are important virulence factors. *Molecular Plant-Microbe Interactions*, *15*(5), 472–480. https://doi.org/10.1094/MPMI.2002.15.5.472

Zambryski, P. C. (1992). Chronicles from the Agrobacterium-plant cell DNA transfer story. *Annual Review of Plant Physiology and Plant Molecular Biology*, *43*(1), 465–490. https://doi.org/10.1146/annurev.pp.43.060192.002341

Zannoni, D., Borsetti, F., Harrison, J. J., Turner, R. J., Zannoni, D., & Al, E. T. (2008). The Bacterial Response to the Chalcogen Metalloids Se and Te. *Advances in microbialphysiology* 53 (2007): 1-31. https://doi.org/10.1016/S0065-2911(07)53001-8

Zhang, Y., Wu, G., Palmer, I., Wang, B., Qian, G., Fu, Z. Q., & Liu, F. (2019). The Role of a Host-Induced Arginase of *Xanthomonas oryzae* pv. oryzae in Promoting Virulence on Rice.

Phytopathology, *109*(11), 1869–1877. https://doi.org/10.1094/PHYTO-02-19- 0058-R

Supplementary Material

Supplementary table 1: Genomes of *Pseudomonas syringae* analyzed in this work

Pseudomonas syringae Pathovars				
Strain	**RefSeq sequence**	**Isolation/Host**	**Localization**	**Pathovar**
B728a	NC_007005.1	leaflet	USA	syringae
Shaanxi_M228	NZ_CP032631.1	Actinidia	China	actinidiae
NZ-45 (ICMP 20586)	NZ_CP017007.1	*Actinidia deliciosa*	New Zealand	actinidiae
MAFF212063	NZ_CP024712.1	*Actinidia chinensis*	Japan	actinidiae
ICMP 18708	NZ_CP012179.1	Actinidia	New Zealand	actinidiae
ICMP 18884	NZ_CP011972.2	*Actinidia deliciosa*	New Zealand	actinidiae
CRAFRU14.08	NZ_CP019732.1	*Actinidia deliciosa*	Portugal	actinidiae
NZ-47	NZ_CP017009.1	*Actinidia chinensis*	New Zealand	actinidiae
CRAFRU12.29	NZ_CP019730.1	*Actinidia deliciosa*	Italy	actinidiae
P155/P220	NZ_CP032871.1	kiwifruit	China	actinidiae
ICMP 9853	NZ_CP018202.1	Actinidia	Japan	actinidiae
inb918	NZ_CP024646.1	soil	Philippines	Unknown
CFBP3846	NZ_LT963402.1	*Prunus avium*	France	avii
B301D	NZ_CP005969.1	*Pyrus communis*	United Kingdom	syringae B301D
LMG5095	NZ_CP028490.1	*Triticum aestivum*	New Zealand	atrofaciens
CFBP4215	NZ_LT962480.1	Unknown	Unknown	syringae
CFBP2116	NZ_LT985192.1	Unknown	Unknown	Unknown
CFBP2118	NZ_LT962481.1	Unknown	Unknown	syringae
CFBP6109	NZ_LT963391.1	*Prunus yedoensis*	Japan	cerasicola
CFBP3840	NZ_LT963409.1	Unknown	Unknown	Unknown
HS191	NZ_CP006256.1	*Panicum miliaceum*	Australia	syringae HS191
Pss9097	NZ_CP026568.1	Prunus sp.	United Kingdom	syringae
ATCC 10859	NZ_CP013183.1	Triticum aestivum	China	lapsa
PP1	NZ_CP034078.1	Pisum sativum	Japan	pisi
UMAF0158	NZ_CP005970.1	Mango tree	Unknown	syringae
CC1557	NZ_CP007014.1	snow	France	Unknown

UB303	NZ_CP047267.1	lake water	France	Unknown
USA011	NZ_CP045799.1	stream water	USA	Unknown

Supplementary table 2: Genomes of *Ralstonia solanacearum* Species complex analyzed in this work.

Ralstonia solacearum Species Complex			
Strain	**RefSeq sequence**	**Isolation/Host**	**Localization**
OE1-1	NZ_CP009764.1	*Solanum melongena*	Japan
FJAT-1458	NZ_CP016554.1	tomato	China
FJAT15252.F50	NZ_CP052102.1	tomato	China
FJAT1458.F1	NZ_CP052120.1	tomato	China
FJAT1458.F50	NZ_CP052118.1	tomato	China
FJAT1463.F50	NZ_CP052114.1	tomato	China
FJAT1463.F1	NZ_CP052116.1	tomato	China
FJAT15249.F50	NZ_CP052106.1	tomato	China
FJAT15252.F1	NZ_CP052104.1	tomato	China
FJAT15249.F1	NZ_CP052108.1	tomato	China
T78	NZ_CP022765.1	*Solanum tuberosum*	South Korea
EP1	NZ_CP015115.1	eggplant	China
T60	NZ_CP022768.1	*Solanum tuberosum*	South Korea
FJAT454.F1	NZ_CP052070.1	tomato	China
FJAT448.F1	NZ_CP052074.1	tomato	China
FJAT448.F50	NZ_CP052072.1	tomato	China
SL3882	NZ_CP022778.1	*Solanum tuberosum*	South Korea
FJAT454.F50-1	NZ_CP060701.1	tomato	China
SEPPX05	NZ_CP060701.1	sesame seedling	China
HA4-1	NZ_CP022481.1	peanut	China
FJAT91.F50	NZ_CP052068.1	tomato	China
FJAT91-F1	NZ_CP056083.1	Unknown	China

FJAT-91	NZ_CP016612.1	healthy tomato plant	China
FJAT91-F8	NZ_CP056085.1	Unknown	China
SL3300	NZ_CP022786.1	*Solanum tuberosum*	South Korea
FJAT15353.F8	NZ_CP052084.1	tomato	China
FJAT15353.F50	NZ_CP052086.1	tomato	China
FJAT15353.F1	NZ_CP052088.1	tomato	China
FJAT1303.F50	NZ_CP052126.1	tomato	China
FJAT1303.F8	NZ_CP052130.1	tomato	China
YC40-M	NZ_CP015850.1	Rhizoma kaempferiae	China
SL3822	NZ_CP022780.1	*Solanum tuberosum*	South Korea
CQPS-1	NZ_CP016914.1	Tobacco	China
B2	NZ_CP049787.1	tobacco substrate	China
204	NZ_CP049793.1	tobacco substrate	China
203	NZ_CP049791.1	tobacco substrate	China
202	NZ_CP049789.1	tobacco substrate	China
FJAT15244.F50	NZ_CP052110.1	tobacco substrate	China
RSCM	NZ_CP025985.1	*Cucurbita maxima*	China
FJAT15244.F1	NZ_CP052112.1	tomato	China
FJAT15244-F8	NZ_CP059376.1	tomato	China
SL3755	NZ_CP022782.1	*Solanum tuberosum*	South Korea
YQ	NZ_CP059489.1	*Casuarina equisetifolia*	China
T117	NZ_CP022755.1	*Solanum tuberosum*	South Korea
T42	NZ_CP022772.1	*Solanum tuberosum*	South Korea
GMI1000	NZ_CP022772.1F	*Solanum lycopersicum*	Unknown
SL3730	NZ_CP022784.1	*Solanum tuberosum*	South Korea
SL2729	NZ_CP022792.1	*Solanum tuberosum*	South Korea
FJAT442.F50	NZ_CP052080.1	tomato	China
FJAT442.F1	NZ_CP052082.1	tomato	China
FJAT1452.F50	NZ_CP052122.1	tomato	China

FJAT1452.F1	NZ_CP052124.1	tomato	China
FJAT445.F50	NZ_CP052076.1	tomato	China
FJAT445.F1	NZ_CP052078.1	tomato	China
SL2330	NZ_CP022794.1	*Solanum tuberosum*	South Korea
FJAT15304.F6	NZ_CP052096.1	tomato	China
FJAT15340.F50	NZ_CP052092.1	tomato	China
FJAT15304.F50	NZ_CP052098.1	tomato	China
FJAT15340.F1	NZ_CP052094.1	tomato	China
FJAT15304.F1	NZ_CP052100.1	tomato	China
FJAT15340.F6	NZ_CP052090.1	tomato	China
UW386	NZ_CP039339.1	soil	Nigeria
FJAT1303.F1	NZ_CP052128.1	tomato	China
T95	NZ_CP022761.1	*Solanum tuberosum*	South Korea
SL2064	NZ_CP022798.1	*Solanum tuberosum*	South Korea
KACC 10722	NZ_CP014702.1	*Solanum tuberosum*	South Korea
SL3022	NZ_CP023016.1	*Solanum tuberosum*	South Korea
SL3175	NZ_CP022788.1	*Solanum tuberosum*	South Korea
T98	NZ_CP022759.1	*Solanum tuberosum*	South Korea
T11	NZ_CP022776.1	*Solanum tuberosum*	South Korea
SL2312	NZ_CP022796.1	*Solanum tuberosum*	South Korea
T82	NZ_CP022763.1	*Solanum tuberosum*	South Korea
T101	NZ_CP022757.1	*Solanum tuberosum*	South Korea
T12	NZ_CP022774.1	*Solanum tuberosum*	South Korea
PSI07	NC_014311.1	Unknown	Unknown
T51	NZ_CP022770.1	*Solanum tuberosum*	South Korea
UW163	NZ_CP012939.1	plantain	Peru
SL3103	NZ_CP022790.1	*Solanum tuberosum*	South Korea
CIAT_078	NZ_CP051296.1	plantain	Colombia
Po82	NC_017574.1	Unknown	Unknown

IBSBF 2571	NZ_CP026307.1	*Musa sp.*	Brazil
KACC10709	NZ_CP016904.1	*Lycopersicon esculentum*	Korea
IBSBF1503	NZ_CP012943.1	*Cucumis sativus*	Brazil
UY031	NZ_CP012687.1	*Solanum commersonii*	Uruguay
RS 488	NZ_CP021652.1	*Solanum lycopersicum*	Brazil
RS 489	NZ_CP021766.1	*Solanum lycopersicum*	Brazil
UA-1617	NZ_CP034199.1	Plantain	Colombia
UA-1591	NZ_CP034195.1	Plantain	Colombia
UA-1611	NZ_CP034196.1	Banana	Colombia
UA-1579	NZ_CP034194.1	Banana	Colombia
UA-1609	NZ_CP034197.1	Banana	Colombia
UA-1612	NZ_CP034198.1	Banana	Colombia
FQY_4	NC_020799.1	Bacterial wilt Nursery	China
T523	NZ_CP022702.1	*Solanum lycopersicum*	Philippines
CMR15	NC_017559.1	Unknown	Unknown
P824	NZ_CP025741.1	*Vaccinium corymbosum*	USA
IBSBF 2570	NZ_CP026090.2	Musa sp.	Brazil
SFC	NZ_CP026092.2	Musa sp.	Brazil
Rs-09-161	NZ_CM002757.1	eggplant	India
Rs-10-244	NZ_CM002755.1	Chilli	India

Supplementary table 3: Genomes of *Agrobacterium tumefaciens* analyzed in this work.

		Agrobacterium tumefaciens		
Strain	**RefSeq sequence**	**Isolation/Host**	**Localization**	**Chromosome**
1D1609	NZ_CP026924.1	*Medicago sativa*	USA	I
1D1609	NZ_CP026925.1	*Medicago sativa*	USA	II
CFBP6625	NZ_CP039910.1	Unknown	Unknown	Unknown
CFBP6625	NZ_CP039911.1	Unknown	Unknown	Unknown
CFBP6623	NZ_CP039903.1	Unknown	Unknown	circular
CFBP6623	NZ_CP039904.1	Unknown	Unknown	linear
12D1	NZ_CP033031.1	Unknown	Unknown	circular
12D1	NZ_CP033032.1	Unknown	Unknown	linear
S33	NZ_CP014259.1	Unknown	Unknown	Unknown
S33	NZ_CP014260.1	Unknown	Unknown	Unknown
CFBP6624	NZ_CP039907.1	Unknown	Unknown	circular
CFBP6624	NZ_CP039908.1	Unknown	Unknown	linear
186	NZ_CP042274.1	*Juglans regia*	USA	circular
186	NZ_CP042275.1	*Juglans regia*	USA	linear
CFBP6626	NZ_CP039916.1	Unknown	Unknown	Unknown
CFBP6626	NZ_CP039917.1	Unknown	Unknown	Unknown
1D1460	NZ_CP032926.1	Rubus sp.	USA	circular
1D1460	NZ_CP032927.1	Rubus sp.	USA	linear
CFBP7129	NZ_CP039922.1	Unknown	Unknown	circular
CFBP7129	NZ_CP039923.1	Unknown	Unknown	linear
CFBP5499	NZ_CP039888.1	Unknown	Unknown	circular
CFBP5499	NZ_CP039889.1	Unknown	Unknown	linear
CFBP5877	NZ_CP039897.1	Unknown	Unknown	circular
CFBP5877	NZ_CP039898.1	Unknown	Unknown	linear
1D1108	NZ_CP032921.1	Euonymus sp.	USA	circular
1D1108	NZ_CP032922.1	Euonymus sp.	USA	linear

1D1108	NZ_CP033027.1	Unknown	Unknown	circular
A6	NZ_CP033028.1	Unknown	Unknown	linear
Ach5	NZ_CP011246.1	*Achillea ptarmica*	USA	circular
Ach5	NZ_CP011247.1	*Achillea ptarmica*	USA	linear
15955	NZ_CP032917.1	Unknown	Unknown	circular
15955	NZ_CP032918.1	Unknown	Unknown	linear
EML4	NZ_CP058525.1	Unknown	Unknown	circular
EML4	NZ_CP058526.1	Unknown	Unknown	linear

Supplementary table 4: Genomes of *Xanhtomonas oryzae* pv. oryzae analyzed in this work.

Xanthomonas oryzae pv. oryzae			
Strain	**RefSeq Sequence**	**Isolation/Host**	**Localization**
PXO99A	NC_010717.2	*Oryza sativa*	Unknown
ICMP3125	NZ_CP031697.1	*Oryza sativa*	India
NX0260	NZ_CP033192.1	*Oryza sativa*	Nepal
PXO145	NZ_CP013961.1	*Oryza sativa*	Philippines
CIAT	NZ_CP033194.1	*Oryza sativa*	Colombia
PXO211	NZ_CP013674.1	*Oryza sativa*	Philippines
PX079	NZ_CP031462.1	*Oryza sativa*	Philippines
PXO83	NZ_CP012947.1	*Oryza sativa*	Philippines
PX086	NZ_CP007166.1	*Oryza sativa* subsp. indica	Philippines
PX086-2	NZ_CP031463.1	*Oryza sativa*	Philippines
JW11089	NZ_CP033193.2	*Oryza sativa*	South Korea
PXO61	NZ_CP020942.1	*Oryza sativa*	Philippines
PXO61-2	NZ_CP021789.1	*Oryza sativa*	Philippines
PX0142	NZ_CP031698.1	*Oryza sativa*	Philippines
PXO61-3	NZ_CP021788.1	*Oryza sativa*	Philippines
KXO85	NZ_CP033197.1	*Oryza sativa*	South Korea

PXO236	NZ_CP013675.1	*Oryza sativa*	Philippines
PXO61-4	NZ_CP033187.3	*Oryza sativa*	Philippines
XF89b	NZ_CP011532.1	*Oryza sativa*	Taiwan
IX-280	NZ_CP019226.1	*Oryza sativa*	India
AUST2013	NZ_CP033196.1	*Oryza sativa*	Australia
PXO524	NZ_CP013677.1	*Oryza sativa*	Philippines
PXO602	NZ_CP013679.1	*Oryza sativa*	Philippines
JP01	NZ_CP031460.1	*Oryza sativa*	Japan
MAFF311018	NC_007705.1	Unknown	Unknown
PXO563	NZ_CP013678.1	*Oryza sativa*	Philippines
SK2-3	NZ_CP019515.1	*Oryza sativa*	Thailand
XM9	NZ_CP020334.1	*Oryza sativa*	Taiwan
PXO513	NZ_CP033188.1	*Oryza sativa*	Philippines
HuN37	NZ_CP031456.1	*Oryza sativa*	China
PXO404	NZ_CP033190.1	*Oryza sativa*	Philippines
PXO421	NZ_CP033189.1	*Oryza sativa*	Philippines
PXO71	NZ_CP013670.1	*Oryza sativa*	Philippines
PXO364	NZ_CP033191.1	*Oryza sativa*	Philippines
JL25	NZ_CP031457.1	*Oryza sativa*	China
JL33	NZ_CP031459.1	*Oryza sativa*	China
YC11	NZ_CP031464.1	*Oryza sativa*	China
ScYc-b	NZ_CP031469.1	*Oryza sativa*	China
ScYc-b	NZ_CP018087.1	*Oryza sativa*	China
CFBP7319	NZ_CP033181.1	*Oryza sativa*	Burkina Faso
CIX298	NZ_CP036378.1	*Oryza sativa*	Burkina Faso
CFBP7340	NZ_CP033174.1	*Oryza sativa*	Niger
mai/01	NZ_CP025609.1	*Oryza sativa*	Mali
CFBP1949	NZ_CP033184.1	*Oryza sativa*	Mali
CFBP7325	NZ_CP033176.1	*Oryza sativa*	Mali

MAI134	NZ_CP019091.1	*Oryza longistaminata*	Mali
CFBP7337	NZ_CP033175.1	*Oryza sativa*	Mali
CIX2374	NZ_CP036377.1	*Oryza sativa*	Senegal
CFBP7323	NZ_CP033178.1	*Oryza sativa*	Niger
Ug11	NZ_CP033170.1	*Oryza sativa*	Uganda
Dak16	NZ_CP033172.1	*Oryza sativa*	Tanzania
T19	NZ_CP033171.1	*Oryza sativa*	Tanzania
CFBP7324	NZ_CP033177.1	*Oryza sativa*	Niger
CFBP7320	NZ_CP033186.1	*Oryza sativa*	Burkina Faso
BAI3	NZ_CP025610.1	*Oryza sativa*	Burkina Faso
CFBP7321	NZ_CP033180.1	*Oryza sativa*	Burkina Faso
CFBP7322	NZ_CP033179.1	*Oryza sativa*	Burkina Faso
MAI106	NZ_CP019089.1	*Oryza sativa*	Mali
mai/95	NZ_CP019087.1	*Oryza sativa*	Mali
CFBP1951	NZ_CP033183.1	*Oryza sativa*	Mali
mai/73	NZ_CP019086.1	*Oryza sativa*	Mali
MAI145	NZ_CP019092.1	*Oryza sativa*	Mali
MAI129	NZ_CP019090.1	*Oryza sativa*	Mali
mai/68	NZ_CP019085.1	*Oryza sativa*	Mali
mai/99	NZ_CP019088.1	*Oryza sativa*	Mali
JL28	NZ_CP031458.1	*Oryza sativa*	China
CFBP1952	NZ_CP033182.1	*Oryza sativa*	Mali
AXO1947	NZ_CP013666.1	*Oryza sativa*	Cameroon
CFBP1948	NZ_CP033185.1	*Oryza sativa*	Cameroon
CFBP8172	NZ_CP033173.1	*Oryza sativa*	Benin
YN24	Unknown	*Oryza sativa*	China
BXO1	Unknown	*Oryza sativa*	India
PXO282	NZ_CP013676.1	*Oryza sativa*	Philippines
KACC10331	NC_006834.1	Unknown	Unknown

OS198	NZ_CP031461.1	*Oryza sativa*	China
IXO1088	NZ_CP040687.1	*Oryza sativa*	India
IXO704	NZ_CP040604.1	*Oryza sativa*	India
K2	NZ_CP050113.1	*Oryzae sativa* L.	South Korea
K1	NZ_CP049205.1	*Oryzae sativa* L.	South Korea
K3	NZ_CP050114.1	*Oryzae sativa* L.	South Korea
K3a	NZ_CP050115.1	*Oryzae sativa* L.	South Korea
ITCCBB0002	NZ_CP046148.1	*Oryza sativa*	India
LN18	Unknown	*Oryza sativa*	China

Supplementary table 5: Genomes of *Xanthomonas campestris* analyzed in this work.

		***Xanthomonas campestris* pahtovars**		
Strain	**RefSeq Sequence**	**Isolation/Host**	**Localization**	**Pathovar**
ATCC 33913	NC_003902.1	*Brassica oleracea*	Unknown	campestris
8004	NC_007086.1	*Brassica oleracea var. botrytis*	United Kingdom	campestris
CN18	NZ_CP017319.1	*Brassica juncea var. foliosa*	China	campestris
CN17	NZ_CP017307.1	*Brassica rapa subsp. Chinensis*	China	campestris
CN12	NZ_CP017310.1	*Brassica napus subsp. Oleifera*	China	campestris
B100	NC_010688.1	Mud	Germany	campestris
3811	NZ_CP025750.1	Cabbage	Unknown	campestris
MAFF302021	NZ_AP019684.1	*Brassica oleracea var. botrytis*	Japan	campestris
CN03	NZ_CP017308.1	*Brassica rapa subsp. Pekinensis*	Japan	campestris
CN14	NZ_CP017317.1	*Brassica juncea var. foliosa*	China	campestris
CN15	NZ_CP017323.1	*Brassica rapa subsp. Chinensis*	China	campestris
MAFF106712		Unknown	Unknown	campestris
NEB122	NZ_CP051651.1	Unknown	Unknown	badrii
756C	NC_017271.1	Unknown	Unknown	raphani
ICMP 21080	NZ_CP012145.1	cabbage	New Zealand	campestris

ICMP 4013	NZ_CP012146.1	*Brassica oleracea var. capitata*	New Zealand	campestris
ICMP 4013	NZ_CP051651.1	Unknown	Unknown	badrii
MAFF106181	NZ_CP058243.1	Japanese radish	Japan	raphani

Supplementary table 6: Genomes of *Xanthomonas axonopodis* analyzed in this work.

		Xanthomonas axonopodis		
Strain	**RefSeq Sequence**	**Isolation/Host**	**Localization**	**Pathovar**
Xac29-1	NC_020800.1	Unknown	Unknown	Unknown
LMG26789	NZ_CP031059.1	*Commiphora wightii*	India	commiphoreae
NCPPB 796	NZ_CP053649.1	sugarcane	Mauritius	vasculorum

Supplementary table 7: Genomes of *Erwinia amylovora* analyzed in this work.

	Erwinia amylovora		
Strain	**RefSeq Sequence**	**Isolation/Host**	**Localization**
E-2	NZ_CP024970.1	Malus sp.	Belarus
CFPB1430	NC_013961.1	Unknown	Unknown
ATCC 49946	NC_013971.1	Unknown	Unknown
FB-86	NZ_CP050258.1	Apple tree	South Korea
FB-207	NZ_CP050263.1	Pear tree	South Korea
TS3238	NZ_CP050244.1	Pear tree	South Korea
TS3128	NZ_CP056034.1	*Pyrus pyrifolia var. culta*	South Korea
FB-307	NZ_CP050242.1	Apple tree	South Korea
FB-20	NZ_CP050240.1	Pear tree	South Korea

Supplementary table 8: Genomes of *Xylella fastidiosa* analyzed in this work.

	Xylella fastidiosa		
Strain	**RefSeq Sequence**	**Isolation/Host**	**Localization**
9a5c	NC_002488.3	Orange Valencia	Brazil
Hib4	NZ_CP009885.1	Hibiscus	Brazil
J1a12	NZ_CP009823.1	Citrus	Brazil
Ann-1	NZ_CP006696.1	Oleander	Unknown
3124	NZ_CP009829.1	coffee	Brazil
U24D	NZ_CP009790.1	*Citrus sinensis*	Brazil
Pr8x	NZ_CP009826.1	Plum	Brazil
Fb7	NZ_CP010051.2	Citrus	Argentina
MUL0034	NZ_CP006740.1	Mulberry	Unknown
M23	NC_010577.1	*Prunus dulcis*	USA
Temecula1	NC_004556.1	Grapevine	USA
De Donno	NZ_CP020870.1	*Olea europaea*	Italy
Salento-2	NZ_CP016610.1	*Olea europaea*	Italy
Salento-1	NZ_CP016608.1	*Olea europaea*	Italy
Bakersfield-1	NZ_CP040799.1	*Vitis vinifera*	USA
GB514	NC_017562.1	grape	USA
M12	NC_010513.1	*Prunus dulcis*	USA
ATCC 35879	NZ_CP044352.1	Grapevine	USA
RH1	NZ_CP052853.1	*Olea europaea*	USA
LM10	NZ_CP052854.1	*Olea europaea*	USA
Fillmore	NZ_CP052855.1	*Olea europaea*	USA

Supplementary table 9: Genomes of *Dickeya (dadantii* and *solani)* analyzed in this work.

Dickeya dadantii			
Strain	**RefSeq**	**Host**	**Localization**
DSM 18020	NZ_CP023467.1	*Pelargonium capitatum*	Comoros
3937	NC_014500.1	African violet	Unknown

Dickeya solani			
Strain	**RefSeq**	**Host**	**Localization**
IOP2222	NZ_CP015137.1	*Solanum tuberosum*	Netherlands
IFB0223	NZ_CP024710.1	*Solanum tuberosum*	Germany
IFB 0099	NZ_CP024711.1	*Solanum tuberosum*	Poland
RNS 08.23.3.1.A	NZ_CP016928.1	*Solanum tuberosum*	France
D s0432-1	NZ_CP017453.1	*Solanum tuberosum*	Finland
PPO 9019	NZ_CP017454.1	Muscari	Netherlands
IFB0223	NZ_CP024710.1	*Solanum tuberosum*	Germany
IFB0421	NZ_CP051460.1	*Solanum tuberosum*	Portugal
IFB0231	NZ_CP051458.1	*Solanum tuberosum*	Finland
IFB0417		*Solanum tuberosum*	Portugal
IFB0167	NZ_CP051457.1	*Solanum tuberosum*	Poland

Supplementary table 10: Genomes of *Pectobacterium carotovorum* (and *P. atrosepticum*) analyzed in this work.

Pectobacterium carotovorum			
Strain	**RefSeq**	**Host**	**Localization**
PC1	NC_012917.1	Unknown	Unknown
PCC21	NC_018525.1	Unknown	Unknown
JR1.1	NZ_CP034237.1	Radish	South Korea
BP201601.1	NZ_CP034236.1	Potato	South Korea
Pectobacterium atrosepticum			
Strain	**RefSeq**	**Host**	**Localization**
SCRI1043	NC_004547.2	Solanum tuberosum	Unknown
JG10-08	NZ_CP007744.1	Solanum tuberosum	China
21A	NZ_CP009125.1	Solanum tuberosum	Belarus
36A	NZ_CP024956.1	Solanum tuberosum	Belarus

Supplementary table 11: ICEs identified in the genomes of the main phytopathogenic bacteria

Dickeya dadantii

Strain	Element	RefSeq	Location (nt)	Length (bp)
3937	ICEDd.1	NC_014500.1	3126494-3201103	74.610
DSM 18020	ICEDd.2	NZ_CP023467.1	866896-926592	40.117

Dickeya solani

Strain	Element	RefSeq	Location (nt)	Length (bp)
IPO2222	ICEDs.1	NZ_CP015137.1	4033605-4081983	48.378
IFB0223		NZ_CP024710.1	1870124-1918502	48.378
IFB 0099		NZ_CP024711.1	1873274-1921652	48.378
RNS 08.23.3.1.A		NZ_CP016928.1	4694369-4742747	48.378
D s0432-1		NZ_CP017453.1	835683-884061	48.378
PPO 9019		NZ_CP017454.1	828904-877282	48.378
IFB0421		NZ_CP051460.1	1873239-1921616	48.377
IFB0231		NZ_CP051458.1	1873279-1921657	48.378
IFB0417		CP051459.1	1872976-1921336	48.360
IFB0223		NZ_CP024710.1	1870124-1918502	48.378
IFB0167		NZ_CP051457.1	1873279-1921657	48.378

Pectobacterium atrosepticum

Strain	Element	RefSeq	Location (nt)	Length (bp)
SCRI1043	ICEPa.1	NC_004547.2	590755-688599	97.845
SCRI1043	ICEPa.2		1867875-1926959	59.085
JG10-08	ICEPa.3	NZ_CP007744.1	3165718-3228840	63.123
JG10-08			980430-1077384	96.954
21A	ICEPa.1	NZ_CP009125.1	975442 -1072594	97.152
36A		NZ_CP024956.1	586814-683968	97.154

36A	ICEPa.4		1863011-1908923	45.912

Pectobacterium carotovorum

Strain	**Element**	**RefSeq**	**Location (nt)**	**Length (bp)**
JR1.1	ICEPc.1	NZ_CP034237.1	1595656-1632307	36.651
BP201601.1	ICEPc.2	NZ_CP034236.1	1663873-1730176	66.303

Agrobacterium tumefaciens

Strain	**Element**	**RefSeq**	**Location (nt)**	**Length (bp)**
1D1609 (Cromossomo I)	ICEAt.1	NZ_CP026924.1	272270-334336	61.887
12D1 (Cromossomo circular)	ICEAt.2	NZ_CP033031.1	2309372-2377994	68.662
12D1 (Cromossomo linear)	ICEAt.3	NZ_CP033032.1	1340555-1455519	114.964
186(Cromossomo circular)	ICEAt.4	NZ_CP042274.1	2013312-2114889	101.577

Pseudomonas syringae

Strain	**Element**	**RefSeq**	**Location (nt)**	**Length (bp)**
B728a	ICEPs.1	NC_007005.1	1604658-1724374	119.716
Shaanxi_M228	ICEPs.2	NZ_CP032631.1	5463863-5614284	150.421
Shaanxi_M228	ICEPs.3		1672240-1753644	81.404
Shaanxi_M228	ICEPs.4		1753496-1862291	108.795
Shaanxi_M228	ICEPs.5		1862882-1911082	48.201
NZ-45 (ICMP 20586)	ICEPs.2	NZ_CP017007.1	5369000-5512238	143.238
NZ-45 (ICMP 20586)	ICEPs.6		1734480-1836698	102.218
NZ-45 (ICMP 20586)	ICEPs.7		1835321-1892784	57.463
NZ-45 (ICMP 20586)	ICEPs.8		5513439-5621120	107.200
MAFF212063	ICEPs.9	NZ_CP024712.1	4717464-4816692	99.228
MAFF212063	ICEPs.10		5548512-5709520	161.009
ICMP 18708	ICEPs.2	NZ_CP012179.1	5266321-5409559	143.238
ICMP 18708	ICEPs.7		1734473-1791958	57.485
ICMP 18884	ICEPs.2	NZ_CP011972.2	5266318-5409556	143.238
ICMP 18884	ICEPs.7		1734470-1784283	49.813

CRAFRU14.08	ICEPs.2	NZ_CP019732.1	1829573-1980910	151.337
CRAFRU14.08	ICEPs.11		1735800-1828308	92.508
CRAFRU14.08	ICEPs.12		5462941-5504692	41.751
NZ-47	ICEPs.2	NZ_CP017009.1	5266318-5409556	143.238
NZ-47	ICEPs.7		1734470-1784322	49.852
NZ-47	ICEPs.13		5410536-5501754	91.218
CRAFRU12.29	ICEPs.2	NZ_CP019730.1	5360196-5503434	143.238
CRAFRU12.29	ICEPs.11		1737471-1829979	92.508
CRAFRU12.29	ICEPs.7		1830465-1880317	49.852
P155/P220	ICEPs.14	NZ_CP032871.1	1689937-1797268	107.331
P155/P220	ICEPs.2		5384241-5527881	143.640
CFBP6109	ICEPs.15	NZ_LT963391.1	1050946-1102190	51.244
CFBP3840	ICEPs.16	NZ_LT963409.1	4987773-5066398	78.625
HS191	ICEPs.17	NZ_CP006256.1	5140209-5226281	85.921
PP1	ICEPs.18	NZ_CP034078.1	865150-953883	89.125
CC1557	ICEPs.19	NZ_CP007014.1	2591336-2746640	155.304
UB303	ICEPs.20	NZ_CP047267.1	5453047-5484042	30.968

Xanthomonas campestris

Strain	**Element**	**RefSeq**	**Location (nt)**	**Length (bp)**
B100	ICEXc.1	NC_010688.1	2798361-2862467	64.106
3811		NZ_CP025750.1	2798845-2863378	64.534
CN03	ICEXc.2	NZ_CP017308.1	2398353-2482109	83.757

Xylella fastidiosa

Strain	**Element**	**RefSeq**	**Location (nt)**	**Length (bp)**
9a5c	ICEXf.1	NC_002488.3	1934170-2022372	88.202
Hib4	ICEXf.2	NZ_CP009885.1	1399531-1497444	97.913
J1a12	ICEXf.3	NZ_CP009823.1	1931410-2069541	158.355
U24D	ICEXf.1	NZ_CP009790.1	1934275 - 2022404	88.129

		R. pseudosolanacearum		
Strain	**Element**	**RefSeq**	**Location (nt)**	**Length (bp)**
GMI1000	Tn4371	NC_003295.1	2780151-2825764	45.613
RS476		NZ_CP021762.1	2780170-2825773	45.603
CRMRs218		NZ_CP021764.1	2780419-2826032	45.613
FJAT-91	ICERps1	NZ_CP016612.1	1283877-1335170	51.293
FQY_4	ICERps2	NC_020799.1	1113417-1173796	60.379
YC40-M		NZ_CP015850.1	2532466-2588165	55.699
HA4I	ICERps3	NZ_CP022481.1	1709954-1756828	46.874
UW386	ICERps4	NZ_CP039339.1	545761-602147	56.386
FJAT91-F1	ICERps1	NZ_CP056083.1	2505206-2556182	50.976
FJAT91-F8		NZ_CP056085.1	2505097-2556073	50.976

		R. syzyzii		
Strain	**Element**	**RefSeq**	**Location (nt)**	**Length (bp)**
T98	ICERsy1	NZ_CP022759.1	1925451-1985747	60.296
SL3175		NZ_CP022788.1	1925467-1985763	60.296

Supplementary table 12: Size and GC content of the identified elements

Dickeya dadantii

Strain	Element	Location (nt)	RefSeq	Genome Length (bp)	Element Length (bp)	Genome %GC	Element %GC
3937	ICEDd.1	3126494-3201103	NC_014500.1	4.922.802	74.610	56.3	52.1
DSM 18020	ICEDd.2	876895-917012	NZ_CP023467.1	4.997.541	40.117	56.4	52.9

Dickeya solani

Strain	Element	Location (nt)	RefSeq	Genome Length (bp)	Element Length (bp)	Genome %GC	Element %GC
IOP2222	ICEDs.1	4033605-4081983	NZ_CP015137.1	4.919.833	48.378	56.20	49.4
IFB0223		1870124-1918502	NZ_CP024710.1	4.937.554			
IFB 0099		1873274-1921652	NZ_CP024711.1	4.932.920			
RNS 08.23.3.1.A		4694369-4742747	NZ_CP016928.1	4.922.468		56.30	
D s0432-1		835683-884061	NZ_CP017453.1	4.919.812		56.20	
PPO 9019		828904-877282	NZ_CP017454.1	4.918.850		56.29	
IFB0421		1873239-1921616	NZ_CP051460.1	4.934.537	48.377	56.20	
IFB0231		1873279-1921657	NZ_CP051458.1	4.924.702	48.378		
IFB0417		1872976-1921336	CP051459.1	4.924.102	48.360		
IFB0223		1870124-1918502	NZ_CP024710.1	4.937.554	48.378		
IFB0167		1873279-1921657	NZ_CP051457.1	4.922.289			

Pectobacterium atrosepticum

Strain	Element	Location (nt)	RefSeq	Genome Length (bp)	Element Length (bp)	Genome %GC	Element %GC
SCRI1043	ICEPa.1	590755-688599	NC_004547.2	5.064.019	97.845	51.00	48.3
SCRI1043	ICEPa.2	1867875-1926959		5.064.019	59.085		40.7

JG10-08	ICEPa.3	3165718-3228840	NZ_CP007744.1	5.004.926	63.123	51.10	48.3
JG10-08		980430-1077384		5.004.926	96.954		48.3
21A	ICEPa.1	975442 -1072594	NZ_CP009125.1	4.991.806	97.152	51.07	48.3
36A		586814-683968	NZ_CP024956.1	4.965.575	97.154	51.10	48.3
36A	ICEPa.4	1863011-1908923		4.965.575	45.912		46.9

Pectobacterium carotovorum

Strain	**Element**	**Location (nt)**	**RefSeq**	**Genome Length (bp)**	**Element Length (bp)**	**Genome %GC**	**Element %GC**
JR1.1	ICEPc.1	1595656-1632307	NZ_CP034237.1	4.872.902	36.651	52.00	45.5
BP201601.1	ICEPc.2	1663873-1730176	NZ_CP034236.1	4.853.176	66.303	52.20	51.3

Agrobacterium tumefaciens

Strain	**Element**	**Location (nt)**	**RefSeq**	**Genome Length (bp)**	**Element Length (bp)**	**Genome %GC**	**Element %GC**
1D1609 (Cromossomo I)	ICEAt.1	272270-334336	NZ_CP026924.1	3.058.772	61.887	59.49	63.7
12D1 (Cromossomo circular)	ICEAt.2	2309372-2377994	NZ_CP033031.1	3.027.766	68.662	59.53	58.7
12D1 (Cromossomo linear)	ICEAt.3	1340555-1455519	NZ_CP033032.1	2.258.260	114.964		58.1
186(Cromossomo circular)	ICEAt.4	2013312-2114889	NZ_CP042274.1	2.943.916	101.577	59.43	61.0

Pseudomonas syringae

Strain	**Element**	**Location (nt)**	**RefSeq**	**Genome Length (bp)**	**Element Length (bp)**	**Genome %GC**	**Element %GC**
B728a	ICEPs.1	1604658-1724374	NC_007005.1	6.093.698	119.716	58.70	54.5
Shaanxi_M228	ICEPs.2	5463863-5614284	NZ_CP032631.1	6.674.594	150.421	58.28	57.0
Shaanxi_M228	ICEPs.3	1672240-1753644		6.674.594	81.404		54.3
Shaanxi_M228	ICEPs.4	1753496-1862291		6.674.594	108.795		54.3
Shaanxi_M228	ICEPs.5	1862882-1911082		6.674.594	48.201		54.4
NZ-45 (ICMP 20586)	ICEPs.2	5369000-5512238	NZ_CP017007.1	6.665.031	143.238	58.37	57.2
NZ-45 (ICMP 20586)	ICEPs.6	1734480-1836698		6.665.031	102.218		54.8
NZ-45 (ICMP 20586)	ICEPs.7	1835321-1892784		6.665.031	57.463		53.9
NZ-45 (ICMP 20586)	ICEPs.8	5513439-5621120		6.665.031	107.200		55.2

MAFF212063	ICEPs.9	4717464-4816692	NZ_CP024712.1	6.556.999	99.228	58.35	54.7
MAFF212063	ICEPs.10	5548512-5709520		6.556.999	161.009		55.3
ICMP 18708	ICEPs.2	5266321-5409559	NZ_CP012179.1	6.555.571	143.238	58.60	57.2
ICMP 18708	ICEPs.7	1734473-1791958		6.555.571	57.485		53.9
ICMP 18884	ICEPs.2	5266318-5409556	NZ_CP011972.2	6.555.569	143.238	58.37	57.2
ICMP 18884	ICEPs.7	1734470-1784283		6.555.569	49.813		53.9
CRAFRU14.08	ICEPs.2	1829573-1980910	NZ_CP019732.1	6.546.028	151.337		57.2
CRAFRU14.08	ICEPs.11	1735800-1828308		6.546.028	92.508		54.4
CRAFRU14.08	ICEPs.12	5462941-5504692		6.546.028	41.751		53.7
NZ-47	ICEPs.2	5266318-5409556	NZ_CP017009.1	6.545.910	143.238	58.40	57.2
NZ-47	ICEPs.7	1734470-1784322		6.545.910	49.852		53.9
NZ-47	ICEPs.13	5410536-5501754		6.545.910	91.218		55.1
CRAFRU12.29	ICEPs.2	5360196-5503434	NZ_CP019730.1	6.545.549	143.238	58.37	57.2
CRAFRU12.29	ICEPs.11	1737471-1829979		6.545.549	92.508		54.4
CRAFRU12.29	ICEPs.7	1830465-1880317		6.545.549	49.852		53.9
P155/P220	ICEPs.14	1689937-1797268	NZ_CP032871.1	6.529.859	107.331		54.8
P155/P220	ICEPs.2	5384241-5527881		6.529.859	143.640		57.2
CFBP6109	ICEPs.15	1050946-1102190	NZ_LT963391.1	6.015.874	51.244	58.10	55.9
CFBP3840	ICEPs.16	4987773-5066398	NZ_LT963409.1	6.013.125	78.625	58.06	54.8
HS191	ICEPs.17	5140209-5226281	NZ_CP006256.1	5.950.211	86.072	58.96	54.6
PP1	ICEPs.18	865150-953883	NZ_CP034078.1	5.883.416	88.734	58.80	54.6
CC1557	ICEPs.19	2591336-2746640	NZ_CP007014.1	5.758.024	155.304	58.56	57.0
UB303	ICEPs.20	5453047-5484042	NZ_CP047267.1	6.141.482	30.968	59.20	50.7

Xanthomonas campestris

Strain	**Element**	**Location (nt)**	**RefSeq**	**Genome Length (bp)**	**Element Length (bp)**	**Genome GC**	**Element GC**
B100	ICEXc.1	2798361-2862467	NC_010688.1	5.079.002	64.106	65.00	61.7
3811		2798845-2863378	NZ_CP025750.1	5.072.566	64.534		

CN03	ICEXc.2	2398353-2482109	NZ_CP017308.1	5.035.984	83.756	65.05	60.1

Xylella fastidiosa

Strain	Element	Location (nt)	RefSeq	Genome Length (bp)	Element Length (bp)	Genome GC	Element GC
9a5c	ICEXf.1	1934170-2022372	NC_002488.3	2.813.297	88.202	52.64	52.9
Hib4	ICEXf.2	1399531-1497444	NZ_CP009885.1	2.813.297	97.913	52.69	66.4
J1a12	ICEXf.3	1931410-2069541	NZ_CP009823.1	2.788.789	158.355	52.81	54.6
U24D	ICEXf.1	1934275-2022404	NZ_CP009790.1	2.681.334	88.129	52.64	53

Ralstonia pseudosolanacearum

Strain	Element	Location (nt)	RefSeq	Genome Length (bp)	Element Length (bp)	Genome GC	Element GC
GMI1000	Tn4371	2780151-2825764	NC_003295.1	3.716.413	45.613	66.96	63.2
RS476		2780170-2825773	NZ_CP021762.1	3.716.422	45.603	66.96	63.2
CRMRs218		2780419-2826032	NZ_CP021764.1	3.716.755	45.613	66.70	63.2
FJAT-91	ICERps1	1283877-1335170	NZ_CP016612.1	3.873.214	51.293	66.87	60.9
FQY_4	ICERps2	1113417-1173796	NC_020799.1	3.715.422	60.379	66.79	63
YC40-M		2532466-2588165	NZ_CP015850.1	3.844.764	55.699	66.70	62.3
HA4I	ICERps3	1709954-1756828	NZ_CP022481.1	3.890.347	46.874	66.68	62.2
UW386	ICERps4	545761-602147	NZ_CP039339.1	3.658.282	56.386	66.93	62.4
FJAT91-F1	ICERps1	2505206-2556182	NZ_CP056083.1	3.873.221	50.976	66.87	60.9
FJAT91-F8		2505097-2556073	NZ_CP056085.1	3.873.088	50.976	66.87	60.9

Ralstonia syzyzii

Strain	Element	Location (nt)	RefSeq	Genome Length (bp)	Element Length (bp)	Genome GC	Element GC
T98	ICERsy1	1925451-1985747	NZ_CP022759.1	3.569.696	60.296	66.33	60.2
SL3175		1925467-1985763	NZ_CP022788.1	3.569.709	60.296	66.33	60.2

Supplementary table 13: Attachment sites

Dickeya dadantii

Element	Strain	Sequence	AttL	AttR
ICEDd.1	3937	ccagtcagaggagccaaa	3126494-3126511	3201086-3201103

Dickeya solani

Element	Strain	Sequence	AttL	AttR
ICEDs.1	IPO2222	tggctcctctgactgg	4033605-4033620	4081968-4081983
ICEDs.1	IFB0223	tggctcctctgactgg	1870124-1870139	1918487-1918502
ICEDs.1	IFB 0099	tggctcctctgactgg	1873274-1873289	1921637-1921652
ICEDs.1	RNS 08.23.3.1.A	tggctcctctgactgg	4694369-4694384	4742732-4742747
ICEDs.1	D s0432-1	ccagtcagaggagcca	835683-835698	884046-884061
ICEDs.1	PPO 9019	ccagtcagaggagcca	828904-828919	877267-877282
ICEDs.1	IFB0421	tggctcctctgactgg	1873239-1873254	1921601-1921616
ICEDs.1	IFB0231	tggctcctctgactgg	1873279-1873294	1921642-1921657
ICEDs.1	IFB0417	tggctcctctgactgg	1872976-1872991	1921321-1921336
ICEDs.1	IFB0223	tggctcctctgactgg	1870124-1870139	1918487-1918502
ICEDs.1	IFB0167	tggctcctctgactgg	1873279-1873294	1921642-1921657

Pectobacterium atrosepticum

Element	Strain	Sequence	AttL	AttR
ICEPa.1	SCRI1043	agtggtgcccggactcgg	590755-590803	688582-688599
ICEPa.2	SCRI1043	ttggctcctctgactggactcgaa	1886342-1886365	1927138-1927161
ICEPa.3	JG10-08	atcgggagaatttggtggtcagatttggggtca	3165718-3165750	3228808-3228840
ICEPa.1	JG10-08	agtggtgcccggactcgg	980430-980447	1077564-1077581
ICEPa.1	21A	agtggtgcccggactcgg	975442-975459	1072576-1072593
ICEPa.1	36A	agtggtgcccggactcgga	586814-586862	683950-683968
ICEPa.4	36A	aatttggctcctctgactgg	1863011-1863030	1908904-1908923

Pectobacterium carotovorum

Element	Strain	Sequence	AttL	AttR
ICEPc.1	JR1.1	aatttggctcctctgactgg	1595656-1595675	1632288-1632307
ICEPc.2	BP201601.1	atttgggggtcactccgtcatcgaaccaa	1663873-1663901	1730148-1730176

Agrobacterium tumefaciens

Element	Strain	Sequence	AttL	AttR
ICEAt.3	12D1 (Cromossomo linear)	gcccccgcaaccaccga	1340555-1340571	1455503-1455519
ICEAt.4	186(Cromossomo circular)	gctccttcagcaccg	2015364-2015378	2114875-2114889

Pseudomonas syringae

Element	Strain	Sequence	AttL	AttR
ICEPs.1	B728a	acaagcagccgtctg	1604658-1604672	1724360-1724374
ICEPs.2	Shaanxi_M228	agttttccggccatt	5463863-5463877	5614270-5614284
ICEPs.3	Shaanxi_M228	ttggcttttaaccaattggtcgtaggttcgaatcccacacgacccaccattt	1672243-1672294	1753593-1753644
ICEPs.4	Shaanxi_M228	tataaagcctctaatggggacattagag	1753503-1753530	1862258-1862285
ICEPs.2	NZ-45 (ICMP 20586)	agttttccggccatt	5369000-5369014	5512224-5512238
ICEPs.6	NZ-45 (ICMP 20586)	ctgacgtaggattttgtggcagcccaatagatctt	1735777- 1735811	1836664-1836698
ICEPs.8	NZ-45 (ICMP 20586)	tgcgacctgatacgagtggcatcagatctttaagattcggctccaaggt	5513439-5513487	5621072-5621120
ICEPs.9	MAFF212063	aaatggtgggtcgtgtgggattcgaacctacgaccaattggttaaaag	4717464-47175511	4816645-4816692
ICEPs.2	ICMP 18708	agttttccggccatt	5266321-5266335	5409545-5409559
ICEPs.2	ICMP 18884	agttttccggccatt	5266318-5266332	5409542-5409556
ICEPs.2	CRAFRU14.08	aatggccggaaaact	1829573-1829587	1980896-1980910
ICEPs.2	NZ-47	agttttccggccatt	5266318-5266332	5409542-5409556
ICEPs.13	NZ-47	gcgttatgtttcgcgacagacggctgcttg	5410536-5410565	5501725-5501754
ICEPs.2	CRAFRU12.29	agttttccggccatt	5360196-5360210	5503420-5503434
ICEPs.11	CRAFRU12.29	ttggcttttaaccaattggtcgtaggttcgaatcccacacgacccaccattt	1737471-1737522	1829928-1829979
ICEPs.14	P155/P220	ttggcttttaaccaattggtcgtaggttcgaatcccacacgacccaccattt	1689937-1689988	1797217-1797268

ICEPs.2	P155/P220	agttttccggccatt	5384241-5384255	5527867-5527881
ICEPs.16	CFBP3840	gtggcatcagatctttaagattcggctccaaggtgaaccttggaaccaaaaatggtgggtcgtgtgggattcgaacctacgaccaattggttaaaag	4987773-7987869	50663302-5066398
ICEPs.17	HS191	tggtcggggtaaggggattcgaactcctgacatcctgctcccaaagcaggcgcgctaccggactgcgctataccccg	5140209-5140285	5226205-5226281
ICEPs.18	PP1	cggggtatagcgcagtccggtagcgcgcctgctttgggagcaggatgtcaggagttcgaatccccttaccccgacca	864759-864835	953807-953883
ICEPs.19	CC1557	gtctcgtttcccgctcca	2591336-2591353	2746623-2746640
ICEPs.20	UB303	actcataatcctttggtccacggttcgagtccgtgtgggcccacca	5453003-5453048	5484451-5484496

Xanthomonas campestris

Element	**Strain**	**Sequence**	**AttL**	**AttR**
ICEXc.1	B100	agcgagcgccgggcttg	2798361-2798377	2862451-2862467
ICEXc.1	3811	agcgagcgccgggcttg	2798845-2798861	2863362-2863378
ICEXc.2	CN03	gagggtcggcagggaatcattac	2398353-2398375	2482087-2482109

Xylella fastidiosa

Element	**Strain**	**Sequence**	**AttL**	**AttR**
ICEXf.2	Hib4	ctcgtttcccgctcca	1399531-1399546	1497429-1497444

Supplementary table 14: Insertion sites, Type of Integrase and Relaxase

Dickeya dadantii

Strain	Element	RefSeq	Insertion site	Integrase	Relaxase
3937	ICEDd.1	NC_014500.1	tRNA-Asn	Phage	MOBC
DSM 18020	ICEDd.2	NZ_CP023467.1	BamE	Tyr	MOBP

Dickeya solani

Strain	Element	RefSeq	Insertion site	Integrase	Relaxase
IOP2222	ICEDs.1	NZ_CP015137.1	tRNA-Asn	Tyr	MOBC
IFB0223	ICEDs.1	NZ_CP024710.1			
IFB 0099	ICEDs.1	NZ_CP024711.1			
RNS 08.23.3.1.A	ICEDs.1	NZ_CP016928.1			
D s0432-1	ICEDs.1	NZ_CP017453.1			
PPO 9019	ICEDs.1	NZ_CP017454.1			
IFB0223	ICEDs.1	NZ_CP024710.1			
IFB0421	ICEDs.1	NZ_CP051460.1			
IFB0231	ICEDs.1	NZ_CP051458.1			
IFB0223	ICEDs.1	CP051459.1			
IFB0417	ICEDs.1	NZ_CP024710.1			
IFB0167	ICEDs.1	NZ_CP051457.1			

Pectobacterium atrosepticum

Strain	Element	RefSeq	Insertion site	Integrase	Relaxase
SCRI1043	ICEPa.1	NC_004547.2	tRNA-Phe	Tyr	TraI
SCRI1043	ICEPa.2		tRNA-Asn	Phage	MOBQ

36A	ICEPa.1	NZ_CP024956.1			
36A	ICEPa.4		tRNA-Asn		MOBC

Pectobacterium carotovorum

Strain	Element	RefSeq	Insertion site	Integrase	Relaxase
JR1.1	ICEPc.1	NZ_CP034237.1	tRNA-Asn	Phage	MOBC
BP201601.1	ICEPC.2	NZ_CP034236.1		Tyr	

Agrobacterium tumefaciens

Strain	Element	RefSeq	Insertion site	Integrase	Relaxase
1D1609 (Cromossomo I)	ICEAt.1	NZ_CP026924.1	guaA	Tyr	MOBP
12D1 (Cromossomo circular)	ICEAt.2	NZ_CP033031.1	tRNA-Met	Recombinase	-
12D1 (Cromossomo linear)	ICEAt.3	NZ_CP033032.1			MOBP
186(Cromossomo circular)	ICEAt.4	NZ_CP042274.1	tRNA-Arg		

Pseudomonas syringae

Strain	Element	RefSeq	Insertion site	Integrase	Relaxase
B728a	ICEPs.1	NC_007005.1		Tyr	MOBH
Shaanxi_M228	ICEPs.2	NZ_CP032631.1	tRNA-Pro		
Shaanxi_M228	ICEPs.3		tRNA-Lys		
Shaanxi_M228	ICEPs.4				
Shaanxi_M228	ICEPs.5				
NZ-45 (ICMP 20586)	ICEPs.2	NZ_CP017007.1			
NZ-45 (ICMP 20586)	ICEPs.6				
NZ-45 (ICMP 20586)	ICEPs.7				
NZ-45 (ICMP 20586)	ICEPs.8				
MAFF212063	ICEPs.9	NZ_CP024712.1	tRNA-Pro		
MAFF212063	ICEPs.10				-
ICMP 18708	ICEPs.2	NZ_CP012179.1			

ICMP 18884	ICEPs.7				
CRAFRU14.08	ICEPs.2				
CRAFRU14.08	ICEPs.11	NZ_CP019732.1	tRNA-Lys		
CRAFRU14.08	ICEPs.12				
NZ-47	ICEPs.2				
NZ-47	ICEPs.7	NZ_CP017009.1	tRNA-Pro		
NZ-47	ICEPs.13				
CRAFRU12.29	ICEPs.2				
CRAFRU12.29	ICEPs.11	NZ_CP019730.1	tRNA-Lys		
CRAFRU12.29	ICEPs.7		tRNA-Pro		
P155/P220	ICEPs.14	NZ_CP032871.1	tRNA-Lys		
P155/P220	ICEPs.2				
CFBP6109	ICEPs.15	NZ_LT963391.1			
CFBP3840	ICEPs.16	NZ_LT963409.1	tRNA-Pro		
HS191	ICEPs.17	NZ_CP006256.1			
PP1	ICEPs.18	NZ_CP034078.1			
CC1557	ICEPs.19	NZ_CP007014.1	tRNA-Gly	Phage	
UB303	ICEPs.20	NZ_CP047267.1	tRNA-Ile	Tyr	-

Xanthomonas campestris

Strain	Element	RefSeq	Insertion site	Integrase	Relaxase
B100	ICEXc.1	NC_010688.1	tRNA-Arg	Phage	MOBH
3811	ICEXc.1	NZ_CP025750.1			
CN03	ICEXc.2	NZ_CP017308.1	tRNA-Gly		

Xylella fastidiosa

Strain	Element	RefSeq	Insertion site	Integrase	Relaxase
9a5c	ICEXf.1	NC_002488.3	ABC-F family ATP-binding cassette	Tyr	

1a12	ICEXf.3	NZ_CP009823.1	tRNA-Lys	Tyr	
U24D	ICEXf.1	NZ_CP009790.1	ABC-F family ATP-binding cassette	Tyr	

Ralstonia pseudosolanacearum

Strain	**Element**	**RefSeq**	**Insertion site**	**Integrase**	**Relaxase**
GMI1000	Tn4371	NC_003295.1	purM	Ser	MOBP
RS476	Tn4371	NZ_CP021762.1			
CRMRs218	Tn4371	NZ_CP021764.1			
FJAT-91	ICERps1	NZ_CP016612.1	tRNA-Lys	Tyr	
FQY_4	ICERps2	NC_020799.1			
YC40-M	ICERps2	NZ_CP015850.1			
HA4I	ICERps3	NZ_CP022481.1	tRNA adenosine		
UW386	ICERps4	NZ_CP039339.1	tRNA-Lys		
FJAT91-F1	ICERps1	NZ_CP056083.1			
FJAT91-F8	ICERps1	NZ_CP056085.1			

Ralstonia syzyzii

Strain	**Element**	**RefSeq**	**Insertion site**	**Integrase**	**Relaxase**
T98	ICERsy1	NZ_CP022759.1	guaA	Tyr	MOBP
SL3175	ICERsy1	NZ_CP022788.1		Tyr	

Supplementary table 15: Putative significant cargo genes carried by ICEs characterized in this work

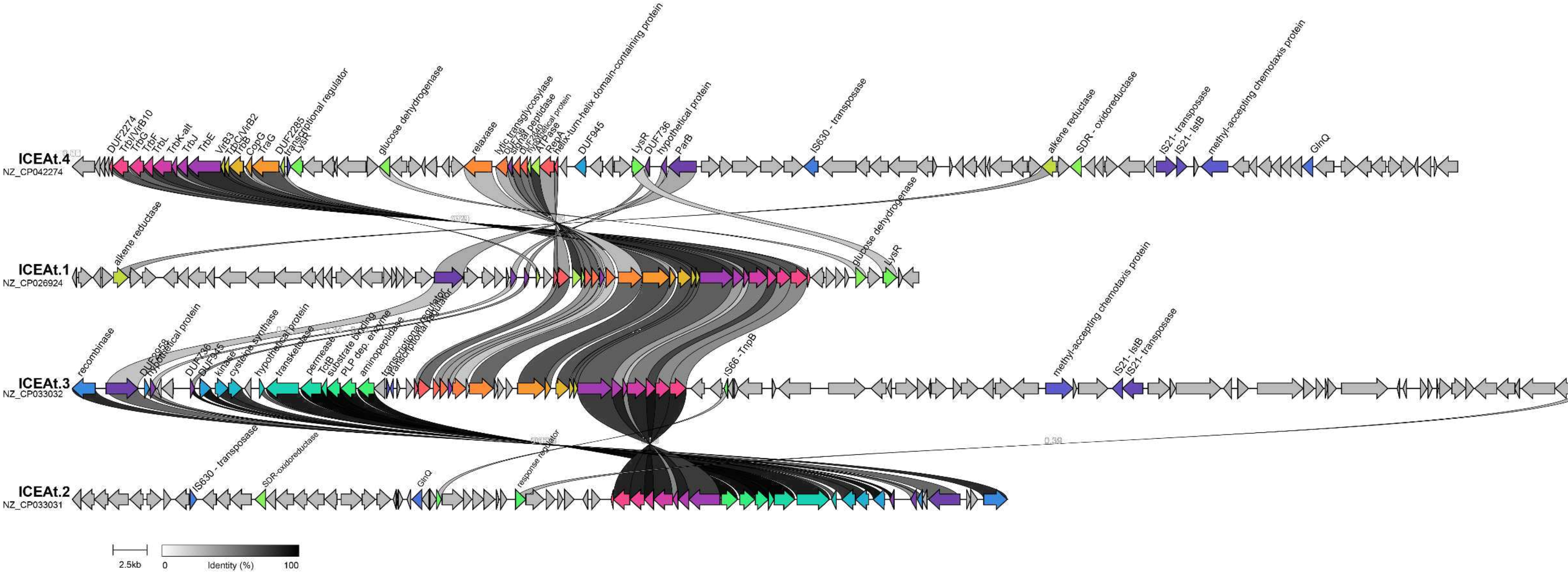
Element	Gene	Putative Function
ICEAt.1	Alkene reductase Glutathione S-transferase	Adaptation
	Cysteine hydrolase Glycosidase	Virulence
ICEAt.3	Universal stress protein aqpZ	Adaptation
	Diguanylate cyclase Invasion associated locus B	Virulence
	L.D - transpeptidase	Antibiotic Resistance
ICEAt.4	Endo-1,4-beta-xylanase Isochorismatase family protein	Virulence
	MBL fold metallo-hydrolase	Antibiotic Resistance
ICEDd.1	tssI - VgrG Hcp effector	Virulence
	TerB	Tellurium resistance
	EexN	Entry exclusion
ICEDd.2	PAAR domain containing protein tssI - VgrG Hcp effector	Virulence
ICEDs.1	tssI - VgrG Hcp effector Amidohydrolase	Virulence
ICEPa.1	Coronafacic acid dehydratase Coronafacic acid synthetase Coronafacic acid synthetase component Coronafacate ligase	Virulence
	Type I polyketide synthase	Secondary metabolism
ICEPa.2	EexN	Entry exclusion
	Phospholipase D	Virulence
ICEPa.3	Glutathione S-transferase	Adaptation
	MBL fold metallo-hydrolase	Antibiotic Resistance
ICEPa.4	Arginase family protein	Virulence
	MipA/OmpV family protein	Antibiotic Resistance
	EexN	Entry exclusion
ICEPc.1	EexN	Entry exclusion
ICEPc.2	Glutathione peroxidase	Antioxidant Resistance
	MBL fold metallo-hydrolase	Antibiotic Resistance
ICEPs.1	Copper resistance cluster	Metal resistance

Virulence

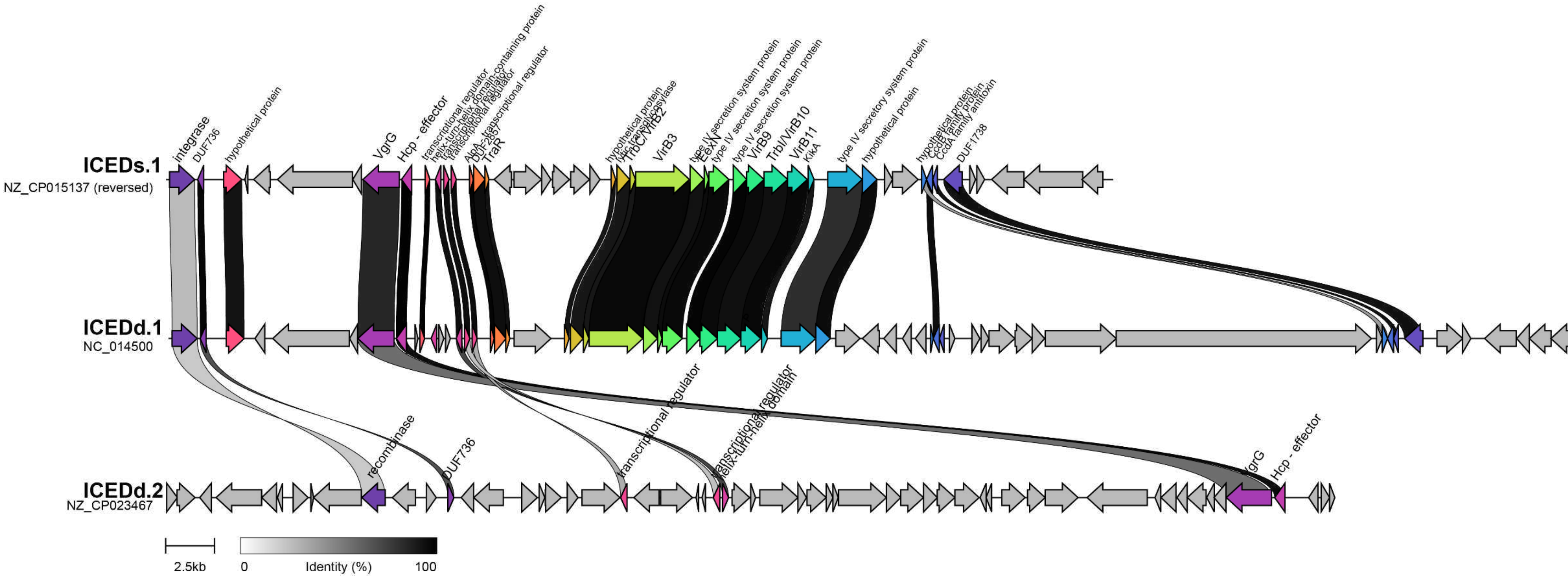
	Arsenic resistance cluster	Arsenic resistance
	CrpP family protein	Antibiotic Resistance
ICEPs.2	Tellurium resistance cluster	Tellurium resistance
	Trehalose phosphatase	Adaptation
	PSPTO0907 Type III secretion system effector phosphothreonine lyase Cellulase family glycosylhydrolase NUDIX hydrolase Alginate lyase Glycoside hydrolase family 32 protein Type III effector HopR1 Effector protein Type III effector Type III chaperone protein ShcF	Virulence
ICEPs.3	DsbA family protein Type VI secretion system tube protein Hcp SEC-C domain-containing protein	Virulence
	CrpP family protein	Antibiotic Resistance
ICEPs.4	Arsenic resistance cluster	Arsenic resistance
	Ultraviolet light resistance protein RulA umuC	UV Resistance
	CrpP family protein	Antibiotic Resistance
ICEPs.5	AvrRpm1	Avirulence
	Type III chaperone protein ShcF Type III effector Peptidase C55 Type III secretion system effector	Virulence
ICEPs.6	PapB Adhesin biosynthesis transcription regulatory protein	Virulence
	CrpP family protein	Antibiotic Resistance
ICEPs.7	TonB-dependent siderophore receptor	Adaptation
	AvrRpm1	Avirulence
	Peptidase C55 Type III secretion system effector	Virulence
ICEPs.8	Copper resistance cluster	Metal resistance
	Arsenic resistance cluster	Arsenic resistance
	CrpP family protein	Antibiotic Resistance
ICEPs.9	Type III effector	Virulence
	HopAU1	Avirulence
	CrpP family protein	Antibiotic Resistance
ICEPs.10	gstA MOSC domain-containing protein	Adaptation
	PSPTO0907	

Supplementary table 16: Elements of this work described in the literature

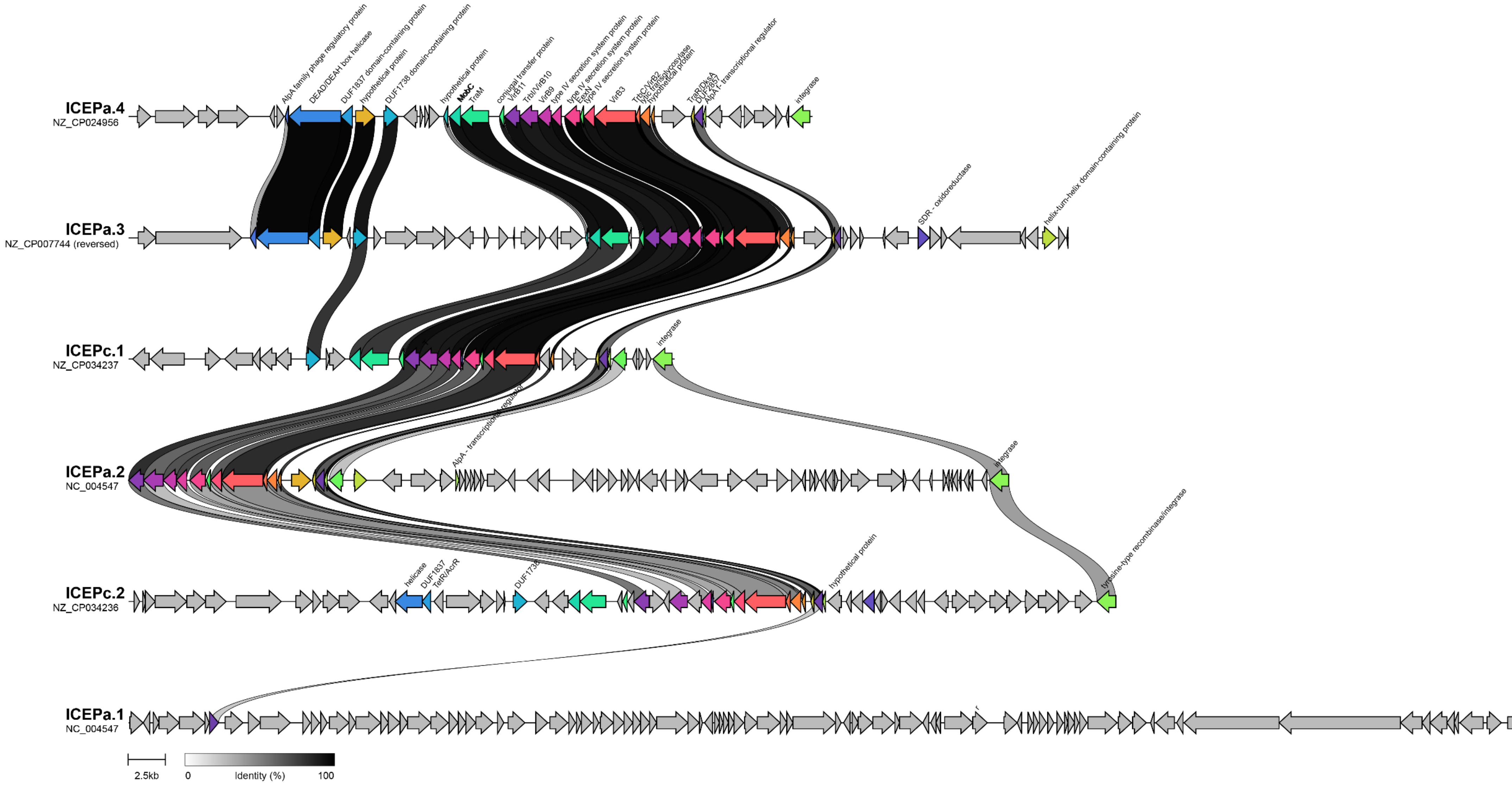
Element	Name	Reference
ICEDd.1	Tn4371	Toussaint et al., 2003
ICEPa.1	HAI2	Bell et al., 2004
ICEXf.2	-	Denancé et al., 2019
ICEPs.1	PsyrGI-6	Feil et al, 2005
ICEPs.6	Pac_ICE1	Butler et al, 2013
ICEPs.4	Pac_ICE2	Butler et al, 2013
ICEPs.8	Psa$_{\mathrm{NZ45}}$ICE_Cu	Colombi et al, 2017
ICEPs.7	Psa$_{\mathrm{NZ13}}$ICE_eno	Colombi et al, 2017
ICEPs.13	Psa$_{\mathrm{NZ47}}$ICE_Cu	Colombi et al, 2017
Tn4371	Tn4371	Salanoubat et al., 2012
ICERps1	ICERps1	Gonçalves et al., 2020
ICERps2	ICERps2	Gonçalves et al., 2020
ICERps3	ICERps3	Gonçalves et al., 2020
ICERsy1	ICERsy1	Gonçalves et al., 2020



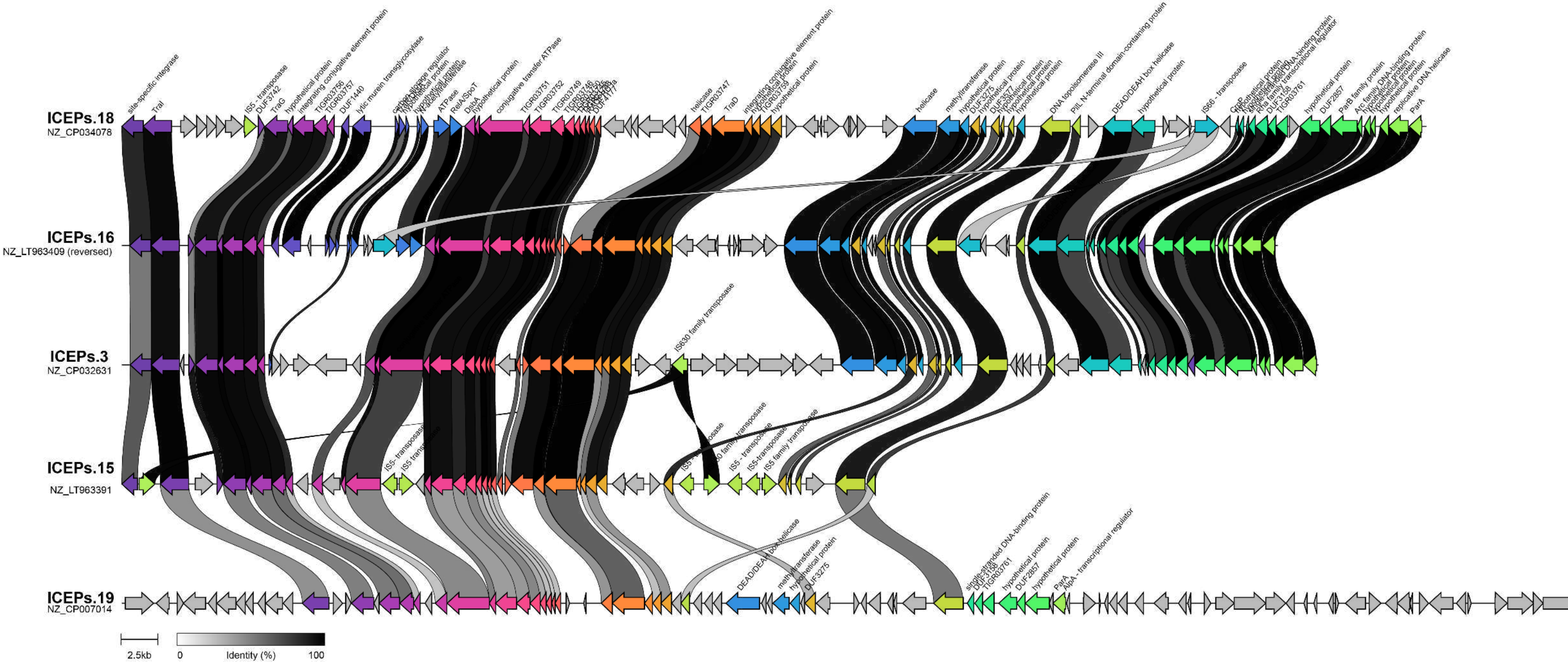
Supplementary Figure 1*:* Gene clusters comparision of *Agrobacterium tumefaciens* ICEs. ORFs are represented by arrows, colloured arrows representsdifferent synthenic gene clusters, genes products are indicated in the representative ORF arrow.



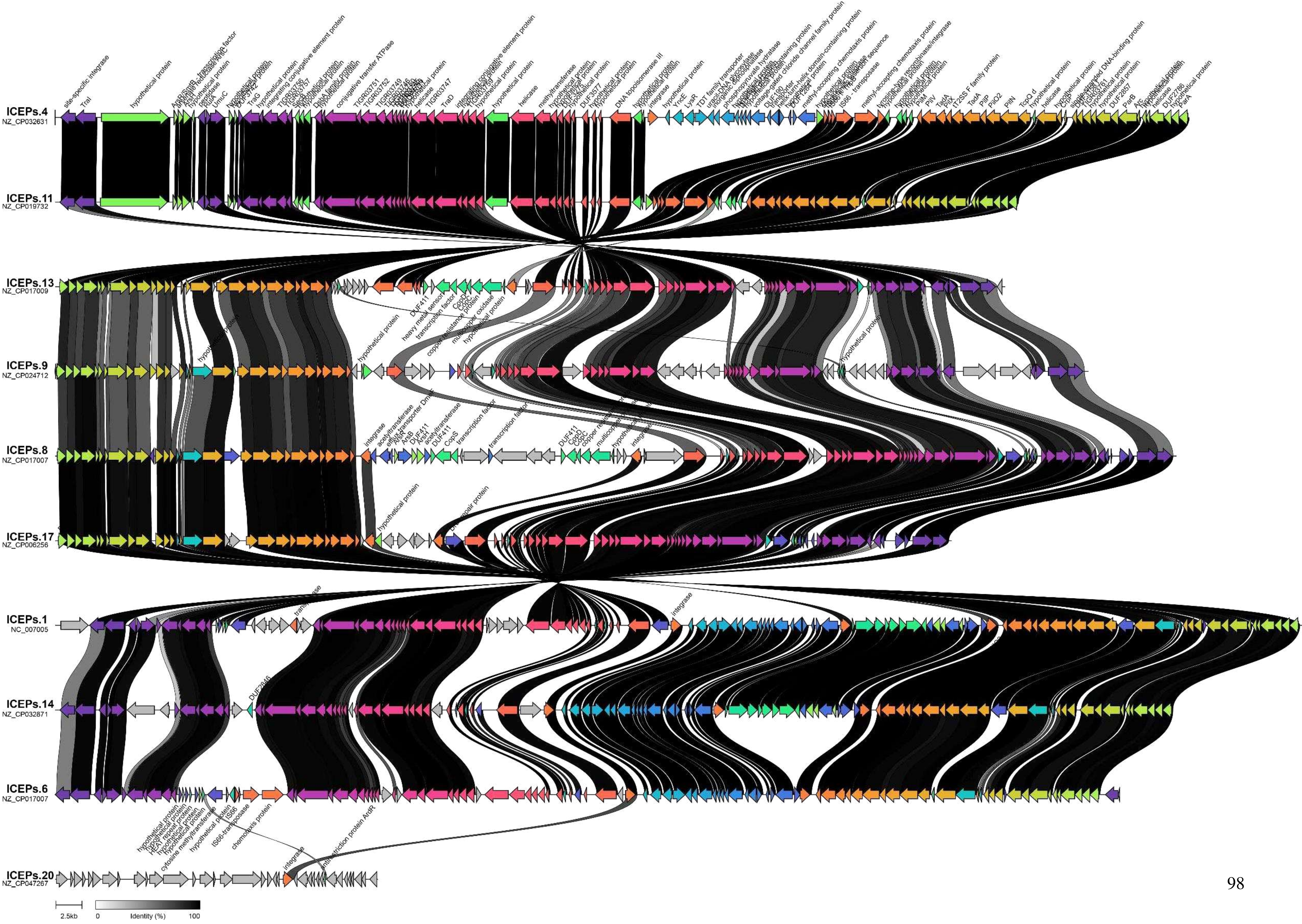
Supplementary Figure 2*:* **Gene clusters comparision of *Dickeya* ICEs.** ORFs are represented by arrows, colloured arrows represents different synthenic geneclusters, genes products are indicated in the representative ORF arrow.



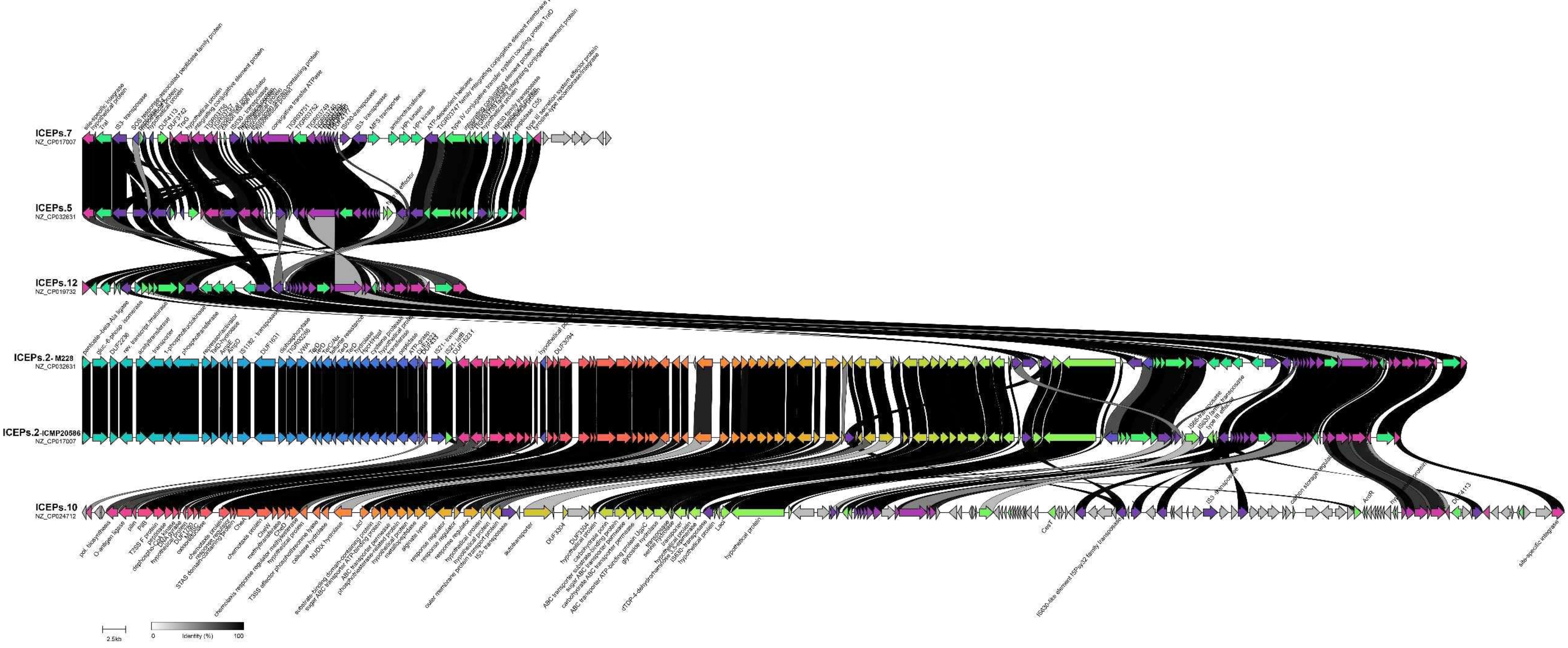
Supplementary Figure 3*:* **Gene clusters comparision of *Pectobacterium carotovorum* (and *atrosepticum*) ICEs.** ORFs are represented by arrows,colloured arrows represents different synthenic gene clusters, genes products are indicated in the representative ORF arrow.



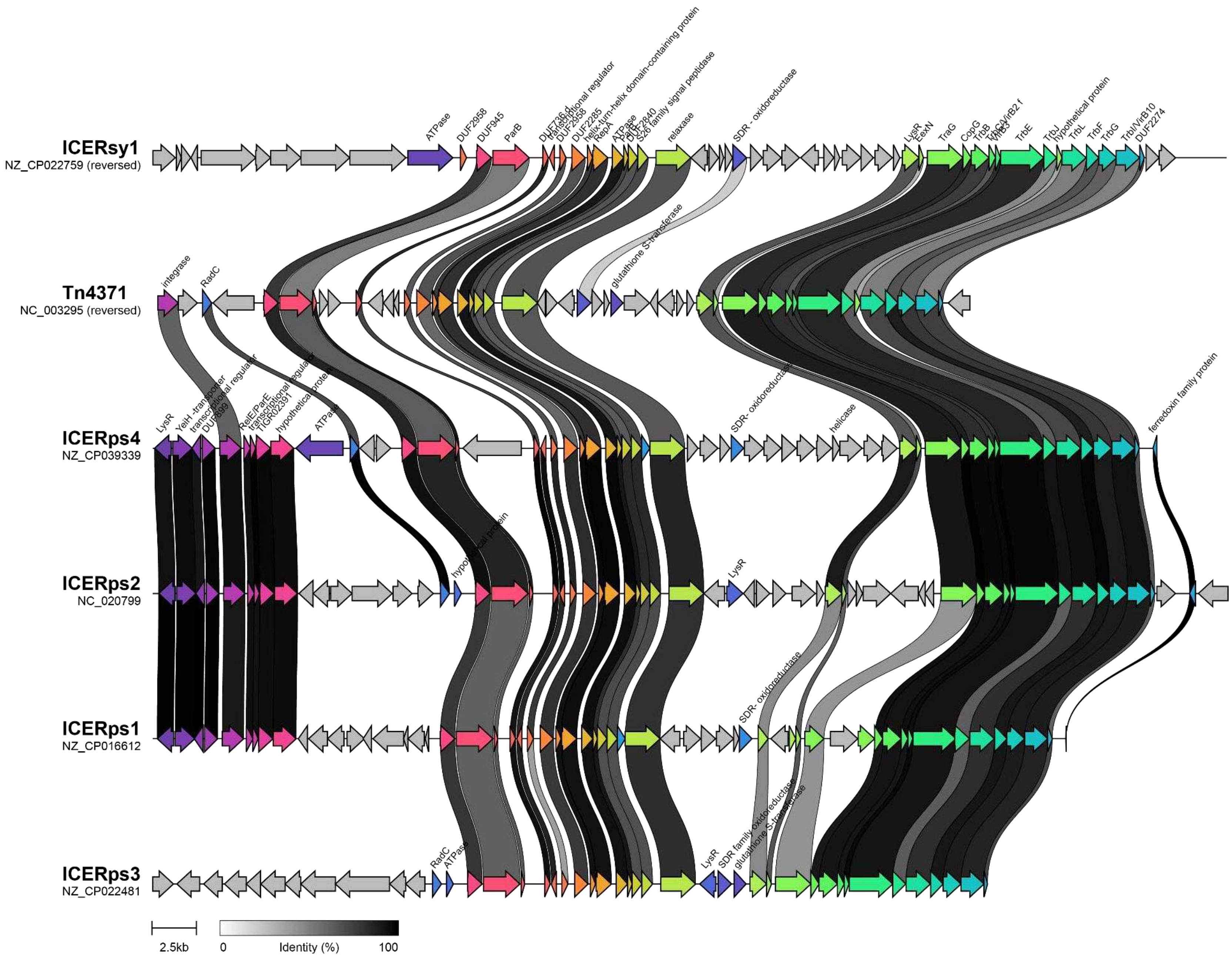
Supplementary Figure 4*:* **Gene clusters comparision of group 1 *Pseudomonas syringae* ICEs.** ORFs are represented by arrows, colouredarrows represents different synthenic gene clusters, genes products are indicated in the representative ORF arrow


ICEPs.4
NZ_CP032631
site-specific integrase
TraI
hypothetical protein
ArsR/SmtB - transcription factor
arsenate reductase ArsC
ArsH
hypothetical protein
peptidase
RulA
UmuC
hypothetical protein
DUF3742
TraG
integrating conjugative element protein
TIGR03756
TIGR03757
SecB
DsbA family protein
conjugative transfer ATPase
TIGR03751
TIGR03752
TIGR03749
TIGR03746
helicase
TIGR03747
TraD
TIGR03759
methyltransferase
DUF3275
DUF3577
DNA topoisomerase III
integrase
YncE
LysR
TDT family transporter
uracil-DNA glycosylase
inorganic diphosphatase
phosphopyruvate hydratase
voltage-gated chloride channel family protein
DUF190
transporter
helix-turn-helix domain-containing protein
DUF1264
methyl-accepting chemotaxis protein
IS66 - transposase
tyrosine-type recombinase/integrase
PilM
PilV
TadA
PilX
tT2SS F family protein
PilP
PilO2
PilN
TcpQ d
CrpP
single-stranded DNA-binding protein
TIGR03761
DUF2857
ParB
Arc
DUF2786
ParA
ICEPs.11
NZ_CP019732
ICEPs.13
NZ_CP017009
DUF411
heavy metal sensor
transcription factor
CopD
CopC
copper resistance protein
multicopper oxidase
ICEPs.9
NZ_CP024712
ICEPs.8
NZ_CP017007
acetyltransferase
efflux transporter DmeF
ArsR
ArsB
ArsH
CopS
copper resistance protein
ICEPs.17
NZ_CP006256
DNA repair protein
ICEPs.1
NC_007005
transposase
ICEPs.14
NZ_CP032871
DUF2846
ICEPs.6
NZ_CP017007
HEAT repeat protein
cytosine methyltransferase
IS66
IS66-transposase
chemotaxis protein
antirestriction protein ArdR
ICEPs.20
NZ_CP047267
2.5kb
0
Identity (%)
100

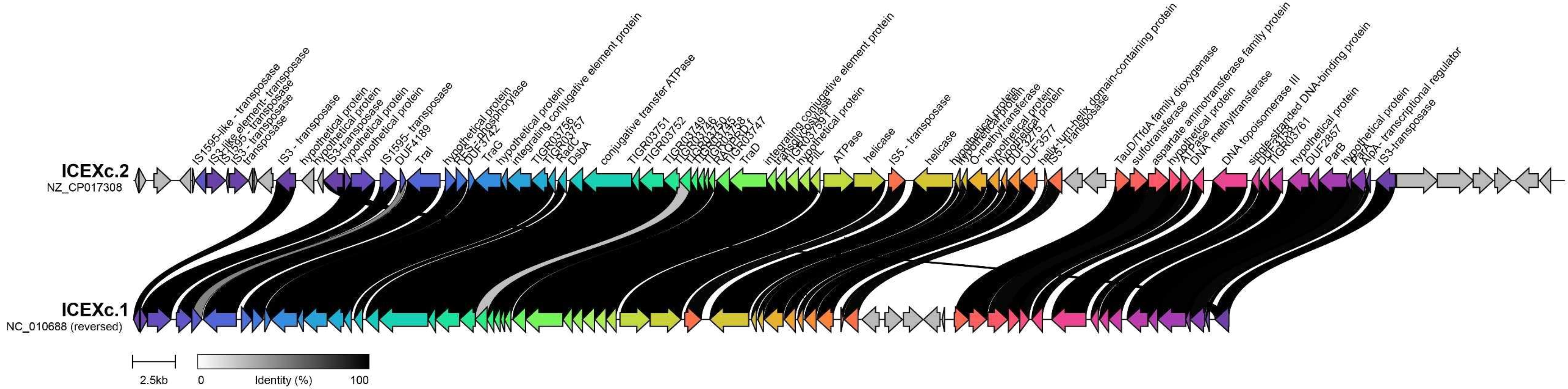
Supplementary Figure 5*:* Gene clusters comparision of group 2 *Pseudomonas syringae* ICEs. ORFs are represented by arrows, collouredarrows represents different synthenic gene clusters, genes products are indicated in the representative ORF arrow.



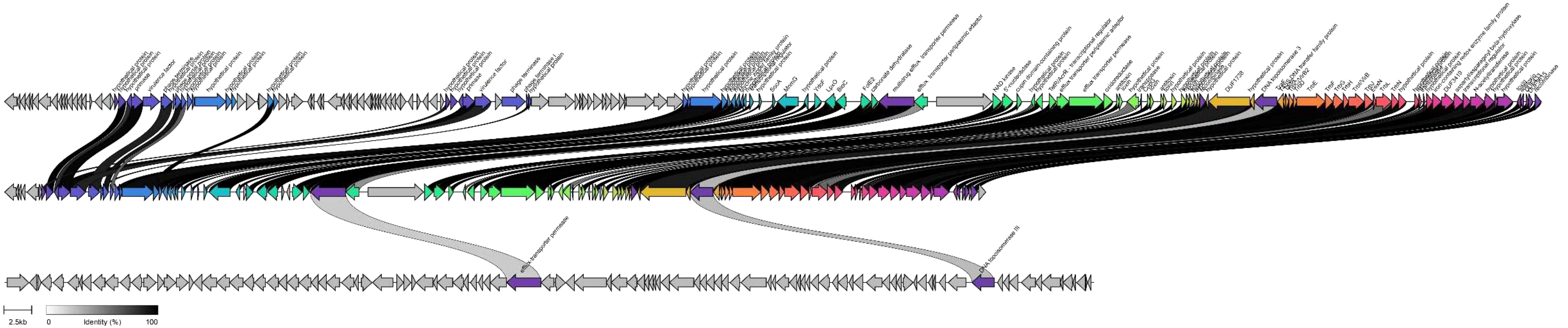
Supplementary Figure 6*:* Gene clusters comparision of group 3 *Pseudomonas syringae* ICEs. ORFs are represented by arrows, collouredarrows represents different synthenic gene clusters, genes products are indicated in the representative ORF arrow.



Supplementary Figure 7*:* Gene clusters comparision of RSSC ICEs. ORFs are represented by arrows, colloured arrows represents different synthenic geneclusters, genes products are indicated in the representative ORF arrow.



Supplementary Figure 8*:* Gene clusters comparision of *Xanthomonas campestris* ICEs. ORFs are represented by arrows, colloured arrows representsdifferent synthenic gene clusters, genes products are indicated in the representative ORF arrow.rs, genes products are indicated in the representative ORF arrow.



Supplementary Figure 9*:* Gene clusters comparision of *Xylella fastidiosa* ICEs. ORFs are represented by arrows, colloured arrows represents differentsynthenic gene clusters, genes products are indicated in the representative ORF arrow.rs, genes products are indicated in the representative ORF arrow